6-20-2016 12:00 AM

Sensory Filtering and Cognitive Function in a Valproic Acid Rat Model of Autism

Theshani A. De Silva, *The University of Western Ontario*

Supervisor: Dr. Susanne Schmid, *The University of Western Ontario*

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

Abstract

Autistic individuals display sensory filtering impairments often correlated with cognitive dysfunction. Studies have shown that both these functions can be modulated by big potassium (BK) channels. Importantly, a subset of individuals with autism have shown BK channel mutations. We assessed sensory filtering and cognitive function through behavioural tests in a valproic acid (VPA) rat model of autism. We hypothesize that the model will display sensory filtering and cognitive impairments and that activation of BK channels may rescue observed cognitive deficits. Results revealed impairments in sensory filtering, hyper-locomotive activity and increased anxiety in VPA animals during adolescence. Although no significant impairments in cognitive function were observed, BK channel modulators were shown to facilitate normal cognitive function. We conclude that the VPA model is valid for displaying sensory filtering impairments associated with autism. However, no cognitive deficits were identified. Our results also provided further evidence for the importance of BK channels in cognition.

Keywords: Autism spectrum disorder, valproic acid, sensory filtering, cognitive function, acoustic startle response, open field, 5-choice serial reaction time task, big potassium channels

Acknowledgments

I would like to thank my supervisor Dr. Susanne Schmid for this opportunity and for her valuable guidance and support. This has been a period of intense learning on both a personal and scientific level. I have gained man tools and learned invaluable lessons that not only helped me complete my thesis, but allowed me to develop skills I will carry with me in my future endeavors. I would like to thank my supervisory committee, Dr. Raj Rajakumar and Dr. Shawn Whitehead for their constructive input and expertise.

To all the members of the Schmid lab, this experience would not have been the same without you. I would like to thank Mitch Cooper, Jason Gopaul, Andrea Louttit and Faraj Haddad for your friendship and support. I want to extend a special thank you to Cleusa De Oliveira and Erin Azzopardi for all your technical support, training and guidance. Finally thank you to my family and to my best friend Alena, for your wise counsel, infinite love and unwavering support. I could not have asked for a more supportive team.

Table of Contents

List of Abbreviations

Abbreviations	Term
5-CSRTT	5-Choice Serial Reaction Time Task
ASD	Autism Spectrum Disorder
ASR	Acoustic Startle Response
BK channels	Big potassium channels
FX	Fragile-X Syndrome
FMRP1	Fragile-X Mental Retardation Protein
I/O function	Input/output function
ISI	Inter-stimulus interval
ITI	Inter-trial interval
LTH	Long-term habituation
PnC	Pontine reticular formation
PPI	Prepulse Inhibition
STH	Short-term habituation

List of Figures

Chapter 1: Introduction

1 Introduction

In 2010, it was estimated that there were 52 million cases of Autism Spectrum Disorders (ASD) worldwide, affecting one in every 132 people with males being more affected than females (Baxter et al. 2014). ASD is a highly prevalent neurodevelopmental disorder primarily characterized by deficits in social interaction and communication, as well as by repetitive behaviours. The new DSM-V introduced large changes to the diagnostic criteria for autism. The major change to consider is the removal of subgroups and the introduction of one general diagnostic term, autism spectrum disorder (Wing, Gould, & Gillberg, 2011). ASD now encompasses autistic disorder, Asperger's disorder and pervasive developmental disorder not otherwise specified. This change in criteria is important to note as literature before the DSM-V refers to autism and its subgroups separately. As a result, studies treated the subgroups as separate disorders and thus the distinct patient populations were investigated individually. However after the introduction of DSM-V, all subgroups are categorized under the term ASD. As a result, current studies investigate ASD as a whole, which includes a spectrum of individuals who show a range of impairments.

The DSM-V has also included sensory processing impairments as a diagnostic feature of autism. With approximately 70-90% of individuals with ASD displaying atypical responses to sensory stimulation (Billstedt, Carina Gillberg, and Gillberg 2007; Kohl et al. 2014; Leekam et al. 2007), sensory processing abnormalities have become an important symptom in the diagnostic process (Rogers and Ozonoff 2005). Several studies have shown that patterns of abnormal sensory filtering have been associated with

impaired academic performance, poor attention to cognitive tasks, high anxiety levels and social skill deficits (Ashburner, Ziviani, and Rodger 2008; B. Pfeiffer, M.Kinnealey, C.Reed 2005; Baker et al. 2008; Takahashi et al. 2015). This emphasizes a correlation between sensory filtering mechanisms and cognitive function.

Recently, it has been shown that mechanisms directly influencing synaptic transmission contribute to sensory filtering. In this context, we have identified widely expressed calcium and voltage-activated big potassium channels (BK channels) throughout the brain that regulate neurotransmitter release and control cell excitability, as important players. It is likely that the activation and/or phosphorylation of these channels play a role in sensory filtering processes and cognitive function. This work aims to gain an understanding of sensory filtering processes and cognitive function and their disruptions in a Valproic Acid (VPA) rat model of autism and to evaluate the potential of BK channel modulators to ameliorate observed cognitive disruptions. This could provide a basis for the development of drugs that enhance sensory filtering and associated cognitive function for mental disorders, such as ASD.

1.1 Sensory Processing in Autism Spectrum Disorder

1.1.1 Sensory processing theories

Abnormalities in sensory processing have been widely noted in ASD. There is also a widespread notion that core symptoms in ASD, such as repetitive behaviours and social impairments, might be a result of sensory dysfunction. Over the years, different theories have been proposed to explain the wide range of symptoms seen in autistic individuals.

The *over arousal theory*, addresses the hypersensitivity to sensory stimuli commonly witnessed in children and adolescents with ASD (Rogers and Ozonoff 2005). Individuals are sensitive to and easily aroused by their sensory environments. These individuals experience detailed focused processing, which produces overreactions to even the most insignificant sensory information. Green and colleagues (2015) noted that in autistic youth the sensory limbic system is hyper-responsive to auditory stimuli, due to a participant's inability to habituate. Increased responsiveness to auditory stimuli is also seen in patients with Fragile X Syndrome (FXS). FXS is the most common known genetic cause of ASD and is often used in research as a window into understanding the biological mechanisms underlying ASD. When exposed to loud sounds for 40-50 seconds, FXS individuals exhibited severe seizures (Chen and Toth 2001). This is consistent with the hypersensitivity to auditory stimulation seen in ASD. Failure to habituate to sensory stimuli in both FXS and ASD in particular, results in constant over-arousal and highly anxious states. Autistic individuals experiencing hypersensitivity are constantly overwhelmed by sensory input and as a result, develop poor compensatory mechanisms to prevent sensory overload including repetitive behaviours and withdrawal from complex and novel environments (Chen, Rodgers, and McConachie 2009; Hilton, Graver, and LaVesser 2007). Studies have shown that activity in the brainstem reticular formation is sustained at high levels during sensory stimulation, which subsequently results in blockade of neural sensory pathways to prevent over stimulation (Rogers and Ozonoff 2005). These compensatory mechanisms prevent autistic children from actively participating in a classroom setting thus preventing an enriching learning experience and impeding academic performance.

The *under arousal theory*, addresses hyposensitivity to sensory stimuli in ASD. These individuals are less responsive to sensory stimulation (Rogers and Ozonoff 2005). They often fail to notice stimuli or exhibit delayed responses when compared to their normally developing peers. Studies have shown deficits in the reticular activating system in these individuals. As a result, their limbic systems are suppressed and reward systems are inactivated. Consequently, they experience sensory deprivation, and this results in isolation from arousing environments simply due to a lack of interest (Rogers and Ozonoff 2005). Affected individuals are uninterested in social situations and isolate themselves from social interactions. Bitsika and colleagues (2015) showed that boys who displayed hyposensitivity to sensory stimuli exhibited depressive symptoms. This lack of optimal arousal in any setting can result in depression (B. Pfeiffer, M.Kinnealey, C.Reed 2005).

Lastly, the *perceptual inconstancy theory* addresses the presence of both hypersensitivity and hyposensitivity (Rogers and Ozonoff 2005). For example, subtle changes in their sensory environment may go unnoticed (hypo-sensitivity), however at the same time there could be an over-reaction to irrelevant information in their environment (hypersensitivity, Baxter et al. 2014). This theory suggests that brainstem abnormalities cause a state of fluctuation between under and over arousal (Rogers and Ozonoff 2005). Therefore, an individual can experience the same stimulus differently. It is important to note that the paradoxical patterns of sensory processing seen in ASD are due to the complex nature of autism being a spectrum disorder.

1.1.2 Impairments in sensory processing

It is evident that autistic individuals display sensory processing impairments. Autistic individuals who show normal hearing will continue to present abnormal auditory processing (Bomba & Pang, 2004; Gomot, Giard, Adrien, Barthelemy, & Bruneau, 2002; Roth, Muchnik, Shabtai, Hildesheimer, & Henkin, 2012). It is important to note that these abnormalities are not a result of physical malformations but problems in the top-down, neural control over auditory structures. Autistic individuals are unable to distinguish relevant information from background noise, a typical process that requires top-down neurological control. For example De Pape et al, (2012) noted that adolescents with ASD showed poorer filtering compared to controls when asked to attend to one speech stream and ignore another. Khalifa and colleagues (2001), further noted that children and adolescents with ASD also displayed auditory filtering impairments when presented with distracting stimuli. These individuals were unable to activate midbrain-filtering mechanisms (medial olivo-cochlear system, MOC). Studies have also gone on to show that autistic individuals are unable to extract salient information from noise when temporal dips are present in the background (Alcántara et al. 2004; Groen et al. 2009). This suggests an inability to properly process auditory information at early stages in the auditory pathway, resulting in impairments in integration at midbrain areas.

Imaging studies have also shown auditory processing impairments occurring as a function of neurological control. Autistic individuals show abnormal development of brain areas, in particular, auditory processing areas such as the temporal lobes, which contain the auditory cortex (Courchesne and Pierce 2005). Furthermore, Diffuse Tensor Imaging studies have provided insight into white matter connectivity in autistic

individuals. Chang and colleagues (2014) noted white matter microstructure is impaired in autistic individuals. In addition, these individuals exhibited decreased connectivity of white matter in parieto-occipital tracts, which are involved in sensory processing and multi-sensory integration. These results correlate with abnormal sensory processing in autistic individuals. Furthermore, other studies have shown decreased white matter connectivity in the auditory cortex and corpus callosum in individuals with ASD (Barnea-Goraly et al. 2004). Overall, we see clear abnormalities in sensory processing in ASD that occurs as a result of impairments in top-down neurological control and not inner ear functionality.

1.2 Sensory Filtering in Autism Spectrum Disorder

1.2.1 Habituation and PPI as sensory measures

It is important to be able to extract salient information from our environment and respond appropriately, which requires filtering out irrelevant input. Two important sensory filtering mechanisms are sensorimotor gating and habituation. Operational measures for these pre-attentive processes are prepulse inhibition and habituation, respectively. They can be easily assessed in human and animal models through the acoustic startle reflex (Koch and Schnitzler 1997; ASR Koch 1999), which is a protective response elicited by a loud, sudden acoustic stimulus. Measuring the startle response amplitudes and latencies to an acoustic stimulus allows for quantification of the ASR. Habituation, a non-associative form of learning, is a reversible decrease in the startle response to a repeated acoustic stimulus (Koch 1999). The reduction in the startle response amplitude occurs as the information presented becomes non-salient over time. Prepulse inhibition (PPI)

provides a measure of sensorimotor gaiting and occurs when a weaker acoustic pre-stimulus attenuates the following response to the stronger acoustic stimulus. PPI reflects the orientation of the animal towards the prepulse, thereby facilitating perceptual processing and reducing aversive responses, such as startle, in the meanwhile (Fendt, Li, and Yeomans 2001; Yeomans 2012). It is a mechanism that is important to adequately process and ultimately react to salient sensory stimuli.

The sensory filtering mechanisms discussed are disrupted in several mental disorders such as Alzheimer's Disease, Huntington's chorea, attention-deficit hyperactivity disorder and disruptions in PPI are an endophenotype for schizophrenia (Braff, Geyer, and Swerdlow 2001; Braff, Grillon, and Geyer 1992; Swerdlow et al. 1995). Moreover sensory filtering mechanisms are disrupted in autistic individuals and animal models of autism (see below).

Overall, the neural circuitry mediating habituation and PPI are highly conserved and well understood. They are proven to be validated objective measures of sensory filtering in both human and animal models and can be used to reveal underlying mechanisms central to the pathogenesis of several neurological disorders including ASD.

1.2.2 Habituation and PPI in Autism Spectrum Disorders

Habituation and prepulse inhibition of the startle reflex have been investigated in individuals with ASD. Studies have shown that short term habituation of startle is significantly slower in individuals with ASD (Ornitz et al. 1993; Perry et al. 2007). A few studies have also found differences in PPI in individuals with ASD when compared to

controls. Perry and colleagues (2007) noted significantly less PPI at the 86db/60 msec inter-stimulus interval (ISI) in adults with autism, which was correlated with increased repetitive behaviours. McAlonan et al. (2002) also noted significant impairment in PPI at the 16dB/120 ms ISI in adults with Asperger's syndrome which also correlated with increased restrictive behaviours. Furthermore, profound PPI deficits have been found in the Fragile X patient population as well (Frankland et al. 2004; Yuhas et al. 2011). The magnitude of PPI deficits in the FXS group also predicted the severity of autistic phenotypes (Frankland et al. 2004). PPI failure found in these two groups indicates a possible dysfunction in normal inhibitory regulation in ASD. Lastly, Madsen and colleagues (2014) looked at autistic children and were able to find increased sensitization of the startle reflex, which correlated with an increase in anxiety levels. They were also the only group to find contradictory results to current literature. They showed increased PPI at the 76dB/120 msec ISI in autistic children when compared to age-matched controls.

Other studies were able to find no differences in PPI or habituation in ASD groups compared to controls (Kohl et al. 2014; Oranje et al. 2012; Ornitz et al. 1993; Yuhas et al. 2011). The large variability in findings could be due to the age group of the populations tested, the methodology used such as differences in prepulse intensity and most importantly, cohort heterogeneity. Despite the variability in findings, habituation and PPI are reliable indicators of abnormal sensory processing in ASD.

1.2.3 Sensory Filtering and Cognitive Function in ASD

Proper sensory processing is critical to daily functioning. It is important to be able to extract salient information from background noise in order to interact and respond appropriately to one's environment. Recent studies have highlighted a significant relationship between sensory processing abnormalities and the degree of maladaptive behaviour in ASD. Poor sensory processing abilities have been shown to strongly correlate with behavioural and emotional problems in individuals with ASD including high irritability, stereotypic behaviours and hyperactivity (Baker et al. 2008; O'Donnell et al. 2012). Tanguay and Edwards (1982) further suggested that impairments in sensory processing early on in life, especially auditory input, leads to impairments in developing complex cognitive abilities later on in life. Studies have shown impairments in auditory filtering mechanisms are correlated with deficits in learning, inattention to cognitive tasks and poor academic performance (Ashburner et al. 2008). The complex, noisy classroom environment often results in cognitive disruption due to sensory overload, and consequently, children with ASD will become withdrawn and are more likely to underachieve academically (Ashburner et al. 2008). Sensory overload is also translated into social settings and as a result, individuals with ASD are not only withdrawn in academic environments but are socially isolated as well (Hochhauser and Engel-Yeger 2010). Studies have also shown sensory processing impairments are associated with anxiety and depressive symptoms (Bitsika et al. 2015; Kohl et al. 2014). Pfeiffer et al., (2005) found that as symptoms of anxiety and depression increased in children and adolescents with Asperger's, their performance in academic and social environments decreased. It is clear that there is a strong correlation between sensory processing impairments and higher order cognitive dysfunction.

1.3 Big Potassium Channels

1.3.1 BK Channel functionality

Big Potassium (BK) channels are extensively expressed throughout the brain such as the amygdala, cerebral cortex, caudate nucleus, hippocampus, hypothalamus, brain stem and spinal cord and are concentrated at axons and synapses. The channel consists of four pore forming alpha subunits and modulatory beta and gamma subunits. The highly preserved *slo1* gene (KCNMA1 gene in humans) encodes for the alpha subunits of the BK channel (Lee and Cui 2010). These channels are unique in the sense that they are synergistically activated by changes in membrane potential and intracellular concentrations of Ca^{2+}(Knaus et al. 1996; Misonou et al. 2008). A depolarization of the membrane or an increase in calcium entry via co-localized voltage dependent calcium channels activate BK channels. As a result, the BK channels open and there is an efflux of K+ through the pore gate. Therefore these channels induce a hyper-polarization of the membrane following an action potential, negatively controlling neurotransmitter release. They are also known to play a key role in hormone release and muscle tone, and are considered to be involved in the integration of both biochemical and electrical signals. Overall these channels exert a powerful control on neuronal excitability and integration (Gribkoff, Starrett, and Dworetzky 2001).

1.3.2 BK channels in sensory filtering and cognition

Several lines of evidence suggest an important function of BK channels in sensory filtering. As mentioned above, BK channels are co-localized with voltage dependent

calcium channels thereby establishing a link between intracellular calcium levels and neurotransmitter release. This makes them ideal candidates for mediating calcium-dependent presynaptic depression in the primary startle pathway (Weber, Schnitzler, and Schmid 2002), thereby potentially mediating short-term habituation of startle. Previous data supports this theory and have shown that short-term habituation is completely abolished in BK channel knockout mice (Typlt, Mirkowski, Azzopardi, Ruth, et al. 2013) and PPI does not improve upon repeated testing sessions (Typlt, Mirkowski, Azzopardi, Ruettiger, et al. 2013). This confirms the possibility of BK channels playing a crucial role in sensory filtering. Studies have also shown the possible role of BK channels in cognition. In another study by Typlt and colleagues (2013), BK channel knockout mice showed spatial learning impairments in the Morris Water Maze task. Hébert and colleagues (2014) further demonstrated the importance of these channels in cognitive function. Through the use of a selective BK channel opener, they were able to completely correct a broad range of cognitive and behavioural disturbances in a transgenic FXS mouse model. Collectively, these results suggest that there is an important function of BK channels in sensory filtering processes and higher cognitive tasks.

1.3.3 BK channels in Autism Spectrum Disorder

An analysis of a *de novo* balanced reciprocal translocation in a subject with autism revealed a haplo-insufficiency of the KCNMA1 gene, which encodes for the alpha subunit in BK channels. Further mutational analyses on 116 autistic subjects led to the identification of an amino acid substitution located in the domain of KCNMA1(Laumonnier et al. 2006). There is a physical disruption and therefore decreased activity of these channels in this subset of autistic individuals. Furthermore, administration of a BK channel opener was able to increase the activity of these channels

in this patient group (Laumonnier et al. 2006). Another report showed that individuals with Timothy Syndrome, a multisystem disorder that includes autism, showed disruption in calcium signaling due to $Ca_v1.2$ channel mutations (Splawski et al. 2004). Other studies have shown impairments in calcium signaling and alterations in BK channel function not only in individuals with autism but in mental retardation and most notably in Fragile X syndrome (FXS) as well (Higgins et al. 2008; Krey and Dolmetsch 2007). Interestingly, BK channel accessory subunit β4 has been shown to directly interact with Fragile X mental retardation protein 1 (FMRP). Loss of FMRP is associated with the intellectual disability and autistic like behaviour found in individuals with FXS. Studies in transgenic FSX mice have shown that loss of FMRP was associated with a decrease in BK channel activity and as a result abnormalities in neurotransmitter release and synaptic information transmission were present (Deng et al. 2013). Conclusively, deficits in synaptic transmission, neuronal excitability and calcium signaling regulated through the activity of BK channels may be involved in the pathogenesis of ASD.

1.4 Animal Models of Autism

1.4.1 Valproic Acid (VPA) Model

Valproic acid is an anti-seizure medication commonly administered to epileptic patients. VPA is a potent teratogen and thus impacts embryonic development however; its exact mechanism of action is unknown. VPA is a direct inhibitor of histone deacetylase (HDAC) and therefore it is hypothesized that its pathogenic effects are caused by changes in gene expression (Phiel et al. 2001). In humans, there have been several risks associated with VPA administration during pregnancy. It has been found that there is a 7-fold

greater incidence of ASD in children born to mothers taking VPA (Ishiura et al. 2008) . Offspring are more likely to show language impairments, social deficits and restricted behaviours. The use of VPA *in utero* in animal studies has resulted in similar impairments in the offspring as seen in ASD and have provided a large insight into the mechanistic action of VPA on neurodevelopment and behaviour. The valproic acid (VPA) model is an example of an environmentally induced model of autism. Over the years it has become a well-established model with high face value and construct validity that encompasses all important anatomical and behavioural similarities to autistic humans (Mabunga et al. 2015).

Schneider and Przeqlocki (2005) showed multiple impairments in the VPA model that demonstrate its validity. They showed that VPA rats exhibited diminished PPI, repetitive stereotypic activity, decreased exploratory behaviour, and decreased sociability (Schneider and Przewłocki 2005). In addition the rats displayed delayed maturation and motor development. Other studies have also shown increased depressive and anxiety like symptoms in VPA rats compared to controls (Mehta, Gandal, and Siegel 2011; Nakasato et al. 2008). These abnormalities are similar to patterns seen in humans, therefore further validating the VPA animal model of autism.

Neuroanatomical and molecular studies have also been conducted in the VPA model. Neuroanatomical features of the VPA model have been compared to post-mortem brains of humans with ASD. Several changes observed are similar to the abnormalities seen in humans, such as large changes in the circuitry and morphology of neurons and increased neuronal connectivity in the somatosensory and medial prefrontal cortex (Rinaldi, Silberberg, and Markram 2008). Electrophysiological recordings revealed that this

increase in connectivity is paralleled by a decrease in the strength of the connections, resulting in a possible hyperactivity of the circuit. Furthermore, disruption in the maturation of serotonergic neurons in rats exposed to VPA is consistent with abnormal serotonin levels observed in the brains of individuals with ASD (Mabunga et al. 2015). Lastly, the molecular data has revealed similarities in genetic anomalies in the VPA model compared to humans with ASD. For example, neuroligin 3 mRNA expression, an autism related gene, is decreased in the somatosensory cortex as well as the hippocampus of VPA animals (Kolozsi et al. 2009).

In conclusion, the VPA animal of model autism is a well-established model and displays behavioural and structural similarities to humans with ASD. It is a cost effective and highly translational model, providing a great framework for developing an understanding of the underlying mechanisms central to the pathogenesis of ASD.

1.5 The Proposed Framework

1.5.1 Rationale

Individuals with ASD display abnormalities in sensory processing. Studies have shown that habituation and sensory gating, mechanisms of sensory filtering, are also impaired in individuals with ASD. Patterns of abnormal sensory filtering in autistic children have been associated with impaired academic performance, poor attention to cognitive tasks, high anxiety levels and social skill deficits (Ashburner et al. 2008). This emphasizes a correlation between sensory filtering mechanisms and cognitive function.

