2010

EFFECTS OF PROPIONIC ACID ON LOCOMOTOR ACTIVITY, ANXIETY- RELATED BEHAVIOUR, SOCIAL INTERACTION, AND ITS INDUCTION OF AVERSIVE INTERNAL CUES, IN MALE & FEMALE RATS: SUPPORT FOR AN ANIMAL MODEL OF AUTISM

Jessica Benzaquen
Western University

EFFECTS OF PROPIONIC ACID ON LOCOMOTOR ACTIVITY, ANXIETY-RELATED BEHAVIOUR, SOCIAL INTERACTION, AND ITS INDUCTION OF AVERSIVE INTERNAL CUES, IN MALE & FEMALE RATS: SUPPORT FOR AN ANIMAL MODEL OF AUTISM

(Spine Title: Effects of Propionic Acid in Adolescent Male & Female Rats)

(Thesis format: Integrated-Article)

By

Jessica Benzaquen

Graduate Program in Neuroscience

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

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

SCHOOL OF GRADUATE AND POSTDOCTORAL STUDIES

CERTIFICATE OF EXAMINATION

Joint-Supervisors

Dr. Klaus-Peter Ossenkopp

Dr. Martin Kavaliers

Advisory Committee Members

Dr. Derrick F. MacFabe

Dr. D. Peter Cain

Dr. Susanne Schmid

Examiners

Dr. David F. Sherry

Dr. Elizabeth Hampson

Dr. Mark R. Cole

The thesis by

Jessica Leigh Benzaquen

entitled:

Effects of Propionic Acid on Locomotor Activity, Anxiety-Related Behaviour, Social Interaction, and its Induction of Aversive Internal Cues, in Male & Female Rats: Support for an Animal Model of Autism

is accepted in partial fulfilment of the
requirements for the degree of
Master of Science

Date_____

Chair of the Thesis Examination Board

ABSTRACT AND KEYWORDS

Autism spectrum disorders (ASDs) are characterized by altered motor activity, restricted interests and social impairment, and are four times more prevalent in males. Co-morbidities such as anxiety disorder, sickness behaviour and gastrointestinal symptoms are present in a subset of patients. Propionic acid (PPA) is a short-chain fatty acid and end-product of enteric bacteria that has been implicated in autism symptoms. Experiment one examined the effects of 14 days of twice daily intraperitoneal (IP) injections of PPA (500mg/kg, 0.26 M, pH 7.5) on locomotor activity and social interaction in juvenile male and female Long-Evans rats. Male and female PPA treated rats exhibited hypoactivity and increased anxiety-related behaviours, as indexed by decreased rearing and stereotypy, and increased thigmotaxis. Only the male PPA treated rats showed social impairment as evidenced by decreased percentage of time in physical contact. These findings suggest that PPA can alter social behavior in a sex-specific manner. Experiment two investigated the effects of IP injections of PPA (500mg/kg, 0.26 M, pH 7.5) in juvenile male rats in a place & taste avoidance paradigm in order to investigate the induction of aversive internal cues by PPA. Over 12 conditioning days rats received alternating IP injections of PPA (or PPA with 2 % saccharin) & vehicle (phosphate buffered saline; 0.15 M) in separate contexts. On a drug-free test day, PPA treated rats did not exhibit a significant avoidance of the treatment-paired chamber, nor did PPA-saccharin treated rats avoid 0.2 % saccharin water. However, PPA rats spent a greater percentage of time rearing in the treatment-paired chamber, a putative index of escape behavior. These results suggest that IP injections of PPA may induce some aversive internal cues. Together, these experiments offer further support towards propionic acid administration as an animal model of autism.

Keywords: sex differences, short chain fatty acid, sickness behavior

ACKNOWLEDGMENT OF CO-AUTHORSHIP

In addition to preparation of the manuscript, all experimental work was conducted and analyzed by Jessica Benzaquen. Dr. Klaus-Peter Ossenkopp and Dr. Martin Kavaliers contributed to the experimental design and preparation of the manuscript. Dr. Derrick MacFabe also contributed to the preparation of the manuscript. Kelly Foley and Lisa Tichenoff assisted with procedural aspects of the experiment in Chapter 2.

DEDICATION

To the memory of my grandparents, Richard & Gaye Gardner.

ACKNOWLEDGMENTS

To begin with, I would like to thank my advisors, Dr. Peter Ossenkopp and Dr. Martin Kavaliers. I thank you both for your guidance, advice, and ability to answer any question I ever came up with over these past two years. I so appreciate your laid back and good-natured attitude, it helped to make this experience such a positive one. You enabled me to take responsibility for my learning and research, and for that I am thankful.

To Derrick MacFabe, your knowledge continuously amazes me, and I thank you for sharing it with me, and letting me be part of your team for these past two years.

Thank you to all the girls in the lab for your help in all things research. More so, thank you for your friendship, and always having time for a chat. Special thanks to Kelly Foley and Lisa Tichenoff, who helped me so much in the early days when I was just the new kid.

Mum and Dad, thank you for always supporting me, and telling me a million times over that I can do anything, and that everything will always work out. You are wonderful and I am so grateful to have you both as my parents. To my beautiful sisters, Danielle, Laurel and Natalie, thanks for gently reminding me that science isn't *that* cool, and making me laugh harder than anyone else.

Alex, I think you are now one of the most knowledgeable economists on the planet regarding PPA and autism. Thank you for always being in my corner, for listening to my endless speeches about the latest thing I find fascinating, and for just being the person who hears about my day, everyday. I am so glad you were with me every step of the way these past two years.

Lastly, a thank you to my grandparents, to whom this thesis is dedicated. I lost you both during this journey, but I am sure Grandpa is bragging on a golf course somewhere while Grandma enjoys a crossword.

TABLE OF CONTENTS

Chapter 3

LIST OF FIGURES

LIST OF ABBREVIATIONS

3-NP	3-nitropropionic acid
ANOVA	Analysis of variance
ASD	Autism Spectrum Disorder
BBB	Blood-brain barrier
CNS	Central nervous system
CFA	Chronic fatigue syndrome
ConA	Conclavin A
CPA	Conditioned place avoidance
CPP	Conditioned place preference
CS	Conditioned stimulus
CSF	Cerebrospinal fluid
CTA	Conditioned taste avoidance
GABA	Gamma-aminobutyric acid
GFAP	Glial fibrillary acidic protein
HLA	Human leukocyte antigen
ICV	Intracerebroventricular
IFN	Interferon
IL	Interleukin
IP	Intraperitoneal
LiCl	Lithium chloride
LPS	Lipopolysaccharide
MDP	Muramyl dipeptide
mtDNA	Mitochondrial DNA
PBS	Phosphate buffered saline
PND	Postnatal day
PPA	Propionic acid
Sac	Saccharin
SCFA	Short-chain fatty acid
S-N-K	Student-Newman-Keuls
SPSS	Statistical package for the social sciences
TNF	Tumor necrosis factor
US	Unconditioned stimulus
VPA	Valproic acid

Chapter 1

General Introduction

1.1 Autism Spectrum Disorders

Autism spectrum disorders (ASDs) are a family of neurodevelopmental disorders present in 1 in every 110 children, four times more often in males (Centers for Disease Control & Prevention, 2009). Symptoms of ASDs include social impairment, altered motor activity, restrictive and repetitive interests, and language problems (Arndt, Stodgell, & Rodier, 2005; DiCicco-Bloom, et al., 2006). Autopsy studies of ASD patients, regardless of their age, reveal a diffuse innate neuroinflammatory response characterized by reactive astrogliosis, activated microglia and an elevation of proinflammatory cytokines in the cerebrospinal fluid (CSF) (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004).

Due to a host of factors, such as incomplete concordance rates for autism amongst monozygotic twins, and a discrepancy in severity of autism in monozygotic twins, research has begun to investigate environmental factors that may play a role in the development of ASD. Environmental agents known to possibly contribute to the development of autism include; prenatal exposure to valproic acid (Ingram, Peckham, Tisdale, & Rodier, 2000), ethanol (Arndt, Stodgell, & Rodier, 2005), and thalidomide (Narita, Kato, Tazoe, Miyazaki, Narita, & Okado, 2002) as well as exposure to some metals and bacterial & viral infections (Fatemi, Cuadra, El Fakahany, Sidwell, & Thuras, 2000). Further support of environmental agents playing a role in the development of autism comes from reports that indicate an induction or exacerbation of symptoms following gastrointestinal abnormalities (Horvath, Papadimitriou, Rabsztyn, Drachenberg, & Tildon, 1999) and routine or antibiotic resistant paediatric infections

(Finegold, Song, & Liu, 2002b; Fallon, 2005). Ingestion of wheat or dairy containing foods have also been found to induce or exacerbate symptoms, as well as elicit bloating, irritability, and lethargy or hypotonia, with a subset of these patients experiencing an improvement in symptoms following elimination of these foods from the diet (Jyonouchi, Sun, & Itokazu, 2002). Additionally, Herbert, et al. (2006) found many environmentally responsive genes involved in functions such as metabolism and the immune and inflammatory response, in linkage regions previously established as being modified or affected in autistic patients. The finding that there are environmentally triggered genes associated with autism lends further support to the idea that the environment may play a role in the development of autism in susceptible individuals. Thus, although autism is considered to be strongly genetically based, emerging research suggests at least some ASDs may be environmentally triggered.

A subset of ASD patients also present with co-morbidities such as anxiety disorder, gastrointestinal symptoms, sickness behaviour and seizure disorder (Skokauskas & Gallagher, 2010; Horvath, Papadimitriou, Rabsztyn, Drachenberg, & Tildon, 1999; Jyonouchi, Sun, & Itokazu, 2002; Canitano, 2007). Additionally, ASD patients have been found to have widespread systemic inflammation, not just limited to the central nervous system (Croonenberghs, Bosmans, Deboutte, Kenis, & Maes, 2002; Croonenberghs, et al., 2002). These co-morbidities suggest that autism may be considered a whole-body disorder systemic in nature, rather than a solely brain-based disorder. In fact, emerging research suggests autism may be a systemic condition involving immune, digestive, and metabolic dysfunction.

1.1.1 Immune Dysfunction

In terms of immune dysfunction and autism, an increased incidence of autoimmune disease has been found in families with an autistic child (Atladóttir, et al., 2009). Allergies have also been associated with autism (Pardo, Vargas, & Zimmerman, 2005; Sperner-Unterweger, 2006). Additionally, studies of those with autism show abnormalities in peripheral immune cells, as well as variations in the expression of genes for cytokines, cytokine receptors, and the human leukocyte antigen (HLA) system (Patterson, 2009). Although what these various associations actually mean has yet to be determined, it does suggest that immune status may play a role in the aetiology of ASDs. A consistent finding in ASD is the presence of an innate neuroinflammatory process, as dictated by activated microglia and reactive astrocytes, as well as up-regulation of cytokines in both the brain and CSF (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004). This finding has been established both in children and the adult brain, suggesting that this immune dysfunction in the brain begins early and is a permanent state maintained in adulthood (Patterson, 2009).

