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Neural and Social Mechanisms Behind the Social Transmission of Food Preference

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NEURAL AND SOCIAL MECHANISMS BEHIND THE SOCIAL TRANSMISSION OF FOOD PREFERENCE

by

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Abstract

Research on the social transmission of food preference (STFP) has shown that preferences for specific foods can be transmitted between conspecifics (Bilkó *et al*., 1994; Wrenn *et al*., 2003; Hikami *et al.,* 1990; Galef *et al.,* 1984; Galef & Wigmore, 1983; Lupfer *et al*., 2003). Although these findings provide an understanding of how food related information is shared, none explore the influence that personality may have on various factors of STFP, as well as how this transmission may occur in a naturalistic foraging setting. In the current thesis, individual personality was assessed and rats were placed into same/different preference foraging pairs within a novel arena, to explore the dynamics of STFP development and transmission between pairs of rats.

In addition to investigating behavioural factors affecting STFP, I examined how development of a preference based on exposure to an odour might alter the representation of that odour within the rodent brain. As shown in previous studies, exposing rat pups to a novel odour paired with tactile stimulation led to that odour being preferred and also being represented by a larger ensemble of mitral cells that is more reliably recruited upon re-exposure to the odour (Shakhawat *et al.,* 2014). Since the pairing of carbon disulfide with a novel food odour will create a preference in the subject during STFP, I hypothesized that this pairing, like tactile stimulation, could alter the olfactory representation of the odour (Galef e*t al*., 1984).

My findings reveal that personality does not affect the strength of an individual's food preference. Bolder individuals spend less time eating while foraging. In addition, less bold individuals spend a large proportion of their foraging time eating while their partner is also

eating, which I defined as an *overlap score*. A larger recruitment of mitral cells within the main olfactory bulb did not occur upon presentation of the preferred odour. However, distinct activation patterns were present upon exposure to different odours, suggesting that differentiation of the stimuli was visible at the neuronal level but integration of social information must take place further downstream.

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Chapter 1: General Introduction

Research on the social transmission of food preference (STFP) demonstrates that food preferences can be transmitted between conspecifics (Galef & Wigmore, 1983; Lupfer *et al.*, 2003). However, no studies that I am aware of have explored the role that personality may have in the transmission of information. As well, how transmission of information occurs between pairs within a naturalistic foraging setting remains largely uninvestigated. Through a battery of personality tests, the current thesis first assesses personality and then evaluates the effect that this may have on STFP. By placing rats into same/different preference foraging pairs within a novel arena, factors acting upon information transmission are also investigated within a more naturalistic foraging setting than ones used during the classic STFP transmission paradigm. Using this procedure, my thesis aimed to explore the dynamics of STFP acquisition and transmission between rat pairs.

1.1 Social Transmission of Food Preferences

In the STFP paradigm originally and nearly simultaneously described by Posadas and colleagues (1983) as well as Galef & Wigmore (1983), naïve rats (observers) were able to acquire a preference for a certain food after smelling this food on the breath of a demonstrator rat. In Galef and Wigmore's (1983) protocol, a demonstrator rat was fed powdered food mixed with a novel flavouring such as cocoa or cinnamon for 30 minutes. Following feeding, the demonstrator was placed in a cage with a food-restricted observer for an additional 30 minutes. After the interaction, the observer underwent a feeding choice test in which two novel foods were made available within the home cage. One of the novel foods available was the flavoured

food consumed by the demonstrator, and the other was a novel flavouring to which the observer had never been exposed. For 24 hours, the observer was left undisturbed to consume the flavoured foods after which the food was removed and consumption of each food was recorded. In numerous replications of this procedure, Galef and his collaborators found that the majority of the total food consumed by the observer was of the flavoured food consumed by the demonstrator prior to the interaction. Thus, a preference for this previously novel food had been learned solely through a social mechanism.

Despite being a reliable demonstration of social learning, STFP is not dependent upon a certain behaviour of the demonstrator (Galef & Kennett, 1987). For example, STFP occurs even if the demonstrator is anaesthetized for the duration of the interaction phase (Galef, 1985). Subsequent studies have shown that the key element for successful STFP is the presence of carbon disulfide, present in small quantities within the digestive tract, in combination with a novel food odour (Galef *et al*., 1988). Replacing the demonstrator with a carbon disulfide-soaked cotton ball rolled in the novel food flavouring is sufficient for STFP to occur. Odours presented on a cotton ball in the absence of carbon disulfide do not lead to the establishment of a preference in the observer (Galef *et al*., 1988).

Since rats are neophobic (Barnett, 1958) and consumption of a novel food is generally avoided (Barnett, 1963), smelling a novel food odour on another rat's breath could indicate that this food is safe to eat, thereby increasing the rodent's repertoire of consumable food. However, there are limits to learning via STFP, as the sharing of food information will only lead to the development of a preference and not an aversion (or avoidance). Following feeding of the demonstrator, if the demonstrator receives an intraperitoneal injection of lithium chloride and exhibits signs of illness during the interaction with the observer, the observer does not

show an avoidance of the demonstrated food but rather consumes a greater proportion of it when given a choice test (Galef, 1985; Coombes *et al*., 1980). This suggests that the effect of social influence on preference is greater than on aversion, therefore STFP may function as a means for sharing information to increase familiarity rather than indicating the safety of a novel food.

Although STFP research to date is considerable, there remain several unanswered questions concerning the effect of personality, the dynamics and utilization of preference information in a foraging setting, and the neurological substrates behind STFP. By first assessing the personality of individuals and then giving them an odour preference via the STFP protocol, the research discussed in Chapter Two of the current thesis examines if personality can affect the strength of an individual's socially-acquired food preference. As well, I explored what effect conflicting preference information from a conspecific had when rats were placed into pairs within a foraging arena. Finally, by combining individual personalities, behaviours exhibited during foraging trials, and results for preference tests at the start and end of each experimental phase, I provide information as to how social and spatial dynamics affect food preference transmission and maintenance.

In Chapter 3, I examine whether the preferred odour acquired via STFP is represented by a larger ensemble of mitral cells than a non-preferred odour, as well as whether that ensemble is more reliably recruited upon exposure to the preferred odour. To conclude the chapter, I provide evidence on whether or not the integration of both social and odour information is present within the main olfactory bulb.

1.2 Personality

Since STFP is an inherently social phenomenon, the food information shared during STFP may not be the only informative signal being received: each individual (the demonstrator and the observer) presumably has additional information concerning the other individual that may alter STFP. For example, perhaps the food preference of shyer rats can be disrupted by conflicting information from a bold conspecific. In considering additional information that may alter STFP, potential measures of personality in the rat should be examined.

Human personality can be defined as a consistent pattern in thinking, feeling, or behaving that is relatively stable over time and across situations (Gosling, 2001). Personalities in animals work in much the same way as they are consistent within an individual across time and influence their behaviour across contexts (Biro & Stamps, 2008). Animal personality is of importance ecologically as well as evolutionarily in that it helps to explain what would otherwise be thought to be maladaptive behaviour (Réale *et al*., 2007).

Depending on the literature's primary audience, species of interest, or the preference of the experimenter, several terms are used when studying animal personality. For example, temperament (Réale *et al*., 2007), individuality (Jonas, 1968), or personality traits (Biro & Stamps, 2008) are commonly used terms. However, throughout my thesis these individual tendencies potentially leading to predictable outcomes in behaviour will be referred to as personality.

Similar to human personalities, there are a variety of animal personalities within a population. For example, often examined under the classification of "behavioural syndromes" (Sih *et al*., 2004), a large body of research examines one such animal personality type referred

to as “aggression syndromes”. Individuals exhibiting an aggression syndrome will behave more aggressively across several situations and contexts than other, less aggressive, individuals (Sih *et al*., 2004). A behavioural syndrome arises from a correlation between several personality traits (Sih *et al*., 2004).

Proposed by Réale *et al*. (2007), animal personality can be described using five general traits: activity, shyness/boldness, exploration, aggression and sociability. When assessing these traits in rodents, several tests are regularly used, including the elevated plus maze, light/dark emergence into an open field, and social preference tests. An elevated plus maze consists of two open and two closed arms arranged in a cross-elevated position above the ground. Predominantly used for drug research, this test is assumed to measure anxiety-like behaviour, under the assumption that a less anxious rat will spend a greater proportion of its time on the open arms of the maze (Montgomery, 1955). Also common in drug research, the light/dark emergence test utilizes a rodent’s natural tendency to avoid brightly lit areas. During this task, a small enclosed compartment, or start box, is placed within a brightly lit arena. Moving from the start box and into the arena, as well as movement between the two over the duration of the trial, has been reported as a measure of activity-exploration (Bourin & Hascoët, 2003; Crawley, 1985). The amount of time spent in the arena is also reported as a measure of a subject’s aversion to the light (Bourin & Hascoët, 2003; Crawley, 1985). The boldness and anxiety-like behaviours of a rodent can also be measured by observing food caching in the presence of a predator stimulus (Herman *et al*., 2000). In an experiment by Dochtermann and Jenkins (2007), hulled sunflower seeds were scattered in an arena where predator urine had been sprayed. Rats of higher boldness were observed collecting and caching more seeds than less bold conspecifics (Dochtermann & Jenkins, 2007).

A social preference test assesses whether a rat will choose to spend a greater proportion of its time in the vicinity of a conspecific than a decoy. During this test, a rat is placed in an arena containing two cages. One of the cages houses a live conspecific and the other houses a stuffed decoy rat. If more time is spent around the live conspecific, the rat is presumably more social (Moy *et al.,* 2004). Mirror-image stimulation (MIS) is also a commonly used test for assessing social behavioural pattern variations amongst individuals of several different species (Dochtermann & Jenkins, 2007; Baenninger, 1966). MIS is preferable to direct conspecific interaction in that it is able to give a measure of approach, avoidance, and sociability without the risks associated with physical conflict (Svendsen & Armitage, 1973). In a study by Dochtermann & Jenkins (2007), behavioural variations in kangaroo rats were assessed. Using MIS, the time to resume activity after a subject saw its reflection (latency) was utilized as a measure of aggressiveness toward a conspecific.

Following assessment of personality, my thesis aimed to determine if variations in preference strength acquired via STFP could be explained by an individual's personality. For example, whether a rat displaying behaviours associated with high sociability would develop a stronger preference following the demonstrator/observer interaction. If a rat scored as being "highly social" perhaps they would be better at detecting and utilizing socially transmitted information than an anti-social conspecific. Perhaps shy rats, due to their anxious tendencies, are less likely to gather information on their own and therefore rely on socially transmitted information more than bold rats. I would hypothesize that if this were true, bold rats would develop weaker preferences following demonstrator/observer interactions as they are more likely to acquire food related information on their own, making socially transmitted information less salient.

1.3 Living in Groups

For individuals, living in a group confers many benefits, including a dilution of predation risk, collective defense, increased mating opportunities, increased anti-predator vigilance, and an increase in the efficiency of foraging (Krause & Ruxton, 2002). For example, individuals in larger groups are less likely to be attacked by a predator both due to a decreased statistical probability of being chosen for attack and, for individuals near the center of the group, due to "shielding" by other members of the group (Williams, 1966; Alexander, 1974). Additionally, the more members in a homogeneous group, the more difficult it is for a predator to single out and attack an individual, which is referred to as *predator confusion*, as the predator is bombarded by stimuli and unable to focus on a target (Landeau & Terborgh, 1986). Some groups are also capable of organizing and implementing collective defense, as the group approaches and attacks a predator *en masse* to deter the predator. Collective defense not only acts to drive away an intruder, it also functions to promote vigilance and alert others in the group of the predator's presence (Krause & Ruxton, 2002; Lima & Dill, 1990).

Although living in a group provides the individual with many benefits, decreases in fitness with an increase in group size are also possible. As the number of individuals in a group increases, there is greater competition for finite resources, resulting in less available for each individual (Krause & Ruxton, 2002). *Kleptoparasitism* in avian species or *food snatching* in rats also occurs as individuals steal food from one another in a presumed attempt to gain information about a novel food source (Brockmann & Barnard, 1979; Galef, *et al*., 2001). Kleptoparasitic events result in costs to some group members and have the potential to become more frequent with increasing group size (Krause & Ruxton, 2002). Additionally, several other costs are possible including an increased parasitic load of contact-transmitted

parasites (Brown & Brown, 2004), a greater risk of misdirected parental care (Hoogland & Sherman, 1976), and larger groups being more easily detectable by predators than individuals (Krause and Ruxton, 2002).

Despite the costs listed above, living in a group confers several foraging benefits. For an individual organism, there is a trade-off between the amount of time spent in search of food and time spent watching for predators (Barnard & Sibly, 1981). When an individual is a part of a group, however, there are more individuals available to detect a predator. This distributes the time spent scanning for predators throughout the group and increases the efficiency of predator detection (which is referred to as the many eyes hypothesis). That is, the probability of detecting a predator increases and the latency to detect a threat is reduced. Importantly, this has the benefit of increasing overall foraging efficiency (Lima, 1995; Godin & Morgan, 1985).

The efficiency of foraging is further increased in a group context because individuals have the opportunity to observe and learn from the foraging behaviours of other members of the group, reducing the need for personal experience. Between individuals, multiple kinds of information about a foraging context can be shared, including information concerning location (Galef & Giraldeau, 2001) and type (Real, 1992) of food available, as well as any predation risks (Ward *et al*., 2008). The sharing of food-related information provides the advantages of locating additional sources of food, while eliminating the energetic demand and predation risk associated with learning about a foraging environment, thus increasing an individual's likelihood of survival (Krause & Ruxton, 2002; Beauchamp, 2013).

The following review is restricted to data on rodents, the order used in the current thesis and for which a wealth of data already exists. Previous research involving rodents includes a classic demonstration of the sharing of food-related information within a rat colony

following the introduction of a poisonous substance to control the pest population (Steiniger, 1950). Steiniger (1950) showed that, following the introduction of a poisoned food, the population of a colony initially declined but then increased following the mating of surviving members. Although a large portion of the colony consumed the food and perished as a result, some of the members consumed only a small amount, leading to illness followed by avoidance. As the elders avoided the poisoned food, subsequent generations also avoided the food source without having sampled it themselves. Further studies investigated how food source information is transmitted between individuals.

According to Galef and Clark (1971), the behaviour described above resulted from a three-stage process: 1) pups followed elders to a safe food source, 2) pups learned to associate cues with the food source, 3) food sources other than those observed being consumed by the elders were then avoided as a result of innate neophobia (Galef & Clark, 1971). Information transmitted by elders is so salient that even the presence of an anaesthetized adult near a food source can lead to preference acquisition in a pup (Galef, 1981; Galef, 1971). During these experiments, rat pups chose to feed at the same novel food site as both a feeding, and later an anaesthetized, adult, suggesting that the adult's presence is sufficient produce a feeding preference (Galef, 1981; Galef,1971; Galef & Clark, 1971). Many other studies have generated data consistent with this social transmission of food preference by observation. For instance, a naïve forager can sometimes estimate the quality of a food patch by observing others foraging (Marler *et al*., 1986) without the energetic expense and risk of going out and finding the source independently.

Unlike learning about food sources through direct observation of a conspecific with a particular food, the utilization of social transmission via STFP means that conspecifics would not

need to be in the same vicinity during foraging as information could be shared following the feeding event. The idea that STFP can act as a mobile exchange of food related information suggests that conspecifics could forage in independent locations, later sharing this information, and thereby increasing the net probability of locating food.

