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The Neuropsychology and Functional Anatomy of Timing

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Thesis submitted for the degree of Doctor of Philosophy
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Abstract

This thesis explores the neural correlates of motor and perceptual timing. Motor timing involves the production of a timed movement (e.g. dancing), whereas perceptual timing requires a perceptual judgement (e.g. deciding which of two events lasted longer). A body of research has investigated this type of timing, concentrating on millisecond- and seconds-range intervals. Patients with Parkinson's disease (PD) and cerebellar pathology exhibit motor and perceptual timing deficits, which has led to the suggestion that both the basal ganglia and cerebellum are involved in this type of temporal processing.

The research presented here uses a variety of techniques (functional imaging, transcranial magnetic stimulation (rTMS) and clinical studies on patients) to investigate the contribution of different neural structures to temporal processing. Using positron emission tomography (PET), the basal ganglia and cerebellum were both found to be active during millisecond- and seconds-range timing. However, only the basal ganglia were active when non-temporal aspects of the task were controlled for. At the cortical level, rTMS was used to show that the right dorsolateral prefrontal cortex was essential to the reproduction of seconds-range intervals, possibly due to a role in memory processes. In a further study, the motor and perceptual timing performance of patients with PD was modulated by dopaminergic medication, with medication improving performance. Patients with cerebellar disease displayed increased variability in timing tasks that included a significant motor component, but did not show impaired accuracy. A second PET study, comparing patients with PD and healthy controls, showed that the basal ganglia were active during motor timing for the control group. Compared to their medicated state, the patients showed decreased coupling between the basal ganglia and dorsolateral prefrontal cortex when 'off' medication. These studies support the notion that the basal ganglia, and not the cerebellum, play a fundamental role in motor and perceptual timing.

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Abbreviations[1]

ACh	Acetylcholine
ADHD	Attention Deficit/Hyperactivity Disorder
AGM	Attentional gate model
ANOVA	Analysis of variance
BA	Brodmann Area
BDI	Beck Depression Inventory
BeT	Behavioural theory of timing
CD	Cerebellar disease
CNV	Contingent negative variation
CV	Clock variance
DBS	Deep brain stimulation
DLPFC	Dorsolateral prefrontal cortex
EEG	Electroencephalography
EMG	Electromyography
ERP	Event related potential
FDI	First dorsal interosseus muscle
fMRI	Functional magnetic resonance imaging
FWE	Familywise error rate
GLM	General linear model
GPi	Internal segment of the globus pallidus
Hz	Hertz
ILOCA	Idiopathic late onset cerebellar ataxia
IRI	Inter-response interval
ISI	Inter-stimulus interval
IVU	Intravenous urograph
LED	Light emitting diode
LTE	Left temporal lobe epilepsy
MMSE	Mini-Mental State Examination
MNI	Montreal Neurological Institute

[1] excluding abbreviations in tables and figures

MRI	Magnetic resonance imaging
ms	Milliseconds
MTS	Multiple time scale model
MSA	Multiple system atrophy
MV	Motor delay variance
N	Newton
NART	National Adult Reading Test
NMDA	N-methyl-D-aspartate
PASAT	Paced Auditory Serial Addition Test
PD	Parkinson's disease
PET	Positron emission tomography
PPI	Psychophysiological interaction
preSMA	Pre-supplementary motor area
RAM	Resource allocation model
RFX	Random effects analysis
RT	Reaction time
RTE	Right temporal lobe epilepsy
rCBF	Regional cerebral blood flow
rTMS	Repetitive transcranial magnetic stimulation
s	Seconds
SBF	Striatal beat frequency model
SD	Standard deviation
SE	Standard error
SET	Scalar expectancy theory
SMA	Supplementary motor area
SNc	Substantia nigra pars compacta
SPM	Statistical parametric mapping
TDT	Temporal discrimination threshold
TMS	Transcranial magnetic stimulation
TV	Total variance
UPDRS	United Parkinson's Disease Rating Scale
WDIN	Wellcome Department of Imaging Neuroscience

Chapter 1

Introduction

Timing is an extremely broad-ranging topic and encompasses biological rhythms through to psychological conceptions of days, months and years. The biological timing systems encompass such oscillatory phenomena as circadian rhythms, brain waves and heart rate and are defined by their precision and accuracy. Perception and judgement of long periods of time, implicitly linked to episodic memory, are relatively inaccurate and subject to considerable variance. The focus of this thesis, and of a considerable body of published research, is on temporal processing within the milliseconds- and seconds-range. This type of timing contributes to the neurophysiological control of movement, for example, enabling us to calculate the split-second adjustments needed for catching a ball, allowing an orchestra to play with synchronicity and ensuring that we dance in complement to music. Timing within this range is not exclusively motoric; subconsciously learnt timing information pervades behaviour (e.g. predicting when a traffic light is about to change) and perceptual timing judgements (e.g. deciding if a kettle might have boiled, returning to watch TV after a commercial break) are a part of everyday life. Both motor and perceptual timing can be conscious or subconscious, depending on the length of interval being timed (i.e. below a certain threshold, time intervals are said to be inaccessible to conscious control) and the type of task or event involved (for example, the timing involved in playing a much-loved piece of music compared to learning a piece for the first time).

By means of an introduction to this topic, this chapter serves to explain the principal research findings in this area, including clinical, pharmacological and functional imaging findings as well as a broad overview of models of timing. The work contained in this thesis is primarily focused on exploring the neuroanatomical location of 'clock' like processes. As the basal ganglia and cerebellum are most commonly hypothesised to provide such a function and the differential roles of these two structures are explored throughout this thesis,

these two structures are focused on in this review. To start, the types of tests used to assess timing performance will be described.

1.1 MEASURING TECHNIQUES AND PROCEDURES

Many different motor and perceptual tasks have been used to capture timing accuracy and variability and are cited throughout this thesis. Accuracy refers to how near (e.g. in milliseconds) to the target an estimated duration is. This is most commonly described by the mean score across a set of trials. A mean score that is an under- or overestimation of the target interval can also be described as a response bias. Accuracy can also be represented by an absolute error score, which is the averaged difference on each trial between the estimated duration and the target duration, regardless of the direction of the error (or bias). Variability is a measure of how varied the repeated estimates of a target duration are, so is an expression of how much difference there is between the values that form the mean accuracy score (or put another way, how well the mean represents the data). In this thesis, variability is most commonly represented by the standard deviation score. Variability across data can be either random (i.e. not following a systematic pattern) or systematic (i.e. caused by a consistent error that comes from a fixed source), for example drift. It is important to consider both accuracy and variability in fully characterising a temporal response. For ease of reference, a description of the most commonly used tasks is included below. It has previously been hypothesised (e.g. Keele et al, 1985) that both types of task are underpinned by a common neural timing mechanism. Keele et al (1985) found a correlation between motor and perceptual timing performance in healthy subjects. Furthermore, the occurrence of both motor and perceptual timing deficits have been observed in patient populations (e.g. Harrington et al, 1998; Ivry and Keele, 1989; Pastor et al, 1992ab).

1.1.1 Motor timing tasks

1.1.1.2 Repetitive tapping

The only widely recognised measure of motor timing is the repetitive tapping paradigm whereby the subject produces a regularly paced rhythm, most commonly with the right index finger. Typically, the subject engages in two tapping phases: the synchronisation phase and the continuation phase. During the synchronisation phase the subject taps in synchrony with a series of tones presented at regular intervals, this enables the frequency to become entrained. After a criterion number of taps, the pacing stimulus is switched off and the subject attempts to maintain the frequency unaided (e.g. Harrington et al, 1998a; Ivry and Keele, 1989; Ivry et al, 1988; Pastor et al, 1992a).

A model devised by Wing and Kristofferson (1973ab) has been used to break down the variance of the inter-response interval during the un-paced continuation phase into variance associated with a central clock and variance associated with motor implementation. In brief, the model proposes that two independent processes underlie timed movements: a central clock and a peripheral motor implementation system. The clock, entrained to the rate of the pacing stimulus, emits a pulse each time the target interval has elapsed, with the clock intervals subject to random temporal variance (clock variance: CV). Emission of a pulse activates the motor implementation system, which executes the motor command. The lag between pulse emission and the motor response is termed the motor delay, which is also subject to random temporal variation (motor delay variance: MV). The model rests on two key assumptions, the independence of the clock and motor components as separate processes and the independence of successive clock intervals and of successive motor delays. The inter-response interval (IRI) between successive taps is the sum of the associated clock interval plus the difference between the motor delays of the current and previous responses. Total (IRI) variance (TV) can be calculated using the simple formula TV = CV + 2MV. The model will be discussed in detail in Chapter 5.

Synchronisation and continuation tapping involve different processes, indeed synchronisation is often studied in isolation (e.g. Lejeune et al, 1997; Peters et al, 1989; Pressing, 1998; Repp, 2003; Vorberg and Wing, 1996). As described by O'Boyle (1997), synchronising to a beat involves i) evaluating, representing and re-producing the interval provided by the metronome, ii) predicting the times of occurrence of both the metronome beat and the associated tap, iii) perceiving any temporal asynchrony between metronome beat and tap and iv) regulating subsequent performance on the basis of that performance. As such, error correction is a considerable demand of this type of timed movement, with 'synchronisation error' describing the difference between response onset and the time of the metronome beat. On the other hand, Wing and Kristofferson do not conceptualise a feedback loop within their model of continuation tapping. An extensive literature has described the corrective processes that enable synchronised tapping (e.g. Pressing, 1998; Repp, 2002, Repp, 2003; Vorberg and Wing, 1996). Synchronisation error tends to be negative (i.e. response onset precedes the metronome beat), one suggestion for this is that undershooting the target enables variance to be kept to a minimum (Vorberg and Wing, 1996), with the Paillard-Fraisse hypothesis (Fraisse, 1980; Paillard, 1949) suggesting that the 'perceptual latency' of sensory inputs varies with modality, such that response onset has to occur before the metronome beat for them to be subjectively judged as occurring at the same time. The two types of tapping can also be compared in terms of internal versus external modulation of temporal processing. The continuation task makes greater demands on internal timing as there is no external guidance for the rhythm being produced.

1.1.2 Perceptual timing tasks

This thesis classifies a perceptual timing task as any task that does not fit the criterion of a classic motor timing task i.e. that does not involve regular, repetitive timed movements. Some of the perceptual timing tasks require that the temporal decision be executed via a motor response (e.g. the subject judges a 3 s period by pressing a button) that is integral to the timing decision. This brings in a motor element. This can be particularly problematic when testing patient groups with motor deficits as the (typically) slowed motor

response can introduce an additional variable to the data. However, some tasks allow a temporal decision to be communicated without relying on a timed movement; this is a very pure test of perceptual timing.

1.1.2.1 Duration discrimination

Traditionally, this task involves the subject listening to two intervals in the milliseconds-range, the first being a standard duration and the second (a comparison interval) varying from the standard by a specified amount. The subject has to decide if the comparison interval is longer or shorter than the standard (e.g. Harrington et al, 2004a; Ivry and Keele, 1989; Mangels et al, 1998). Commonly, parameter estimation by sequential testing (PEST) (Pentland, 1980), an adaptive psychophysical procedure, is used. This technique uses the subject's previous response to determine the length of a comparison interval presented on a given trial. Upper and lower thresholds can be calculated in which the subjects responds correctly ('longer' or 'shorter', respectively) on a criterion percentage of trials. The average of these two thresholds is the point of subjective equality, i.e. the comparison interval at which subjects are equally likely to respond short or long. This produces a measure of accuracy and indicates a bias towards over- or underestimation.

1.1.2.2 Temporal discrimination threshold

The temporal discrimination threshold (TDT) technique measures the subject's ability to temporally discriminate between two closely occurring stimuli. In a typical example, subjects listen to very short pairs of stimuli (e.g. 0.2 ms clicks), separated by very short millisecond intervals; the smallest interval between the stimuli that still allows the subject to recognise them as separate is taken as the TDT (e.g. Artieda et al, 1992). This task simply requires perception of the temporal qualities of a sensory input and, unlike the majority of perceptual timing tasks, there are minimal cognitive demands (e.g. no involvement of temporal memory). It has been argued that TDT engages brain regions involved in temporal processing (Nichelli, 1993). However, it may be that the TDT is a distinct temporal task, dependent primarily on auditory processing, which is largely unrelated to the timing processes tapped by other perceptual timing tasks (for further discussion see page 65).

1.1.2.3 Time estimation

In this type of task the subject is presented with a temporal interval (e.g. marked with an onset and offset stimulus) and is asked to estimate its duration (e.g. to the nearest second) (e.g. Koch et al, 2002).

1.1.2.4 Time reproduction

A time reproduction task is an estimation task in which the subject has to reproduce a target interval that has previously been presented to them. For example, the subject judges the length of a visual cue (Estimation Phase) and when the visual cue disappears starts to reproduce the interval, pressing a response button when they think that an identical period of time has elapsed (Reproduction Phase) (e.g. Pastor et al, 1992b).

Time estimation and time reproduction tasks can also be divided by whether they are *retrospective* (the subject is asked to time an interval that has passed, such as time taken to complete a task, that they were not asked to time) or *prospective* (the subject is aware that time estimation will be asked of them and have attended to the interval) (e.g. Brown, 1985; Hicks, et al 1976; Predebon, 1995).

1.1.2.5 Time production

Unlike time reproduction, a time production task requires the subject to produce a period of time that they have not been given an example of. For example, a subject might be asked to press a button when they think that 20 seconds have passed. This task is similar to the time estimation task since, unlike the reproduction task, it is not based on the accuracy of temporal memory but is a measure of subjective time sense.

The intervals for time estimation, time reproduction and time production can be metered by internal counting (e.g. Pastor et al, 1992b), filled with distracter stimuli to inhibit counting (e.g. Koch et al, 2003) or have counting neither directly encouraged nor inhibited (e.g. Wahl and Sieg, 1980).

The following studies were developed for the study of timing performance in animals (typically rats), though all now have human corollaries. Typically, the durations are much shorter in the human studies, mainly to prevent chronometric counting. Procedures (e.g. distracter stimuli, such as reading random numbers) have been developed for human subjects to minimise counting at longer ranges (e.g. Rakitin et al, 1998; Malapani et al, 1998ab; 2002).

1.1.2.6 Peak-interval procedure

The peak-interval procedure (Roberts, 1981) consists of fixed interval and probe trials. During fixed interval trials, a sound or light signal is introduced and the animal is rewarded with a food pellet when it presses the response lever after a fixed interval (e.g. 20 s) of the signal has elapsed; the lever press also terminates the signal. On approximately half the trials (probe trials), the food reward is not made available after the fixed interval elapses, regardless of the number of lever presses made, and the signal will typically last at least three-to-four times the duration of the fixed interval. Thus, the probe trials are a measure of the animal's ability to predict the time of the food reward. For these trials, a response-rate function is generated that plots the number of responses as a function of time since stimulus onset (normally divided into time bins). The time at which maximum responding occurs is the peak time, and reflects the animal's judgement of the fixed interval. Rakitin et al (1998) produced a human equivalent of the peak-interval procedure by requiring subjects to press a button as many times as necessary around the time they thought the fixed interval had elapsed, with the aim of placing a response at the exact time. Malapani et al (1998ab; 2002) produced something similar, although the subjects were asked to press the button just before they thought that the fixed interval would elapse and to hold it down until after they thought it would have ended.

1.1.2.7 Temporal bisection

In the temporal bisection task (Church and Deluty, 1977), the animal is trained to discriminate between two durations (e.g. specified by a light or sound) of different length (e.g. 2 s and 8 s). The animal learns to press a certain lever in response to the short duration and to press a different lever in response to the

long duration. Selection of the correct lever delivers a food reward. During the testing phase the short and long intervals are presented (with a probability of 0.25) along with intervals of intermediate durations (spaced at equal logarithmic intervals between the two standard durations). None of the intermediate signals are rewarded. The data produces a psychometric function reflecting the probability of making a 'long' response as a function of stimulus duration. The bisection point (or point of subjective equality) is that duration at which long and short responses occur with equal probability. The task has been adapted for humans, whereby subjects have to classify each presented duration as more similar to the short or long interval (presented at the beginning of the trial) (Wearden, 1991). A modified version of the task does not present short and long standards (Wearden and Ferrara, 1995).

1.1.2.8 Temporal generalization

The temporal generalization task (Church and Gibbon, 1982) is very similar to the temporal bisection task, with only the decision process differing. Through food reward, the animal is trained to press a lever after the presentation of an interval (e.g. light or sound) of a specific length (the criterion duration). Following this, the animal is presented with a variety of durations, including the criterion duration and both shorter and longer intervals. If the animal presses a lever after hearing the criterion duration (typically occurring on 50% of trials) a food reward is delivered. No reward is delivered following a lever press in response to any of the other durations. The data can be plotted as a temporal generalization gradient, which illustrates the probability of a response as a function of signal duration i.e. with responses peaking at the reinforced duration. When testing humans (e.g. Wearden, 1992; Wearden et al, 1997), subjects are initially presented with examples of a standard duration. During the testing phase, a range of durations are presented consecutively with the standard duration being presented with a probability of 0.25. After each interval presentation the subject responds 'yes' if they believe that the interval is the standard duration and 'no' if they think otherwise. Feedback is given. Analogous to the animal data, the proportion of 'yes' responses for each presented duration are plotted to create a temporal generalization gradient.

1.1.2.9 Filled vs unfilled durations

Filled and unfilled durations refer to the manner of presentation of the stimuli that are to be timed and have been used by researchers looking at the ways in which timing judgements can be biased by stimulus factors. Whether a stimulus variable is filled or unfilled is a question that can be asked of virtually any of the tasks that have been previously outlined. To clarify, a filled interval is a period of time that is 'filled' in some way. Within the literature this has varied from a tone or simple visual display to asking the subject to carry out a task. An unfilled interval (sometimes referred to as an empty interval) is typically one in which nothing occurs, for example, onset and offset tones denote the boundaries of the interval but the actual interval is empty. Traditionally, it has been hypothesised that filled intervals are overestimated compared to unfilled intervals (e.g. Thomas and Brown, 1974), although certain studies have found the opposite effect, with unfilled intervals being judged longer (e.g. Zakay et al, 1983). A direct way of measuring filled and unfilled durations is to present a filled interval and an empty interval of equal duration and ask the subject which is longer. Or, timing accuracy can be compared for two different sets of stimuli, one filled and one unfilled, which are otherwise identical. Typically, researchers using a battery of tests should use either unfilled or filled durations so that comparisons between tasks are not affected by this fairly powerful variable.

1.1.2.10 Dual tasks

Another useful tool in delineating the mechanisms used in timing is the dual task paradigm, which is a measure of divided attention. The paradigm requires two tasks to be carried out concurrently and the extent to which the tasks interfere (e.g. degrade performance) is a measure of the extent to which the two tasks involve common processes. Typically, subjects are asked to make time estimates whilst simultaneously engaging in a non-temporal task (e.g. card sorting, anagram solving, mental arithmetic). This approach has helped researchers evaluate the independence or dependence of the timing mechanisms thought to be involved in the temporal task (e.g. Brown, 1997; Casini and Ivry, 1999; Hicks et al, 1977).

1.2 MODELS OF TEMPORAL PROCESSING

The purpose of this section is to describe the most prominent theoretical accounts of temporal processing. These theories seek to explain the type of timing behaviour evidenced in a wide range of paradigms in both humans and animals. Particular attention is given to the theory that over twenty years since its first conception still dominates the field: Scalar expectancy theory (SET). This model, and others before and since, presents the appealing hypothesis that an 'internal clock' directly meters the passing of time. SET has shaped peoples' conception of the cognitive and timing processes involved in the judgement of millisecond and seconds-range intervals and its influence can be seen in the interpretations afforded in many experimental, clinical and functional studies.

1.2.1 Scalar expectancy theory

Scalar expectancy theory (SET, Gibbon, 1977; Gibbon et al, 1984), sometimes referred to as scalar timing theory, is the most influential and widely cited model of temporal processing. It has stemmed from the observation in animal data (e.g. fixed interval trials) that the standard deviation of judgements of time grows in proportion to the mean of the interval being timed (Gibbon, 1977). This observation is known as the scalar property (or sometimes scalar timing). Framed a different way, the scalar property means that the coefficient of variation (standard deviation divided by the mean) remains constant across different timed intervals (a form of Weber's law) and that data obtained in the timing of different durations will superimpose when plotted on the same relative scale (superimposition).

SET has also been conceptualised within an information processing framework (Gibbon et al, 1984), which has been influenced by an earlier model by Treisman (1963). In this model the principals of the scalar property are combined with three processing stages: clock, memory and decision, to offer a more complete hypothesis of temporal processing (see Figure 1.1). The clock stage consists of a pacemaker that emits pulses, with the pacemaker being

connected to an accumulator via a switch. At the onset of an interval that is to be timed, the switch is closed by a timing signal and the pulses are gated from the pacemaker to the accumulator. At the end of the interval the timing signal goes off and the switch opens, causing the accumulation of pulses to cease. The value recorded in the accumulator reflects the number of pulses gated to it, i.e. the pulse rate multiplied by the duration the switch was closed. The accumulator is conceived as a part of working memory, such that 'working memory directly reflects the accumulated count' (Gibbon et al, 1984). Later work describes working memory as an extended buffer for the accumulator, which is used under certain circumstances (Meck et al, 1984). In fact, the accumulator is now more commonly described as part of the clock process, although they are sometimes considered as a single system (see Wearden, 1999). Important time values (e.g. those associated with reinforcement in animal studies or a standard interval in a human study) are transferred from working memory (or the accumulator) to reference memory, a more permanent store. The value that is transferred is multiplied by a memory storage constant, making current time (the present value in the accumulator or working memory) and remembered time dissociable. The decision stage uses a comparator to compare the current time in working memory with a random sample of remembered time in reference memory. The judgement of when to respond is based on a ratio comparison of the two values.

Variance can occur independently at the clock, memory and decision stages, although, in most timing tasks all three processes act in concert, making disambiguating the source(s) of the variance difficult. It should be noted that the pacemaker is conceived as producing a Poisson distribution, i.e. the variance of the inter-pulse intervals (rather then the standard deviation, as seen with the scalar property) grows in proportion to the mean of the interval being timed. Although, this seems incompatible with scalar predictions of the model, as well as with timing data that show the scalar property, it can be assumed that the Poisson variance from the pacemaker is relatively small or that it is only observable within-trials (Gibbon et al, 1984).

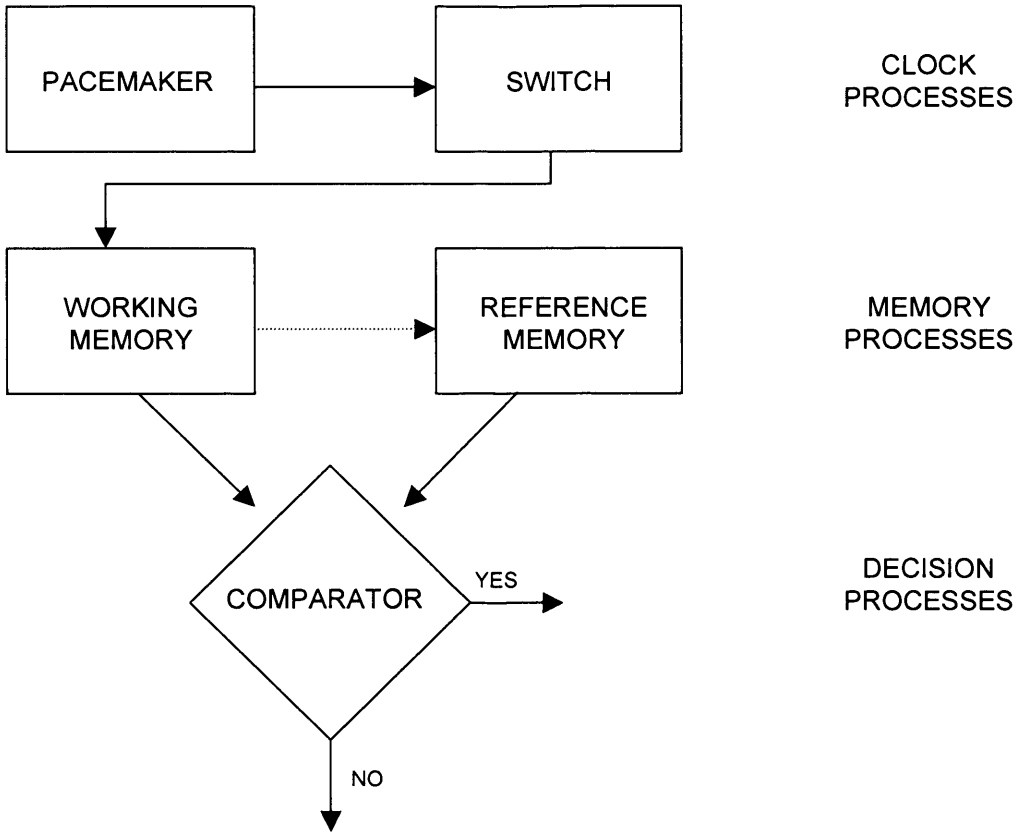

Figure 1.1: SET information processing model (Gibbon et al, 1984)

As has been mentioned, SET sprang from observation of the scalar property in animals, and has fitted animal data well (e.g. Church and Gibbon, 1982; Church et al, 1994; Gibbon et al, 1984). In more recent years, the predictions of SET have also been tested in humans (e.g. Rakitin et al., 1998; Wearden, 1992, 1991; Wearden et al, 1997) using tasks that have been adapted from the animal paradigms. As chronometric counting is known to produce vary different variance properties to pure timing, with data producing a Poisson distribution, estimates of intervals in the seconds-range tend to include counting distracters such as reading a list of random numbers aloud (e.g. Wearden et al, 1997). As an aside, Grondin and colleagues report that the cut off above which explicit counting improves performance is 1.184 s (Grondin et al, 1999). The human data has led to some modifications of SET (e.g. the suggestion that animals use a ratio rule and humans use a difference rule in the decision process), but with the principal tenets of the model remaining intact (see Allan, 1998 for a review).

1.2.2 Connectionist version of scalar expectancy theory

Church and Broadbent (1990) argued that some of the cognitive concepts within the information processing version of SET do not have a known biological corollary. They also questioned the large capacity needed to accommodate the distribution of values in reference memory, which could be storing several types of interval at the same time. They proposed a connectionist version of SET in which the connections and representations within the system are more characteristic of the nervous system of animals (see Figure 1.2). Instead of a single pacemaker there are a set of harmonically related oscillators set at different frequencies that span a range of possible durations to be timed (from circadian rhythms to interval timing at nearly all time ranges). Thus durations of different lengths are represented by individual oscillators and, in a departure from the classic SET model, there is no underlying fundamental frequency. At the start of the interval to be timed, the oscillators are reset to zero. Instead of the accumulator are 'status indicators', one per oscillator, which record information about the half phase (+ or -) of the oscillator (rather than the number of cycles, which would be more similar to the accumulator in the classic version of the model). Instead of a working memory and a reference memory that hold sample distributions, here both processes are represented by matrices of connection weights. This means that instead of values being held as one dimensional numbers, they can be distributed throughout a matrix. In using such a distribution, the memory matrices can store information about an infinite number of samples of a value, rather than (as in classic SET) the system having to increase in size to accommodate additional samples. In terms of the decision process, a similarity measure is computed between an input vector (the current representation of time) and an output vector (the product of the reference memory matrix and the input vector i.e. the remembered representation of reinforced time) and if it is above a given threshold, a response is made.

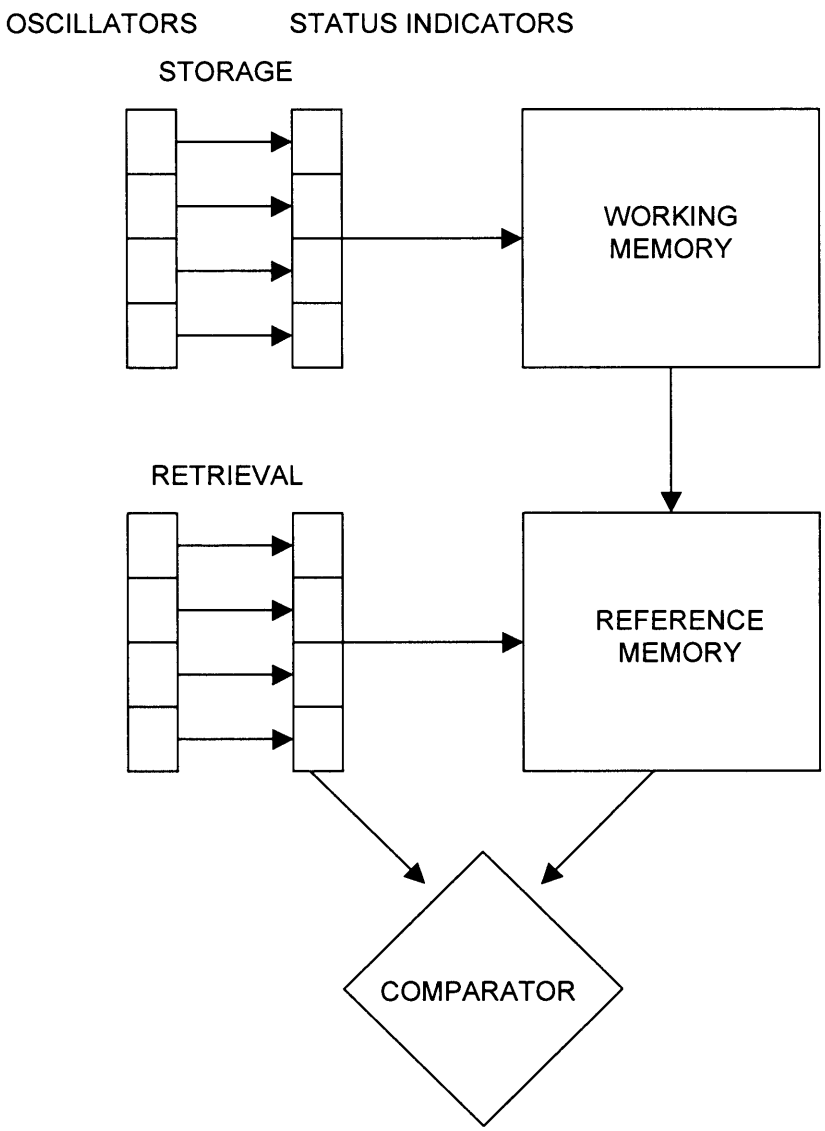

Figure 1.2: Connectionist version of SET (Church and Broadbent, 1990)

1.2.3 Behavioural theory of timing

The behavioural theory of timing (BeT, Killeen and Fetterman, 1988) also proposes that a Poisson pacemaker and accumulator underpin timing. However, in contrast to the more cognitive approach of SET, this theory considers behaviour to be the mediator of temporal judgements. It is hypothesised that animals meter time by moving through a series of behavioural states (e.g. running to the back of the cage, sitting, scratching its leg and so on)

and that this series of behavioural states can become reinforced and act as conditional stimuli. For example, in the temporal bisection procedure the animal would respond 'short' if its current behavioural state is one that has become associated with reward following short responses and would respond 'long' if it is in a later behavioural state that has previously been associated with reward following 'long' responses. The accumulator, interpreted in behavioural terms, is essentially this ability to use behavioural states as conditional cues for responses. The pacemaker is presented as a biological oscillator and each pulse that is registered moves the animal onto the next behavioural state, a transfer that occurs with a constant probability. Furthermore, the pacemaker speed is proportional to the amount of reinforcement, unlike the pacemaker conceived in SET. In outlining a timing theory in which responses are based on reinforced behaviours and with disregard for more complex processes such as the isolation and comparison of stimuli, BeT is limited to explaining the data of unsophisticated organisms.

1.2.4 Multiple time scale model

The multiple time scale model (MTS) (Staddon and Higa, 1999) provides a pacemaker-free account of timing. Like SET, the model assumes a separation between estimates of current time and memory for times reinforced in the past and similarly proposes that interval timing is based on a comparison of these two types of times. However, instead of a pacemaker-accumulator system, temporal information is derived from memory data and depends on the same mechanism as habituation, which can be defined as the waning of a reflex response as a stimulus is repeatedly presented. A behavioural event (such as the onset of an interval to be timed) induces a memory trace (represented by multiple traces set at different rates), which decays over time. The value (or strength) of the trace at any given point provides information about the extent of elapsed time and this memory trace can be seen to be providing clock-like functions (i.e. particular strengths correspond to particular durations). As a consequence of reinforcement, specific actions can become associated with specific strengths of this memory trace enabling learnt timed behaviour.

However, the model has not stood up well to some of the animal timing data (e.g. Matell and Meck, 1999).

1.2.5 Neural network models

Miall (1989; 1992; 1996) has proposed neural network accounts of timing which, like Church and Broadbent, are based on the premise that timing is represented across a distributed set of oscillators. Miall's earliest formulation (Miall, 1989; 1992) consisted of a large population of oscillatory pacemaker neurons with a narrow distribution of oscillation periods (5-15 Hz). The network is able to select a small population of oscillators that are synchronously active at the start and end of an interval that is being timed. These neurones have a beat frequency (the lowest common multiple of the periods of the different oscillations) that is in phase with the length of the interval. This calculation process enables just a few hundred oscillators, regardless of the small range of oscillator frequencies, to encode a wide range of time intervals. The network has an output neuron, which reaches threshold when the subset of oscillators are in phase. The oscillators are then simultaneously reset, such that they will be in phase (i.e. simultaneously active) again after exactly the same interval of time, enabling accurate and replicable timing. Miall (1996) critiqued this initial model and highlighted that the simulations to not produce output that resemble data from psychophysiological studies, i.e. the data do not conform to the scalar property. Rather, the network is either accurate or fails, with no distributions of values around the target interval. Further questions regarding biological plausibility were prompted by the observation that the network does not withstand even small fluctuations in the period of the whole population of oscillators. As a final point, a reasonably powerful reset mechanism would be required to reset the oscillators prior to recall of the interval.

Taking inspiration from pacemaker-accumulator accounts of timing, a second model proposed by Miall (1996) suggests that an integration mechanism, which considers the total activity within a population of neurons, may provide an accumulation or integration mechanism. The population of neurons all receive inputs from an internal clock, with each neuron having a low probability of being

activated by any given pulse. Once a pulse from the clock activates a neuron it remains on, with a small probability of switching itself off. As a consequence of these competing effects, individual neurons will not show a clear increase or decrease in activity over time. However, assuming that each neuron is able to project excitatory inputs to another neuron or network of neurons, the total activity within the population could show evidence of modulation over time, in effect representing an accumulated measure of time. This model provides an output that displays the scalar property, making it compatible with human and animal data. Both models are thought provoking in that, from a biological perspective, they suggest that networks of neurons can operate on time scales that are very different from the time scales of the individual neurons.

1.2.6 Cognitive models

Cognitive models of temporal processing seek to explain the influence of cognitive mechanisms on accurate timing; both memory and attentional mechanisms have been described. An attentional allocation model proposed by Thomas and Weaver (1975) asserts that a temporal processor (directly timing stimulus duration) works in parallel with a non-temporal processor (processing all other aspects of the stimulus, such as colour and size as well as encoding of the time spent processing these aspects) and that both processors have to compete for limited attentional resources. Thus, the estimate of a duration is the weighted average of the output of the temporal processor *and* the encoding of the time spent processing non-temporal information. The type of task being engaged in influences the amount of attention allocated to each processor and their relative influence on any given duration judgement. For example, if the non-temporal aspects of the task were particularly complex, more attention would be allocated to the non-temporal processor and the relative influence of the non-temporal processor on the duration judgement would increase (i.e. because of the increasing unreliability of the temporal processor). The applicability of this model is limited as it was proposed only for intervals of less than 1 s and has only been applied to duration estimates of less than 100 ms.

Zakay (1989) presents a revised version of this model, the resource allocation model (RAM), that attempts to explain temporal estimates greater than 1 s and which rejects the idea that the temporal and non-temporal processors work in parallel. The information accumulated in the temporal processor is stored in short term memory, which stores time values for finite periods, without transferring them to a longer term store. Information collated in the non-temporal processor is also transferred to a short term memory, but with elements then being transferred to long term memory. The more attention allocated to the non temporal processor, the more information that will eventually form part of the long term memory. Attentional resources are shared between the two types of processor with the number of 'subjective time units' accumulated in the temporal processor being dependent on the degree of attentional resources available. Rather than the two processors working in parallel, only the output of the most reliable processor is used to compute duration estimates.

This model explains the differential effects of task load predicted in prospective and retrospective tasks. In prospective tasks, the temporal processor is used to estimate durations and estimation increases linearly with the number of pulses accumulated. This means that the more resources that are allocated to the non-temporal processor the shorter the eventual estimate will be. This is neatly reflected in dual task studies in which the more complex the non temporal task (which has to be processed simultaneously with a temporal task) the shorter the duration judgements on the temporal task (Hicks et al, 1977). Moreover, duration estimates decrease when the subjects are instructed to pay more relative attention to the non-timing task than the timing task (Macar et al, 1994). This effect would not be predicted in Thomas and Weaver's model, in which it can be assumed that the two parts of the output (output of the temporal processor and time spent engaged in non-temporal processing) would cancel each other out. In a retrospective task, in which the subject is told after an interval/task has elapsed that they have to time it, the pulse count in short term memory would not be available as it would have already decayed or have been overwritten (the temporal processor is automatically started and reset by 'starting signals' in the environment, the task demands merely dictate whether

attention is paid to it). Therefore, information in the non-temporal processor, which would amount to stored information that is available from the long term memory, is used. This means that retrospective estimations increase linearly with non-temporal storage size, such that the more resources allocated to the non-temporal processor, the more information that is stored and the longer the estimations will be. Thus, the complexity of the non-temporal information has differential effects under prospective and retrospective conditions. In addition, whereas prospective timing can be explained in an attentional framework and with reference to a timing mechanism, retrospective timing is dependent on memory processes and is a bi-product of general information processing.

The previous models do not incorporate a timer per se, making them incompatible with pervasive 'internal clock' theories and scalar findings. As such, a later model by Zakay (e.g. Zakay and Block, 1996), the attentional gate model (AGM), merged ideas from Thomas and Weaver's attentional allocation model, SET and the early timing model of Triesman (1963). Broadly speaking, the model adapts SET to include the modulation of temporal processing by attention and also accounts for the prospective/retrospective dissociation outlined above. A 'gate' is added between the pacemaker and the switch which, when open, allows the flow of pulses from the pacemaker to the switch. The opening of the gate is mediated by the allocation of attention to time, with increased attention enabling the gate to be opened more widely (or more frequently), allowing more pulses to pass. Conversely, when time is not relevant to the task (a retrospective or non-temporal task) the gate narrows, allowing fewer pulses to pass through. The number of pulses that are transferred is also dependent on the pulse rate, which is described as being affected by arousal, both general (e.g. circadian rhythms) and specific (e.g. stimulus induced). Whereas the gate is concerned with attention allocation, the switch (the start signal for timing a duration) is conceived as being under the influence of selective attention as it is responsive to the temporal meaning of a stimulus. The authors also outline how other processes within SET, such as summing the number of pulses representing current time, transferring pulses from the accumulator to working memory or the decision making (comparison) process, require attentional resources for their effective operation. However, it has been

argued that the switch process defined in SET is as able as the two module approach (gate and switch) of AGM to explain attentional effects (Lejeune, 1998).

1.2.7 Neurobiological models

Although psychological models of timing abound, neurobiological models and theories of timing are less prominent. Many clinical, animal and functional imaging studies interpret their data in terms of SET. For example, pharmacological data collated by Meck (see Meck, 1996) has been used to suggest that dopaminergic activity in the basal ganglia provides the pacemaker-accumulator system, with the substantia nigra as the pacemaker and the dorsal striatum functioning as an accumulator. Furthermore, the data also suggest that acetylcholine function in the frontal cortex is linked to temporal memory. However, arguably the first formalised model to combine neurobiology and theoretical accounts of timing is the striatal beat frequency model (SBF) (Matell and Meck, 2000; 2004). The authors argue that oscillatory models, e.g. proposed by Church and Broadbent (1990) and Miall (1989; 1992; 1996), are the most biologically plausible of the approaches and, as a result, the SBF model adapts Miall's early model (Miall, 1989 and 1992) to fit the neurophysiological constraints of the cortico-striatal-thalamic loop. Essentially, it is proposed that the detection of coincident neural activity, known to be a function of the striatum, encodes temporal durations. Cortical input to the striatum serves as the oscillatory activity (or clock signal) proposed by Miall, whereas striatal spiny neurons act as 'coincidence detectors' (or integrators of the clock signal to produce a temporal estimate), firing when a set of oscillating neurons oscillate with the same beat frequency (defining a temporal duration). Dopaminergic activity in the substantia nigra pars compacta is posited, among other things, to reset the coincidence detection neurons at the onset of a stimulus to be timed. In a further reach for biological plausibility, the model has had variance added to it and simulations of the model find that it matches psychophysical data, unlike in Miall's original conception.

1.3 NEURAL STRUCTURES IMPLICATED IN TIMING

Two main areas, the basal ganglia and the cerebellum, have been hypothesised to play a role in timing in the milliseconds- and seconds-range. Support for involvement of both areas in temporal processing will be discussed with relation to evidence from animal work, clinical studies, pharmacological investigations, functional imaging experiments and transcranial magnetic stimuliation.

1.3.1 Animal studies

1.3.1.1 Lesions to the cerebellum

Much of the animal work investigating the role of the cerebellum has come from classical conditioning studies. Rabbits exposed to a light or tone (conditioned stimulus) followed by a puff of air directed at the eyes (unconditioned stimulus) quickly learn to respond to the conditioned stimulus with a conditioned eyeblink response. Rabbits with unilateral lesions to the cerebellum lose the conditioned eyeblink response in the ipsilesional eye, although the unconditioned eyeblink reaction remains intact when the air puff is presented (Yeo et al, 1985ab). This conditioning paradigm demands that the temporal relationship between the conditioned and unconditioned stimulus is learnt, as the conditioned eyeblink response must be initiated at a specific time point to be effective. Therefore, the efficacy of the cerebellum is necessary for this precise temporal reaction. Perrett and colleagues (1993) conditioned rabbits to produce two differently timed eyeblink responses to two different tones. Following lesions to the cerebellar cortex, not only were the eyeblink responses occurring at much shorter latencies but there was no longer any differentiation in the time of the response to the two different frequencies. These types of animal studies have contributed to Ivry (1996) arguing that the cerebellum is preferentially involved in the timing of millisecond-range intervals.

Clarke et al (1996) found that rats with bilateral lesions of the cerebellar dentate and interpositus nuclei displayed decreased 'consistency' in a temporal bisection task with intervals ranging from 300 – 1200 ms, but not when the intervals ranged from 20 – 45 s. Breukelaar and Dalrymple-Alford (1999) found

a similar short/long dissociation using intervals of 200 – 800 ms and 2 – 8 s in rats with lesions to the cerebellar hemispheres. However, rats with lesions to the cerebellar vermis were unimpaired at both interval ranges, suggesting further dissociation between lateral and midline regions. Further work has shown that rats with stunted cerebellar development, leading to a 10% cerebellar weight reduction in adulthood, were not impaired at making a timed movement in a 10 – 14 s time window in order to receive reinforcement (Ferguson et al, 2001). Conversely, Lurcher mutant mice, who have a degenerated cerebellum, were unable to learn a time dependant avoidance response that needs to be performed either 5 – 10 s or 10 -15 s after task onset (Monfort et al, 1998). Bruekelaar and Dalrymple-Alford (1999) suggest that the cerebellar damage could be adding constant variability to timing operations. When durations in the seconds-range are being estimated other sorts of variability mask the finding and as such the temporal processing of the rats can appear unimpaired.

An additional line of evidence comes from work in which cooling of the dentate nucleus in monkeys was studied (Flament and Hore, 1986). The lesions induced hypermetric movements without tremor that, compared to control movements, had smaller magnitudes of acceleration and larger magnitudes of deceleration. The disruption to acceleration was ascribed to agonist muscle activity that was late in onset, smaller in magnitude and prolonged in duration. The disruption to deceleration was associated with delayed onset of antagonist muscle activity. This research suggests the importance of the cerebellum in gauging the timing and amplitude of muscle activity.

1.3.1.2 Lesions of the basal ganglia

Lesions to the rat substantia nigra cause impaired temporal discrimination of intervals, which is restored with administration of levodopa (see Meck, 1996). Damage to the dorsal striatum also affected discrimination, although levodopa did not improve performance. Neurotoxic depletions of dopamine within the dorsal striatum produced a relative increase the rate of responding to stimulus paired with shorter (20 s) compared to longer (60 s) intervals. This led Meck (1996) to suggest a role for the substantia nigra in generating clock pulses and

for the dorsal striatum in accumulating (gating) the pulses. Further research (Matell et al, 2000) has shown that lesioning the left, but not right, substantia nigra pars compacta (SNc) in the rat causes lasting temporal deficits on the peak interval procedure. Bilateral lesions cause the most extensive damage to temporal control.

Although limited evidence from lesions studies has been produced supporting the role of the basal ganglia in timing, it is worth describing physiological studies that compliment the lesion work. Matell et al (2003) recorded from the left anterior dorsal striatum in rats during a temporal task in which they learnt that responding (lever press) to two different durations (10 s and 40 s) could provide a food reward. As no signal was given as to which duration would be rewarded on any given trial, on long (40 s) trials the animals would produce a high press rate at both 10 s and 40 s. This enabled comparison of neural firing in the striatum to two different durations, with 22% of neurons only showing a modulation in firing rate at *one* of the two durations despite a behavioural response for both durations. It was suggested that the neurons may encode specific signal durations as a direct function of their firing rate. Temporally specific firing patterns were also recorded in the frontal cortex, although the pattern of activity was not indicative of these neurons representing signal duration directly. In primate research, increased striatal activity is observed during the delay preceding an anticipated stimuli (Apicella et al, 1992; Schultz et al, 1992) and neurons in the SNc fire at the time of an expected, but undelivered, reward (Hollerman and Schultz, 1998).

1.3.1.3 Lesions of the cortex

Olton and colleagues have found an interesting dissociation between the performance of rats with frontal and hippocampal lesions on the peak-interval procedure task. Lesions to both regions cause a disruption to the reference memory for the timed event but with the direction of the error occurring in opposite directions (Olton et al, 1988). Further research has found that rats with lesions to the frontal cortex, unlike those with hippocampal lesions, are unable to time two intervals simultaneously; indicative of a failure of attentional mechanisms. Moreover, rats with hippocampal lesions, unlike those with frontal

lesions, are unable to perform adequately when a 'gap' (10 s delay) occurs in the signal of the probe trial, suggesting dysfunction of working memory mechanisms (Olton et al, 1988). This research has been challenged by Dietrich et al (1998) who re-tested both types of lesioned rat and found no evidence of dysfunction on the peak-interval procedure or on 'gap' trials for rats with lesions to the hippocampus. In addition, although deficits on the peak-interval procedure were observed in rats with frontal lesions, they did not induce the same pattern of deficits with the main finding being that of delayed learning. However, it should be noted that the frontal lesion sites in this study were more anterior and that the target interval was 40 s as opposed to 10 s and 20 s in the previous study.

Functional imaging has also been used in animal studies. Onoe et al (2001) were interested in deciphering regions of the brain activated on a duration discrimination task (pairs ranging from 400-600 ms to 1000-1500 ms) in two monkey subjects. The study used positron emission tomography (PET) and found that the left dorsolateral prefrontal cortex (DLPFC), bilateral inferoposterior parietal cortex, right posterior cingulate cortex and basal ganglia (right putamen in one monkey and left caudate nucleus in the other monkey) were consistently active across both subjects. The cerebellum (left) was only active in one subject.

1.3.2 Pharmacological studies

1.3.2.1 Animal data

Investigators interested in the effects of different drugs on timing performance in animals have made clever use of behavioural procedures such as peak-interval and temporal bisection. In plotting a response curve representing the probability of a response at a particular duration, they can observe horizontal shifts in the curve as a result of pharmacological manipulation (see Meck, 1996 for a review). The results are interpreted in terms of SET, with evidence suggesting that dopamine agonist and antagonists have differential effects on clock speed. To briefly explain the theoretical background: in the training (no drug) phase, standard or fixed interval values become reinforced in reference memory and

are represented by a certain number of clock pulses (in this example let X pulses = 2 s). During the testing phase, under pharmacological manipulation, the reference memory values are compared to current time (number of pulses in the accumulator) in order to make a judgement about when to respond. If the clock speed has been slowed down then X pulses in current time will elapse in over 2 s. This discrepancy with the values stored in reference memory means that the animal will overestimate intervals, reflected in a rightward shift in the response curve. Similarly, if the clock speed has been sped up then X pulses will elapse in under 2 s, leading to underestimation and a leftward shift in the response curve. The shift should be proportional to the length of the interval being timed and the dose of drug given, which indicates that the rate of temporal processing has been affected.

Using the temporal bisection task, rats given a dopamine antagonist (e.g. a neuroleptic such as haloperidol) show a response curve that is shifted to the right, indicative of a slowed clock (Maricq and Church, 1983; Meck, 1986). Additionally, the dose of drug needed to produce a 15-20% shift is correlated with its affinity for dopamine D2 receptors, whereas no correlation is found for D1 and D3 receptor affinity (Meck, 1986). Haloperidol produces a similar result on the peak-interval procedure, with rats overestimating the time of the fixed interval on probe trials (i.e. a rightward shift), again suggestive of a slowed clock (Drew et al, 2003). A D1 receptor antagonist did not affect the timing judgement on this task, further suggesting the importance of D2 receptor activity. Methamphetamine, a dopamine agonist, shifts the response curve in the temporal bisection task to the left, indicating an increase in clock speed (Maricq and Church, 1983).

Pharmacological investigation of the temporal bisection task and the peak-interval procedure has been extended to drugs that affect levels of acetylcholine (ACh), with this neurotransmitter argued to influence temporal memory. When clock speed is affected by dopaminergic drugs, the curve-shift occurs abruptly but the effects only last as long as it takes for a rescaling of reference memory to occur. As reference memory is constantly being updated then it will be eventually become dominated by values with the new pulse rate, i.e. those

obtained during the testing phase. Cholinergic drugs are seen to induce a very different pattern of activity, with curve-shift changes occurring gradually and with long-lasting effects. This is said to reflect changes in memory storage speed as the values (e.g. X pulses) transferred from the accumulator to reference memory depend on the integrity of the speed of transfer. A decreased speed of transfer will mean that a value of X pulses will be encoded in reference memory as being longer than it actually is (i.e. greater than 2 s), resulting in overestimation and a rightward shift in the response curve. Whereas, an increased speed of transfer will mean that a value of X pulses will be encoded as being shorter than 2 s, resulting in underestimation and a leftward shift on the response curve. Again, the shift of the response curve should be dose-dependent and in proportion to the interval being timed.

Changes in memory storage speed will not have an immediate effect on the response curve in the testing stage as the values stored in memory were acquired during the training phase. However, as the testing phase continues distorted intervals become dominant in the reference memory storage system and are likely to be used with increasing probability. As such, accurate current time values are compared to inaccurate values from memory, resulting in shifts in the response curve. Clearly, this distortion will not dissipate as although the clock is running normally it is independent of the dysfunctional memory storage speed, which cannot be compensated for (unlike the eventual effects of clock speed on reference memory).

Rats administered drugs that reduce levels of ACh (e.g. the ACh antagonist antropine) show a gradual and persistent rightward shift in the response curve on both the temporal bisection task (Meck, 1983) and peak-interval procedure (Meck and Church, 1987). This suggests that the speed at which values are transferred into reference memory has been decreased and that overestimation is occurring. Conversely, when rats are treated with pyhsostigmine, which increases cholinergic activity, a leftward shift gradually occurs in the response curve, once again both for the temporal bisection task (Meck, 1983) and the peak-interval procedure (Meck and Church, 1987). This suggests that the speed at which values are transferred into reference memory has been increased and

that underestimation is occurring. More recently, measures of sodium-dependent high-affinity choline uptake in the frontal cortex of rats have been found to be proportional to discrepancies in the remembered times of reinforcement stored in temporal memory. A similar correlation between temporal memory error and choline uptake in the hippocampus was also observed, but only for aged rats (Meck, 2002).

1.3.2.2 Human data

Rammsayer has conducted a comprehensive range of experiments investigating the effect of different drugs on human timing. The administration of haloperidol, a dopamine receptor antagonist, adversely affects duration discrimination in the milliseconds- (50 ms standard) and seconds- (1000 ms standard) range, whereas remoxipride (another dopamine receptor antagonist) only disrupts duration discrimination in the seconds-range (Rammsayer, 1993; 1997). This led to the conclusion that temporal processing in the millisecond-range is dependent on D2 receptor activity in the basal ganglia, a circuit that is not affected by remoxipride. Furthermore, it is proposed that the processing of seconds-range durations is dependent on D2 receptor activity in the mesolimbocortical system, a target of both drugs, which mediates memory functions. In support of this, Rammsayer (1999) found that the benzodiazapine midazolam, known for its impairment of memory processes, disrupts duration discrimination in the seconds-range, but not the milliseconds-range. Reboxetine, a selective noradrenaline reuptake inhibitor, selectively improves performance on seconds-range duration discrimination and the influence on this drug on attention further suggests the importance of cognitive processing in seconds-range timing (Rammsayer et al, 2001). Rammsayer purports that very short durations (such as the 50 ms used in his studies) are below the threshold for cognitive control and are dependent on dopaminergic activity in the basal ganglia. In contrast, seconds-range timing depends on the efficacy of working memory processes, with directed attention also being influential. It is difficult to directly compare the animal and human work as the extent of cognitive involvement is very different as well as the actual tasks used. Nevertheless, both sets of results suggest that temporal memory is frontally mediated, with the work of Rammsayer suggesting that D2 receptor activity in mesolimbocortical

regions is important in long-interval judgments in humans and Meck and colleagues proposing that ACh, specifically in the frontal cortex, is required for the integrity of temporal 'memory transfer'. Both groups suggest that the dopamine activity in the nigrostriatal system is important, Rammsayer for brief, non-cognitive durations in humans and Meck and colleagues for 'clock speed' over longer durations.

1.3.3 Clinical studies

Using a similar range of tests, data from patients with cerebellar pathology and Parkinson's disease have significantly contributed to understanding of cerebellar and basal ganglia involvement in motor and perceptual timing. Patient studies provide invaluable information as observed deficits can be attributed to the lesion site or neurochemistry affected by the disease process. To aid comparison of the two patient groups, Table 1.1abc summarises the results from the studies discussed in this introductory chapter and throughout this thesis. Only significant results are reported. Data showing a non significant trend or trends that were not statistically verified are not included. Patients with Parkinson's disease and cerebellar disease are the two patient groups for which the most convincing evidence of a specific temporal processing deficit exists. However, other patient groups have been investigated and have provided some interesting findings. Of these, the most commonly investigated are briefly discussed in this section.

Authors	Temporal task	Intervals used	Patient group	CP vs controls: Main Findings *	PD vs controls: Main Findings **	PD: Improved 'on' vs 'off' medication? ***
Wing et al (1984)	Repetitive tapping	450 ms and 550 ms	PD (case study)	-	Total variance (TV) and clock variance (CV) increased on impaired side	-
Ivry et al (1988)	Repetitive tapping	550 ms	CP	CV increased (lateral lesions) Motor variance (MV) increased (medial lesions) (no control group)	-	-
Ivry & Keele (1989)	Repetitive tapping	550 ms	CP and PD ('on' & 'off')	TV, MV and CV increased IRI unimpaired	Variability unimpaired Faster IRI (tested 'on')	No
O'Boyle et al (1996)	Repetitive tapping	550 ms	PD ('on' & 'off')	-	CV, MV and TV increased	Yes
Pastor et al (1992a)	Repetitive tapping	400 ms - 2 s	PD ('on' & 'off')	-	CV, MV and TV increased Slower IRI (400 and 500 ms)	Yes (IRI)
Harrington et al (1998)	Repetitive tapping	300 ms and 600 ms	PD ('on')	-	CV and TV increased Faster IRI	-
Harrington et al (2004a)	Repetitive tapping	300 ms and 600 ms	CP	CV increased (superior lesions only) IRI unimpaired	-	-

Table 1.1a: Clinical motor timing studies

** Reports performance of patients with cerebellar pathology (CP) compared to control group, unless otherwise specified*

*** Reports performance of patients with Parkinson's disease (PD) compared to control group. Studies with 'on' and 'off' conditions are assumed to use the 'off' condition for this comparison, unless otherwise specified*

****Reports whether PD group showed improved performance in 'on' condition compared to 'off' condition, unless otherwise specified*

KEY: CP = cerebellar pathology, CV = clock variance, IRI = inter-response interval, MV = motor variance, PD = Parkinson's disease, TV = total variance,

Authors	Temporal task	Intervals used	Patient group	CP vs controls: Main Findings *	PD vs controls: Main Findings **	PD: Improved 'on' vs 'off' medication? ***
Ivry & Keele (1989)	Duration discrimination	400 ms	CP and PD ('on')	Impaired	Not impaired	-
Ivry and Diener (1991)	Velocity discrimination	Not relevant	CP	Impaired	-	-
Artieda et al (1992)	Temporal discrimination threshold (TDT)	= 5 ms (subject dependent)	PD ('on' & 'off')	-	Impaired	Yes
Nichelli et al (1996)	Temporal bisection	100 ms - 32 s	CP	Impaired	-	-
Harrington et al (1998)	Duration discrimination	300 ms and 600 ms	PD ('on')	-	Impaired	-
Mangels et al (1998)	Duration discrimination	400 ms and 4 s	CP	Impaired	-	-
Casini and Ivry (1999)	Duration discrimination	400 ms	CP	Impaired	-	-
Riesen and Schnider (2001)	Duration discrimination	200 ms and 1 s	PD ('on')	-	Impaired	-
Harrington et al (2004a)	Duration discrimination	300 ms and 600 ms	CP	Not impaired	-	-

Table 1.1b: Clinical perceptual timing studies: Discrimination tasks

** Reports performance of patients with cerebellar pathology (CP) compared to control group, unless otherwise specified*

*** Reports performance of patients with Parkinson's disease (PD) compared to control group. Studies with 'on' and 'off' conditions are assumed to use the 'off' condition for this comparison, unless otherwise specified*

****Reports whether PD group showed improved performance in 'on' condition compared to 'off' condition, unless otherwise specified*

KEY: CP = cerebellar pathology, PD = Parkinson's disease, TDT = temporal discrimination threshold

Authors	Temporal task	Intervals used	Patient group	CP vs controls: Main Findings *	PD vs controls: Main Findings **	PD: Improved 'on' vs 'off' medication? ***
Pastor et al (1992b)	Time estimation	3, 9 and 27 s	PD ('on' & 'off')	-	Underestimated	Yes
Pastor et al (1992b)	Time reproduction	3 - 9 s	PD ('on' & 'off')	-	Overestimated	Yes
Lange et al (1995)	Time estimation	10, 30 and 60 s	PD ('on' & 'off')	-	Underestimated	Yes (comparison with controls only)
Lange et al (1995)	Time production	10, 30 and 60 s	PD ('on' & 'off')	-	Overestimated	Yes (comparison with controls only)
Malapani et al (1998a)	Peak-interval procedure	8, 12 and 21 s	CP	Increased variability for patients with lateral lesions compared to medial lesions. Normal accuracy	-	-
Malapani et al (1998b)	Peak-interval procedure	8 and 21 s	PD ('on' & 'off')	-	Increased variability (8 s) Impaired accuracy (21 s)	Yes
Riesen and Schnider (2001)	Time estimation	12, 24 and 48 s	PD ('on')	-	Unimpaired	-
Malapani et al (2002)	Peak-interval procedure	6 and 17 s	PD ('on' & 'off')	-	-	Yes
Koch et al (2004)	Time reproduction	5 and 15 s	PD ('on' & 'off' DBS and 'on' & 'off' medication)	-	Overestimated 5 s Underestimated 15 s (tested whilst 'off' DBS and 'off' medication)	Yes

Table 1.1c: Clinical perceptual timing studies: Other tasks

** Reports performance of patients with cerebellar pathology (CP) compared to control group, unless otherwise specified*

*** Reports performance of patients with Parkinson's disease (PD) compared to control group. Studies with 'on' and 'off' conditions are assumed to use the 'off' condition for this comparison, unless otherwise specified*

****Reports whether PD group showed improved performance in 'on' condition compared to 'off' condition, unless otherwise specified*

KEY: CP = cerebellar pathology, DBS = deep brain stimulation, PD = Parkinson's disease

1.3.3.1 Patients with cerebellar pathology

The cerebellum is primarily engaged in motor-related functions, for example modulating the force and range of movement (i.e. 'fine tuning' muscle movement), maintaining posture, coordinating head and eye movements and learning motor skills. The cerebellum forms part of the central nervous system and receives somatosensory information from the spinal cord, balance information from the inner ear, as well as motor information from the motor cortex. Loss of functioning in the cerebellum can occur for a variety of reasons including stroke, tumours, long-term alcohol abuse and cerebellar disease. Hereditary genetic defects can lead to cerebellar disease, for example autosomal regressive cerebellar ataxia (e.g. Friedreich's ataxia) and autosomal dominant cerebellar ataxia (e.g. spinocerebellar ataxia types), although some of these pathologies affect regions outside of the cerebellum as well. Other patients who can be defined as having cerebellar disease are those with idiopathic late onset cerebellar ataxia (ILOCA). ILOCA is a neurodegenerative disorder of unknown cause and is a syndrome rather than a well-defined disease. Some of the patients have multiple system atrophy (MSA) and it is unknown whether the remaining ILOCA patients represent a single disease process or a collection of clinically similar disorders with different aetiology (Klockgether et al, 1998). ILOCA results from degeneration of the cerebellar cortex with loss of Purkinje cells, with additional degenerative changes in other parts of the central nervous system sometimes observed. These patients, who are studied in Chapter 5, are characterised by disease onset occurring after the age of 25 and a progressive cerebellar ataxia. The aetiological heterogeneity of cerebellar pathology is matched by clinical heterogeneity, but with the classic symptoms including ataxia (i.e. impaired coordination of movements and poor timing, clumsiness and unsteadiness), intention tremor (not seen when the limb is at rest and probably resulting from continual hypermetric corrections of position), nystagmus (involuntary eye movements) and dysarthria (poorly articulated speech e.g. slurring). In light of these deficits, it is perhaps not surprising that timing deficits have been observed in this patient group.

With reference to the previously cited classical conditioning work in animals, humans with cerebellar lesions fail to learn the timed conditioned eyeblink

response in the tone-airpuff conditioning paradigm and, in concordance with the animal research, the deficit is limited to the ipsilesional eye only (Woodruff-Pak et al, 1996). In parallel to the previously reported animal study (Flament and Hore, 1986), at another basic physiological level, patients with cerebellar lesions show impairments in the timing of the activation of agonist and antagonist muscles during rapid limb movements (Hallett et al, 1991; Hore et al, 1991).

Ivry et al (1988) applied the Wing and Kristofferson (1973ab) model of repetitive tapping to cerebellar patients in the interests of identifying the effects of different lesion sites on clock and motor variance. They concluded that the lateral regions of the cerebellum are involved in accurate timing since increased clock variability was observed in these patients whereas the medial regions are involved in the implementation and execution of motor responses because increased variance in the motor implementation system was observed. They argue that these findings fit the known dissociation within the cerebellum, with the lateral cerebellum being associated with the planning and preparation of movements and the medial cerebellum being associated with actual motor response. Further work with the repetitive tapping paradigm in patients with cerebellar pathology replicated the finding of clock and motor impairments for this group of patients (Ivry and Keele, 1989). Indeed, Ivry and colleagues have been the key proponents of the hypothesis that the cerebellum underlies timing operations. However, a more recent study by Harrington and colleagues has contradicted this hypothesis by reporting evidence of increased clock variability *only* in a group of patients with stroke-induced lesions in a superior location (those with lesions in a more inferior location were not affected). The dissociation between patients with medial and lateral damage was not observed (Harrington et al, 2004a). In addition, the increased clock variability was seen to correlate with poorer working memory. Mean accuracy, seen as a reflection of the rate of the internal clock, was not impaired as indeed it was not in the data collected by Ivry and Keele (1989).

The cerebellum is traditionally perceived as part of the motor system; however, an increasing body of evidence suggest that its role is far broader and even

encompasses cognitive operations (e.g. Rapoport et al, 2000). In parallel to this, evidence of non-motor timing deficits have been found. Patients with cerebellar pathology are impaired at judging the velocity of a moving stimulus (Ivry and Diener, 1991), at temporal bisection (Nichelli et al, 1996) and also at making duration discrimination judgements (Casini and Ivry, 1999; Ivry and Keele 1989; Mangels et al, 1998). Mangels et al (1998) were able to illustrate that the poor performance could not be explained by deficits in working memory as the group, unlike a group of patients with prefrontal lesions, were insensitive to the length of the inter-stimulus interval (ISI). In fact, cerebellar damage has to be accompanied by brainstem pathology for memory deficits to emerge (Daum et al., 1993). However, although Harrington et al (2004a) found evidence of impairment to clock variability in patients with damage to superior regions of the cerebellum; they found little evidence to suggest impairment on a duration discrimination task. A small subset of patients (again, with superior damage) showed a non-significant trend for increased variability on the task, but this was interpreted as reflecting a deficit in processing speed, as evidenced by correlated slowed contralateral tapping speed and slowed performance on the Trail Making Task Part A, a measure of visual scanning and motor speed. Evidence of impairment of patients with cerebellar lesions on a frequency discrimination task (Casini and Ivry, 1999), i.e. making pitch judgements, also raises questions as to whether a more general perceptual or sensory discrimination deficit may explain the results.

In fact, alternative, non-temporal explanations for the deficits of patients with cerebellar degeneration on timing tasks have been a theme of other research. In a time bisection study, Nichelli and co-workers (1996) found impairments in the bisection of short (100 ms and 600 ms standards, and 100 ms and 900 ms standards, although not the shorter range of 100 ms and 325 ms standards) and long (8 s and 32 s standards) intervals. However, although the short interval deficits appeared robust, precision was impaired when the intervals were in the longer range of 8-32 s. As such, it was concluded that disruption to non-temporal functions such as sustained attention or strategy use could be underpinning the time discrimination impairment at the more cognitively demanding longer interval range. Malapani and colleagues used the peak-

interval procedure to test reproduction of intervals of 8, 12 and 21 s and found increased variability in patients with lateral lesions of the cerebellum compared to those with medial lesions (Malapani et al, 1998a). However, accuracy was normal for both groups and variability was scalar across durations for both groups, suggesting the data aligns with the predictions of SET, in contrast to the non-scalar increases in variance (rather than remaining constant, coefficient of variation is significantly larger in the 'off' state than the 'on' state and is significantly larger for the 8 s target than the 21 s target in the 'off' state) observed in patients with Parkinson's disease on the same task (Malapani et al, 1998b). As a result, the authors concluded that the cerebellum has a secondary role in temporal performance.

A caveat to these data is that as the cerebellum is not a homogenous entity with different lesion sites producing different symptoms, as the finding of lateral-medial and superior-inferior distinctions indicates. Researchers investigating this group of patients can find that isolating a common pathology can be difficult. Typically, researchers test patients with an idiopathic diagnosis and/or those with damage due to stroke or tumour; patients with, among other things, alcohol-related degeneration or an identified genetic cause tend to be avoided. However, it is known that patients with stroke can display remarkable recovery of function as they enter a more chronic stage. Thus, many of the possible timing deficits that occur after a stroke affecting the cerebellum may only be observable, or more sensitive to testing, in a critical period, diluting the impact of stroke-based studies (see Ivry et al, 1988). Patients with idiopathic degeneration bring their own limitations, as this type of atrophy is seldom focal and may affect other brain regions (Harrington et al, 2004a). To this effect, the degenerative nature of Parkinson's disease, with its *relatively* homogenous time-course and symptomatology, automatically biases itself towards the detection of timing dysfunction.

Despite some contradictory findings, evidence for the role of the cerebellum in timing has clearly been amassed. A substantial body of work has suggested that these results reflect a secondary, non-temporal role in timing, yet no clear consensus exits to suggest what that might be.

1.3.3.2 Patients with Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative condition primarily affecting motor behaviour. Typically, patients present with increased muscle tome (rigidity/muscle stiffness), bradykinesia (slowness of movement), akinesia (poverty or absence of movement), tremor (4-5 per second at rest) and balance and walking problems (a shuffling gait). The genesis of the disease is the depletion of dopamine producing neurones in the substantia nigra pars compacta. The substantia nigra is one of the main structures that constitute the basal ganglia, the others being the striatum (including the putamen, caudate nucleus and nucleus accumbens), globus pallidus and subthalamic nucleus (see Figure 1.3). The basal ganglia play a significant role in movement. A series of circuits connect the basal ganglia to various parts of the cortex, via the thalamus (Alexander et al, 1986) (see Figure 1.4). The dopamine deficiency in PD causes the balance of inhibitory and excitatory flow within these circuits (or frontostriatal loops) to be altered. Each circuit has a direct and indirect pathway from the striatum to the output nuclei of the basal ganglia.

Depleted dopamine levels are most prevalent in the putamen, which is the primary basal ganglia component in the frontostriatal motor loop, the circuit that connects the basal ganglia to the motor cortex, supplementary motor area (SMA) and lateral premotor cortex and which has a primary role in movement. Consequently, the SMA, which plays a key role in the initiation of internally generated movements (Jahanshahi et al, 1995), receives excess inhibitory outflow from the thalamus and initiation of movement is severely affected. As the disease progresses dopamine depletion in the basal ganglia affects the other loops, the impact on the frontal cortex becomes more widespread and cognitive deficits emerge. Nevertheless, the motor loop remains the most affected circuit. The most common medical treatment for PD is the administration of levodopa, the precursor of dopamine, which provides a short-lasting reversal of symptoms.

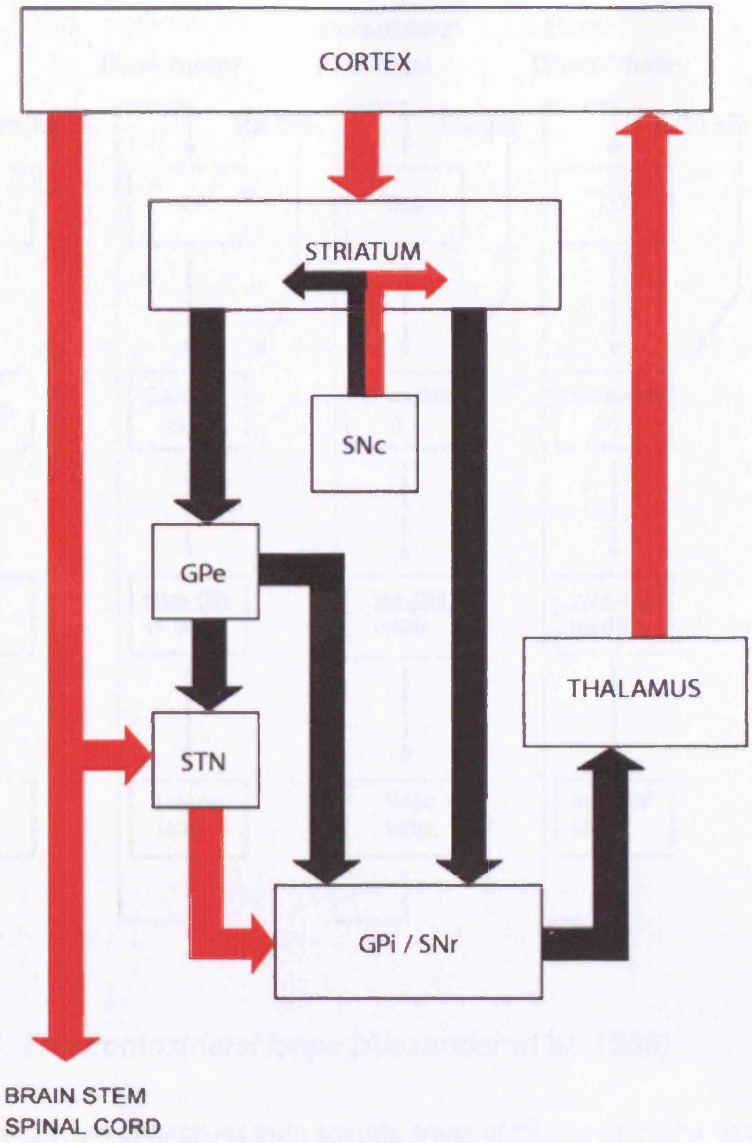

Figure 1.3: The basal ganglia

The direct and indirect pathways between the basal ganglia and the frontal cortex are represented. The direct pathway from the striatum to the internal segment of the globus pallidus (GPi)/substantia nigra pars reticulata (SNr) and the indirect pathway via the external segment of the globus pallidus (GPe) and subthalamic nucleus (STN). Red arrows represent excitatory connections; black arrows represent inhibitory connections. SNc = substantia nigra pars compacta.