Research also suggests that maternal infection, which is a risk-factor associated with autism (Ciaranello & Ciaranello, 1995; Hyman, Arndt, & Rodier, 2006; Moy & Nadler, 2008), may be responsible for this immune dysfunction early in life. Maternal infection has been shown to alter the immune status of the fetal brain, and perhaps even the fetus' peripheral immune system (Patterson, 2009). Activation of the maternal immune system elevates cytokines in the fetal environment, and it is the pro- and anti-inflammatory cytokine balance that then mediates the effects that this activation has on

the fetus (Patterson, 2009). Investigating the effects of this cytokine balance has largely been done by upregulating or blocking cytokines during pregnancy in animals. For instance, intraperitoneal injection of interleukin-6 (IL-6) in pregnant rats for three days resulted in elevated IL-6 mRNA levels in the offspring at 4 and 24 weeks of age, as well as astrogliosis and elevated GFAP levels, signifying an ongoing state of immune dysfunction reminiscent of that seen in autism (Samuelsson, Jennische, Hansson, & Holmang, 2006). Studies suggest that IL-6 and other cytokines may act directly on developing neurons and glia. For example, over-expression of IL-6 in astrocytes early in life causes neuropathology as well as decreases in the seizure threshold, which is of significance as seizures are a common co-morbidity seen in autism (Samland, Huitron-Resendiz, Masliah, Criado, Henriksen, & Campbell, 2003). IL-6 and related cytokines are also known to influence brain development and neural repair, along with regulate neuronal excitability, stress responses, feeding and depressive behaviours, and long-term potentiation and learning (Bauer, Kerr, & Patterson, 2007). Additionally, Croonenberghs, et al. (2002) found significantly increased production of interferon- γ (IFN-γ) and IL-1 receptor agonist, as well as a trend toward significantly increased levels of IL-6 and tumour necrosis factor-α (TNF-α) in patients with autism. Researchers concluded that autism may be associated with a specific activation of the immune response, and hypothesized that increased production of these proinflammatory cytokines may play a role in the pathophysiology of autism (Croonenberghs, Bosmans, Deboutte, Kenis, & Maes, 2002).

Further support for the suggestion that immune dysregulation is a substantial aspect of ASD comes from a preliminary study of 25 autistic children that demonstrated treatment with anti-inflammatory drugs led to a significant decrease, especially in younger patients, in symptoms such as irritability, lethargy, stereotypy, and hyperactivity (Boris, et al., 2007). Together, these studies suggest that immune dysfunction, not only in the brain but also systemically, is an ever present aspect of autism spectrum disorders, and offer further support for the proposal that autism is not just a brain-based disorder.

1.1.2 Gastrointestinal & Digestive Dysfunction

Gastrointestinal problems in a subset of children with ASD are also well documented. Horvath, et al. (1999) examined a group of patients with autism who had gastrointestinal symptoms. The most frequent complaints were chronic diarrhea, gaseousness, and abdominal discomfort and distension (Horvath, Papadimitriou, Rabsztyn, Drachenberg, & Tildon, 1999). Findings from this study included increased rates of gastrointestinal disorders that were previously undiagnosed in these individuals, and included reflux esophagitis, disaccharide malabsorption, chronic gastritis, and chronic duodenitis. Furthermore, Finegold, et al. (2002a) found late-onset autism individuals to have higher counts of clostridia in their fecal flora, as well as a greater number of clostridial species. Clostridia are the principal bacteria that produce both enterotoxins and neurotoxins, and are very metabolically active, enabling them to produce a variety of toxic metabolites, as well as PPA (Finegold, et al., 2002a). They can be very difficult to eradicate and overgrowth of some species of clostridia may be

triggered by antibiotic use (Finegold, et al., 2002a). This is significant since there is a known association between antibiotic use and late-onset autism (Parracho, Bingham, Gibson, & McCartney, 2005). Furthermore, ASD patients were found to have nine species of *Clostridium* in their stools not found in controls, whereas controls only had three species not found in autism patients. Additionally, in gastric and duodenal specimens from controls there was a total absence of non-spore forming anaerobes and microaerophilic bacteria, where there were a significant number of such bacteria in children with ASD. These findings suggest that children with late-onset ASD have significant alterations in their upper and lower intestinal flora, which may be able to explain certain aspects of the development of ASD. Additionally, they offer further support that at least in a subset of patients, autism may not be a solely brain-based disorder.

1.1.3 Metabolic Dysfunction

Although most cases of autism are not thought to be associated with known metabolic disorders, there are a number of known neurometabolic and mitochondrial disorders which present with autistic features (Zecavati & Spence, 2009). Such disorders include; phenylketonuria, disorders of purine metabolism, creatine deficiency, biotinidase deficiency, cerebral folate deficiency, succinic semialdehyde dehydrogenase (SSADH) deficiency, and Smith-Lemli-Opitz syndrome (Zecavati & Spence, 2009; Manzi, Loizzo, Giana, & Curatolo, 2008). The prevalence of these disorders in the autistic population, however, is unknown. It is expected that they often go undiagnosed, especially since research has shown routine neurometabolic testing often does not

uncover these specific disorders (Zecavati & Spence, 2009). Some researchers suggest the possibility that it is underlying neurometabolic dysfunction that may be mild and unrecognized, which may predispose some to express the autism phenotype (Zecavati & Spence, 2009). Autism as a metabolic disorder, at least in a subset of patients, would offer further support that autism may be a systemic disorder implicating many areas in the body.

The role of mitochondrial disorders in autism is also a new area of research. Thus far, research suggests that mitochondrial disorders are a rare cause of autism (Lerman-Sagie, Leshinsky-Silver, Watemberg, & Lev, 2004), although diagnosing mitochondrial disorders has proven difficult. Nonetheless, a number of case reports have implicated mitochondrial abnormalities in autism (Pons, et al., 2004; Filiano, Goldenthal, Rhodes, & Marin-Garcia, 2002; Tsao & Mendell, 2007; Oliveira, et al., 2005). For instance, one study analyzed five patients with A3243G mitochondrial DNA (mtDNA) mutations, as well as mtDNA depletion, and found three of the five patients to fulfill diagnostic criteria for autism. This led researchers to suggest that the autistic phenotype may be the expression of mitochondrial dysfunction in the developing brain (Pons, et al., 2004). Another study found mtDNA deletions in children with hypotonia, epilepsy, autism, and developmental delay (Filiano, Goldenthal, Rhodes, & Marin-Garcia, 2002), while a case study of two children with autism found them to have deficiencies in respiratory chain enzymes after muscle biopsy (Tsao & Mendell, 2007). Together these studies suggest that mitochondrial dysfunction may play a role in autism. Only one population-based study has examined the prevalence of this phenomenon. Oliveira, et

al. (2005) tested 120 children with autism and concluded that mitochondrial respiratory chain disorder may be one of the most common disorders associated with autism, with 7.2% of children testing positive for one (Oliveira, et al., 2005). Notably, none of these children had known mtDNA mutations or deletions associated with established mitochondrial disorders, suggesting that some kind of mitochondrial *dysfunction*, rather than recognized mitochondrial *disease*, may be associated with autism (Zecavati & Spence, 2009). Furthermore, it has been suggested that this dysfunction may have less severe signs and symptoms, and may not be elucidated on traditional muscle biopsy due to a unique and as of yet unestablished mitochondrial pathology (Filiano, Goldenthal, Rhodes, & Marin-Garcia, 2002; Lombard, 1998)

1.2 Propionic Acid

One factor that may influence the expression or incidence of the differential immune, metabolic, and possibly behavioural dysfunction present in ASDs is propionic acid. Propionic acid is a short-chain fatty acid, and in the human body about 50% of it is produced from catabolism of amino acids and about 30% of it comes from the metabolism of odd-chain fatty acids. The remaining 20% is produced by a subpopulation of opportunistic enteric bacteria (i.e.clostridia) as a fermentation by-product (Finegold, et al., 2002a). It is also used as a food preservative in refined wheat and dairy products (Brock & Buckel, 2004). It is mostly metabolized in the liver, and as a weak acid, it can exist in both water-soluble and lipid-soluble forms, thus allowing it to readily enter systemic or central nervous system (CNS; through the blood-brain barrier) environments, either passively via diffusion, or actively via monocarboxylate

transporters, which have a high affinity for SCFAs (Niederman, Zhang, & Kashket, 1997; Maurer, Canis, Kuschinsky, & Duelli, 2004). PPA and other SCFAs can affect a variety of physiological processes in the body such as cell signaling (Nakao, Moriya, Furuyama, Niederman, & Sugiy, 1998), mitochondrial function (Wagner, Bayir, Ren, Puccio, Zafonte, & Kochanek, 2004), lipid metabolism (Hara, Haga, Aoyama, & Kiriyama, 1999), neurotransmitter synthesis and release (DeCastro, et al., 2005), immune function (Le Poul, Loison, & Struyf, 2003), modulation of gene expression (Suzuki, Nagao, Tokunaga, Katayama, & Uyeda, 1996; Nankova, Tichenoff, Taylor, Mohammad-Asef, La Gamma, & MacFabe, 2008), and gap junctional gating (Rorig, Klausa, & Sutor, 1996). Together, these widespread effects suggest PPA may provide a plausible link to the disparate aspects of autism.

1.2.1 Propionic Acid & Autism

Metabolic disorders of altered PPA metabolism have been shown to have similar symptoms to ASDs (Knerr, Gibson, Jakobs, & Pearl, 2008; Pearl, et al., 2003; Ching, et al., 2010; Filiano, Goldenthal, Rhodes, & Marin-Garcia, 2002). For instance, in propionic acidemia, individuals experience an accumulation of PPA due to an error in metabolism. This error can occur in any of the enzymes involved in the metabolism of PPA. Initial symptoms of propionic acidemia include hypotonia, lethargy, vomiting, and loss of appetite. In the long term, however, symptoms include seizures, decreased muscle tone, movement disorder, and language delays (Kaya, et al., 2008). Individuals with propionic acidemia demonstrate that accumulation of PPA can elicit symptoms reminiscent of autism.

Results of recent investigations have revealed that male rats receiving intracerebroventricular (ICV) infusions of propionic acid display behavioural, physiological and metabolic changes similar to those seen in ASD. Published studies using PPA to model autism in the rat have all used ICV infusions of PPA directly into the lateral cerebral ventricles of adult male rats. Findings include reversive bouts of hyperactivity, impairments in social behaviour, perseveration in a water maze task, caudate spiking, and limbic kindled seizures, effects consistent with those seen in ASD (MacFabe, et al., 2007; MacFabe, et al., 2008; Shultz S. R., et al., 2008; Shultz S. R., et al., 2009; Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010). Furthermore, neuropathological findings reveal an innate neuroinflammatory response (i.e. reactive astrogliosis and activated microglia) and an increase in oxidative stress markers, as well as altered lipid and acylcarnitine profiles, findings once again consistent with ASD autopsy studies (Chauhan & Chauhan, 2006; Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004; James, et al., 2006; Chauhan, Chauhan, Brown, & Cohen, 2004; Kuratsune, et al., 2002). Furthermore, collaborating labs at various institutions are investigating other aspects of this model (Finegold, et al., 2002a; Herbert, et al., 2006; Nguyen, Rauch, Pfeifer, & Hu, 2010).

