THE EFFECT OF PRENATAL VALPROIC ACID EXPOSURE ON THE DEVELOPING BRAIN: FACE, CONSTRUCT, AND PREDICTIVE VALIDITY OF AN ANIMAL MODEL OF AUTISM

SARAH RAZA
BSc (Biological Sciences), University of Alberta, 2012

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfillment of the
Requirements for the Degree

MASTER OF SCIENCE

Department of Neuroscience
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

THE EFFECT OF PRENATAL VALPROIC ACID EXPOSURE ON THE DEVELOPING BRAIN: FACE, CONSTRUCT, AND PREDICITIVE VALIDITY OF AN ANIMAL MODEL OF AUTISM

SARAH RAZA

Date of Defense: April 22, 2015

Robbin Gibb Supervisor	Associate Professor	PhD
Bryan Kolb Supervisor	Professor	PhD
Sergio M. Pellis Thesis Examination Committee Member	Professor	PhD
Paul Vasey Thesis Examination Committee Member	Professor	PhD
David Euston Chair, Thesis Examination Committee	Assistant Professor	PhD

ABSTRACT

Autism is a behaviorally defined neurodevelopmental disorder characterized by qualitative impairments in social interaction, communication, and aberrant repetitive behaviors. Behavioral phenotyping in laboratory animals to simulate core symptomology has been a large research focus with the aim to not only elucidate the behavioral basis and neural underpinnings of the disorder, but also study potential remedial treatments. The present thesis evaluated the validity of the valproic acid (VPA) rat model of autism by examining perturbations in the neurodevelopmental trajectories – behavioral and neurobiological – of juvenile and adult rats prenatally exposed to VPA, as well as the therapeutic effects of early tactile stimulation on the synergistic brain-behavior relationship. Behavioral and neuroanatomical findings confirmed the *face, construct,* and *predictive validity* of the VPA rat model, given the ethological, etiological, and therapeutic similarities to facets of human autism; thus, reinforcing the VPA model as a viable model for the study of autism in rats.

ACKNOWLEDGEMENTS

First and foremost, I would like to express my gratitude to my supervisors, Dr. Robbin Gibb and Dr. Bryan Kolb, for their unwavering support, guidance, patience, and encouragement throughout my undergraduate and graduate education. Your friendly demeanors and constant mentorship has made my master's years very enjoyable and I cannot say thank you enough for inspiring me to pursue my goals. Your vision of excellence has been of tremendous importance to both my academic and personal development. To Dr. Sergio Pellis, I thank you not only for your advice and encouragement, but also your great expertise. The knowledge I have gained from you has been fundamental to my thesis work and pursuit of my career path. Additionally, I would also like to acknowledge Dr. Paul Vasey; your support, enthusiasm, and constructive feedback have been invaluable throughout my research process.

I owe a great debt of gratitude to the Gibb and Kolb labs for their continuous support, expertise, and assistance over the years. A special thank you to Allonna Harker, Stephanie Himmler, and Brett Himmler – your constant encouragement, support, and willingness to lend a helping hand was very much appreciated. I will miss our daily conversations and humorous antics.

Finally, I would like to extend a heartfelt thank you to my family for their unconditional love. To my mom and dad, Naila and Ahmad Raza, you are an inspiration. You have encouraged me to push forward, work hard, and pursue my goals despite any circumstance. You have not only been there to celebrate my accomplishments, but also to console me through any "bumps along the road," and for that, I cannot thank you enough for your unconditional love, support, and encouragement. I could never have made it this

far without you. To my brother and sister, Hassan and Yamin, I am so grateful to have siblings as wonderful as you two. You are the best role models a girl could ask for. And finally to my grandfather, Bashir Ahmed Javaid, you are my strength. Thank you for always being there and inspiring me to become the best student, granddaughter, daughter, sister, and friend I can be.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AID	dorsal agranular insular cortex (used to represent OFC)
ASD	autism spectrum disorders
Cg3	cingulate area 3 (used to represent mPFC)
DSM-V	diagnostic and statistical manual of mental disorders
EPM	elevated plus maze
E/I	excitatory/inhibitory
FGF-2	fibroblast growth factor 2
G	gestational day
GABA	γ-aminobutyric acid
OFC	orbital prefrontal cortex
mPFC	medial prefrontal cortex
NTS	non-tactile stimulation
PFC	prefrontal cortex
PN	postnatal day
VPA	valproic acid
TS	tactile stimulation
USV	ultrasonic vocalizations

CHAPTER 1

General Introduction

1.1. Autism Spectrum Disorders (ASD)

1.1.1. Definition and Prevalence

Leo Kanner introduced the diagnostic concept of autism in 1943, based on his original case series of 11 children exhibiting severe deficits in sociability, communicative skills, and restricted and repetitive behaviors and/or interests. Kanner described autism as the child's innate inability "to relate themselves in an ordinary way to people and situations from the beginning of life" and "to form the usual, biologically provided affective contact with people," establishing the first clinical description of the pathognomonic disorder (Kanner, 1943). Owing to the heightened awareness over the past two centuries, the diagnostic criteria and clinical phenotype of autism has significantly shifted; originally being defined as 'early-onset schizophrenia,' then 'infantile autism,' and later renamed 'autism disorder' (Eisenberg & Kanner, 1956; Weintraub, 2011). It has only been in the past few decades that the common name autism has incorporated a wide repertoire of symptomology and related disorders, given the tremendous heterogeneity in the genotype-phenotype relationship. That is, the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) has termed autism spectrum disorders (ASD) as the single diagnostic dimension to characterize classic autistic disorder, Asperger's disorder, and pervasive developmental disorder not otherwise specified (PDD) (American Psychiatric Association, 2013). Within this diagnostic dimension, primarily two domains of symptoms define ASD: social-communicative deficits, and restricted and repetitive interests and/or behaviors (Table

1.1). Symptom severity within these domains vary from mild to severe, including complete absence of social functioning and fixated rituals and/or repetitive motor behaviors to subtle dysfunctions in social interactions, verbal and nonverbal communication, and motor stereotypies (Schneider & Przewlocki, 2011). Despite the detailed analysis of the neurobehavioral derangements and the identification of possible diagnostic biomarkers, the etiology of autism still remains elusive.

As postulated by Kanner in 1943, ASD forms "a 'unique syndrome', not heretofore reported, which seems to be rare enough, yet is probably more frequent than is indicated by the paucity of observed cases" (Kanner, 1943). Since then, Kanner's prediction has been fulfilled. In fact, since the first epidemiological studies conducted in the late 1960s, the global prevalence of ASD has increased twenty to thirtyfold. More specifically, the prevalence of ASD is progressively on the rise, currently affecting 1 in 68 children, with a 5 times greater likelihood of occurrence in the male population (Centers for Disease Control and Prevention, 2014). Interestingly, a recent study on parent-reported ASD by the National Center for Health Statistics identified a prevalence estimate closer to 1 in 50 children (Blumberg et al., 2013). In part to the broadened diagnostic criteria, service availability, and increased awareness for ASD, a reported 29% increase in the prevalence of ASD was observed between 2008 and 2010, and 123% increase between the 2002 and 2010 in the United States (Centers for Disease Control and Prevention, 2014). Despite the extensive clinical research on defined ASD populations, these prevalence estimates cannot be taken as absolute. The influence of extrinsic factors such as geographic area, race or ethnicity, socioeconomic disparities, and

level of intellectual ability on ASD prevalence ought to also be explored, in order to better understand the etiological mechanisms underlying the origin of ASD.

1.1.2. Behavioral Characterization

As purported by the DSM-V, ASD is a behaviorally defined disorder with early childhood onset. Characterized principally by impairments in social interaction, communication, and restricted, stereotypic patterns of behaviors and interests, the behavioral spectrum for ASD is heterogeneous in its presentation, ranging from mild to severe symptomology (American Psychiatric Association, 2013). For instance, clinical patterns in low-functioning children may present a complete absence of social interest and spoken language, whereas those with high-functioning ASD possess a milder symptom profile (Geschwind & Levitt, 2007). As such, it is evident that the severity of impairment varies along the spectrum and is individual-specific, where no two individuals with ASD possess the same behavioral profile (Lord, Cook, Leventhal, & Amaral, 2000). Generally speaking, deficits in social interaction and social communication may include impaired peer initiation, engagement, and response; poor pragmatic and social use of verbal and nonverbal communication; lack of theory of mind (inability to ascribe mental states to others); and difficulties in developing and maintaining social relationships. Restricted and repetitive behaviors and/or interests, on the other hand, may include atypical or pedantic speech; idiosyncratic language; stereotyped hand and whole body movements; preoccupations with objects and topics; preference for sameness; and complex routines or rituals, which are associated with distress when they cannot be fulfilled (Table 1.1; American Psychiatric Associaton, 2013;

Ventola, Oosting, Anderson, & Pelphrey, 2013; Baron-Cohen, Leslie, & Frith, 1985).

Table 1.1. Diagnostic criteria for ASD (American Psychiatric Association, 2013).

Diagnostic Criteria for Autism Spectrum Disorder:
A. Persistent deficits in social interaction and social communication across various contexts, as manifested by 3 of the following symptoms: 1. Deficits in social-emotional reciprocity; *including abnormal social approach, failure to initiate and respond to social interaction, inability to engage in normal back-and-forth conversation, and reduced sharing of affect, interests, and emotions.* 2. Deficits in nonverbal communicative behaviors used in social interaction; *including abnormal eye contact and body language, lack of facial expressions and/or gestures, inability to integrate verbal and nonverbal communication during social interaction, and deficits in the use and understanding of nonverbal communication.* *3.* Deficits in the development, maintenance, and comprehension of relationships (appropriate to developmental level and relationships beyond those with caregivers); *including absence of interest in peers, difficulties adjusting behaviors to suit social contexts, and difficulties making friends.* **B. Restrictive, repetitive patterns of behavior or interests, as manifested by 2 of the following:** 1. Stereotyped or repetitive motor movements, speech, or use of objects; *including idiosyncratic phrases, motor stereotypies, and echolalia.* 2. Excessive resistance to change, adherence to routines, and ritualized patterns of

verbal and nonverbal behavior; *including difficulties with transitions, insistence on sameness, and motoric rituals.*

3. Highly fixated interests that are abnormal in intensity; *including perseverative interests and preoccupation with unusual objects.*
4. Hyper- or hypo-reactivity to sensory input and/or abnormal interest in sensory features of the environment; *including indifference to pain/cold/heat, excessive touching and smelling of objects, fascination with moving objects and lights, and adverse responses to certain sounds.*

C. Symptoms are present in early childhood, but absolute manifestation of symptoms may not emerge until social demands exceed limited capacities.

D. Together, symptoms result in clinically significant impairments in social, occupational, and everyday functioning.

E. Disturbances cannot be better explained by intellectual disability (i.e. intellectual developmental disorder) or global developmental delay.

Additionally, a range of comorbid symptoms may also be exhibited, including hypersensitivity, abnormal pain reactivity, self injury, seizures, reduced joint attention, socially inappropriate behaviors, mental retardation, anxiety, and sleep disturbances (Sandman, 1990; Volkmar & Nelson, 1990; Bågenholm & Gillberg, 1991; Rapin, 1991; Realmuto & Ruble, 1999; Muris, Steerneman, Merckelbach, Holdrinet, & Meesters, 1998; Kim, Szatmari, Bryson, Streiner, & Wilson, 2000; Militerni et al., 2000; Dawson et al., 2004; Liu, Hubbard, Fabes, & Adam, 2006; Baron-Cohen, Ashwin, Ashwin, Tavassoli, & Chakrabarti, 2009; Schneider & Przewlocki, 2011). Intellectual capabilities

also appear to be compromised in ASD, where approximately half of individuals with ASD display intellectual disability and less than one in five display moderate to severe intellectual disability (as measured by IQ) (Charman et al., 2011).

1.1.3. Brain Abnormalities and Pathology

Given the multifaceted nature of ASD symptomology, it has been difficult to delineate a single unified neurological mechanism that may underlie the core features of ASD. Although an abundance of pathological studies – aimed at linking changes in brain structure and cellularity with atypical behavior – have emerged, findings are inconsistent with regard to the location and the alterations in the neural systems involved. The scope of this chapter is not to examine all findings contributing to the pathogenesis of ASD, but rather focus on two brain regions consistently implicated in ASD research and of the studies presented in this thesis: the *prefrontal cortex (PFC)* and the *amygdala*.

1.1.3.1. Prefrontal Cortex (PFC)

The PFC constitutes the highest level of cortical hierarchy with regard to the representation and execution of actions. Memory, planning, social cognition, and executive functioning, all encompass the wide repertoire of higher-order cognitive, social, language, and emotional processes regulated by the PFC (Fuster, 2001; Stuss & Knight, 2002; Kolb & Whishaw, 2009). Given that many of these PFC-dependent functions are disrupted in ASD, it is likely that alterations in the neural circuitry underlie these impairments. That is, abnormal functioning within the PFC and/or diminished functional connectivity between the PFC and other brain areas may underlie the deficits

in sociality, attention, communication, multi-tasking, and stereotypic behaviors characteristic of ASD (Courchesne & Pierce, 2005; Rinaldi, Perrodin, & Markram, 2008). In the present thesis, two PFC areas are of particular interest: the medial prefrontal cortex (mPFC) and orbital prefrontal cortex (OFC). Well connected with the brainstem and limbic formations, both the mPFC and OFC play a major role in emotion, social processes, and the control of basic drives (Fuster, 2001) – all of which are thought to be implicated in ASD.

Located medially in the PFC, the mPFC surrounds the most anterior part of the dorsal cingulate gyrus. Comprised of five main subdivisions – anterior cingulate cortex, prelimbic cortex, infralimbic cortex, shoulder cortex, and lateral orbital areas – the mPFC receives projections from the hippocampus, amygdala, ventral tegmental area, and nucleus accumbens, and projects to most subcortical regions of the brain (Zilles, 1985). Traditionally, the mPFC has been implicated in attentional processes, working memory, general motility, processing and integrating emotion/affect, theory of mind, and behavioral flexibility (Fuster, 2001; Heidbreder & Groenewegen, 2003). Interestingly, numerous imaging studies of the mPFC in clinical ASD populations have identified abnormal activity. More specifically, reduced or deviant activation of the mPFC has been shown during a variety of social (Happe et al., 1996; Pierce, Haist, Sedaghat, & Courchesne, 2004; Neuhaus, Beauchaine, & Bernier, 2010), executive function (Gilbert, Bird, Brindley, Frith, & Burgess, 2008), and cognitive tasks (Kennedy, Redcay, & Courchesne 2006), leading to the idea of altered mPFC activation and neocortical connectivity in ASD. Moreover, abnormally patterned and increased short-distance functional connectivity within the mPFC, but reduced long-distance reciprocal

connectivity between the PFC and other brain regions, is postulated to underlie the impaired executive and other higher-order processes seen in ASD (Courchesne & Pierce, 2005; Rinaldi et al., 2008). For the purpose of this thesis, the Cg3 subdivision of the mPFC, as determined by Zilles (1985), will be examined.

The OFC is located along the rhinal fissure, forming the anterior insular region of the PFC, as well as the tissue on the ventral surface of the frontal pole. Subdivided into medial and lateral portions; the OFC is well connected with the mPFC, as well as outside – including limbic areas (hippocampus and amygdala), sensory areas (somatic sensory, olfaction, vision, and taste) and midbrain structures (Price, 2006). The OFC is believed to play a major role in response inhibition, the exclusionary aspect of attention, filtering extraneous cognitive information, control of mood, social regulation, empathy, and aspects of reward and decision making mechanisms with a special emphasis on olfactory inputs (Cavada & Schultz, 2000; Fuster, 2001; Ventola et al., 2013). Moreover, the highly functional and reciprocal relationship between the OFC and amygdala permits the modulation and maintenance of intra-specific social bonding, as well as the self-regulation of emotional states, thus, subserving the processes of social-cognition and emotion functioning which are thought to be highly dysregulated in ASD (Bachevalier & Loveland, 2006). In fact, a number of studies in clinical ASD populations have identified abnormalities in OFC (amid a series of interconnected brain structures), suggesting a dysfunctional neural network (Kawasaki et al., 1997; Dawson, Meltzoff, Osterling & Rinaldi, 1998; Schultz, Romanski, & Tsatsanis, 2000; Zilbovicius et al., 2001; Ashwin, Baron-Cohen, Wheelwright, O'Riordan, & Bullmore, 2007). Interestingly, Bachevalier & Loveland (2006) posit that dysfunction of the OFC may lead to an observed loss of social

skills around the second year of life – an idea that is consistent with the manifestation/diagnosis of social impairment in children with ASD. For the purpose of this thesis, the AID subdivision of the OFC, as determined by Zilles (1985), will be examined.

1.1.3.2. Amygdala

Considered the epicenter of emotion, the amygdala is located in the anterior portion of the medial temporal lobe and is comprised of thirteen interconnected nuclei, including the lateral, central, and basal nuclei. Briefly, the lateral nucleus receives visual (facial expressions, gaze direction, body postures) and auditory (vocal sounds and intonations) information and can, reciprocally, modulate the cortical processing of sensory information (affective and emotional states). The central nucleus of the amygdala, in contrast, is deemed to influence the endocrine and autonomic manifestations of emotion via projections to the brainstem and hypothalamus. Namely, this pathway can elicit activation of emotional reactions and responses. Finally, the basal and accessory basal nuclei project to the ventral striatum, enabling action by subcortical elements of the motor system in response to incoming affective information from these nuclei (Amaral, 1992; Rolls, 1999; Bachevalier & Loveland, 2006).

Given the large role of amygdalar nuclei in various aspects of social and emotional processing, it is likely that dysregulation of this structure is involved in the genesis of ASD. In fact, lack of regional activation during facial processing and recognition (Baron-Cohen et al., 2000; Critchley et al., 2000), and increased grey matter amygdalar volume (Abell et al., 1999; Waiter et al., 2004) have consistently been

demonstrated in clinical ASD populations. Moreover, increases in amygdalar volume have been shown to be highly correlated with impaired facial recognition and symptom severity (Howard et al., 2000; Sparks et al., 2002). As such, disordered patterns of socio-emotional behavior in ASD may, in part, be attributed to amygdalar dysfunction and/or alterations in circuitry.

1.1.4. Treatment Options for ASD

Despite the plethora of research surrounding the possible neurobiological causes of ASD, the etiology of the disorder still remains elusive. As a result, current treatment options have only been targeted treatments of ASD symptomology, as opposed to the underlying cause. In the past few decades, a myriad of treatment options and interventions have been developed with the aim to improve social, communicative, and motor functioning in ASD populations. Additionally, given the likelihood of comorbid behavioral issues associated with ASD, clinicians often adopt a multifaceted approach to treatment. Behavioral intervention and pharmacological treatment constitute the two major therapeutic approaches to ASD.

Psychopharmacological treatments seek to alleviate core ASD symptomology, as well as concomitant comorbid behaviors (Ventola et al., 2013). Atypical antipsychotics, such as risperidone, have proven effective in tempering aggression, self-injury, and irritable behaviors often associated with ASD (McDougle et al., 1998; McCracken et al., 2002; Schneider & Przewlocki, 2011). Additionally, other medications including psychostimulants (e.g. methylphenidate), serotonin reuptake inhibitors (e.g. fluoxetine, clomipramine), and presynaptic noradrenergic blocking agents (e.g. clonidine) have also

been shown to attenuate symptoms of hyperactivity, self-injury, and irritability (Myers, 2007). More recently, intranasal oxytocin administration in children and adults with ASD has exhibited great efficacy as a pharmacological agent. Marked improvements in social cognition (Bartz & Hollander, 2008), social motivation (Andari et al., 2010), and a reduction in repetitive behaviors (Hollander et al., 2003) were observed, suggesting enhanced social functioning and salience as a consequence of oxytocin administration.

In contrast, behavioral, or psychosocial, intervention seeks to target and improve behavioral functioning by focusing on the techniques of learning and behavioral change. Treatments have been aimed at attenuating restricted interests and impaired social behaviors while reinforcing the proper use of language and social strategies (Lovaas, Koegel, Simmons, & Long, 1973; Ventola et al., 2013). Applied behavioral analysis (ABA), for instance, adopts a systematic approach of skill acquisition in children with ASD to reinforce desired behaviors and reduce undesired behaviors (Lovaas et al., 1973; Vismara & Rogers, 2010; Ventola et al., 2013). Additionally, pivotal response treatment (PRT), another intensive behavioral therapy, builds upon the foundations of ABA and promotes naturalistic skill acquisition in social and communicative domains, by addressing the child's social motivation (Koegel & Koegel, 2012; Ventola et al., 2013).

While several pharmacological treatments and behavioral modification therapies have proven to be effective in the attenuation of ASD symptomology, limited success of any treatment modality – individual or combined – to completely reverse core autistic traits have been met.

1.1.4.1. Massage/Touch Therapy

Infant massage, or touch therapy, constitutes another alternative behavioral approach that has been shown to be effective in attenuating autistic symptoms. Physical in nature, touch therapy has been demonstrated to have a positive physiological and psychological impact on infant development that far exceeds the immediate sensation (Field et al., 1996). During development, exposure to touch not only influences the mother-infant attunement and co-regulation, but also infant brain development (Jones & Mize, 2007). More specifically, touch therapy during infancy profoundly influences cognitive, motor, social, and physiological development, and may play a pivotal role in the socialization of higher-order emotional processes (Wyschograd, 1981; Ottenbacher et al., 1987; Weber, 1990) – further illuminating touch as a potent avenue for intervention.

As revealed similarly in premature infants, infants of depressed mothers, and other high-risk groups (e.g. orphans and infants suffering from Cerebral Palsy), touch therapy in children with ASD proves to be beneficial by promoting neurobehavioral development, thus improving the child's overall outcome (Jones & Mize, 2007). Improvements in verbal and nonverbal communication skills, sleeping patterns, increased on-task behavior and social relatedness, increased attentiveness in the classroom, fewer stereotypical autistic behaviors, and decreased touch aversion were reported in ASD children following touch intervention in early childhood (Field et al., 1997; Escalona, Field, Singer-Strunck, Cullen, & Hartshorn, 2001; Cullen, Barlow, & Cushway, 2005a; Jones & Mize, 2007). Thus, it appears that massage therapy is an effective intervention in attenuating various social and communicative behavioral issues in children with ASD, further insinuating the importance of early sensory input on the developing autistic brain.

1.2. Animal Models of ASD

Despite decades of research, the etiology of ASD remains undetermined. In recent years, however, converging lines of evidence suggest that most cases of ASD arise from a complex interaction of genetic predisposition and strong environmental influence. Thus, in order to elucidate the neural basis of this complicated and unique human disorder, it is important to pursue all avenues – molecular, cellular, genetic, and behavioral levels – underlying ASD. Animal models, particularly rodents, have proven to be a highly valuable tool for the study of ASD. More specifically, animal models may be utilized to study the neural pathways, cellular and molecular anomalies, roles of genes and the environment, and the application of therapeutic treatments – all of which may be implicated in the pathogenesis of ASD. Currently, animal models of ASD can be categorized into three general approaches: 1) models based on neurobiological anomalies – neurophysiological or neurochemical in nature; 2) genetically modified animal models – targeted mutations in genes; and 3) environmentally induced animal models – pre- and perinatal exposure to teratogens and/or immunological infections (Schneider & Przewlocki, 2011). In the present chapter, we will briefly examine genetic and insult-based etiological animal models of ASD to illustrate the translational value of such models in reproducing behavioral and pathological symptoms characteristic of human ASD. Moreover, the valproic acid (VPA) rodent model of ASD – an environment-insult based model – will be discussed in detail, as the behavioral and neuroanatomical characterization of this model constitutes the studies presented in this thesis.

It is important to note that given the tremendous phenotypic variability in ASD, an ongoing challenge for the development of a translational animal ASD model is the

extent to which the model encompasses the many clinical hallmarks of ASD. That is, should animal models replicate all pathophysiological aspects (behavioral, genetic, and neuroanatomical), or particular features of the disorder? Resolution of this conundrum lies primarily in the goal or question the model wishes to validate. For instance, modeling ASD symptomology (*face validity*) versus simulation of the underlying mechanisms (*construct validity*) ought to be considered when evaluating criterion for animal models of ASD and other psychiatric disorders (Tordjman et al., 2006; Schneider & Przewlocki, 2011).

1.2.1. Genetic Characterization

Given the extraordinarily heritable nature of ASD, genetically altered animal models offer promising new developments in studying the etiology of this intrinsically complex disorder. Most genetically induced rodent models of ASD are based on the manipulation of candidate genes or pathways that have been identified and characterized in subsets of human ASD populations (Moy, Nadler, Magnuson, & Crawley, 2006). Moreover, the customary strategy for genetic modification involves the creation of a null allele, where targeted disruption of a loci segment prevents the production of functional proteins (i.e. chromosomal deletions and knockout mutations). Additionally, generation of an ASD-associated allele (i.e. knock-in mutation) is an alternative approach to generate analogous mutations in the rodent genome (Moy et al., 2006; Silverman, Yang, Lord, & Crawley, 2010).

In developing genetically engineered rodent models of ASD, it is important to note that despite the strong genetic component, ASD is not a monogenic disorder. That is,

whereas single-gene mutants do offer insight into some features of ASD, they are not absolute or directly relevant models. In fact, it has been suggested that the number of loci associated with ASD in clinical populations exceeds 15 (Risch et al., 1999), leading to the idea that ASD is far more complex than initially thought, and that a single loci or factor cannot account for the multifaceted nature of the disorder (Moy et al., 2006). Nonetheless, there are several genetically modified mice and rat models that share behavioral features analogous to human ASD symptomology.

For instance, the BTBR T^{+}tf/J (BTBR) mouse model is a genetically homogenous inbred strain that displays great behavioral similarity to the two diagnostic domains of symptoms in ASD. Reductions in sociality and reciprocal social interactions, altered ultrasonic vocalizations in pups and adults, and increased frequency of repetitive self grooming all encompass the behavioral phenotype of BTBR mice that is relevant to human ASD (Silverman et al., 2010; Meyza et al., 2013). Additionally, whereas neuroanatomical and physiological similarities to human ASD have also been demonstrated in BTBR mice – such as the *Disc1* mutation, for example – only a fraction of human ASD cases appear to possess these alterations (Kilpinen et al., 2008; Crepel et al., 2010; Meyza et al., 2013).

Nlgn4 mice constitute another genetically altered animal model of ASD that exhibits a behavioral phenotype reflecting social deficit. Mutation in the human *NLGN4* gene is implicated in the pathogenesis of ASD, as *NLGN4* encodes an important post-synaptic cell adhesion protein (Jamain et al., 2003). As such, a null mutation in the murine orthologue of the *NLGN4* gene produces mutant mice exhibiting an ASD-like behavioral phenotype, particularly in the social and communicative domains (Jamain et

al., 2003; Jamain et al., 2008; El-Kordi et al., 2013; Wohr & Scattoni, 2013a, b). In sum, these two genetic mice models of ASD provide a sample of the wide range of rodent models available for elucidating the origins of ASD. It is evident that manipulation of candidate genes may reproduce behavioral and neuroanatomical phenotypes comparable to human ASD.

1.2.2. Environmental Characterization

Exposure to a multitude of environmental factors during embryogenesis has long been implicated in the pathogenesis of ASD. Given the degree of ecological validity associated with environmental risk factors, insult-based animal models of ASD have been valuable in elucidating the complex relationships underlying ASD. Ranging from pre- and perinatal exposure to thalidomide (Narita et al., 2002), VPA (Ingram, Peckham, Tisdale, & Rodier, 2000), ethanol (Arndt, Stodgell, & Rodier, 2005), and pre- and postnatal viral infections (Lancaster, Dietz, Moran, & Pletnikov, 2007; Chess, Fernandez, & Korn, 1978), environmentally-induced animal models of ASD have been proposed and evaluated on the basis of behavioral, genetic, and neuropathological similarities to the human disorder.

An extensive body of epidemiological research has clearly established the link between maternal use of VPA during pregnancy and the increased incidence of ASD – a 4.42% absolute risk (Williams et al., 2001; Bromley et al., 2013; Christensen et al., 2013; Roullet, Lai, & Foster, 2013). Given the many health risks associated with VPA use during pregnancy and the childbearing years, simulation of clinical endpoints and behavioral phenotyping in animals models have provided further insight into the impact

of VPA on the developing brain and the emergence of ASD (Roullet et al., 2013). Several independent laboratories have demonstrated that *in utero* exposure to VPA induces abnormal patterns of somatic and physiological development across a variety of species including rabbits (Petrere, Anderson, Sakowski, Fitzgerald, & de la Iglesia, 1986), rhesus monkeys (Mast, Cukierski, Heinz, & Hendrickx, 1986), and especially rodents (Brown, Kao, & Fabro, 1980; Bruckner, Lee, O'Shea, & Henneberry, 1983; Nau, Hauck, & Ehlers, 1991; Ehlers, Sturje, Merker, & Nau, 1993; Sonoda, Ohdo, Ohba, Okishima, & Hayakawa, 1993; Mengola, Broccia, Prati, & Giavini, 1998). In fact, in 1996, Rodier and colleagues established the VPA rodent model of ASD to further elucidate the mechanism of its teratogenic action (Rodier, Ingram, Tisdale, Nelson, & Romano, 1996; Rodier, Bryson, & Welch, 1997a). This model exhibits striking behavioral, neuropathological, cellular, and immunological similarities to the ASD phenotype, validating the multidimensional approach of the VPA model in elucidating the clinicopathology of ASD (Rodier, Ingram, Tisdale, & Croog, 1997b; Dufour-Rainfray et al., 2011; Schneider & Przewlocki, 2011; Roullet et al., 2013). Decreases in social interactions, increased repetitive/stereotypic activity, abnormal responses to painful and non-painful stimuli, increased anxiety, alterations in pup ultrasonic vocalizations (USVs), and early signs of neurodevelopmental delay all encompass the wide repertoire of impaired behavioral functioning consequent of prenatal VPA exposure (Schneider & Przewlocki, 2005; Roullet et al., 2013). Moreover, on an anatomical and cellular level, rats prenatally exposed to VPA exhibit reduced cranial nerve motor nuclei, significant diminution of cerebellar Purkinje cells, hypoplasia of brainstem structures, reduced volume of posterior portions of cerebellar vermis and hemispheres, injury of cerebellar deep nuclei, and

decreases in the spine density of forebrain structures, paralleling many neuropathological findings reported in human ASD (Rodier et al., 1996; Rodier et al., 1997a, b; Courchesne, 1997; Ingram et al., 2000). Thus, given the remarkable similarities between the VPA rodent model of ASD and the human condition, it is evident that this model is a viable experimental platform to further elucidate the neurobiological mechanisms underlying the origins of ASD.