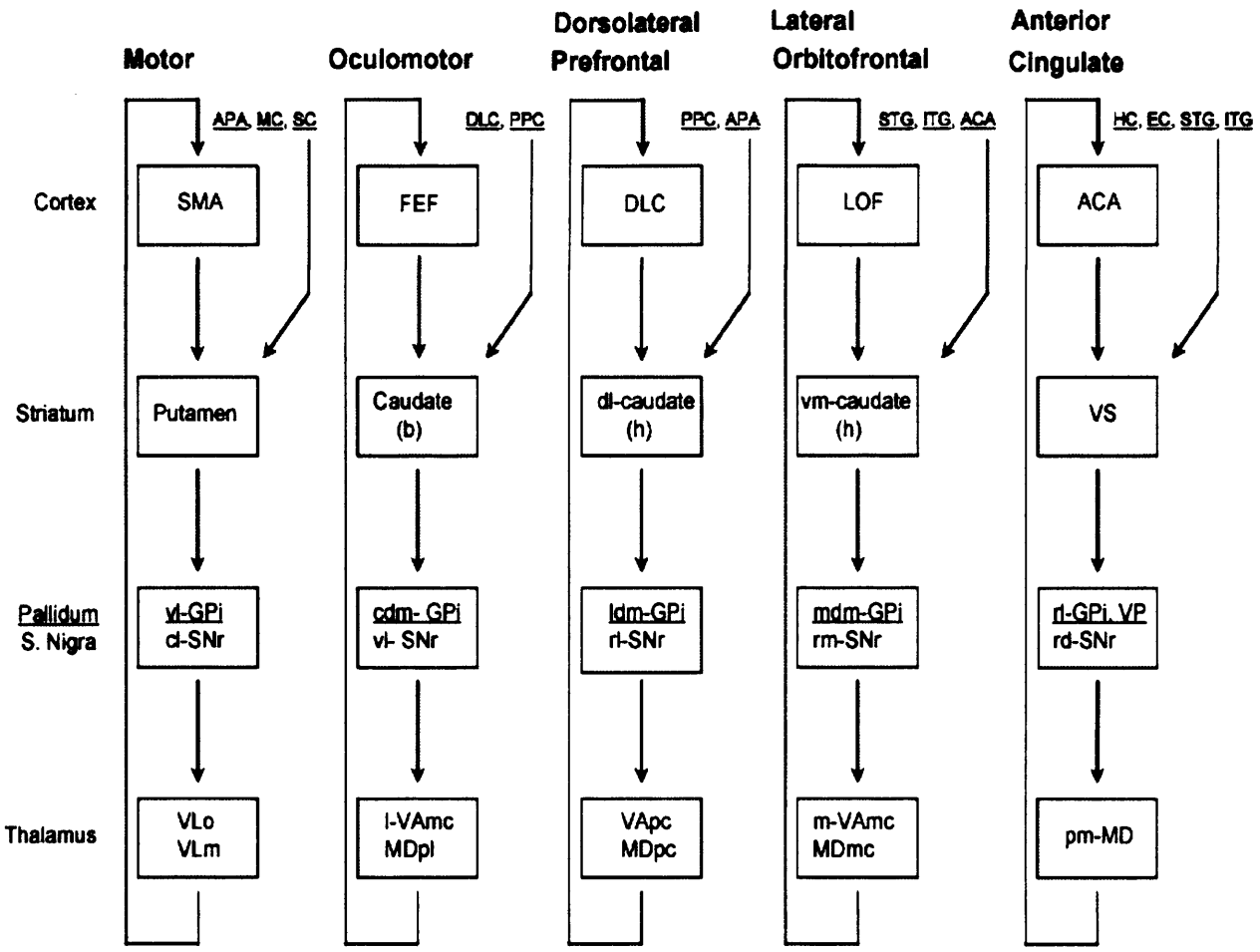

Figure 1.4: The frontostriatal loops (Alexander et al, 1986)

The five circuits show projections from specific areas of the frontal cortex to specific areas of the striatum, which project back to the frontal regions via particular output sections of the basal ganglia and thalamus. KEY: ACA = anterior cingulate, APA = acruate premotor area, caudate (b) = caudate body, caudate (h) = caudate head, DLC = dorsolateral prefrontal cortex, EC = entorhinal cortex, FEF = frontal eye fields, GPi = internal segment of the globus pallidus, HC = hippocampus, ITG = inferior temporal gyrus, LOF = lateral orbitofrontal gyrus, MC = motor cortex, MDpl = medialis dorsalis pars paralamellaris, MDmc = medialis dorsalis pars magnocelluaris, MDpc = medialis dorsalis pars parvocellularis, PPC = posterior parietal cortex, PUT = putamen, SC = somatosensory cortex, SMA = supplementary motor area, SNr = substantia nigra pars reticulata, STG = superior temporal gyrus, VAmc = ventralis anterior pars magnocellularis, VApc = vantralis anterior pars parvocellularis, VLm = ventralis lateralist pars medialis, VLo = ventralis lateralis pars oralis, VP = ventral pallidum, VS = ventral striatum, cl = caudolateral, cdm = caudal dorsomedial, dl = dorsolateral, l = lateral, ldm = lateral dorsomedial, m = medial, mdm = medial dorsomedial, pm = posteromedial, rd = rostrodorsal, rl = rostralateral, rm = rostromedial, vm = ventromedial, vl = ventrolateral

Many studies have used the Wing and Kristofferson (1973ab) model of repetitive tapping. The results are varied, but with the majority of studies finding evidence of some degree of motor timing impairment. The first investigation was a single case study in which a patient with unilateral symptoms showed increased inter-response interval (IRI) and clock variability when finger tapping with the affected hand (target interval 450 and 550 ms). There was no difference in motor variability between the two hands (Wing et al, 1984; Wing and Miller, 1984). A study by Ivry and Keele (1989) found no evidence of differences in IRI, clock or motor variance between a group of patients 'on' their normal levodopa medication and a group of control subjects. Neither was medication seen to modify performance in a subset of patients tested both 'on' and 'off' medication. Taken together, these results led Ivry and colleagues to conclude that the basal ganglia do not have a direct role in motor timing, but with Ivry (1996) later suggesting that the basal ganglia are involved in seconds-range timing. However, the patients with PD showed significantly shorter mean IRIs compared to the control group, indicating impaired accuracy. Also, a subset of four patients showed increased clock variability when performing with their impaired hand compared to their unaffected hand.

Other research groups draw different conclusions. First, Pastor et al (1992a), using 80° flexion-extension movements of the wrist on a wider range of target intervals (400 ms – 2 s) than previously used, found that IRI, clock and motor variability were higher for patients 'off' medication than for a control group and that this was true for all rates of tapping. The mean IRI was significantly slower for the patient group at the two shortest target intervals (400 ms and 500 ms) for both the synchronisation and the continuation data. Medication, tested in a smaller subset of the patients, was considered to significantly improve the accuracy of the mean IRI at the three shorter target intervals used (400 ms, 500 ms, 666 ms), but not when the inter-stimulus intervals were longer (1 s and 2 s). Unfortunately, there was not sufficient data to statistically compare the variability measures of the Wing and Kristofferson model under the 'on' and 'off' conditions. O'Boyle and colleagues were able to report such statistics using a similar experimental design in which subjects finger tapped every 550 ms, and

found that IRI, motor and clock variability were increased in the 'off' medication condition compared to the normal medication state (O'Boyle et al, 1996). Compared to controls, in complement to the findings of Pastor and colleagues, all three types of variance were higher for the patient group 'off' medication. When 'on' medication, only the clock variance remained elevated. However, in contradiction to the previous study but reflecting the Ivry and Keele (1989) study, the patients tended to tap with a faster IRI than the controls, and this was significant in the 'on' medication condition. O'Boyle et al (1996) also compared patients with unilateral symptoms on their 'worse' and 'better' hand. IRI, clock and motor variance were all worse in the affected hand, compared to both the better hand and the control group. In a further study, Harrington et al (1998a) reported increased IRI and clock variability in patients tested 'on' medication compared to controls (rate 300 ms and 600 ms) as well as an increased mean IRI, but with no significant difference in motor variability.

Further evidence that the basal ganglia may play a fundamental role in timing comes from observations of deficits in non-motor timing tasks in patients with PD. Patients with PD tested 'off' medication have an impaired temporal discrimination threshold (TDT) for distinguishing between closely occurring visual, auditory and tactile stimuli, which is improved, although not to the level of controls, 'on' medication (Artieda et al, 1992). Non-medicated patients trained to count at a 1 s rate show underestimation when timing a presented interval (3 s, 9 s and 27 s) using the learnt rate. In addition, the same patients overestimate in a variety of time reproduction tasks, in which a presented interval (range 3 – 9 s) had to be reproduced (Pastor et al, 1992b). In a similar study, Lange and colleagues required patients with PD to estimate presented intervals of 10 s, 30 s and 60 s using a pre-trained inter-count interval of 1 s, or to produce the same intervals (again, using counting) from a given start signal. When non-medicated, the patients underestimated compared to controls on the time estimation task and overestimated on the time production task (Lange et al, 1995). These pattern of results are argued to be indicative of a slowed 'internal clock' in patients with PD and are supported by a significant improvement in performance when the patients were tested 'on' medication (although significant effects were not found for all types or rates of time reproduction in the study of

Pastor et al, 1992b). It should be mentioned that subvocalisation (internal counting) of intervals was required in both studies, which introduces a timed motor element. As well as medication improving performance, patients treated with electrode implantation for subthalamic deep brain stimulation (DBS) are better at reproducing 5 and 15 s intervals when the stimulator is switched on than when it is switched off ('off' medication in both cases) (Koch et al, 2004). The authors suggest that the role of deep brain stimulation in reducing inhibition of the thalamo-cortical projections to the DLPFC is likely to explain the improvement.

The duration discrimination task, which does not involve internal counting, has also been used to test this patient group. Ivry and Keele (1989) found no impairment for patients in the 'on' medication state, whereas Harrington et al (1998a) reported significant impairment for patients 'on' medication, with preserved performance on a frequency discrimination task suggesting that non-temporal factors were unlikely to underpin the deficit. In a fairly complex version of the duration discrimination task, Riesen and Schnider (2001) presented visual intervals for discrimination but with the second interval appearing at a variable time during the presentation of the first interval, rather than sequentially. Medicated patients with PD were significantly worse than controls; both when the shorter interval (variably presented first or second) was either 200 ms or 1 s. The fairly high attentional and working memory demands of this task may have contributed to this result, but as no independent measure of attention or working memory was recorded, it is not possible to determine if the patients may have been affected by cognitive demands. On a second measure of perceptual timing, subjects verbally estimated different stimulus intervals (12 s, 24 s and 48 s) whilst pressing a space bar at a self-paced rate of once per second. To prevent the counting of the presses, subjects read aloud random numbers (1-9) presented on a computer screen (yoked to the press-rate). Both controls and medicated patients underestimated by approximately 30%, with no significant difference between the groups. However, any differences in performance may have been masked by the strategic help that the tapping provided. The patients were reported as tapping at a similar rate (0.8 Hz) to the

controls, suggesting the lack of a motor timing deficit. However, reading the numbers aloud could have provided a salient pacing cue.

As mentioned previously, the peak-interval procedure has also been tested in patients with PD. When tested 'off' medication compared to 'on' medication these patients show increased variability as well as inaccuracy, with the data not conforming to the scalar property. Furthermore, in the 'on' medication state the patients were more comparable to healthy controls (Malapani et al, 1998b). These results were followed up in a later study. By requiring patients to encode and reproduce intervals under different medication states; the group were able to establish a dysfunction in the storing and retrieval of temporal memories (Malapani et al, 2002). This is in contrast to the testing of the peak-interval procedure in rats, in which dopamine-related performance fluctuations are interpreted as indicative as a speeding or slowing of an 'internal clock'. Interestingly, a recent functional imaging study has found that caudate activity in healthy subjects is not correlated with the 'migration effect' (overestimation of long intervals and underestimation of short intervals) on a duration discrimination task (Harrington et al, 2004b). This migration effect was central to the memory-related distortions in temporal processing observed in the PD patients studied by Malapani and colleagues and suggests that such an effect may be cortically driven (albeit as a result of basal ganglia dysfunction), rather then relating to the basal ganglia per se.

The different conclusions reached in these studies are likely to partly reflect different methodologies. Moreover, the severity of the patients has been shown to affect performance (Pastor et al, 1992ab) as well as whether the more affected hand is tested (O'Boyle et al, 1996). However, overall, patients with PD demonstrate impaired motor timing performance, and this is reflected in increases in clock and motor variance. Furthermore, although there are some contradictory studies, the majority of the work on perceptual timing in PD suggests the presence of a non-motor timing deficit. The much replicated finding of modulation of performance following dopaminergic medication is a convincing indicator that pathology within the basal ganglia disrupts motor and perceptual timing function. The known cognitive deficits in late-stage PD must

be kept in mind given the suggestion that seconds-range timing is cognitively mediated.

1.3.3.3 Other pathologies

The preponderance of data from patients with cerebellar pathology and Parkinson's disease has dominated the literature. However, other neurological conditions and some psychiatric conditions have also provided an insight into the neural correlates of timing. This section offers an overview of the main findings.

Prefrontal/frontal lesions

Patients with lesions to the frontal cortex have been tested on a variety of temporal processing tasks. Most lesions are the result of stroke, which can have the advantage of providing a discrete lesion location unlike degenerative diseases such as Parkinson's disease and idiopathic late onset cerebellar ataxia that can impact on regions of the brain outside of the primary focus of the disease. However, it is known that that stroke can lead to neural recovery and reorganisation, which brings a different challenge to the interpretation of results. Motor timing has been assessed in patients with lesions to the cortex, including the posterior frontal lobes, using the repetitive tapping task. The patients were found to have increased variability compared to healthy controls, limited to motor, rather than clock, variance (Ivry and Keele, 1989). However, as the lesions were described as extending to the posterior frontal lobe, they were likely to have included motor regions, which could explain the deficits in motor-related variability. The same patients were not impaired on a duration discrimination task (standard tone 400 ms), which suggests that performance deficits could not have been the result of a faulty 'internal clock'; if it is assumed that both types of task are subserved by similar clock processes. A further study has shown that frontal patients are slowed at initiating a repetitive tap sequence of four self-paced taps, despite a normal execution rate and normal initiation of single taps (Lepage et al, 1999). This suggests a higher-level impairment in the programming of repetitive trains, with the lesion sites causing most disruption including the anterior cingulate and premotor cortex. Further analysis of patients with lesions to posterior frontal areas revealed that damage to the SMA or

premotor cortex disrupts the ability to produce rhythms from memory (Halsband et al, 1993). Thus impaired motor timing performance in patients with frontal lesions is most likely to reflect problems with the programming of movements, including temporal parameters, and is mainly the preserve of posterior frontal regions that have known motor roles.

Further studies have found evidence of deficits in time perception in patients with frontal lesions. For example, such patients showed diminished accuracy in a temporal bisection study when the standard intervals were 100 ms and 900 ms and when they were 8 s and 32 s (Nichelli et al, 1995). When the standard intervals were in the longer range precision was also affected, which could be interpreted as increased cognitive demands further compromising performance. In fact, Mangels and colleagues found perceptual timing deficits in prefrontal patients on a duration discrimination task, but only for intervals of 4 s (Mangels et al, 1998). At the shorter interval range of 400 ms, in which cognitive demands were less substantial, performance was preserved. To corroborate this finding, the patients were also more impaired at a frequency discrimination task when the comparison frequencies were separated by a long interval of 4 seconds rather than a shorter one of 1 second. This would suggest that a common mechanism, such as working memory, underlies the performance deficits of the frontal patients. Casini and Ivry (1999) required that patients with prefrontal lesions (DLPFC region) carry out a duration discrimination (400 ms standard) task and a frequency discrimination task simultaneously in a dual task paradigm. Compared to the results for both tasks performed separately, the patients showed deteriorated performance on both tasks, whereas patients with cerebellar lesions and healthy controls only showed impaired performance on the duration task. This suggests that the timing deficits observed in the patients with prefrontal lesions can, unlike the cerebellar group, be explained by general deficits such as attention.

Harrington et al (1998b) employed a duration discrimination task (standard interval of 300 ms and 600 ms) and a frequency perception task in order to delineate the processing problems in patients with right and left hemisphere cortical lesions. Only the right hemisphere group displayed time perception

deficits, once patients with substantial frequency perception deficits were excluded. Duration discrimination performance, although not frequency discrimination performance, correlated with attention switching problems in the right hemisphere group and the authors proposed a right prefrontal-inferior parietal network that influences temporal processing via its role in attention and working memory.

The weight of evidence seems to suggest that the efficacy of the prefrontal cortex is necessary for accurate timing because of contribution to cognitive functions that support timing operations. It should be noted that the deficits that are said to underpin timing dysfunction tend to reflect the cognitive demands of the task, such that attention problems are cited when the intervals are < 1000 ms (Casini and Ivry, 1999; Harrington et al, 1998b) and working memory disruption is found when the intervals are in the seconds-range (Mangels et al, 1998). Indeed, evidence of an inability to use strategic support (e.g. subdividing the intervals) was also found in the study of Mangels et al (1998), which suggests executive problems also become apparent as the task becomes more cognitively demanding. Moreover, patients with lesions to the orbitofrontal cortex overestimate periods of time and underestimate on a time reproduction task, both of which suggest a fast 'clock', but are arguably related to orbitofrontal characteristics such as impulsivity (Berlin et al, 2004). Increased variability on measures of time production and time reproduction in patients with closed head injury are reflected in problems with attention, working memory and processing speed (Perbal et al, 2003). It is clear that a range of non-temporal processes affect the timing problems of patients with prefrontal lesions and that further work to delineate the exact contribution of these processes will give greater insight into the network of temporal and non-temporal processes that are essential for efficient timing.

Right and left hemisphere asymmetry

Harrington et al (1998b) (above) propose that the right hemisphere is preferentially important in perceptual timing, possibly due to an attentional role. In fact, there have been several studies that have presented evidence for right or left hemisphere dominance in different aspects of temporal processing.

Results from the analysis of duration discrimination performance in a split brain patient, in which the visual field of presentation and hand used in responding was manipulated, suggested that the right hemisphere is important for working memory representation; with the durations themselves probably represented subcortically (Handy et al, 2003). Kagerer et al (2002) found that patients with left and right hemisphere lesions showed preserved performance for temporal reproduction of intervals between 1 – 3 s, but patients with right hemisphere damage showed underestimation of intervals between 3.5 – 5.5 s. The pattern of results did not suggest a confounding attentional problem, but as the longer intervals placed greater cognitive demand on the subjects, it is conceivable that a failure of cognitive operations underpinned the deficit.

A patient with a lesion to the right DLPFC demonstrated significant underestimation of a presented 90 s period, indicative of a slowed internal clock, a finding which was supported by anecdotal evidence of the patient having problems judging time in everyday life (Koch et al, 2002). Estimation of shorter intervals did not cause impairment, but a normal score on the Trail Making Test argues against a deficit in attention and the interval is too long to have been affected by working memory. The research group speculate that the right DLPFC may receive input from subcortical timing areas and form a conscious representation of time intervals in the manner of an accumulator. They suggest that this process applies to long intervals outside of the working memory boundary, although the patient was statistically unimpaired at the time estimation of a 60 s period. Evidence of a slowed clock is also apparent in a patient with a lesion to the left superior prefrontal cortex who showed extreme overestimation in the production of a 60 s period (average estimate 286 s), in stark contrast to the underestimation typically observed in healthy subjects (Binkofski and Block, 1996). Although no formal measure was reported, the subject was said to show evidence of withdrawal, apathy and fatigue, factors that could have contributed to his result. A comparison of patients with right and left temporal lobe epilepsy (RTE and LTE) was made on two measures of time perception: time reproduction (500 ms – 8 s) and temporal bisection (1 s and 2 s standards) (Vidalaki et al, 1999). A dissociation in performance was found,

with the RTE group showing increased variability on both tasks, and the LTE group significantly underestimating intervals on the time bisection task.

The previous studies have concentrated on measures of perceptual timing. Two patients with lesions to the corpus callosum, preventing communication between the two hemispheres, were only able to make normal repetitive movements with their right hands (Kashiwagi et al, 1989). However, the ability of one of the patients to use their left hand efficiently at a very high rate of movement (5 beats/s) led to the conclusion that the right hemisphere was less able to process the temporal aspects of the repetitive movements. Furthermore, patients with left hemisphere damage were more impaired than patients with right hemisphere damage on measures of rhythm perception and production (Nakamura, 1990). This left hemisphere dominance for the performance of repetitive movements reflects the left sided focus of the lesions to the SMA in the patients studied by Halsband et al (1993).

There is clearly evidence of a right hemisphere advantage for timing, probably reflecting secondary cognitive operations, which has been supported by evidence from functional imaging studies (e.g. Rao et al, 2001). However, evidence of timing deficits, particularly motor timing deficits, in patients with dysfunction in the left hemisphere do exist and warrants an explanation. One possible hypothesis is that the left hemisphere is engaged in a qualitatively distinct type of timing, related to the known functional role of this hemisphere.

First, evidence has accumulated for a left hemisphere advantage in the discrimination of fine temporal events, for example as seen in the temporal discrimination threshold task and in gap detection (e.g. detecting an 8 – 14 ms gap in stimuli) (see Nicholls, 1996 for a review). Second, the left hemisphere is known to be dominant in motor processing, including the temporal aspects of motor programming (Halsband et al, 1993). As an example, Ibbotson and Morton (1981) showed that the left hemisphere (i.e. right hand) is superior at rhythmic tapping in both right- and left-handers. The type of timing investigated in the work of Nicholls and colleagues (i.e. the perception of small millisecond intervals) is argued to be a reflection of both the language and motor processing

preference in the left hemisphere, and is a form of temporal processing that cannot be explained within the cognitive information-processing model of SET. Deficits on a variant of the temporal discrimination task (judging temporal order rather than distinguishing a threshold) are observed in subjects with dyslexia (Virsu et al, 2003) but performance on a duration discrimination task is preserved (range 400 ms – 2 s) (Ramus et al, 2003). In addition, stutterers do not seem to show impaired repetitive tapping (Hulstijn et al, 1992). These two studies suggest that there is a dissociation between deficits related to timing relevant to reading (e.g. processing of rapidly changing stimulus sequences) and speech timing, which are likely to be driven by the left hemisphere, and the classic conception of motor and perceptual timing that is supported by cognitive operations in the right hemisphere.

Schizophrenia

Patients with schizophrenia also display perceptual timing deficits. This is particularly interesting because the dysfunction of dopaminergic mechanisms has been cited as a principal component of the pathophysiology of schizophrenia (Davis et al, 1991). Furthermore, patients with schizophrenia are also described as having neurological signs of cerebellar dysfunction (e.g. Ho et al, 2004), reflected in neuroimaging studies that find abnormalities in cerebellar volume and blood flow (e.g. Okugawa et al, 2003; Wiser et al, 1998). Indeed, patients with schizophrenia behave differently from control subjects on an eyeblink conditioning paradigm (previously referred to in this Introduction) that depends on learning a timed conditioned response and which is known to depend on the integrity of the cerebellum (Brown et al, 2005; Sears et al, 2000). However, one of the studies found evidence of impaired learning of the conditioned response (Brown et al, 2005) and the other found evidence of facilitated learning of the conditioned response (Sears et al, 2000), suggesting that further studies are warranted. Measures of time estimation and time production have indicated that the patients overestimate time (range 5 – 60 s) compared to healthy controls, suggesting a slowed 'internal clock' (Wahl and Sieg, 1980). A further study found that patients with schizophrenia were typically more variable than healthy subjects in the time production of a 30 s interval. The pattern of over- and underestimation was varied, perhaps due to

the heterogeneity of the patients. However, the patients with chronic schizophrenia showed a tendency to overestimate, which was speculated to be a consequence of the 'hypo-frontal" activity in these patients (Tysk, 1984). Indeed, hypoactivity of the putamen, anterior thalamus and right medial prefrontal cortex is observed in patients with schizophrenia during a duration discrimination task (Volz et al, 2001). More recently, Davalos and colleagues have found a duration discrimination deficit in patients with schizophrenia when the standard interval was just 400 ms, suggesting deficits are apparent even in the presence of relatively minor cognitive demands (Davalos et al, 2003a). Performance on the task did not deteriorate when the ISI was increased from 500 ms to 3000 ms, which further suggests that working memory and attention deficits are unlikely to explain the results. Rammsayer found deficits in patients with schizophrenia when discriminating durations using a standard interval of just 50 ms, with measures of attention and vigilance being normal (Rammsayer, 1990). Another study found deficits in patients with schizophrenia on a temporal generalization and a temporal bisection task (125 – 875 ms range), deficits that did not correlate with working memory performance (Elvevag et al, 2003).

Davalos and colleagues also measured mismatch negativity (MMN) waveforms (reflecting preattentive recognition of deviant stimuli) in response to temporal stimuli and found that patients with schizophrenia show reduced MMN amplitude to irregular ISIs in a regular auditory rhythm (Davalos et al, 2003b). As the patients did not have to attend to or respond to the stimuli this result suggests that temporal deficits in this group occur at the physiological level. It would be interesting to see if such performance correlates with temporal deficits on cognitively-loaded tasks. Unfortunately, motor timing is less investigated, although Elvevag et al (2003) cite their unpublished data that found evidence of deficits on the repetitive tapping task. It is clear that patients with schizophrenia have deficits in temporal processing; however, there is one major obstacle to the interpretation of these findings. Patients are always tested in their medicated state and at present little is known of the moderating effect of the varying types of medication on performance, though typical neuroleptics have been shown to decrease 'clock' speed (e.g. Meck, 1986). Interestingly, of the two studies that looked at the conditioned eyeblink response and which found

opposite findings, one tested patients who were taking antipsychotic medication at the time testing and one tested patients who had not had medication for three weeks (Brown et al, 2005; Sears et al, 2000). Further work with de novo patients or groups of patients with different drug regimes is needed to allow clearer interpretation of the results obtained to date. Whether it is possible to tease apart the specific pathology underlying the temporal deficits, particularly relating to the cerebellum and the dopaminergic system, is another challenge for future research.

Depression

The reduction of psychomotor activity in clinically depressed patients has both motor and cognitive underpinnings and immediately suggests that these patients may have difficulty with temporal processing. The monoamines norepinephrine and serotonin are the neurotransmitters most commonly believed to play a role in depression (e.g. Meyers, 2000). However, dopamine abnormalities within the striatum have been observed in those with depression (Meyer et al, 2001) and recent animal work has shown that serotonin induced dopamine release in the nucleus accumbens is critical to the behavioural effect of nefazodone, a fast-onset antidepressant (Dremencov et al, 2004).

Compared to healthy controls, patients with depression have a slowed sense of subjective time, as measured on a visual analogue scale of how fast or slow time has passed on a given day (Bschor et al, 2004). However, these patients also produced significantly shorter estimates on a time production task of 90 s (although non-significant differences were found for 35 s and 7 s), which conversely suggests a speeded sense of time. Patients with manic depression were more consistent and had a speeded sense of subjective time using the visual analogue scale and significantly underestimated the production of the 35 s and 90 s intervals compared to the control group. Neither group were significantly different to the controls on a standard time estimation task. This finding mirrors an earlier study, in which control subjects and patients with depression did not differ statistically on time production (counting to 30 s) or time estimation tasks (5 – 240 s). Interestingly, performance on the time estimation task negatively correlated with a measure of psychomotor retardation

for both patients and controls (Kitamura and Kumar, 1983). It should be noted that significant differences in time estimation have been noted in previous studies, for example Kuhs et al (1991) found significant underestimation of a 30 s interval, which correlated with a feeling of being unwell. Interestingly, the dissociation found in Bschor's study with the differential scores on the measure of time sense for the depressed and manic depressive patients, in contrast to the similar results on the time production task, suggest that these two measures tap different mechanisms.

In a variant of the duration discrimination task, depressed patients were significantly less accurate than control subjects using intervals of around 1.2 s, but not for shorter durations (Sevigny et al, 2003). Repetitive tapping (synchronisation only) was also impaired, with the ISI being 1 s and 10 s. The patients were also worse at a task requiring sustained attention and as the deficits were only apparent at intervals > 1 s, this suggests that cognitive factors may influence the results. However, patients with depression have also been found to be impaired at duration discrimination in a short interval range (standard interval of 50 ms) that is too short to be explained by cognitive deficits (Rammsayer, 1990). Again, in this group, it is not possible to test patients who are not under the influence of medication (very rarely is clinical depression not treated with medication), which limits interpretation. Medication, along with methodological differences, the heterogeneity found among depressed patients (e.g. presence/absence of psychomotor retardation) and the varying ways of rating depression, probably accounts for the inconsistent results that have been found. Indeed, differential results as a consequence of disease severity (Munzel et al, 1988) and the number of episodes of depression (Bschor et al, 2004) have been recorded. Bschor et al (2004) noted that different research groups have found evidence of underestimation of time, overestimation of time and no impairment of timing in depressed patients, suggesting that a clear consensus of opinion has yet to emerge.

Attention Deficit/Hyperactivity Disorder (ADHD)

ADHD is a developmental disorder that is best described by overactive behaviour (i.e. hyperactivity), impulsive behaviour and difficulty in paying

attention. Interestingly, fMRI technology has revealed that children with ADHD have atypical activation in the putamen (Teicher et al, 2000) and lesions of the putamen increase the risk of ADHD traits (Max et al, 2002). In addition, it has been shown that the head of the caudate nucleus in children with ADHD shows reversed asymmetry and that the left head is reduced in size (Semrud-Clikeman et al, 2000). This suggests that basal ganglia dysfunction could underlie any temporal deficits found in this patient group. Indeed, deficits in perceptual timing have been recorded in children with ADHD, for example in a variant of the duration discrimination task (requiring judgement of which of two circles (standard 1 s) appeared for a longer period) and in a time reproduction task of 12 s, in which significant underestimation was recorded (Smith et al, 2002). However, the significant finding for the time reproduction task was reduced to a trend after controlling for IQ and digit span scores and no significant effect was found for the time reproduction of 5 s intervals or the time estimation of 10 s intervals. The authors conclude that the length of the interval being estimated is likely to be the most important factor for performance because of problems with attention or motivational delay aversion. Digit span was equivalent across the control and ADHD group, making working memory problems an unlikely factor.

Indeed, motivation is a definite contender for explaining the results, as children with ADHD are better at a time reproduction task when the paradigm includes positive sham feedback and the possibility of a reward. However, as the children still performed more badly than the control children (who incidentally, were no more accurate in the motivating condition), this does not provide a full explanation (McInerney and Kerns, 2003). Furthermore, Rubia et al (1999) observed that children with hyperactive behaviour were more impulsive and variable on a timed motor anticipation task (anticipating a target occurring every 6 s), but were not impaired on a measure of perceptual timing. Rubia and colleagues also established that children with ADHD were not impaired on a repetitive tapping task (synchronisation only, ISI 700 ms) despite showing deficits in motor response inhibition (Rubia et al, 2001). If the hypothesis that motor and perceptual timing share common neural mechanisms is accepted, then the null result for motor tapping argues against a central timing deficit. Moreover, problems with attention are likely to underpin timing deficits for longer

intervals as attention, particularly mediated by the right hemisphere, is argued to be important for the accurate timing of intervals in the seconds-range (e.g. Lewis and Miall, 2003a). Indeed, ADHD is associated with a smaller volume of white matter in the right frontal lobe, which has been correlated with problems in sustained attention (Semrud-Clikeman et al, 2000). Brown and Vickers (2004) tested adolescents with ADHD and found no dysfunction on a temporal discrimination task (similar to the TDT, but judging whether two stimuli appeared at the same time), nor was medication seen to moderate performance (Brown and Vickers, 2004). This result is used to suggest that timing deficits in patients with ADHD recover with age, although if the timing deficits are cognitively mediated then the low-level demands of this task may be masking the dysfunction.

Clearly, deficits in temporal processing do occur in children and adolescents with ADHD. However, the range of traits that underlie ADHD provide suitable explanation for these deficits and make the existence of a primary timing deficit unlikely. Indeed, this group of patients serve to illustrate that the disruption to timing processes is possible by many means and that interpretation of any clinical data as representing timing deficits must be treated with caution.

1.3.4 Functional imaging studies

1.3.4.1 PET and fMRI

Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have made a significant contribution to furthering understanding of the differential brain areas involved in motor and perceptual timing. A detailed discussion of functional imaging and particularly PET (used in Chapters 3 and 6) can be found in Chapter 2. The data are somewhat confused by the variety of tasks used and by the changeable parameters selected, for example, the length of the duration being estimated, the type of control task and the modality of presentation. To provide an easy reference point, a summary of the different tasks has been provided in Table 1.2abc and Table 1.3abc, with the former presenting perceptual timing tasks and the latter presenting motor timing data. The studies presented are a comprehensive list of the range of PET and fMRI

studies of temporal processing, all of which use healthy subjects. It should also be noted that the control tasks and findings are only reported in the Tables if directly relevant to this introductory chapter. Furthermore, functional imaging studies discussed in this thesis that are not primarily focused on motor or perceptual timing are not included. First to be discussed are perceptual timing tasks, particularly enlightening as a certain subset do not include a timed motor element, a factor that can make interpreting basal ganglia and cerebellar activity difficult.

Duration discrimination tasks (Table 1.2a)

Maquet et al (1996) compared a visual version of the duration discrimination task (standard interval of 700 ms) to a control task involving passive listening to similar stimuli and a random button press. Areas of activation included the right prefrontal cortex, right anterior cingulate, right inferior parietal lobule, left fusiform gyrus and left cerebellar vermis. However, similar areas were also found when comparing an intensity discrimination task (comparing the brightness of a comparison LED to a standard) to the control, suggesting that these regions are involved in general visual attention and working memory processes. The comparison of the duration discrimination task with the intensity discrimination task (which controls for the cognitive aspects of the task) yielded no significant results, limiting any conclusions regarding the functional anatomy of perceptual timing. Ferrandez et al (2003) replicated the study of Maquet et al (1996), but used fMRI rather than PET. Using this more sensitive technique, the team found activation of the bilateral SMA and left putamen, specific to the duration discrimination task when compared to the intensity discrimination task. They suggest that these activations reflect 'clock' processes. Furthermore, cortical areas were also uncovered in this comparison. A subset were interpreted as providing attentional processes, enabling the temporal representations to be held in working memory (left premotor cortex, ventrolateral prefrontal cortex (BA 45/47) and inferior parietal cortex (BA 40)). Further areas were argued to provide an internal representation of the temporal duration (Broca's area (BA 44) and superior temporal gyrus (BA 21/22)), for example by use of subvocalisation or the creation of internal auditory representations of the visually presented stimuli.

Authors	Method	Temporal task	Relevant Control task(s)	Intervals used	Modality	Relevant findings
Jueptner et al (1995)	PET	Duration discrimination (D)	Auditory cued finger lift task (AC)	300ms standard	auditory	(D - AC): b anterior cingulate (BA 24) b thalamus b putamen/globus pallidus r caudate b cerebellar hemipshere l cerebellar vermis
Maquet et al (1996)	PET	Duration discrimination (D)	Intensity (LED) discrimination (I) Control random button press task (C)	700 ms standard	visual	(D - I): no significant results (D - C): r PFC (BA 45) r anterior cingulate (BA 32) r inferior parietal lobule l fusiform gyrus l cerebellar vermis
Rao et al (2001)	fMRI	Duration discrimination (D)	Frequency (pitch) discrimination (F)	1.2 s standard	auditory	(D - F): r DLPFC r insula/frontal operculum r putamen r caudate nucleus
Ferrandez et al (2003)	fMRI	Duration discrimination (D)	Intensity (LED) discrimination (I) Visually cued button press task (VC)	700 ms standard	visual	(D - VC) - (I - VC): b PFC (BA 45/47 & 44) b SMA l PMC (BA 4/6) r insula b superior temporal gyrus (BA 21/22) b inferior parietal cortex (BA 40) l putamen
Lewis and Miall (2003)	fMRI	Duration discrimination (D)	Length discrimination (L)	600 ms and 3 s standards	visual	(D(600 ms) - L) & (D(3 s) - L): b DLPFC r frontal pole b insula r preSMA r infeior parietal cortex (angular gyrus)
Nenadic et al (2003)	fMRI	Duration discrimination (D)	Frequency (pitch) discrimination (F)	1 s standard	auditory	(D - F): r putamen
Smith et al (2003)	fMRI	Duration discrimination (D)	Order discrimination (O)	1 s	visual	(D - O): r middle and inferior frontal gyrus (BA 9/44) r DLPFC (BA 9/46) b SMA l cerebellum
Harrington et al (2004)	fMRI	Duration discrimination (D)	Rest (R)	1.2 and 1.8 s standard	auditory	(encoding phase of D - R): r medial frontal cortex (BA 9) r inferior frontal cortex (BA 44/45) l anterior cingulate (BA 24) r posterior cingulate (BA 23) r pre-SMA (BA 6) b superior temporal cortex (BA 21, 22, 40, 41, 42) l superior pareital cortex (BA 7) r precuneus (BA 7) l angular gyrus (BA 39) b inferior parietal cortex (BA 40) r lingual gyrus (BA 18) b caudate l putamen b cerebellum

Table 1.2a: PET/fMRI perceptual timing studies: Duration discrimination tasks

KEY: b = bilateral, r = right, l = left, SMA = supplementary motor area, PMC = premotor cortex, DLPFC = dorsolateral prefrontal cortex, PFC = prefrontal cortex

Smith et al (2003) compared duration discrimination (standard 1 s) to making a temporal order judgement about the same stimuli (i.e. which of two coloured circles appeared first). The contrast elicited right middle and inferior frontal gyrus, right DLPFC, bilateral SMA and left cerebellum. The authors suggest that the cerebellum is involved in temporal processing, though point out that the absence of basal ganglia activation may be because they were activated during the processing of temporal order judgements.

When the stimuli are auditory, comparing the duration discrimination task with a frequency discrimination task (comparing the pitch of tones) has uncovered timing-specific activation in the right putamen (Nenadic et al, 2003) and right DLPFC, right insula/frontal operculum, right putamen, and right caudate nucleus (Rao et al, 2001). These tasks used similar standard intervals of 1 s and 1.2 s, respectively. An earlier study, using auditory intervals of a shorter duration (standard of 300 ms), found similar activation of the bilateral putamen/globus pallidus and right caudate nucleus, but also activation of the bilateral anterior cingulate (BA 24), bilateral thalamus and bilateral cerebellar hemispheres (Jueptner et al, 1995). However, the control task involved passive listening to stimuli and lifting a finger, thus it did not contain the same level of cognitive processing as better matched control tasks.

The study of Rao and colleagues (Rao et al, 2001) is particularly interesting in that it uses event related fMRI technology to disambiguate activation during different parts of the duration discrimination task (Rao et al, 2001). For this interpretation, activation during the duration discrimination task was compared to a second control task similar to that used by Jueptner et al (1995) (involving listening passively to stimuli and making a button press), which enabled analysis of the temporal and non-temporal cognitive processes in the timing task. Basal ganglia activation occurred early, associating it with the encoding of the time intervals (right putamen and bilateral caudate nucleus), suggestive of a central role in timing processes. Bilateral cerebellar vermis was also activated, but this only occurred at the end of the task, just before and during the discrimination task. As such, they suggest the cerebellum could be optimizing sensory input from auditory systems, facilitating the comparison of durations in

working memory. The right intraparietal sulcus/angular gyrus (BA 40) was active throughout the task and is interpreted in terms of a role in attention. Rao and colleagues go as far as suggesting that it could be accumulating pulses. The bilateral premotor cortex (BA 6) and right DLPFC were also activated and were implicated in working memory processes, with the late activation of the DLPFC suggesting that it is specifically involved in the comparison of the two intervals, enabling response selection. As with Jueptner et al (1995), the thalamus, although only in the right hemisphere, was also active with right superior and left middle temporal activation also being reported. Emphasis was placed on the dominance of right hemisphere activation, suggesting that this hemisphere is preferentially involved in time processing. Indeed, areas that were commonly active during both the duration and frequency discrimination task tended to be in the left hemisphere.

The design of Rao et al (2001) draws attention to an important methodological variation; some studies present the standard interval prior to scanning (e.g. Ferrandez et al, 2003; Lewis and Miall, 2003b), whereas others present the standard interval prior to each comparison interval (e.g. Jueptner et al, 1995; Rao et al, 2001). As well as affecting the breadth of data collected, the former design places greater demands on temporal working memory. The caudate nucleus has been implicated in working memory processes, such that the caudate activation found during the encoding phase in the study of Rao and colleagues (Rao et al, 2001) could be reflecting maintenance of a representation of the standard interval in memory. However, a follow up study, in which two standard intervals (1.2 s and 1.8 s) were randomly presented (preventing rehearsal of the same interval and encouraging encoding on each trial), found similar caudate activation during the encoding phase when compared to rest (Harrington et al, 2004b). Furthermore, the study found that greater activity in the right caudate was associated with reduced timing sensitivity, further suggesting a role in clock processes. Activation of the putamen was seen during both the encoding and decision phases and did not correlate with any measures of timing performance. This suggests differential roles for different regions of the basal ganglia in timing. In a potential challenge to the conclusions of their earlier study, cerebellar activity was seen to correlate

with temporal processing efficiency during the encoding phase. However, the authors argue that this finding is not inconsistent with a role for the cerebellum in monitoring and optimising input from sensory (in this case, auditory) systems that are essential to the encoding of intervals. The right medial temporal lobe (hippocampus and parahippocampus) was also active during the encoding phase and it correlated with increases in the bisection point, this was said to reflect the encoding and representation of output from the internal clock in memory. A particularly important conclusion was that areas that were active during the decision phase (including frontal, parietal and temporal regions) did not correlate with timing sensitivity and that areas that *did* correlate with timing sensitivity (right caudate, right inferior parietal cortex and left cerebellum) were not active during the decision phase. This result supports the idea that clock and decision processes are independent.

A caveat to the majority of studies investigating duration discrimination is that it is difficult to achieve a control task that is similarly attentionally demanding. For example, an intensity discrimination task can be solved as soon as the comparison LED is switched on; the subject would not have to pay attention to the whole of the duration. In contrast, a decision about a comparison temporal interval in the duration discrimination task can only be attempted after the entire comparison interval has been attended to. Lewis and Miall (2003b) circumvented this problem by presenting a control task that required the length discrimination of a visually displayed fluctuating line, which was being compared to a previously presented standard line (i.e. of a standard length). The length of the comparison line varied throughout its presentation and the judgement had to be made about the mean length of the line at the end of the presentation. The duration discrimination task involved two interval lengths, a 600 ms standard and a 3 s standard. Similar regions were identified for both interval lengths when compared separately to the length discrimination task, but with a larger network of regions being identified for the shorter interval. The areas activated by both intervals included the bilateral DLPFC, right frontal pole, bilateral insula, right preSMA and right angular gyrus, suggesting that these areas are implicated in a general timing system, regardless of stimulus characteristics (i.e. duration). The short interval task also activated additional regions; those that

survived a direct comparison of the two interval durations included parts of the motor system (the right frontal operculum and left cerebellar hemisphere). This is presented as evidence that the motor system is used for measurement of intervals in the milliseconds-range.

All but three of the eight duration discrimination studies have found activation of the striatum, with two of those three finding SMA activation. In fact, the right SMA (pre or proper), the right DLPFC and the right parietal region appear in at least three studies. There has been less evidence for the involvement of the cerebellum in central timing processes, particularly in well controlled comparisons. Although, it should be noted that Jueptner and colleagues (Jueptner et al, 1995) suggested the cerebellar activation in their study reflects timing processes.

Other discrimination tasks (Table 1.2b)

Another variation of the discrimination task is the velocity discrimination task, in which subjects have to monitor two velocities (in this instance presented as tactile stimuli on the right hand) and judge if the second is faster or slower than the first. Compared to a somatosensory control involving passive exposure to stimuli at constant velocities, the discrimination task produced contralateral cerebellar hemisphere and vermis activation, suggesting a role for these regions in temporally-defined judgements, even in the absence of motor output (Jueptner et al, 1996). Further activation was found in the right DLPFC, attributed to decision making processes.

As well as duration and velocity discrimination, researchers have also investigated the temporal discrimination threshold task. Pastor et al (2004) found that the right preSMA and right anterior cingulate were significantly more active during a tactile version of the TDT than a spatial control in which subjects had to decide whether two tactile stimuli were presented to the left or right of an imaginary line on the forearm. When both tasks were compared separately to a control task involving detection and response to stimuli, a broad cortical and subcortical network was uncovered including the head of the caudate nucleus, substantia nigra, subthalamic nucleus and bilateral cerebellar vermis and

hemispheres. This suggests the importance of basal ganglia and cerebellar regions in tasks involving discrimination, regardless of a temporal component. The cerebellar activity was interpreted as reflecting its role in optimizing sensory inputs through interactions with the cerebral cortex.

Authors	Method	Temporal task	Relevant Control task(s)	Intervals used	Modality	Relevant findings
Jueptner et al (1996)	PET	Velocity discrimination (V)	Presentation of stimuli of equal velocity (C) Rest (R)	not relevant	tactile	(V - C): l cerebellar hemisphere l cerebellar vermis r temporal cortex (BA 37) r insula r DLPFC (BA 10/46)
Schubotz et al (2000)	fMRI	Rhythm discrimination (monitor rhythm violations) (R)	Frequency (pitch) discrimination (F) Colour discrimination (C)	blocks of 2.4 s divided into three intervals	auditory and visual	(auditory R - F) & (visual R - C) : b frontal opercular cortex l SMA b PMC b intraparietal sulcus l putamen r putamen/caudate b cerebellar hemispheres
Schubotz et al (2001)	fMRI	Rhythm discrimination (monitor rhythm violations) (R)	Monitor for changes in the content of presented stimuli (S) Monitor for changes in spatial location of presented stimuli (SL)	blocks of 2.4 s divided into three intervals	visual	(R - (S + SL)): l SMA b frontal opercular cortex r caudate nucleus
Pastor et al (2004)	fMRI	Temporal discrimination Threshold (TDT)	Spatial discrimination (SD)	5-140 ms discrimination difference	tactile	(TDT - SD): r preSMA r anterior cingulate

Table 1.2b: PET/fMRI perceptual timing studies: Other discrimination tasks

KEY: b = bilateral, r = right, l = left, SMA = supplementary motor area, PMC = premotor cortex, DLPFC = dorsolateral prefrontal cortex

A further type of perceptual timing task that involves no timed motor element is that involving the monitoring of rhythms for deviants. The left SMA, bilateral frontal opercular cortex and right caudate nucleus were all activated by a task that required subjects to spot a deviation in a three-part rhythm over a 2.4 s interval (Schubotz et al, 2001). The task was compared to two controls that used the same stimuli but required discrimination of different elements (stimuli type or stimuli location), controlling well for cognitive elements of the experimental task. A further task found similar regions activated in monitoring for rhythmic deviants in both visual and auditory stimuli (albeit with a slightly

different basal ganglia focus in left putamen and right putamen/caudate) (Schubotz et al, 2000). Additional non-modality specific activations were found in the bilateral premotor cortex, bilateral intraparietal sulcus and bilateral cerebellar hemispheres.

Time production and time reproduction tasks (Table 1.2c)

Moving on to tasks in which a timed motor response is required, the time production study of Tracy et al (2000) required subjects to produce intervals varying from 12-24 s, following a start signal that indicated the interval to be produced. The data from this task was compared to both a silent counting task (the subjects internally counted, going up in ones) and a counting backwards task (subjects had to count backwards from a given number, taking seven off each time). Given the large intervals being estimated, these control tasks were argued to control for time monitoring strategies and numeric manipulation, respectively. To further control for non-temporal processes, most specifically attention, the contrast was masked by activation elicited by a dual task (the subject produced time intervals as well as counting backwards). This complex contrast produced activation in the right lateral cerebellum and the right inferior temporal gyrus. These regions were thus interpreted as reflecting 'primary timekeeper function', with further analysis showing that accuracy was associated with bilateral prefrontal cortex, left posterior parietal cortex and right lateral cerebellum activity.

A time production task using much shorter intervals was more recently conducted by Basso et al (2003), in which subjects had to time a 1.5 s delay before providing a response to a simple working memory task (deciding if a probe digit had been in a previous array of five digits). Feedback regarding timing accuracy was given. Compared to a basic working memory task, in which the decision regarding the probe was made as quickly as possible, the task activated the bilateral DLPFC and the right inferior parietal lobule. However, the regions activated may have been influenced by the on-going working memory demands of the task, indeed, interval estimates were significantly longer if the probe did not appear in the array.

Authors	Method	Temporal task	Relevant Control task(s)	Intervals used	Modality	Relevant findings
Brunia et al (2000)	PET	Time production with feedback (TPF)	Time Production with false feedback (TPFF)	3 s	not relevant	(scans 3&4) - (scans 1&2) for both tasks: r SMA r DLPFC
Tracy et al (2000)	fMRI	Time production (TP)	Silent couting (SC) Counting backwards in sevens (CB) Dual Task (TP and CB	12 - 24 s	not relevant	(TP - (SC CB)) masked by DT: r lateral cerebellum r inferior temporal gyrus (BA 20)
Lewis and Miall (2002)	fMRI	Time production (TP)	Force production (FP)	3 s	not relevant	(TP - FP): r DLPFC r anterior cingulate r preSMA r PMC r insula r intraparietal sulcus r supramarginal gyrus
Macar et al (2002)	PET	Time reproduction (TR)	Auditory cued button press task (AC)	2.2, 2.7 & 3.2 s & 9, 11 & 13 s	tactile	(TR - AC): b DLPFC (BA 9/46) r anterior cingulate l precentral gyrus r SMA r inferior parietal lobule
Basso et al (2003)	fMRI	Time production (TP)	Working memory task (W)	1.5 s	not relevant	(TP - W): b DLPFC (BA 46) r inferior parietal lobule (BA 40)
Kudo et al (2004)	fMRI	Time reproduction (atypical) (TRA)	Reaction time (RT)	target with moving at 12-26 cm . s^{-1}	visual	(TRA - RT): l postcentral sulcus (BA 7) r fusiform gyrus (BA 7) l cuneus (BA 19) l precuneus (BA 7) r precuneus (BA 7) r superior parietal (BA 7)
Macar et al (2004)	fMRI	Time production (TP)	Force production (FP)	2.5 s	not relevant	(TP - FP): r SMA (BA 6) l primary motor cortex (BA 4)

Table 1.2c: PET/fMRI perceptual timing studies: Time production and reproduction tasks

KEY: b = bilateral, r = right, l = left, SMA = supplementary motor area, PMC = premotor cortex, DLPFC = dorsolateral prefrontal cortex

A third time production study required subjects to produce intervals of 3 s. Compared to a force production task, time production was associated with right DLPFC, right preSMA, right premotor cortex, right anterior cingulate, right insula, and left supramarginal gyrus and intraparietal sulcus (Lewis and Miall, 2002). In a similarly designed study, in which the produced intervals were 2.5 s,

only right SMA and left premotor cortex were related to time production (Macar et al, 2004). A final study required the production of 3 s intervals with feedback either being false or correct. Behaviourally, subjects improved in the second half of the scanning session regardless of the type of feedback given. This improvement was associated with right SMA and right DLPFC activation (Brunia et al, 2000). The authors suggest that these areas are associated with the creation of an internal standard of the interval and with the related temporal programming of movement.

The time reproduction paradigm, which differs from the time production paradigm in providing an example of the interval to be produced, has also been tested. Compared to a task in which auditory-cued responses were made, the reproduction of seconds-range (2.2 – 13 s) intervals produced activation in the bilateral DLPFC, right anterior cingulate, left precentral gyrus, right SMA and right inferior parietal cortex (Macar et al, 2002). Activation of the left putamen was also found for a subset of shorter intervals (2.2 - 3.2 s), although there were no significant results for the interaction between task and interval duration. The authors suggest a key role for the SMA in time reproduction, probably via its connections with the basal ganglia. It is suggested that non-motor cortical activity could be reflecting the high attentional demands of this type of task, particularly in relation to the control task. The DLPFC and anterior cingulate are implicated in comparison and decision processes, with the authors also drawing attention to the dominance of the right hemisphere amongst the activations. The final task in this section is that of Kudo et al (2004), which uses a design tentatively labelled in this thesis as atypical time reproduction, although contains some element of rhythm perception and synchronisation (albeit with limited movement). In the timing task, the subjects observed seven horizontally placed LEDs that were lit in sequence at a given speed, giving the impression of a horizontally moving light. They had to judge the timing of the moving stimulus, such that they performed an index finger-thumb tap at the time they expected the last LED to be lit. This task activated several posterior regions, with activation of the intraparietal sulcus causing particular interest. The authors refer to the proposed role of this region in the perception of action and in the attentive tracking of moving targets. However, the control task required a

reaction time movement to the *first* LED, meaning that the stimuli could be ignored for the remainder of the task. So, whether the parietal activity represents timing-specific activity related to tracking a moving object or more non-specific attentional mechanisms cannot be disambiguated. Certainly, the study raises interesting questions about the relationship between temporal and spatial information (see Walsh (2003) for further discussion).

Activation of the right SMA and right (or bilateral) DLPFC are the most consistent findings of the time production and reproduction tasks, occurring in four of the seven studies. Activation of the SMA is perhaps not surprising as the tasks involve a self-initiated movement. However, the control task typically includes a volitional movement that controls for SMA activation, thus the data is suggestive of a role for this region in timing processes. The SMA activation provides evidence for the role of the frontostriatal motor loop in timing, which is particularly interesting as basal ganglia activation is notably absent in time production and reproduction tasks compared to the discrimination tasks. Right parietal activity, in different regions, occurs in over half the tasks. Indeed, the parietal cortex is the area of posterior cortex that is most consistently activated across the range of functional imaging studies of timing. It is more common in the perceptual timing tasks than the motor timing tasks, which supports a role for this region in providing necessary attentional mechanisms: the motor timing tasks often require regular tapping and therefore place limited demands on attentional processing. Indeed, the only motor timing task whose main comparison shows parietal activity is that of Lejeune et al (1997), where the subjects had to tap in time to a cue presented at the relatively slow rate of every 2.7 s. It should be noted that recent discussion has suggested that the parietal cortex could encode both space and time within the same neurons (see Walsh (2003)), but that there is little empirical evidence to support this at present.

Synchronisation and continuation tasks (Table1.3a)

Motor timing processes have been investigated through exploration of the repetitive tapping paradigm. Rao et al (1997) compared both the synchronisation and continuation task (ISI 300 ms and 600 ms) to rest and found left sensorimotor cortex, right superior temporal gyrus and right cerebellar

activation common to both tasks. This led to the proposal that the cerebellum is engaged in sensorimotor functions during both tasks, particularly as the area of activation was within the vicinity of the dorsal dentate nucleus, known to share connections with the sensorimotor cortex. Additional activation of Broca's area, left SMA, left ventrolateral thalamus and left putamen was found in the continuation task. The activation of the latter three areas suggests that the frontostriatal motor loop is engaged when explicit, internally generated timing is required. The activation of Broca's area reflects the requirement for the rehearsal of auditory information. This study did not directly compare the synchronisation and continuation data, limiting the strength of the conclusions.

More recently, Jantzen et al (2004) performed such a comparison on data that was collapsed across synchronised and syncopated tapping (ISI of 800 ms), with the task involving finger-thumb opposition movements, rather than the more typical single finger button press. Compared to the continuation task, the synchronisation task elicited bilateral superior temporal gyrus activation, which is probably reflecting the presence of tones in this task. The opposite contrast (continuation > synchronisation) was not reported. Bilateral superior temporal gyrus activity was also reported in the synchronisation > continuation contrast in a task presenting tones every 400 ms (Jancke at al, 2000), although they did not find any significant results for the continuation > synchronisation contrast. This study also included a visual version of the task that presented visual pacing stimuli in the synchronisation task. The four tasks were separately compared to rest and all activated the left premotor cortex (ventral in the two auditory tasks and dorsal in the two visual tasks), left primary motor cortex, left sensorimotor cortex, SMA (left in the two auditory tasks and midline in the two visual tasks), bilateral inferior parietal lobe and right cerebellum. Interestingly, there were no 'consistent differences' in activity between the synchronisation and continuation tasks when the two presentation modalities were collapsed across.

Authors	Method	Temporal task	Relevant Control task(s)	Intervals used	Modality	Relevant findings
Rao et al (1997)	fMRI	Synch task (S) Cont task (C)	Rest (R)	300 ms and 600 ms	auditory	(S - R): l sensorimotor cortex (BA 4) r superior temporal gyrus (BA 22) r cerebellum (C - R): r inferior frontal gyrus (BA 44) l sensorimotor cortex (BA 4) l SMA (BA 6) r superior temporal gyrus (BA 22) l thalamus l putamen r cerebellum
Jancke et al (2000)	fMRI	Synch task: auditory (SA) Synch task: visual (SV) Cont task: auditory (CA) Cont task: visual (CV)	-	400 ms	auditory and visual	(SA - CA): b superior temporal gyrus (CA - SA): no significant results (SV - CV): r dorsal and ventral PMC b inferior occipital gyrus (CV - SV): l dorsal PMC l primary motor cotex l primary somatosensory cortex l inferior parietal lobe
Jantzen et al (2004)	fMRI	Synch task: synchronised (SC) Synch task: syncopated (SP) Cont task: synchronised (CC) Cont task: syncopated	-	800 ms	auditory	(SC + SP) - (CC + CP): b superior temporal gyrus
Lewis et al (2004)	fMRI	Tone presentation and initation of tapping (I) Synch task (S) Cont task (C)	-	500 ms for isochronous tapping, 174 - 936 ms for complex rhythms	auditory	(I) Correlation with temporal complexity: b DLPFC r anterior cingulate b SMA b preSMA b PMC r primary sensorimotor cortex l thalamus b caudate b putamen

Table 1.3a: PET/fMRI motor timing studies: Synchronisation and continuation tasks

KEY: b = bilateral, r = right, l = left, SMA = supplementary motor area, PMC = premotor cortex, DLPFC = dorsolateral prefrontal cortex, Synch task = synchronisation task, Cont task = continuation task

Synchronisation tasks (Table 1.3b)

A small subset of studies has looked at the synchronisation task in isolation. Rubia et al (1998) found right medial prefrontal cortex activation, including the anterior cingulate, during synchronisation tasks with 600 ms and 5 s ISIs. During a synchronisation task with an ISI of 2.7 s, Lejeune et al (1997) also found activation in the right anterior cingulate, as well as in the right ventrolateral prefrontal cortex, right inferior parietal lobule and right vermis. The cerebellar activation was found despite a control task that involved an equivalent amount of button pressing, in reaction to irregular presentations of a tone. A conjunction analysis with the duration discrimination task of Maquet et al (1996) found left putamen and left cerebellar hemisphere common to both types of timing task, with the left hemisphere focus of the cerebellum suggestive of a non-motor role in timing. Further activation was found in a right hemisphere network of DLPFC, insula/ventrolateral prefrontal cortex, anterior cingulate and inferior parietal lobule, perceived as being important for working memory and attentional processes.

Authors	Method	Temporal task	Relevant Control task(s)	Intervals used	Modality	Relevant findings
Lejeune et al (1997)	PET	Synch task (S)	Visually cued button press task (AC)	2.7 s	visual	(S - AC): r insula/ventrolateral PFC (BA 47) r anterior cingulate (BA 32) r inferior parietal lobule (BA 40) r cerebellar vermis
Jancke et al (1998)	fMRI	Synch task (S)	Rest (R)	200 ms - 2 s	visual	linear relationship between BOLD signal and tapping rate (200 - 666.67 ms): l sensorimotor corex
Rubia et al (1998)	fMRI	Synch task (S)	-	600 s and 5 s	visual	(S(600 ms) and S(5 s)): r anterior cingulate (BA 10/32)

Table 1.3b: PET/fMRI motor timing studies: Synchronisation task only

KEY: b = bilateral, r = right, l = left, Synch task = synchronisation task

The DLPFC activation in the conjunction analysis is particularly interesting as most tasks that find DLPFC activity use perceptual timing tasks. Perceptual timing tasks are highly cognitive, whereas motor timing tasks, once learnt, can be performed with little demand on cognitive processes and have been termed

'automatic' (Lewis and Miall, 2003a). It may be that this distinction accounts for the differential DLPFC activity, rather than the motor/non-motor distinction. Indeed, DLPFC activity in timing tasks is typically interpreted in terms of the cognitive demands of these tasks such as working memory and decision making. Lewis and Miall (2002) found right DLPFC activity during a time production task that they describe as motoric and non-automatic. The subjects had to modify each successive timed press in accordance with a visual cue (making it longer or shorter than their previous production, with the production of the first press being 3 s). It is suggested that the right DLPFC is important in motor timing tasks, at least if they contain a non-automatic element. However, as with most time production tasks, each trial was separated by an inter-trial delay, which meant that the task did not have the continuous element of the classic motor timing task.

The speed of tapping in the motor timing task is likely to influence neural activity. Indeed, Jancke et al (1998) found a linear relationship between increased tapping rate on the synchronisation task (for ISIs of 200 ms – 666.67 ms) and activity in the left sensorimotor cortex. BOLD activity within this region was different for longer ISIs (1 and 2 s), leading to the suggestion that motor control is managed differentially dependent on rate.

Other motor timing tasks (Table 1.3c)

In addition to the repetitive tapping tasks, research has also investigated the production of more complex rhythms. Penhune et al (1998) found cerebellar activity during the unpaced reproduction of a simple, isochronous rhythm (that had been presented either visually or auditorily), compared to passively listening to or observing the rhythm. However, even greater cerebellar activity was found during the reproduction of a more complex (non-isochronous) rhythm that was novel, compared to reproducing a non-isochronous rhythm that was presented repeatedly. This suggests that cerebellar activity is augmented by the complexity or novelty of a timed motor response. It is argued that the cerebellum, rather than providing clock functions, holds the necessary circuitry for the 'sensory system to extract temporal information and for the motor system to learn to produce the timed response'. Although, globus pallidus activation

was evident in the simple isochronous task, basal ganglia activation (putamen) was only found in the novel vs repeated comparison when the stimuli was presented in the auditory modality. Penhune et al (1998) conclude that the basal ganglia may be important for the implementation of the motor response. Further research, focusing on motor learning, found that the cerebellum is active during the early learning of a complex motor sequence but is not active when the sequence is learnt (Penhune and Doyon, 2002). Ramnani and Passingham (2001) found increases in cerebellar activation associated with the learning of a complex rhythm, compared to producing random rhythms (no learning possible). However, there were no learning-related differences in activation found for a simple synchronisation task, which doesn't favour a motor learning hypothesis for cerebellar activity during classic motor timing tasks (Penhune and Doyon, 2002). Moreover, in a study of overlearned rhythms fitted within a synchronisation-continuation framework, Lewis et al (2004) found no correlation between cerebellar activity and the temporal complexity of the rhythms, which suggests a lack of sensitivity to temporal demands. Areas including the bilateral DLPFC, SMA and preSMA, rostral dorsal premotor cortex and caudate and putamen were correlated with complexity during an 'initiate' stage in which the learnt rhythm was presented and initiated. The authors suggest that the preSMA and dorsal premotor cortex activation is related to the selection of timing parameters.

Another variation on the theme of motor timing has been Kawashima et al's (2000) 'memory-timed' (MT) finger movement task in which subjects learn to press a response button every 1.5 s (in beat with a metronome) and in the scanner produce this rhythm from memory (no cues). Thus, the most distinguishing feature of this motor timing design is the emphasis on temporal memory. A conjunction analysis was performed on the MT task minus a visually cued button press task (pressing a button when a visual stimuli changed in brightness) compared with the same MT task minus rest. Activation was found in the left DLPFC, left inferior frontal cortex and the bilateral cerebellar lobules. Interestingly, the number of movements was greater in the visually cued task, suggesting that the cerebellar activation is not reflecting the execution of movement. Rather, the results are consistent with the cerebellar hypothesis of

clock function. The DLPFC activation is interpreted as reflecting the 'willed', or internally generated, component of the production task, or the dominant memory component. The left inferior frontal cortex is argued to reflect subvocalisation, particularly as it was also activated in a fourth task that involved silent articulation.

Authors	Method	Temporal task	Relevant Control task(s)	Intervals used	Modality	Relevant findings
Penhune et al (1998)	PET	Perception and reproduction of isochronous sequence (I) P and R of a non-isochronous repeated sequence (R) P and R of a non-isochronous novel sequence (N)	Perception of isochronous sequence (P)	250 ms and 750 ms	auditory and visual	(I - P) for both auditory and visual stimuli: l primary motor/sensorimotor cortex l globus pallidus r cerebellum (N-R) for both auditory and visual stimuli: b cerebellar hemispheres b cerebellar vermis
Kawashima et al (2000)	fMRI	Memory timed rhythm production (MT)	Visually cued button press task (VC) Rest (R)	1.5 s	not relevant	Conjunction analysis of (MT - VC) and (MT - R): l DLPFC l inferior frontal cortex b cerebellar lobules (IV and V)

Table 1.3c: PET/fMRI motor timing studies: other tasks

KEY: b = bilateral, r = right, l = left, DLPFC = dorsolateral prefrontal cortex

Looking across the range of motor timing studies, cerebellar activity certainly occurs although it is often afforded a non-temporal explanation. The greater tendency for cerebellar activation to occur in motor timing tasks suggests that its role in motor processing may in some way underlie the activity. However, some results, such as Kawashima's well-controlled motor timing study (Kawashima et al, 2000), suggest that easy dismissal of the cerebellar contribution to timing is not possible. It has also been found that the right cerebellar hemisphere is active during a letter discrimination task in which the time interval between successive stimuli is randomised, compared to when they occur regularly, suggesting a role in 'timing adjustment' (Dreher and Grafman, 2002). Basal ganglia activation is less apparent in the motor timing tasks

compared to the perceptual timing tasks, although it is only in the discrimination tasks in which it makes a consistent appearance. It should be remembered that the control tasks in motor timing studies often include a timed motor element (e.g. synchronisation vs continuation or synchronisation vs cued button press), which may inhibit the activation of certain timing-related regions.

Summary

If the entire spectrum of timing-related functional imaging studies is considered, it is clear that both basal ganglia and cerebellar activity appear with relative consistency. The basal ganglia activations found across the studies are perhaps more convincing, particularly with regard to their relative dominance in the discrimination tasks. Lewis and Miall (2003a) reviewed the current functional imaging literature on timing and concluded that there are two distinct timing systems, one is the 'automatic' timing system that is primarily involved in continuous timing of milliseconds-range intervals that are defined by movement and the second is a 'cognitively controlled' timing system that is preferentially involved in the measurement of discrete seconds-range intervals that are non-motor. 'Automatic' type tasks are found to activate the motor system, with the over-learned nature of the tasks removing the need for attentional modulation, whereas 'cognitively controlled' type tasks activate prefrontal and parietal areas associated with working memory and attention. Lewis and Miall (2003a) serve to underline a very important point that was touched on earlier, i.e. differential results in the timing literature reflect the range of tasks (and control tasks) studied. Taking a different perspective, the review of Macar et al (2002) looks for common areas of activation across the tasks (and also electrophysiological tasks) and notes that the basal ganglia, SMA and cerebellum as well as the DLPFC, anterior cingulate and right parietal cortex, are active across a range of perceptual and motor timing tasks. Certainly, involvement of these areas are persistently observed across the timing tasks presented in this Introduction, with a right hemisphere dominance being evident.

1.3.4.2 Electrophysiology

Electrophysiological research using EEG recordings enables measurement of neural activity. Although the spatial resolution of EEG is not as impressive as

PET and fMRI, this procedure offers superior temporal resolution. Furthermore, whereas interpretation of PET and fMRI results rely on the observation that areas of high blood flow reflect areas of high neural activity, EEG *directly* measures electrical activity in the brain, with output reflecting the summated activity of populations of neurons.

In an event-related potential (ERP) study, subjects were tested on a temporal generalization paradigm (standard interval 200 ms) compared to a pitch generalization paradigm (Gibbons et al, 2003), with both sets of stimuli being identical. The temporal task activated a broader network, including areas corresponding to the prefrontal cortex, reflecting greater working memory activity. A similar region was implicated in a duration judgement task, in which subjects had to decide if two successive durations (range 236 – 650 ms) were the same or different (Schubotz and Friederici, 1997) and also in a temporal reproduction task (3 or 4 s) (Casini and Macar, 1996). Monfort et al (2000) have used ERP recordings to illustrate that the right frontal cortex plays a crucial role in time perception (range 560 ms – 3 s). In a further EEG study, Mohl and Pfurtscheller (1991) found involvement of the right parietal cortex prior producing a button press of 500 ms or 1.3 s in length was stronger when performance was accurate. Although the authors suggest that the activity could also reflect visual attention (the hand to be used and the duration to be produced were both presented visually). Macar et al (1999) found activation over the mesio frontocentral cortex, mainly incorporating the SMA, during a time production task and a duration discrimination task (target/standard 2.5 s). The activity was greater if the duration produced was longer and if the duration judged was longer (regardless of the actual duration). In accordance with internal clock theories, the authors propose a role for the SMA in either providing the pulse accumulation process or in receiving such output from the striatum. These data are particularly interesting as it assigns the SMA a role in temporal processing that is not dependent on motor activity. A follow up study that manipulated the feedback given to the subjects has suggested that the performance related activity over the SMA is the result of temporal memory consolidation (based on the accumulation of pulses that are necessary for

encoding a duration) rather trial-to-trial memory updating controlled by feedback (Macar and Vidal, 2002).