When a rat is introduced into a novel environment, it will typically start exploring the environment by making several short trips or excursions and returning to one specific location, referred to as the home base (Eilam & Golani, 1989). The home base is the site of a highest proportion of grooming and rearing compared to behaviours outside of this location and can be used as a reference when examining exploratory behaviour (Eilam & Golani, 1989). Rats placed in a novel environment will quickly establish a home base, at a sheltered location if available. Eventually, as they habituate to the environment, secondary home bases may be established (Drai *et al*., 2000).

Consistent with the Information Center Hypothesis, during foraging a home base might act as a safe location used by the rats in which they can interact and share information with one another (Galef & Giraldeau, 2001). In a natural setting, the rat colony acts as an information center where information, particularly about the presence and safety of a food source, is shared through sniffing of a returning foragers' breath.

As discussed at the beginning of the previous section, individuals involved in transmission of information either in the context of STFP or within a naturalistic foraging setting presumably have access to information concerning the other individual. For the current thesis, I was interested in studying if personality could alter information acquisition, transmission and maintenance. I hypothesized that during foraging with a conspecific, perhaps the food preference of shyer rats could be disrupted by conflicting information from a bold conspecific. If

this was the case, the preference of a shy individual could potentially be readily and largely degraded, or a preference for a partner's preferred food may have developed following the social presentation of conflicting information. Conversely, I presumed it would be likely for bold individuals to be less likely to acknowledge and utilize food related information from a shy conspecific as they could be more likely to discover novel food information through exploration on their own. In addition to this, perhaps bold individuals displayed lower latency when sampling novel foods. Lower latency to sample by bolder individuals may also suggest that they rely less on social information than shy individuals in the context of foraging. Preference strength following demonstrator observer interactions may largely depend on the sociability of the subject whereas sampling of a new food and preference degradation in the absence of social interactions may rely more on an individual's boldness.

1.4 Neurobiology of Social Learning

When studying behaviours indicative of learning within a group or at the level of the individual, it is important to also consider the neurological substrates involved. More specifically, the neurobiological mechanisms associated with STFP has been studied extensively, at least in part because the procedure makes two steps essential for social learning easily identifiable. The first step being that of acquisition of the transmitted information during the demonstrator/observer interaction and the other step being the point of retention following this interaction. By manipulating the observer prior interaction, mechanisms involved in acquisition can be studied. Additionally, mechanisms involved in memory, retention, and recall of the information can be examined by manipulation of the observer following the interaction, during what is referred to as the "post-social" phase (Choleris & Kavaliers, 1999). In a study by

Winocur (1990) observer rats underwent lesions of either the hippocampus or dorsomedial thalamic regions prior to interactions. Animals from either lesion group were comparable to control rats as they exhibited a preference during a choice test following the demonstrator/observer interaction. However, Winocur (1990) revealed a difference between the two groups when the choice test following interaction was delayed. Rats with hippocampal lesions exhibited a preference for a period of only 1-2 days following the interaction while rats with dorsomedial thalamic lesions exhibited a preference for up to 8 days after. In a second experiment, Winocur (1990) tested the effects of lesions to these regions either immediately, 2, 5, or 10 days following a demonstrator/observer interaction. Rats with dorsomedial thalamic lesions showed no preference recall when the lesions were done immediately but displayed a preference when lesions were done following the 2, 5, or 10 day delay. Similarly, hippocampal-lesioned groups showed no preference recall when lesioned immediately but displayed gradual improvement when lesions were delayed. Findings comparable to that of the temporally graded retrograde amnesia shown by Winocur's post-social hippocampal lesions have also been investigated in the context of fear-conditioning as well as spatial learning (Anagnostaras *et al.*, 1999; Cain & Saucier, 1996)

An additional study by Bunsey and Eichenbaum (1995) investigated the impact of hippocampal lesions (including subiculum) prior to demonstrator/observer interaction. Lesioned animals acquired a food preference but long term retention was impaired. Localized lesion studies to either the hippocampus proper, dentate gyrus, or subiculum resulted in no short- or long-term preference memory deficits, indicating that the entire hippocampus is essential for retention of STFP related memory.

Studies have not only been conducted examining the role of various brain regions in STFP, but underlying neurobiological mechanisms have also been investigated. Acquisition of food preference in a mutant strain of mice lacking cAMP responsive element-binding protein (CREB) showed no short term deficits in preference recall (Kogan *et al*., 1997). These mice however, did display long term deficits in food preference memory as well as fear conditioning.

The results of studies discussed in this section provide a variety of ways by which social learning occurs at the neural level. In Chapter 3 of my thesis, I investigated social learning within the main olfactory bulb of adult rats by examining mitral/tufted cell recruitment upon presentation of an odour which was made preferred by means of STFP. The role of social learning in establishing a food preference was investigated by comparing various demonstrator/observer interaction types. One of which was the classic social demonstrator/observer interaction while the other, although shown to create a preference, was conducted entirely lacking of social cues by use of a surrogate demonstrator.

1.5 Mechanisms of Olfaction

To investigate the neurobiology behind learning through STFP, it is important to understand functions and mechanisms of the sensory system on which it is acting, the olfactory system. Olfaction provides animals with the ability to detect information that guides behaviour critical for not only assessing novel foods, but also for locating food (Whishaw & Tomie, 1989; Rieger & Jakob, 1988), predator avoidance (Amo *et al*., 2008; Flowers & Graves, 1997), mating (Murphy & Schneider, 1970), establishing social hierarchies (Barata *et al*., 2007), and recognition of the mother and littermates (Gelhaye *et al*., 2011). While a great deal of work has been done investigating the behavioral and physiological mechanisms behind STFP (Galef *et al*.,

1988), as well as how preferences are established within the rat pup (Rangel & Leon, 1995; Sullivan *et al*., 2000; Yuan *et al*., 2003), few studies have examined the neurological substrates supporting STFP in the adult rat. Chapter three of the current thesis examined Arc expression within the main olfactory bulb (MOB) to see if the preferred odour was represented by a larger ensemble of mitral cells, which were more reliably recruited upon exposure.

In order for an odour to be perceived, a substance must release a volatile chemical called an odourant. To begin this perceptive path, the odourant must enter through the nares or nostrils of the animal and into the nasal cavity. Once in the nasal cavity, the odourant will pass over a mucus layer lining the nasal cavity, called the olfactory mucosa. Within the olfactory mucosa are the cilia of olfactory receptor neurons (ORN's) to which the odourants can bind. However, each ORN only expresses one of a possible 1500 different olfactory receptor genes, meaning each neuron expresses only one kind of G-protein coupled receptor, referred to as the one neuron-one receptor rule (Ardiles *et al*., 2007; Serizawa *et al*., 2004). Once the odourant binds to the receptor, the G-protein breaks away, activating sodium gated ion channels on the surface of the cell, causing depolarization and firing of an action potential. The action potential continues up through the axon of the ORN which extends through the cribriform plate of the animal's skull and terminates onto neurons in the glomeruli of the olfactory bulb (OB). The axons of ORN's expressing the same receptor converge onto a particular set of glomeruli and due to this axonal convergence, a single odourant molecule can activate several glomeruli within the set (Ardiles *et al*., 2007; Egana *et al*., 2005). Following convergence of ORN's onto a specific set of glomeruli, a second synaptic connection is made with the dendrites of mitral/tufted (M/T) cells (Ardiles *et al*., 2007). Within the olfactory bulb, tufted cells respond to a wide range of odour concentrations while mitral cells only respond when the odour

concentration is high (Igarashi *et al*., 2012). Additionally, tufted cells project densely to targets in anterior areas of the olfactory cortex while mitral cells project dispersedly to all olfactory cortex areas (Igarashi *et al*., 2012). Unless neuronal markers are used, mitral and tufted cells are difficult to differentiate and are found in close proximity of one another, generally leading to a combined classification (Pinching & Powell, 1971). Once synaptic connections are made with the M/T cells of the glomeruli, M/T axonal projections travel through the lateral olfactory tract (LOT) and terminate on the olfactory cortex (OC) where the odour is then perceived (Apicella *et al*., 2010).

To perceive different odours, each odour must have a specific signal that is transmitted to the OC. In addition to the one neuron-one receptor rule (Ardiles *et al*., 2007), each type of receptor is capable of detecting multiple odourants, and each odourant can be detected by multiple types of receptors (Malnic *et al.,* 1999; Araneda *et al*., 2004). Odours can then be differentiated based on the unique ensemble of receptors that they bind to and activate, this activation pattern (also referred to as an "olfactory image") is called combinatorial coding (Malnic *et al*., 1999). Combinatorial coding maximizes the number of discriminable odours based on the number of receptors within the olfactory epithelium.

1.5.1 Neural Representation of Preferred Odours in Rat Pups

Until the second week of life, rat pups, unable to see or hear, rely heavily on their sense of smell and touch to gather information within their environment. As an essential precursor to nipple attachment, social behaviour, and approach to the mother, a rat pup must learn to identify and utilize odour cues (Raineki *et al*., 2010). As young as post-natal day 1, pups are able to demonstrate odour preference for a novel odour stimulus associated with their mother

(Moriceau *et al*. 2010). When given a choice in a Y-maze between bedding from their mother who had been fed a specific diet and bedding of another dam fed a differing diet, pups tended to prefer bedding with a scent of their mother (Sullivan *et al*., 1990). In addition to this, Galef and Henderson (1972) demonstrated that rat pups were able to utilize odour information about the dam's diet contained in the milk, to later exhibit a food preference when given a choice test during their first solid food meal. A pup's ability to form an odour preference is so robust that an otherwise aversive olfactory stimulus can become preferred when paired with tactile stimulation such as stroking, warmth, licking, and milk delivery (Amiri et al., 1998; Yuan et al., 2003). The pairing of tactile stimulation with a novel odour not only elicits a behaviourally observable preference but pairing is also associated with electrical and metabolic neural changes within the olfactory bulb via a beta-adrenergic receptor-dependent mechanism (Rangel & Leon, 1995; Sullivan et al., 2000; Yuan et al., 2003). When tactile stimulation is delivered, noradrenergic neurons of the locus coeruleus (LC) are activated causing the release of norepinephrine (NE) and subsequent delivery of the neuromodulator to the MOB. When tactile stimulation is paired with an odour, the presence of NE in the MOB from the LC causes plastic changes within the activated population, resulting in larger and more reliable M/T cell recruitment upon re-exposure to the odour (Rangel & Leon, 1995). In other words, activation of the LC due to tactile stimulation in combination with odour specific MOB activation results in a cellularly observable change indicative of a preference.

1.5.2 Neural Representation of Preferred Odours in Adult

To date, little research has been done examining how or if a preferred odour is represented within the main olfactory bulb of the adult rat brain. However, research has been

conducted investigating odour representations within the piriform cortex that are governed by excitatory and inhibitory synaptic input. The work of Poo & Isaacson (2009) shows that odour exposure results in sparse spiking activity across the affected neuronal ensemble. Within the cortical population of the piriform cortex as well as the MOB, global inhibition occurs in a widespread, nonspecific, and broadly tuned manner, while excitation is more conserved and highly specific to certain odours (Spors & Grinvald, 2002). In order for an odour to be detected, the olfactory receptor neuron that the odourant binds to needs to fire an action potential (AP; Hopfield, 1995). Both a preferred and non-preferred odour can elicit an AP and both receive equal amounts of inhibition (average inhibitory postsynaptic charge: 78.6 ± 3.7 pC), however, by measuring excitatory post synaptic charge (EPSC), a preferred odour evokes greater excitation (average EPSC: 46.5 ± 1.5 pC) compared to a non-preferred (EPSC: 16.9 ± 0.7 pC; Poo & Isaacson, 2009). The results of Poo and Isaacson (2009) suggest that excitation associated with the presentation of an odour must be strong enough to overcome global inhibition activated upon binding of any odourant, to generate APs within the olfactory cortex.

1.5.3 Hypothesized Mechanisms of Odour Representation

Although the exact neuronal mechanism supporting preference learning in the adult rat brain is relatively unknown, a large body of research has contributed to the understanding of preference learning in rat pups. Several of these studies have examined how the pairing of tactile stimulation with a novel odour can result in formation of a preference. When physical stimulation is applied to a rat pup, the noradrenergic system of the locus coeruleus (LC) is activated which directly projects to the M/T cells of the OB, thereby increasing norepinephrine (NE) levels. Increasing NE levels has three important effects, 1) NE reduces inhibition in the

granule-mitral cell connection, 2) NE increases excitability in M/T cells allowing for activation of NMDA receptors, and finally, 3) NE decreases M/T habituation to odours (Sullivan *et al.*, 2000; Yuan *et al*., 2003). Due to the influx of NE, subsequent exposure to a novel odour promotes long-term potentiation-like modifications in the granule-mitral cell response patterns, as well, upon exposure to the preferred odour, a specific ensemble of granule-mitral cells is more reliably recruited (Sullivan *et al*., 2000; Yuan *et al*., 2003; Shakhawat *et al*., 2014). When NE is made unavailable by blocking noradrenergic receptors, M/T habituation to odours occurs as well as an inability to form odour preferences (Sullivan *et al.*, 2000). In the absence of tactile stimulation, pairing a novel odour with a beta noradrenergic agonist produces both behavioural and neural changes observable in stimulated pups (Sullivan *et al*., 2000). It is plausible that a comparable mechanism may drive preference learning in the adult during STFP. During social interaction with a demonstrator, tactile stimulation occurs which could lead to NE release in the observer brain in combination with the novel odour. In the absence of tactile stimulation, perhaps the presence of a conspecific alone is enough to activate comparable mechanisms in the adult rat brain, explaining how preferences can be established even when a demonstrator is anesthetized or a mesh barrier is placed between the demonstrator and observer (Galef *et al*., 1988; Galef & Wigmore, 1983). Similar to the rat pup studies, this would recruit a larger and more reliably represented ensemble of granule-mitral cell activity within the MOB following re-exposure to the demonstrated odour.

In addition to ORNs, the olfactory epithelium also contains a different type of chemosensory receptor called trace amine-associated receptors (TAARs). Like ORNs, TAARs are G-protein coupled but have a very different protein sequence (Liberles & Buck, 2006). Studies in rats have classified the presence of 17 different olfactory TAARs which recognize volatile

amines (Lewin, 2006). Volatile amines are present in the urine of mice, suggesting that TAARs may play a distinct role from odour detecting ORNs, possibly related to social cues (Liberles, 2009). Two amines found in rat urine with notable social cue significance are isoamylamine, and 2-phenylethylamine (Liberles & Buck, 2006). While 2-phenylethylamine is found in the urine of stressed animals, isoamylamine which is more prevalent in male urine, has been shown to accelerate puberty in female mice (Grimsby *et al*., 1997; Paulos & Tessel, 1982; Snoddy *et al*., 1985; Nishimura *et al*., 1989). Perhaps volatile amines are present in rodent breath and are utilized during detection of a preferred odour following STFP.

1.6 Conclusion

By combining individual personalities, behaviours exhibited during foraging trials, and results of preference tests throughout experimentation, the current thesis contributes to the understanding of how social and spatial dynamics affect food preference transmission and maintenance. By first assessing the personality of individuals and then giving them an odour preference via the STFP protocol, I determined if personality can affect the strength of an individual's socially-acquired food preference. As well, I explored what effect conflicting preference information from a conspecific has when rats were placed into pairs within a foraging arena. The neurobiological substrate that supports STFP in the olfactory system was also investigated by examining Arc expression within mitral cells of the MOB to determine where in the olfactory sensory pathway the integration of both social and odour information occurs.