1.3. Objective of Thesis

The present studies were aimed at investigating the validity of *in utero* VPA exposure in simulating the brain developmental disturbances equivalent to those seen in human ASD. Specific objectives achieved in the present thesis include:

1. To investigate the effect of *in utero* VPA exposure on offspring lifelong behavior in social, communicative, emotional, cognitive, and motor domains;
2. To investigate the plastic changes associated with *in utero* VPA exposure, in brain regions implicated in ASD; and,
3. To investigate the therapeutic effect of early tactile stimulation – an enriching experience – in attenuating the behavior and neural underpinnings of *in utero* VPA exposure.

1.4. Hypotheses

The following hypotheses guided the research:

1. Prenatal exposure to a single dosage of VPA will produce behavioral and neuroanatomical characteristics equivalent to human ASD (*face and construct validity*); and,
2. An early enriching experience of tactile stimulation will attenuate or remediate the VPA-induced behavioral and neuroanatomical changes in rats (*predictive validity*).

1.5. Organization of Thesis

The present thesis includes two experiments, both utilizing an acute high dosage paradigm of prenatal VPA to simulate a translational model of ASD. Experiments are presented as individual manuscripts, each of which has been submitted to a journal for publication.

CHAPTER 2

The Effects of Prenatal Exposure to Valproic Acid on the Development of Juvenile-Typical Social Play in Rats∗

2.1. Abstract

Autism is a severe neurodevelopmental disorder characterized by qualitative impairments in social behavior, communication, and aberrant repetitive behaviors. A major focus of animal models of autism has been to mimic the social deficits of the disorder. The present study assessed whether rats exposed prenatally to valproic acid (VPA) exhibit deficits in social play as juveniles that are consistent with the social deficits seen in autism. Dams were exposed to an acute dose of VPA on gestational day 12.5. Later, the playful interactions and associated ultrasonic vocalizations (USVs) of the juveniles were examined. It was predicted that VPA-treated rats should play less than the controls. Characteristic of neurobehavioral insult at this early age, the VPA-treated juveniles exhibited significant increases in the frequency of body shakes and sexual mounting, but played at the same frequency as the controls. However, when playing, they were less likely to use tactics that facilitated bodily contact and vocalized less. These data suggest that prenatal VPA exposure disrupts some aspects of being able to communicate effectively and engage partners in dynamic interactions – deficits that are consistent with those seen in autism.

∗Raza, S., Himmler, B.T., Himmler, S.M., Harker, A., Kolb, B., Pellis, S.M., & Gibb, R. (2015). The Effects of Prenatal Exposure to Valproic Acid on the Development of Juvenile-Typical Social Play in Rats. *Submitted to Behavioral Pharmacology.*

2.2. Introduction

Autism is a pervasive neurodevelopmental disorder characterized by impairments in social interaction and communication, and by highly stereotypic behaviors and/or restricted interests. Owing to the heightened awareness and shifting diagnostic criteria over the past decade, the prevalence of autism has seen a significant rise: seemingly affecting 1 in 68 children (Centers for Disease Control and Prevention, 2014). Despite decades of research, the etiology of autism remains elusive. Recently, however, several lines of converging evidence strongly suggest that the interplay between environmental and genetic factors may be contributing to the behavioral pathologies seen in autism (Hallmayer et al., 2011; Ronald & Hoekstra, 2011). For this reason, preclinical research is now focusing on the development of transgenic and environmental animal models of autism to understand the genotype-phenotype relationship better, as well as the neurobiological mechanisms that underlie the disorder (Bolivar, Walters, & Phoenix, 2007; Banerjee et al., 2014). Validation of these animal models of autism is contingent upon the ability to simulate the distinctive clinical features of autism, such as the deficits in aspects of social function (Wöhr & Scattoni, 2013a, b).

Retrospective studies on human subjects have demonstrated that *in utero* exposure to VPA –a teratogenic anticonvulsant – during the first trimester of pregnancy leads to an increased incidence (4.42% absolute risk) of autism and intellectual disability (Christianson, Chesler, & Kromberg, 1994; Moore et al., 2000; Rasalam et al., 2005; Christensen et al., 2013). Owing to this correlation between *in utero* VPA exposure and the increased risk of autism, the VPA rodent model was developed (Rodier et al., 1996; Arndt et al., 2005; Bromley, Baker, & Meador, 2009; Bath & Sharfman, 2013; Bromley

et al., 2013). Pregnant dams exposed to a single dose of VPA on gestational day 12.5 produce progeny that exhibit behavioral and neuroanatomical anomalies that are similar to those present in humans with autism (Rodier et al., 1996; Rodier et al., 1997a, b; Schneider & Przewlocki, 2005; Rinaldi et al., 2008; Snow, Hartle, & Ivanco, 2008; Mychasiuk, Richards, Nakahashi, Kolb, & Gibb, 2012; Chomiak, Turner, & Hu, 2013).

A potentially useful behavioral assay of the kinds of social deficits seen in autism is rough-and-tumble play or play fighting (Pellis & Pellis, 1998). Play fighting is an affiliative form of behavior that involves many neural systems for its execution and regulation (Siviy & Panksepp, 2011; Vanderschuren, Niesink, & Van Ree, 1997), and involves complex patterns of inter-animal coordination and communication (Palagi et al., 2015). That is, play fighting involves the kinds of social abilities thought to be compromised in autism. Thus, it is likely that play fighting may not only offer insight into the dynamics of social interactions, but may also highlight some of the neural mechanisms that are altered or compromised in VPA-exposed rats. Interestingly, Schneider and Przewlocki (2005) and Chomiak and colleagues (2010) have demonstrated attenuated social play behavior in rats prenatally exposed to VPA, indicating impaired sociality. However, while these studies indicate reduced sociality, the measures used to evaluate play fighting do not allow for the characterization of altered behavioral mechanisms (e.g., Bell, McCaffrey, Forgie, Kolb, & Pellis, 2009; Pellis, Pellis, & Whishaw, 1992) that led to this reduction in play. Also, rats emit high frequencies of ultrasonic vocalizations (USVs) in the 50-kHz range during play (Brunelli et al., 2006; Burgdorf et al., 2008), which may have a potential communicatory role during play (Himmler, Kisko, Euston, Kolb, & Pellis, 2014; Kisko, Himmler, Himmler, Euston, &

Pellis, 2015). Therefore, in the present study, a detailed analysis of play fighting involving the scoring of the attack and defense tactics used (Himmler, Pellis, & Pellis, 2013) and the frequency of the 50-kHz calls emitted (Burgdorf et al., 2008; Wright, Gourdon, & Clarke, 2010) was conducted on prenatally exposed VPA rats. Pregnant dams were exposed to an acute high dose of VPA on gestational day 12.5, and the resultant female progeny were tested between 29 and 34 days of age, which is within the peak period of playful interactions in rats (Thor & Holloway, 1984). Females were utilized to avoid potential dominance effects typically seen in male rats (Pellis, Field, Smith, & Pellis, 1997).

It was hypothesized that if exposing rats prenatally to VPA rats is a suitable animal model of autism, the reduced playfulness previously reported (Schneider & Przewlocki, 2005; Chomiak, Karnik, Block, & Hu, 2010) should arise from a reduction in the use of playful tactics that promote sustained body contact and there should be a reduction in the emission of 50-kHz calls associated with play. That is, the rats should have impairments in the skills that lead to fast-paced and coordinated playful interactions.

2.3. Methods

2.3.1. Subjects

Thirty-two female Long-Evans rats (16 VPA and 16 control) born to 3 VPA-exposed and 3 control dams were used. All subjects remained with their mothers until weaning on postnatal (PN) day 22, when they were randomly paired with a group- and age-matched (within 3 days) partner. Pairs were housed in standard polycarbonate shoebox cages (46cm X 25cm X 20cm) and maintained on a 12hr light/12hr dark diurnal

cycle. Food and water were available *ad libitum*. Animals were born and raised in the vivarium maintained at the Canadian Centre for Behavioural Neuroscience, University Lethbridge. All experimental protocols were approved in accordance with the Canadian Council of Animal Care and the University of Lethbridge Animal Care Committee.

2.3.2. VPA Administration

Pregnant dams were administered VPA orally on gestational (G) day 12.5. Prior to VPA administration, all dams were spoon-fed 1.5g of peanut butter for three consecutive days. On G12.5, half of the dams received 800mg/kg of VPA mixed with peanut butter, while the remaining dams received peanut butter alone.

2.3.3. Testing Enclosure and Measurement of Ultrasonic Vocalizations

Play trials were conducted in a Plexiglas® box, measuring 50cm X 50cm X 50cm, with 1-2cm of CareFresh® bedding. The Plexiglas® box was enclosed in a soundproofed chamber (inside measurements: 59cm X 65.5cm X 81.5cm). The soundproofed chamber contained sound-attenuating foam and an ultrasonic microphone (Model 4939, Brüel & Kjaer, Denmark) located 32-38cm above the chamber floor. The microphone was connected to a Soundconnect^{TM} amplifier (Listen, Inc, Boston, MA) that processed all recordings via a multifunction processor (RX6, Tucker-Davis Technologies, Alachua, CA) on a desktop in the testing room. The multifunction processor was programmed with MATLAB. The receiver recorded frequencies ranging from 4- and 100-kHz and all files were, subsequently, converted to .wav files. The play trials were filmed using a DVD103 Sony Handycam with night-shot capacity, elevated at a 45° angle. Off-line analysis was

performed using Raven Pro 1.4 software (Cornell University, Ithaca, NY) for audio files, while Avidemux 2.6 software was used for video files.

2.3.4. Procedure

Play fighting was assessed between PN29-PN34, the age at which playful interactions are most frequent in rats (Thor, & Holloway, 1984). Three days prior to testing, the rats were habituated, in pairs, to the play enclosure for 30 min/day, then prior to testing, the rats were socially isolated for 24 hours, to ensure enhanced playfulness when introduced to the experimental enclosure (Panksepp & Beatty, 1980; Niesink & Van Ree, 1989; Pellis & Pellis, 1990). Test sessions lasted for 10-min, providing sufficient time to capture a large sample of playful interactions (Pellis & Pellis, 1990). Pairs of rats were placed in the test enclosure, one at a time, and recording began once both rats were inside the enclosure. Given that the vocalizations and play behavior were recorded on separate devices, the audio and video files were synchronized via a device emitting a simultaneous light/sound cue. At the beginning of each trial, three consecutive cues were emitted. Trials were considered to have begun during the onset of the third beep (Himmler et al., 2014). Each pair was tested twice, with a 24-hour rest interval between the two trials. Both habituation and testing occurred in complete darkness, as the frequency of social behaviors has been demonstrated to increase in the dark (Pellis & Pellis, 1987, 1990, 1997; Smith, Forgie, & Pellis, 1998; Foroud & Pellis, 2002).

2.3.5. Behavioral Analyses

Following collection of behavioral data, each 10-min play session was scored for

attack and defense (Pellis & Pellis, 1987). A playful attack was scored when one rat moved its snout towards the nape of the other, or actually contacts the nape with the snout. The response of the recipient of the attack was then scored. The recipient could either show no response and simply continue with its preceding activity (e.g., walking, manipulating the substrate) or defend against the imminent contact. Playful defense involves either evasion, whereby the defender moves its nape away from the attacker while simultaneously also turning its face away from the partner, such as by running, pivoting, or leaping, or by facing defense, whereby the defender moves its nape away from the attacker while turning to face the partner. In doing so, the defender opposes its teeth between its attacker and its own nape, thereby blocking access to the nape. Facing defense may take one of three forms: (a) complete rotation, where the defender rolls completely over onto its back, (b) partial rotation, where the defender rotates its forequarters, but ground contact with one or both of its hind feet is maintained, and (c) other, where rotations or movements in other dimensions are executed (Pellis et al., 1992). The defensive maneuver that was first attempted by the defending rat (as seen in the first 2-3 frames) was scored (Himmler et al., 2013). In addition, given that that the studies by Schneider and Przewlocki (2005) and Chomiak et al. (2010) used the frequency of pinning as their measure of play, this was also scored. A pin is scored when one rat lies on its back with all four paws freed from contact with the ground and the partner stands over it (Panksepp, 1981). The absolute frequency of this on-bottom/on-top configuration over the trial was scored. To investigate the effects of prenatal VPA exposure on play fighting, the frequency of pins and playful attacks per trial was calculated, as was the probability of defense against attacks. When a defense did occur,

the probability of using each of the different defensive tactics was also calculated.

Given the previous findings on the effects of VPA on play (Schneider & Prezwlocki, 2005; Chomiak et al., 2010), it was predicted that all the measurements that reflect the motivation to play (frequency of playful attacks, frequency of pinning, probability of defense) (Panksepp, 1981; Reinhart, Pellis, & McIntyre, 2004; Thor & Holloway, 1983), should decrease in the VPA-treated rats as compared to the controls. Alternatively, if the reduction in pinning revealed by the previous studies arose because of changes in how the VPA-treated rats defended themselves (Bell et al., 2009; Pellis et al., 1992), then it was predicted that the use of the complete rotation tactic would be reduced, and either evasion would increase if the rats were avoiding contact (Varlinskaya, Spear, & Spear, 1999) or one of the standing defenses would increase if the rats stood their ground to fend off the partner's attack (Reinhart et al., 2004).

In addition to playful attack and defense, four other behaviors were scored during the 10-min trials. In rats, as in other murid rodents (Pellis, 1993), play fighting involves competition for a body target, which for rats is the nape (Pellis & Pellis, 1987; Siviy & Panksepp, 1987), a target that is contacted by males during pre-copulatory interactions in adults. Sometimes, especially as the rats approach sexual maturity, the likelihood that playful interactions include mounting increases (Pellis & Pellis, 1990). Similar to copulatory behaviors in sexually mature male and female rats, mounting involves one animal approaching the partner from the rear, grasping its partner's flanks with its forepaws and making ventro-dorsal body contact. Such contact may also include pelvic thrusting, but without intromission. Both the playful competition for the nape and mounting is present in both males and females (Meaney & Stewart, 1981; Pellis & Pellis,

1990). Higher frequencies of mounting during play trials may indicate the precocial maturation of sex, a characteristic of autism reported in humans, especially in females (Dalldorf, 1983; Realmuto & Ruble, 1999). Therefore, the frequency of mounting was also scored during the play trials and it was predicted that the VPA-treated rats would mount more frequently.

During playful interactions, rats will occasionally jump, rotate, and shake their heads and bodies (Pellis & Pellis, 1983). However, when stressed, rats will show exaggerated rates of head and body shaking. In addition, stressed rats will increase their frequency of scratching the anterior of their body with their hind paws, and will engage in short duration, highly stereotyped bouts of grooming. These behaviors have been used in rats and other animals as markers of stress (Redmond & Huang, 1979; Van Hooff & Aureli, 1994; Van Den Bos, 1998; Komorowska & Pellis, 2004; Reinhart et al., 2004; Norscia & Palagi, 2011). Given that changes in play may result from changes in anxiety or stress, rather than to changes in being able to follow social rules and communicate effectively, head and body shakes, scratches, and stereotyped grooming were scored for their frequency over the duration of the trials. Moreover, increased head and body shaking has been reported as a side effect of postnatal treatment with VPA (Handley, & Singh, 1986; Fletcher, & Harding, 1981; Loscher & Honack, 1996) and may also be indirectly associated with prenatal treatments, given the influence of VPA on serotonergic systems which also increase the frequency of such shakes (Narita et al., 2002; Kuwagata, Ogawa, Shioda, & Nagata, 2009; Dufour-Rainfray et al., 2010). If this shaking increased along with scratching and stereotyped grooming, then it would likely signify that it may have arisen as a by product of stress, but if it alone increased, it would

reflect the VPA treatment did have a direct teratogenic effect. Therefore, it was predicted that shaking would increase in the VPA-treated rats, whether the other markers for stress did so or not.

Higher frequencies of play are correlated with higher rates of emitting 50-kHz calls (e.g., Brunelli et al., 2006; Burgdorff et al., 2008; Knutson, Burgdorf, & Panksepp, 1998), therefore, conversely, if VPA-treatment reduces play, there should be an associated reduction in the frequency of calling. To evaluate this possibility, the overall frequency of calling was scored. Due to the high number of calls, for quantitative comparisons only the calls emitted in the first 5-min of each trial were scored. Given that the highest frequency of play by rats is within the first five minutes after introduction to the test enclosure (Panksepp & Beatty, 1980; Panksepp, 1981) and that calling is most frequent when rats play the most, this truncated sample interval should suffice to evaluate any VPA-induced changes. Overall, however, if the frequency of calling follows frequency of play, the rate of calling per bout of play fighting (i.e., total number of calls/total number of play fights) should remain the same. To confirm that the frequency of calling fluctuated with the frequency of playing, the minute-by-minute occurrence of calling was compared to the minute-by-minute occurrence of play. It was predicted that if play was reduced in the VPA-treated rats, then the frequency of emitting ultrasonic calls would also be reduced, and that this reduction would be reflected in the above measurements.

2.3.6. Statistical Analysis

All statistical analyses were performed on SPSS 21 for Mac. For pairwise

comparisons with a predicted direction of treatment effect, independent one-tailed Student's t-tests were used and for cases in which a direction of effect was not predicted, two-tailed t-tests were used. For multiple pairwise comparisons that did have a predicted outcome, a Bonferroni correction was used to correct the alpha value. For the minute-by-minute comparison of the frequency of playful attacks with the frequency of calling, a repeated measures two-way ANOVA with group and time as the factors of interest was used. The significance level was set at ≤ 0.05 for all the analyses. All graphical representations of the data represent the means and standard errors.

2.3.7. Inter-Observer Reliability

The videotapes were all scored by one observer (S.R). To ensure a high degree of consistency in scoring, a subset of trials (6 pairs; 3 VPA, 3 control) were re-scored for playful attack, probability of defense, total number of pins, complete rotation defense, and evasive defense by another observer (S.M.H.), as were the frequency of vocalizations occurring in these trials (B.T.H.). The values obtained by these second scorers were compared to those of the primary scorer (S.R.) using the Pearson's correlation, which revealed a significantly high degree of inter-observer reliability for all measures: values ranged between 0.774 and 0.986, with five of six being significant, $p < 0.05$. For the purposes of this paper, all data shown are values derived from the primary scorer (S.R.).

2.4. Results

2.4.1. Frequency of Play

VPA and control rats did not differ significantly either in their frequency of

launching nape attacks [$t(14) = 1.149$, $p = 0.270$] (Figure 2.1A) or in the probability of defending against a nape attacks [$t(14) = 0.0270$, $p = 0.979$] (Figure 2.1B). Similarly, there was no significant difference in the frequency of pins [$t(14) = 0.784$, $p = 0.446$] (Figure 2.1C).

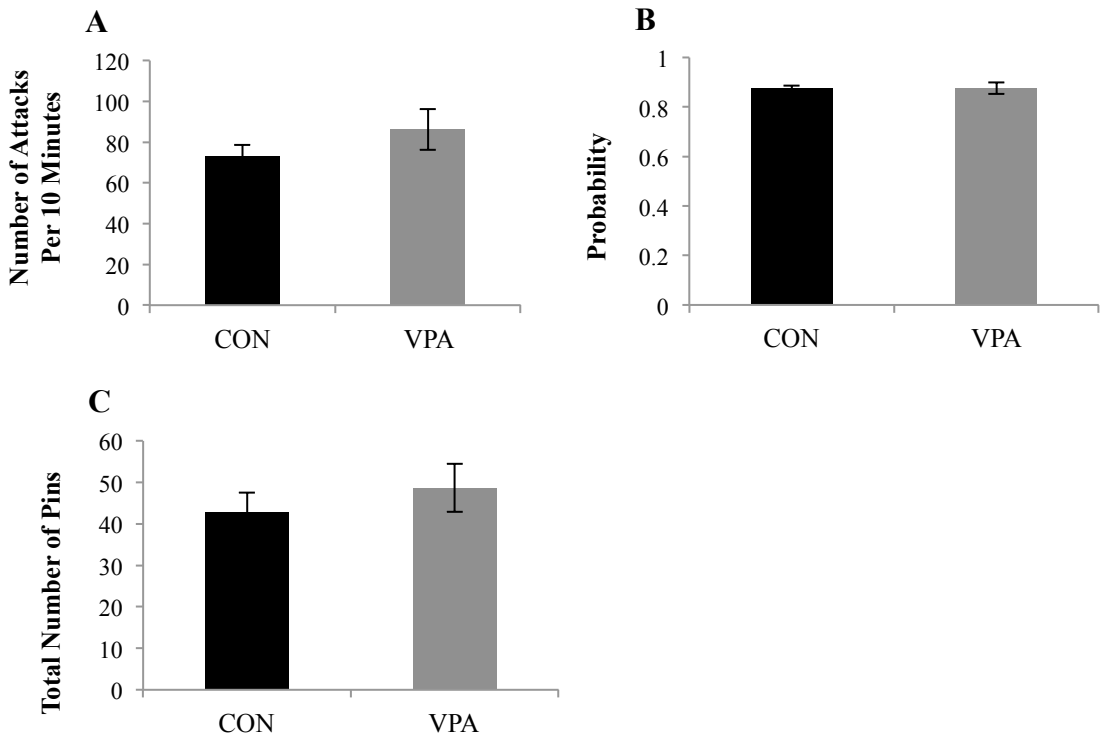

Figure 2.1. (A) The total number of playful attacks per 10 minute play trial. (B) The probability of defending against a playful attack. (C) The total number of pins per 10 minute play trial.

2.4.2. Frequency of Defensive Tactics

The VPA and control rats did not differ in their use of evasion [$t(14) = 0.986$, $p = 0.341$] (Figure 2.2A). With regard to the turning to face defensive tactics, VPA rats were significantly less likely to turn to supine [$t(14) = -2.513$, $p = 0.025$] (Figure 2.2B) and significantly more likely to remain standing, using "other" defense tactics [$t(14) = 2.822$, $p = 0.014$] (Figure 2.2C). There was no significant difference in the probability of using

the partial rotation tactic [$t(14) = -1.984$, $p = 0.067$].

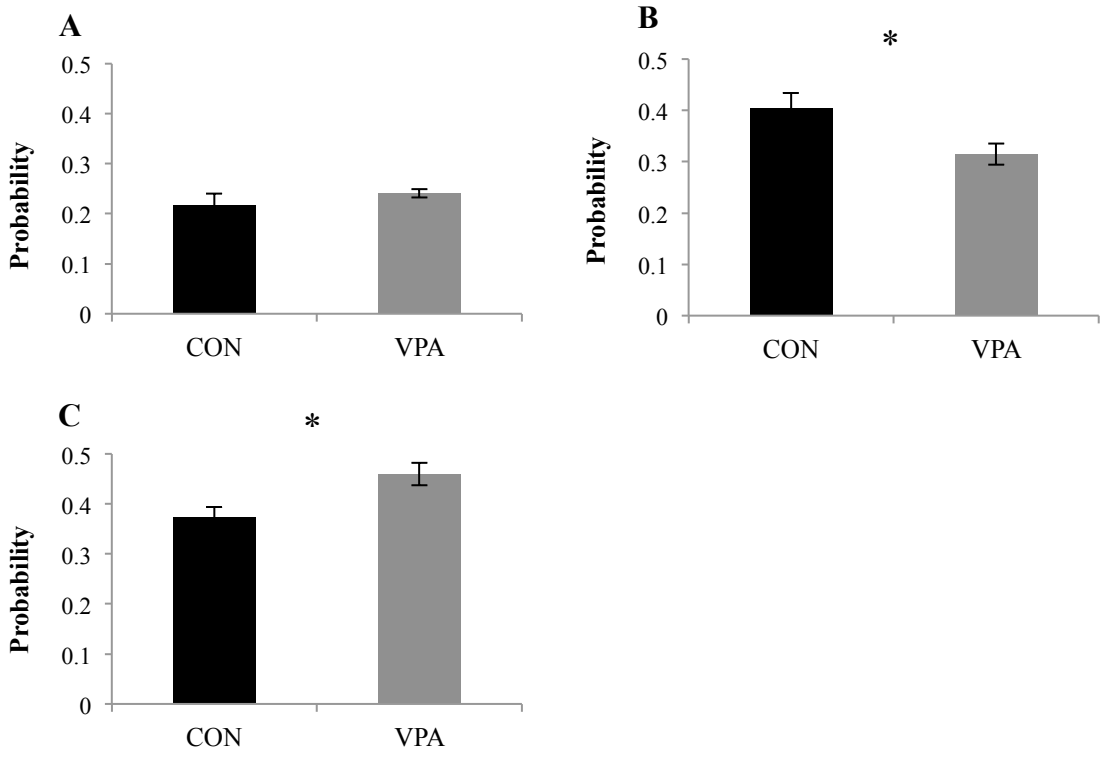

Figure 2.2. (A) The probability of using evasive tactics when defending against a playful attack. (B) The probability of using the complete rotation tactic when defending against a playful attack. (C) The probability of using standing, or other tactics when defending against an attack.

2.4.3. Sexual and Anxiety-Like Behaviors

VPA rats mounted each other significantly more often than did the control rats [$t(14) = 4.241$, $p = 0.001$] and were significantly more likely to perform head and body shakes [$t(14) = 2.570$, $p = 0.022$], but there were no significant differences for either scratching [$t(14) = -0.132$, $p = 0.897$] or grooming [$t(14) = 0.863$, $p = 0.403$] (Table 2.1).

Table 2.1. Mean (± SE) values for measures of anxiety and sexual behavior in pairs of VPA and control rats

Behavior (#/10min)	**VPA Pairs**	**Control Pairs**	***t*-tests**
Head and Body Shakes	10.25 ± 1.66	5.63 ± 0.71	$p = 0.022$
Scratching	3.75 ± 0.49	3.88 ± 0.81	*NS*
Grooming Bouts	5.25 ± 1.31	4.00 ± 0.63	*NS*
Mounting	12.37 ± 2.59	1.25 ± 0.41	$p = 0.001$

2.4.4. Ultrasonic Vocalizations

While the VPA rats tended to call less frequently overall, the difference was not statistically significant [$t(14) = -1.882$, $p = 0.081$] (Figure 2.3A). The rate of calling, however, revealed that the VPA rats called significantly less often than expected for the number of times they initiated play [$t(14) = -2.683$, $p = 0.018$] (Figure 2.3B). This latter finding was corroborated by the minute-by-minute analyses. Repeated measures ANOVAs revealed that there was a significant difference in the frequency of play, with VPA rats initiating more playful attacks in the first ($p = 0.05$), second ($p = 0.018$), and

third ($p = 0.014$) minutes (Figure 2.4A), but emitting fewer calls over these time points, an especially in the fifth minute ($p = 0.021$) (Figure 2.4B).

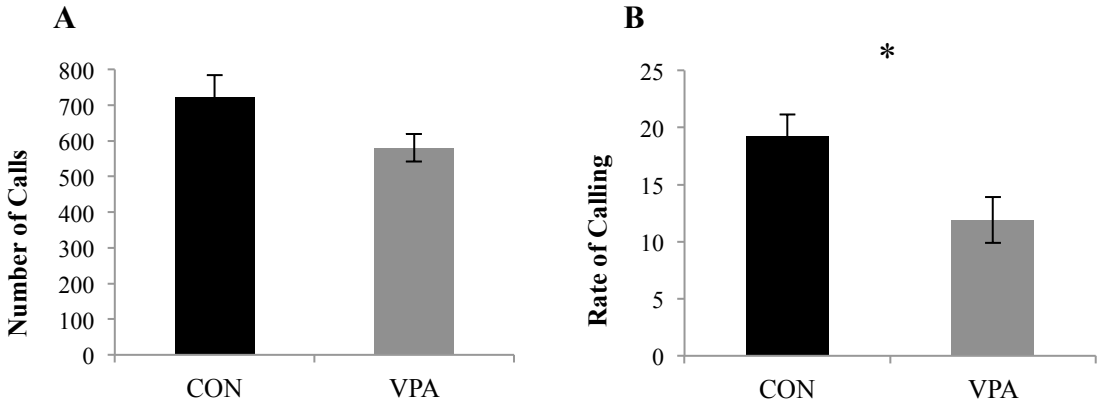

Figure 2.3. (A) The total number of calls emitted per 5 minute play trial. (B) The rate of calling per bout of play fighting (total number of calls/total number of play fights).