Research has suggested that the contingent negative variation (CNV), a slow negative wave generated over several seconds between two associated stimuli, may be the cortical electrophysiological correlate of temporal processing (see Pouthas (2003) and Macar and Vidal (2004) for reviews). This is a consistent finding, observed in both auditory and visual temporal tasks (N'Diaye et al, 2004) and during both filled and unfilled intervals in both randomised and blocked designs (Gibbons and Rammsayer, 2004). Interestingly, the amplitude of the CNV is reduced in patients with Parkinson's disease and moderated by levodopa medication (e.g. Ikeda et al, 1997; Oishi et al, 1995). Matell and Meck (2004) discuss how ramp activity, including evidence from the CNV, is a potential neural clock source. Single cell studies have shown that cells in the prefrontal cortex show monotonically increasing firing rates during a delay period (e.g. Fuster et al, 1982). However, Matell and Meck (2004) comment that these types of studies tend to use delay tasks in which a stimulus must be held in working memory; whether these cells would show similar activity in a temporal task is not yet apparent. Another potential electrophysiological correlate of temporal processing is oscillatory activity (see Matell and Meck, 2004), reflecting the neural network and neurobiological models discussed earlier in this chapter. For example, Lebedev and Wise (2000) have found oscillatory activity in the premotor cortex during a delay period preceding movement. The pattern of the oscillatory activity suggested that the oscillations may be coding the movement that is being prepared, which could include the timing of the movement. Matell and Meck (2004) suggest that both ramp and oscillatory activity in the cortex could produce firing patterns that serve as clock signals that are integrated by the striatum, in keeping with their striatal beat frequency model (Matell and Meck, 2000; 2004). Contrary to this, a recent study by Matell and colleagues failed to find evidence of oscillatory patterns in frontal cortex neurons that were active during the timing of learnt intervals, although such activity may be apparent in other areas of the cortex (Matell et al, 2003). It should also be remembered that clinical research in patients with lesions to the

cortex (see section 1.3.3.3) does not produce convincing evidence that the cortex acts as a timekeeper.

Research using EEG has also contributed to the debate regarding a possible dissociation between automatic and cognitively controlled timing. The observation of mismatch negativity (MMN) waveforms in response to incongruent temporal durations has indicated that duration discrimination can occur at the automatic, preattentive level (Näätänen et al, 2004). Interestingly, Grimm et al (2004) found that the MMN was absent when the standard temporal interval was 1 s, compared to when it was 200 ms. Conversely, when the subjects were instructed to attend to the stimuli and judge when they heard the deviant, the MMN was present in both conditions. This suggests that intervals of 1 s are not automatically detected on a sensorial level, supporting the suggestion that millisecond-range intervals are processed automatically whereas seconds-range intervals depend on cognitive processes such as working memory and attention (e.g. Rammsayer 1993; 1997; 1999; 2001). Contradicting this research, Näätänen et al (2004) found evidence of the MMN when the standard interval was 200 ms, 400 ms, 800 ms and 1600 ms. However, the MMN for the two longer intervals was significantly smaller than for the two shorter intervals.

Electrophysiological research clearly supports a role for the cortex in temporal processing, particularly the prefrontal cortex and SMA, although the exact nature of that contribution still remains unclear. Indeed, Macar and Vidal (2004) have recently described how the CNV may reflect accumulator processes, memory processes, decision processes or learning processes.

1.3.5 Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) can be used to temporarily disrupt the neural functioning in a discrete area of cortex. Thus, if the application of TMS affects performance on a task, then the area being stimulated can be said to be essential to the task performance. The technique is explained in detail in Chapter 2. To date, only two published studies have used TMS to investigate

the neural correlates of timing. In the first, Theoret and colleagues (2001) used 5 minutes of 1 Hz repetitive TMS (rTMS) in a 'before and after' paradigm to investigate its effect on repetitive tapping (synchronisation only) with an ISI of 475 ms. After applying rTMS over the medial cerebellum variability on the task was affected, with accuracy remaining unimpaired. Conversely, rTMS over the lateral cerebellum and motor cortex did not affect either dimension. This suggests that the medial cerebellum plays a necessary role in milliseconds-range motor timing. Koch at al (2003) tested subjects on a time reproduction task before and after 10 minutes of 1 Hz rTMS over the right DLPFC and left DLPFC. Stimulation over the right DLPFC resulted in underestimation of intervals of 5 s and 15 s duration, whereas stimulation over the left DLPFC did not alter timing behaviour. The authors concluded that the right DLPFC plays a specific role in seconds range timing and speculate that its function is related to memory or decision processes.

1.4 PURPOSE OF THE THESIS

To date, evidence has amassed that links both the cerebellum and basal ganglia to millisecond- and seconds-range motor and perceptual timing. However, it seems unlikely that basal ganglia and cerebellum are both primary timing systems. Certainly, if they were independent timing systems then patients with PD and cerebellar pathology would be unimpaired on timing tasks as they would be able to recruit the second, non-impaired timing system. Alternatively, timing functions may be primarily dependent on one structure, with the second being integral to accurate timing by providing necessary, but secondary functions. The impressive pharmacological work with dopamine presents a fairly convincing argument for the role of the basal ganglia in 'clock' functions. Functional imaging work that has found clear evidence of SMA activity during temporal processing further suggests the importance of the frontostriatal motor loop. Overall, the data relating to the cerebellum seems more varied. It has been suggested that damage to this structure may cause deregulation of areas of the thalamus that are convergence points for striatal and cerebellar cortical connections (Gibbon et al, 1997; Malapani et al, 1998a).

Alternatively, the cerebellum may be necessary for acquiring task-relevant sensory and cognitive information and, also, in coordinating this with motor output (Harrington et al, 2004a). The contribution of the cortex to temporal processing has also emerged, with most data suggesting that it provides additional cognitive support.

This thesis seeks to add to the data that has been collected to date by further exploring the differential contributions of the basal ganglia, cerebellum and cortex to motor and perceptual timing in the milliseconds- and seconds-range. To clarify the differential contributions of the basal ganglia and cerebellum to timing, Chapter 3 addresses a prominent hypothesis proposed by Ivry (1996) that suggests that the cerebellum is engaged in the timing of milliseconds-range intervals whereas the basal ganglia is engaged in the timing of seconds-range timing. PET is used to look at the differential activation elicited by a short (milliseconds-range) and a long (seconds-range) time reproduction task. In Chapter 4, a study using repetitive TMS is reported in which the role of the right dorsolateral prefrontal cortex in timing is assessed. Chapter 5 assesses patients with PD and patients with cerebellar disease on a range of timing tasks, with the aim of dissociating the contribution of the basal ganglia and cerebellum to perceptual and motor timing. The study includes a warned and unwarned reaction time task as well as a task assessing memory for temporal order, two tasks that have not yet been compared with measures of motor and perceptual timing. Chapter 6 presents a second PET study in which the role of apomorphine (a dopamine agonist) in modulating the brain activity of patients with PD during motor timing is assessed. The patients are compared to a group of healthy controls to provide a comprehensive exploration of patterns of neural activity associated with the repetitive tapping task in patients 'on' and 'off' medication relative to normals.

Chapter 2
Methods

The functional imaging technique positron emission tomography (PET) is used in Chapters 3 and 6 of this thesis. Magnetic resonance imaging (MRI) is used in conjunction with PET to provide structural scans of the subjects' brains, to aid in interpretation of the data. The data are analysed using the statistical parametric mapping (SPM) method devised at the Wellcome Department of Imaging Neuroscience (WDIN), Institute of Neurology. Chapter 4 uses repetitive Transcranial Magnetic Stimulation (rTMS). This chapter gives a detailed description of these methods.

2.1 FUNCTIONAL IMAGING

Functional imaging techniques provide a unique opportunity for scientists to investigate the human brain *in vivo*. Researchers in the field of cognitive neuroscience are typically interested in regionally specific brain areas activated by a particular cognitive process (or process of interest). Using PET and fMRI, data reflecting blood flow is measured, following the observation that areas of high blood flow represent areas in which neurons are more active (Raichle, 1998). Broadly speaking, functional imaging investigates the two principles of brain organisation: *functional specialisation* and *functional integration*. Functional specialisation is concerned with *where* in the brain activity relating to specific processes occur and rests on the assumption that a cortical (or subcortical) area can be specialised for a particular task and that this specialisation can be anatomically segregated within the (sub)cortex. A cognitive process can be subserved by several functionally specialised areas; functional integration describes the unique pattern of connections established between the specialised areas. As such, functional specialisation and functional integration are intrinsically linked. Functional integration can be further subdivided into *functional connectivity* and *effective connectivity*. Functional connectivity refers to the temporal correlations that occur between regions of

brain activity, but does not say anything about how these correlations are mediated. On the other hand, effective connectivity refers explicitly to the influence that one neural system exerts over another (e.g. Friston, 1994).

2.1.1 Design of experiments

Interpretation of imaging data most often relies on the principal of *cognitive subtraction*. By this method, data collected during experimental task (A), involving the processes of interest, is compared to data collected during a control task (B), which is identical to A except for the process of interest. In subtracting B from A, it is proposed that the regions (identified as voxel co-ordinates) involved in the process of interest will be revealed. It should be noted that the assumption that the A and B are identical in every way except for the process of interest is often difficult to meet. For example, in Chapter 1 the inadequacy of an intensity discrimination task (control task) for controlling for sustained attention in a duration discrimination task (experimental task) was discussed. This type of subtraction method also rests on the assumption of 'pure insertion', whereby an extra cognitive component can be introduced without affecting existing components (i.e. an interaction between the old and new components) (Friston et al, 1996). One way to avoid the assumption of pure insertion is to use an alternative method, *conjunction analysis* (Price and Friston, 1997). Conjunction analysis takes two or more subtractions (e.g. B-A and C-D) that both contain the same process of interest in their difference and looks for areas of activation that are common to the task pairs. Thus, the method extracts common brain areas across two tasks that are involved in a particular, shared process. These brain areas should be uniquely associated with the process of interest and not with any interactions specific to each subtraction.

Factorial designs combine two or more factors within the same experiment. In an example from the literature, Ramnani et al (2001) presented a 2 X 2 factorial design that compared arm movement and finger movement (main effect of effector) at two levels of motor activity (main effect of movement). Looking at interactions between the factors identifies regions of the brain in which the

effect of one factor varies depending on the presence or absence of another factor. In *parametric designs*, the process of interest is treated as a dimension and brain areas that vary as a function of this dimension are uncovered. This dimension can either be manipulated externally by the experimenter (e.g. tapping rate) or determined by the subject (e.g. accuracy of temporal reproduction). This latter manipulation is only possible in fMRI, where event-related techniques allow detailed post-hoc manipulation of the classification of the data.

2.1.2 PET

2.1.2.1 Principles of PET

PET is an invasive procedure and relies on the injection of a small amount of a radioactive isotope (or tracer), which has been combined with a compound normally found in the body, into the subject's bloodstream. A variety of these radioactively labelled biological probes are used (e.g. $H_2{}^{15}O$, ^{18}F-FDG, ^{18}F-Dopa) for the detection of changes in physiological (e.g. blood flow), metabolic (e.g. glucose metabolism) or neurotransmitter processes (e.g. dopamine receptors). For the studies reported in this thesis, radiolabelled water ($H_2{}^{15}O$) is used, allowing measurement of the flow of oxygen to different parts of the brain, which provides an indirect measure of rCBF (regional cerebral blood flow). During its decay process, the ^{15}O tracer emits a positron that quickly annihilates with an electron, producing a pair of gamma ray photons in opposite directions. The photons are detected by paired photomultipliers arranged around the subject's head. This allows their origin in the brain to be plotted, as the coincident photons define a line that intersects the position of the annihilation event. Furthermore, the intensity of the emission indicates the focal concentration of the isotope at any particular position in the head. The data can be reconstructed to provide a count density that reflects the concentration of the tracer in the tissue. Photons that do not occur in a pair (coincident within several nanoseconds) are ignored. As the tracer has a relatively short half-life (approximately 2 minutes), the isotope is produced in a cyclotron close to the scanner.

For Chapters 3 and 6, a Siemens/CPS ECAT EXACT HR+ PET scanner (Siemans/CTI Inc., Knoxville, TN) in 3D mode with inter-detector collimating septa retracted, is used. An axial field of view of 155 mm provides coverage of the whole brain, including the cerebellum. The subject lies supine on the scanner bed with their head in the scanner, a foam padded helmet is worn and secured to the scanner bed to minimise discomfort. Prior to data collection, a transmission scan is collected to correct for attenuation effects. Following on from this, each measurement is enabled by giving approximately 9mCi of $H_2{}^{15}O$ intravenously through a forearm cannula over 20 s, followed by a 20 s saline flush. Subsequent rCBF data is acquired during a 90 second activation period that begins 5 s before the rising phase of the count curve. Following this procedure, 12 measurements are collected, with an 8 minute rest period occurring between each successive scan to allow for the radioactivity to decay. The images are reconstructed using 3D filtered back projection into 63 transverse planes and into a 128 x 128 pixel image matrix (pixel size 2.4 mm x 2.1 mm x 2.1 mm), with a resolution of 6 mm at full-width half maximum.

2.1.2.2 Safety

The use of a radioactive material necessarily means that tight controls surround the use of PET. The amount of radioactivity that a subject is exposed to in any one experiment is comparable with the radioactive dose involved in an intravenous urograph (IVU) or to living in Devon for 10 months (where the granite rocks provide higher natural levels of radioactivity than other parts of the country). Subjects are only permitted a limited number of scans for research purposes (one in a lifetime at the WDIN). Females of childbearing age are not scanned.

2.1.3 MRI

2.1.3.1 Principles of MRI

For this thesis, MRI is not used as a functional imaging tool (i.e. fMRI) but as a means to provide structural information about the neuroanatomy of the subjects tested. It will be discussed in this context.

Simply put, MRI works by measuring radio frequency signals that provide information about the anatomical structure brain. Protons, the nuclei of hydrogen atoms, spin very fast which means that they produce a small magnetic field. They can be conceptualised as tiny magnets all pointing in different directions. A subject in the MRI scanner is in the presence of a very strong external magnetic field; this magnet causes protons to align in the direction of its field (NB. in fact some of the protons will align against the field in the opposite direction, but the *net* result is an alignment in the direction of the field). When an external radio frequency is applied the protons change alignment and when the radio frequency is switched off they slowly realign to their original position. During this process of realignment they emit a radio frequency signal that can be detected by the MRI coil, which is placed over the subjects head. Differences in proton density and the rate of realignment enable identification of different tissue types. In receiving a radio signal from each point in the brain, a structural image of the whole brain can be reconstructed.

In Chapters 3 and 6 a Siemens VISION MR scanner operating at 2 Tesla (Siemans, Erlangen, Germany) is used to acquire structural MRI images that give information about the neuroanatomy of the brain to complement the PET data. As with PET, the subject lies supine on a scanner bed although more of the subject's body enters the MRI scanner. As a result of scanner noise, earplugs are given to the subject. A head support is used and is adjusted to minimise subject discomfort. A hand held 'emergency' buzzer is given to the subject since the scanner noise makes hearing the subject impossible during scanning.

2.1.3.2 Safety

A significant advantage of MRI over PET is that it does not require exposing the subject to a radioactive substance. However, MRI does use a very strong magnet, requiring that subjects be carefully screened so that there is nothing in (e.g. pacemaker, cochlear implants, metal) or on (e.g. tattoo, jewellery, coins) the subject that could be heated up by or attracted to the magnet. Providing these criteria are adhered to, MRI poses no safety risk to the participant.

2.1.4 SPM

The PET procedure produces maps of the brain plotted as voxels, with each voxel representing the activity of a particular co-ordinate in 3D space. For this thesis, the PET images were analysed using statistical parametric mapping software (SPM99, Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) executed in Matlab 6.0 (Mathworks Inc., Sherborn, MA). Simply put, SPM enables hypotheses about regionally specific effects to be tested on each voxel. Before statistical analysis of the data is possible, the data must be spatially preprocessed to remove unwanted variance components. The structural MRI scans were processed using the spatial preprocessing techniques of the software.

2.1.4.1 Spatial preprocessing

There are three main components to spatial preprocessing for functional imaging data: realignment, spatial normalisation and smoothing. Although both PET and MRI scanners provide head support, head movement is still possible and the realignment process seeks to address this. For each subject, all of the scans acquired are realigned to the first scan. Specifically, this involves the processes of coregistering (the estimation of six parameters (three translations and three rotations) of an affine rigid body transformation that minimizes the differences between each successive scan and the first scan) and reslicing (applying the transformation by re-sampling the data using an interpolation method) (Friston et al, 1995a). At this stage, within-subject analysis seems feasible; however, comparison between subjects would be meaningless due to inter-subject differences in neuroanatomy. The process of spatial normalisation (Friston et al, 1995a) estimates warping parameters that transforms each subject's data into a template based upon the MNI (Montreal Neurological Institute) reference brain that conforms to a standard anatomical space (Talairach and Tournoux, 1988). The final process, smoothing, is used to spatially 'spread out' the data. This process accommodates for intersubject differences in anatomy, increases the signal to noise ratio and allows for subsequent statistical inference using Gaussian random field theory. In

Chapters 3 and 6 the data are smoothed using an isotropic Gaussian kernel of 12mm at full width at half maximum (FWHM).

For the structural MRI data, each subjects' individual image was coregistered to their mean functional image and then normalised into standard anatomical space.

2.1.4.2 Statistical analysis

To estimate activation effects at each voxel in the brain, subsequent analysis is performed as a multiple linear regression, a special case of the general linear model (GLM) (Friston et al, 1995b). The experimental design is represented in a mathematical structure known as the design matrix. Within this design matrix, the data that have been collected are partitioned into columns (or regressors) representing activations of interest (e.g. a column for task A would typically include all scans in which task A was performed) and activations that are not of interest and which may confound the results (e.g. scan to scan differences in global blood flow). Essentially, the design matrix should include all known variables that may explain the evoked neural responses. The contribution of columns in the design matrix to the observed rCBF can be estimated using the GLM and standard least squares. These estimated contributions are known as parameter estimates and hypotheses about regionally specific condition effects can be examined by looking for differences between these parameter estimates, specified using linear contrasts (e.g. Task A > Task B). For each contrast, a t statistic is computed for every voxel to form a statistical parametric map (SPM {t}). For convenience, the SPM {t} values are then transformed to the unit normal distribution to give an SPM {z}. The p values are corrected according to Gaussian random field theory, which controls the familywise error rate (FWE), accounting for the search volume of a SPM in much the same way as Bonferroni correction accounts for multiple discrete statistical tests (Worsley et al, 1992; Worsley et al, 1996). The adjustment of p values based on Gaussian random field theory depends on the inference being made. Where no prior anatomical hypothesis exists about the regional specificity of an experimental effect, it is necessary to correct for multiple comparisons across

the entire brain (with p typically < 0.05). If a more constrained anatomical hypothesis exists, then it is possible to restrict the correction for multiple comparisons to either a single voxel (using an uncorrected p value) or a restricted volume of interest.

It is also worth mentioning the inferences possible in functional imaging experiments. Originally, experiments used scan to scan error variance, such that variability was within-subjects. This means that although inferences can be made about the particular subjects studied, the inference cannot be generalised to the population from which the subjects were selected. This type of analysis is called fixed effects. Random effects analysis (RFX) seeks to circumvent this problem by using subject to subject error variance (using the contrast of parameter estimates for each subject) i.e. between-subjects variance (e.g. Penny et al, 2003). In practical terms, the contrasts of parameter estimates from a first level (or fixed effect) analysis are entered into a second level (or random effects) analysis where (typically) a one sample t test is used (i.e. both between-subjects and within-subjects variance are considered). This means that there is only one observation (i.e. contrast) per subject in the second design matrix meaning that the number of observations is the number of subjects, rather than the number of scans, as with the fixed effect approach. RFX allows the inferences to be generalized to the population (i.e. as if the subjects are 'randomly' drawn from the population) but is a more conservative procedure, partly as the number of degrees of freedom has reduced and partly because subject to subject variability is greater. Both types of approach are valid providing the inferences made are appropriate. For both functional imaging techniques, a compromise between these two procedures is to used a fixed effects design and to then use conjunction analysis to establish that every subject studied showed the relevant activation, this allows the inference that at least a 'certain proportion' of the population would have shown the effect (Friston et al, 1999).

2.1.4.3 Analysis of effective connectivity

The statistical analysis described above is concerned with functional specialisation, which is the cornerstone of functional imaging analysis. Chapter

6 of this thesis also explores functional integration, in the form of effective connectivity. This was investigated using the method of psychophysiological interaction (PPI), as described by Friston et al (1997). PPIs aim to explain regionally specific responses in terms of an interaction between activity in a particular cortical area (index area) and the influence of an experimental parameter. Explained simply, if the activity of one region was regressed on to the activity of a second region, the slope of the regression would reflect the influence that the second area could be having over the first area. If this regression was then repeated on data acquired in a different context (e.g. during different task conditions), the slope of the regression might change. This change in slope represents a PPI. The PPI analysis therefore tests for differences in the regression slope of the activity in the index area on the activity in all remaining areas under the different experimental conditions. PPIs are limited to testing regions for which there is an a priori hypothesis about decreased responsiveness or increased influence under given conditions. A significant result can either be interpreted as a change in the influence of the index area on other brain regions, or as a change in the responsiveness of the index area to inputs from other brain regions. The PPI does not allow these interpretations to be disambiguated.

2.1.5 Anatomical localisation

Anatomical localisation of the significant voxel coordinates were determined using the subjects' structural MRIs, a group average MRI and the T1 canonical brain from the MNI series and with reference to the atlases of Durvenoy (1999) and Schmahmann et al (2000). In addition, the standard stereotactic atlas of Talairach and Tournoux (1988) was used for further reference with regard to Brodmann areas. For certain regions, probabilistic cytoarchitectonic atlases have been produced and these were also used (Geyer et al, 1996; Geyer et al, 1999; Geyer et al, 2000).

2.2 TMS

The appeal of TMS to cognitive neuroscientists is that it can safely and temporarily disrupt neural activity in a discrete portion of the brain, in effect creating a 'virtual lesion' in healthy subjects (e.g. Jahanshahi and Rothwell, 2000). In applying TMS during task performance, exploration of the task-relevant roles of specific areas of cortex is possible. PET and fMRI give insight into the breadth of regions active during a particular task (i.e. that are related to that task) and provide impressive spatial localisation. However, whether a region is *essential* to the task is not clear and often further interpretation of the data relies on relevant clinical studies. TMS is an attractive complement to functional imaging as if stimulation of a given brain region (i.e. a virtual lesion) can disrupt performance on a task, the region can be said to be essential to task performance, much the same as in clinical studies. TMS also circumvents some of the problems inherent in clinical studies as the characteristics of subjects can be better controlled. Comorbidity is not always easy to avoid in patient-based studies and it may be difficult to find patients with lesions limited to the area of interest or with functional deficits that directly reflect the discrete lesion site. Furthermore, naturally occurring lesions can lead to plastic (compensatory) changes in the brain, which may create misleading conclusions (Pascual-Leone et al, 1999).

2.2.1 Design of experiments

TMS can be applied in different ways to investigate cortical functioning. Researchers in the field of cognitive neuroscience typically measure performance on a task and look for TMS-induced changes in the latency, accuracy or variability of responses, and sometimes in the response bias. In a 'before and after' paradigm investigators compare performance on a task before and after TMS has been applied. Giving TMS for a prolonged period results in prolonged disruption, allowing researchers to calculate a time window in which the brain will still be disrupted by the stimulation. Alternatively, stimulation can occur during the task. This technique allows greater flexibility in the design, but has the disadvantage of performance potentially being affected by the

distracting nature of the stimulation (the stimulation produces loud clicks and can be felt on the scalp). However, these effects can be controlled for, either by stimulating a control site that is not involved in the task of interest or by including a control task that is identical to the experimental task bar the particular process of interest. These control elements are also used to establish the specificity of the effect in relation to the brain region of interest and are therefore also common in 'before and after' designs. In addition, the time at which the TMS occurs can also be manipulated such that the effect of a brain region(s) on different parts of a task (e.g. encoding and retrieval) can be investigated. This design is obviously not possible if a 'before and after' paradigm is used. It is also worth noting that sham TMS can also be used as a control condition; it has the stimulus properties of TMS without actually using a magnetic field. See Jahanshahi and Rothwell (2000) for a more complete review.

2.2.2 Principles of TMS

TMS works by applying a coil (or coils) of insulated copper wire that has been encased in plastic over a specific region of the cortex. Once in place, brief pulses of a current are passed through the coil, which has the effect of generating a rapidly changing magnetic field. The magnetic field is able to pass unattenuated through the subject's scalp and skull into the brain and the rapid fluctuations enable electrical currents to be induced in the brain. Thus, the magnetic field is not directly stimulating the brain, but is a means by which electrical currents, which do stimulate, can pass into the brain. The induced current will depolarise some of the neurons (it may hyperpolarise others) and cause them to fire an action potential. The synchronous depolarisation of a large population of neurons usually leads to repetitive activity lasting 5-10 ms. This is then terminated by a long-lasting inhibitory post-synaptic potential that occurs because the excitatory events have triggered activity in inhibitory neurons. Another way of conceptualising TMS is to think of the stimulation as applying random 'neural noise' to organised neural activity in a prescribed cortical region (Walsh and Rushworth, 1999). The rate of change of the magnetic field (a direct reflection of the rate of change of the electrical current

provided to the coil) determines the degree of electrical activity in the cortex, thus the degree of stimulation (and thus, disruption to neural functioning) can be carefully controlled. TMS can be used in a variety of ways (e.g. exploring intracortical connectivity, exploring brain-muscle relationships), but this chapter concentrates on it use as a virtual lesion.

Limiting the stimulation to a discrete area can be challenging as the current has a sphere of influence beyond its target area that can extend to several centimetres. A coil designed in a figure-of-eight shape is the most effective design, with the magnetic field being strongest at the junction of the two circles, a feature that enables enhanced spatial accuracy (e.g. Cohen et al, 1990). Deciding the positions on the scalp that denote given brain areas can be decided by various techniques. Often the international 10-20 EEG system is used to guide locations and, if available, a programme such as Brainsight (Brainsight Frameless Stereotaxic system, Rogue Research, Montreal, Canada) can be used to integrate a structural MRI scan of the subject with their head and coil position to ensure accuracy. If stimulation of a given area produces a known physiological effect then this can also be used (e.g. stimulation of the hand motor area in the motor cortex induces muscle contractions in the hand) and confirmed, if appropriate, by EMG recordings. The spatial resolution of TMS can be as small as 0.5 cm for structures near the surface of the scalp (at least with reference to the point of maximum stimulation), though is less impressive for deeper structures. Currently, stimulation of very deep structures (e.g. the basal ganglia) is not possible as it is impossible to prevent depolarization of the neurons nearer the cortical surface. As a final point on localisation, the orientation of the coil is also important as the direction of the electric currents affects the neural elements that are stimulated.

2.2.3 Repetitive TMS

Initially, TMS techniques enabled a magnetic pulse lasting 1 ms to be delivered every few seconds. However, for the past fifteen years researchers have been able to apply a train of pulses in quick succession over a period of several milliseconds and up to a rate of 50-60 Hz; this technique is otherwise known as

repetitive TMS (rTMS). The advantage of this technique, which is used in Chapter 4, is that brain function can be disrupted for longer periods of time and at a higher frequency, essentially rendering it more effective. The temporal resolution of the disruptive effect of one shock is about 50-100 ms, but this is increased for rTMS where the repetitive trains have an obvious cumulative effect.

2.2.4 Safety

Clearly, disrupting neural activity in the human brain introduces safety concerns. The consensus of research has not found evidence of significant side effects of single pulse TMS in subjects with no neurological history. However, rTMS carries a higher risk of adverse effects than single pulse TMS with several (albeit rare) examples of rTMS inducing epileptic seizures in healthy subjects being documented. Furthermore, side effects including skin burns in the presence of electrodes, headache, temporary auditory threshold shifts and tinnitus have also been recorded (e.g. see Pascual-Leone et al, 1997, Pascual-Leone et al, 1999, Wasserman, 1998 for reviews). Clear guidelines regarding the maximum safe durations of single trains of rTMS at different frequencies and intensities have been published (see Wasserman, 1998). Furthermore, screening of subjects is important, with anyone with a history (or family history) of seizures being excluded. A similar approach is taken for a history of head injury, neuropsychiatric disorders and the taking of medication that alters depolarization thresholds (e.g. antidepressants, antieplileptics). Furthermore, the presence of metal in the head and cardiac pacemakers/implanted medication pumps are also reasons for exclusion.

There has been no evidence that either single or repetitive TMS damage the cortex and there has been no consistent evidence to suggest that stimulation causes harmful long term effects in terms of neurological, physical or cognitive performance. However, paradoxically, research into the possible benefits of several sessions of rTMS in treating depression indicate rTMS can have long-term carry over effects suggesting that caution should be advised over the

amount of rTMS any one individual receives (e.g. see Pascual-Leone et al, 1997, Pascual-Leone et al, 1999 for reviews).

2.3 STATISTICAL DATA ANALYSIS

Analysis of all behavioural data was performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois, USA). First, the variables were checked for the presence of outliers (> 3 SD from the mean) and also for departures from a normal distribution (p value < 0.05 in the Shapiro-Wilk test was used to indicate non-normality). Data that were not normally distributed were tested using non-parametric statistics or log transformed data to correct the non-normality to allow use of parametric statistics. The logarithmic transform option was typically implemented when the use of analysis of variance (ANOVA) was optimal (i.e. more than two factors were being tested). Repeated measures ANOVAs were tested for sphericity using Mauchly's Test of Sphericity. If the data violated the assumption of sphericity then the Greenhouse-Geisser correction was applied and reported. For parametric tests, Levene's test for equality of variances was also checked. If this statistic was significant then the variances were assumed to be significantly different and a corrected statistic (adjusted for equal variances not being assumed) was reported if appropriate.

Throughout the thesis, the significance level used was $\alpha \leq 0.05$, with the null hypothesis being rejected for all data reaching this criterion. For the functional imaging studies (Chapters 3 and 6), established procedure was followed and an uncorrected threshold of $p < 0.001$ was used where an a priori hypothesis existed. For the remaining studies (Chapters 4 and 5), where multiple tests were run on the same data, the data were corrected for multiple comparisons using the Bonferroni correction. An alpha greater than the significance level was cautiously interpreted as reaching threshold if it was within 0.005 of the significance level and there were strong a priori reasons for accepting the data. This occurred once in Chapter 4 and once in Chapter 5, where further discussion of using this slightly lowered threshold can be found.

2.4 SAMPLE SIZE

Both pragmatic and empirical reasoning influenced the sample sizes used in this thesis; no formal power calculations were performed. For the PET studies (Chapters 3 and 6) the number of subjects reflected those used in similar studies and followed the advice of collaborators with expertise in the field. Chapter 6 included patients with Parkinson's disease who took apomorphine; apomorphine is not a drug that is prescribed commonly and those given the drug do not often meet standard inclusion criteria. This had an influencing effect on the number of subjects in this study. For the rTMS study presented in Chapter 4 it was decided to only test people who were used to the procedure. This is because rTMS can be felt on the scalp and be distracting, more so to those not familiar and comfortable with the sensation. Consequently, nine subjects who were familiar with rTMS were tested; no other such subjects were available. For the clinical study described in Chapter 5 it was decided to test twelve subjects in each group, reflecting the sample size of similar studies. The cerebellar patients were a relatively rare group, so it was only possible to include eight in the final analysis. The group of de novo patients with Parkinson's disease were also only eight in size, reduced for pragmatic reasons. As the recruitment of the patients took a long time, it was possible to test a total of twenty healthy controls during this period.

2.5 ETHICS APPROVAL AND INFORMED CONSENT

All studies had the approval of the Joint Medical Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. With respect to the PET experiments, the administration of radioactivity was covered under a licence from the Administration of Radioactive Substances Advisory Committee (ARSAC) held at the WDIN. Written, informed consent was obtained from all subjects prior to testing.

Chapter 3

Estimation of long vs short intervals: The functional anatomy of time reproduction studied with positron emission tomography

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3.1 INTRODUCTION

As was described in Chapter 1, experimental studies on clinical populations have provided strong evidence that both the basal ganglia and cerebellum play a role in timing. Patients with cerebellar pathology have difficulty with motor timing (e.g. repetitive tapping of specific frequencies) and perceptual timing (e.g. discriminating the duration of two intervals) (e.g. Ivry and Keele, 1989; Ivry et al, 1988; Mangels et al, 1998). Patients with Parkinson's disease also display significant deficits in both motor (e.g. Harrington et al, 1998a; O'Boyle et al, 1996; Pastor et al 1992a) and perceptual timing tasks (e.g. Harrington et al, 1998a; Pastor et al, 1992b), deficits which are ameliorated with dopaminergic medication (O'Boyle et al, 1996; Pastor et al, 1992ab). As a result, it is proposed that the principal anatomical structures affected by these disorders must be crucial to the effective running of an 'internal clock' (e.g. Ivry, 1996). The basal ganglia are linked to the prefrontal cortex via five distinct circuits (Alexander et al, 1986) and projections from the cerebellum to the prefrontal cortex have also been described (Middleton and Strick, 1994). Patients with lesions to the frontal lobes are also impaired on measures of perceptual timing (Casini and Ivry, 1999; Harrington et al, 1998b; Mangels et al 1998), though this is commonly thought to be due to attention and working memory problems, rather than damage to the internal clock per se. The involvement of the cerebellum, basal ganglia and the prefrontal cortex in motor and perceptual timing has been confirmed by a number of more recent imaging studies using various timing tasks such as the repetitive tapping paradigm (e.g. Lejeune et al, 1997; Rao et al 1997; Rubia et al, 1998), duration discrimination (e.g. Jueptner et al, 1995; Ferrandez et al, 2003; Harrington et al, 2004b; Lewis and Miall, 2003b; Maquet et al, 1996; Nenadic et al, 2003; Rao et al, 2001; Smith et al, 2003), velocity discrimination (Jueptner et al, 1996), rhythm discrimination

(Schubotz et al, 2000; Scubotz et al, 2001) and time production and reproduction (e.g. Brunia et al, 2000; Tracy et al, 2000; Macar et al, 2004; Macar et al, 2002).

What is so far unclear is what the specific role of the cerebellum vs basal ganglia in timing may be. Ivry (1996) has suggested that the cerebellum controls the timing of short intervals (milliseconds range) whereas the basal ganglia are involved in the timing of long intervals (seconds range). This hypothesis is consistent with the commonly held view that the role of the cerebellum in the precise timing of short intervals reflects its role in motor co-ordination and movement control. Empirical support for Ivry's hypothesis comes from several sources. First, classical conditioning work has established that a learnt conditioned eyeblink response is not maintained in the ipsilesional eye of rabbits with unilateral cerebellar lesions (Yeo et al, 1985ab). The paradigm requires the animal to correctly time a conditioned eyeblink response following a conditioned stimulus (e.g. tone) so as to avoid an aversive unconditioned stimulus (airpuff to the eye). Such a deficit has also been demonstrated in patients with cerebellar lesions (Woodruff-Pak et al, 1996). In contrast, patients with Parkinson's disease, despite obvious deficits in the timing of movements, show no impairment of the conditioned eyeblink response (Daum et al, 1996). This suggests a specific role for the cerebellum in the timing of very brief durations. Interestingly, Green et al (1999) have noted similarities in the pattern of variability for the conditioned eyeblink task and the repetitive tapping task in healthy human subjects, suggesting that the two tasks are subserved by a common neural system.

A second line of evidence in support of Ivry's (1996) hypothesis comes from animal studies of timing. Rats with bilateral lesions of the cerebellar dentate and interpositus nuclei showed poorer performance on a measure of consistency in a temporal bisection task with intervals ranging from 300 – 1200 ms, but not when the intervals ranged from 20 – 45 s (Clarke et al, 1996). In a study that used intervals of 200-800 ms and 2-8 s, Breukelaar and Dalrymple-Alford (1999) found a similar dissociation in rats with lesions of the cerebellar hemispheres. Rats with lesions of the cerebellar vermis were unimpaired at

both interval ranges, which further suggests that the lateral cerebellum is of key importance in the timing of short intervals.

It is clear that the precise and differential roles of the basal ganglia and cerebellum in timing need clarification. Thus, the primary aim of this study was to test Ivry's (1996) hypothesis that the cerebellum is primarily engaged in timing short intervals and that the basal ganglia is concerned with the timing of long intervals. This was done using a time reproduction task that necessitated the reproduction of either short (500 ms) or long (2 s) intervals. Although evidence supports involvement of both the basal ganglia and cerebellum in temporal processing, perceiving or producing a duration of time involves a network of brain areas engaged in supportive processes such as attention and memory with the hypothesised 'clock'-like structures only one part. Therefore, in an attempt to further tease apart the differential contributions of the two structures, another focus of this study was to use a third, control task that tightly controlled for non-temporal aspects of the timing tasks.

3.1.1 Aims of the study

1. To investigate Ivry's (1996) hypothesis that the cerebellum is involved in the timing of short (millisecond-range) intervals and that the basal ganglia is involved in the timing of long (seconds-range) intervals.

2. To compare short and long interval timing tasks to a well matched control task to identify brain regions *specifically* involved in temporal processing and not in supportive processes.

3.2 MATERIALS AND METHOD

3.2.1 Subjects

8 male, right-handed volunteers with an average age of 27.5 years (SD 6.8; range 19-40) participated in the study. All of the subjects were healthy and

without a history of neurological or psychiatric disease or head injury. Prior to the experiment, the extent of right handedness was measured with a modified version of the Handedness Inventory (Oldfield, 1971). The subjects were all strongly right-handed (mean = 94.7; SD = 8.07). Estimates of verbal IQ were obtained from the National Adult Reading Test (NART: Nelson, 1982). The average score was 119 (SD = 4.24) indicating that all the sample had IQs in the high average range.

3.2.2 Design

The study used a within subject repeated measures design. There were three experimental conditions: short interval reproduction (SHORT), long interval reproduction (LONG) and a control reaction time (RT) task. During the PET scan each condition was repeated four times, resulting in 12 scans per subject. The order of presentation was pseudo-randomised across subjects using a Latin Square procedure.

The intervals chosen to represent 'short' and 'long' timing were based on previous literature. Michon (1985) has described 500 ms as the cut off between interval estimation that is highly perceptual and interval estimation that is cognitively mediated. The interval was considered to be suitably short, without being at risk of eliciting simple reaction times. The interval of 2000 ms was seen to be long enough to qualify for Ivry's (1996) definition of a long interval as well as requiring cognitive mediation, with minimal risk of more elaborate strategy use or waning attention.

3.2.3 Procedure

Approximately 30 minutes before the PET scan the subjects practised the three experimental tasks, each twice. The purpose of the practice blocks was to ensure that the subjects had understood the requirements of the tasks and that they had reached a criterion level of accuracy on the time estimation conditions. For the short interval condition, the mean of each trial was required to be within 100 ms of the target (i.e. ±100ms) and for the long interval condition the mean

of each trial was to be within 400 ms of the target (i.e. ±400ms). All subjects achieved criterion performance within the two blocks of practice trials.

3.2.3.1 Reproduction of a SHORT interval

Subjects were instructed that they would be required to reproduce a 'short' interval. The duration of the interval was 500ms, although the precise value was not explicitly communicated to the subjects. First, the duration of the interval was demonstrated to the subject, with presentation of two tones (1000 Hz, duration 50 ms) marking its onset and offset. After five presentations of the interval, the subjects began a practice block. They were told that a tone would be presented which would mark the beginning of the short interval. They should immediately start estimating and reproducing the duration of the target interval and press the response button to mark its end. A block consisted of 50 trials. The inter-tone-intervals varied between 3-4 seconds (mean 3.5 seconds). During the scan each experimental block was preceded by three demonstrations of the duration of the target interval. This allowed subjects to re-acquaint themselves with the duration, encouraging optimal performance.

3.2.3.2 Reproduction of a LONG interval

Subjects were informed that they would be reproducing a 'long' interval. The duration of the interval was 2000ms, but this value was not explicitly communicated to the subject. The instructions and procedures were identical to that used for the short intervals.

3.2.3.3 Control reaction time task

This was a simple reaction time (RT) task. Subjects were instructed that when a tone was presented they should press the response button as quickly as possible in response to it. The reaction time condition matched the time estimation conditions in terms of the characteristic of the tone (1000 Hz, duration 50ms), the number of responses (50 trials) and the inter-tone-interval (3-4 s, mean 3.5 s).

The tasks were programmed in Quick Basic and run on a Dell laptop. The same response box was used in all three conditions. It measured 15 cm x 8 cm x 5 cm and had two identical circular response buttons (diameter 2.5 cm) positioned at either end. All the subjects were instructed to use the same button and to ignore the second button. The travel of the button (i.e. distance the button travelled when pressed fully) was 2.5 mm and the operating force (i.e. force needed to press button fully) was 0.8 N. The button had a flat plastic surface and made a 'click'-type sound when pressed. All responses were made with the right index finger. The response times were recorded to the nearest millisecond. During the practice trials, the tones were presented through a loudspeaker. When the subjects were in the scanner the tones were presented through earphones, with adjustment made for optimal volume for each subject. When in the scanner, the box was attached to the arm rest for the right arm. Subjects positioned their right index finger over the button during each run of trials, so the only movement required was for that finger. The height of the box meant that the hand was resting at an angle of approximately 45° onto the box.

3.2.4 PET

Measurements of rCBF were obtained using a Siemens/CPS ECAT EXACT HR+ PET scanner (Siemans/CTI Inc., Knoxville, TN). Twelve separate measurements were taken, with four measurements being acquired for each of the three tasks. Additionally, T1 weighted structural magnetic resonance imaging (MRI) scans were obtained for each subject using a Siemens Magnetom VISION MRI scanner operating at 2 Tesla (Siemans, Erlangen, Germany). A description of these methods is provided in Chapter 2.

Subsequent reconstruction and analysis of the images was undertaken using statistical parametric mapping software (SPM99, Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) executed in Matlab (Mathworks Inc., Sherbon, MA), as described in Chapter 2. The general linear model (GLM) was used to estimate condition and subject effects at each voxel point in the brain (Friston et al, 1995b). Scan to scan differences in global blood flow were modelled as a confounding covariate. The statistical

analysis was aimed at identifying regions of the brain specific to short and long interval reproduction. This was tested using simple SHORT > LONG and LONG > SHORT contrasts. Areas of the brain that were common to both tasks were elicited in a (SHORT + LONG) > RT comparison. The level of significance was set to $p < 0.05$, corrected for multiple comparisons. Cortical and subcortical areas, about which there was an a priori hypothesis, were reported at $p < 0.001$, uncorrected. The design used a fixed effects model. In addition, conjunction analysis was used to check that areas that were important to the hypothesis were present in all or a majority of subjects (Friston et al, 1999).

Anatomical localisation of the significant voxel coordinates was determined by rendering them onto the subjects' structural MRIs and the MNI reference brain and with reference to the atlas of Durvenoy (1999). In addition, the standard stereotatic atlas of Talairach and Tournoux (1988) was used for further reference, particularly to aid determining Brodmann areas. Detailed information about the location of voxels in the cerebellum was gained with reference to an MRI atlas of the cerebellum (Schmahmann et al, 2000). For the primary motor cortex and somatosensory area, probabilistic cytoarchitectonic atlases have been produced and these were also used (Geyer et al, 1996; Geyer et al, 1999; Geyer et al, 2000).

3.3 RESULTS

3.3.1 Behavioural results

For the two practice blocks, the average reproduced duration of the short interval was 555.21ms (SD 26.10) and the long interval was 2027.36ms (SD 81.89). Both of these values indicate that subjects were accurate in the time reproduction and had reached the required level of competence prior to the scanning. The mean reaction time across the two practice blocks was 209.29ms (SD 36.59). During scanning, the mean reproduced duration for the short interval was 561.96ms (SD 68.47) and for the long interval was 2065.86 (SD 110.30). The mean reaction time was 202.14 (SD 44.05). Once again, the

subjects maintained a high degree of accuracy in time estimation for both the long and short intervals. The data collected during the scanning sessions are presented in Figure 3.1.

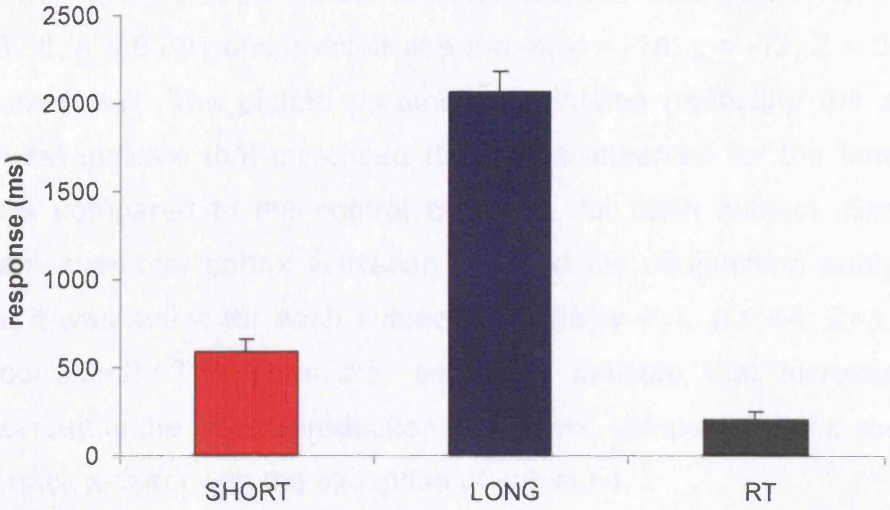


Figure 3.1: Average response times for the SHORT interval reproduction task (target 500 ms), LONG interval reproduction task (target 2000 ms) and control reaction time task (± SE)

3.3.2 PET results

3.3.2.1 Time reproduction tasks vs control reaction time task

Areas corresponding to the region of the left substantia nigra and red nucleus (x = -4, y = -12, z = -8; Z = 3.39; p < 0.001 uncorrected; with a further foci at x = -14, y = -18, z = -2; Z =2.45; p = 0.007 uncorrected) and the left lateral premotor cortex (BA 6, x = -24, y = 2, z = 44; Z = 3.14; p = 0.001 uncorrected; with a further foci at x = -22, y = 10, z = 38; Z = 2.96; p = 0.002 uncorrected) were more activated during the time reproduction tasks than the RT task. The results are shown in Figures 3.2 and 3.3. The subcortical co-ordinate plotted in Figure 3.2 is anatomically very close to both the red nucleus and the substantia nigra (e.g. Oikawa et al, 2002). The plotted co-ordinate was shown to various experts in basal ganglia and midbrain anatomy, including a functional neurosurgeon and an anatomist. Armed with this knowledge and theoretical understanding of the

role of the substantia nigra in temporal processing, it is proposed that this region approximates the left substantia nigra pars compacta (SNc). A conjunction analysis performed across all subjects showed that activation of the left substantia nigra pars compacta was common to all subjects ($x = -14$, $y = -16$, $z = -4$; $Z = 3.59$; $p < 0.001$ uncorrected; with sub-foci $x = -2$, $y = -6$, $z = -6$; $Z = 3.58$; $p < 0.001$ uncorrected and $x = -4$, $y = -14$, $z = -12$; $Z = 3.52$; $p < 0.001$ uncorrected). The plotted parameter estimates (reflecting the adjusted rCBF values) indicate that increased rCBF was observed for the time reproduction tasks compared to the control condition, for each subject. Similarly, the left lateral premotor cortex activation survived the conjunction analysis, indicating that it was active for each subject ($x = -28$, $y = 4$, $z = 44$; $Z=3.46$; $p < 0.001$ uncorrected). The parameter estimates indicate that increased rCBF was observed in the time reproduction conditions, compared to the control condition, for each subject with the exception of subject 4.

Relative to the time reproduction tasks, the only area that showed significant activation (at the corrected level) during the RT task was the right precuneus (BA 7, $x = 6$, $y = -72$, $z = 56$; $Z = .75$; $p = 0.030$).

3.3.2.2 SHORT > LONG interval reproduction

This contrast elicited significant activation in cortical regions including the left anterior cingulate (BA 32), left middle temporal gyrus (BA 21) and left superior temporal gyrus (BA 22), right superior frontal gyrus, spreading mesially (BA 6/8), the left middle frontal gyrus (BA 8), the left superior frontal gyrus (BA 8 and 10), and the right superior and mesial frontal gyrus (BA 9/10). Subcortically, activation was observed in the left caudate nucleus and in the right cerebellar hemisphere. The results are illustrated in Figure 3.4 and presented in Table 3.1. Figure 3.5 illustrates the left caudate nucleus and right cerebellar hemisphere activation. The conjunction analysis found significant left caudate activation ($x = -12$, $y = -8$, $z = 22$; $Z = 3.60$; $p < 0.001$ uncorrected) in an analogous location, indicating that the finding is robust across all subjects. In addition, the plotted parameter estimates indicate that the neural activity in this area is higher in the SHORT than the LONG condition across all subjects. The conjunction analysis for the right cerebellar hemisphere also revealed significant activation in a

similar region (x = 40, y = -72, z = -42; Z = 3.38; p < 0.001 uncorrected). Parameter estimates illustrated that the right cerebellar hemisphere activation was greater in the SHORT than LONG condition in all subjects.

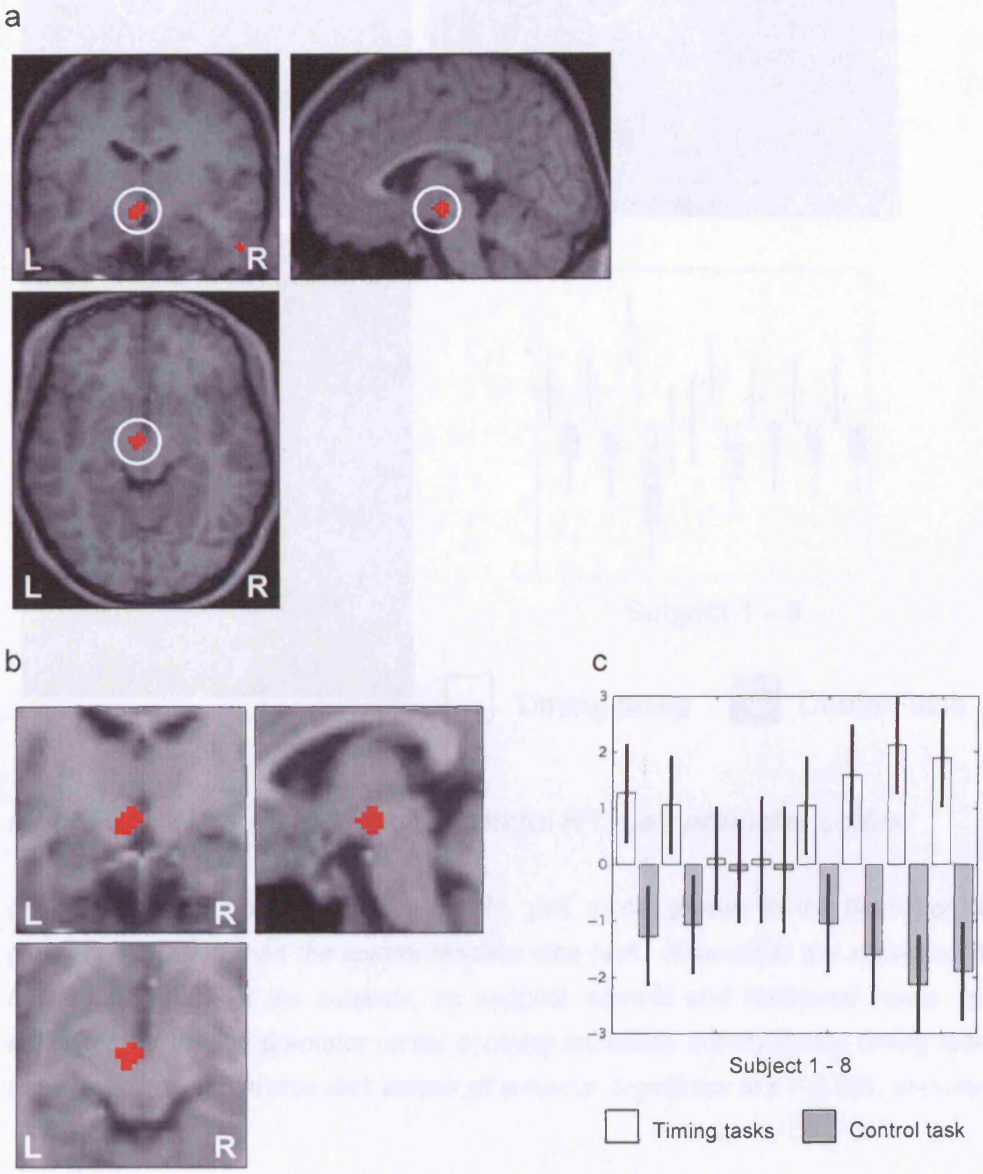


Figure 3.2: Time reproduction > control RT: Left SNc

(a+b) Left substantia nigra pars compacta activation (x=-4, y=-12, z=-8) greater in the time reproduction tasks (SHORT + LONG) than the control reaction time task. Activations are shown on the structural MRI scan of one of the subjects, on saggital, coronal and horizontal views. (c) Parameter estimates for the left substantia nigra pars compacta showing increased activity during timing tasks compared to the control reaction time task across all subjects. Significant at p > 0.001 uncorrected.

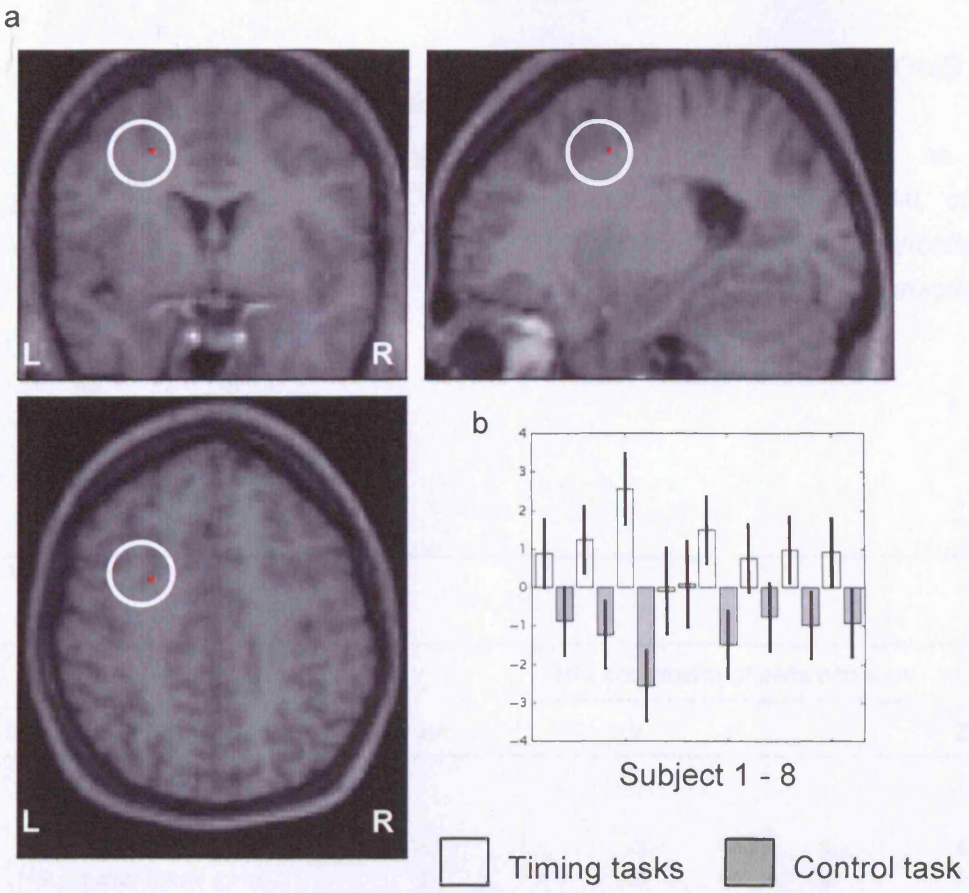


Figure 3.3: Time reproduction > control RT: Left premotor cortex

(a) Left premotor cortex activation (x=-24, y=2, z=44) greater in the time reproduction tasks (SHORT + LONG) than the control reaction time task. Activations are shown on the structural MRI scan of one of the subjects, on saggital, coronal and horizontal views. (b) Parameter estimates for the left premotor cortex showing increased activity during timing tasks compared to the control reaction time task across all subjects. Significant at p > 0.001, uncorrected.

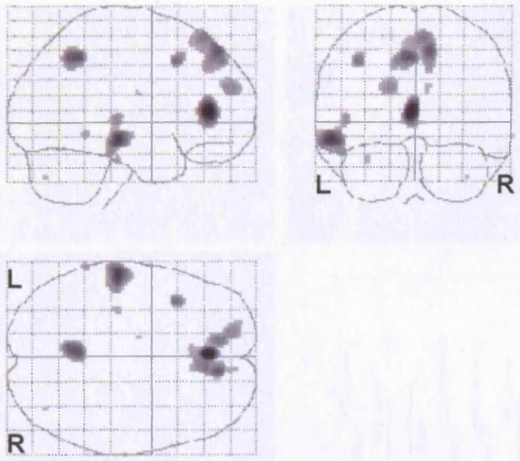


Figure 3.4: SHORT > LONG contrast

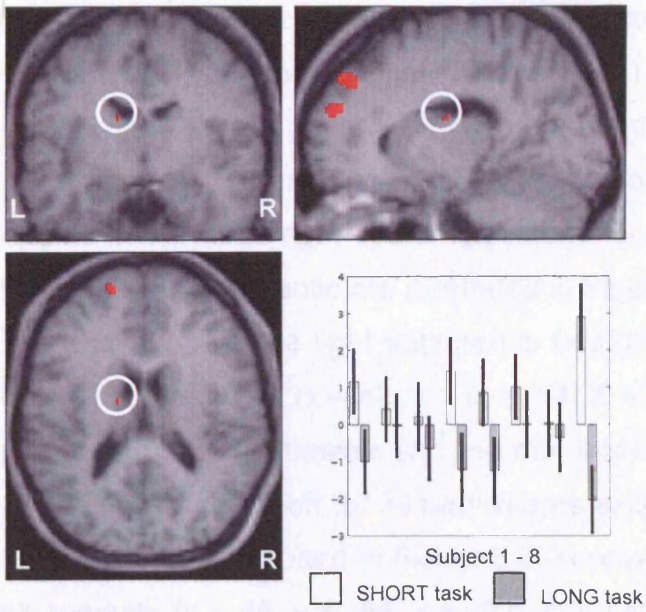
Results are displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space. Significant at p > 0.001 uncorrected.

	BA	MNI coordinates of peak activation x	y	z	Z score	p value*
Frontal cortex						
L anterior cingulate	32	-2	40	8	4.8	0.025**
R superior frontal gyrus	8	10	46	52	3.99	< 0.001
R superior and mesial frontal gyrus	6/8	6	38	60	3.97	< 0.001
L middle frontal gyrus	8	-40	18	44	3.79	< 0.001
L superior frontal gyrus	8	-12	48	44	3.71	< 0.001
L superior frontal gyrus	10	-18	54	24	3.54	< 0.001
R superior and mesial frontal gyrus	9/10	8	56	24	3.18	0.001
Temporal cortex						
L middle temporal gyrus	21	-58	-24	-12	4.21	< 0.001
L superior temporal gyrus	22	-48	-24	2	3.71	< 0.001
Basal ganglia						
L caudate nucleus		-14	-10	20	3.15	0.001
Cerebellum						
R cerebellar hemisphere (Crus I)		36	-74	-38	3.12	0.001

* all significant at p > 0.001, uncorrected
** significant at p > 0.05, FWE

Table 3.1: Areas of greater activation with the SHORT interval reproduction task compared to the LONG interval reproduction task

a



b

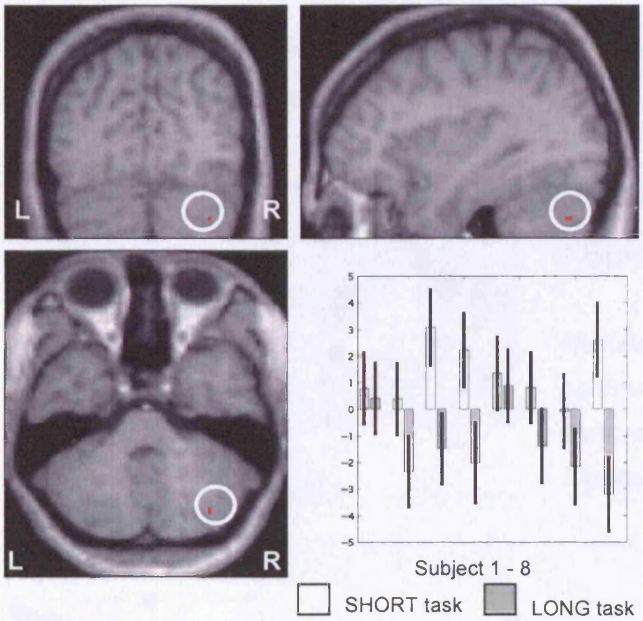


Figure 3.5: SHORT > LONG contrast: Significant subcortical activations

(a) Left caudate nucleus (x = -14, y = -10, z = 20) activation greater in the SHORT reproduction task than in the LONG reproduction task. Parameter estimates showing mean activation for each subject are also displayed. (b) Right cerebellar hemisphere (x = 36, y = -74, z = -38) activation greater in the SHORT reproduction task then in the LONG reproduction task. Parameter estimates showing mean activation for each subject are also displayed. Activations are shown on the structural MRI scan of one of the subjects, on saggital, coronal and horizontal views. Significant at p > 0.001, uncorrected.

3.3.2.3 LONG > SHORT interval reproduction

This contrast produced significant rCBF increases in the right superior parietal cortex (BA 7), lateral premotor cortex (BA 6) bilaterally, right SMA (medial BA 6), the right inferior parietal (BA 40) cortex, the right cuneus (BA 17), the right primary motor cortex (BA 4), the right dorsolateral prefrontal cortex (BA 9/46 and 10/46), the right putamen/insula border, and the right cerebellar hemisphere. The results are illustrated in Figure 3.6 and presented in Table 3.2. The activation for the right putamen is illustrated in Figure 3.7 and survived the conjunction analysis (x = 42, y = 6, z = 4; Z = 3.22, p = 0.001 uncorrected). The plot of parameter estimates showed that this area was more active in the LONG than SHORT condition for all participants except subject 1. The right cerebellar hemisphere, also plotted in Figure 3.7, survived the conjunction analysis across all subjects (x = 48, y = -64, z = -22; Z = 3.40, p < 0.001 uncorrected) and the plotted parameter estimates showed that the area is more active in the LONG than SHORT condition in 6 of the 8 subjects, with limited discernable difference in the other 2 subjects.

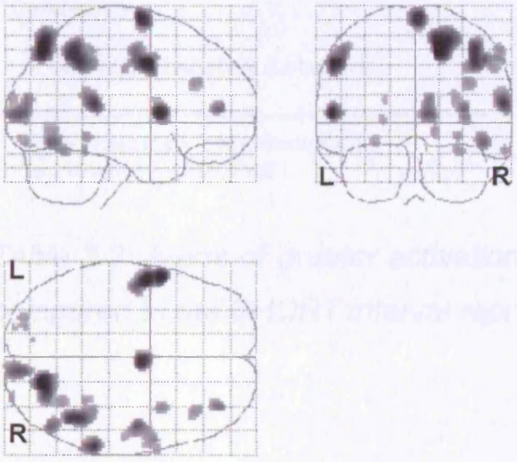


Figure 3.6: LONG > SHORT contrast

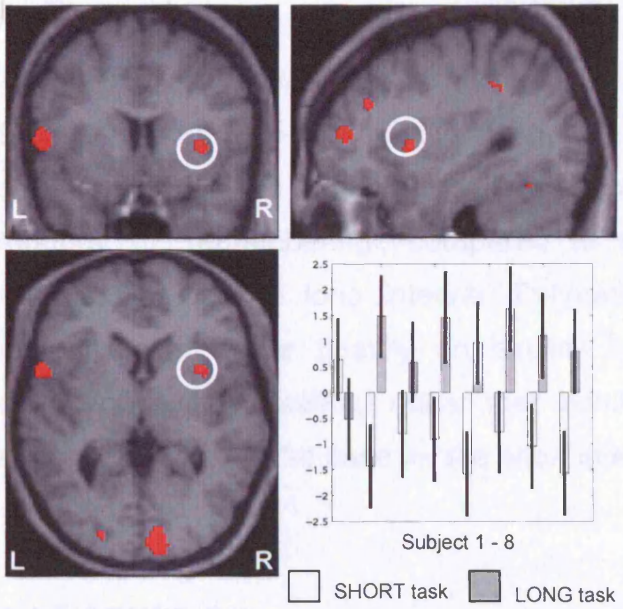
Results are displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space. Significant at p > 0.001 uncorrected.

	BA	MNI coordinates of peak activation x	y	z	Z score	p value*
Frontal cortex						
L lateral premotor cortex	6	-54	-2	42	4.59	< 0.001
L lateral premotor cortex	6	-56	8	8	4.49	< 0.001
R SMA	medial 6	2	-6	74	4.35	< 0.001
R primary motor cortex	4	48	-8	44	3.92	< 0.001
R lateral premotor cortex	6	58	2	46	3.48	< 0.001
R somatosensory area	3	54	-16	38	3.25	< 0.001
R dorsolateral prefrontal cortex	10/46	34	46	10	3.82	< 0.001
R dorsolateral prefrontal cortex	9/46	38	32	28	3.40	< 0.001
R lateral premotor cortex	6	64	4	16	3.23	0.001
Parietal cortex						
R superior parietal cortex	7	18	-74	52	4.74	0.033**
R inferior parietal cortex (intraparietal sulcus/angular gyrus)	40	42	-52	50	4.26	< 0.001
Occipital gyrus						
R cuneus	17	10	-90	6	3.95	< 0.001
Basal ganglia						
R putamen/insula border		34	8	4	3.55	< 0.001
Cerebellum						
R cerebellar hemisphere (Lobule VI)		30	-60	-18	3.32	< 0.001

* all significant at p > 0.001, uncorrected
** significant at p > 0.05, FWE

Table 3.2: Areas of greater activation with the LONG interval reproduction task compared to the SHORT interval reproduction task

a



b

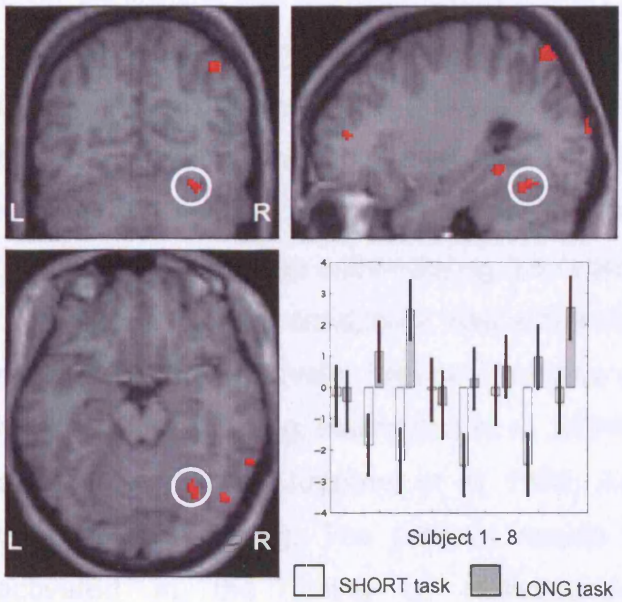


Figure 3.7: LONG > SHORT contrast: Significant subcortical activations

(a) Right putamen (x = 34, y = 8, z = 4) activation that greater in the LONG reproduction task than in the SHORT reproduction task Parameter estimates showing mean activation for each subject are also displayed. (b) Right cerebellar hemisphere (x = 30, y = -60, z = -18) activation greater in the LONG reproduction task then in the SHORT reproduction task. Parameter estimates showing mean activation for each subject are also displayed. Activations are shown on the structural MRI scan of one of the subjects, on saggital, coronal and horizontal views Significant at p > 0.001, uncorrected.

3.3.3 Self-reported methods of time estimation

At the end of the experiment, the subjects completed a form to indicate how they estimated and reproduced the target intervals. For the majority of the subjects (88%) the short interval was estimated and reproduced using a strategy of 'remembering', compared to only 25% using this strategy for reproduction of the long interval. Estimation and reproduction of the long interval relied more heavily on explicit use of conscious strategies (e.g. counting, rhythm creation) rather than having a 'template' or 'memory' of the interval, which was the case for the short interval.

3.4 DISCUSSION

This study tested Ivry's (1996) hypothesis that the cerebellum is involved in the timing of short (millisecond) intervals and that the basal ganglia is involved in the timing of long (second) intervals. The results refuted this hypothesis. The left caudate nucleus was active during the reproduction of 500 ms intervals and the right putamen was active during the reproduction of 2000 ms intervals, while the right cerebellar hemisphere was active during the reproduction of both 2000 ms and 500 ms intervals. Previous imaging studies tend to conclude that either the basal ganglia (e.g. Harrington et al, 2004b; Rao et al, 1997; Rao et al, 2001) or cerebellum (e.g. Jueptner et al, 1995; Jueptner et al, 1996) is important in temporal processing. The present results suggest that both structures are activated in the timing of millisecond- and seconds-range intervals. Furthermore, the study also revealed activations in the left substantia nigra pars compacta (SNc) and left lateral premotor cortex that were specific to temporal processing compared to a control task, this suggests that the basal ganglia plays a more fundamental role in temporal processing than the cerebellum.

3.4.1 Time reproduction activates the motor frontostriatal circuit

The left SNc and the left lateral premotor cortex were more activated across the timing tasks (SHORT + LONG) compared to the control RT task. The SHORT

vs LONG interval comparisons revealed areas associated with either short- or long-range time reproduction. However, these areas are not limited to temporal processing and a direct comparison of the timing tasks with the control task was important for indicating areas that are specific to time reproduction once additional processes such as attention, anticipation/preparation of/for the tone, response initiation and motor execution were controlled. The symptoms of Parkinson's disease manifest following the degeneration of dopamine producing neurones in the SNc (e.g. Lang and Lozano, 1998). These neurones have been argued to act as 'pacemaker' units i.e. to provide clock-like processes in timing behaviour (Meck, 1996). Indeed, dopamine neurones within the SNc are known to fire rhythmically as a result of pacemaker-like slow depolarisation (Kang and Kitai, 1993ab). The moderating effect of dopaminergic medication on timing performance in Parkinson's disease (e.g. O'Boyle, 1996; Pastor et al, 1992ab) and the differential effect of dopamine agonists and antagonists on 'clock speed' in pharmacological studies (e.g. Maricq et al, 1981; Maricq and Church, 1983; Meck, 1996) is well documented. Furthermore, lesions to the rat SNc cause impaired interval discrimination (e.g. Matell et al, 2000). This has led to the proposal that dopaminergic input from the SNc to the striatum is fundamental to temporal processing.

In a neurobiological model of temporal processing (the striatal beat frequency model), it is hypothesised that the SNc resets activity in striatal neurons integral to the timing process, acting as a 'perceptual starting gun' at the onset of an interval to be timed (Matell and Meck, 2000; 2004), a function that is essential for the two timing tasks used in this study. The SNc is also known to receive projections from the subthalamic nucleus, a region that, along with the external globus pallidus, has been proposed to provide 'clock'-like functions (Beurrier et al, 2002; Plenz and Kitai, 1999). In fact, stimulation of the rat subthalamic nucleus during rhythmic firing has been shown to induce arrhythmic firing in the dopaminergic neurones of the SNc, which in turn may cause the desynchronisation of dopamine neurons (Kang and Futami, 1999). Thus, connections between the SNc and other regions of the basal ganglia are likely to be key components of a timing network and the SNc, with its dopamine rich neurones, is likely to play a fundamental role in temporal processing.