1.4 Present Studies

As discussed above, emerging research suggests that autism is a whole-body disorder rather than solely brain-based disorder. Since much of the research in this propionic acid animal model has used direct infusion of PPA into the ventricles of the brain, the aim of the current studies were to examine the effects of systemically

administered PPA through repeated intraperitoneal injections. Furthermore, as autism is four times more prevalent in males, this was the first study to investigate the effect of PPA in males compared to females. Lastly, these experiments investigated the effects of PPA not in adult animals as previous studies have done, but also in adolescents. This is important as autism is a lifelong disorder, necessitating examination of the effects of PPA at different points in development.

An effective animal model incorporates face validity (has aspects strongly analogous to the endophenotypes in humans), construct validity (is due to the same biological dysfunction that causes the human disease), and predictive validity (has an analogous response to preventative or symptom treatments in humans) (Silverman, Yang, Lord, & Crawley, 2010). Autism diagnosis is based entirely on behavioural criteria as there are no consistent biological markers of this disorder. Presence of core elements in three categories are required for diagnosis by the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV, the diagnostic manual of the American Psychiatric Association). These categories are; abnormal reciprocal social interactions, impaired communication, and repetitive behaviours (Diagnostic and Statistical Manual of Mental Disorders, 2000). Some research is currently working towards generating new behavioural tasks for animals that can most closely maximize face validity for these core symptoms (Silverman, Yang, Lord, & Crawley, 2010), as there is no known cause or consistently effective treatment of autism. The current studies aimed to further establish face validity in this PPA animal model of autism by investigating social interaction and locomotor activity (including repetitive behaviour) in adolescent rats

treated with PPA. It is expected that systemic administration of PPA will result in hyperactivity and social impairment, similar to that seen in animals infused with PPA via ICV administration. It is also expected that behavioural abnormalities due to PPA administration will be more severe in males than females. This is expected as in a valproic acid (VPA) model of autism, researchers demonstrated that males exposed to VPA exhibit abnormalities in a variety of behavioural and immunological tests, where females perform normally in nearly all tasks (Schneider, et al., 2008). One of the metabolites of valproic acid is propionic acid (Sztajnkrycer, 2002). Furthermore, there is some evidence that PPA metabolism differs in males and females (Wolever, Fernandes, & Rao, 1996). Also, autism is four times more prevalent in males. Lastly, as adolescence is a developmental period in rats marked by hyper-sensitivity to the environment as well as synaptic reorganization and plasticity (Ono, Sakamoto, & Sakura, 2001), it is hypothesized that the effect of PPA on adolescents might be more severe than in adult rats.

In summary, Chapter 2 specifically examined the effects of repeated IP injections of PPA on locomotor activity, anxiety-related behaviour, and social interaction in male and female juvenile rats. In light of Chapter 2's findings, Chapter 3 then investigated the presence of aversive internal cues due to IP injections of PPA using a place and taste avoidance paradigm. These studies allow for further validation of this rodent model of autism, an important endeavour, as a well established animal model will permit the investigation of this disorder in a safe and effective way.

1.5 References

Arndt, T. L., Stodgell, C. J., & Rodier, P. M. (2005). The teratology of autism. International Journal of Developmental Neuroscience , 23, 189-199.

Atladóttir, H. O., Pedersen, M. G., Scient, C., Thorsen, P. M., Deleuran, B., Eaton, W. W., et al. (2009). Association of family history of autoimmune diseases and autism spectrum disorders. Pediatrics , 124 (2), 687-694.

Bauer, S., Kerr, B. J., & Patterson, P. H. (2007). The neuropoietic cytokine family in development, plasticity, disease and injury. *Nature Reviews Neuroscience , 8*, 221-232.

Boris, M., Kaiser, C. C., Goldblatt, A., Elice, M. W., Edelson, S. M., Adams, J. B., et al. (2007). Effect of pioglitazone treatment on behavioral symptoms in autistic children. *Journal of Neuroinflammation , 4*, 3.

Brock, M., & Buckel, W. (2004). On the mechanism of action of the antifungal agent propionate. *European Journal of Biochemistry , 271*, 3227-3241.

Canitano, R. (2007). Epilepsy in autism spectrum disorders. *European Child & Adolescent Psychiatry , 16* (1), 61-66.

Centers for Disease Control & Prevention. (2009). Prevalence of Autism Spectrum Disorders - Autism & Developmental Disabilities Monitoring Network. *MMWR Surveillance Summaries , 58 (SS-10)*, 1-20.

Chauhan, A., & Chauhan, V. (2006). Oxidative stress in autism. *Pathophysiology , 13*, 171-181.

Chauhan, A., Chauhan, V., Brown, W. T., & Cohen, I. (2004). Oxidative stress in autism: increase lipid peroxidation and reduced serum levels of ceruloplasmin and transferrin - the antioxidant proteins. *Life Sciences , 75*, 2539-2549.

Ching, M. S., Shen, Y., Tan, W. H., Jeste, S. S., Morrow, E. M., Chen, X., et al. (2010). Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics , 153B* (4), 937-47.

Ciaranello, A. L., & Ciaranello, R. D. (1995). The neurobiology of infantile autism. *Annual Reviews in Neuroscience , 18*, 101-128.

Croonenberghs, J., Bosmans, E., Deboutte, D., Kenis, G., & Maes, M. (2002). Activation of the inflammatory response system in autism. *Neuropsychobiology , 45*, 1-6.

Croonenberghs, J., Wauters, A., Devreese, K., Verkerk, R., Scharpe, S., Bosmans, E., et al. (2002). Increased serum albumin, y globulin, immunoglobulin IgG, and IgG2 and IgG4 in autism. *Psychological Medicine , 32* (8), 1457-1463.

DeCastro, M., Nankova, B. B., Shah, P., Patel, P., Mally, P. V., Mishra, R., et al. (2005). Short chain fatty acids regulate tyrosine hydroxylase gene expression through a cAMP-dependent signaling pathway. *Brain Research Molecular Brain Research , 142*, 28-38.

Diagnostic *and Statistical Manual of Mental Disorders* (4th ed.). (2000). Washington DC: American Psychiatric Association.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S. R., Schmitz, C., et al. (2006). The developmental neurobiology of autism spectrum disorder. *The Journal of Neuroscience , 62*, 6897-6906.

Fallon, J. (2005). Could one of the most widely prescribed antibiotics amoxicillin/clavulanate "augmentin" be a risk factor for autism? *Medical Hypotheses , 64*, 312-315.

Fatemi, S. H., Cuadra, A. E., El Fakahany, E. E., Sidwell, R. W., & Thuras, P. (2000). Prenatal viral infection causes alterations in nNOS expression in developing mouse brains. *Neuroreport , 11*, 1493-1496.

Filiano, J. J., Goldenthal, M. J., Rhodes, C. H., & Marin-Garcia, J. (2002). Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. *Journal of Child Neurology , 17*, 435-439.

Finegold, S. M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M.-L., Bolte, E., et al. (2002a). Gastrointestinal microflora studies in late-onset autism. *Clinical Infectious Diseases , 35*, S6-S16.

Finegold, S. M., Song, Y., & Liu, C. (2002b). Taxonomy-general comments and update on taxonomy of Clostridia and Anaerobic cocci. *Anaerobe , 8*, 283-285.

Hara, H., Haga, S., Aoyama, Y., & Kiriyama, S. (1999). Short-chain fatty acids suppress cholesterol synthesis in rat liver and intestine. *Journal of Nutrition , 129*, 942-948.

Herbert, M. R., Russo, J. P., Yang, S., Roohi, J., Blaxill, M., Kahler, S. G., et al. (2006). Autism and environmental genomics. *Neurotoxicology , 27*, 671-684.

Horvath, K., Papadimitriou, J. C., Rabsztyn, A., Drachenberg, C., & Tildon, J. T. (1999). Gastrointestinal abnormalities in children with autistic disorder. *Journal of Pediatrics , 135*, 559-563.

Hyman, S. L., Arndt, T. L., & Rodier, P. M. (2006). Environmental agents and autism: once and future associations. *International Review of Research in Mental Retardation , 30*, 171-194.

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.

James, S. J., Melnyk, S., Jernigan, S., Cleves, M. A., Halsted, C. H., Wong, D. H., et al. (2006). Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics , 141*, 947-956.

Jyonouchi, H., Sun, S., & Itokazu, N. (2002). Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. *Neuropsychobiology , 46*, 76-84.

Kaya, N., Al-Owain, M., AlBakheet, A., Colak, D., Al-Odaib, A., Imtiaz, F., et al. (2008). Array compatative genomic hybridization (aCGH) reveals the largest novel deletion in PCCA found in a Saudi family with propionic acidemia. *European Journal of Medical Genetics , 51*, 558-565.

Knerr, I., Gibson, K. M., Jakobs, C., & Pearl, P. L. (2008). Neuropsychiatric morbidity in adolescent and adult succinic semialdehyde dehydrogenase deficiency patients. *CNS Spectrums , 13* (7), 598-605.

Kuratsune, H., Yamaguti, K., Lindh, G., Evengard, B., Hagberg, G., Matsumura, K., et al. (2002). Brain regions involved in fatigue senstation; Reduced acetylcarnitine uptake into the brain. *NeuroImage , 17*, 1256-1265.

Le Poul, E., Loison, C., & Struyf, S. e. (2003). Functionaly characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *The Journal of Biological Chemistry , 278*, 25481-25489.

Lerman-Sagie, T., Leshinsky-Silver, E., Watemberg, N., & Lev, D. (2004). Should autistic children be evaluatied for mitochondrial disorders? *Journal of Child Neurology , 19*, 379-381.

Lombard, J. (1998). Autism: a mitochondrial disorder? *Medical Hypotheses , 50*, 497-500.

MacFabe, D., Cain, D., Rodriguez-Capote, K., Franklin, A., Hoffman, J., Boon, F., et al. (2007). Neurobiological effects of intraventicular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. *Behavioural Brain Research , 176*, 149-169.

MacFabe, D., Rodriguez-Capote, K., Hoffman, J., Franklin, A., Mohammad-Asef, Y., Taylor, R., et al. (2008). A novel rodent model of autism: intraventricular infusions of propionic acid increase locomotor activity and induce neuroinflammation and ocxidative stress in discrete regions of adult rat brain. *American Journal of Biochemistry and Biotechnology , 4* (2), 146-166.

Manzi, B., Loizzo, A. L., Giana, G., & Curatolo, P. (2008). Autism and metabolic diseases. *Journal of Child Neurology , 23* (3), 307-314.

Maurer, M. H., Canis, M., Kuschinsky, W., & Duelli, R. (2004). Correlation between local monocarboxylate transporter 1 (MCT1) and glucose transporter 1 (GLUT1) densities in the adult rat brain. *Neuroscience Letters , 355*, 105-108.

Moy, S. S., & Nadler, J. J. (2008). Advances in behavioral genetics: mouse models of autism. *Molecular Psychiatry , 13*, 4-26.

Nakao, S., Moriya, Y., Furuyama, S., Niederman, R., & Sugiy, H. (1998). Propionic acid stimulates superoxide generation in human neutrophils. *Cell Biology International , 22*, 331-337.