Chapter 2: Factors Affecting the Social Transmission of Food Preference

2.1 Introduction

It is well-known that preferences for specific foods can be transmitted between pairs of rats (Galef *et al.,* 1984, see also Chapter 1, above), a phenomenon referred to as social transmission of food preference (STFP), but there is little research on other factors that can affect this transmission. For example, no studies have addressed the potential role of personality on information transmission or how transmission may occur within a naturalistic foraging setting. This chapter experimentally answers questions concerning personality, exploration of novel environments, and STFP, to situate STFP in the ecology and cognition of rats more generally. Rats were individually assayed on their personalities, trained to prefer a particular food flavour (using STFP), and then allowed to explore a novel environment in pairs. Rats were paired, on different trials, with partners that had either the same or a different food preference.

The transmission of foraging related information between individuals and groups has been extensively studied within the context of rat colonies (Steiniger, 1950). From foetal development (Smotherman, 1982) through adulthood (Galef & Clark, 1971; Galef, 1981), the sharing of food source information leads to predictable behavioural outcomes in the form of food preferences (Galef *et al.*, 1984). Novel food information is carried on the breath of a demonstrator, resulting in a conspecific's preference for the novel food (Galef *et al.*, 1984).

During STFP, novel food information does not exist in isolation. Other factors between the interacting pair, external to the food itself affect the transmission of information, some of which include of dominance (Nicol, 1995), kinship and familiarity (Valsecchi *et al.,* 1996), and age (Galef & Kennett, 1987). However, little is known about the effect of personality on STFP. Personality, or "behavioural syndromes" in animals, are consistent patterns of a subject's

behavioural tendencies that are unchanged over time (Gosling, 2001; Sih *et al*., 2004). Assessed through a multitude of testing methods (Montgomery, 1955; Bourin & Hascoët, 2003; Crawley, 1985; Herman *et al*., 2000; Dochtermann, & Jenkins, 2007; Moy *et al*., 2004; Baenninger, 1966; Svendsen & Armitage, 1973) animal personality has been shown to predict behaviour within rodent pairs (Dochtermann, & Jenkins, 2007). Since the strength of preference acquired during STFP is variable between subjects, I hypothesized that personality might influence the transmission of information during STFP. For example, perhaps a rat of higher sociability would acquire a stronger preference following an interaction, as measured by resistance to preference degradation over time or even a higher proportion of preferred food being consumed during a choice test. In addition to this, perhaps the preference information being transmitted on the breath of a bold demonstrator elicits a stronger preference in a shy rat than if the information were coming from another shy conspecific.

The sharing of food related information can be accomplished within, and is a major benefit to, living in a group. For group living individuals, not only is there social transmission of novel food odours, information shared can also increase the efficiency of foraging (Krause & Ruxton, 2002). In addition to more foraging time due to a decreased need for individual vigilance, information may be shared amongst group members regarding food location (Galef & Giraldeau, 2001), type (Real, 1992), predation risks (Ward *et al*., 2008), and quality of the patch (Marler *et al.,* 1986). While sharing of this information can increase an individual's likelihood of survival (Krause & Ruxton, 2002; Beauchamp, 2013), foraging costs such as greater competition for finite resources (Krause & Ruxton, 2002), and food snatching (Galef, *et al*., 2001) are also associated with group living. Within my thesis, I explored the dynamics of sharing food related information between pairs of rats. I was interested in determining if food preferences

established via STFP could affect foraging behaviour and if preferences were maintained or altered within a foraging setting when subjects were partnered with a conspecific of the same or differing preference.

During exploration of a novel environment, rats engage in several excursions and return to one specific location, referred to as the home base (Eilam & Golani, 1989). Consistent with the Information Center Hypothesis, a home base might act as a safe location in which conspecifics can interact and share information (Galef & Giraldeau, 2001). After establishing a preference using STFP, my thesis examined how preferences may be altered by introducing a same/different preference partner within a novel environment foraging arena. In addition to alteration of preferences, my thesis explores a conflict between personal versus social information. During the demonstrator observer interaction, a subject is acquiring knowledge of a food by means of social interaction. However, when a subject enters the SF arena with a preference, this socially transmitted information becomes personal information for the subject as it is (or is not) applying the information within a novel foraging context. The animal's ability to choose to ignore new social information from the paired conspecific may be analyzed within this experiment, as well, the point at which personal information is replaced by new socially transmitted information can also be investigated. . I hypothesized that shy individuals would be more likely to consume a larger portion of a novel food if their foraging partner were bold and of a differing preference, therefore more readily disregarding personal information in favour of social information. During social foraging bold individuals would perhaps be more likely to disregard conflicting preference information from a shy conspecific therefore maintaining their initial preference and largely feeding from their preferred food source. Alternatively, bold individuals may be more likely to engage in risk taking behaviour by sampling from the novel

food, perhaps to the point where it reaches equipalatibility with the preferred food. This may indicate that bold individuals rely more on personal information that they gather independently while foraging.

Using a combination of both a social food preference task and exploration of a novel environment, my research provides additional information as to how transmission of social information may take place. Factoring in individual personalities and their additional effect on transmission of social information, the experiment provides an explanation of this behaviour that is more applicable to what might be observed in a naturalistic setting.

2.2 Methods

2.2.1 General Overview of Procedure

The experimental timeline for each observer rat was as follows (*Figure 2.1*):

1) Personality testing
    i. Light Dark Emergence into an Open Field (3 trials of 30 minutes over 3 consecutive days)
    ii. Social Preference (1 trial of 10 minutes)
    iii. Elevated Plus Maze (1 trial of 10 minutes)
2) Establish food preference (30-minute interaction with a demonstrator followed by a 4-hour food preference test)
3) Foraging (same/different preference partners) (5 trials of 30 minutes each on 5 consecutive days).
4) Re-test food preference (4-hour food preference test).

5) Re-establish food preference (30 minute interaction with demonstrator followed by another 4-hour food preference test).

6) Foraging (different/same preference partners) (5 trials of 30 minutes each for 5 consecutive days.

7) Food preference test (4-hour food preference test)

8) Re-assess personality

   i. Light Dark Emergence into an Open Field (1 trial of 30 minutes)

   ii. Social Preference (1 trial of 10 minutes)

   iii. Elevated Plus Maze (1 trial of 10 minutes)

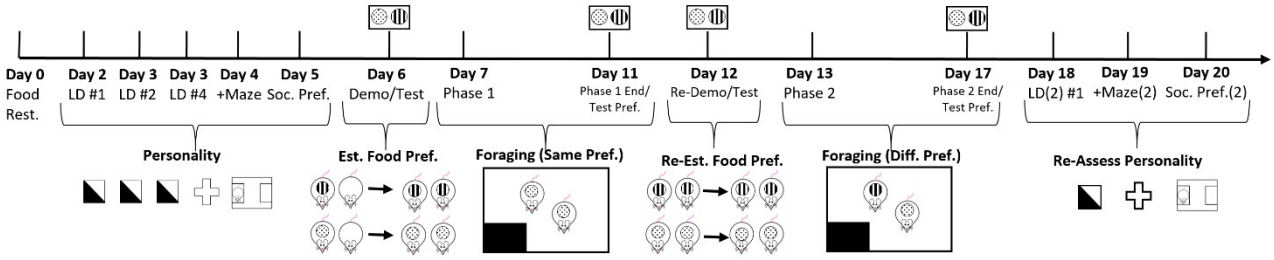

Figure 2.1: Experimental timeline. The experiment began with five days of testing to assess the personality of each subject. The first three days consisted of a light/dark emergence task, followed by one day of elevated plus maze, and ending with one day of social preference testing. On day six, rats underwent STFP during which they had one 30 minute interaction with a demonstrator rat who had consumed either cocoa or cinnamon flavoured food. Following the interaction, rats were tested for preference to ensure successful transmission. On days seven through eleven, rats were placed into a foraging arena with a conspecific (whether a subject's first foraging phase was with a partner of same or differing preference was counterbalanced). After five days of social foraging, food preference was tested once again. On day twelve, food preferences were re-established by having subject interact with a demonstrator having consumed the same flavouring that they were initially exposed to. Following this demonstration,

preference was once again tested to ensure successful transmission. From day thirteen until day seventeen, pairs of rats once again entered the foraging arena, paired with a partner of a same or differing preference (whichever they had not had at first). After a further five days of social foraging, preference was once again tested. Finally, personality was re-assessed over the course of three days. On day eighteen, rats underwent a light/dark emergence task, followed by elevated plus maze on day nineteen, and social preference on day twenty.

2.2.2 Subjects

Subjects were 80 male Sprague-Dawley rats (Charles River Breeding Farms, St. Constant, QC, Canada), approximately 50-52 days old and 201-225g upon arrival. Rats were tested in 4 separate batches of 20 rats each, with 16 observers per batch, 8 of which were given a cocoa preference, and 8 a cinnamon preference. The remaining four animals per batch were used only as demonstrators (2 cocoa, 2 cinnamon). Demonstrator rats did not undergo personality testing or social foraging.

Subjects were pair housed upon arrival and, a week later, were transferred to individual housing units for the duration of the experiment. Rats were handled for 10 days prior to the start of the experiment. The colony room was maintained at 21–22 °C on a 12-h reversed light–dark cycle (lights off at 0700h). Testing was done during the dark cycle, however all tests (other than social interactions) were completed under full florescent lighting. Animals were fed a restricted diet of standard rat chow (20g per day during experimentation) and given water ad libitum.

The procedures used followed the Canadian Council on Animal Care guidelines and were approved by the Wilfrid Laurier University Animal Care Committee.

2.2.3 Apparatus

2.2.3.1 Light Dark Emergence into an Open Field

The light dark emergence task took place in a 122cm x 122cm x 44cm (length, width, height) arena constructed from white melamine-laminated particle board with a 122cm x 122cm sheet of black haircell ABS for the flooring. Within the arena, a 41cm x 24cm x 18cm (length, width, height) start box was placed, constructed from black melamine-laminated particle board with a hinged top and a 10cm x 15cm opening in the center of the front face. The start box was placed in the middle of one of the walls within the arena (see Figure 2.2A).

2.2.3.2 Social Preference

Two cages, identical to those in which the rats were housed, were placed halfway down the length of the wall on opposite sides of the arena previously used for the Light Dark Emergence Task. Cages were clear acrylic measuring 45cm × 25 cm × 20 cm (length, width, height), with a wire lid. The height of the cages made it possible for the subject to climb on top of either cage, allowing for access to scent cues. Each cage contained woodchip bedding as well as a piece of PVC piping used for environmental enrichment. In one of the cages, a decoy stuffed rat was placed. In the other cage, a live novel rat, which was not otherwise involved in the experiment and had not been previously exposed to the test rats, was placed (see Figure 2.2B).

2.2.3.3 Elevated Plus Maze

The elevated plus maze consisted of two open sided and two closed sided black PVC arms, each measuring 57cm x 10cm x 42cm (length, width, height), with black ABS haircell as

flooring within the arms. Arms were arranged in a cross-elevated position, 53 cm from the ground (see Figure 2.2C).

2.2.3.4 Demonstrator/Observer Interaction Cage

Demonstrator/observer interactions were carried out in a cage identical to those described in the *Social Preference* apparatus description above. Cages were clear acrylic measuring 45cm × 25 cm × 20 cm (length, width, height) and contained woodchip bedding. A sheet of Plexiglas® measuring 48cm x 28cm with several small air holes drilled in it was secured over the top of the cage using large metal binder clips.

2.2.3.5 Social Foraging Arena

A 183cm x 183cm open-field arena with black ABS haircell flooring was constructed in which the social foraging took place (see Figure 2.3). Using three walls of the room to surround the arena, a fourth wall was constructed out of 23.5 cm high PVC. Across the outside of the arena where the fourth wall was constructed, a plastic shower curtain was suspended from the ceiling to isolate the testing area. In one corner of the arena, a 35.5cm x 20cm overhang of white gator board was attached to the wall approximately 20cm from the floor, providing a shelter for the rats.

Within the foraging arena were 2 pairs of bowls. One bowl per pair contained cinnamon flavoured food while the other contained cocoa flavoured food. A video camera was mounted to the ceiling with the entire arena in frame and operated remotely using a tablet. Within the shelter, a GoPro camera was secured to record behaviour under the shelter not visible to the ceiling mounted camera.

2.2.3.6 Testing Bowls

During training of the demonstrators, a flavoured food was made available to them for 1 hour. The bowls used for training were the container portion of Rubbermaid® 7J55 Easy Find Lid square food storage containers (1/2 cup). The lid for the container was adhered to a 7cm x 7cm x 6cm (length, width, height) metal water dish so that the container portion could be snapped into and out of the secured lid for ease of weighing. The metal dish was then secured to a 9cm x 9cm Plexiglas® base. Following the demonstrator observer interaction, the observer food preference was tested. In this case, two foods were presented simultaneously. Bowls used were two Rubbermaid®/metal water dish units adhered to a 15cm x 9cm sheet of Plexiglas®. The same type of bowl unit pairings used for the observer testing, were also used during social foraging as well as during equipalatibility testing.

2.3 Procedure

2.3.1 Personality Testing

2.3.1.1 Light Dark Emergence into an Open Field (Figure 2.2A)

Rats were placed within the start box and left undisturbed to explore the arena for 30 minutes. The movement of the rat over the course of the trial was recorded using a webcam mounted on the ceiling and tracked using custom software. Time spent out of the start box as a proportion of the total duration of the trial was extracted from the trajectories for each subject. Each rat had one trial per day for three consecutive days. On each day, the order in which the rats participated in the trials was counterbalanced. The arena and start box were sanitized using spray disinfectant and wiped dry after each subject had completed a trial.

2.3.1.2 Social Preference (Figure 2.2B)

The subject was placed into the arena and allowed to explore undisturbed for 10 minutes. The subject's movement was recorded using a webcam mounted to the ceiling and later tracked. The proportion of time that the subject spent near the cage of the living conspecific was calculated as a proportion of the total amount of time spent near both cages. Time spent in the area between the two cages was ignored. To control for side preference effects, the position of the decoy and living rat were changed for each subject. The arena was sanitized using spray disinfectant and wiped dry after each subject had completed a trial.

2.3.1.3 Elevated Plus Maze (Figure 2.2C)

Rats were placed into the maze and left undisturbed for 10 minutes. The rat's movement was recorded using a webcam mounted on the ceiling and later tracked. The amount of time that the individual spent on the open arms of the maze was calculated. The maze was sanitized after each subject had completed a trial.

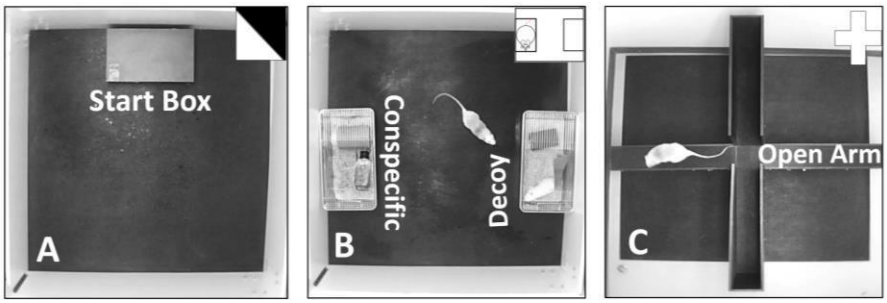

Figure 2.2*:** The three tasks used for personality assessment. ***A) Light/Dark Emergence into an open field; time spent exploring the arena was measured. ***B)*** Social Preference Test; the proportion of time spent near the conspecific was measured. ***C)*** Elevated Plus Maze; the proportion of time spent exploring the open arms was measured.