Several lines of evidence have suggested an important function of BK channels in sensory filtering and cognitive function. Studies have shown that short-term habituation

is completely abolished, PPI does not improve, and spatial learning impairments are present in BK channel knockout mice (Typlt, Mirkowski, Azzopardi, Ruettiger, et al. 2013; Typlt, Mirkowski, Azzopardi, Ruth, et al. 2013). BK channel dysfunction has also been implicated in several neurological disorders including ASD and Fragile X syndrome. Hébert and colleagues (2014) went on to show that a selective BK channel opener (BMS-204352) in FSX mice (often associated with autistic features) completely corrected a broad range of cognitive and behavioural disturbances. The evidence presented thus far highlights the central role of BK channels in proper cognitive function and sensory filtering mechanisms.

The proposed research seeks to test sensory filtering and cognitive impairments in an established valproic acid (VPA) rat model of autism. The effects of a BK channel modulator on cognitive function will also be tested. Furthermore, in the human population of ASD, males are more commonly affected than females. As a result, we expect to see sex differences in our study, with males displaying a stronger phenotype than females.

1.5.2 Hypothesis

We hypothesize that the VPA rat model of autism is a valid model to assess sensory filtering impairments and cognitive dysfunction. Additionally, we hypothesize that positive BK channel modulators may influence cognitive outcome in the VPA model.

1.5.3 Specific Aims

The aims of this research project are:

1) Confirm sensory filtering deficits in a putative valproic acid (VPA)-induced rat model of autism.

2) Measure cognitive deficits (learning, attention, behavioural anxiety) that occur as a result of sensory filtering impairments.

3) Determine the effects of a positive BK channel modulator on cognition.

Chapter 2: Methodology

2 Methods

2.1 Valproic Acid (VPA) Model

Dosage and time of exposure to valproic acid (VPA) has shown to impact the severity of symptoms relating to the core signs of autism. A dose of 600 mg/kg at gestation day (GD) 12.5 has shown to consistently produce hallmark behavioural changes associated with ASD (Chan et al. 2011; Markram and Foster 2013; Roullet et al. 2013; Schneider and Przewłocki 2005). Long Evans rats (Charles River, Canada) were housed on a 12h:12h light dark cycle with food and water *ad libitum*. Male and female rats of approximately 3 months of age were mated overnight. Next day, the female was examined for the presence of a copulatory plug, indicating successful mating. This was considered the first day of gestation (Banerjee et al. 2014; Markram and Foster 2013; Olexová et al. 2013; Schneider and Przewłocki 2005). Sodium valproate (Sigma Aldrich, Oakville, ON) was dissolved in 0.9% saline at a concentration of 250 mg/ml. At GD 12.5, during the middle of the light phase, pregnant females received a single intraperitoneal injection of 600 mg/kg sodium valproate (pH=7.3). Control pregnant mothers were injected with saline at 12.5 days of gestation.

After the offspring were born, both VPA and control litters remained with their mothers until weaning on postnatal day (PND) 21. After weaning they were housed in groups of 2-4 rats per cage, separated by sex. All animal procedures were according to approved animal use protocols by the Animal Care Committee at the University of Western Ontario and in accordance with the guidelines and rules of the Canadian Council on Animal Care (CCAC).

2.2 Project Timeline

All animals underwent open field and startle testing during adolescence, starting at 6 weeks of age. Animals were then placed on food restriction for one week (8 weeks of age) before beginning the 5 Choice Serial Reaction Time Task (5-CSRTT) training. Upon completion of the 5-CSRTT, animals were then taken off food restriction for at least one week and re-tested on the open field and startle tests during adulthood (around 4 months of age; Figure 1).

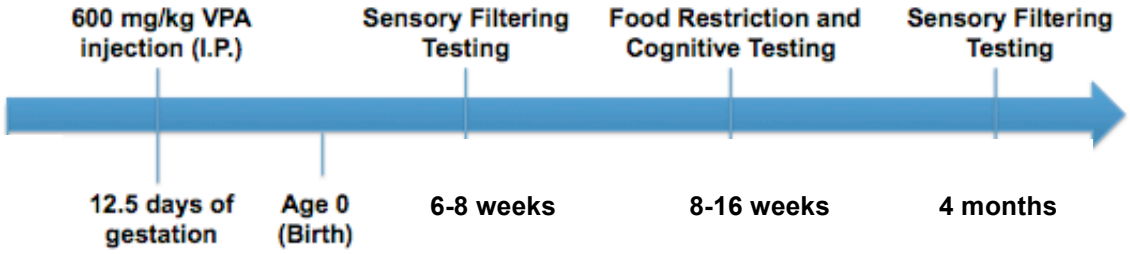

Figure 1. Project Timeline. Pregnant mothers were injected at 12.5 days of gestation with VPA. Sensory filtering testing began 6 weeks after birth (adolescence). Open field box was tested for one week. Startle testing was completed immediately afterwards for one week. Animals were then placed on food restriction for one week and started on the 5 Choice Serial Reaction Time Task (5-CSRTT) training and testing. The effects of big potassium channel modulators on the task were tested after initial training and testing were completed. The 5-CSRTT lasted for approximately 8 weeks. Upon completion of the 5-CSRTT, animals were taken off food restriction. Animals were re-tested on the open field box task and on startle at approximately 4 months of age (adulthood).

2.3 Acoustic Startle Response

Startle boxes were used to measure the ASR (Med Associates Inc., St. Albans, VT, Figure 2). Animals were placed in a clear, cylindrical holder with holes on the side that faces the speaker. The cylinders were placed on a platform that is mounted on a transducer. When the animal startles, the movement causes a vertical dislocation of the platform, which the transducer converts into a voltage signal. This is then translated into a digital startle amplitude readout on a computer where the startle responses were measured as peak-to-peak values within a 100 ms time window after the startle stimulus (Valsamis and Schmid 2011).

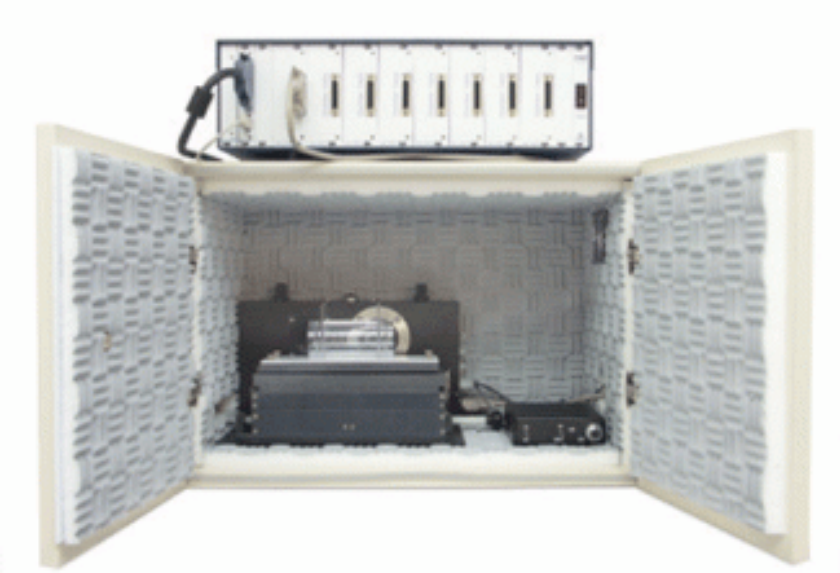

Figure 2. Med Associates Startle Box. The speaker is located at the back of the box. The platform with the animal holder is mounted on the transducer and located directly in front of the speaker.

2.3.1 Startle Procedure

Before an animal was tested, they underwent acclimation to the startle boxes, the holders, and the background noise. The acclimation period lasted for 5 minutes where the animal was presented with 65 dB of white background noise with no startle stimuli. This procedure was repeated three times.

Next, input/output (i/o) function was established. After an acclimation period of 5 minutes, 12 startle stimuli of increasing intensity (75 dB-130 dB, with 5dB steps, white noise, 20 ms duration) were presented on the background noise every 20 sec. The I/O function provided approximate startle intensities for each animal allowing the sensitivity of the platform to be decreased or increased, respectively. Changing the sensitivity of the platform was accomplished through adjusting the gain on the transducer amplifier.

Short Term habituation (STH) and prepulse inhibition (PPI) were measured in one session. On testing day the animal underwent a 5 minute acclimatization period with a white background noise of 65dB followed by Block I: Habituation and Block II: PPI. During Block I, 50 startling acoustic stimuli of 110 dB white noise and 20 ms duration were presented every 20 seconds on the background noise. This was followed by Block II which consisted of pseudo randomized trials of startle stimuli alone and startle stimuli presented with a prepulse of 4 ms white noise and intensities of 75 dB or 85 dB that were presented either 30 ms or 100 ms before the startling pulse. Each trial type was presented 10 times to a total of 50 trials in Block II.

In order to measure long-term habituation (LTH), the entire protocol was run for five consecutive days. The overall testing procedure for startle occurred as follows:

Day 1: Acclimation 2X (at least one hour in-between)

Day 2: Acclimation + I/O Function (at least one hour in-between)

Day 3: STH and PPI

Day 4: STH and PPI

Day 5: STH and PPI

Day 6: STH and PPI

Day 7: STH and PPI

2.3.2 Startle Data Analysis

Baseline startle: Baseline startle was calculated by averaging the first two responses of each animal on the first day of testing. This was then averaged per group and plotted.

Short Term Habituation: For short term habituation analysis, the responses of each animal in Block I testing day 1 were normalized to the average of the first two responses per animal. The normalized responses were then averaged across all animals within each group and plotted to view the course of habituation. Short term habituation ratios provide a quantitative assessment of the amount of habituation and are calculated by averaging the last ten responses of each animal divided by the average of the first two responses for each animal and for each day. These ratios are then averaged over five days in order to determine an overall STH ratio.

Long term habituation: In order to analyze long-term habituation, normalized startle amplitudes were calculated by normalizing every trial on every day to the average of the first two responses on the first day of testing. The resulting normalized startle amplitudes for each animal were averaged and then plotted for each day. To provide a quantitative assessment for LTH, normalized responses for each animal were averaged across animals of a group and plotted.

Prepulse Inhibition: Prepulse inhibition (PPI) is calculated independently for each trial type (sorted according to prepulse intensity and respective inter-stimulus interval). The ten traces per trial type were averaged for each animal, divided by its respective baseline startle and multiplied by 100%, which provides the amount of remaining startle. The

percentage of PPI is then calculated by subtracting the amount of remaining startle from 100%. This is then averaged across animals per group and plotted.

Latency to Startle: Latency to startle is calculated for each animal by analyzing the time taken to reach peak startle amplitude (in msec).This is then averaged for each group and plotted. Latency to startle was assessed within a testing session (for STH effects) using day 1 data only, and was assessed across days (for LTH effects).

2.4 Open Field Box

The open field maze test is a measure of exploratory behaviour and can also give some indication about anxiety levels. The open field consists of a large box with tall walls to prevent the animal from escaping. The dimensions of the open filed maze boxes are 45.7 cm x 45.7 cm x 40.6 cm (Figure 3). Animals were placed into the box and allowed to explore for an allotted amount of time.

Exploratory behaviour was measured as total ambulatory distance. Habituation of locomotive behaviour was indicated by a decrease in exploratory behaviour over time. STH was measured across blocks of time within a testing session while LTH was measured across days. Anxiety was measured as a ratio of time spent in the center vs. the periphery.

2.4.1 Open Field Maze Procedure

The boxes were cleaned with 70% ethanol before and after use. An overhead camera above the boxes tracked the animal's movements and time spent in center vs. periphery zones. One animal was placed in each box for a total of 20 minutes. Total distance travelled was measured in 5-minute block intervals. Analysis was performed using video tracking software (ANY-MAZE software version 4.82, Wood Dale, IL).

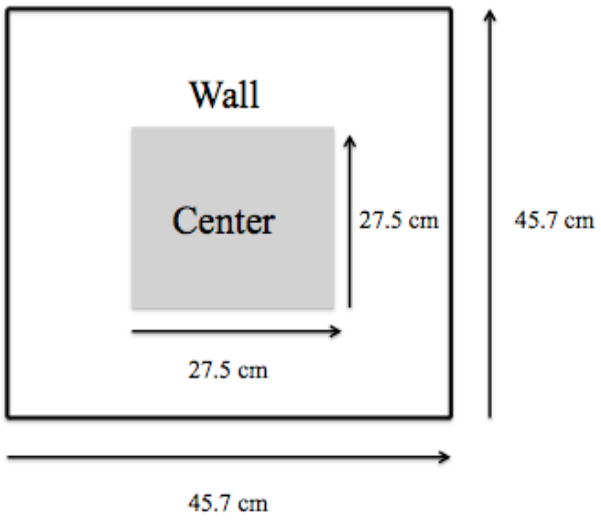

Figure 3. Open Field Maze Schematic. The box is 45.7 cm X 45.7 cm and is enclosed by walls 40.6 cm in height. The dark zone represents the conceptual center of approximately 27.5 cm x 27.5 cm.

2.5 5-Choice Serial Reaction Time Task (5-CSRTT)

The 5-Choice Serial Reaction Time Task is a classical test used for measuring various aspects of attention control and motor impulsivity.

Apparatus:

Bussey-Sakida Touch Screen Chambers (Lafayette Instrument Company, Lafayette, IN) were used to run the 5-CSRTT (Figure 4). The touch screen is present at the front of a trapezoid box and is 15 inches (portrait). Five response locations are on the touch screen. Recording of the response on the touch array uses infra-red technology. At the back of the box, the reward system delivers a 45 mg grain based food pellet. Each box is located in a separate sound attenuated chamber. An external computer controls the entire set of four chambers. Software responsible for image presentation and touch capture are Whisker and Abet II Touch v.2.15.1 (Lafayette Instrument Company, Lafayette, IN). The software was also used to design, manage and execute experiments as well as for data analysis.

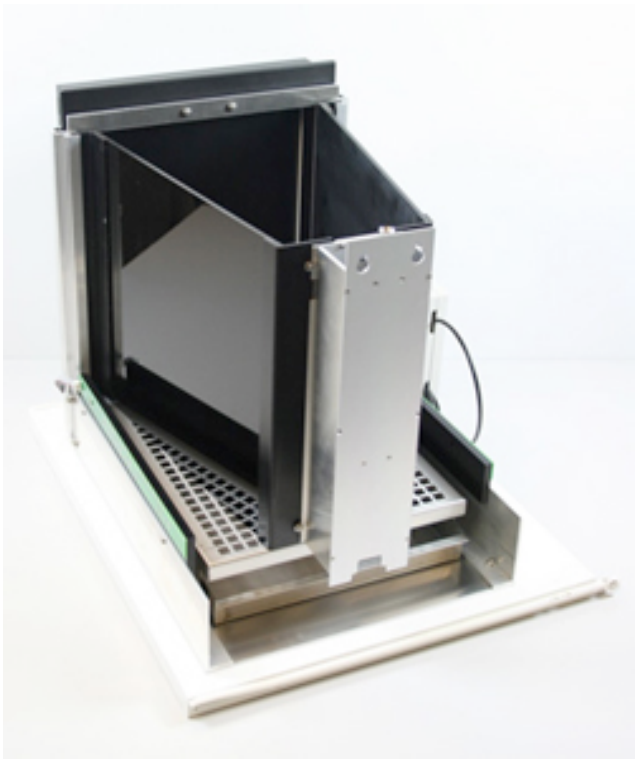

Figure 4. Bussey Saksida Touch Screen Boxes. The touch screen is located at the front of the box. The reward trough is located near the back. The trapezoidal walls prevent escape and are sound attenuating. The pull out tray located underneath the perforated floor collects urine and feces.

2.5.1 5-CSRTT Procedure

Food restrictions were started one week before testing. Animals were restricted to 85% of the average weight for their age and strain. Animals were weighed three times a week to ensure appropriate weight gains with increasing age. Animals were trained at approximately the same time every day for a minimum of 5 days a week. They were placed in the same testing chamber throughout the entirety of testing. Chambers were cleaned with soap and water between testing sessions.

2.5.2 5-CSRTT Training

Animals first learned to habituate to their chamber and to retrieve the food pellets. Five sugar pellets were placed in the reward trough. No light stimulus was presented. At the end of the session, the trough was checked to ensure all pellets were eaten. After the animal learned to obtain the reward pellet, they underwent several training sessions, which required accurately recognizing the spatial location of the light stimulus in order to receive the food pellet reward. The schematic for a single trial was as follows (Figure 4):

1. The animal was placed in the operant chamber and allowed to habituate.
2. At the end of habituation, the reward trough is illuminated and one sugar pellet was delivered. The first trial began once the animal retrieved the pellet.
3. Each trial began with an inter-trial interval (ITI) during which only the light in the chamber is on. At the end of the ITI a light stimulus in one of the five response locations was presented for the pre-established stimulus duration. The rat was allotted a prescribed amount of time to respond to the stimulus by touching the correct spatial location. This is referred to as responding within the limited hold (LH) period.

4. Responding in the correct location resulted in the delivery of a sugar pellet at the reward trough, which was paired with a tone. This was referred to as a correct response. If the animal responded at an unlit response location (incorrect response) or failed to respond within the LH period (omission), this resulted in a time out (TO): The house light turned off and the animal had to wait 5 seconds for the next trial, which began with an ITI, and the light in the chamber illuminated. If the animal touched the response location during the ITI, this was considered to be a premature response which was also followed by a TO period. The same trial was then reinitiated at the end of the TO.

5. Each training session finished after completion of all 60 trials or after 60 minutes, whichever came first. Animals were progressed onto the next training stage once they reached criteria (minimum 80% accuracy). Progressing through the training stages, stimulus duration decreased from 60 seconds to 1.5 seconds and LH decreased from 60 seconds to 5 seconds, while ITI and TO remained at 5 seconds.

At the end of training, all animals reached the same baseline performance, which entailed all 60 trials completed within one hour at an accuracy level of at least 80%. This ensured all animals were performing at a similar level before test days. The rate at which the animal progressed through the training stages provided a measure for their learning ability.

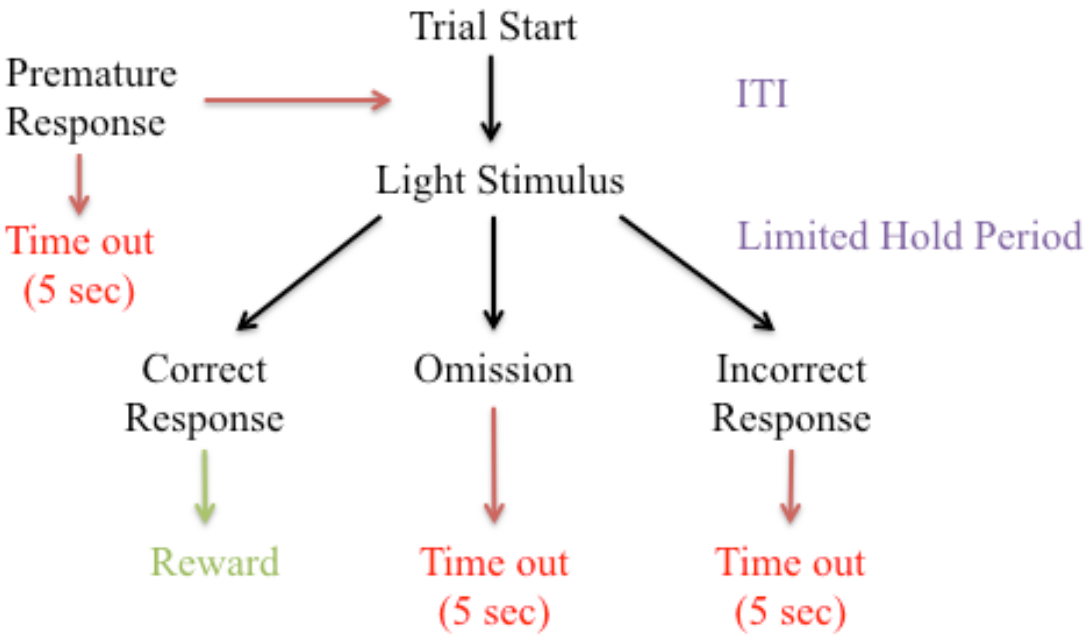

Figure 5. 5-CSRTT Trial Schematic Trials began with a 5 second inter-stimulus interval (ITI) in which the house light was illuminated. After 5 seconds a light stimulus was presented in one of the response locations. If the rat touched the correct response location within the limited hold period (LH,) a sugar pellet was delivered in the reward trough. Retrieval of the reward started the next trial. Omissions (failure to respond within LH) and incorrect responses resulted in a 5 second time out (TO) and a new trial was initiated. A premature response (responding during an ITI) also resulted in a TO and the same trial was reinitiated.

2.5.3 5-CSRTT Test Days

Progressing through test days, stimulus durations were decreased to challenge the animals. Additionally, on separate test days, an acoustic distractor was incorporated into testing during the ITI. The distractor was the same acoustic tone that was played to indicate a food reward. The protocol used during testing days was as follows:

Testing Day 1: Stimulus duration, 1.0 s

Testing Day 2: Stimulus duration, 0.8 s

Testing Day 3: Stimulus duration, 0.6 s

Testing Day 4: Stimulus duration, 0.5 s

Testing Day 5: With Distractor. Stimulus duration, 1.0 s

Testing Day 6: With Distractor. Stimulus duration, 0.8 s

Testing Day 7: With Distractor. Stimulus duration, 0.6 s

Testing Day 8: With Distractor. Stimulus duration, 0.5 s

2.5.4 Drug Administration

A positive BK channel modulator BMS-204352 was tested to determine its efficacy in improving cognitive outcome. Behavioural tests (5CSRTT) were performed at the maximum BMS-204352 brain concentration after systemic injections (i.e. 30 min after injection). BMS-204352 (TOCRIS, Avonmouth, Bristol) was diluted in a vehicle solution (DMSO 1/80; Tween 80 1/ 80; 0.9% NaCl) and was administered via a 10 ml/kg single intraperitoneal injection. This dose was administered as it is has shown to reverse behavioural and structural abnormalities in the FX mouse model (Hébert et al. 2014).

Four test groups were present:

1) VPA + BMS-204352

2) VPA + vehicle (DMSO 1/80; Tween 80 1/ 80; 0.9% NaCl)

3) Control + BMS-204352

4) Control + vehicle (DMSO 1/80; Tween 80 1/ 80; 0.9% NaCl)

Animals were tested with drugs using two different paradigms:

1) Basic testing day (stimulus duration 0.8 sec)

2) Testing day with distractor (stimulus duration 0.8 sec + distractor)

All animals acted as their own control. Animals were tested first with the vehicle, then with the BK channel modulator. Following BMS-204352 administration, subsequent drug test days were one week apart.

2.6 Statistical Analysis

Statistical Package for the Social Sciences (SPSS, Version 20.0.0, IBM Corporation) was used for statistical analysis. Data is expressed as group means ± the standard error of the mean (SEM). A 2-way ANOVA or a repeated measurement ANOVA was used to compare groups. For repeated measures ANOVA, in order to test if the data violated the sphericity assumption, the Mauchley test was used. In case of violation the degrees of freedom were corrected using the Greenhous-Geisser (if $\varepsilon < 0.75$) or the Huynh-Feldt method (if $\varepsilon > 0.75$). Post hoc paired t-tests with Bonferroni corrections were performed

where appropriate. Differences were considered statistically significant when $p<0.05$. In figures, significance levels were indicated as followed: *$p< 0.05$, **$p< 0.01$, ***$p<0.001$.