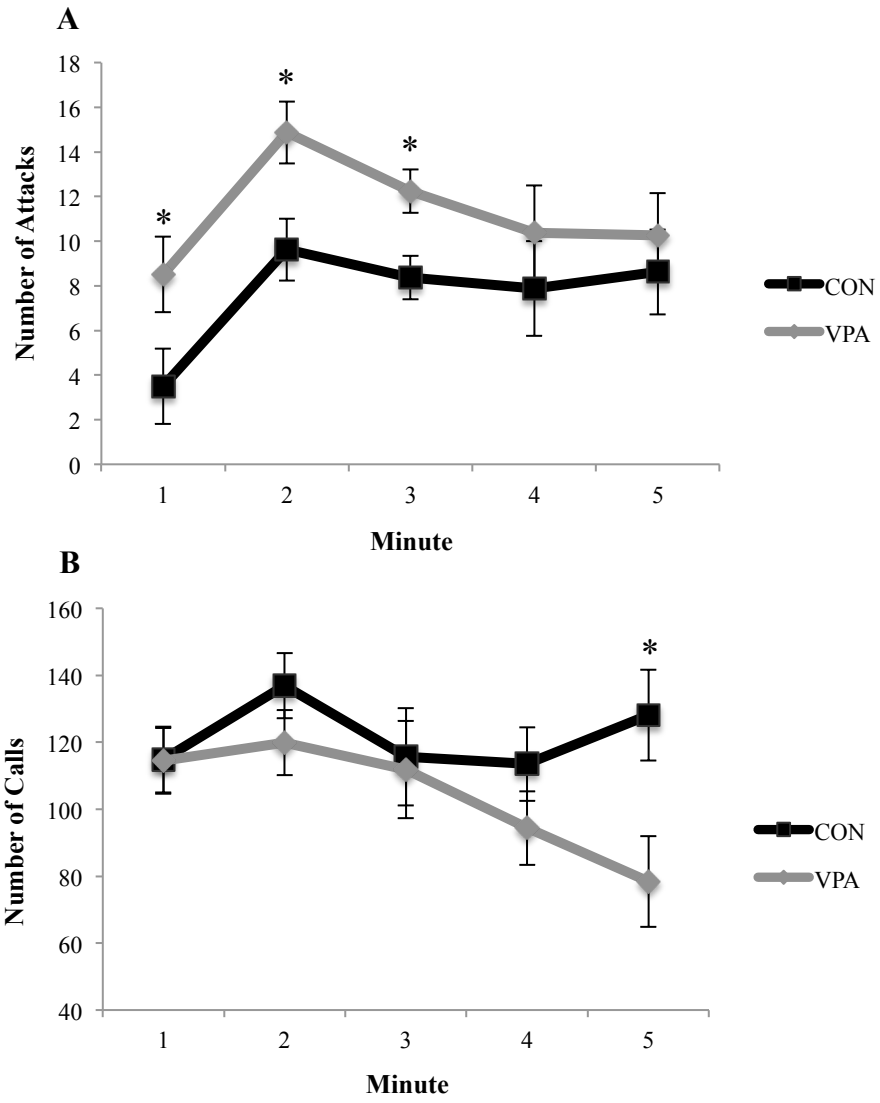

Figure 2.4. (A) Minute-by-minute occurrence of play fighting per 5 minutes. (B) Minute-by-minute occurrence of calling per 5 minutes.

2.5. Discussion

The first diagnostic criterion for autism, described in the Diagnostic and Statistical Manual of Mental Disorders (DSM-V), involves qualitative impairments in social interaction (American Psychiatric Association, 2013). Poor peer relationships, lack of social reciprocity, refusal of body contact, inability to empathize, and unusual/inappropriate social approach behaviors all encompass the wide repertoire of impaired social functioning in autism (Adrien et al., 1991; Gillberg, Nordin, & Ehlers, 1996; Seltzer et al., 2003; Sigman, Dijamco, Gratier, & Rozga, 2004; Trevarthen & Daniel, 2005; Auyeung et al., 2009; American Psychiatric Association, 2013). As such, purported animal models of autism, like fetal exposure to VPA in rodents, need to be able to produce similar impairments in social behavior.

While reduced sociality, measured by a range of behavioral indices, has consistently been demonstrated in animal models of autism (Schneider & Przewlocki, 2005; Markram, Rinaldi, La Mendola, Sandi, & Markram, 2008; Dufour-Rainfray et al., 2010; Olexová, Talarovicová, Lewis-Evans, Borbélyová, & Krsková, 2012; Wellmann, Varlinskaya, & Mooney, 2014), how sociality is reduced is not well understood. Given the complex cognitive, emotional, and communicative demands on animals during social play (Graham & Burghardt, 2010; Palagi et al., 2015; Pellis, Pellis, & Reinhart, 2010), the present study provides a detailed analysis of the social play of rats exposed to VPA as fetuses. It was predicted that the previously reported decreases in social play (Schneider & Przewlocki, 2005; Chomiak et al., 2010) should be correlated with a reduction in contact promoting maneuvers during the play and also a reduction of play-associated ultrasonic vocalizations in the 50-kHz range. The data, in the present study, did not

support the previous studies, but did reveal changes in the dynamics of play and the communication occurring therein.

The marker revealing a reduction in the frequency of play in the previously published papers (Schneider & Przewlocki, 2005; Chomiak et al., 2010) was pinning, in which one rat lays supine and the partner stands over it (Panksepp, 1981). Using the same measure, we did not find a comparable reduction in play in the present study. Similarly, we did not find a significant reduction in playful attacks nor was there a significant reduction in the likelihood that playful attacks elicited playful defense. That is, on all of these measures of playfulness (Himmler et al., 2013), the VPA-treated rats did not differ from the untreated controls, suggesting that the tendency of VPA-treated rats to socialize was not diminished. One explanation for this failure to replicate the previous findings is that the mode of treatment, ingestion versus injection of VPA, was unsuccessful. This is unlikely for several reasons.

First, mounting was significantly increased in the VPA-treated rats, suggesting that the VPA was effective in altering development. Research on autistic populations has shown that socially inappropriate behaviors, such as self-stimulatory behaviors, are prevalent and may indicate deviations in sexual development (Realmuto & Ruble, 1999). The elevated frequency of mounting, especially at this early developmental stage (Pellis, 1993), suggests that the VPA accelerated the maturation of sexual behavior. Moreover, given that the subjects used were female, and females mount much less frequently than males (Meaney & Stewart, 1981; Pellis & Pellis, 1990), the VPA may have altered the normal development of the neural circuitry involved in regulating sexual behavior.

Second, some previous research has shown the occurrence of elevated frequencies

of stress-related responses, such as excessive scratching, in rats prenatally exposed to VPA when tested in anxiety provoking paradigms (Schneider, Ziółkowska, Gieryk, Tyminska, & Przewlocki, 2007; Raza, Harker, Richards, Kolb, & Gibb, 2015). The absence of exaggerated rates of either scratching or stereotyped grooming in the present study suggests that the VPA exposure did not increase anxiety in the rats but we did find a significantly higher frequency of body shaking. Postnatal exposure to VPA has been shown to increase the frequency of body shakes and this increase is thought to arise from increased serotonin (5-HT) activity (Handley & Singh, 1986; Fletcher & Harding, 1981). There is debate, however, on the influence of VPA on the occurrence of body shakes via 5-HT transmission, given the absence of increased body shakes following exposure to trans-2-en-VPA, the major metabolite of VPA (Nau & Loscher, 1984; Loscher, Honack, Nolting, & Fassbender, 1991; Loscher & Honack, 1996). Nonetheless, it seems reasonable to hypothesize that prenatal exposure to VPA may indirectly influence 5-HT transmission, given the evidence for hyperserotonemia and altered serotoninergic neurons following prenatal VPA exposure – both of which are consistent with epidemiological studies of disrupted serotonergic systems in autistic populations (Anderson et al., 1987; Anderson, Horne, Chattergee, & Cohen, 1990; Cook et al., 1993; Betancur et al., 2002; Miyazaki, Narita, & Narita, 2005; Hadjikhani, 2010). Whatever the mechanism involved, the present findings of an increase in body shaking suggest that the VPA treatment was effective.

Third, examination of the VPA-induced effects utilizing an oral dosing paradigm identical to the one used in the current study, revealed long-term behavioral and neuroanatomical alterations in rats that are consistent with symptoms of autism

(Mychasiuk et al., 2012; Raza et al., 2015). For instance, impaired performance was detected on several behavioral tasks – delayed non-match-to-sample T-maze, skilled reaching, elevated plus maze, and open field activity – offering interesting parallels to human autism literature, including compromised problem solving skills, delayed motor skill development, high levels of anxiety, and hyperactivity, respectively (McEvoy, Rogers, & Pennington, 1993; Bellini, 2004; Simonoff et al., 2008; Papadopoulous et al., 2011). On an anatomical level, marked decreases in dendritic complexity of the medial prefrontal cortex (mPFC) and orbitofrontal (OFC) cortex were reported, consistent with substantial evidence of prefrontal cortex-dependent deficits in autism (Prior & Hoffman, 1990; McEvoy et al., 1993). Thus, the studies conducted by Mychasiuk and colleagues (2012), and Raza and colleagues (2015) support the validity of the oral administration of VPA to pregnant dams as a means of inducing autistic-like anomalies in the developing offspring. Therefore, it is reasonable to conclude that the route of drug administration, in the present study, was similarly effective.

Fourth, although the measures of play that reflect playful motivation were not altered by the VPA treatment, other measures that reflect how playful interactions are negotiated were changed. These changes may be indicative of the second DSM-IV diagnostic criterion for autism - impairment in social communication (American Psychiatric Association, 2013). During play fighting, rats maneuver so as to enhance body contact (Panksepp, 1981), which is highly rewarding (Panksepp, 1998). The VPA-treated rats were less likely to use the complete rotation tactic, a tactic that promotes close body contact, and more likely to use defensive tactics involving remaining standing, tactics that block the partner's access to the defender's body (Pellis & Pellis, 1987; Pellis,

Pellis, & McKenna, 1994). Thus, while the rats may have been motivated to play, they limited the contact involved in playful wrestling. Again, this kind of ambivalence to playing with a social partner has been reported in autistic children, and may reflect deficits in their ability to engage in turn taking (Jordan, 2003). In addition to using maneuvers during play that promote reciprocal exchanges (Pellis et al., 2010), effective communication is essential to avoid the risk of escalation to serious fighting (Bekoff, 1972, 1995). In rats, a likely source of signaling during play is the 50 kHz calls that are emitted during these interactions (Burgdorff et al., 2008; Brunelli et al., 2006; Himmler et al., 2014; Kisko et al., 2015; Knutson et al., 1998). The rate of calling per bout of play fighting was significantly reduced in the VPA-treated rats compared to controls. Indeed, contrary to what was found in previous studies (Knutson et al., 1998), and in the controls of the present study, the minute-by-minute analysis of the occurrence of play and the frequency of calling revealed that the frequency of vocalizing was at its lowest during the minutes when play was most frequent. This discordance between vocalizing and play, and the reduction in contact promoting tactics during play, may reflect altered socio-communicative function, as well as dysregulation of pragmatics in social relations, both of which are thought to be characteristic of autism (Baron-Cohen, 1988). Irrespective of the mechanisms involved, these VPA-induced changes in play and vocalizing further support the interpretation that the VPA treatment was effective.

The difference between the present study and the two previous ones reporting reductions of pinning during play (Schneider & Przewlocki, 2005; Chomiak et al., 2010) could be a matter of degree rather than one showing an effect on play and one not. First, the different routes of VPA administration may have resulted in a difference in the

dosage actually delivered to the fetuses. Given the use of intraperitoneal injections as the route of administration in the preceding two studies, it is plausible that oral administration of VPA, in the present study, may not have entered fetal circulation at the intended dose. It is difficult to estimate the proportion of VPA that crosses the placenta, given the variability in the metabolism and bioavailability of the VPA ingested by the dam (Favre et al., 2013; Raza et al., 2015). As such, it is likely that only a proportion of the total dosage entered fetal circulation, resulting in reduced toxicant exposure. Alternatively, the likelihood of fetal resorption may also account for the dampened behavioral impairments. As established by Vorhees (1987), the frequency of embryonic resorption is correlated with simultaneous increases in VPA dosage. It is plausible that *in utero* VPA exposure, at 800mg/kg (upper limit), may have induced a sample bias, in which only the embryos resistant to the VPA insult survived. Consequently, the number of viable offspring available for testing was reduced (Raza et al., 2015).

Second, the kinds of changes characterized in play in the present study and the previous two have some similarity. During play fighting, the most common way for rats to end in the pin configuration is for the defender of a nape attack to rotate to supine (Pellis & Pellis, 1987). Thus, a reduction in the frequency of pinning could arise from a reduced frequency of playful attack or a reduced likelihood of using the rotation to supine tactic (S.M. Himmler, Lewis, & Pellis, 2014; Pellis et al., 1992). Thus, our findings that the VPA-treated rats were less likely to roll over to supine are consistent with the previous findings showing a reduction in pinning. In our case, the degree of VPA-induced change may not have been sufficient to suppress the rotation to supine tactic sufficiently for this to be reflected in the pinning measure. Nonetheless, all these studies

point in the same direction of effect by the VPA. Thus, like previous studies, the present paper shows that in rats, early exposure to VPA induces changes in some aspects of sociality, aspects that mimic the situation in humans with autism.

2.5.1. Conclusion

The present study supports the validity that *in utero* exposure to VPA produces a viable rodent model for the study of autism. The changes in play, at least at the doses delivered in our method of administration, do not seem to affect the motivation to play, and so presumably the desire to socialize in general. However, the changes in how they play suggest that what rewards they may be gaining from play are altered. Like the broader distinction between 'wanting' and 'liking' in motivated behavior (Berridge, 1996; Berridge, Robinson, & Aldridge, 2009), dopaminergic systems are involved in rats seeking out the opportunity to play, but opioid systems are involved in rewarding the act of playing (Panksepp, 1998; Siviy, 1998; Siviy & Panksepp, 2011). The VPA-treated rats in the present study were just as motivated to engage in play, but the rewards offered by playing may be altered, hence reducing the pleasure to be gained from the physical contact usually associated with play (Vandershuren, 2010). This could account for the shift away from defense involving rotating to supine to defense involving keeping the partner at arms' length. Such a change may also explain the reduced rate of emitting 50-kHz calls relative to the amount of play initiated, as higher rates of emission are associated with greater positive affect (Knutson, Burgdorf, & Panksepp, 2002).

Alternatively, given the evidence that at least some of the 50-kHz calls may be used tactically to communicate with the partner during play (Himmler et al., 2014), the

reduced calling rate may reflect deficits in communication. Indeed, as negotiating close quarter contact, which occurs when one rat rolling over onto its back is attacked by another, appears to involve more sophisticated inter-animal coordination than other tactics not involved in such close-quarter action (Bell et al., 2009; Pellis & Pellis, 1987), a reduced ability to communicate could diminish the use of such tactics during play. Given the diversity of 50-kHz calls (Wright et al., 2010, Wright, Dodosiewiez, & Clarke, 2012), a detailed analysis of the temporal structure of when different calls are emitted during the course of the moment-to-moment maneuvers performed during play may shed light on such deficits in communication. These communicatory effects of VPA need not be exclusive with the ones influencing the motivation and reward systems for social contact. As indicated by the present study and two previous ones (Schneider & Przewlocki, 2005; Chomiak et al., 2010), both may be compromised, depending on dose, and so mimic different facets of autism.

CHAPTER 3

Tactile Stimulation Improves Neuroanatomical Pathology But Not Behaviour in Rats Prenatally Exposed to Valproic Acid∗

3.1. Abstract

Autism is a severe neurodevelopmental disorder with a population prevalence of 1 in 68, and dramatically increasing. While no single pharmacologic intervention has successfully targeted the core symptoms of autism, emerging evidence suggests that postnatal environmental manipulations may offer greater therapeutic efficacy. Massage therapy, or tactile stimulation (TS), early in life has repeatedly been shown to be an effective, low-cost, therapeutic approach in ameliorating the cognitive, social, and emotional symptoms of autism. While early TS treatment attenuates many of the behavioral aberrations among children with autism, the neuroanatomical correlates driving such changes are unknown. The present study assessed the therapeutic effects of early TS treatment on behavior and neuroanatomy using the valproic acid (VPA) rodent model of autism. Rats were prenatally exposed to VPA on gestational day 12.5 and received TS shortly following birth. Whereas TS reversed almost all the VPA-induced alterations in neuroanatomy, it failed to do so behaviorally. The TS VPA animals, when compared to VPA animals, did not exhibit altered or improved behavior in the delayed non-match-to-sample T-maze, Whishaw tray reaching, activity box, or elevated plus maze tasks. Anatomically,

---

 Published as: Raza, S., Harker, A., Richards, S., Kolb, B., & Gibb, R. (2015). Tactile Stimulation Improves Neuroanatomical Pathology but not Behaviour in Rats Prenatally Exposed to Valproic Acid. *Behavioural Brain Research, 282,* 25-36. doi:10.1016/j.bbr.2014.12.055

however, there were significant increases in dendritic branching and spine density in the medial prefrontal cortex, orbital frontal cortex, and amygdala in VPA animals following early TS treatment, suggesting a complete reversal or remediation of the VPA-induced effects in these regions. The results suggest that postnatal TS, during a critical period in development, acts as a powerful reorganization tool that can ameliorate the neuroanatomical consequences of prenatal VPA exposure.

3.2. Introduction

Autism is a severe neurodevelopmental disorder that develops in the first 3 years of life. Characterized by impairments in social interactions, communication, and repetitive behaviors, the etiology of autism is not entirely known, but genetic and environmental components have been hypothesized to be involved (American Psychiatric Association, 1994; Schneider & Przewlocki, 2005). There is an accumulating body of evidence that *in utero* exposure to valproic acid (VPA), a teratogenic anticonvulsant, leads to an increased risk and incidence of autism (Bromley et al., 2009; Bath & Scharfman, 2013; Bromley et al., 2013). In fact, several retrospective human and case studies have documented difficulties in attentional, social, language, and motor abilities among children prenatally exposed to VPA, leading to the idea that valproate exposure during fetal development greatly alters neurodevelopment, including emotional and cognitive functioning (Christianson et al., 1994; Williams et al., 2001; Dean et al., 2002; Adab et al., 2004; Alsdorf & Wyszynski, 2005; Rasalam et al., 2005; Bromley et al., 2009; Ornoy, 2009).

In view of the correlation between *in utero* VPA exposure and the incidence of autism in humans – a 4.42% absolute risk (Christensen et al., 2013) – the VPA rodent model of autism was developed (Rodier et al., 1996; Rasalam et al., 2005; Ornoy, 2009). Prenatal exposure to VPA on gestational day 12.5 has proven to be a viable rodent model of autism, as it appears to parallel the anatomical, functional, and behavioral pathology reported in human studies of autism (Mychasiuk et al., 2012). More specifically, rats prenatally exposed to VPA have been shown to exhibit structural and cellular features in brain similar to those observed in autistic patients, including physical malformations (Dufour-Rainfray et al., 2011), brainstem and cerebellar anomalies (Rodier et al., 1996; Rodier et al., 1997a), altered morphology of motor cortex neurons (Snow et al., 2008), and hyper-connectivity (Rinaldi et al., 2008). On a behavioral level, VPA rats have been shown to display many autistic-specific deficits, including decreased social interactions and behaviors, repetitive or stereotypic behaviors, low sensitivity to painful stimuli, and increased anxiety (Schneider & Przwelocki, 2005; Schneider et al., 2007; Bambini-Junior et al., 2011; Chomiak et al., 2013).

In the US alone, autism is estimated to affect 1 in 68 children (Weintraub, 2011; Centers for Disease Control and Prevention, 2014) and is believed to be on the rise (Snow et al., 2008). In fact, rates are considerably higher than those 20 years ago (Fombonne, 2002). Given the recent rise in the incidence of autism, the need for remedial and preventative strategies is crucial. Studies aimed at attenuating core autistic behavioral symptoms have primarily undertaken a pharmacological route (Buitelaar, 2003; Schneider et al., 2006). Although novel pharmacotherapies – such as risperidone and other atypical antipsychotics – have been central in managing related symptoms of

autism, treatment of the core symptoms remains a large area of unmet need (Wink, Erickson, & McDougle, 2010). As a result, intensive behavioral therapy has recently taken the forefront in numerous interventions targeted at autistic patients (Lovaas, 1987; Harris, Handleman, Gordon, Kristoff, & Fuentes, 1991; Ozonoff & Cathcart, 1998; Scheinkopf & Siegel, 1998; Josefi & Ryan, 2004; Lopata, Thomeer, Volker, & Nida, 2006; Kim, Wigram, & Gold, 2008; Woo & Leon, 2013). In fact, the efficacy of behavioral therapy is related to its positive lifelong implications (McEachin, Smith, & Lovaas, 1993). For instance, Fields et al. (1997) have demonstrated the positive influence of massage therapy among individuals with autism. Given twice a week for 20 min, massage therapy resulted in fewer stereotypical behaviors, reduced touch aversion, and greater social relatedness (in the classroom) among autistic children. Improvements in sleeping patterns, sensory impairments, and social and basic living skills have also been reported following massage therapy (Escalona et al., 2001; Cullen et al., 2005a; Hughes, 2008).

Massage therapy involves kinesthetic or sensory stimulation (Mathai, Fernandez, Mondkar, & Kanbur, 2001), an intervention equivalent to tactile stimulation (TS) in animal studies. Interestingly, animal studies have shown TS to be an effective measure of protection against cortical injury (Gibb, Gonzalez, Wegenast, & Kolb, 2010; Kolb & Gibb, 2010) and anxiety (Imanaka et al., 2008). TS has also been shown to stimulate maturation in preterm and newborn animals (Field et al., 1986; Schanberg & Field, 1987), and even alter the behavioral and neuroanatomical organization in non-brain injured rats (Richards, Mychasiuk, Kolb, & Gibb, 2012). Given the abundance of

literature reinforcing TS as positive enriching experience, it is plausible that such an experience may offer preventative or remedial intervention in animal models of autism.

The purpose of this study was to investigate the magnitude and extent, if any, of the behavioral and neuroanatomical changes induced by prenatal exposure to VPA and whether an early TS treatment can remediate such behavioral and anatomical pathologies. Using a within litter design, half of the rat pups derived from VPA and vehicle-treated dams were given TS treatment. To elucidate the therapeutic effects of TS on behavior in VPA animals, a battery of behavioral assessments were employed: delayed non-match-to-sample T-maze, Whishaw tray reaching task, activity box, and elevated plus maze (EPM). The dendritic organization of the medial prefrontal cortex (mPFC; Cg3), orbitofrontal cortex (OFC; AID), and amygdala was quantified using Golgi methodology.

3.3. Methods

3.3.1. Subjects

All experimental protocols were approved in accordance with the Canadian Council of Animal Care and the University of Lethbridge Animal Care Committee. Animals were born and raised in an accredited animal care facility at the University Lethbridge. Twelve dams and twelve male Long-Evans rats were utilized in this study. The control animals were a subset of a larger study conducted by Richards et al. (2012) and the same data was utilized. All procedures among the VPA and control animals – from gestation to adult behavioral testing – were identical and conducted during the same time period, to ensure no significant effect of time or any observable behavioral differences. A single male was paired with a single female in a shoebox cage and mating

behaviors were observed for 20 min. If mating behaviors were observed during this time interval, the male remained with the female over the next 24 hrs. If not, the male was removed. The breeding procedure was repeated the next morning and was continued until all male-female pairs were determined to have mated. Throughout the duration of the pregnancy, female rats were housed in pairs. However, upon the birth of rat pups, each mother was housed individually with her litter. The neonates remained with their mothers until weaning on postnatal day 21 (PN21), when they were, consequently, housed with their same-sex siblings. Eighty-two pups were born to 7 VPA dams (46 female, 36 male) and sixty-nine pups to 5 control dams (37 female, 32 male). Behavioral testing of pups commenced on PN65. Animals were housed in standard polycarbonate shoebox cages and maintained on a 12hr light/12hr dark diurnal cycle. Food and drinking water were available *ad libitum*, with the exception of food restriction during the duration of the Whishaw tray-reaching task.

3.3.2. VPA Administration

Pregnant dams were administered VPA on G12.5. Three days prior to VPA administration (G9-G11), all dams were given 1.5g/day of peanut butter. The peanut butter was spoon-fed to each individual rat. On G12.5, half of the dams were given peanut butter mixed with 800mg/kg of VPA, whereas the remaining control dams received peanut butter alone.

3.3.3. Tactile Stimulation

Tactile stimulation (TS) was performed three times a day (09:00, 13:00, and 16:00) for 15 min intervals. Seven VPA and five control litters were utilized, where equal numbers of male and female rat pups, within each litter, were randomly assigned to the tactile stimulation (TS) and non-tactile stimulation (NTS) groups. TS commenced on PN3 and continued until weaning. All animals were transported in their home cage to a testing room for the TS session. Dams were removed from the home cage and placed in a transport cage. The home cage, containing all the rat pups, was placed on a heating pad set to 24°C. During each session, a partition was used to separate the TS and NTS groups. A Swiffer® duster was used to stimulate the TS group. At the end of each session, mothers were returned to the home cage.

3.3.4. Behavioral Methods

3.3.4.1. Delayed Non-Match-to-Sample T-maze

Testing on the non-match-to-sample T-maze task occurred between PN65 and PN75. For 10 consecutive days, ten trials were run per day. The task consisted of 2 trials, separated by a 10 sec delay. Trial 1 was a forced trial: one arm was blocked, whereas the other arm was open and contained a food reward. Trial 2 was a choice run: an animal had the choice of entering either arm (both arms were open), but the food reward was located in the arm opposite to that of trial 1. The open arm was randomly assigned and altered between trials. That is, a semi-random schedule was employed (for instance, day 1: RLRRLRLLRL; day 2: LRLLRLRRLR). The number of times the animal entered each arm on trial 2 was recorded and analyzed. When rats performed at 80% or better for 3 consecutive days, training stopped.

3.3.4.2. Whishaw Tray Reaching Task

Training and testing on the Whishaw tray-reaching task occurred successively from PN75 and PN95. Animals were food restricted (25g rat chow/day) on training days 1 to 7 to encourage reaching behavior. It was ensured that the animals did not lose more than 10% of their pre-test body weight. Food restriction was increased slightly (that is, greater food deprivation; 20g rat chow/day) on days 8 to 21. The animals were placed in the reaching cages for 30 min per day. Following reaching and/or retrieval of the chicken pellets by the rats, pellets were consistently replaced to encourage further attempts. On test day (PN95), animals were video recorded for 5 min and, subsequently, the reaching behavior was scored. The attempted reaches, misses, and hits (that is, the successful reach, grasp, and consumption of food) were analyzed. A percent correct hit score (number of hits/total number of attempts) was determined.

3.3.4.3. Activity Box

On PN96, the exploratory behavior of the animals was recorded. Rats were individually placed in Accusan® activity-monitoring boxes. Each Plexiglas® box measured 41 X 41 X 30.5 cm, which recorded the movements of each rat. Rats were introduced to the activity box for 10 minutes and their activity level was recorded in two 5-minute intervals, using the VersaMax^{TM} computer software. The intervals were combined and the overall activity/distance travelled was determined.

3.3.4.4. Elevated Plus Maze

Testing on the EPM occurred approximately on PN105. The EPM apparatus consisted of 3 main components: a base (94 cm), two open arms (10 X 40 cm), and two closed arms (10 X 40 X 40 cm). The apparatus, made of black Plexiglas, was housed in a well-lit empty testing room. On testing day, rats were introduced to the maze, where their forepaws were placed in the center square of the maze facing a closed arm. Time spent in the open and closed arms were scored. An animal was deemed to be occupying an arm when the first half of their body entered the arm. Animals were video recorded for 5 minutes, with the camera elevated at the end of an open arm.

3.3.5. Anatomical Methods

3.3.5.1. Histological Procedures

Following behavioral testing, animals (~PN106) were administered an overdose of sodium pentobarbital and perfused with 0.9% saline. The brains were removed, weighed, and transferred to a Golgi-Cox solution for at least 14 days. Following 14 days of preservation in the Golgi-Cox solution, the brains were transferred to a 30% sucrose solution for at least 3 days. The brains were then sectioned on a Vibratome, at 200 μm, and mounted on gelatin-coated slides. Brains were stained following a protocol as described by Gibb and Kolb (1998).

The dendritic architecture of pyramidal cells from layer III of the mPFC (Cg3) and OFC (AID), and amygdala were examined. Neurons, in each region of interest, were identified and traced at 250X with a camera lucida. It was ensured that the neuronal dendritic trees and branching were intact and not obscured by dendrites of other neurons, blood vessels, or astrocytes. Three measures were employed: 1) branch order (Cg3: apical

and basilar fields, AID: basilar), where the dendritic complexity was calculated by measuring the number of bifurcations from the cell body; 2) Sholl analysis (Cg3: apical and basilar fields, AID: basilar), where dendritic length was calculated using an overlay of concentric rings to count the number of intersections; and 3) spine density (Cg3: apical and basilar fields, AID: basilar; amygdala: basilar) to estimate the synaptic density along a dendritic segment based on the exact length and number of spines along that segment (spines/10μm). For each area of interest, 10 neurons (5 from each hemisphere) were analyzed using the three measures. An average value among the 5 neurons was calculated for each area in each hemisphere.