2.3.2 Selecting Foraging Pairs

Following completion of personality testing, the 5 raw personality measures (proportion of total time spent on the open arms of the plus maze, proportion of total time spent near the conspecific during social preference, and the proportion of total time spent in the arena on each of the three days of light dark emergence), were reduced using principal components analysis (PCA), which resulted in a set of personality scores for each rat (see below). To maximize our chances of observing a behavioral difference between the two animals, foraging pairs were created by selecting rats with the greatest possible difference in personality scores.

2.3.3 Social Transmission of Food Preference

Rats were placed on food restriction for 24 hours before the start of the experiment. Observer rats remained on food restriction (20g per day) for the remainder of the experiment, with the exception of the personality testing phases.

2.3.3.1 Testing for Equipalatibility

Prior to starting the experiment, both foods were tested for 'equipalatibility' using rats that were not involved in the study. By presenting naïve rats with a pair of food bowls containing cocoa and cinnamon flavoured food, the foods are considered 'equipalatable' if the animals consume equal proportions of both (Galef & Whiskin, 1998). This ensures that one of the foods used during experimentation is not more desirable to the naïve animal and preferences acquired are indeed caused by STFP rather than an innate preference.

To test equipalatibility, demonstrator rats were given 60g of cocoa flavoured powdered food (2% w/w), as well as 60g of cinnamon flavoured powdered food (1% w/w). Subjects were

left undisturbed to consume the food over a 4-hour period, after which the food was removed from the demonstrators' cages and weighed.

2.3.3.2 Training the Demonstrators

The previously designated demonstrator rats underwent food preference training. During this time, rats were fed a diet of either cinnamon or cocoa flavoured food and left undisturbed for 1 hour. The food was then removed from the demonstrator's cage and weighed. If the demonstrator had consumed at least 3g of the flavoured food, this was considered a sufficient amount to successfully transmit this information in the following STFP interaction. In the event that the demonstrator did not consume 3g of the flavoured food, an additional 30 min was given for the demonstrator to feed. If, after the additional time, the demonstrator did not consume the required amount of food, food restriction for this subject was re-assessed or, as a final option, a different demonstrator was used.

2.3.3.3 Demonstrator/Observer Interaction

Carried out in dim lighting, immediately following the training of the demonstrators, in a cage novel to both rats, a demonstrator was placed with an observer rat and allowed to interact undisturbed for 30 min. The preference of the demonstrator matched the predetermined desired preference to be given to the observer. Rats were then removed from the testing cages and returned to their individual home cages. Each demonstrator was only used for one interaction per day.

2.3.3.4 Observer Food Preference Test

Immediately following the demonstrator/observer interactions, observers were given a 4-hour preference test. As described above, rats were given 60 g of cocoa flavoured food as well as 60 g of cinnamon flavoured food. After 4 hours, the remaining food was weighed. If >50% of the total food consumed was that of the flavoured food also consumed by the demonstrator, then the transmission of food preference was said to have been successful. If >50% of the total consumed was of the non-demonstrated flavour, the observer was given another demonstrator/observer interaction of the desired preferred food and re-tested until a preference was achieved.

2.3.4 Social Foraging

Once observer preferences had been established, subjects proceeded to the next phase of the experiment, social foraging. Rats of the same or differing food preferences (the order of trials was counterbalanced between groups) were placed in the foraging arena (Figure 2.3). Each pair of rats was allowed to explore the foraging arena for 30 min per day for five consecutive days. Between trials the arena was sanitized using spray disinfectant and wiped dry. Foraging sessions were recorded using a video camera mounted on the ceiling. Once the trials were completed, the videos were manually coded.

Each rat underwent two phases of social foraging, each consisting of five consecutive days, as described above. Half the rats were first paired with a partner of the same food preference for the initial 5-day SF phase and then a partner of the opposite food preference for the second 5-day SF phase; the other half had a partner of the opposite food preference first. Each rat was paired with the same partner over the foraging phases, switching partners only

once between phase 1 and phase 2. In between these social foraging phases, rats' preferences were re-tested and re-established, as described above.

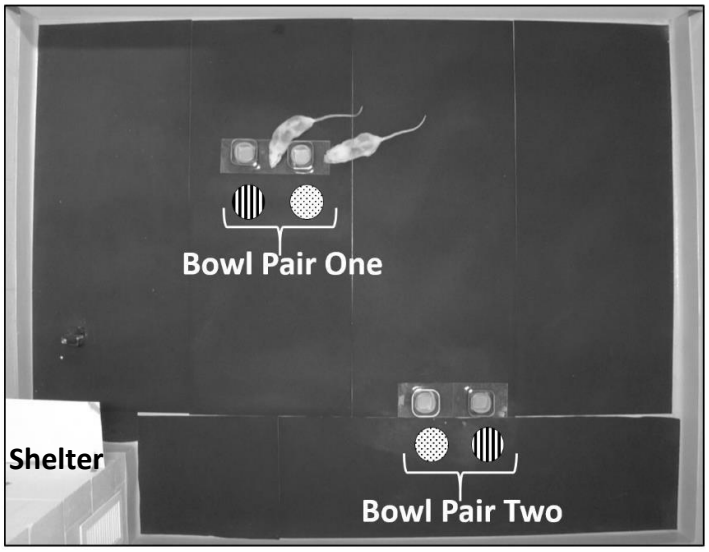

***Figure 2.3*:** The pair foraging arena. A 183 x 183 cm foraging arena. Two bowl pairs contained one of each flavoured food (one cocoa and one cinnamon). Behaviour was recorded using a video camera mounted to the ceiling and footage was later analyzed.

2.3.5 Re-Assess Personality

Identical to the procedure described in *Personality Testing*, rats underwent three days of experimentation to re-assess their personality at the end of the experiment. The first day consisted of one trial of light dark emergence into an open field (1 trial of 30 minutes). On the following day, their performance was once again tested on an elevated plus maze (1 trial of 10 minutes), and finally, on the last day, social preference was again tested (1 trial of 10 minutes).

2.3.6 Data Analysis

Social foraging videos were manually coded by recording the time (start and stop), type of food (cocoa or cinnamon), and bowl pair (A or B) for every feeding event of each rat.

Personality testing videos were tracked using custom in-house software that extracted the position of the rat in every frame of the video. These trajectories were then analyzed in *Mathematica* (v. 9, Wolfram Research) to extract the following measures: duration of time spent on the open arms (elevated plus maze), proportion of time spent near the live conspecific (social preference test), and total time spent outside the start box (3 light/dark emergence tests). Individual scores on all these measures were entered into a PCA.

2.4 Results

Following demonstrator/observer interactions, occasionally the observer would not exhibit a preference (~20%). In this case, the observer underwent the same demonstrator/observer interaction the following day until a preference was established, usually following the second or third interaction. Further analysis of the data could determine if there is a correlation between failure to establish a food preference and the personality of the observer or demonstrator. Additionally, as mentioned in the methods section of this chapter, if after additional feeding time was given, the demonstrator did not consume the required amount of food, as a final option, a different demonstrator was used. This only happened once throughout the course of the experiment.

2.4.1 Personality

The PCA results of the personality measures indicated that the first two components were significant. The eigenvalues of the correlation matrix were: 3.2, 0.94, 0.46, 0.21, and 0.18, explaining 64.2%, 18.8%, 9.2%, 4.1%, and 3.7% of the variance, respectively. It is generally accepted that eigenvalues above (or near) 1 are significant. Table 2.1 gives the loadings of the first two factors onto the raw measures. The loadings show a correlation between the proportion of time spent on the open arms of the plus maze (T_{open}) and the proportion of time spent out in the arena over each session of light dark emergence (T_{out1}, T_{out2}, T_{out3}). All four of these measures load most heavily on Factor (F1). Since each of these measures could be interpreted as a reflection of risk-taking behaviour and all load most heavily on F1, the correlation between them suggests that F1 may reflect 'boldness'. Time spent near the conspecific is not correlated with any of the other 4 measures and loads most heavily on Factor 2 (F2). Given that social preference is used to assess sociability, this suggests that F2 reflects 'sociability'.

In the context of my thesis, personality testing measures commonly used to measure anxiety-like behaviours (Elevated-Plus and Light Dark Emergence) were considered to be measures of "shyness". Therefore instead of my results being discussed as high-anxiety versus low-anxiety, behaviour was considered to be a marker of boldness versus shyness.

Test	*Measure*	*Factor 1*	*Factor 2*
Elevated Plus	T_{Open}	0.8049	-0.1054
Social Preference	T_{Con}	0.3126	0.9484
Light Dark Emerg. (1)	T_{out1}	0.9178	-0.1514
Light Dark Emerg. (2)	T_{out2}	0.8932	-0.0590
Light Dark Emerg. (3)	T_{out3}	0.9088	-0.0219

Table 2.1. Loadings of personality measures onto principal components Factor 1 (Boldness) and Factor 2 (Sociability). Measures shown include the proportion of time spent on the open arms of the Elevated Plus Maze, T_{open}, the proportion of total time that the subject spends in the vicinity of the conspecific during the Social Preference Test, T_{Con}, as well as the amount of time spent out of the start box in all three Light Dark Emergence trials T_{Out}.

To assess the accuracy of our personality tests, as well as to see if personality had changed over the course of social foraging, correlations for personality testing both pre and post social foraging (SF) were completed (Figure 2.4). Since the post-social foraging personality testing consisted of only one light dark emergence session (instead of the 3 pre-SF sessions), the PCA used to assess pre-SF personality tests could not be applied. To correlate performance in the personality testing battery before and after social foraging, raw scores were instead used. Scores were strongly correlated for time spent out of the shelter on the light-dark test ($r = 0.72$, $t_{41} = 6.66$, $p < 0.0001$) as well as time on the open arms of the elevated plus maze ($r= 0.67$, $t_{41} = 5.71$, $p < 0.0001$). However, social preference scores pre- and post-SF were not correlated ($r= 0.13$, $t_{41} = 0.83$, $p = 0.21$).

Due an inconsistency in the number of testing measures pre- and post-SF (three LD trials pre-SF versus one LD trial post-SF), when analysing the effect of personality, my results utilize only pre-SF personality scores for boldness and sociability.

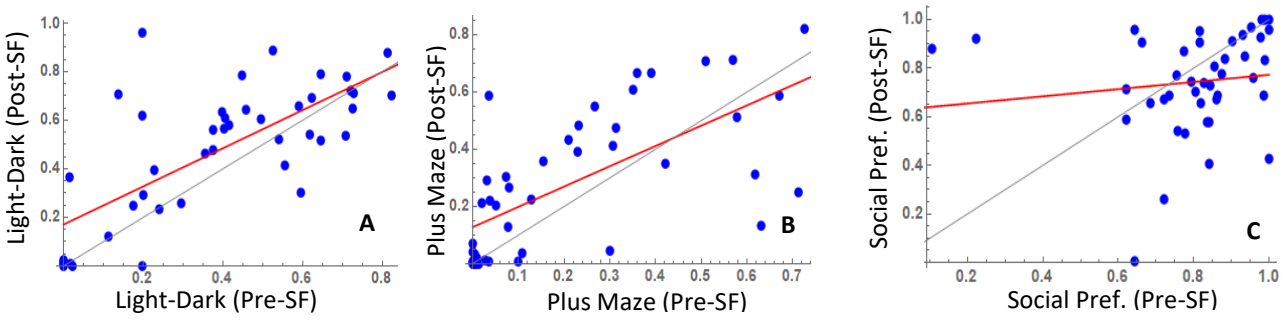

Figure 2.4: Correlation of pre- and post-social foraging (SF) scores for each personality test. Light-Dark (**A**) and Plus Maze (**B**) scores are positively correlated pre- and post-SF. However, Social Preference scores (**C**) are not correlated. Each blue dot represents one rat. The red line in each panel indicates the best-fit linear regression to the data; the grey line shows the diagonal.

2.4.2 STFP

Food preference tests were given four times over the course of the experiment (before SF_1, after SF_1, before SF_2, and after SF_2). This was done in order to test preference transmission (post demonstrator/observer interaction) as well as preference maintenance (post foraging). Means and standard deviations (SD) for each of the four preference tests are given in the table below (Table 2.2), showing that preferences were successfully established following each demonstrator/observer interaction. Means and SDs are also given for cocoa and cinnamon separately, showing that the flavours are equipalatable and one does not create a stronger preference than the other. Using a series of Two-Sample Kolmogorov–Smirnov (KS) tests, preference strength between cocoa and cinnamon rats was compared for all four preference tests; no effect was found (all $D < 0.32$, all $p > 0.21$).

	Before SF_1		*After SF_1*		*Before SF_2*		*After SF_2*	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Cocoa	75.03	17.14	62.47	26.39	75.49	17.42	60.52	24.11
Cinnamon	78.25	12.04	52.47	21.80	69.17	15.54	51.03	19.40
Overall	76.64	14.70	57.47	24.41	72.33	16.60	55.78	22.12

Table 2.2: Means and standard deviations for the proportion of preferred food consumed by the observer following food preference tests. Tests were given four times throughout the experiment, before SF_1, after SF_1, before SF_2, after SF_2. Results are given according to the food flavour demonstrated to the observer during the demonstrator/observer interaction as well as the overall results following each test.

To answer our first question, whether personality can affect the strength of an individual's food preference, I compared consumption of the demonstrated food flavour following the initial demonstrator/observer interaction to an individual's boldness (Figure 2.5A) and sociability (Figure 2.5B), as determined pre-SF. The correlations between boldness and preferred food consumption ($r = 0.20$, $t_{41} = 1.33$, $p = 0.10$) and between sociability and preferred food consumption ($r = -0.12$, $t_{41} = -0.80$, $p = 0.21$) were both not significant.

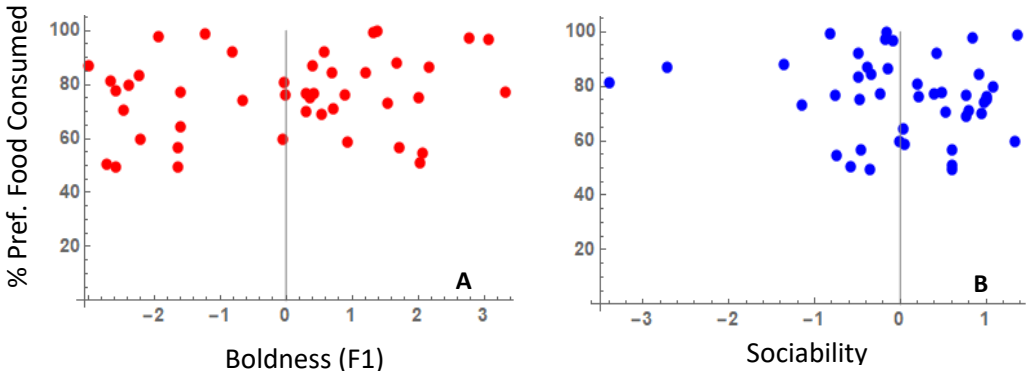

Figure 2.5: Strength of STFP is not predicted by personality. There is no correlation between percentage of preferred food consumed and **boldness** (**A**) or **sociability** (**B**).