Within the motor circuit, the lateral premotor cortex is one of the three main cortical projection sites of the putamen, the other two being the SMA and the primary motor cortex (Alexander et al, 1986; Middleton and Strick, 2000). The results suggest that the lateral premotor cortex plays a primary role in timing. Significant activation of the lateral premotor cortex is also found in the LONG > SHORT contrast. Indeed, previous clinical evidence has established that patients with lesions of the premotor cortex or SMA display difficulties in rhythm reproduction from memory, particularly if left-sided (Halsband et al, 1993). Ramnani and Passingham (2001) used fMRI to investigate neuronal changes during rhythm learning. They found activation of the dorsal premotor and pre-SMA regions, which they concluded represented the preparation of the timed response. Premotor activity has been found in several fMRI studies of perceptual timing in which no motor component is present, or is controlled for (e.g. Ferrandez et al, 2003; Rao et al, 2001; Schubotz et al, 2000). Schubotz et al (2000) found that the premotor cortex was significantly activated when subjects had to monitor visual and auditory rhythms. They concluded that the function of the premotor cortex extends beyond its traditional movement-related role and that it also supports non-motor sequencing and timing activities. In their fMRI study of duration discrimination, Rao et al (2001) suggest that the premotor area may have a working memory function in maintaining the standard interval during the trial. This explanation fits well with the current results, as the timing task used in this study demanded that the learnt interval be stored and maintained over the scanning period. Furthermore, the significantly greater activation of the lateral premotor activation in the LONG than the SHORT condition may reflect the differentially greater demands of storage and maintenance of longer intervals.

The absence of cerebellar activation in the timing vs control task contrast argues against the suggestion that this structure plays a 'clock'-like role in temporal processing. The control task required a motor response and was carefully selected to provide adequate control for the attentional, tone anticipation/preparation, response initiation and motor execution components in the time reproduction tasks. It is true that any motor task contains a timed

element; however, the time reproduction tasks were distinct in requiring the subjects to consciously engage in temporal activity. Thus, although the SHORT>LONG and LONG>SHORT comparisons revealed that the cerebellum was activated during millisecond and seconds-range timing, the absence of cerebellar activation in the comparison between the timing tasks and the control task suggested that the cerebellar activation is not specific to 'clock' processes.

Some caution must be taken with interpreting the absence of cerebellar activation as a small sample size was used, reflected in the limited significant results from this contrast. An uncorrected p value of < 0.001 was used, which is conventionally the most lenient p threshold that it is acceptable to report. However, this finding is in agreement with previous research results. For example, cerebellar lesions in humans lead to increased scalar variability on the peak- interval procedure (as one would expect according to scalar expectancy theory e.g. Gibbon, 1977; Gibbon et al, 1984), whereas Parkinson's disease leads to non-scalar increases in variance as well as reduced accuracy (Malapani et al, 1998ab). A recent clinical study has also failed to find any convincing deficits in non-motor, time perception in patients with cerebellar lesions (Harrington et al, 2004a). Using the Wing and Kristofferson (1973ab) model to decompose the variability on a repetitive tapping task, a subset of the patients were observed to display greater 'clock'-related variability on the task, however this was seen to correlate with working memory performance. The authors concluded that the cerebellum may be involved in processing task-relevant sensory or cognitive information as well as being important for motor-output. In terms of evidence from functional imaging, Ferrandez et al (2003) found no evidence of cerebellar activation in a duration discrimination task when it was contrasted with an intensity discrimination task, after motor activity had been controlled for. Furthermore, neither Lewis and Miall (2002) nor Macar et al (2004) found cerebellar activation when a time production task was compared to a force production task. Lewis and Miall (2002) did find cerebellar activity when their time production task was compared to a basic motor control task, which suggests that the cerebellar activity was related to non-temporal processes that are common to both the time production and force production tasks. In a study of time reproduction, in which seconds-range intervals were

produced, no cerebellar activity was found when the task was compared to a stimulus-matched control (Macar et al, 2002). Finally, recordings of cerebellar spike activity in monkeys have failed to reveal periodic oscillatory discharge that would resemble a clock-like timing signal (Keating and Thach, 1997).

3.4.2 Reproduction of long vs short intervals

Early work investigating timing performance in patients with cerebellar lesions solely concentrated on intervals in the milliseconds range (e.g. Ivry and Keele, 1989; Ivry et al, 1988; Mangels et al, 1998). However, more recent work has used longer time ranges and supports the findings of this study. For example, patients with lateral cerebellar lesions show increased variability when reproducing long intervals in the range of 8-21 seconds (Malapani et al 1998a). Nichelli et al (1996) reported that patients with cerebellar degeneration were significantly impaired on a temporal bisection task at both the 100-600 ms and 8-32 s ranges; although they concluded that the timing deficit in the 8-32 s range might be reflect additional cognitive deficits (e.g. sustained attention/strategy use). However, Mangels et al (1998) found that patients with cerebellar lesions were impaired in the discrimination of both short (400 ms) and long (4 s) intervals and that performance was not aided by cognitive strategies (e.g. subdividing the interval). Additionally, unlike frontal patients, patients with cerebellar lesions were not sensitive to the length of the inter-stimulus interval (1s or 4s) in a frequency discrimination task. This suggests that cognitive demands, such as working memory or attention, are not contributing to the poor performance of cerebellar patients. On a related note, Lurcher mutant mice, who have a degenerated cerebellum, are unable to learn a time dependant avoidance response that needs to be performed either 5 – 10 s or 10 -15 s after task onset (Monfort et al, 1998). Within the functional imaging literature, cerebellar activation is found when subjects discriminate between durations in the milliseconds (300 ms) range (Jeuptner et al, 1995) but also during the discrimination of tones of 1.2 s in length (Rao et al, 2001). Additionally, Lejeune et al (1997) noted cerebellar activation during repetitive tapping for a long inter-response interval of 2.7 s. The results presented in this study also support previous animal and clinical work suggesting that the lateral

cerebellum, rather than medial regions, are the key cerebellar structures involved in temporal processing (e.g. Breukelaar and Dalrymple-Alford, 1999; Ivry et al 1998; Malapani et al, 1998a).

The specific role for the cerebellum in milliseconds- and seconds-range timing is difficult to determine from this study. The absence of such activation in the timing vs control task contrast argues against a primary role in timing. Using PET, Penhune et al (1998) found that the cerebellum was active during the production of rhythmic sequences, particularly when they were complex or novel. They suggest that the cerebellum may not provide a 'clock' function, but that it may be involved in the learning of timed motor responses and also in sensory integration, including extracting temporal parameters from sensory inputs. Rao et al (2001) used fMRI with the duration discrimination paradigm and found basal ganglia activity throughout the task but cerebellar activity only just before and during execution of the response (button press). This was considered to reflect a role for the cerebellum in optimising sensory information. It seems feasible that the cerebellar activity found in both the LONG and SHORT tasks could reflect different processes as a function of the specific demands of the two tasks. Mangels et al (1998) found no evidence that cognitive deficits underpinned timing dysfunction in patients with cerebellar pathology. However, Harrington et al (2004a) have argued that the timing deficits of patients with cerebellar lesions may be in part related to working memory dysfunction and poor speed of processing, depending on task demands. It may be that the cerebellar activity in the LONG condition is related to cognitive demands such as memory processes, whereas activity in the SHORT condition reflects sensory processing. Further work would is needed to disambiguate this result.

Evidence from various sources suggests that the basal ganglia are fundamental to the timing of both short and long intervals. Patients with Parkinson's disease are impaired in the estimation and reproduction of intervals of 8 and 21 s (Malapani et al, 1998b) and 3, 9 and 27 s (Pastor et al, 1992b), with performance being improved by the administration of levodopa. However, patients with Parkinson's disease also display deficits on the repetitive tapping

task, which typically requires tapping in the milliseconds range (Harrington et al, 1998a; O'Boyle et al, 1996; Pastor et al, 1992a). Once again, administration of levodopa improved performance in these patients (O'Boyle et al, 1996; Pastor et al, 1992a) suggesting that the integrity of the basal ganglia, and its dopaminergic connections, is essential for the timing of movements. Basal ganglia activation was evident in a functional imaging study requiring discrimination of intervals of 1.2 ms (Rao et al, 2001) and 1000 ms (Nenadic et al, 2003), but is also observed with a short interval (300ms) duration discrimination task (Jueptner et al, 1995) and in the repetitive tapping study of Rao et al (1997), which used inter-stimulus intervals of 300 and 600 ms. The timing of intervals in the millisecond- and seconds-range was compared by (Lewis and Miall, 2003b). They required subjects to compare probe intervals with a visually presented standard interval to judge if they were longer or shorter than the standard. The left cerebellar hemisphere was active in the short interval condition (600 ms standard) compared to the long interval condition (3 s standard). However, no basal ganglia activity was found in either task. The different pattern of results may be related to the fact that in Lewis and Miall's study the comparison stimuli contained visual subdivisions (different length lines); whereas the task presented in the present study did not include any markers that could have aided temporal judgements.

Previous research lends favour for a primary role of the basal ganglia in temporal processing and the SNc activation in the timing vs control task contrast supports this. As such, it is proposed that the basal ganglia activation in this study is related to timing processes, although the different foci found in each task provide interesting evidence for fundamental differences in the way that millisecond and seconds range intervals are processed. A previous study has found a dissociation between activation of the caudate and putamen on a duration discrimination task (Harrington et al, 2004b). Caudate activation was apparent during the encoding phase (compared to rest) and was associated with reduced timing sensitivity, suggesting a key role in timing processes. Activation of the putamen was seen during both the encoding and decision phases and did not correlate with any measures of timing performance; this is

not inconsistent with the possibility of a non-specific role in perceptual timing processes, although further research is needed.

3.4.2.1 Frontostriatal activity

The long interval condition also activated the right SMA, right sensorimotor cortex and bilateral lateral premotor cortex, which, along with the putaminal activation, provides evidence that the frontostriatal motor loop is involved in seconds-range temporal estimation. The results provide evidence against a recent hypothesis, proposing that the motor system is preferentially involved in an 'automatic' timing system concerning the measurement of 'predictable sub-second intervals defined by movement' (Lewis and Miall, 2003a). An alternative, 'cognitively controlled' timing system is described as timing seconds-range durations that typically occur as discrete events, and which are not defined by movement. Despite containing a motor element, the LONG task better fits the 'cognitively controlled' timing system, and indeed activates the prefrontal and parietal regions predicted to be involved in this cognitive style of timing. The cortical motor activation cannot easily be explained by the motor demands, as they did not differ between the two timing tasks. Indeed, recent evidence from functional imaging studies suggests a role for motor cortical areas in non-motor timing tasks (e.g. Ferrandez et al, 2003; Harrington et al, 2004b; Lewis and Miall, 2003b; Rao et al, 2001; Schubotz et al, 2000; Schubotz et al, 2001; Smith et al, 2003). Evidence of SMA activation in long (5 s), but not short (600 ms), intervals has been found once before in a study of externally-cued, rhythmic tapping, although a direct comparison for the two tasks was not made (Rubia et al, 1998).

The reason for the additional motor cortex activation in the long interval condition may in part come from the subjects' own reports of how they timed the intervals. Michon (1985) has described how perception of durations below 500ms is highly perceptual and not under cognitive control. The SMA, which was active in the long interval condition, is known to be important in self-initiated, or 'willed' actions (e.g. Jahanshahi et al, 1995). This could reflect the greater demand on conscious processing and conscious strategies in the long interval condition. The short interval condition demands an intuitive reaction to

the tone (e.g. 'I remembered the tone'), which the subjects found difficult to explain, rather than a more considered implementation of a response. However, a considerable body of functional imaging research has suggested that the SMA has a primary role in timing (e.g. Ferrandez et al, 2003; Macar et al, 2002; Macar et al, 2004) and certainly this is consistent with the frontostriatal activation found for the timing tasks compared to the control task. Macar et al (2004) found that the right SMA and left primary motor cortex were more active in a time production task (target 2.5 s) compared to a force production task. Using EEG recordings, Macar et al (1999) found activation over the SMA during a time production task and a duration discrimination task (target/standard 2.5 s). Furthermore, a positive relationship was found between the length of duration (produced or judged) and the degree of activity. Furthermore, Brunia et al (2000) found that right SMA and right DLPFC activation was associated with improved accuracy over time on a time production task (target 3 s). Certainly, this study presents considerable evidence that motor areas, arguably through connections with the basal ganglia, play an important role in temporal processing.

3.4.2.2 Prefrontal activity

In addition to the activation of the frontostriatal motor loop in the LONG condition, different prefrontal structures were also activated by the two conditions. The LONG condition activated the right dorsolateral prefrontal cortex (BA 9/46 and 10/46), whereas the SHORT condition elicited bilateral mesial activation in the region of the superior and middle frontal cortex as well as left anterior cingulate. The reason for the differential areas of activation may once again lie in the non-temporal differences between the two tasks. Rammsayer (1997) used healthy subjects to study the effects of different dopamine antagonists on the discrimination of short (50ms) and long (1s) durations. They found that disruption of judgements of short intervals was due to D2 receptors being blocked in mesostriatal areas, whereas long interval timing was also disrupted by the blocking of D2 receptors in mesolimbic and mesocortical areas. This suggests that timing long intervals is moderated by dopamine levels in cortical areas, perhaps due to the memory load involved in the processing of longer intervals. Indeed, Rammsayer also found that the benzodiazapine

midazolam, which impairs memory processes, disrupts duration discrimination in the seconds-range, but not the milliseconds-range (Rammsayer, 1999). Thus, it is proposed that the right DLPFC activation found in this study reflects the additional cognitive demands of the long interval, most probably attributable to working memory processes. Furthermore, functional imaging studies of timing that find DLPFC activation tend to use longer intervals and tasks that are more 'cognitive' than short-range, automatic timing tasks (Lewis and Miall, 2003a). In fact, clinical research has found that patients with frontal lesions show decreased accuracy on duration discrimination tasks when they involve intervals of 4s, compared to intervals of 400 ms (Mangels et al, 1998). These results suggest that the increased working memory demands of the long interval discrimination tasks may have mediated the observed deficits.

Prefrontal activation in the short interval condition was more anterior and medial and also occurred bilaterally. These areas of activation may reflect the different properties of milliseconds-range interval estimation and reproduction. Although the short interval was held in memory, the task was far less demanding upon memory processes and subjects described using intuition, rather than cognition, to time the short intervals. The dominance of left hemisphere prefrontal activation perhaps provides a clue to what is happening in the short interval condition. Motor variability on a repetitive tapping task (as assessed by the Wing and Kristofferson (1973ab) model of repetitive tapping) is constantly lower in the right hand of right handed subjects (Sergent et al, 1993). Sergent et al (1993) consequently proposed a significant role for the left hemisphere in motor timing for right handed individuals. Additionally, Ibbotson and Morton (1981) and Wolff et al (1977) both provided evidence that the left hemisphere (i.e. right hand) was superior at rhythmic tapping in both right and left handers. This suggests that the left hemisphere provides key motor timing processes, regardless of handedness. Thus, it is proposed that the frontal activation in the SHORT task reflects the processing of sensory and motoric aspects of the stimuli as the task encourages subconscious motor timing. In addition, the anterior cingulate has been implicated in modulating attentional focus to regulate cognitive processing (e.g. Bush et al 2000) and is believed to be of particular importance when there are competing inputs and actions (e.g. Pardo

et al, 1990; Corbetta et al, 1991). At 500 ms, the interval being produced in this study is close enough to reaction time that frontal cortex may have been involved in inhibiting an immediate response. Certainly, the anterior cingulate cortex is active when we attend to our actions (Frith, 2002). Further work is needed to ascertain the different areas of frontal cortex associated with different types of timing strategy.

3.4.2.3 Posterior activity

Only the LONG interval condition activated parietal regions (right superior parietal cortex (BA 7) and right inferior parietal cortex (BA 40)). When comparing short vs long interval timing, Lewis and Miall (2003b) found left inferior parietal activation during timing of the long interval, although they reserve caution with regards to this finding as activation of adjacent areas of the parietal cortex were present during short interval timing at a lower significance threshold. Considering the established role of the parietal cortex in attention (e.g. Posner et al, 1987; Posner and Presti, 1987; Robinson et al, 1995), the most parsimonious explanation is that the parietal activity is reflecting the increased attentional load of the long intervals. Indeed, parietal activation is commonly observed in functional imaging studies that have investigated the timing of long intervals (Lewis and Miall, 2003a). In an fMRI study, the presence of parietal activity throughout a duration discrimination task led Rao et al (2001) to propose an attentional role for the region. Bilateral parietal activity is also evident in functional imaging studies requiring the monitoring and learning of rhythms, tasks in which attentional demand is high (Ramnani and Passingham, 2001; Schubotz et al, 2000). Clinical work has shown that right hemisphere lesions, including the parietal lobe, can cause timing disturbances that are correlated with attention switching (Harrington et al, 1998b). In dual task experiments in healthy subjects, attending to a non-temporal task reduces temporal accuracy, further suggesting the importance of attentional processing in timing calculations (e.g. Sergent et al, 1993). Indeed, several psychological models have been formulated to reflect the close link between timing processes and attentional resources (e.g. Thomas and Weaver, 1975; Zakay, 1989; Zakay and Block, 1996).

Only the SHORT interval condition elicited temporal cortex activation, specifically in the left middle and superior temporal cortex. This mirrors the previous study comparing short and long intervals; in which right middle/superior temporal gyrus and left superior temporal gyrus were active in the short condition (Lewis and Miall, 2003b). This suggests that auditory processing is more paramount in milliseconds-range timing. Although subjects were exposed to the same number of tone presentations in all conditions, it may be that the significant temporal cortex activation in the short interval reflects the salient way in which the tones were processed in this condition, using a form of auditory template to reproduce the short interval. Similarly, Rao et al (1997) have discussed the importance of 'auditory imagery' in temporal processing.

3.4.2.4 Lateralisation effects

All of the cortical areas in the LONG interval, with the exception of bilateral premotor cortex activity (BA 6), were in the right hemisphere. This right cortical dominance did not occur in the SHORT condition, where activation was spread bilaterally, with posterior cortical areas being entirely located in the left hemisphere. Harrington et al (1998b) suggest that a right prefrontal-inferior parietal network is crucial to effective temporal processing; the key sites include the lateral premotor cortex, the middle and superior gyri of the dorsolateral prefrontal cortex (BA 9, 46) and the supramarginal gyrus. A previous PET investigation of time reproduction compared reproducing intervals of 2.2-11 s to a stimulus-matched auditory cued response task (Macar et al, 2002). Unlike in the present study, the to-be-produced interval was presented immediately prior to each reproduction. Compared to the activation found for the LONG condition in this study, a similar right-hemisphere focused cortical network, including the bilateral DLPFC, right SMA, right anterior cingulate, right inferior parietal lobule and left precentral gyrus, was activated for the time reproduction task compared to the control task. Rao et al (2001) report a similar right hemisphere bias in the duration discrimination of 1.2 s intervals. Mohl and Pfurtscheller (1991) used EEG recordings investigate patterns of activity when subjects were instructed to press a button for an estimated 500 ms or 1.3 s. They found EEG changes in the right parietal region prior to movement; these were increased when the estimations were more accurate. A study that used ERP recordings over both

hemispheres during the encoding and recognition of intervals in the 560 ms – 3 s range also concluded that the right frontal cortex plays a critical role in time perception (Monfort et al, 2000).

Clinical work also provides evidence of a right hemisphere involvement in temporal processing. Harrington et al (1998b) compared right and left hemisphere patients and found that once patients with substantial frequency perception deficits had been excluded only the right hemisphere group displayed deficits in a duration discrimination task. Additionally, this deficit was worse for longer intervals (600ms standard) than shorter intervals (300ms standard). Performance on the time perception task correlated with problems with attention switching in the right hemisphere group, but not in the left hemisphere group. Furthermore, rTMS stimulation of the right, but not left, DLPFC disrupted temporal reproduction in the seconds range (Koch et al, 2003). Timing performance has been studied in a split-brain patient to determine if either hemisphere held an advantage (Handy et al, 2003). A standard interval (570 ms or 150 ms) was presented to both cerebral hemispheres whereas the comparison interval (with the instruction to judge whether it was longer or shorter) was presented to just one hemisphere. The patient was more accurate when responding with his left hand, but there was no interaction between hand used to respond and the visual field in which the comparison interval was presented. The superiority of the right hemisphere in retaining and comparing duration information may have given an advantage when the patient used his left hand. In short, 'the working memory capacities of the right hemisphere may be more optimally tuned to duration judgements than the working memory capacities of the left hemisphere'. Despite these laterality effects on temporal processing, the authors suggested that temporal representations are not likely to be lateralised and are probably subcortically based. Overall, the literature currently supports the proposal that a network of right hemisphere activation is commonly found in the temporal processing of intervals in the seconds range because of the increased attentional and working memory demands.

The more bilateral activation in the short interval condition, with a bias towards left hemisphere activation, indicates the different ways in which the two intervals are processed. As has been discussed previously, the predominance of left hemisphere activation during the short interval condition reflects reliance on sensory/motor information when processing short, sub-cognitive intervals. These hypothesis fit with the left hemisphere's known dominance in processing aspects of movement (e.g. Haaland et al, 2000; Rushworth et al, 1998; Schluter et al, 1998).

3.4.3 Conclusions

1. Contrary to the hypothesis of Ivry (1996), both the basal ganglia and cerebellum are involved in short and long interval time estimation and reproduction. The specific subcortical and cortical areas activated in the two conditions suggest fundamental differences in the way short and long intervals are timed.

2. The activation of left lateral premotor cortex and the left substantia nigra pars compacta in the two timing conditions when compared to the control task suggest that these regions play fundamental roles in temporal processing.

3. In the long interval condition, the activation of prefrontal and parietal areas lends further support to the existence of a right fronto-parietal network that contributes essential working memory and attentional processes to seconds-range temporal calculations.

Chapter 4

The role of the right dorsolateral prefrontal cortex in time reproduction studied with repetitive transcranial magnetic stimulation

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4.1 INTRODUCTION

The PET study reported in Chapter 3 found that the right DLPFC was active during the time reproduction of long (2 s) intervals but not during the time reproduction of short (500 ms) intervals. It is suggested that the right DLPFC provides necessary cognitive support during the reproduction of long intervals, most probably through providing memory processes. However, the limitations of PET make it impossible to break down activity during different parts of the task or disambiguate essential regions, inhibiting a more concrete conclusion. As has been mentioned, these results concur with the observation that functional imaging studies showing DLPFC activation tend to use longer intervals and tasks that are more 'cognitive', rather than short-range, automatic timing tasks (Lewis and Miall, 2003a). In complement to this, patients with lesions to the prefrontal cortex are impaired in the duration discrimination of long (4 s) but not short (400 ms) intervals (Mangels et al, 1998). Similar patients show increased impairment on a duration discrimination task (400 ms) and frequency discrimination task when they are combined in a dual task paradigm. The authors argue that inadequate attentional resources underpinned the prefrontal patients' deficits (Casini and Ivry, 1999), although Mangels et al (1998) suggested that the prefrontal cortex was providing working memory operations. In fact, there is no clear consensus regarding the specific role of the right DLPFC in timing, with some arguing that it may even provide primary timing functions (Lewis, 2002).

Clinical studies provide powerful conclusions, as a brain area can be argued as essential to a particular process if that process is disrupted in a patient with a lesion to that area. One further way of identifying the areas that are 'essential' to temporal processing is to use transcranial magnetic stimulation (TMS). As

described in Chapter 2, this technique uses a magnetic field to create a safe, temporary disruption of neural functioning in a discrete area. Thus, in the manner of a clinical lesion, behavioural disruption following TMS indicates that the targeted brain area is essential to the task, a conclusion not possible in the PET study of Chapter 3. A key advantage of TMS over patient studies is that the disruption or 'lesion' can be turned off and on such that the effect of the region during different parts of a task can be explored. This flexibility offers the possibility of honing the role of the DLPFC in temporal processing, in a way that is not possible in clinical studies and which was also not possible in the earlier PET study.

To date, there have been few investigations of temporal processing using TMS. After 5 minutes of 1 Hz repetitive TMS (rTMS) over the medial cerebellum, Theoret and colleagues (2001) found that the variability on a repetitive tapping task (synchronised tapping with a visual cue presented every 475 ms) was affected. Conversely, rTMS over the lateral cerebellum and motor cortex had no significant effect. Koch at al (2003) looked at the effect of 10 minutes of 1 Hz rTMS on subsequent performance on a time reproduction task. Stimulation over the right DLPFC, but not left DLPFC, resulted in an underestimation of intervals of 5 s and 15 s duration. In line with previous data, the authors suggest that the right DLPFC plays a specific role in seconds-range timing, possibly providing memory or decision processes. However, the researchers instructed the subjects to read a random sequence of numbers aloud (presented on a computer screen) whilst they were completing the task. This additional instruction was proposed to prevent subvocal counting and to therefore provide a more realistic representation of interval timing. However, the addition of the counting task creates a dual-task paradigm, which is known to affect temporal performance (e.g. Fortin et al, 1993; Sergent et al, 1993) and is likely to place additional demands on frontal areas such as the DLPFC. Furthermore, in using long intervals only, the possibility of the DLPFC being essential during millisecond estimation was not investigated. A PET study has also used the time reproduction paradigm to investigate seconds-range interval timing. In agreement with Koch and colleagues, Macar et al (2002) discovered a right hemisphere network, including the right DLPFC. However, they also found

evidence of SMA activity. Considering its role as the main projection site of the frontostriatal motor loop led the authors to suggest that the SMA forms a key role in the timing process. Previous functional imaging studies, including the investigation of long and short interval estimation reported in Chapter 3 (e.g. Brunia et al, 2000; Kawashima et al, 2000; Ramnani and Passingham, 2001; Schubotz et al, 2000), have also found that the SMA is activated during temporal processing and the projections it receives from the basal ganglia clearly make this assumption attractive.

This study uses rTMS to further investigate the role of the right DLPFC in a time reproduction task. In addition, the functional imaging evidence for the role of the SMA in time reproduction suggested that it would also be interesting to explore this region further. Indeed, to date rTMS has not been used to investigate whether the SMA is essential to temporal processing. As Koch and colleagues only looked at seconds-range timing, both millisecond- and second-range intervals were looked at to determine if the short/long dichotomy supported by the results of Chapter 3 is a key issue in the differential roles of the SMA and the right DLPFC in temporal processing. Additionally, as a time reproduction task involves two distinct phases, an Estimation Phase and a Reproduction Phase, the brain was stimulated at both phases allowing the influence of the SMA and right DLPFC on the component timing processes occurring in each phase to be investigated. A potential problem with rTMS is that the auditory and sensory component of the stimulation can disrupt timing behaviour and that this can be difficult to disentangle from real, neural effects. For example, listening to a train of clicks during timing is known to increase arousal and distort time estimation (e.g. Penton-Voak, 1996). Therefore, a control site, the leg motor area, was also included.

4.1.1 Aims of the study

1. To establish the differential roles of the right DLPFC and SMA in time reproduction.

2. To further the results of Chapter 3 and investigate the dissociation between milliseconds- and seconds-range interval timing for the right DLPFC and SMA.

4.2 MATERIALS AND METHODS

4.2.1 Subjects

9 right-handed, university educated subjects with a mean age of 30.6 years (SD 6.19; range 24-41) participated in the study. Three were female and all were right handed. All of the subjects were healthy and without a history of neurological or psychiatric disease or head injury.

4.2.2 Design

The study used a repeated measures 3 (Site) x 2 (Duration) x 2 (Phase) design. Each subject performed a time estimation and reproduction task at both SHORT and LONG interval lengths. For each interval length there were three rTMS sites tested (SMA, right DLPFC and leg motor area), with rTMS delivered at one of two time points: Estimation Phase and Reproduction Phase. The order of conditions was pseudo-randomised using a Latin Square procedure.

4.2.3 Procedure

Subjects were seated opposite a computer screen with a response button placed at a comfortable distance in front of them. The task was first described to the subjects and they then attempted 5 practice trials (no rTMS) to ensure that they fully understood it. The task involved reproducing an interval of time that was visually presented to the subjects. A light blue circle (Circle 1) was flashed in the centre of a grey screen for 100ms, after a specified period a dark blue circle (Circle 2) appeared for 100ms. The subjects were instructed to estimate the period *between* the appearances of the two circles (Estimation Phase). As soon as the dark blue circle disappeared the subjects were asked to start

reproducing the interval that they had just estimated (Reproduction Phase). When they considered that the same amount of time had elapsed then they were to press the response button. Their response initiated the presentation of a black circle (Circle 3), which also appeared for 100 ms. No feedback was given. All subjects used their right index finger to respond. The task was programmed in Visual Basic 6.0 and run on a Toshiba laptop, which was connected to a computer screen that the subjects viewed.

The response button measured 1 cm x 1cm and was mounted on a response box measuring 3 cm x 2 cm x 2 cm. The travel of the button (i.e. distance the button travelled when pressed fully) was 0.3 mm and the operating force (i.e. force needed to press button fully) was 2.4 N (+/- 25%). The button had a flat plastic surface and made a 'click'-type sound when pressed. The response times were recorded to the nearest millisecond. Subjects positioned their right index finger over the button during each run of trials, with their hand resting flat on the table.

For each rTMS site, a complete run consisted of 50 trials (split into two 25 trial blocks) in which the subjects estimated SHORT intervals and 50 trials (split into two 25 trial blocks) in which the subjects estimated LONG intervals. SHORT trials had a standard interval of 400 ms, 450 ms, 500 ms, 550 ms or 600 ms (average 500 ms). LONG trials had a standard interval of 1600 ms, 1800 ms, 2000 ms, 2200 ms or 2400 ms (average 2000 ms). The computer programme selected interval lengths pseudo-randomly, such that each subject received 5 presentations of each interval length within a 25 trial block. The inter-trial intervals were one of five randomly selected lengths (2000 ms, 2500 ms, 3000 ms, 3500 ms or 4000 ms). The different interval lengths were used to prevent learning. A baseline condition was also included in which subjects completed two 25 trial bocks (one SHORT, one LONG) without any rTMS occurring.

4.2.4 rTMS

The rTMS was delivered at one of two time points during the task; at the beginning of the Estimation Phase (i.e. at the onset of Circle 1) and at the

beginning of the Reproduction Phase (i.e. at the onset of Circle 2). In the SHORT and LONG conditions, one block of 25 trials consisted of stimulation during the Estimation Phase and the other block of 25 trials consisted of stimulation during the Reproduction Phase.

rTMS was delivered with a flat figure-of-eight coil (90 mm outer winding diameter) connected to a Magstim rapid stimulator (Magstim, Whitland, Dyfed, UK). Each time four stimuli were given at a rate of 20 Hz. The three sites for the rTMS were the SMA, the right DLPFC and the leg motor area. The leg motor area was determined as the spot in which maximum muscle activity was observed in the legs when held out in front of the subject with ankles dorsiflexed (all areas were established using single stimulus pulses). To localize the SMA site, the coil was moved 4 cm forward from the leg motor site (approx. FCz). The DLPFC is a broad area; the site used is similar to that used by other research groups using TMS (e.g. Epstein et al, 2002; Zheng, 2000). The coil was placed 5 cm anterior from the hand motor area on the right hemisphere and held parallel to the midsaggital line. The hand motor area was located by finding the lowest threshold spot for activating the contralateral first dorsal interosseus (FDI) muscle. For both the leg motor area and right DLPFC, rTMS was applied at an intensity equal to the resting hand motor threshold. The latter was established visually by finding the threshold at which a motor twitch was observed approximately 50% of the time, whilst the hand was in a resting state. To ensure that the rTMS penetrated deep enough at the SMA site, 90% of the active leg motor threshold was used. This was determined by finding the threshold at which 50% of pulses induced a twitch in the legs when held in the position described above (i.e. when leg muscles were active). For all three sites the coil handle was pointing backwards. The study used rTMS parameters within established guidelines (Wasserman, 1998).

4.3 RESULTS

Although the main focus of the experiment was to compare the effects of rTMS at different scalp sites, a baseline condition was also included in which no TMS

was applied. As expected (Vierordt's Law), subjects tended to overestimate the duration of the SHORT interval (mean 595ms rather than 500ms) whereas they tended to underestimate the LONG interval (mean 1860ms rather than 2000ms). When rTMS was applied over the leg motor area, all estimates were longer than the no stimulation condition. Since the leg motor area is not known to play any role in time estimation this overestimation is interpreted as due to factors such as the noise of the stimulus and the scalp sensation produced by rTMS interfering with performance of the task. As a result, further analysis was confined to comparison of rTMS over the leg motor area with rTMS over DLPFC or SMA.

4.3.1 Site specific effects of rTMS

A three factor ANOVA on the data (see section 4.2.2) revealed, as expected, a main effect of Duration ($F(1, 8) = 386.15$; $p < 0.001$), and also a significant effect of Site of stimulation ($F(1, 8) = 3.82$; $p = 0.044$). There was no significant main effect of Phase. The analysis also showed that there was a three way interaction that approached significance (Site X Duration X Phase: $F(1, 8) = 3.55$; $p = 0.053$), none of the other interactions were significant. The three way interaction is 0.003 short of the significance level used in this thesis. It was decided to cautiously accept and interpret this result as significant due to the strong a priori prediction that the site of rTMS would modulate temporal performance. Furthermore, the use of only nine subjects may have limited the power (a discussion on the justification of sample sizes can be found in the Methods sections), which further justifies interpretation of this interesting borderline significant result.

The main effect of Site is explored in Figure 4.1 where data has been collapsed over both phases and durations of the task. A priori contrasts showed that the main effect was due to rTMS over the right DLPFC causing subjects to underestimate time intervals compared with rTMS over the leg motor area ($F(1,8) = 15.18$; $p = 0.005$).

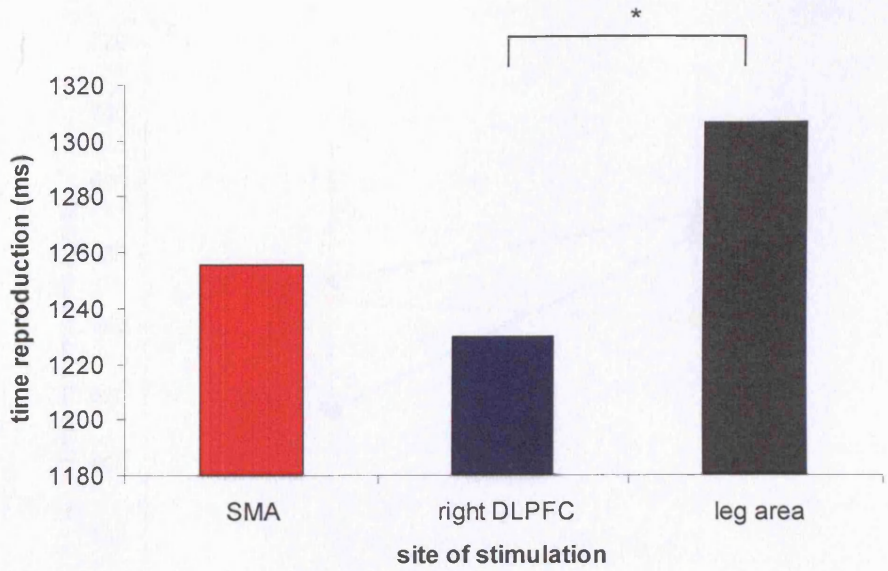

Figure 4.1: Mean differences in time reproduction of the subjects collapsed across Phase and Duration

** significant effect of difference between rTMS over the right DLPFC and rTMS over the leg motor area*

The three way interaction was explored by separate 2 factor ANOVAs for SHORT and LONG intervals, with a Bonferroni correction for multiple comparisons of $\alpha = 0.025$ (Figure 4.2ab). The ANOVA for the SHORT interval was not significant for the main effects of Site and Phase, or for the interaction of Site X Phase. To ensure that no effects in the SHORT condition could be contributing to the significant three way interaction, a paired samples t test was used to compare the time reproduction values for rTMS over the right DLPFC compared to rTMS over the leg motor area in the Estimation Phase. This test was not significant and as rTMS over these two areas showed the greatest difference within a Phase, no data from the SHORT condition could be explaining the three way interaction.

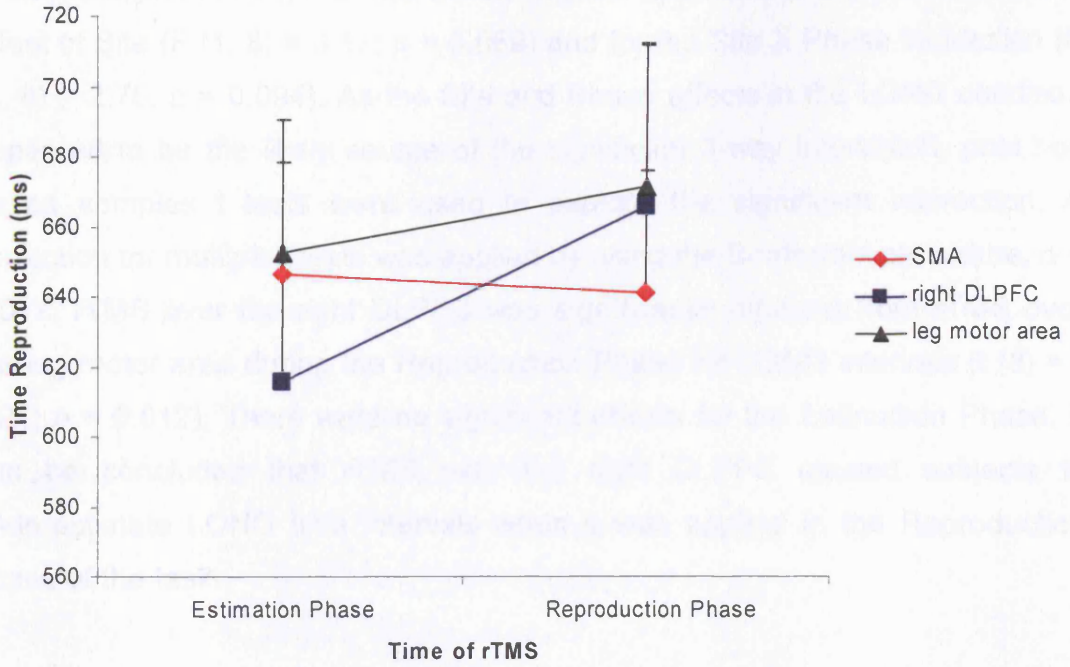

Figure 4.2a: Mean differences (± SE) in time reproduction of the subjects in the SHORT condition

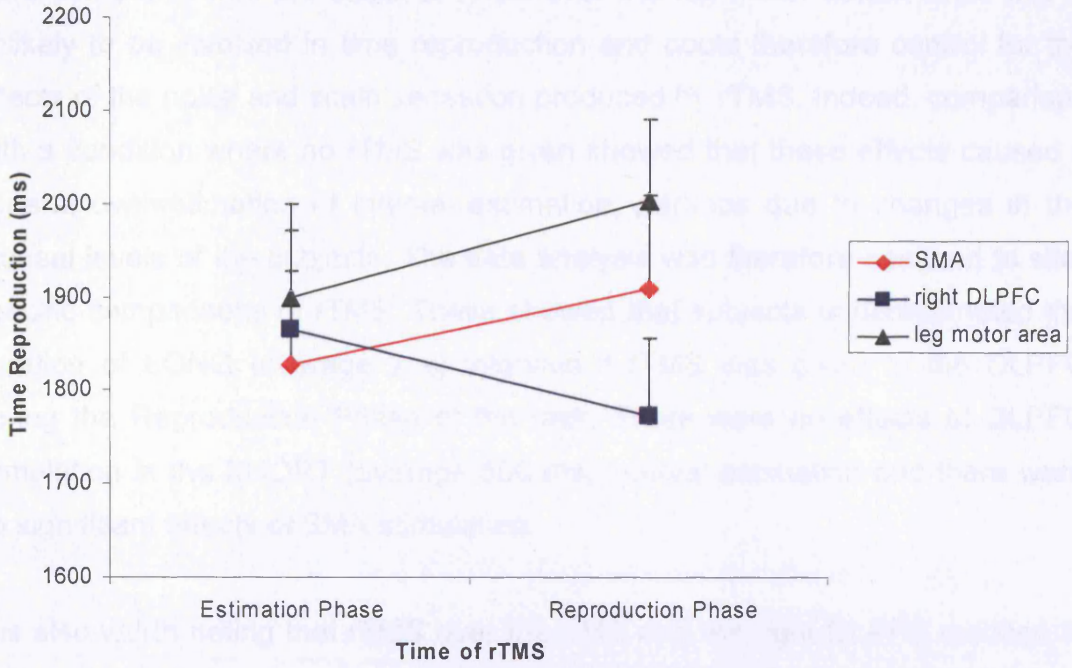

Figure 4.2b: Mean differences (± SE) in time reproduction of the subjects in the LONG condition

In contrast, the ANOVA for the LONG interval approached significance for the effect of Site ($F(1, 8) = 3.17$; $p = 0.069$) and for the Site X Phase interaction ($F(1, 8) = 2.75$; $p = 0.094$). As the Site and Phase effects in the LONG condition appeared to be the likely source of the significant 3-way interaction, post hoc paired samples t tests were used to explore the significant interaction. A correction for multiple t tests was applied by using the Bonferroni procedure, $\alpha = 0.017$. rTMS over the right DLPFC was significantly different from rTMS over the leg motor area during the Reproduction Phase for LONG intervals ($t(8) = -3.21$; $p = 0.012$). There were no significant effects for the Estimation Phase. It can be concluded that rTMS over the right DLPFC caused subjects to underestimate LONG time intervals when it was applied in the Reproduction phase of the task.

4.4 DISCUSSION

The present experiment explores the effect of disrupting function in the right DLPFC and the SMA with rTMS during a time reproduction task. The results were compared with the effect of rTMS over the leg motor cortex since this is unlikely to be involved in time reproduction and could therefore control for the effects of the noise and scalp sensation produced by rTMS. Indeed, comparison with a condition where no rTMS was given showed that these effects caused a general overestimation of interval estimation, perhaps due to changes in the arousal levels of the subjects. The data analysis was therefore confined to site-specific comparisons of rTMS. These showed that subjects underestimated the duration of LONG (average 2 s) intervals if rTMS was given to the DLPFC during the Reproduction Phase of the task. There were no effects of DLPFC stimulation in the SHORT (average 500 ms) interval estimation and there were no significant effects of SMA stimulation.

It is also worth noting that rTMS over the SMA and the right DLPFC resulted in a decrease in the time reproduction values when compared to rTMS over the leg motor area. This implies that the natural bias towards underestimating long intervals (Vierordt's law; see Woodrow, 1951) is increased when rTMS is used

at these sites. The significantly increased effect on a pre-existing response tendency with rTMS over the right DLPFC in the Reproduction Phase (when compared to rTMS over the leg motor area) implies that this modulation of a pre-existing response bias is particularly related to the right DLPFC. Modulation of an existing response bias using rTMS has also been found in a study using rTMS to investigate random number generation; in this study rTMS over the left DLPFC altered the direction of the subject's response bias (Jahanshahi et al, 1998).

4.4.1 Right dorsolateral prefrontal cortex

The data suggest that in long interval timing, the right DLPFC performs a function at the beginning of the Reproduction Phase that is essential to temporal reproduction. As was highlighted in the Results section, the significant post hoc analyses that support this conclusion were interpreted following a borderline significant three way interaction. Therefore, although the post hoc t test that underpins the right DLPFC finding was highly significant, this result must still be interpreted with some caution. In support of the finding, the pattern of results complement the PET study in Chapter 3 in which subjects reproduced previously learned intervals of 500 ms and 2 s. Right DLPFC activation was only observed in the long interval condition, which led to the conclusion that it was involved in the additional cognitive processes that seconds-range timing requires, possibly working memory. The PET study also found right SMA activation in the long interval condition, although evidence to suggest that the SMA is *essential* to temporal processing is not clear in the present study. Additionally, the findings partially concur with the PET study of Macar et al (2002) who found SMA and DLPFC activation in a similar temporal reproduction paradigm. However, the intervals used were, on average, 2.7 s and 11 s, which are much longer than those used here.

The results also confirm the findings of Koch et al (2003) who found underestimation in a seconds range temporal reproduction task with rTMS over the right, but not left, DLPFC. The present study extends this conclusion in showing that rTMS during the Reproduction Phase, but not the Estimation

Phase, has a significant effect on temporal processes. Koch et al (2003) suggested that the underestimation could reflect memory or decision making processes. The results presented here argue against the second hypothesis as the effect of rTMS was only significant when it occurred at the onset of Circle 2, which is unlikely to significantly impact upon the decision to respond. The onset of Circle 2 is also the point at which the temporal reproduction occurs, i.e. 'clock' processes are initiated to reproduce a period of time. However, it seems unlikely that these clock processes are being disrupted, as clock processes are also initiated at the onset of Circle 1. Alternatively, it can be proposed that the disruption produced by rTMS over the right DLPFC at this time point reflects interference with memory processes since at the onset of the Circle 2, subjects would be consolidating the time interval presented during the Estimation Phase (marked by Circle 1 and Circle 2) in memory. Thus, a disruption to the encoding of information is occurring. This reflects the pharmacological work of Meck and colleagues (Meck, 1983; Meck and Church, 1987) as well as a rat lesion study (Olton, 1989), both of which suggest that the frontal cortex is involved in the transfer of temporal intervals to memory.

The lack of a significant effect of rTMS on estimation of SHORT intervals suggests that the right DLPFC plays a differential role in millisecond- and seconds-range timing. This concurs with the assertion that, unlike millisecond range timing, seconds range time intervals are calculated using cognitive processes and recruit cortical areas such as the DLPFC and parietal cortex (Lewis and Miall, 2003a). In corroboration of this, Michon (1985) has proposed that information processing below 500 ms is highly perceptual and not accessible to cognitive control. Rammsayer (1999) found that duration discrimination of long intervals (1000 ms) was affected by midazolam, which is known to affect working memory functions, whereas short interval discrimination (50 ms) was not. Indeed, a concurrent short-term memory task causes a lengthening of the reproduced interval in a time reproduction task when it occurs in the Reproduction Phase. Whilst, when the concurrent task occurs during the Estimation Phase, temporal reproductions decrease (Fortin and Rousseau, 1998). This suggests that timing tasks share working memory

resources with non-temporal tasks, particularly as concurrent tasks that don't have a short-term memory component do not affect timing (e.g. Fortin et al, 1993; Fortin and Breton, 1995). Overall, this implies that longer intervals are more vulnerable than short intervals to non-specific, task oriented memory processes subserved by prefrontal areas.

The key question that remains is whether the working memory components are storing the temporal information or providing timing calculations themselves? Fletcher and Henson (2001) suggest that the DLPFC is involved in selecting, manipulating and monitoring the items held in working memory. Certainly, many theorists dismiss the working memory aspects of the timing process as being non-specific, for example patients with frontal lesions are unable to execute a temporal (duration discrimination) or non-temporal (frequency discrimination) task when the intervals are too long and the memory load too demanding (e.g. Mangels et al, 1998). However, other research suggests that memory may be the key to timing. The multiple time scale model of Staddon and Higa (1999) proposes that temporal judgements are based on memories of different 'strengths' i.e. a memory decays as time passes and this change is quantified in a systematic, predictable way by the organism. Indeed, inhibitory cell pairs have been identified in the DLPFC that appear to show a delay in activity between them of 200 to 1400 ms, which has been presented as evidence of timing-like behaviour in the prefrontal cortex (Constantinidis et al, 2002). In fact, Lewis (2002) goes as far as proposing that this evidence suggests that the internal clock may be located within the prefrontal cortex, arguing that patient's with Parkinson's disease who display temporal deficits tend to be in an advanced stage of illness and thus have a deterioration in the dopaminergic projections to the prefrontal cortex. It is also worth noting that the original conceptions of working memory, derived from animal work with the delayed response task, considered working memory as holding information 'on line' over a period of time (e.g. Goldman-Rakic, 1996). Regardless of the exact nature of the contribution of the prefrontal cortex to timing processes, the data suggest that rTMS over the right DLPFC has a differential effect on the timing of SHORT and LONG intervals, and that this difference is in some way underpinned by the cognitive nature of estimating and reproducing long intervals. This leads to the

conclusion that the right DLPFC is essential to memory transfer and storage in seconds-range time reproduction. Reiterating an earlier point, the borderline significance of the three way interaction requires that some caution be taken with the conclusion. The testing of further subjects or a follow-up study may enable the finding, which complements the study of Koch et al (2003) and the data in Chapter 3, to be reported at the standard significance level of $\alpha \leq 0.05$.

It is also worth commenting on the interesting non-significant pattern of results for rTMS over the right DLPFC in the SHORT condition. rTMS over the right DLPFC during the Estimation Phase caused underestimation of the target interval compared to rTMS during the Reproduction Phase. As only nine subjects were used, it could be argued that a greater n may have resulted in this effect being significant. This would clearly cause a problem for the existing interpretation, which suggests the right DLPFC provides seconds-range specific cognitive processes. Although, to support the current conclusions, even a t test comparing rTMS over the right DLPFC compared to rTMS over the leg motor area in the Estimation Phase was not significant in the post hoc analysis.

4.4.2 Supplementary motor area

The results showed that rTMS over SMA had no significant effect, compared with rTMS over the leg area on interval estimation in any of the tasks. At first sight this might lead to the conclusion that the SMA is not essential for time estimation. However, there is one limitation in the present experimental design that prevents the interpretation of any negative results. Although rTMS was given over the approximate area of the SMA, there was no independent measure at the site and stimulus intensities used, that the rTMS was actually successful in disrupting activity in the SMA. Unlike the motor cortex, where effective stimulation can be verified by the presence of muscle twitches in contralateral body muscles, there is no test for effective stimulation of SMA. In fact, considerable evidence suggests that the SMA plays a non-motor role in timing, for example, SMA activation was found throughout the various stages of a duration discrimination task (Rao et al, 2001) and Macar et al (1999) found EEG changes in the SMA during both duration discrimination and time

reproduction tasks. Additionally, this study had a rhythmic presentation across trials whilst previous research has shown that lesions to the SMA result in impairments in reproducing rhythms from memory (Halsband et al, 1993) and SMA activation has been identified in an fMRI study of auditory and visual monitoring of rhythms (Schubotz et al, 2000). In addition, the data presented in Chapter 3 show that the SMA is active during the time reproduction of 2000 ms intervals. Clearly further work is needed to test these hypotheses with rTMS.

4.4.3 Conclusions

1. The different pattern of results in the SHORT and LONG conditions supports the results of Chapter 3 and the hypothesis that short and long interval timing involves different neural structures (e.g. Lewis and Miall, 2003a).

2. Chapter 3 found evidence of right DLPFC activation during the reproduction of 2 s intervals. This study confirms and adds to this finding by providing evidence that the right DLPFC is *essential* to the accurate reproduction of intervals in the 2 seconds range. This corroborates with the hypothesis that the right hemisphere, including the right DLPFC, is involved in the timing of long (seconds) durations.

3. The study also furthers the conclusions of Chapter 3 by finding a pattern of results that suggest the role of the right DLPFC in the time reproduction of long intervals is likely due to its role in the consolidation and transfer of temporal memory.

Chapter 5

A clinical investigation of the differential roles of the basal ganglia and cerebellum in motor and perceptual timing

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5.1 INTRODUCTION

The PET study reported in Chapter 3 found evidence of basal ganglia and cerebellar activation in short (500 ms) and long (2 s) time reproduction. This suggests that both structures play a role in timing. The activation of the substantia nigra pars compacta in the two timing tasks compared to the control reaction time task suggests that the basal ganglia plays a more fundamental role, providing 'clock'-like processes. Like the rTMS study presented in Chapter 4, clinical studies can complement functional imaging work as they can give valuable insight into the necessity of a given region for performance on a particular task. In particular, clinical research affords the opportunity of selecting a range of complementary tasks, such that specific pattern of deficits can give a relatively sensitive measure of the contribution of a given brain region to a particular process. Research studies that have tested patients with PD and patients with cerebellar pathology on a range of timing tasks have therefore provided additional insight into the function of the basal ganglia and cerebellum in temporal processing.

To date, clinical studies have found timing deficits in both patient groups. The literature for the patients with cerebellar pathology indicates deficits in motor timing, as measured by the repetitive tapping task (Ivry et al, 1988; Ivry and Keele, 1989). The Wing and Kristofferson model (1973ab) has been used to break down the variability into that representing clock function and motor-implementation, with the pattern of results revealing significant deficits in cerebellar patients on both measures compared to control subjects (Ivry and Keele, 1989). Further, it is suggested that lateral regions of the cerebellum result in increased clock variability whilst medial regions result in increased motor variability (Ivry et al, 1988). However, the role of the cerebellum in motor

timing has recently been challenged by Harrington et al (2004a) who found that only clock variability was impaired in a group of cerebellar patients, a deficit that correlated with working memory performance. Interestingly, none of the studies report deficits in the mean inter-response interval (IRI), which suggests that accuracy is not impeded in this patient group.

Evidence relating to perceptual timing measures in patients with cerebellar pathology is also divided, with evidence of impaired (Casini and Ivry, 1999; Ivry and Keele 1989; Mangels et al, 1998) and normal performance on a duration discrimination task (Harrington et al, 2004a) being found. Although the weight of evidence suggests that duration discrimination deficits exist, it may be that non-temporal factors explain the poor performance. Harrington et al (2004a) found a subset of cerebellar patients with a non-significant trend for increased variability on the duration discrimination task. However, this correlated with slowed contralateral tapping speed and slowed performance on the Trail Making Task Part A, a measure of visual scanning and motor speed. This suggests that the patients may have had difficulty with the processing speed required to compete the task competently. Cerebellar patients have been shown to be proficient at a discrimination task that involves loudness rather than duration judgement (Ivry and Keele, 1989). However, cerebellar patients have also shown significant deficits on a frequency discrimination task (Casini and Ivry, 1999) as well as a trend towards frequency discrimination deficits in a further study (Mangels et al, 1998). Therefore, it cannot be dismissed that impairments on the duration discrimination task may be attributable to basic perceptual or sensory deficits. Malapani et al (1998a) found increased variability on the peak-interval procedure for patients with lateral cerebellar lesions compared to patients with medial cerebellar lesions. However, accuracy was normal and, in agreement with scalar expectancy theory (SET: Gibbon, 1977; Gibbon et al, 1984), variability was scalar across durations. A range of timing-related tests have been tested on patients with cerebellar pathology, for example, the patients have also shown impairments in judging the velocity of a moving stimulus (Ivry and Diener, 1991) and in temporal bisection (Nichelli et al, 1996). However, measures of time estimation, time production and time reproduction (although

the peak-interval procedure could be seen to be a variant of this) have not been tested in this group.

For patients with PD, the results have also been varied. One study found no evidence of deficits in motor or clock variability on the repetitive tapping task for patients with PD tested 'on' medication when compared to an elderly control group (Ivry and Keele, 1989). The patients tapped at a significantly faster rate than the control group. However, interpretation of this result was made difficult as a group of college-aged controls also tapped significantly faster than the elderly controls, and four other patient groups also appeared to tap at a faster rate. The reported results did not statistically compare the mean ages of the elderly control group and the PD group, although the PD group appeared slightly younger.

In contrast, Pastor et al (1992a) found that IRI, clock and motor variability were all higher for patients 'off' medication than for a matched control group, for rates of repetitive movement varying from 400 ms to 2 s. The mean IRI (for both synchronisation and continuation data) was significantly slower for the patient group at the two shortest target intervals (400 ms and 500 ms). Some patients were also tested 'on' medication and this significantly improved the accuracy of the mean IRI at the three shorter target intervals used (400 ms, 500 ms, 666 ms), but not for the longer target intervals (1 s and 2 s). Unfortunately, variance, as measured by the Wing and Kristofferson model (1973ab), could not be statistically compared for the data collected 'on' and 'off' medication. The study was slightly atypical as it used repetitive 80° flexion-extension movements of the wrist rather than finger tapping. In a more standard design, O'Boyle and colleagues found that tapping every 550 ms produced increased IRI, motor and clock variability for patients with PD 'off' medication compared to 'on' medication and to a control group (O'Boyle et al, 1996). When the PD patients were compared 'on' medication to the control group, only the clock variance remained elevated. This group replicated one of the findings of Ivry and Keele (1989), reporting that the patients tapped with a faster IRI (continuation phase) than the controls, with this group difference being significant 'on' medication. Harrington

et al (1998a) found increased IRI and clock variability in patients tested 'on' medication compared to controls, with the patients also tapping at a significantly faster rate.

Taken together, there seems evidence of increased variability on the repetitive tapping task both 'on' and 'off' medication, with O'Boyle and colleagues suggesting that being 'on' medication significantly reduces the variability compared to the 'off' medication condition. However, the data clearly produce inconsistencies, with varying results for the effect of the disease on mean accuracy and with the components of variability that are affected varying between studies.

Pastor and colleagues have also tested patients with PD on a range of perceptual timing tasks. Compared to controls, patients tested 'off' medication and trained to count at a 1 s rate showed underestimation on a time estimation task (3, 9 and 27 s) when using the learnt counting rate. Furthermore, the same patients overestimated on a variety of time reproduction tasks (range 3 – 9 s) in which the presented interval was divided by numeric time markers that had to be internally counted at the same rate to reproduce the interval (Pastor et al, 1992b). In a similar study, Lange and colleagues found that compared to controls, patients with PD tested 'off' medication underestimated when estimating presented intervals of 10 s, 30 s and 60 s using a pre-trained inter-count interval of 1 s, and overestimated when producing the same intervals from a start signal (Lange et al, 1995). These patterns of results are considered to be indicative of a slowed 'internal clock' in patients with PD and are supported by a significant improvement in performance when the patients were tested 'on' medication. In both studies, instructing patients to use subvocalisation (internal counting) introduces a timed motor element. In the Pastor et al (1992b) study, a condition that did not explicitly instruct internal counting did not significantly differ from a task in which internal counting occurred. However, the patients may have elected to use subvocalisation in the non-instructed condition as they were told to use their 'own preferred strategy'.

Another study using estimation of long intervals (12 s, 24 s and 48 s) required the subjects to press a space bar at a self-paced rate of once per second (Riesen and Schnider, 2001). The subjects also read aloud random numbers (1-9) presented on a computer screen (yoked to the press-rate) to prevent counting of the number of presses. No differences were found between patients with PD ('on' medication) and a group of controls. However, it is difficult to interpret these data as reflecting perceptual timing performance because of the requirement of a timed movement. Furthermore, reading numbers aloud could provide a salient cue for deciding the length of the intervals.

Typically, the duration discrimination task does not involve pacing stimuli and uses much shorter intervals. Ivry and Keele (1989) found no impairment for PD patients in the 'on' medication state, whereas Harrington et al (1998a) found significant impairment for patients 'on' medication. Riesen and Schnider (2001) found that medicated patients with PD were significantly worse that controls, but used a modified version of the task that included substantial working memory and attentional demands. Finally, patients with PD when tested 'off' medication compared to 'on' medication on the peak-interval procedure, showed increased variability as well as inaccuracy, with the data not conforming to the scalar property (Malapani et al, 1998b). In a follow up study, patients with PD were required to encode and reproduce intervals under different medication states. The data reflected a dysfunction in the storing and retrieval of temporal memories (Malapani et al, 2002).

Taken together, the interpretation of the results of time estimation, reproduction and production tasks for patients with PD is hampered by the inclusion of motor-dependent pacing cues. Also, Lange et al (1995) and Pastor et al (1992b) both used chronometric counting, which means that interval timing is being supported by a language-based strategy (e.g. Hinton et al, 2004). Using chronometric counting provides information about the ability to utilise pacing cues, i.e. to divide a seconds-range interval into consecutive estimates of timed millisecond durations, which although interesting, is not the target of this study. Not surprisingly, the psychophysical properties of chronometric counting and interval timing are very different, with the variance of timing based on

chronometric counting not conforming to the scalar property (the standard deviation of the response distribution increasing with the mean) as in standard interval timing (Hinton and Rao, 2004). Although chronometric counting may still utilise internal timing processes (e.g. to generate individual counts), it is a less pure measure of internal timing processes and results in more precise estimations (Hinton et al, 2004). As such, counting was not used in this study. Although some studies have used measures such as reading randomly presented numbers aloud to prevent counting (e.g. Malapani et al, 1998ab; 2002), this study will not employ such a manipulation as the inclusion of a low-level cognitive task may cause a differential effect on performance for patients and controls (e.g. Brown and Marsden, 1991).

The lack of consistency in the results as well as the methodological variations suggests that testing patients with PD and cerebellar pathology on a range of motor and perceptual timing tasks would be timely. To date, only Ivry and Keele (1989) have directly compared patients with PD and patients with cerebellar pathology on the same range of tests. These researchers found that the patients with cerebellar pathology had significantly higher clock and motor variability than the patients with PD on the repetitive tapping task, whereas the difference between the poorer performance of the group with cerebellar disease on a duration discrimination task only approached significance. The current study directly compared the two groups of patients on a broader range of tasks, such that the differential contributions of the basal ganglia and cerebellum to motor and perceptual timing could be more thoroughly dissected. As with previous studies the patients with PD were compared 'on' and 'off' medication and in a novel contribution to the timing literature, non-medicated PD patients were also compared to a group of de novo patients.

To complement the earlier chapters, the patients were tested on a range of durations to see if duration (millisecond- and seconds-range) has a differential effect in the two patient groups. The standard repetitive tapping task was used as well as measures of time production and time reproduction. A fourth timing task was novel to the clinical timing literature and requires the subject to produce a button press in response to a tone, in the presence or absence of

variously timed warning tones. In particular, this is used as an index of how well the subjects can use the timed warning cue to enhance their reaction time. A further novel task included was the memory for temporal order task[1]. This measures a subject's ability to remember the temporal order in which stimuli are presented and patients with PD have previously been shown to be impaired on this task (Vriezen and Moscovitch, 1990).

5.1.1 Aims of the study

1. To compare the effects of dopamine medication on performance for patients with PD on measures of motor and perceptual timing.

2. To compare the performance of chronically medicated patients with PD to de novo patients with PD on measures of motor and perceptual timing, to investigate the effects of disease severity and duration of illness.

3. To compare the performance of patients with PD, patients with cerebellar disease and healthy controls on measures of motor and perceptual timing.

5.2 MATERIALS AND METHOD

5.2.1 Subjects

21 patients with Parkinson's disease, 9 patients with cerebellar disease (CD group) and 21 healthy controls (control group) were recruited. All patients had been diagnosed by a neurologist following attendance at a movement disorders clinic. The clinical diagnosis of idiopathic PD was established according to the

[1] In keeping with Vriezen and Moscovitch (1990) the task, which requires memory for a presented sequence, is labelled 'memory for temporal order'. This should not be confused with similarly labelled psychophysical tasks (e.g. temporal order judgement) that require the order of presentation of rapidly presented stimuli to be determined.

criteria of the UK Parkinson's Disease Society Brain Bank (Hughes et al, 1992). The diagnosis of idiopathic late onset cerebellar ataxia was based on clinical, neurological and radiological evidence, with a progressive cerebellar ataxia observed in all patients. Diagnosis included the exclusion of other possible causes for the ataxia, including genetic mutations and multiple system atrophy. 9 of the patients with Parkinson's disease had not yet started taking medication for the control of their PD (PD-de novo group). The remaining 12 patients were all chronically treated with dopaminergic medication (PD-drug group) and were tested both 'on' (PD-drug-on) and 'off' (PD-drug-off) medication. None of the subjects had a history of psychiatric or (additional) neurological disease or head injury. Participants were screened for cognitive impairment using the Mini-Mental State Examination (MMSE: Folstein et al, 1975). One subject from each of the CD, PD-de novo and control groups were removed as they had MMSE scores below 27 (23, 23 and 26, respectively), the cut-off indicating presence/absence of cognitive impairment. All subsequent results and tables refer to: 20 healthy controls (7 male, 13 female), 12 PD-drug (5 male, 7 female), 8 PD-de novo (5 male, 3 female) and 8 CD (5 male, 3 female). All subjects were right handed except one patient from the CD group and two of the control group participants.

The demographic and clinical details of the patients are tabulated in Table 5.1. Stages of illness for the PD groups was assessed with the Hoehn and Yahr rating scale (Hoehn and Yahr, 1967) and disease severity with the United Parkinson's Disease Rating Scale (UPDRS: Fahn et al, 1987) and for the CD group with a measure of ataxia (see Jahanshahi et al, 1993).

Table 5.1: Demographic and clinical characteristics of PD-drug, PD-de novo and CD groups

*Standard deviation (SD) in brackets *Dose of Sinemet followed by relative amount of levodopa in brackets*

	Patient number	Gender	Age (years)	Duration of illness	Hoehn & Yahr (ON)	Hoehn & Yahr (OFF)	Ataxia score	Dose of medication/day*
PD-drug	1	M	65	9	2	2.5		Sinemet 550 mg (500 mg) Ropinirole 6 mg
	2	F	56	10	2	3		Sinemet Plus 250 mg (200 mg) Sinemet CR 375 mg (300 mg) Amantadine 100 mg
	3	F	69	10	2	2.5		Pramipexole 2.16 mg
	4	F	51	10	1	2.5		Ropinirole 12 mg
	5	F	69	13	2	3		Sinemet Plus 375 mg (300 mg) Selegiline 10 mg
	6	M	73	7	1	2		Sinemet CR 625 mg (500 mg) Pergolide 3 mg
	7	F	58	3	1	2		Ropinirole 24 mg
	8	F	61	11	1	3		Pergolide 4.5 mg
	9	F	59	4	2	3		Sinemet 775 mg (605 mg) Sinemet CR 250 mg (200 mg) Cabergoline 4 mg
	10	M	67	10	1	2		Sinemet Plus 125 mg (100 mg) Sinemet CR 250 mg (200 mg) Pramipexole 2.16 mg Amantadine 100 mg
	11	M	58	10	2.5	3		Sinemet Plus 750 mg (600 mg) Sinemet CR 250 mg (200 mg) Ropinirole 21 mg
	12	M	68	4	2	2		Sinemet Plus 375 mg (300 mg) Cabergoline 3 mg
			62.83 (6.6)	**8.42 (3.18)**	**1.63 (0.57)**	**2.54 (0.45)**		
PD-de novo	1	M	69	2		1		
	2	M	71	3		1.5		
	3	F	52	7		2.5		
	4	F	50	5		1		
	5	M	69	2		2		
	6	F	72	2		2		
	7	M	69	4		1		
	8	M	49	2		2		
			62.63 (10.27)	**3.38 (1.85)**		**1.63 (0.58)**		
CD	1	M	71	10			11	
	2	M	61	45			6	
	3	M	82	22			17	
	4	M	62	10			9	
	5	F	59	8			7	
	6	F	53	10			9	
	7	M	46	6			19	
	8	F	55	4			8	
			61.13 (11.15)	**14.38 (13.48)**			**10.75 (4.74)**	

5.2.2 Design

A mixed within subject and between groups design was used. Three motor timing tasks and one perceptual timing task were used, with all subjects performing all tasks. For the PD-drug group, the CD group and a subset of the control group, a test of memory for temporal order was also used. Patients in the PD-de novo group, patients with cerebellar disease and healthy controls were tested on all timing tasks once. The PD-drug group were tested on two occasions, once 'on' medication and once 'off' medication after overnight withdrawal from all PD-related medication (average length of withdrawal = 14.25 hours (SD 3.47)). Patients were randomly assigned to being tested 'on' or 'off' medication first. Data analysis was focused on the following issues:

I) *Medication effects*
PD-drug compared 'on' and 'off' medication to test for the effect of dopamine on timing performance in PD.

II) *Duration of illness and disease severity effects*
PD-drug-off compared to the PD-de novo group to test for the effect of duration of illness and disease severity.

III) *Disease specific effects*
PD-drug-off vs CD vs controls, to compare the effect of PD and cerebellar disease on timing performance relative to controls.

5.2.3 Procedure

Subjects were seated at a table with a response box placed at a comfortable distance in front of them. The response box was identical to the one described in Chapter 3, with subjects again using just one of the buttons. The response box was used in the time reproduction, warned and unwarned reaction time task and repetitive tapping tasks. During the time reproduction and repetitive tapping tasks the subjects placed their index finger over the response button. The position of the finger for the warned and unwarned reaction time task was slightly different and is described below. The height of the box meant that the hand was resting at an angle of approximately 45° onto the box.

The four timing tasks (programmed in Quick Basic) were run on a Dell laptop, which was placed on the table facing the experimenter, away from the subject. The four timing tasks were presented in a pseudo-randomised order (using a Latin Square procedure) and were interleaved with the non-temporal tasks. The order of the blocks within each task was also pseudo-randomised. Each task was fully explained to the subject and the subjects were told not to use counting during the timing tasks. All tasks were performed with the subjects' dominant hand, unless otherwise specified.

5.2.3.1 Time production task

Subjects were instructed to estimate a set period of time using intuitive judgement, rather than strategy. Three periods of time were estimated; 30 s, 60 s and 120 s. The subject indicated the beginning of the interval by pressing the space bar on the computer and indicated when they considered the period had elapsed by pressing the space bar a second time. They were instructed to estimate the selected time interval five times consecutively, with a short pause between estimates.

5.2.3.2 Time reproduction task

In this task the subject reproduces a timed interval that they have just heard. The subject was presented with Tone 1 (1000 Hz, duration 50 ms), which was followed after the target interval (250, 500, 1000 or 2000 ms) by a second identical tone (Tone 2) (Estimation Phase). The subject then had to press a response key after the same inter-tone interval had elapsed (Reproduction Phase). The button press elicited the third tone - Tone 3 (with characteristics identical to the first two tones). The set of three tones constituted one trial, for each target interval there was a total of 10 consecutive trials (1 run). The task consisted of 2 runs of each target interval, divided into two blocks.

5.2.3.3 Warned and unwarned reaction time task

This task consists of five blocks of a reaction time (RT) task; one block was 'unwarned' and four were 'warned'. In the unwarned block subjects were instructed to press the response button as quickly as possible after hearing a tone (Go-tone) (1000 Hz, duration 50 ms). In the four warned blocks the Go-

tone was preceded by a higher pitch warning tone (1500 Hz, duration 50 ms), although the subjects had to wait for the Go-tone before responding. The interval between the warning tone and Go-tone varied for each block of trials and was either 250, 500, 1000, or 2000 ms. The number of trials in each block was 25. The subjects were instructed that it was important to wait for the 'Go' tone before responding, with RTs < 100 ms and > 2000 ms being rejected as error trials. For all trials, the subjects' finger was positioned next to the response button (in front and below it); with responding requiring that the finger be lifted and moved to press the button (height of response button was 1.5 cm).

5.2.3.4 Repetitive tapping task

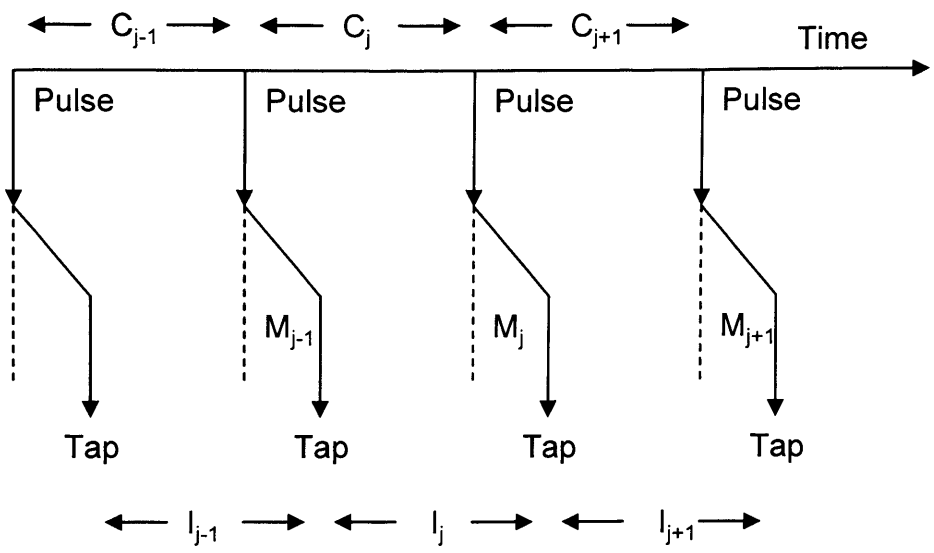
Subjects were instructed to tap in synchrony with a tone (1000 Hz, duration 50 ms) presented with a constant inter-stimulus interval (ISI) (synchronisation phase). After 31 taps (30 intervals) the tone stopped and subjects continued to tap and maintain the rhythm for a further 30 intervals (continuation phase). The subjects performed the task over two blocks. Each block consisted of four inter-tone intervals set either at 250, 500, 1000 or 2000 ms. For both the synchronisation phase and continuation phase, the first 5 responses were removed to ensure that the data were limited to responses where the required response rate was fully entrained. This procedure has been adopted in previous research (e.g. Pastor et al, 1992a).