Nankova, B. B., Tichenoff, L., Taylor, A. R., Mohammad-Asef, Y., La Gamma, E. F., & MacFabe, D. F. (2008). Intracerebroventricular infusions of propionic and butyric acid induce behavioural, neuropathological and epigenetic changes in rats - possible relevance to autism spectrum disorders. *International Meeting for Autism Research.* London, UK: International Society for Autism Research.

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.

Nguyen, A., Raugh, T. A., Pfeifer, G. P., Hu, V. W. (2010). Global methylation profiling of lymphoblastoid cell lines reveals epigenetic contributions to autism spectrum disorders and a novel autism candidate gene, RORA, whose protein product is reduced in autistic brain. *The FASEB Journal, 24* (8), 3036-3051.

Niederman, R., Zhang, J., & Kashket, S. (1997). Short-chain carboxylic-acid-stimulated, PMN-mediated gingival inflammation. *Critical Reviews in Oral Biology & Medicine , 8*, 269-290.

Oliveira, G., Diogo, L., Grazina, M., Garcia, P., Ataide, A., Marques, C., et al. (2005). Mitochondrial dysfunction in autism spectrum disorders: a population-based study. *Developmental Medicine & Child Neurology , 47*, 185-189.

Ono, H., Sakamoto, A., & Sakura, N. (2001). Plasma total glutathione concentrations in healthy pediatric and adult subjects. *Clinica Chimica Acta , 312* (1-2), 227-229.

Pardo, C. A., Vargas, D. L., & Zimmerman, A. W. (2005). Immunity, neuroglia and neuroinflammation in autism. *International Review of Psychiatry , 17*, 485-495.

Parracho, H. M., Bingham, M. O., Gibson, G. R., & McCartney, A. L. (2005). Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. *Journal of Medical Microbiology , 54*, 987-991.

Patterson, P. H. (2009). Immune involvement in schizophrenia and autism: Etiology, pathology, and animal models. *Behavioural Brain Research , 204*, 313-321.

Pearl, P. L., gibson, K. M., Acosta, M. T., Vezina, L. G., Theodore, W. H., Rogawski, M. A., et al. (2003). CLinical spectrum of succinic semialdehyde dehydrogenase deficiency. *Neurology , 60* (9), 1413-1417.

Pons, R., Andreu, A. L., Checcarelli, N., Vila, M. R., Engelstad, K., Sue, C. M., et al. (2004). Mitochondrial DNA abnormalities and autistic spectrum disorders. *Journal of Pediatrics , 144*, 81-85.

Rorig, B., Klausa, G., & Sutor, B. (1996). Intracellular acidification reduced gap junction coupling between immature rat neocortical pyramidal neurons. *Journal of Physiology , 490 (pt-1)*, 31-49.

Samland, H., Huitron-Resendiz, S., Masliah, E., Criado, J., Henriksen, S. J., & Campbell, I. L. (2003). Profound increase in sensitivity to glutamatergic- but not cholinergic agonist-induced seizures in transgenic mice with astrocyte production of IL-6. *Journal of Neuroscience Research , 73*, 176-187.

Samuelsson, A. M., Jennische, E., Hansson, H. A., & Holmang, A. (2006). Prenatal exposure to interleukin-6 results in inflammatory neurodegeneration in hippocampus with NMDA/GABA(A) dysregulation and impaired spatial learning. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology , 290*, 1345-1356.

Schneider, T., Roman, A., Basta-Kaim, A., Kulbera, M., Budziszewska, B., Schneider, K., et al. (2008). Gender-specific behavioural and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. *Psychoeuroendocrinology , 33*, 728-740.

Shultz, S. R., MacFabe, D. F., Ossenkopp, K.-P., Scratch, S., Whelan, J., Taylor, R., et al. (2008). Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behaviour in the rat: Implications for an animal model of autism. *Neuropharmacology , 54*, 901-911.

Shultz, S. R., MacFabe, D. K., Martin, S., Jackson, J., Taylor, R., Boon, F., et al. (2009). Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. *Behavioral Brain Research , 200*, 33-41.

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

Skokauskas, N., & Gallagher, L. (2010). Psychosis, affective disorders and anxiety in autism spectrum disorder: prevalence and nosological considerations. *Psychopathology , 43* (1), 8-16.

Sperner-Unterweger, B. (2006). Immunological aetiology of major psychiatric disorders. Evidence and therapeutic implications. *Drugs , 65*, 1493-1520.

Suzuki, K., Nagao, K., Tokunaga, J., Katayama, N., & Uyeda, M. (1996). Inhibition of DNA methyltransferase by microbial inhibitors and fatty acids. *Journal of Enzyme Inhibition , 10*, 271-280.

Sztajnkrycer, M. D. (2002). Valproic acid toxicity: overview and management. *Journal of Toxicology: Clinical Toxicology , 40* (6), 789-801.

Thomas, R. H., Foley, K. A., Mepham, J. R., Tichenoff, L. J., Possmayer, F., & MacFabe, D. F. (2010). Altered brain phospholipid and acylcarnitine profiles in propionic acid

infused rodents: further development of a potential model of autism spectrum disorders. *Journal of Neurochemistry , 113* (2), 515-529.

Tsao, C. Y., & Mendell, J. R. (2007). Autistic disorder in 2 children with mitochondrial disorders. *Journal of Child Neurology , 22*, 1121-1123.

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2004). Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of Neurology , 57*, 67-81.

Wagner, A. K., Bayir, H., Ren, D., Puccio, A., Zafonte, R. D., & Kochanek, P. M. (2004). Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: the impact of gender, age, and hypothermia. *Journal of Neurotrauma , 21*, 125-136.

Wolever, T. M., Fernandes, J., & Rao, A. V. (1996). Serum acetate: propionate ratio is related to serum cholesterol in men but not in women. *Journal of Nutrition , 126*, 2790-2797.

Zecavati, N., & Spence, S. (2009). Neurometabolic Disorders and Dysfunction in Autism Spectrum Disorders. *Current Neurology and Neuroscience Reports , 9*, 129-136.

Chapter 2:

The Effects of Intraperitoneal Injections of Propionic Acid on Locomotor Activity, Anxiety-Related Behaviour and Social Interaction in Adolescent Male & Female Rats

2.1 Introduction

Autism spectrum disorders are a family of lifelong disorders of restricted interests, social impairment, altered motor activity, and language problems (Arndt, Stodgell, & Rodier, 2005; DiCicco-Bloom, et al., 2006). A subset of patients also present with co-morbidities such as anxiety disorder, gastrointestinal symptoms, and sickness behaviour (Skokauskas & Gallagher, 2010; Horvath, Papadimitriou, Rabsztyn, Drachenberg, & Tildon, 1999; Jyonouchi, Sun, & Itokazu, 2002). Additionally, autopsy studies of patients with autism demonstrate increased CNS immune activity, regardless of age, as demonstrated by increases in reactive astrocytes and activated microglia, as well as the elevation of proinfammatory cytokines in the cerebral spinal fluid (CSF) (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004).

There has been a dramatic rise in the number of children with ASD. The latest study by the U.S.A. Center for Disease Control and Prevention reports prevalence of autism in 2006 as averaging 1 in 110 children, and being four times more prevalent in males (Centers for Disease Control & Prevention, 2009). Although it has been suggested that this rise in prevalence is due solely to an increase in awareness and diagnostic criteria, many publications suggest that these increases cannot be fully explained in this manner (Newschaffer, Falb, & Gurney, 2005; Rutter, 2005; King & Bearman, 2009).

While there is much evidence for a strong genetic contribution to ASD, incomplete concordance rates amongst monozygotic twins, as well as variation in disorder severity when both twins do have autism, suggest environmental factors may play a role in ASD (Hu, Frank, Heine, Lee, & Quackenbush, 2006). Furthermore,

environmental agents already identified to possibly contribute to the development of autism include; prenatal exposure to valproic acid (Ingram, Peckham, Tisdale, & Rodier, 2000), ethanol (Arndt, Stodgell, & Rodier, 2005), and thalidomide (Narita, Kato, Tazoe, Miyazaki, Narita, & Okado, 2002). Additional support that environmental agents may play a role comes from reports of an induction or exacerbation of symptoms following gastrointestinal abnormalities (Horvath, Papadimitriou, Rabsztyn, Drachenberg, & Tildon, 1999) and routine or antibiotic resistant pediatric infections (Finegold, Song, & Liu, 2002; Fallon, 2005). Additionally, Herbert, et al. (2006) found many environmentally responsive genes in linkage regions previously established in autistic patients, suggesting a mechanism to explain how the environment may play a role in the development of autism in susceptible individuals. A variety of animal studies are now addressing how environmental factors may influence the expression of ASD (Ingram, Peckham, Tisdale, & Rodier, 2000; Narita, Kato, Tazoe, Miyazaki, Narita, & Okado, 2002; Kamitani, Ono, & Yoshino, 2003; Fatemi, Pearce, Brooks, & Sidwell, 2005).

The administration of propionic acid (PPA) has recently been proposed as a way to model ASDs in the rat (MacFabe, et al., 2007; MacFabe, et al., 2008; Shultz S. R., et al., 2008; Shultz S. R., et al., 2009; Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010). PPA is a short-chain fatty acid (SCFA) that is an important intermediate of cellular metabolism and is also produced by a subpopulation of enteric bacteria (i.e. clostridia & propionibacteria) as a fermentation by-product (Finegold, et al., 2002). It is a weak acid, and can thus exist in two forms, water-soluble and lipid-soluble. Thus, PPA can readily enter systemic or CNS environments (through the blood-brain barrier), either

passively via diffusion, or actively via monocarboxylate transporters (Niederman, Zhang, & Kashket, 1997; Maurer, Canis, Kuschinsky, & Duelli, 2004).

It is hypothesized that PPA and related SCFAs may provide a link among the disparate behavioural, dietary, gut, metabolic and immune factors implicated in ASD due to their ability to affect a variety of physiological processes. Published studies using PPA to model autism in the rat have used ICV infusions of PPA directly into the lateral cerebral ventricles of adult male rats. Findings include behavioural, biochemical, neuropathological and electrophysiological effects consistent with those seen in ASD (MacFabe, et al., 2007; MacFabe, et al., 2008; Shultz S. R., et al., 2008; Shultz S. R., et al., 2009; Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010).

As ASD is systemic in nature, investigating the peripheral administration of PPA is necessary to further validate this rodent model, as is investigating the effects of PPA at varying points in development, since autism is a lifelong disorder. There is a known sex difference in autism, with the prevalence being four times larger in males than females (Centers for Disease Control & Prevention, 2009). Therefore, it is important to investigate the effects of PPA on females in order to further establish this animal model of ASD. Thus, the aim of the present experiment was to further investigate the effect of systemic IP injections of PPA on locomotor activity and social behaviour, while investigating potential sex differences in the behavioural alterations of rats exposed to PPA. Hence, animals were investigated both individually, as well as in pairs, immediately following an injection of PPA to identify abnormalities in locomotor activity, anxiety, and social behaviour.