Figure 2.6 shows the consumption of the demonstrated food both before and after SF. The blue and green lines show the percentage consumption of the demonstrated food following the demonstration interaction or classic STFP protocol (blue shows preference before any social foraging; green shows preference after re-acquisition, following the first set of social foraging trials). In both cases, there is a high percentage consumption of the preferred food (see Table 2.2). The red and orange lines show percentage consumption of demonstrated food following social foraging (yellow after the first set of trials; red after the second). In both cases, food preferences have been largely degraded.

Since the first SF partner pairing preference (same or different) was counterbalanced, I was able to assess whether a same- or different-preference partner had a different effect on the subject's post-SF1 STFP test. Results from a KS test show that there is no difference ($D = 0.19$, $p=0.75$). In other words, preference for a demonstrated odour decreases after social foraging, but this decrease does not depend on whether the foraging partner has the same or a different food preference.

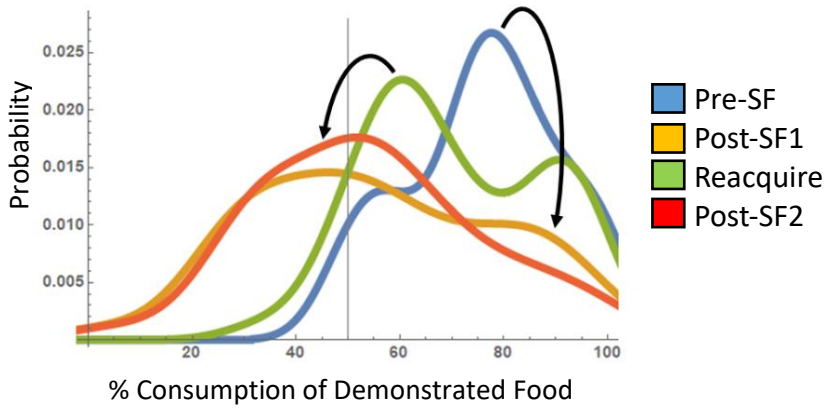

Figure 2.6: Consumption of demonstrated food by observer rats following **initial STFP (blue)**, **first SF phase (yellow)**, **re-acquisition of STFP (green)**, and **second SF phase (red)**. Following demonstrations,

consumption of the demonstrated food was above chance (Pre-SF and Reacquire). However, following both phases of SF, preferences were largely degraded (Post-SF1, Post-SF2).

2.4.3 Social Foraging

I looked next at rat pairs' behaviour within the foraging arena. During SF trials, rats were placed into pairs within a foraging arena where they explored freely for 30 minutes. Each rat received two five day blocks of SF sessions, SF_1 and SF_2. During each SF block, rats were paired with a partner of the same of differing food preference for SF_1 (counterbalanced) and then a different partner of same or differing preference for SF_2. Two pairs of bowls containing cocoa and cinnamon flavoured food were placed in the arena and feeding behaviour was recorded. Data collected from SF was complex as several factors can be examined: rat personalities, preference types and strengths, differences between partners, bowl pairs at which a subject is feeding, and food type consumed. As well, there are 5 days of data for each SF session. In order to address these factors, the data analysis has been broken down into a series of small questions.

Behaviour over each 5-day SF session was averaged. Figure 2.7 shows whether or not the average amount of time spent eating while foraging can be predicted by individual personality (as determined pre-SF). The left panel indicates that with increasing boldness, individuals spend less time eating ($r = -0.58$, $t_{41} = -4.57$, $p < 0.0001$). This could be because they are spending more time in exploration compared to shy individuals. Further analysis using tracking software will reveal if the animals are spending their time in exploration or under the shelter. The right panel indicates that sociability does not have an effect on the mean time the animal spends eating ($r = -0.30$, $t_{41} = -2.06$, $p = 0.02$).

In addition to the individual personalities and their potential effect on behaviour in the foraging arena, I also looked at the mean time spent eating as a function of the difference in pre-SF personality between members of the pair (difference in boldness, for example, has been shown to be important in exploration of a novel environment by pairs of zebrafish; Guayasamin *et al*., 2017). The difference in personality is calculated as the distance between a pair of rats when plotted on a graph with Factor 1 as the x-axis and Factor 2 as the y-axis, as depicted in Figure 2.8. The results, for both same-preference and different-preference pairs, were not significant (r_{same} = 0.08, t_{41}= 0.53, p = 0.30; $r_{different}$ = 0.13, t_{41}= 0.86, p = 0.20). From this I can conclude that differences in personality between individuals within a pair are not predictive of their behaviour during social foraging.

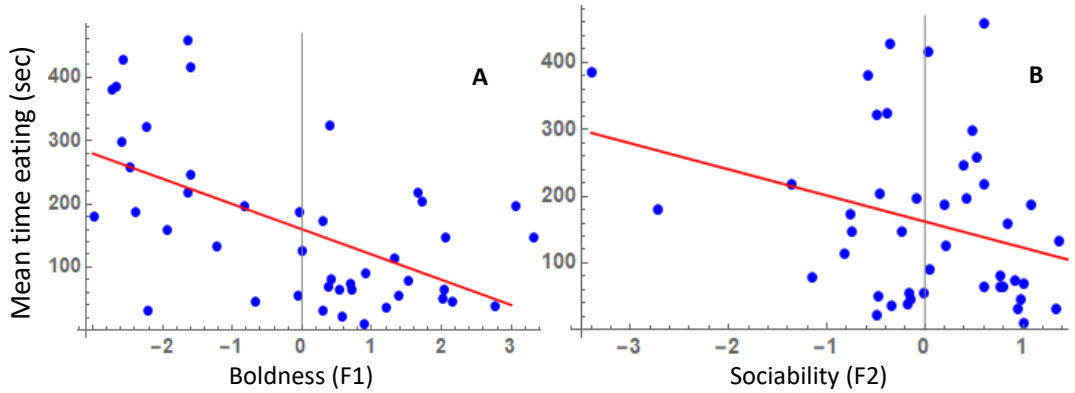

Figure 2.7: During social foraging, the amount of food eaten depends on boldness (**A**) but not sociability (**B**). Bolder rats eat less than less bold rats.

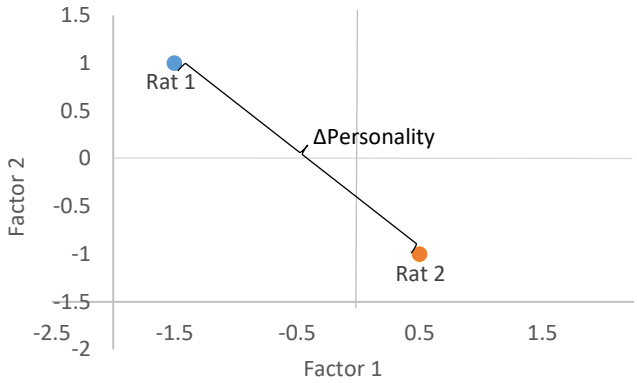

***Figure 2.8*:** Calculating differences in personality. The personality scores for Factor 1 and Factor 2 were plotted for each rat. The distance between two rats was considered to be the difference in personality (Δpersonality) between subjects. The data shown are for illustration of the method only.

I also examined how much of an individual's preferred food was consumed as a proportion of their total food consumption during SF. The proportion of preferred food consumed (PPF) was assessed as a function of pre-SF personality, same or different preference partner, as well as the pair's Δpersonality score. Whether a partner had the same or different preference had no effect on PPF (KS-test, D = 0.09, p = 0.37). Additionally, the total amount eaten by the pair during SF was unaffected by the preference of the partner (KS-test, D = 0.36, p = 0.11). Next, I examined if a pair's Δpersonality score had an effect on PPF; it did not (r_{same} = -0.20, t_{41} = -1.29, p = 0.10; $r_{different}$ = 0.13, t_{41}= 0.86, p = 0.20). In summary, PPF does not appear to be a function of personality, same or different preference partner, or the pair's Δpersonality score.

Based on the findings shown in Table 2.2, preferred food consumed during an STFP test declines following SF sessions. To explain this, I examined if having a same- or different-preference partner during SF had an effect on preference test performance. To do so, a KS-test was conducted on the distribution of the change in preference test scores, or Δpreference

(difference between pre-SF scores and post-SF scores). No significant findings were found (D = 0.16, p = 0.64). Finally, I asked if Δpreference could be explained by PPF consumed during SF. Results were calculated based on the presence of same- or different-preference partners and, once again, results were not significant (r_{same} = 0.07, t_{41} = 0.43, p = 0.33; $r_{different}$ = -0.01, t_{41} = -0.10, p = 0.46). From this I can conclude that Δpreference is unaffected by the preference of a partner during SF and is also unaffected by PPF consumed during SF.

2.4.4 Overlap

Next, I looked at the timing of feeding events to examine the potential for coordination during social foraging. I observed a large amount of overlap between pairs during individual feeding events. I defined 'overlap' as the proportion of the time of one rat's feeding during which the other rat was also feeding, regardless of which bowl pair either rat was at. The overlap score for each individual rat was calculated for every foraging trial and then averaged, giving each individual a single overlap score. For example, if an individuals' overlap score is 1, then they always engage in feeding at the same time as their partner. Conversely, if an individuals' overlap score is 0 then they never engage in feeding at the same time as their partner.

In Figure 2.9, the distribution of mean feeding overlap per rat is shown in blue. This distribution appears to be bimodal. To test whether it is or not, I used a maximum likelihood estimation procedure that compared the goodness-of-fit (using the Akaike Information Criterion, AIC) of Gaussian mixture models with 1, 2, or 3 modes to the data distribution (Everitt, 1981). The AIC values were: -37.096, -37.901, and -32.455, respectively, suggesting that the distribution is most likely bimodal (the model with the lowest AIC score is preferred).

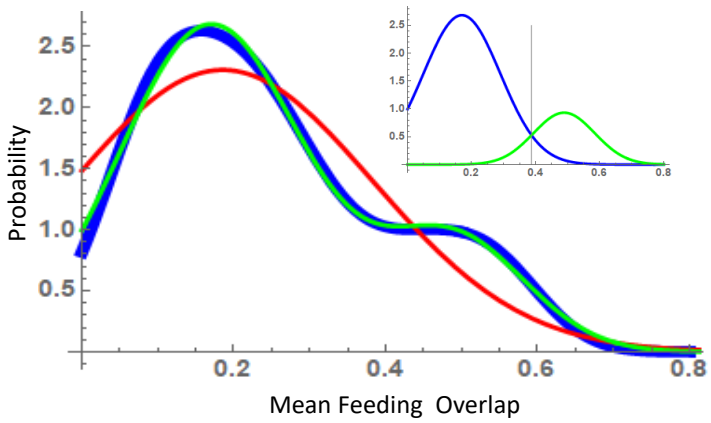

Figure 2.9: The **distribution of mean overlap per rat** (blue). The distribution is bimodal: the best-fit **single Gaussian model (**in red) fits less well than a **2-Gaussian model** (green; see text for details). The inset shows the two modes of the best-fit bimodal model and the criterion for assigning rats to a mode (overlap = 0.39).

I next divided my subjects by which mode of the overlap distribution they inhabited (I used the intersection of the two components, at overlap = 0.387, to assign individuals to a mode) to see how these populations may differ. Ten of my 41 experimental subjects fell into the high-overlap mode. The pre-SF boldness and sociability scores of rats in the high-overlap mode (green in Figure 2.10) were compared to those of animals in the low-overlap mode (blue in Figure 2.10). Rats with higher overlap scores tended to have lower boldness scores (KS-test, $D = 0.91$, $p = 2.3 \times 10^{-7}$). There was no significant correlation of sociability with overlap mode ($D = 0.25$, $p = 0.62$).

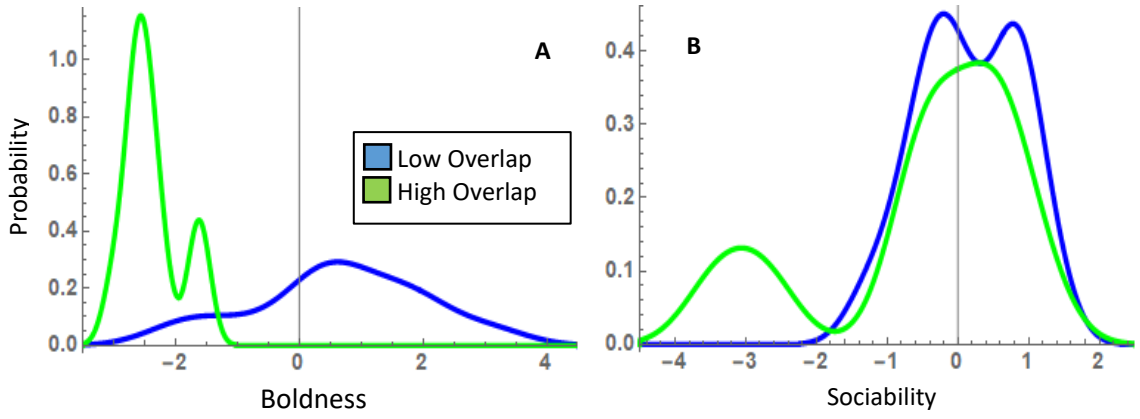

Figure 2.10: Overlap mode is predicted by boldness (**A**) but not by sociability (**B**). Rats in the **low-overlap mode** tend to have higher boldness scores than rats in the **high-overlap mode**, i.e., bolder rats overlap less in their feeding. Sociability scores do not correlate significantly with overlap mode.

The same effect can be shown by correlating the individual overlap scores for each rat with their personality scores (Figure 2.11). As the boldness score for an individual decreases, the mean overlap in feeding events increases significantly ($r= -0.64$, $t_{41} = -5.35$ $p < 0.0001$). Sociability is not significantly correlated ($r= -0.32$, $t_{41} = -2.19$, $p = 0.02$). In other words, the bolder a rat, the less likely it is to feed at the same time as its partner.

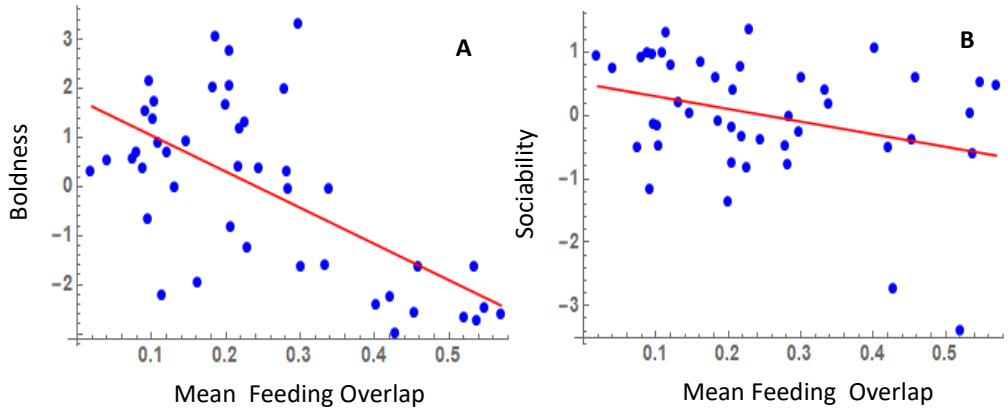

Figure 2.11: Overlap scores. Boldness (**A**) but not sociability (**B**) correlates negatively with feeding overlap. Like Figure 2.10, this shows that bolder rats overlap less in their feeding. Each blue dot represents one rat; the red line represents the best-fit linear regression to the data.