3.3.6. Statistical Analyses

All statistical analyses were performed on SPSS 21 for Mac. Two-way ANOVAs with group (VPA or CON) and treatment (TS or NTS) as variables were performed to determine behavioral outcomes and any interactions. All neuroanatomical statistical analyses performed were three-way ANOVAs, to determine the interaction(s) among group (VPA or control), treatment (TS or NTS), and hemisphere (right or left) in each brain region. If an ANOVA did not reveal a significant effect of hemisphere, the data were collapsed. Sexes were analyzed independently for clarity of results description. The significance level was set at ≤ 0.05. All graphical representations of the data represent the means and standard error of measurement.

3.4. Results

3.4.1. Behavioral Results

3.4.1.1. Delayed Non-Match-to-Sample T-maze

VPA increased the days to criterion but there was no significant effect of TS treatment (Figure 3.1).

A two-way ANOVA of the days to reach criterion in the male group revealed a significant effect of group [$F(1,24) = 4.462$, $p = 0.047$], where VPA male animals took greater number of days to reach criterion compared to control counterparts. There was no effect of TS [$F(1,24) = 3.790$, $p = 0.065$] nor an interaction between Group and Treatment [$F(1,24) = 0.139$, $p = 0.713$].

A two-way ANOVA of the females revealed no main effect of group [$F(1,25) = 2.238$, $p = 0.149$], treatment [$F(1,25) = 0.131$, $p = 0.721$], nor the interaction [$F(1,25) = 3.790$, $p = 0.064$]. The interaction reflected a non-significant trend towards an increase in the number of days to reach criterion in the NTS VPA females, compared to NTS controls. Given that the data in Figure 3.1 appears to show a clear difference between the NTS groups in both sexes, we did a posthoc comparison of the NTS control and NTS VPA group, finding a significant difference [$F(1,11) = 5.510$, $p = 0.041$].

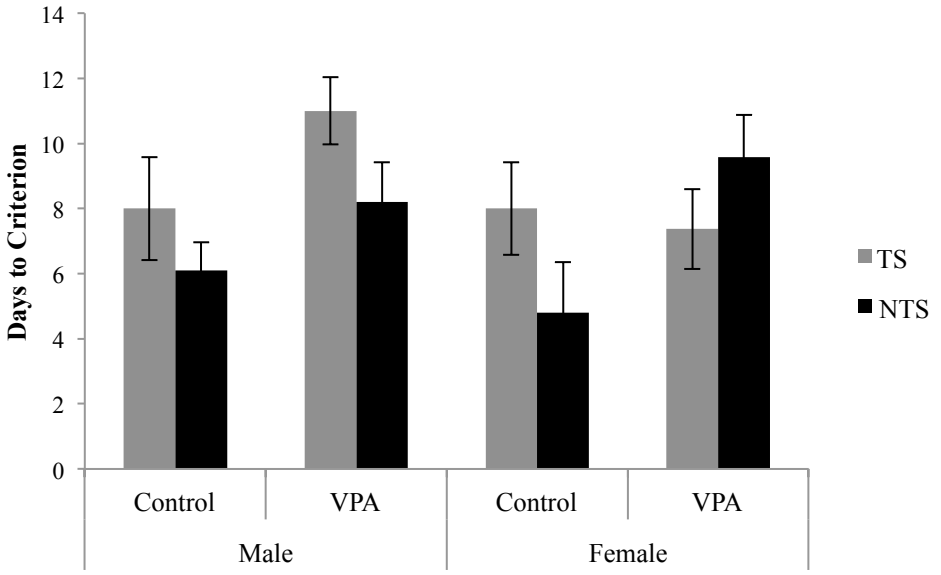

Figure 3.1. Prenatal VPA exposure increased the number of days to reach criterion in the non-match-to-sample T-maze in NTS male, but not female, animals. Both sexes were unaffected by TS treatment.

3.4.1.2. Whishaw Tray Reaching Task

While VPA female animals exhibited significant motor impairments in retrieving food pellets when compared to control females, male animals were unaffected by prenatal VPA exposure. TS treatment did not influence performance on this task in either sex (Figure 3.2).

A two-way ANOVA of percent hit score among the female group revealed a significant effect of group [$F(1,78) = 5.011$, $p = 0.028$], where VPA females exhibited impaired performance compared to controls. There was no significant effect of treatment [$F(1,78) = 0.325$, $p = 0.570$], nor an interaction between the Group and Treatment [$F(1,78) = 1.993$, $p = 0.162$].

In contrast to females, a two-way ANOVA for males revealed no main effect of group [$F(1,57) = 0.890$, $p = 0.350$], treatment [$F(1,57) = 0.074$, $p = 0.787$], nor an interaction [$F(1,57) = 0.878$, $p = 0.353$].

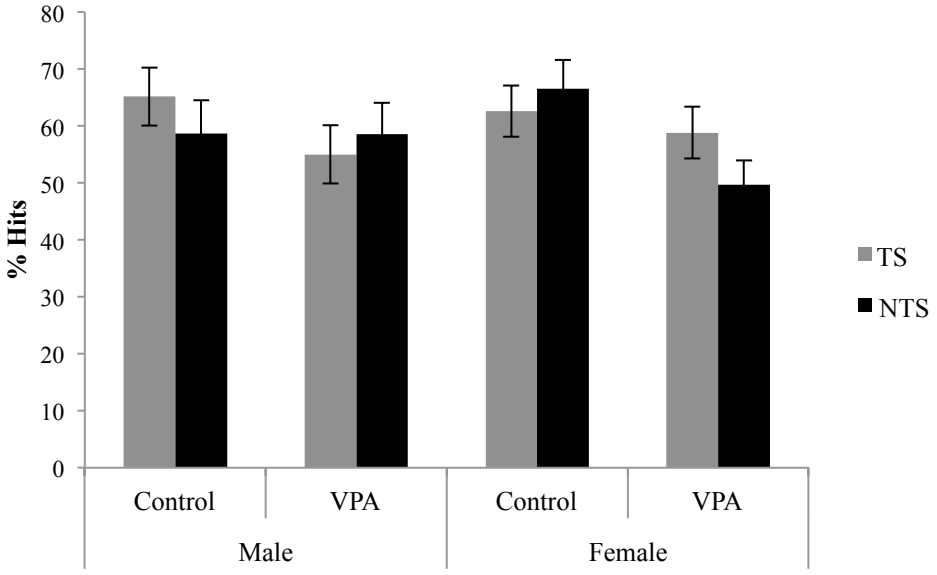

Figure 3.2. Prenatal VPA exposure significantly decreased the percent hit score among female, but not male, animals in the Whishaw tray reaching task. Reaching performance in both sexes was unaffected by TS treatment.

3.4.1.3. Activity Box

Prenatal VPA exposure increased the overall activity level in both sexes, although the effect of TS treatment was apparent only in female VPA animals. Male animals were unaffected by TS treatment (Table 3.1).

There was no significant effect of group [$F(1,74) = 3.734$, $p = 0.057$] or treatment [$F(1,74) = 0.200$, $p = 0.656$] for female animals. There was a significant interaction of Group and Treatment [$F(1,74) = 4.650$, $p = 0.034$], owing to higher activity among the TS VPA female animals ($p = 0.004$). Moreover, TS VPA females were significantly more active than NTS VPA females ($p = 0.049$).

There was a significant group effect [$F(1,55) = 9.804$, $p = 0.003$] among male animals, as VPA increased activity. There was no effect of TS treatment [$F(1,55) = 0.547$, $p = 0.463$] nor an interaction [$F(1,55) = 0.008$, $p = 0.929$] on locomotor activity.

3.4.1.4. Elevated Plus Maze

In the EPM task, VPA female animals spent less time in the closed arms, but not open arms, compared to female controls. TS treatment had no effect among female animals. Males were unaffected by either VPA exposure or TS on this task (Table 3.1).

ANOVA of time spent in the closed arms by females revealed a main effect of group [$F(1,83) = 7.474$, $p = 0.008$], where control females spent greater time in the closed arms compared to VPA females. There was no effect of treatment [$F(1,83) = 0.001$, $p = 0.947$] nor an interaction [$F(1,83) = 1.014$, $p = 0.317$] on closed arm time. ANOVA on time spent in the open arms, revealed no main effects of group [$F(1,83) = 0.788$, $p = 0.377$], treatment [$F(1,83) = 0.064$, $p = 0.801$], nor an interaction [$F(1,83) = 0.258$, $p = 0.613$].

ANOVA on the time spent in the closed arms in the male group revealed no significant effect of group [$F(1,61) = 0.700$, $p = 0.406$], treatment [$F(1,61) = 0.284$, $p = 0.596$], nor an interaction [$F(1,61) = 0.407$, $p = 0.526$]. Time spent in the open arms revealed a similar trend among males animals: no significant effect of group [$F(1,61) = 0.274$, $p = 0.602$], treatment [$F(1,61) = 0.104$, $p = 0.748$], nor an interaction [$F(1,61) = 0.783$, $p = 0.380$].

3.4.2. Summary of Behavioral Results

The behavioral results are summarized in Table 3.1. The VPA-treated rats exhibited significant behavioral changes (or, in some cases, significant interactions) on an adult battery of behavioral tests. Whereas impaired behavior was observed among female VPA animals on all tasks, behavioral alterations among VPA males were exhibited only on the T-maze and activity box tasks. Compared with the control group, VPA-treated rats demonstrated: (1) impaired performance on the delayed non-match-to-sample T-maze; (2) motor dysfunction on the Whishaw tray reaching task; (3) hyperactive locomotor behavior; and (4) reduced propensity to remain in the closed arms of the EPM, suggesting altered anxiety. Surprisingly, early TS treatment did not alter behavior on any task, with the exception of the increased activity in female VPA animals in the activity box.

Table 3.1. Main effects of VPA and TS treatment from two-way ANOVAs for all behavioral tasks measured. Sexes were analyzed independently.

Behavioural Task	VPA		TS	
	Male	Female	Male	Female
T-Maze				
Days to Criterion	↑	↑+	-	-
Tray Reaching				
% Hits	-	↓	-	-
Activity Box				
Activity Level	↑	↑+	-	↑+
EPM				
Time Spent in Closed Arms	-	↓	-	-
Time Spent in Open Arms	-	-	-	-

+, Trend towards significance or significant interaction

3.4.3. Anatomical Results

3.4.3.1. Medial Prefrontal Cortex (Cg3)

3.4.3.1.1. Dendritic Branching

Apical. The apical dendritic branching of pyramidal cells in the mPFC was reduced in VPA female and male animals, compared to controls. In contrast, TS

treatment increased apical dendritic branching in all groups, suggesting a remediating effect of the TS treatment in the VPA group (Figure 3.3A).

Female animals demonstrated main effects of group [$F(1,75) = 4.623$, $p = 0.035$] and treatment [$F(1,75) = 53.232$, $p \leq 0.001$] but no interaction [$F(1,75) = 1.836$, $p = 0.180$]. Thus, whereas VPA decreased branching, TS treatment increased it in all groups.

ANOVA in males showed a significant effect of treatment [$F(1,59) = 8.973$, $p = 0.004$], where TS increased apical dendritic branching, but there was no main effect of group [$F(1,59) = 2.865$, $p = 0.096$]. There was a significant interaction [$F(1,59) = 13.208$, $p = 0.001$], demonstrating that NTS VPA males exhibited reduced apical dendritic branching ($p = 0.001$) relative to NTS controls. In addition, TS VPA males exhibited greater apical dendritic branching compared to NTS VPA counterparts ($p \leq 0.001$).

Basilar. TS treatment increased the basilar dendritic branching of pyramidal cells in the mPFC in both sexes. In addition, both female and male animals exhibited greater dendritic branching in the right hemisphere, as opposed to the left. VPA only decreased basilar dendritic branching in males and this effect was remediated by TS treatment.

A three-way ANOVA (Group X Treatment X Hemisphere) of basilar dendritic branching among female animals demonstrated a significant effect of treatment [$F(1,75) = 28.932$, $p \leq 0.001$] and hemisphere [$F(1,75) = 13.743$, $p \leq 0.001$], but no main effect of group [$F(1,75) = 0.624$, $p = 0.432$]. TS treatment significantly increased basilar dendritic branching compared to NTS females. Greater basilar dendritic branching was also observed in the right hemisphere, as opposed to the left. The basilar dendritic branching in the right hemisphere for both VPA females ($p = 0.002$) and control females ($p = 0.039$)

was greater than the left hemisphere. There was no Group X Treatment X Hemisphere interaction [$F(1,75) = 0.152$, $p = 0.698$] (Figure 3.3B).

When subjected to a three-way ANOVA (Group X Treatment X Hemisphere), basilar dendritic branching among male animals revealed a main effect of group [$F(1,59) = 3.847$, $p = 0.05$], treatment [$F(1,59) = 20.002$, $p \leq 0.001$], and hemisphere [$F(1,59) = 4.393$, $p = 0.041$]. While VPA decreased basilar dendritic branching along males ($p = 0.05$), it appeared that TS treatment produced an increase in basilar dendritic branching compared to NTS counterparts in both sexes ($p \leq 0.001$). In addition, the right hemisphere exhibited an increase in basilar dendritic branching compared to the left hemisphere ($p = 0.041$). A two-way interaction between Group and Treatment [$F(1,59) = 8.562$, $p = 0.005$] was also observed, where branching in right hemisphere of NTS control ($p \leq 0.001$) and TS VPA ($p \leq 0.001$) males was greater than branching in NTS VPA males of the same hemisphere. This effect was also observed in the left hemisphere of TS VPA animals ($p = \leq 0.001$). There was no three-way interaction (Group X Treatment X Hemisphere) [$F(1,59) = 0.133$, $p = 0.717$] (Figure 3.3C).

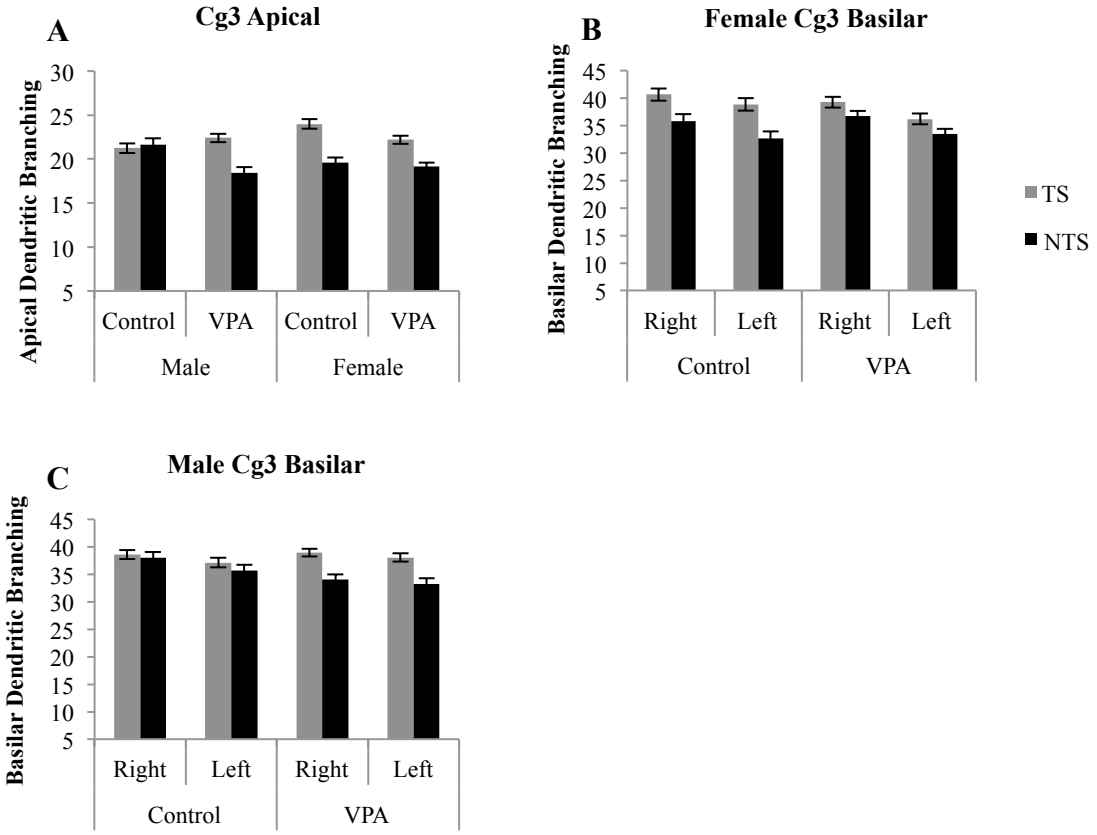

Figure 3.3. Changes in dendritic branching of pyramidal cells in Cg3 in response to prenatal exposure to VPA and TS. (A) Dendritic branching of apical dendrites. VPA significantly reduced apical dendritic branching of both sexes. This effect was remediated by TS treatment. (B) Dendritic branching of basilar dendrites in female animals. TS increased basilar dendritic branching in female animals. Greater basilar dendritic branching was observed in the right hemisphere, as opposed to the left. Branching was unaffected by VPA. (C) Dendritic branching of basilar dendrites in male animals. While VPA decreased Cg3 basilar dendritic branching in males, this effect was remediated by TS treatment. Greater basilar dendritic branching was observed in the right hemisphere, as opposed to the left.

3.4.3.1.2. Dendritic Length

Apical. TS treatment significantly increased the apical dendritic length of all groups, female and male, compared to the NTS counterparts. TS treatment appeared to block the effect of VPA and increase the apical dendritic length in both female and male cohorts (Figure 3.4A).

A two-way ANOVA of the apical dendritic length in female animals revealed a main effect of treatment [$F(1,119) = 90.617$, $p \leq 0.001$], but not of group [$F(1,119) = 3.662$, $p = 0.058$] nor the interaction [$F(1,119) = 0.008$, $p = 0.928$]. Thus, TS treatment significantly increased apical dendritic length among all female groups. Prenatal VPA exposure produced a trend towards a reduction in dendritic length.

ANOVA on male animals revealed a main effect of treatment [$F(1,81) = 60.173$, $p \leq 0.001$] and a significant interaction [$F(1,81) = 7.953$, $p = 0.006$], but no effect of group [$F(1,81) = 3.412$, $p = 0.069$]. More specifically, the apical dendritic length in NTS VPA males was reduced compared to NTS controls ($p = 0.004$). This was reversed by TS, as TS VPA males exhibited an increase in apical dendritic length when compared to NTS VPA counterparts ($p \leq 0.001$), a TS result that was also observed among TS controls ($p = 0.002$).

Basilar. The basilar dendritic length of pyramidal cells in the mPFC was reduced in VPA animals, whereas TS treatment increased basilar dendritic length in all groups. The length of cells in the right hemisphere was significantly longer than those in the left hemisphere.

ANOVA on the basilar dendritic length in the Cg3 region of female animals revealed a significant effect of treatment [$F(1,114) = 66.000$, $p \leq 0.001$] and hemisphere

[$F(1,114) = 15.798$, $p \leq 0.001$], where TS treatment significantly increased basilar dendritic length. Basilar dendritic branching was also greater in the right hemisphere. There was a non-significant trend for an effect of group [$F(1,114) = 3.253$, $p = 0.074$], where prenatal exposure to VPA decreased Cg3 basilar dendritic length. There was no significant interaction [$F(1,114) = 0.335$, $p = 0.564$] (Figure 3.4B).

ANOVA of basilar dendritic length in males revealed a main effect of group [$F(1,81) = 6.271$, $p = 0.014$], treatment [$F(1,81) = 27.148$, $p \leq 0.001$], and hemisphere [$F(1,81) = 6.579$, $p = 0.012$]. VPA males exhibited decreased basilar dendritic length compared to control males ($p = 0.014$), whereas TS-treated males had greater basilar dendritic length ($p \leq 0.001$). Interestingly, the right hemisphere exhibited significantly greater basilar dendritic length than that of the left hemisphere ($p = 0.012$). A significant two-way interaction between Group and Treatment was revealed [$F(1,81) = 5.166$, $p = 0.026$], where the basilar dendritic length in the right hemisphere of NTS VPA males was reduced compared to the NTS control males of the same hemisphere ($p = 0.046$). The same effect was observed in the left hemisphere ($p = 0.023$). In addition, VPA males that underwent TS treatment displayed increased basilar dendritic length in the right ($p \leq 0.001$) and left ($p \leq 0.001$) hemispheres compared to the NTS VPA males. There was no Group X Treatment X Hemisphere interaction [$F(1,81) = 0.073$, $p = 0.788$] (Figure 3.4C).

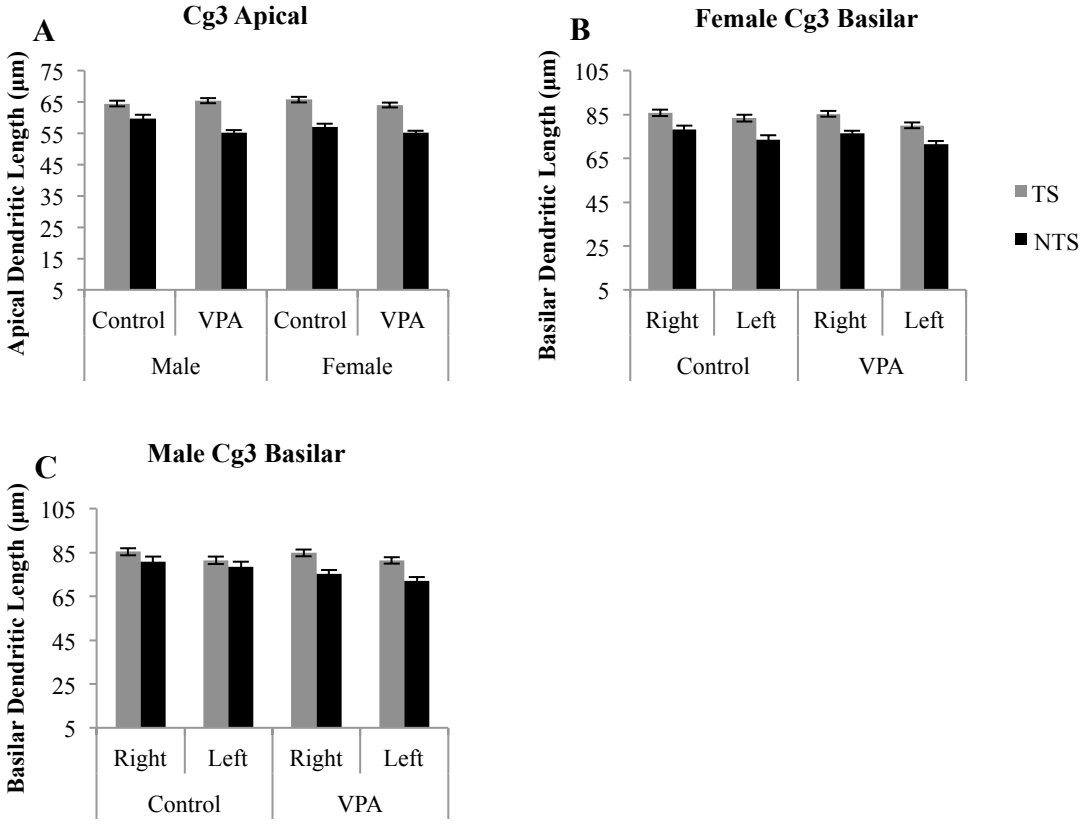

Figure 3.4. Changes in dendritic length of pyramidal cells in Cg3 in response to prenatal exposure to VPA and TS. (A) Dendritic length of apical dendrites. TS treatment significantly increased apical dendritic length of both sexes, blocking the VPA-induced reduction in length. (B) Dendritic length of basilar dendrites in female animals. TS treatment significantly increased basilar dendritic branching, remediating the VPA-induced trend of decreased dendritic length. The length of cells in the right hemisphere was significantly longer than those in the left hemisphere. (C) Dendritic branching of basilar dendrites in male animals. VPA significantly reduced basilar dendritic length, whereas TS treatment significantly increased length. The length of cells in the right hemisphere was significantly longer than those in the left hemisphere.

3.4.3.1.3. Spine Density

Apical. Prenatal exposure to VPA significantly reduced the apical spine density of Cg3 pyramidal cells among male animals. TS treatment, on the other hand, significantly increased apical spine density in both sexes, irrespective of group. In fact, among VPA female and male animals, it appears that TS treatment may have remediated or blocked the VPA-induced changes in the apical spine density in both sexes (Figure 3.5A).

A two-way ANOVA of the apical spine density among female animal revealed a main effect of treatment [$F(1,119) = 36.400$, $p \leq 0.001$], but no main effect of group [$F(1,119) = 0.033$, $p = 0.857$] or interaction [$F(1,119) = 3.312$, $p = 0.071$] of both groups.

In males, there was a main effect of group [$F(1,83) = 9.458$, $p = 0.003$] and treatment [$F(1,83) = 20.055$, $p \leq 0.001$], as well as an interaction [$F(1,83) = 7.319$, $p = 0.008$]. The interaction reflected the fact that TS increased spine density in the VPA-treated group, but not in the controls. In fact, the TS treatment in VPA animals raised the spine density to control levels.

Basilar. The basilar spine density of pyramidal cells of the mPFC was significantly increased in VPA female and male animals following TS treatment. Basilar spine density was decreased as a result of prenatal VPA exposure, which was more pronounced in males, but TS treatment remediated the prenatal drug-induce decrease in basilar spine density (Figure 3.5B).

ANOVA on the spine density for female animals revealed no significant effect of group [$F(1,119) = 0.825$, $p = 0.366$], but a main effect of treatment [$F(1,119) = 91.011$, $p \leq 0.001$] and a significant two-way interaction [$F(1,119) = 15.649$, $p \leq 0.001$]. Both

control and VPA groups showed increased spine density after TS, the effect being larger in the VPA group.

ANOVA on the spine density for males revealed a main effect of group [$F(1,83) = 16.123$, $p \leq 0.001$], treatment [$F(1,83) = 29.395$, $p \leq 0.001$], and an interaction [$F(1,83) = 19.767$, $p \leq 0.001$]. As with the apical spines, the interaction reflected the fact that the TS treatment increased spine density in the VPA-treated group but not in the controls.

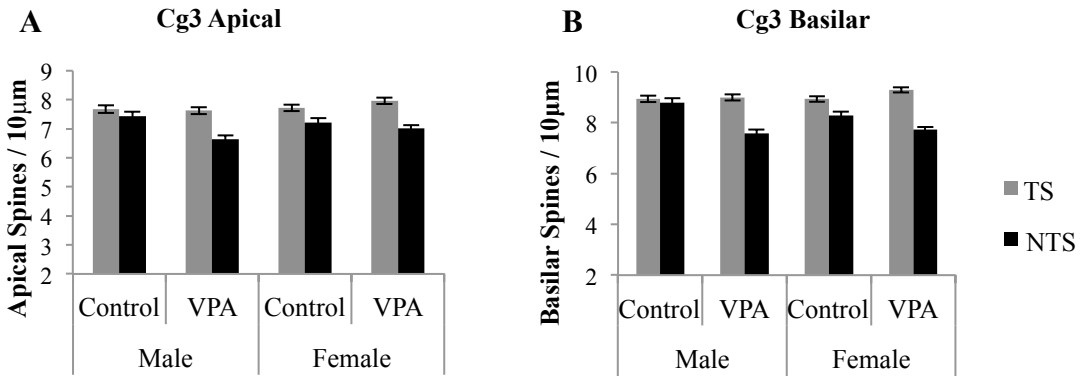

Figure 3.5. Changes in spine density of pyramidal cells in Cg3 in response to prenatal exposure to VPA and TS. (A) Spine density on segment of apical dendrites. Prenatal exposure to VPA significantly reduced the apical spine density of male animals. Females were unaffected by prenatal VPA exposure. TS treatment significantly increased apical spine density in VPA males and female groups. (B) Spine density on segment of basilar dendrites. The basilar spine density of males was significantly reduced in response to prenatal VPA exposure. TS treatment significantly increased basilar spine density in both sexes, which was more pronounced in the VPA groups.

3.4.3.2. Orbital Prefrontal Cortex (AID)

3.4.3.2.1. Dendritic Branching

VPA reduced branching in male animals, but TS increased branching in the VPA-treated rats and the female controls (Figure 3.6).

A two-way ANOVA of dendritic branching in females revealed a main effect of treatment [$F(1,115) = 47.681$, $p \leq 0.001$], but no effect of group [$F(1,115) = 0.546$, $p = 0.462$], nor an interaction [$F(1,115) = 1.361$, $p = 0.246$]. TS treatment significantly increased dendritic branching of AID pyramidal cells in both VPA and control groups.

A two-way ANOVA of dendritic branching in males revealed a main effect of group [$F(1,79) = 10.040$, $p = 0.002$], treatment [$F(1,79) = 28.725$, $p \leq 0.001$], and an interaction [$F(1,79) = 32.694$, $p \leq 0.001$]. The interaction resulted from the large group-specific effect of TS. In fact, TS increased the dendritic branching in the VPA animals above control levels.