2.2 Methods

2.2.1 Subjects

Subjects were 16 male and 16 female Long-Evans hooded rats (Charles River Laboratories, Quebec, Canada) who arrived on postnatal day (PND) 21. Rats were housed in groups of four until PND 37 when groups were split and housed in same-sex, same-treatment groups of two. Animals were housed in standard acrylic cages (26 cm x 48 cm x 21 cm) with wood shavings at a controlled temperature (21 ± 1°C) in a 12:12 light/dark cycle with lights on from 7:00 to 19:00h. Animals were allowed access to food (Rat Chow) and water *ad libitum*. All experimental procedures were in accordance with guidelines of the Canadian Council on Animal Care and approved by the University of Western Ontario Animal Use Committee.

2.2.2 Treatment Groups

Rats were randomly assigned to one of two treatment groups, propionic acid (PPA; 500mg/kg, 0.26 M solution, n = 16, 8 males and 8 females) or vehicle, phosphate buffered saline (PBS; 0.1 M solution of phosphate, n = 16, 8 males and 8 females). PPA was buffered to a physiological pH of 7.5 before injection using hydrochloric acid or sodium hydroxide. Doses were chosen based on past dose-response findings that used repeated intracerebroventricular infusions of 0.26 M solution of PPA (MacFabe, et al., 2007). Rats were injected intraperitoneally twice daily, 4 hours apart, for fourteen consecutive days with all behavioural testing occurring immediately following the second injection of the day.

2.2.3 Apparatus

2.2.3.1 Individual Locomotor Activity. Locomotor activity (Ossenkopp & Kavaliers, 1996) was measured using eight *VersaMax Animal Activity Monitors* (Model NVMA16TT/W, Accuscan Instruments Inc., Columbus, OH). Each monitor consisted of a Plexiglas open field (40 cm x 40 cm x 30.5 cm) with a Plexiglas lid with air holes. Horizontal activity was measured by a set of infrared beams located every 2.54 cm for a total of 16 beams on each side of the monitor, creating a grid at a height of 2.5 cm. To measure vertical activity, two additional sets of beams were located 14 cm above the floor of the open-field. A *VersaMax Analyser* (Accuscan Model VSA-16, Columbus, OH) recorded data from each monitor to a computer in an adjacent room.

2.2.3.2 Paired Social Interaction. Social behaviour was evaluated in a circular open-field (90 cm diameter, 40 cm high) with Beta Chip bedding covering the floor of the arena. A CD camera (WV-CP470, Panasonic) connected to a computer was mounted above the arena. Behaviour was recorded using *EthoVision 3.0.15 Behavioural Monitoring and Analysis System* at a rate of 5.994 frames/s. Each animal's behaviour was recorded in terms of x-y coordinates. Behaviour was also video recorded on a VHS cassette for later analysis.

2.2.4 Experimental Procedures (Fig 2.1)

2.2.4.1 Baseline Individual Locomotor Activity. Rats were handled for an hour on three separate days before testing. Starting one week after arrival (PND 28) animals were habituated to the locomotor activity apparatus for three days for a period of thirty minutes each day, commencing at 13:00h. On the third habituation day (PND 30) data were recorded to provide baseline measures.

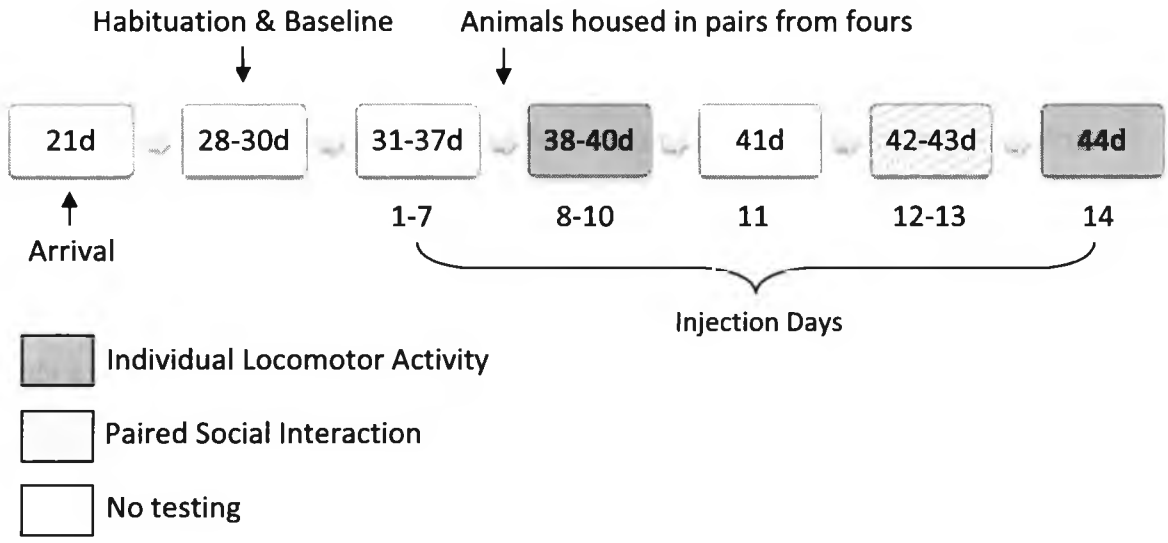

Figure 2.1 Methods timeline, where d = days old. On injection days, IP injections occurred at 09:00 h and 13:00 h. On behavioural testing days (individual locomotor activity & paired social interaction), testing occurred immediately after the second injection for 30 minutes.

2.2.4.2 Individual Locomotor Activity. The day after baseline measures were recorded, rats began treatment, receiving twice-daily intraperitoneal injections for 14 days. Injections were completed at 09:00 and 13:00h during the light period. Prior to each morning injection, rats were weighed. Locomotor activity was recorded on injection days 8, 9, 10 and 14 for thirty minutes immediately following the second injection. Thirty minutes was chosen based on pilot work, as well as past studies, that show that the majority of behavioural effects occur in the first 30 minutes following infusion or injection of PPA (MacFabe, et al., 2007; Shams, 2009). Each monitor was cleaned with a mild detergent solution (Alconox Inc., New York) and rinsed with baking soda solution after each behavioural testing session.

2.2.4.3 Paired Social Interaction. On injection day 11 animals received no behavioural testing. On injection days 12 and 13 animals were tested in the social behaviour apparatus. The day before social testing commenced the dorsal surface of one rat from each cage was coloured black using a black non-toxic permanent marker (Staples Business Depot, Staples Inc.) ensuring the marker's scent would not be novel to any of the rats on subsequent test days. Rats were also coloured again on each social testing day. This enabled the *EthoVision* tracking system to distinguish and track each rat separately (Lazar, Rajakumar, & Cain, 2008). Immediately following the 13:00h injection two same-treatment rats from different home cages were placed into the open field and behavioural data were collected for 30 minutes.

For social interaction testing, unfamiliar, same-sex, same-treatment pairs were used. Same-treatment pairs were utilized for a variety of reasons. Shams (2009)

examined social interaction in adolescent male rats and observed that PPA treated rats showed no differences in social interaction, as measured by frequency of approaching, sniffing and allogrooming partner, whether they were paired with a same- or different-treatment animal. Further, it was previously observed was that regardless of treatment pairings (PPA – PPA or PPA – PBS) PPA treated animals showed reduced social interaction compared to PBS – PBS pairs. Thus, regardless of their partner's treatment, PPA treated rats approached their partners less. The exception to this was frequency of avoidance, where PPA treated animals demonstrated a greater frequency of avoidance in PPA – PBS pairs than in PPA – PPA pairs. However, this could be explained due to PBS treated rats making more approaches towards their partners than PPA treated rats. Additionally, another study in a different animal model of autism compared data from both same- and different-treatment and no significant difference was found between the two experimental designs on measures of social behaviour (Pletnikov, Rubin, Vasudevan, Moran, & Carbone, 1999). This further helps demonstrate why same-treatment pairs, as opposed to different-treatment pairs, may be used while exploring social behaviour in an animal model of autism.

2.2.5 Behavioural Measures

2.2.5.1 Individual Locomotor Activity. As described, *VersaMax Animal Activity Monitors* automatically collected data using a grid of infrared beams. Variables reflecting locomotor and thigmotaxis activity (Ossenkopp & Kavaliers, 1996) were collected and are described below. For thigmotaxis measures, the centre square region consisted of the inner $25cm^2$ square area.

- Total Distance (cm) - the total horizontal distance (cm) travelled.
- Frequency of horizontal movements - the number of individual horizontal movements made with a minimum stop time of 1 s to separate movements.
- Frequency of centre entries – the number of entries to the centre region of the open field.
- Frequency of rearing - the number of individual vertical movements made with a minimum stop time of 1 s to separate movements.
- Frequency of stereotypy - the number of times a rat breaks the same beam without breaking an adjacent beam, with a minimum stop time of 1 s to separate movements.
- Distance (cm) travelled in centre – the distance (cm) travelled while in the center region of the open field.
- Time (s) in centre – the time (s) spent in the centre region of the open field.

2.2.5.2 Paired Social Interaction – Automated Analysis. As described, *Ethovision Behavioural Monitoring and Analysis system* automatically collected data for measures of locomotor activity, thigmotaxis, and social interaction. These are described below. For thigmotaxis, the centre was defined as the 70 cm diameter circle in the centre of the open-field, 10 cm from the wall.

- Total distance (cm) – the total horizontal distance (cm) travelled.
- Frequency of centre entries – the number of entries to the centre region of the open field.
- Time (s) in centre – the time (s) spent in the centre region of the open field.

- Mean distance (cm) apart – the distance (cm) between the centers of gravity of the two animals.
- Percentage of time in proximity – the percentage of time (s) when the distance between the two animals is smaller than 5 cm while in the open field.

2.2.5.3 Paired Social Interaction – Video Analysis. As mentioned, VHS recordings of animals were made while animal pairs were in the open-field being monitored by *EthoVision*. The first 10 minutes of video for each animal was scored using *The Observer* (Noldus Information Technology, Sterling, VA) event recording software while watching the VHS tapes. Additional measures of locomotor activity and social interaction were visually scored. A subset of videos was also scored by an individual blind to treatment conditions. These additional measures are defined below;

- Frequency of approach – the number of times the animal approached their partner
- Frequency of evasive defense – the number of times an animal withdraws its nape from the partner's snout by either leaping, running, or turning away from the partner.
- Frequency of facing defense – the number of times an animal withdraws its nape from a partner's snout by turning to face the partner.
- Percentage of time in physical contact – the time (s) an animal spends touching, its partner divided by the total time in the open field, multiplied by 100.
- Frequency of rearing – the number of times an animal stands on only its hind legs.

2.2.6 Statistical Analysis of Behaviour

All statistical tests were calculated using SPSS 17.0 (SPSS, Inc.) for Windows. Tests were completed using $\alpha = .05$ as the criterion for significant effects.

2.2.6.1 Individual Locomotor Activity, Baseline. One-way analysis of variance (ANOVA), with PPA treatment and sex as the between-subjects variable, was used to analyze group differences of all locomotor variables on the baseline test day.