Analysis of the data involved initial investigation of the mean inter-response interval and standard deviation for each interval length for both the synchronisation phase and continuation phase. Further analysis of the continuation phase was carried out by application of the Wing and Kristofferson (1973ab) model. As only two runs were collected per target interval, only limited data did not violate the assumptions of the Wing and Kristofferson model. The analysis of the Wing and Kristofferson model was confined to the analysis across patients with cerebellar disease, patients with PD (PD-drug-off) and healthy controls.

The Wing and Kristofferson model (1973ab)

To reiterate the description of the model from the Introduction, the model proposes that two independent processes underlie timed movements: a central clock and a peripheral motor implementation system. The clock, entrained to the rate of the pacing stimulus, emits a pulse each time the target interval has elapsed, with the clock intervals (C_j) subject to random temporal variance (clock variance: CV). Emission of a pulse activates the motor implementation system, which executes the motor command. The lag between pulse emission and the motor response is termed the motor delay (M_j), which is also subject to random temporal variation (motor delay variance: MV). The model rests on two key assumptions, the independence of the clock and motor components as separate processes and the independence of successive clock intervals and of successive motor delays. The inter-response interval (IRI or I_j) between successive taps is the sum of the associated clock interval plus the difference between the motor delays of the current and previous responses (i.e. $I_j = C_j+M_j-M_{j-1}$). Total variance (TV) is the variance of the IRI data and can be calculated directly. See Figure 5.1.

Neighbouring inter-response intervals (i.e. intervals at lag 1) are negatively correlated, such that a short IRI tends to be followed by a long IRI and vice versa. Wing and Kristofferson (1973b) suggest that this negative correlation is the result of variability in the motor implementation process; a long motor delay would increase the current IRI and decrease the next IRI (assuming the central clock remained constant), whereas a short motor delay would have the opposite effect. As is illustrated in Figure 5.1, the length of the clock interval only affects the current IRI, so cannot explain the negative correlation. This observed effect means that MV can be calculated as the negative of the autocovariance at lag 1. As TV is the variance of the IRI data (TV = CV + 2MV), the CV can be calculated indirectly: CV = TV – 2MV.



$$\mathbf{I_j = C_j + M_j - M_{j-1}}$$

Figure 5.1: Wing and Kristofferson's model for the timing of repetitive movements

KEY: C = clock interval, M = motor delay, I = inter-response interval

The autocorrelation function at lag 1 can be defined as the normalised measure of the statistical dependence between successive intervals. It is calculated by normalising the lag 1 autocovariance function (-MV) by the lag 0 autocovariance function (TV), summarised as:

$$\text{Lag 1 autocorrelation} = -\frac{1}{2 + \left[\frac{CV}{MV}\right]}$$

Following from the assumptions of the model, Wing and Kristofferson (1973b) illustrate that the lag 1 autocorrelation must lie between 0 and –0.5; this is the main prediction of the model. In addition, for lags greater than 1, the predicted autocovariance is 0.

Analysis of the Wing and Kristofferson model (1973ab)

For the 25 IRIs recorded during the continuation phase, autocovariance estimates were calculated at lags 0-5. The autocorrelation function at lag 1 was then calculated for each trial by normalising lag 1 autocovariance by lag 0 autocovariance. The Wing and Kristofferson model requires that the IRI data is statistically stationary, as non-stationarity may distort the autocovariance values. Data is stationary if its mean remains constant over time, so linear regression analysis was used to calculate linear trends in the IRI data. Each IRI for each subject and for each trial was analysed separately. Averaging across the data, the mean regression slope and r^2 was calculated for each group under each trial condition. The mean percentage of trials that produced a significant regression was also calculated as well as calculating the percentage of positive and negative slopes for each trial-type.

The principal tenet of the model is that the lag 1 autocorrelation function should lie between 0 and -0.5. Any data that did not fit this prediction was discarded. For lags greater than 1, the model predicts that the autocovariance estimates should equal 0. However, in practice violations of this prediction are not removed from the data (e.g. Collins et al, 1998; O'Boyle et al, 1996) and often not reported (e.g. Harrington et al, 2004a; Pastor et al, 1992a), so this calculation was not performed.

For each trial, the lag 0 autocovariance (TV) was used to calculate the MV and the CV, using the formulae described above. The values were then transformed by taking the square root, and thus expressed as standard deviations. For each subject, a mean IRI, TV, CV and MV was calculated by averaging across the two trials for each target interval, providing they both met the lag 1 prediction. Group means for each target interval were then calculated by averaging across subjects.

5.2.3.5 Memory for temporal order task

This task was a modified version of the task used by Vriezen and Moscovitch (1990). Subjects were shown ten cards, presented at the rate of one every 3 s, depicting simple line drawn objects such as a watch or car. They were asked to

remember the order of presentation, as they would be asked to recall it later. In addition, upon presentation of each card presentation they had to name the object depicted out loud to ensure attention to and processing of each stimulus card. After all the cards had been presented a 3 minute distracter task was completed. This involved the subjects looking at a separate series of cards that had line drawn objects on them. They had to decide whether the picture was of something man made (e.g. a comb) or natural (e.g. an apple). The cards were presented at a rate of 1 every 3 seconds. After 3 minutes had elapsed the subjects were shown the initial 10 test cards, which had been arranged into a pseudo-random order. They were asked to rearrange them into the order they were previously presented. After another 20 minutes, during which time the subjects completed other tasks from the battery, the subjects completed a delayed recognition task. They were shown 20 cards, presented a the rate of one every 30 s, depicting simple line drawn objects, which included the 10 original items plus 10 'foils'. They had to indicate with a 'yes' or 'no' response whether each card was one of the original 10 test cards or not.

The following scores were obtained from the data:

Total Recall Score 1: The number of items placed in the correct position
Total Recall Score 2: The number of intact pairs of items recalled. Credit was given for correctly placing the first item and the last item, and for any sequence of two items corresponding to contiguous pairs of items in the original presentation.
Total Recall Score 3: An absolute deviation score. This was derived by calculating the distance between each item's presentation position and recall position. The overall absolute deviation score was then calculated by summing the scores across the 10 items.
Total Recall Score 4: A relative deviation score. This was derived by summing the distance between each item's presentation position and presentation positions of the items placed before and after it on recall. The overall relative deviation score was then calculated by summing the scores across the 10 items.

Total Recognition Score 1: The number of items correctly identified

Total Recognition Score 2: The number of false positives (i.e. falsely identified as previously seen)

Total Recognition Score 3: Corrected recognition score. This was the subtraction Total Recognition Score 2 from Total Recognition Score 1.

5.2.3.6 Additional tests

National Adult Reading Test (NART: Nelson, 1982)

This was included as a measure of pre-morbid verbal IQ. Subjects were asked to read aloud a list of 50 words. None of the words followed the common rules of pronunciation, such that word recognition was necessary for the word to be pronounced correctly.

Paced Auditory Serial Addition Test (PASAT: Gronwall and Wrightson, 1981)

Competent performance on this task requires focused attention. A tape recorder was used to play a series of 33 numbers between 1 and 9 at a rate of one every 4 seconds. The subjects were instructed to add each number being spoken to the number that was presented immediately before it and to say out loud the sum. This mental arithmetic was performed for each pair of consecutive numbers. If the subject lost their train of thought at any point they were instructed to clear their head and re-start the calculation process with the next presented number. This task therefore enabled a measure of attentional ability to be obtained.

Purdue Pegboard (Tiffin and Asher, 1948)

This test was used to assess motor speed and finger dexterity. It comprises of a set of metal pegs and a pegboard with two parallel lines of holes. Subjects were instructed to pick up the pegs one at a time and place them one by one in one of the lines of holes as quickly as possible. This was done three times: with the right hand (using the line of holes on the right of the board), with the left hand (using the line of holes on the left of the board) and with both hands (using the lines of holes on both sides). The number of pegs placed in the holes in 30 seconds was recorded on each occasion.

Beck Depression Inventory (BDI: Beck et al, 1961)

This was a questionnaire used to assess the subjects' current mood, to screen for moderate or severe self-reported depression.

Measure of self-reported stress and arousal (Mackay et al, 1978)

As medication is known to affect arousal, which could impact on performance on the tasks, a questionnaire exploring separate measures of self-reported levels of stress and arousal (Mackay et al, 1978) was administered to the PD-drug patients. The questionnaire consists of a list of adjectives (e.g. active, drowsy) and the patients had to rate themselves on each item. The questionnaire was administered during the testing and 'on' and 'off' medication. Although the motivation for using the questionnaire was to assess arousal, the stress data were also analysed.

5.2.3.7 Clinical assessment

Parkinson's disease

As previously mentioned, stage of illness was assessed using the Hoehn and Yahr rating scale (Hoehn and Yahr, 1967). Part III of the United Parkinson's Disease Rating Scale (UPDRS: Fahn et al, 1987) was used to quantify the severity of motor symptoms of PD. For patients taking medication, this was done both 'on' and 'off' medication.

Cerebellar disease

Level of movement-related disability was assessed using an ataxia rating scale (see Jahanshahi et al, 1993) that assessed rapid alternating movements, dysmetria and intention tremor, sway and postural stability, gait and speech. Scores ranged from 0 to 32, with a higher score indicating greater disability.

All clinical assessments were conducted by a neurologist.

5.3 RESULTS

Prior to analysis, the different groups were compared in terms of age, National Adult Reading Test (NART) derived estimates of premorbid verbal IQ scores and Beck Depression Inventory (BDI) scores. Group means for these measures are given in Table 5.2. The non-parametric Kruskal-Wallis test was used to establish if there was a significant difference in the scores between groups for the different measures. There was no significant difference in age, NART IQ score (with all groups showing an average IQ within the high average to superior range) or BDI scores, even when the PD-drug group scores used were those from the 'off' medication condition (where the mean score indicated mild self-reported depression). For the other groups, all BDI scores were within the normal range (0-9), indicating minimal self-reported depression, although the PD-drug-on group were on the cusp of mild self-reported depression.

	n	Age		MMSE		NART IQ		BECK	
Control	20	67.65	(8.87)	28.45	(0.95)	120.05	(6.49)	7.2	(5.68)
PD-drug-on	12	62.83	(6.60)	29.42	(0.67)	122.67	(3.52)	9.5	(4.95)
PD-drug-off								10.75	(5.83)
PD-de novo	8	62.63	(10.27)	28.71	(0.95)	119.71	(4.15)	4.71	(2.93)
CD	8	61.13	(11.15)	29.13	(0.84)	114	(10.93)	8.75	(9.05)

Table 5.2: Profiles of the four groups of subjects
Standard deviation (SD) in brackets

For the patients, differences in duration of illness were statistically tested using the Kruskal-Wallis test. A significant difference was found (Chi-Square (2) = 12.39; $p = 0.002$). Not surprisingly, post-hoc Bonferroni corrected ($\alpha = 0.017$) comparisons revealed that this was due to a significantly shorter duration of illness for the PD-de novo group than the PD-drug group (Mann-Whitney U = 9.00; $z = -3.05$; $p = 0.002$) and for the PD-de novo group than the CD group (Mann-Whitney U = 3.50; $z = -3.03$; $p = 0.002$). There was no significant difference between duration of illness for the PD-drug group and CD group.

5.3.1 Medication effects for PD-drug-on vs PD-drug-off

First, the performance of the PD-drug group 'on' and 'off' medication on various motor and psychological variables were compared, to ensure that any reported drug-related differences on the timing tasks could not be explained by non-temporal factors. The average scores on the BDI ('on' = 9.5 (SD 4.95); 'off' = 10.75 (SD 5.83)) did not significantly differ between 'on' and 'off' medication states (t (11) = -0.78; p = 0.451), suggesting that the drug state did not have a significant affect on mood. The differences in PASAT scores ('on' = 5.41 (SD 5.23); 'off' = 6.08 (SD 6.10)) were similarly non significant (t (11) = -0.80; p = 0.438), suggesting medication did not affect attentional capabilities.

Subjective measures of arousal and stress were compared. As would be expected, the measure of stress (max. score possible = 19) was slightly higher when 'off' medication ('on' = 2.93 (SD 3.07); 'off' = 6 (SD 5.64)) and the measure of arousal (max. score possible = 15) was slightly lower ('on' = 7.17 (SD 4.53); 'off' = 5.58 (SD 3.78)). The change in the arousal score was not significant (t (11) = 1.028; p = 0.33), but the patients identified significantly more with stress-related adjectives when 'off' medication (Wilcoxon signed ranks test: z = -2.03; p = 0.043).

	on		**off**	
Hoehn & Yahr	1.63	(0.57)	2.54	(0.45)
UPDRS Part III	17.5	(9.58)	36.92	(8.61)
Purdue left hand	10.17	(1.90)	9.58	(1.78)
Purdue right hand	12.5	(2.47)	10.58	(1.51)
Purdue bilateral	8.58	(1.56)	7.17	(2.17)

Table 5.3: Stage of illness, disease severity and motor speed for the PD-drug group when tested 'on' and 'off' medication

Standard deviation (SD) in brackets

Of further interest was how much measures of disability relating to PD would vary as a function of medication (see Table 5.3). The average Hoehn and Yahr score was significantly higher when 'off' medication (Wilcoxon signed ranks test: $z = -2.99$; $p = 0.003$), suggesting a more advanced stage of illness. The Part III score of the UPDRS, reflecting severity of motor symptoms, was also significantly higher 'off' medication ($t(11) = -9.07$; $p < 0.001$). A further measure was the Purdue Pegboard, which reflects motor speed and manual dexterity. In a 3 (hand used (right, left, bilateral)) x 2 (medication state) repeated measures ANOVA, a significant main effect of hand used ($F(2, 22) = 46.16$; $p < 0.001$) and medication state ($F(1, 11) = 27.77$; $p < 0.001$) was found. This indicates that the patients were significantly slower when 'off' medication on this task. The interaction between the two main effects was non significant ($F(2, 22) = 2.12$; $p = 0.144$). Breaking down the main effect of hand used, post hoc pairwise comparisons showed that patients were significantly slower when using their left hand compared to their right hand (mean difference –1.67; $p = 0.005$)), when using both hands compared to their left hand (mean difference 2.00; $p < 0.001$)) and when using both hands compared to their right hand (mean difference 3.67; $p < 0.001$)).

5.3.1.1 Time production task

These results can be seen in Table 5.4. Medication resulted in an overestimation for the 30 s and 60 s intervals and an underestimation for the 120 s interval. To enhance comparison between the three durations, an absolute error score (i.e. the difference between the estimated duration and the target duration, regardless of the direction of the error) was also calculated for each of the intervals. This calculation showed that the absolute error, or deviation from the target duration, increased linearly with the length of the interval being produced and that the error margin improved when the patients were 'on' mediation. Variability was also assessed by finding the average SD for each subject for each interval length (i.e. the SD of the five attempts at each interval), reflecting within-subject variability. The SD score was higher when the subjects were 'off' medication and increased with interval length. As the relative degree of over- and underestimation, the absolute error and the measure of variability were all important for teasing apart the pattern of deficits, three

ANOVAs were performed. A Bonferroni correction of $\alpha = 0.017$ was used to account for the three analyses.

Mean production

For the mean data, a repeated measures ANOVA (3 (duration) X 2 (medication state)) revealed a main effect of duration ($F(2, 22) = 88.70$; $p < 0.001$) but a non significant main effect of medication. The interaction between duration and medication state only approached significance ($F(2, 22) = 3.00$; $p = 0.070$). With regard to the significant duration effect, a priori polynomial comparisons revealed a significant linear relationship between the estimates of the three target durations ($F(1, 11) = 109.30$; $p < 0.001$). These results suggest that both the degree and pattern of over- and underestimations is not significantly affected by medication.

	Mean production (s)	Mean absolute error (s)	Mean variability measure (SD)
	ON	ON	ON
30 s	**27.41** (10.61)	**7.98** (7.17)	**3.30** (2.78)
60 s	**52.10** (22.17)	**17.83** (14.58)	**8.48** (5.35)
120 s	**94.06** (31.34)	**31.00** (25.83)	**17.03** (7.52)
	OFF	OFF	OFF
30 s	**30.72** (13.36)	**10.52** (7.64)	**4.37** (3.72)
60 s	**57.15** (29.49)	**24.83** (14.35)	**10.75** (8.97)
120 s	**86.58** (35.45)	**39.77** (27.38)	**17.27** (12.52)

Table 5.4: Time production scores for PD-drug group when tested 'on' and 'off' medication

Standard deviation (SD) in brackets

Absolute error

Following log transformation to normalise the distribution, a 3 (duration) X 2 (medication) repeated measures ANOVA was performed on the absolute error scores. A significant effect of duration (F (1.991, 21.90) = 9.11; p = 0.001) was found. A priori polynomial contrasts revealed a significant linear relationship between the absolute error scores for the three durations (F (1, 11) = 17.15; p = 0.002). The effect of medication was only 0.001 away from reaching the threshold for Bonferroni significance (F (1, 11) = 7.75; p = 0.018), so it was decided to report this as a marginally significant effect considering the conservative nature of the Bonferroni correction and the minute statistical difference. Thus, the data suggest that the absolute error was significantly worse for the patients in the PD-drug group when 'off' medication. The interaction between the two factors was not significant.

Variability

The data were subjected to a log transformation to normalise the distribution and were analysed with a 3 (duration) X 2 (medication) repeated measures ANOVA. A significant effect of duration (F (2, 22) = 30.52; $p < 0.001$), a non significant effect of medication and a non significant interaction were obtained. A priori polynomial contrasts revealed a significant linear relationship between the within-subject variability in estimations for the three durations (F (1, 11) = 42.08; $p < 0.001$). Thus, subjects showed significantly increased variability as the durations increased, but medication did not have a significant effect on that variability.

5.3.1.2 Time reproduction task

These results can be seen in Table 5.5. There is very little difference between the mean reproduction values 'on' and 'off' medication, with the reproductions being longer in the 'off' medication condition for the 250 ms and 2 s conditions only. Mean absolute error scores are also tabulated, with the 'off' medication condition only causing an increase in the 500 ms and 2 s conditions. The variability of the responses was investigated by looking at the SD for the twenty measures recorded for each duration condition, both 'on' an 'off; medication. Variability was higher for the 250 ms, 500 ms and 2 s conditions in the 'off'

medication condition, but not for the 1 s condition. As with the time production task, relative over- and underestimation, absolute error and variability were investigated in separate ANOVAs. A Bonferroni correction of $\alpha = 0.017$ was used to account for three comparisons. None of the data were normally distributed, so a log transformation was used on all three ANOVAs.

Mean reproduction

A 4 (duration) X 2 (medication) repeated measures ANOVA for the relative mean scores revealed a significant effect of reproduction duration ($F(2.069, 22.757) = 1126.74$); $p < 0.001$) but no significant effect of medication or duration X medication interaction. The main effect of duration was explained by a priori polynomial comparisons, which revealed a significant linear relationship between the reproductions at the different target durations ($F(1, 11) = 2071.54$; $p < 0.001$). A significant departure from the linear trend was also observed in a cubic trend in the data ($F(1, 11) = 28.59$; $p < 0.001$).

Absolute error

A 4 (duration) X 2 (medication) repeated measures ANOVA for the absolute error scores revealed a significant effect of reproduction duration ($F(1.406, 15.47) = 37.15$; $p < 0.001$), with the effect of medication and the interaction between these two factors being non significant. A priori polynomial contrasts reveal a significant linear relationship between the absolute error scores for the four durations ($F(1, 11) = 47.40$; $p < 0.001$). Significant quadratic ($F(1, 11) = 14.39$; $p = 0.003$) and cubic ($F(1, 11) = 12.63$; $p = 0.005$) deviations from this trend were also noted. The data suggest that medication did not have a significant effect on the degree and pattern of absolute error on a time reproduction task.

Variability

A 4 (duration) X 2 (medication) repeated measures ANOVA found a significant effect of duration ($F(1.593, 17.523) = 28.11$; $p < 0.001$), but no significant effect of medication. The interaction of the two factors approached uncorrected significance ($F(3, 33) = 2.81$; $p = 0.055$). A priori polynomial contrasts revealed

a significant linear relationship between the SD scores for the four durations ($F(1, 11) = 66.02$; $p < 0.001$).

	Mean reproduction (s)	Mean absolute error (s)	Mean variability measure (SD)
	ON	ON	ON
250 ms	**247.38** (44.15)	**72.09** (56.36)	**58.84** (26.55)
500 ms	**468.45** (49.13)	**73.17** (23.96)	**71.70** (32.14)
1000 ms	**930.41** (90.86)	**121.86** (79.49)	**128.80** (99.15)
2000 ms	**1685.06** (242.77)	**330.21** (226.24)	**140.72** (49.01)
	OFF	OFF	OFF
250 ms	**253.63** (51.64)	**69.42** (34.82)	**77.33** (59.54)
500 ms	**442.72** (43.58)	**86.29** (20.51)	**73.51** (30.04)
1000 ms	**913.50** (67.24)	**114.69** (33.73)	**85.39** (32.44)
2000 ms	**1715.96** (285.87)	**356.21** (215.43)	**197.94** (80.88)

Table 5.5: Time reproduction scores for PD-drug group when tested 'on' and' off' medication

Standard deviation (SD) in brackets

5.3.1.3 Warned and unwarned reaction time task

Figure 5.2 shows that the unwarned RT task produced the slowest RTs. The cued warning tones improved performance, with the tone 250 ms prior to the Go-tone producing the biggest improvement and the tone 2 s prior to the Go-tone producing the least speeding of RTs. The patients were slower 'off' medication across all durations. Figure 5.3 shows the average SD across the different trials for each condition. The data suggest that variability does not change a great deal 'off' medication although it seems to vary more broadly in the 'on' medication condition.

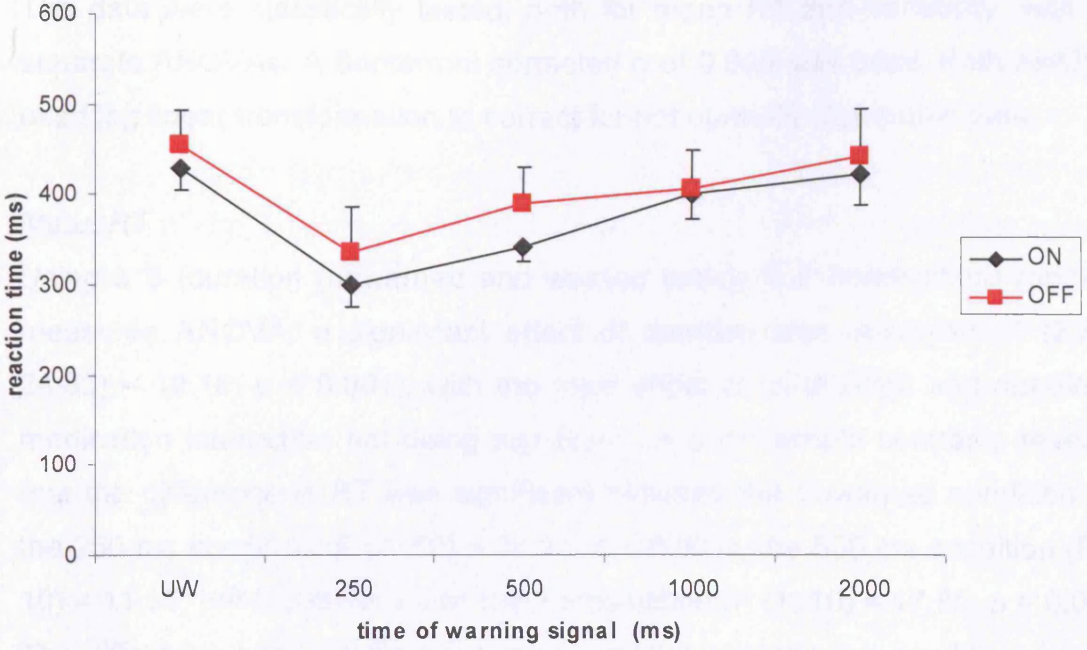


Figure 5.2: Warned and unwarned RTs (± SE) for the PD-drug group when tested 'on' and 'off' medication

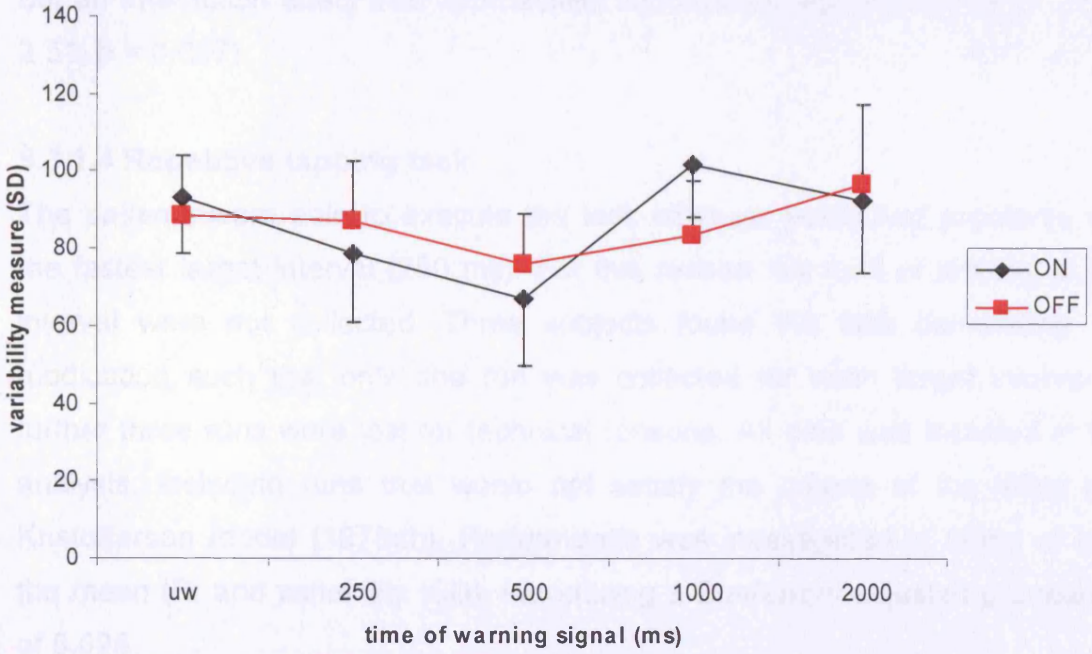


Figure 5.3: Variability measure for warned and unwarned RTs (± SE) for the PD-drug group when tested 'on' and 'off' medication

The data were statistically tested, both for mean RT and variability, with two separate ANOVAs. A Bonferroni corrected α of 0.025 was used. Both ANOVAs used log linear transformation to correct for not normally distributed data.

Mean RT

Using a 5 (duration (unwarned and warned trials)) X 2 (medication) repeated measures ANOVA, a significant effect of duration was observed ($F(2.463, 24.63) = 12.16$; $p < 0.001$), with the main effect of medication and duration X medication interaction not being significant. A priori simple contrasts revealed that the difference in RT was significant between the unwarned condition and the 250 ms condition ($F(1, 10) = 26.97$; $p < 0.001$), the 500 ms condition ($F(1, 10) = 11.95$; $p = 0.006$) and also the 1 s condition ($F(1, 10) = 17.89$; $p = 0.002$). The difference between the unwarned condition and the 2 s condition was not significant.

Variability

A 5 (duration (unwarned and warned trials)) X 2 (medication) repeated measures ANOVA revealed no significant main effect of duration or medication but an interaction effect that approached uncorrected significance ($F(4, 40) = 2.51$; $p = 0.057$).

5.3.1.4 Repetitive tapping task

The patients were able to execute the task although some had problems with the fastest target interval (250 ms). For this reason, six runs of tapping at this interval were not collected. Three subjects found the task demanding 'off' medication such that only one run was collected for each target interval. A further three runs were lost for technical reasons. All data was included in this analysis, including runs that would not satisfy the criteria of the Wing and Kristofferson model (1973ab). Performance was investigated in terms of both the mean IRI and variability (SD), introducing a Bonferroni adjusted p threshold of 0.025.

Mean IRI

The mean IRI for each of the durations in the synchronisation and continuation phases was calculated, collapsed across the two runs. The patients were able to tap at roughly the desired rate, suggesting the task was within the motor capabilities of the patients. Overall, the pattern of results suggests a tendency towards underestimation, with patients underestimating slightly more when 'off' medication compared to when 'on' medication, although this is notably not the case at the longest target interval (2000 ms) (see Table 5.6).

	Synchronisation Phase	Continuation Phase
	ON	ON
250 ms	**249.56** (2.95)	**248.33** (16.08)
500 ms	**500.67** (3.56)	**486.38** (17.97)
1000 ms	**1004.00** (11.75)	**966.21** (60.79)
2000 ms	**1995.38** (11.66)	**1911.92** (206.90)
	OFF	OFF
250 ms	**249.65** (12.87)	**244.15** (9.37)
500 ms	**495.55** (7.37)	**483.45** (30.42)
1000 ms	**997.64** (3.80)	**949.91** (47.72)
2000 ms	**2002.14** (8.82)	**1918.64** (185.99)

Table 5.6: Mean IRI scores in the repetitive tapping task for PD-drug group when tested 'on' and 'off' medication

Standard deviation (SD) in brackets

The data would be most appropriately analysed using a 2 (medication) X 2 (phase) X 4 (duration) ANOVA. However, the majority of the data for the synchronisation phase were not normally distributed and were not corrected

when a log transformation was applied. It was decided to limit statistical analysis of the synchronisation phase to non-parametric tests concerning the comparison of most interest, that of the effect of medication, whilst investigating the continuation phase separately using a 4 (duration) X 2 (medication) repeated measures ANOVA. For the synchronisation phase, using the Wilcoxon-signed ranks test each task was compared at each rate 'off' and 'on' medication. As four tests were carried out the Bonferroni corrected p value was set at 0.0125. One comparison was significant, that for the mean IRI during the synchronisation phase with a target interval of 1000 ms ($z = -2.536$; $p = 0.011$). Another comparison was significant (mean IRI during the synchronisation phase with a target interval of 500 ms ($z = -2.077$; $p = 0.038$)), but only at the uncorrected level so it was not considered to have reached the appropriate threshold.

For the continuation phase data, the repeated measures ANOVA revealed a significant effect of duration ($F(1.244, 8.709) = 1650.91$; $p < 0.001$), but a non significant main effect of medication and non-significant duration X medication interaction. As would be expected, a priori polynomial comparisons revealed a significant linear relationship between the target interval and the mean response ($F(1, 7) = 1938.61$; $p < 0.001$).

Variability

Although the Wing and Kristofferson model (1973ab) was not applied to the data, a measure of variability was obtained for each trial (SD, equivalent to the square root of the total variability in the Wing and Kristofferson model) and averaged across the runs for each interval and for each phase (Table 5.7). The most striking feature of the variability was that, for both phases, the measure is elevated in the 250 ms condition compared to the 500 ms condition when the patients are 'off' medication, despite the data following a linear trend when the patients are 'on' medication (the more typical data pattern). Overall, the patients appear to show greater variability 'on' medication. The data were then analysed using a 2 (medication) X 2 (phase) X 4 (duration) ANOVA (log transformed to normalise). The data revealed only one significant main effect, that of duration ($F(3, 21) = 75.21$; $p < 0.001$), with the medication and phase effects not

reaching significance. However, a significant duration X medication interaction ($F(3, 21) = 3.82$; $p = 0.025$) was observed.

	Synchronisation Phase	Continuation Phase
	ON	ON
250 ms	**12.72** (3.86)	**18.56** (22.92)
500 ms	**33.44** (21.35)	**44.99** (48.11)
1000 ms	**70.62** (58.42)	**68.12** (59.76)
2000 ms	**130.33** (24.60)	**101.09** (33.59)
	OFF	OFF
250 ms	**36.72** (35.13)	**28.93** (28.94)
500 ms	**23.81** (10.40)	**24.02** (7.48)
1000 ms	**51.78** (18.12)	**49.43** (18.03)
2000 ms	**117.63** (44.79)	**115.80** (62.16)

Table 5.7: Mean variability measure (SD) in the repetitive tapping task for PD-drug group when tested 'on' and 'off' medication

Standard deviation (SD) in brackets

The significant main effect of duration can be explained using a priori polynomial comparisons, which found a significant linear trend across the durations ($F(1, 7) = 113.89$; $p < 0.001$). This was accompanied by a significant quadratic relationship ($F(1, 7) = 5.77$; $p = 0.047$). The duration X medication interaction has been plotted in Figure 5.4, with the data averaged across the two phases to provide better illustration of the effect. The elevated variability for the 250 ms target interval for the PD-drug-off data can be clearly seen, with the variability when 'off' medication being lower compared to the 'on' medication condition for the other three target intervals (although only marginally so at the

2000 ms target). The effect can be further statistically illustrated if two post hoc ANOVAs are run, one for the 'on' medication data (collapsed across phase) and one for the 'off' medication data (collapsed across phase), with a Bonferroni corrected p of 0.025. Both ANOVAs show a main effect of duration ('on' medication: $F(3, 24) = 68.926$; $p < 0.001$, 'off' medication: $F(1.169, 10.525) = 30.440$; $p < 0.001$). However, whereas planned polynomial comparisons show that this effect is explained by a significant linear trend in the 'on' medication data ($F(1, 8) = 201.14$; $p < 0.001$). For the 'off' medication data, a significant linear effect ($F(1, 9) = 35.21$; $p < 0.001$) *and* significant quadratic ($F(1, 9) = 15.08$; $p = 0.004$) and cubic ($F(1, 9) = 11.31$; $p = 0.008$) effects were found.

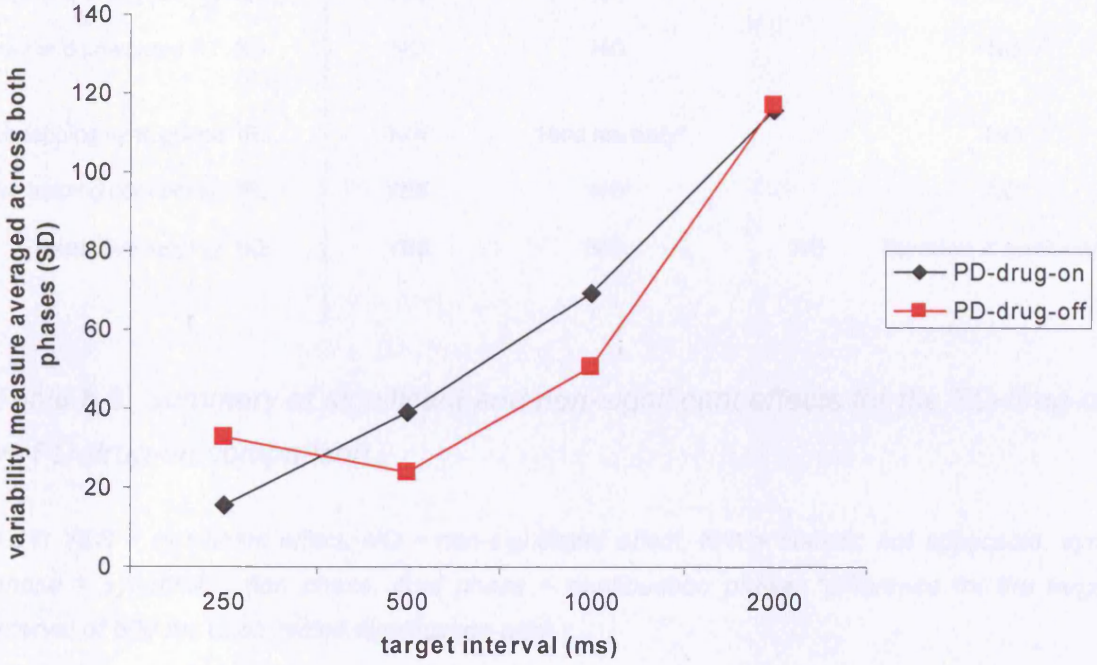


Figure 5.4: Duration X medication interaction collapsed across the synchronisation phase and continuation phase for the PD-drug group

5.3.1.5 Summary of results for PD-drug-on vs PD-drug-off

A summary of the results is provided in Table 5.8. To present a more sensitive impression of the data, significant results that reached conventional significance, but not Bonferroni corrected significance are marked with a *.

	Main effect of duration	Main effect of medication	Main effect of phase	Interaction
Time Production: relative eror	**YES**	NO		NO
Time Production:absolute eror	**YES**	**YES**		NO
Time Production: SD	**YES**	NO		NO
Time Reproduction: relative eror	**YES**	NO		NO
Time Reproduction:absolute eror	**YES**	NO		NO
Time Reproduction: SD	**YES**	NO		NO
Warned and unwarned RT: RT	**YES**	NO		NO
Warned and unwarned RT: SD	NO	NO		NO
Repetitive tapping sync phase: IRI	N/A	**1000 ms only***		N/A
Repetitive tapping cont phase: IRI	**YES**	NO		NO
Repetitive tapping: SD	**YES**	NO	NO	**duration X medication**

Table 5.8: Summary of significant and non-significant effects for the PD-drug-off vs PD-drug-on comparison

*KEY: YES = significant effect, NO = non-significant effect, N/A = statistic not applicable, sync phase = synchronisation phase, cont phase = continuation phase, *difference for the target interval of 500 ms uncorrected significance only*

A significant effect of medication was found for the time production task, with a significantly longer absolute error score in the time production task 'off' medication compared to 'on' medication. A significant effect was found for the repetitive tapping in the synchronisation task; with the patients being faster (and less accurate) 'off' medication at the 1000 ms target interval. Furthermore, the patients showed differential variability on the repetitive tapping task, depending on their medication state. When 'on' medication the patients showed a steady, linear increase in variability, but in the 'off' medication condition the patients showed higher variability than in the 'on' medication condition at the 250 ms

target interval, but lower comparative variability at the 500 ms and 1000 ms target intervals with the variability appearing almost identical at the 2000 ms target. The effect of duration was significant for all mean response scores, indicating the subjects were able to differentiate between the different values on the time production, time reproduction and repetitive tapping tasks. On the warned and unwarned RT task the result indicated that subjects were significantly slower on the task when their RT response was unwarned compared to when the 250 ms, 500 ms and 1000 ms warning tones were included. The significant main effect of duration for the variability measure generally reflected a linear increase in variability with the mean of the interval being timed. The lack of a significant effect for the warned and unwarned RT task reflects that the durations were being used as timing cues, rather then as intervals to be timed.

5.3.2 Duration of illness and disease severity effects for PD-drug-off vs PD-de novo

The twelve PD-drug patients were directly compared with the eight PD-de novo patients to explore the effect of disease severity and duration of illness. For the PD-drug group, the data collected during the 'off' medication condition was used as this removes the effect of being 'on' medication as a confounding covariate. All the patients in the PD-de novo group were right handed but three of the patients used their left had to perform the task, following a clinical decision made by the neurologist conducting the experiment. To ensure that this variable didn't affect the results a repeated measures ANOVA was conducted on each of the four timing tasks, with the target duration as a repeated measures factor and with the effect of hand used as a between subjects factor. The main effect of hand used and the hand X duration interaction were not significant for the mean estimate on the time estimation task, the mean reproduction value on the time reproduction task and the mean reaction time on the warned and unwarned reaction time task. The repetitive tapping task was investigated using a 2 (phase) X 4 (duration) X 2 (hand) ANOVA. No significant effects were found. Furthermore, there was no difference in performance on the Purdue Pegboard as a function of hand used. Taken together these results suggest that the hand

used (whether the dominant or non-dominant hand) did not have a significant effect on performance.

The two groups were initially compared on a range of motor and attentional variables, to establish if any subsequent differences in timing performance could be explained by non-temporal factors (see Table 5.9). The difference in the PASAT scores (PD-drug-off = 6.08 (SD 6.10); PD-de novo = 5.17 (SD 5)) were not significant ($t(16) = 0.32$; $p = 0.755$). For the Hoehn and Yahr scores, the difference between the two groups was significant (Mann-Whitney $U = 11.50$; $Z = -2.92$; $p = 0.004$). Similarly, the difference between the UPDRS Part III scores of two groups was also significant ($t(17) = 2.68$; $p = 0.16$). This confirms that the PD-drug-off group are at a significantly more severe stage of illness than the PD-de novo group and also show significantly greater disease severity than the PD-de novo group. On the Purdue Pegboard a 3 (hand used (right, left, bilateral)) X 2 (group) mixed design ANOVA revealed a significant main effect of hand used ($F(1, 16) = 20.89$; $p < 0.001$) but a non-significant main effect of group and a non-significant interaction. To break down the main effect of hand used, post hoc pairwise comparisons were conducted which showed a significant difference between using the right hand and both hands (mean difference = 2.79; $p < 0.001$) as well as between using the left hand and both hands (mean difference = 2.71; $p < 0.001$). However, across both groups of patients the difference between using the left hand or the right hand was not significant. Thus, although the UPDRS Part III score differed significantly between groups, the groups did not significantly differ on a specific measure of motor speed and dexterity.

For the comparison between the two groups, the hypothesis is that any group differences are related to disease severity (i.e. the degree of basal ganglia dysfunction). However, disease severity correlates with duration of illness and it has already been established that the PD-drug group have a significantly longer duration of illness compared to the PD-de novo group. To help tease apart these related factors, it was decided to perform additional analysis that used the UPDRS Part III scores and the duration of illness as covariates, to determine if these factors explain part of the variance in the data.

	PD-drug-off		PD-de novo	
Hoehn & Yahr	2.54	(0.45)	1.63	(0.58)
UPDRS Part III	36.92	(8.61)	26.00	(8.52)
Purdue left hand	9.58	(1.78)	10.33	(2.42)
Purdue right hand	10.58	(1.51)	9.17	(1.72)
Purdue bilateral	7.17	(2.17)	7.17	(1.33)

Table 5.9: Stage of illness, disease severity and motor speed for the PD-drug group 'off' medication and the PD-de novo group
Standard deviation (SD) in brackets

5.3.2.1 Time production task

The results are presented in Table 5.10. The mean estimates showed slight underestimation of the 30 s and 60 s intervals in the PD-de novo group compared to the PD-drug-off group, with the reverse pattern observed at 120 s. As with the previous investigation of these data, absolute error scores were also measured to allow better comparison of deviation from the target interval. The mean absolute error was lower for the PD-de novo group compared to the PD-drug-off group, particularly at the two higher target intervals. Within-subject variability for the two groups, as measured by the SD across the five repetitions of each interval for each subject, was higher in the PD-drug-off group compared to the PD-de novo group at all interval lengths. Three ANOVAs were used analyse the data, with a Bonferroni correction of $\alpha = 0.017$. The majority of the data included in the three analyses, including the duration of illness data used as a covariate, were not normally distributed. Subsequently, all data were log transformed to create a normally distributed data set.

Mean production

For the mean data, a mixed design 3 (duration) X 2 (group) ANOVA was used. A main effect of duration was found ($F(2, 36) = 244.86$; $p < 0.001$) but the main effect of group was not significant. The interaction between duration and group was only significant at the uncorrected level ($F(2, 36) = 3.49$; $p = 0.041$),

therefore it was not explored further. The data therefore suggest that there is no difference in the degree of over- and underestimation between groups. As expected, a priori polynomial comparisons revealed a significant linear relationship between the duration of the target interval and the patients' estimates (F (1, 18) = 380.25; p < 0.001).

	Mean production (s)	Mean absolute error (s)	Mean variability measure (SD)
	PD-drug-off	PD-drug-off	PD-drug-off
30 s	**30.72** (13.36)	**10.52** (7.64)	**4.37** (3.72)
60 s	**57.15** (29.49)	**24.83** (14.35)	**10.75** (8.97)
120 s	**86.58** (35.45)	**39.77** (27.38)	**17.27** (12.52)
	PD-de novo	PD-de novo	PD-de novo
30 s	**27.47** (9.20)	**9.26** (7.38)	**2.63** (1.06)
60 s	**52.68** (9.32)	**9.26** (7.08)	**5.89** (4.01)
120 s	**99.70** (21.37)	**25.34** (13.93)	**15.21** (5.86)

Table 5.10: Time production scores for the PD-drug 'off' medication group and PD-de novo group

Standard deviation (SD) in brackets

When duration of illness was included as a covariate, the main effects remained the same (main effect of duration F (2, 34) = 16.02; p < 0.001; no significant main effect of group) but with the interaction between duration and group only approaching uncorrected significance (F (2, 34) = 2.69; p = 0.083). Using the UPDRS Part III score as a covariate meant that the main effect of duration was no longer significant, nor was the main effect of group. The group X duration interaction was only significant at the uncorrected level (F (1.428, 22.855) = 4.030; p = 0.044)). These data suggest that neither the duration of illness nor

disease severity significantly influence the lack of a significant group effect. The significant effect of duration was lost when the UPDRS Part III score was included as a covariate; this suggests that motor-related disease severity does effect the differences between the production of different durations.

Absolute error

A mixed design ANOVA (3 (duration) X 2 (group)) revealed a significant effect of duration ($F(2, 36) = 9.47$; $p < 0.001$) and group ($F(1, 18) = 7.32$; $p = 0.014$), with the interaction between these factors being non-significant. A priori polynomial contrasts revealed a significant linear relationship between the absolute error scores for the three durations ($F(1, 18) = 13.56$; $p = 0.002$). The data therefore suggest that the PD-drug-off group are significantly worse than the PD-de novo group at producing estimates of seconds-range durations.

To explore whether the differential duration of illness between groups contributed to this effect, the ANOVA was re-run with duration of illness as a covariate. This eliminated the significant effect of duration, whilst the main effect of group failed to reach the Bonferroni corrected significance ($F(1, 17) = 5.21$; $p = 0.036$). The interaction effect remained non-significant. The analysis was then run with the UPDRS Part III score as a covariate; all significant effects were lost. Thus, both duration of illness and motor severity contributed to the significant effects that were found.

Variability

A mixed design ANOVA showed that variability significantly differed across durations ($F(2, 36) = 51.51$; $p < 0.001$) but the main effect of group was not significant. The duration X group interaction was not significant. A priori polynomial contrasts revealed a significant linear relationship between the three durations, explaining the main effect of duration ($F(1, 18) = 95.46$; $p < 0.001$).

When duration of illness was included as a covariate, the pattern of significant results remained unchanged, with the main effect of estimation duration being significant ($F(2, 34) = 5.79$; $p = 0.007$) and the main effect of group and group X duration interaction being non-significant. Including the UPDRS Part III as a

covariate meant that the main effect of duration was reduced to subthreshold significance (F (2, 32) = 2.59; p = 0.091). The effect of group was also not significant, however, the duration X group interaction was significant (F (2, 32) = 4.63; p = 0.017). This interaction effect was due to the lower relative variability for the PD-de-novo group for the 60 s interval, suggesting that this group did not show the same relative increase in response variability for the 60 s intervals, although variability better matched the PD-drug-off group at the 120 s interval (see Figure 5.5). An independent samples t test was used to establish whether the difference in variability for the 60 s production was significant for the two groups. This did not reach significance.

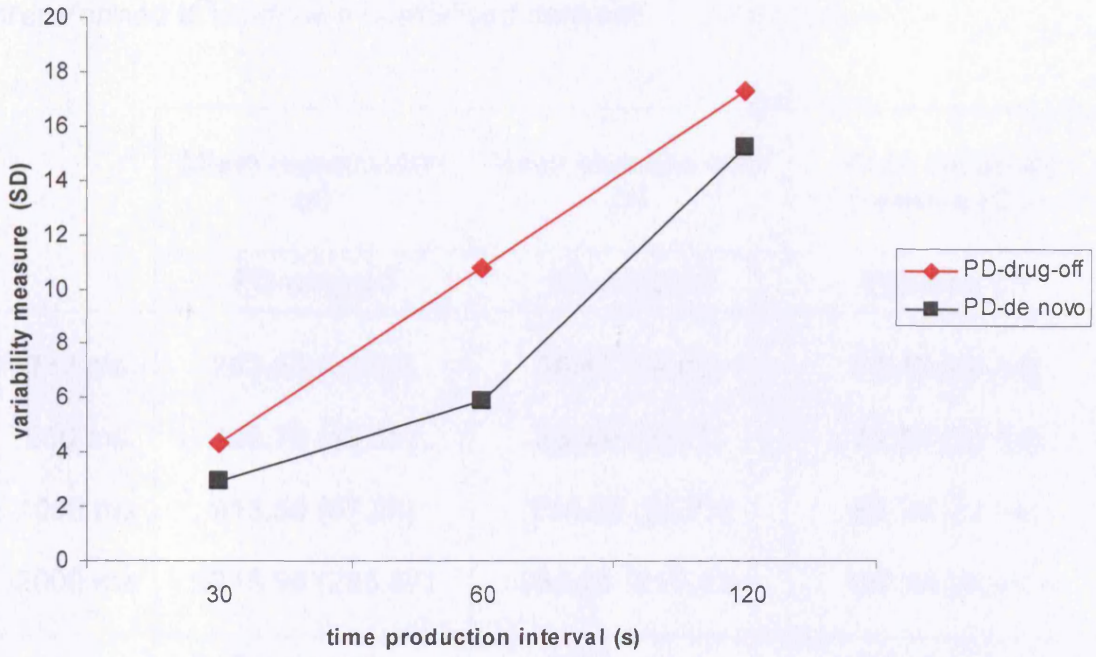


Figure 5.5: Interaction between duration and group for the measure of variability (± SE, not visible), significant when UPDRS Part III was included as a covariate

These data suggest that duration of illness has no effect on the pattern of the scores. However, severity of illness affects the degree of difference between the duration scores and also results in a significant interaction between duration and group.

5.3.2.2 Time reproduction task

These results can be seen in Table 5.11. Apart from when reproducing 250 ms intervals, the PD-de novo group underestimated compared to the PD-drug-off group. Mean absolute error scores indicated that the PD-de novo group showed a greater degree of error at all four target intervals. The variability of the responses at each interval length for each patient was investigated by looking at the SD across the twenty trials for each duration. Variability was higher across all interval ranges for the PD-de novo group. As previously, three ANOVAs were used to explore the data using a Bonferroni correction of $\alpha = 0.017$. The mean reproduction, absolute error and variability data, as well as the duration of illness data, were not normally distributed. Subsequently, all data were log transformed to produce a normalised data set.

	Mean reproduction (s)	Mean absolute error (s)	Mean variability measure (SD)
	PD-drug-off	PD-drug-off	PD-drug-off
250 ms	**253.63** (51.64)	**69.42** (34.82)	**77.33** (59.54)
500 ms	**442.72** (43.58)	**86.29** (20.51)	**73.51** (30.04)
1000 ms	**913.50** (67.24)	**114.69** (33.73)	**85.39** (32.44)
2000 ms	**1715.96** (285.87)	**356.21** (215.43)	**197.94** (80.88)
	PD-de novo	PD-de novo	PD-de novo
250 ms	**267.17** (168.91)	**114.63** (151.17)	**85.63** (57.97)
500 ms	**427.64** (151.42)	**144.38** (77.40)	**84.96** (41.66)
1000 ms	**784.18** (217.45)	**244.76** (179.99)	**144.86** (68.63)
2000 ms	**1580.78** (49.05)	**443.12** (88.00)	**217.48** (121.00)

Table 5.11: Time reproduction scores for the PD-drug group 'off' medication and the PD-de novo group

Standard deviation (SD) in brackets

Mean Reproduction

A 4 (duration) X 2 (group) repeated measures ANOVA for the mean reproduction scores revealed a significant effect of reproduction duration ($F(1.513, 27.227) = 231.452$; $p < 0.001$). The main effect of group was not significant, nor was the interaction between duration and group. A priori polynomial comparisons revealed a significant linear relationship between mean reproduction scores for the different durations ($F(1, 18) = 404.26$; $p < 0.001$).

When duration of illness was included as a covariate, the pattern of significant results remained identical (main effect of duration: $F(1.520, 25.832) = 12.52$; $p < 0.001$). When the UPDRS Part III score was used as a covariate, no significant effects were found. This suggests that motor severity, but not duration of illness affected the pattern of data.

Absolute error

A 4 (duration) X 2 (group) repeated measures ANOVA for the absolute error scores revealed a significant effect of reproduction duration ($F(1.531, 26.023) = 44.07$; $p < 0.001$) and a significant effect of group ($F(1, 18) = 7.84$; $p = 0.012$), but the interaction between these two factors was not significant. A priori polynomial contrasts revealed a significant linear relationship between the absolute error scores for the four durations ($F(1, 18) = 72.64$; $p < 0.001$). A significant departure form the linear trend, in the form of a quadratic ($F(1, 18) = 5.43$; $p = 0.032$) and cubic ($F(1, 18) = 7.99$; $p = 0.011$) relationship was also observed. These data suggest that the PD-de novo group were significantly worse on this task, as evidenced by significantly higher absolute error scores.

The ANOVA was re-run with duration of illness as a covariate. The effect of reproduction duration did not reach significance at the corrected F value ($F(1.627, 27.659) = 2.93$; $p = 0.080$). The main effect of group approached significance ($F(1, 17) = 3.392$; $p = 0.083$) and the duration X group interaction failed to reach significance. UPDRS Part III score was also used as a covariate. This had the effect of eliminating all significant effects, with the main effect of group approaching significance ($F(1, 16) = F = 3.712$; $p = 0.072$). These data suggest that both the duration of illness and UPDRS scores contributed to the significant effects previously reported.

Variability

A 4 (duration) X 2 (group) repeated measures ANOVA found a significant effect of duration (F (1.764, 31.750) = 23.90; $p < 0.001$), but no significant effect of group or interaction effect. A priori polynomial contrasts revealed a significant linear relationship between the SD scores for the four durations (F (1, 18) = 43.80; $p < 0.001$), with a significant departure from the linear trend also being observed (F (1, 18) = 6.46; $p = 0.020$).

When the duration of illness was used as a covariate the effect of the reproduction interval remained significant (F (1.855, 31.537) = 5.98; $p = 0.007$). The main effect of group was not significant, nor was the duration X group interaction. Including the UPDRS Part III score eliminated all significant results. This suggests that the duration of illness did not affect the results, whereas disease severity did.

5.3.2.3 Warned and unwarned reaction time task

Figure 5.6 shows that both groups showed a similar pattern of results, with unwarned tones producing the slowest RTs. It is also clear that the PD-drug-off patients produced slower RTs than the PD-de novo patients across all of the conditions. Figure 5.7 shows the average SD for the different trials for each condition with the data suggesting greater variability for the PD-drug-off group than the PD-de novo group, but with the groups being similar on the 1000 ms condition (reduced variability for PD-drug-off and increased variability for PD-de novo). Differences in the mean RT and variability data were tested statistically, using a Bonferroni adjusted p value of 0.025. Both sets of data, as well as the duration of illness data, were not normally distributed. As such, all data was log transformed to produce a normally distributed data set.

Mean RT

Using a 5 (duration (unwarned and warned trials)) X 2 (group) repeated measures ANOVA, a significant effect of duration was observed (F (2.946, 53.034) = 13.54; $p < 0.001$), but the main effect of group and duration X group interaction were not significant. A priori simple contrasts revealed that the difference in RT was significant between the unwarned condition and the 250

ms condition (F (1, 18) = 27.10; p < 0.001) and the 500 ms condition (F (1, 18) = 5.98; p = 0.025), with the difference between the unwarned and the 1 s condition only approaching significance (F (1, 18) = 3.53; p = 0.077). The difference between the unwarned condition and the 2 s condition was not significant.

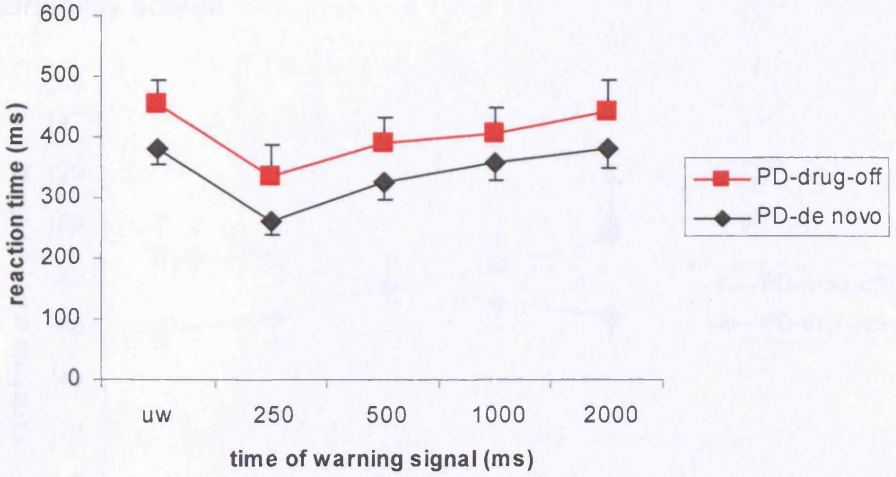


Figure 5.6: Warned and unwarned RTs (± SE) for the PD-drug group 'off' medication and the PD-de novo group

When duration of illness was used as a covariate, the results were identical with the main effect of duration being significant (F (2.882, 49.991) = 3.02; p = 0.040) and the main effect of group and duration X group interaction not being significant. To break down the main effect of duration, a priori simple contrasts were carried out that revealed that the difference in RT was only significant between the unwarned condition and the 250 ms condition (F (1, 17) = 4.39; p = 0.052). None of these significant effects survived when the UPDRS Part III 'off' medication score was used as a covariate. These data suggest that the patients' duration of illness affected the degree to which the unwarned condition significantly differed from the warned condition and that when the patients' motor disability is considered, differences between the groups and the different durations were abolished.

Variability

A repeated measures ANOVA revealed no significant main effect of duration or group, with the interaction also failing to reach significance. When the data were reanalysed with duration of illness as a covariate, none of the effects were significant. The same was true when UPDRS Part III 'off' medication was used as a covariate. This suggests that neither factor significantly influenced the RT variability scores.

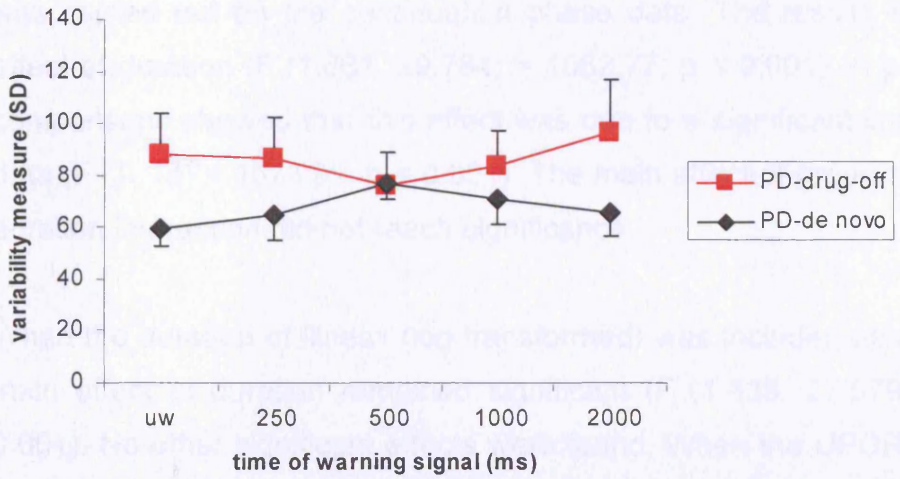


Figure 5.7: Variability measure for warned and unwarned RTs (± SE) for the PD-drug 'off' medication and the PD-de novo group

5.3.2.4 Repetitive tapping task

All data was included in this analysis, including runs that would not satisfy the criteria of the Wing and Kristofferson model (1973ab). Analysis of the mean inter-response interval data and a measure of its variability (SD) were used, resulting in a Bonferroni adjusted p threshold of 0.025. The data were averaged across the two runs and compared for the synchronisation phase and the continuation phase.

Mean IRI

The results are plotted in Table 5.12. The data appeared roughly similar, the PD-de novo group were slower than the PD-drug-off group at the 250 ms target interval for both phases and were marginally slower at the 2000 ms target interval for the continuation phase, otherwise they underestimated in

comparison to the PD-drug-off group. The data best suited a mixed design 2 (duration) X 2 (medication) X 4 (rate) ANOVA. However, the data was not normally distributed and a log linear transformation did not normalise the synchronisation phase data. As a result, the analysis for the synchronisation phase (non transformed) data was limited to the main contrast of interest, i.e. the difference between groups on the different measures, using the non-parametric Mann-Whitney U test for independent samples. None of the comparisons were significant. A 4 (duration) X 2 (group) mixed design ANOVA was carried out on the continuation phase data. The results showed a main effect of duration ($F(1.861, 29.784) = 1062.77$; $p < 0.001$). A priori polynomial comparisons showed that this effect was due to a significant linear trend in the data ($F(1, 16) = 1673.91$; $p < 0.001$). The main effect of group and the group X duration interaction did not reach significance.

When the duration of illness (log transformed) was included as a covariate, the main effect of duration remained significant ($F(1.838, 27.579) = 91.24$; $p < 0.001$). No other significant effects were found. When the UPDRS Part III score (log transformed) was used as a covariate the main effect of duration remained significant ($F(1.878, 26.286) = 4.80$; $p = 0.018$), with no other significant effects. Thus the factors of duration of illness and motor-related disability had no influence on the pattern of results.

	Synchronisation Phase	Continuation Phase
	PD-drug-off	PD-drug-off
250 ms	**249.65** (12.87)	**244.15** (9.37)
500 ms	**495.55** (7.37)	**483.45** (30.42)
1000 ms	**997.64** (3.80)	**949.91** (47.72)
2000 ms	**2002.14** (8.82)	**1918.64** (185.99)
	PD-de novo	PD-de novo
250 ms	**260.19** (24.96)	**257.15** (9.37)
500 ms	**496.31** (6.50)	**474.13** (13.78)
1000 ms	**994.94** (12.82)	**930.44** (89.23)
2000 ms	**1983.31** (30.65)	**1919.00** (348.12)

Table 5.12: Mean IRI scores in the repetitive tapping task for PD-drug group 'off' medication and the PD-de novo group

Standard deviation (SD) in brackets

Variability

The data are plotted in Table 5.13. The PD-de novo group appear to show a similar pattern to the PD-drug-off group, with both having elevated variability for the 250 ms condition compared to the 500 ms condition. However, the PD-de novo group appear to show increased variability for the 2000 ms interval compared to the PD-drug-off group. The data were tested statistically using a 2 (group) X 2 (phase) X 4 (duration) ANOVA (log transformed to normalise). A main effect of phase ($F(1, 16) = 5.33$); $p = 0.035$) and duration ($F(3, 48) = 75.49$; $p < 0.001$) was found, with the group effect being non-significant. No interactions reached threshold. Using a priori polynomial contrasts, the main effect of phase was explained by a significant linear trend ($F(1, 16) = 96.45$; $p < 0.001$), as well as by significant quadratic ($F(1, 16) = 32.87$; $p < 0.001$) and

cubic (F (1, 16) = 12.61; p = 0.003) effects. The main effect of phase could be explained by significantly higher variability in the synchronisation phase than in the continuation phase, across both groups (a priori simple contrast: F (1, 16) = 5.33; p = 0.035).

	Synchronisation Phase	Continuation Phase
	PD-drug-off	PD-drug-off
250 ms	**36.72** (35.13)	**28.93** (28.94)
500 ms	**23.81** (10.40)	**24.02** (7.48)
1000 ms	**51.78** (18.12)	**49.43** (18.03)
2000 ms	**117.63** (44.79)	**115.80** (62.16)
	PD-de novo	PD-de novo
250 ms	**37.84** (40.75)	**31.70** (30.16)
500 ms	**23.95** (6.80)	**28.07** (12.51)
1000 ms	**61.35** (15.48)	**52.24** (18.98)
2000 ms	**197.31** (89.24)	**140.23** (52.40)

Table 5.13: Mean variability measure (SD) in the repetitive tapping task for PD-drug group 'off' medication and the PD-de novo group
Standard deviation (SD) in brackets

When duration of illness (log transformed) was included as a covariate the significant effect of phase was lost, but the significant effect of duration remained (F (1.329, 19.939) = 18.28; p < 0.001). When the UPDRS Part III score (log transformed) was used as a covariate all the significant effects were lost. Thus, both duration of illness and motor-related disability influenced the results, with the difference between the phases being partly explained by

duration of illness and the differences in phase and duration being influenced by disease severity.

5.3.2.4 Summary of results for PD-drug-off vs PD-de novo

A significant main effect of duration was found for all responses, reflecting that the patients differentiated between the different intervals on the production, reproduction and repetitive tapping tasks. For the warned and unwarned RT task, the significant duration effect reflected the significantly longer RTs for the unwarned condition compared to when a warning tone was played 250 ms or 500 ms prior to the 'Go' tone. Variability increased with duration on the production, reproduction and repetitive tapping tasks. Variability did not vary with warning tone duration in the RT task. For the main effect of group, a significant difference was found for the absolute errors on the time production task; the PD-drug-off group were significantly worse. In contrast, the PD-de novo group had significantly worse absolute error scores on the time reproduction task. No significant effect of group was found for the RT task or for the repetitive tapping task, although a main effect of phase in the repetitive tapping task indicated that the patients showed significantly higher variability in the synchronisation task.

The covariates had an effect on the pattern of significant results and these are summarised in Table 5.14 for ease of reference. To present a more sensitive impression of the data, significant results that reached conventional significance, but not Bonferroni corrected significance are marked with a *. The UPDRS Part III score clearly had a larger effect on the significance of results, eliminating the main effect of duration on 7 out of 8 possible analyses. When used as a covariate, duration of illness removed the effect on 2 occasions, on the time production and time reproduction absolute error scores. This suggests that disease severity had a greater impact on the scores of the patients than duration of illness.

	Main effect of duration			Main effect of group			Main effect of phase			Interaction		
covariate:	None	Illness	UPDRS	None	Illness	UPDRS	None	Illness	UPDRS	None	Illness	UPDRS
Time Production: relative eror	**YES**	**YES**	NO	NO	NO	NO				NO*	NO	NO*
Time Production:absolute eror	**YES**	NO	NO	**YES**	NO*	NO				NO	NO	NO
Time Production: SD	**YES**	**YES**	NO	NO	NO	NO				NO	NO	**YES**
Time Reproduction: relative eror	**YES**	**YES**	NO	NO	NO	NO				NO	NO	NO
Time Reproduction:absolute eror	**YES**	NO	NO	**YES**	NO	NO				NO	NO	NO
Time Reproduction: SD	**YES**	**YES**	NO	NO	NO	NO				NO	NO	NO
Warned and unwarned RT: RT	**YES**	**YES**	NO	NO	NO	NO				NO	NO	NO
Warned and unwarned RT: SD	NO	NO	NO	NO	NO	NO				NO	NO	NO
Repetitive tapping sync phase: IRI	N/A	N/A	N/A	NO	N/A	N/A				N/A	N/A	N/A
Repetitive tapping cont phase: IRI	**YES**	**YES**	**YES**	NO	NO	NO				NO	NO	NO
Repetitive tapping: SD	**YES**	**YES**	NO	NO	NO	NO	**YES**	NO	NO	NO	NO	NO

Table 5.14: Summary of significant and non-significant effects for the PD-drug-off vs PD-de novo comparison, with the results for the effects of the two covariates compared to no covariate

*KEY: YES = significant effect, NO = non-significant effect, N/A = statistic not applicable, None = ANOVA results without a covariate, Illness = ANOVA with duration of illness covariate, UPDRS = ANOVA with UPDRS Part III score covariate, sync phase = synchronisation phase, cont phase = continuation phase, *effects that reached uncorrected significance only*

For the two significant main effects of group that were found, including the UPDRS score as a covariate eliminated the effect on both occasions. The effect was only eliminated on one occasion when the duration of illness covariate was used. This suggests that the significantly worse absolute error score of the PD-drug-off group for the time production task can be partly explained by their more severe motor symptoms, but not by duration of PD. For the significantly worse absolute error scores for the PD-de novo group in the time reproduction task, this effect can partly be explained by both the difference in the duration of illness and the difference in motor severity between the two groups. Including the UPDRS score as a covariate on the time production task also caused a significant interaction between duration and group for the variability score.

Again, this suggests that the severity of the motor symptoms had an effect on scores in the time production task. The significant effect of phase in the repetitive tapping task was also eliminated when either the duration of illness or UPDRS score were used as a covariate, suggesting that these factors influenced the significant effect.

5.3.3 PD-drug-off vs cerebellar disease vs controls

As some significant group differences were found between the PD-de novo group and the PD-drug-off group, it was decided to only include the PD-drug group in the comparison with the CD and the control groups rather than collapse across the two PD data sets. The PD-drug group were chosen rather than the PD-de novo patients as they were the larger group, represent greater disease severity and were more similar to the PD groups used in previous studies (i.e. not medication naive). It was decided to use the 'off' medication data as this better compared the pure disease processes of the two patient groups. The PD-de novo data was not included as a separate group, partly because of the difference in the duration of illness compared to the other two patient groups.

First, the performance between the three groups on a range of motor and psychological variables was compared. The PASAT scores for the three groups appeared similar (PD-drug-off = 6.08 (SD 6.10); CD group = 4.25 (SD 3.54); control group = 5.60 (SD 5.81)). The control group data was not normally distributed and as some of the scores were 0, log transformation was not possible. Consequently, the Kruksal-Wallis test was used and showed that the groups were not significantly different (Chi-Square (2) = 0.13; $p = 0.935$).

All groups of subjects completed the Purdue Pegboard and the means for each group are plotted in Table 5.15. A 3 (hand used) X 3 (group) repeated measures ANOVA showed a significant main effect of hand used ($F(2, 72) = 49.65$; $p < 0.001$), a significant main effect of group ($F(2, 36) = 21.26$; $p < 0.001$) and a non significant interaction between hand used and group. Breaking down the main effect of group, post hoc pairwise comparisons

revealed a significant difference between the CD group and PD-drug-off group (mean difference = 0.10; p = 0.054) and the control group and PD-drug-off group (mean difference = -0.14; p = 0.001) as well as between the CD group and the control group (mean difference = -0.24; p < 0.001) i.e. suggesting that a significant difference in performance existed between all the groups. Post hoc pairwise comparisons on the main effect of hand used revealed that the significant main effect was a reflection of a significant difference between the right hand performance and the left hand performance (mean difference = -0.04; p = 0.032), between the left hand performance and the bilateral performance (mean difference = 0.12; p < 0.001) and between the right hand performance and the bilateral performance (mean difference = 0.15; p < 0.001).