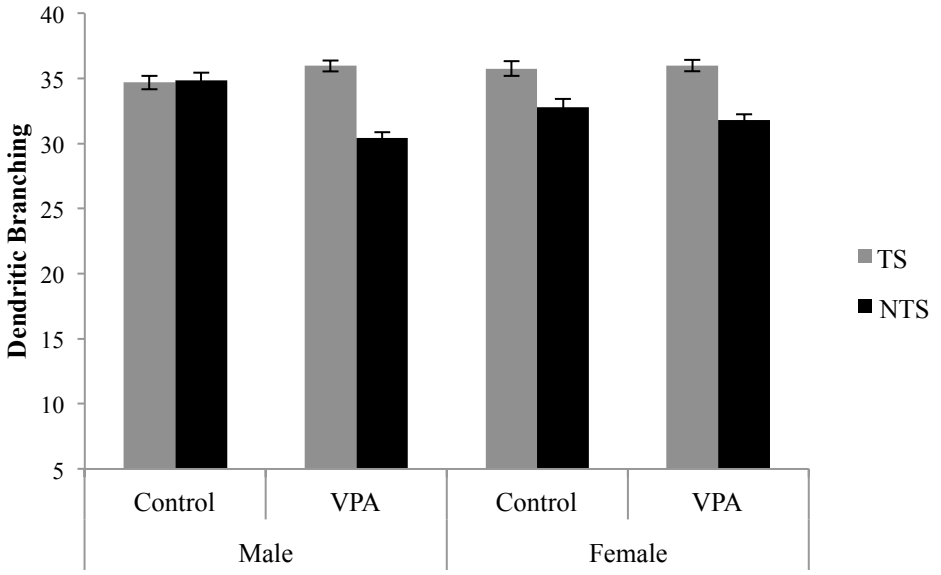

Figure 3.6. VPA reduced dendritic branching in the AID region of male, but not female animals. TS treatment significantly increased branching in the VPA-treated animals and female groups.

3.4.3.2.2. Dendritic Length

VPA did not significantly reduce dendritic length in either sex. TS treatment increased dendritic length in the male VPA and both female groups (Figure 3.7).

A two-way ANOVA of dendritic length in females revealed a significant effect of treatment [$F(1,121) = 83.432$, $p \leq 0.001$] and the interaction [$F(1,121) = 9.301$, $p = 0.003$], but there was no main effect of group [$F(1,121) = 1.093$, $p = 0.298$]. The interaction reflected a trend towards a VPA-induced effect and a larger TS effect in the VPA group than the control groups.

A two-way ANOVA of dendritic length in the male group revealed a significant effect of treatment [$F(1,79) = 19.855$, $p \leq 0.001$] and the interaction [$F(1, 79) = 14.728$, $p \leq 0.001$], but no main effect of group [$F(1,79) = 2.657$, $p = 0.107$]. The interaction reflected the group-specific effects of TS shown in Figure 3.7.

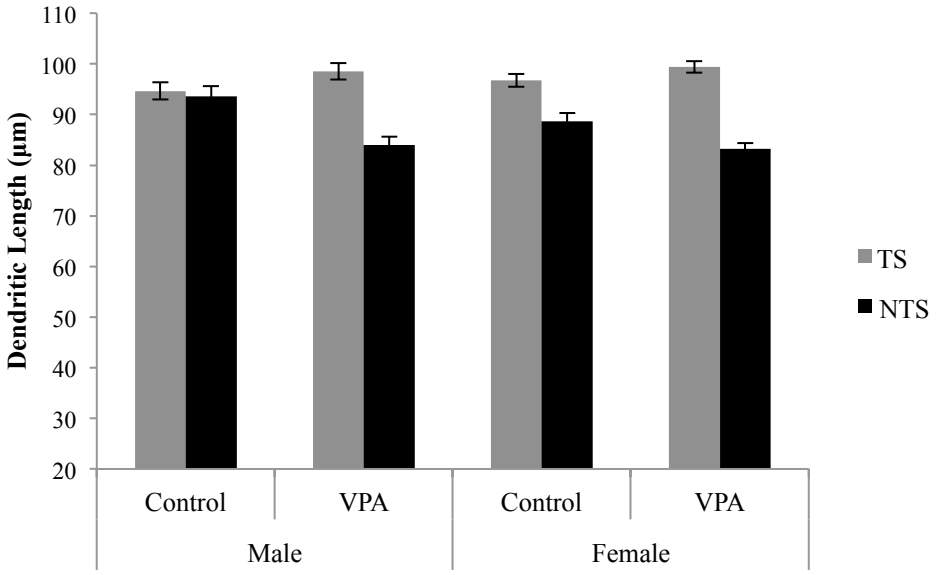

Figure 3.7. Prenatal exposure to VPA decreased dendritic length in the AID region of NTS male and female animals. Interestingly, early TS treatment increased dendritic length in male VPA and female groups.

3.4.3.2.3. Spine Density

While prenatal exposure to VPA did not alter spine density in females, TS treatment significantly decreased spine density in the control group, but not in the VPA group. In contrast to females, VPA induced a reduction in spine density in males. While TS treatment produced a trend towards an increase in spine density among male animals, this effect was most prominent among VPA males, where TS treatment reversed the effects of VPA (Figure 3.8).

A two-way ANOVA of spine density in females revealed a main effect of treatment [$F(1,107) = 5.683$, $p = 0.0019$] and the interaction [$F(1,107) = 14.229$, $p \leq 0.001$], but no effect of group [$F(1,107) = 0.940$, $p = 0.334$]. In this case, the interaction demonstrated that there was no effect of VPA and that TS treatment actually decreased spine density in the control animals ($p \leq 0.001$), but did not affect the VPA group.

ANOVA on the male animals revealed a significant effect of group [$F(1,81) = 24.806$, $p \leq 0.001$] and the interaction [$F(1,81) = 11.362$, $p = 0.001$], but no effect of treatment [$F(1,81) = 3.239$, $p = 0.0076$]. The interaction reflected the fact that TS only increased spine density in the VPA group ($p \leq 0.001$).

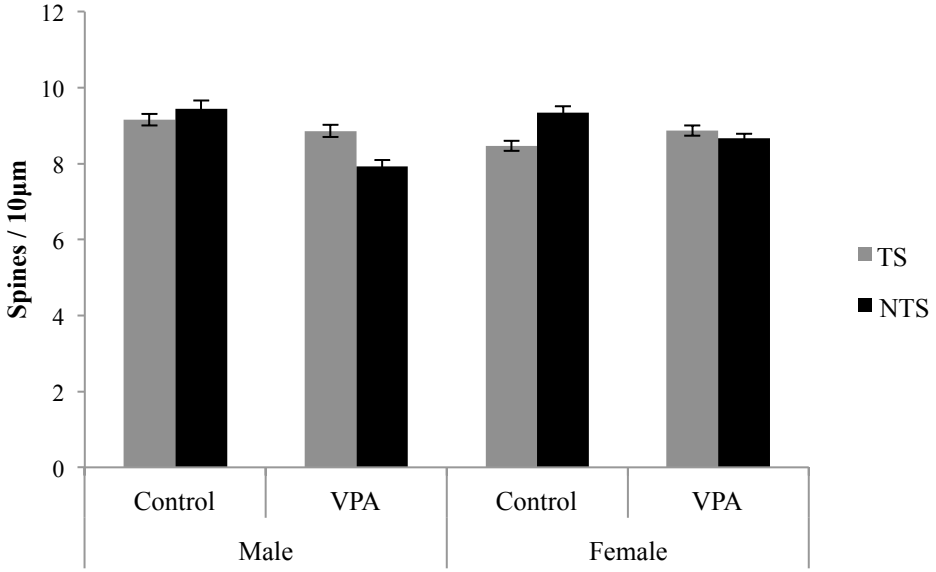

Figure 3.8. AID spine density was significantly reduced in males, but not females, following prenatal VPA exposure. While TS decreased spine density in female controls, an increase in density was observed in the male VPA group.

3.4.3.3. Amygdala

3.4.3.3.1. Spine Density

The effect of VPA was exhibited only among female animals, where prenatal VPA exposure increased amygdalar spine density. TS treatment, on the other hand, stimulated an increase in amygdalar spine density among all animals (Figure 3.9).

A two way ANOVA on females revealed a significant effect of group [$F(1,125) = 9.718$, $p = 0.002$] and treatment [$F(1,125) = 122.121$, $p \leq 0.001$], but no interaction [F

(1,125) = 1.311, $p = 0.254$]. More specifically, amygdalar spine density was shown to increase (independently) in response to VPA, as well TS treatment.

In contrast, ANOVA on males revealed a significant effect of TS treatment [$F(1,92) = 50.266$, $p \leq 0.001$], but not for group [$F(1,92) = 1.600$, $p = 0.209$] or the interaction [$F(1,92) = 0.302$, $p = 0.584$].

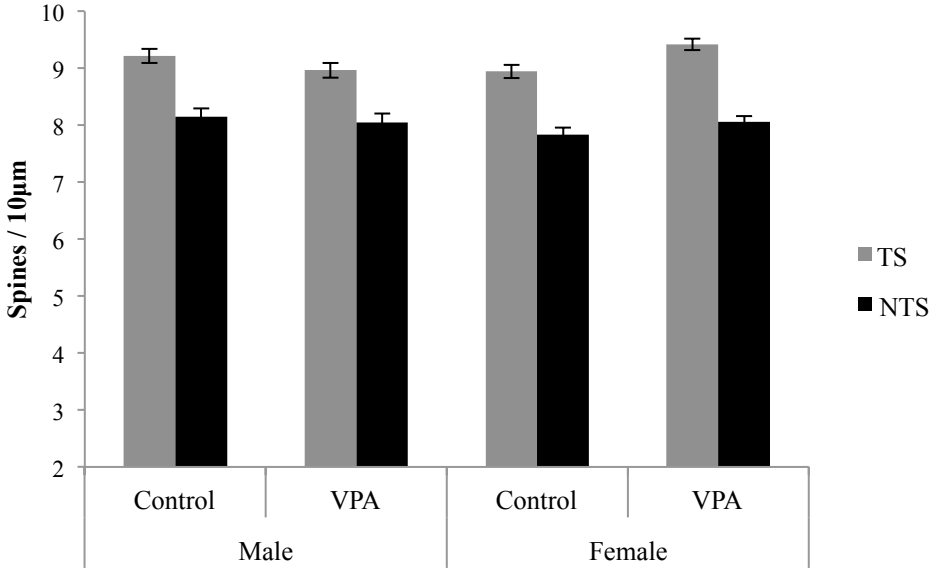

Figure 3.9. Prenatal exposure to VPA significantly increased amygdalar spine density among female animals, but not males. TS treatment significantly increased amygdalar spine density in both sexes.

3.4.4. Brain Weight

Statistical analyses of adult brain weight revealed a significant reduction in brain weight in VPA females, but not VPA males. There was no effect of TS on brain weight.

A two-way ANOVA of female brain weight revealed a main effect of group [$F(1,81) = 3.796$, $p = 0.055$], but no significant effect of treatment [$F(1,81) = 1.235$, $p = 0.270$], nor an interaction [$F(1,81) = 1.503$, $p = 0.224$].

A two-way ANOVA for males found no significant effect of group [$F(1,65) = 3.047$, $p = 0.086$], treatment [$F(1,65) = 0.025$, $p = 0.874$], nor an interaction [$F(1,65) = 0.000$, $p = 0.992$].

3.4.5. Body Weight

Prenatal VPA exposure significantly increased body weight among female VPA animals. TS treatment increased body weights among VPA, but not control, females. Male body weight was unaffected by either prenatal VPA exposure or TS treatment.

A two-way ANOVA of female body weight revealed a significant effect of group [$F(1,81) = 7.216$, $p = 0.009$] and the interaction [$F(1,81) = 5.258$, $p = 0.025$], but no effect of TS treatment [$F(1,81) = 0.237$, $p = 0.627$]. The interaction reflected the fact that TS increased body weight among VPA females ($p \leq 0.001$).

A two-way ANOVA of male body weight revealed no significant effects of group [$F(1,65) = 0.266$, $p = 0.608$], treatment [$F(1,65) = 0.450$, $p = 0.505$], nor an interaction [$F(1,65) = 0.002$, $p = 0.966$].

3.4.6. Summary of Neuroanatomical Results

The anatomical results are summarized in Table 3.2. Analysis of neuroanatomical measures indicates that early TS treatment reversed almost all VPA-induced changes in dendritic morphology in male and female animals. Among the three brain regions examined – Cg3, AID, and amygdala – 10 measurements (dendritic complexity, dendritic length, and spine density) per sex were analyzed. Among female animals, 2 measures were significantly affected by prenatal VPA exposure, while 6 measures exhibited an

interaction or trend towards significance. For male animals, 6 measures were significantly altered in response to VPA and 3 measures demonstrated a significant interaction. Only female brain and body weight was altered in response to prenatal VPA exposure. Early TS treatment significantly reversed all changes in dendritic morphology in VPA animals. More specifically, TS increased dendritic complexity, length, and spine density in all male and female animals (with the exception of AID spine density) in all brain regions. Although TS did not alter brain weight, an increase in body weight was observed among VPA female animals.

Table 3.2. Main effects of VPA and TS treatment from three-way ANOVAs for all neuroanatomical measurements examined. Sexes were analyzed independently.

Anatomical Measurement	VPA		TS	
	Male	Female	Male	Female
Branch Order				
Cg3 apical	↓+	↓	↑	↑
Cg3 basilar	↓	-	↑	↑
AID	↓	-	↑	↑
Sholl				
Cg3 apical	↓+	↓+	↑	↑
Cg3 basilar	↓	↓+	↑	↑
AID	↓+	↓+	↑	↑
Spines				
Cg3 apical	↓	-	↑	↑
Cg3 basilar	↓	↓+	↑	↑
AID	↓	↓+	↑+	↓
Amygdala	-	↑	↑	↑

+, Trend towards significance or significant interaction

3.5. Discussion

The findings of the current study are in concordance with previous reports that prenatal exposure to VPA on gestational day 12.5 produces long-term selective effects on behavior and neuroanatomy in male and female rats (Schneider & Przewlocki, 2005; Schneider et al., 2007; Mychasiuk et al., 2012). The therapeutic effects of early TS treatment were detected in the anatomical pathology of rats prenatally exposed to VPA, completely reversing the effects of VPA in the regions measured. Surprisingly, however, TS treatment failed to remediate or alter behavior in VPA animals.

3.5.1. Effects of VPA and early TS on behavioral outcomes

Prenatal exposure to VPA altered behavior among animals. Consistent with the findings of Mychasiuk and colleagues (Mychasiuk et al., 2012), VPA-treated animals exhibited impaired performance on the delayed non-match-to-sample T-maze, Whishaw tray reaching, and EPM tasks. With respect to activity level, increased activity or hyperactivity was demonstrated among male and female VPA animals. Interestingly, however, VPA female animals exhibited alterations in behavior on all tasks, whereas behavioral changes among VPA-treated males were evident only on the T-maze and activity box tasks. Given that autism occurs predominately in males – a 4 to 1 male/female ratio – the behavioral differences among the sexes in the current study were surprising (Currenti, 2010; Rubenstein & Merzenich, 2003). Although impairments in behavior among VPA animals were detected on a multitude of tasks, the magnitude of such impairments were not as robust as anticipated. While prenatal exposure to VPA did alter behavior, the effects were sex-dependent and varied depending on the measures

scored. Given that the bioavailability, metabolism, and pharmacokinetics of *in utero* VPA exposure vary among rodents (Favre et al., 2013), it is difficult to estimate the proportion or level of VPA that enters fetal circulation to induce autistic-like abnormalities. While the current literature does demonstrate that *in utero* VPA exposure at higher doses (upper limit of 800mg/kg) is sufficient to produce autistic-like anomalies in rodents (Vorhees, 1987), the bulk of such studies utilize intraperitoneal injections as the route of administration. As such, in the current study, it is plausible that the oral dose of VPA administered, 800mg/kg, may not have entered fetal circulation in entirety, therefore dampening toxicant exposure. Alternatively, it is also quite plausible that the 800mg/kg dose of VPA induced a sample bias through embryonic resorption. Vorhees (1987) has previously established a dose-dependent relationship for resorption rate and fetal outcome following prenatal VPA exposure, where the frequency of fetal resorption increases – in a step-wise fashion – with excessive doses of VPA. In the current study, we may hypothesize that perhaps only a subset of the embryos survived the VPA insult given the large dose. It is plausible that only the embryos resistant to the VPA insult survived, reducing the number of viable offspring for behavioral testing. Consequently, the magnitude of the observed behavioral impairments may not have been robust or severe as originally anticipated. As a result, establishing a precise oral dose threshold to produce specific autistic-like features may prove to be useful to control for *in utero* VPA exposure in future studies.

Contrary to expectation, early TS treatment did not significantly alter behavior on the majority of tasks. Surprisingly, no significant differences among TS and NTS animals, irrespective of prenatal VPA exposure or sex, were observed. While early TS

treatment does, indeed, induce neuroplastic changes among animals – VPA and control – it may be the case that the behavioral tasks employed were not sensitive enough to detect TS-related modifications to behavior. More specifically, many of the behavioral tasks utilized have been designed to highlight deficits in animal models of brain damage (Kolb & Gibb, 1990; Kolb, Cioe, & Whishaw, 2000; Kolb & Gibb, 2007), rather than therapeutic effects, so it may be the case that the protocols utilized are not sensitive to the more subtle effects of TS. As previously mentioned, it is likely that the behavioral deficits produced by prenatal VPA exposure are mild, especially when compared to the deficits produced in lesions studies; as such, TS-induced changes in behavior among VPA animals may not be large enough to be detected. The lack of TS induced modification to behavior among all animals is further supported by the study conducted by Richards and colleagues (2012) where early TS treatment failed to alter normal adult rat behavior on the Whishaw tray reaching, EPM, and activity box tasks. While dendritic reorganization in the prefrontal cortex (PFC) and amygdala were demonstrated following early TS treatment, alterations in behavior were not exhibited. This further supports that idea that the therapeutic effects of TS escape detection on such behavioral tasks and future studies may need to rethink how to demonstrate this.

Although a heterogeneous neurodevelopmental disorder, autism is characterized principally by impaired social interactions. Impairments in reciprocal interactions, emotional recognition, and expressions are commonly manifested during peer-play among individuals with autism (American Psychiatric Association, 1994; Volkmar, Carter, Grossman, & Klin, 1997). Given that early social play is imperative to the proper development of social competency and emotional regulation in humans (Pellis & Pellis,

2009), a rat model of peer play may prove to be an ideal system to better understand the etiology of autism. In fact, Schneider & Przewlocki (2005) have demonstrated attenuated social play among VPA rats in juvenile and adulthood, suggesting an inability to express intraspecific communicative signals, as well as inadequate performance of social behaviors. Given the validity of the VPA rodent model of autism in detecting disturbances in social play behavior, investigation into the efficacy of early therapeutic treatments in attenuation or reversal of these social impairments may offer a new avenue for research. More specifically, investigating the enriching effects of early TS treatment on social play behavior in the VPA model of autism may prove to be a more sensitive measure of the therapeutic effects of such a treatment. In fact, high levels of maternal licking and grooming – a form of TS – have been shown to alter play fighting in periadolescent rats, suggesting profound alterations in the development of sociality and competency (Parent & Meaney, 2008). As such, social play may serve as a good model for exploring the therapeutic effects of TS in the VPA rodent model of autism.

3.5.2. Effects of VPA and early TS on dendritic morphology

Despite the lack of change in behavior, there were significant neuroanatomical changes induced by both prenatal VPA exposure and early TS treatment. Consistent with the findings of Mychasiuk and colleagues (2012), prenatal VPA exposure significantly altered the neuronal morphology of the PFC, reducing dendritic branching, length, and spine density in the mPFC and OFC. A significant increase in amygdalar spine density was also exhibited among female VPA animals. Remarkably, however, early TS treatment reversed all neuroanatomical alterations induced by prenatal VPA exposure,

completely remediating the effects of VPA alone. More specifically, a general increase in dendritic complexity, length, and synaptic contact was observed in all cortical areas (with the exception of spine density in the OFC of females) and the amygdala, resulting in anatomical morphology comparable to that of control animals. Given the abundance of literature demonstrating the permanent and widespread effects of early TS treatment on the morphological and neurochemical structures in the cortex of normal and brain-injured animals (Schanberg & Field, 1987; Kolb et al., 2000; Gibb et al., 2010; Kolb & Gibb, 2010; Kolb, Mychasiuk, Williams, & Gibb, 2011), it is no surprise that the autistic brain is susceptible to such changes in response to early enriching experiences. It has been well established that the developing brain is a highly plastic and malleable structure, capable of restructuring neuronal circuits in response to environmental influences, especially during critical periods (Greenough, Black, & Wallace, 1987; LeBlanc & Fagiolini, 2011). Given that TS treatment, in the current study, encompassed these sensitive periods of development, it is likely that changes in neural circuitry will emerge. Even more, in autism, it has been hypothesized that the expression and/or timing of critical periods in several brain regions are disrupted early in development, also resulting in altered plasticity and miswired circuits that are unable to respond optimally to future experiences (LeBlanc & Fagiolini, 2011). Taken together, it is apparent that both prenatal VPA exposure and early TS treatment differentially affect brain plasticity during development. Although the precise mechanism of the additive effect of both experiences is not yet known, we may postulate that postnatal TS is perhaps recreating plasticity in the VPA brain, recovering proper function – at least in the case of the brain regions measured here. It is possible, of course, that other brain regions were altered by the VPA treatment and

these regions were not normalized by the TS, which could account for the lack of behavioral effects of the TS.

Our finding of greater dendritic branching and length in the right hemisphere of the basilar dendrites in Cg3 is consistent with an earlier report that T-maze training produces increased length in the right hemisphere in both Cg3 and Par1 (Comeau, McDonald, & Kolb, 2010). This result does not result from spatial maze training in general, however, as training rats in a spatial reversal task or the Morris water task did not produce this asymmetrical result (Kolb, Cioe, & Comeau, 2008; Comeau et al., 2010). Perhaps it is the working memory aspect of the T-maze training that is critical.

3.5.3. Potential underlying mechanisms

In elucidating the attenuating neuroanatomical effects of early TS treatment in VPA animals, potential underlying cellular and molecular mechanisms should be considered. It has been well established that balanced cortical excitatory and inhibitory (E/I) neurotransmission is crucial in the developing brain, given the tight regulation of critical periods. Central to the establishment of this homeostatic E/I transmission is the GABAergic system, wherein γ-aminobutyric acid (GABA) – an inhibitory neurotransmitter that also has excitatory effects in the developing brain – plays an important role in the maturation of inhibitory circuits and the establishment of neuronal connectivity (Manent et al., 2005; Represa & Ben-Ari, 2005; Wang & Kriegstein, 2008; LeBlanc & Fagiolini, 2011; Kilb, Kirischuk, & Luhmann, 2013). GABA is imperative to brain development, as it influences a wide range of processes including neurogenesis, neurite growth and axon elongation, neuronal migration, and synaptic connectivity (Kilb,

2012; Kilb et al., 2013). GABA is also known to regulate the expression of $GABA_A$ receptor subunits and down-regulate GABA receptor binding (Tehrani & Barnes, 1988; Hablitz, Tehrani, & Barnes, 1989; Schousboe & Redburn, 1995). As previously mentioned, an imbalance in the E/I transmission may cause, in some cases, excessive plasticity or in other cases, insufficient plasticity. With respect to altered critical period plasticity in the autistic brain, it has been demonstrated that cortical E/I neurotransmission is greatly disrupted and is likely contributing to the anomalies in behavior and neural circuitry (Rubenstein & Merzenich, 2003; LeBlanc & Fagiolini, 2011). More specifically, it has been hypothesized that this E/I imbalance may be related to suppression in GABAergic signaling, leading to a reduction in inhibition and alterations in cortical circuitry (Hussman, 2001; Rubenstein & Merzenich, 2003). In fact, human studies have, indeed, demonstrated decreased GABA levels in the frontal lobe of autistic patients (Harada et al., 2011), reduction in protein levels of enzymes that synthesize GABA in the cerebellar and parietal cortices (Fatemi et al., 2002), and down-regulation of $GABA_A$ receptors in the superior frontal cortex, parietal cortex, and cerebellum (Fatemi, Reutiman, Folsom, & Thuras, 2009). A reduction in GABA receptor binding can severely impair brain development, given the crucial role of GABA in numerous developmental processes (Kilb, 2012; Kilb et al., 2013; Ben-Ari, 2002). Additionally, down-regulation of $GABA_A$ receptors can also negatively impair dendrite formation (Kilb et al., 2013). In summary, these data suggest that a derangement in the developing GABAergic system is likely contributing to the abnormal cortical interneuronal circuitry in autism. Interestingly, it has been demonstrated that prenatal exposure to VPA, in rodents, also perturbs GABAergic transmission during early

developmental stages. Classically, VPA exerts its pharmacological effects by increasing brain concentrations of GABA (Mychasiuk et al., 2012). Because VPA is readily transported across the placenta (Alsdorf & Wyszynski, 2005; Kaneko et al., 1983), this appears to also be the case among rat offspring prenatally exposed to VPA (Mesdjian et al., 1982; Miyazaki, Matsuyama, Ichikawa, & Goto, 1988; Laeng et al., 2004; Manent et al., 2007). Based on these findings, we speculate that these elevated levels of GABA may result in attenuated GABAergic transmission at later stages in development (Schneider et al., 2007) via down-regulation of $GABA_A$ receptors and receptor binding. In fact, reduced GABAergic inhibition in the temporal cortex has been demonstrated (Banerjee et al., 2013; Chomiak et al., 2013), giving rise to the possibility of a compromised GABAergic system in VPA animals. As a result, in the current study, we hypothesize that the changes in neuronal morphology/circuitry among our VPA animals may be attributed to disturbances in E/I neurotransmission and, consequently, result in altered development of GABAergic neuronal populations, similar to human cases of autism. Taking into account the suggested decrease in GABAergic tone in VPA animals, TS during the postnatal period may attenuate such effects, given its profound impact on the forebrain GABAergic system (Caldji, Diorio, & Meaney, 2000). Meaney and colleagues have demonstrated significantly higher levels of $GABA_A$ receptor binding in adult offspring of high licking/grooming mothers, suggesting that early TS treatment does, indeed, produce lifelong alterations in the $GABA_A$ receptor complex of animals (Francis & Meaney, 1999; Kaffman & Meaney, 2007; Caldji et al., 2000). Additionally, significant increases in the expression of enzymes that synthesize GABA in the mPFC were also exhibited among adult offspring, following early postnatal TS (Caldji et al., 2000). Because

prenatal exposure to VPA may result in down-regulation of $GABA_A$ receptor binding and reduction in protein levels of GABA-synthesizing enzymes, it seems plausible that early TS treatment may be reversing such effects. In fact, early TS may be preventing or interfering with the cellular sequelae of events following prenatal VPA exposure, resulting in 'normalized' brain circuitry. While a plethora of literature supports a disturbed or suppressed GABAergic system in the autistic brain, it appears that early TS treatment allows for increased GABAergic inhibition, potentially countering not only the E/I imbalance, but also the consequent neuroanatomical effects following VPA insult. Although the proposed cascade of cellular events during development is highly speculative, it is obvious that a plastic interaction between prenatal VPA exposure and early TS treatment is occurring.

One difficulty with the GABA-related changes in E/I balance is that the increase in excitation might be expected to increase excitatory synapses. The decrease in spine density, and thus the likely decrease in excitatory synapses, in the current study thus would appear to be at odds with at least one prediction of the E/I imbalance hypothesis. The difficulty is that given that GABA has an excitatory role in the developing brain as well as an inhibitory role the mature brain, our seemingly paradoxical spine density results could reflect changes in the E/I balance during development and during different critical periods, with less predictable consequences on cerebral morphology, possibly related to epigenetic effects that influence synaptic organization. This remains to be seen.

Another explanation underlying the plastic interaction between prenatal VPA exposure and early TS treatment may be related to the endogenous expression of fibroblast growth factor-2 (FGF-2) – a potent neurotrophic growth factor – during this

critical period. Gibb et al. (Gibb & Kolb, submitted for publication) have reported that early TS treatment leads to increased FGF-2 expression in both the skin and brain. Given that FGF-2 is a key facilitator of recovery following early cortical injury (Gibb et al., 2010; Comeau, Hastings, & Kolb, 2007; Gibb & Kolb, submitted for publication), we speculate that increased expression of FGF-2 in response to early TS would likely contribute to alterations in cortical plasticity in our VPA animals. It is plausible that the consequential increases of FGF-2 following TS during the postnatal period is, indeed, altering the neuroanatomical sequelae in the VPA brain, producing long-lasting changes. Further studies are warranted to elucidate the proposed relationship between E/I neurotransmission, GABAergic signaling, neurotrophic growth factors, and neuronal circuitry in VPA rats, which may lay a basis for understanding the therapeutic effects of TS treatment in this model, as well as in human autism studies of infant massage.