Chapter 3: Results

3 Results

3.1 Acoustic Startle Response

Two separate litters of VPA animals (total n=16) and one control litter (n=12) underwent startle and open filed testing at 6 weeks. Animals were then placed on food restriction before beginning training and testing on the 5 Choice Serial Reaction Time Task (5-CSRTT). At 4 months of age, animals were re-tested on the startle and open field tests.

Baseline Startle

Baseline startle provides a control measure and determines the startle response without an effect of habituation or sensitization. To calculate baseline startle, the first two startle responses on Day 1 of testing were averaged. At 6 weeks, a main effect of group was present ($F(1,24)=10.519$, $p=0.004$, Figure 6). VPA animals showed lower baseline startle responses when compared to controls indicating lower startle reactivity. No main effects of sex ($F(1,24)=1.889$, $p=0.182$) and no significant interaction of group and sex ($F(1,24)=1.588$, $p=0.220$) were present. At 4 months, there were no significant differences between VPA and control animals. No main effects of group ($F(1,24)=1.598$, $p=0.218$), or sex ($F(1,24)=2.902$, $p=0.101$) and no significant interaction of group and sex ($F(1,24)=0.458$, $p=0.505$) were present.

In summary, at 6 weeks, VPA animals showed significantly lower baseline startle, suggesting lower startle reactivity. At 4 months of age, VPA and control animals showed no differences in baseline startle.

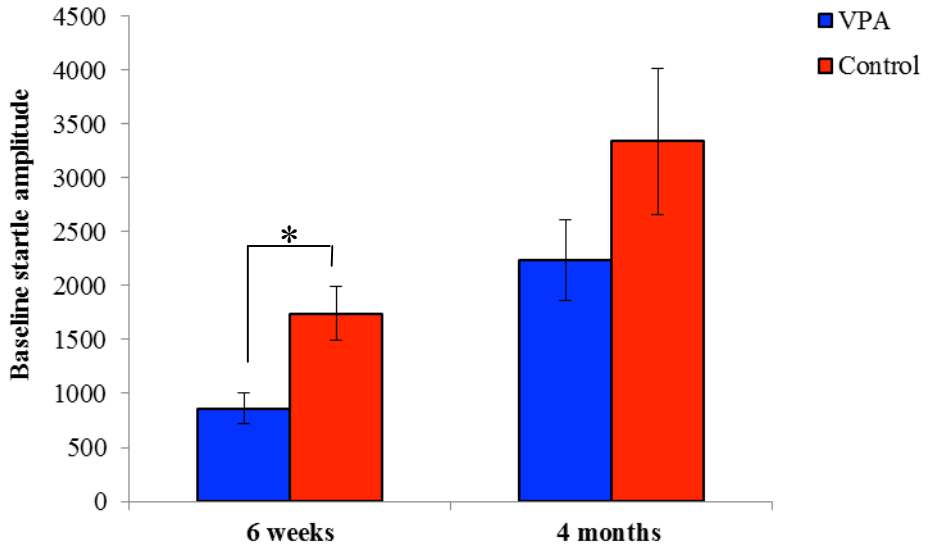

Figure 6. Day 1 baseline startle amplitudes at 6 weeks and 4 months of age. At 6 weeks a main effect of group was present (p=0.004). VPA animals showed lower baseline startle on day 1 of testing when compared to controls. At 4 months there were no significant differences between the groups in baseline startle (p=0.218, VPA n=16, Control n=12).

Short Term Habituation

Habituation is an important sensory filtering mechanism that can be easily assessed through the acoustic startle reflex. It corresponds to a decrease in the startle amplitude upon repeated exposure to the startle stimulus. Short term habitation (STH) refers to a decrease in startle response within a single testing session. Figure 7 shows normalized short term habituation curves over 50 trials on Day 1 of testing (Figure 7). A 3-way repeated measures ANOVA revealed a significant effect of trial at 6 weeks ($F(49,1176)=2.760$, $p<0.001$), indicating that animals did habituate. Furthermore, there was no main effect of group ($F(1,24)=0.187$,$p=0.669$), and no significant interaction of trial and group ($F(49,1176)=1.026$, $p=0.426$), indicating that both control animals and VPA animals exhibited a similar decrease in startle responses over trials (Figure 7). At 4 months, there was a significant effect of trial ($F(49,1176)=5.879$, $p<0.001$). No main effect of group ($F(1,24)=0.187$,$p=0.669$), and no significant interaction of trial and group ($F(49,1176)=2.302$, $p=0.142$) were presented. In summary, normalized STH curves showed a decrease in startle responses over trials relatively to the same degree in both groups indicating STH (Figure 7).

In order to quantify STH, short-term habituation ratios were calculated by taking the average of the last ten responses and dividing them by the average of the first two responses for each animal and for each day. These ratios are then averaged over five days in order to determine an overall STH ratio. A greater STH ratio indicates that less habituation is occurring. At 6 weeks of age, a 2-way ANOVA revealed a main effect of group ($F(1,24)=0.976$, $p=0.041$). STH ratios were greater in the VPA animals suggesting an impairment in STH when compared to controls. At 4 months of age, there were no

significant differences between the groups ($F(1,24)=0.795$, $p=0.382$, Figure 8).

Overall, Day 1 STH curves showed no significant difference between VPA and control animals at any time point. STH ratios however, revealed a significant impairment in STH in VPA animals at 6 weeks of age. At 4 months, STH ratios showed no significant differences between the VPA and control groups. Thus, the impairment in STH in VPA animals seemed to ameliorate when the animals reached adulthood.

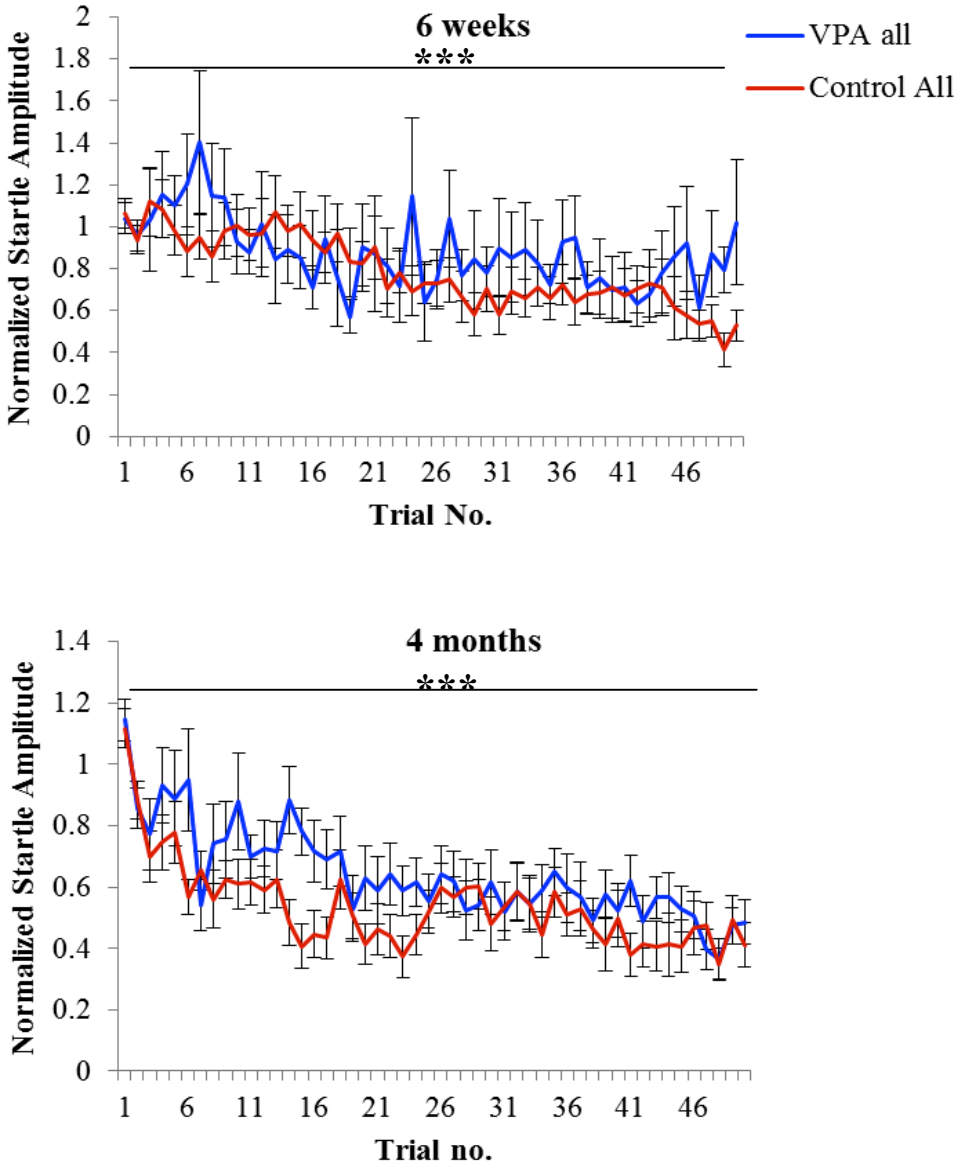

Figure 7. Normalized short term habituation curves on Day 1. A. At 6 weeks of age, there was a significant effect of trial (p<0.001). VPA and control animals showed a decrease in startle responses over time, indicating STH. **B.** At 4 months of age, there was a significant effect of trial (p<0.001). Both groups showed a decrease in startle response indicating STH (VPA n=16, Control n=12).

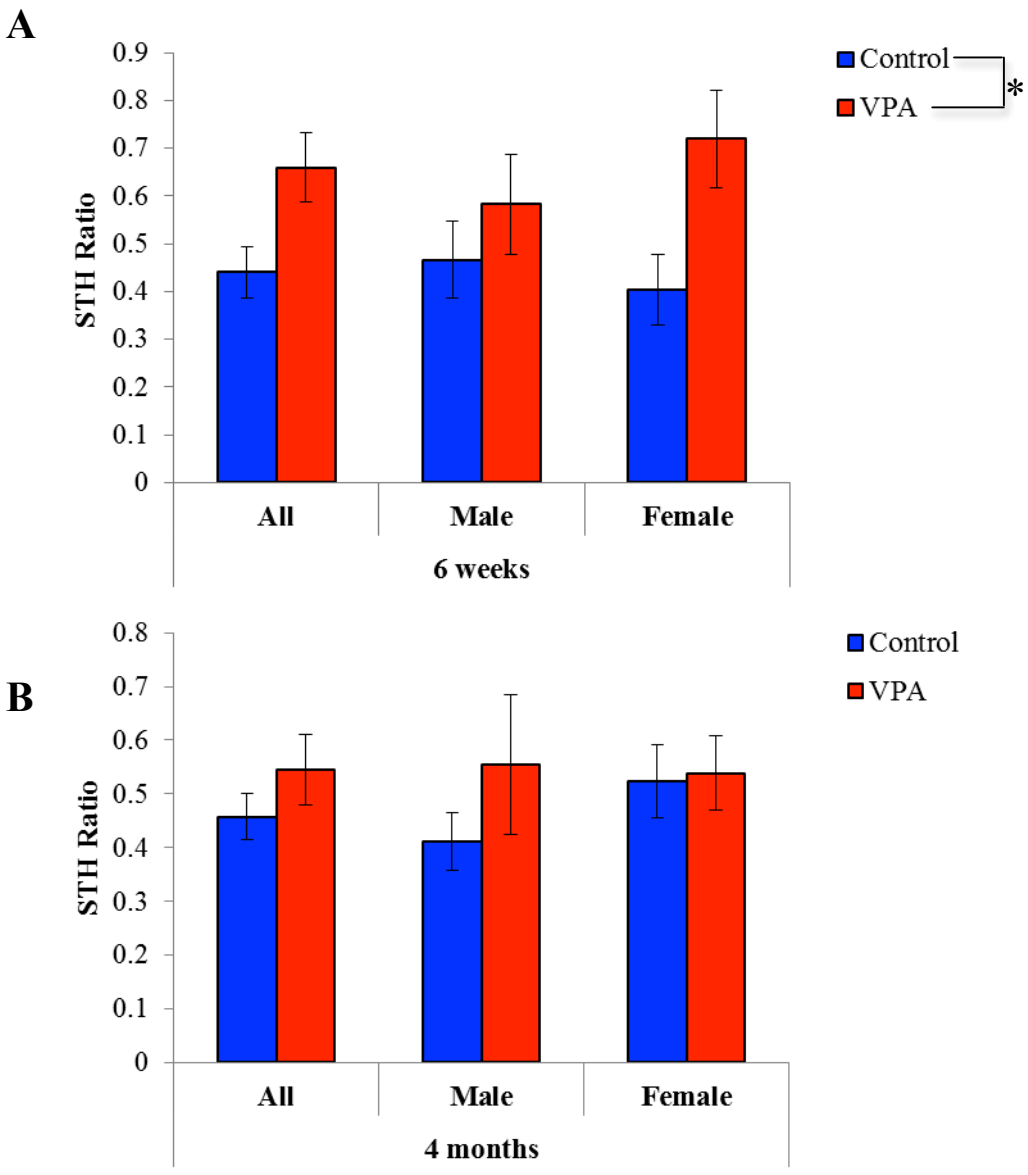

Figure 8. Short term Habituation ratios. A. At 6 weeks of age, a main effect of group was present (p=0.041). STH ratios were greater in the VPA group, indicating greater impairments in STH when compared to controls. **B.** At 4 months, there were no significant differences between the groups (p=0.382, VPA n=16, Control n=12).

Long Term Habituation

Long term habituation refers to the decrease in startle amplitudes across testing days. Normalized long-term habituation (LTH) curves are depicted in Figure 9A &Figure 10A. Normalized startle amplitudes are calculated by normalizing every trial on every day to the average of the first two responses on the first day of testing. The resulting normalized startle amplitudes for each trial within each testing session can then be plotted for each day (Figure 9A &Figure 10A).

At 6 weeks, 3-way repeated measures ANOVA revealed a significant effect of day ($F(4,96)=10.393$, $p<0.001$), a main effect of group ($F(1,24)=4.743$, $p=0.039$), as well as an interaction between day and group ($F(4,96)=10.393$), $p<0.001$, Figure 9A). This suggests that LTH across days was different in VPA from controls. These results were further supported when normalized responses were averaged for each testing day (Figure 9B &Figure 10B). At 6 weeks, a 3-way repeated measures ANOVA revealed a no significant effect of day ($F(4,96)=0.105$, $p=0.981$) and no main effects of group ($F(1,24)=2.542$, $p=0.124$). However, a significant interaction between day and group ($F(4,96)=4.195$, $p=0.004$) was present, thus further supporting a difference in LTH between groups over testing days (Figure 9B). Post-hoc paired t-test analysis with Bonferroni corrections revealed a significant difference in averaged normalized startle responses between Day 1 and Day 5 in control animals ($t_{11}=4.620$, $p=0.001$) suggesting LTH. However no significant differences in VPA animals between Day 1 and Day 5 were observed ($t_{15}=-1.725$, $p=0.105$), indicating a lack of LTH in VPA animals during adolescence.

At 4 months, a 3-way repeated measures ANOVA revealed a significant effect of day ($F(4,96)=18.634$, $p<0.001$), no main effects of group ($F(1,24)=0.155$, $p= 0.697$) and no significant interaction of day and group ($F(4,96)=2.290$, $p= 0.065$). Both groups displayed a decrease in startle amplitude across days, indicating LTH (Figure 10A). This was further supported when comparing averaged normalized startle responses across days (Figure 10B). A 3-way repeated measures ANOVA revealed a main effect of day ($F(4,96)=7.973$, $p<0.001$), no main effect of group ($F(1,24)=0.397$, $p=0.078$), and no significant interaction of day and group ($F(4,96)=2.207$, $p=0.074$). Both groups showed a decrease in averaged normalized startle responses over testing days, indicating LTH.

In summary, VPA animals displayed a lack of LTH at 6 weeks of age. The impairment in LTH in VPA animals seemed to normalize when the animals reached adulthood.

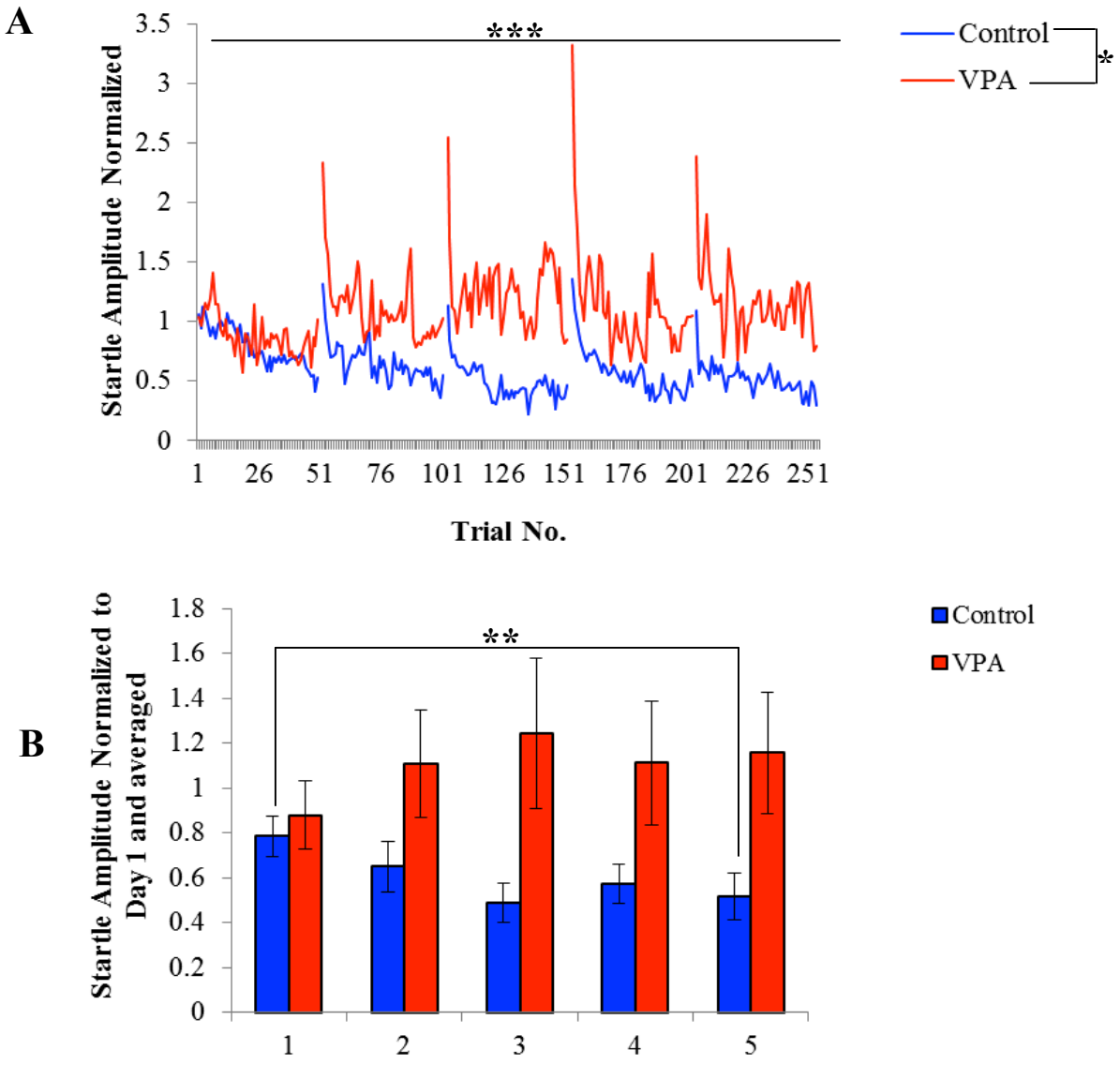

Figure 9. Long Term Habituation at 6 weeks of age. A. Normalized startle amplitudes over five days of testing at 6 weeks of age. A main effect of group was present (p=0.039). Control animals showed a decrease in startle amplitude over days indicating LTH. VPA animals seemed to show sensitization in startle responses over time. **B.** Normalized and averaged startle amplitudes over five days of testing at 6 weeks of age. Post hoc analysis revealed a significant difference in averaged normalized startle responses between Day 1 and Day 5 in control animals only (p=0.001), indicating that control animals showed LTH while VPA animals did not (VPA n=16, Control n=12).

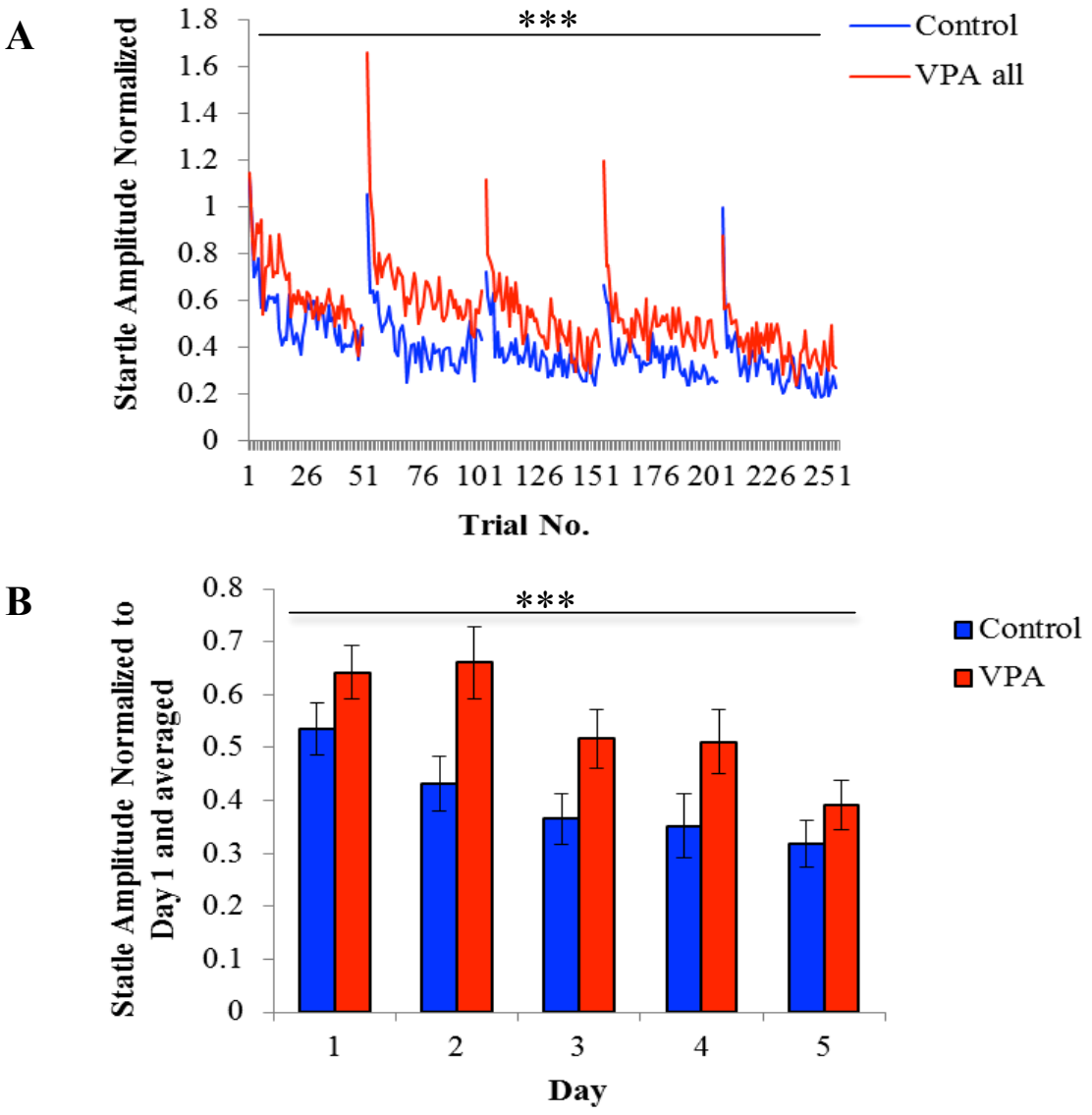

Figure 10. Long Term Habituation at 4 months of age. A. Normalized startle amplitudes over five days of testing at 4 months of age. At 4 months, a main effect of day was present ($p<0.001$). Both groups showed a decrease in startle amplitude over testing days indicating LTH. **B.** Normalized and averaged startle amplitudes over five days of testing at 4 months of age. A main effect of day was present ($p<0.001$), both groups showed LTH (VPA n=16, Control n=12).

