6-26-2013 12:00 AM

Cognitive Function in BK Channel Knock-Out Mice

Magdalena N. Mirkowski
The University of Western Ontario

Supervisor
Dr. Susanne Schmid
The University of Western Ontario

Graduate Program in Anatomy and Cell Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science

COGNITIVE FUNCTION IN BK CHANNEL KNOCK-OUT MICE

by

Magdalena Natalia Mirkowski

Graduate Program in Anatomy and Cell Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

The School of Graduate and Postdoctoral Studies
Western University
London, Ontario, Canada

Abstract

BK channels are large conductance potassium channels activated by both Ca^{2+} concentration and membrane depolarization. They recently have been implicated to be involved in sensorimotor gating and higher cognitive function. However, it is not yet clear to what extent these channels affect these functions. The purpose of this study is to assess the role of BK channels in sensorimotor gating and cognitive function. We used a C57BL6 and SV129 hybrid mouse model in which the pore forming alpha subunit was genetically deleted. We employed the acoustic startle response to assess mechanisms of sensory gating (habituation and PPI), and the Y-maze and Morris water maze to test higher cognitive function. We found that BK channel knock-out mice had impaired sensorimotor gating as well as deficits in spatial learning; however their working and spatial reference memory was intact. Thus BK channels seemingly have a crucial role in basic and higher cognitive function.

Keywords

BK channel, cognition, sensorimotor gating, habituation, prepulse inhibition, working memory, spatial reference learning and memory, anxiety, motor function

Acknowledgements

I am indebted to my many colleagues who supported me in completing this work. I wish to thank, first and foremost, my supervisor Dr. Susanne Schmid for her continuous help and support, as well as the members of my advisory committee, Drs. Rajakumar, Prado, and Whitehead, for all of their help and advice throughout this research project. I would also like to extend my sincere gratitude to all of my fellow lab members, who have been a constant source of support, encouragement, and reassurance, especially at times when I needed it most.

Table of Contents

List of Figures

1 Introduction

Large conductance calcium-activated potassium channels (BK channels, also known as Maxi-K, BK_{Ca}, or *Slo1* channels) are a specialized subfamily of K^+ channels (Gribkoff et al., 2001; Lee and Cui, 2010), characterized by their high single-channel conductance (Salkoff et al. 2006), in addition to their unique dual requirement of being activated by both elevation of intracellular Ca^{2+} concentration and membrane depolarization (Cui et al. 2009; Kaczorowski et al., 1995). BK channels are expressed throughout most tissues of the body, and are abundantly expressed in the mammalian central nervous system, with high levels of expression in the brain compared to peripheral tissues (Salkoff et al., 2006; Gribkoff et al., 2001; Knaus et al., 1996). Within the brain, BK channels have been found to be strongly expressed in synaptic terminals in many types of neurons (Knaus et al., 1996; Salkoff et al., 2006; Hu et al., 2010; Wang et al., 2001). BK channels have been proposed to be physiologically important in the control of neuronal excitability as well as in the control of neurotransmitter release (Faber and Sah, 2003).

Among the diverse physiological roles that BK channels have been implicated to be important players in, specific roles for BK channels have been indicated in the control of movement (Sausbier et al., 2004; Imlach et al., 2008), hearing (Ruttiger et al., 2004), sensory gating (Engel and Wu, 2008; C. Rankin, personal communication), and higher cognitive function (Zhang et al., 2006; Laumonnier et al., 2006, Deng et al., 2013; Matthews and Disterhoft, 2009). In terms of higher cognitive function, BK channels have been found to be associated with several neurological disorders associated with cognitive disruptions, including schizophrenia (Zhang et al., 2006), autistic disorder and mental retardation (Laumonnier et al., 2006), and fragile X syndrome (FXS, Deng et al., 2013). BK channels have also been suggested to play a role in the learning process, specifically in the acquisition of a trace eyeblink conditioning task (Matthews and Disterhoft, 2009). To what extent the disruption of higher cognitive function is caused by sensory gating disruptions or is an independent pathology is not known.

The present study aimed to understand the role BK channels have in sensorimotor gating and cognition. We used a genetic α subunit BK channel knock-out mouse model in order to assess the role of BK channels in sensorimotor gating and cognitive function. We employed the acoustic startle response as a tool to assess mechanisms of sensory gating (habituation and PPI), and the Y-maze and Morris water maze to test higher cognitive function in the mice. We hypothesized that non-functional BK channels will lead to the observed deficits in measures of sensory gating, specifically in short-term habituation. We also hypothesized that if loss of BK channel function results in impairment of sensorimotor gating, then this will also result in deficits in higher cognitive function.

2 Literature Review

This study used a global BK channel knock-out mouse model for investigating BK channel function, and focuses on the physiological role that BK channels play in mechanisms of sensorimotor gating, and in higher cognitive function.

2.1 BK channels: molecular structure, physiological role, and distribution

Potassium channels encompass a large diverse group of integral membrane proteins (Atkinson et al., 1991). Within the family of potassium (K^+) ion channels, which generally function to control the electrical excitability of cells (Knaus et al., 1994) and to open and allow K^+ to flow out of the cell and thus hyperpolarize the neuron (Gribkoff et al., 2001), there is a specialized subfamily of K^+ channels referred to as BK channels (also known as Maxi-K, BK_{Ca}, or *Slo1* channels, Gribkoff et al., 2001; Lee and Cui, 2010). This class of 'big potassium' channels is characterized by their high single-channel conductance (Salkoff et al. 2006), in addition to their unique dual requirement of being activated by both elevation of intracellular Ca^{2+} concentration and membrane depolarization (Cui et al. 2009; Kaczorowski et al., 1995).

The pore forming alpha subunit of BK channels (tetramers) are encoded by a single gene termed *Slo1* (Cui et al., 2009), a name derived from the *slowpoke* (*slo*) gene which was first identified and cloned in *Drosophila melanogaster* and shown to encode the structural component of Ca^{2+} - and voltage-activated K^+ channel protein (Salkoff et al., 2006; Cui et al., 2009; Atkinson et al., 1991). The mammalian mouse and human homologs encoding this gene were later isolated and cloned, and the human homolog is also called KCNMA1 (Pallanck and Ganetzky, 1994; Butler et al., 1993). BK channels consist of two dissimilar types of subunits – the Alpha (α) subunit, encoded by the *Slo1* gene, which forms the ion conduction pore, as well as the Beta (β) subunit, a protein contributing to channel gating properties and pharmacology (Lee and Cui, 2010; Kaczorowski et al., 1996). There are four types of β-subunits, encoded by the KCNMB1-4 genes,

each displaying distinctive expression in tissue and altering Ca^{2+} sensitivity and gating kinetics, therefore contributing to BK channel diversity (Lee and Cui, 2010; Orio et al., 2002). β4 subunits are primarily expressed in the brain, whereas β1 subunits are primarily expressed in smooth muscle cells. β2 and β3 subunits tend to be neuronally expressed (Lee and Cui, 2010). β-subunits are also implicated in modification of the BK channel pharmacological properties, such as toxin binding and acting as drug receptors (Orio et al., 2002).

Slo1 α-subunits have seven membrane-spanning domains, S0-S6, that surround an ion-selective pore. They have a cytosolic carboxy-terminal extension and calcium bowl containing sites that sense cytosolic factors which can modify gating (Salkoff et al., 2006). The *Slo1* α-subunit comprises three main structural domains, each serving a distinctive function – a voltage sensing domain (VSD) which senses membrane potential, a cytosolic domain which senses calcium ions, and a pore-gate domain (PGD) which controls potassium permeation by opening and closing (Lee and Cui, 2010). Collectively, the voltage sensing domain and the pore-gate domain are referred to as the membrane spanning domains, formed by S1-S4 and S5-S6, respectively (Meera et al., 1997; Lee and Cui, 2010). Two RCK (regulator of K^+ conductance) domains (RCK1 and RCK2) make up the cytosolic domain, which together contain two high-affinity calcium binding sites (one in the RCK1 domain, and one in the calcium bowl region which is located in the RCK2 domain, Lee and Cui, 2010). The S0 transmembrane segment which is unique to *Slo1* channels is important in the functional interaction between the α and β subunits, and is thought to play a role in modulation of the equilibrium between resting and active states of the voltage sensing domain (Koval et al., 2007, Figure 2.1)

BK channels are expressed throughout most tissues of the body, and are abundantly expressed in the mammalian central nervous system, as well as in many other organs including the pancreas, hair cells, and smooth muscle (Salkoff et al., 2006). However, BK channel mRNA has been found to have prevalent levels of expression in the brain compared to peripheral tissues, and is highly expressed in the neocortex, olfactory cortex, hippocampus, and cerebellum of the adult mammalian rat brain (Gribkoff et al., 2001; Knaus et al., 1996). BK channel mRNA has also been observed to be enriched in termination areas of major projection tracts, suggesting that the BK channel protein is found most abundantly in synaptic terminals (Knaus et al., 1996; Salkoff

et al., 2006). Further research to localize BK channels on a cellular level revealed that BK channels are located in the presynaptic terminal in many types of neurons (Hu et al., 2010; Wang et al., 2001). Investigations of BK channel expression in the mouse brain revealed high levels in the neocortex, olfactory bulb, basal ganglia, hippocampus, and cerebellum (Sausbier et al., 2006).

BK channels have been implicated to be physiologically important in the control of neuronal excitability as well as in the control of neurotransmitter release (Faber and Sah, 2003). They have been proposed to contribute to the fast phase of the afterhyperpolarization (AHP) potential after an action potential, thereby also contributing to the refractory period, excitability and firing rate of a neuron (Salkoff et al., 2006; Faber and Sah, 2003). BK channels have also been suggested to play an important role in controlling neurotransmitter release by primarily serving as negative regulators of neurotransmitter secretion (Wang, 2008; Wang et al., 2001). It has been suggested that BK channels may function as an emergency brake under conditions of excessive depolarization and amassing of intracellular calcium by reducing calcium influx and therefore neurotransmitter release upon their activation (Hu et al., 2001; Sailer et al., 2006). They may as well function as modulators of synaptic efficacy resulting for e.g. in synaptic depression, which normally involves a reduction in the amount of neurotransmitter released (Robitaille and Charlton, 1992; Zucker 1989).

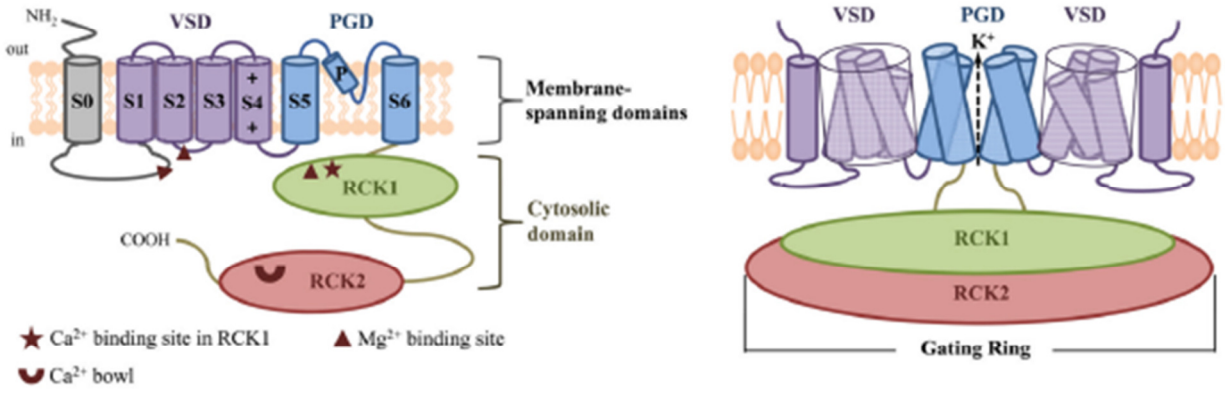


Figure 2.1 BK channel *Slo1* subunit structure. The Slo1 subunit is comprised of a membrane spanning domain and a cytosolic domain. The membrane spanning domain includes the S0 segment, the voltage sensing domain (VSD, S1-S4) and the pore-gate domain (PGD, S5-S6). The cytosolic domain is formed by two RCK domains (modified after Lee and Cui, 2010).

2.2 BK channels and their involvement in hearing and motor function

Among the diverse physiological roles that large conductance calcium-activated potassium (BK) channels have been implicated to be important players in, specific roles for BK channels have been indicated in the control of movement (Sausbier et al., 2004; Imlach et al., 2008), as well as in hearing (Ruttiger et al., 2004).

Evidence for the involvement of BK channels in motor function has been shown in studies with *Caenorhabditis elegans*. BK channels are present in both neurons and muscles of *C. elegans*. Through the genetic removal of the BK channels, a functional role of this channel was identified in the presynaptic regulation of neurotransmitter release from motor neurons at the neuromuscular junction when *Slo1* mutants were associated with increased quantal content of neurotransmitter secretion (Wang et al., 2001). A critical role for BK channels in muscle tissue was established in *C. elegans* in relation to the dystrophin homologue *dys-1*, mutations of which

lead to Duchenne muscular dystrophy (DMD), a progressive muscle wasting disease in humans (Carre-Pierrat et al., 2006). The absence of dys-1 down-regulates the activity of the BK channel, putting the muscle in a deleterious hyperactive state, which may contribute to the pathogenicity of *dys-1* mutations (Carre-Pierrat et al., 2006).

Moreover, inhibition of BK channels has been proposed as a mechanism underlying "ryegrass staggers", a neurological disease of livestock that impairs motor function involving ataxia and tremors, based on results showing that intraperitoneal BK channel inhibitor (paxilline) administration in wild-type mice induced motor impairment in the form of tremors and limb incoordination but had no effect in mice lacking BK channels (Imlach et al., 2008). Furthermore, motor impairment has been observed in mice deficient for the pore forming subunit of the BK channel in the form of tremor as well as abnormal interlimb coordination (e.g., in gait). These effects were suggested to be due to cerebellar dysfunction through fast afterhyperpolarization (AHP) suppression and slowed action potential repolarization in purkinje cells, as shown in recordings of PCs in cerebellar slices of wild-type animals treated with the BK channel blocker iberiotoxin (Sausbier et al., 2004). In the present study, we used a different BK channel knock-out mouse for behavioural testing, so we carefully re-assessed motor function of our BK channel knock-out mouse before we analyzed cognitive tests that rely on motor function.

Another physiological role that BK channels have been suggested to be involved in is hearing. Auditory hair cells are sensory receptors performing three main functions: transduction of a stimulus into an electrical signal, filtering of the stimulus or response to augment behaviourally salient frequencies, and synaptic transmission of information to the central nervous system (Roberts et al., 1990). BK channels have been found to contribute to the electrical tuning of auditory hair cells in conjunction with calcium channels whereby they maximize the responsiveness of the cells to particular sound stimulation frequencies (Jones et al., 1999; Roberts et al., 1990). Differential expression of alternatively spliced α subunit combined with a β subunit gradient has been suggested to possibly underlie the tonotopic organization of the turtle cochlea (Jones et al., 1999). Moreover, BK channels in inner hair cells of the cochlea have been implicated to be crucial in central auditory processing by coding the temporal structure of sound, as well as in signal detection in a noisy environment, as was shown in mice with hair-cell

specific deletion of the pore forming α subunit (Kurt et al., 2012). Additionally, an experiment using knock-out mice lacking the pore forming α subunit of the BK channel has shown a critical role of this subunit to the development of normal hearing function and cochlear phenotype, due to observed progressive high-frequency hearing loss in these mice beginning four weeks postnatally (Ruttiger et al., 2004).

Unperturbed hearing function is essential for our experiments using modulations of the acoustic startle response. In contrast to the previously published studies, we used an F1 generation hybrid mouse, generated by mating heterozygous C57BL6 and SV129 mice. Auditory brainstem responses (ABRs) were recorded from these animals and click-evoked ABRs revealed hearing thresholds that were not significantly different between control and knock-out mice, as was true for tone-evoked ABR thresholds for low and middle frequency ranges of hearing. High frequency (32 kHz and above) hearing loss was detected in knock-out mice, however, these frequencies don't play any role in our startle testing and no progressive age related hearing deficits were found in knock-out mice from 4-15 weeks of age (Unpublished data, personal communication Lukas Ruttiger, Figure 2.2).

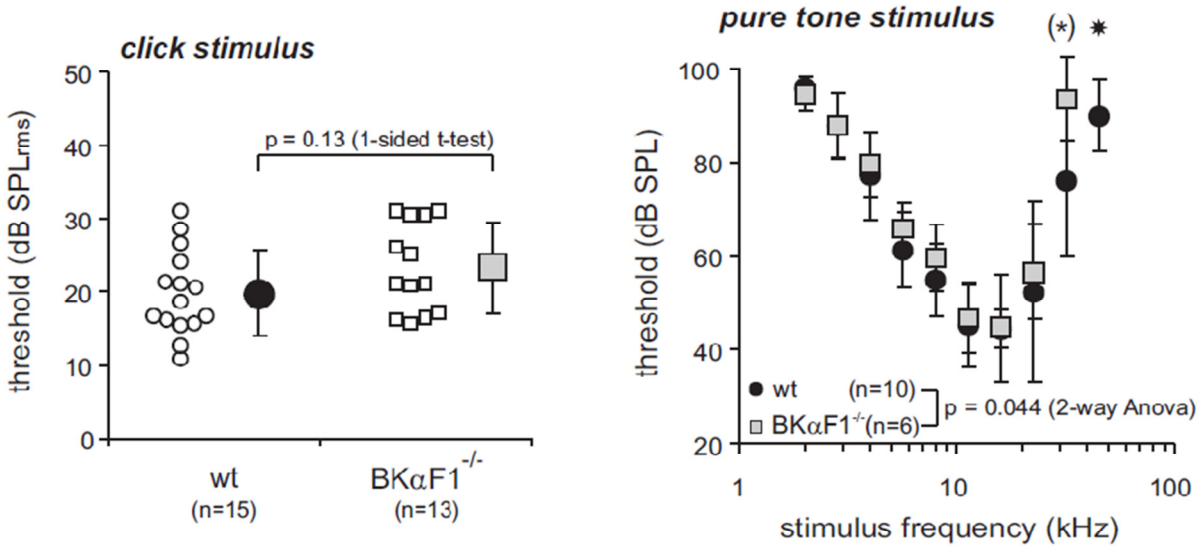


Figure 2.2 Auditory brainstem responses recorded from F1 generation α subunit homozygous knock-out and wild-type littermate control mice. Click-evoked ABRs revealed hearing thresholds that were not significantly different between control and knock-out mice. Tone-evoked ABR thresholds for low and middle frequency ranges of hearing were not significantly different between control and knock-out mice. High frequency (32 kHz and above) hearing loss was detected in knock-out mice (Unpublished data, personal communication Lukas Ruettiger).