Overlap scores were then calculated for each SF session separately, so that each rat received two scores: one for the same-preference SF trials and one for different. In Figure 2.12A, the distributions of the mean feeding overlap during both social foraging phases are shown. Overlap distributions for both same and different preference sessions were similar, indicating that overlap was equally likely in both foraging phases (KS-test, D = 0.27, p = 0.08), though there was a trend for overlap to be lower when foraging partners had different food preferences.

Showing the same effect in another way, Figure 2.12B demonstrates that the overlap scores for both foraging phases for the same rat are correlated (r = 0.40, t_{41} = 2.82, p = 0.004). This indicates that the timing of a subject's foraging behaviours is mostly determined by the personality of the individual, not the personality or food preference of their partner.

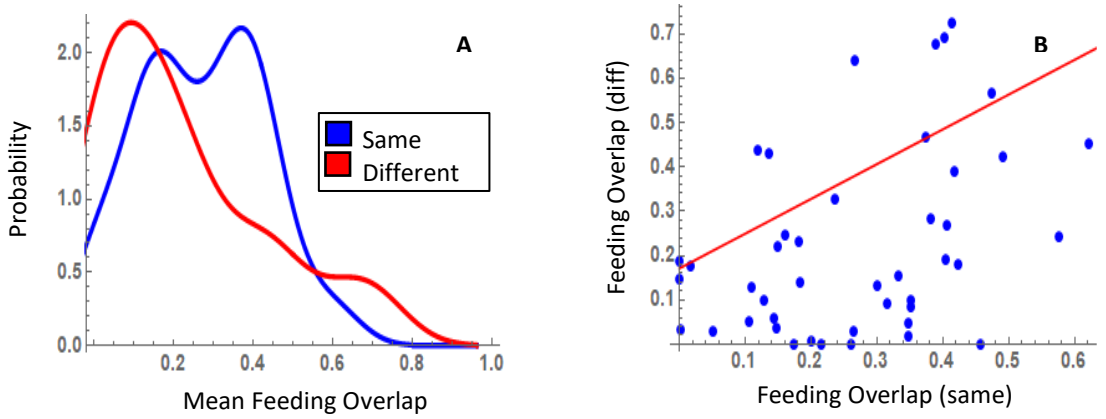

Figure 2.12: Overlap is consistent across same and different SF sessions. **A**. Distributions of mean overlap during **same-preference** (blue) and **different-preference** (red) SF sessions. **B**. Individual overlap is significantly correlated across same- and different-preference SF sessions. Each blue dot indicates one rat; the red line is the best-fit linear regression to the data.

2.5 Discussion

Using a combination of STFP, personality assessment, and social foraging, this experiment examined how personality may affect STFP both transmitted during classic observer/demonstrator interactions and within a naturalistic setting. Our results show that the strength of food preference, defined as the proportion of the preferred food that was consumed, is not correlated with either boldness or sociability. Based on our personality testing battery, the ability to acquire a preference for a certain food following a social interaction appears to be driven by mechanisms that could be independent of the calculated personality scores of the observer.

To test whether the personality of one individual with a certain odour preference could alter the preference of a conspecific, animals were placed in pairs into a foraging arena and food choices were recorded during foraging trials. Additionally, each individual was given a preference test following the final foraging session to see if their preference had changed compared to pre-foraging results. Overall, food preferences largely disappeared following social foraging. I found that food choices during foraging trials, as well as food preference results following foraging, were not affected by the personality or preference of the foraging partner. However, independent of food preference, individuals assessed as being bolder spent less time eating. Although further video analysis would need to be completed to confirm this explanation, it is likely that the bolder individuals are spending more time in exploration and less time eating. In our personality tests, bolder individuals spent more time outside of the start box in the light dark emergence test, which indicates that they were spending more time exploring the open field. The same could also be true during the foraging trials. In addition to

this, Kurvers *et al.* (2010) provided evidence that bolder individuals explore a novel environment more readily, which supports our findings.

During this experiment, animals that were shyer tended to spend more time eating at the same time as their partner, which I called *overlap*. Higher overlap in shy individuals could occur because shy individuals tend to rely on social foraging information, in this case, the decision to engage in an eating event, more than personal information (Kurvers *et al*., 2010). Additionally, a number of studies have shown that shy individuals largely copy the decisions of bolder individuals (Harcourt *et al*., 2009; Kurvers *et al*., 2009; Nakayama *et al*., 2012). The likelihood for shy individuals to copy decisions of bold is interesting in the context of my thesis because it seems as though food choices were not copied in terms of type of food, but rather in terms of the decision to engage in eating more generally.

A possible hypothesis potentially independent of personality is that satiety of the preferred food stimulus may be occurring (Berridge, 1991) or perhaps progressively larger samplings of the non-preferred novel food both during SF and the choice test, leads to post-SF choice test results comparable to those of equipalatibility. This hypothesis could be tested by presenting an observer with the choice test and weighing both foods at various time intervals to determine at which point equal portions of the foods are being consumed. When taking into account that the degradation of preference occurs following social foraging, perhaps there is an interaction between personal versus social information. Upon the first day of SF, each rat is entering the arena with a preference (personal information). During social foraging, as pairs are interacting, additional and potentially conflicting social information is being made present possibly leading to the degraded preference evident following choice tests. Social information is once again successfully transmitted as a preference is re-establishes pre-SF2. Following

completion of my experiment, when and if social information becomes more important than personal information remains unclear. Examination of this could be done by tracking non-preferenced feeding of a partner and seeing when sampling of that food is exhibited by a specific rat. Additionally, rats could undergo social foraging individually to see if/when sampling of the non-preferred food occurs.

Personality was tested both at the beginning of the experiment and again at the end. For the elevated plus maze, scores pre and post experiment were correlated. This finding is unusual when compared to the phenomenon of "one trial tolerance" normally exhibited during plus maze trials (File *et al*., 1990). According to File *et al.* (1990), after exposure to the elevated plus maze, re-exposure showed a marked decrease in anxiety related behaviour as the rats explored the open arms as frequently as an anxiolytic treated animal. However, one trial tolerance effects associated with the elevated plus maze have been shown to last only up to 14 days between exposures (Rodgers & Shepherd, 1992; File *et al.* 1990). Since personality testing on the plus maze was conducted on day 4 and day 19 of the experiment, the 15 day waiting period may have been sufficient to negate the effects of the one trial tolerance phenomenon.

Correlation of pre and post personality scores showed no correlation between the social preference tests, indicating that rats spent less time around the conspecific during the final assessment. This could be explained by habituation to the social stimulus. In a study by Niesink & Van Ree (1982), rats that were housed in isolation showed an increase in social interactions, increasing to its maximum after 4-7 day of isolation. One week following arrival in our facility, rats used in the current thesis were placed into isolation and then handled for 10 days. Once the experiment began, rats continued to be tested in isolation until the 5th day of experimentation when they were tested for social preference. Applying the findings of Niesink

& Van Ree (1982), this would be well into the period of isolation that elicits maximal interactions between conspecifics. Following the completion of social foraging, rats received their second social preference test 3 days after the final pair foraging trial. Based on Niesink & Van Ree (1982), an increase in interaction frequency is not demonstrated until day 4 thus potentially explaining lack of correlation that I found between pre and post social preference scores. Since performance on the social preference test seems to differ depending on the length of time between testing sessions, perhaps this is not a good measure of personality. As described previously, personality is defined as remaining unchanged over time, this suggests that alternatives to the social preference test used in my thesis should be considered.

Future directions and manipulations to this experiment could look at implementing automated lids on the food bowls present in the foraging arena. Lids could be controlled by a computer running automated tracking software (Pérez-Escudero *et al.,* 2014; Miller & Gerlai, 2012) to open and close upon approach of a certain rat. This could allow us to artificially create a leader, allowing one food source to only be made available to one rat, in which case the other rat in the pair would need to follow this rat to have access to the desired food.

The experiment could also be conducted using social foraging groups of larger sizes. During pilot studies for our experiment, groups of three rats in an open field foraging setting consistently exhibited the same roles within a group: one rat would lead in exploration and bring food back to the home base (in these pilot studies, the provided food was whole Froot Loops®, rather than powdered food as used in the final experiment, which tends to be eaten at the bowl), one would follow, and one would remain in the home base. Rats shared the food that was deposited in the shelter.

Another interesting phenomenon to investigate would be the variance in preference strength amongst individuals. Perhaps some rats consume a greater proportion of the demonstrated food because they have a lower latency to begin eating from the food bowl. Although the scent of the food has been introduced via a demonstrator, when the food is presented during the choice test, it is novel to the observer in terms of direct experience with the stimulus. This could be assessed by recording how long it takes each individual to start feeding. As well, preferred food consumption may be predicted by implementing additional personality testing such as a novel object exploration task. Subjects that have a lower latency of exploration of the object or even spend a higher proportion of time investigating the object, may be less hesitant to consume the demonstrated food.

2.6 Conclusions

Using a combination of individual personality and STFP within a novel environment, this chapter has provided a greater understanding into how transmission of social information takes place in a more naturalistic setting. Our results suggest that personality does not affect the strength of an individual's food preference. As well, conflicting information from a partnering conspecific does not seem to alter an individual's initial food preference during foraging trials. However, following social foraging individual preferences are largely degraded. Bold individuals tend to spend less time eating and have a lower overlap score when compared to shy individuals. These findings provide evidence that personality, food preference, and social foraging all interact in very complex ways.

Chapter 3: Modulation of Arc expression in the olfactory bulb by social preference

3.1 Introduction

While a great deal of behavioral work has been done using the STFP paradigm, relatively few experiments have investigated the neurobiological substrate that supports this form of social learning within the main olfactory bulb of adult rats. Although several studies have been conducted, work to date has focused on demonstrating the involvement of the hippocampal formation and surrounding medial temporal lobe structures in mediating STFP (Bunsey & Eichenbaum, 1995; Alvarez *et al*., 2001; Clark *et al.*, 2002; Countryman *et al*., 2005; Ross & Eichenbaum, 2006; but see Burton *et al*., 2000). It remains unknown if the integration of social information and the identity of the odour to be preferred may be integrated "upstream" of the hippocampus. As reviewed in section 1.4.3, extrapolating what is known about the development of odour preferences in other paradigms can provide a rationale to hypothesize that integrative information processing required to support STFP may occur within the olfaction system, prior to information being transmitted to the hippocampus. Although any number of locations within the olfaction system could potentially have the network properties necessary to support STFP, the current thesis looked for changes in the information processing of M/T cells of the MOB, as this is the first set of synapses to process incoming odour information. By doing so, I was able to investigate how socially-learned preferences may alter the representation of odours in this brain region.

The MOB is a plausible locus for the integration of odour and social information because responses in this region change reliably in a different paradigm for socially transmitted preferences – the pairing of an odour with tactile stimulation. When paired with tactile

stimulation, a preferred odour comes to be represented by a larger ensemble of M/T cells that are more reliably recruited upon re-exposure to that preferred odour (Sullivan *et al.*, 2000; Yuan *et al*., 2003; Shakhawat *et al*., 2014). When an odour is detected, a distinct population of M/T cells will be activated (Malnic *et al*., 1999). Upon re-exposure to the odour, the same population will be activated, however there will be some variance in the population (Vazdarjanova & Guzowski, 2004). The proportion of cells being activated during both presentations of the odour is called the similarity score (Vazdarjanova & Guzowski, 2004). A similarity score of 1 indicates complete overlap of the activated cellular population where a similarity score of 0 indicates that the activation overlap of the cellular populations is no more than one would expect due to random chance (Vazdarjanova & Guzowski, 2004). As shown by Yuan and Harley (2014), if an odour is preferred two exposures to that odour will have a higher similarity score than if a non-preferred odour is presented twice. It is reasonable to hypothesize that STFP may cause the same changes in the representation of odours in M/T cells of the MOB.

This hypothesis can be tested by examining the expression of an activity-dependent cytoskeletal protein (*Arc*, also known as *Arg3.1*). By capitalizing on the unique properties of immediate-early genes such as *Arc* (Guzowski *et al*., 1999) through the use of compartmental analysis of temporal activity by fluorescence in situ hybridization (catFISH), it is possible to generate a histological record of the activity pattern of neurons during two distinct behavioural epochs (Figure 3.1). Generating such a histological record is possible because of the tight coupling that occurs between neuronal activity and *Arc* transcription. Following neuronal activity sufficient to induce synaptic plasticity, *Arc* is transcribed nearly immediately. If tissue is collected, these gene products (*Arc* mRNA) can be visualized in the nucleus of the cell within 30 seconds of activity and remain detectable at distinct transcriptional intra-nuclear foci (INF) for

approximately 5 minutes. As *Arc* production continues, *Arc* m-RNA transcripts migrate from within the nucleus to the surrounding cytoplasm, where they are detectable by *in situ* hybridization for a period of approximately 10 minutes, peaking 30 minutes after neuronal activity. Because the expression of *Arc* within these two compartments (i.e., within and outside of the nucleus) can be linked to discrete time frames, a report of the activity history of a large section of neuropil can then be generated.

Although catFISH has been used primarily to detect hippocampal activity, this technique has been successfully used in several brain regions (reviewed in Guzowski *et al.,* 2001; 2005) including M/T cells of the MOB (Shakhawat *et al*., 2014). Generating comparable data by examining M/T cells of the MOB following exposure to a preferred odour established by means of the STFP paradigm will provide valuable insight into the neural mechanisms underlying this form of social learning. I hypothesized that upon exposure to an odour, a greater proportion of M/T cells would express *Arc* and the M/T cellular population would be more reliably activated (higher similarity score) following re-exposure to the odour if an odour preference has been pre-established using STFP. When comparing a preference transmitted via live demonstration to a preference transmitted via surrogate, I hypothesized that results would be comparable to those found in rat pups (Sullivan *et al.*, 2000; Yuan *et al*., 2003; Shakhawat *et al*., 2014). As well, since the demonstrator and observer would be physically interacting, NE dependant mechanisms may also be driving this neuronal change.