2.2.6.2 Individual Locomotor Activity & Paired Social Interaction (Automated & Video). Data were analyzed for main effects using a mixed design ANOVA with treatment and sex being the between-subjects factors and test day as the within-subject factor. If a main effect of treatment or sex was found, a Student-Newman-Keuls (S-N-K) post-hoc was carried out using group as the between-subjects factor to reveal specific differences between groups on each test day. All paired social interaction variables scored by video analysis were also scored by an observer blinded to all experimental procedures. Inter-rater reliability statistics were conducted using a Pearson correlation.

Variables that were analyzed slightly differently were time in proximity, and percentage of time in physical contact. For time in proximity, an automated variable, each rat had a value identical to its pair. All of the rats were included in statistical analysis, meaning each value was represented twice (once for each animal in the pair). However, for percentage of time in physical contact, a visually scored variable, each rat had a value that was very similar, but not identical (due to human error during scoring) to its pair. To control for this error, these two similar values were averaged, creating a new value that was given to each animal of the pair. However, unlike the time in

proximity variable, where this identical value was counted twice (once for each animal of the pair), for percentage of time in physical contact, one animal of the pair was eliminated from statistical analysis, thus resulting in an n value half the size of all other variables.

2.3 Results

2.3.1 Baseline

2.3.1.1 Individual Locomotor Activity. There were no significant group differences among the four treatment groups for any of the variables during baseline testing.

2.3.2 Individual Locomotor Activity

There was no effect of drug treatment or sex on the total distance travelled (Fig. 2.2a), frequency of horizontal movements (Fig. 2.2b), or frequency of centre entries (Fig. 2.4a).

For frequency of rearing, a significant main of effect of both drug treatment, $F(1, 24) = 11.336$, $p < .005$, and sex, $F(1, 24) = 5.323$, $p < .05$, was found. Post-hoc analyses revealed significant group differences for frequency of rearing on injection days 8 and 10. On injection day 8 male and female PPA animals exhibited less rearing compared to male PBS animals, and on day 10 male PPA animals exhibited less rearing than female PBS animals (*ps* < .01; Fig. 2.3a).

There was a significant main effect of drug treatment but not sex for frequency of stereotypy, $F(1, 24) = 60.546$, $p < .001$. Post-hoc analyses revealed significant group

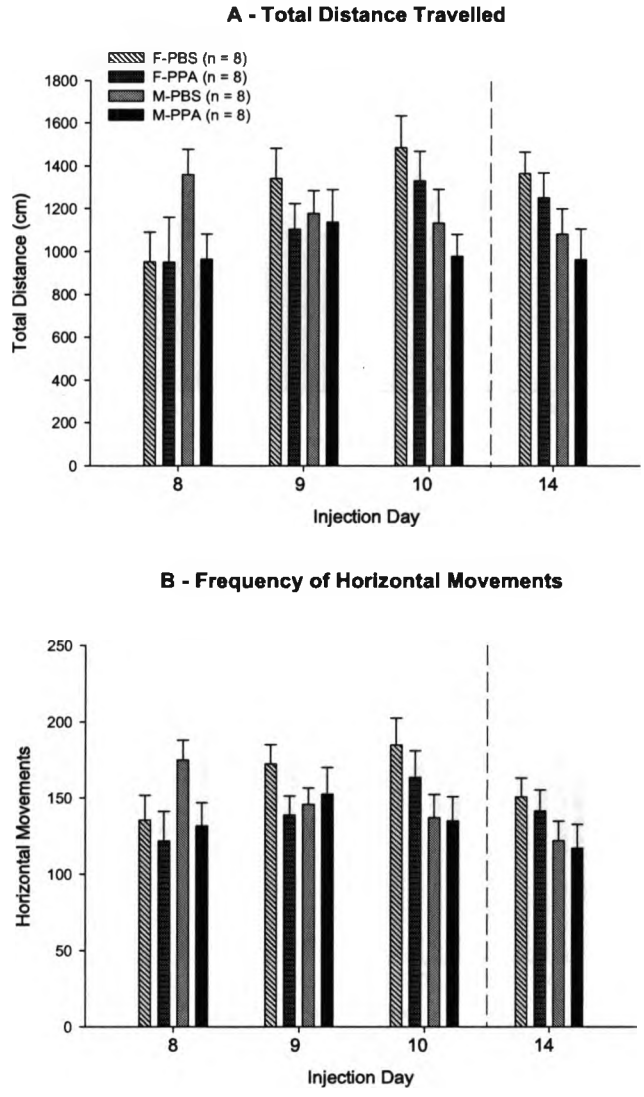

Figure 2.2 Total distance (cm) travelled **(A)** and frequency of horizontal movements **(B)** in an open field across individual locomotor activity test days for female and male adolescent rats, injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean data for the 30 minutes immediately following injection. Error bars represent + SEM. There was no significant main effect of drug or sex found for either variable.

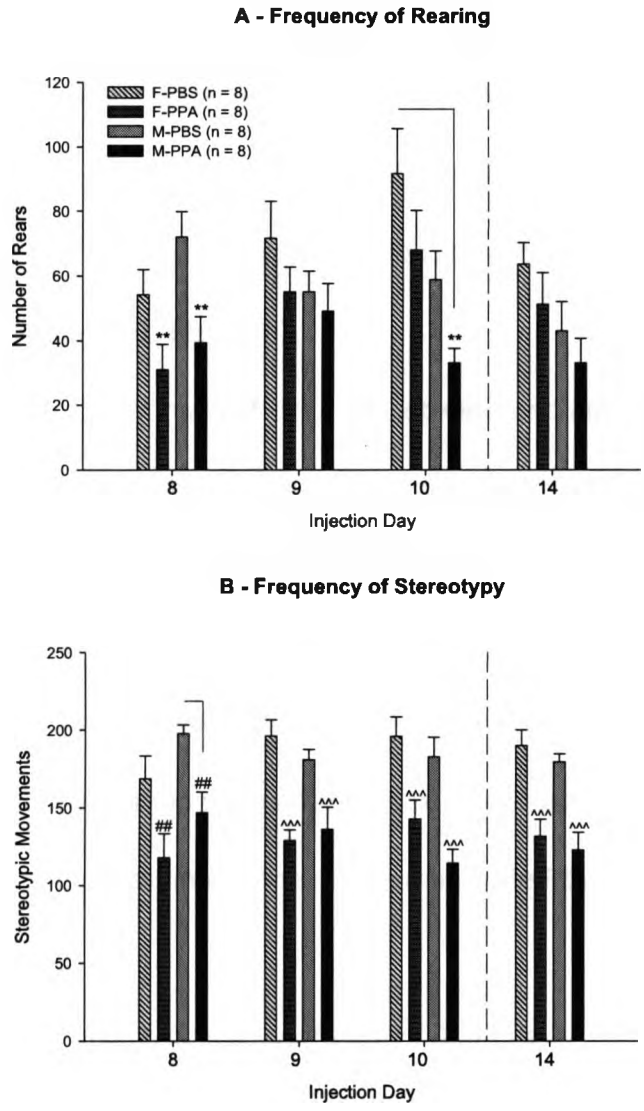

Figure 2.3 Frequency of rearing **(A)** and frequency of stereotypy **(B)** in an open field across individual locomotor activity test days for female and male adolescent rats, injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean data for the 30 minutes immediately following injection. Error bars represent + SEM. ** $p < .01$, female PPA and male PPA significantly fewer than male PBS (unless indicated otherwise). ## $p < .01$, female PPA significantly less than female and male PBS groups (unless indicated otherwise). ^^^ $p < .001$, female and male PPA significantly less than female and male PBS groups.

differences across days. On injection day 8 female PPA animals exhibited less stereotypic behaviour compared to female and male PBS animals ($p< .005$) and male PPA animals exhibited less stereotypic behaviour than male PBS animals. On days 9, 10, and 14 female and male PPA animals exhibited less stereotypic movements than female and male PBS animals ($p<.001$; Fig. 2.3b).

There was a significant main effect of drug treatment, but not sex, for distance (cm) travelled in centre, $F (1, 24) = 4.920$, $p<.05$. Post-hoc analyses revealed significant group differences on day 8, with female PPA animals travelling a smaller distance in the centre than male PBS animals ($p<.05$; Fig. 2.4b).

For time (s) in centre a significant main effect of drug treatment was found, $F (1, 24) = 5.771$, $p<.05$. There was no main effect of sex. Post-hoc analyses revealed that female PPA animals spent less time in the centre than male PBS animals on the eighth injection day ($p<.05$; Fig. 2.4c).

2.3.3 Paired Social Interaction – Automated Analysis

There was no main effect of drug or sex for total distance (cm) travelled (Fig. 2.5a), or frequency of centre entries (Fig. 2.5b).

For time in centre, a main effect of sex, but not drug, was found, $F (1, 28) = 7.050$, $p < .05$. Post-hoc analyses revealed on day 13, male PPA animals averaged a shorter time in the centre than female PBS animals ($p < .05$; Fig 2.5c).

For mean distance apart, a main effect of sex, but not drug, was found, $F (1, 24) = 28.137$, $p <.001$. Post-hoc analyses revealed significant group differences for both days of social testing. On day 12, both female groups (PPA & PBS) averaged a