2.3 The acoustic startle response

We used measurements of the acoustic startle response to assess sensorimotor gating in wild-type and BK channel knock-out animals. The startle reflex is a fast reaction to sudden and intense stimuli generally characterized by a fast twitch of facial and body muscles, and can be described as a response pattern serving a protective function against injury. It can be elicited by a variety of stimuli and stimulus combinations, including acoustic, tactile, visual, and vestibular, in various species of animals as well as in humans (for review see Koch 1999). The startle response can exist in many different forms across species; for instance, the nematode *Caenorhabditis elegans* swims backwards in response to a mechanical stimulus in the form of a tap to the side of the Petri dish where it resides, which is termed as the tap withdrawal response. The distance travelled backwards in response to each stimulus is a measure of the magnitude of this response (Rankin et al. 1990). *Drosophila* exhibit a giant fibre escape pathway in response to a visual stimulus, the intensity of which can be measured by recording from the target muscle fibre (Engel and Wu, 1996). Similarly, the mollusc *Aplysia* displays a gill-withdrawal reflex in response to a tactile stimulus presented to the skin, and this response can be quantified with recordings of postsynaptic potentials (EPSPs) in the gill motor neurons (Castellucci et al., 1970).

The mammalian acoustic startle response (ASR) is the most commonly studied startle reflex, characterized by eye-lid closure as well as facial, neck, and skeletal muscle contraction producing a crouch-like movement (Koch 1999; Fleshler 1965), and was first evoked and described in rats by Prosser and Hunter (1936). These experiments consisted of concentric needle electrodes being inserted into the leg muscles (predominantly the gastrocnemius) of rats which recorded the electrical response of the muscle fibres in response to a sudden and loud click acoustic stimulus that was presented repeatedly to the animals, resulting in contraction of the muscle. The whole body startle response is used frequently in small animals to assess the magnitude of the response to acoustic stimuli, whereas in humans, the acoustic startle response is most commonly measured by observing the eye blink reflex component of this response, as blinking is considered to be the most reliable constituent of startle (Geyer and Braff, 1982; Graham, 1975; Landis and Hunt, 1939). The universality of this relatively simple motor

response pattern has been observed across a wide variety of species of animals as well as in humans (Landis and Hunt, 1939; Fleshler, 1965), allowing studies with animals to be generalizable to humans and aid in our further understanding of human sensorimotor integration (Koch, 1999).

The neural circuitry underlying the acoustic startle response pathway was first studied in rats by Davis et al., (1982a) through a series of experiments involving tracing, electrical stimulation, and electrolytic lesions. The short latency of the acoustic startle reflex suggested that this response is mediated by a simple neural circuit involving few synapses (Davis et al., 1982a), and in accordance with this idea, the results of these experiments yielded that the acoustic startle pathway consists of the auditory nerve, ventral cochlear nucleus (VCN), ventral nucleus of the lateral lemniscus (VLL), caudal pontine reticular nucleus (PnC), and ends with spinal interneurons and spinal motor neurons (Koch, 1999; Davis et al., 1982a). The critical involvement of the PnC in mediating the ASR was further supported when a subpopulation of cells within the PnC, termed giant neurons, were found to respond to short-latency auditory input and project to the spinal cord, making these cells good candidates for mediating the ASR (Koch et al., 1992; Lingenhohl and Friauf, 1994). Furthermore, lesion studies in rats revealed a close correlation between the reduction in the amplitude of the startle response and the loss of PnC giant neurons, as well as a marked reduction or even abolition of the ASR observed after large lesions of the PnC (Koch et al., 1992). The primary acoustic startle circuit originally proposed by Davis et al. (1982a) was later re-evaluated using discrete cell-specific chemical lesions; the results of this study revealed that lesioning of the VLL did not eliminate startle, but when the lesion invaded a portion of the ventrolateral part of the PnC, startle was significantly reduced, suggesting that only the PnC and not the VLL is critical in the mediation of the reflex (Lee et al., 1996). Specific lesions of the ventrolateral part of the PnC were found to completely block startle, further supporting the importance of this structure in ASR mediation. Additionally, cochlear root neurons (CRNs - neurons embedded in the auditory nerve) receive direct auditory input from the cochlea and project axon collaterals that synapse mainly in the ventrolateral part of the PnC. This is suggestive of their potential involvement in the acoustic startle reflex, most likely by providing the auditory input to the PnC that is required to elicit a startle response

(Lingenhohl and Friauf, 1994). Lee et al. (1996) confirmed the involvement of CRNs in the ASR by lesioning these neurons, which resulted in a complete abolishment of the startle reflex. A role for the cochlear nucleus (CN) in startle response mediation remains unclear, although it does project auditory input to the PnC (Lee et al., 1996; Weber et al., 2002). Taken together, these results show that the neural circuitry underlying the ASR is simpler and may involve less synapses than previously thought, which include the spiral ganglion cells that innervate the cochlear hair cells, CRNs, the ventrolateral part of the PnC, and spinal motor neurons (Lee et al., 1996, Figure 2.3). Additionally, PnC giant neurons can be considered as the sensorimotor interface of the startle reflex, since these neurons receive sensory information from other modalities as well and project directly onto facial, cranial, and spinal motor neurons (Lingenhohl and Friauf, 1994; Koch, 1999).

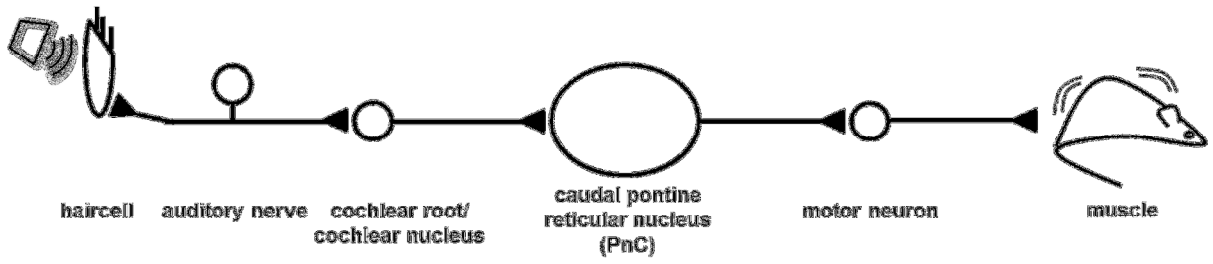


Figure 2.3 Hypothetical primary pathway of the acoustic startle reflex. The neural circuitry underlying the ASR includes the spiral ganglion cells that innervate the cochlear hair cells, CRNs, the ventrolateral part of the PnC, and spinal motor neurons (modified after Koch, 1999).

2.4 Operational measures of sensorimotor gating: habituation and prepulse inhibition

The acoustic startle response can be modulated by a variety of factors such as repetitive presentation of a startling stimulus (sensitization/habituation), as well as by prior presentation of a non-startling prepulse stimulus (prepulse inhibition), and thereby shows a number of forms of plasticity that reflect sensorimotor gating mechanisms. Therefore the ASR serves as a valuable response model in furthering our understanding of the mechanisms underlying sensorimotor gating and how they alter other behaviour (Koch, 1999; Lingenhohl and Friauf, 1994).

Sensory gating is a pre-attentive process that allows us to filter out extraneous irrelevant stimuli and extract only the salient information important for survival, allowing us to respond appropriately through efficient information processing. Once the distinction between unimportant and relevant stimuli has been made, cognitive resources can then be allotted to process the salient stimuli (Graham, 1975; Braff and Light, 2004). Two operational measures which reflect sensorimotor gating mechanisms are habituation and prepulse inhibition (PPI) of startle, discussed below.

Habituation is defined as the reversible decrease in a behavioural response resulting from repeated presentation of a stimulus (Thompson and Spencer, 1966). It is considered to be the most basic form of non-associative learning, since only a single stimulus is thought to be involved rather than an association between multiple stimuli, and it does not involve sensory or motor fatigue (Rankin et al., 2009; Rose and Rankin, 2001). The characteristics of habituation (for review see Thompson and Spencer, 1966; Rankin et al., 2009) have been found to be universally present across a wide variety of behavioural responses differing in degrees of complexity, with one of the simpler and most commonly used responses to study habituation being that of the startle reflex (Thompson and Spencer, 1966; Leaton et al., 1985). Habituation is independent of a simultaneous parallel process termed sensitization, which is characterized by an increase in behavioural responsiveness to a repeated stimulus, and together these processes yield the net behavioural outcome in response to the repeated presentation of a stimulus (Groves and

Thompson, 1970). Additionally, habituation exists in two forms: short-term, which is the decrement in a behavioural response observed within a testing session, and long-term, the reduction of a behavioural response observed across days (Leaton et al., 1985). Habituation is a phenomenon and form of learning that allows organisms to focus on important stimuli by filtering out irrelevant stimuli, and is considered to be a prerequisite to understanding other more complex forms of learning (Rankin et al., 2009).

Prepulse inhibition (PPI) is defined as the reduction in the magnitude of a startle response evoked by a startling stimulus when this stimulus is preceded by a non-startling stimulus (Ison and Hammond, 1971). This phenomenon reflects another aspect of pre-attentive information processing and a mechanism of sensorimotor gating, allowing an organism to attend to salient stimuli and ignore those that are irrelevant, thereby reducing information overload and demand for cognitive resources and allowing an organism to function efficiently (Fendt et al., 2001; Ellenbroek 2004; Braff and Light, 2004). Prepulse inhibition does not require learning, as it occurs on the first prepulse-pulse trial, and repeated exposure to a prepulse stimulus does not impede the ability of subsequent presentations of the prepulse to reduce the magnitude of the startle response (Fendt et al., 2001; Koch, 1999). The amount of prepulse inhibition observed is dependent on the intensity and modality of the prepulse stimulus, as well as the time interval between presentation of the prepulse and startle stimulus (interstimulus interval, ISI, Fendt et al., 2001; Ison and Hammond, 1971). ISIs for maximum prepulse inhibition have been found to be 120 msec in humans (Braff 1978; Koch, 1999) but shorter in rats and mice (30-50 msec), likely due to their smaller brain size. Maximum ISIs where prepulse inhibition can be observed are between 1000 and 2000 msec (Yeomans et al., 2010).

Deficits in sensory gating result in information overload received by the brain and this is present in many neurological disorders such as Huntington's disease (Swerdlow et al., 1995), Tourette's syndrome (TS), attention-deficit hyperactivity disorder (Castellanos et al., 1996), fragile X syndrome (FXS) (Frankland et al., 2004), autistic disorder (AD) (Perry et al., 2007), and most notably in schizophrenia (Geyer and Braff, 1982, Braff et al., 1978). Abnormalities in sensorimotor gating have been observed in schizophrenic patients in studies showing impaired habituation of the eye blink reflex as well as impaired prepulse inhibition of this response (Geyer

and Braff, 1982; Braff et al., 1978; Graham, 1975), which is in line with schizophrenic patients having reported to experience oversensitivity to sensory stimulation. (Braff and Geyer, 1990) Such deficits in sensorimotor gating result in sensory information overload, and may lead to cognitive disintegration in the form of attention-dependent cognitive deficits (Braff and Light, 2004), as well as perceptual and reasoning thought disturbances (Perry et al., 1999), suggesting an association between deficits in sensorimotor gating with other cognitive deficits.

In summary, habituation and prepulse inhibition represent sensory gating mechanisms that exemplify an organism's ability to filter incoming sensory information. Understanding the role of BK channels in the basic mechanisms underlying habituation and PPI allows us to heighten our understanding of sensorimotor gating mechanisms, as well as how disturbance of these mechanisms leads to impairment of sensorimotor gating and associated cognitive deficits potentially caused by deficits in sensorimotor gating.

2.5 Circuitry mediating habituation and prepulse inhibition of the ASR

Short-and long-term habituation of the ASR are independent processes that require different underlying neuronal mechanisms; short-term habituation is hypothesized to be inherent to the acoustic startle response pathway, whereas long-term habituation is thought to be mediated extrinsically (Leaton et al., 1985). A study involving decerebrated rats revealed that these animals displayed robust short-term habituation but no long-term habituation of the ASR, supporting the notion that short-term habituation, but not long-term habituation of the acoustic startle response is mediated in the lower brainstem (Leaton et al., 1985). Short-term habituation of the ASR is presumed to be mediated intrinsically by the acoustic startle response pathway itself, as Davis et al. discovered (1982b) by electrically stimulating rats at various points of the neural circuit underlying this response. The results showed that elicited startle responses decreased across repetitive stimulation of the cochlear nucleus, but not with repetitive

stimulation of the caudal pontine reticular nucleus (PnC), indicating that habituation seems to occur in the earlier sensory parts of the acoustic startle pathway and specifically at the synapse between the cochlear nucleus and the PnC (Davis et al., 1982b). Furthermore, electrical stimulation of the PnC produced startle responses that did not habituate to repeated stimulus presentation (Lingenhohl and Friauf, 1994). Intracellular recordings of PnC giant neurons, thought to represent the sensorimotor interface of the acoustic startle reflex, have revealed decreased postsynaptic potentials during repeated acoustic stimulation (Lingenhohl and Friauf, 1994). Moreover, repetitive electrical stimulation of auditory afferents was observed to induce synaptic depression in the synapse with PnC giant neurons, providing further evidence for the site of short-term habituation to be along the sensory pathways to the PnC (Weber et al., 2002; Pilz et al., 2004), and this mechanism responsible for the synaptic depression thought to underlie short-term habitation has been suggested to be localized presynaptically (Simons-Weidenmaier et al., 2006). To summarize, although little is still known about the molecular and cellular mechanisms underlying its behavioural expression (Rose and Rankin, 2001), short-term habituation appears to be due to depression of synaptic input from sensory afferents to giant neurons in the PnC through a presynaptically located mechanism.

On the other hand, long-term habituation has been proposed to be mediated extrinsically of the acoustic startle response pathway. Decerebrated rats with intact lower brain stems and therefore intact primary acoustic startle response neural pathways showed abolished long-term habituation yet robust short-term habituation, providing evidence for long-term habituation not being mediated solely within this pathway (Leaton et al., 1985). The neuronal substrates that have been implicated to be involved in the mediation of long-term habituation of the acoustic startle response include the mesencephalic reticular formation, the medial cerebellum, the ventral periaqueductal gray, as well as various cortical areas. It is not yet clear which site in the acoustic startle response pathway is relevant to mediation of long-term habituation (Koch, 1999).

The neuronal circuitry underlying the mediation of prepulse inhibition is also thought to be extrinsic to the acoustic startle pathway, although is not yet fully understood. The acoustic PPI pathway, consisting of midbrain structures, runs parallel to and modulates the primary acoustic startle pathway by providing feed-forward inhibition onto the giant neurons in the PnC through

inhibitory projections from the pedunculopontine tegmental nucleus (PPTg), presumably through both cholinergic and GABAergic inhibition (Koch, 1999; Yeomans et al., 2010). Acoustic prepulse stimuli are projected from the cochlear nucleus and are received as auditory input by the inferior colliculus (IC), a critical component of the acoustic PPI pathway, and from there this sensory information is relayed to the superior colliculus (SC). The SC not only receives auditory input, but also visual and somatosensory input, and projects to the PPTg, therefore acting as a structure capable of relaying multi-modal prepulse stimuli to the PPTg (Fendt et al., 2001). As mentioned previously, the PPTg then projects to and inhibits PnC giant neurons (Figure 2.4). A critical role for the PPTg was established through electrical stimulation studies of this area, which led to an observed reduction in startle response and therefore enhancement of prepulse inhibition (Saitoh et al., 1987). Additionally, lesioning of the PPTg as conducted by Leitner et al. (1981) resulted in attenuated prepulse inhibition, further supporting a role for this structure in the mediation of prepulse inhibition. However, lesioning of the PPTg has not completely abolished prepulse inhibition, suggesting that there may be other pathways involved in modulating the primary acoustic startle pathway. It should also be mentioned that the PPTg itself is modulated by several other brain regions, thus contributing another layer of complexity to the neuronal circuitry underlying prepulse inhibition mediation (Koch, 1999).

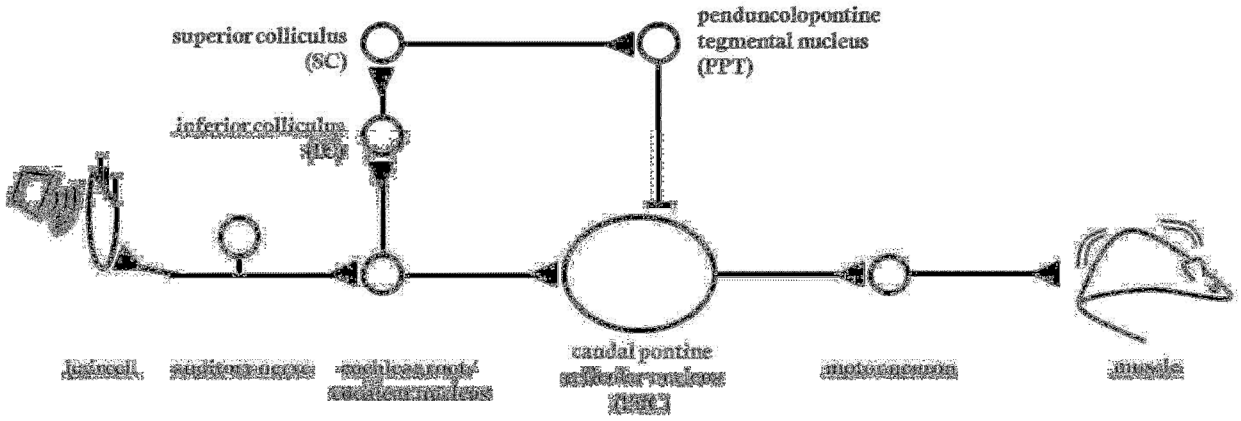


Figure 2.4 Hypothetical primary pathway of the acoustic startle reflex and extrinsic prepulse inhibition circuitry. Short-term habituation is thought to occur between the cochlear root/nucleus sensory neurons and PnC giant neurons. Prepulse inhibition is thought to be mediated extrinsically of this primary startle pathway by sending inhibitory projections to the PnC. (modified after Koch, 1999).

2.6 Evidence for the importance of BK channels in sensorimotor gating

A requirement for the biological analysis of learning is that the neural circuitry of the behaviour undergoing modification by the learning process be correctly specified in order to associate cellular function with learning and memory (Bailey and Chen, 1988). Habituation in different animals and across various response modalities likely cannot be explained by a common single neuronal mechanism, however certain types of habituation that share common parameters may potentially also share a specific set of mechanisms underlying these processes (Castelluci and Kandel, 1974). Although little is still known about the molecular and cellular mechanisms underlying the behavioural expression of habituation (Rose and Rankin, 2001), forms of habituation with common parametric characteristics have been established for a variety of behavioural and reflex responses in animals with well-differentiated central nervous systems (Thompson and Spencer, 1966), and similarity across species suggests a common neuronal mechanism mediating short-term habituation of startle (Pinsker et al., 1970).

Assessment of the gill-withdrawal reflex evoked by a tactile stimulus presented to the same spot on the skin in the mollusc *Aplysia* by Pinsker et al. (1970) revealed habituation of the gill responses to an average of 25 percent of control amplitudes with repetition of the stimulus (a jet of seawater). The neuronal mechanisms of habituation of the gill-withdrawal reflex were investigated using a simplified neural circuit of the reflex in the form of an isolated ganglion joined to a piece of skin from the tactile receptive field. A weak electrical stimulus was applied to the skin which produced excitatory postsynaptic potentials (EPSPs) in the gill motor neurons, and this response was observed to decrease with repeated stimulation (Castellucci et al., 1970). This data suggests that short-term habituation of the gill-withdrawal reflex involves plastic changes in the form of a decrease in excitatory synaptic transmission (homosynaptic depression) between the sensory and motor neuron (Castellucci et al., 1970; Castellucci and Kandel, 1974). Further analysis of the synaptic depression underlying short-term habituation of the gill-withdrawal reflex in *Aplysia* was done to assess the amount of neurotransmitter release at the presynaptic terminal of the sensory neuron, revealing that an important change associated with the habituation of the gill-withdrawal reflex is that of a decrease in the number of neurotransmitter quanta (multimolecular packets) released, suggesting a presynaptic mechanism may participate in short-term habituation (Castellucci and Kandel, 1974).