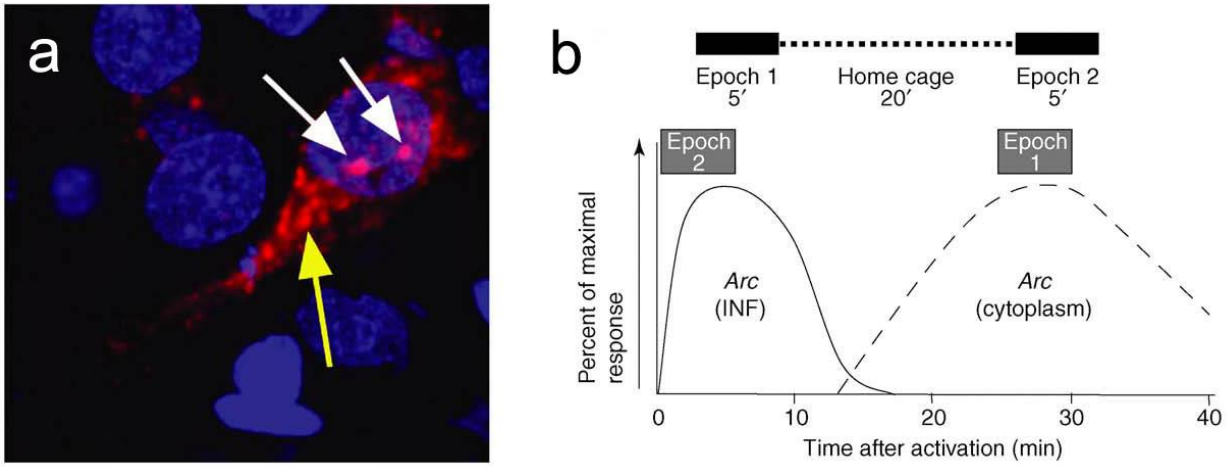

Figure 3.1*:* Imaging neural activity with temporal and cellular resolution using IEG catFISH. On the left, (**A**) a projection image generated from a confocal image stack from a rat hippocampus shows a cell containing *Arc* mRNA both within INF (white arrows) and the surrounding cytoplasm (yellow arrow). Nuclei were counterstained with DAPI (blue) and *Arc* RNA was detected with cyanine 3 (red). On the right, (**B**) a behavioural timeline for a typical catFISH imaging experiment is shown. In addition, the approximate time course for the detection of *Arc* in INF (solid line) as well as the cytoplasm (dotted line) following neuronal activation in a two experience *Arc* catFISH experiment is also depicted (below). The behavioural epochs for which *Arc* in INF or cytoplasm provide 'readout' are shown above the curves. Adapted from Guzowski *et al.,* 2001.

3.2 General Overview of Procedure

To test how STFP may alter the activity of MOB M/T cells, adult male Sprague Dawley rats were exposed to two novel odours (cocoa and cinnamon) and a preference for one of the odours (counterbalanced) was induced using a standard STFP protocol (Figure 3.2). This procedure produced a preferred (P) odour, while the novel odour to which they were not exposed during the demonstrator/observer interaction was considered to be the non-preferred (NP) odour. The following day, groups of rats (n = 6/group) were exposed to either the

preferred odour twice (P/P), the non-preferred odour twice (NP/NP), or both odours (P/NP), with each exposure spaced 30 minutes apart. Size and pattern of the ensemble activated by each odour could then be determined by examining compartmental expressions of *Arc*. If, as hypothesized, STFP causes the preferred odour to induce a larger and more specific pattern of mitral cell activity, this would suggest that the large body of data on the mechanisms of preference development through tactile stimulation may also apply to the STFP paradigm.

3.3 Methods

3.3.1 Subjects

For this experiment, I used 32 male Sprague-Dawley rats (Charles River, St. Constant, Quebec), purchased at approximately 50-52 days old and 201-225 g. Subjects were pair housed upon arrival and a week later were transferred to individual housing units.

The procedures used followed the Canadian Council on Animal Care guidelines and were approved by the Wilfrid Laurier University Animal Care Committee.

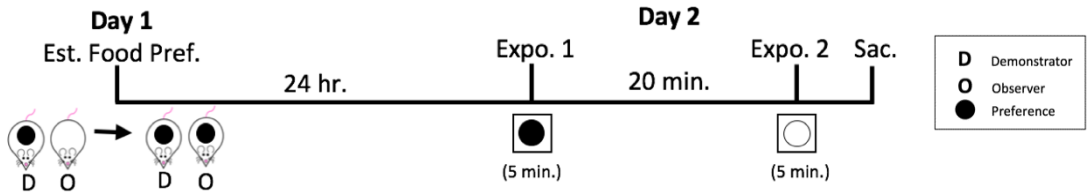

Figure 3.2: Experimental timeline for each subject. Observer rats were given a preference by demonstrator/observer interaction following STFP protocol. 24 hours later, observers were exposed to one of two food flavours, cocoa or cinnamon (Expo. 1), given a 20 minute ITI, and exposed to another flavour (Expo.2). Immediately following the second exposure, rats were sacrificed and olfactory bulbs were removed and flash frozen.

3.3.2 Test for Equipalatibility in Naïve Rats

Prior to starting the experiment, both foods were tested for 'equipalatibility' using rats that were not involved in the study. By presenting naïve rats with a pair of food bowls containing cocoa and cinnamon flavoured food, 'equipalatibility' is said to be present if the animals consume equal portions of both foods (Galef & Whiskin, 1998). This ensures that one of the foods used during experimentation is not more desirable to the naïve animal and preferences acquired are indeed caused by STFP rather than an innate preference.

To test equipalatibility, demonstrator rats were given 60 g of cocoa flavoured powdered food (2% w/w), as well as 60 g of cinnamon flavoured powdered food (1% w/w). Subjects were left undisturbed to consume the food over a 4-hour period, after which, the food was removed from the demonstrators' cages and weighed. If the mean percent intake by the demonstrators was roughly 50% for each of the food flavours (in the range of 43% to 57%), the social interaction portion of the experiment could then take place (Galef & Whiskin, 1998). Previous literature has demonstrated the following food pairings to be equipalatable: 2% (w/w) ground marjoram and 1% (w/w) ground anise, 0.4% (w/w) ground cloves and 0.5% ((w/w) ground cumin, and finally, 0.5% (w/w) ground rosemary and 0.5% (w/w) ground cardamom (Galef *et al.*, 1984).

3.3.3 Training the Demonstrators

Demonstrator rats were fed a diet of either cinnamon or cocoa flavoured food and left undisturbed for 1 hour. Food was then removed and weighed. If the demonstrator had consumed at least 3 g of the flavoured food, then they had consumed a sufficient amount to successfully transmit this information in the following STFP interaction (Galef *et al.*, 1984). In

the event that the demonstrator did not consume 3 g of the flavoured food, an additional half an hour was given for the demonstrator to feed. If after the additional time, the demonstrator did not consume the required amount of food, food restriction for this subject was re-assessed or a different demonstrator was used.

3.3.4 Demonstrator-Observer Interaction

Observer rats were exposed to a demonstrator using one of three social paradigms (Figure 3.3). Some rats received "live demonstration with preference", where the demonstrator/observer interaction was with a demonstrator rat that had been feed a specific diet of either powdered cocoa or cinnamon flavoured food. A second paradigm was also implemented, "live demonstration no preference", involving a demonstrator rat that had been fed unflavoured powdered food. The third and final paradigm, "surrogate demonstration with preference" consisted of interaction with a cotton ball surrogate that had been rolled in either cocoa or cinnamon flavoured food and dampened with diluted carbon disulfide (1:1000 ppm)(VWR).

Immediately following training of the demonstrator, one of the three social paradigms described above was implemented. In a cage novel to both the demonstrator (or surrogate) and the observer, a demonstrator (or surrogate) was placed with an observer rat and allowed to interact undisturbed for 30 minutes (Figure 3.2). Over the course of the interaction, behaviours were filmed using a video camera. After the allotted 30-minute interaction, rats were taken out of the testing cages and placed back into their individual home cages. Demonstrators were only used for one interaction per day.

There were 24 observers in total for the experiment, 12 of which were given a live demonstration of cocoa or cinnamon (Group A and B). An additional group of 6 was given a live demonstration during which the demonstrator was feed an unflavoured powdered food (Group C). The final group of 6 was given surrogate demonstrations (Group D). Following demonstrations, the food flavouring consumed by the demonstrator was then referred to as the preferred flavouring of the observer.

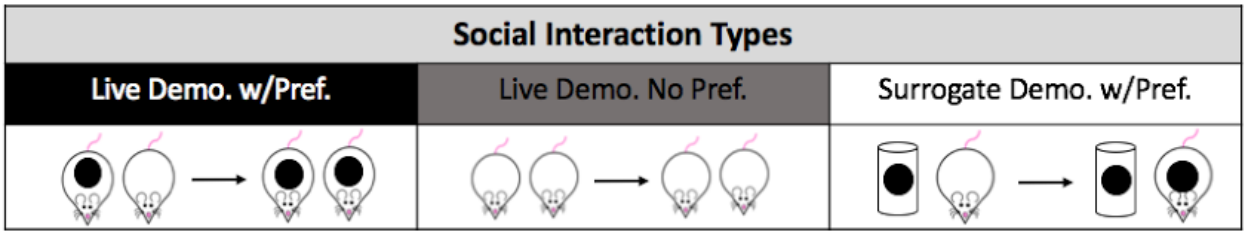

Figure 3.3: Social interaction paradigm. Observer rats (right animal in the pair) were placed in a cage with a demonstrator conspecific (left) and left undisturbed to interact with one another for 30 minutes. Immediately prior to the interaction, demonstrators were fed a flavoured (black dot), or unflavoured food (no dot) for 1 hour or until 3 grams had been consumed. 24 hours later, observer rats were exposed to two flavours of food (one having been demonstrated and the other being novel), as described in Figure 3.2.

3.3.5 Observer Testing

Twenty four hours after the social interaction had occurred, rats underwent two five minute exposures to the flavoured food (Figure 3.4). Groups (n = 6/group) were exposed to either the preferred (P) odour twice (P/P) (Exposure Condition 1), the non-preferred (NP) odour twice (NP/NP) (Exposure Condition 2), or both odours (P/NP) (Exposure Condition 3) during the course of the testing phase (Table 3.1).

For the first exposure, while in their individual housing units, a food cup containing 60 g of either the cocoa or cinnamon flavoured food was placed in the home cage. Once 5 minutes had passed, the food was removed and weighed to record the amount consumed over the testing duration. A 20 minute inter-trial interval during which subjects remained undisturbed in their home cages followed exposure one. The second exposure occurred by placing a food cup containing 60 g of either the same or different food flavouring that was introduced during exposure one.

Immediately following completion of the 5 minute exposure to the second food, the subjects were sacrificed, and OBs were extracted and flash frozen for analysis of *Arc* expression using catFISH by established protocols (Shakhawat *et al*., 2014, Vazdarjanova and Guzowski, 2004).

An additional group (n=4) of caged controls rats were sacrificed immediately out of their homecages. They did not undergo any of the demonstration types nor were they exposed to any odours during observer testing.

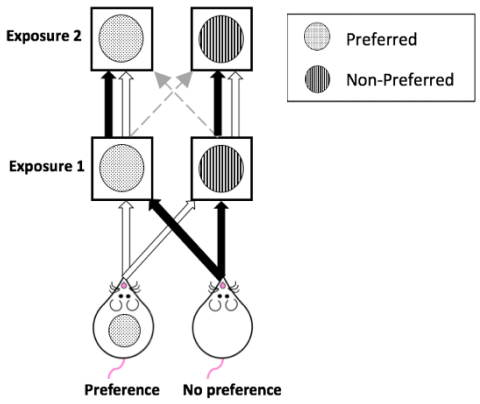

Figure 3.4: Preference testing. 24 hours after social interactions, observer rats could be divided into 2 groups; those with a preference (dots) and those that did not. These groups of rats were exposed to either the preferred flavour (dots) or a non-preferred flavour (stripes) for 5 minutes (Exposure 1). After a

20 minute delay, these rats were wither exposed to the same flavour again or the opposite flavour from their first exposure (Exposure 2).

Group	*Demo. Type*	*Demo. Pref.*	*Exposure 1*[1]	*Exposure 2*
A	Live	Yes	C(N)	N(C)
B	Live	Yes	C(N)	C(N)
C	Live	No	C(N)	N(C)
D	Surrogate	Yes	C(N)	N(C)

Table 3.1: Description of the different observer groups. [1] C = cocoa, N = cinnamon

3.3.6 Histology

Immediately following the final 5 minute exposure, animals were anesthetized by isoflurane inhalation. Animals were decapitated and brains were extracted in under 3 minutes to maintain mRNA integrity. If a brain was not extracted within the 3 minute window, the tissue was discarded. Following extraction, brains were quick-frozen in a beaker of isopentane bathed in a dry ice/ethanol slurry. Brains were stored at -80°C until sectioning. Prior to sectioning, brains were mounted together with OCT compound (Fisher Scientific, Whitby, ON). Sectioning was completed in a -20°C cryostat slicing coronally at 20 µm. Sections were thaw-mounted onto Superfrost Plus slides (Fisher), dried, and stored at -80°C until processing via catFISH (Shakhawat *et al*., 2014, Vazdarjanova and Guzowski, 2004).

3.3.7 Fluorescence *in situ* hybridization

This technique was conducted as previously described (Guzowski *et al*., 1999). A riboprobe for *Arc* was synthesized using a commercially available transcription kit (Ambion, Austin, TX). The *Arc* riboprobe was conjugated to digoxigenin-labeled UTP. Riboprobes were purified on a mini-quick spin column (Roche Applied Sciences, Montreal, PQ) and verified by gel electrophoresis. Slides were thawed, fixed in 4% formaldehyde, bathed in 0.5 % acetic

anhydride, methanol/acetone, equilibrated in SSC (saline-sodium citrate), and treated with 100µl pre-hybridization buffer (Sigma-Aldrich Canada, Oakville, ON) at room temperature for 30 minutes. The slides were then diluted in hybridization buffer (Sigma-Aldrich), denatured at 90˚C, chilled on ice, treated with ~100ng of the riboprobe and then incubated in a humid chamber at 56˚C overnight. The slides were then bathed in a series of SSC washes, placed in RNase A (10 mg/ml, Sigma-Aldrich) at 37˚C, quenched with H_2O_2 and blocked with tyramide signal amplification blocking buffer (Perkin Elmer, Boston, MA) containing 5% normal sheep serum (Sigma-Aldrich). Slides were then incubated with anti-digoxigenin-HRP antibody (Roche) for 2 hours at room temperature, washed with Tris-buffered saline containing 0.05% Tween 20. The HRP-antibody conjugate was labeled with CY3 (cyanine-3) signal amplification kit (Perkin Elmer). The slides were counterstained with 4'-6-Diamidino-2-phenylindole (DAPI), coverslips were applied with Vectashield anti-fade mounting medium (Vector Laboratories, Burlington, ON) and sealed with nail polish.

3.3.8 Image Analysis

The size (total number of M/T cells expressing *Arc*) and pattern of the ensemble activated (which M/T cells expressed Arc in either their INF, cytoplasm or both) by each odour was determined by examining the compartmental expression of *Arc*. If, as hypothesized, STFP caused the preferred odour to induce a larger and more specific pattern of M/T cell activity, this suggests that the large body of data on the mechanisms of preference development through tactile stimulation may also apply to the STFP paradigm.

Images of four different regions of the MOB were collected using an FV-1000 laser scanning confocal microscope (Olympus Canada, Mississauga, ON) at 40x magnification. For

consistency, photomultiplier tube assignments, pinhole size, and contrast values remained the same for each slide. Images were acquired by taking z-stacks (optical thickness, 1.1 µm; interval, 0.7 µm) in 4 random locations in the MOB in each of 3-4 slides per animal. Activation was quantified by examining the proportion of cells transcribing *Arc* during each odour epoch [i.e., cells containing *Arc* solely in INF, those containing *Arc* solely in the surrounding cytoplasm (CYTO) or both nucleic and cytoplasmic *Arc* (DOUBLE)] and generating a similarity score as described in previous literature (Vazdarjanova and Guzowski, 2004). Briefly, the similarity score could be calculated from the *Arc* expression during each epoch as follows: (1) Epoch 1 active cells = fraction of total cytoplasmic-positive cells [(CYTO + DOUBLE)/total cells]. (2) Epoch 2 active cells = fraction of total nucleus-positive cells [(INF + DOUBLE)/total cells]. (3) p(E1E2) = epoch 1 active cells × epoch 2 active cells. This is the probability of cells being active in both epochs (i.e., DOUBLE), assuming the two epochs activated statistically independent neuronal ensembles. (4) diff(E1E2) = (DOUBLE) - p(E1E2). This is a measure of deviation from the independence hypothesis. (5) Least epoch = the smaller of the ensembles activated by epoch 1 or epoch 2. (6) Similarity score = diff(E1E2)/(least epoch - p(E1E2). This normalizes the diff(E1E2) fraction to a perfect overlap. That is, complete overlap in the cell population recruited to epoch 1 and the population recruited to epoch 2 will yield a similarity score of 1. A similarity score of 0 would result if the probability of recruiting the same cell to become active during both epochs is equal to simple random chance with replacement.