	PD-drug-off		CD		Control	
Purdue left hand	9.58	(1.78)	7.38	(2.39)	13.53	(2.76)
Purdue right hand	10.58	(1.51)	8.50	(2.45)	13.53	(2.91)
Purdue bilateral	7.17	(2.17)	6.13	(1.81)	9.80	(1.29)

Table 5.15: Purdue Pegboard scores for the PD-drug-off, CD and control groups

Standard deviation (SD) in brackets

5.3.3.1 Time production task

The results are displayed in Table 5.16. The mean estimates show that all groups tended towards underestimation (the exceptions being the 30 s estimates for the PD-drug-off and CD groups), with this being most marked for the control group. The pattern of mean absolute errors, representing the degree of error regardless of direction, does not present an obvious pattern, although it is interesting to note that the control group showed the largest errors at the 120 ms interval. Variability, as measured by mean SD, was elevated in the patient groups when compared to the control group. As before, three ANOVAs were used to explore the data fully, using a Bonferroni correct p value of 0.0017.

	Mean production (s)	Mean absolute error (s)	Mean variability measure (SD)
	PD-drug-off	PD-drug-off	PD-drug-off
30 s	**30.72** (13.36)	**10.52** (7.64)	**4.37** (3.72)
60 s	**57.15** (29.49)	**24.83** (14.35)	**10.75** (8.97)
120 s	**86.58** (35.45)	**39.77** (27.38)	**17.27** (12.52)
	CD	CD	CD
30 s	**32.27** (12.52)	**13.70** (13.37)	**4.37** (4.46)
60 s	**53.37** (22.19)	**20.40** (8.17)	**6.92** (5.41)
120 s	**101.60** (43.77)	**34.64** (24.61)	**17.09** (13.70)
	Control	Control	Control
30 s	**25.74** (13.71)	**11.20** (8.39)	**4.03** (3.35)
60 s	**43.48** (17.47)	**21.50** (13.97)	**6.53** (3.08)
120 s	**77.06** (29.05)	**44.04** (29.66)	**14.48** (11.40)

Table 5.16: Time production scores for the PD-drug-off, CD group and control groups

Standard deviation (SD) in brackets

Mean production

A 3 (duration) X 3 (group) mixed factor ANOVA was used to explore the mean date. A significant main effect of duration was found (F (1.454, 50.891) = 137.02; $p < 0.001$) but no significant effect of group or group X duration interaction was revealed. A priori polynomial comparisons showed a significant linear relationship between the three estimation durations (F (1, 35) = 171.32; $p < 0.001$), and also a significant quadratic departure from the linear trend (F (1,

35) = 15.17; $p < 0.0001$). This suggests that the differential pattern of over and underestimation does not vary significantly between the groups.

Absolute error

A mixed factorial 3 (duration) X 3 (group) ANOVA was employed (log transformed to normalise), with the results revealing a significant main effect of duration (F (1.554, 54.375) = 18.25; $p < 0.001$), but with no significant effect of group or duration X group interaction. A priori polynomial comparisons revealed a significant linear relationship between the different durations (F (1, 35) = 22.19; $p < 0.001$), with a significant departure from the linear trend also being observed (significant quadratic relationship: F (1, 35) = 5.31; $p = 0.027$). This suggests that the degree of absolute error does not vary significantly between the groups.

Variability

Using the same 3 X 3 ANOVA (log transformed to normalise) as before, there was a significant effect of duration (F (2, 70) = 58.41; $p < 0.001$). A priori polynomial contrasts showed a significant linear trend between variability and estimation duration (F (1, 35) = 97.40; $p < 0.001$). No other effects were significant. This suggests that variability does not vary significantly between the groups.

5.3.3.2 Time reproduction task

The mean data for the time reproduction task are shown in Table 5.17. For the mean reproduction values, the CD group showed greater overestimation relative to the other two groups, although their actual estimates were still underestimations of the target interval for the 1 s and 2 s targets. The mean absolute error results indicated that the PD-drug-off group showed less error across all trials than the control group. However, this apparent advantage for the PD-drug group is difficult to interpret, since if 'normal' performance is characterised by a certain degree of error, then it is the *difference* between the PD-drug group and the control group that is important, not the direction of the difference. In terms of variability (SD), the CD group were the most variable. As before, the data was explored using three ANOVAs, using a Bonferroni

corrected p value of 0.0017. None of the data were normally distributed, so a log transformation was used on all three ANOVAs.

	Mean reproduction (ms)	Mean absolute error (ms)	Mean variability measure (SD)
	PD-drug-off	PD-drug-off	PD-drug-off
250 ms	**253.63** (51.64)	**69.42** (34.82)	**77.33** (59.54)
500 ms	**442.72** (43.58)	**86.29** (20.51)	**73.51** (30.04)
1000 ms	**913.50** (67.24)	**114.69** (33.73)	**85.39** (32.44)
2000 ms	**1715.96** (285.87)	**356.21** (215.43)	**197.94** (80.88)
	CD	CD	CD
250 ms	**296.02** (81.76)	**118.95** (71.81)	**118.90** (60.39)
500 ms	**510.79** (73.99)	**109.57** (55.51)	**129.13** (50.60)
1000 ms	**922.42** (107.43)	**136.21** (86.41)	**136.99** (73.11)
2000 ms	**1844.05** (257.87)	**340.64** (172.26)	**353.06** (213.99)
	Control	Control	Control
250 ms	**259.70** (64.10)	**74.87** (69.42)	**71.66** (50.16)
500 ms	**447.95** (63.29)	**108.07** (63.07)	**96.41** (63.70)
1000 ms	**942.25** (121.14)	**160.49** (97.72)	**192.20** (139.63)
2000 ms	**1602.57** (192.86)	**425.84** (164.57)	**238.52** (72.70)

Table 5.17: Time reproduction scores for the PD-drug-off, CD group and control groups

Standard deviation (SD) in brackets

Mean reproduction

A 4 (duration) X 3 (group) mixed design ANOVA was carried out on the mean reproduction data. The data revealed a main effect of duration (F (2.025, 68.845) = 1113.10; $p < 0.001$). A priori polynomial comparisons showed this to be due to a significant linear trend across the different durations (F (1, 34) = 1712.19; $p < 0.001$). A significant departure from the linear trend was also observed in the form of a significant cubic relationship (F (1, 34) = 7.22; p = 0.011). The main effect of group and the duration X group interaction were not significant, suggesting that the pattern of mean reproductions did not significantly differ between groups.

Absolute error

A 4 (duration) X 3 (group) mixed design ANOVA was carried out on the absolute error data. The main effect of duration was significant (F (2.422, 82.351) = 67.65; $P < 0.001$), but no other effects reached threshold. The main effect of duration can be explained by a priori polynomial comparisons that showed a significant linear trend (F (1, 34) = 132.53; $P < 0.001$). Significant departures from this linear trend were also observed (quadratic: F (1, 34) = 18.14; $p < 0.001$), cubic: F (1, 34) = 10.10; p = 0.003).

Variability

Using the same ANOVA as above, a significant effect of duration was found (F (2.322, 78.933) = 46.67; $p < 0.001$) and a significant duration X group interaction (F (4.643, 78.933) = 2.88; P = 0.012). Further investigation of these effects revealed that the main effect of duration was explained by a significant linear relationship between the durations (F (1, 34) = 101.94; $P < 0.001$), with a significant quadratic departure from the linear trend also being observed (F (1, 34) = 12.41; p = 0.001). The main effect of group only reached uncorrected significance (F (2, 34) = 3.47; P = 0.043), so was not investigated any further.

To explore the significant duration X group interaction, the variability score was plotted against duration for each group (Figure 5.8). The control group showed a steady linear increase in variability with increasing duration, whereas the two patient groups showed a flatter relationship between the 250, 500 and 1000 ms

durations, followed by a sharp increase in variability at 2000 ms. To further explore this effect statistically, the relationship between the first three reproduction durations for each group was explored using a priori polynomial contrasts. A significant linear relationship was found between the reproductions of 250 ms, 500 ms and 1000 ms for the control group ($F(1, 17) = 23.98$; $p < 0.001$) but not for the two patient groups. The results also predicted that the difference between the mean reproduction for 1000 ms and for 2000 ms would only be statistically significant for the two patient groups, where a sharp increase in variability was observed. This was confirmed using repeated measures t tests (Bonferroni corrected to $\alpha = 0.017$) to compare the mean reproduction at the two intervals for each of the three groups (PD-drug-off: $t(11) = -7.08$; $p < 0.001$; CD: $t(6) = -4.53$; $p = 0.004$: controls: ns).

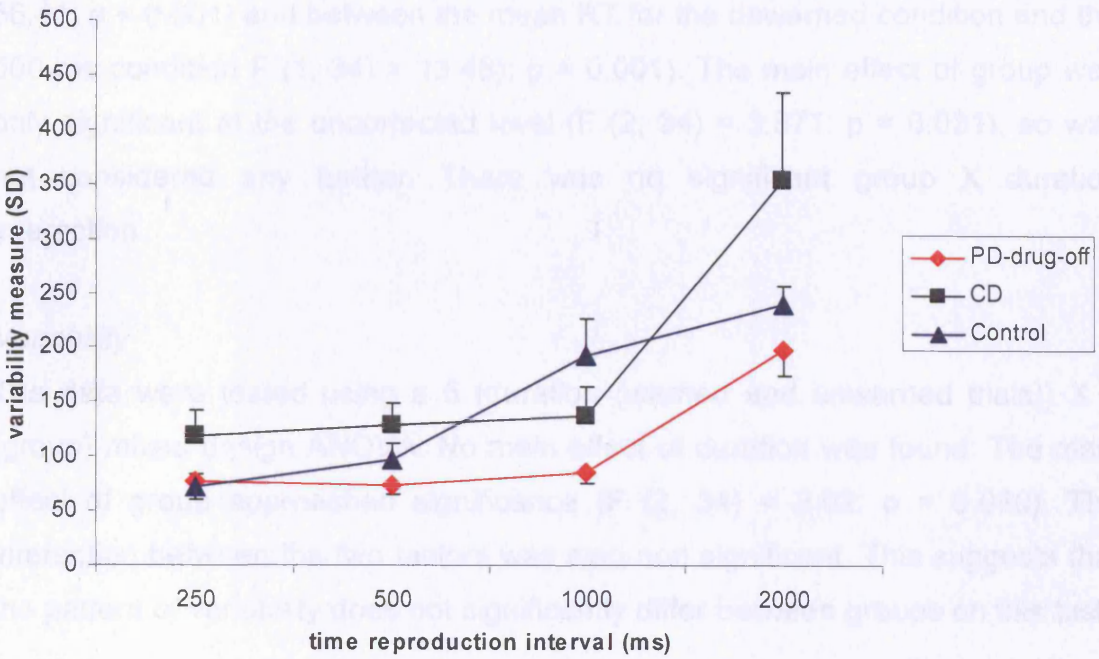


Figure 5.8: Group X duration interaction for the variability measure (SD) (± SE), for the PD-drug-off, CD and control groups

5.3.3.3 Warned and unwarned reaction time task

Three of the CD patients were excluded from this analysis due to a variation in the way they conducted the task that would have enhanced their RTs (finger held above the response button rather than in front and below it). The plotted mean RTs (Figure 5.9) show that the CD group had the slowest RTs and the

control group the fastest RTs, with all groups showing roughly the same pattern of response across the different intervals. The variability data (Figure 5.10) was slightly less clear; particularly as variability for the CD group seemed to be smallest at the longest interval (2000 ms). Both mean RTs and variability were explored statistically with ANOVAs, with a Bonferroni corrected p threshold of 0.025.

Mean RT

The data were tested using a 5 (duration (warned and unwarned trials)) X 3 (group) mixed design ANOVA (log transformed to normalise). The data revealed a main effect of duration ((F (2.924, 99.418) = 17.50; $p < 0.001$), which a priori simple comparisons showed was due to a significant difference between the mean RT for the unwarned condition and the 250 ms condition (F (1, 34) = 56.41; $p < 0.001$) and between the mean RT for the unwarned condition and the 500 ms condition F (1, 34) = 13.48); p = 0.001). The main effect of group was only significant at the uncorrected level (F (2, 34) = 3.871; p = 0.031), so was not considered any further. There was no significant group X duration interaction.

Variability

The data were tested using a 5 (duration (warned and unwarned trials)) X 3 (group) mixed design ANOVA. No main effect of duration was found. The main effect of group approached significance (F (2, 34) = 2.92; p = 0.068). The interaction between the two factors was also non significant. This suggests that the pattern of variability does not significantly differ between groups on this task

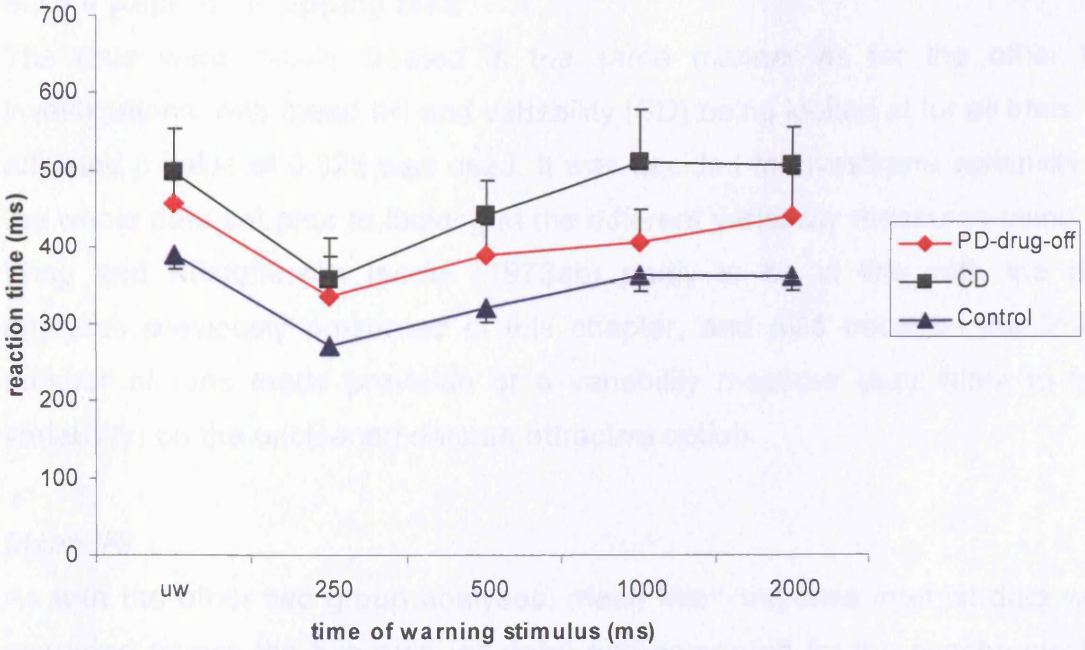


Figure 5.9: Warned and unwarned RTs (± SE) for the PD-drug-off, CD and control groups

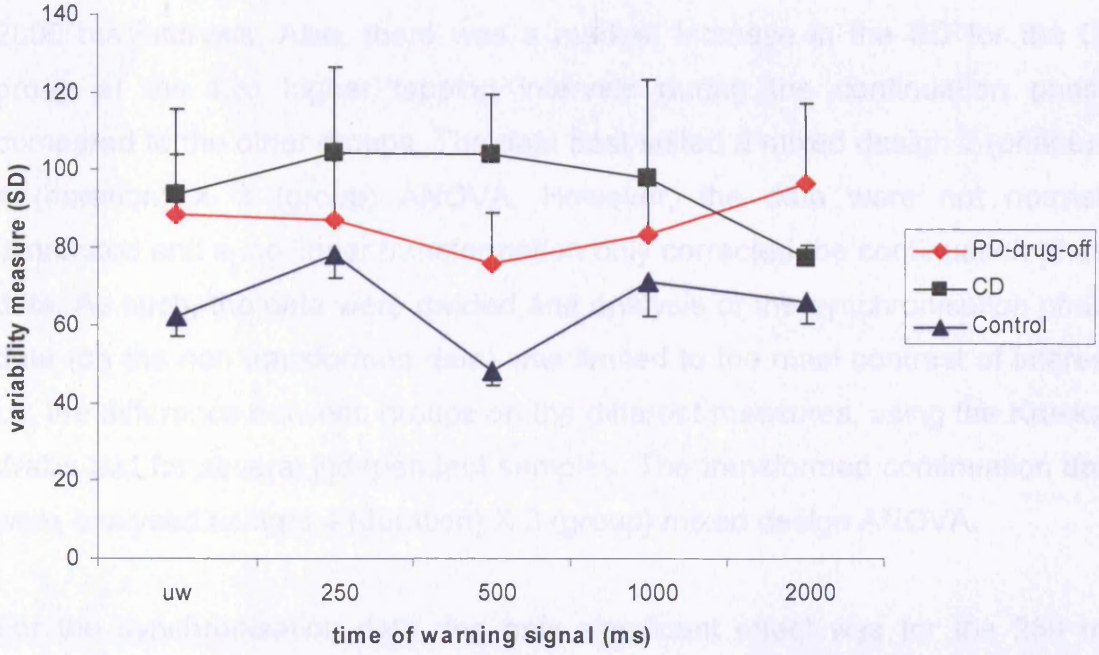


Figure 5.10: Variability measure for warned and unwarned RTs (± SE) for the PD-drug-off, CD and control groups

5.3.3.4 Repetitive tapping task

The data were initially treated in the same manner as for the other two investigations, with mean IRI and variability (SD) being looked at for *all* trials. An adjusted p value of 0.025 was used. It was decided to investigate variability for the whole data set prior to looking at the different variability measures using the Wing and Kristofferson model (1973ab) partly to be in line with the data analyses previously presented in this chapter, and also because the limited number of runs made provision of a variability measure (equivalent to total variability) on the uncleaned data an attractive option.

Mean IRI

As with the other two group analyses, mean inter-response interval data were averaged across the two runs (all data) and compared for the synchronisation phase and the continuation phase. The results are plotted in Table 5.18. The data does not seem greatly different, with perhaps the most notable feature being the relative underestimation for the continuation phase compared to the synchronisation phase not being observed for the CD group at the 250 and 2000 ms intervals. Also, there was a marked increase in the SD for the CD group at the two higher tapping intervals during the continuation phase, compared to the other groups. The data best suited a mixed design 2 (phase) X 4 (duration) X 3 (group) ANOVA. However, the data were not normally distributed and a log linear transformation only corrected the continuation phase data. As such, the data were divided and analysis of the synchronisation phase data (on the non transformed data) was limited to the main contrast of interest, i.e. the difference between groups on the different measures, using the Kruskal-Wallis test for several independent samples. The transformed continuation data were analysed using a 4 (duration) X 3 (group) mixed design ANOVA.

For the synchronisation data, the only significant effect was for the 250 ms target interval (Chi-Square (2) = 7.77; p = 0.021). However, as four comparisons were used it was decided that a conservative Bonferroni correction of p = 0.0125 should be used for this particular data, thus none of the between groups comparisons reached appropriate significance for the synchronisation phase.

	Synchronisation Phase	Continuation Phase
	PD-drug-off	PD-drug-off
250 ms	**249.65** (12.87)	**244.15** (9.37)
500 ms	**495.55** (7.37)	**483.45** (30.42)
1000 ms	**997.64** (3.80)	**949.91** (47.72)
2000 ms	**2002.14** (8.82)	**1918.64** (185.99)
	CD	CD
250 ms	**295.44** (47.58)	**302.00** (63.84)
500 ms	**492.63** (14.25)	**480.00** (21.76)
1000 ms	**1011.19** (43.70)	**992.25** (128.80)
2000 ms	**1997.92** (5.39)	**2011.75** (319.40)
	Control	Control
250 ms	**255.89** (12.22)	**253.89** (13.06)
500 ms	**499.34** (3.19)	**484.53** (14.55)
1000 ms	**1000.16** (8.74)	**949.00** (84.35)
2000 ms	**2004.92** (24.29)	**1924.82** (194.69)

Table 5.18: Mean IRI scores in the repetitive tapping task for the PD-drug-off, CD and control groups

Standard deviation (SD) in brackets

For the continuation phase, a significant main effect of duration (F (2.289, 93.843) = 3166.17; $p < 0.001$) was found, which a priori polynomial contrasts revealed was due to a significant linear relationship between the data (F (1, 41) = 5846.61 $p < 0.001$). A significant quadratic departure from this trend was also

observed (F (, 1, 41) = 16.60; $p < 0.001$). No significant main effect of group was found, nor a significant duration X group interaction.

Variability

The data are presented in Table 5.19. Clearly, the CD group showed greater variability compared to the other two groups. Furthermore, both patient groups showed greater variability in the 250 ms condition compared to the 500 ms condition, whereas the expected linear increase in variability with duration was only observed in the control group.

The data were tested statistically using a 3 (group) X 2 (phase) X 4 (duration) mixed design ANOVA (log transformed to normalise). The results showed a significant main effect of group (F (2, 34) = 11.63; $p < 0.001$), a significant effect of phase (F (1, 34) = 8.27; $p < 0.007$) and a significant effect of duration (F (1.843, 62.68) = 157.93; $p < 0.001$). In addition, a significant duration X group interaction was found (F (6, 102) = 4.82; $p < 0.001$). The phase X rate interaction was significant at the uncorrected level only (F (3, 102) = 2.88; $p < 0.040$). The main effect of group was explored using independent samples t tests, with the data collapsed across duration and phase (Bonferroni correction of $\alpha = 0.025$). The results revealed that the main effect could be explained by significantly greater variability in the CD group compared to the control group (t (25) = 6.15; $p < 0.001$) and significantly greater variability in the CD group compared to the PD-drug-off group (t (17) = -2.95; $p = 0.009$). A priori polynomial comparisons showed that the main effect of duration could be explained by a significant linear trend (F (1, 34) = 235.20; $p < 0.001$), as well as by significant departures from that trend (quadratic: F (1, 34) = 42.00; $p < 0.001$, cubic: F (1, 34) = 9.09; $p = 0.005$). The significant effect of phase was explained using an a priori simple comparison, in which the variability for the synchronisation phase was shown to be significantly greater than the variability for the continuation phase (F (1, 34) = 8.27; $p = 0.007$). Figure 5.11 shows the group X duration interaction, collapsed across phase. The significant interaction appeared to be the result of the control group showing less variability than the PD-drug-off group at the 250 ms target duration but showing greater variability

than the PD-drug-off group at the other target intervals, particularly the 2000 ms target.

	Synchronisation Phase	Continuation Phase
	PD-drug-off	PD-drug-off
250 ms	**36.72** (35.13)	**28.93** (28.94)
500 ms	**23.81** (10.40)	**24.02** (7.48)
1000 ms	**51.78** (18.12)	**49.43** (18.03)
2000 ms	**117.63** (44.79)	**115.80** (62.16)
	CD	CD
250 ms	**63.24** (32.36)	**42.18** (33.40)
500 ms	**42.44** (27.89)	**36.37** (10.09)
1000 ms	**85.46** (39.53)	**66.85** (182.72)
2000 ms	**156.54** (59.51)	**182.72** (55.17)
	Control	Control
250 ms	**15.31** (5.57)	**14.62** (4.11)
500 ms	**27.29** (14.52)	**25.54** (7.08)
1000 ms	**60.39** (22.16)	**50.47** (12.86)
2000 ms	**148.09** (56.83)	**144.03** (86.50)

Table 5.19: Mean variability measure (SD) in the repetitive tapping task for the PD-drug-off, CD and control groups

Standard deviation (SD) in brackets

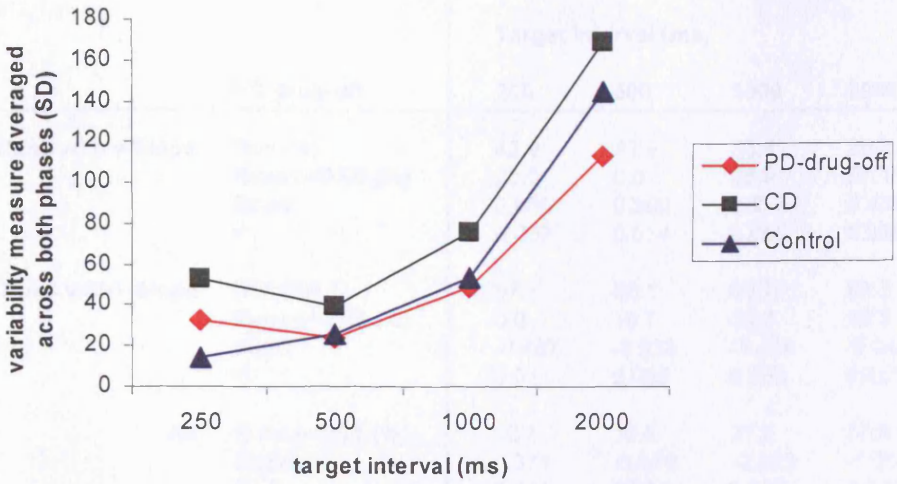


Figure 5.11: Group X duration interaction collapsed across the synchronisation and continuation phases, for the PD-drug-off, CD and control groups

The Wing and Kristofferson model

The Wing and Kristofferson model (1973ab) was used to fractionate the variance in inter-response intervals, specifically into 'clock' and 'motor' related components.

Stationarity of the data during the continuation phase

The results of the linear regression analysis of the IRI are shown in Table 5.20. There was a significant linear trend in over 50 % of trials for the three groups of subjects for the target IRI of 2000 ms. For the 250 ms target interval the percentage of trials with a significant linear trend varied from 7.9 % (control group) to 10.5 % (PD-drug-off group) to 18.8 % (CD group). The two intermediate IRI targets produced an intermediate percentage of significant runs, with the lowest percentage being for the PD-drug-off group at the 500 ms IRI (10.5 %) and highest percentage being for the control group at the 1000 ms IRI (35.1 %). For the PD-drug-off group, the patients showed more negative than positive runs for all target intervals, indicating that the IRI tended to decrease rather than increase in the course of the continuation phase. For the CD group, two of the target intervals showed more negative runs and for the control group three of the target intervals showed such a pattern.

		Target interval (ms)			
	PD-drug-off	**250**	**500**	**1000**	**2000**
Runs with + Slope	Run (%)	42.9	42.9	33.3	36.8
	Runs p<0.05 (%)	20.0	0.0	25.0	57.1
	Slope	0.980	0.300	2.273	5.456
	r^2	0.067	0.014	0.098	0.203
Runs with - Slope	Run (%)	57.1	57.1	66.7	63.2
	Runs p<0.05 (%)	0.0	16.7	33.3	58.3
	Slope	-0.467	-0.933	-1.434	-5.245
	r^2	0.011	0.093	0.083	0.201
All	Runs p<0.05 (%)	10.5	10.5	27.8	57.9
	Slope	0.371	-0.478	-0.385	-1.303
	r^2	0.051	0.064	0.097	0.202
	CD	**250**	**500**	**1000**	**2000**
Runs with + Slope	Run (%)	31.3	60.0	42.9	62.5
	Runs p<0.05 (%)	20.0	11.1	16.7	50.0
	Slope	0.957	0.794	2.526	10.247
	r^2	0.048	0.069	0.111	0.162
Runs with - Slope	Run (%)	68.8	40.0	57.1	37.5
	Runs p<0.05 (%)	18.2	16.7	25.0	66.7
	Slope	-1.186	-1.201	-2.892	-9.319
	r^2	0.068	0.056	0.143	0.151
All	Runs p<0.05 (%)	18.8	13.3	21.1	56.3
	Slope	-0.382	-0.004	-0.570	3.054
	r^2	0.060	0.064	0.129	0.200
	Control	**250**	**500**	**1000**	**2000**
Runs with + Slope	Run (%)	65.8	29.7	32.4	42.1
	Runs p<0.05 (%)	12.0	18.2	33.3	43.8
	Slope	0.345	0.442	2.871	8.697
	r^2	0.060	0.047	0.160	0.214
Runs with - Slope	Run (%)	34.2	70.3	67.6	57.9
	Runs p<0.05 (%)	0.0	7.7	36.0	54.6
	Slope	-0.242	-0.464	-1.948	-5.693
	r^2	0.016	0.036	0.143	0.214
All	Runs p<0.05 (%)	7.9	10.8	35.1	50.0
	Slope	-0.385	-0.208	-0.385	0.234
	r^2	0.097	0.041	0.149	0.215

Table 5.20: Linear regression analysis of IRI values of each target interval. Slope values represent mean of all subjects and all runs

Clearly, drifts in the stationarity of the data are more apparent at longer intervals, reflecting the greater difficulty in accurately producing longer intervals.

The data were not corrected for these linear trends as previous research has shown that such correction (across control and patient data) has 'minimal' effect on the resultant variability values (Ivry and Keele, 1989). Further research has also refrained from adjusting the data in this way (e.g. O'Boyle et al, 1996; Pastor et al, 1992a), with O'Boyle et al, (1996) noting that the statistical procedure for de-trending the data is complicated in that at least two different procedures can be used, to varying effect.

Violations of the predictions of the Wing and Kristofferson model at lag 1

The model's prediction that the lag 1 autocorrelation function in the continuation phase should lie between 0 and -0.5 was not observed on all runs. Table 5.21 shows the percentage of runs that met this prediction across all subjects at the four tapping rates. Clearly the shorter intervals led to fewer violations. The grand mean percentage across all tapping rates for the different groups suggests that approximately 1 in 2 of all runs met the predictions of the model. All of the intervals that did not fit the prediction were removed from the analysis.

	Target interval (ms)				Mean
	250	**500**	**1000**	**2000**	
PD-drug off	77.78	84.21	26.32	26.32	53.65
CD	75.00	73.33	60.00	25.00	58.33
Control	75.68	65.79	48.65	23.68	53.45

Table 5.21: Percentage of runs that fit the prediction of the Wing and Kristofferson model that the lag 1 correlation falls between 0 and -0.5

Statistical analysis

Mean IRI

The mean IRI scores were re-calculated for the 'cleaned' data. This was to monitor that the adjusted means were similar to the means calculated prior to the data being 'cleaned'. The data are shown in Table 5.22 and are roughly similar to the means taken across the entire dataset; there is no striking pattern of relative over- or underestimation compared to the original dataset although cleaning the data has reduced the variability. The amount of missing data leant

analysis of the data to the Kruskal-Wallis test, with group differences at each duration being compared. None of the results were significant.

Variability measures

The data (see Figure 5.12abc) clearly showed that for total variability, clock variability and motor variability, the groups were most distinct at the 2000 ms target interval with the CD group showing greatly increased variability. Unfortunately, the amount of missing data reduced the number of subjects with a compete dataset to a level that is not compatible with parametric multifactorial analysis. Thus, comparison of the data used the Kruskal-Wallis test. The performance of the three groups was compared for each type of variability at each interval length. Significant results were found for the following: total variability for 250 ms (Chi-Square (2) = 9.412; $p = 0.009$), clock variability for 250 ms (Chi-Square (2) = 8.395; $p = 0.015$), total variability for 500 ms (Chi-Square (2) = 9.027; $p = 0.011$), clock variability for 500 ms (Chi-Square (2) = 7.143; $p = 0.028$) and total variability for 2000 ms (Chi-Square (2) = 6.684; $p = 0.035$). However, as 12 statistical comparisons were made, the corrected p threshold is $p < 0.005$, which means that none of the above comparisons reached the adjusted level of significance. However, it could also be argued that finding 5 out of 12 comparisons with a significant effect (41.67 %) is in itself suggestive of 'real' effects in the data, with the Bonferroni correction not taking into account that the more statistical effects that are found, the *less* likely that any of them could have occurred by chance.

	Continuation Phase: All data	Continuation Phase: W&K data
	PD-drug-off	PD-drug-off
250 ms	**244.15** (9.37)	**243.40** (10.80)
500 ms	**483.45** (30.42)	**481.75** (31.44)
1000 ms	**949.91** (47.72)	**960.00** (19.11)
2000 ms	**1918.64** (185.99)	**1904.20** (151.59)
	CD	CD
250 ms	**302.00** (63.84)	**309.19** (82.20)
500 ms	**480.00** (21.76)	**476.00** (19.77)
1000 ms	**992.25** (128.80)	**935.17** (89.07)
2000 ms	**2011.75** (319.40)	**2041.17** (118.38)
	Control	Control
250 ms	**253.89** (13.06)	**251.94** (13.09)
500 ms	**484.53** (14.55)	**481.60** (22.07)
1000 ms	**949.00** (84.35)	**946.42** (60.82)
2000 ms	**1924.82** (194.69)	**1873.14** (102.06)

Table 5.22: Mean IRI repetitive tapping scores for the continuation phase for the PD-drug-off, CD and control groups, for all data and for data with violations of the lag 1 autocorrelation function prediction removed (W&K data)

Standard deviation (SD) in brackets

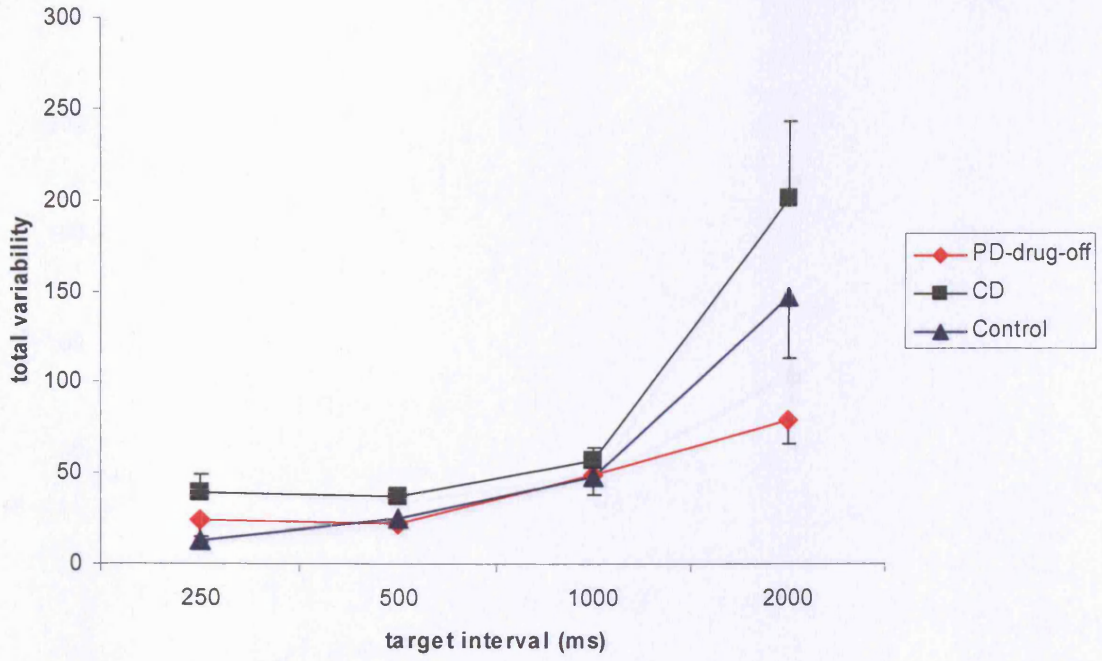


Figure 5.12a: Total variability (ms) at each target interval for each group (± SE)

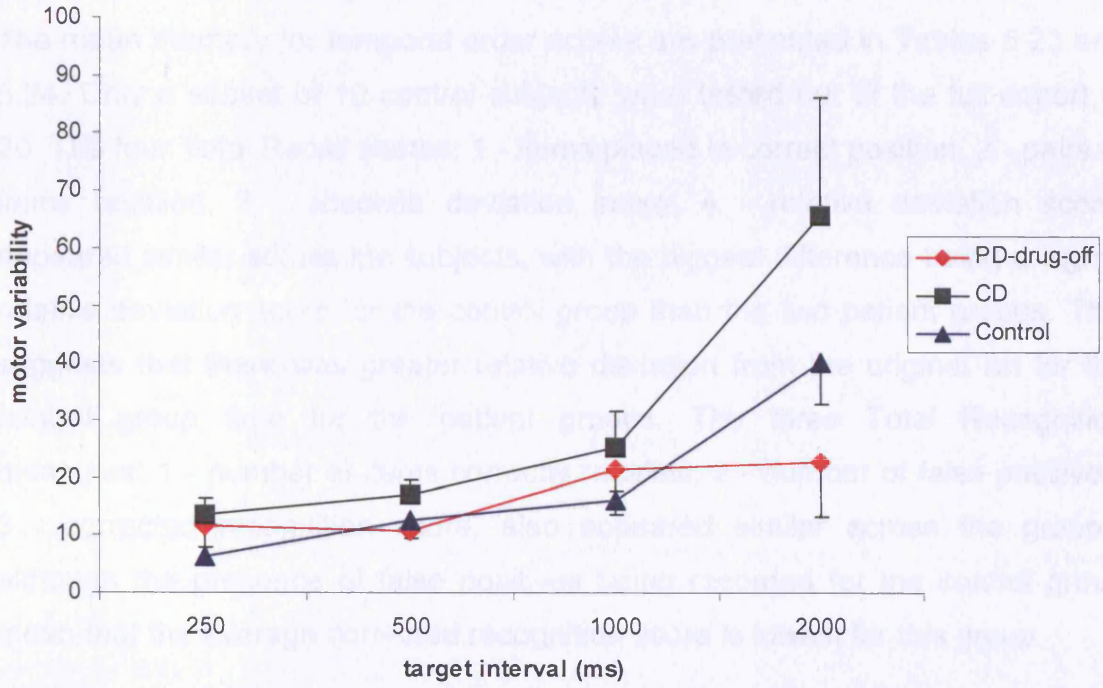


Figure 5.12b: Motor variability (ms) at each target interval for each group (± SE)

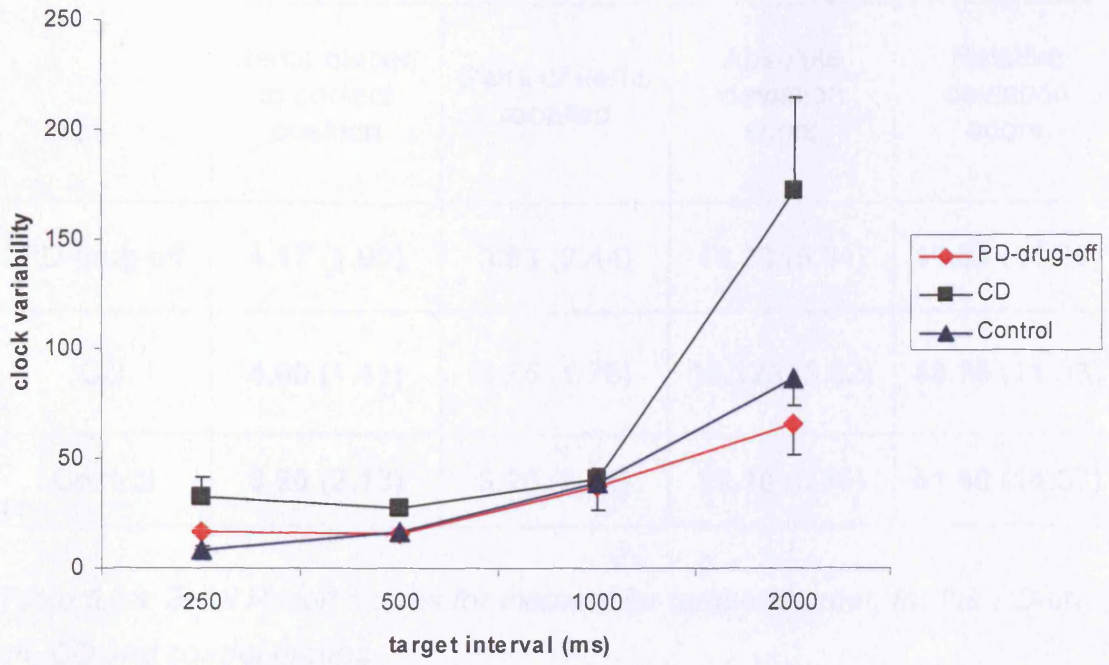


Figure 5.12c: Clock variability (ms) at each target interval for each group (± SE)

5.3.3.5 Memory for temporal order task

The mean memory for temporal order scores are presented in Tables 5.23 and 5.24. Only a subset of 10 control subjects were tested out of the full cohort of 20. The four Total Recall scores: 1 - items placed in correct position, 2 - pairs of items recalled, 3 - absolute deviation score, 4 - relative deviation score, appeared similar across the subjects, with the biggest difference being a higher relative deviation score for the control group than the two patient groups. This suggests that there was greater relative deviation from the original list for the control group than for the patient groups. The three Total Recognition measures: 1 - number of items correctly recalled, 2 - number of false positives, 3 - corrected recognition score, also appeared similar across the groups, although the presence of false positives being recorded for the control group mean that the average corrected recognition score is lowest for this group.

	Items placed in correct position	Pairs of items recalled	Absolute deviation score	Relative deviation score
PD-drug-off	**4.17** (1.90)	**3.83** (2.44)	**13.33** (5.94)	**41.33** (11.29)
CD	**4.00** (1.41)	**3.75** (1.75)	**15.125** (8.92)	**43.75** (11.93)
Control	**3.90** (2.13)	**3.20** (2.35)	**15.10** (5.86)	**51.60** (14.57)

Table 5.23: Total Recall Scores for memory for temporal order, for the PD-drug-off, CD and control groups

Standard deviation (SD) in brackets

	Number of items correct	False positives	Corrected recognition score
PD-drug-off	**9.83** (0.39)	**0.00** (0.00)	**9.92** (0.29)
CD	**10.00** (0.00)	**0.00** (0.00)	**10.00** (0.00)
Control	**9.90** (0.32)	**0.50** (0.53)	**9.40** (0.70)

Table 5.24: Total Recognition Scores for memory for temporal order, for the PD-drug-off, CD and control groups

Standard deviation (SD) in brackets

Group differences in each of the four recall measures was measured with a univariate ANOVA (Bonferroni correction of $\alpha = 0.0125$). No significant effects were found. The temporal memory recognition scores were not normally distributed and the zero scores in the total recognition 2 and 3 do not lend

themselves to log transformation, so the data were tested using the Kruskal-Wallis test (Bonferroni correction of $\alpha = 0.017$). The number of items correctly identified were not significantly different, but the number of false positives were (Chi-Square (2) = 11.60; $p = 0.003$) as was the corrected recognition score (Chi-Square (2) = 11.29; $p = 0.004$). Post hoc tests using the Mann-Whitney U (Bonferroni correction of $\alpha = 0.017$) test showed that the significant number of false positives was due to the increased number of false positives in the control group compared to the PD-drug-off group (Mann-Whitney U = 30.00; Z = -2.72; $p = 0.006$). The control group compared to the CD group showed a difference that was only significant at the uncorrected level (Mann-Whitney U = 20.00; Z = -2.29; $p = 0.022$). The significant corrected recognition score was due to a significantly lower corrected recognition score for the control group compared to the PD-drug-off group (Mann-Whitney U = 28.00; Z = -2.58; $p = 0.010$) and for the control group compared to the CD group (Mann-Whitney U = 4.00; Z = -3.46; $p = 0.001$), as well as a significantly lower score for the PD-drug-off group compared to the CD group (Mann-Whitney U = 0.000; Z = -4.22; $p < 0.001$). This suggests that the CD group had the significantly better scores, followed by the PD-drug-off group, with the control group performing the worst. This reflects the larger number of false positives for the control group compared to the patient groups (no false positives recorded). Indeed, the PD-drug-off performed the worst correctly identifying items (total recognition score 1), but the group did not make any false positive errors.

5.3.3.6 Summary of results for PD-drug-off vs cerebellar disease vs controls

The data are summarised in Table 5.25. To enable a more sensitive impression of the data, significant results that reached conventional significance, but not Bonferroni corrected significance are marked with a *.

	Main effect of duration	Main effect of group	Main effect of phase	Interaction
Time Production: relative eror	**YES**	NO		NO
Time Production:absolute eror	**YES**	NO		NO
Time Production: SD	**YES**	NO		NO
Time Reproduction: relative eror	**YES**	NO		NO
Time Reproduction:absolute eror	**YES**	NO		NO
Time Reproduction: SD	**YES**	NO*		**YES**
Warned and unwarned RT: RT	**YES**	NO*		NO
Warned and unwarned RT: SD	NO	NO		NO
Repetitive tapping sync phase: IRI	N/A	NO*		N/A
Repetitive tapping cont phase: IRI	**YES**	NO		NO
Repetitive tapping: SD	**YES**	**YES**	**YES**	**duration X group*** **
W&K total variability	N/A	NO**		N/A
W&K clock variability	N/A	NO**		N/A
W&K motor variability	N/A	NO		N/A
Memory for temporal order: recall	N/A	NO		N/A
Memory for temporal order: recog	N/A	**YES**		N/A

Table 5.25: Summary of significant and non-significant effects for the PD-drug-off vs CD vs control group comparison

*KEY: YES = significant effect, NO = non-significant effect, N/A = statistic not applicable, sync phase = synchronisation phase, cont phase = continuation phase, W&K = Wing and Kristofferson model, *ANOVA-related effects that reached uncorrected significance only, **non-parametric effects that reached uncorrected significance only, ***additional interaction of phase X rate reached uncorrected significance only)*

There were no group differences in the time production task. For the time reproduction task, the patient groups failed to show a linear increase in the variability of their time reproduction scores across the durations. Instead, there

was no linear increase in variability across the 250 ms, 500 ms and 1000 ms intervals, with a significant increase in variability between the 1000 ms interval and the 2000 ms interval. This suggests that both the PD-drug-off and the CD group were differentially affected by whether the reproduction interval is longer or shorter than 1000 ms, whereas the control group showed a linear increase in variability across the four target intervals. For the repetitive tapping task (all data), the CD group were significantly more variable in their responses than the PD-drug-off group and the control group. Furthermore, a significant duration X phase interaction indicated that the control group showed less variability than the PD-drug-off group at the 250 ms target duration but showed greater variability than the PD-drug-off group at the other target intervals, particularly the 2000 ms target.

The effect of duration was significant for all mean response scores, indicating the subjects were able to differentiate between the different values on the time production, time reproduction and repetitive tapping tasks. On the warned and unwarned RT task the results indicated that subjects were significantly slower on the task when the Go-tone was unwarned compared to when the 250 ms and 500 ms warning tones were included. The significant main effect of duration for the variability measure mainly reflected a linear increase in variability with the mean of the interval being timed. This effect was not apparent in the warned and unwarned RT task, where the different warning durations did not significantly alter variability. For the repetitive tapping task, a significant effect of phase was found for the variability score, this was because variability for the synchronisation phase was greater than the variability for the continuation phase.

It should also be noted that significant main effects of group failed to reach corrected significance for the variability for the time reproduction task and the mean RT on the warned and unwarned RT. Furthermore, for the repetitive tapping task, variability measures decomposed using the Wing and Kristofferson model found group differences in clock (250 ms, 500 ms) and total (200 ms, 500 ms, 2000 ms) variability that failed to reach corrected significance. These data are particularly interesting because the CD group show the greatest

degree of variability on all three tasks. Furthermore, there were no corrected or uncorrected significant effects for variability on the time production task, in which the motor demands were negligible. Lastly, the memory for temporal order task found that recall did not significantly differ between groups. For the recognition scores, the corrected recognition score revealed that PD-drug-off group were significantly worse than the CD group, with the control group performing worst of all, due to a higher degree of false positive identifications.

5.3.4 Correlations between the temporal data and measures of attention, motor speed and disease severity

Linear regression was used to explore the relationship between cognitive and motor measures and performance on the timing tasks. These data were limited to exploration of the PD-drug-off and control data as the CD and PD-de novo groups had $n < 10$, making the data incompatible with regression analysis. The following predictor variables were investigated: PASAT score, right hand Purdue Pegboard score, UPDRS Part III score. To limit the number of statistical tests run, it was decided to regress these measures on just the time production task, representing the most pure measure of perceptual timing, and the repetitive tapping task, representing motor timing. For the time production task, the scores for the three target durations were averaged across and for the repetitive tapping task the two extreme target intervals (250 ms and 2000 ms) were investigated separately, because of the significant group X duration interaction. The data were not averaged across phase because of the phase X duration interaction (at the uncorrected level). The extent of the analysis was limited by just testing the absolute error score and variability score for the time production task, and the continuation data (mean IRI and SD) for the repetitive tapping task (all log transformed).

For the time production task (Table 5.26), attention, as measured by the PASAT, accounted for a negligible (i.e. < 1 %) amount of variance for the PD-drug-off group. However, for the control group attention accounted for 46 % of the variance of the absolute error and for 23 % of the variance of the SD scores. Whereas motor speed and manual dexterity (represented in the Purdue

Pegboard right hand score) accounted for 14 and 17 % of the absolute error and SD scores for the control group, they accounted for a small amount of the absolute error score and a significant 42 % of the SD score for the patient group. For the patient group, the UPDRS Part III score (clinical measure of disease severity) accounted for a negligible amount of the variance for both measures. The results suggest that attentional proficiency predicts performance on the time production task for the control group but not for the PD-drug-group. Furthermore, for the PD group motor speed and dexterity accounted for a considerable portion of the variability (SD) observed in the time production task, despite the task involving minimal motor demands.

Time Production task

	PD-drug-off		
PASAT			
AE	F (1, 10) = 0.62	p = 0.451	r^2 = 0.058
SD	F (1, 10) = 0.42	p = 0.523	r^2 = 0.042
Pegboard			
AE	F (1, 10) = 0.30	p = 0.596	r^2 = 0.029
SD	**F (1, 10) = 7.25**	**p = 0.023**	**r^2 = 0.420**
UPDRS			
AE	F (1, 10) = 0.51	p = 0.492	r^2 = 0.048
SD	F (1, 10) = 0.14	p = 0.719	r^2 = 0.014
	Control		
PASAT			
AE	**F (1, 18) = 15.32**	**p = 0.001**	**r^2 = 0.460**
SD	**F (1, 18) = 5.60**	**p = 0.029**	**r^2 = 0.237**
Pegboard			
AE	F (1, 17) = 2.31	p = 0.147	r^2 = 0.120
SD	F (1, 17) = 2.04	p = 0.171	r^2 = 0.107

Table 5.26: Linear regression between the absolute error and SD scores for the time production task and predictor variables PASAT error score, right hand Purdue Pegboard score and UPDRS Part III score (PD-drug-off group only).

KEY: AE = absolute error score, SD = standard deviation score. Significant results are highlighted in bold

For the continuation phase of the repetitive tapping task (Table 5.27), none of the regressions reached significance. However, whereas the PASAT error score accounted for a negligible degree of the variance for the mean and SD for both tapping intervals for the control group, it accounted for 23 % of the variance of the mean IRI for the 2000 ms interval for the PD-drug-off group. The Purdue Pegboard score accounted for a negligible degree of variance for most of the timing measures, apart from the variability at the 2000 ms interval where it accounted for 15 % and 16 % of the variance in the data of the patient and control groups, respectively. For the patient group, the UPDRS Part III score accounted for 18 % of the mean IRI and 32 % of the SD for the 250 ms target interval and a negligible amount for the 2000 ms scores.

These results suggest that attentional factors are more important for the performance of the PD-drug-off group than the control group for ensuring an accurate response on the continuation phase of the repetitive tapping task. Motor impairment in the PD-drug-off group, as assessed by the UPDRS, predicted part of the mean and SD data at the short (250 ms) interval range only, suggesting that disease severity was less of a factor when the intervals were longer and less challenging physically (if not temporally). Interestingly, the motor speed and dexterity measure predicted a proportion of the variability for both groups on the SD measure at the more long (2000 ms) interval range only, suggesting a dissociation from the UPDRS measure.

Repetitive Tapping task: continuation phase

	PD-drug-off		
PASAT			
250:Mean	F (1, 8) = 0.31	p = 0.592	r^2 = 0.038
250:SD	F (1, 8) = 0.47	p = 0.511	r^2 = 0.056
2000:Mean	F (1, 9) = 0.27	p = 0.136	r^2 = 0.229
2000:SD	F (1, 9) = 0.55	p =0.476	r^2 = 0.057
Pegboard			
250:Mean	F (1, 8) = 0.37	p = 0.559	r^2 = 0.044
250:SD	F (1, 8) = 0.04	p = 0.839	r^2 = 0.005
2000:Mean	F (1, 9) = 0.48	p = 0.507	r^2 = 0.050
2000:SD	F (1, 9) = 1.54	p = 0.246	r^2 = 0.146
UPDRS			
250:Mean	F (1, 8) = 1.71	p = 0.227	r^2 = 0.176
250:SD	F (1, 8) = 3.68	p = 0.091	r^2 = 0.315
2000:Mean	F (1, 9) = 0.04	p = 0.851	r^2 = 0.004
2000:SD	F (1, 9) = 0.10	p = 0.755	r^2 = 0.011
	Control		
PASAT			
250:Mean	F (1, 17) = 0.48	p = 0.499	r^2 = 0.027
250:SD	F (1, 17) = 0.05	p = 0.833	r^2 = 0.052
2000:Mean	F (1, 17) = 0.02	p = 0.884	r^2 = 0.001
2000:SD	F (1, 17) = 0.40	p =0.553	r^2 = 0.023
Pegboard			
250:Mean	F (1, 16) = 0.21	p = 0.653	r^2 = 0.013
250:SD	F (1, 16) = 0.01	p = 0.922	r^2 = 0.001
2000:Mean	F (1, 16) = 0.06	p = 0.814	r^2 = 0.004
2000:SD	F (1, 16) = 3.12	p = 0.097	r^2 = 0.163

Table 5.27: Linear regression between the absolute error and SD scores for the continuation phase of the repetitive tapping task and predictor variables PASAT error score, Purdue Pegboard right hand score and UPDRS Part III score (PD-drug-off group only)

5.4 Discussion

Patients with PD were tested 'on' and 'off' medication to explore the effects of dopamine on motor and perceptual timing. Furthermore, data collected while the patients were in the 'off' medication condition were compared to a de-novo

group of patients who were in an earlier stage of illness. As disease severity is correlated with duration of illness, these two factors were both used as covariates during analysis. To date, the comparison of such PD sub groups has not been reported in the timing literature. Finally, the PD patients, tested in their 'off' medication state, were compared to a group of patients with cerebellar disease and healthy controls to directly investigate the differential roles of the basal ganglia and cerebellum in temporal processing. The data are discussed for each of the timing tasks in turn.

5.4.1 Time production task

The time production task was used as a measure of subjective sense of time, as no example of the duration was provided. Both the degree of error and the pattern of over- and under-estimation were of interest. Overestimation indicates a slowed sense of subjective time (i.e. a slowed 'internal clock') whereas underestimation indicates a speeded sense of subjective time (i.e. a speeded 'internal clock'). This is the first study to investigate time production in patients with cerebellar disease and the results suggest that these patients do not differ from controls and do not show deficits in seconds-range time production. The PD-drug-off group were also not significantly different from the control group, who showed underestimation compared to the two patient groups. Lange et al (1995) found that patients with PD tested 'off' medication overestimated to a significant degree on a time production task (intervals of 10 s, 30 s and 60 s) compared to a control group, when the production of the intervals involved internal counting at a pretrained rate. It is possible that the timed motor element introduced with the counting increases the timing-related dysfunction for the patients with PD on this task, despite the pattern of results remaining similar.

The patients with PD tested 'on' and 'off' medication tended to overestimate (relatively) when 'off' medication at the two shorter target intervals (30 s and 60 s) but underestimate (relatively) when 'off' medication at the longer interval (120 s) compared to when 'on' medication. An identical pattern was found for the PD-drug-off group when compared to the PD-de novo group, with the PD-drug-off group showing relative overestimation of the 30 s and 60 s target intervals and

relative underestimation of the 120 s target interval compared to the PD-de novo group. However, none of these differences, for either the PD-drug-on vs PD-drug-off or PD-drug-off vs PD-de novo, reached corrected significance. This suggests that the effective level of dopamine (either through within-group manipulation of dopaminergic medication or between group differences in dopamine loss) does not systematically or significantly alter the speed of the 'internal clock', contrary to the conclusions of Pastor et al (1992b). This result also fails to reflect pharmacological work, such as the finding that haloperidol, a dopamine antagonist, causes rats to overestimate on the peak-interval procedure (Drew et al, 2003). Pharmacological studies with animals are arguably a purer measure of the influence of drugs on timing processes as higher-level influences (e.g. cognition, strategy, motivation) on performance are removed or better controlled. Consideration must also be given to the sample size used in this study; it may be that testing more patients would bring this pattern of results to above the threshold for statistical significance. As such, easy dismissal of the speed of internal clock hypothesis in relation to PD is certainly not possible. Although, the lack of a systematic pattern of results (classic internal clock predictions would suggest overestimation on all intervals) is difficult to reconcile, regardless of significance levels.

A measure of absolute error, which disregards the direction of the error and concentrates on the degree of error, showed that the patients deviated significantly further from the target duration when they were 'off' medication. Furthermore, an identical pattern of results was found when the PD-drug group tested 'off' medication were compared to the PD-de novo group, with the PD-drug-off group showing significantly greater absolute errors. This suggests that, regardless of the direction of the error, the subjective sense of time is significantly mediated by dopamine, either within- or between-subjects. For the PD-drug-off and PD-de novo comparison, when duration of illness and the UPDRS Part III score were used as covariates, the significant main effect was lost, suggesting that the timing-related effects can be accounted for by these two highly correlated factors that both reflect effective levels of dopamine. When duration of illness was used as a covariate the significance of the main group effect only dropped to a marginally non-significant level (significant at the

uncorrected level), suggesting that duration of illness is a less potent contributor to timing effects than the measure of motor symptoms.

This result complements previous research that found time perception of seconds-range intervals to be improved by dopaminergic medication (e.g. Lange et al, 1995; Malapani et al, 1998b; Pastor et al, 1992b). Our study was unique in not including chronometric counting or a secondary task to inhibit counting, thus, showing that time perception deficits (or more specifically, time production deficits) persist in PD in the absence of either pacing stimuli or distracter stimuli.

Dopaminergic medication did not seem to affect a measure of the patients' variability across several repetitions of the tasks. A null result was also obtained for the PD-drug-off vs PD-de novo variability comparison. However, when group differences in the UPDRS Part III score was taken into account, the significant main effect of duration was eliminated and a significant duration X group interaction was introduced. This was because the PD-de novo group showed more similar variability for the two shorter estimates followed by a sharp increase in variability for the 120 s estimate, compared to the PD-drug-off group where a linear effect was observed i.e. the 60 s estimate was produced with greater relative variability. This suggests that motor-related disease severity affects the relationship between target duration and group for the variability measure, with increased disease severity causing the effect of intervals less than or equal to 60 s being estimated with relatively reduced variability being lost.

Despite the time production task involving a minimal motor component, the severity of motor symptoms still explained differences found between the two groups. One hypothesis is that the motor symptoms reflect the basal ganglia dysfunction and it is this dysfunction that underpins the performance on the timing tasks, with both perceptual and motor timing being mediated by the same regions of the frontstriatal motor loop. This hypothesis has been previously suggested, for example by Keele et al (1985) who found that accuracy of perceptual judgments correlated significantly with regularity of motor tapping

performance for healthy subjects. Regression analysis showed that performance on the Purdue Pegboard explained some of the variance on the variability measure of the time production task but that the UPDRS Part III score explained neither the absolute error nor variability scores for the PD-drug-off data, despite accounting for some of the difference between the two groups. Although this task used very long intervals, the subjects were required to use 'time sense' rather than any strategy to time the interval. This suggests that the data most likely reflect a problem with an internal 'timer' system, rather than any cognitive dysfunction. Indeed, although the PASAT score explained variability on this task for the control group, it did not for the PD-drug group tested 'off' medication and the patients performed similarly on the PASAT task 'on' and 'off' medication. This suggests that performance on the task is not being influenced by levels of attention for the patient group and in the absence of strategic support or cognitive load (e.g. self-paced counting or distractor stimuli) the result reflects a fundamental timing dysfunction.

5.4.2 Time reproduction task

The time reproduction task provided an example of the interval to be estimated and required that the subjects reproduce it, thus the task measures the subjects' ability to accurately measure and reproduce an interval (250 ms, 500 ms, 1000 ms and 2000 ms). This task is similar to the task presented in the PET study of Chapter 3, although presenting the interval prior to each reproduction places less demand on temporal memory. The findings of Chapter 3 suggest that the basal ganglia, rather than the cerebellum, is fundamental to the timing processes engaged by the task and would predict that the patients with PD should show poor performance on this task. Furthermore, the rTMS study presented in Chapter 4 used an almost identical set of stimuli (visual rather than auditory mode of presentation) and found that the reproduction of 2 s intervals placed greater demand on temporal memory than the reproduction of 500 ms, implicating the right DLPFC in this process.

For the cerebellar disease, PD-drug-off and control groups no group differences in mean reproduction error and absolute error was observed. It should be

mentioned, however, that the patients with CD did show overestimation at three of the intervals (not 1000 ms) compared to the control group and on four of the intervals compared to the PD-drug-off group. Given the small number of CD patients that were tested, it is interesting to speculate as to whether the small n is masking a significant effect. Previous researchers (e.g. Pastor et al, 1992b) have suggested overestimation on this task indicates a slowed internal clock. However, this is unlikely to be the case as the clock would have to be slow during either the Estimation Phase *or* the Reproduction Phase for overestimation to occur for this reason; a slowed clock during both parts of the task would still enable an accurate result as long as the clock rate remained steady. An alternative explanation is that the motor dysfunction of the group produced delayed responding, complimenting the longer RTs seen for this group in the warned and unwarned RT task. Both patient groups showed a significantly different pattern of variability on the task compared to the control group. Whereas the control group showed a linear increase in variability across the intervals, the patients showed a non-linear relationship between the three shorter intervals, indicative of a static level of variability across the three intervals. Furthermore, variability increased significantly between the 1 s and 2 s intervals, unlike the control group. First, this is interesting as the patient groups don't seem to differ for the shorter intervals in terms of their variability scores and secondly, the increased variability for the 2 s interval suggests that longer intervals are disproportionately more difficult for the patients to time consistently. In effect, the patients' variability is affected by whether the interval is ≤ 1 s or > 1s, whereas the control group show no such differentiation. This result is particularly interesting as previous researchers have proposed that intervals over a particular threshold may be timed in a qualitatively different way (e.g. Ivry, 1996; Michon et al, 1985). As both patient groups were affected it may be that this finding is a disease non-specific consequence of brain damage or that neural dysfunction in both disease processes impacts on the circuitry that underlie this effect.

No analysis was made of the variability in responding to the time reproduction tasks reported in Pastor et al (1992b). However, Malapani et al (1998ab) have shown that patients with PD tested 'off' medication and patients with cerebellar

lesions show increased variability on the peak-interval procedure, which involved the reproduction of seconds-range intervals (between 8 and 21 s). A follow up study found that the dysfunction in the PD group was due to deficits in the storage and retrieval of temporal memories (Malapani et al, 2002). The study reported here presented the target intervals immediately prior to reproduction and also used shorter intervals (250 – 2000 ms), such that fewer demands were placed on temporal memory. Indeed, in the study of Malapani and colleagues (2002) increased variability for the PD group was only found when two timed intervals were held in memory, not when only one temporal interval had to be reproduced. The rTMS study in Chapter 4 showed that the right DLPFC is important in temporal memory processes in the time reproduction task for intervals of 2000 ms, but not those of 500 ms. Right DLPFC activation was also found in a PET study of time reproduction in the seconds-range (2.2 – 13 s) (Macar et al, 2002). It is clear that seconds-range intervals are more demanding of the cognitive processes dependent on prefrontal function, regions that are influenced by dopaminergic levels in the basal ganglia. Thus, the differential accuracy results between this study and the work of Malapani and colleagues may reflect differences in the intervals assessed.

The absence of any significant medication effects for the PD-drug group is in contrast to previous work. In fact, the patients showed a greater degree of absolute error 'off' medication for the 500 ms and 2000 ms target intervals only and showed relative overestimation in the 'off' medication condition for the 250 ms and 2000 ms target intervals only, which does not suggest a consistent sub-threshold trend in the data. Pastor et al (1992b) tested patients with PD 'off' medication on a comprehensive range of time reproduction tasks (range 3 – 9 s) and found that the patients had a greater absolute error than a control group across the tasks as well as showing a greater percentage of overestimation. However, these tasks were different from the one presented here as for most the presented interval was divided by fifteen numeric time markers presented at a rate of 1.6 Hz, 3.3 Hz or 5 Hz. Reproduction of the intervals required internal counting of the numbers at the rate at which they were presented. Thus, the task required chronometric counting, which is known to alter timing (e.g. Gibbon

et al, 1977), as well as a motor component. Indeed, the patients were found to have greater absolute error when tested 'off' medication compared to 'on' medication when the time markers were presented at the rate of 5 Hz or 3.3 Hz (i.e. the fastest presentation times) only. This suggests that when the motor demands were highest the patients were susceptible to greater error. Although a version of the task in which no time markers were used did not produce significantly different results for the PD group tested 'off' medication and the control group than when the time markers were included, it is difficult to conclude that the counting strategy was not used by the subjects as the instructions advised the subjects to use their 'own preferred strategy'. Furthermore, the PD group did not differ on the task without time markers when compared 'on' and 'off' medication, suggesting that the medication effect is not robust when counting and motor demands are not present. Therefore, the medication-related findings of Pastor et al (1992b) mimic the results reported in this study for an appropriately matched task. Interestingly, the task with no time markers that Pastor and colleagues used required subjects to reproduce intervals of 6 s and 9 s. This suggests that dopaminergic medication does not affect the reproduction of intervals longer than those used in the present study.

However, the PD-de novo patients showed significantly greater absolute error on this task than the PD-drug group tested 'off' medication, which suggests that severity of striatal dysfunction influences time reproduction. This finding therefore reflects the PET study presented in Chapter 3 and the study of Macar et al (2002), both of which found basal ganglia activation during time reproduction. Both the duration of illness and disease severity explained this effect, with both covariates eliminating the significant result. This finding is interesting as it suggests that shorter duration of illness and milder disease severity contribute to the *greater* degree of error on this task for the PD-de novo group. The task requires other processes such as attention, movement preparation and execution and response inhibition. A dysfunction in motor execution or response inhibition would result in significant over- or underestimation, respectively, but this was not found. Also, the two groups did not differ on the PASAT task, a measure of attentional capabilities. The results suggest that the contribution of the basal ganglia to temporal processing is

complex. One possibility is that the chronic use of medication has positively affected the performance of the PD-drug group compared to the PD-de novo group. Alternatively, the group differences may relate to duration of illness and disease severity. It is not possible to separate these alternatives from the data collected. As with the time production task and as previously discussed, the results do not suggest that PD patients have a slowed internal clock, or at least a slowed internal clock would not prove adequate explanation for the results found in the PD-drug-off vs PD-de novo comparison. The clock would have to have been running at a slowed pace during just one of the phases (e.g. due to drug manipulation) for its effects to be reflected in the data. As with the CD group, the small sample size for the PD-de novo group is problematic and further research is needed to fully establish the pattern of results reported here.

As a final point, the data also suggest a dissociation between the effects of medication on time production (in which medication affected absolute error) and time reproduction (in which medication had no effect, although differences dependent on disease severity were found). Whether this is related to the use of longer intervals, different timing techniques or the absence of an example of the timed interval needs to be answered in further research. Interestingly, for both tasks the effective level of dopamine was seen to affect patient performance, but the patients did not perform significantly differently to the control group. This indicates that dopamine plays a role in both tasks but, in this study at least, this does not cause a significant deterioration in performance compared to the performance of healthy individuals. This pattern of results, i.e. medication effects in the company of less striking between group effects, has been previously found in the timing literature for patients with PD (Malapani et al, 1998b).

5.4.3 Warned and unwarned reaction time task

The warned and unwarned reaction time task was a test of how well the subjects were able to use timing cues to enhance preparation for and reaction to a 'Go-tone' that required a simple button press response. This is an implicit temporal task as, rather than having to explicitly attend to and judge a temporal

interval, the subjects have to engage in temporal processing in order to produce an accurate and fast RT.

The CD group displayed longer RTs when compared to the control group and the PD-drug-off group. However, the main effect of group failed to reach corrected significance. Delayed RTs across all the intervals is clearly a reflection of general motor slowing and does not indicate a problem for the cerebellar group with timing per se as this would be expressed in differential responsiveness to the warning cues. Across all groups, the subjects showed significantly shorter RTs for the 250 ms warning tone and the 500 ms warning tone compared to the unwarned condition. This suggests that all groups were helped by the shorter interval warning tones, enabling the RT to be predicted. Crucially, no group X duration interaction was found, indicating that the patient groups were not affected by the presence of a warning cue, or the length of the warning cue, in a significantly different way to the control group. Jahanshahi et al (1992a) used visual warning stimuli occurring 200 ms, 800 ms, 1600 ms or 3200 before the 'Go' stimuli, compared to trials in which no warning cue was present. Unlike in this study, the PD group (tested 'on' medication) performed with significantly slower RTs compared to the control group. As in this study, no group X duration interaction was found. In a study that included both patients with PD ('on' medication) and patients with cerebellar disease, the presence of a warning signal significantly decreased RTs for both groups on a choice RT task (the 'Go' stimuli indicated which of four response buttons to press), with an enhanced effect of the 200 ms warning interval compared to the other warning intervals for the PD group, which was not apparent for the CD group (Jahanshahi et al, 1993). However, for the simple RT task the CD group did not show a significant difference in RTs as a function of warning interval when the interval was presented in a block (as in this study). The difference was only significant when the warned/unwarned intervals were intermixed randomly. The current study, unlike previous work, also investigated the variability of responding, but no significant effects were found.

No significant group effects were found for the PD-drug-off vs PD-drug-on or PD-drug-off vs PD-de novo comparisons. Furthermore, the covariates of illness

duration and UPDRS Part III scores had no impact on the group effects, although the significant main effect of duration was reduced to a non-significant level by the UPDRS score for the mean RT results. This suggests that the efficiency with which the patients used the temporal cue to enhance their RTs was not moderated by disease severity. This is in contrast to the explicit time production and reproduction tasks in which drug or disease severity effects were found. Previously, both a choice reaction time task and the simple reaction time task have been paired with warning visual stimuli for patients tested both 'off' and 'on' medication (Jahanshahi et al, 1992b). The effects of giving the different warned and unwarned intervals (200 ms, 800 ms, 1600 ms or 3200 ms) in blocks or randomly mixed were compared. Presenting blocks of trials in the 'off' medication condition (as in this study) resulted in the unwarned simple RT being significantly longer than the warned RTs, with this effect not being apparent 'on' medication. In this study, RTs were significantly shorter for the 250 ms and 500 ms warning tone compared to the unwarned condition for both 'on' and 'off' medication conditions, a pattern also found in the control group.

To conclude, neither of the patient groups were impaired in their response to the temporal cues. Both PD and CD groups have shown timing dysfunction in previous studies and it may be that the patients are better at responding to temporal information when it is presented implicitly and does not involve the timing information being cognitively processed. However, the deficit shown by both groups on timing of millisecond-range tasks that are relatively cognitively undemanding (and arguably performed 'automatically') (e.g. Ivry et al, 1988; O'Boyle et al, 1996) suggest against this proposal. These results raise interesting questions about what temporal processes the warned and unwarned RT task are tapping, considering that some sort of timing information is being processed in order to produce the enhanced RTs. If clock processes were engaged in this task then it could be speculated that they were initiated at the onset of the warning tone and switched off at the onset of the 'Go-tone'. In theory this would enable this interval to be timed and predicted on subsequent trials, enabling non-motor preparation prior to the 'Go' signal (subjects were instructed to initiate their RT only *after* the 'Go-tone'). As with the time reproduction task, a clock simply running too slow or too fast would not affect

performance on this task, assuming the clock was running at a constant rate during the task. A clock that runs irregularly would cause an atypical response profile across the different warning intervals, as the prediction of the 'Go-tone' would be too variable to allow systematic enhancement of the RT in the warned condition. However, it may be that this task does not engage a 'clock' as is conceptualised for other forms of motor and perceptual timing, a suggestion that warrants further investigation given the null result for the patient populations studied here.