BK channels, which are suggested to have important roles in controlling neurotransmitter release (Wang, 2008), were first proposed to play a role in habituation in an experiment by Engel and Wu (1998), who attempted to link behavioural changes to cellular or molecular defects by altering the *slowpoke* (*Slo*) gene encoding the α pore forming subunit of the BK channel in *Drosophila* and observing the functional role that this null mutation had in terms of habituation. To examine habituation behaviour, long-latency giant fibre responses were analyzed in response to electrical stimulation. Pulses from electrodes in the eyes were used to trigger the cervical giant fibre, and responses were recorded from their target muscle fibre (dorsal longitudinal muscle). The results revealed *Slo* mutants to have a markedly reduced rate of habituation compared to controls. These results suggest that *Slo*-type Ca^{2+} -activated K^+ channels have distinct functional consequences on short-term habituation, linking this behavioural phenotype to specific K^+ channel physiology.

Similar results were found in an experiment using the nematode *Caenorhabditis elegans*. This organism swims backwards in response to a mechanical stimulus in the form of a "tap to the side of the Petri dish" where it resides, termed as the tap withdrawal response, and the distance travelled backwards in response to each stimulus is measured and used to quantify habituation (Rankin et al. 1990). A *Slo1* channel mutant was used in this study in which the α pore forming subunit of the BK channel was abolished, and these worms were tested for habituation alongside controls. Results were then compared to wild type worms, which showed that while wild type worms habituated to the mechanical stimulus, the *Slo1* channel mutants did not display habituation. These results imply that BK channels are involved in sensory gating in the form of short-term habituation (Unpublished data, personal communication Catharine Rankin).

In summary, short-term habituation appears to be mediated through a presynaptic mechanism involving synaptic depression and reduced neurotransmitter release. Specifically, BK channels have been suggested to play an important role in short-term habituation in studies with *Drosophila* and *C. elegans*, implying that this behavioural phenotype may be explained on a cellular and molecular level through potassium channel physiology.

2.7 BK channels and their implication in higher cognitive function

A relatively under-researched area of knowledge in neuroscience is that of BK channel physiology influencing higher cognitive function. To date, BK channels have been found to be associated with a few neurological disorders that are associated with cognitive impairments, including schizophrenia, autistic disorder, and fragile X syndrome (FXS). BK channels were also directly implicated in the acquisition and learning of a task, discussed below.

The BK channel has been hypothesized to play a role in the schizophrenia, largely based on the known function of these channels in neuronal excitability and neurotransmitter release, in combination with the fact that commonly used neuroleptics work by altering calcium-activated potassium conductance among central neurons (Zhang et al., 2006; Salkoff et al., 2006; Faber

and Sah, 2003). A study conducted on schizophrenic patients investigated the effects of diazoxide, a potassium channel opener, and found that this could be an effective adjuvant agent in the treatment of this disorder (Akhondzadeh et al., 2002). Additionally, a post-mortem analysis of brains from schizophrenic patients revealed lower BK channel mRNA levels in the prefrontal cortex, suggesting a possible role for BK channels in schizophrenia (Zhang et al., 2006).

Laumonnier et al. (2006) found a possible association between autistic disorder and the BK channel upon discovering that the KCNMA1 gene, which codes for the pore forming α subunit of the BK channel (see above), is expressed at a lower level in comparison to healthy control subjects, suggesting a haploinsufficiency of this gene associated with autism. Therefore these authors provided evidence for autism and mental retardation being linked to a channelopathy involving a defect of the BK channel, and proposed that this may be due to deficiencies in neuronal excitability and synaptic transmission, both of which are functions that the BK channel has been implicated to be involved in (Laumonnier et al., 2006; Salkoff et al., 2006; Faber and Sah, 2003).

BK channels have also recently been found to be involved in the molecular basis of fragile X syndrome (FXS), a mental disorder which develops with loss of the fragile X mental retardation protein (FMRP, Deng et al., 2013). Deng et al. (2013) found that FMRP is involved in the regulation of action potentials in cortical and hippocampal pyramidal neurons, concluded after recordings from knock-out mice lacking this protein revealed increased action potential duration compared to wild-type control mice. Further experimentation involving reintroduction of FMRP to the neurons on the presynaptic side of the synapse resulted in restoration of normal action potentials, and also led to the discovery that FMRP acts presynaptically to regulate action potential duration. Moreover, this regulation by FMRP was found to be mediated specifically by BK channels, to which FMRP binds and regulates their level of sensitivity to calcium, thereby affecting synaptic function and consequently action potential duration.

Aside from being involved in several neurological disorders, BK channels have also been suggested to play a role in learning. Matthews and Disterhoft (2009) conducted a study on rats

whereby animals received bilateral infusions of paxilline, a BK channel blocker, into the CA1 region of the hippocampus, and were then trained to perform a trace eyeblink conditioning learning task. Results showed that blocking of BK channels slowed learning in these animals, as they showed slower acquisition of the task compared to control animals; however, hippocampal-dependent learning was not abolished as these animals were able to learn the task eventually. Similar results have also been found by Sausbier et al. (2004) in knock-out mice deficient for the pore forming α subunit of the BK channel. However, these mice reportedly showed no learning of the task at all compared to wild-type control mice, that were able to learn the task rapidly, which the authors suggested could be attributed in part to cerebellar/auditory dysfunction.

3 Objectives and hypotheses

The main objectives of the present study are to establish a role for BK channels in sensorimotor gating, as well as aim to determine an association between BK channels and higher cognitive function. We hypothesized that non-functional BK channels will lead to deficits in measures of sensory gating, specifically in short-term habituation. We also hypothesized that if loss of BK channel function results in impairment of sensorimotor gating, then this will also result in deficits in higher cognitive function.

4 Materials and methods

Fifty-six C57BL6/SV129 (F1 generation) male (n=31) and female (n=25) mice were used for this study; 20 homozygous (-/-) Slo1 knockout (10 male, 10 female), 17 heterozygous (-/+) Slo1 knockout (10 male, 7 female), and 19 wild-type littermates (11 male, 8 female). These animals were generated and genotyped in the Pharmaceutical Institute of the University of Tübingen, Germany, and shipped to Western University at the age of 2-3 months. Mice were housed in a 12 hour light/dark cycle in groups of 2, 3, or 4 animals with mixed genetic background per cage. All behavioural testing was done during the light phase, unless otherwise stated. All animal procedures were done according to NIH and institutional guidelines (see Appendix).

4.1 Motor Function Tests

Grip Force Test Procedure

In order to test front limb strength, 16 animals from each genotype (Knock-out: 9 male, 7 female; Heterozygous: 10 male, 6 female; Wild type: 9 male, 7 female) were randomly selected for this test and order of animal testing was randomized. Animals were held by the base of their tail and their front limbs were positioned near the bar of the recording unit of the Grip Strength Meter (Columbus Instruments, Columbus, Ohio, USA). Animals gripped the bar with their front limbs and were pulled back until they released their grip. The strength of their grip was measured in lbF (pound force). Each animal was subjected to 5 trials, all within one testing session which were then averaged for each animal. Animals were returned to their home cage following trials.

Catwalk Procedure

11 animals from each genotype (KO: 7 male, 4 female; HET: 7 male, 4 female; WT: 6 male, 5 female) were randomly selected for this test and order of animal testing was randomized. Animals were placed at one end of the CatWalk (Noldus Information Technology Inc., Leesburg, Virginia, USA) runway and allowed to walk across back and forth (until a consistent recording could be obtained from the animal). Each animal was subjected to only 1 trial. Animals were returned to their home cage following each trial. The CatWalk was cleaned with soap and water, and disinfected with 70% EtOH following each trial to avoid olfactory cues. Animal activity was monitored and analyzed with CatWalk™ 7.0 software (Noldus Information Technology Inc., Leesburg, Virginia, USA) to assess measures of gait including regularity index (a measure of interlimb coordination) and stride length.

Activity Monitor/Open Field Procedure

Locomotor boxes (AccuScan Instruments Inc., Columbus, Ohio, USA) were used to assess general locomotor activity. 16 animals from each genotype (KO: 9 male, 7 female; HET: 10 male, 6 female; WT: 9 male, 7 female) were randomly selected for this test and order of animal testing was randomized.

Animals were placed into the open field boxes and were allowed 2 hours of exploration time. Animals were tested for five consecutive days, with 1 trial per day. Open field box and compartment was kept constant for each animal throughout the five days of testing. Animals were returned to their home cage following each trial. Open field boxes were cleaned with soap and water, and disinfected with 70% EtOH following each trial to avoid olfactory cues. Animal activity was monitored throughout the 2 hour period using VersaMax™ software (AccuScan Instruments Inc., Columbus, Ohio, USA) for locomotor activity and number of rearing movements. Additionally, centre vs. margin activity was analyzed as an indirect measure of anxiety-like behaviour.

4.2 Anxiety Tests

Elevated Plus Maze Procedure

11 animals from each genotype (KO: 4 male, 7 female; HET: 8 male, 3 female; WT: 8 male, 3 female) were randomly selected for this test. A plus-shaped maze was used with four arms, two of which were open and two of which were enclosed by walls. The order of animal testing was randomized. Animals were placed in the centre of the maze and were allowed 5 minutes of exploration time while being videotaped by a webcam. Each animal was subjected to only 1 trial. Animals were returned to their home cage following each trial. All arms of the maze were cleaned with soap and water, and disinfected with 70% EtOH following each trial to avoid olfactory cues. The number of closed arm/open arm entries was analyzed using ANY-maze™ software (Stoelting Co., Wood Dale, Illinois, USA) for each animal, as well as time spent in centre, open arms, and closed arms.

Light/Dark Box Procedure

The light/dark box was used as another measure of anxiety-related behaviour. 12 animals from each genotype (KO: 5 male, 7 female; HET: 9 male, 3 female; WT: 9 male, 3 female) were randomly selected for this test. Animals were tested during their dark cycle. The testing room was kept dark at all times other than when the experiment was in progress, at which time the lights were turned on. The order of animal testing was randomized. Animals were placed in the enclosed "dark" section of the light/dark box. Once inside the box, the lights were turned on inside the testing room, and the animals were allowed 10 minutes of exploration time. Each animal was subjected to only 1 trial. Four animals were tested simultaneously in four light/dark boxes. Animals were returned to their home cage following each trial. Light/dark boxes were cleaned with soap and water, and disinfected with 70% EtOH following each trial to avoid olfactory cues. Animal activity was monitored using VersaMaxTM software (AccuScan Instruments Inc., Columbus, Ohio, USA) and duration of time spent in the light and/or dark was recorded, as well as latency to enter the light.

4.3 Basic Cognitive Function

Startle Testing Procedure

Habituation and prepulse inhibition of the acoustic startle response were used as a measure of sensorimotor gating. All 56 animals were used for this testing; 20 KO animals (10 male, 10 female), 17 heterozygous animals (10 male, 7 female), and 19 wild-type animals (11 male, 8 female). Animals were subjected to three days of acclimation: *Acclimation Day 1*: Animals from all three genotypes were placed in holders and then placed into startle boxes (Med Associates Inc., St. Albans, Vermont, USA) in a randomized order. Animals stayed in the startle boxes for 5 minutes. No acoustic stimulation was administered. *Acclimation Day 2*: Animals were placed in holders and then placed into startle boxes, ensuring that each animal was being placed in the same startle box that they had used previously. Animals stayed in the startle boxes for 5 minutes. White background noise was administered at 65 dB. *Acclimation Day 3*: Animals were placed in holders and then placed into the same startle boxes. Animals were then tested for their input/output function; they were exposed to 5 minutes of white background noise at 65 dB, immediately followed by 12 stimuli of increasing volume, ranging from 65-120 dB (65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120 dB).

Following the acclimation period of three days, animals were placed into the same startle boxes that they had used previously. Animals were exposed to a 5 minute acclimation period of white background noise (65 dB), immediately followed by:

Block I (Habituation): Animals were exposed to 100 pulse-alone trials of 105 dB stimuli (white noise, 20 ms duration, with a varying intertrial interval of 10-20 seconds).

Block II (Prepulse Inhibition): Animals were exposed to 60 prepulse followed by startle pulse trials, and 10 startle pulse alone trials. 75 dB and 85 dB prepulse stimuli were used (4 ms white noise) and 105 dB startle pulse stimuli (see above). Interstimulus intervals varied between 10, 30, and 100 ms. Each trial type (75 dB and 85 dB, at three different intervals, respectively) was presented 10 times, in a pseudo-randomized order.

Each animal was subjected to the testing blocks as described above (acclimation, Block I, Block II) once a day for 5 consecutive days. Four animals were tested simultaneously in four startle boxes. Animal holders were cleaned in between startle box testing. Animals were returned to their home cages following startle box testing. In a subsequent experiment testing for long-term habituation, the same experiment was conducted on the same animals approx. 4 months later, but with a shortened block I of 30 pulse-alone trials of 105 dB stimuli only, and without block II in order to avoid sensitization by over-stimulation. Data was analyzed using the Startle Reflex 5.95 software (Med Associates, St. Albans, Vermont, USA). The startle response amplitude (peak-to-peak) to the 105 dB pulse was analyzed for each trial. PPI was calculated from block II using the following formula: % prepulse inhibition = [1- (prepulse-pulse trial amplitudes)/ (pulse alone trial amplitude)]* 100. Short-term habituation scores were calculated by dividing the average of the last 5 trials by the average of the first 5 trials.

4.4 Higher Cognitive Function

Morris Water Maze Procedure

14 animals from each genotype (KO: 7 male, 7 female; HET: 10 male, 4 female; WT: 9 male, 5 female) were randomly selected for this test in the Morris water maze. Genotypes were counterbalanced so that each genotype received an equal opportunity to search for the platform in each quadrant of the pool. Learning trials were conducted over 4 days, with 4 trials per day. Order of animal testing was randomized. A clockwise semi-random set of four start positions relative to the location of the platform was used for each animal. One trial each day was from each of these four positions. Animals were released into the water at water-level. Each learning trial lasted 90 seconds or until the animal reached the platform. Animals' search patterns were tracked using ANY-maze^{TM} software (Stoelting Co., Wood Dale, Illinois, USA). If the animal did not reach the platform after the allotted amount of time (90 seconds), the animal was picked up and placed onto the platform for ~15 seconds. Animals were returned to a cage following

each trial to dry off, and were returned to their home cage following completion of all 4 learning trials each day. A probe trial without a platform was given ~ 24 hours after the last acquisition day for each animal. Each probe trial lasted 60 seconds. Each animal used the same start position for the probe trials.

Y Maze Spontaneous Alternation

This test was used as a measure of working memory. 15 animals from each genotype (KO: 7 male, 8 female; HET: 10 male, 5 female; WT: 9 male, 6 female) were randomly selected for this test. A Y-shaped maze was used with three white opaque plastic arms at a 120º angle from each other. The order of animal testing was randomized. Animals were placed in the centre of the maze and were allowed 5 minutes of exploration time. Each animal was subjected to only 1 trial. Animals were returned to their home cage following each trial. All arms of the maze were cleaned with soap and water, and disinfected with 70% EtOH following each trial to avoid olfactory cues. The number of arm entries and number of triads were recorded using a webcam (as to not interfere with the animals' maze exploration) by the experimenter in order to calculate the percentage of alternation.

4.5 Statistical Analysis

IBM SPSS Statistics 20 software was used for all statistical analyses. Outliers for all behavioural data were determined using a range of two standard deviations from the mean and were removed from the data set. In the case of a behavioural test lasting across multiple trials or days, outlier data was replaced with the respective genotype mean for that trial or day if the animal was not eliminated from the entire dataset in order to allow for repeated measures ANOVA statistical assessment. Two-way ANOVAs, multivariate ANOVAs, and two-way repeated measures

ANOVAs were used to assess statistical significance between genotypes as well as any main effects of gender and any interactions. If warranted, Bonferroni and Least Significant Difference (LSD) post-hoc tests were used to determine significance between genotypes. Results were considered statistically significant at a *p*-value of 0.05 (α=0.05).

5 Results

5.1 Effects of deficiency of the α-subunit of the BK channel on motor function

In order to assess motor function, mice underwent a grip force test, their gait was analyzed using a CatWalk runway, and they were monitored for two hours each in a locomotor box. $BK^{-/-}$ animals showed significantly reduced forelimb grip force [BK^{WT} average=0.41 ± 0.01, $BK^{+/-}$ average=0.38 ± 0.02, $BK^{-/-}$ average=0.17 ± 0.01, ANOVA, $F(2,42)=119.03$, $p<0.01$, Figure 5.1].

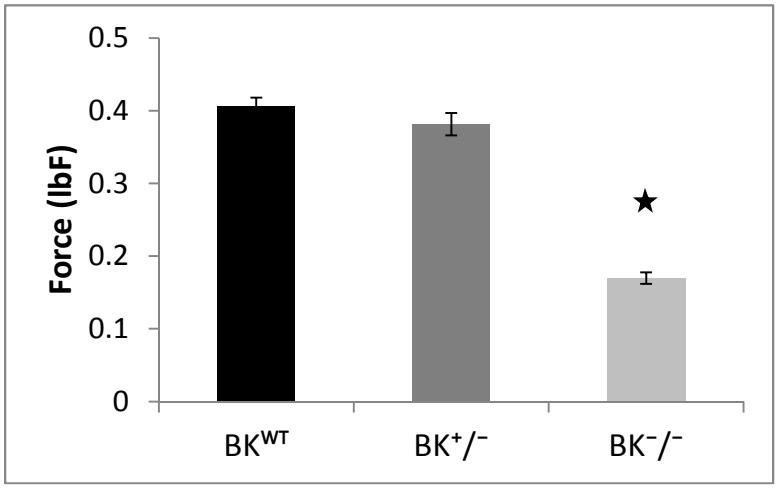


Figure 5.1 Grip force measurement. $BK^{-/-}$ animals showed significantly reduced forelimb grip force compared to $BK^{+/-}$and BK^{WT} animals. Bonferroni post-hoc analysis used to determine significance between genotypes. No main effect of gender was present [F(1,42)=3.97, p=0.053]. BK^{WT} n=16, $BK^{+/-}$ n=16, $BK^{-/-}$ n=16.

$BK^{-/-}$ animals seemed to walk funny. Indeed, when their gait was analyzed, $BK^{-/-}$ animals showed a significantly impaired regularity index [BK^{WT} average=99.66 ± 0.35, $BK^{+/-}$ average=97.92 ± 0.70, $BK^{-/-}$ average=91.54 ± 2.18, ANOVA, $F(2,25)=13.36$, $p<0.01$, Figure 5.2] compared to $BK^{+/-}$and BK^{WT} animals.