3.5.4. Conclusion

Analogous to infant massage or touch therapy, several reports have suggested the effectiveness of TS treatment in attenuating core autistic behaviors and touch aversion in autistic patients (Fields et al., 1997; Escalona et al., 2001; Cullen, Barlow, & Cushway, 2005a, b). It has been speculated that TS enhances parasympathetic (vagal) activity in autistic patients, suggesting that a physiological change is taking place following the experience (Field, 1998). While this may be the case, no detailed mechanism has been established, especially in terms of brain plasticity. To date, the current study is the first of its kind to examine the neuroanatomical correlates of early TS treatment in a rodent model of autism. Despite the lack of remediation or improvement in behavior among our

VPA animals, we offer insight into the powerful remediating abilities of early TS treatment on brain anatomy in the regions measured here. Understanding the underlying mechanisms that restore plasticity in affected neuronal circuits may be the first step to better identify the neuroanatomical changes that mediate these effects in the autistic brain. This may yield wider implications not only for further development of therapeutic interventions that enhance brain activity and behavior in animal models of autism, but also for the application of such therapies to attenuate autistic symptoms in humans.

CHAPTER 4

General Discussion

This thesis contributes to the large literature investigating the utility of maternal challenge with VPA as an animal model of ASD. Although clinical relevance of the VPA rodent model has been documented extensively (Rodier et al., 1997b; Roullet et al., 2013), further examination of the association between behavioral and neurobiological profiles would confirm the *face, construct*, and *predictive validity* of this model in simulating human ASD. That is, analysis of the ethological, etiological, and therapeutic similarities to human ASD would provide proof-of-principle for the VPA rodent model. As such, the objective of this thesis was to examine the perturbations in the neurodevelopmental trajectories – during juvenile and adulthood – as a consequence of *in utero* VPA exposure. The synergistic interaction between brain and behavior was considered and compared to facets of human ASD, to evaluate the utility of the VPA rodent model. Furthermore, the *predictive validity* of the VPA rodent model was also assessed by examining the therapeutic effects of early TS in rats prenatally exposed to VPA, on both behavioral and neuroanatomical levels. The results of these studies produced a number of findings that offer insight into the behavioral and neural underpinnings of ASD, as well as shed light on the validity and limitations of the VPA rodent model. The findings are summarized below.

4.1. Translational Value of the VPA Rat Model of ASD

Developing an animal model for ASD poses a challenge for researchers, as the etiological basis of the disorder remains to be determined. However, environmentally

induced rodent models have appeared to provide a unique avenue to explore the possible mechanisms underlying ASD. More specifically, the VPA rat model of ASD has undergone rigorous behavioral and neuropathological evaluation, providing a strong basis for the relevance of this model to human ASD. The studies presented in the current thesis further contribute to this large body of rodent research, supporting the conclusion that *in utero* exposure to VPA produces a viable and translational model for the study of ASD, as many of the behavioral and neuroanatomical findings appear to be consistent with human literature. A summary of the observed behavioral changes is presented in table 4.1.

As described in the DSM-V, qualitative impairments in social interaction and communication constitute two of the core clinical features of ASD. Failure to develop and maintain peer relationships, lack of social reciprocity and theory of mind, a paucity of nonverbal communication, and reduced eye contact all encompass various aspects of impaired social functioning in ASD (Adrien et al., 1991; Jordan, 2003; Seltzer et al., 2003; American Psychiatric Association, 2013; Ventola et al., 2013). Although comparison of sociality in ASD patients and rodents is not directly feasible, interesting parallels can be drawn to evaluate the validity of the VPA model in simulating impaired social functioning characteristic of human ASD (chapter 2). For instance, in our paradigm of social play, VPA rats were less likely to engage in defensive tactics that promote bodily contact and reciprocal exchanges, thereby implying a refusal of body contact and an inability to facilitate social interactions, both of which have been reported in the human ASD literature (Adrien et al., 1991; Gillberg et al., 1996). Furthermore, a reduction in the rate of USV calling per bout of play fighting – that is, discordance

between vocalizing during playful interactions – suggest that aspects of being able to communicate effectively while engaging in dynamic social interactions have been disrupted or altered as a consequence of *in utero* VPA exposure. Dysregulation of socio-communicative abilities and/or social pragmatics may be driving such changes, as exhibited in clinical ASD populations (Baron-Cohen, 1988). Furthermore, ASD patients also present with several comorbid symptoms, some of which parallel the behavioral impairments observed in the present studies. Executive function difficulties were observed in the non-match-to-sample T-maze task, where VPA animals took significantly longer to reach criterion than controls (chapter 3). It is plausible that VPA animals imparted a greater number of perseverative responses or possessed difficulties in problem solving, both of which are characteristic of cognitive impairment in ASD (McEvoy et al., 1993; Mychasiuk et al., 2012). In the Whishaw tray reaching task, female VPA animals exhibited a reduction in the percent hits – defined as the total number of successful reaches over the total number of attempts – implying motor skill dysfunction, once again paralleling the reported delay in motor skill development in clinical ASD populations (chapter 3; Papadopoulos et al., 2011; Mychasiuk et al., 2012).

Table 4.1. Summary of findings for juvenile (A) and adult behavior (B-E) in VPA rats

	Prenatal VPA	
	Male	**Female**
A. Play		
Attacks	n/a	-
Defence	n/a	-
Pins	n/a	-
Complete Rotation	n/a	↓
Partial Rotation	n/a	-
Evasion	n/a	-
Other	n/a	↑
Mounting	n/a	↑
Body Shakes	n/a	↑
Scratching	n/a	-
Stereotyped Grooming	n/a	-
Rate of USVs	n/a	↓
B. T-Maze		
Days to Criterion	↑	↑+
C. Whishaw Tray Reaching		
% Hits	-	↓

D. Locomotor Activity	↑	↑+
E. EPM		
Time in Closed Arms	-	↓
Time in Open Arms	-	-

+, Trend towards significance or significant interaction

At the neuroanatomical level, significant reductions in dendritic branching, complexity, and spine density were exhibited in the mPFC and OFC in rats prenatally exposed to VPA (chapter 3). Although abnormal assembly of synapses and dendritic spines has been reported in the pathogenesis of ASD (Watts, 2008), the type and location of such alterations vary with age and from case to case. Moreover, a thorough examination of the dendritic morphology in the mPFC and OFC of ASD patients has not yet, to our knowledge, been quantified, but evidence of frontal lobe cellular abnormalities and behavioral deficits suggest that the PFC circuitry is likely implicated (Prior & Hoffman, 1990; Carper & Courchesne, 2005). Reports of mild disruptions of laminar organization (Bailey et al., 1998), reduced spine density (Williams, Hauser, Purpura, DeLong, & Swisher, 1980), and patches of decreased pyramidal cell density (Belichenko, Hagberg, & Dahlstrom, 1997) in the frontal lobes of ASD patients have been demonstrated, lending to the idea that alterations in the dendritic organization of the PFC are likely to emerge.

4.2. Face and Construct Validity of the VPA Rat Model of ASD

ASD is a behaviorally defined disorder characterized principally by social-communicative deficits, and restrictive and repetitive behaviors and/or interests (American Psychiatric Association, 2013). With the emergence of symptoms in early childhood, the diagnosis of ASD is solely based on behavioral assessment, thus, highlighting the importance of comparable behavioral endpoints in potential animal models of ASD. Validation of rodent models of ASD, therefore, lies primarily in the phenomenological similarity between the behavioral features exhibited in the animal model to those observed in human ASD (*face validity*) and, the accuracy by which the animal model mimics the etiopathogenic mechanisms involved in the pathogenesis of the human disorder (*construct validity*) (Belzung, Leman, Vourc'h, & Andres, 2005; Tordjman et al., 2006). The evidence from this thesis corroborates previously published work suggesting that the VPA rat model of ASD is, indeed, a viable tool and relevant model of ASD. Namely, the VPA model of ASD exhibits significant *face* and *construct* validity, suggesting that the observed behaviors in the rodents are etiologically and ethologically relevant and comparable to human ASD (Patterson, 2011; Schneider & Przewlocki; 2011; Chomiak et al., 2013; Roullet et al., 2013).

With respect to the principle of *face validity*, reproduction of core ASD symptomology constitutes the primary criterion in validating the VPA rat model. Qualitative impairments in social interaction and social communication were exhibited among VPA juvenile rats during rough-and-tumble play – as outlined in chapter 2 – implying impaired sociality as a consequence of *in utero* VPA exposure. More specifically, disrupted facilitation of dynamic social interactions, marked by reductions in contact-promoting maneuvers and rate of USVs emitted per bout of play fighting, were

thought to reflect ambivalence in social relations, dysregulation of pragmatics, deficits in turn taking, and altered socio-communicative function – all of which are characteristic of impaired social functioning in ASD (Baron-Cohen, 1988; Adrien et al., 1991; Jordan, 2003; Seltzer et al., 2003; American Psychiatric Association, 2013). Furthermore, an extensive body of rodent literature investigating the effects of *in utero* VPA exposure on social and communicative abilities using alternate behavioral paradigms and assays, has consistently replicated and confirmed the findings of impaired sociality and communication (Schneider & Przewlocki, 2005; Markram et al., 2008; Chomiak et al., 2010; Gandal et al., 2010; Kataoka et al., 2013; Kim et al., 2011), further validating the ethological relevance of the VPA model to the human correlate. With respect to repetitive stereotypical patterns of behavior – another domain of core ASD symptomology – studies in the current thesis were unable to delineate and characterize such impairments among prenatally exposed VPA animals in the behavioral paradigms employed. Although impaired motor performance in VPA females was revealed on the Whishaw tray reaching task (chapter 3), it is unclear whether this impairment reflects motor stereotypies or an inability to execute reaching abilities. That is, a motor stereotypy may be expressed as rapid multiple reaches towards the food, whereas a failure to execute reaches may be expressed as uncoordinated movements. Interestingly, however, despite the absence of sensitive behavioral measures of repetitive/stereotyped patterns of behavior in the present thesis, several independent laboratories have concluded *face validity* related to this core symptom. That is, increased patterns of repetitive/stereotyped movements, measured on a variety of behavioral paradigms, has been shown in rodents prenatally exposed to VPA (Schneider & Przewlocki, 2005, Schneider et al., 2008; Markram et al., 2008; Gandal et

al., 2010; Mehta, Gandal, & Siegel, 2011). Even more, as postulated by Tordjman and collegues (2006), the greater number of features represented by the VPA model, the more viable and pertinent animal model of ASD it will be. As such, additional comorbid behavioral deficits, unrelated to core symptomology but consistent with human ASD, were exhibited in the present studies, including altered anxiety-like behaviors, hyperactivity, reduced cognitive function, and sexual behavior – further exemplifying the VPA rodent model as a multi-trait approach to better understand the clinicopathology of ASD. Thus, corroborated with the ever-growing avenue of VPA rodent research, the behavioral studies in this thesis confirm that the VPA rodent model of ASD demonstrates significant *face validity*, whereby the observed rodent behaviors are ethologically pertinent to the behavioral deficits in clinical ASD populations.

Despite being elusive in nature, ASD is a multifactorial disorder that is variable in the expressed phenotype. In spite of the heterogeneous etiology – genetic and/or environmentally induced – when evaluating pertinent animal models of ASD, demonstration of *construct validity* is another necessary and important component. That is, the accuracy by which the animal model replicates the underlying causes of the disease (Belzung et al., 2005; Schneider & Przewlocki, 2005). With respect to the VPA rodent model of ASD, translation of human epidemiological data informed the creation of this model, whereby *in utero* exposure to VPA during the first trimester of pregnancy increases the incidence of ASD and intellectual disability (Christianson et al., 1994; Moore et al., 2000; Rasalam et al., 2005; Christensen et al., 2013). On the basis of etiological relevance to human ASD, the VPA model does demonstrate *construct validity* to ASD. Moreover, of the vast number of animal models that adopt a multifactorial

approach to the study of ASD, the VPA model exhibits behavioral, genetic, immunological, biochemical, neuroanatomical, and cellular similarities to human ASD (for review, see Schneider & Przewlocki, 2005), further validating the viability of this model. Although general consensus has been met regarding the *construct validity* of the VPA rodent model (Patterson, 2011; Roullet et al., 2013), it is important to keep in mind that this model represents a proportion of individuals with ASD; that is, those exposed to VPA *in utero*. Although aspects of the VPA rodent model may be translational to other populations of ASD, it does not entirely mimic the etiology of children unexposed to VPA.

4.3. Predictive Validity of the VPA Rat Model of ASD: Implications for Remediation

In reference to psychopathological animal models, the principle of *predictive validity* refers to the model's ability to make precise predictions about the disorder in question. That is, in a more narrow sense, the ability of an animal model to predict and produce treatments of therapeutic value in both animals and humans similarly (Belzung et al., 2005; Schneider & Przewlocki, 2005; Tordjman et al., 2006). In addition to *face* and *construct validity, predictive validity* constitutes an important component in the validation of animal models of ASD.

This thesis examined the therapeutic effects of early TS treatment in attenuating the behavioral and neuroanatomical aberrations in rats prenatally exposed to VPA (chapter 3). Given the known benefits of touch therapy – an intervention equivalent to TS in animals – in promoting neurobehavioral development and improving core

symptomology in pediatric ASD populations (Field et al., 1997; Escalona et al., 2001; Cullen et al., 2005b; Jones & Mize, 2007), studies in the present thesis sought to evaluate the beneficial effects of early TS on the behavioral expression and neuropathological deficits in animals prenatally exposed to VPA. Remediation or reversal of the VPA-induced changes in behavior and neuroanatomy would confirm the *predictive validity* of the VPA model, under the assumption of comparable therapeutic efficacy in both humans and animal models. While the therapeutic effects of early TS treatment were detected in the neuroanatomical pathology of rats prenatally exposed to VPA, completely reversing the VPA-induced alterations in dendritic organization in the mPFC and OFC, this was not the case behaviorally. That is, contrary to expectation, early TS failed to remediate or alter behavior in all rats, VPA or control. Given the conflicting behavioral and neuroanatomical findings, one might suggest that the *predictive validity* of the VPA model is questionable. That is, if an animal model is considered *predictive* based on its ability to characterize a therapeutic treatment for human ASD (Tordjman et al., 2006), it is plausible that the VPA model is *predictive* but only to an extent. However, based on the significant alterations in the PFC (post-TS) in our rodent study, and the numerous reports of improvements in core symptomology following touch therapy in children with ASD (Field et al., 1997; Escalona et al., 2001; Cullen et al., 2005a; Jones & Mize, 2007), we have reason to believe that the VPA model does, indeed, exhibit *predictive validity*. The lack of TS-induced modification to behavior among all animals may rather be attributed to the behavioral measures employed. More specifically, it is likely that the behavioral tasks utilized were not sensitive enough to detect the subtle effects of TS treatment. This proposition is further confirmed by Richards and colleagues (2012),

where TS treatment failed to be detected on a range of behavioral tasks identical to the ones utilized in this thesis. At the very least, we propose that TS is a potent avenue for intervention, given the pronounced therapeutic effects on neuroanatomy (and quite possibly behaviorally as well, in the case that more detailed behavioral tasks are employed instead) in the VPA rat model of ASD. This leads us to confirm, at least in part, the *predictive validity* of the VPA model, given the capacity of this model to validate behavioral interventions with relevance to human ASD.

4.4. Limitations and Future Directions

While the present thesis assessed the validity of the VPA rat model in simulating the neurodevelopmental disturbances as seen in human ASD, numerous future experiments could be conducted to remedy the limitations present in the current studies, as well as address novel questions of interest. One limitation to the study of sociality in rats prenatally exposed to VPA is the absence of male subjects. Given the confirmed male bias in ASD – a 4 to 1 male/female ratio (Rubenstein, & Merzenich, 2003; Currenti, 2010) – examination of the playful interactions and associated USVs in VPA-treated juvenile male rats may shed light on the sex-specific phenotypes seen in human ASD. Furthermore, comparison of the playful dynamics of VPA male and female subjects would further evaluate the hypothesis of masculinized-typical play in females, as postulated by the 'extreme male brain' theory (Baron-Cohen, 2002; Knickmeyer, Wheelwright, & Baron-Cohen, 2008). Given the VPA-induced changes in the dynamics of play and communication occurring therein during the juvenile period, it would also be

interesting to assess whether these alterations in sociality propagate into adulthood (as exhibited in human cases of ASD).

One of the most significant limitations in this thesis was the inability to detect the therapeutic effects of early TS on a multitude of behavioral tasks. That is, although TS treatment appeared to significantly alter (and remediate, in the case of VPA animals) the neuronal morphology in the PFC, no significant differences were observed on the behavioral tasks employed. As previously suggested (chapter 4.3), it is likely that the behavioral protocols utilized were not sensitive to the subtle effects of TS and, thus, more thorough tests are required for future experiments. Detailed behavioral paradigms, such as social play fighting, skilled reaching, or even measurement of 50-kHz and 22-kHz USVs may prove to better highlight the therapeutic effects of TS in both VPA and control animals.

While the current studies assessed behavior in the juvenile and adult periods, it would have been interesting to implement behavioral testing in the early, preweaning period. Seeing as how manifestation of atypical behaviors and subsequent diagnosis of ASD typical occurs around the age of three (Chawarska et al., 2014), behavioral testing of rats during preweaning period may allow for the identification of early sensory-motor, social-emotional, and communicative impairments predictive of ASD. Furthermore, by assessing the earliest manifestations of atypical behavioral development, targeted interventions during these critical periods could be adopted to counter the abnormal developmental cascade that eventually leads to full-blown ASD. Additionally, anatomical and epigenetic analysis during the preweaning, juvenile, and adult periods could be also assessed to examine the synergistic relationship between brain and behavior, and if these

changes are persistent lifelong. Owing to the fact that interplay of genetic, epigenetic, and environmental processes are suspected to be involved in the pathogenesis of ASD (Schneider & Przewlocki, 2011), significant changes in DNA methylation and the epigenetic processes regulating cellular development would be predicted.

Finally, in the future, it would be valuable to adapt the VPA rat model of ASD to better reflect the human clinic. That is, examination of the effects of long-term fetal VPA exposure, at clinically relevant doses, on the functional and anatomical outcomes of offspring would be of interest. Given the seemingly high degree of ecological validity associated with this paradigm of maternal epilepsy, one would predict that prenatal exposure to small daily dosages of VPA, throughout gestation and during the lactation period, will produce behavioral and neuropathological characteristics equivalent to those observed in human ASD, as well as simulate a greater translational model of autism than the previous acute high dosage paradigm.

4.5. Conclusion

VPA maternal challenge in rodents has long been proposed as a viable animal model to study ASD. The present thesis examined the utility of the VPA rodent model of ASD by assessing the behavioral and neurodevelopmental trajectories in rats prenatally exposed to VPA, as well as the therapeutic effects of an enriching environmental manipulation on behavioral expression and pathology. Corroborated with the large VPA rodent literature, we conclude that the VPA model exhibits all elements of a translational animal model, namely, *face, construct,* and *predictive validity,* given the striking behavioral and neuropathological similarities to human ASD profiles. Nonetheless,

evidence from this thesis further illustrates how environmental challenges can play a significant role in the etiology of ASD, producing persistent changes in the structure and functioning of the developing brain.

4.6. References

Abell, F., Krams, M., Ashburner, J., Passingham, R., Friston, K., Frackowiak, R., Happe, F., Frith, C., & Frith, U. (1999). The neuroanatomy of autism: A voxel-based whole brain analysis of structural scans. *NeuroReport, 10,* 1647.

Adab, N., Kini, U., Vinten, J., Ayres, J., Baker, G., Clayton-Smith, J., (…) Chadwick, D.W. (2004). The longer term outcome of children born to mothers with epilepsy. *Journal of Neurology, Neurosurgery, & Psychiatry, 75,* 1575-1583. doi: 10.1136/jnnp.2003.029132

Adrien, J.L., Faure, M., Perrot, A., Hameury, L., Garreau, B., Barthelemy, C., & Sauvage, D. (1991). Autism and family home movies: Preliminary findings. *Journal of Autism and Developmental Disorders, 21,* 43-49. doi: 10.1007/BF02206996

Alsdorf, R., & Wyszynski, D.F. (2005). Teratogenicity of sodium valproate. *Expert Opinion on Drug Safety, 4,* 345-353. doi: 10.1517/14740338.4.2.345

Amaral, D.G. (1992). Anatomical organization of the primate amygdaloid complex. In: *The amygdala: Neurobiological aspects of emotion, memory, and dysfunction.* J.P. Aggleton (editor). New York, Wiley-Liss; pp. 1-66.

American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders (4th ed., rev). Washington, DC: American Psychiatric Association.

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed., rev). Washington, DC: American Psychiatric Association.

Andari, E., Duhamel, J.R., Zalla, T., Herbrecht, E., Leboyer, M., & Sirigu, A. (2010). Promoting social behavior with oxytocin in high-functioning autism spectrum disorders. *Proceedings of the National Academy of Sciences of the United States of America, 107,* 4389-4394. doi: 10.1073/pnas.0910249107

Anderson, G.M,. Freedman, D.X., Cohen, D.J., Volkmar, F.R., Hoder, E.L., McPhedran, P., (…) Young, J.G. (1987). Whole blood serotonin in autistic and normal subjects. *Journal of Child Psychology and Psychiatry, 28,* 885-900. doi: 10.1111/j.1469-7610.1987.tb00677.x

Anderson, G.M., Horne, W.C., Chatterjee, D., & Cohen, D.J. (1990). The hyperserotonemia of autism. *Annals of the New York Academy of Sciences, 600,* 331–340. doi: 10.1111/j.1749-6632.1990.tb16893.x

Arndt, T.L., Stodgell, C.J., & Rodier, P.M. (2005). The teratology of autism. *International Journal of Developmental Neuroscience, 23,* 189-199. doi: 10.1016/j.ijdevneu.2004.11.001

Ashwin, C., Baron-Cohen, S., Wheelwright, S., O'Riordan, M., & Bullmore, E.T. (2007). Differential activation of the amygdala and the 'social brain' during fearful face-processing in Asperger syndrome. *Neuropsychologia, 45,* 2-14. doi: 10.1016/j.neuropsychologia.2006.04.014

Auyeung, B., Baron-Cohen, S., Ashwin, E., Knickmeyer, R., Taylor, K., & Hackett, G. (2009). Fetal testosterone and autistic traits. *British Journal of Psychology, 100,* 1-22. doi: 10.1348/000712608X311731

Bachevalier, J. & Loveland, K.A. (2006). The orbitofrontal-amygdala circuit and self-regulation of social-emotional behavior in autism. *Neuroscience and Biobehavioral Reviews, 30,* 97-117. doi: 10.1016/j.neubiorev.2005.07.002

Bågenholm, A., & Gillberg, C. (1991). Psychosocial effects on siblings of children with autism and mental retardation: A population-based study. *Journal of Intellectual Disability Research 35,* 291-307. doi: 10.1111/j.1365-2788.1991.tb00403.x

Bailey, A., Luthert, P., Dean, A., Harding, B., Janota, I., Montgomery, M., (…) Lantos, P. (1998). A clinicopathological study of autism. *Brain, 121,* 889-905. doi: http://dx.doi.org/10.1093/brain/121.5.889

Bambini-Junior, V., Rodrigues, L., Behr, G.A., Moreira, J.C.F., Riesgo, R., & Gottfried, C. (2011). Animal model of autism induced by prenatal exposure to valproate: Behavioral changes and liver parameters. *Brain Research, 1408,* 8-16. doi: 10.1016/j.brainres.2011.06.015

Banerjee, A., Engineer, C.T., Sauls, B.L., Morales, A.A., Kilgard, M.P., & Ploski, J.E. (2014). Abnormal emotional learning in a rat model of autism exposed to valproic acid in utero. *Frontiers Behavioral Neuroscience, 8,* 1-13. doi: 10.3389/fnbeh.2014.00387

Banerjee, A., Garcia-Oscos, F., Roychowdhury, S., Galindo, L.C., Hall, S., Kilgard, M.P., & Atzori, M. (2013). Impairment of cortical GABAergic synaptic transmission in an environmental rat model of autism. *The International Journal of Neuropsychopharmacology, 16*, 1309-1318. doi: http://dx.doi.org/10.1017/S1461145712001216

Baron-Cohen, S. (1988). Social and pragmatic deficits in autism: Cognitive or affective? *Journal of Autism Developmental Disorders, 18*, 379-402. doi: 10.1007/BF02212194

Baron-Cohen, S. (2002). The extreme male brain theory of autism. *Trends in Cognitive Sciences, 6,* 248–254. doi: 10.1016/S1364-6613(02)01904-6

Baron-Cohen, S., Ashwin, E., Ashwin, C., Tavassoli, T., & Chakrabarti, B. (2009).

Talent in autism: Hyper-systemizing, hyper-attention to detail and sensory hypersensitivity. *Philosophical Transactions of the Royal Society B: Biological Sciences 364,* 1377-1383. doi: 10.1098/rstb.2008.0337

Baron-Cohen, S., Leslie, A.M., & Frith, U. (1985). Does the autistic child have a "theory of mind" ? *Cognition, 21,* 37-46. doi: 10.1016/0010-0277(85)90022-8

Baron-Cohen, S., Ring, H.A., Bullmore, E.T., Wheelwright, S., Ashwin, C., & Williams, S.C.R. (2000). The amygdala theory of autism. *Neuroscience and Biobehavioral Reviews, 24,* 355-364. doi: 10.1016/S0149-7634(00)00011-7

Bartz, J.A., & Hollander, E. (2008). Oxytocin and experimental therapeutics in autism spectrum disorders. *Progress in Brain Research, 170*, 451-462. doi: 10.1016/S0079-6123(08)00435-4

Bath, K.G., & Scharfman, H.E. (2013). Impact of early life exposure to antiepileptic drugs on neurobehavioral outcomes based on laboratory animal and clinical research. *Epilepsy Behavior, 26,* 427-439. doi: 10.1016/j.yebeh.2012.10.031

Bekoff, M. (1972). The Development of Social Interaction, Play, and Metacommunication in Mammals: An Ethological Perspective. *The Quarterly Review of Biology, 47,* 412-434.

Bekoff, M. (1995). Play signals as punctuation: The structure of social play in canids. *Behaviour, 132,* 419-429. doi: 10.1163/156853995X00649

Belichenko, P.V., Hagberg, B., & Dahlstrom, A. (1997). Morphological study of neocortical areas in Rett syndrome. *Acta Neuropathologica, 93,* 50-61. doi: 10.1007/s004010050582

Bell, H.C., McCaffrey, D., Forgie, M.L., Kolb, B., & Pellis, S.M. (2009). The role of the medial prefrontal cortex in the play fighting in rats. *Behavioral Neuroscience, 123*, 1158-1168. doi: http://dx.doi.org/10.1037/a0017617

Bellini, S. (2004). Social skill deficits and anxiety in high-functioning adolescents with autism spectrum disorders. *Focus on Autism and Other Developmental Disabilities, 19,* 78-86. doi: 10.1177/10883576040190020201

Belzung, C., Leman, S., Vourc'h, P., & Andres, C. (2005). Rodent models for autism: A critical review. *Drug Discovery Today: Disease Models, 2,* 93-101. doi: 10.1016/j.ddmod.2005.05.004

Ben-Ari, Y. (2002). Excitatory actions of GABA during development: The nature of the nurture. *Nature Reviews Neuroscience, 3*, 728–739. doi: 10.1038/nrn920

Berridge, K.C. (1996). Food reward: Brain substrates of wanting and liking.

Neuroscience & Biobehavioral Reviews, 20, 1-25. doi: 10.1016/0149-7634(95)00033-B

Berridge, K.C., Robinson, T.E., & Aldridge, J.W. (2009). Dissecting components of reward: 'Liking', 'wanting', and learning. *Current Opinions in Pharmacology*, 9, 65-73. doi: 10.1016/j.coph.2008.12.014

Betancur, C., Corbex, M., Spielewoy, C., Philippe, A., Laplanche, J.L., Launay, J.M., (…) Leboyer, M. (2002). Serotonin transporter gene polymorphisms and hyperserotonemia in autistic disorder. *Molecular Psychiatry, 7,* 67-71. doi: 10.1016/j.coph.2008.12.014

Blumberg, S.J., Bramlett, M.D., Kogan, M.D., Schieve, L.A., Jones, J.R., & Lu, M.C. (2013). Changes in prevalence of parent-reported autism spectrum disorder in school-aged US children: 2007 to 2011–2012: *National Health Statistics Reports, 65*, 1-11.

Bolivar, V.J., Walters, S.R., & Phoenix J.L. (2007). Assessing autism-like behavior in mice: Variations in social interactions among inbred strains. *Behavioral Brain Research, 176,* 21-26. doi: 10.1016/j.bbr.2006.09.007

Bromley, R.L., Baker, G.A., & Meador, K.J. (2009). Cognitive abilities and behavior of children exposed to antiepileptic drugs in utero. *Current Opinion in Neurology, 22,* 162-166. doi: 10.1097/WCO.0b013e3283292401

Bromley, R.L., Mawer, G.E., Briggs, M., Cheyne, C., Clayton-Smith, J., Garcia-Finana, M., (…) & Baker, G.A. (2013). The prevalence of neurodevelopmental disorders in children prenatally exposed to antiepileptic drugs. *Journal of Neurology, Neurosurgery, & Psychiatry, 84, 637*-643. doi: 10.1136/jnnp-2012-304270

Brown, N.A., Kao, J., & Fabro, S. (1980). Teratogenic potential of valproic acid. *Lancet, 1,* 660-661. doi: 10.1111/j.1552-6909.1986.tb01376.x

Bruckner, A., Lee, Y.J., O'Shea, K.S., & Henneberry, R.C. (1983). Teratogenic effects of valproic acid and diphenylhydantoin on mouse embryos in culture. *Teratology, 27,* 29-42. doi: 10.1002/tera.1420270106

Brunelli, S.A., Nie, R., Whipple, C., Winiger, V., Hofer, M.A., & Zimmerberg, B. (2006). The effects of selective breeding for infant ultrasonic vocalizations on play behavior in juvenile rats. *Physiology Behavior, 87,* 527-536. doi: 10.1016/j.physbeh.2005.11.020

Buitelaar, J.K. (2003). Why have drug treatments been so disappointing? *Novartis Foundation Symposia, 251,* 235- 244.