Latency to Startle

Latency to startle refers to the time between onset of the startle stimulus and either the onset or the peak of the startle response. We here measured the time (msec) to reach peak startle amplitude. At 6 weeks of age, a 3-way repeated measures ANOVA revealed a significant effect of trial, ($F(49,1176)=1.759$, $p=0.001$), main effect of group ($F(1,24)=11.3$, $p=0.003$) and a significant interaction between trial and group. Overall, latency to startle peak changed differently over trials between the two groups ($F(49,1176)=2.003$, $p=0.000$, Figure 11A). VPA animals showed a significantly lower response time when compared to controls. At 4 months, there was a main effect of trial ($F(49,1176)=1.462$, $p=0.022$), but no main effect of group $F(1,24)=0.729$, $p=0.402$) and no significant interaction of trial and group ($F(49,1176)=1.055$, $p=0.372$), indicating that both groups displayed STH and exhibited no differences in response time (Figure 11B).

Long-term habituation (LTH) refers to a decrease in latency to startle between testing sessions. It was measured by averaging the latencies within each testing day. At 6 weeks, 3-way repeated measures ANOVA revealed no main effects of group ($F(1,24)=0.065$, $p=0.801$). However, a main effect of day ($F(4,96)=3.801$, $p=0.007$) and a significant interaction of day and group ($F(4,96)=10.262$, $p=0.000$, Figure 12A) was present. Post hoc paired t-tests with Bonferroni corrections revealed a significant difference in latency to peak startle between Day 1 and Day 5 of testing in control animals ($t_{11}=5.880$, $p=0.000$) , indicating LTH of latency to startle. No significant differences in latencies between Day 1 and Day 5 in VPA animals were observed indicating an impairment of LTH of startle latencies at 6 weeks ($t_{15}=-2.290$, $p=0.037$). At 4 months, there was a main effect of day ($F(4,96)=11.810$, $p=0.000$, Figure 12B), but no

effect of group ($F(1,24)=0.281$, $p=0.601$) or interaction of group and day ($F(4,96)=0.848$, $p=0.498$), indicating that both groups showed a similar decrease in latency to startle across days.

Overall, at 6 weeks both groups showed a decrease in latency to startle within a testing session. However, only control animals showed a decrease in latency to startle across testing days, whereas VPA animals did not exhibit any reduction of peak startle latencies. Furthermore at 6 weeks, VPA animals showed an overall lower response time when compared to controls. At 4 months, both groups displayed no differences in response time and exhibited both STH and LTH of latency to startle.

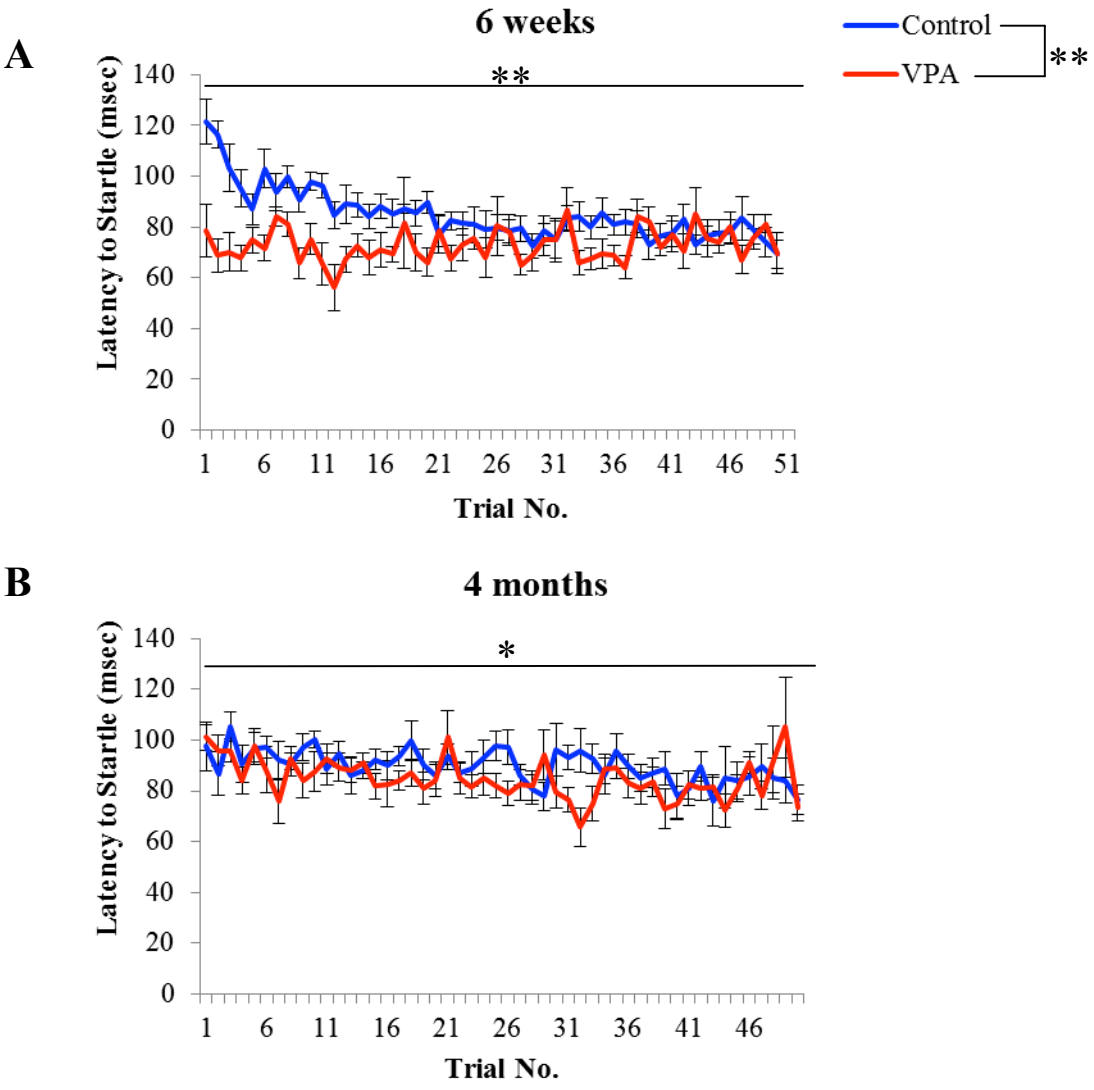

Figure 11. Short Term Habituation of Latency to Startle on Day 1. A. At 6 weeks of age, both groups showed STH of latency to startle (p=0.001). A significant effect of group was also present (p=0.003). VPA animals showed a significantly lower response time when compared to controls. **B.** At 4 months, both groups showed STH of latency to startle (VPA n=16, Control n=12).

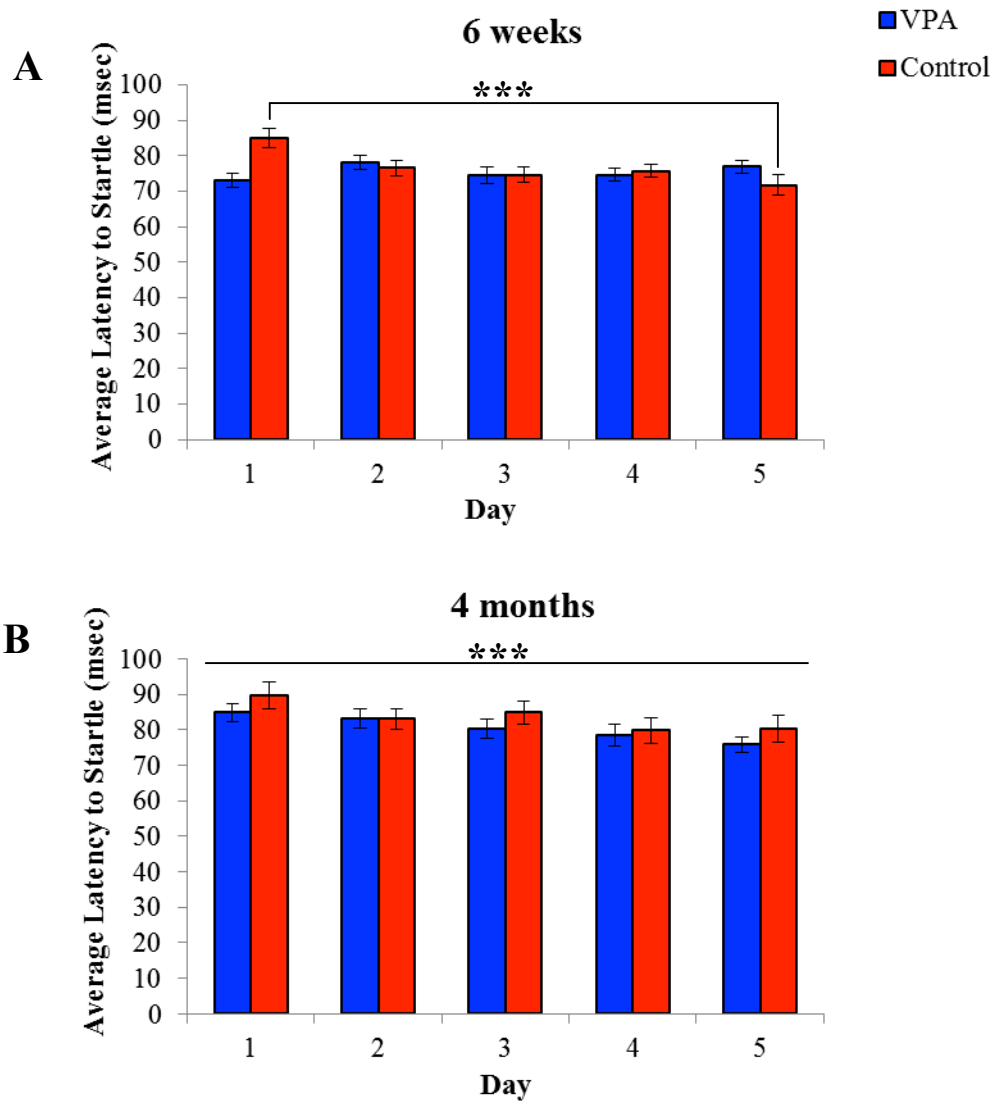

Figure 12. Long Term Habituation of Latency to Startle. A. At 6 weeks of age, post hoc analysis revealed a significant difference in average latency to startle between Day 1 and Day 5 in control animals ($p<0.001$) indicating LTH of startle latencies. However no significant differences between responses on Day 1 and Day 5 were present in VPA animals indicating impairment in LTH of startle latencies. **B**. At 4 months, a main effect of day was present ($p<0.001$). Both groups displayed a decrease in latency to startle over testing days indicating LTH (VPA n=16, Control n=12).

Prepulse Inhibition of acoustic startle response

Prepulse inhibition (PPI) provides a measure of sensorimotor gating and occurs when a weaker acoustic pre-stimulus attenuates the following response to a stronger acoustic stimulus. Prepulse inhibition was tested at 30 and 100 ms inter-stimulus intervals (ISI) with 75 and 85 dB prepulse intensities. At 6 weeks at the 30 ms ISI with a 75 dB prepulse, 2-way ANOVAs revealed no main effects of group ($F(1,24)=0.130$, $p=0.722$) or sex ($F(1,24)=2.026$, $p=0.167$) as well as no significant interaction of sex and group ($F(1,24)=0.149$, $p=0.703$,Figure 13A). Additionally at the 100 ms ISI with a 75 dB prepulse, there was no main effect of group ($F(1,24)=2.590$, $p=0.121$), no main effect of sex ($F(1,24)=0.677$, $p=0.419$) and no significant interaction of sex and group ($F(1,24)=2.305$, $p=0.142$, Figure 13C). However, the 85dB prepulse at the 100 ms ISI produced significantly different PPI between the groups ($F(1,24)=4.852$, $p=0.037$, Figure 13D) but no significant difference between sexes ($F(1,24)=3.370$, $p=0.079$) as well as no significant interaction of sex and group were observed ($F(1,24)=1.185$, $p=0.287$) . Overall, VPA animals displayed less inhibition of the startle response following a prepulse when compared to controls, indicating impairment in PPI. Lastly, no group differences ($F(1,24)=2.389$, $p=0.135$), no sex differences ($F(1,24)=1.212$, $p=0.282$) and no significant interaction of sex and group ($F(1,24)=0.011$, $p=0.918$) were observed at the 30 ms ISI with an 85 dB prepulse (Figure 13B).

At 4 months, at the 30 ms ISI with a 75 dB prepulse, 2-way ANOVAs revealed no main effects of group ($F(1,24)=0.299$, $p=0.590$), no main effect of sex ($F(1,24)=0.643$, $p=0.430$), as well as no significant interaction of sex and group ($F(1,24)=1.964$, $p=0.174$, Figure 14A). Furthermore, at the 100 ms ISI with a 75 dB prepulse, there were also no

main effects of group ($F(1,24)=0.037$, $p=0.850$) or sex ($F(1,24)=0.012$, $p=0.912$) and no significant interaction of sex and group ($F(1,24)=0.423$, $p=0.522$, Figure 14C). However, the 85dB prepulse at the 100 ms ISI showed main effects of both group ($F(1,24)=6.28$, $p=0.020$) and sex ($F(1,24)=4.643$, $p=0.041$), but no significant interaction of sex and group was present ($F(1,24)=0.658$, $p=0.425$, Figure 14D). Overall, this indicates that VPA animals showed less inhibition of the startle response following a prepulse, indicating impairment in PPI was equal across sexes. Lastly, no group differences ($F(1,24)=0.104$, $p=0.750$), no sex differences ($F(1,24)=0.522$, $p=0.477$) as well as no significant interaction of sex and group ($F(1,24)=1.885$, $p=0.182$, Figure 14B) were observed at the 30 ms ISI with an 85 dB prepulse.

In summary, mild impairments in PPI were seen with a prepulse intensity of 85 dB at the 100 ms ISI. VPA animals displayed these impairments in PPI at 6 weeks, and they continued into adulthood.

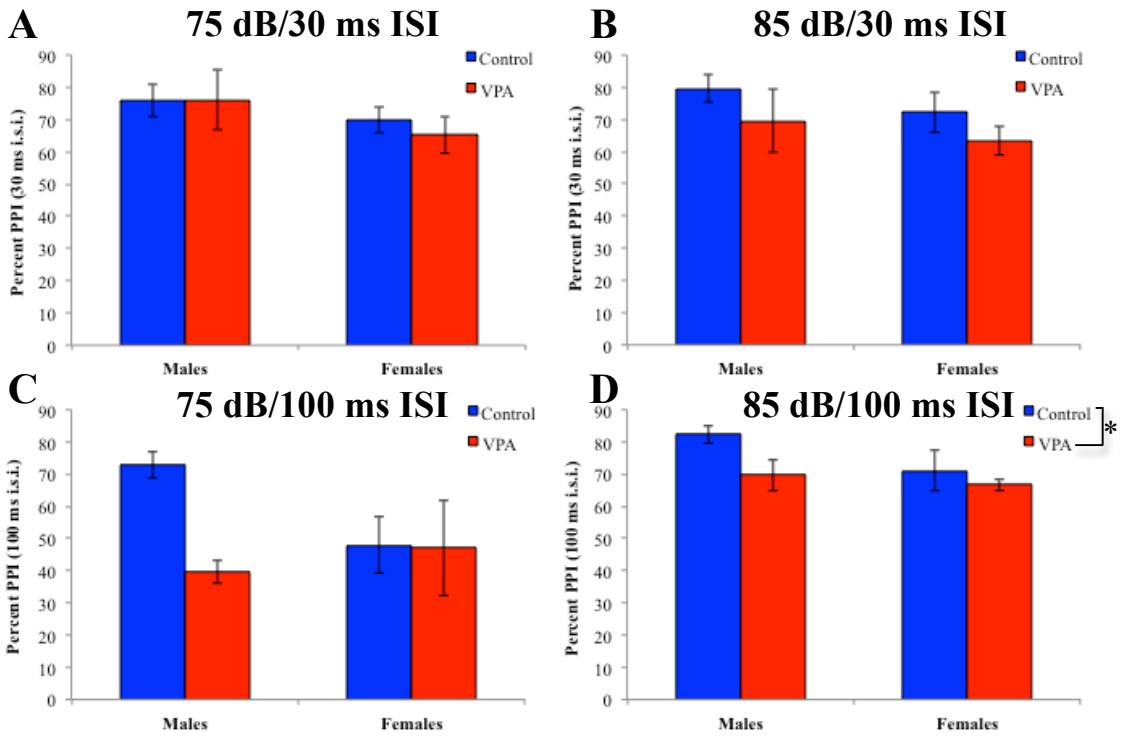

Figure 13. Prepulse Inhibition of the Acoustic Startle Response at 6 weeks of age. A. At 6 weeks at the 75 dB prepulse/30 msec inter-stimulus interval, there were no significant differences observed between VPA and control groups (p=0.722). **B.** At 6 weeks at the 85 dB prepulse/30 ms inter-stimulus interval, there were no significant differences between VPA and control animals (p=0.135). **C.** At 6 weeks at the 75 dB prepulse/100 msec inter-stimulus interval, no significant differences between the groups were observed (p=0.121). **D.** At 6 weeks at the 85 dB prepulse/100 ms inter-stimulus interval, a main effect of group was present (p=0.037). VPA animals displayed less PPI when compared to controls (VPA females n=9, VPA males n=7, control females n=5, control males n=7).

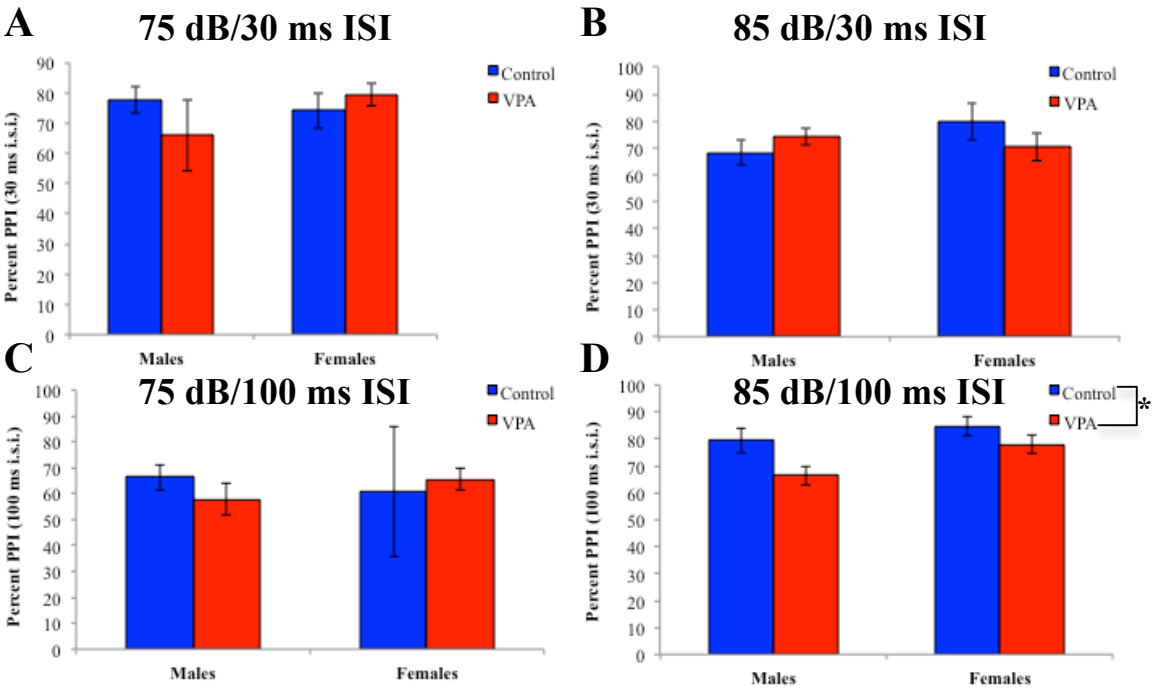

Figure 14. Prepulse Inhibition of the Acoustic Startle Response at 4 months of age. **A.** At 4 months at the 75 dB prepulse/30 msec inter-stimulus interval, there were no significant differences observed between VPA and control groups (p=0.590). **B.** At 6 weeks at the 85 dB prepulse/30 ms inter-stimulus interval, there were no significant differences between VPA and control animals (p=0.182). **C.** At 4 months at the 75 dB prepulse/100 msec inter-stimulus interval, no significant differences between the groups were observed (p=0.850). **D.** At 4 months at the 85 dB prepulse/100 ms inter-stimulus interval, a main effect of group was present (p=0.020). VPA animals displayed less PPI when compared to control (VPA females n=9, VPA males n=7, control females n=5, control males n=7).

3.2 Open Field Testing

Rats were subjected to open field testing in order to examine exploratory and anxiety—like behaviour. At 6 weeks of age, animals were tracked for 20 minutes in the locomotor boxes. Data was binned into 5 minute blocks. 3-way repeated measures ANOVA revealed main effects of group ($F(1,21)=67.047$, $p<0.001$), indicating that VPA animals showed hyper-locomotion when compared to controls. We also observed a main effect of sex ($F(1,21)=6.624$, $p=0.018$) Both groups displayed sex differences, with females across both groups displaying greater locomotive activity when compared to their male counter parts. There was also a significant interaction of day, block and group ($F(3,63)=0.543$, $p=0.048$) , Figure 15A). Furthermore, VPA and control animals showed both STH and LTH, as there was a significant effect of block ($F(1,21)=174.208$, $p<0.001$) and day ($F(1,21)=7.030$, $p=0.015$; Figure 15A).