3.3.9 Data Analysis

Arc expression (total # of cells activated as well as location of expression cyto/INF/both) was analyzed using a 3 x 3 x 2 general factorial analysis of variance (ANOVA) using

demonstration type (i.e., live, surrogate, or none), number of odours experienced (i.e., 0, 1, or 2), and the presence of social learning (i.e., yes, meaning a preference was imparted via live demonstration; or no, meaning that animals were exposed either to surrogates or conspecifics without an odour matching any of the flavours to be tested) as factors. Alpha was set to 0.05 and all post-hoc tests were conducted using Tukey's HSD, where appropriate. All statistical analyses were carried out using the R statistical package (R core team, 2013).

3.4 Results

A total of 28,742 M/T cells were counted for the present analysis (1,029 ± 123 per animal). The collected results demonstrate that, although odour identity seems to be encoded by odour specific patterns of MOB M/T cell activity, in agreement with previous data collected in this region (i.e., Shakhawat *et al*., 2014), the preference induced by STFP does not seem to alter these odour-specific patterns. These results are discussed in further detail below.

3.4.1 *Arc* Expression within MOB M/T Cells is Odour-Specific

Comparable to previous studies, the current study shows that exposure to novel odours elicited *Arc* expression within significantly more M/T cells when compared to animals that remained within the home cage (Figure 3.5a). This was demonstrated by a significant main effect of the number of odours experienced on the number of *Arc*-expressing cells during both epoch 1 ($F_{2,18} = 7.885$; $p = 0.012$) and epoch 2 ($F_{2,18} = 7.981$; $p = 0.011$). Post-hoc analyses show that during both epochs, the number of cells expressing *Arc* in caged controls was significantly lower than animals exposed to 1 odour (epoch 1: $p = 0.029$; epoch 2: $p = 0.030$) or 2 (epoch 1: $p = 0.024$; epoch 2: $p = 0.025$). The number of cells expressing *Arc* was not significantly different, however, between animals exposed to 1 vs 2 odours in either epoch 1 ($p = 0.090$) or epoch 2 (p

= 0.185). This pattern is consistent with previous work examining MOB Arc expression (i.e., Shakhawat *et al*., 2014). No significant differences were observed in the number of cells expressing *Arc* during either epoch on the basis of social learning (epoch 1: $F_{1,18} = 0.588$; $p = 0.453$; epoch 2: $F_{1,18} = 0.700$, $p = 0.794$), or on the type of demonstration animals received (epoch 1: $F_{2,18} = 0.091$; $p = 0.766$; epoch 2: $F_{2,18} = 0.015$, $p = 0.944$). The most critical observation among animals, however, is not the number of cells that express *Arc*, but rather the pattern of that expression between the two epochs (shown in Figure 3.5c). Similarity scores were found to differ significantly as a function of how many odors an animal was presented with ($F_{2,18} = 10.237$; $p = 0.005$). Post-hoc tests showed that when animals were exposed to the same odour during both behavioral epochs, they were significantly more likely to express *Arc* in the same M/T cells during both time points ($p = 0.003$) relative to animals exposed to two distinct odours. These data are consistent with previous observations that the identity of an odour is encoded, in part, by the population of M/T cells recruited.

3.4.2 Socially Transmitted Odour Preferences do not alter *Arc* Expression within MOB M/T Cells

Social transmission of food preference with a live demonstrator had no significant effect on any measure of M/T cell activity recorded (Figure 3.5b). Social transmission had no significant effect on the number of M/T cells active during either epoch 1 ($F_{1,18} = 0.588$, $p = 0.453$), or epoch 2 ($F_{1,18} = 0.070$, $p = 0.794$). Moreover, the similarity scores generated from animals ($F_{1,18} = 0.144$, $p = 0.745$) showed no significant differences as a result of the presence of a live demonstrator versus a surrogate during the demonstration phase of STFP. Collectively, these data make it unlikely that STFP-induced odour preference are encoded by the pattern of recruitment in populations of M/T MOB cells.

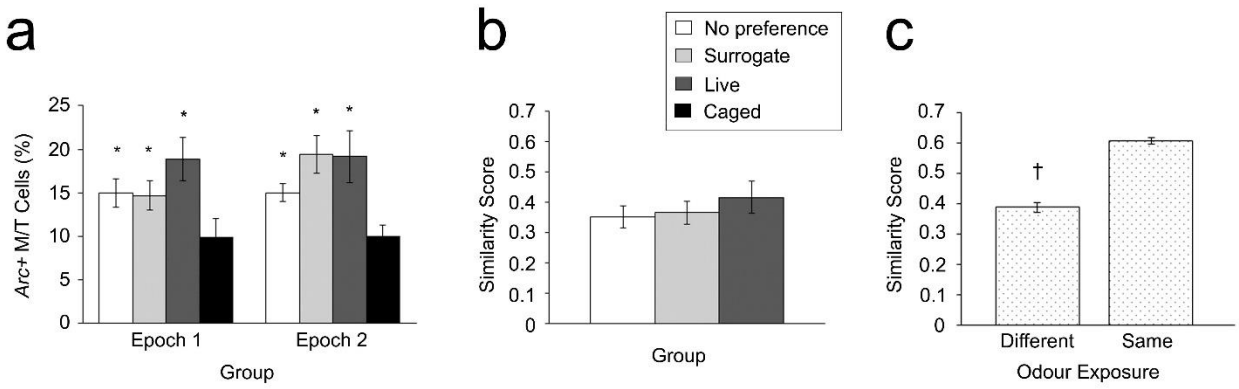

Figure 3.5: *Arc* expression in the olfactory bulb encodes odour identity but not social preference. Analysis of the total number of M/T cells expressing *Arc* (**A**) show that either exposing animals to odours in the STFP paradigm induces significantly more *Arc* expression than can be seen in animals that remained in the home cage (black). This was true of animals that were not given a preference (white), animals exposed to a surrogate (light grey), and animals that were exposed to a live demonstrator (dark grey). Moreover, the induction of a social preference has no significant effect on the pattern of M/T cell activity among animals in the three exposure groups (**B**), as similarity scores generated from the three behavioral group were comparable. Unlike social preference training, however, the number of odours to which an animal was exposed significantly affected the pattern of *Arc* expression in M/T cells (**C**). Similarity scores show that significantly more M/T cells express *Arc* during both exposures if the exposures were to the same flavour of food, showing that the pattern of *Arc* expression in M/T cells is odour-specific. Data are presented as mean ± SEM, * = $p < 0.05$ vs caged control; † = $p < 0.05$ vs repeated exposure to the same odour.

3.5 Discussion

Based on my experimental findings, the development of a preference through STFP did not induce a change in the number or pattern of M/T cells activated in response to an odour. This suggests that mechanisms involved in adult STFP may be different from those involved in

rat pup preference induction through the pairing of an odour with tactile stimuli. Thus, STFP is unlikely to be norepinephrine-dependent. Examination of mitral cell activity in my experiment showed odour specific patterns of *Arc* transcription, demonstrating that odour identity is encoded at the cellular level within the MOB. Since my findings revealed that preference information acquired by means of social interaction is not encoded within the MOB, further activity analysis of downstream processing within the accessory olfactory bulb (AOB) and piriform cortex (PC) may reveal where social information is integrated, therefore changing the cellular representation of a preferred odour upon re-exposure.

In a study by Ross & Eichenbaum (2006), ventral hippocampal lesions (including subiculum) resulted in temporally graded retrograde amnesia if lesions were administered prior to day 21 following demonstrator/observer interaction. Since damage to the hippocampus has been shown to cause deficits in a rat's ability to exhibit a preference following STFP, the integration of social (i.e., the presence of CS_2) and odour information must occur somewhere between the MOB and the hippocampus (Bunsey & Eichenbaum, 1995; Alvarez *et al.*, 2001; Clark *et al*., 2002). Since my MOB results did not show evidence of social information integration via a larger M/T cell ensemble, subsequent experiments should examine odour representations downstream of the MOB. Possible regions of interest could include the ipsilateral olfactory tubercle, pre-pyriform and pyriform cortices, ventrolateral entorhinal area, and anterior and posterolateral divisions of the cortical amygdaloid nucleus where MOB projections terminate (Scalia & Winans, 1975).

Within the main olfactory epithelium, Munger *et al*. (2010) identified a specialized type of ORN vital for establishing a preference via STFP that express the receptor guanylyl cyclase ($GC\text{-}D^+$). Receptors of this type were found to be highly sensitive to volatile CS_2 and project

from main olfactory epithelium to the necklace glomeruli within the MOB. Mice lacking GC-D$^+$ ORNs did not exhibit a food preference following STFP (Munger, *et al*., 2010). Although GC-D$^+$ ORNs were found to be vital for detection of CS_2, little is known about where this information is integrated with odour identity information processed via canonical glomeruli. Leinders-Zufall *et al*. (2007) showed that GC-D$^+$ expressing ORNs project almost exclusively to the necklace glomeruli. However, the way that M/T cells of the necklace glomeruli respond to odourants makes it is unlikely that the integration of odour and social information happens in this region. The presence of a single peptide to which GC-D$^+$ ORNs respond causes calcium transients in approximately 75% of necklace glomeruli neurons, and natural stimulants, such as dilute rodent urine, reliably elicit calcium transients from nearly 100% of these cells (Leinders-Zufall *et al*., 2007). This suggests that the information contained in this network is binary (i.e., simply encodes the presence or absence of socially transmitted preference cues), and this information needs to be passed on and assimilated into odour representations elsewhere. It would be reasonable to assume that chemical information indicating saliency of an odour stimuli (CS_2) may be integrated with odour information via canonical glomeruli at sites of MOB projection termination, such as the pyriform cortex.

Operating in a parallel system to the canonical and necklace glomeruli of the MOB, there is a similar relationship between the vomeronasal organ (VO) and AOB as chemosensory information is detected and processed. Primary receptor neuron axons of the VO terminate in the AOB (Døving & Trotier, 1998). In the rat, the VO has primarily been studied in order to understand its role as a chemosensory organ for detection of socially relevant compounds, i.e. pheromones (Døving & Trotier, 1998). Located in the foremost part of the nasal cavity, the VO's pheromone detecting abilities are thought to aid in facilitation and regulation of social

interactions (Døving & Trotier, 1998). In the context of reproductive behaviour, male guinea pigs with damage to their VO's failed to mount conspecifics, while female guinea pigs had little interest in sexual partners, rarely reproduced, and did not show lordosis (Døving & Trotier, 1998). Since the VO acts as a chemosensory organ and relays important social information, it is plausible that the ORNs located in this region could also detect the presence of CS_2. If analysis downstream from the AOB is required, the medial amygdaloid nucleus and posteromedial portions of the cortical amygdaloid nucleus should be considered. Following lesion studies, these regions exhibited degradation indicative of their potential as sites of AOB termination (Scalia & Winans, 1975).

The lack of significant differences in M/T cell recruitment following STFP may result from potential limitations of catFISH analysis. Although a larger recruitment of M/T cells is indicative of a preferred odour within the rat pup brain, perhaps the adult brain represents a preference through greater excitation (i.e., higher firing rates) of the already recruited cells. As described in this section 3.1, following neuronal activity that is sufficient enough to induce synaptic plasticity, *Arc* is transcribed. Using catFISH to analyse *Arc* expression, activity is analyzed based on the presence or absence of fluorescently labelled *Arc* within a cellular compartment, however, further increases in firing rates will not alter this observation. Based on a recent paper by Witharana and colleagues (2016), the level of brightness of fluorescence following catFISH may coincide with the amount of firing in an individual cell, although these data have yet to be replicated.

3.6 Conclusions

Analysis of *Arc* expression within the MOB revealed that the induction of a social food preference does not alter the recruitment of M/T cells in response to the preferred odour. However, distinct activation patterns were present upon exposure to different odours. These data suggest that while differentiation of individual odours occurs reliably within the MOB, the integration of this information with social information likely takes place elsewhere.

Chapter 4: General Discussion

By considering the significant and nonsignificant findings within my thesis, it is clear that factors affecting STFP likely interact in complex ways. In Chapter 2, following SF trials, individual preferences were largely degraded. This suggests that during SF an additional or alternate association between a food odour and a stimulus may have been formed resulting in a behavioural change. If by continuing the work done in Chapter 3, I am able to identify the neurological characteristics associated with a preferred odour in the pyriform cortex or AOB, I could investigate how the neural representation of a preferred odour may change before and after SF trials.

There is experimental evidence to suggest that socially-derived preferences could reasonably continue to be established and altered by various social stimuli present during SF. Previous work by Leinders-Zufall *et al*. (2007) has established that the same olfactory subsystem that responds to CS_2 also responds reliably to urine and feces, by binding specifically to guanylin and uroguanylin (peptides produced in the kidneys that are excreted in urine and feces). If during SF, a conspecific urinates or defecates in close proximity to the food dishes, these compounds in combination with the food odours present have the potential to alter food preferences previously established by STFP. For example, if a rat with a cocoa preference is exposed to a conspecific's urine and cinnamon, as well as CS_2 from a conspecific's breath and cocoa during SF (or vice versa), these conflicting signals may lead to the observed degradation of food preference. Preference may then be re-established following SF as only one socially relevant chemical signal is being transmitted in combination with the food odour via demonstrator/observer interactions. Even if urination and/or defecation occurs during these

interactions, only one specific food odour is present, therefore the type of interference present during SF from two conflicting odours will not occur.

In naturalistic settings, the marking of food sites by conspecifics can impart preferences in naïve rats (Laland & Plotkin, 1991). The results of preference tests pre- and post-SF in Chapter 2 may be consistent with this idea. To investigate this in an experimental setting, I could present a rat with two surrogate demonstrators simultaneously. One surrogate would be dipped in a combination of urine and a novel food odour while the other would be dipped in CS_2 with an additional novel food odour. The resulting preference (or lack thereof) would help us to understand if one socially relevant chemical stimulus leads to the formation of a more robust food preference or if they are equally effective.

Additionally, available data on the neurobiology of STFP indicates that the same system involving GC-D^+ expressing ORNs, the necklace glomeruli, and ultimately the hippocampus, should drive both preference acquisition via CS_2 and acquisition via urine peptides (although the site of the conjunctive information processing remains unknown). If the mechanisms responsible for updating preferences in SF and STFP are in fact the same, then knockdown of the ventral hippocampus and subiculum should ablate the updating and alteration of preferences during SF.

Through analysis of personality, foraging behaviour, and gene expression within M/T cell ensembles, my thesis contributes to the understanding of both the social and neurological mechanisms behind STFP. As well, suggested future directions based on my findings may provide interesting avenues for extending this understanding.

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