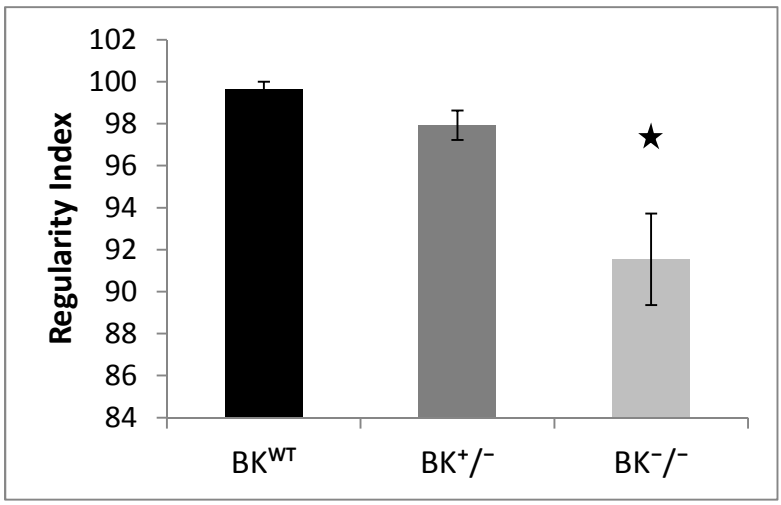
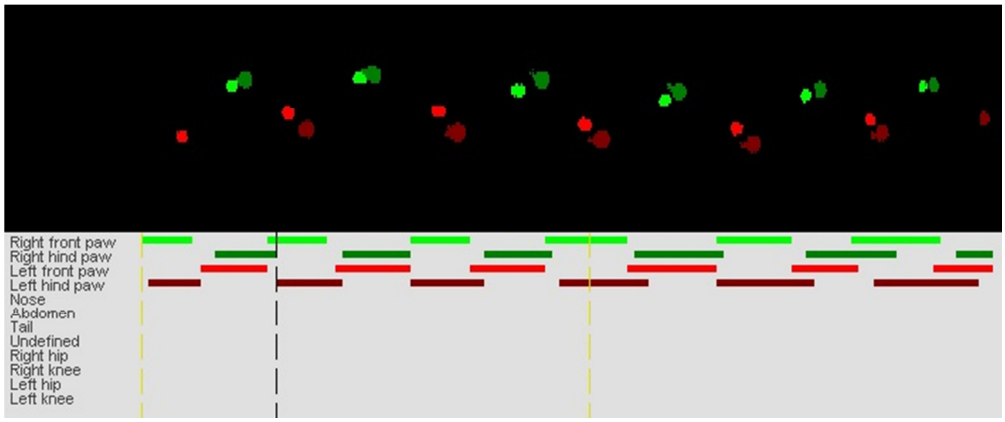


Figure 5.2 Regularity index (a measure of interlimb coordination) assessment. $BK^{-/-}$ animals showed significantly impaired regularity index compared to $BK^{+/-}$and BK^{WT} animals. Bonferroni post-hoc analysis used to determine significance between genotypes. No main effect of gender was present [F(1,25)=0.96, p=0.34]. BK^{WT} n=10, $BK^{+/-}$ n=10, $BK^{-/-}$ n=11.

a)



b)

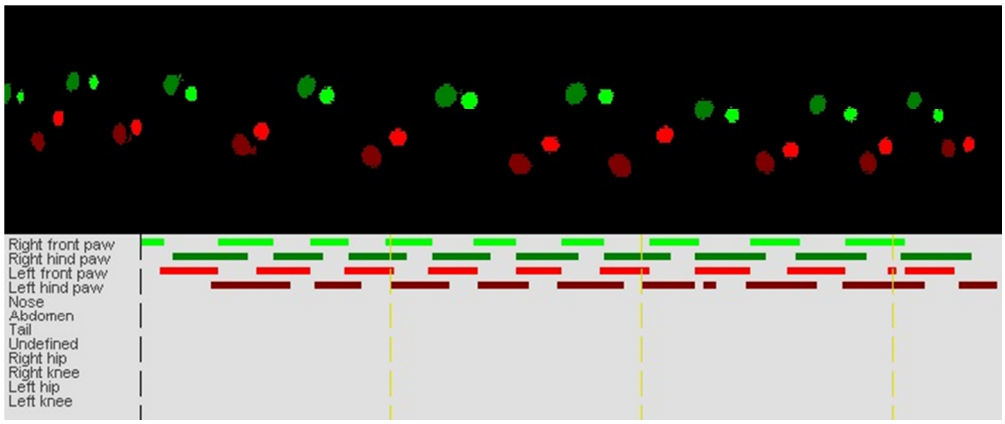


Figure 5.3 Examples of Catwalk footprint analyses from a BK^{WT} animal (a) and a $BK^{-/-}$ animal (b). Right front and hind paws are pictured in light and dark green, respectively; left front and hind paws are pictured in light and dark red, respectively. a) Footprint pattern of a BK^{WT} animal scoring a 100% regularity index. b) Footprint pattern of a $BK^{-/-}$ animal scoring an 88.89% regularity index.

Stride length was also significantly reduced in $BK^{-/-}$ animals as compared to $BK^{+/-}$and BK^{WT} animals in the right front [BK^{WT} average=8091.4 ± 160.12, $BK^{+/-}$ average=7891 ± 149.97, $BK^{-/-}$ average=5755.1 ± 172.61, ANOVA, $F(2,24)=57.48$, $p<0.01$], left front [BK^{WT} average=8085.6 ± 173.86, $BK^{+/-}$ average=7749.1 ± 160.14, $BK^{-/-}$ average=5333.5 ± 221.27, ANOVA, $F(2,24)=55.79$), $p<0.01$], right hind [BK^{WT} average=7978.5 ± 119.01, $BK^{+/-}$ average=7815.2 ± 185.45, $BK^{-/-}$ average=5155.5 ± 187.2, ANOVA, $F(2,24)=86.25$, $p<0.01$], and left hind [BK^{WT} average=7823.1 ± 182.95, $BK^{+/-}$ average=7585.2 ± 131.02, $BK^{-/-}$ average=5383.2 ± 206.79, ANOVA, $F(2,24)=53.21$, $p<0.01$] paws (Figure 5.4).

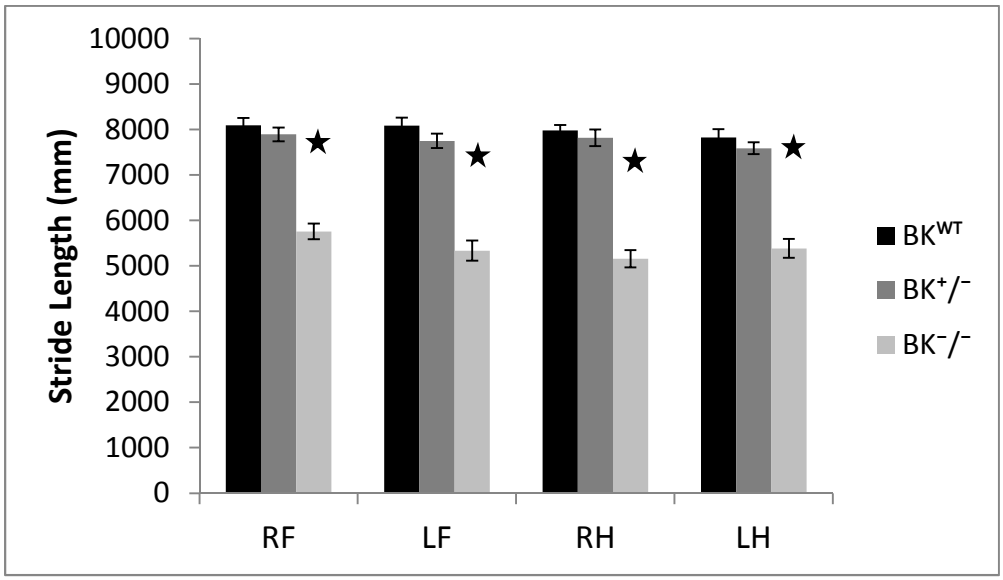


Figure 5.4 Stride length assessment. $BK^{-/-}$ animals showed significantly reduced stride length compared to $BK^{+/-}$and BK^{WT} animals in the right front, left front, right hind, and left hind paws. Bonferroni post-hoc analysis used to determine significance between genotypes. No main effect of gender was present in the right front [F(1,24)=0.40, p=0.54], left front [F(1,24)=0.20, p=0.66], right hind [F(1,24)=0.95, p=0.34], or left hind [F(1,24)=0.02, p=0.88] paw measures. BK^{WT} n=10, $BK^{+/-}$ n=9, $BK^{-/-}$ n=11.

When tested in the locomotor box, however, genotypes did not significantly differ in terms of total distance travelled [ANOVA, $F(2,40)=0.37$, $p=0.70$, Figure 5.5], sum of total activity [ANOVA, $F(2,41)=2.57$, $p=0.09$, Figure 5.6], or number of rearing movements [ANOVA, $F(2,39)=1.71$, $p=0.19$, Figure 5.7], indicating that $BK^{-/-}$ animals despite their funny gait and reduced grip force seemed to be as active as their heterozygous and wild-type littermates.

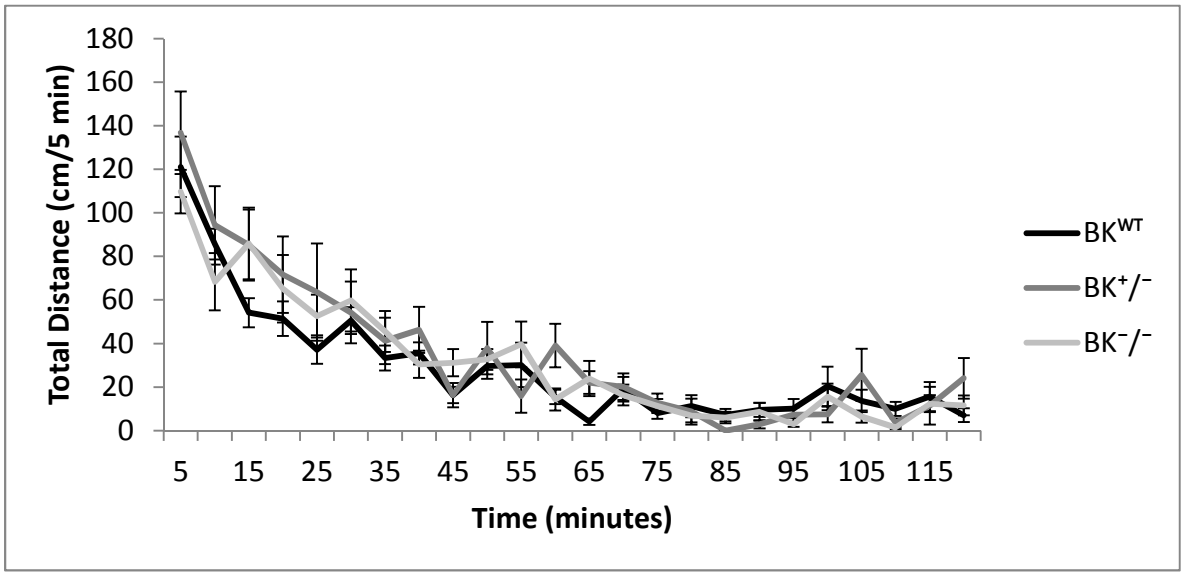


Figure 5.5 Total distance travelled over a two hour period in the open field box. Genotypes did not significantly differ overall in terms of total distance travelled. A main effect of time interval was present [$F(23,920)=42.34$, $p<0.01$]. No time interval by genotype by gender interaction [$F(46,920)=0.89$, $p=0.68$] or main effect of gender [$F(1,40)=2.99$, $p=0.09$] was present. BK^{WT} n=16, $BK^{+/-}$ n=15, $BK^{-/-}$ n=15.

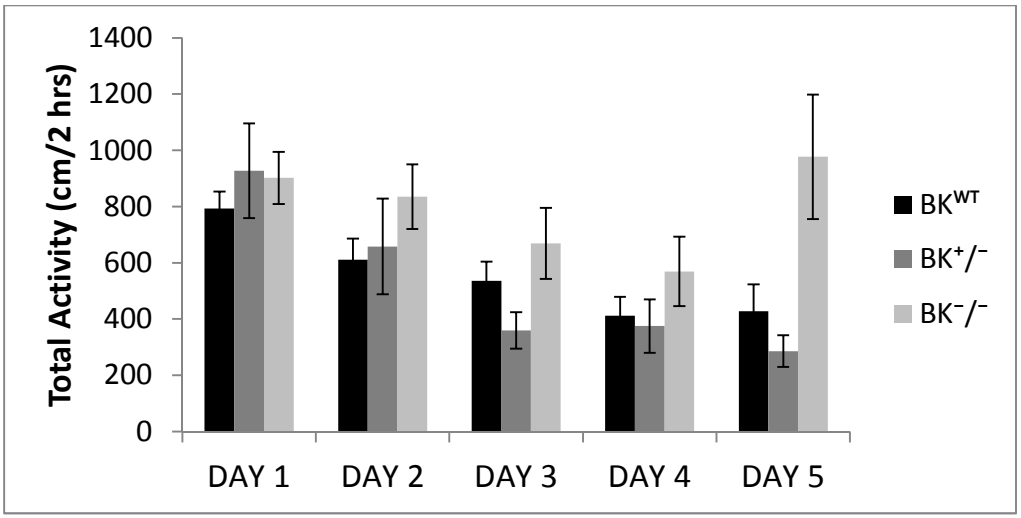


Figure 5.6 Sum of total activity occurring in the open field box over a two hour period across five days. Genotypes did not significantly differ overall in terms of total activity. A main effect of day was present $F(4,164)=13.64$, $p<0.01$ as well as a main day by genotype interaction [$F(8,164)=3.37$, $p<0.01$]. No day by genotype by gender interaction [$F(8,164)=0.24$, $p=0.98$] or main effect of gender [$F(1,41)=0.73$, $p=0.40$] was present. BK^{WT} n=16, $BK^{+/-}$ n=15, $BK^{-/-}$ n=16.

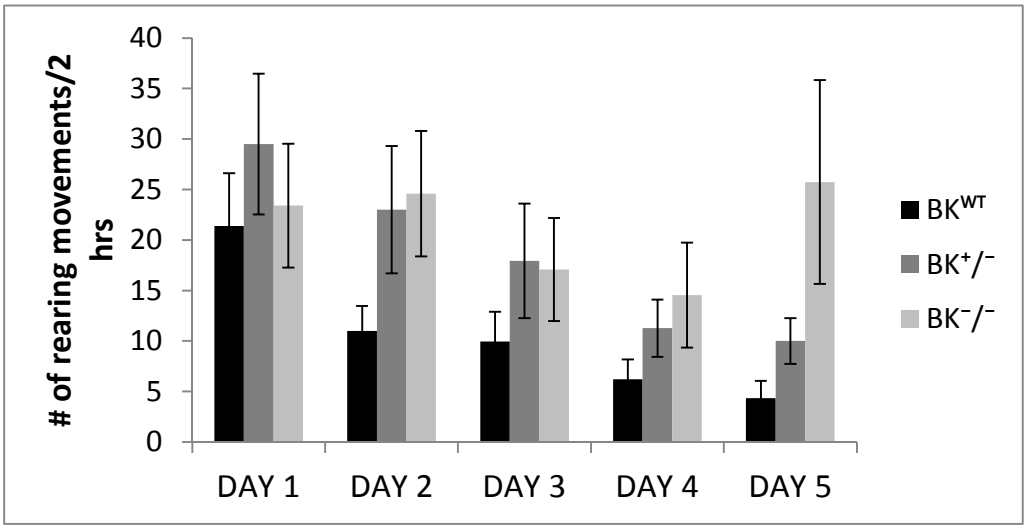
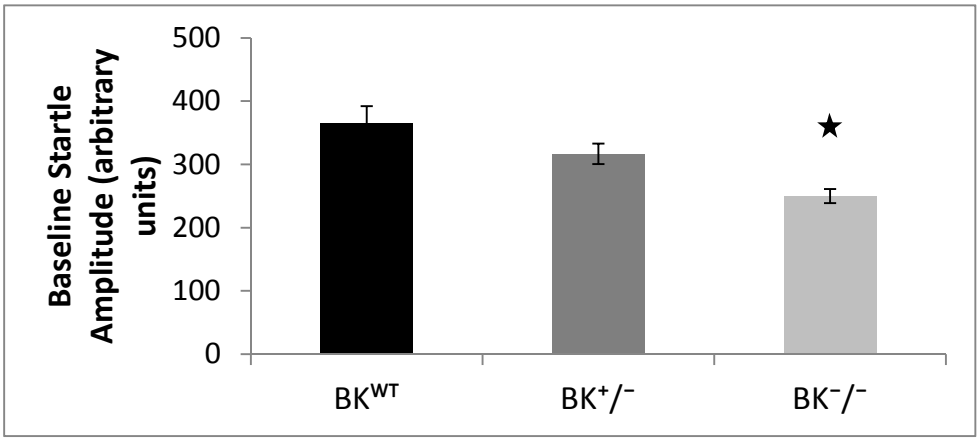


Figure 5.7 Number of rearing movements occurring over a two hour period across five days. Genotypes did not significantly differ in terms of number of rearing movements. A main effect of day was present [F(4,156)=5.29, p<0.01], as well as a main day by gender interaction [F(4,156)=2.52, p<0.05]. No day by genotype by gender interaction [F(8,156)=0.69, p=0.70] or main effect of gender [F(1,39)=3.27, p=0.08] was present. BK^{WT} n=15, $BK^{+/-}$ n=15, $BK^{-/-}$ n=15.

5.2 Effects of deficiency of the α-subunit of the BK channel in measures of sensory gating

$BK^{-/-}$ animals showed a significantly lower baseline startle compared to $BK^{+/-}$and BK^{WT} animals in block I (habituation block) [BK^{WT} average=364.99± 27.29, $BK^{+/-}$ average=316.72 ± 15.99, $BK^{-/-}$ average=249.83 ± 11.20, ANOVA, $F(2,47)=9.01$, $p<0.01$, Figure 5.8] but genotypes were equal in block II [PPI block, see Materials and Methods, BK^{WT} average=245.96 ± 22.16, $BK^{+/-}$ average=232.33 ± 17.40, $BK^{-/-}$ average=207.66 ± 6.71, ANOVA, $F(2,48)=1.14$, $p=0.33$, Figure 5.8].

a)



b)

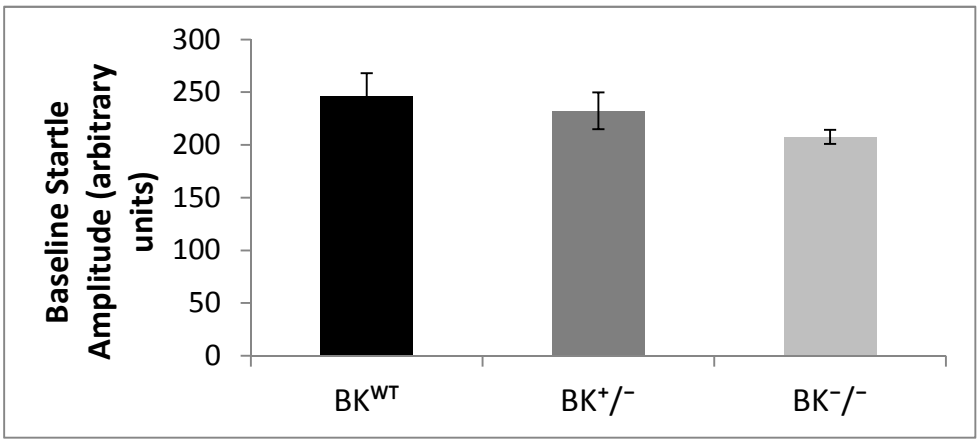


Figure 5.8 Baseline startle measures from block I (a) and block II (b). a) $BK^{-/-}$ animals showed a significantly lower baseline startle compared to $BK^{+/-}$and BK^{WT} animals. LSD *post-hoc* analysis used to determine significance between genotypes. No main effect of gender was present $F(1,47)=1.48$, $p=0.23$. BK^{WT} n=18, $BK^{+/-}$ n=15, $BK^{-/-}$ n=20. b) Baseline startle was equal among genotypes. No main effect of gender was present [$F(1,48)=1.94$, $p=0.17$]. BK^{WT} n=19, $BK^{+/-}$ n=16, $BK^{-/-}$ n=19.