At 4 months, 3-way repeated measures ANOVA revealed no significant effect of day ($F(1,16)=0.728$, $p=0.406$), no main effect of group ($F(1,16)=0.239$, $p=0.631$), and no significant differences between sex ($F(1,16)=0.015$, $p=0.903$). However, a significant interaction of day, block and group ($F(3,48)=3.542$, $p=0.021$, Figure 15B) was present, suggesting that both groups behaved differently across blocks and testing days. Post hoc paired t-tests with Bonferroni corrections revealed a significant difference between Day 1 Block 1 and Day 5 Block 1 in VPA females ($t_6=11.693$, $p< 0.001$) and control males ($t_5=10.492$, $p< 0.001$), indicating LTH of locomotion in these two groups. No differences were seen in VPA males ($t_4=3.968$, $p=0.017$) and control females ($t_1=7.416$,

P=0.085), suggesting that these two groups failed to show LTH of locomotor behaviour. Lastly, a significant effect of block was present ($F(3,16)=124.761$, $p<0.001$) indicating that STH persisted into adulthood in both groups.

We also analyzed the time spend in the center of the box versus along the walls as an indicator for anxiety. A 2-way ANOVAs revealed that VPA animals exhibited greater anxiety levels at 6 weeks when compared to controls as indicated by lower center: wall ratios ($F(1,21)=13.632$, $p=0.001$,Figure 16A). However, no main effects of sex ($F(1,21)=0.071$, $p=0.793$) and no significant interaction of sex and group ($F(1,21)=0.003$, $p=0.959$) were observed. At 4 months, no significant group differences ($F(1,16)=0.143$, $p=0.710$,Figure 16B) or sex differences ($F(1,16)=0.123$, $p=0.730$) as well as no significant interaction of sex and group ($F(1,16)=0.000$, $p=0.994$) were observed.

In summary, VPA animals showed hyper-locomotive activity and higher levels of anxiety at 6 weeks of age. This group difference was no longer present at 4 months. Furthermore, both groups showed STH of locomotive behaviour at both time points. LTH was seen in both groups at 6 weeks, but at the 4 month period, only VPA females and control males exhibited LTH of exploratory behaviour.

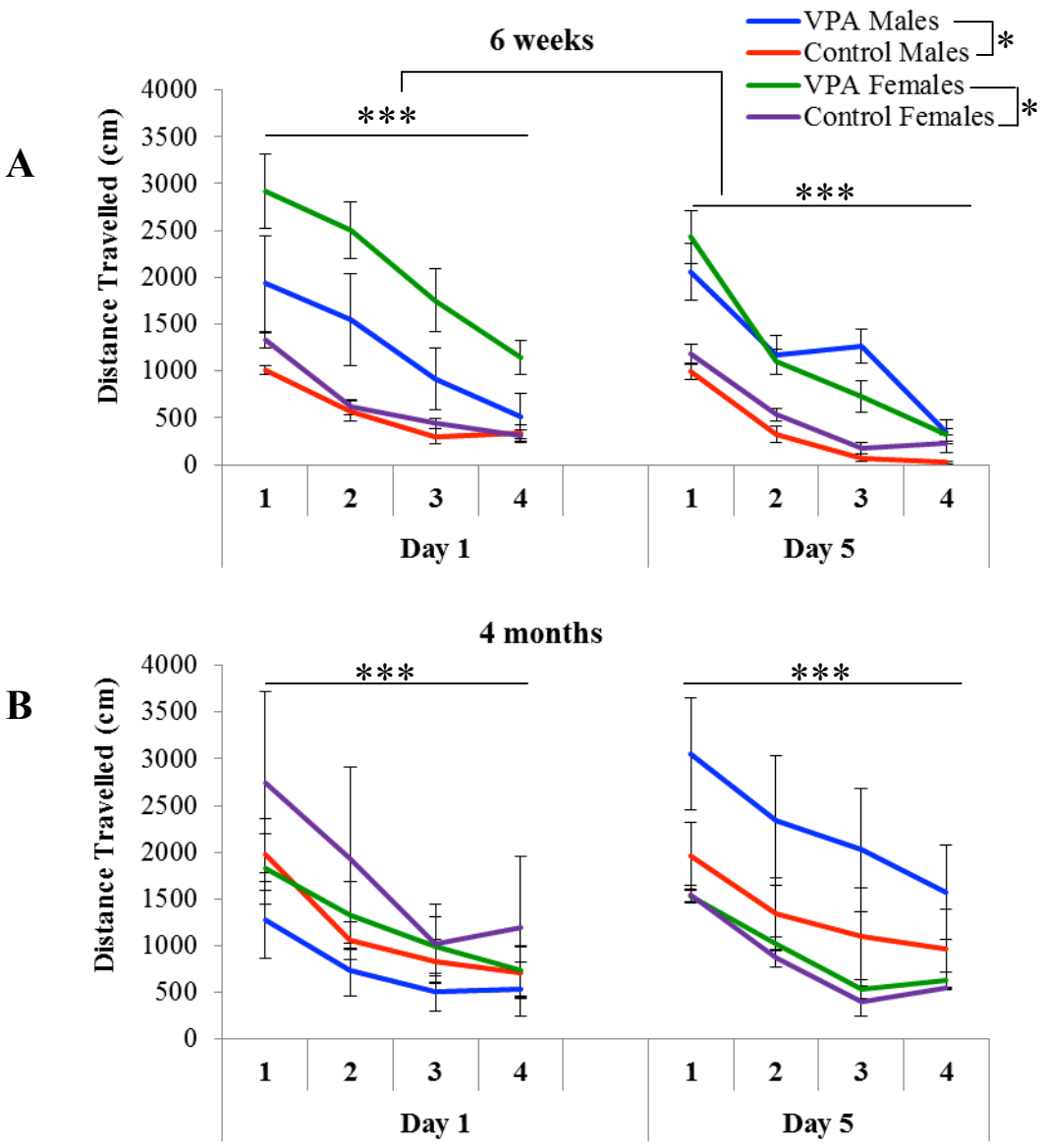

Figure 15. Habituation of Locomotive Behaviour. Graphs show locomotor activity at Day 1 and Day 5 of testing. Testing was carried out for 20 minutes which was divided into 4 blocks of 5 minute intervals. **A.** At 6 weeks, a main effect of trial (p<0.001) and day (p=0.015) were present. Both groups displayed STH and LTH of locomotor behaviour. Furthermore, a main effect of group was present (p<0.001). VPA animals showed hyper-locomotion when compared to controls. A main effect of sex was also present (p=0.018). Females across both groups displayed greater locomotive activity when compared to male counter parts (VPA females n=9, VPA males n=7, control females n=5, control males n=7). **B.** At 4 months, a main effect of trial was present (p<0.001) both groups displayed STH of locomotor behaviour (VPA females n=9, VPA males n=7, control females n=5, control males n=7).

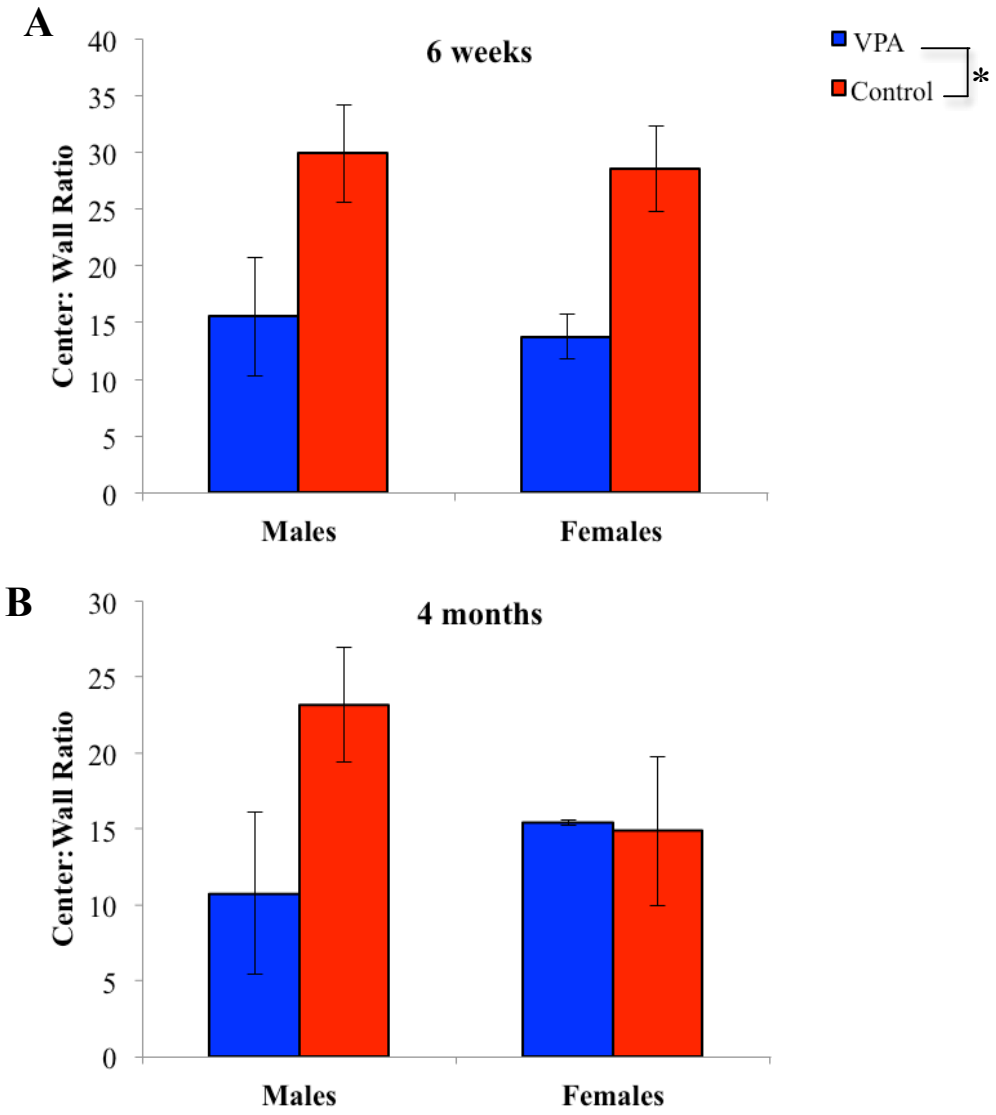

Figure 16: Center: Wall Ratio as an anxiety measure. A. At 6 weeks of age a main effect of group was present (p=0.001). VPA animals displayed greater levels of anxiety when compared to control animals (VPA n=12, Control n=13). **B.** At 4 months no significant group differences were observed (p=0.730, VPA females n=9, VPA males n=7, control females n=5, control males n=7).

3.3 Five Choice Serial Reaction Time Task (5-CSRTT)

Several aspects of cognitive function were measured through the 5-Choice Serial Reaction Time Task. Learning was measured by the time taken to progress through training stages (Figure 17). A 3-way repeated measures ANOVA revealed a significant effect of day ($F(20,480)=840.299$, $p=0.000$), a main effect of group ($F(1,24)=18.822$, $p=0.000$), no main effect of sex ($F(1,24)=0.264$, $p=0.612$) and a significant interaction of day and group ($F(20,480)=8.953$, $p=0.000$). VPA animals advanced through the learning stages significantly quicker than controls suggesting that VPA animals learned at a faster rate.

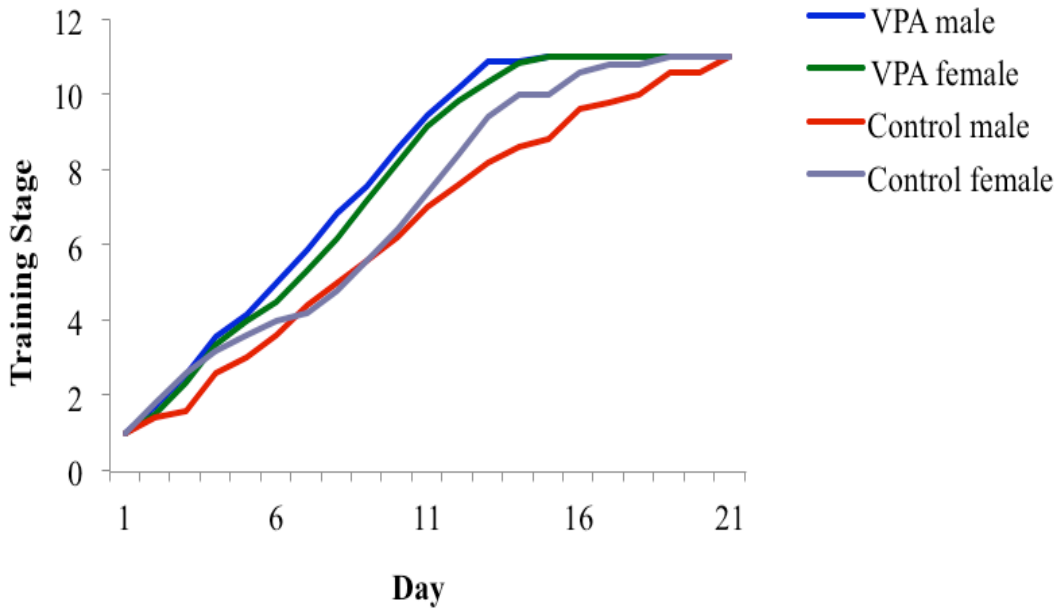

Figure 17. Progress through learning stages over time. A main effect of group was present ($p<0.001$). VPA animals advanced through the learning stages quicker than controls as indicated by steeper learning curves (VPA females n=9, VPA males n=7, control females n=5, control males n=7).

Basic Test Days

At basic testing days decreasing stimulus durations were delivered over four subsequent test days (see Material and Methods). Attentional control was measured through the percent accuracy of responses. 3-way repeated measures ANOVAs revealed no significant group ($F(1,23)=0.215$, $p=0.647$) or sex ($F(1,23)=0.570$, $p=0.458$) differences in accuracy of responses in the 5-choice serial reaction time task (Figure 18A). However, a main effect of day was present ($F(3,69)=32.982$, $p=0.000$). Accuracy in both groups decreased as stimulus duration decreased, indicating a decline in accuracy as the task gets more challenging with shorter stimulus durations. A significant interaction of day and group was also present ($F(3,69)=2.747$, $p=0.049$) indicating that VPA and control animals behaved differently across test days. Post hoc paired t-tests with Bonferroni corrections revealed no significant differences between VPA females and control females or between VPA males and control males at the 0.6 and 0.5 second stimulus duration (females $t_4=0.266$, $p=0.803$, $t_4=0.325$, $p=0.762$; males $t_5=1.392$, $p=0.223$, $t_5=0.198$, $p=0.850$ respectively).

Omissions indicate a failure to respond to a trial and is another indicator for attention. No main effects of group ($F(1,23)=0.791$, $p=0.383$), of sex ($F(1,23)=0.007$, $p=0.936$), or day ($F(3,69)=0.103$, $p=0.958$) nor any significant interaction of day and group ($F(3,69)=0.043$, $p=0.988$) were present (Figure 18B).

Impulsivity was measured through the amount of premature responses. Although there were no significant differences in premature responses between control and VPA animals ($F(1,23)=1.937$, $p=0.177$, Figure 18C), the number of premature responses increased

significantly over testing days ($F(3,69)=6.335$, $p=0.001$). No main effects of sex ($F(1,23)=0.227$, $p=0.638$) or significant interaction of day and group $F(3,69)=1.326$, $p=0.273$) were present.

Lastly, reward collection latency was measured (Figure 18D). The time taken to retrieve the reward provides a measure of motivation. Reward collection latency decreased in both groups as stimulus duration decreased ($F(3,69)=2.823$, $p=0.045$). No main effects of group ($F(1,23)=3.262$, $p=0.084$), sex ($F(1,23)=1.335$, $p=0.260$) or significant interaction of day and group ($F(3,69)=1.956$, $p=0.129$) were present.

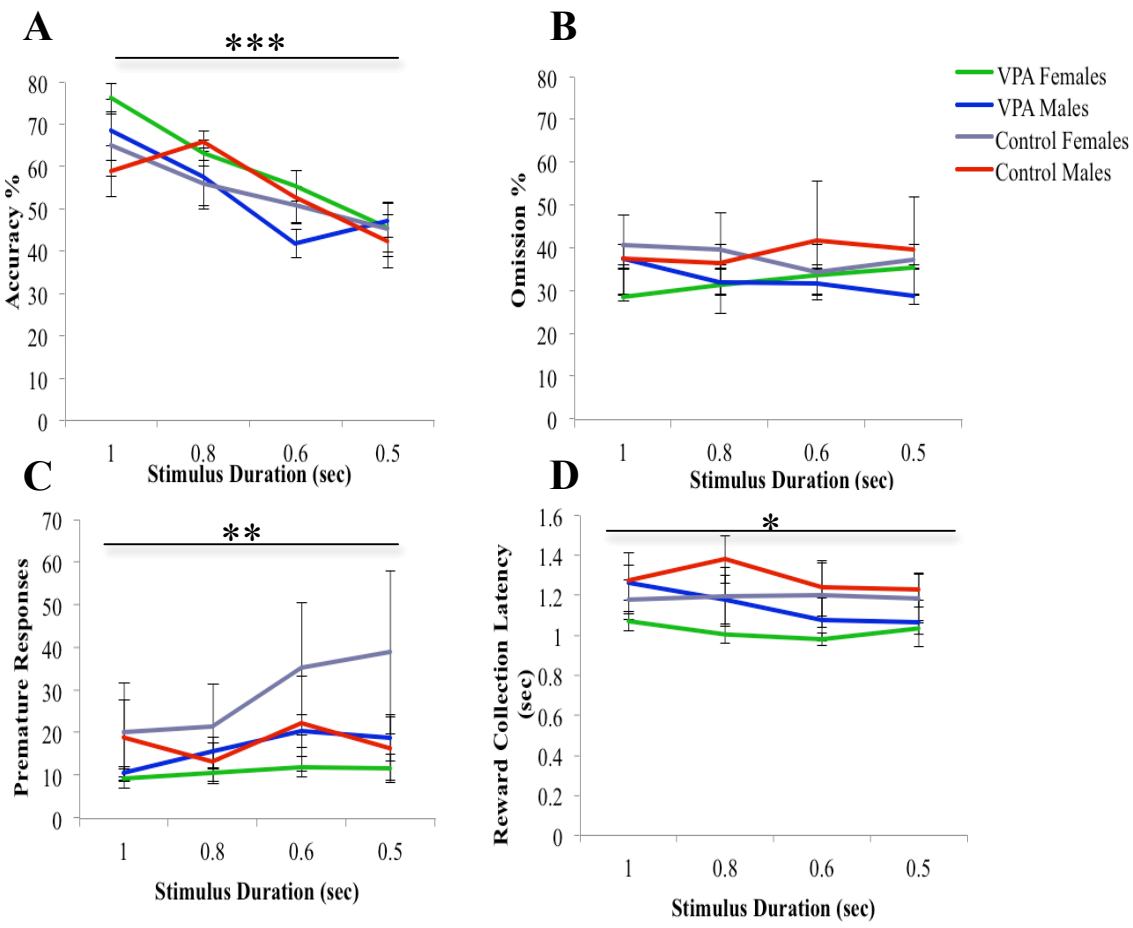

Figure 18 Performance on 5-CSRTT on basic test days. A. A main effect of stimulus duration was present ($p<0.001$). Accuracy in both groups decreased as stimulus duration decreased across testing days. **B.** No significant differences in omissions between VPA and controls were present ($p=0.383$). **C.** Impulsivity, measured through premature responses, increased significantly as stimulus duration decreased in both groups ($p=0.001$). **D.** Motivation, measured through reward collection latency, decreased in both groups as stimulus duration decreased ($p=0.045$, VPA females n=9, VPA males n=7, control females n=5, control males n=7).

Test days with a distractor

The second testing protocol was similar to the basic test days, but included an auditory distractor during the time the animal need to pay attention. A 3-way repeated measure ANOVA revealed a decreasing accuracy on the testing days with the distractor in both groups as stimulus duration decreased ($F(3,66)=8.521$, $p<0.001$, Figure 19A). A main effect of group was also present ($F(1,22)=12.967$, $p=0.002$) with VPA animals performing with higher accuracy than controls, indicating better attentional control. No main effect of sex ($F(1,22)=0.780$, $p=0.387$) and no significant interaction of day and group ($F(3,66)=0.911$, $p=0.441$) were observed.

In terms of omissions, there were no significant effects of day ($F(3,66)=1.091$, $p=0.359$), group ($F(1,22)=2.184$, $p=0.154$), or sex ($F(1,22)=0.221$, $p=0.643$), and no significant interaction of day and group were present ($F(3,66)=1.420$, $p=0.245$, Figure 19B).

For premature responses no significant effects of day ($F(3,66)=0.428$, $p=0.733$) or group, ($F(1,22)=2.953$, $p=0.100$) and no significant interaction of day and group ($F(3,66)=0.329$, $p=0.804$), were present (Figure 19C). However a significant effect of sex was present ($F(1,22)=6.522$, $p=0.018$) with females in both groups displaying a greater number of premature responses.

Lastly, analysis of reward collection latency revealed no significant effects of day ($F(3,69)=0.917$, $p=0.437$), group ($F(1,23)=0.251$, $p=0.621$), or sex ($F(1,23)=3.790$, $p=0.064$), and no significant interaction of day and group ($F(3,69)=0.458$, $p=0.712$, Figure 19D).