Burgdorf, J., Kroes, R., Moskal, J., Pfaus, J., Brudzynski, S., & Panksepp, J. (2008).

Ultrasonic vocalization of rats during mating, play and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. *Journal of Comparative Psychology, 122,* 357-367. doi: http://dx.doi.org/10.1037/a0012889

Caldji, C., Diorio, J., & Meaney, M.J. (2000). Variations in maternal care in infancy regulate the development of stress reactivity. *Biological Psychiatry, 48*, 1164-1174. doi: 10.1016/S0006-3223(00)01084-2

Carper, R.A., & Courchesne, E. (2005). Localized enlargement of the frontal cortex in early autism. *Biological Psychiatry, 57,* 126-133. doi: 10.1016/j.biopsych.2004.11.005

Cavada, C., & Schultz, W. (2000). The mysterious orbitofrontal cortex. *Cerebral Cortex, 10*, 205-209. doi: 10.1093/cercor/10.3.205

Centers for Disease Control and Prevention. (2014). Prevalence of autism spectrum disorder among children aged 8 years – autism and developmental disabilities monitoring network, 11 sites, United States, 2010. *Morbidity and Mortality Weekly Report, 63,* 1-21.

Chawarska, K., Shic, F., Macari, S., Campbell, D.J., Brian, J., Landa, R., (…) Bryson, S. (2014). 18-month predictors of later outcomes in younger siblings of children with autism spectrum disorder: A baby siblings research consortium study. *Journal of the American Academy of Child & Adolescent Psychiatry, 53,* 1317-1327.e1. doi: 10.1016/j.jaac.2014.09.015

Chess, S., Fernandez, P., & Korn, S. (1978). Behavioral consequences of congenital rubella. *Journal of Pediatrics, 93,* 699-703. doi: 10.1016/S0022-3476(78)80921-4

Chomiak, T., Karnik, V., Block, E., & Hu, B. (2010). Altering the trajectory of early postnatal cortical development can lead to structural and behavioural features of autism. *BioMed Central Neuroscience, 11,* 1-10. doi: 10.1186/1471-2202-11-102

Chomiak, T., Turner, N., & Hu, B. (2013). What we have learned about autism spectrum disorder from valproic acid. *Pathology Research International, 2013,* 1-8. doi: http://dx.doi.org/10.1155/2013/712758

Christianson, A.L., Chesler, N., & Kromberg, J.G. (1994). Fetal valproate syndrome: Clinical and neurodevelopmental features in two sibling pairs. *Developmental Medicine & Child Neurology, 36,* 361-369. doi: 10.1111/j.1469-8749.1994.tb11858.x

Christensen, J., Gronborg, T.K., Sorensen, M.J., Schendel, D., Parner, E.T., Pederen, L.H., & Vestergaard, M. (2013). Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. *Journal of the American Medical*

Association, 309, 1696-1703. doi: 10.1001/jama.2013.2270

Comeau, W., Hastings, E., & Kolb, B. (2007). Pre- and postnatal FGF-2 both facilitate recovery and alter cortical morphology following early medial prefrontal cortical injury. *Behavioral Brain Research, 180,* 18-27. doi: 10.1016/j.bbr.2007.02.026

Comeau, W., McDonald, R. J., & Kolb, B. (2010). Learning-induced alterations in prefrontal cortical circuitry. *Behavioural Brain Research, 214*, 91–101. doi: 10.1016/j.bbr.2010.04.033

Cook, Jr. E.H., Arora, R.C., Anderson, G.M., Berry-Kravis, E.M., Yan, S.Y., Yeoh, H.C., (…) Leventhal, B.L. (1993). Platelet serotonin studies in hyperserotonemic relatives of children with autistic disorder. *Life Science, 52,* 2005-2015.

Courchesne, E. (1997) Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. *Current Opinions in Neurobiology, 7,* 269-278. doi: 10.1016/S0959-4388(97)80016-5

Courchesne, E., & Pierce, K. (2005). Why the frontal cortex in autism might be talking only to itself: Local over-connectivity but long-distance disconnection. *Current Opinion in Neurobiology, 15,* 225-230. doi: 10.1016/j.conb.2005.03.001

Crepel, A., Breckpot, J., Fryns, J.P., De la Marche, W., Steyaert, J., Devriendt, K., & Peeters, H. (2010). DISC1 duplication in two brothers with autism and mild mental retardation. *Clinical Genetics, 77,* 389-394. doi: 10.1111/j.1399-0004.2009.01318.x

Critchley, H.D., Daly, E.M., Bullmore, E.T., Williams, S.C.R., Van Amelsvoort, T., Robertson, D.M., (…) & Murphy, D.G.M. (2000). The functional neuroanatomy of social behavior: Changes in cerebral blood flow when people with autistic disorder process facial expressions. *Brain, 123,* 2203-2212. doi: http://dx.doi.org/10.1093/brain/123.11.2203

Cullen, L.A., Barlow, J.H., & Cushway, D. (2005a). Positive touch, the implication for parents and their children with autism: An exploratory study. *Complementary Therapies in Clinical Practice, 11,* 182-184. doi: 10.1016/j.ctcp.2004.12.004

Cullen, L.A., Barlow, J.H., & Cushway, D. (2005b). Exploring a massage intervention for parents and their children with autism: The implications for bonding and attachment. *Journal of Child Health Care, 9*, 245-255. doi: 10.1177/1367493505056479

Currenti, S.A. (2010). Understanding and determining the etiology of autism. *Cellular and Molecular Neurobiology, 30*, 161 – 171. doi: 10.1007/s10571-009-9453-8

Dalldorf, J.S. (1893). Medical needs of the autistic adolescent. In: *Autism in adolescents*

and adults. E. Schopler, & G.B. Mesibov (editors). New York: Plenum Press; pp. 150-167.

Dawson, G., Meltzoff, A.N., Osterling, J., & Rinaldi, J. (1998). Neuropsychological correlates of early symptoms of autism. *Child Development, 69,* 1276. doi: 10.1111/j.1467-8624.1998.tb06211.x

Dawson, G., Toth, K., Abbott, R., Osterling, J., Munson, J., Estes, A., & Liaw, J. (2004). Early Social Attention Impairments in Autism: Social Orienting, Joint Attention, and Attention to Distress. *Developmental Psychology, 40,* 271-283. doi: http://dx.doi.org/10.1037/0012-1649.40.2.271

Dean, J., Hailey, H., Moore, S., Lloyd, D., Turnpenny, P., & Little, J. (2002). Long term health and neurodevelopment in children exposed to antiepileptic drugs before birth. *Journal of Medical Genetics, 39*, 251-259. doi: 10.1136/jmg.39.4.251

Dufour-Rainfray, D., Vourc'h, P., Le Guisquet, A., Garreau, L., Ternant, D., Bodard, S., (…) Guilloteau, D. (2010). Behavior and serotonergic disorders in rats exposed prenatally to valproate: A model for autism. *Neuroscience Letters, 470,* 55-59. doi: 10.1016/j.neulet.2009.12.054

Dufour-Rainfray, D., Vourc'h, P., Tourlet, S., Guilloteau, D., Chalon, S., & Andres, C.R. (2011). Fetal exposure to teratogens: Evidence of genes involved in autism. *Neuroscience and Biobehavioral Reviews, 35*, 1254-1265. doi: 10.1016/j.neubiorev.2010.12.013

Ehlers, K., Sturje, H., Merker, H.J., & Nau, H. (1992). Valproic acid-induced spina bifida: A mouse model. *Teratology, 45,* 145-154. doi: 10.1002/tera.1420450208

Eisenberg, L., & Kanner, L. (1956). Childhood schizophrenia symposium, 1955. 6. Early infantile autism, 1943-55. *American Journal of Orthopsychiatry, 26*, 556-566. doi: http://dx.doi.org/10.1111/j.1939-0025.1956.tb06202.x

El-Kordi, A., Winkler, D., Hammerschmidt, K., Kästner, A., Krueger, D., Ronnenberg, A., (…) & Ehrenreich, H. (2013). Development of an autism severity score for mice using Nlgn4 null mutants as a construct-valid model of heritable monogenic autism. *Behavioural Brain Research, 251,* 41-49. doi: 10.1016/j.bbr.2012.11.016

Escalona, A., Field, T., Singer-Strunck, R., Cullen, C., & Hartshorn, K. (2001). Brief Report: Improvements in the Behavior of Children With Autism Following Massage Therapy. *Journal of Autism and Developmental Disorders, 31,* 513-516.

Fatemi, S.H. Halt, A., Stary, J., Kanodia, R., Schulz, S., & Realmuto, G. (2002). Glutamic acid decarboxylase 65 and 67 kDa proteins are reduced in autistic parietal and cerebellar cortices. *Biological Psychiatry, 52*, 805-810. doi: 10.1016/S0006-3223(02)01430-0

Fatemi, S.H., Reutiman, T.J., Folsom, T.D., & Thuras, P.D. (2009). GABAA receptor downregulation in brains of subjects with autism. *Journal of Autism and Developmental Disorders, 39*, 223-230. doi: 10.1007/s10803-008-0646-7

Favre, M.R., Barkat, T.R., LaMendola, D., Khazen, G., Markram, H., & Markram, K. (2013). General developmental health in the VPA-rat model of autism. *Frontiers in Behavioral Neuroscience, 7,* 1-11. doi: 10.3389/fnbeh.2013.00088

Field, T. (1998). Massage therapy effects. *American Psychologist, 53*, 1270–1281. doi: http://dx.doi.org/10.1037/0003-066X.53.12.1270

Field, T., Grizzle, N., Scafidi, F., Abrams, S., Richardson, S., Kuhn, C., & Schanberg, S. (1996). Massage therapy for infants of depressed mothers. *Infant Behavior & Development, 19,* 107-112. doi: 10.1016/S0163-6383(96)90048-X

Field, T., Lasko, D., Mundy, P., Henteleff, T., Talpins, S., & Dowling, M. (1997). Autistic children's attentiveness and responsivity improved after touch therapy. *Journal of Autism and Developmental Disorders, 27,* 329-334. doi: 10.1023/A:1025858600220

Field, T., Schanberg, S.M., Scafidi, F., Bauer, C.R., Vega-Lahr, N., Garcia, R., (…) & Kuhn, C.M. (1986). Tactile/kinesthetic stimulation effects on preterm neonates. *Pediatrics, 77*, 654-658.

Fletcher, A., & Harding, V. (1981). An examination of the 'wet dog' shake behaviour in rats produced by acute administration of sodium n-dipropylacetate, *Journal of Pharmacy and Pharmacology, 33,* 811. doi: 10.1111/j.2042-7158.1981.tb13945.x

Fombonne, E. (2002). Epidemiological trends in rates of autism. *Molecular Psychiatry, 7,* S4–S6. doi: 10.1038/sj.mp.4001162

Foroud, A., & Pellis, S.M. (2003). The development of "roughness" in the play fighting of rats: A laban movement analysis perspective. *Developmental Psychobiology, 42*, 35-43. doi: 10.1002/dev.10088

Francis, D., Meaney, M.J. (1999). Maternal care and the development of stress responses. *Current Opinion in Neurobiology, 9*, 128–134. doi: 10.1016/S0959-4388(99)80016-6

Fuster, J.M. (2001). The prefrontal cortex – an update: Time is of the essence. *Neuron, 30,* 319-333. doi: 10.1016/S0896-6273(01)00285-9

Gandal, M.J., Edgar, J.C., Ehrlichman, R.S., Mehta, M., Roberts, T.P., & Siegel, S.J. (2010). Validating gamma oscillations and delayed auditory responses as translational biomarkers of autism. *Biological Psychiatry, 68,* 1100-1106. doi:

10.1016/j.biopsych.2010.09.031

Geschwind, D.H., & Levitt, P. (2007). Autism spectrum disorders: Developmental disconnection syndromes. *Current Opinions in Neurobiology, 17,* 103-111. doi: 10.1016/j.conb.2007.01.009

Gibb, R., Gonzalez, C.L.R., Wegenast, W., & Kolb, B. (2010). Tactile stimulation promotes motor recovery following cortical injury in adult rats. *Behavioural Brain Research, 214,* 102-107. doi: 10.1016/j.bbr.2010.04.008

Gibb, R., & Kolb, B. (1998). A method for vibratome sectioning of Golgi–Cox stained whole rat brain. *Journal of Neuroscience Methods, 79*, 1-4. doi: 10.1016/S0165-0270(97)00163-5

Gibb, R., & Kolb, B. Tactile stimulation of functional recovery after perinatal damage is mediated by FGF-2. Submitted for publication.

Gilbert, S.J., Bird, G., Brindley, R., Frith, C.D., & Burgess, P.W. (2008). Atypical recruitment of medial prefrontal cortex in autism spectrum disorders: An fMRI study of two executive function tasks. *Neuropsychologia, 46,* 2281-2291. doi: 10.1016/j.neuropsychologia.2008.03.025

Gillberg, C., Nordin, V., & Ehlers, S. (1996). Early detection of autism. Diagnostic instruments for clinicians. *European Child & Adolescent Psychiatry, 5,* 67-74. doi: 10.1007/BF01989498

Graham, K.L., & Burghardt, G.M. (2010). Current perspectives on the biological study of play: Signs of progress. *The Quarterly Review of Biology, 85***,** 1-24. doi: 10.1086/656903

Greenough, W.T., Black, J.E., & Wallace, C.S. (1987). Experience and brain development. *Child Development, 58,* 539-559.

Hablitz, J.J., Tehrani, M.H., & Barnes, E.M. Jr. (1989). Chronic exposure of developing cortical neurons to GABA down-regulates GABA/benzodiazepine receptors and GABA-gated chloride currents. *Brain Research, 501*, 332–338. doi: 10.1016/0006-8993(89)90650-1

Hadjikhani, N. (2010). Serotonin, pregnancy and increased autism prevalence: Is there a link? *Medical Hypothesis, 74,* 880-883. doi: 10.1016/j.mehy.2009.11.015

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe T., (…) & Risch, N. (2011). Genetic heritability and shared environmental factors among twin pairs with autism. *Archives of General Psychiatry, 68,* 1095-1102. doi: 10.1001/archgenpsychiatry.2011.76

Handley, S.L., & Singh, L. (1986). Neurotransmitters and shaking behaviour - more than a 'gut-bath' for the brain? *Trends in Pharmacological Sciences, 7,* 324-328. doi: 10.1016/0165-6147(86)90371-8

Happe, F., Ehlers, S., Fletcher, P., Frith, U., Johansson, M., Gillberg, C., (…) & Frith, C. (1996). 'Theory of mind' in the brain. Evidence from a PET scan study of Asperger syndrome. *Neuroreport 8,* 197-201. doi: 10.1097/00001756-199612200-00040

Harada, M., Taki, M.M., Nose, A., Kubo, H., Mori, K., Nishitani, H., & Matsuda, T. (2011). Non-invasive evaluation of the GABAergic/glutamatergic system in autistic patients observed by MEGA-editing proton MR spectroscopy using a clinical 3 tesla instrument. *Journal of Autism and Developmental Disorders, 41*, 447-454. doi: 10.1007/s10803-010-1065-0

Harris, S., Handleman, J., Gordon, R., Kristoff, B., & Fuentes, F. (1991). Changes in cognitive and language functioning of preschool children with autism. *Journal of Autism and Developmental Disorders, 21,* 281–290. doi: 10.1007/BF02207325

Heidbreder, C.A., & Groenewegen, H.J. (2003). The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. *Neuroscience & Biobehavioral Reviews, 27*, 555-579. doi: 10.1016/j.neubiorev.2003.09.003

Himmler, B.T., Kisko, T.M., Euston, D.R., Kolb, B., & Pellis, S.M. (2014). Are 50-kHz calls used as play signals in the playful interactions of rats? I. Evidence from the timing and context of their use. *Behavioral Processes, 106,* 60-66. doi: 10.1016/j.beproc.2014.04.014

Himmler, B.T., Pellis, V.C., Pellis, S.M. (2013). Peering into the dynamics of social interactions: Measuring play fighting in rats. *Journal of Visual Experiments, 71,* e4288. http://dx.doi.org/10.3791/4288

Himmler, S.M., Lewis, J.M,, Pellis, S.M. (2014). The development of strain typical defensive patterns in the play fighting of laboratory rats. *International Journal of Comparative Psychology, 27*, 385-396.

Hollander, E., Novotny, S., Hanratty, M., Yaffe, R., DeCaria, C.M., Aronowitz, B.R., & Mosovich, S. (2003). Oxytocin infusion reduces repetitive behaviors in adults with autistic and Asperger's disorders. *Neuropsychopharmacology, 28,* 193-198. doi: 10.1038/sj.npp.1300021

Howard, M.A., Cowell, P.E., Boucher, J., Broks, P., Mayes, A., Farrant, A., & Roberts, N. (2000). Convergent neuroanatomical and behavioural evidence of an amygdala hypothesis of autism. *NeuroReport, 11,* 2931.

Hughes, J.R. (2008). A review of recent reports on autism: 1000 studies published in 2007. *Epilepsy & Behavior, 13,* 425-437. doi: 10.1016/j.yebeh.2008.06.015

Hussman, J.P. (2001) Suppressed GABAergic inhibition as a common factor in suspected etiologies of autism. *Journal of Autism and Developmental Disorders, 2,* 247-248.

Imanaka, A., Morinobu, S., Toki, S., Yamamoto, S., Matsuki, A., Kozuru, T., & Yamawaki, S. (2008). Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague-Dawley rats. *Behavioral Brain Research, 186,* 91–97. doi: 10.1016/j.bbr.2007.07.039

Ingram, J.L., Peckham, S.M., Tisdale, B., & Rodier, P.M. (2000). Prenatal exposure of rats to valproic acid reproduces the cerebellar anomalies associated with autism. *Neurotoxicology and Teratology, 22,* 319-324. doi: 10.1016/S0892-0362(99)00083-5

Jamain, S., Quach, H., Betancur, C., Råstam, M., Colineaux, C., Gillberg, I.C., (…) & Bourgeron, T. (2003). Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. *Nature Genetics, 34,* 27-29. doi: 10.1038/ng1136

Jamain, S., Radyushkin, K., Hammerschmidt, K., Granon, S., Boretius, S., Varoqueaux, F., (…) & Brose, N. (2008). Reduced social interaction and ultrasonic communication in a mouse model of monogenic heritable autism. *Proceedings of the National Academy of Sciences of the United States of America, 105,* 1710-1715. doi: 10.1073/pnas.0711555105

Jones, N.A., & Mize, K.D. (2007). Touch interventions positively affect development. In: *Low-cost approaches to promote physical and mental health*. L. L'Abate (editor). New York, NY: Springer New York; pp. 353-369.

Jordan, R. (2003). Social play and autistic spectrum disorders. *Autism, 7*, 347-360. doi: 10.1177/1362361303007004002

Josefi, O., & Ryan, V. (2004). Non-directive play therapy for young children with autism: A case study. *Clinical Child Psychology and Psychiatry, 9*, 533-551. doi: 10.1177/1359104504046158

Kaffman, A., & Meaney, M.J. (2007). Neurodevelopmental sequelae of postnatal maternal care in rodents: Clinical and research implications of molecular insights. *Journal of Child Psychology and Psychiatry, 48*, 224-244. doi: 10.1111/j.1469-7610.2007.01730.x

Kaneko, S., Otani, K., Fukushima, Y., Sato, T., Nomura, Y., & Ogawa, Y. (1983). Transplacental passage and half-life of sodium valproate in infants born to

epileptic mothers. *British Journal of Clinical Pharmacology, 15,* 503-505. doi: 10.1111/j.1365-2125.1983.tb01541.x

Kanner, L. (1943). Autistic disturbances of affective contact. *Nervous Child, 2*, 217- 250.

Kataoka, S., Takuma, K., Hara, Y., Maeda, Y., Ago, Y., & Matsuda, T. (2013). Autism-like behaviors with transient histone hyperacetylation in mice treated prenatally with valproic acid. *International Journal of Neuropsychopharmacology, 16,* 91-103. doi: http://dx.doi.org/10.1017/S1461145711001714

Kawasaki, Y., Yokota, K., Shinomiya, M., Shimizu, Y., & Niwa, S. (1997). Electroencephalographic paroxysmal activities in the frontal area emerged in middle childhood and during adolescence in a follow-up study of autism. *Journal of Autism and Developmental Disorders, 27*, 605.

Kennedy, D.P, Redcay, E., & Courchesne, E. (2006). Failing to deactivate: Resting functional abnormalities in autism. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 8275-8280. doi: 10.1073/pnas.0600674103

Kilb, W. (2012). Development of the GABAergic system from birth to adolescence. *Neuroscientist, 18*, 613–630. doi: 10.1177/1073858411422114

Kilb, W., Kirischuk, S., & Luhmann, H.J. (2013). Role of tonic GABAergic currents during pre- and early postnatal rodent development. *Frontiers in Neural Circuits, 7,* 1-13. doi: 10.3389/fncir.2013.00139

Kilpinen, H., Ylisaukko-Oja, T., Hennah, W., Palo, O.M., Varilo, T., Vanhala, R., (…) & Peltonen, L. (2008). Association of DISC1 with autism and Asperger syndrome. Molecular Psychiatry, 13, 187-96.

Kim, K.C., Kim, P., Go, H.S., Choi, C.S., Yang, S.I., Cheong, J.H. (…) & Ko, K.H. (2011). The critical period of valproate exposure to induce autistic symptoms in Sprague–Dawley rats. *Toxicology Letters, 201,* 137-142. doi: 10.1016/j.toxlet.2010.12.018

Kim, J.A., Szatmari, P., Bryson, S.E., Streiner, D.L., & Wilson, F.J. (2000). The prevalence of anxiety and mood problems among children with autism and Asperger syndrome. *Autism, 4,* 117-132. doi: 10.1177/1362361300004002002

Kim, J., Wigram, T., & Gold, C. (2008). The effects of improvisational music therapy on joint attention behaviors in autistic children: A randomized controlled study. *Journal of Autism and Developmental Disorders, 38,* 1758-1766. doi: 10.1007/s10803-008-0566-6

Kisko, T.M., Himmler, B.T., Himmler, S.M., Euston, D.R., & Pellis, S.M. (2015). Are

50-kHz calls used as play signals in the playful interactions of rats? II. Evidence from the effects of devocalization. *Behavioral Processes, 111,* 25-33. doi: 10.1016/j.beproc.2014.11.011

Knickmeyer, R.C., Wheelwright, S., & Baron-Cohen, S. (2008). Sex-typical play: Masculinization/defeminization in girls with an autism spectrum condition. *Journal of Autism and Developmental Disorders, 38,* 1028-1035. doi: 10.1007/s10803-007-0475-0

Knutson, B., Burgdorf, J., & Panksepp, J. (1998). Anticipation of play elicits high frequency ultrasonic vocalizations in rats. *Journal of Comparative Psychology, 112,* 65-73. doi: http://dx.doi.org/10.1037/0735-7036.112.1.65

Knutson, B., Burgdorf, J.A., & Panksepp, J. (2002). Ultrasonic vocalizations as indices of affective states in rats. *Psychological Bulletin, 128,* 961-977. doi: http://dx.doi.org/10.1037/0033-2909.128.6.961

Koegel, R.L., & Koegel, L.K. (2012). *The PRT Pocket Guide*. Baltimore, MD: Brookes Publishing.

Kolb, B., Cioe, J., & Comeau, W. (2008). Contrasting effects of motor and visual learning tasks on dendritic arborization and spine density in rats. *Neurobiology of Learning and Memory, 90,* 295–300. doi: 10.1016/j.nlm.2008.04.012

Kolb, B., Cioe, J., & Whishaw, I.Q. (2000). Is there an optimal age for recovery from motor cortex lesions? Behavioral and anatomical sequelae of bilateral motor cortex lesions in rats on postnatal days 1, 10, and in adulthood. *Brain Research, 882,* 62-74. doi: 10.1016/S0006-8993(00)02828-6

Kolb, B., & Gibb, R. (1990). Anatomical correlates of behavioral change after neonatal prefrontal lesions in rats. *Progressive Brain Research, 85,* 241-256.

Kolb, B., & Gibb, R. (2007). Brain plasticity and recovery from early cortical injury. *Developmental Psychobiology, 49*, 107-118. Doi: 10.1111/j.1469-8749.2011.04054.x

Kolb, B., & Gibb, R. (2010). Tactile stimulation after frontal or parietal cortical injury in infant rats facilitates functional recovery and produces synaptic changes in adjacent cortex. *Behavioural Brain Research, 214,* 115-120. doi: 10.1016/j.bbr.2010.04.024

Kolb, B., Mychasiuk, R., Williams, P., & Gibb, R. (2011). Brain plasticity and recovery from early cortical injury. *Developmental Medicine & Child Neurology, 53,* 4-8. doi: 10.1111/j.1469-8749.2011.04054.x

Kolb, B., & Whishaw, I. (2009). *Fundamentals of human neuropsychology* (6th ed.).

New York, NY: Worth Publishers.

Komorowska, J., & Pellis, S.M. (2004). Regulatory mechanisms underlying novelty-induced grooming in the laboratory rat. *Behavioral Processes, 67,* 287-293. doi: 10.1016/j.beproc.2004.05.001

Kuwagata, M., Ogawa, T., Shioda, S., & Nagata, T. (2009). Observation of fetal brain in a rat valproate-induced autism model: A developmental neurotoxicity study. *International Journal of Developmental Neuroscience, 27*, 399-405. doi: 10.1016/j.ijdevneu.2009.01.006

Lancaster, K., Dietz, D.M., Moran, T.H., & Pletnikov, M.V. (2007). Abnormal social behaviors in young and adult rats neonatally infected with Borna disease virus. *Behavioral Brain Research, 176,* 141-148. doi: 10.1016/j.bbr.2006.06.013

LeBlanc, J.J., & Fagiolini, M. (2011). Autism: A "critical period" disorder? *Neural Plasticity, 2011,* 1-17. doi: 10.1155/2011/921680

Liu, X., Hubbard, J.A., Fabes, R.A., & Adam, J.B. (2006). Sleep disturbances and correlates of children with autism spectrum disorders. *Child Psychiatry & Human Development, 37,* 179-191. doi: 10.1007/s10578-006-0028-3

Lopata, C., Thomeer, M.L., Volker, M.A., & Nida, R.E. (2006). Effectiveness of a cognitive-behavioral treatment on the social behaviors of children with Asperger disorder. *Focus on Autism and Other Developmental Disabilities, 21,* 237-244. doi: 10.1177/10883576060210040501

Lord, C., Cook, E.H., Leventhal, B.L., & Amaral, D.G. (2000). Autism spectrum disorders. *Neuron, 28*, 355-363. doi: 10.1016/S0896-6273(00)00115-X

Loscher, W., & Honack, D. (1996). Valproate and its major metabolite E-2-en-valproate induce different effects on behaviour and brain monoamine metabolism in rats. *European Journal of Pharmacology, 299,* 61-67. doi: 10.1016/0014-2999(95)00831-4

Loscher, W., Honack, D., Nolting, B., & Fassbender, C.P. (1991). Trans-2- en-valproate: Reevaluation of its anticonvulsant efficacy in standardized seizure models in mice, rats and dogs. *Epilepsy Research, 9,* 195. doi: 10.1016/0920-1211(91)90053-I

Lovaas, O.I. (1987). Behavioral treatment and normal educational and intellectual functioning in young autistic children. *Journal of Consulting and Clinical Psychology, 55,* 3-9. doi: 10.1037/0022-006X.55.1.3

Lovaas, O.I., Koegel, R., Simmons, J.Q., & Long, J.S. (1973). Some generalization and follow up measures on autistic children in behavior therapy. *Journal of Applied*

Behavioral Analysis, 6, 131-165. doi: 10.1901/jaba.1973.6-131

Manent, J.B., Demarque, M., Jorquera, I., Pellegrino, C., Ben-Ari, Y., Aniksztein, L., & Represa, A. (2005). A noncanonical release of GABA and glutamate modulates neuronal migration. *Journal of Neuroscience, 25,* 4755-4765. doi: 10.1523/JNEUROSCI.0553-05.2005

Manent, J.B., Jorquera, I., Mazzucchelli, I., Depaulis, A., Perucca, E., Ben-Ari, Y., & Represa, A. (2007). Fetal exposure to GABA-acting antiepileptic drugs generates hippocampal and cortical dysplasias. *Epilepsia, 48*, 684–693. doi: 10.1111/j.1528-1167.2007.01056.x

Markram, M., Rinaldi, T., La Mendola, D., Sandi, T., & Markram, H. (2008). Abnormal fear conditioning and amygdala processing in an animal model of autism. *Neuropsychopharmacology, 33,* 901–912. doi: 10.1038/sj.npp.1301453

Mast, T.J., Cukierski, M.A., Heinz, N., & Hendrickx, A.G. (1986). Predicting the human teratogenic potential of the anticonvulsant, valproic acid, from a nonhuman primate model. *Toxicology, 39,* 111-119.