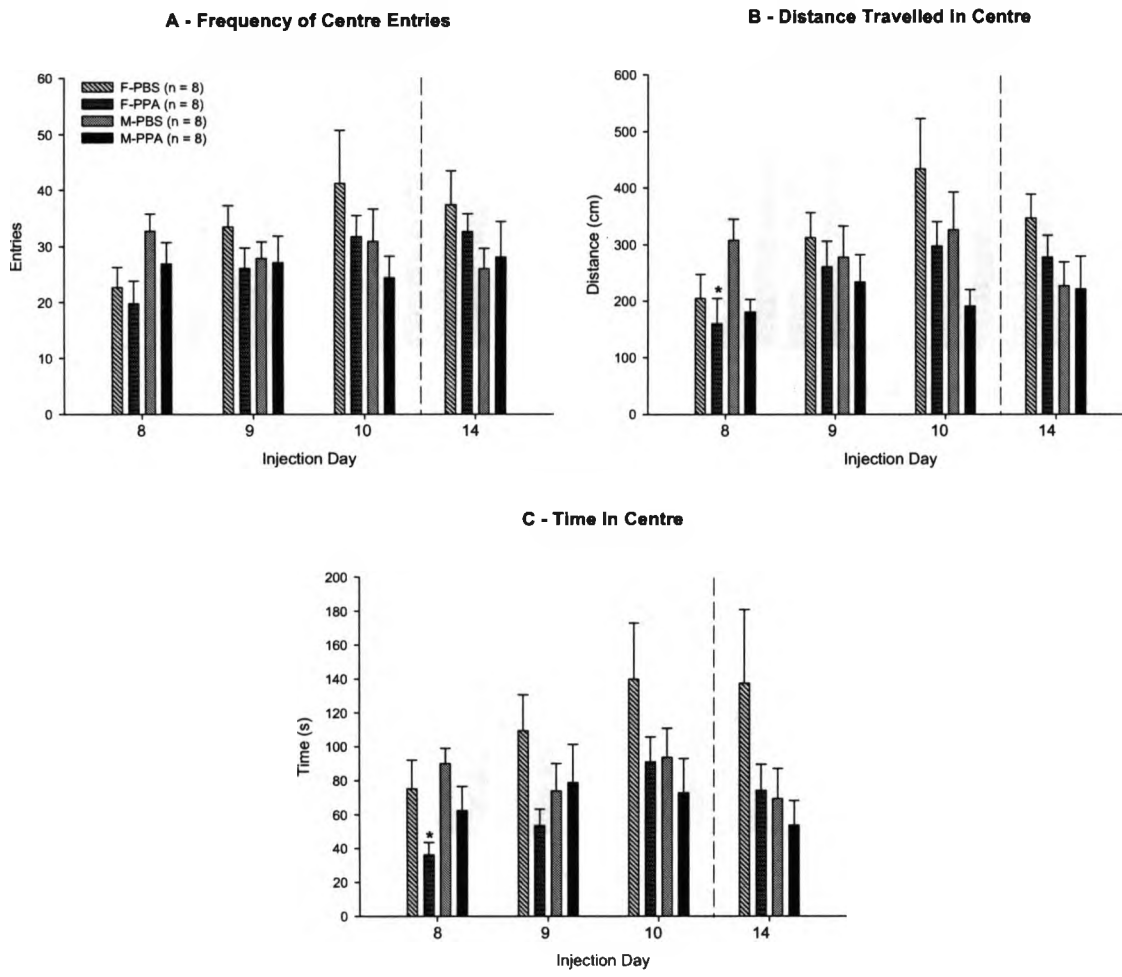

Figure 2.4 Frequency of centre entries **(A)**, distance (cm) travelled in centre **(B)**, and time (s) in centre **(C)** in an open field across individual locomotor activity test days for female and male adolescent rats, injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean data for the 30 minutes immediately following injection. Error bars represent + SEM. * $p < .05$, female PPA less than male PBS.

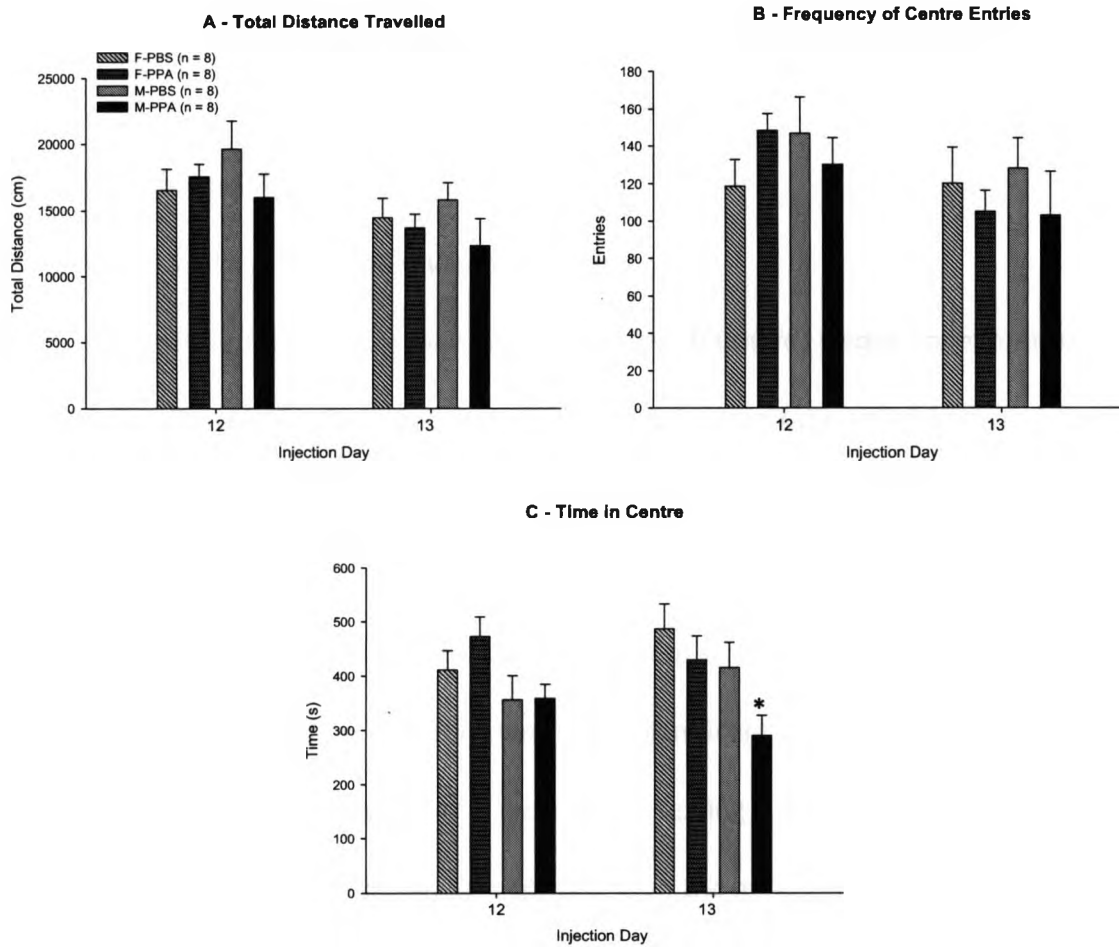

Figure 2.5 Social interaction automated analysis of total distance (cm) travelled **(A)**, frequency of centre entries **(B)**, and time (s) in centre **(C)** in a large open field. Animals were in pairs of female or male adolescent rats and injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean of data for the 30 minutes immediately following injection. Error bars represent + SEM. * $p < .05$, male PPA less than female PBS. No significant group differences in **(A)** and **(B)**.

larger distance apart than male PBS animals. On day 13, female PPA animals averaged a larger distance apart compared to male animals, both PPA & PBS (*ps* < .05; Fig. 2.6a).

For percentage of time in proximity, once again a main effect of sex, but not drug, was found, F (1, 24) = 6.193, $p<.05$. However, post-hoc analyses revealed no significant group differences (Fig. 2.6b).

2.3.4 Paired Social Interaction – Video Analysis

No main effect of drug treatment or sex was found for frequency of approach (Fig.2.8a) or frequency of evasive defense (Fig. 2.8c). Inter-rater reliability was found to be; r (16) = 0.91, $p < .001$ and r (16) = 0.79, $p < .001$, respectively.

For percentage of time in physical contact, the main effect of drug treatment was F (1, 12) = 17.037, $p<.005$ and the main effect of sex was F (1, 12) = 6.371, $p<.05$. The drug by sex interaction was *not* found to be significant, F (1, 12) = 4.501, $p= .055$. Post-hoc analyses revealed on both days of social testing the male PBS group spent a greater percentage of time in physical contact than the male PPA group and the female PPA and PBS groups. ($p<.05$ and $p<.01$ on day 12 & 13, respectively; Fig. 2.7a). Inter-rater reliability was; r (16) = 0.83, $p < .001$.

For frequency of rearing, a main effect of drug treatment, F (1, 28) = 5.270, $p<.05$, and sex, F (1, 28) = 4.804, $p<.05$, was found. Post-hoc analyses, however, revealed no significant group differences (Fig. 2.7b). Inter-rater reliability was found to be; r (16) = 0.66, $p < .01$.

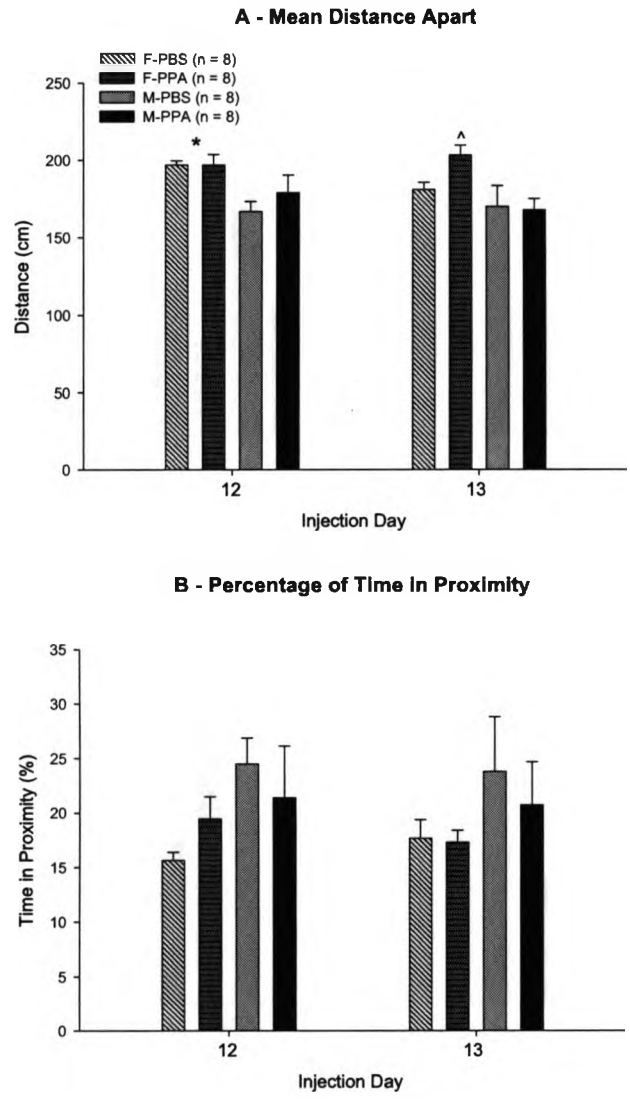

Figure 2.6 Social interaction automated analysis of mean distance (cm) apart **(A)** and percentage of time in proximity **(B)** in a large open field. Animals were in pairs of female or male adolescent rats and injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean of data for the 30 minutes immediately following injection. Error bars represent + SEM. * $p <$ .05, female PPA & PBS greater than male PBS. ^ $p <$ .05, female PPA > male PBS & PPA. No group differences for **(B)**.