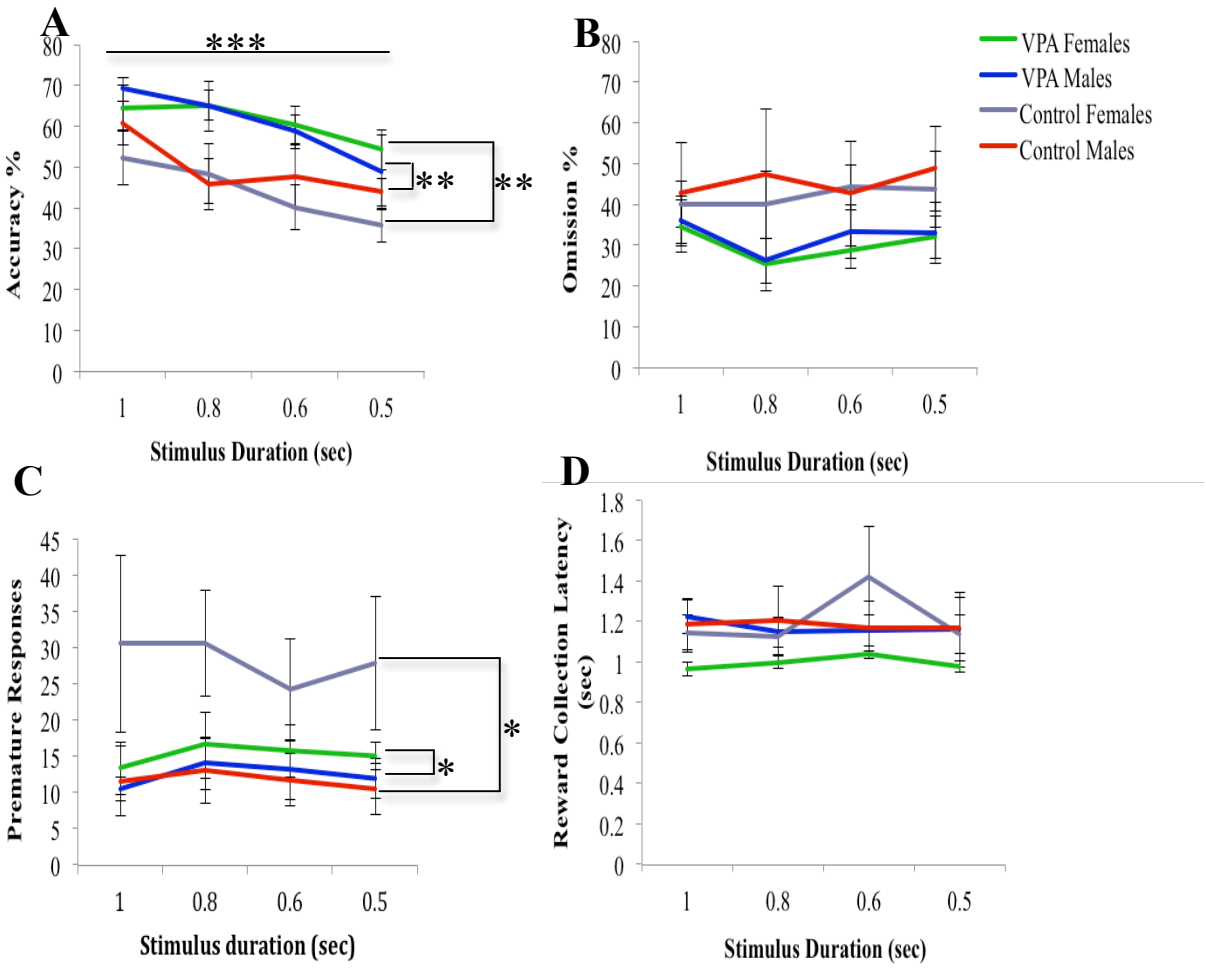

Figure 19. Performance on 5-CSRTT on test days with a distractor. A. A main effect of group was present (p=0.002). VPA animals performed with greater accuracy when compared to controls. A main effect of stimulus duration was also present (p<0.001). Accuracy decreased as stimulus duration decreased in both groups. **B.** No significant differences in omissions between VPA and controls were present (p=0.154). **C.** No significant group differences were present (p=0.100). A main effect of gender however was present (p=0.018). Females showed greater impulsivity across testing days in both groups. **D.** No significant differences in motivated behaviour were present between both groups (p=0.621, VPA females n=9, VPA males n=7, control females n=5, control males n=7).

Test days with and without distractor

In order to determine the effects of a distractor on cognitive outcome, performance on basic test days and distractor test days were compared in control animals. 3-way repeated measures ANOVA revealed decreasing accuracy in both groups as stimulus durations decreased ($F(3,54)=12.680$, $p<0.001$, Figure 20A). However, no main effects of sex ($F(1,18)=0.447$, $p=0.512$) or type of test (basic vs. distractor) ($F(1,18)=2.755$, $p=0.114$) and no significant interaction of day and type of test ($F(3,54)=1.034$, $p=0.385$) were present. Statistical analysis on omissions revealed similar results. No main effect of day ($F(3,54)=0.174$, $p=0.914$), sex ($F(1,18)=0.043$, $p=0.937$) or type of test ($F(1,18)=0.271$, $p=0.1609$) and no significant interaction of day and type of test ($F(3,54)=0.282$, $p=0.838$, Figure 20B) were observed. In terms of premature responses, there were no main effects of day ($F(3,54)=0.961$, $p=0.418$), sex ($F(1,18)=2.614$, $p=0.123$) or type of test ($F(1,18)=0.146$, $p=0.707$), however a significant interaction of stimulus duration and type of test ($F(3,54)=3.313$, $p=0.027$, Figure 20C) was present. Post hoc paired t-tests with Bonferroni corrections revealed no significant differences in premature responses between the 1 and 0.5 second stimulus duration in neither control females nor control males between basic test days and test days with distractor respectively (females $t_4=2.030$, $p=0.112$, $t_4=0.337$, $p=0.753$; males $t_5=0.952$, $p=0.385$, $t_5=0.208$, $p=0.844$ respectively). Lastly, no statistical differences in reward collection latency were present. There were no main effects of day ($F(3,54)=0.724$, $p=0.542$), sex ($F(1,18)=0.061$, $p=0.807$) or type of test ($F(1,18)=0.120$, $p=0.733$) and no significant interaction of day and type of test ($F(3,54)=1.039$, $p=0.383$; Figure 20D).

Overall, there were no significant differences in performance between basic test days and test days with a distractor, indicating that in contrast to our expectations, the distractor did not impact attentional control in any of the animals. As a result, the effects of the BK channel modulator are shown only for basic test days.

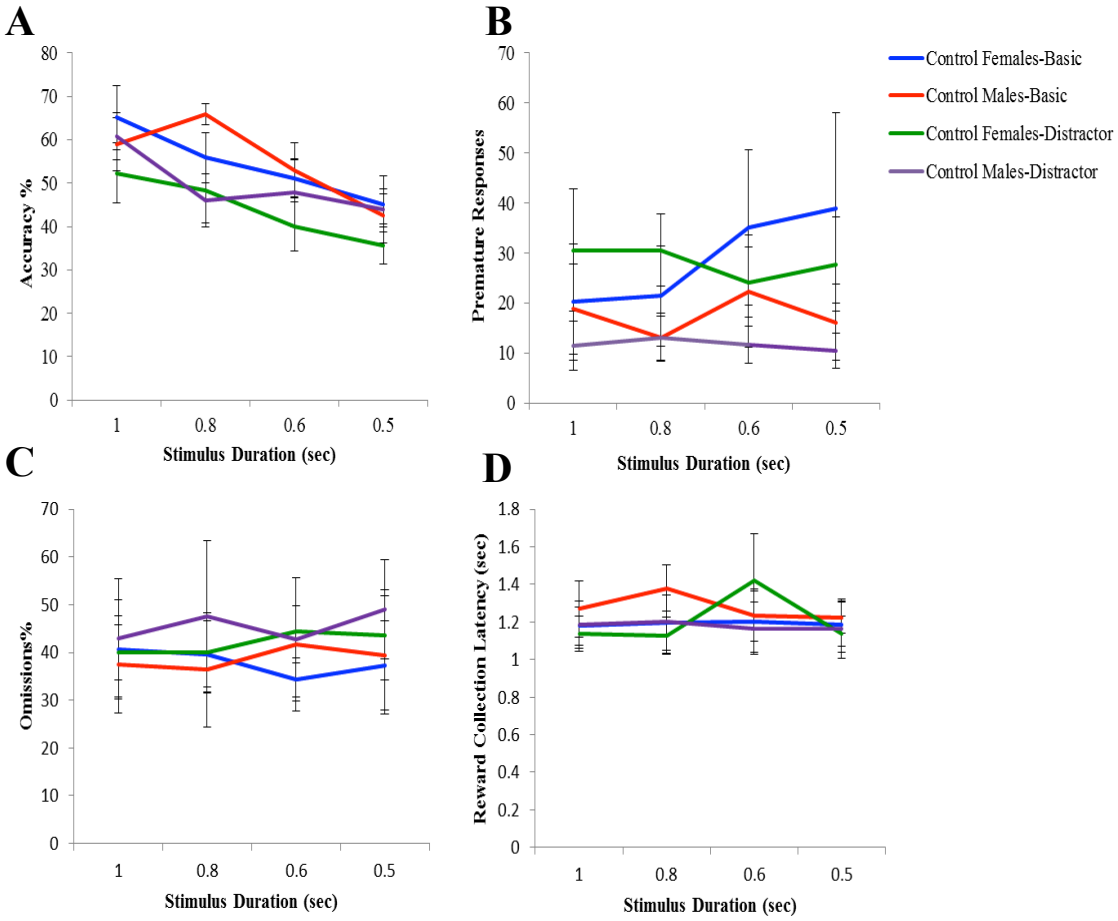

Figure 20. Performance on 5-CSRTT on basic test days vs. distractor test days in control animals. A. No significant differences in accuracy between the test types were present (p=0.114). **B.** No significant differences in premature responses between the test types were present (p=0.418). **C.** No significant differences in omissions between the test types were seen (p=0.160). **D**. No significant differences in reward collection latency between the test type were observed (p=0.733, VPA females n=9, VPA males n=7, control females n=5, control males n=7).

BK Channel Modulator

The effect of the positive BK channel modulator was measured on one basic testing day at stimulus duration of 0.8 seconds. 3-way repeated measures ANOVA revealed that the BK channel modulator increased accuracy across both groups when compared to saline conditions, thus improving attentional capacity ($F(1,23)=6.105$, $p=0.021$, Figure 21A). A main effect of group was also present, indicating that VPA animals performed with an overall greater accuracy when compared to controls ($F(1,23)=8.509$, $p=0.008$). Furthermore, no main effects of sex ($F(1,23)=0.094$, $p=0.762$) and no significant interaction of drug and group ($F(1,23)=0.137$, $p=0.715$) were observed. The BK channel modulator also affected impulsive behaviour (Figure 21C). Although no main effects of drug ($F(1,23)=0.254$, $p=0.619$), group ($F(1,23)=0.108$, $p=0.745$), or sex ($F(1,23)=0.277$, $p=0.604$) were present, statistical analysis revealed a significant interaction of drug and group ($F(1,23)=29.404$, $p<0.001$). Post hoc paired t-test with Bonferroni corrections revealed a significant difference in premature responses in VPA animals ($t_{15}=3.907$, $p=0.001$) and control animals ($t_{10}=4.000$, $p=0.003$) before and after drug administration. Therefore, BK channel modulator significantly decreased impulsivity in VPA animals but had the opposite effect in controls. In terms of omissions, no main effects of drug ($F(1,23)=1.824$, $p=0.190$) , group ($F(1,23)=1.886$, $p=0.183$) or sex ($F(1,23)=0.122$, $p=0.730$), and no significant interaction of drug and group ($F(1,23)=1.968$, $p=0.174$, Figure 21B) were present. Similar results were seen with reward collection latency. No effects of drug ($F(1,23)=0.128$, $p=0.724$), group ($F(1,23)=0.020$, $p=0.888$) or sex ($F(1,23)=1.824$, $p=0.190$) and no significant interaction of drug and group ($F(1,23)=2.189$, $p=0.153$, Figure 21D) were observed.

Overall, VPA animals showed no significant impairments in cognition when compared to controls. Interestingly, VPA animals learned at a faster rate than control animals on training days. On both basic test days and test days with a distractor, attentional control and impulsivity decreased in both groups as stimulus duration decreased. However, when comparing performance on basic test days and test days with a distractor, no significant differences were observed. As a result, the effects of a BK channel modulator were tested on a basic test day only. The BK channel modulator was able to improve cognitive outcomes in both groups as measured in an increase of accuracy across both test days, with VPA animals performing at an overall greater accuracy than controls. Interestingly, the relative high impulsivity in VPA animals was improved by administering the BK channel modulator across both test days, whereas it had the opposite effect on the relative low impulsivity in control animals. Lastly, no effects of the drug on omissions or reward collection latency were seen.

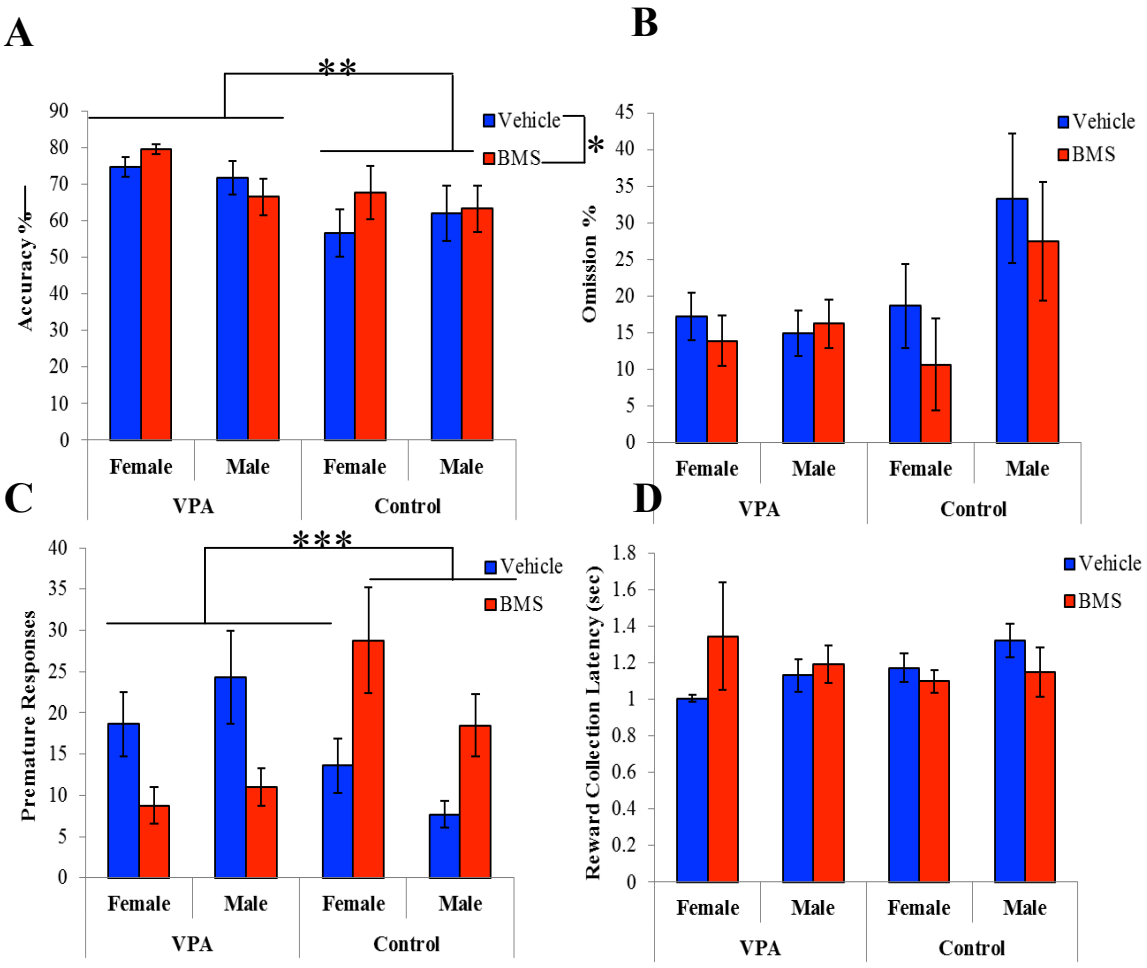

Figure 21. BK Channel modulator effects on basic test days. A. A main effect of drug was present (p=0.021). Accuracy was greater in the BMS condition indicating that the BK channel modulator improved attentional capacity. A main effect of group was also present (p=0.008). VPA animals performed with a greater accuracy when compared to controls. **B.** No main effects of drug (p=0.190) or group (p=0.183) were observed. **C.** There was a significant difference in premature responses in VPA animals and control animals before and after drug administration. The BK channel modulator significantly decreased impulsivity in VPA animals (p=0.001) and increased impulsivity in the control group (p=0.003). **D.** No main effects of drug (p=0.724) or group (p=0.888) were present (VPA females n=9, VPA males n=7, control females n=5, control males n=7).

Chapter 4: Discussion

4 Discussion

4.1 Habituation and Prepulse Inhibition of the Acoustic Startle Response

Proper sensory filtering mechanisms are critical to interacting and responding appropriately to one's environment. Disruptions in filtering mechanisms have been shown to be disrupted in several neurological disorders (Braff et al. 2001, 1992; Swerdlow et al. 1995). Moreover these mechanisms are disrupted in autistic individuals and putative animal models of autism.

Two important sensory filtering mechanisms are sensorimotor gating and habituation, and can reliably assessed through the acoustic startle response (ASR). ASR is a protective response elicited by a loud, sudden acoustic stimulus. Measuring the startle response either by EMG measurement of eye muscle contraction in humans or by whole body twitch in rodents, allows for quantification of the ASR (Valsamis and Schmid 2011). Overall, the neural circuitry mediating the acoustic startle pathway are highly conserved and well understood. They are proven to be validated objective measures of sensory filtering in both human and animal models and can be used to reveal underlying mechanisms central to the pathogenesis of several neurological disorders including ASD.

Baseline Startle

Baseline startle is an important control measure used to determine the level of sensitivity to auditory stimulation in an animal. It is calculated by averaging the first two startle responses on Day 1 of testing, which allows determining the startle magnitude of each animal without an effect of habituation or sensitization. Therefore, increased baseline startle indicates increased acoustic startle reactivity. At 6 weeks, VPA animals showed

significantly lower baseline startle when compared to control animals. This suggests lower startle reactivity in the VPA animals in adolescence. The differences in baseline however seem to subside once VPA animals reached adulthood (Figure 6). There results do not coincide with existing studies concerning acoustic startle reactivity in subjects with autism.

Most studies have not found a difference in startle magnitude in individuals with ASD (Bernier et al. 2005; Mcalonan et al. 2002; Yuhas et al. 2011). A few studies however have shown that there is an increased startle magnitude in both adults and children with ASD (Kohl et al. 2014; Takahashi et al. 2015) and that startle amplitudes and can be dependent on the severity of symptoms in different cohorts (Kohl et al. 2014). The reason for the discrepancy in our results can be explained by potential differences in weight between VPA and control animals. In contrast to measuring human startle, the rat startle measurements using a motion-sensitive platform are directly influenced by the weight of an animal, leading to larger startle amplitude with increasing weights.

Habituation

The first sensory filtering mechanism tested was habituation, a non-associative form of learning. It corresponds to decrease in the startle response following repeated presentations of an acoustic stimulus (Koch 1999). Short-term habituation (STH) refers to a decrease in response within a single testing session and is (partly) reversible. At 6 weeks of age, STH curves show no differences between VPA and control animals. Both groups displayed a similar decrease in startle responses over trials (Figure 7). STH ratios however, revealed an impairment of STH in VPA animals at 6 weeks of age (Figure

8Figure 8. At four months, there were no differences between VPA animals and control animals, which was supported by both STH curves and ratios. The impairment in STH in VPA animals seemed to be normalized when the animals reached adulthood.

Evidently, there is a discrepancy in the results provided by STH curves and ratios at 6 weeks of age. STH curves display startle magnitude as a function of trial and are therefore sensitive to the progression of startle amplitude over time. STH ratios however provide a smaller frame of reference comparing only initial and final startle amplitudes. This suggests that VPA animals have a higher final startle amplitude than controls but the manner of progressive decrease across trials in both groups is similar. Therefore, it is reasonable to assume that there is a difference in STH between control and VPA animals at 6 weeks of age. These results agree with the existing literature examining STH deficits in both humans and other animal models of ASD.

Studies have shown that STH of startle is slower in individuals with ASD when compared to controls (Ornitz et al. 1993; Perry et al. 2007). This was further supported by a study done by Guiraud and colleagues (2011), where the observed reduced neural habituation to repeated acoustic stimuli in infants at high risk for autism (Guiraud et al. 2011). Animal models of autism have provided similar results. Tadpoles exposed to valproic acid in-utero exhibited decreased acoustic startle habituation (James et al. 2015). FMR1 knockout mice, a mouse model of FXS, did not exhibit STH of the acoustic startle response (Nielsen et al. 2002).

As mentioned above, the neural circuitry underlying the acoustic startle response is highly conserved. An acoustic stimulus activates the auditory neurons in the cochlear

nucleus (cochlear root in mice and rats), which innervate the giant neurons in the caudal pontine reticular formation (PnC). The PnC acts as the sensorimotor interface and integrates the sensory input from cochlear nuclei and directly project onto the cranial and spinal motor neurons, eliciting a startle response (Koch 1999). The mechanisms underlying the changes in STH appear to be mediated by intrinsic mechanisms that lie within the startle response pathway (Davis et al. 2016; Simons-Weidenmaier et al. 2006). STH has shown to be induced by repeated activation of synapses in the ASR pathway resulting in synaptic depression in the PnC. This occurs as a result of a decrease in the intensity of presynaptic transmitter release or decreased sensitivity of post-synaptic receptors (Koch and Schnitzler 1997; Weber et al. 2002). An impairment of STH at 6 weeks implies possible dysregulation of synaptic transmission and receptor activity. The lack of impairment of STH at 4 months signifies a return to normal synaptic function at the PnC.

Long-term habituation (LTH) refers to a decrease in startle response across testing days. LTH results reveal a similar pattern to STH data. At 6 weeks, VPA animals failed to show LTH (Figure 9). VPA animals displayed a slight increase in the final startle responses when progressing across testing days, indicating sensitization. At 4 months, VPA animals showed LTH but not to the same effect as controls (Figure 10). These results do not corroborate with previously published studies.

Existing literature concerning LTH of startle in both human and animal models of autism remains inconsistent. Ornitz and colleagues (1993) looked at LTH in a cohort of individuals with autism and found no significant differences in LTH of the startle response (Ornitz et al. 1993). In animal models of autism, tadpoles exposed to valproic

acid in-utero exhibited impairments in LTH when compared to controls (James et al. 2015). Although, LTH provides valuable insight into mechanisms underlying sensory filtering that are different to STH, research into LTH in individuals with ASD remains limited. This could be due to the difficulty in assessing LTH due to the requirement of repeated testing procedures across several days.

Unlike STH, both sensitization and LTH have shown to be mediated by mechanisms extrinsic to the primary startle pathway (Koch 1999). Possible structures that have shown to play a role in LTH most commonly include the pedunculopontine tegementum, the cerebellar vermis and cortical areas (Koch and Schnitzler 1997; Leaton and Supple 1991, 2016). An impairment of LTH at 6 weeks provides support for abnormities in brainstem tegmental pathways or cerebellar vermal regions. Existing neuroanatomical data in ASD reveal cerebellar vermal structural deficiencies (Courchesne 1991) and a consistent loss of Purkinje cells in the cerebellar cortex (Bauman 1991), which support the LTH impairments seen in our VPA model at 6 weeks. Sensitization on the other hand has shown to be most commonly mediated by the amygdala and the bed nucleus of stria terminalis (Davis, Walker, and Lee 1997; Koch 1999). It has been shown that sensitization influences the primary startle circuit at the level of the PnC possibly through direct projections from the medial region of central amygdaloid nucleus (Koch 1999). Madesen and colleagues (2014) showed increased sensitization of the acoustic startle reflex in young children with autism (Madsen et al. 2014). Increased sensitization at 6 weeks in VPA rats, which is analogous to a juvenile age, supports these results found in young children with ASD. It is important to note that the aversive startle can induce a state of anxiety. In fact, Madsen and colleagues (2014)showed a strong correlation

between sensitization and anxiety levels in the participants. Studies have shown that this aversive character of the startling stimulus occurs as a result of amygdala activation. The amygdala excites the PnC and is involved in fear conditioning/sensitization (Davis et al. 1997). Furthermore, existing literature supports hyperactivity of the amygdala in patients with ASD (Monk et al. 2010). Therefore over-responsive activity of the amygdala could influence the startle reflex through enhancement of afferents to the PnC or could be due to a deficit in the inhibitory system of the amygdala. Animal models of autism have provided similar results. One study found a disruption of synaptic excitatory/inhibitory balance in the lateral amygdala in VPA rats thus providing evidence for physiological alterations in amygdala function in an animal model of autism (Lin et al. 2013). Markram and colleagues (2008) demonstrated enhanced and overgeneralized conditioned fear memories in a VPA rat model of autism. It has been thought that the basolaterla amgydala plays a key role in mechanisms underlying fear memories. This theory was supported as Markram and colleagues (2008) further demonstrated that the observed behavioural alterations could be a result of abnormal pathology in the lateral amygdala. They found decreased inhibition and a hyper-reactive circuit upon electrical stimulation. Therefore, sensitization of the acoustic startle reflex in VPA rats at 6 weeks of age suggests hyperactivity in the amygdala and as a result exaggerated potentiation of the startle response is present.