In terms of short-term habituation, $BK^{-/-}$ animals showed significantly less attenuation of startle responses during block I compared to BK^{WT} animals [ANOVA, $F(2,50)=4.56$, $p<0.05$, Figure 5.9], as well as significantly larger short term habituation scores compared to BK^{WT} and $BK^{+/-}$ animals [BK^{WT} average=0.77 ± 0.03, $BK^{+/-}$ average=0.79 ± 0.05, $BK^{-/-}$ average=0.92 ± 0.03, ANOVA, $F(2,47)=4.91$, $p<0.05$, Figure 5.10], indicating that they did not habituate. Genotypes did not differ in terms of long-term habituation across training sessions. Indeed, none of the genotypes showed any long-term habituation, see Figure 5.11 [ANOVA, $F(2,50)=0.49$, $p=0.62$].

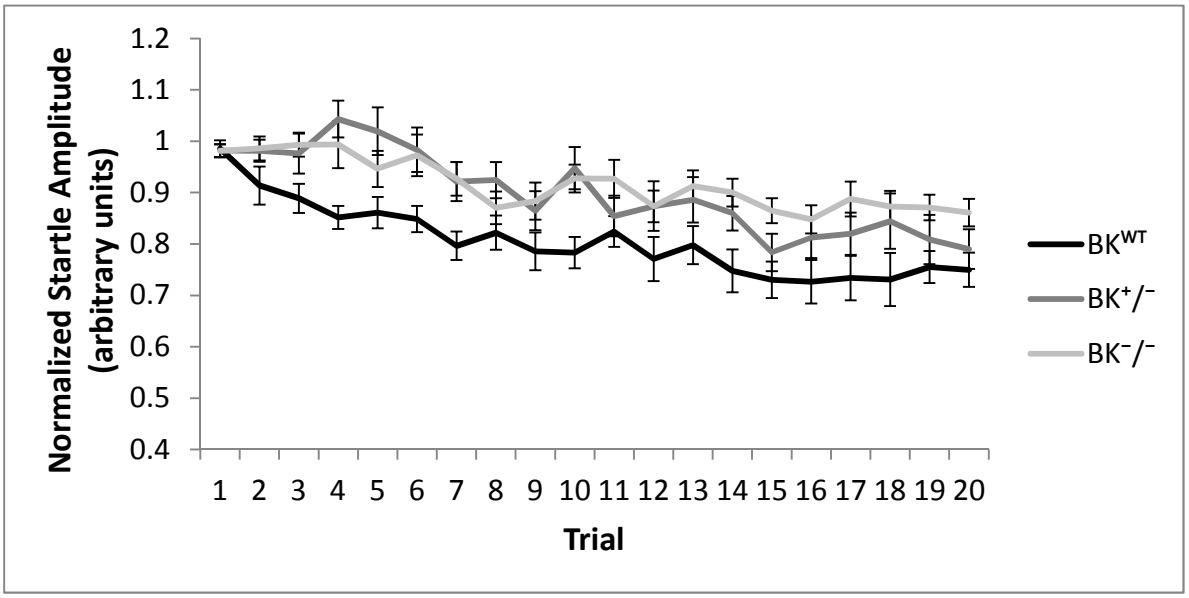


Figure 5.9 Short-term habituation of the acoustic startle response. $BK^{-/-}$ animals habituated to 86% of their initial three startle responses, while BK^{WT} and $BK^{+/-}$animals habituated to 75% and 79%, respectively. BK^{WT} animals showed significant attenuation of startle amplitudes compared to $BK^{-/-}$ animals. Bonferroni *post-hoc* analysis used to determine significance between genotypes. A main effect of trial was present [$F(19,950)=21.95$, $p<0.01$], as well as a main interaction of trial by genotype [$F(38,950)=1.61$, $p<0.05$]. No trial by genotype by gender interaction was present [$F(38,950)=1.18$, $p=0.22$]. No main effect of gender was present [$F(1,50)=0.29$, $p=0.60$]. BK^{WT} n=19, $BK^{+/-}$ n=17, $BK^{-/-}$ n=20.

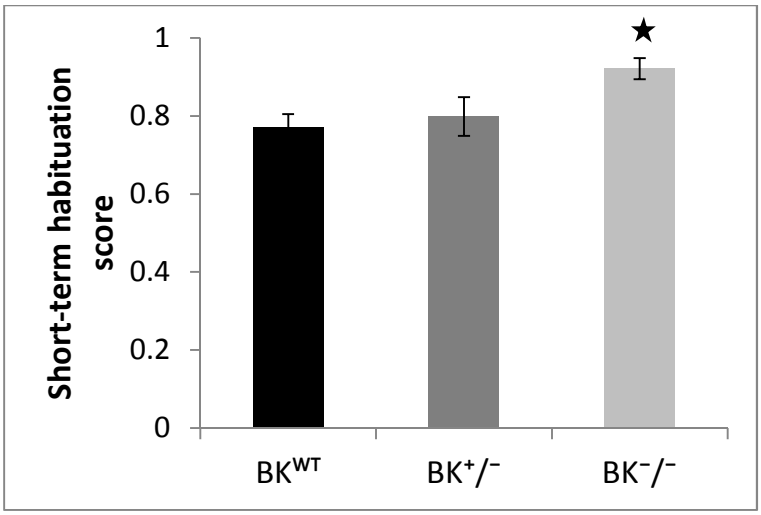


Figure 5.10 Short-term habituation scores of the last five startle responses vs. the first five startle responses. $BK^{-/-}$ animals showed significantly larger short term habituation scores compared to BK^{WT} and $BK^{+/-}$ animals. LSD *post-hoc* analysis was used to determine significance between genotypes. No main effect of gender was present [$F(1,47)=0.04$, $p=0.84$]. BK^{WT} n=18, $BK^{+/-}$ n=16, $BK^{-/-}$ n=19.

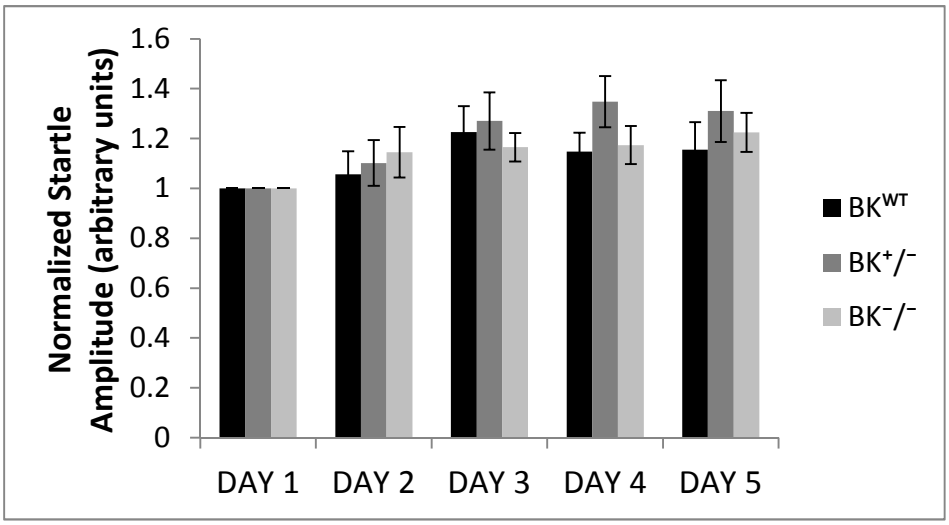
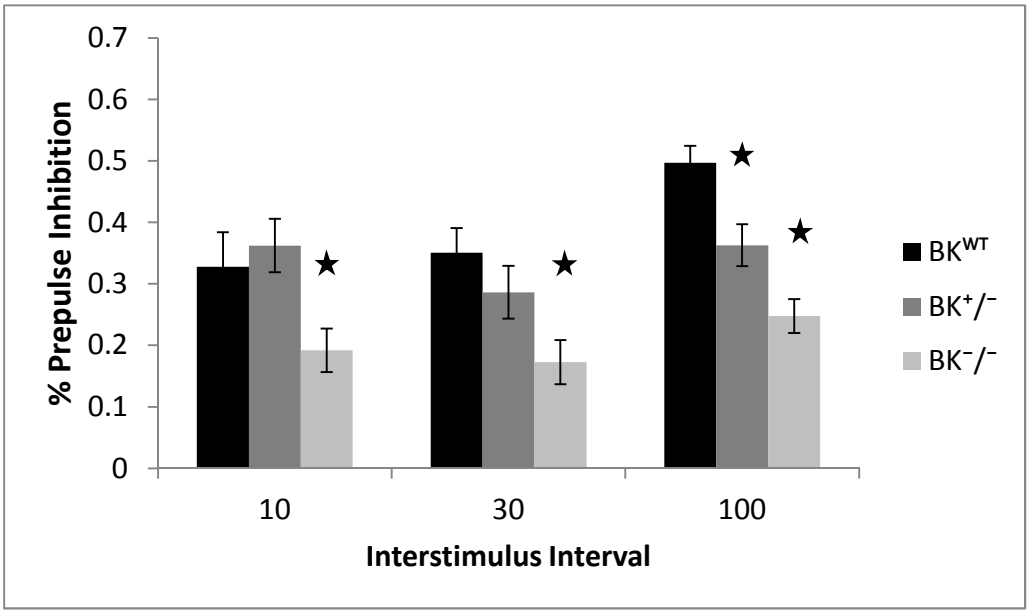


Figure 5.11 Long-term habituation of the acoustic startle response. Genotypes did not significantly differ overall across days. No day by genotype by gender interaction was present [$F(8,200)=0.24$, $p=0.98$]. No main effect of gender was present [$F(1,50)=0.87$, $p=0.36$]. BK^{WT} n=19, $BK^{+/-}$ n=17, $BK^{-/-}$ n=20.

Prepulse inhibition in $BK^{-/-}$ animals was significantly impaired compared to $BK^{+/-}$and BK^{WT} animals at 10 ms [BK^{WT} average=0.33 ± 0.06, $BK^{+/-}$ average=0.36 ± 0.04, $BK^{-/-}$ average=0.19 ± 0.03, ANOVA, $F(2,50)=3.42$, $p<0.05$], 30 ms [BK^{WT} average=0.35 ± 0.04, $BK^{+/-}$ average=0.29 ± 0.04, $BK^{-/-}$ average=0.17 ± 0.04, ANOVA, $F(2,50)=5.42$, $p<0.01$], and 100 ms [BK^{WT} average=0.50± 0.03, $BK^{+/-}$ average=0.36 ± 0.03, $BK^{-/-}$ average=0.25 ± 0.03, ANOVA, $F(2,50)=18.38$, $p<0.01$] interstimulus intervals with both the 75dB prepulse and the 85 dB prepulse [10 ms [BK^{WT} average=0.42 ± 0.03, $BK^{+/-}$ average=0.34 ± 0.03, $BK^{-/-}$ average=0.20 ± 0.04, ANOVA, $F(2,50)=10.91$, $p<0.01$], 30 ms [BK^{WT} average=0.42± 0.03, $BK^{+/-}$ average=0.34 ± 0.03, $BK^{-/-}$ average=0.20 ± 0.03, ANOVA, $F(2,50)=13.23$, $p<0.01$], and 100 ms [BK^{WT} average=0.57± 0.03, $BK^{+/-}$ average=0.52 ± 0.02, $BK^{-/-}$ average=0.33 ± 0.02, ANOVA, $F(2,50)=23.83$, $p<0.01$] (Figure 5.12)]. $BK^{+/-}$animals significantly differed from BK^{WT} animals at the 100 ms interstimulus level with the 75 dB prepulse [ANOVA, $F(2,50)=18.38$, $p<0.01$, Figure 5.12].

a)



b)

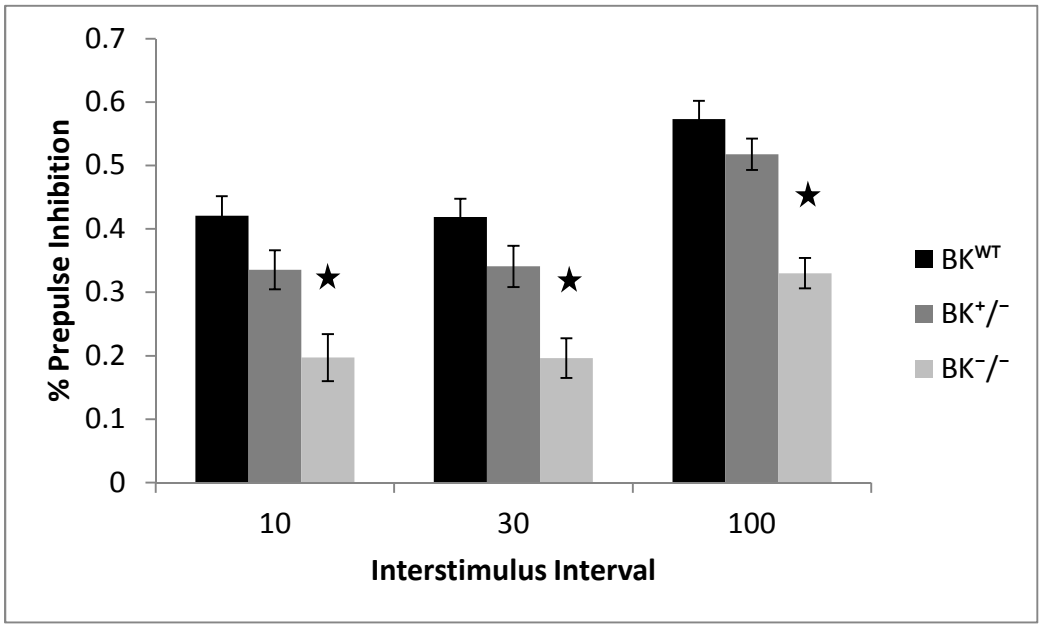


Figure 5.12 Percent prepulse inhibition of the acoustic startle response with 75dB prepulse (a) and 85 dB prepulse (b). a) Prepulse inhibition in $BK^{-/-}$ animals was significantly impaired compared to $BK^{+/-}$and BK^{WT} animals with the 10, 30, and 100 ms interstimulus intervals. $BK^{+/-}$ animals significantly differed from BK^{WT} animals in the 100 ms ISI level condition. LSD *Post-hoc* analysis used to determine significant differences between genotypes in each ISI level condition. No main effect of gender was present at the 10 [$F(1,50)=2.05$, $p=0.16$], 30 [$F(1,50)=0.19$, $p=0.67$] or 100 [$F(1,50)=3.21$, $p=0.08$] interstimulus intervals. b) Prepulse inhibition in $BK^{-/-}$ animals was significantly impaired compared to $BK^{+/-}$and BK^{WT} animals in the 10, 30, and 100 ms interstimulus intervals. LSD *post-hoc* analysis used to determine significant differences between genotypes in each ISI level condition. No main effect of gender was present at the 10 ($F(1,50)=0.55$,$p=0.46$), 30 ($F(1,50)=0.17$, $p=0.68$), or 100 ($F(1,50)=0.56$, $p=0.46$) interstimulus intervals. BK^{WT} n=19, $BK^{+/-}$ n=17, $BK^{-/-}$ n=20.

5.3 Effects of deficiency of the α-subunit of the BK channel in measures of higher cognitive function

We used the Y-maze to test for working memory. Genotypes did not significantly differ in terms of percent spontaneous alternation in the Y-maze [BK^{WT} average=52.16 ± 3.41, $BK^{+/-}$ average=51.95 ± 3.17, $BK^{-/-}$ average=53.89 ± 3.25, ANOVA, $F(2,38)=0.03$, $p=0.97$, Figure 5.13], indicating normal working memory function in $BK^{-/-}$ mice.

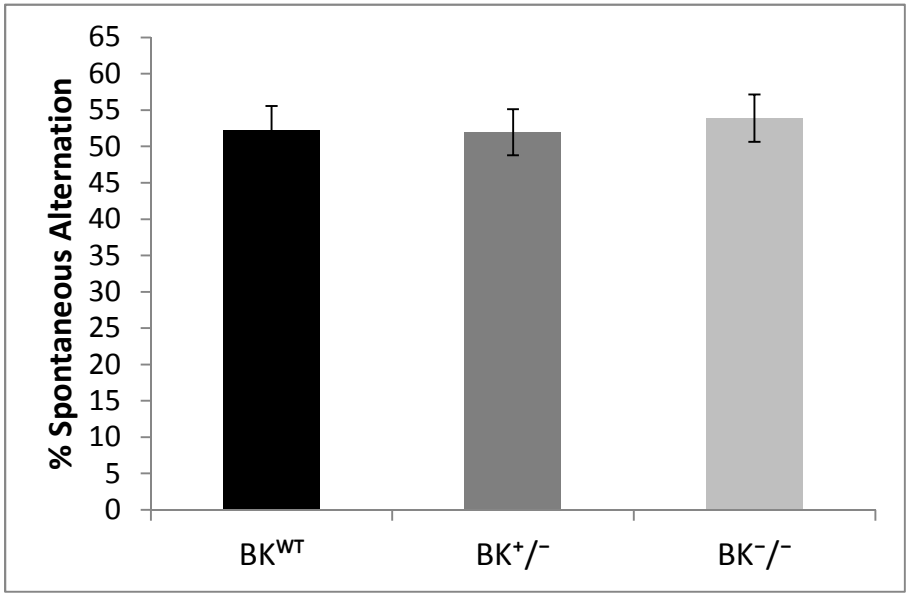


Figure 5.13 Percent spontaneous alternation in the Y-maze. Genotypes did not significantly differ in percentage of spontaneous alternation. No main effect of gender was present [$F(1,38)=2.12$, $p=0.15$]. BK^{WT} n=15, $BK^{+/-}$ n=15, $BK^{-/-}$ n=14.

Spatial learning and memory was tested in the Morris water maze. $BK^{-/-}$ animals showed a significantly longer distance [ANOVA, $F(2,35)=9.38$, $p<0.01$] as well as latency [ANOVA, $F(2,35)=14.18$, $p<0.01$] to reach the target in the Morris water maze compared to $BK^{+/-}$ and BK^{WT} animals (Figure 5.15), indicating slower learning of the task (see Figure 2.16 for learning curves from earlier vs. later days of training). Mean swimming speed was equal among genotypes [ANOVA, $F(2,36)=1.20$, $p=0.31$, Figure 5.14].