5.4.4 Repetitive tapping task

The repetitive tapping task was used to assess motor timing, including the capacity to tap in time with a tone and to maintain the rhythm in the absence of the tone.

For the cerebellar group, PD-drug group tested 'off' medication and the control group, no significant group effects were found for the mean IRI. These results suggest that statistically the three groups performed equally well and that the patient groups could entrain the target duration and produce an accurate response. Care should always be taken in interpreting this type of result when the sample sizes are small. Though, the only consistent pattern in the data that would suggest a possible true effect is being masked by low power is that of the PD-drug-off group tapping slightly faster than the control group. Indeed, for the cerebellar patients, these data fit previous work in which the mean IRI was not impaired (Harrington et al, 2004a; Ivry and Keele, 1989). However, Harrington et al (1998a), Ivry and Keele (1989) and O'Boyle et al (1996) all found evidence that patients with PD tested 'on' medication tapped at a significantly faster rate during the continuation phase than controls when the target interval was between 300 and 600 ms (elevated tapping rates were also found when 'off' medication in the study of O'Boyle et al (1996) but did not reach significance). Contrary to this, Pastor et al (1992a) found that patients with PD tested 'off' medication tapped at a significantly slower rate than controls with similar IRIs (400 ms and 500 ms), although they showed a more inconsistent pattern at higher rates of tapping. In this study, the patients tested 'off' medication tapped

faster than the controls during both the synchronisation phase and the continuation phase, apart from the 1000 ms target interval in the continuation phase where they were marginally (0.91 ms) slower. Thus, the pattern of data reflects the majority of previous findings in finding that the PD group tap at a faster rate; a larger sample size may have rendered this result significant.

Total variability (i.e. SD) was also examined across all the data, with the results showing that the CD group had higher levels of variability than both the PD-drug-off and the control groups. This is in contrast to the study of Harrington et al (2004a) who found no significant differences in total variability for a group of patients with lesions to the cerebellum compared to a group of healthy controls. However, Ivry and Keele (1989) found increased variability for a group of cerebellar patients, compared to a control group and also to a group of patients with PD. The lack of deficits in variability for the PD group is in contrast to the findings of Pastor et al (1992a) who compared patients with PD tested 'off' medication to healthy controls and also the findings of Harrington et al (1998a) who tested patients 'on' medication. O'Boyle et al (1996) found that variability was higher when the patients with PD were 'off' medication, but not when they were 'on' medication. In the present study, the PD-drug-off group only show greater variability compared to the controls at the 250 ms target duration. The two groups showed similar levels of variability at the 500 ms and 1000 ms target intervals, but the control group clearly showed greater variability at the 2000 ms target interval, a pattern reflected by the significant interaction. The reason for this result is unclear but the poorer performance of the CD than the PD group is a robust finding and confirms the previous findings of Ivry and Keele (1989).

Using the Wing and Kristofferson (1973ab) model, the data for the total, clock and motor variance failed to reach corrected significance, although the CD group clearly showed greater variability. Only eight trials could be collected from each subject (across 4 interval types), which falls short of the number collected by other research groups (e.g. O'Boyle et al, 1996). The failure to find significant effects is doubtless a consequence of the limited data that was left once trials that violated the key assumption of the Wing and Kristofferson model (lag 1 autocovariance should be between 0 and -0.5) had been eliminated and

the reliance on multiple Bonferroni corrected tests. The principal aim of this study was to test patients with PD and cerebellar disease on a range of timing tests and across a range of interval lengths. The problem of fatigue meant that the number of trials collected for the repetitive tapping task had to be limited in order to be able to collect data from a range of motor and perceptual timing tasks. No significant effects were found for motor variability, although both patients with PD (O'Boyle et al, 1996; Pastor et al, 1992a) 'off' medication and patients with cerebellar pathology (Ivry and Keele, 1989) have previously shown deficits on this measure. Uncorrected group effects were found for clock variability for the 250 ms and 500 ms intervals, and in previous studies both patients with PD 'on' medication (Harrington et al, 1998a; O'Boyle et al, 1996) and 'off' medication (Pastor et al, 1992a; O'Boyle et al, 1996) and patients with cerebellar pathology (Ivry and Keele, 1989; Harrington et al, 2004a) have shown deficits on this measure.

Despite some of the effects not reaching corrected significance, the patients with cerebellar disease showed increased variability when repetitive tapping compared to healthy controls. These patients also showed greater variability on the warned and unwarned RT task, although a reduced variability for the 2000 ms warning tone condition probably accounted for the lack of a significant effect. Both the PD-drug-off and CD group showed a different pattern of variability to the control group on the time reproduction task, with the CD group showing enhanced variability to the PD-drug-off group. Thus, although not all the effects were significant, a pattern of increased variability on these tasks for the patients with cerebellar disease is apparent. Interestingly, the group show either less or equal variability at different durations to the PD-drug-off group on the time production task, in which the motor component is minimal. Although it seems likely that the greater variability in CD is a function of motor-related factors, elevated clock-related variability on the repetitive tapping task suggests that clock-related factors contribute to it. Harrington et al (2004a) suggest that increases in clock variability may be due to deficits in 'acquiring auditory or cognitive input relevant to an intended temporal goal and coordinating it with an impaired motor-output system'. The cerebellum is known to engage in a wide range of sensory, motor and cognitive processes (e.g. Thach, 1998), which

makes alternative explanations for any timing-related dysfunction associated with cerebellar patients important to explore. For example, Penhune et al (1998) suggest the cerebellum may be engaged in the learning of timed motor responses and also in sensory integration. Other research suggests that dysfunction in the cerebellum may cause deregulation of thalamic control, which affects striato-thalamo-cortical loops or even cerebellar cortical connections (Gibbon et al, 1997; Malapani et al, 1998a). It is suggested that the increased variability in the CD group is related to the motor demands of the tasks, particularly as no deficits are observed in this group on the time production task. Furthermore, accuracy is not impaired on any of the tasks and increases in variability alone are typically attributed to processes that support timing, rather than to timekeeper dysfunction per se (see Harrington et al, 2004 for a review). This finding also complements the results from Chapter 3, in which the cerebellum was not active during two time reproduction tasks compared to a well-matched control task. However, it can be proposed that the intact functioning of the cerebellum is necessary for the operation of a fully efficient timing system, such that in CD sensory, motor or cognitive functions are being disrupted that contribute to consistent timing performance. The limited number of cerebellar patients assessed made regression analysis unavailable, which does not enable the teasing apart of these different influences. Indeed, comment must also be made that the limited number of patients with CD reduces the statistical power of this group. It may be that a larger sample size would have produced more convincing results, although there was no suggestion in the data of a systematic pattern of impaired accuracy. Furthermore, it could also be said that the greater number of statistical comparisons carried out across the patients with PD (patients with PD were included in all three main analyses; the patients with CD were included in only one) naturally biases the finding of significant effects towards the PD group. For this reason it has been important to discuss sub-threshold trends, such as the pattern of variability across the CD data sets.

In terms of the effect of dopamine on timing performance, the mean IRI did not differ between the PD-drug-off and PD-de novo groups. The two covariates had no effect on the continuation phase data. Variability was significantly higher in

the synchronisation phase than the continuation phase. This is interesting as in the synchronisation phase a pacing cue is provided. This implies that the two groups found it difficult to adapt to the task demands in the synchronisation phase and predict the tone onset systematically. The use of duration of illness as a covariate eliminated this effect and the UPDRS score eliminated both this effect and the significant effect of duration. As with all of the tasks, disease severity had a greater impact on the results than the duration of illness. This suggests that duration of illness has a less consistent effect on timing performance.

Comparing the PD-drug-off with PD-drug-on with a more sensitive within-subject design, gives an idea of the effects of dopamine on timing performance. The mean IRI for the repetitive tapping task only varied significantly as a function of medication state for the synchronisation phase at the 1000 ms target interval. This target interval was significantly underestimated 'off' medication compared to 'on' medication, although the 'on' medication response was marginally less accurate. The relative underestimation of the 500 ms interval in the 'off' medication condition compared to the 'on' medication condition (accuracy being greater 'on' medication) reached uncorrected significance. Interestingly, the patients showed the same pattern of results for the synchronisation phase data as in the time reproduction task, with only the 250 ms and 2000 ms target intervals being overestimated 'off' medication. It could be that the differential motor demands of the very short interval caused the overestimation, whereas the effect for the 2000 ms interval was due to a more real timing effect. Only the 2000 ms interval showed overestimation in the 'off' medication condition for the continuation phase, where no effects were significant. In fact, the data collected from the regression analyses for the PD group tested 'off' medication support this finding, with the UPDRS Part III score reflecting 18 % of the variance for the 250 ms interval and a negligible amount for the 2000 ms interval and conversely the PASAT score explaining variance on the mean IRI at the 2000 ms target interval and not at the 250 ms target interval. The same pattern was also found for the patients variability score at the two intervals. Interestingly, the PASAT score did not contribute to the performance of the control group at either interval, which suggests that the

patient group found the task more demanding as the repetitive tapping task is meant to be performed 'automatically', without cognitive involvement (e.g. Lewis and Miall, 2003a). Pastor et al (1992a) found that for the continuation phase data, the PD patients produced significantly slower IRIs 'off' medication when the target intervals were 400 ms, 500 ms and 666 ms, but not when they were 1000 ms and 2000 ms. However, in parallel to this study, O'Boyle et al (1996) failed to find that medication significantly altered performance in a group of PD patients tapping with a target interval of 550 ms (continuation phase), despite the patients tapping significantly faster than the control group when 'on' medication. The Purdue Pegboard score accounted for a negligible degree of variance for most of the timing measures, apart from the variability at the 2000 ms interval where it accounted for 15 % and 16 % of the variance for the patient and control groups, respectively. As with the time production task, the Purdue Pegboard and UPDRS scores did not explain equivalent amounts of variance, which confirms the dissociation between these two measures and also the complexity of the processes underlying motor and perceptual timing.

For the variability of performance on the repetitive tapping task, a significant duration X medication interaction was found (collapsed across both phases). This effect was the result of variability being higher in the 'off' medication condition for the 250 ms condition and lower in the 'off' medication condition for the other three target intervals (although negligibly at the 2000 ms interval). Other groups found results inconsistent with this such that variability was higher 'off' medication compared to 'on' medication for intervals ranging from 500-666 ms (O'Boyle et al, 1996; Pastor et al, 1992a). However, the fixed order effect ('off' followed by 'on') in the study of O'Boyle et al (1996) could predict the reduced variability in the 'on' condition as a function of practice, regardless of the medication effect. Ivry and Keele (1989) found 'minimal' differences in total variability for patients with PD as a function of medication state when tapping with an IRI of 550 ms. It can be suggested that the impaired motor function of the patients when 'off' medication causes increased variability at the most motorically demanding interval (250 ms). Indeed, the UPDRS Part III score accounted for about a third of the variability of the 250 ms interval during the

continuation phase ('off' medication), but a negligible amount of the variance of the 2000 ms interval.

This is the first study to statistically compare variability across four target intervals. The results suggest that the target interval had a significant effect on the variability of the responses. Taken together, the results demonstrated that the cerebellar patients showed increased variability compared to the PD and control groups and that both IRI and variability can be modulated by dopaminergic medication for patients with PD. Rao et al (1997) found that left SMA and left putamen were more active during the continuation phase than the synchronisation phase for healthy subjects, this implicates the frontostriatal motor loop in motor timing particularly when internally generated timing is required. Furthermore, in a functional imaging study of repetitive tapping in PD, patients were found to show increased activity in the left putamen, left thalamus and SMA during the continuation phase when 'on' medication compared to when 'off' medication (Elsinger et al, 2003).

5.5.5 Memory for temporal order task

The memory for temporal order task was used to measure the subjects' ability to remember the temporal order in which items were presented to them. At a behavioural level, the ability to reconstruct the order in which stimuli occur relies on both the retrieval of temporal information and estimating and sequencing the temporal framework of the presented stimuli (Vriezen and Moscovitch, 1990). Failure on the task would indicate frontal lobe dysfunction (Vriezen and Moscovitch, 1990).

The two patient groups did not show any deficits on this task. The control group made false positive errors, which resulted in significantly lower 'corrected recognition scores' although the PD group actually identified less items than the control group. This result is difficult to interpret as there is no hypothesis as to why the patient groups may be less vulnerable to false positive identifications and the result cannot be explained by cognitive impairment in the control group. The findings suggest that temporal order judgement is not impaired in patients

with CD or patients with PD tested 'off' medication. Therefore, a dissociation between temporal processing of millisecond- and seconds-range intervals and processing of the temporal order of items exists. This is in contrast to the findings of Vriezen and Moscovitch (1990), who found that patients with PD showed a greater relative deviation score compared to controls when picture stimuli were used, with the patients also showing deficits on further measures when word stimuli were used. The patients with PD used in the study of Vriezen and Moscovitch (1990) had a duration of illness that ranged from 1-22 years (compared to 3-13 years in the present study). As more severe PD (reflected in duration of illness) is more likely to affect frontal lobe function, it is possible that a subgroup of patients in the Vriezen and Moscovitch study with a longer duration of illness may have also had frontal dysfunction.

The task is clearly cognitively demanding and processed using high-level cognitive strategies. Indeed, deficits on this task by patients with lesions to the frontal lobes have been previously documented (e.g. Shimamura et al, 1990). Mangels et al (1997) required patients with frontal lobe lesions (primarily DLPFC) to learn the temporal order of a series of 24 words, a more demanding task than the one used in this study although the items were presented with a slower inter-stimulus interval (6 s). The patients were less able than controls to use serial associative strategies as well as their ordering being confused by semantic relatedness between items (i.e. semantically-related items being clustered together in recall, regardless of the temporal order). However, although performance their recall performance was worse than controls when learning a list of semantically related words, they performed similarly to controls when the words were semantically unrelated and when the temporal order of the words was processed automatically (i.e. without intention). This indicates that dysfunction in the DLPFC may account for disruption to memory for temporal order by affecting the monitoring and organising of temporal information but that memory for temporal order must rely on functioning in other brain regions. It is difficult to conceive how clock processes, conceptualised within the traditional sense (e.g. SET), could be applied to this task. If some sort of 'clock' process underlies the task then perhaps the it would be activated at the onset of the learning phase, with each presented picture being paired with a

different clock value (cumulative from the onset) that can later be retrieved. It is difficult to see how dysfunction to clock processes would result in increased errors in the task, as long as the clock counter did not stop or slow to such an inordinate degree that stimuli shared the same clock value. It is questionable whether clock processes as defined in SET would meter this type of task, given that memory is intrinsically linked with time in a way that is qualitatively and quantitatively outside the milliseconds- and seconds- range of the phenomena investigated by internal clock theorists. Also considering the lack of any dysfunction on this task in the patient groups, the results of this study suggest that the memory for temporal order task taps processes different from the temporal processing required for remembering target intervals, which rely on an 'internal clock' system.

5.5.6 Conclusions

Basal ganglia

1. Reduced levels of dopamine (i.e. PD patients 'off' medication vs 'on' medication and PD patients 'off' medication vs less severe de novo PD patients) resulted in increased absolute error on the time production task. When group differences in the UPDRS scores were taken into account, the PD patients 'off' medication and the PD de novo patients showed significantly different patterns of variability. Taken together, this suggests that the efficacy of dopamine levels is important for producing intervals of time in the seconds-range. The limited cognitive demands of the task and the lack of difference in attentional processing 'on' and 'off' medication suggest that this result reflects a 'timer' dysfunction.

2. The greater absolute error on the time reproduction task for the group of less severe de novo PD patients compared to a more severe PD group tested 'off' medication, suggests against a simple linear relationship between severity of basal ganglia dysfunction and timing deficits on this task. The lack of an effect of medication for the PD-drug group ('on vs 'off') suggests different processes underlie performance compared to the time production task.

3. Medication also had a differential effect on some measures of IRI and variability for the PD-drug group on the repetitive tapping task. This suggests that striatal dopamine levels affect a range of motor and perceptual timing tasks and presents compelling evidence for the role of the basal ganglia in temporal processing.

Cerebellum

4. There is evidence of significantly increased variability for patients with cerebellar disease on the repetitive tapping task, and an abnormal pattern of variability on the time reproduction task that was also observed in the PD-drug-off group. Furthermore, variability was elevated (although not necessarily at the corrected significance level) for the CD group in the three tasks that included the greatest motor demands, but not in the time production task in which the motor demands were negligible. This suggests that the pathological pattern of variability may be intrinsically tied with motor execution and not with timer function per se.

Additional findings

5. On the warned and unwarned reaction time tasks all groups showed enhanced RTs when a warning tone was provided. However, the lack of any significant group or medication effects suggests that perhaps this task does not tap motor or perceptual timing processes in a similar fashion to the other tasks employed.

6. The memory for temporal order was not deficient in either the CD or PD-drug groups. This suggests that organising and sequencing temporal information does not rely on the same processes as in motor and perceptual timing.

7. Further investigation of the differential effects of target interval length is required, with the data suggesting that variability on the time reproduction task is influenced by whether the target interval is greater or less than 1 s for the CD and PD group tested 'off' medication.

Furthermore, for the PD-drug-off group on the repetitive tapping task, the variance for the 250 ms interval (continuation phase) was explained by disease severity and the variance for the 2000 ms interval by attentional proficiency.

Chapter 6

Motor timing in Parkinson's disease and the effect of apomorphine studied with positron emission tomography

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6.1 INTRODUCTION

The functional imaging study reported in Chapter 3 suggests that the basal ganglia are involved in millisecond- and seconds-range timing. Furthermore, the clinical study presented in the pervious study has shown that effective levels of dopamine influence the timing performance of patients with Parkinson's disease (PD). Indeed, previous research has also shown that dopaminergic medication ameliorates motor and perceptual timing problems in patients with PD (O'Boyle et al, 1996; Pastor et al, 1992ab). These results are compatible with the hypothesis that the basal ganglia play a fundamental role in metering timing behaviour. This study is interested in further exploring the effect of dopamine on timing behaviour, using PET to explore how dopamine moderates neural activity related to motor timing.

The neural correlates of motor behaviour in PD have previously been investigated using functional imaging. Relative to matched controls, unmedicated patients with PD show underactivation of mesial fronto-striatal circuitry including the putamen, SMA (particularly the rostral part, or pre-SMA), anterior cingulate and DLPFC during simple motor tasks (Haslinger et al, 2001; Jahanshahi et al, 1995; Jenkins et al, 1992; Playford et al, 1992; Sabatini et al, 2000; Samuel et al, 1997). The underactivation is thought to be the result of excessive inhibitory output from the internal globus pallidus (GPi), which projects to motor cortical areas including the lateral premotor cortex, SMA and primary motor cortex (Alexander et al, 1986; Middleton and Strick, 2000). In addition, in some studies compensatory overactivity has been observed in the premotor area and parietal cortex (e.g. Catalan et al, 1999; Samuel et al, 1997). Overactivity of the ipsilateral cerebellum has been observed by others during the performance of self-generated movements by patients with PD tested 'off'

medication (Rascol, 1997). The premotor, parietal and cerebellar overactivity in PD relative to normals are considered to represent the recruitment of compensatory parallel motor circuits, specifically cerebellar-lateral parietal-lateral premotor connections, interpreted as a switch to using intact neural circuits (Brooks, 2001). The parietal cortex is known to be important for sensory-motor integration, intention and attention (e.g. Andersen and Buneo, 2002) whereas the role of the premotor cortex in externally generated movements has been established (e.g. Mushiake et al, 1991; Passingham, 1985), thus the circuitry could be providing sensory-guided movement generation (Samuel et al, 1997). This hypothesis concurs with the clinical observation of deficits in self-initiated movements coupled with preserved performance of externally guided movements in patients with Parkinson's disease (Martin, 1967). Levodopa has been found to partially normalise the dysfunctional activation found during simple motor tasks, including underactivation in the SMA and cerebellum, and overactivity in the primary motor cortex, lateral premotor cortex and superior parietal cortex (Haslinger et al, 2001; Rascol et al, 1997). Similarly, apomorphine (a dopamine receptor agonist) has shown to reverse the underactivation of the SMA in these patients as well as eliminate the overactivity in the cerebellar hemispheres (Jenkins et al, 1992; Peters et al, 2003; Rascol et al, 1992).

Despite the wealth of research investigating motor performance in PD, to date only a single study has used functional imaging to investigate motor timing in PD (Elsinger et al, 2003). As dopamine is known to influence temporal performance in patients with PD, the study presented here used PET to investigate the difference in neural activity in a motor timing task with patients 'on' vs 'off' medication. This is of particular interest as the motor circuit between SMA/lateral premotor cortex and the putamen that is underactive during simple motor tasks involves the same circuitry that has been implicated in temporal processing. The study used the most well-known measure of motor timing, the repetitive tapping paradigm (Wing and Kristofferson, 1973ab). Data from the synchronisation and continuation phases (presented separately as a synchronisation task and a continuation task) allowed the investigation of internally paced timing and timing that is driven by an external cue. In contrast

to the study of Elslinger et al (2003), in which the subjects were assessed on dopamine agonist medication (only levodopa medication was withdrawn), this study compared motor timing after withdrawal of medication for 12 hours ('off' condition) and after injection of apomorphine ('on' condition).

6.1.1 Aims of the study

1. To compare motor timing (synchronisation task and continuation task) to a well-matched control task for both patients with PD and healthy controls, to identify regions active during motor timing.

2. To investigate the differences in activity elicited by externally-paced (synchronisation task) and internally-paced (continuation task) motor timing for both patients with PD and healthy controls.

3. To investigate the effect of apomorphine injections on task performance, particularly motor timing, for the patients with PD, including apomorphine-mediated striato-frontal coupling.

6.2 MATERIALS AND METHOD

6.2.1 Subjects

8 patients with idiopathic PD (7 male; 1 female) and 8 healthy controls (4 male; 4 female) participated. The clinical diagnosis of idiopathic PD was established according to the criteria of the UK Parkinson's Disease Society Brain Bank (Hughes et al, 1992). All participants were right handed. The subjects' right-handedness was formally assessed using a modified version of the Handedness Inventory (Oldfield, 1971). All subjects were found to be strongly right handed, with a mean score of 86 (SD = 7.7) in the PD group and a mean score of 84 (SD = 5.2) in the control group. Mean age was 57.88 years (SD 6.79; range 49-70) in the patient group and 61 years (SD 10.39; range 40-75) in the healthy control group. This difference was not statistically significant. There

was no history of additional neurological disease in the PD group. There was no history of neurological illness, head injury or psychiatric illness in the control group. The Mini-Mental State Examination (MMSE: Folstein et al, 1975) was used for cognitive screening, with all subjects scoring above the cut-off of 27, indicating absence of cognitive impairment (PD: mean = 28.63 (SD 1.06); control: mean = 29.13 (SD 0.99)).

The patients had been diagnosed with idiopathic Parkinson's disease by a neurologist following attendance at a movement disorders clinic. They were assessed as Hoehn and Yahr grade 3-4 whilst 'off' medication (mean 3.5; SD 0.53) (Hoehn and Yahr, 1967). The average duration of the disease was 15.25 years (range 11 – 20 years; SD 3.62). All 8 PD subjects were receiving apomorphine drug therapy. Apomorphine is a dopamine receptor agonist that is administered through subcutaneous injection ('rescue therapy' offering short lasting effect) or subcutaneous infusion (for symptom relief during waking hours) (Frankel et al, 1990; Richardson et al, 1999). Five patients took apomorphine through subcutaneous injection and used it intermittently to relieve 'off' periods, which varied between 3 per day and 2 per week. Two patients used a subcutaneous apomorphine infusion pump throughout the waking day. Seven of the eight patients were chronically exposed to apomorphine, with one patient being new to the drug (average duration of use 20.56 months (SD 25.66)). This patient was taking domperidone, a peripheral D2 antagonist, to protect against the emetic side effects of apomorphine. Domperidone has the additional effect of reducing the global increases in regional cerebral blood flow (rCBF) that can occur with patients who are not chronically exposed to the drug. All patients were also prescribed levodopa. A full summary of the clinical details can be found in Table 6.1.

Table 6.1: Information about the patients with Parkinson's disease

Patient number	Gender	Age (years)	Hoehn & Yahr (OFF)	Duration of illness (years)	Duration of apomorphine therapy (months)	Dose of apomorphine	Dose of additional medication/day[1]	Apomorphine after scan 6 (half way)
1	M	49	4	11	19	7.5 mm/hr*	Madopar 500 mg (400 mg)	5 mg
2	M	70	3	18	0.1	2.5 mg (N/A)**	Sinemet Plus 1000 mg (800 mg) Amantadine 200 mg Cabergoline 2 mg	2.5 mg
3	M	56	4	13	6	4 mm/hr*	Madopar 625 mg (500 mg)	6 mg, 8 mg
4	M	61	3	18	11	4 mg (0-2/day)**	Sinemet CR 1000 mg (800 mg) Entacapone 800 mg Pergolide 4 mg	5 mg
5	M	54	3	13	4	4 mg (2/week)**	Madopar 1000 mg (800 mg) Madopar dispersible 250 mg (200 mg) Madopar CR 125 mg (100 mg) Entecapone 1200 mg Cabergoline 6 mg	4 mg
6	M	51	4	11	2.5	3 mg (3/day)**	Madopar 1125 mg (900 mg) Madopar CR 1125 mg (900 mg) Selegeline 10 mg Cabergoline 5 mg	3 mg, 3 mg, 5 mg
7	M	60	4	18	59	6 mg (2-3/day)**	Sinemet Plus 1000 mg (800 mg) Sinemet CR 250 mg (200 mg) Entacapone 400 mg Pergolide 0.75 mg	6 mg
8	F	62	3	20	63	4 mg (2/week)**	Madopar 870 mg (700 mg) Cabergoline 2 mg	4 mg
Mean		57.88	3.50	15.25	20.58			6.44
SD		6.79	0.53	3.62	25.64			3.00

** Subcutaneous infusion in mm/hr given each day during waking hours ** 'Rescue injection': frequency of administration given in brackets [1]Dose of Sinemet followed by relative amount of levodopa in brackets*

6.2.2 Design

Participants completed three tasks, synchronisation, continuation and reaction time. Two groups of subjects, PD and control, were included. During the PET scanning, each task was repeated four times, culminating in 12 scans per subject. To assess the influence of dopamine on motor timing, the PD group were tested 'off' medication for the first six scans and 'on' medication for the last six. The patient group completed each of the three tasks twice in both drug states. The order of task presentation was pseudo-randomised using a Latin Square procedure.

6.2.3 Procedure

The PD group were scanned following approximately 12 hours overnight withdrawal of their anti-parkinsonian medication (levodopa and apomorphine). The subjects were familiarised with the three tasks prior to the scanning.

6.2.3.1 Synchronisation task

Participants were instructed that they would be required to tap in synchrony with a tone (1000 Hz, duration 50 ms), which would be presented at regular intervals with an inter-stimulus interval of 1000 ms. They were asked to remain in synchrony with the tone and not to pre-empt it or produce a delayed response. The participants were instructed to listen, without responding, to the first few tones to establish a rhythm. A block ended when the participant had made 150 responses.

6.2.3.2 Continuation task

As in the synchronisation task, participants were instructed to tap in time with a tone (1000 Hz, duration 55 ms) that was presented at regular intervals (1000 ms). However, after 30 button presses the pacing tone ceased. Participants were instructed that when the pacing tone stopped, they should continue tapping and try and maintain the rhythm as accurately as possible. The participants were instructed to listen, without responding, to the first few tones

to establish a rhythm. To control for the auditory component in the synchronisation task, button presses produced by the participants were followed by a tone of a lower frequency (950 Hz, duration 55 ms). A block consisted of 150 responses, 30 with the pacing tone and 120 without. PET data was acquired during the Continuation Task only.

6.2.3.3 Control reaction time task

This was a simple reaction time (RT) task. A tone (1000 Hz, duration 50 ms) was presented at a mean rate of every 1000 ms. Participants were instructed to press the response button as quickly as possible in response to each tone. The inter-stimulus interval varied randomly between 850 ms and 1150 ms to prevent the subjects anticipating the tone. A block consisted of 150 responses.

The tasks were programmed in Quick Basic and run on a Dell laptop. The same response box was used exactly as described in Chapter 3. The response times were recorded to the nearest millisecond. All responses were made with the right index finger. During the practice trials, the tones were presented through a loudspeaker. When the subjects were in the scanner the tones were presented through earphones, with the sound level adjusted for maximum comfort.

6.2.3.4 Apomorphine administration

Apomorphine is suitable for use in functional imaging experiments, as it produces no significant change in resting rCBF in patients who have been chronically exposed to it (Jenkins et al, 1992). rCBF increases are observed in patients who are apomorphine-naive, but this effect is not observed if domperidone is administered (Sabatini et al, 1991). A further reason for using apomorphine is its fast-acting effect, which had practical benefits in the context of an imaging study. Prior to the scanning sessions, consultation with each patient established his or her optimal apomorphine dose to be administered during scanning (see Table 6.1). Subjects who used 'rescue' injections were given their normal dose; those who used a pump were given a dose established after a discussion with the patient and the result of their initial apomorphine 'challenge' test. The dose was given after scan 6, half way through the scanning session. When the patients subjectively felt they were 'on' and this agreed with

the neurologist's motor assessment, the scanning was continued. During the final six scans the patient was given more apomorphine if they started to turn 'off'. Before the patient entered the scanner, severity of their motor symptoms were assessed using a modified version (items 20, 22, 23, 24 and 25 of Part III: Motor Examination) of the United Parkinson's Disease Rating Scale (UPDRS: Fahn et al, 1987). The modified version of the UPDRS was used as the items selected could all be assessed while the subject was lying in the scanner. They were also assessed on the UPDRS after the apomorphine injection when in the 'on' state, before the latter half of the scans commenced, and immediately at the end of the scanning session. All of the procedures described were conducted by a neurologist.

6.2.3.5 Additional tests

The National Adult Reading Test (NART: Nelson, 1982), Paced Auditory Serial Addition Test (PASAT: Gronwall and Wrightson, 1981) and Beck Depression Inventory (BDI: Beck et al, 1961) were administered to all subjects. These tasks, and their motivation for inclusion in the study, are described in Chapter 5. The measure of self-reported stress and arousal (Mackay et al, 1978), also described in Chapter 5, was also used. This was completed by all participants three times: immediately prior to scanning, before the start of scan 7 (after the patients had been given apomorphine and been assessed as being 'on' medication) and at the end of the scanning session.

6.2.4 PET

Measurements of rCBF and structural magnetic resonance imaging (MRI) scans were obtained in an identical manner to those described in Chapter 3.

The PET images were reconstructed and analysed using statistical parametric mapping software (SPM99, Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm) executed in Matlab (Mathworks Inc., Sherbon, MA), as described in Chapter 2.

As in Chapter 3, the general linear model (GLM) was used to estimate effects at each voxel point in the brain (Friston et al, 1995b). Scan to scan differences in global blood flow were modelled as a confounding covariate. The statistical analysis was aimed at identifying regions of the brain specific to motor timing, to internally-paced (continuation task) versus externally-paced (synchronisation task) motor timing and to the effect of apomorphine injections on motor timing, both within- and between-subjects. The level of significance was set to $p < 0.05$, corrected for multiple comparisons. Regions of the brain for which there was an a priori hypothesis, were reported at $p < 0.001$, uncorrected.

In addition to the primary analysis, another area of interest is how the apomorphine would modulate effective connectivity between the basal ganglia and the rest of the brain in the PD group. This was investigated using the method of psychophysiological interaction (PPI), as described by Friston et al (1997). As described in Chapter 2, PPIs aim to explain regionally specific responses in terms of an interaction between activity in a particular cortical area (index area) and the influence of an experimental parameter. PPIs are limited to testing regions for which there is an a priori hypothesis about decreased responsiveness or increased influence under given conditions. The physiological variable was defined as the first eigenvariate of the rCBF signal from a sphere (radius 8 mm) centred on the voxel in the left head of the caudate nucleus that showed increased activation in the 'off medication' compared to the 'on' medication collapsed across all three experimental tasks (see Table 6.7). The experimental variable was whether the patients were in the 'on' or 'off' medication state. Modelled within SPM, these two regressors were multiplied together to create a third regressor (covariate of interest), which represented the interaction between the two variables. The resulting SPM {t} reflected the significance of the PPI, where a significant value reflects a difference in the regression slopes linking the activity in the left caudate nucleus to activity in other brain areas, depending on whether the patients were 'on' or 'off' medication. A significant increase in the regression slope (positive interaction) was interpreted as an increase in effective connectivity between the left caudate nucleus and other brain regions in the 'off' medication condition compared to the 'on' medication condition. Whilst a significant decrease in the regression

slope was interpreted as a decrease in effective connectivity between the left caudate nucleus and other brain regions in the 'off' medication condition compared to the 'on' medication condition.

Anatomical localisation of the significant voxel coordinates was determined using the participants structural MRIs and a group average MRI and with reference to the atlas of Durvenoy (1999). In addition, the standard stereotatic atlas of Talairach and Tournoux (1988) was used for further reference with regard to Brodmann areas. Detailed information about the location of voxels in the cerebellum was gained with reference to an MRI atlas of the cerebellum (Schmahmann et al, 2000). For the primary motor cortex and somatosensory area, probabilistic cytoarchitectonic atlases have been produced and these were also used (Geyer et al, 1996; Geyer et al, 1999; Geyer et al, 2000).

6.3 RESULTS

6.3.1 Behavioural results

The two groups scored similarly on the PASAT (average error score: PD = 10.5 (SD 8.28); control = 3.5 (SD 5.45), not sig.), which suggests that they did not significantly differ in ability related to attention. For the BDI, the PD patients averaged a higher score (mean = 13; SD = 4.57) than the control group (mean = 6.5; SD = 4.14). This difference was statistically significant ($t(14) = 2.98$; $p = 0.01$) and suggests mild self-reported depression in the patient group. The NART results showed that the control group (mean = 119; SD = 3.82) had significantly higher verbal IQs than the PD group (mean = 110.29; SD = 5.12) ($t(13) = 5.46$; $p > 0.001$), although the scores put both groups in the high average range. Levels of arousal and stress were measured at three time points throughout the scanning period using a questionnaire (Mackay et al, 1978). Figure 6.1ab illustrates the scores obtained from the two groups, with arousal scores and stress scores plotted separately.

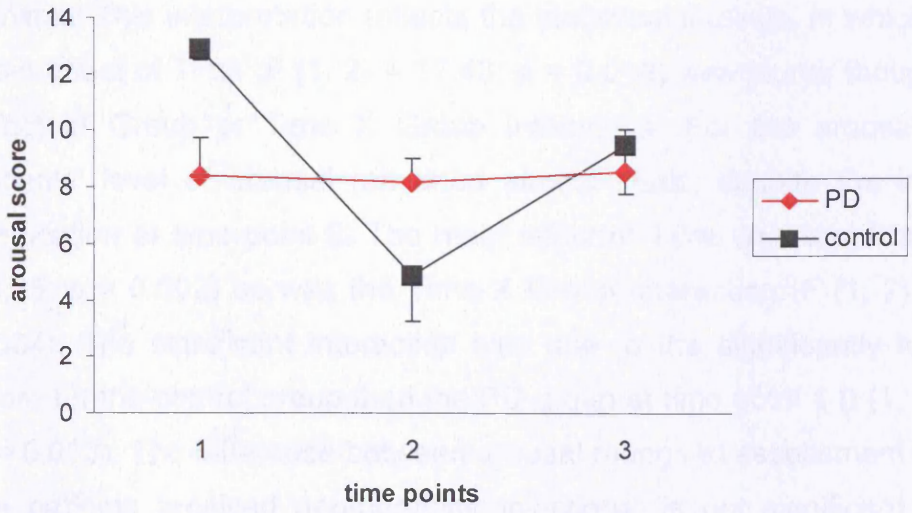

Figure 6.1a: Self-reported arousal scores for the PD and control subjects, taken at time points 1 (prior to scanning), 2 (just before Scan 7) and 3 (at the end of scanning) (± SE)

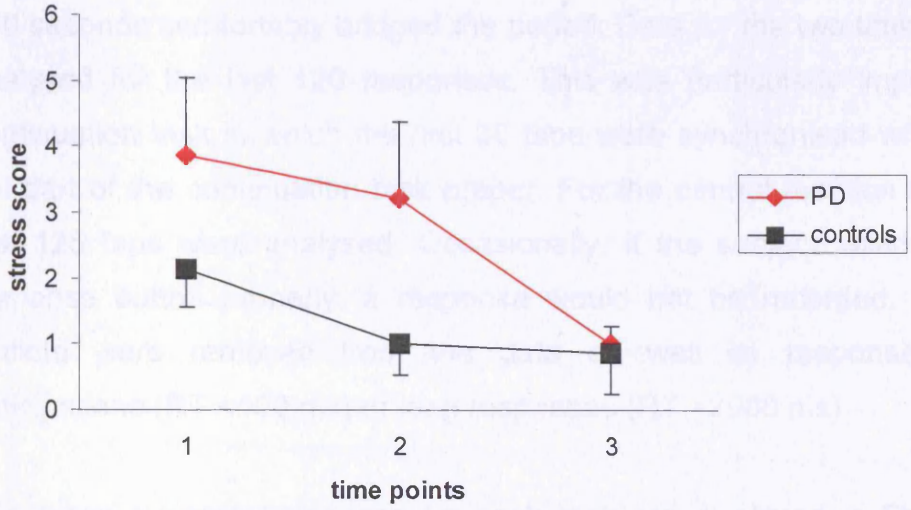

Figure 6.1b: Self-reported stress scores for the PD and control subjects, taken at time points 1 (prior to scanning), 2 (just before Scan 7) and 3 (at the end of scanning) (± SE)

Two mixed-factorial ANOVAs (one for the arousal scores and one for the stress scores) were used to compare scores over the three time points for the two

groups. As can be seen, both groups' self-reported stress levels reduced during the scanning period, although the difference between the two groups was minimal. This interpretation reflects the statistical findings, in which a significant main effect of Time ($F(1, 2) = 17.43$; $p = 0.018$) was found, though not a main effect of Group or Time X Group interaction. For the arousal ratings, the patients' level of arousal remained almost static, despite the introduction of medication at time point 2. The main effect of Time was significant ($F(1, 2) = 68.28$; $p = 0.002$) as was the Time X Group interaction ($F(1, 2) = 60.60$; $p = 0.004$). The significant interaction was due to the significantly higher arousal score for the control group than the PD group at time point 1 ($t(1, 7.92) = -3.19$; $p = 0.013$). The difference between arousal ratings at assessment point 2, when the patients received apomorphine injections, is not significant. As a result, changes in arousal in the PD group were not confounding medication-dependent effects.

The mean response time was measured for all three tasks. The 'active' window during PET scanning was 90 seconds, and the performance for each task for 150 seconds comfortably bridged the period. Data for the two timing tasks were analysed for the last 120 responses. This was particularly important for the continuation task in which the first 30 taps were synchronised with a tone and not part of the continuation task proper. For the control reaction time task, the first 125 taps were analysed. Occasionally, if the subject failed to press the response button properly, a response would not be recorded. The resulting outliers were removed from the data as well as responses that were anticipations (RT <100 ms) or long responses (RT >2000 ms).

The mean average responses for each task are displayed in Figure 6.2. The Control data is collapsed across all scans (there was no significant difference in performance between the first and second six scans for this group), whereas the PD data shows the different results for the first six scans ('off' medication) and the second six scans ('on' medication). Both groups were more accurate on the synchronisation task than the continuation task. Additionally, the PD group underestimated and were less accurate than the healthy controls. Performance in the PD group appeared to be augmented (i.e. became more similar to

controls) after the administration of apomorphine. The data was tested statistically using a mixed factorial ANOVA: a 2 (Task) x 2 (Scan: 1st six or 2nd six scans) repeated measures ANOVA, with an additional between group factor of Group (PD or control). The main effect of Task was significant ($F(1, 14) = 29.88$, $p < 0.0001$), indicating the less accurate performance in the continuation task. However, the main effect of Scan and Group were not significant and none of the resulting interactions were significant. For the control reaction time task, the control subjects were faster than the PD group and the patients' reaction times were faster in the 'on' than the 'off' condition. Once again, these data were statistically tested using a mixed factorial ANOVA, with the within group factor of Scan (1st six or 2nd six) and the between group factor of Group (PD or control). There was no statistically significant difference between reaction times for the first six and second six scans. However, there was a main effect of Group ($F(1, 14) = 16.87$, $p < 0.001$), reflecting significantly slower reaction times in the patient group. The interaction of the two main effects was not significant.

As well as analysing accuracy, there was also interest in measuring variability in performance, as represented by the standard deviation score for each task. Figure 6.3 displays the data and it can be seen that the data reflect the pattern of the mean responses, with the control subjects having lower variability than the patient group and with variability being smaller for the synchronisation task than the continuation task. A mixed factorial ANOVA (2 (Task) x 2 (Scan) x 2 (Group)) showed that there was a significant main effect of Task ($F(1, 14) = 21.43$, $p < 0.001$) but not of Scan or Group, nor were there any significant interactions. For the control reaction time task, data were analysed with an ANOVA with Scan and Group as the factors. A significant main effect of Group was found ($F(1, 14) = 11.55$, $p < 0.004$). The main effect of Scan and the interaction were not significant.

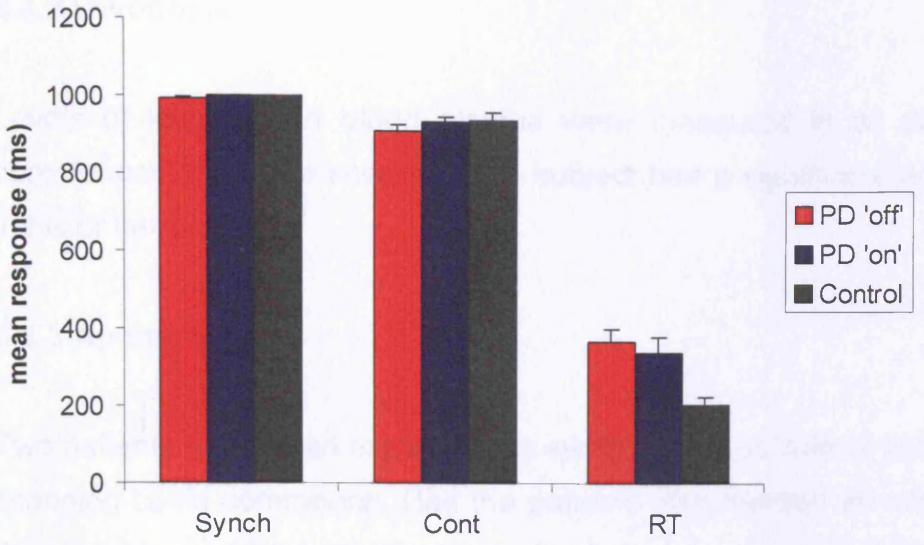

Figure 6.2: Mean inter-response interval in the synchronisation (Synch) and continuation (Cont) tasks and mean reaction time (ms) in the control RT task (± SE)

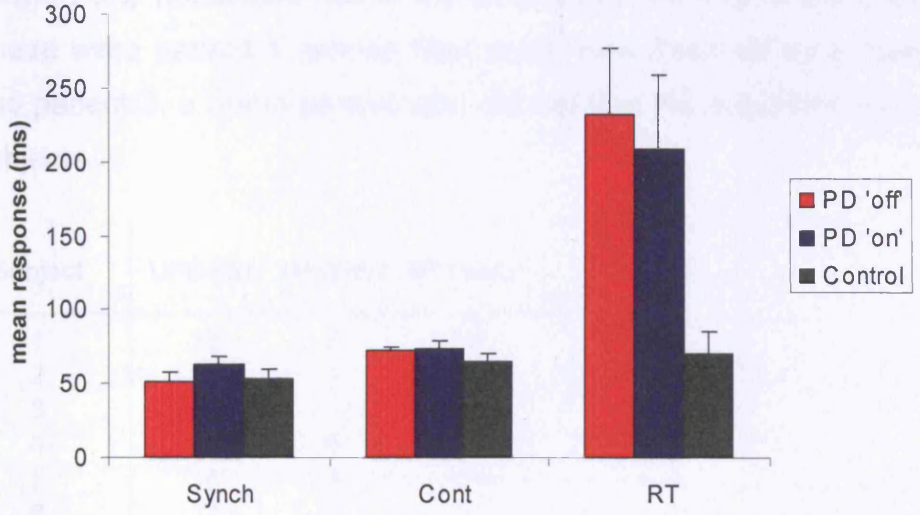

Figure 6.3: Mean standard deviation for the inter-response interval in the synchronisation (Synch) and continuation (Cont) tasks and mean standard deviation for the mean reaction time (ms) in the control RT task (± SE)

6.3.2 Levodopa

Levels of levodopa in blood plasma were measured in all patients prior to commencement of the scanning. No subject had a significant level of levodopa in his or her blood.

6.3.3 Apomorphine

Two patients needed an extra dose to switch them 'on' before the second half of scanning could commence. Half the patients also needed an extra dose during the six 'on' scans because they were beginning to show signs of the medication effects wearing off. The UPDRS was competed at three time points. These data are presented in Table 6.2. As evident from Table 6.2, the UPDRS scores drop following the administration of apomorphine, indicating the patients were in an 'on' state when the second half of scanning began. Furthermore, patients remained in this 'on' state to the end of the scanning session. Only two patients showed any noticeable rise in the UPDRS by the end of the scanning session. These were patient 1, whose final score was distorted by a 'freezing' episode and patient 3, a pump patient who did not find the injections as potent as other subjects.

Subject	UPDRS1	UPDRS2	UPDRS3
1	36	8	24
2	20	6	5
3	24	10	20
4	16	6	9
5	14	3	3
6	17	1	0
7	19	6	2
8	18	5	8
Mean	**20.50**	**5.63**	**8.88**
SD	**6.93**	**2.77**	**8.69**

Table 6.2: UPDRS scores for the patients with Parkinson's disease at the three time points

6.3.3 PET results

Analysis was centred on three topics of interest:

1. The functional anatomy of motor timing (synchronisation task and continuation task) versus control task, both within and between groups.

2. The effect of internally-paced (continuation task) versus externally-paced (synchronisation task) timing, both within and between groups.

3. The effect of apomorphine injections on task performance, particularly motor timing, in the PD group and striato-frontal coupling relative to the dopamine-depleted state.

6.3.4.1 The effect of the timing tasks on rCBF

A comparison between the two timing tasks (synchronisation task and continuation task) and the control reaction time task was used to determine areas of the brain that are significantly more active during a motor timing task, once non-temporal factors such as attention, anticipation, response initiation and execution have been controlled for.

Within group effect:

Healthy controls

Significantly greater activation in the timing tasks that the control task was found in the bilateral angular gyrus (BA 39), right hippocampus, left superior frontal gyrus (BA 9/10) as well as a more caudal medial frontal region (BA 10), left posterior cingulate and left nucleus accumbens. The left nucleus accumbens activation was further explored following the a priori prediction that the basal ganglia are involved in temporal processing. At the more generous threshold of $p < 0.01$, this area was extended to include additional striatum, particularly the caudate nucleus. Conversely, the areas more active in the control task than the timing tasks included the right insula (boarding on the putamen), the left anterior cingulate (BA 32), and the right superior temporal gyrus (BA 22). Subcortical regions for the latter contrast included the right mediodorsal thalamic nucleus,

an area approximating the left medial geniculate body and left substantia nigra and extending to the left ventral lateral thalamic nucleus as well as the cerebellar vermis and left cerebellar hemisphere. These data are presented in Figure 6.4 and 6.5 and Table 6.3.

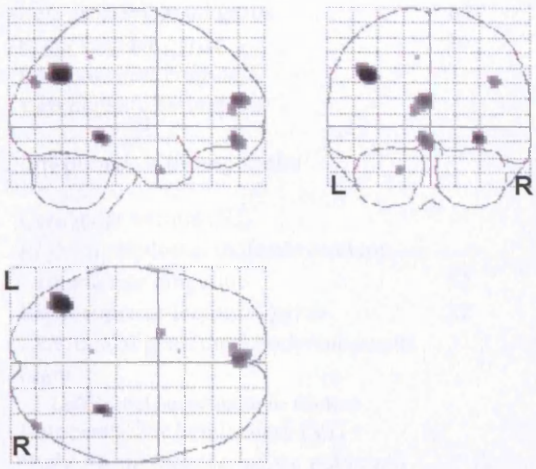

Figure 6.4: Main effect of timing tasks (timing tasks > control) for the control subjects

Results are displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space. Significant at p < 0.001, uncorrected.

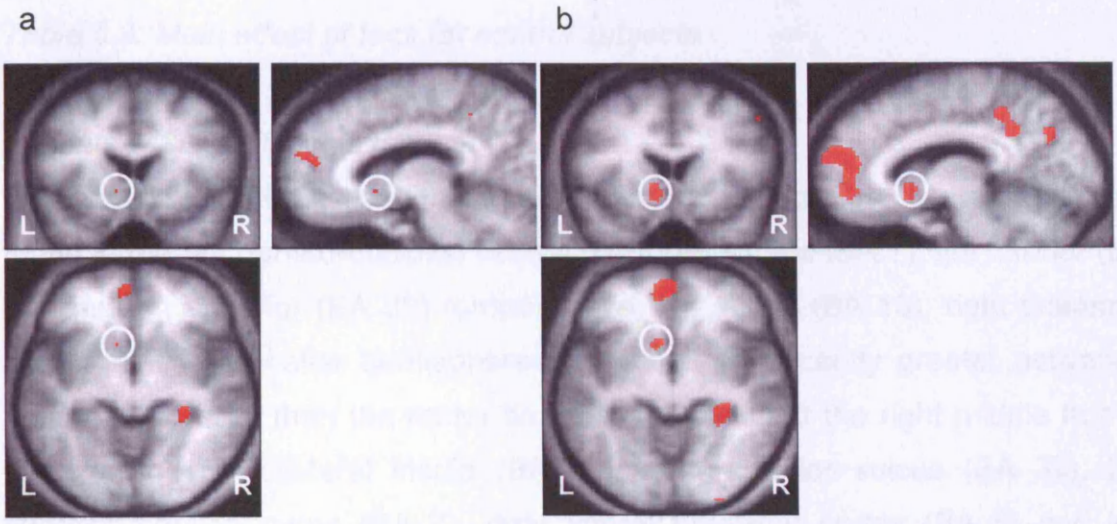

Figure 6.5: Basal ganglia activation in the main effect of timing tasks (timing tasks > control) for the control subjects

(a) significant at p < 0.001, uncorrected (b) significant at p < 0.01, uncorrected. Results are displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space.

	BA	MNI coordinates of peak activation x	y	z	Z score*
Timing tasks > control task					
Left angular gyrus	39	-42	-64	36	4.59
Right hippocampus		32	-38	-8	4.05
Left superior frontal gyrus	9/10	-2	54	18	3.93
Left superior frontal gyrus	10	-10	50	12	3.39
Left medial frontal gyrus	10	-4	52	-8	3.74
Right angular gyrus	39	42	-84	32	3.52
Left posterior cingulate		-8	-44	48	3.13
Left nucleus accumbens		-8	14	-8	3.10
Control task > timing tasks					
Cerebellar vermis (VI)		0	-72	-24	4.04
Right mediodorsal thalamic nucleus		6	-18	4	3.95
Left anterior cingulate	32	-4	12	46	3.84
Right superior temporal gyrus	22	66	-36	12	3.58
Left medial geniculate body/substantia nigra		-12	-22	-6	3.55
Left ventral lateral thalamic nucleus		-10	-20	4	3.53
Left cerebellar hemisphere (VI)		-28	-60	-28	3.31
Right insula (boardering on putamen)		36	8	-4	3.30

* all significant at $p < 0.001$, uncorrected

Table 6.3: Main effect of task for control subjects

Patients with Parkinson's disease

Significantly greater activation for the timing tasks than the control task was found in the left parieto-occipital fissure, right precuneus (BA 7), left inferior (BA 20) and left superior (BA 38) temporal gyri, left insula (BA 13), right thalamus and bilateral cerebellar hemispheres. Areas of significantly greater activation during the control than the motor timing tasks included the right middle frontal gyrus (BA 8/6), bilateral insula (BA 13), right anterior sulcus (BA 32), left superior parietal gyrus (BA 7), right lateral premotor cortex (BA 6) and left inferior (BA 20) and bilateral middle (BA 20/21) temporal gyri. These data are presented in Figure 6.6 and Table 6.4.

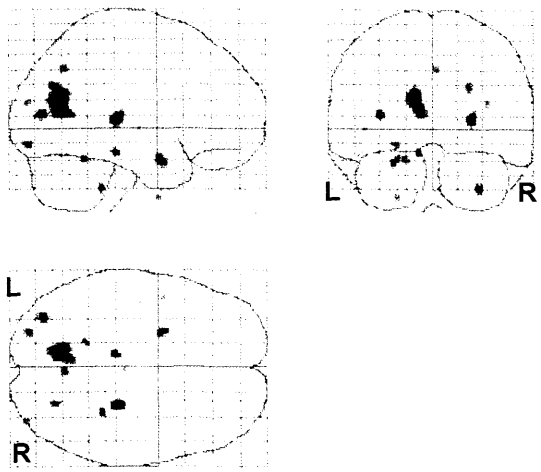

Figure 6.6: Main effect of timing tasks (timing tasks > control) for the patients with Parkinson's disease

Results are displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space. Significant at p < 0.001, uncorrected.

		MNI coordinates of peak activation			
	BA	x	y	z	Z score
Timing tasks > control task					
Left parieto-occipital fissure		-12	-66	20	4.23
Left parieto-occipital fissure		-8	-64	12	3.73
Right thalamus		26	-26	6	3.96
Right cerebellar hemisphere (VI)		32	-26	-42	3.36
Left cerebellar hemisphere (V)		-18	-50	-20	3.30
Left parieto-occipital fissure		24	-70	28	3.27
Right precuneus	7	4	-62	40	3.20
Left inferior temporal gyrus	20	-22	0	-48	3.18
Left superior temporal gyrus	38	-48	4	-18	3.14
Left insula	13	-44	-12	0	3.11
Control task > timing tasks					
Right middle frontal gyrus	8/6	56	18	42	3.76
Right insula		48	16	-10	3.67
Right anterior cingulate sulcus	32	2	16	42	3.60
Left superior parietal gyrus	7	-22	-72	58	3.53
Right lateral premotor cortex	6	54	-2	42	3.30
Right superior temporal gyrus	22/42	68	-38	6	3.29
Left insula	13	-30	4	12	3.20
Left inferior temporal gyrus	20	-68	-28	-16	3.19
Left middle temporal gyrus	21	-64	-46	0	3.16
Right middle temporal gyrus	20/21	66	-44	-10	3.15

* all significant at p > 0.001, uncorrected

Table 6.4: Main effect of task for patients with Parkinson's disease

Between group effect:

Group X Task interaction

Data for the two subject groups were then combined to create a 2 (Task) x 2 (Group) ANOVA. Data was collapsed across the two timing tasks (synchronisation task and continuation task) and compared to the control reaction time task. First, areas more active for the control group than the PD group in the timing task than the control task were examined. This was masked by the timing tasks > control task contrast for the control group to limit the interpretation of the results. A significant result was found in the right middle frontal gyrus (BA 6/8) (x = 56, y = 16, z = 44; Z = 3.42; p = 0.0001, uncorrected), left middle temporal gyrus (BA 21) (x = -68, y = -28, z = -14; Z = 3.41; p = 0.0001, uncorrected), left middle temporal gyrus (BA 21) (x = -58, y = -44, z = -4; Z = 3.27; p = 0.001, uncorrected), right inferior temporal gyrus (BA 20) (x = 70, y = -34, z = -22; Z = 3.20; p = 0.001, uncorrected), left middle temporal gyrus (BA 20/21) (x = -62, y = -52, z = -14; Z = 3.20; p = 0.001, uncorrected) and left head of caudate nucleus (x = -8, y = 12, z = -4; Z = 3.10; p = 0.001, uncorrected). Second, areas more active for the PD than the control group in the timing tasks than the control task were examined. This was masked by the timing tasks > control task contrast for the PD group. Significant activation was found in the right thalamus (x = 26, y = -24, z = 6; Z = 3.57; p = 0.0001, uncorrected) and left cerebellar hemisphere (V) (x = -18, y = -50, z = -20; Z = 3.71; p = 0.0001, uncorrected) and midline vermal area (IV) (x = 6, y = -48, z = -4; Z = 3.82; p = 0.0001, uncorrected). The significant interactions are shown in Figure 6.7 and 6.8.

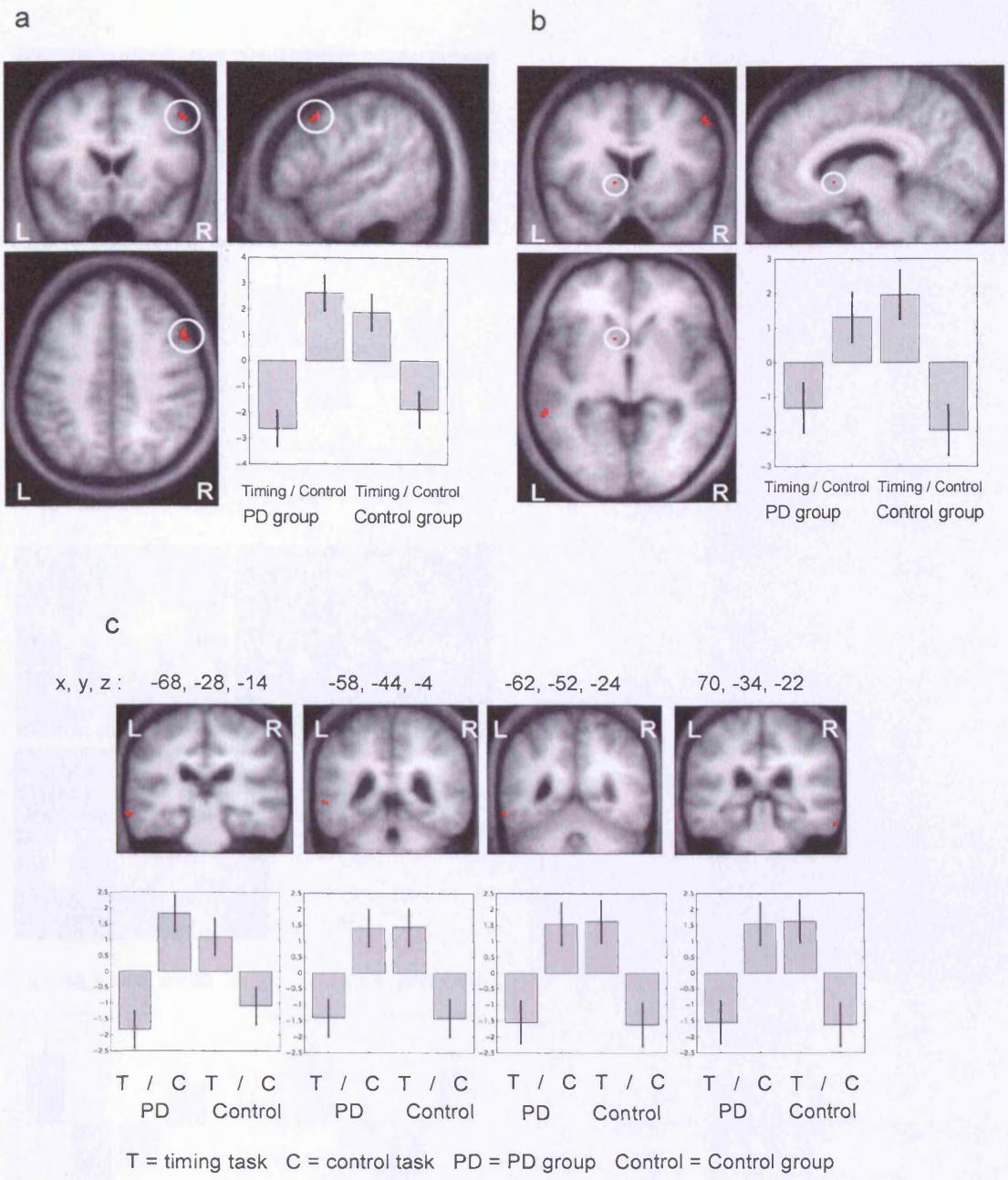

Figure 6.7: Interaction of Task x Group for the control subjects

(a) increased right middle frontal gyrus (BA 6) ((x = 56, y = 16, z = 44) (b) increased left caudate nucleus (x = -8, y = 12, z = -4) and (c) increased left middle temporal gyrus (3 foci: x = -68, y = -28, z = -14; x = -58, y = -44, z = -4; x = -62, y = -52, z = -14) and right inferior temporal gyrus (x = 70, y = -34, z = -22) activation for control subjects, compared to PD subjects, during the timing tasks. Significant at p < 0.001, uncorrected. Parameter estimates showing mean activation during the timing tasks and control task, for each group, are also displayed.

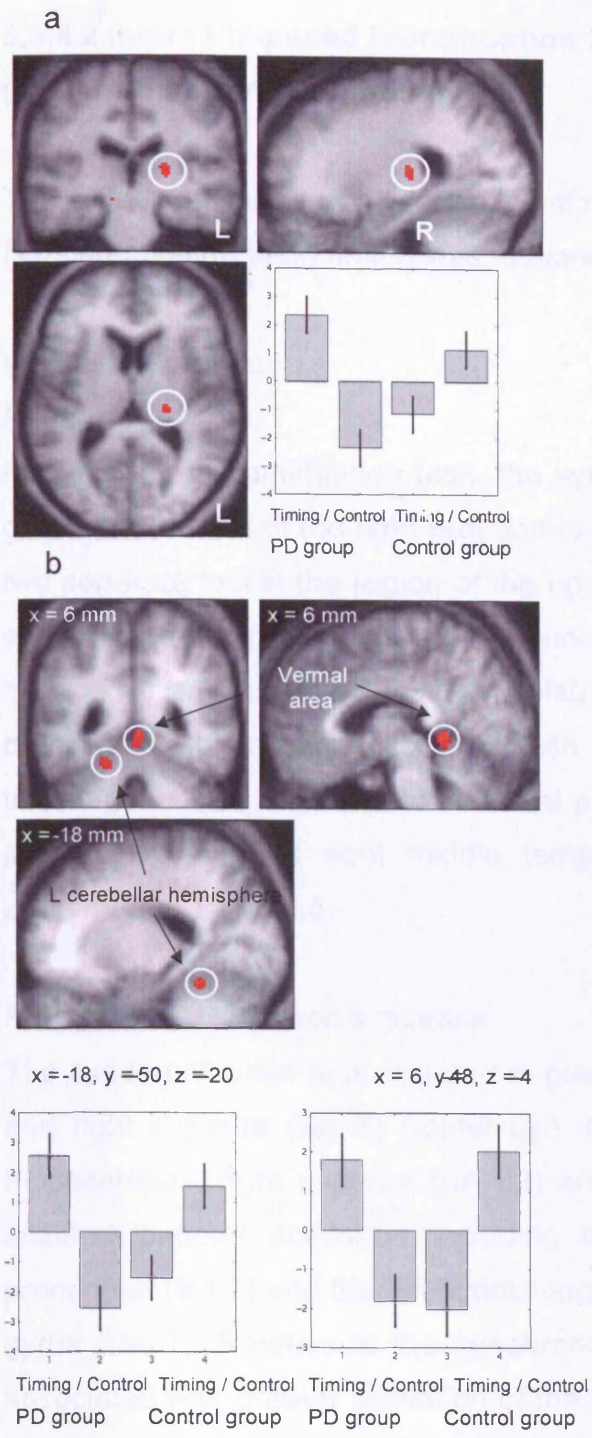

Figure 6.8: Interaction of Task x Group for the patients with Parkinson's disease

(a) increased right thalamus (x = 26, y = -24, z = 6), and (b) increased left cerebellar hemisphere (V) (x = -18, y = -50, z = -20) and midline vermal area (IV) (x = 6, y = -48, z = -4) activation for PD subjects, compared to control subjects, during the timing tasks. Significant at p < 0.001, uncorrected. Parameter estimates showing mean activation during the timing tasks and control task, for each group, are also displayed.

6.3.4.2 Internally-paced (continuation task) versus externally-paced (synchronisation task) timing

The effect of internally-paced (continuation task) versus externally-paced (synchronisation task) timing was explored, both within and between groups.

Within group effect:

Healthy controls

Relative to the continuation task, the synchronisation task was associated with greater activation of the right precuneus (BA 7), left orbitofrontal cortex (BA 11), two separate foci in the region of the right parieto-occipital fissure, one of which extended to the precuneus, right hippocampus, right somatosensory area (BA 1/2) and left insula (BA 13). Relative to the synchronisation task, the continuation task was associated with greater activation of the right inferior frontal gyrus (BA 44), the dorsolateral prefrontal cortex (BA 9 and 46), the left insula (BA 13) and right middle temporal gyrus (BA 21). These data are presented in Table 6.5.

Patients with Parkinson's disease

The synchronisation task resulted in greater activation of the left middle (BA 6) and right superior (BA 6) frontal gyri, right primary motor cortex (BA 4), left hippocampus, right superior (BA 22) and middle (BA 21) temporal gyrus, and bilateral parietal activation including the right parieto-occipital fissure and precuneus (BA 7) and the left supramarginal gyrus (BA 40) and superior parietal gyrus (BA 7). Relative to the synchronisation task, the continuation task was associated with greater activation of the right supramarginal gyrus (BA 40), right dorsolateral prefrontal cortex (BA 9/46), right insula (BA 13), right anterior cingulate (BA 10/32), right orbitofrontal cortex (BA 11), bilateral superior temporal gyrus (BA 22/42), right superior parietal lobe (BA 7) and several foci in the left cerebellar hemisphere was activated. These data are presented in Table 6.6.

	BA	MNI coordinates of peak activation x	y	z	Z score*
Synchronisation > Continuation					
Right precuneus	7	4	-56	42	3.85
Left orbitofrontal cortex	11	-4	52	-10	3.43
Right parieto-occipital fissure		16	-62	14	3.42
Right hippocampus		30	-20	-20	3.41
Right hippocampus		26	-16	-26	3.12
Right parieto-occipital fissure/precuneus		6	-66	24	3.32
Right somatosensory area	1/2	32	-50	62	3.21
Left insula	13	-34	-24	-6	3.12
Continuation > Synchronisation					
Right inferior frontal gyrus	44	60	8	14	3.54
Right middle temporal gyrus	21	68	-40	-10	3.49
Left insula	13	-32	16	-4	3.47
Right dorsolateral prefrontal cortex	9	42	40	30	3.32
Right dorsolateral prefrontal cortex	46	58	38	6	3.14

* all significant at $p < 0.001$, uncorrected

Table 6.5: Synchronisation versus continuation task contrast for the control subjects

Between group effect:

Group X Task interaction

Data for the two tasks and two groups were then compared with a 2 (synchronisation vs continuation) X 2 (Group) ANOVA. There was particular interested in the differences in activation between the two groups for the continuation task, i.e. during internally paced timing. Therefore, areas more active for the control group than the PD group in the continuation than the synchronisation task were examined. This was masked with the continuation versus synchronisation contrast for the control group to limit the interpretation of the results. Significant activation was found in the left superior parietal gyrus (BA 7) ($x = -20$, $y = -82$, $z = 46$; $Z = 3.74$; $p = 0.0001$, uncorrected), the right lateral premotor cortex (BA 6) ($x = 64$, $y = 8$, $z = 36$; $Z = 3.33$; $p = 0.0001$), the left insula (BA 13) ($x = -36$, $y = 16$, $z = -6$; $Z = 3.11$; $p = 0.001$) and the left cerebellar hemisphere/midline (V) ($x = -12$, $y = -56$, $z = -10$; $Z = 3.44$; $p = 0.0001$). Areas that were more active for the PD group than the control group in the continuation than the synchronisation task contrast were also examined.

This was masked by the continuation versus synchronisation contrast for the PD group. The patients showed significantly greater activation in the right cerebellar hemisphere (Crus I) (x = 28, y = -92, z = -24; Z = 3.35; p = 0.0001) and in the left cerebellar hemisphere (Crus II) (x = -12, y = -90, z = -32; Z = 3.20; p = 0.001) than the controls for the continuation than the synchronisation task.

		MNI coordinates of peak activation			
	BA	x	y	z	Z score*
Synchronisation > Continuation					
Right primary motor cortex	4	42	-10	40	3.42
Left hippocampus		-20	-16	-24	3.37
Right superior temporal gyrus	22	60	-10	4	3.29
Right middle temporal gyrus	21	58	0	-24	3.28
Left supramarginal gyrus	40	-48	-52	30	3.28
Right parieto-occipital fissure		24	-72	26	3.72
Right precuneus	7	6	-72	32	3.21
Left middle frontal gyrus	6	-32	-2	44	3.14
Right superior frontal gyrus	6	30	-4	66	3.12
Left superior parietal gyrus	7	-18	-80	48	3.10
Continuation > Synchronisation					
Right supramarginal gyrus	40	70	-42	40	4.29
Right dorsolateral prefrontal cortex	46	52	46	8	4.04
Right dorsolateral prefrontal cortex	9/46	44	40	28	3.52
Right insula		56	6	2	3.93
Left cerebellar hemisphere (Crus I)		-34	-78	-24	3.73
Left cerebellar hemisphere (Crus I)		-34	-88	-22	3.3
Right anterior cingulate	10/32	18	42	-8	3.56
Left cerebellar hemisphere (Crus II)		-12	-90	-32	3.47
Right orbitofrontal cortex	11	36	46	-12	3.27
Right superior temporal gyrus	22/42	50	-28	6	3.24
Left superior temporal gyrus	22/42	-66	-40	20	3.15
Right superior parietal gyrus	7	48	-52	60	3.11
Left cerebellar hemisphere (Crus I)		44	-60	-30	3.09

* all significant at p < 0.001, uncorrected

Table 6.6: Synchronisation versus continuation task contrast for the patients with Parkinson's disease

6.3.4.3 The effect of apomorphine injections on rCBF

To examine the effect of apomorphine injections in the patient group, a separate 2 (Task) x 2 (Drug state) ANOVA was used with data collapsed across the two timing tasks (synchronisation task and continuation task).

Main effect of task

This was reported above in (1)

Main effect of drug

For the 'off' medication versus 'on' medication contrast, significant increases were dominated by subcortical and cerebellar structures including the bilateral cerebellar hemispheres, right head of the caudate nucleus spreading to the putamen and globus pallidus, left head of caudate nucleus and the left thalamus. Significant cortical increases were found in the right middle frontal gyrus (BA 10), right superior frontal gyrus (BA 9), left orbitofrontal gyrus (BA 11), right frontopolar gyri, and left inferior temporal gyrus (BA 22). These data are presented in Figure 6.9 and Table 6. 7.

For the 'on' medication versus 'off' medication contrast, significant increases were found in a widespread, right hemisphere dominant, cortical network including the left middle frontal gyrus (BA 10/46), right superior frontal gyrus (BA 9 and 10), left inferior frontal gyrus (BA 44), right anterior cingulate (BA 32), right lateral premotor cortex (BA 6) and left primary motor cortex (BA 4), right superior temporal gyrus (BA 41/42 and 21/22 and 38), left middle temporal gyrus (BA 21), bilateral parahippocampal gyri, left posterior cingulate, right superior parietal gyrus (BA 7) and left occipital lobe (BA 17). These data are reported in Figure 6.10 and Table 6.8.