Latency to startle refers to the time taken to produce a startle response. Short term and long term habituation of the latency to startle can also be analyzed. At 6 weeks VPA animals showed a significantly lower response time when compared to control animals. LTH of latency to startle were also impaired at 6 weeks in the VPA group (Figure

11Figure 12). At 4 months, VPA rats showed no differences in response time when compared to control animals. VPA animals also exhibited STH and LTH of latency to startle relatively to the same effect as control animals. These results are inconsistent with current literature.

When examining high functioning individuals with autism, normal startle latencies have been reported (Bernier et al. 2005; Mcalonan et al. 2002). No changes in startle latency were shown in FXS individuals either (Frankland et al. 2004; Yuhas et al. 2011). Any changes in startle latency that have been reported in ASD individuals have shown a prolonged onset (Ornitz et al. 1993; Takahashi et al. 2014, 2015; Yuhas et al. 2011). This contradicts our results where we saw a decrease in startle latency in VPA rats as well as impaired LTH of startle onset, when compared to control animals at 6 weeks of age. Ison and colleagues (1973) have suggested that decreased startle latencies have the same functional significance as increased startle amplitudes as they both define reflex facilitation (Ison, McAdam, and Hammond 1973). However, the exact neural circuitry underlying differences in latency to startle have not been well defined.

Overall, VPA animals show decreased baseline startle when compared to controls at 6 weeks of age but by 4 months, differences in startle reactivity subside. Furthermore, impairments in habituation of both the startle response and startle latency are seen at 6 weeks in VPA rats. These sensory filtering impairments seem to subside when rats reach adulthood. This finding is consistent with existing literature. Typically, abnormal sensory filtering processes are classified as distinguishing symptoms more commonly in young children with autism (Wiggins, Robins, Bakeman, & Adamson, 2009).

Prepulse inhibition

Another important sensory filtering mechanism is sensorimotor gating. Prepulse inhibition (PPI) provides a measure of sensorimotor gaiting and occurs when a weaker acoustic pre-stimulus attenuates the following response to the stronger acoustic stimulus. At 6 weeks and 4 months of age, VPA animals displayed less PPI when compared to control animals at the 85 dB prepulse and 100 ms inter-stimulus interval (Figure 13Figure 14). Evidence for PPI deficits in ASD has been provided in other studies.

Although some research groups have not been able to elucidate differences in PPI in ASD (Kohl et al. 2014; Oranje et al. 2012; Ornitz et al. 1993; Yuhas et al. 2011), other studies have shown reduced PPI in adults with ASD which was correlated with increased repetitive behaviours (Mcalonan et al. 2002; Perry et al. 2007). Furthermore, profound PPI deficits have been found in the Fragile X patient population (Frankland et al. 2004; Yuhas et al. 2011). The magnitude of PPI deficits in the FXS group also predicted the severity of autistic phenotypes (Frankland et al. 2004). Decreased PPI has also been observed in the VPA rat model of autism by others (Schneider and Przewłocki 2005).

PPI has shown to be mediated by a feed forward inhibitory loop that runs parallel to the startle pathway. The inferior colliculus (IC) is activated by the acoustic prepulse and in turn activates the superior colliculus (SC). The SC connects to the pedunculopontine tegmental nucleus, which in turn activates inhibitory cholinergic projections to the PnC, that mediate PPI. This midbrain circuit that mediates PPI is modulated by higher brain structures (Fendt et al. 2001; Swerdlow et al. 1995; Swerdlow, Geyer, and Braff 2001). Therefore, reduced PPI found in certain individuals with autism

indicates a possible dysfunction in normal inhibitory regulation in ASD. Furthermore, the extent to which prepulse inhibition occurs depends heavily upon the prepulse intensity used (Fendt et al. 2001). Typically, an increase in prepulse intensity results in greater prepulse inhibition. However, our results show an impairment of PPI in VPA animals at the higher prepulse intensity. A possible explanation for these results could be that the lower prepulse intensity used (75 dB) was not strong enough to show a deficit. The strongest inhibition of startle, up to 80%, is observed with a greater prepulse intensity. PPI mechanisms are more robust at this intensity and therefore deficits would also be more pronounced. The amount of PPI is also strongly dependent on the inter-stimulus interval (ISI), the time in-between the prepulse and startle stimulus (Fendt et al. 2001). Prepulses first activate ionotropic receptors in the PnC that have been shown to mediate PPI at ISI's of 8-30 ms. They are often involved in fast, transient inhibition of the startle reflex. Long lasting inhibition of the startle response occurs via subsequent activation of metabotropic muscarinic and GABA receptors in the PnC and has been shown to be implicated at longer ISI's of 100-1000 ms. Long lasting inhibition of the startle reflex allows for processing of the prepulse at higher brain areas (Yeomans et al. 2010). VPA rats showed impaired PPI at the longer ISI (100 ms), which indicates either a decreased sensitivity of metabotropic receptors at the level of the PnC giant neurons, decreased intensity of presynaptic transmitter release, or a dysfunction in higher brain areas modulating the midbrain PPI circuit.

In summary, large variability exists in the current literature in terms of sensory filtering disruptions in ASD. This can be attributed to differences in methodology used, age group of the populations tested and most importantly differences in symptom severity between

individuals. Overall, we were able to confirm sensory filtering deficits in the VPA rat model of autism thereby confirming our hypothesis. However, no sex differences in sensory filtering were observed.

4.2 Open Field Box

The open field box test is typically used for measuring the natural exploratory behaviour of rodents. It can also be used as a measure of animal emotionality, in particular anxiety,. Evolutionarily, rats will avoid open environments, a concept which is incorporated into the open field maze. The open field consists of a large box in which the animal is allowed to roam free and explore its environment for an allotted amount of time. The inside of the box is conceptually divided into a center and periphery zone. Exploratory behaviour is measured as total ambulatory distance. Anxiety is measured as ratio of time spent in center vs. the periphery. Rats displaying high anxiety levels will typically display decreased exploratory behaviour and will remain near the walls or in the periphery zone for security (thigmotaxis, Seibenhener and Wooten 2015). Habituation of exploratory behaviour was also investigated.

VPA animals showed hyper-locomotion when compared to controls at 6 weeks of age (Figure 15), which was paralleled with increased anxiety levels (Figure 16). Both VPA animals and control animals showed LTH and STH of locomotive behaviour. At 4 months, no group differences were present however only VPA females and control males exhibited LTH of locomotive behaviour (Figure 15). Although increased anxiety levels have been frequently reported in human and animal models of autism (Bitsika et al. 2015;

Kohl et al. 2014; Markram et al. 2008), which supports the results in our study, the patterns of exploratory behaviour observed in our study contradict existing literature.

Recent studies have shown a link between autism and reduced environmental exploration. Children with autism have shown to spend less time actively exploring when compared to controls (Neill and Happe 2000). Animal models of autism provide further support for reduced exploratory behaviour (Schneider and Przewłocki 2005). In contrast, our results indicate hyper-locomotive activity in VPA animals when compared to controls at 6 weeks of age, which correlated with increased levels of anxiety. Alteration in structures involved in regulating fear, such as hyperactivity of the amygdala, have been shown to be implicated in human and animal models of ASD (Markram et al. 2008; Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, Lammers CR, Reiss AL 2004). This provides a possible explanation for the increased anxiety levels found in VPA animals at 6 weeks of age. Lastly, habituation of exploratory behaviour was measured. Although current literature shows increased habituation of locomotive behaviour in VPA rats when compared to controls (Olexová et al. 2013), our results show normal levels of habituation in VPA animals at both time points. Habituation of locomotive behaviour is contingent upon self-dependent stimuli and has shown to be modulated by cholinergic activity (Brown 1976). Therefore, normal habituation suggests relatively normal cholinergic activity in the VPA group. Overall, differences in exploratory behaviour and anxiety levels found at 6 weeks in VPA rats are indicative of pathological alterations in brain areas mediating or modulating exploratory behaviour. Although sex differences were present, females exhibited greater exploratory behaviour thereby disproving our hypothesis that males would be more severely affected.

4.3 Cognitive Function

Recent studies have highlighted a strong correlation between sensory processing impairments and higher order cognitive dysfunction in ASD. Tanguay and Edwards (1982) have suggested that impairments in sensory processing early on in life, especially auditory input, leads to impairments in developing complex cognitive abilities later on in life (Tanguay and Edwards 1982). Poor sensory processing abilities have been shown to strongly correlate with behavioural and emotional problems in individuals with ASD including high irritability, stereotypic behaviours and hyperactivity (Baker et al. 2008; O'Donnell et al. 2012). Complex sensory environments often result in sensory overload and as a result, individuals with ASD are withdrawn from academic and social environments which prevent the development of normal social and cognitive skills (Ashburner et al. 2008; Hochhauser and Engel-Yeger 2010). It is clear that there is a strong correlation between sensory processing impairments and higher order cognitive dysfunction. After confirming the presence of sensory filtering deficits in our model, cognitive function was assessed.

The 5-Choice Serial Reaction Time Task (5-CSRTT) provided a measure of cognition. The task was developed to understand the behavioural deficits in individuals with Attention Deficit Hyperactivity Disorder. It was initially made for humans and has since then been applied to animals. This makes the task highly translational and creates a bidirectional relationship between human and animal studies as you can run the exact same test in both populations (Bari, Dalley, and Robbins 2008). The 5CSRTT confers several advantages over other forms of behavioural testing. The tests provide a high level

of construct validity. Testing results have been consistent across laboratories providing increased reliability and easily replicable experimentation. Animals can be trained and sustained at stable levels of performance for long periods of time and can be easily retrained on the task. Though long training periods are required, sustained performance provides flexibility to the type of experiments that can be run, for example chronic drug injections. Additionally, behaviour is strictly regulated. The operant chambers are controlled through an external computer. Stimulus delivery is always precise, and data acquisition is automatic and unbiased (Bari et al. 2008).

One aspect of cognitive function that was assessed through the 5-CSRTT is learning. Learning was measured by the time taken to progress through learning stages. VPA animals advanced through the learning stages quicker than controls, indicating faster learning rates in the VPA group (Figure 17). Once all animals reached the same baseline performance, they were tested in two different testing protocols. The first protocol consisted of basic testing days. These test days were similar to the training stages and consisted of decreasing stimulus durations over four test days. Attentional control was measured through the accuracy of responses. The greater the accuracy on testing days, the greater the attentional control. Through the 5-CSRTT, no significant group differences in attentional control were obvious (Figure 18). Omissions indicate a failure to respond to a trial. No significant group differences in omissions were seen across testing days either. Impulsivity was measured through premature responses. If an animal displayed a greater number of premature responses, they displayed greater impulsivity. No significant differences in premature responses between control and VPA animals were observed. Lastly, reward collection latency, the time taken to retrieve the reward,

provides a measure of motivation. No differences in reward collection latency were observed. However, both groups did show a decrease in accuracy and motivated behaviour as well as an increase in impulsivity across testing days. This indicates that the test was sensitive to disruptions, since performance decreased as stimulus duration decreased thus as cognitive demand increased. The second testing protocol was similar to the basic test days however involved an auditory distractor. This allowed measuring not only sustained and divided attention, but selective attention as well. No significant differences in omissions, premature responses and reward collection latency were observed, however (Figure 19). A group difference in accuracy however was present, with VPA animals having performed with higher accuracy than controls, indicating greater attentional control. Accuracy also decreased across both groups as stimulus durations decreased. Additionally, a sex difference was observed with females exhibiting greater premature responses than males. Overall, no consistent differences in cognitive function between VPA and control animals were observed. The differences that were present, increased learning and attentional control, indicated better cognitive function in the VPA animals. This contradicts existing literature.

Typically individuals with autism that exhibited impairments in auditory filtering, displayed deficits in learning and inattention to cognitive tasks which often contributed to poor academic performance (Ashburner et al. 2008; Belmonte and Yurgelun-Todd 2003; Landry and Bryson 2004). Delayed emotional and social learning is also exhibited in the VPA models of autism (Banerjee et al. 2014; Schneider and Przewłocki 2005). Although our model displayed deficits in sensory filtering, they were not correlated with impairments in the 5-CSRTT, thereby disproving our hypothesis. It might be possible, of

course, that the VPA animals still have cognitive impairments, but they are different from what the 5-CSRTT tests for.

Differences in performance in control animals between basic test days and test days with a distractor, was also determined. We expected a greater impairment in performance on test days with a distractor as it increases cognitive demand. However, no significant differences were observed (Figure 20). As a result, the effect of the big potassium (BK) channel modulator on cognitive performance was analyzed on a basic test day only (no auditory distractor).

As mentioned earlier, big potassium (BK) channels exert a powerful control on neuronal excitability and integration. Several lines of evidence have also suggested an important function of BK channels in sensory filtering mechanisms and cognition (Hébert et al. 2014; Typlt, Mirkowski, Azzopardi, Ruth, et al. 2013). Furthermore, subsets of individuals with ASD are haplo-insufficient for the KCNMA1 gene, which encodes for the alpha subunit in BK channels. As a result, there is a physical disruption and therefore decreased activity of these channels in this subset of autistic individuals (Laumonnier et al. 2006). Therefore the current literature highlights the central role of BK channels in proper sensory filtering mechanisms as well as cognitive function and their implications in ASD. As a result, the effects of a BK channel opener on cognition were measured.

The effect of the positive BK channel modulator on one basic testing day at stimulus duration of 0.8 seconds was successful in improving accuracy, therefore attentional capacity in both VPA and control animals (Figure 21). The BK channel modulator also significantly decreased premature responses in VPA animals thus impulsivity was

improved. Control animals however exhibited the opposite effect with an increase in impulsivity as a result of drug exposure.

The improved attentional control in both groups as a result of drug administration provides evidence that BK channels play a prominent role in cognition. As mentioned previously, BK channels are involved in controlling neuronal excitability mainly by negatively controlling neurotransmitter release. However, BK channels may also increase neuronal excitability by either reducing inhibitory tone or by decreasing GABA effect on glutamatergic terminals (Samengo et al. 2014). As a result, these channels exert preferential control on excitatory vs. inhibitory synapses. Alterations in excitatory/inhibitory balance have been shown to be characteristic of several neuropsychiatric disorders (Ramocki and Zoghbi 2008). Activating these channels in normal functioning circuits might disturb existing excitatory/inhibitory homeostasis, which could explain the increase in impulsivity measured in controls as a result of the BK channel opener.

Although impaired cognition was not present in our VPA model, the BK channel modulator was able to improve attentional capacities and was able to decrease impulsivity in the VPA animals. These results provide additional evidence to the role of BK channels in cognitive function and identifies BK channels as a potential target for developing drugs that improve sensory filtering and cognitive function.

4.4 Study Limitations

Although we aimed to characterize sensory filtering and cognition in the VPA model in

this study, the use of an environmentally induced model of autism is often criticized. The VPA model of autism has been well established and displays both structural and behavioural similarities to humans with ASD; however, studies show a large variability in results. This could be explained by VPA administration. Dosage and time of exposure to valproic acid (VPA) has shown to impact the severity of symptoms relating to the core signs of autism. Our study administered a dose of 600 mg/kg at GD 12.5 since it has shown to consistently produce hallmark behavioural changes associated with ASD (Chan et al. 2011; Markram and Foster 2013; Roullet et al. 2013; Schneider and Przewłocki 2005). Furthermore, the underlying pathology that occurs as a result of VPA administration is not well understood. As a result, it is argued that the variability seen in the VPA model makes the results less reliable when compared to a genetic model where the underlying changes can be attributed to a single controlled factor that is uniform in all animals. However, the large variability in the VPA model is more representative of the spectrum seen in autism and as a result, can be more insightful in understanding the several possible underlying mechanisms that are central to the pathogenesis of ASD.

A second limitation could be that the BK channel modulator was delivered systemically instead of directly into the brain. BK channels are extensively located throughout the body, including neuromuscular junctions. As a result, motor control could have been adversely affected upon drug administration, which could have affected performance on the 5-CSRTT. Although we found no evidence for this in our results, if there was an effect on motor function, it would affect both VPA and control animals to the same extent and therefore should not confound the comparison between the groups.

Another potential limitation is the use of outbred Long Evans rats. Autism is a complex spectrum disorder affected by various genetic and environmental factors. Therefore using outbred Long Evans rats can further increase genetic variability as a result increasing the variability in the observed pathogenesis in the model. Despite this, Long Evans rats were used due to better visual acuity, which is necessary to undergo 5-CSRTT testing.

4.5 Future Directions

In our study, we looked at cognitive function, mainly attention, in the 5-CSRTT. Since no differences were found between the VPA and control group, the next logical step would be to test other aspects of cognition in order to confirm the presence of cognitive dysfunction in the model.

As mentioned earlier, BK channels play a role in sensory filtering mechanisms as well as cognitive function, however only their effect on improving cognitive outcome was measured. BK channels are co-localized with voltage dependent calcium channels thereby establishing a link between intracellular calcium levels and neurotransmitter release. This makes them ideal candidates for mediating calcium dependent presynaptic depression in the primary startle pathway (Weber et al. 2002), thereby potentially mediating short-term habituation of startle. We have already confirmed sensory filtering impairments in the VPA model in this study. Therefore, it would be interesting to determine the effects of positive BK channel modulators on improving sensory filtering mechanisms especially in adolescent animals; more specifically their effects on short-term habituation of the acoustic startle response. This could eventually lead to the development of pharmaceutical targets that enhance sensory filtering mechanisms.

Another possible study is determining if the behavioural changes seen in the model were paralleled by molecular changes in the brain. For example increased neuronal connectivity has shown to be paralleled by a decrease in the strength of the connections in both human and animal models of autism, resulting in a possible hyperactivity of the circuit (Rinaldi et al. 2008). Abnormal circuitry has been shown to directly influence higher order cognitive function in autism (Courchesne and Pierce 2005). Therefore, determining either the presence of increased neuronal connectivity or decreased connections in the VPA model could strengthen our behavioural results.

5 Summary and Conclusions

5.1 Summary of Findings

- Impairments in short term habituation of the acoustic startle response were detected at 6 weeks of age in VPA animals. The STH deficit seemed to ameliorate once animals reached adulthood.

- Lack of long term habituation was detected in VPA animals at 6 weeks of age. The impairment seemed to be normalized once animals reached adulthood.

- VPA animals displayed mild impairments in prepulse inhibition with a prepulse intensity of 85 dB at the 100 ms inter-stimulus interval. These impairments were observed at 6 weeks of age and continued into adulthood.

- VPA animals showed hyper-locomotive activity which were correlated with high levels of anxiety at 6 weeks of age. Hyper locomotive activity was also more prominent in females. VPA animals also showed short term and long term habituation of exploratory behaviour at both time points.

- VPA animals advanced through 5-CSRTT training at a faster rate than controls suggesting faster learning in the VPA group.

- VPA animals showed no significant impairments in cognition when compared to controls. VPA animals tended to perform at an overall greater accuracy than control animals.

- No differences between basic test days and test days with a distractor were seen.
- A positive BK channel modulator was successful in improving attention control and impulsivity in both VPA and control animals providing evidence for the important role of these BK channels in cognitive function.
- Sex differences were not consistently present throughout the behavioural tests. However when they were, VPA females were more adversely affected than their male counterparts.

5.2 Conclusion

This study demonstrated sensory filtering impairments in the VPA rat model of autism during the adolescent period. Furthermore, hyper-locomotive activity, which was paralleled with, increased anxiety levels were also observed in the VPA model at 6 weeks of age. These impairments observed during the adolescent period only coincide with the pattern and progression of symptomology in humans with ASD. Although no significant impairments in cognitive function were observed, we revealed that the use of a positive BK channel modulator was successful in improving cognitive outcomes in both the VPA and control group. This provides increased evidence for a role of these channels in cognitive behaviour. Furthermore, when sex differences were present, VPA females seemed to be more adversely affected than their male counterparts. It is important to note that the exact mechanism of VPA action is unknown.

Overall, our findings provide evidence for the validity of the VPA model to study underlying mechanisms central to the pathogenesis of ASD. Although no significant impairments in attention were observed, existing literature provides evidence for

cognitive dysfunction in this model and therefore, it would prove interesting to study alternate forms of cognition. Lastly, these results also provide evidence for the important role of BK channels in cognitive function thereby highlighting a promising pharmaceutical target. This could provide a basis for the development of drugs that enhance sensory filtering and associated cognitive function for mental disorders, such as ASD.

6 References

Alcántara, José I., Emma J. L. Weisblatt, Brian C. J. Moore, and Patrick F. Bolton. 2004. "Speech-in-Noise Perception in High-Functioning Individuals with Autism or Asperger's Syndrome." *Journal of Child Psychology and Psychiatry and Allied Disciplines* 45:1107–14.

Ashburner, J., J. Ziviani, and S. Rodger. 2008. "Sensory Processing and Classroom Emotional, Behavioral, and Educational Outcomes in Children with Autism Spectrum Disorder 27." *Am.J Occup.Ther.* 62(0272-9490 (Print)):564–73.

B. Pfeiffer, M.Kinnealey, C.Reed, G. Herzberg. 2005. "Sensory Modulation and Affective Disorders in Children and Adolescents with Asperger's Disorder.pdf." 335–45.

Baker, Amy E. Z., Alison Lane, Manya T. Angley, and Robyn L. Young. 2008. "The Relationship between Sensory Processing Patterns and Behavioural Responsiveness in Autistic Disorder: A Pilot Study." *Journal of Autism and Developmental Disorders* 38(5):867–75.

Banerjee, Anwesha et al. 2014. "Abnormal Emotional Learning in a Rat Model of Autism Exposed to Valproic Acid in Utero." *Frontiers in Behavioral Neuroscience* 8(November):1–13.

Bari, Andrea, Jeffrey W. Dalley, and Trevor W. Robbins. 2008. "The Application of the 5-Choice Serial Reaction Time Task for the Assessment of Visual Attentional Processes and Impulse Control in Rats." *Nature protocols* 3(5):759–67.

Barnea-Goraly, Naama et al. 2004. "White Matter Structure in Autism: Preliminary Evidence from Diffusion Tensor Imaging." *Biological Psychiatry* 55(3):323–26.

Bauman, Margaret L. 1991. "Microscopic Neuroanatomic Abnormalities in Autism." *Pediatrics* 87(5 Pt 2):791–96.