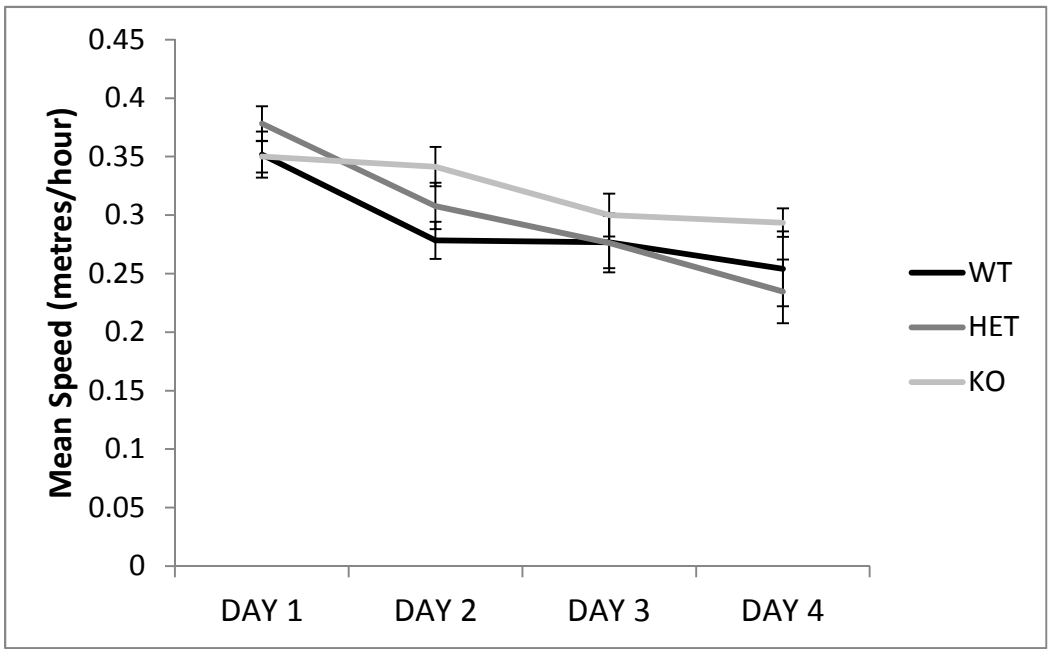
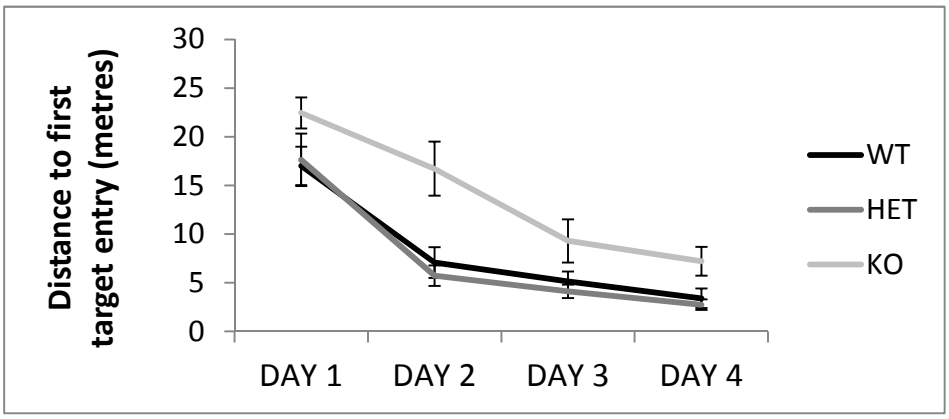


Figure 5.14 Mean swimming speed of four trials per day across four days in the Morris water maze. Genotypes did not significantly differ overall in terms of mean speed. A main effect of day was present [$F(3,108)=14.81$, $p<0.01$]. No day by genotype by gender interaction [$F(6,108)=0.37$, $p=0.89$] or main effect of gender [$F(1,36)=0.41$, $p=0.53$] was present. BK^{WT} n=14, $BK^{+/-}$ n=14, $BK^{-/-}$ n=14.

a)



b)

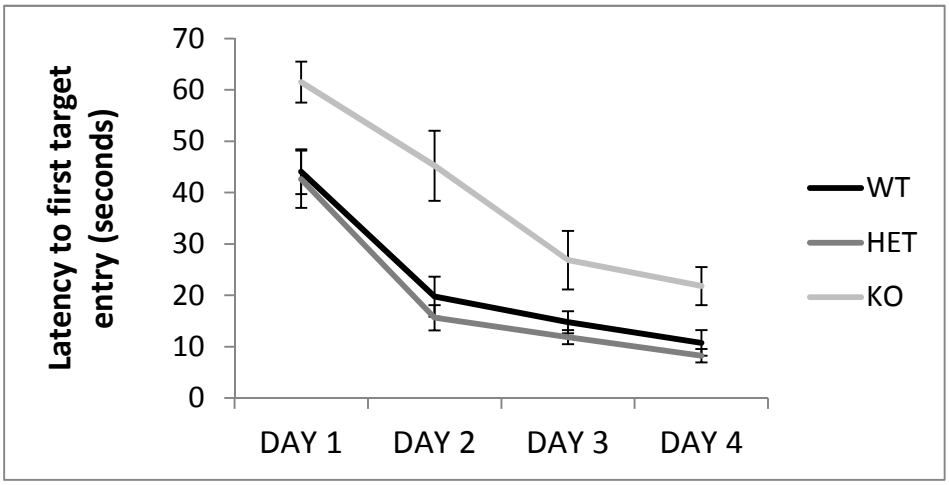
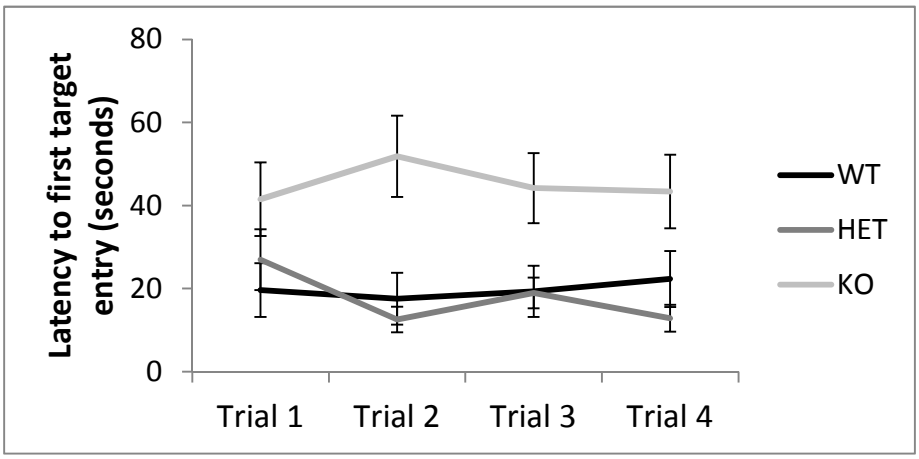


Figure 5.15 Average distance (a) and latency (b) to target of four trials per day across four days in the Morris water maze. a) $BK^{-/-}$ animals showed a significantly longer distance to reach the target compared to $BK^{+/-}$and BK^{WT} animals. LSD *post-hoc* analysis used to determine significant differences between genotypes. A main effect of day was present [$F(3,105)=43.17$, $p<0.01$]. No day by genotype by gender interaction [$F(6,105)=0.23$, $p=0.97$] or main effect of gender [$F(1,35)=0.01$, $p=0.91$] was present. BK^{WT} n=14, $BK^{+/-}$ n=13, $BK^{-/-}$ n=14. b) $BK^{-/-}$ animals showed a significantly longer latency to reach the target compared to $BK^{+/-}$and BK^{WT} animals. LSD *post-hoc* analysis used to determine significant differences between genotypes. A main effect of day was present [$F(3,105)=49.62$, $p<0.01$]. No day by genotype by gender interaction [$F(6,105)=0.13$, $p=0.99$] or main effect of gender [$F(1,35)=0.87$, $p=0.36$] was present. BK^{WT} n=14, $BK^{+/-}$ n=13, $BK^{-/-}$ n=14.

a)



b)

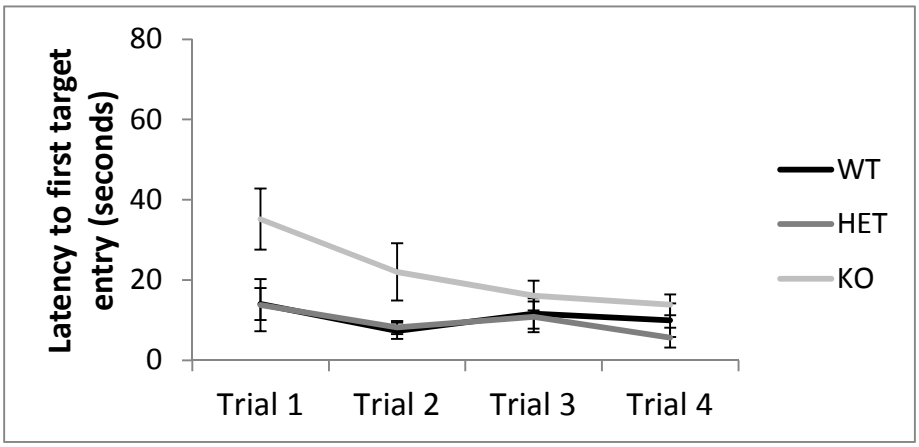


Figure 5.16 Learning curves shown in terms of latency to target across four trials on day 2 (a) and day 4 (b) of training in the Morris water maze. a) $BK^{-/-}$ animals showed a significantly longer latency to reach the target compared to $BK^{+/-}$and BK^{WT} animals [$F(2,36)=8.21$, $p<0.01$]. LSD *post-hoc* analysis used to determine significant differences between genotypes. No trial by genotype by gender interaction [$F(6,108)=1.82$, $p=0.10$] or main effect of gender [$F(1,36)=2.34$, $p=0.74$] was present. BK^{WT} n=14, $BK^{+/-}$ n=14, $BK^{-/-}$ n=14. b) $BK^{-/-}$ animals showed a significantly longer latency to reach the target compared to $BK^{+/-}$and BK^{WT} animals [$F(2,36)=5.82$, $p<0.01$]. LSD *post-hoc* analysis used to determine significant differences between genotypes. A main effect of trial was present [$F(3,108)=4.98$, $p<0.01$]. No trial by genotype by gender interaction [$F(6,108)=0.64$, $p=0.70$] or main effect of gender [$F(1,36)=0.53$, $p=0.47$] was present. BK^{WT} n=14, $BK^{+/-}$ n=14, $BK^{-/-}$ n=14.

$BK^{-/-}$ animals spent significantly more time in the target quadrant on probe trial day than $BK^{+/-}$ animals [BK^{WT} average=47.5 ± 4.51, $BK^{+/-}$ average=39.96 ± 2.87, $BK^{-/-}$ average=56.18 ± 4.90, ANOVA, $F(2,36)=3.34$, $p<0.05$, Figure 5.17], indicating that once they learned the task they had no deficits in memory and remembered the task. Genotypes did not differ in terms of percentage of time spent per quadrant in the opposite [$F(2,36)=2.31$, $p=0.11$], left [$F(2,36)=0.70$, $p=0.50$] or right [$F(2,36)= 3.20$, $p=0.053$] quadrant conditions.

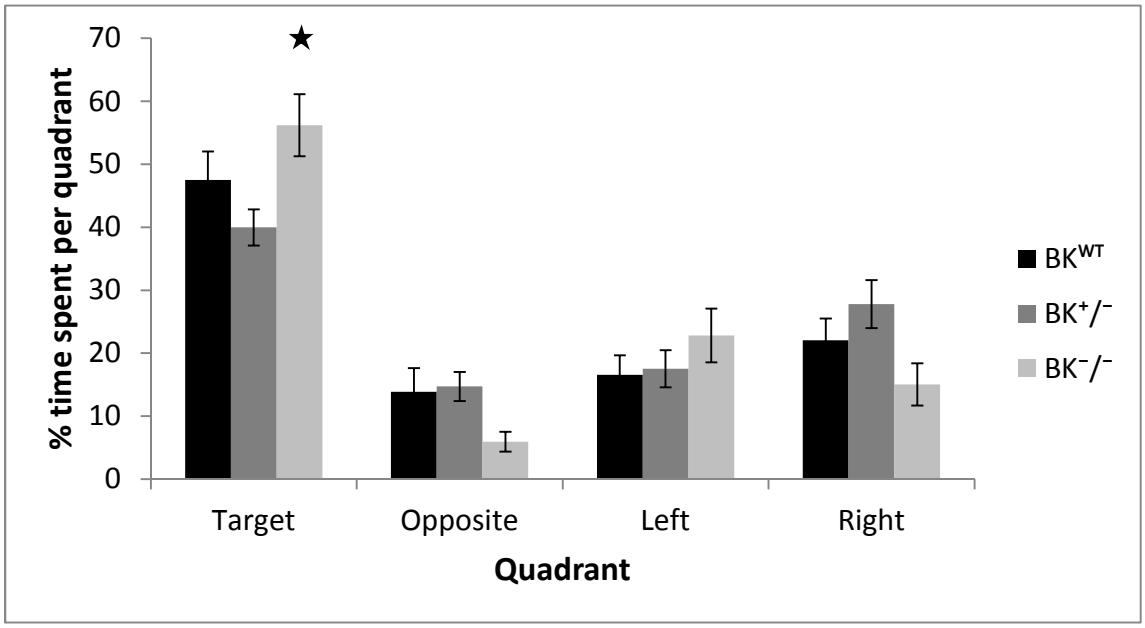


Figure 5.17 Percentage of time spent per quadrant of the Morris water maze on probe trial day. $BK^{-/-}$ animals spent significantly more time in the target quadrant than $BK^{+/-}$animals but did not differ from BK^{WT} animals. LSD *post-hoc* analysis used to determine significant differences between genotypes. Genotypes did not differ in terms of percentage of time spent per quadrant in the opposite, left, or right quadrants. No main effect of gender was present for the target [$F(1,36)=1.28$, $p=0.27$], opposite [$F(1,36)=1.54$, $p=0.22$], left [$F(1,36)=0.01$, $p=0.92$], or right [$F(1,36)=0.09$, $p=0.77$] quadrants. BK^{WT} n=14, $BK^{+/-}$ n=14, $BK^{-/-}$ n=14.

5.4 Effects of deficiency of the α-subunit of the BK channel on measures of anxiety-like behaviour

$BK^{-/-}$ animals spent significantly more time in the centre of the open field box than $BK^{+/-}$and BK^{WT} animals [ANOVA, $F(2,40)=5.98$, $p<0.01$, Figure 5.18], indicating a lower level of anxiety than their wild-type counterparts.

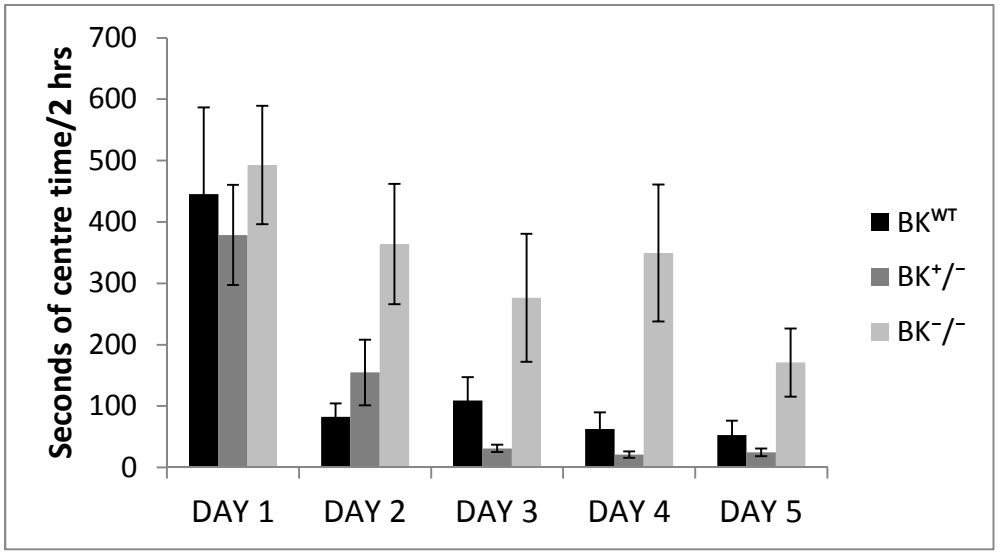


Figure 5.18 Amount of time spent in the centre of the open field box over a period of two hours across five days. $BK^{-/-}$ animals spent significantly more time in the centre than $BK^{+/-}$and BK^{WT} animals. Bonferroni *post-hoc* analysis used to determine significant differences between genotypes. A main day by gender interaction was present [$F(1,40)=6.91$, $p<0.01$], as was a main effect of gender [$F(1,40)=8.36$, $p<0.01$]. No day by genotype by gender interaction [$F(8,160)=0.53$, $p=0.83$] was present. BK^{WT} n=15, $BK^{+/-}$ n=15, $BK^{-/-}$ n=16.

In order to test for differences in anxiety levels, animals were tested in two classical anxiety paradigms, the elevated plus maze and the light/dark boxes. Number of open arm entries in the elevated plus maze was higher in $BK^{-/-}$ animals compared to BK^{WT} animals [BK^{WT} average=4.82 ± 0.74, $BK^{+/-}$ average=8.09 ± 1.38, $BK^{-/-}$ average=11.5 ± 1.63, ANOVA, $F(2,26)=6.06$, $p<0.01$, Figure 5.19]. Closed vs. open arm time ratios in the elevated plus maze were significantly lower in $BK^{-/-}$ animals than in BK^{WT} animals [BK^{WT} average=23.23 ± 7.0, $BK^{+/-}$ average=12.56 ± 4.38, $BK^{-/-}$ average=1.45 ± 0.23, ANOVA, $F(2,25)=8.32$, $p<0.01$, Figure 5.20], confirming potential lower anxiety levels in $BK^{-/-}$ mice.

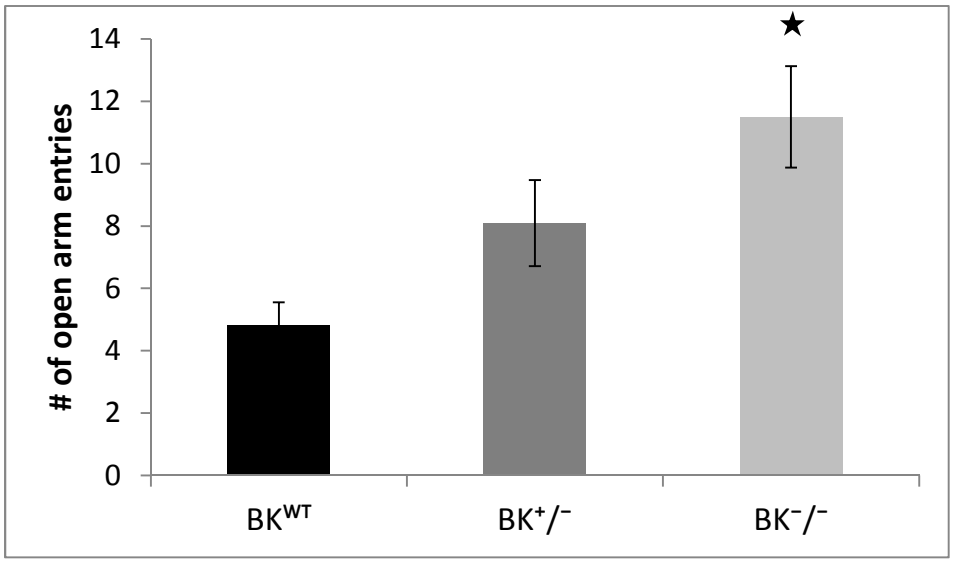


Figure 5.19 Sum of open arm entries in the elevated plus maze. Number of open arm entries was significantly higher in $BK^{-/-}$ animals compared to BK^{WT} animals. Bonferroni *post-hoc* analysis used to determine significant differences between genotypes. No main effect of gender was present [$F(1,26)=1.27$, $p=0.27$]. BK^{WT} n=11, $BK^{+/-}$ n=11, $BK^{-/-}$ n=10.