Mathai, S., Fernandez, A., Mondkar, J., & Kanbur, W. (2001). Effects of tactile-kinesthetic stimulation in preterms: A controlled trial. *Indian Pediatrics, 38,* 1091-1098.

McCracken, J.T., McGough, J., Shah, B., Cronin, P., Hong, D., Aman, M.G., (…) & McMahon, D. (2002). Risperidone in children with autism and serious behavioral problems. *The New England Journal of Medicine, 347,* 314-321. doi: 10.1056/NEJMoa013171

McDougle, C.J., Holmes, J.P., Carlson, D.C., Pelton, G.H., Cohen, D.J., & Price, L.H. (1998). A double-blind, placebo-controlled study of risperidone in adults with autistic disorder and other pervasive developmental disorders. *Archives of General Psychiatry, 55,* 633-641. doi: 10.1001/archpsyc.55.7.633

McEachin, J.J., Smith, T., & Lovaas, O.I. (1993). Long-term outcome for children with autism who received early intensive behavioral treatment. *American Journal on Mental Retardation, 97,* 359–372.

McEvoy, R., Rogers, S., & Pennington, B. (1993). Executive function and social communication deficits in young autistic children. *Journal of Child Psychology and Psychiatry, 34,* 563-578. doi: 10.1111/j.1469-7610.1993.tb01036.x

Meaney, M.J., & Stewart, J. (1981). A descriptive study of social development in the rat (Rattus norvegicus). *Animal Behavior, 29*, 34-45. doi: 10.1016/S0003-3472(81)80149-2

Mehta, M.V., Gandal, M.J., & Siegel, S.J. (2011). mGluR5-antagonist mediated reversal of elevated stereotyped, repetitive behaviors in the VPA model of autism. *PLoS One, 6,* e26077. doi: 10.1371/journal.pone.0026077

Mengola, E., Broccia, M.L., Prati, M., & Giavini, E. (1998). Stage-dependent skeletal malformations induced by valproic acid in rat. *International Journal of Developmental Biology, 42,* 99-102.

Mesdjian, E., Ciesielski, L., Valli, M., Bruguerolle, B., Jadot, G., Bouyard, P., & Mandel, P. (1982). Sodium valproate: Kinetic profile and effects on GABA levels in various brain areas of the rat. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 6*, 223-233. doi: 10.1016/S0278-5846(82)80172-3

Militerni, R., Bravaccio, C., Falco, C., Puglisi-Allegra, S., Pascucci, T., & Fico, C. (2000). Pain reactivity in children with autistic disorder. *The Journal of Headache and Pain, 1,* 53-56. doi: 10.1007/s101940050011

Miyazaki, C., Matsuyama, K., Ichikawa, M., & Goto S. (1988). Effect of sodium valproate (VPA) on cerebral amino acids: mechanism of gamma-aminobutyric acid (GABA) elevation and possible causal relation of VPA-induced encephalopathy and glutamine level. *Chemical and Pharmaceutical Bulletin, 36*, 3589-3594. doi: 10.1248/cpb.36.3589

Miyazaki, K., Narita, N., & Narita, M. (2005). Maternal administration of thalidomide or valproic acid causes abnormal serotonergic neurons in the offspring: Implication for pathogenesis of autism. *International Journal of Developmental Neuroscience, 23,* 287-297. doi: 10.1016/j.ijdevneu.2004.05.004

Moore, S.J., Turnpenny, P., Quinn, A., Glover, S., Lloyd, D.J., Montgomery, T., & Dean, J.C. (2000). A clinical study of 57 children with fetal anticonvulsant syndromes. *Journal of Medical Genetics, 37,* 489-497. doi: 10.1136/jmg.37.7.489

Moy, S.S., Nadler, J.J., Magnuson, J.R., &, Crawley, J.N. (2006). Mouse models of autism spectrum disorders: The challenge for behavioral genetics. *American Journal of Medical Genetics Part C, 142,* 40-51. doi: 10.1002/ajmg.c.30081

Muris, P., Steerneman, P., Merckelbach, H., Holdrinet, I., & Meesters, C. (1998). Comorbid anxiety symptoms in children with pervasive developmental disorders. *Journal of Anxiety Disorders, 12*, 387-393. doi:10.1016/S0887-6185(98)00022-X

Mychasiuk, R., Richards, S., Nakahashi, A., Kolb, B., & Gibb, R. (2012). Effects of rat prenatal exposure to valproic acid on behavior and neuro-anatomy. *Developmental Neuroscience, 34,* 268-276. doi: 10.1159/000341786

Myers, S.M. (2007). The status of pharmacotherapy for autism spectrum disorders. *Expert Opinions in Pharmacotherapy, 8,* 1579-1603. doi:

10.1517/14656566.8.11.1579

Narita, N., Kato, M., Tazoe, M., Miyazaki, K., Narita, M., & Okado, N. (2002). Increased monoamine concentration in the brain and blood of fetal thalidomide- and valproic acid-exposed rat: Putative animal models for autism. *Pediatric Research, 52,* 576-579. doi: 10.1203/00006450-200210000-00018

Nau, H., Hauck, R.S., & Ehlers, K. (1991). Valproic acid-induced neural tube defects in mouse and human: Aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. *Pharmacology Toxicology, 69,* 310-321. doi: 10.1111/j.1600-0773.1991.tb01303.x

Nau, H., & Loscher, W. (1984) Valproic acid and metabolites: Pharmacological and toxicological studies. *Epilepsia, 25,* 14. doi: 10.1111/j.1528-1157.1984.tb05632.x

Neuhaus, E., Beauchaine, T.P., & Bernier, R. (2010). Neurobiological correlates of social functioning in autism. *Clinical Psychology Review, 30,* 733-748. doi: 10.1016/j.cpr.2010.05.007

Niesink, R.J.M., & Van Ree, J.M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharamcology, 28,* 411-418. doi: 10.1016/0028-3908(89)90038-5

Norscia, I., & Palagi, E. (2011). When play is a family business: Adult play, hierarchy, and possible stress reduction in common marmosets. *Primates, 52,* 101-104. doi: 10.1007/s10329-010-0228-0

Olexová, L., Talarovicová, A., Lewis-Evans, B., Borbélyová, V., & Krsková, L. (2012). Animal models of autism with a particular focus on the neural basis of changes in social behavior: An update article. *Neuroscience Research, 74,* 184-194. doi: 10.1016/j.neures.2012.10.004

Ornoy, A. (2009). Valproic Acid in Pregnancy: How much are we endangering the embryo and fetus? *Reproductive Toxicology, 28,* 1-10. doi: 10.1016/j.reprotox.2009.02.014

Ottenbacher, K.J., Muller, L., Brandt, D., Heintzelman, A., Hojem, R., & Sharpe, P. (1987). The effectiveness of tactile stimulation as a form of early intervention: A quantitative evaluation. *Developmental and Behavioral Pediatrics, 8,* 68-76.

Ozonoff, S., & Cathcart, K. (1998). Effectiveness of a home program intervention for young children with autism. *Journal of Autism and Developmental Disorders, 28,* 25–32. doi: 10.1023/A:1026006818310

Palagi, E., Burghardt, G.M., Smuts, B., Cordoni, G., Dall'Olio, S., Fouts, H.N., (…) Pellis, S.M. (2015). Rough-and-tumble play as a window on animal

communication. *Biological Reviews*, in press. doi: 10.1111/brv.12172

Panksepp, J. (1981). The ontogeny of play in rats. *Developmental Psychobiology, 14,* 327-332. doi:10.1002/dev.420140405

Panksepp, J. (1998). *Affective neuroscience*. New York: Oxford University Press.

Panksepp, J., & Beatty, W.W. (1980). Social deprivation and play in rats. *Behavioral and Neural Biology, 30*, 197-206. doi:10.1016/SO163-1047(80)91077-8

Papadopoulos, N., McGinley, J., Tonge, B., Bradshaw, J., Saunders, K., & Rinehart, N. (2011). An investigation of upper limb motor function in high functioning autism and Asperger's disorder using a repetitive Fitt's aiming task. *Research in Autism Spectrum Disorders, 6,* 286-292. doi: 10.1016/j.rasd.2011.05.010

Parent, C.I., & Meaney, M.J. (2008). The influence of natural variations in maternal care on play fighting in the rat. *Developmental Psychobiology, 50*, 767-776. doi: 10.1002/dev.20342

Patterson, P.H. (2011). Modeling autistic features in animals. *Pediatric Research, 69,* 34R-40R. doi: 10.1203/PDR.0b013e318212b80f

Pellis, S.M. (1993). Sex and the evolution of play fighting: A review and a model based on the behavior of muroid rodents. *Journal of Play Theory and Research, 1,* 56-77.

Pellis, S.M., Field, E.F., Smith, L.K., & Pellis, V.C. (1997). Multiple differences in the play fighting of male and female rats. Implications for the causes and functions of play. *Neuroscience and Biobehavioral Reviews, 21,* 105-120. doi: 10.1016/0149-7634(95)00060-7

Pellis, S.M., & Pellis, V.C. (1983). Locomotor-rotational movements in the ontogeny and play of the laboratory rat Rattus norvegicus. *Developmental Psychobiology, 16,* 269-286. doi:10.1002/dev.420160403

Pellis, S.M., & Pellis, V.C. (1987). Play-fighting differs from serious fighting in both target of attack and tactics of fighting in the laboratory rat *Rattus norvegicus. Aggressive Behavior, 13*, 227-242. doi:10.1002/1098-2337(1987)13:4<227::AID-AB2480130406>3.0.C);2-C

Pellis, S.M., & Pellis, V.C. (1990). Differential rates of attack, defense and counterattack during the developmental decrease in play fighting by male and female rats. *Developmental Psychobiology, 23,* 215-231. doi:10.1002/dev.420230303

Pellis, S.M., & Pellis, V.C. (1997). The prejuvenile onset of play fighting in laboratory rats (*Rattus norvegicus*). *Developmental Psychobiology, 31*, 193-205.

doi:10.1002/(SICI)1098-2302(199711)31:3<193::AID-DEV4>3.0.CO;2-N

Pellis, S.M., & Pellis, V.C. (1998). Play fighting of rats in comparative perspective: A schema for neurobehavioral analyses. *Neuroscience & Biobehavioral Reviews, 23*, 87-101. doi: 10.1016/S0149-7634(97)00071-7

Pellis, S.M., & Pellis, V.C. (2009). *The playful brain: Venturing to the limits of neuroscience.* Oxford, UK: Oneworld Publications.

Pellis, S.M., Pellis, V.C., & McKenna, M.M. (1994). Evidence for a feminine dimension in the play fighting of rats (Rattus norvegicus) and its defeminization neonatally by androgens. *Journal of Comparative Psychology, 108,* 68-73. doi: http://dx.doi.org/10.1037/0735-7036.108.1.68

Pellis, S.M., Pellis, V.C., & Reinhart, C.J. (2010). The evolution of social play. *In: Formative experiences: The interaction of caregiving, culture, and developmental psychobiology.* C. Worthman, P. Plotsky, & D. Schechter (editors). Cambridge, UK: Cambridge University Press; pp. 406-433.

Pellis, S.M., Pellis, V.C., & Whishaw, I.Q. (1992). The role of the cortex in play fighting by rats: Developmental and evolutionary implications. *Brain, Behavior, and Evolution, 39*, 270-284. doi:10.1002/1098-2337(1992)18:6<449::AID-AB2480180607>3.0.CO;2-T

Petrere, J.A., Anderson, J.A., Sakowski, R., Fitzgerald, J.E., & de la Iglesia, F.A. (1986). Teratogenesis of calcium valproate in rabbits. *Teratology, 34,* 263-269. doi: 10.1002/tera.1420340305

Pierce, K., Haist, F., Sedaghat, F., & Courchesne, E. (2004). The brain response to personally familiar faces in autism: Findings of fusiform activity and beyond. *Brain, 127,* 2703-2716. doi: http://dx.doi.org/10.1093/brain/awh289

Price, J.L. (2006). Connections of orbital cortex. In: *The orbitofrontal cortex.* D.H. Zald, & S.L. Rauch (editors). New York, NY: Oxford University Press; pp. 39-55.

Prior, M., & Hoffmann, W. (1990). Neuropsychological testing of autistic children through an exploration with frontal lobe tests. *Journal of Autism and Developmental Disorders, 20*, 581-590. doi: 10.1007/BF02216063

Rapin, I. (1991). Autistic children: Diagnosis and clinical features. *Pediatrics, 87,* 751-760.

Rasalam, A.D., Hailey, H., Williams, J.H., Moore, S.J., Turnpenny, P.D., Lloyd, D.J., & Dean, J.C. (2005). Characteristics of fetal anticonvulsant syndrome associated autistic disorder. *Developmental Medicine & Child Neurology, 47*, 551–555. doi: http://dx.doi.org/10.1017/S0012162205001076

Raza, S., Harker, A., Richards, S., Kolb, B., & Gibb, R. (2015). Tactile stimulation improves neuroanatomical pathology but not behavior in rats prenatally exposed to valproic acid. *Behavioral Brain Research, 282,* 25-36. doi: 10.1016/j.bbr.2014.12.055

Realmuto, G.M., & Ruble, L.A. (1999). Sexual behaviors in autism: Problems of definition and management. *Journal of Autism and Developmental Disorders, 29,* 121-127.

Redmond, D.E., & Huang, Y.H. (1979). New evidence for a locus coeruleus-norepinephrine connection with anxiety. *Life Science, 25*, 2149-2162.

Reinhart, C.J., Pellis, S.M., & McIntyre, D.C. (2004). Development of play fighting in kindling-prone (FAST) and kindling-resistant (SLOW) rats: How does the retention of phenotypic juvenility affect the complexity of play? *Developmental Psychobiology, 45*, 83-92. doi: 10.1002/dev.20016

Represa, A., & Ben-Ari, Y. (2005). Trophic actions of GABA on neuronal development. *Trends in Neurosciences, 28*, 278-283. doi: 10.1016/j.tins.2005.03.010

Richards, S., Mychasiuk, R., Kolb, B., & Gibb, R. (2012). Tactile stimulation during development alters behaviour and neuroanatomical organization of normal rats. *Behavioural Brain Research, 231*, 86-91. doi: 10.1016/j.bbr.2012.02.043

Rinaldi, T., Perrodin, C., & Markram, H. (2008). Hyper-connectivity and hyper-plasticity in the medial prefrontal cortex in the valproic acid animal model of autism. *Frontiers in Neural Circuits, 2,* 1-7. doi: 10.3389/neuro.04.004.2008

Risch, N., Spiker, D., Lotspeich, L., Nouri, N., Hinds, D., Hallmayer, J., (…) & Myers R.M. (1999). A genomic screen of autism: Evidence for a multilocus etiology. *American Journal of Human Genetics, 65,* 493-507. doi: 10.1086/302497

Rodier, P.M., Bryson, S.E., & Welch, J.P. (1997a). Minor malformations and physical measurements in autism: Data from Nova Scotia. *Teratology, 55,* 319-325. doi: 10.1002/(SICI)1096-9926(199705)55:5<319::AID-TERA4>3.0.CO;2-U

Rodier, P.M., Ingram, J.L., Tisdale, B., & Croog, V.J. (1997b). Linking etiologies in humans and animal models: Studies of autism. *Reproductive Toxicology, 11,* 417-422. doi: 10.1016/S0890-6238(97)80001-U

Rodier, P.M., Ingram, J.L., Tisdale, B., Nelson, S., & Romano, J. (1996). An embryological origin for autism: Developmental anomalies of the cranial nerve motor nuclei. *Journal of Comparative Neurology, 370,* 247-261. doi: 10.1002/(SICI)1096-9861(19960624)370:2<247::AID-CNE8>3.0.CO;2-2

Rolls, E.T. (1999). *The brain and emotion.* Oxford University Press, Oxford.

Ronald, A., & Hoekstra, R.A. (2011). Autism spectrum disorders and autistic traits: A decade of new twin studies. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 156,* 255-274. doi: 10.1002/ajmg.b.31159

Roullet, F.I., Lai, J.K., & Foster, J.A. (2013). In utero exposure to valproic acid and autism - a current review of clinical and animal studies. *Neurotoxicology and Teratology, 36,* 47-56. doi: 10.1016/j.ntt.2013.01.004

Rubenstein, J.L.R., & Merzenich, M.M. (2003). Model of autism: Increased ratio of excitation/inhibition in key neural systems. *Genes, Brain and Behavior, 2,* 255-267. doi: 10.1034/j.1601-183X.2003.00037.x

Sandman, C.A. (1990). The opiate hypothesis in autism and self-injury. *Journal of Child and Adolescent Psychopharmacology, 1,* 237-248. doi: 10.1089/cap.1990.1.237

Schanberg, S.M., & Field, T.M. (1987). Sensory deprivation, stress, and supplemental stimulation in the rat pup and preterm human neonate. *Child Development, 58,* 1431–1447.

Scheinkopf, S.J., & Siegel, B. (1998). Home based behavioural treatment for young autistic children. *Journal of Autism and Developmental Disorders, 28,* 15–23. doi: 10.1177/1362361300004004007

Schousboe, A., & Redburn, D.A. (1995). Modulatory actions of gamma aminobutyric acid (GABA) on GABA type A receptor subunits expression. *Journal of Neuroscience Research, 41*, 1-7. doi: 10.1002/jnr.490410102

Schneider, T., & Przewlocki, R. (2005). Behavioral alterations in rats prenatally to valproic acid: Animal model of autism. *Neuropsychopharmacology, 30,* 80-89. doi: 10.1038/sj.npp.1300518

Schneider, T., & Przewlocki, R. (2011). Environmental factors in the aetiology of autism: Lessons from animal models. In: *Autism - a Neurodevelopment Journey from Genes to Behavior*. V. Eapen (editor). InTech Open Access Publisher; pp. 213-250.

Schneider, T., Roman, A., Basta-Kaim, A., Kubera, M., Budziszewska, B., Schneider, K., & Przwelocki, R. (2008). Gender-specific behavioral and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoneuroendocrinology, 33,* 728-740. doi: 10.1016/j.psyneuen.2008.02.011

Schneider, T., Ziółkowska, B., Gieryk, A., Tyminska, A., & Przewlocki, R. (2007). Prenatal exposure to valproic acid disturbs the enkephalinergic system

functioning, basal hedonic tone, and emotional responses in an animal model of autism. *Psychopharmacology, 193,* 547-555. doi: 10.1007/s00213-007-0795-y

Schultz, R.T., Romanski, L.M., & Tsatsanis, K.D. (2000). Neurofunctional models of autistic disorder and Asperger syndrome: Clues from neuroimaging. In: *Asperger Syndrome.* A. Klin, F.R. Volkmar, & S. Sparrow (editors). The Guilford Press, New York; pp. 172-209.

Seltzer, M.M., Krauss, M.W., Shattuck, P.T., Orsmond, G., Swe, A., & Lord, C. (2003). The symptoms of autism spectrum disorders in adolescence and adulthood. *Journal of Autism and Developmental Disorders, 33,* 565-81. doi: 10.1023/B:JADD.0000005995.02453.0b

Sigman, M., Dijamco, A., Gratier, M., & Rozga, A. (2004). Early detection of core deficits in autism. *Mental Retardation and Developmental Disabilities Research Reviews, 10,* 221–233. doi: 10.1002/mrdd.20046

Silverman, J.L., Yang, M., Lord, C., & Crawley, J.N. (2010). Behavioural phenotyping assays for mouse models of autism. *Nature Review Neuroscience, 11,* 490-502. doi: 10.1038/nrn2851

Simonoff, E., Pickles, A., Charman, T., Chandler, S., Loucas, T., & Baird, G. (2008). Psychiatric disorders in children with autism spectrum disorders: prevalence, comorbidity, and associated factors in a population-derived sample. *Journal of the American Academy of Child and Adolescent Psychiatry, 47,* 921-929. doi: 10.1097/CHI.0b013e318179964f

Siviy, S.M. (1998). Neurobiological substrates of play behavior: Glimpses into the structure and function of mammalian playfulness. In: *Animal play: Evolutionary, comparative, and ecological Perspectives*. M. Bekoff, & J.A. Byers (editors). Cambridge, UK: Cambridge University Press; pp. 221-242.

Siviy, S.M., & Panksepp, J. (1987). Sensory modulation of juvenile play in rats. *Developmental Psychobiology, 20*, 39-55. doi: 10.1002/dev.420200108

Siviy, S.M., & Panksepp, J. (2011). In search of the neurobiological substrates for social playfulness in mammalian brains. *Neuroscience & Biobehavioral Reviews, 35,* 1821-1830. doi: 10.1016/j.neurbiorev.2011.03.006

Smith, L.K., Forgie, M.L., & Pellis, S.M. (1998). Mechanisms underlying the absence of the pubertal shift in the playful defense of female rats. *Developmental Psychobiology, 33,* 147-156. doi:10.1002/(SICI)1098-2302(199809)33:2<147::AID-DEV5>3.3.CO;2-H

Snow, W., Hartle, K., & Ivanco, T. (2008). Altered morphology of motor cortex neurons in the VPA rat model of autism. *Developmental Psychobiology, 50,* 633–639. doi:

10.1002/dev.20337

Sonoda, T., Ohdo, S., Ohba, K.I., Okishima, T., & Hayakawa, K. (1993). Sodium valproate-induced cardiovascular abnormalities in the Jcl:ICR mouse fetus: Peak sensitivity of gestational day and dose-dependent effect. *Teratology, 48,* 127-132. doi: 10.1002/tera.1420480206

Sparks, B.F., Friedman, S.D., Shaw, D.W., Aylward, E.H., Echelard, D., Artru, A.A., (…) & Dager, S.R. (2002). Brain structural abnormalities in young children with autism spectrum disorder. *Neurology, 59,* 184. doi: 10.1002/tera.1420480206

Stuss, D., & Knight, R. (2002). *Principles of frontal lobe function*. Oxford, Oxford University Press.

Tehrani, M.H., & Barnes, E.M. Jr. (1988). GABA down-regulates the GABA/ benzodiazepine receptor complex in developing cerebral neurons. *Neuroscience Letters, 87*, 288-292. doi: 10.1016/0304-3940(88)90463-6

Thor, D.H., & Holloway, W.R. (1983). Play solicitation behavior in juvenile male and female rats. *Animal Learning & Behavior, 11*, 173-178. doi:10.3758/BF03199645

Thor, D.H., & Holloway, W.R. (1984). Developmental analysis of social play behavior in juvenile rats. *Psychonomic Bulletin & Review, 22,* 587–590. doi: 10.1016/0149-7634(84)90004-6

Tordjman, S., Drapier, D., Bonnot, O., Graignic, R., Fortes, S., Cohen, D., (…) & Roubertoux, P.L. (2006). Animal models relevant to schizophrenia and autism: Validity and limitations. *Behavioral Genetics, 37,* 61-78. doi: 10.1007/s10519-006-9120-5

Trevarthen, C., & Daniel, S. (2005). Disorganized rhythm and synchrony: Early signs of autism and Rett syndrome. *Brain & Development, 27,* S25-S34. doi: 10.1016/j.braindev.2005.03.016

Van Den Bos, R. (1998). Post-conflict stress-response in confined group-living cats (Felis silvestris catus). *Applied Animal Behaviour Science, 59,* 323-330. doi: 10.1016/S0168-1591(98)00147-6

Van Hooff, J.A.R.A.M., & Aureli, F. (1994). Social homeostasis and the regulation of emotion. In: *Emotions: Essays on emotional theory.* SHM. van Goozen, NE. van de Poll, & J.A. Sergeant (editors). Hillsdale, NJ: Lawrence Erlbaum; pp. 197-218.

Vanderschuren, L.J. (2010). How the brain makes play fun. *American Journal of Play, 2,* 315-337.

Vanderschuren, L.J., Niesink, R.J., & Van Ree, J.M. (1997). The neurobiology of social

play behavior in rats. *Neuroscience & Biobehavioral Reviews, 21,* 309-326. doi:10.1016/S0149-7634(96)00020-6

Varlinskaya, E.I., Spear, L.P., & Spear, N.E. (1999). Social behavior and social motivation in adolescent rats: Role of housing conditions and partner's activity. *Physiology & Behavior, 67,* 475-482. doi: 0.1016/S0031-9384(98)00285-6

Ventola, P.E., Oosting, D., Anderson, L.C., & Pelphrey, K.A. (2013). Brain mechanisms of plasticity in response to treatments for core deficits in autism. In: *Changing brains, applying brain plasticity to advance and recover human ability.* M. Merzenich, M. Nahum, T.M.V. Vleet (editors). Great Britain: Elsevier; pp. 225-266.

Vismara, L.A., & Rogers, S.J. (2010). Behavioral treatments in autism spectrum disorder: What do we know? *Annual Review of Clinical Psychology, 6,* 447-468. doi: 10.1146/annurev.clinpsy.121208.131151

Volkmar, F. R., Carter, A., Grossman, J., & Klin, A. (1997). Social development in autism. In: *Handbook of autism and developmental disorders.* D.J. Cohen, & F.R. Volkmar (editors). New York: Wiley; pp. 173-194.

Volkmar, F.R., & Nelson, D.S. (1990). Seizure Disorders in Autism. *Journal of the American Academy of Child & Adolescent Psychiatry, 29,* 127-129. doi: 10.1097/00004583-199001000-00020

Vorhees, C.V. (1987). Teratogenicity and developmental toxicity of valproic acid in rats. *Teratology, 35,* 195-202. doi: 10.1002/tera.1420350205

Waiter, G.D., Williams, J.H.G., Murray, A.D., Gilchrist, A., Perrett, D.I., & Whiten, A. (2004). A voxel-based investigation of brain structure in male adolescents with autism spectrum disorder. *NeuroImage ,22,* 619. doi: 10.1016/j.neuroimage.2004.02.029

Wang, D.D., & Kriegstein, A.R. (2008). GABA regulates excitatory synapse formation in the neocortex via NMDA receptor activation. *Journal of Neuroscience, 28,* 5547-5558. doi: 10.1523/JNEUROSCI.5599-07.2008

Watts, T.J. (2008). The pathogenesis of autism. *Clinical Medicine: Pathology, 1,* 99-103.

Weber, R. (1990). A philosophical perspective on touch. In: *Touch: The Foundation of Experience*. : K. Barnard & T.B. Brazelton (editors). Madison, CT: International Universities Press; pp. 11-43.

Weintraub K. (2011). Autism counts. *Nature, 479,* 22-24. doi: 10.1038/479022a

Wellmann, K.A., Varlinskaya, E.I., & Mooney, S.M. (2014). d-Cycloserine ameliorates

social alterations that result from prenatal exposure to valproic acid. *Brain Research Bulletin, 108,* 1-9. doi: 10.1016/j.brainresbull.2014.08.001

Williams, R.S., Hauser, S.L., Purpura, D.P., DeLong, G.R., & Swisher, C.N. (1980). Autism and mental retardation: Neuropathologic studies performed in four retarded persons with autistic behavior. *Archives of Neurology, 37,* 749-753. doi: 10.1001/archneur.1980.00500610029003

Williams, G., King, J., Cunningham, M., Stephan, M., Kerr, B., & Hersh, J.H. (2001). Fetal valproate syndrome and autism: Additional evidence of an association. *Developmental Medicine & Child Neurology, 43*, 202-206.

Wink, L.K., Erickson, C.A., & McDougle, C.J. (2010). Pharmacological treatment of behavioral symptoms associated with autism and other pervasive developmental disorders. *Current Treatment Options in Neurology, 12,* 529-538. doi: 10.1007/s11940-010-0091-8

Wöhr, M., & Scattoni, M.L. (2013a). Behavioural methods used in rodent models of autism spectrum disorders: Current standards and new developments. *Behavioral Brain Reseach, 251,* 5-17. doi: 10.1016/j.bbr.2013.05.047

Wöhr, M., & Scattoni, M.L. (2013b). Neurobiology of autism. *Behavioural Brain Research, 251*, 1-4.

Woo, C.C., & Leon, M. (2013). Environmental enrichment as an effective treatment for autism: A randomized controlled trial. *Behavioral Neuroscience, 127,* 487-497. doi: 10.1037/a0033010

Wright, J.M., Dodosiewiez, M.R., & Clarke, P.B. (2012). Alpha- and beta-adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. *Neuropsychopharmacology, 37,* 808-821. doi: 10.1038/npp.2011.258

Wright, J.M., Gourdon, J.M., & Clarke, P.B. (2010). Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: Effects of amphetamine and social context. *Psychopharmacology, 211,* 1-13. doi: 10.1007/s00213-010-1859-y

Wyschograd, E. (1981). Empathy and sympathy as tactile encounter. *Journal of Philosophy, 6*, 25-43. doi: 10.1093/jmp/6.1.25

Zilbovicius, M., Garreau, B., Samson, Y., Remy, P., Barthelemy, C., Syrota, A., & Lelord, G. (2001). Delayed maturation of the frontal cortex in childhood autism. *American Journal of Psychiatry, 152,* 248. doi: http://dx.doi.org/10.1176/ajp.152.2.248

Zilles, K. (1985). *The cortex of the rat: A stereotaxic atlas*. Berlin: Springer-Verlag.