Baxter, a J. et al. 2014. "The Epidemiology and Global Burden of Autism Spectrum Disorders." *Psychological medicine* (Cd):1–13.

Belmonte, Matthew K. and Deborah A. Yurgelun-Todd. 2003. "Functional Anatomy of Impaired Selective Attention and Compensatory Processing in Autism." *Cognitive Brain Research* 17(3):651–64.

Bernier, Raphael, Geraldine Dawson, Heracles Panagiotides, and Sara Webb. 2005. "Individuals with Autism Spectrum Disorder Show Normal Responses to a Fear Potential Startle Paradigm." *Journal of Autism and Developmental Disorders* 35(5):575–83.

Billstedt, Eva, I. Carina Gillberg, and Christopher Gillberg. 2007. "Autism in Adults:

Symptom Patterns and Early Childhood Predictors. Use of the DISCO in a Community Sample Followed from Childhood." *Journal of Child Psychology and Psychiatry and Allied Disciplines* 48(11):1102–10.

Bitsika, Vicki, Christopher F. Sharpley, and Richard Mills. 2015. "Are Sensory Processing Features Associated with Depressive Symptoms in Boys with an ASD?" *Journal of Autism and Developmental Disorders* (Apa 2013).

Bomba, Marie D. and Elizabeth W. Pang. 2004. "Cortical Auditory Evoked Potentials in Autism: A Review." *International Journal of Psychophysiology* 53(3):161–69.

Braff, D. L., M. A. Geyer, and N. R. Swerdlow. 2001. "Human Studies of Prepulse Inhibition of Startle: Normal Subjects, Patient Groups, and Pharmacological Studies." *Psychopharmacology* 156(2-3):234–58.

Braff, David L., C. Grillon, and M. A. Geyer. 1992. "Gating and Habituation of the Startle Reflex in Schizophrenic Patients." *Archives of General Psychiatry* 49(3):206.

Brown, C. P. 1976. "Two Types of Habituation in Chicks: Differential Dependence on Cholinergic Activity." *Pharmacology, biochemistry, and behavior* 4(3):235–38.

Chan, Ki et al. 2011. "The Critical Period of Valproate Exposure to Induce Autistic Symptoms in Sprague – Dawley Rats." *Toxicology Letters* 201(2):137–42.

Chang, Yi Shin et al. 2014. "Autism and Sensory Processing Disorders: Shared White Matter Disruption in Sensory Pathways but Divergent Connectivity in Social-Emotional Pathways." *PLoS ONE* 9(7):1–17.

Chen, L. and M. Toth. 2001. "Fragile X Mice Develop Sensory Hyperreactivity to Auditory Stimuli." *Neuroscience* 103(4):1043–50.

Chen, Yu H., Jacqui Rodgers, and Helen McConachie. 2009. "Restricted and Repetitive Behaviours, Sensory Processing and Cognitive Style in Children with Autism Spectrum Disorders." *Journal of Autism and Developmental Disorders* 39(4):635–42.

Courchesne, E. 1991. "Neuroanatomic Imaging in Autism." *Pediatrics* 87(5 Pt 2):781–90.

Courchesne, Eric and Karen Pierce. 2005a. "Brain Overgrowth in Autism during a Critical Time in Development: Implications for Frontal Pyramidal Neuron and Interneuron Development and Connectivity." *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience* 23(2-3):153–70.

Courchesne, Eric and Karen Pierce. 2005b. "Why the Frontal Cortex in Autism Might Be Talking Only to Itself: Local over-Connectivity but Long-Distance Disconnection." *Current Opinion in Neurobiology* 15(2):225–30.

Davis, M., T. Parisi, Gendelman D, M. Tischler, and Kehne J. 2016. "Habituation and Sensitization of Startle Reflexes Elicited Electrically from the Brainstem." *Science* 218(4573):688–90.

Davis, M., D. L. Walker, and Y. Lee. 1997. "Amygdala and Bed Nucleus of the Stria Terminalis: Differential Roles in Fear and Anxiety Measured with the Acoustic Startle Reflex." *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 352(1362):1675–87.

Deng, Pan-Yue et al. 2013. "FMRP Regulates Neurotransmitter Release and Synaptic Information Transmission by Modulating Actional Potential Duration via BK Channels." 77(4):696–711.

DePape, Anne-Marie R., Geoffrey B. C. Hall, Barbara Tillmann, and Laurel J. Trainor. 2012. "Auditory Processing in High-Functioning Adolescents with Autism Spectrum Disorder." *PLoS ONE* 7(9):e44084.

Fendt, M., L. Li, and J. S. Yeomans. 2001. "Brain Stem Circuits Mediating Prepulse Inhibition of the Startle Reflex." *Psychopharmacology* 156(2-3):216–24.

Frankland, P. W. et al. 2004. "Sensorimotor Gating Abnormalities in Young Males with Fragile X Syndrome and Fmr1-Knockout Mice." *Molecular psychiatry* 9(4):417–25.

Gomot, Marie, Marie-Hélène Giard, Jean-Louis Adrien, Catherine Barthelemy, and Nicole Bruneau. 2002. "Hypersensitivity to Acoustic Change in Children with Autism: Electrophysiological Evidence of Left Frontal Cortex Dysfunctioning." *Psychophysiology* 39(OCTOBER 2002):577–84.

Green, Shulamite A. et al. 2015. "Neurobiology of Sensory Overresponsivity in Youth With Autism Spectrum Disorders." *JAMA Psychiatry* 72(8):778.

Gribkoff, V. K., J. E. Starrett, and S. I. Dworetzky. 2001. "Maxi-K Potassium Channels: Form, Function, and Modulation of a Class of Endogenous Regulators of Intracellular Calcium." *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 7(2):166–77.

Groen, Wouter B. et al. 2009. "Intact Spectral but Abnormal Temporal Processing of Auditory Stimuli in Autism." *Journal of Autism and Developmental Disorders* 39(5):742–50.

Guiraud, Jeanne A. et al. 2011. "Differential Habituation to Repeated Sounds in Infants at High Risk for Autism." *NeuroReport* 1.

Hébert, Betty et al. 2014. "Rescue of Fragile X Syndrome Phenotypes in Fmr1 KO Mice by a BKCa Channel Opener Molecule." *Orphanet journal of rare diseases* 9:124.

Higgins, Joseph J., Jin Hao, Barry E. Kosofsky, and Anjali M. Rajadhyaksha. 2008. "Dysregulation of Large-Conductance Ca2+-Activated K+ Channel Expression in

Nonsyndromal Mental Retardation due to a Cereblon p.R419X Mutation." *Neurogenetics* 9(3):219–23.

Hilton, Claudia, Kathleen Graver, and Patricia LaVesser. 2007. "Relationship between Social Competence and Sensory Processing in Children with High Functioning Autism Spectrum Disorders." *Research in Autism Spectrum Disorders* 1(2):164–73.

Hochhauser, Michal and Batya Engel-Yeger. 2010. "Sensory Processing Abilities and Their Relation to Participation in Leisure Activities among Children with High-Functioning Autism Spectrum Disorder (HFASD)." *Research in Autism Spectrum Disorders* 4(4):746–54.

Ishiura, H. et al. 2008. "Autism Spectrum Disorders Following in Utero Exposure To Antiepileptic Drugs." 1921–23.

Ison, J. R., D. W. McAdam, and G. R. Hammond. 1973. "Latency and Amplitude Changes in the Acoustic Startle Reflex of the Rat Produced by Variation in Auditory Prestimulation." *Physiology and Behavior* 10(6):1035–39.

James, Eric J. et al. 2015. "Valproate-Induced Neurodevelopmental Deficits in Xenopus Laevis Tadpoles." *The Journal of neuroscience : the official journal of the Society for Neuroscience* 35(7):3218–29.

Khalfa, S. et al. 2001. "Peripheral Auditory Asymmetry in Infantile Autism." *The European journal of neuroscience* 13(3):628–32.

Knaus, H. G. et al. 1996. "Distribution of High-Conductance Ca(2+)-Activated K+ Channels in Rat Brain: Targeting to Axons and Nerve Terminals." *The Journal of neuroscience* 16(3):955–63.

Koch, M. 1999. "The Neurobiology of Startle." 59(98).

Koch, M. and H. U. Schnitzler. 1997. "The Acoustic Startle Response in Rats--Circuits Mediating Evocation, Inhibition and Potentiation." *Behavioural brain research* 89(1-2):35–49.

Kohl, Sina et al. 2014. "Prepulse Inhibition of the Acoustic Startle Reflex in High Functioning Autism." *PloS one* 9(3):e92372.

Kolozsi, E., R. N. Mackenzie, F. I. Roullet, D. Decatanzaro, and J. a. Foster. 2009. "Prenatal Exposure to Valproic Acid Leads to Reduced Expression of Synaptic Adhesion Molecule Neuroligin 3 in Mice." *Neuroscience* 163(4):1201–10.

Krey, Jocelyn F. and Ricardo E. Dolmetsch. 2007. "Molecular Mechanisms of Autism: A Possible Role for Ca2+ Signaling." *Current Opinion in Neurobiology* 17(1):112–19.

Landry, Reginald and Susan E. Bryson. 2004. "Impaired Disengagement of Attention in Young Children with Autism." *Journal of child psychology and psychiatry, and*

allied disciplines 45(6):1115–22.

Laumonnier, Frédéric et al. 2006. "Association of a Functional Deficit of the BK Channel, a Synaptic Regulator of Neuronal Excitability, With Autism and Mental Retardation." *The American Journal of Psychiatry* 163(9):1622–29.

Leaton, R. N. and W. F. Supple. 1991. "Medial Cerebellum and Long-Term Habituation of Acoustic Startle in Rats." *Behavioral neuroscience* 105(6):804–16.

Leaton, R. N. and W. F. Supple. 2016. "Cerebellar Vermis : Essential for Long-Term Habituation of the Acoustic Startle Response." *Science* 232(4749):513–15.

Lee, Urvi S. and Jianmin Cui. 2010. "BK Channel Activation : Structural and Functional Insights." *Trends Neurosci.* 33(9):415–23.

Leekam, Susan R., Carmen Nieto, Sarah J. Libby, Lorna Wing, and Judith Gould. 2007. "Describing the Sensory Abnormalities of Children and Adults with Autism." *Journal of Autism and Developmental Disorders* 37(5):894–910.

Lin, Hui Ching, Po Wu Gean, Chao Chuan Wang, Yun Han Chan, and Po See Chen. 2013. "The Amygdala Excitatory/Inhibitory Balance in a Valproate-Induced Rat Autism Model." *PLoS ONE* 8(1).

Mabunga, Darine Froy N., Edson Luck T. Gonzales, Ji-woon Kim, Ki Chan Kim, and Chan Young Shin. 2015. "Exploring the Validity of Valproic Acid Animal Model of Autism." 24(4):285–300.

Madsen, Gitte Falcher, Niels Bilenberg, Cathriona Cantio, and Bob Oranje. 2014. "Increased Prepulse Inhibition and Sensitization of the Startle Reflex in Autistic Children." *Autism research : official journal of the International Society for Autism Research* 7(1):94–103.

Markram, Kamila and Jane A. Foster. 2013. "General Developmental Health in the VPA-Rat Model of Autism." 7(July):1–11.

Markram, Kamila, Tania Rinaldi, Deborah La Mendola, Carmen Sandi, and Henry Markram. 2008. "Abnormal Fear Conditioning and Amygdala Processing in an Animal Model of Autism." *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 33(4):901–12.

Mcalonan, Grainne M. et al. 2002. "Brain Anatomy and Sensorimotor Gating in Asperger ' S Syndrome." 1594–1606.

Mehta, Mili V., Michael J. Gandal, and Steven J. Siegel. 2011. "mGluR5-Antagonist Mediated Reversal of Elevated Stereotyped, Repetitive Behaviors in the VPA Model of Autism." *PLoS ONE* 6(10).

Misonou, Hiroaki et al. 2008. "NIH Public Access." 496(3):289–302.

Monk, C. S. et al. 2010. “Neural Circuitry of Emotional Face Processing in Autism Spectrum Disorders.” *Journal of Psychiatry and Neuroscience* 35(2):105–14.

Nakasato, Akane et al. 2008. “Swim Stress Exaggerates the Hyperactive Mesocortical Dopamine System in a Rodent Model of Autism.” *Brain Research* 1193:128–35.

Neill, Daniela K. O. and Francesca G. E. Happe. 2000. “Noticing and Commenting on What ’ S New : Differences and Similarities among 22-Month-Old Typically Developing Children , Children with Down Syndrome and Children with Autism.” 4:457–78.

Nielsen, Darci M., William J. Derber, Danielle A. McClellan, and Linda S. Crnic. 2002. “Alterations in the Auditory Startle Response in Fmr1 Targeted Mutant Mouse Models of Fragile X Syndrome.” *Brain Research* 927(1):8–17.

O’Donnell, Shelley O., Jean Deitz, Deborah Kartin, Theresa Nalty, and Geraldine Dawson. 2012. “Sensory Processing, Problem Behavior, Adaptive Behavior, and Cognition in Preschool Children with Autism Spectrum Disorders.” *The American Journal of Occupational Therapy* 66(5):586–94.

Olexová, Lucia, Tomáš Senko, Peter Stefánik, Alžbeta Talarovičová, and Lucia Kršková. 2013. “Habituation of Exploratory Behaviour in VPA Rats: Animal Model of Autism.” *Interdisciplinary toxicology* 6(4):222–27.

Oranje, Bob, Bertine Lahuis, Herman van Engeland, Rutger Jan van der Gaag, and Chantal Kemner. 2012. “Sensory and Sensorimotor Gating in Children with Multiple Complex Developmental Disorders (MCDD) and Autism.” *Psychiatry Research* 206(2-3):287–92.

Ornitz, E. M., Shelly J. Lane, T. Sugiyama, and J. De Traversay. 1993. “Startle Modulation Studies in Autism.” *Journal of Autism and Developmental Disorders* 23(4):619–37.

Perry, William, Arpi Minassian, Brian Lopez, Leeza Maron, and Alan Lincoln. 2007. “Sensorimotor Gating Deficits in Adults with Autism.” *Biological psychiatry* 61(4):482–86.

Phiel, Christopher J. et al. 2001. “Histone Deacetylase Is a Direct Target of Valproic Acid, a Potent Anticonvulsant, Mood Stabilizer, and Teratogen.” *Journal of Biological Chemistry* 276(39):36734–41.

Ramocki, Melissa B. and Huda Y. Zoghbi. 2008. “Failure of Neuronal Homeostasis Results in Common Neuropsychiatric Phenotypes.” *Nature* 455(7215):912–18.

Rinaldi, Tania, Gilad Silberberg, and Henry Markram. 2008. “Hyperconnectivity of Local Neocortical Microcircuitry Induced by Prenatal Exposure to Valproic Acid.” *Cerebral Cortex* 18(4):763–70.

Rogers, Sally J. and Sally Ozonoff. 2005. "Annotation: What Do We Know about Sensory Dysfunction in Autism? A Critical Review of the Empirical Evidence." *Journal of Child Psychology and Psychiatry* 46(12):1255–68.

Roth, Daphne Ari-Even, Chava Muchnik, Esther Shabtai, Miinka Hildesheimer, and Yael Henkin. 2012. "Evidence for Atypical Auditory Brainstem Responses in Young Children with Suspected Autism Spectrum Disorders." *Developmental Medicine & Child Neurology* 54(1):23–29.

Roullet, Florence I., Jonathan K. Y. Lai, and Jane a Foster. 2013. "In Utero Exposure to Valproic Acid and Autism--a Current Review of Clinical and Animal Studies." *Neurotoxicology and teratology* 36:47–56.

Samengo, Irene, Diego Curr??, Vincenzo Barrese, Maurizio Taglialatela, and Maria Martire. 2014. "Large Conductance Calcium-Activated Potassium Channels: Their Expression and Modulation of Glutamate Release from Nerve Terminals Isolated from Rat Trigeminal Caudal Nucleus and Cerebral Cortex." *Neurochemical Research* 39(5):901–10.

Schneider, Tomasz and Ryszard Przewłocki. 2005. "Behavioral Alterations in Rats Prenatally Exposed to Valproic Acid: Animal Model of Autism." *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 30(1):80–89.

Schumann CM, Hamstra J, Goodlin-Jones BL, Lotspeich LJ, Kwon H, Buonocore MH, Lammers CR, Reiss AL, Amaral DG. 2004. "The Amygdala Is Enlarged in Children But Not Adolescents with Autism; the Hippocampus Is Enlarged at All Ages." *The Journal of neuroscience* 24(28):6392–6401.

Seibenhener, Michael L. and Michael C. Wooten. 2015. "Use of the Open Field Maze to Measure Locomotor and Anxiety-like Behavior in Mice." *Journal of visualized experiments : JoVE* (96):1–6.

Simons-Weidenmaier, Nadine S., Maruschka Weber, Claudia F. Plappert, Peter K. D. Pilz, and Susanne Schmid. 2006. "Synaptic Depression and Short-Term Habituation Are Located in the Sensory Part of the Mammalian Startle Pathway." *BMC neuroscience* 7:38.

Splawski, Igor et al. 2004. "CaV1.2 Calcium Channel Dysfunction Causes a Multisystem Disorder Including Arrhythmia and Autism." *Cell* 119(1):19–31.

Swerdlow, N. R. et al. 1995. "Impaired Prepulse Inhibition of Acoustic and Tactile Startle Response in Patients with Huntington ' S Disease." *Dementia* 58(2):192–200.

Swerdlow, N. R., M. A. Geyer, and D. L. Braff. 2001. "Neural Circuit Regulation of Prepulse Inhibition of Startle in the Rat: Current Knowledge and Future Challenges." *Psychopharmacology* 156(2-3):194–215.

Takahashi, Hidetoshi et al. 2014. “Hyperreactivity to Weak Acoustic Stimuli and Prolonged Acoustic Startle Latency in Children with Autism Spectrum Disorders.” *Molecular Autism* 5(1):23.

Takahashi, Hidetoshi, Sahoko Komatsu, Takayuki Nakahachi, Kazuo Ogino, and Yoko Kamio. 2015. “Relationship of the Acoustic Startle Response and Its Modulation to Emotional and Behavioral Problems in Typical Development Children and Those with Autism Spectrum Disorders.” *Journal of autism and developmental disorders*.

Tanguay, P. E. and R. M. Edwards. 1982. “Electrophysiological Studies of Autism: The Whisper of the Bang.” *Journal of autism and developmental disorders* 12(2):177–84.

Typlt, Marei, Magdalena Mirkowski, Erin Azzopardi, Peter Ruth, et al. 2013. “Habituation of Reflexive and Motivated Behavior in Mice with Deficient BK Channel Function.” *Frontiers in integrative neuroscience* 7(November):79.

Typlt, Marei, Magdalena Mirkowski, Erin Azzopardi, Lukas Ruettiger, et al. 2013. “Mice with Deficient BK Channel Function Show Impaired Prepulse Inhibition and Spatial Learning, but Normal Working and Spatial Reference Memory.” *PloS one* 8(11):e81270.

Valsamis, Bridget and Susanne Schmid. 2011. “Habituation and Prepulse Inhibition of Acoustic Startle in Rodents.” *Journal of visualized experiments : JoVE* (55):e3446.

Weber, Maruschka, Hans Ulrich Schnitzler, and Susanne Schmid. 2002. “Synaptic Plasticity in the Acoustic Startle Pathway: The Neuronal Basis for Short-Term Habituation?” *European Journal of Neuroscience* 16(7):1325–32.

Wiggins, Lisa D., Diana L. Robins, Roger Bakeman, and Lauren B. Adamson. 2009. “Breif Report: Sensory Abnormalities as Distinguishing Symptoms of Autism Spectrum Disorders in Young Children.” *Journal of Autism and Developmental Disorders* 39(7):1087–91.

Yeomans, J. S. 2012. “Muscarinic Receptors.” Pp. 243–59 in *Muscarinic Receptors*. Handb Exp Pharmacol.

Yeomans, John S. et al. 2010. “GABA Receptors and Prepulse Inhibition of Acoustic Startle in Mice and Rats.” *European Journal of Neuroscience* 31(11):2053–61.

Yuhas, Jennifer et al. 2011. “Brief Report: Sensorimotor Gating in Idiopathic Autism and Autism Associated with Fragile X Syndrome.” *Journal of Autism and Developmental Disorders* 41(2):248–53.

Curriculum Vitae

EDUCATION

Undergraduate Degree in Science at McMaster University
Honors Specialization in Biology

Graduate Degree in Anatomy and Cell Biology at Western University
Master's Degree in Anatomy and Cell Biology
Neurological Sciences, 2016 Candidate

RESEARCH EXPERIENCE

McMaster University, Hamilton, ON *Sep 2013-April 2014*
Independent Thesis Study
Thesis Title: Identification of a novel nematode species-C.amalkius as a possible sister to *Caenorhabditis elegans*.

Hamilton General Hospital, Hamilton, ON *May-Aug 2013*
Advanced Lab Placement/Summer Research Student

RELATED WORK EXPERIENCE

Graduate Teaching Assistant- Western University
- Integrative Neuroscience 4451, *September-December 2014&2015*

Graduate Teaching Assistant- Western University
- Biology 1002B, *January-April 2016*

CONFERENCES/POSTER PRESENTATIONS

London Health Research Day, London, ON, April 2015
"The use of a positive big potassium channel modulator, BMS-204352, to improve cognitive deficits related to sensory filtering in a rat model of autism."
A. De Silva, & S. Schmid

Southern Ontario Neuroscience Association (SONA), Hamilton, ON, May 2015
"Sensory Filtering and Cognitive Impairments in a VPA rat model of autism."
A. De Silva, & S. Schmid

Society for Neuroscience (SfN), Chicago, IL, October 2015
"Sensory Filtering and Cognitive Impairments in a VPA rat model of autism."
A. De Silva, & S. Schmid

Anatomy and Cell Biology Research Day, London, ON, October 2015
"Sensory Filtering and Cognitive Impairments in a VPA rat model of autism."
A. De Silva, & S. Schmid

London Health Research Day, London, ON, April 2016
"The use of a positive big potassium channel modulator, BMS-204352, to improve cognitive deficits related to sensory filtering in a rat model of autism."
A. De Silva, & S. Schmid

Southern Ontario Neuroscience Association (SONA), Hamilton, ON, May 2016
"Sensory Filtering and Cognitive Impairments in a VPA rat model of autism."
A. De Silva, & S. Schmid

AWARDS AND ACCOMPLISHMENTS

Western Graduate Research Scholarship, 2014-2016
One-year $4500 Scholarship x2

Deans Honors List, McMaster University, 2012-2014

Queen Elizabeth scholarship for academic excellence, 2010
One year, $4500

Entrance Scholarship McMaster University, 2010
One year, $1000

COMMITTEE/LEADERSHIP EXPERIENCES

ACB Student Council, University of Western Ontario, London, ON, September 2015-April 2016
Committee Member

Organizational Team, Frontier College, London, ON, September 2015-December 2015
Volunteer Tutor for underprivileged youth

Organizational Team, Sexual Assault Centre London, London, ON, January 2016-present
Crisis Line Worker