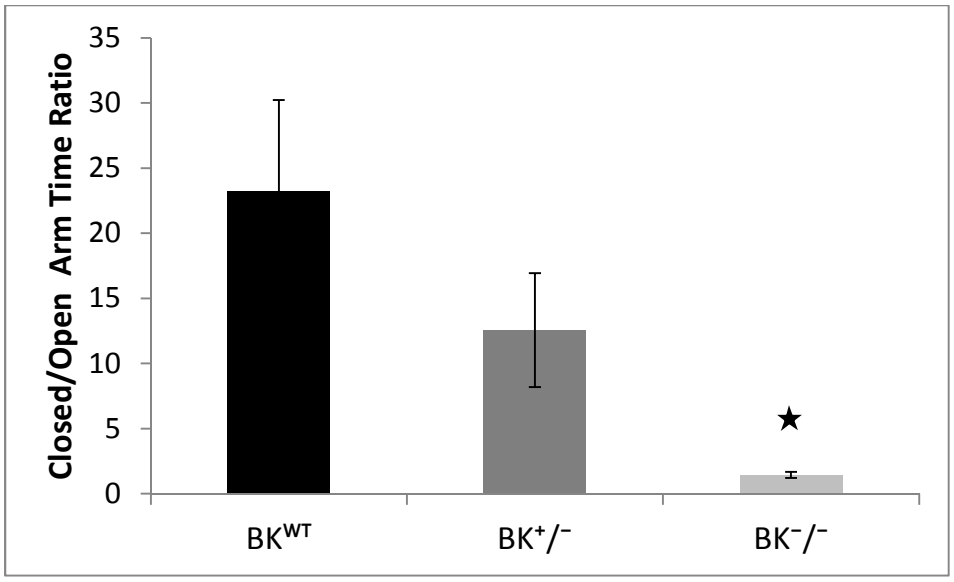
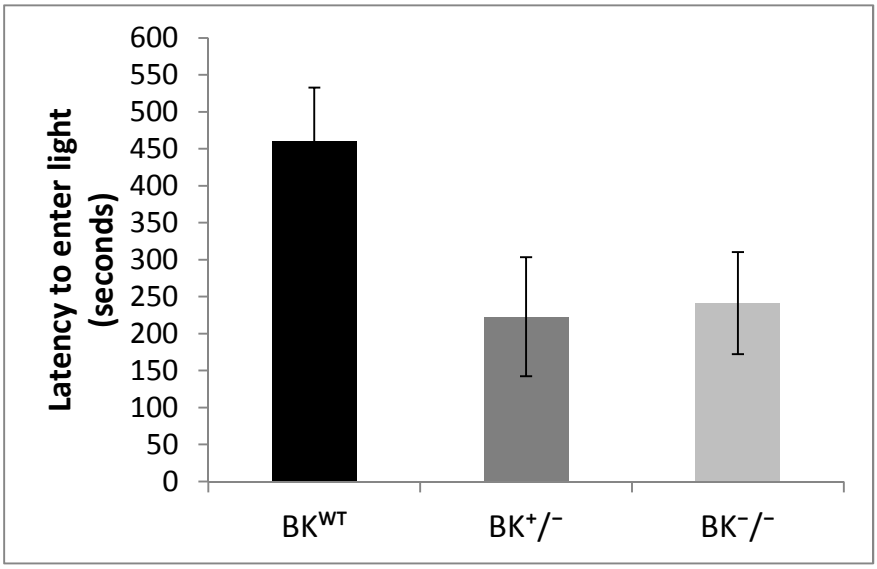


Figure 5.20 Time spent in closed vs. open arms of the elevated plus maze. Closed vs. open arm time ratio was significantly lower in $BK^{-/-}$ animals than in BK^{WT} animals. Bonferroni *post-hoc* analysis was used to determine significant differences between genotypes. A main effect of gender was present [$F(1,25)=8.30$, $p<0.01$]. BK^{WT} n=11, $BK^{+/-}$ n=10, $BK^{-/-}$ n=10.

In the light/dark boxes, latency to enter the light section from starting position in dark section [BK^{WT} average=460.38 ± 72.56, $BK^{+/-}$ average=222.78 ± 80.61, $BK^{-/-}$ average=241.2 ± 69.09, ANOVA, $F(2,30)=2.26$, $p=0.12$] as well as duration of time spent in the light section [BK^{WT} average=4.91 ± 1.69, $BK^{+/-}$ average=31.75 ± 10.08, $BK^{-/-}$ average=36.5 ± 11.46, ANOVA, $F(2,30)=2.76$, $p=0.08$] was not significantly different between genotypes (Figure 5.21). Light vs. dark time ratios also did not significantly differ between genotypes [BK^{WT} average=0.01 ± 0.003, $BK^{+/-}$ average=0.06 ± 0.02, $BK^{-/-}$ average=0.07 ± 0.02, ANOVA, $F(2,30)=2.59$, $p=0.09$, Figure 5.22], which does not support a difference in anxiety levels between genotypes.

a)



b)

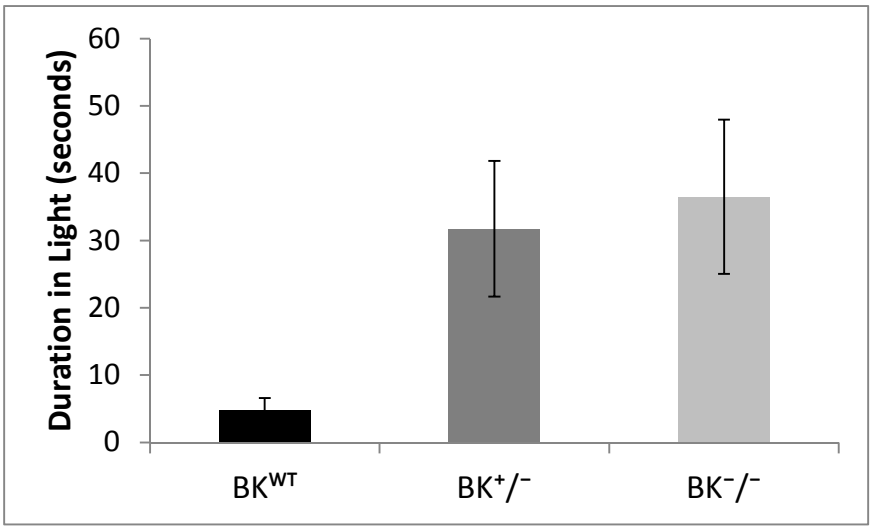


Figure 5.21 Latency to enter the light section (a) and duration spent in light section (b) of the light/dark box. a) Latency to enter the light section from starting position in dark section was not significantly different between genotypes. No main effect of gender was present [$F(1,30)=0.05$, $p=0.82$]. BK^{WT} n=12, $BK^{+/-}$ n=12, $BK^{-/-}$ n=12. b) Duration of time spent in the light section did not differ significantly between genotypes. No main effect of gender was present [$F(1,30)=0.01$, $p=0.93$]. BK^{WT} n=12, $BK^{+/-}$ n=12, $BK^{-/-}$ n=12.

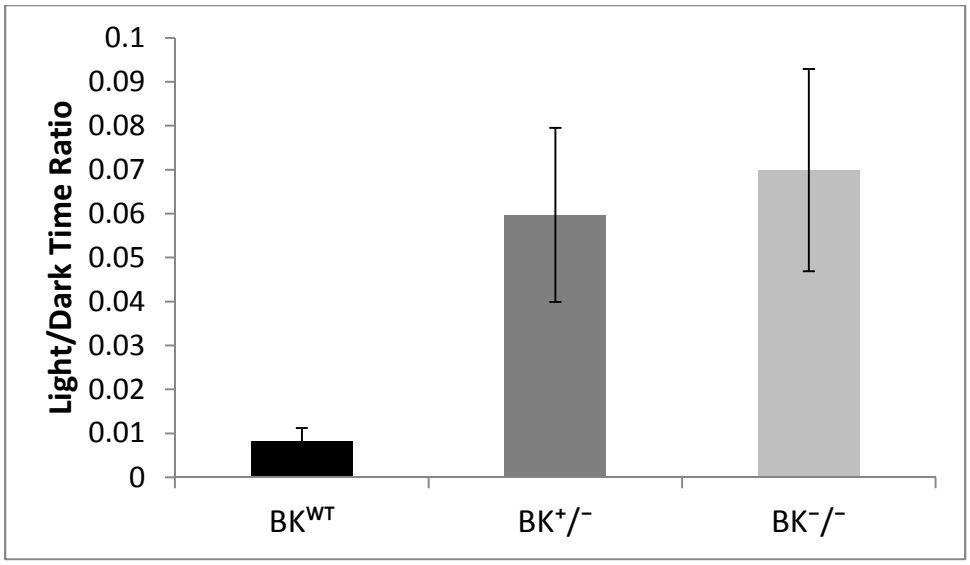


Figure 5.22 Time spent in the light section vs. the dark section of the light/dark box. Light vs. dark time ratios did not significantly differ between genotypes. No main effect of gender was present [$F(1,30)=0.002$, $p=0.97$]. BK^{WT} n=12, $BK^{+/-}$ n=12, $BK^{-/-}$ n=12.

6 Discussion

The results of this research project aim to aid in the further understanding of some of the various physiological roles of BK channels, as investigated in BK channel α subunit homozygous and heterozygous knock-out mice. The work presented here showed that homozygous knock-out animals displayed significantly reduced forelimb grip force, significantly impaired inter-limb coordination, as well as significantly shorter stride lengths in front and hind paws as compared to heterozygous and wild-type animals. However, even with these motor function deficits being present, homozygous knock-out animals were observed to be no less active compared to heterozygous and wild-type animals, as measured in the open field box where no significant differences were found among genotypes in terms of the total distance travelled over a two hour period, the sum of total activity occurring over a two period, as well as the recorded number of rearing movement occurrences. Additionally, homozygous knock-out animals' activity was observed as being comparable to that of heterozygous and wild-type animals in the Morris water maze, as the analysis of mean swimming speeds revealed no significant difference among genotypes. Measures of sensorimotor gating mechanisms showed that homozygous animals displayed significantly less attenuation of startle responses compared to wild-type animals across 100 trials in response to repetitive acoustic stimulation, as well as significantly larger short-term habituation scores compared to heterozygous and wild-type animals, indicating that homozygous knock-out animals did not habituate (short-term). Long-term habituation across days of testing was not significantly different among genotypes, and in fact none of the genotypes were observed to display long-term habituation. Furthermore, measures of prepulse inhibition revealed significantly less percent inhibition in homozygous knock-out animals compared to heterozygous and wild-type animals with both prepulse intensities used (75 and 85 dB), at all three interstimulus intervals tested (10, 30, and 100 ms), indicating impairment of sensorimotor gating in this measure in addition to deficits in short-term habituation. Analysis of higher cognitive function included measures of spatial acquisition of a task, spatial memory, and working memory. Homozygous knock-out animals displayed both a significantly longer latency as well as distance to reach the target (platform) in the Morris water maze compared to

heterozygous and wild-type animals, suggesting that these animals were slower to acquire and learn the task. When tested immediately following four days of training in the Morris water maze, homozygous knock-out animals spent significantly more time in the target quadrant than heterozygous animals but did not differ from wild-type animals, showing that once the task had been acquired, homozygous knock-out animals were able to remember it and did not display deficits in spatial reference memory. There was no significant difference among genotypes in terms of percent spontaneous alternation in the Y-maze, indicating normal working memory function in homozygous knock-out mice. Assessment of anxiety-like behaviour revealed a lower level of anxiety in homozygous knock-out animals, as measured in the open field box where homozygous knock-out animals spent significantly more time in the centre of the box than heterozygous and wild-type animals, as well as in the elevated plus maze where homozygous knock-out animals were recorded to have significantly more open arm entries and significantly lower closed vs. open arm time ratios compared to wild-type animals. However, no dissimilarity in anxiety levels was found as measured in light/dark boxes, where latency to enter the light section, duration of time spent in the light section, as well as light vs. dark time ratios did not significantly differ among genotypes. Outliers for all behavioural data were determined using a range of two standard deviations from the mean and were removed from the respective data set. Each data set for each behavioural test was treated individually and any outliers determined for a particular data set did not affect these animals' probability of being an outlier in a different data set. In other words, if an outlier was removed from one data set, this same animal may be present in another data set, provided that this animal was not determined as being an outlier in this subsequent data set.

6.1 Choice of mouse model

BK channels have been implicated to be of importance in auditory processing and in the development of normal hearing function (Kurt et al., 2012; Ruttiger et al., 2004). Former research with BK channel knock-out mice using an SV129 mouse line has reported these animals

to have hearing deficits (Ruttiger et al., 2004). Hearing function measurements have also been conducted on F1 generation hybrid α subunit homozygous knock-out and control mice, generated by mating heterozygous C57BL6 and SV129 mice, which is strictly relevant and crucial to this research project as this genetic mouse model was used for the current research project. Auditory brainstem responses revealed that hearing thresholds were not significantly different between control and knock-out mice, as was true for tone-evoked ABR hearing thresholds for low and middle frequency ranges of hearing (Unpublished data, personal communication Lukas Ruettiger). This data indicates that F1 generation hybrid BK channel knock-out mice generated by mating heterozygous C57BL6 and SV129 mice have no reported hearing deficits, allowing these animals to be used in behavioural tests requiring normal hearing function, and specifically to this research, in measures of sensorimotor gating of the acoustic startle reflex.

The results of this study showed that the homozygous *Slo1* knock-out mice used here displayed an abnormal motor function phenotype as seen through reduced forelimb grip force and impairment in inter-limb coordination as well as in stride length, confirming a physiological role for BK channels in the control of movement. This finding has also already been established through previous research with mice lacking the α subunit of the BK channel that were bred on a C57BL6 and SV129 background (Sausbier et al., 2004). Surprisingly, despite these motor function deficits being present, homozygous knock-out animals were observed to be no less active at both walking and swimming than heterozygous or wild-type animals, indicating normal locomotion and allowing for all three genotypes to be subjected to and compared in a multitude of behavioural tests requiring movement. In summary, the control experiments on hearing, motor function, and locomotion indicate that the hybrid F1 BK channel knock-out mouse used in this study is a suitable model for testing sensorimotor function and learning and memory performance.

6.2 Sensorimotor gating

Findings from previous research with *Aplysia* (Castellucci et al., 1970), *Drosophila* (Engel and Wu, 1998), and *Caenorhabditis elegans* (C. Rankin, personal communication), had suggested short-term habituation to be mediated through a presynaptic mechanism involving synaptic depression and reduced neurotransmitter release and had proposed the BK channel to be a key player in the mediation of this process. The results of the current research project have indicated that *Slo1* homozygous knock-out mice lack short-term habituation, as seen through these animals' less attenuation of startle responses compared to wild-type animals in response to repetitive acoustic stimulation, as well as larger short-term habituation scores compared to wild-type and heterozygous animals. Of potential importance to this research is the anxiety level of the animal subjects, which could possibly influence the animals' performance during behavioural testing. The results of this work have suggested a novel finding in that α subunit homozygous knock-out mice may have lower levels of anxiety than their heterozygous and wild-type counterparts. However, these results have been inconclusive; measures of anxiety as shown through open field box and elevated plus maze testing have suggested knock-out animals to be less anxious, while light/dark testing did not reveal a difference in anxiety levels among genotypes. According to the dual-process theory by Groves and Thompson (1970), the processes of habituation and sensitization interact with one another and together yield the net behavioural outcome in response to repetitive stimulus presentation. Sensitization of the startle reflex has been investigated in studies of anxiety and appears to be pronounced by anxiety-evoking factors such as fear and stress (Liang et al., 1992). Accordingly, the sensorimotor gating mechanism of habituation is potentially occluded by the simultaneous process of sensitization and thus by anxiety-evoking factors. The results of this research have shown that homozygous knock-out animals displayed less habituation, which could generally mean that these mice might just display stronger sensitization overlying the habituation. However, homozygous knock-out animals were observed to display lower levels of anxiety. Therefore, it is not feasible that the lack of startle attenuation during repeated testing is due to increased sensitization, as the less anxious knock-out animals should show less sensitization. Instead, the results indicate that short-term habituation of startle is mediated by the BK channel, confirming findings of impaired

habituation previously shown in *C. elegans* and *drosophila Slo1* knock-out animals. It further suggests that short-term habituation is mediated through a highly conserved mechanism.

Ca^{2+} - and voltage-activated potassium (BK) channels have been implicated to be physiologically important in the control of neuronal excitability and neurotransmitter release (Salkoff et al., 2006; Faber and Sah, 2003; Wang, 2008), as well as in the modulation of changes in synaptic efficacy such as synaptic depression (Robitaille and Charlton, 1992; Zucker 1989). Additionally, BK channels have been proposed to be found most abundantly in synaptic terminals in many types of neurons (Knaus et al., 1996; Salkoff et al., 2006; Hu et al., 2010; Wang et al., 2001). Accordingly, research on the neuronal circuitry underlying short-term habituation of the acoustic startle response has found this process to be mediated intrinsically by the acoustic startle pathway itself and specifically at the synapse between the cochlear root neurons and the PnC giant neurons (Davis et al, 1982b). Further investigation revealed that electrical stimulation of these auditory afferents induced synaptic depression in the synapse with PnC giant neurons (Weber et al., 2002; Pilz et al., 2004). Additionally, the mechanism responsible for the synaptic depression underlying this process has been suggested to be localized presynaptically (Simons-Weidenmaier et al., 2006), confirming short-term habituation as being mediated along the sensory pathways to the PnC. This study therefore strongly supports the hypothesis that activation of BK channels that are located at the pre-synaptic sensory terminals in the PnC mediate synaptic depression underlying short-term habituation of startle. Taken together, the BK channel seems to fulfill the requirements for the highly conserved mechanism mediating short-term habituation, and thus appears to be a well-suited candidate to explain the cellular and molecular mechanisms underlying this behavioural process, which have been relatively unknown thus far. The results of this research have also yielded measures of prepulse inhibition of the acoustic startle response to be impaired in homozygous knock-out animals compared to heterozygous and wild-type animals with both prepulse intensities used (75 and 85 dB), at all three interstimulus intervals tested (10, 30, and 100 ms). This novel finding indicates that BK channels may possibly function to mediate mechanisms of sensorimotor gating not only in terms of short-term habituation, but also in prepulse inhibition. The neuronal circuitry underlying prepulse inhibition of the acoustic startle response is comprised of several midbrain structures,

and this pathway is thought to run parallel to the primary acoustic startle pathway, providing feed-forward inhibition from the PPTg onto the giant neurons in the PnC. Additionally, the PPTg itself is thought to be modulated by several other brain regions, such as the ventral pallidum, nucleus accumbens, hippocampus, and amygdala (Koch, 1999; Yeomans et al., 2010; Fendt et al., 2001). BK channels have been suggested to be expressed in many structures within the brain where they are regulators of neuronal excitability and neurotransmitter release (Salkoff et al., 2006; Faber and Sah, 2003; Wang, 2008; Wang et al., 2001), including several structures implicated in modulating PPI. In contrast to the role of BK channels in short-term habituation, where there is a clear hypothetical mechanistic model in place, it is impossible to hypothesize at this point how BK channels modulate PPI. Future research will need to focus on localizing where in the proposed PPI circuitry BK channels are affecting this mechanism of sensorimotor gating.