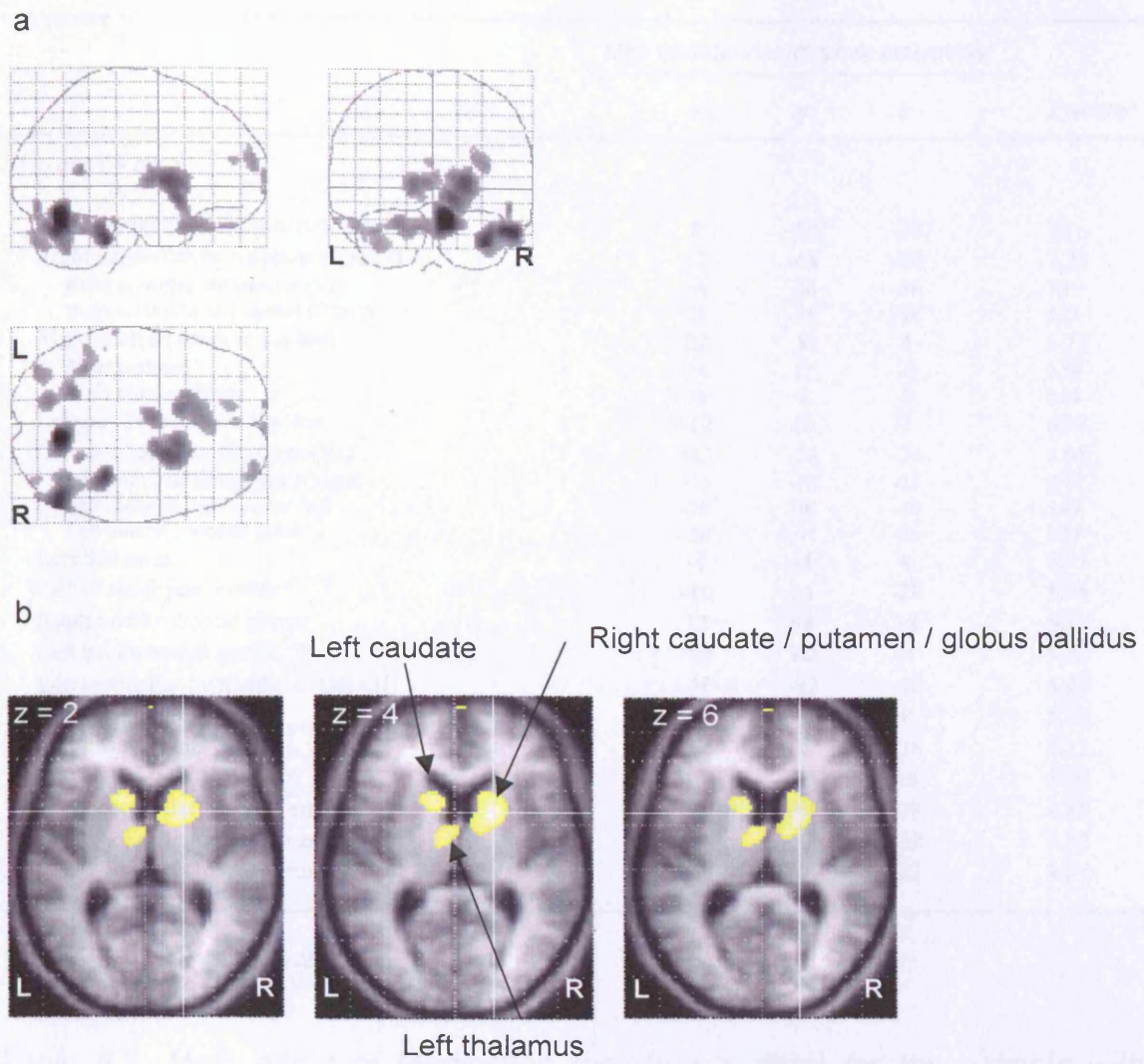

Figure 6.9: Main effect of medication (no drug > drug) for the patients with Parkinson's disease

(a) Results displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space. (b) Transverse image of basal ganglia (left head of caudate nucleus: x = -12, y = 16, z = 2 and a cluster including the right head of caudate nucleus: x = 22, y = 8, z = 4, right putamen: x = 14, y = 12, z = 6 and right globus pallidus: x = 16, y = -2, z = 8) and thalamic activation (left thalamus: x = -4, y = -4, z = 4) in the (no drug > drug) contrast. Significant at p < 0.05, FWE.

	BA	MNI coordinates of peak activation			Z score*
		x	y	z	
No drug > drug					
Right cerebellar hemisphere/midline		8	-66	-22	>8
Right cerebellar hemisphere (Crus I)		52	-68	-28	7.25
Right cerebellar hemisphere (VI)		36	-36	-36	7.1
Right cerebellar hemisphere (Crus I)		38	-64	-38	5.71
Right head of caudate nucleus		22	8	4	6.77
Right putamen		14	12	-6	6.54
Right globus pallidus		16	-2	8	6.08
Left head of caudate nucleus		-12	16	2	6.22
Left cerebellar hemisphere (VI)		-22	-58	-24	5.98
Left cerebellar hemisphere (Crus I)		-16	-76	-28	5.74
Left cerebellar hemisphere (VI)		-38	-50	-30	5.43
Left inferior temporal gyrus		-50	-48	-26	5.15
Left thalamus		-4	-4	4	5.93
Left orbitofrontal cortex		-10	24	-28	5.75
Right middle frontal gyrus	10	32	64	18	5.47
Left orbitofrontal cortex	11	-16	70	-4	5.43
Left cerebellar hemisphere (Crus II)		-36	-82	-40	5.41
Right frontopolar gyrus	10	2	70	8	5.31
Left orbitofrontal cortex	11	-12	48	-28	5.12
Left superior frontal gyrus	9	-2	50	18	5.09
Right cerebellar hemisphere (Crus I)		38	-84	-38	4.88
Left inferior temporal gyrus	20	-64	-30	-22	4.82
Left inferior temporal gyrus	20	-52	-36	-22	4.67

* all significant at $p < 0.05$, FWE

Table 6.7: Main effect of Medication (no drug > drug) for the patients with Parkinson's disease

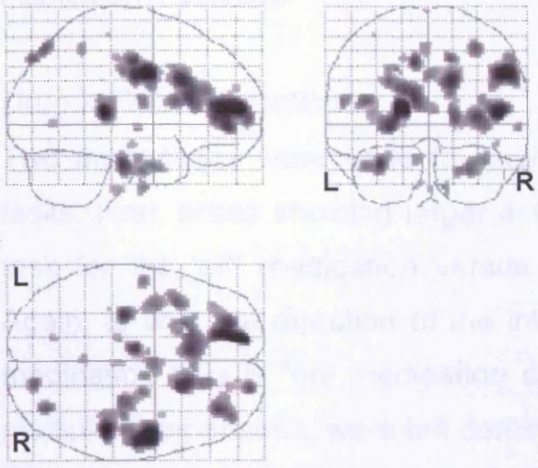

Figure 6.10: Main effect of medication (drug > no drug) for the patients with Parkinson's disease

Results are displayed as statistical parametric maps in sagittal, coronal and transverse projections in stereotactic space. Significant at $p < 0.05$, FWE.

	BA	MNI coordinates of peak activation x	y	z	Z score*
Drug > no drug					
Left middle frontal gyrus	10	-16	56	10	6.81
Left middle frontal gyrus	10	-18	48	12	6.61
Left middle frontal gyrus	10	-26	48	0	6.11
Left middle frontal gyrus	46	-36	38	16	4.79
Right lateral premotor cortex	6	52	-4	36	6.8
Right superior temporal gyrus	41/42	46	-32	8	6.63
Right anterior cingulate	32	16	20	30	6.22
Right anterior cingulate	32	16	34	24	5.49
Left parahippocampal gyrus		-32	-20	-28	6.09
Left posterior cingulate sulcus		-8	-12	44	6.01
Right superior frontal gyrus	10	18	62	6	5.85
Right superior frontal gyrus	10	18	50	18	4.82
Right superior parietal gyrus	7	32	-74	52	5.71
Right parahippocampal gyrus		28	-20	-30	5.7
Right parahippocampal gyrus		22	-8	-32	5.46
Right parahippocampal gyrus		36	-22	-24	5.38
Right superior parietal gyrus	7	16	-82	46	5.7
Left inferior frontal gyrus	44	-42	16	16	5.67
Left middle temporal gyrus	21	-40	0	-30	5.47
Left lateral premotor cortex	6	-56	-6	16	5.42
Left posterior cingulate, marginal sulcus		-2	-48	54	5.11
Right superior temporal gyrus	21/22	60	-10	-2	5.06
Left occipital lobe, near (below) anterior calcarine sulcus	17	-16	-68	2	4.84
Right superior frontal gyrus	9	14	54	24	4.75
Right superior temporal gyrus	38	38	4	-26	4.73
Left primary motor cortex	4	-36	-30	62	4.66

* all significant at $p < 0.05$, FWE

Table 6.8: Main effect of Medication (Drug > No Drug) for the patients with Parkinson's disease

Drug X Task interaction

Two interactions were used to disambiguate the effects of drug state on the tasks. First, areas showing larger activation in the timing tasks than the control task for the 'off' medication versus 'on' medication contrast were examined. Again, to limit the direction of the interpretation, this was masked with the 'off' medication versus 'on' medication contrast. The areas found, uncorrected for multiple comparisons, were left cerebellar hemisphere (Crus II) ($x = -16$, $y = -88$, $z = -40$; $Z = 3.93$; $p < 0.001$), left globus pallidus ($x = -18$, $y = -2$, $z = -4$; $Z = 3.75$; $p < 0.001$) and the left thalamus ($x = -4$, $y = -24$, $z = 2$; $Z = 3.13$; $p =$

0.000). The second interaction concerned larger activations in the timing tasks than the control task for the 'on' medication versus 'off' medication contrast. This was masked with the 'on' medication versus 'off' medication contrast, to limit the direction of the interpretation. The areas showing greater activation in the timing than control tasks for the drug versus no drug contrast were left insula (x = -42, y = 12, z = -6; Z = 3.82; p < 0.001), right dorsolateral prefrontal cortex (BA 9) (x = 14, y = 58, z = 30; Z = 3.34; p < 0.001), left inferior frontal gyrus (BA 45) (x = -40, y = 32, z = 4; Z = 3.29; p < 0.001), left middle temporal gyrus (BA 21) (x = -38, y = 2, z = -30; Z = 4.03; p < 0.001), right superior temporal gyrus (BA 22) (x = 52, y = -14, z = -8; Z = 3.68; p < 0.001) and the left intraparietal sulcus (x = -28, y = -52, z = 44; Z = 3.40; p < 0.001).

Changes in effective connectivity between the left head of the caudate nucleus and the rest of the brain

For the investigation of the PPI, a focus in the head of the left caudate nucleus (x = -12, y = 16, z = 2), as found in the no drug > drug main effect, was used as the physiological variable. This region was selected due to interest in the modulating effects of apomorphine on basal ganglia connectivity, particularly the effect of dopamine on striato-frontal coupling. The basal ganglia activity in the 'off' medication versus 'on' medication condition led to the hypothesis that the left caudate nucleus and basal ganglia would show greater coupling in the 'off' than 'on' medication condition and that the left caudate nucleus and DLPFC would show less coupling in the 'off' than 'on' medication condition (discussed in more detail in the Discussion). Figure 6.11 illustrate the areas activated in the PPI, with 6.11a illustrating regions that showed significantly *increased* coupling with the left caudate nucleus in the 'off' medication condition, relative to the 'on' condition, and 6.11b illustrating regions that showed significantly *decreased* coupling with the left caudate nucleus in the 'off' medication condition, relative to the 'on' condition. In particular, there was a significant increase in coupling of the left caudate nucleus with subcortical regions in the 'off' medication condition relative to the 'on' medication condition, including the right globus pallidus (x = 20, y = 2, z = -4; Z = 4.34; p < 0.001, uncorrected) and the left thalamus (x = -12, y = -18, z = 6; Z = 4.47; p < 0.001, uncorrected) (Figure 6.12ab). Significant decreases in coupling were found between the left caudate nucleus and

prefrontal regions when the PD patients were 'off' medication compared to when they were 'on' medication, including the left DLPFC (BA 46) (x = -48, y = 40, z = 14; Z = 4.91; p = 0.018, FWE) (Figure 6.12c).

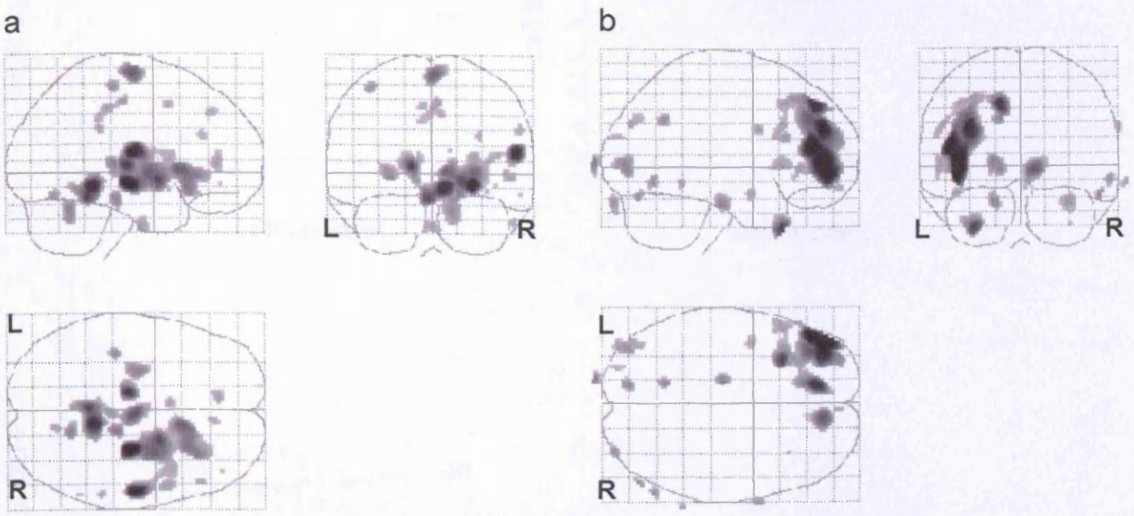

Figure 6.11: Changes in effective connectivity (psychophysiological interaction) with the activation of the left head of the caudate nucleus in patients with PD

(a) Areas showing increased coupling with the left caudate nucleus (x = -12, y = 16, z = 2) in the 'off' medication condition relative to the 'on' medication condition. (b) Areas showing decreased coupling with the left caudate nucleus in the 'off' medication condition relative to the 'on' medication condition. The results are displayed as statistical parametric maps in saggital, coronal and transverse projections in stereotactic space. Significant at p < 0.001, uncorrected.

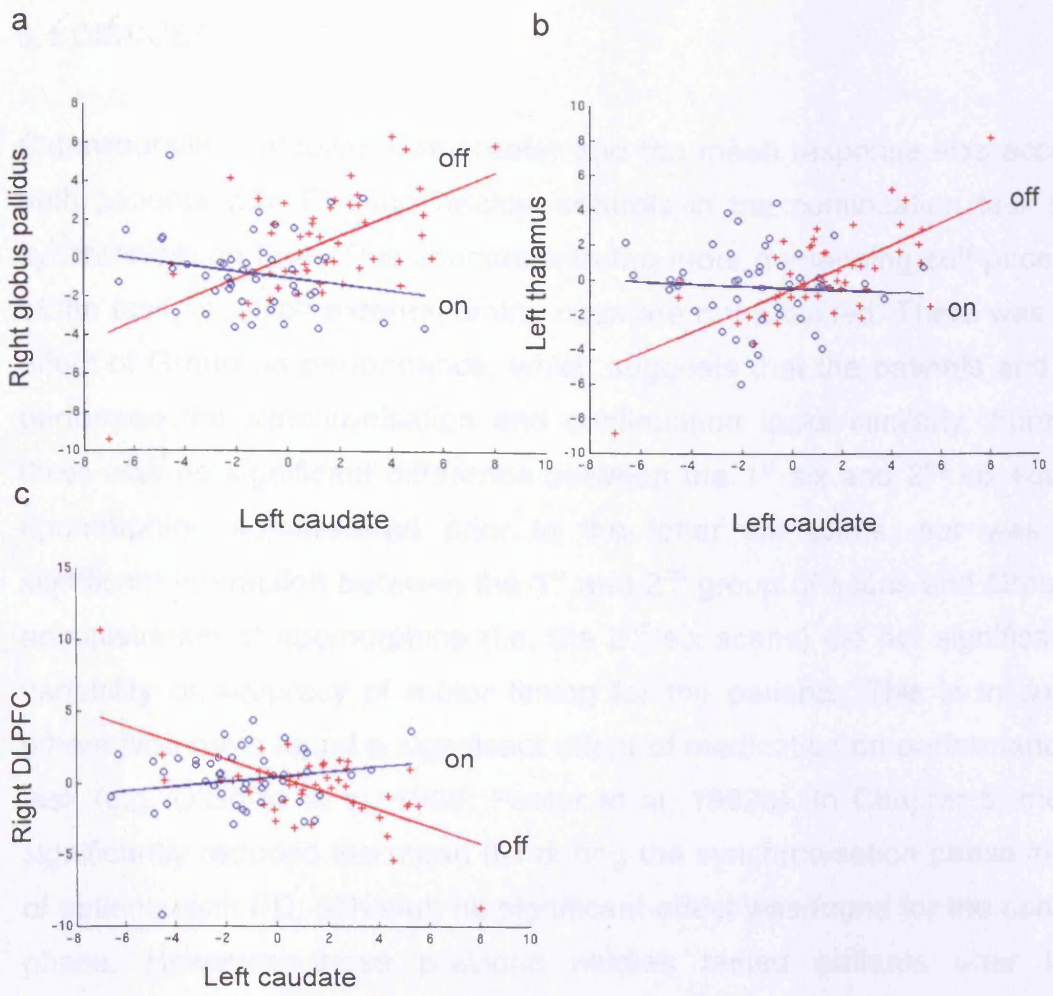

Figure 6.12: Plotted regions of interest for the psychophysiological interaction

(a) Activity in the left caudate nucleus plotted against the right globus pallidus (region of interest) (x = 20, y = 2, z = -4). (b) Activity in the left head of the caudate nucleus plotted against the left thalamus (region of interest) (x = -12, y = -18, z = 6). (c) Activity in the left caudate nucleus plotted against the right DLPFC (region of interest) (x = -48, y = 40, z = 14). The red crosses indicate the correlation between the two regions in the 'off' medication state and the blue circles indicate the correlation between the two regions in the 'on' medication state. Regression lines have been fitted.

6.4 DISCUSSION

Behaviourally, variability was greater and the mean response less accurate for both patients with PD and healthy controls in the continuation task than the synchronisation task. This concurs with the more demanding self-paced nature of the task, in which external timing cues are not provided. There was no main effect of Group on performance, which suggests that the patients and controls performed the synchronisation and continuation tasks similarly. Furthermore, there was no significant difference between the 1st six and 2nd six scans, with apomorphine administered prior to the latter six scans, nor was there a significant interaction between the 1st and 2nd group of scans and Group. Thus, administration of apomorphine (i.e. the 2nd six scans) did not significantly alter variability or accuracy of motor timing for the patients. This is in contrast to others who have found a significant effect of medication on performance of this task (e.g. O'Boyle et al, 1996; Pastor et al, 1992a). In Chapter 5, medication significantly reduced the mean IRI during the synchronisation phase in a group of patients with PD, although no significant effect was found for the continuation phase. However, these previous studies tested patients after levodopa administration, whereas the present study uses apomorphine, a dopamine agonist. The absence of a group or medication effect on performance means that the difference in neural activity for the patients 'on' and 'off' medication and from controls cannot be ascribed to differential performance, e.g. differing variability or tapping rate. For the control reaction time task, there was a main effect of Group, with the patients having a slower mean reaction time and greater variability than the control subjects. Administration of apomorphine did not alter the patients' performance.

6.4.1 Neural correlates of motor timing

6.4.1.1 Motor timing in healthy controls

The two motor timing tasks, when contrasted with the control task, elicited activation in the left superior frontal gyrus (BA 9/10), left medial frontal gyrus (BA 10), bilateral angular gyrus (BA 39/19), right hippocampus, left posterior cingulate and left nucleus accumbens. These are areas specifically associated

with motor timing, once areas involved in motor preparation and execution and tone anticipation had been controlled for. The result concurs with previous functional imaging research that has suggested the basal ganglia play a key role in temporal processing (e.g. Rao et al, 2001). Furthermore, the study presented in Chapter 3 found activation of the substantia nigra pars compacta when two time reproduction tasks were compared with a control reaction time task. As the control used in the present study and previous study controlled for the basic motor components this provides convincing evidence for the role of the basal ganglia in timing. Reflecting the pattern of activation found in this contrast, the nucleus accumbens receives inputs from both the prefrontal cortex and hippocampus (Goto et al, 2002) and has been implicated in selecting appropriate motor plans (Grace, 2000). This region is part of the ventral striatum but is not typically activated in timing tasks, although Harrington et al (2004b) found that a region approximating the putamen and nucleus accumbens was active in association with the increased difficulty of a duration discrimination task. When the threshold for significant activation was lowered the foci in the nucleus accumbens could clearly be seen to spread to other regions of the striatum, particularly the caudate nucleus.

The parietal and frontal activations concur with previous studies that also found such cortical activation during timing tasks, activity that is typically attributed to the attention and working memory demands of the timing tasks (e.g. Lejeune et al, 1997; Maquet et al, 1996; Nenadic et al, 2003; Rao et al, 2001). Similarly, the perceptual timing deficits of patients with frontal lesions are considered to reflect attention and working memory problems, rather than impairment of fundamental timing mechanisms (e.g. Casini and Ivry, 1999; Mangels et al, 1998). In complement to this, the data presented in Chapter 3 show that cortical activation is greater in seconds-range compared to milliseconds-range timing, reflecting the greater demands in cognitive processing. Furthermore, rTMS was used in Chapter 4 to show that the right DLPFC is exclusively essential to time reproduction in the seconds-range and its role is likely to be memory-related. As in Chapter 3, the most parsimonious explanation for the parietal activation is that it is involved in attentional mechanisms (Posner et al 1987ab; Posner and Presti, 1987; Robinson, 1995). Timing performance is known to deteriorate

under dual task conditions (Sergent et al, 1993), thus maintaining attention is a crucial part of effective timing performance. Additionally, the anterior parietal lobe has been implicated in motor attention and motor preparation (Decety et al, 1992; Deiber et al, 1997).

Despite previously published clinical work suggesting the contrary (e.g. Ivry et al, 1988; Ivry and Keele, 1989), the lack of significant cerebellar activation specific to the timing tasks does not support the role of this region in motor timing. Although the cerebellum has been activated in previous functional imaging studies of temporal processing, debate still remains as to whether it plays a fundamental role in 'clock'-like processes. Although cerebellar activation was found in the PET study presented in Chapter 3 during both seconds- and milliseconds-range timing, it was not found in the crucial comparison that compared the two time reproduction tasks to the tightly matched control reaction time task. Penhune et al (1998) found that the cerebellum was active during the production of rhythmic sequences, particularly when they were complex or novel. They suggested that the cerebellum may not provide a clock function, but rather that it may be involved in the learning of timed motor responses and also in sensory integration, including extracting temporal parameters from sensory inputs. The tasks used in this study were rhythmically simple and also pre-practiced, thus the lack of cerebellar activity for the control subjects is consistent with the suggestion that the cerebellum is important for rhythm learning rather than temporal processing per se. Indeed, Penhune and Doyon (2002) demonstrated that the cerebellum is preferentially active during the initial learning phases of rhythm learning.

The predominance of left hemisphere activation in the timing tasks reflects the motoric element, as this hemisphere is known for its role in motor processing (e.g. Haaland et al, 2000; Rushworth et al, 1998; Schluter et al, 1998). Sergent et al (1993) has suggested that the left hemisphere plays a significant role in motor timing by right-handed individuals. Reflecting the data presented in Chapter 3, it has previously been suggested that the timing of seconds-range intervals activates a right hemisphere fronto-parietal network that is different to the type of neural activity elicited by more 'automatic' milliseconds-range timing

(Lewis and Miall, 2003a). Studies investigating the difference between milliseconds- and seconds-range timing tend to avoid intervals in the 1000 ms range and pick less ambiguous intervals (e.g. Lewis and Miall, 2003b; Mangels et al, 1998; Rubia et al, 1998). In fact, no clear consensus exists regarding the threshold for moving from 'automatic' milliseconds-range timing to more 'cognitive' seconds-range timing. The results here suggest that motor timing at the rate of 1 Hz does not produce the dominant right cortical network typically engaged in 'seconds-range' timing. Whether this reflects the strong motor element in the task or the length of the interval remains open to further investigation.

6.4.1.2 Motor timing in patients with Parkinson's disease

For the patients with Parkinson's disease, no basal ganglia activation was found during the timing tasks compared to the control task, reflecting the basal ganglia pathology in these patients. In addition, frontal activity was limited to the left insula (BA 13), which suggests that there was little increase in frontal activity during the timing tasks for the patients, as was the case for the controls. The pattern of activation observed for the patients engaged more posterior cortical areas (left inferior (BA 20) and superior (BA 38) temporal gyrus, left parieto-occipital fissure, right precuneus (BA 7)) as well as the right thalamus and bilateral cerebellar hemispheres and it is possible that the deficiency in activating the striato-frontal areas has resulted in a reliance on the cerebellum and more posterior cortical areas. Indeed, the Group X Task interaction, which provided a direct measure of the significant differences in activation between the control and patient groups for the timing and control tasks, revealed that the right thalamus and left cerebellar hemisphere and midline were more active for the PD patients in the timing tasks compared to the control task than for the control subjects. This suggests that compared to healthy subjects, the patients are relying on the cerebellum as a substitute for the malfunctioning striato-frontal circuits during timing tasks. Furthermore, the interaction also revealed that the right middle frontal gyrus (BA 6/8), left middle temporal gyrus (BA 20/21), right inferior temporal gyrus (BA 20) and left head of caudate nucleus were more active for the controls than the patients during the timing tasks than the control reaction time task. This suggests that the significant frontostriatal

activation present for the controls during motor timing is absent from the patients.

The patients were better able to activate frontal regions during the control reaction time task, including the right middle frontal gyrus (BA 8/6), right anterior cingulate (BA 32), bilateral insula and right lateral premotor cortex (BA 6). As the patients were significantly slower during the reaction time task than the healthy controls but produced a similar performance on the timing tasks, it could be argued that the greater frontal activation in the RT task for the patients reflects the greater effort required by the patients to programme their response through and act of will in the RT task. For the control group, the RT task had a greater dominance of subcortical areas than the timing tasks, perhaps reflecting more automatic nature of this simple stimulus-driven task for a healthy population.

6.4.2 Neural correlates of externally and internally paced motor timing

6.4.2.1 Synchronisation vs continuation tasks for the healthy controls

The comparison of the synchronisation and continuation tasks allows exploration of externally paced and internally timed temporal processing. In providing a tone in response to the subjects' self-generated taps in the continuation task, it was ensured that both tasks provided auditory stimuli and auditory feedback. For the healthy subjects, the ipsilateral somatosensory cortex (BA 1/2) was more active in the synchronisation task. Contralateral primary somatosensory cortex is known to be active during paced tapping. Using MEG, a source occurring at around tap onset was associated with kinesthetic feedback of the finger movement, whereas a source occurring ~100 ms after tap onset was associated with tactile-kinesthetic feedback, with the tactile feedback being related to the tap (Pollok et al, 2004). There was no evidence that the sources localised in the somatosensory cortex reflected the processing of temporal information per se, such as monitoring the delay between the pacing tone and the subject response. Based on these data, the greater activation of the somatosensory cortex in the synchronisation task may reflect the sensory integration between the presented stimuli and the subjects'

attempt at keeping in pace with the stimuli, something that is not pertinent to the continuation task in which the auditory stimuli simply reflect the end of the subjects' response. The synchronisation task also invoked greater parietal activation, which may be related to the somatosensory activity and the transformation of sensory information into motor output, perhaps the principal demand of this task. Ramnani and Passingham (2001) note how the parietal cortex is involved in the mapping of sensory representations into motor representations and suggest that this is true for both spatial and temporal information.

Greater activation of the hippocampus was seen during the synchronisation task than the continuation task and this was also true for the patient group. The hippocampus is traditionally seen as having a role in memory processes (e.g. Squire et al, 2004) and it may be that the activation is reflecting memory demands that are unique to the synchronisation task. Certainly, the presence of the tone, metering the target interval, throughout the task would allow more ready encoding of the interval duration to memory, whereas the continuation task places demands the retrieval of the previously learned interval from memory. In support of this, the hippocampus is known to be involved in selecting the necessary features of a stimulus for encoding into memory (Hampson et al, 2004). Indeed, Harrington et al (2004b) found right parahippocampal and hippocampal activation during the encoding phase of a duration discrimination task and this activation was correlated with a measure of timing accuracy, suggesting the regions were specifically sensitive to the temporal characteristics of the interval presented during the encoding phase. Event-related potential (ERP) recordings in rats (Onoda et al, 2003) have found evidence of hippocampal involvement in a seconds-range temporal bisection task and there has been some evidence of disruption to temporal working memory in rats with hippocampal lesions (Meck et al, 1984; Olton et al, 1988), though not all studies support this (Dietrich and Allen, 1998, Dietrich et al, 1997). More recently, mice lacking NMDA receptors in hippocampal CA1 pyramidal cells have shown a disruption of temporal memory, as evidenced by a failure to memorise a conditioned delay in a trace fear conditioning paradigm (Huerta et al, 2000). The firing of hippocampal neurons during the learning

stage compared to subsequent stages of an eyeblink conditioning paradigm, which involves establishing a timed eyeblink, suggests that the role of this structure in learning may underpin its involvement in conditioning paradigms (McEchron and Disterhoft, 1997). Thus, reflecting the finding of Harrington et al (2004b), it may be that the hippocampus is active during the synchronisation task as it is encoding and learning the relationship between the pacing stimuli and the initiation of the timed response. The precuneus (BA 7) was also more active for both groups in the synchronisation task and interestingly, the left precuneus is activated during tapping to a regularly paced visual cue (every 667 ms) compared to tapping to an irregularly paced cue (Lutz et al, 2000).

Conversely, areas of greater activation for the continuation task were dominantly frontal (right DLPFC (BA 9/46), right inferior frontal gyrus (BA 44), left insula (BA 13)), with one focus in the middle temporal gyrus. This greater frontal activity possibly reflects the greater cognitive demands, as well as the greater demands on volitional processes in the continuation task. Behaviourally, both groups were less accurate and more variable in the continuation task, suggesting that the reliance on internal pacing makes it more demanding. The DLPFC has been previously implicated in 'willed' or internally generated movements. For example, comparison of self-initiated movements (tapping at a pretrained rate of once every 3 s) to externally triggered movement (lifting a finger in response to a tone approximately every 3 s) resulted in greater activation of an area of right DLPFC in healthy subjects (Jahanshahi et al, 1995). Chapter 4 found that right DLPFC was associated with temporal memory processes and it may be that this region is engaged in some of the memory processing relevant to continuation tapping, for example, comparing the current interval to a standard in working memory, as would be predicted by SET (Gibbon et al, 1984). Indeed, the right DLPFC is commonly associated with working memory demands in temporal tasks (e.g. Rao et al, 2001), a finding that is supported by the results of Chapter 4. The greater activation of Broca's area (BA 44) in the continuation task perhaps reflects the use of sub-vocalisation to aid the more demanding internally-timed task. This is consistent with Crosson et al (2001) who found that activity in Broca's area decreased as the silent production of words (freely chosen from a semantic category)

progressed from being produced at a self-generated pace to being produced in response to an external cue. The greater activation of the right middle temporal gyrus (BA 21) may reflect additional auditory demands of the continuation task, such as an auditory temporal template. Rao et al (1997) also found temporal activation related to the continuation task and suggested that it reflected auditory imagery, i.e. auditory representation of the tone interval duration. Rao et al (1997) and Elsinger et al (2003) both found additional SMA activity in the continuation task, which has led to the suggestion that the SMA is important in the internally-paced timing of repetitive movements. However, these studies only compared each task to rest, whereas the study presented here compared the two tasks directly and did not find SMA activity.

Different foci in the left insula showed greater differential activation across both tasks in the control group. The insula is implicated in speech motor control and in auditory processing (see Ackermann and Riecker, 2004 and Bamiou et al, 2003 for a review) and could be complementing the activation of Broca's area found in the control group for the continuation task. Bilateral insula activity is evidenced when subjects passively listen to trains of clicks in the range of 2-6 Hz (Ackermann et al, 2001). Thus, the insula may have a role in the perception and analysis of sequences of auditory stimuli and may have been involved in processing the pacing tone present during the synchronisation task and produced after each finger movement in the continuation task.

Jancke et al (2000), Jantzen et al (2004) and Lewis et al (2004) have also used functional imaging to directly compare activity relating to the synchronisation task and continuation task, although the comparison in the Jantzen study was collapsed across two types of tapping rhythms (synchronised and syncopated) and the Lewis study was collapsed across rhythms of varying complexity. The study of Jancke et al (2000) used auditory and visual stimuli. All of the studies found significantly greater activity in bilateral superior temporal gyrus during the synchronisation task, though this is probably reflecting the absence of auditory stimuli in their continuation tasks. For the continuation task compared to the synchronisation task, Lewis et al (2004) found greatly increased cortical activation, including the right DLPFC, as well as bilateral putamen and

cerebellar activity. However, Jancke et al (2000) found no additional activation in the continuation task when auditory stimuli were used, whereas Jantzen et al (2004) did not report the contrast. It should also be noted that the previous studies used different inter-stimulus intervals (Jancke et al (2000) ISIs of 400 ms, Jantzen et al (2004) ISIs of 800 ms, Lewis et al (2004) ISIs of 500 ms) and, as previously noted, millisecond and seconds-range timing elicit different patterns of neural activity, so directly comparing these results with that of previous studies is difficult.

6.4.2.2 Synchronisation vs continuation tasks for the patients with Parkinson's disease

Patients with PD are known to be better at externally triggered tasks than tasks that require an internal representation of 'when' to move (e.g. Jahanshahi et al, 1995). The continuation task is conceived to make greater demands on internal timing as there is no external guidance for the rhythm being produced. This greater demand is reflected in the larger network of regions, including the cerebellum, found in the continuation task, suggesting that the patients had to recruit areas in the internally timed task that they did not require in the less demanding synchronisation task. Although, the two PD groups (withdrawn from levodopa medication or de novo) in Chapter 5 showed significantly higher variability during the synchronisation phase than the continuation phase of the repetitive tapping task. As with the controls, the patients activated the right DLPFC (BA 9/46) in the continuation task compared to the synchronisation task, further confirming the importance of this region in internally paced timing. The right insula was more active during the continuation task compared to the synchronisation task for the patient group, whereas for the controls the left insula was active during both types of task. Conversely, whereas only the continuation task contrast elicited temporal activation for the controls, the right superior (BA 22) and right middle (BA 21) temporal gyrus were more active during the synchronisation task compared to the continuation task and bilateral superior temporal gyrus (BA 22/42) was more active during the continuation task compared to the synchronisation task. This perhaps suggests differences in activation related to auditory processing, although conclusions are limited as the results are not the product of a direct comparison.

There was particular interest in exploring the difference between the two groups for the continuation task, due to the reported difficulties of patients with Parkinson's disease with internally paced movements. Areas more active for the control group than the PD group in the continuation than synchronisation task included the left superior parietal gyrus (BA 7), right lateral premotor cortex (BA 6), left insula (BA 13) and the left cerebellar hemisphere/midline (V). However, the patients only showed greater activation of the bilateral cerebellar hemispheres when compared to the control group for the continuation than synchronisation task. Thus, despite activating more cortical regions in the continuation task than the synchronisation task, the patient group were less able to activate the cortical network activated in internally paced motor timing for the controls. Taken together, these results suggest that in healthy subjects and those with PD, different brain areas are involved in motor timing if there is a pacing stimulus or not. Timing based on an internal representation, results in prefrontal activation in both combined with a particular reliance on the cerebellum in PD.

6.4.3 The effect of Apomorphine on timing for patients with Parkinson's disease

This study also investigated the effects of apomorphine upon motor timing behaviour in patients with PD. These drug-specific effects are particularly interesting given that medication improves the UPDRS scores and fundamentally influences task-related rCBF, but does not significantly change performance on the timing tasks. Compared to the 'off' state the absence of any significant change in self-reported arousal levels suggest that increase in cortical activation following medication is not a consequence of a generalised increase in alertness for the patient group.

Compared to 'on' medication, when the patients were in the 'off' medication condition, bilateral prefrontal regions and the left inferior temporal cortex (BA 20) were more active together with the left head of the caudate nucleus and a region encompassing the right head of the caudate nucleus, right putamen and right globus pallidus, and left thalamus and the bilateral cerebellar hemispheres.

As far as can be established, this is the first study to show this interesting finding of elevated basal ganglia activation in unmedicated patients. Typically, basal ganglia activity levels are seen to increase following the introduction of dopaminergic drugs (e.g. Elsinger et al 2003). The study of Elsinger et al (2003) is the only previous functional imaging study to explore medication effects during the synchronisation and continuation tasks in patients with PD. The 'off' and 'on' medication effects were not compared directly and the two tasks were not collapsed across, rather they were each compared to rest. Levodopa medication during the continuation task caused increased activity in the left putamen, left thalamus and SMA, which was not present for the continuation task in the 'off' medication condition. Conversely, the 'off' medication condition caused additional activation of the left superior temporal gyrus and right precentral gyrus. Clearly, the data in the current study presents a different picture, with thalamic and basal ganglia activation being observed in the 'off' medication condition when directly compared to the 'on' medication condition. Reasons for this difference are likely to come from methodological sources. The patients used in the study of Elsinger et al (2003) had milder PD than those in the current study (3-7 years duration compared to 11-20 years duration, and not more that Hoehn and Yahr Stage II when 'off') and the subjects were primarily tested with their unaffected right hands. Whether the differential results reflect the deteriorating basal ganglia function that underlines more severe PD cannot be properly explored as 8 of the 10 patients in the study of Elsinger et al (2003) remained on dopamine agonist medication when in the 'off' state, with the medication given in the 'on' state being levodopa, not apomorphine). This means that although the on/off comparison compared the patients at different levels of drug-dependent functioning, the majority still had elevated levels of dopamine compared to their unmedicated state and furthermore, levodopa rather than apomorphine was used during the study.

Previous research has found decreased activity in the DLPFC in PD patients 'off' medication compared to healthy controls (Sabatini et al, 2000) as well as evidence of increased cerebellar activation 'off' medication (e.g. Rascol et al, 1997). However, the previously reported finding of decreased SMA activation in the 'off' medication condition, which is normalised with the administration of

medication, was not found (e.g. Jenkins et al, 1992). Neither was increased lateral premotor cortex and parietal cortex activation in the 'off' medication condition observed (e.g. Catalan et al, 1999; Samuel et al, 1997), in fact greater lateral premotor and parietal activity was observed in the 'on' medication condition. This could be reflecting the differential task demands of this study; for example, the tasks did not elicit SMA or lateral premotor activation in the healthy controls, though the lack of comparison with a rest condition limits the inferences that can be made. A further reason could be the advanced stage of PD of the patients, whereby activation of motor cortical regions is severely compromised, regardless of drug state.

The interaction for the 'off' medication condition compared to the 'on' medication condition found that the left cerebellar hemisphere (Crus II), left globus pallidus and the left thalamus were significantly activated during timing tasks, compared to the control task. This suggests that these regions are implicated in motor timing in the PD group and that they are more active when the patients are depleted of dopamine. The cerebellum has been hypothesised to play a role in temporal processing (e.g. Ivry and Keele, 1989) and it cannot be discounted that this region may be enabling accurate timing in the patient group, despite no evidence to suggest the importance of this region for the healthy control group during motor timing.

When apomorphine was administered and the patients were in the 'on' state, far more extensive regions of frontal cortex, including prefrontal and motor areas, and temporal cortex were activated as well as the left posterior cingulate sulcus, right superior parietal gyrus (BA 7), bilateral parahippocampal gyrus and left occipital lobe (BA 17). Interestingly, no basal ganglia regions were activated although it cannot be discounted that there is basal ganglia activation in the 'on' medication condition in this study that is being masked by the extensive basal ganglia activation in the 'off' medication condition. The interaction of drug and task found that the right dorsolateral prefrontal cortex (BA 9), left inferior frontal gyrus (BA 45), left insula, left superior (BA 22) and middle (BA 21) temporal gyrus and left intraparietal sulcus were all associated with larger activation for timing tasks than control tasks when the patients were 'on' medication. This

suggests that tasks with a motor timing component require activation of discrete frontal and parietal areas and that these areas are not active in patients with PD when they are 'off' medication or performing the more simple control reaction time task. Indeed, frontal and parietal areas were more common in the control group during motor timing than the control task. Elsinger et al (2003) found left superior frontal gyrus activation during the synchronisation task in the 'on' medication condition compared to rest but not in the 'off' medication condition, which mirrors the present findings of augmented frontal activation following medication, although no prefrontal activity was reported for the continuation task in either the 'on' or 'off' medication state.

Comparing performance on simple motor tasks for patients with PD both 'on' and 'off medication has illustrated the imbalance within the unmedicated basal ganglia in this patient group. It is suggested that the observed hyperactivity of the basal ganglia during the 'off' medication condition reflects the inability of this region to project to cortical regions in the dopamine depleted state, which is reflected in the limited cortical activation in this contrast. The activation of the globus pallidus reflects the known excessive inhibitory output that travels from the substantia nigra pars compacta and internal segment of the globus pallidus to the ventrolateral thalamus in patients with Parkinson's disease. In effect, the activity in the basal ganglia is 'stuck', with the activation of parietal regions as well as the extent of activation in frontal, particularly superior frontal, and temporal regions being inhibited. The patients have to recruit alternative pathways, including the cerebellum, in order to complete the tasks. The study found contralateral cerebellar activation as well as ipsilateral activity, suggesting that the cerebellum is not merely aiding motor execution. Indeed, the lack of activity in the sensorimotor cortex during the 'off' medication condition does not suggest that additional sensorimotor circuitry was being activated in the dopamine depleted state. The competence of the PD group at this task when 'off' medication, despite gross under-activation of frontal regions, certainly suggests the diversity of the cerebellum, particularly with regards to its hypothesised role in cognition (Rapoport et al, 2004). Furthermore, it is proposed that the increased cortical activity for the PD patients 'on' medication compared to 'off' medication reflects a 'normalising' of basal ganglia function,

particularly frontostriatal connectivity. Certainly, the control group were observed to activate basal ganglia and cortical regions during the motor timing tasks. It is also worth referring to the work of Bullmore et al (2003), who found greater activation of the right caudate and right putamen in association with increased load in an object-location learning task. It may be that the increased striatal activity in the 'off' medication condition reflects the increased effort required (either as a result of, or reflected in, the greater basal ganglia activation). For this hypothesis to be explored more fully, neural activity would need to be compared with self-reported effort ratings, which unfortunately were not recorded in this study.

6.4.3.1 Effective connectivity of the left head of the caudate nucleus

This study was able to explore the differential connectivity between the left caudate nucleus and the rest of the brain when the PD group were 'on' and 'off' medication. This region was of interest as extensive regions of the basal ganglia, including the left caudate nucleus, were more active for the PD group 'off' medication than 'on' medication. In addition, the frontal cortex, a target of the caudate nucleus, was active to a limited degree in the 'off' medication condition, compared to the widespread frontal activation in the 'on' medication condition. The healthy control group showed significantly greater activation in the left caudate nucleus than the PD group in the timing tasks, an effect that can be attributed to the role of the basal ganglia in timing in healthy subjects. It was hypothesised that the caudal activity in the PD group was related to disrupted functioning within the basal ganglia, rather than the effective activation of the basal ganglia (as with the control group for the timing tasks) during the three motor tasks in the 'off' medication condition. In particular, greater coupling between the left caudate nucleus and basal ganglia was predicted 'off' medication, with the increased dopamine available in the 'on' medication condition facilitating coupling between the left caudate nucleus and DLPFC.

In accordance with the hypothesis, PPI analysis revealed that both the left thalamus and right globus pallidus showed significantly increased coupling with the left caudate nucleus in the 'off' than 'on' condition. This suggests that

subcortical connectivity is enhanced when the subjects are not compensated for their depleted levels of dopamine. Furthermore, there was significantly decreased coupling between the left DLPFC and the left caudate nucleus in the 'off' than 'on' condition. There are known frontostriatal connections between these two areas (Alexander et al, 1986) and it can be suggested that the decreased coupling arises as the non-medicated basal ganglia are ineffective at projecting to cortical regions, although the PPI does not enable the directionality of the influence between the index region and other brain regions to be disambiguated. The decreased coupling leads to excess basal ganglia activity as the patient relies on subcortical activity, coupled with alternative networks (including the cerebellum), to complete the task. This finding is reflected in a study of connectivity in the cortico-striato-thalamic system (Honey et al, 2003). The authors found that a dopaminergic antagonist (sulpiride) increased functional connectivity between the caudate nucleus and the thalamus and ventral midbrain. The volunteers in this study were in the elderly age range (61-80 years) and thus comparable with the data presented in this study.

As has been briefly mentioned, results from this study must be interpreted with regard to the profile of the patients with PD who were tested. The patients were all prescribed apomorphine, which by definition tends to indicate advanced PD (e.g. Pietz et al, 1998). It is perhaps preferable to study less disabled patients, as the degree of frontostriatal dysfunction is better contained to the putamen and associated motorcortical circuitry, making interpretation of results easier. Whether such striking results would be found when comparing less disabled patients in the 'on' and 'off' medication conditions is an interesting question. Apomorphine is typically given to manage incapacitating motor fluctuations and 'off' periods that do not respond to other drugs (Pietz et al, 1998). Two of the patients received apomorphine via continuous subcutaneous infusion, a type of administration which is given in more severe cases (e.g. Chaudhuri and Clough, 1998). Indeed, these patients had higher UPDRS scores and showed less response to the apomorphine injections (see Table 6.2, subjects 1 and 3). As is often the case with patient studies, achieving an optimal sample size can prove difficult given the limited availability of suitable patients. The heterogeneity of the patients perhaps suggests that $n > 8$ would have been circumspect. That

aside, the pattern of data across the different analyses, with lack of basal ganglia activation for the PD group during motor timing and dysfunctional activation of the basal ganglia for the PD group when compared 'on' and 'off' medication, are consistent both with each other and with established theory.

6.4.4 Conclusions

1. In healthy subjects, motor timing was associated with frontostriatal activation, which confirmed the hypothesised role of the basal ganglia in timing.

2. This frontostriatal activation pattern was not observed in PD during motor timing; instead, the patients relied on the cerebellum, reflecting reliance on compensatory neural circuits.

3. 'Off' medication, the patients showed greater activation of the basal ganglia as well as the cerebellum. The greater striatal/thalamic/pallidal activation coupled with the less extensive frontal activation when 'off' medication, may reflect the failure to transfer striatal/pallidal activity to the frontal cortex. When 'on' medication, the patients show increased activity in cortical regions, particularly the frontal cortex.

4. PPI analysis showed that coupling between the left caudate nucleus and the left DLPFC was decreased when the PD group were 'off' medication compared to 'on' medication. Furthermore, coupling between the left caudate nucleus and the basal ganglia was increased when this group were 'off' medication compared to 'on' medication.

5. Tapping in synchrony with a pacing tone and in the absence of a pacing cue activate different brain regions for both patients and controls, with the right DLPFC being active during internally paced motor timing for both groups.

Chapter 7

Discussion

The aim of this thesis was to explore the contributions of the basal ganglia, cerebellum and cortex to motor and perceptual timing in the milliseconds- and seconds-range. Three different techniques, PET, rTMS and the testing of patient populations, were used in a complementary fashion in an attempt to further current understanding. The principal findings of the thesis will be discussed with respect to the three brain areas under investigation.

7.1 PRINCIPAL FINDINGS

7.1.1 The contribution of the basal ganglia to millisecond- and seconds-range motor and perceptual timing

The convincing animal pharmacological work illustrating the effect of dopamine on temporal processing (e.g. Meck, 1996) as well as the timing deficits reported in patients with Parkinson's disease (e.g. O'Boyle et al, 1996; Pastor et al, 1992ab) and more recent physiological work (Matell et al, 2003) have provided strong evidence for the role of the basal ganglia in timing functions. This thesis supports a fundamental role of the basal ganglia in temporal processing.

Chapter 3 tested the hypothesis that the basal ganglia may be preferentially involved in the timing of seconds-range intervals (Ivry, 1996). However, the left caudate was active during the reproduction of a 500 ms interval and the right putamen was active during the reproduction of a 2000 ms interval. This suggests that the role of the basal ganglia in timing also extends to milliseconds-range intervals. However, as different regions of the basal ganglia were found to be more active during each interval range, with greater activation of the frontostriatal motor loop apparent during the reproduction of the longer interval, this also suggests that some differentiation in activity occurs as a result of stimulus length. When the two timing tasks were compared to a well-matched

control task, the left substantia nigra pars compacta (SNc) and lateral premotor cortex were more activated during the timing tasks. PET was also used to investigate the regions of the brain active during a repetitive tapping task (Chapter 6). The control group activated the left nucleus accumbens, expanding to the left caudate nucleus, during motor timing compared to a carefully matched control task. This region, as well as regions of the frontal and temporal cortex, was significantly more active for the control group than for a group of patients with PD. This functional imaging data provides convincing evidence that not only are the basal ganglia active across a range of temporal intervals but that the basal ganglia are active during a timing task even after all other processes critical to the task (e.g. attention, motor preparation and response production) have been controlled.

Further support for the critical role of the basal ganglia in temporal processing comes from the clinical study presented in Chapter 5. The data suggest that dopaminergic medication in patients with PD modulates performance on a time production task in the seconds range (30 – 120 s) and also on some measures of a repetitive tapping task (the mean IRI for synchronised tapping at 1000 ms and the interaction between medication and IRI for variability). The data suggest that the basal ganglia are important for both motor and perceptual timing in both the millisecond- and seconds-range. For both the patients with PD (tested 'off' medication) and patients with cerebellar disease, variability on the time reproduction task was influenced by whether the target interval was greater or less than 1 s. This was not apparent for the control group who showed a straightforward, linear relationship. For the patients with PD tested 'off' medication, disease severity contributed to the variance for the repetitive tapping task at the 250 ms IRI (continuation phase). Furthermore, the variability for the 250 ms target interval was greater than the variability for the 500 ms target interval in both the time reproduction and repetitive tapping task. This can be explained by the greater relative motor demands of tapping with such a short IRI for these patients. Attentional proficiency affected the variance of the 2000 ms interval in the repetitive tapping task for the PD patients tested 'off' medication. However, it did not explain performance for the 2000 ms interval for the control group, in keeping with the idea that continuous timing may be

executed 'automatically' (e.g. Lewis and Miall, 2003a). This suggests that the patients find the longer time interval more cognitively demanding than the control group and also that the 250 ms and 2000 ms intervals engage different processes. These clinical findings support the evidence in Chapter 3 that different regions of the basal ganglia and cortex are activated as a function of interval length.

Surprisingly, attentional proficiency did not influence the error seen in the patients with PD (tested 'off' medication) on the time production task, although the performance of the control group was related to their attentional performance. This hints at the pathology underpinning the performance of the patients on this task. As the task was carefully designed to minimise cognitive load and strategic support, it seems likely that dysfunction is related to a fundamental timing problem, mediated by striatal activity. The patients with PD tested 'off' medication also performed worse on this task than a group of more mildly affected de novo patients and the de novo patients were worse than the patients with PD tested 'off' medication on the time reproduction task. These results further suggest that striatal dopamine levels are important for temporal processing.

Striatal activity was investigated in-depth in Chapter 6. In this study regions of brain activity during a repetitive tapping task were explored, particularly with relation to the effects of a dopamine agonist (apomorphine) on the performance of patients with PD. When 'off' medication (across all tasks), PD patients showed greater activation, among other areas, in the bilateral cerebellar hemispheres, right head of the caudate nucleus spreading to the putamen and globus pallidus, left head of caudate nucleus and the left thalamus. In contrast, the 'on' state was associated with significant increases in bilateral frontal, parietal and temporal activation. At first glance this seems confusing as activation of the basal ganglia is associated with motor activity and motor timing. However, effective connectivity analysis found that during the 'off' medication state, relative to the 'on' medication state, coupling between the left caudate nucleus and the right globus pallidus and left thalamus was increased, whereas coupling between the left caudate nucleus and the left DLPFC was

decreased. It is possible that 'off' medication the basal ganglia are ineffective at adequately activating cortical regions, which leads to reliance on subcortical activity to complete the task. Indeed, the left cerebellum and midline and right thalamus were significantly more activated for the PD group than the control group during the timing tasks. 'On' medication, higher activation of cortical areas, particularly the DLPFC, one of the main output sites of the caudate nucleus, suggests a less pathological pattern of activity. This finding provides compelling evidence as to the pathology of the basal ganglia in motor tasks and also in motor timing tasks in particular.

It is clear that the majority of data provide a convincing case that the basal ganglia play a fundamental role in both motor and perceptual timing in the milliseconds- and seconds-range. It can be speculated that these nuclei comprise the 'internal clock' that is responsible for metering time. The striatal beat frequency model (Matell and Meck, 2000; 2004) suggests that the striatum encodes temporal durations, with the SNc acting as a 'trigger' to start the timing of a given interval. The SNc and striatal activation found in Chapters 3 and 6 is consistent with this theory. It is interesting that the two different studies activated different regions of the basal ganglia in their timing tasks > control contrast. This may be a function of the different tasks used, with different parts of a complex structure being more or less activated depending on task demands. Indeed, when the SHORT and LONG conditions were compared in the PET study in Chapter 3, different regions of the basal ganglia (left caudate nucleus and right putamen, respectively) were activated. To better disambiguate the differential roles of basal ganglia structures using functional imaging, a technique such as dynamic causal modelling (Friston et al, 2003), which uses a realistic neuronal model of cortical regions to estimate and make inferences about the coupling between these regions and the influence of experimental manipulations on that coupling, or correlating the activation in different regions with behavioural performance (e.g. Harrington et al, 2004b) would be useful.

7.1.2 The contribution of the cerebellum to millisecond- and seconds-range motor and perceptual timing

This thesis presents evidence that the cerebellum is involved in processes pertinent to temporal tasks, but evidence that this structure is engaged in temporal processing per se is not apparent. First, in the PET study presented in Chapter 3, the cerebellum was active during millisecond- and seconds-range time reproduction, refuting Ivry's (1996) suggestion that it is only pertinent to timing millisecond-range intervals. However, when the timing tasks were compared to a tightly matched control task, no cerebellar activity was present. Furthermore, no cerebellar activity was found for healthy controls during two motor timing tasks compared to a tightly controlled control task in the second PET study presented in Chapter 6. Cerebellar activation was present for the PD group across the two timing tasks compared to the control task, i.e. the cerebellum is activated only in the presence of pathology in the basal ganglia. Indeed, later analysis showed that the cerebellar activity was only present when the patients were 'off' medication and not when they were 'on' medication.

Some timing deficits were observed for the patients with cerebellar disease in the clinical study presented in Chapter 5. Increased variability on the repetitive tapping task and a pathological pattern of variability on the time reproduction task were observed, as well as a trend towards elevated variability in the warned and unwarned reaction time task. However, there was no evidence of timing deficits on the time production task, which had a minimal motor component, nor was there any evidence of accuracy being compromised. Cerebellar disease is by nature more heterogenous and more rare than Parkinson's disease (hence only 8 patients were tested), which makes finding a robust effect more challenging. Nevertheless, the negative result from the functional imaging studies concurs with the clinical timing result that the cerebellum does not play a direct role in timing processes.

The cerebellum is known to project to motor and prefrontal cortex (Middleton and Strick, 1998) and is hypothesised to carry out a range of functions including motor learning, fine movement control and coordination, sensory analysis and

cognition (e.g. Thach, 1998). Thus, given the complexity of its functions it is possible that a secondary process may underlie the increased variability observed in the cerebellar patients in Chapter 5. Indeed, Malapani et al (1998a) suggest that the evidence for involvement of the basal ganglia *and* cerebellum in timing may be explained by 'clock function' being associated with the basal ganglia, but with intact functioning of the cerebellum being necessary for a 'fully integrated, efficient temporal performance'. This hypothesis fits with the pattern of results obtained in this study, with patients with cerebellar disease showing evidence of increased variability on some timing measures but with cerebellar activity not being apparent in functional imaging studies of healthy controls when a tightly matched control task is used. Malapani et al (1998a) suggest cerebellar pathology could lead to a loss of precision in information transfer or threshold placement, either in striato-thalamo-cortical or cerebellar-thalamo-cortical circuits. Continuing a hypothesis first outlined in Gibbon et al (1997), they suggest the possibility that cerebellar damage may cause a deregulation of thalamic control at convergence points for striatal and cortical connections.

Harrington et al (2004ab) describe the known role of the cerebellum in monitoring and adjusting information from the cortex, particularly its role in signalling inconsistencies between an intended action and the actual sensory consequences (Blakemore et al, 2001). As such, the cerebellum may be monitoring input from sensory (e.g. auditory) systems involved in encoding intervals and then be optimising this input in accord with internal representations of a target interval i.e. acting on sensory information to optimise either sensorimotor or cognitive operations within the cortex. This means that if sensory acquisition is slowed following damage to the cerebellum then there will be disruption to the acquisition of input that is necessary for calculating a temporal interval, with this information then being coordinated with an already dysfunctional motor-output system. In complement to Harrington and colleagues, Penhune et al (1998) suggest that the cerebellum may be involved in the learning of timed motor responses and also in sensory integration, including extracting temporal parameters from sensory inputs. Clearly, a pathological system of this kind would show greater dysfunction when inputs and outputs to the cerebellar system occur at a faster pace, which explains why

the repetitive tapping task was particularly problematic for the patients with cerebellar disease. If temporal deficits are explained by sensory dysfunction, then this also explains why the cerebellum is particularly active when rhythmic sequences are complex or novel (Penhune et al, 1998). These hypotheses also explain why timing is not disrupted on a time production task for patients with cerebellar disease as the lack of sensory input in the form of a target interval means that performance is not degraded by a dysfunctional sensory system.

To conclude, this thesis presents evidence of limited involvement of the cerebellum in motor and perceptual timing in the millisecond- and seconds-range timing, once other contributory processes have been accounted for. In accordance with previously proposed hypotheses, it is suggested that this region contributes necessary sensory functions that cause a break down in precision on particularly demanding tasks.

7.1.3 The contribution of the cortex to millisecond- and seconds- range motor and perceptual timing

This thesis suggests a role for the cortex in supporting timing operations, but does not propose a role as a 'timer'. Right frontal and parietal regions were more common during the time reproduction of 2000 ms than 500 ms intervals in the PET study reported in Chapter 3. Timing a 2000 ms interval is clearly more cognitively demanding and these regions of the right hemisphere have previously been implicated in temporal processing by providing attention and working memory processes (Harrington et al, 1998b). The known role of the parietal cortex in attention suggests that this region is necessary for the attentional demands of the task (e.g. Posner et al, 1987; Posner and Presti, 1987; Robinson et al, 1995). Indeed, it is believed that timing performance is closely linked to the degree of attention afforded to the task (e.g. Thomas and Weaver, 1975; Zakay, 1989; Zakay and Block, 1996). The results of the rTMS study presented in Chapter 4 suggest that the activation of the right DLPFC reflects temporal memory processes. Specifically, it was shown that the right DLPFC was essential to accurate performance of the reproduction of a 2000 ms, but not a 500 ms, interval. The disruptive effects of the rTMS only occurred

when it was presented at the onset of the reproduction period and, as such, the right DLPFC was ascribed a role in the consolidation and transfer of temporal memory. Conversely, the PET study presented in Chapter 6 found mainly left hemisphere cortical activation for the control group during the timing tasks compared to the control task. The task involved repetitive tapping, a task that is highly motoric, and the left hemisphere is known for its role in motor processing (e.g. Haaland et al, 2000; Rushworth et al, 1998; Schluter et al, 1998). Thus, the precise cortical contribution depends on stimulus factors.

The exact nature of the cognitive contribution of the cortex to temporal processing is a source of debate. On the one hand, the working memory contribution to temporal processing might be non-specific. This is suggested by patients with frontal lesions who show poor performance on duration and frequency discrimination tasks only when the working memory demands of the task are increased (e.g. increasing the inter-stimulus interval) (Mangels et al, 1998). However, it is not inconceivable that working memory functions may be providing timing calculations. Inhibitory cell pairs in the DLPFC show a delay in activity between them of 200 to 1400 ms, which has been presented as evidence of timing-like behaviour in the prefrontal cortex (Constantinidis et al, 2002). Lewis (2002) proposes that this evidence suggests that the internal clock may be located within the prefrontal cortex, and that the deterioration of dopaminergic projections to the prefrontal cortex underpin the timing deficits seen in PD. A recent review article, supportive of the striatal beat frequency model (Matell and Meck, 2000; 2004), proposes that working memory and interval timing may rely on the same neural representation of a given stimulus event i.e. the pattern of active neurons gives information about stimulus identity and the oscillatory state of these neurons over time gives temporal information (Lustig et al, 2004). The multiple time scale model (Staddon and Higa, 1999) has also recognised the link between memory and time, suggesting that temporal judgements are based on decaying memories of different 'strengths'. The finding of gross underactivation of the frontal cortex during the motor timing tasks presented in Chapter 6 are not incompatible with the idea that striatally-driven frontal dysfunction may influence deficits in temporal processing in PD. However, the finding of greater errors on a time production task, a task that

requires 'time sense' and does not involve memorising an interval, for patients with PD tested 'off' compared to 'on' medication (Chapter 5) suggests that timing deficits can occur without activating memory processes. This is in keeping with the idea that the cortex provides 'supportive' and non-specific operations.

7.2 COMPARISONS WITH PREVIOUS LITERATURE

With respect to the previous findings described in the Introduction, the results of this thesis are compatible with a wide range of findings although do produce some inconsistencies. With respect to evidence for the role of the basal ganglia in timing, the results presented in both Chapter 5 and Chapter 6 did not replicate the degree of impairment on the repetitive tapping task for the patients with Parkinson's disease that other researchers have reported (e.g. Harrington et al, 1998a, Pastor et al, 1992a; O'Boyle et al, 1996). Also, the results of Chapter 6 did not find evidence of a 'slowed' internal clock in the patients with PD, such as would be indicated by consistent overestimation on a time production task, unlike previous research (e.g. Pastor et al, 1992). Despite this, significantly increased absolute error was found. Discussion has already been made of how the small sample size may have affected the significance levels reported in this thesis. Dopaminergic medication did have the effect of improving performance on certain tasks, supporting previous clinical (e.g. O'Boyle et al, 1996; Pastor et al, 1992b) and pharmacological work (e.g. Rammsayer, 1993; 1997).

The finding of SNc activation during a timing task in the PET study presented in Chapter 3 strongly supports lesion and physiological work in animals that has implicated the SNc in temporal processing (e.g. Matell et al, 2000; 2003). Basal ganglia activation was found in both PET studies (Chapters 3 and 6) and this reflects previous functional imaging studies that have supported a key role for the basal ganglia in timing (e.g. Ferrandez et al, 2003; Harrington et al, 2004b; Rao et al, 2001).

With regards to the cerebellum, as with previous studies on patients with cerebellar pathology, there was no evidence of accuracy being compromised in temporal tasks (e.g. Harrington et al, 2004a; Ivry and Keele, 1989). Concurring with this thesis, a recent clinical study also concluded that patients with cerebellar lesions did not exhibit true perceptual or motor timing deficits (Harrington et al, 2004a) and only one lab has published consistent evidence of timing-specific deficits in patients with cerebellar pathology (e.g. Casini and Ivry, 1999; Ivry et al, 1988; Ivry and Keele, 1989; Mangels et al, 1998). Some studies have found evidence of dysfunctional performance on the duration discrimination task for patients with cerebellar pathology (e.g. Casini and Ivry, 1999; Mangels et al, 1998), a task that was not used in the series of studies presented in this thesis. Although this data may appear convincing, there is also evidence that these patients may be impaired on other (non-temporal) types of discrimination task (Casini and Ivry, 1999). In support of this, most well controlled functional imaging studies of duration discrimination fail to find cerebellar activation (e.g. Ferrandez et al, 2003; Lewis and Miall, 2003b; Nenadic et al, 2003; Rao et al, 2001). There has been convincing evidence that the cerebellum is involved in the learning of a timed response in eyeblink conditioning studies (e.g. Perrett et al, 1993; Yeo et al, 1985ab). However, it does not follow that the regions involved in the subconscious temporal modification of a reflex response are necessarily engaged during typical millisecond- and seconds-range motor and perceptual temporal processing.

There was no evidence in the functional imaging studies (Chapters 3 and 6) that the cerebellum plays a central role in temporal processing. This reflects the findings of previous functional imaging studies in which motor activation has also been tightly controlled for (e.g. Ferrandez et al, 2003; Lewis and Miall, 2002; Macar et al, 2002; 2004). Cerebellar activation occurs more often in motor timing tasks (e.g. Kawashima et al, 2000; Lejeune et al, 1997; Rao et al, 1997), perhaps suggesting that its role in motor processing may in some way underlie the activity. As a final point, there is no evidence of a 'clock-like' timing signal in the deep cerebellar nuclei (Keating and Thach, 1997), indicating the neurophysiology of the cerebellum does not support a role in timing processes.

The finding that the cortex supports cognitive operations necessary for timing reflects previous animal work in which cortical lesions have been found to disrupt memory processes during timing (Olton et al, 1988). Both Chapters 3 and 4 support the idea of a right fronto-parietal network engaged in supportive cognitive operations in the seconds-range, reflecting the findings in previous functional imaging work (see Lewis and Miall, 2003a). As discussed in the Introduction, patient studies have failed to find consistent or convincing evidence for cortical involvement in key temporal processes (e.g. Casini et al, 1999; Mangels et al, 1998). As part of an exploration of their striatal beat frequency model, Matell and Meck (2004) have presented evidence that ramp and oscillatory activity in the cortex could produce firing patterns that serve as clock signals that are integrated by the striatum. This thesis does not produce any results that could categorically refute this hypothesis, although Matell and Meck also acknowledge that 'the striatum and the substantia nigra pars compacta are the only brain areas that have been shown to be necessary for interval timing' (Matell and Meck, 2004).

7.3 LIMITATIONS OF THE THESIS

There are several limitations to the thesis, which need to be considered when interpreting the results. First, the sample sizes used could have been larger, as has been commented on in the relevant chapters. For example, it could be considered that some sub-threshold trends in the clinical study (Chapter 5) may have reached significance if the sample sizes were larger and that the limited regions found in the timing tasks > control task comparison in Chapter 3 may have been more extensive. Attempts have been made to account for this limitation when interpreting the results. In addition, further detail about the specific location of the cerebellar degeneration in the patients with cerebellar disease would have been useful is teasing apart the timing functions of the cerebellum, but unfortunately such information was not available.

Care was taken to use a wide range of temporal tasks but one key task, the duration discrimination task, was not used. This is an important task as it

measures perceptual timing without the involvement of a timed movement, allowing a pure measure of perceptual timing to be established. Although the time production task included in the clinical study presented in Chapter 5 contained limited motor demands, it involved the estimation of seconds-range periods > 30 s. Assessing the performance of the patients on a duration discrimination task spanning the milliseconds- and seconds-ranges used in the other timing tasks in that chapter would have proved informative.

Although the time reproduction tasks used in Chapters 3 and 4 were similar, the limitations of the particular techniques (PET and rTMS) meant that different versions of the tasks had to be used. The PET version of the task has far greater reliance on memory for an estimated period, whereas the rTMS version follows a more typical time reproduction design. The joint impact of the two studies would have been greater if identical versions of the same task were used. This could have been achieved if fMRI had been used, as an event-related design would have leant itself to having Estimation and Reproduction phases separately analysed (not possible in PET). Unfortunately, the PET study was designed and data collected and analysed before the rTMS study.

More evaluation of the findings relating to variability would have perhaps added to the thesis. Comment was not made on whether variability increased in proportion with the mean of the interval being timed, which would suggest a clock effect (see the description of SET in the Introduction) or whether effects on variance are an additive constant (suggesting a sensory registration or motor implementation effect).

7.4 FUTURE RESEARCH

The results presented in this thesis raise interesting possibilities for future research. First, temporal processing is a ubiquitous process, yet it is misleading to suggest that the many varied types of timing are underpinned by one mechanism. In Chapter 5, both the unwarned and warned RT task and the memory for temporal order task required the processing of timing-related

information, yet performance was preserved in patients with PD and patients with cerebellar disease. Future research should investigate the different ways in which time can be represented. For example, a functional imaging study could use identical stimuli for a time reproduction task and an unwarned and warned reaction time task, enabling an investigation of the task-dependent neural activity occurring between the two tones (either denoting the interval to be reproduced or the warning tone and Go-tone). This would provide interesting information about how these types of temporal information are differentially processed. On a similar note, although the data presented in this thesis are not inconsistent with the idea that motor and perceptual timing mechanisms are underpinned by shared neural mechanisms, there is little direct investigation of this hypothesis.

A rather surprising result from this thesis is that the mildly affected de novo patients (Chapter 5), who had not yet received dopamine-therapy, were worse on a time reproduction task than more severely affected patients with PD tested 'off' medication. De novo patients have not previously been compared to more severely affected patients on motor and perceptual timing tasks and the data suggest a complex relationship between temporal processing and basal ganglia pathology. It is difficult to tease apart whether the results from the time reproduction task reflect the effects of duration of illness or disease severity or the effects of being chronically exposed to levodopa medication. A possible solution would be to use a previously employed design (Owen et al, 1997) that tests three groups of patients: i) a PD-de novo group with mild symptoms ii) a PD-drug-off group with mild symptoms iii) a PD-drug-off group with severe symptoms. The present study had groups i) and iii), but the inclusion of group ii) would help disambiguate whether symptom severity or medication underpinned the effect. Following the interesting effect of apomorphine on neural activity during motor performance in Chapter 6, it would also be informative to repeat a similar design using a perceptual timing measure, such as the duration discrimination task. Currently, functional imaging has not been used to investigate the neural correlates of perceptual timing in patients with PD.

The results reported in this thesis do not support the hypothesis that the cerebellum provides a 'clock'-like function during temporal processing. Further work is needed to establish the exact nature of its contribution, with current hypotheses suggesting a role in processing sensory information. Prompted by the finding that patients with cerebellar lesions are impaired at both duration and frequency duration (Casini and Ivry, 1999; with impairments in frequency duration approaching significance in Mangels et al, 1998) it may be useful to investigate this type of task further. Testing patients with cerebellar pathology on auditory and visual versions of the duration discrimination task as well as other types of discrimination task (e.g. frequency, loudness, intensity and tactile (spatial or force)) would help establish whether a fundamental discrimination deficit related to the processing of sensory information exits. As already mentioned, cerebellar patients are considerably heterogeneous. However, as the classification of patients with cerebellar degeneration into different genetic subtypes becomes more common, it may be interesting, and produce a more consistent set of results, to test and compare different genetic subgroups. Finally, as mentioned in the previous section, future work could concentrate more on characterising the variability being observed.

7.5 SUMMARY

In summary, the results presented in this thesis lead to the conclusion that the basal ganglia are the main component of the temporal processing network in the brain. The findings do not support a central role for the cerebellum in temporal processing, although it is suggested that this region provides supportive processes that are necessary for optimal timing calculations. The cortex plays a role in providing necessary cognitive functions.

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