6.3 Higher cognitive function

The work presented here has shown that *Slo1* homozygous knock-out mice displayed both a longer latency as well as distance to reach the target platform in the Morris water maze compared to heterozygous and wild-type animals, suggesting that these animals were slower to learn the task and therefore have impaired task acquisition processes. These results are supported by findings from previous research whereby BK channels in the CA1 region of the hippocampus of rats were blocked with paxilline, a BK channel blocker, and these animals were subsequently trained to perform a trace eyeblink conditioning learning task. These animals displayed slower acquisition of the task compared to control animals (Matthews and Disterhoft, 2009). However, these animals were able to remember the task after eventually learning it, suggesting that hippocampal-dependent learning was not completely abolished, but impaired. Similarly, the results of the current research project showed that *Slo1* homozygous knock-out mice were able to remember the correct location of the target platform when tested after four days of training as well as wild-type animals, suggesting that while acquisition of the task was impaired, spatial

reference memory in these animals was intact. Additionally, assessment of working memory in these animals revealed no differences between genotypes, suggesting normal working memory function in *Slo1* homozygous knock-out mice. Taken together, a role for BK channels in the acquisition of a task has been indicated through the results of this research project, and this is supported by results found through previous research. It should be noted however that assessment of anxiety-like behaviour suggested a lower level of anxiety in homozygous knock-out animals, which is a potential confounding variable as spatial learning in the Morris water maze is aversively motivated and lower levels of stress and anxiety may result in impaired task performance (D'Hooge and De Deyn, 2001). BK channels have been suggested to be important regulators of neuronal excitability as well as playing a role in contributing to the fast phase of the afterhyperpolarization (AHP) potential (Salkoff et al., 2006; Faber and Sah, 2003), and in controlling neurotransmitter release (Wang, 2008), which is a potential mechanism through which BK channels may mediate synaptic plasticity and thereby task acquisition processes. The blocking of BK channels has been shown to result in increased neuronal excitability and firing rate in pyramidal neurons, resulting in excessive excitability and neuronal noise and this in turn may be responsible for disrupting acquisition processes and therefore result in impaired hippocampal-dependent learning of an eyeblink conditioning task (Matthews and Disterhoft, 2009). This suggests that normal BK channel activity is necessary for acquisition of this task, and since functional BK channels are also necessary for the acquisition of a spatial learning task, as suggested by the results of the current research project, these channels may be mediating the learning of tasks in a similar way. Spatial learning in the Morris water maze depends not only on the hippocampus, but also on several other brain regions including the cerebellum, which interact with one another and with neurotransmitter systems to form a network that underlies the learning process (D'Hooge and De Deyn, 2001). BK channels have have been suggested as playing important roles in controlling neurotransmitter release (Wang, 2008), and have also been shown to have high levels of expression in both the hippocampus and cerebellum in mice (Sausbier et al., 2006). Therefore, BK channels may be mediating spatial learning processes by regulating neuronal excitability and neurotransmitter release, and future research is needed in order to localize where this effect is being mediated.

6.4 Implications

The results of this research project suggest an important role for BK channels in sensorimotor gating mechanisms, as well as in spatial learning processes, indicating that BK channels have physiological roles in both basic and higher cognitive function. Deficits in sensorimotor gating result in information overload received by the brain, which is present in many neurological disorders such as fragile X syndrome (FXS, Frankland et al., 2004), autistic disorder (AD) (Perry et al., 2007), and schizophrenia (Geyer and Braff, 1982, Braff et al., 1978). Additionally, BK channels have been found to be involved in the molecular basis of FXS through regulation of normal action potential duration (Deng et al., 2013), as well as being associated with autism due to lower levels of expression of the gene coding for the pore forming α subunit of the BK channel (Laumonnier et al., 2006), and have also been suggested to be part of the etiology of schizophrenia (Zhang et al., 2006). Cognitive deficits observed in neurological disorders are believed to be at least in part due to deficits in sensorimotor gating mechanisms (Braff and Light, 2004; Perry et al., 1999; Singer et al., 2013). Therefore if the BK channel is indeed playing a role in maintaining efficient sensory gating, as has been suggested by the results of this research project, then it may also be exerting effects on higher cognitive function through these mechanisms, in addition to already contributing to the etiology of a number of neurological disorders. However, further research is required to understand to what extent the disruption of higher cognitive function is caused by sensory gating disruptions or rather by an independent pathology. Thus, functional BK channels seem to be of great importance in basic and higher cognitive processes, and therefore seem to be a useful pharmaceutical target aimed at enhancing these processes.

7 References

Akhondzadeh, S., et al. (2002). Diazoxide in the treatment of schizophrenia: novel application of potassium channel openers in the treatment of schizophrenia. *J Clin Pharm Ther*, 27 pp.453-459.

Atkinson, N., et al. (1991). A component of calcium-activated potassium channels encoded by the Drosophila slo locus . *Science*, 253 (5019), pp.551-555.

Bailey, C., and Chen, M. (1988). Morphological basis of short-term habituation in Aplysia. *J Neurosci*, 8 (7), pp.2452-2459.

Braff, D., and Geyer, M. (1990). Sensorimotor gating and schizophrenia: human and animal model studies. *Arch Gen Psychiatry*, 47 (2), pp.181-188.

Braff, D., and Light, G. (2004). Preattentional and attentional cognitive deficits as targets for treating schizophrenia. *Psychopharmacology*, 174 pp.75-85.

Braff, D., et al. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics . *Psychophysiology*, 15 (4), pp.339-343.

Butler, A., et al. (1993). mSlo, a complex mouse gene encoding "maxi" calcium-activated potassium channels. *Science*, 261 pp.221-224.

Carre-Pierrat, M., et al. (2006). The slo-1 BK channel of Caenorhabditis elegans is critical for muscle function and is involved in dystrophin-dependent muscle dystrophy. *J Mol Biol*, 358 pp.387-395.

Castellanos, F., et al. (1996). Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. *Biol Psychiatry*, 39 pp.33-41.

Castellucci, V., and Kandel, E. (1974). A quantal analysis of the synaptic depression underlying habituation of the gill-withdrawal reflex in Aplysia. *Proc Natl Acad Sci U S A* , 71 (12), pp.5004-5008.

Castellucci, V., et al. (1970). Neuronal mechanisms of habituation and dishabituation of the gill-withdrawal reflex in Aplysia. *Science*, 167 (3926), pp.1745-1748.

Cui, J., et al. (2009). Molecular mechanisms of BK channel activation. *Cell Mol Life Sci*, 66 pp.852-875.

Davis, M., et al. (1982a). A primary acoustic startle circuit: lesion and stimulation studies. *J Neurosci*, 2 (6), pp.791-805.

Davis, M., et al. (1982b). Habituation and sensitization of startle reflexes elicited electrically from the brainstem. *Science* , 218 (4573), pp.688-690.

Deng, P., et al. (2013). FMRP regulates neurotransmitter release and synaptic information transmission by modulating action potential duration via BK channels. *Neuron*, 77 pp.696-711.

D'hooge, R. and De Deyn, P. (2001) Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev*, 36 p.60-90.

Ellenbroek, B. (2004). Pre-attentive processing and schizophrenia: animal studies. *Psychopharmacology*,

174 pp.65-74.

Engel, J., and Wu, C. (1998). Genetic dissection of functional contributions of specific potassium channel subunits in habituation of an escape circuit in Drosophila. *J Neurosci*, 18 (6), pp.2254-2267.

Faber, E., and Sah, P. (2003). Calcium-activated potassium channels: multiple contributions to neuronal function. *Neuroscientist*, 9 pp.181-194.

Fendt, M., et al. (2001). Brain stem circuits mediating prepulse inhibition of the startle reflex. *Psychopharmacology*, 156 pp.216-224.

Fleshler, M. (1965). Adequate acoustic stimulus for startle reaction in the rat . *J Comp Physiol Psychol* , 60 (2), pp.200-207.

Frankland, P., et al. (2004). Sensorimotor gating abnormalities in young males with fragile X syndrome and Fmr1-knock-out mice. *Mol Psychiatry*, 9 pp.417-425.

Geyer, M., and Braff, D. (1982). Habituation of the blink reflex in normals and schizophrenic patients. *Psychophysiology*, 19 (1), pp.1-6.

Graham, F. (Presidential Address, 1975). The more or less startling effects of weak prestimulation. *Psychophysiology*, 12 (3), pp.238-248.

Gribkoff, V., et al. (2001). Maxi-K potassium channels: form, function, and modulation of a class of endogenous regulators of intracellular calcium. *Neuroscientist*, 7 (2), pp.166-177.

Groves, P., and Thompson, R. (1970). Habituation: a dual-process theory. *Psychol Rev*, 77 (5), pp.419-450.

Hu, H., et al. (2001). Presynaptic Ca2+ -activated K+ channels in glutamatergic hippocampal terminals and their role in spike repolarization and regulation of transmitter release. *J Neurosci*, 21 (24), pp.9585-9597.

Imlach, W., et al. (2008). The molecular mechanism of "ryegrass staggers," a neurological disorder of K+ channels. *J Pharm Exp Ther*, 327 (3), pp.657-664.

Ison, J., and Hammond, G. (1971). Modification of the startle reflex in the rat by changes in the auditory and visual environments. *J Comp Physiol Psychol* , 75 (3), pp.435-452.

Jones, E., et al. (1999). The role of Ca2+ -activated K+ channel spliced variants in the tonotopic organization of the turtle cochlea . *J Physiol*, 518 (3), pp.653-665.

Kaczorowski, G., et al. (1996). High-conductance calcium-activated potassium channels; structure, pharmacology, and function. 28 (3), pp.255-267.

Knaus, H., et al. (1994). Pharmacology and structure of high conductance calcium-activated potassium channels. *Cell Signal*, 6 (8), pp.861-870.

Knaus, H., et al. (1996). Distribution of high-conductance Ca2+ -activated K+ channels in rat brain: targeting to axons and nerve terminals. *J Neurosci*, 16 (3), pp.955-963.

Koch, M. (1999). The neurobiology of startle. *Prog Neurobiol*, 59 pp.107-128.

Koch, M., et al. (1992). Loss of the acoustic startle response following neurotoxic lesions of the caudal pontine reticular formation: possible role of giant neurons. *Neuroscience*, 49 (3), pp.617-625.

Koval, O., et al. (2007). A role for the S0 transmembrane segment in voltage-dependent gating of BK channels. *J Gen Physiol*, 129 pp.209-220.

Kurt, S., et al. (2012). Critical role for cochlear hair cell BK channels for coding the temporal structure and dynamic range of auditory information for central auditory processing. *FASEB J*, 26 (9), pp.3834-3843.

Landis, C., and Hunt, W. (1939). *The startle pattern*. Oxford: Farrar and Rinehart.

Laumonnier, F., et al. (2006). Association of a functional deficit of the BKCa channel, a synaptic regulator of neuronal excitability, with autism and mental retardation. *Am J Psychiatry*, 163 pp.1622-1629.

Leaton, R., et al. (1985). Short-term and long-term habituation of the acoustic startle response in chronic decerebrate rats. *Behav Neurosci*, 99 (5), pp.901-912.

Lee, U., and Cui, J. (2010). BK channel activation: structural and functional insights. *Trends Neurosci*, 33 (9), pp.415-423.

Lee, Y., et al. (1996). A primary acoustic startle pathway: obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis. *J Neurosci*, 16 (11), pp.3775-3789.

Leitner, D., et al. (1981). Midbrain reticular formation involvement in the inhibition of acoustic startle. *Physiol Behav*, 26 (2), pp.259-268.

Liang, K., et al. (1992) Corticotropin-releasing factor: long-lasting facilitation of the acoustic startle reflex. *J Neurosci*, 12 (6), p.2303-2312.

Lingenhohl, K., and Friauf, E. (1994). Giant neurons in the rat reticular formation: a sensorimotor interface in the elementary acoustic startle circuit?. *J Neurosci*, 14 (3), pp.1176-1194.

Matthews, E., and Disterhoft, J. (2009). Blocking the BK channel impedes acquisition of trace eyeblink conditioning . *Learn Mem*, 16 pp.106-109.

Meera, P., et al. (1997). Large-conductance voltage- and calcium-dependent K+ channel, a distinct member of voltage-dependent ion channels with seven N-terminal transmembrane segments (S0-S6), an extracellular N terminus, and an intracellular (S9-S10) C terminus. *Proc Natl Acad Sci U S A* , 94 (25), pp.14066-14071.

Orio, P., et al. (2002). New disguises for an old channel: maxiK channel ß-subunits. *Physiology*, 17 pp.156-161.

Pallanck, L., and Ganetzky, B. (1994). Cloning and characterization of human and mouse homologs of the Drosophila calcium-activated potassium channel gene, slowpoke. *Hum Mol Gen* , 3 (8), pp.1239-1243.

Perry, W., et al. (2007). Sensorimotor gating deficits in adults with autism. *Biol Psychiatry*, 61 pp.482-486.

Perry, W., et al. (1999). Sensorimotor gating and thought disturbance measured in close temporal proximity in schizophrenic patients. *Arch Gen Psychiatry*, 56 pp.277-281.

Pilz, P., et al. (2004). Habituation of the acoustic and the tactile startle responses in mice: two independent sensory processes. *Behav Neurosci*, 118 (5), pp.975-983.

Pinsker, H., et al. (1970). Habituation and dishabituation of the gill-withdrawal reflex in Aplysia. *Science*, 167 (3926), pp.1740-1742.

Prosser, C., and Hunter, W. (1936). The extinction of startle responses and spinal reflexes in the white rat. *Amer J Physiol*, 117 pp.609-618.

Rankin, C., et al. (1990). Caenorhabditis elegans: a new model system for the study of learning and memory. *Behav Brain Res*, 37 (1), pp.89-92.

Rankin, C., et al. (2009). Habituation revisited: an updated and revised description of the behavioral characteristics of habituation . *Neurobiol Learn Mem*, 92 pp.135-138.

Roberts, W., et al. (1990). Colocalization of ion channels involved in frequency selectivity and synaptic transmission at presynaptic active zones of hair cells. *J Neurosci*, 10 (11), pp.3664-3684.

Robitaille, R., and Charlton, M. (1992). Presynaptic calcium signals and transmitter release are modulated by calcium-activated potassium channels . *J Neurosci*, 12 (1), pp.297-305.

Rose, J., and Rankin, C. (2001). Analyses of habituation in Caenorhabditis elegans. *Learn Mem*, 8 pp.63-69.

Ruttiger, L., et al. (2004). Deletion of the Ca2+-activated potassium (BK) a-subunit but not the BKß1-subunit leads to progressive hearing loss. *Proc Natl Acad Sci U S A* , 101 (35), pp.12922–12927.

Sailer, C., et al. (2006). Immunolocalization of BK channels in hippocampal pyramidal neurons. *Eur J Neurosci*, 24 pp.442-454.

Saitoh, K., et al. (1987). Possible role of the brainstem in the mediation of prepulse inhibition in the rat. *Neurosci Lett*, 75 pp.216-222.

Salkoff, L., et al. (2006). High-conductance potassium channels of the SLO family. *Nature Reviews Neuroscience*, 5 pp.921-931.

Sausbier, M., et al. (2004). Cerebellar ataxia and purkinje cell dysfunction caused by Ca2+ -activated K+ channel deficiency. *Proc Natl Acad Sci U S A* , 101 (25), pp.9474–9478.

Sausbier, U., et al. (2006). Ca 2+ -activated K+ channels of the BK-type in the mouse brain. *Histochem Cell Biol* , 125 pp.725-741.

Simons-Weidenmaier, N., et al. (2006). Synaptic depression and short-term habituation are located in the sensory part of the mammalian startle pathway. *BMC Neuroscience*, 7 (38).

Singer, P., et al. (2013) Prepulse inhibition predicts working memory performance whilst startle habituation predicts spatial reference memory retention in C57BL/6 mice. *Behav Brain Res*, 242 p.166-177.

Swerdlow, N., et al. (1995). Impaired prepulse inhibition of acoustic and tactile startle response in patients with Huntington's disease. *J Neurol Neurosurg Psychiatry*, 58 pp.192-200.

Thompson, R., and Spencer, W. (1966). Habituation: a model phenomenon for the study of neuronal substrates of behaviour. *Psychol Rev*, 73 (1), pp.16-43.

Wang, Z. (2008). Regulation of synaptic transmission by presynaptic CaMKII and BK channels. *Mol Neurobiol*, 38 pp.153-166.

Wang, Z., et al. (2001). Slo-1 potassium channels control quantal content of neurotransmitter release at the C. elegans neuromuscular junction. *Neuron*, 32 pp.867-881.

Weber, M., et al. (2002). Synaptic plasticity in the acoustic startle pathway: the neuronal basis for short-term habituation?. *Eur J Neurosci*, 16 pp.1325-1332.

Yeomans, J., et al. (2010). GABA receptors and prepulse inhibition of acoustic startle in mice and rats . *Eur J Neurosci*, 31 pp.2053-2061.

Zhang, L., et al. (2006). Possible role of potassium channel, big K in etiology of schizophrenia. *Med Hypotheses*, 67 pp.41-43.

Zucker, R. (1989). Short-term synaptic plasticity. *Annu Rev Neurosci*, 12 pp.13-31.

Appendix

Western

2011-077::1:

AUP Number: 2011-077
AUP Title: Studying cognitive function in rodents
Yearly Renewal Date: 02/01/2013

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2011-077 has been approved, and will be approved for one year following the above review date.

1. **This AUP number must be indicated when ordering animals for this project.**
2. **Animals for other projects may not be ordered under this AUP number.**
3. **Purchases of animals other than through this system must be cleared through the ACVS office.**
   Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Kinchlea, Will D
on behalf of the Animal Use Subcommittee

Curriculum vitae

Name:	Magdalena Mirkowski
Post-secondary Education and Degrees:	Western University London, Ontario, Canada 2005-2010 H.BSc.
Honours and Awards:	Western Graduate Research Scholarship 2011-2013 Graduate Thesis Research Award 2013
Related Work Experience	Teaching Assistantship, Integrative Neuroscience 4451a, 4451F, 4451G Western University 2011-2013
Publications:	Mice with deficient BK channel function show impaired prepulse inhibition and spatial learning, but normal working and spatial reference memory, submitted Habituation of reflexive and motivated behaviour in mice with a deficiency of the α-subunit of the BK channel, in preparation The role of BK channels in habituation and prepulse inhibition, published abstract for the International Behavioural and Neural Genetics Society (IBANGS) 2012 meeting Important role of BK channels in sensory gating and cognition, published abstract for the Canadian Association for Neuroscience (CAN) 2012 annual meeting BK potassium channels play an important role in sensory gating, published abstract for the Society for Neuroscience (SfN) 2012 annual meeting Mice deficient for BK potassium channels show sensory gating deficits and impaired spatial memory, published abstract for the Canadian Association for Neuroscience (CAN) 2013 annual meeting