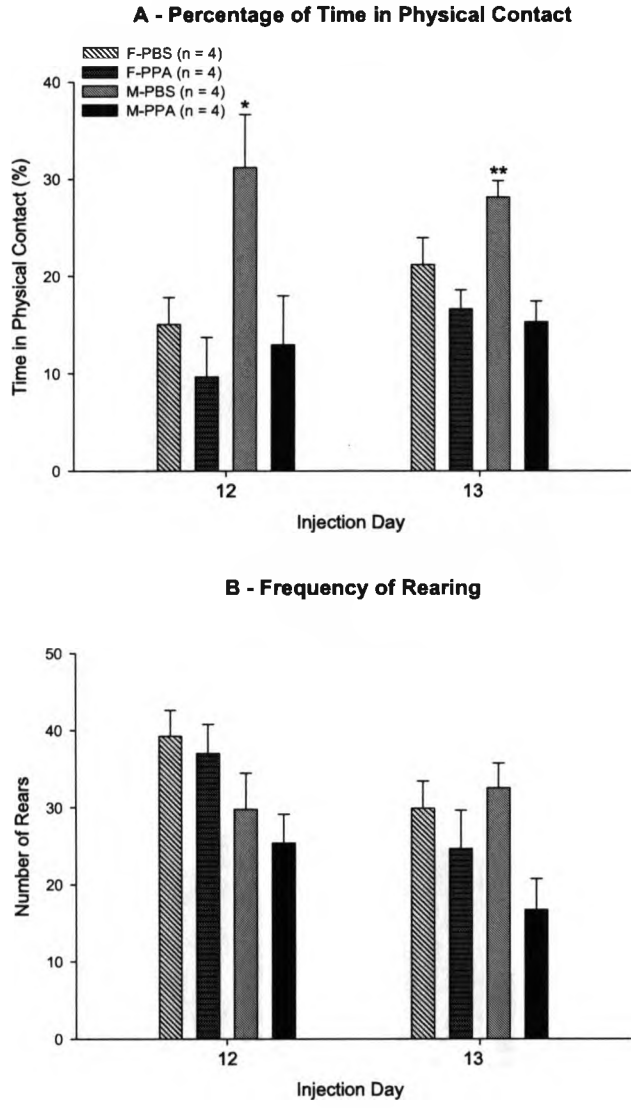

Figure 2.7 Social interaction video analysis of percentage of time in physical contact **(A)** and frequency of rearing **(B)** in a large open field. Animals were in pairs of female or male adolescent rats and injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean of data for the 30 minutes immediately following injection. Error bars represent + SEM. * $p < .05$, ** $p < .01$, all groups less than male PBS. Drug X sex interaction showed a non-significant trend (p = .055). No group differences in **(B)**.

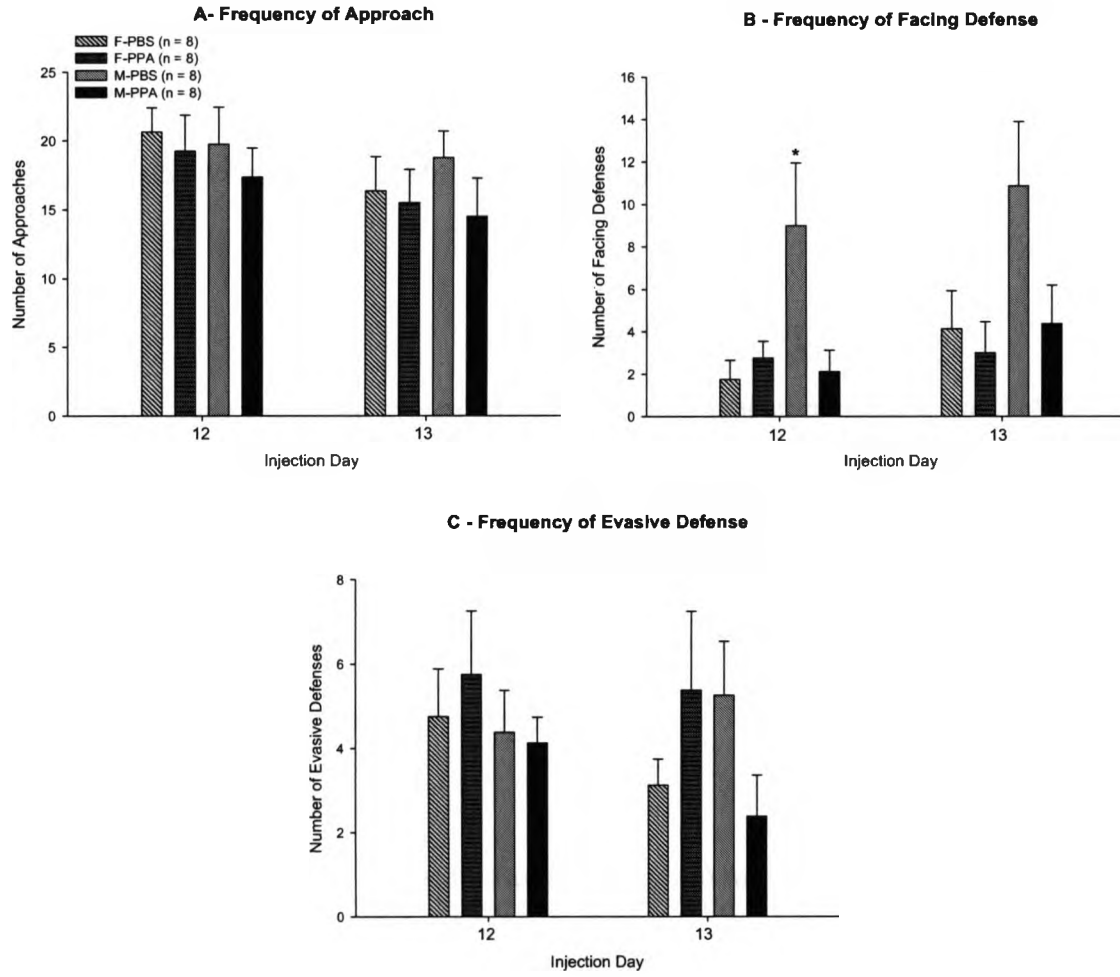

Figure 2.8 Social interaction video analysis of frequency of approach **(A)**, facing defense **(B)** and evasive defense **(C)** in a large open field. Animals were in pairs of female or male adolescent rats and injected (IP) with either PBS (vehicle) or PPA (0.26M). Each bar represents group mean of data for the 30 minutes immediately following injection. Error bars represent + SEM. * $p <$ .05, all groups less than male PBS. Significant drug X sex interaction, $p < .05$. No group differences in **(A)** or **(C)**.

For frequency of facing defense, a main effect of drug treatment, F (1, 28) = 4.595, $p<.05$, and sex, F (1, 28) = 5.485, $p<.05$, was found. There was also a significant drug by sex interaction, F (1, 28) = 5.485, $p<.05$. Post-hoc analyses revealed that on the first day of social testing, all groups performed fewer facing defenses than the male PBS group ($p<.05$ on day 12; Fig. 2.8b). Inter-rater reliability was found to be; r (16) = 0.97, $p < .001$.

2.4 Discussion

This study examined the effects of intraperitoneal administration of PPA on the behaviour of adolescent male and female rats. It was found that PPA treatment resulted in reduced locomotor activity and increased anxiety-related behaviours in both males and females. PPA administration also decreased social interaction in males, while females did not differ significantly from controls.

2.4.1 Locomotor Activity

The variables analyzed for locomotor activity were total distance, stereotypy and rearing. There was no effect of drug treatment or sex for total distance travelled in either the Versamax monitors, where animals were tested individually, or in the large open-field monitored by EthoVision, where animals were tested in pairs. In both Versamax and EthoVision, an effect of drug treatment and sex was found for rearing. However, in EthoVision, post-hoc analyses revealed no group differences. In Versamax, male PPA treated animals were found to rear less than control animals. For stereotypy, which was only measured in Versamax, a strong effect of drug treatment was found. On all test days male and female PPA treated animals exhibited less stereotypic movements

than control animals. The findings from these variables seem to reflect hypoactivity in rats treated with PPA.

Previous studies have reported hyperactivity in response to ICV infusions of PPA (MacFabe, et al., 2007; MacFabe, et al., 2008), while the current study found hypoactivity. This difference in findings may be due to an array of factors. In both the 2007 & 2008 studies by MacFabe, et al., rats received twice daily ICV infusions of PPA for seven days. Locomotor activity testing took place in Versamax on each of the treatment days following the second infusion. This differs from the present study in three important ways; injection and testing schedule, mode of administration, and age of the animals. To begin with, in the current study, rats did not begin behavioural testing until after their second injection on the 8th injection day. Thus, perhaps the effect of PPA on locomotor activity differs according to the amount of PPA exposure over time. Rats may possibly initially present with hyperactivity, but only after extended exposure do rats begin to show hypoactivity. However, this is unlikely as Shams (2009) injected (IP) adolescent and adult male rats daily for seven days and found hypoactivity starting on the first injection day as measured by moving a smaller total distance, spending less time moving, and rearing less. Thus, hypoactivity seen in the current study does not seem to be due to 'loading' of PPA for the seven days before testing. A second way the present study differs from the MacFabe studies of 2007 & 2008 is in mode of administration. In MacFabe's studies PPA was administered centrally, directly into the lateral ventricle of the brain, while in the current study rats received systemic injections into the intraperitoneal cavity. As PPA is a volatile fatty acid located

intracellularly, and rapidly metabolized in the citric acid cycle, the concentration of PPA in the brain is difficult to measure (Leng & Annison, 1963). In fact, no literature has reported the amount of propionic acid in the brains of individuals with propionic acidemia (Brusque, et al., 1999; Hoffmann, Meier-Augenstein, Stocker, Surtees, Rating, & Nyhan, 1993), a genetic disorder where individuals, due to an error in metabolism, produce large amounts of propionic acid and cannot metabolize it (Brusque, et al., 1999). Systemic (IP) administration of PPA requires PPA to gain access to the brain by first passing through the blood-brain barrier (BBB), which is differentially permeable to PPA depending on age. The BBB becomes less permeable to PPA as age increases (Brusque, et al., 1999). Conversely, ICV infusions allow PPA to have direct access to the brain. Thus, it is reasonable to assume that that the concentration reaching the brain following systemic (IP) administration of PPA is different than following ICV infusion of PPA directly into the ventricles of the brain. Consequently, the varying effects of PPA on locomotor activity in ICV and IP studies may be due to a difference in the concentration of PPA reaching the brain during testing. Notably, however, not all ICV experiments have found hyperactivity in PPA treated rats. A study investigating the effects of ICV infusions of PPA on social behaviour (Shultz S. R., et al., 2008) found PPA treated rats to be either the same or slightly hypoactive compared to controls. This was substantially different from other ICV studies as rats only received a total of two infusions, meaning the brain was exposed to less PPA than previous ICV research. This suggests that not only mode of administration, but also dosing and timing, can variably alter the effects of PPA. In fact, a mitochondrial toxin and derivative of PPA used in an animal model of

Huntington's disease, 3-nitropropionic acid (3-NP), has been found to produce both age- and dose-dependent effects on striatal lesions and locomotor activity (Beal, et al., 1993; Borlongan, Koutouzis, Freeman, Hauser, Cahil, & Sanberg, 1997). Beal, et al. (1993) found striatal lesions caused by sub-acute systemic administration of 3-NP were markedly age-dependent, with four month old animals being much more vulnerable than one month old animals. This is notable as all past ICV studies have been conducted in adults, while the current study used adolescents. Furthermore, they also found dose-dependent striatal lesions and reductions in markers for striatal intrinsic neurons (e.g. GABA) and markers for striatal afferents (e.g. dopamine and its metabolites). Borlongan, et al. (1997) found that manipulating the number of intraperitoneal 3-NP injections can result in either increased or decreased locomotor activity. It is possible that PPA exposure could be similar to 3-NP exposure. These studies would then suggest that the effects of PPA on locomotor activity can vary depending on the dosing and testing schedule, as well as the age of the animals. Further dose-dependent studies investigating locomotor behaviour and using both ICV and IP administration of PPA would need to be conducted to establish if this is the case.

Decreased activity, hypotonia, and lethargy are well documented initial symptoms for a subset of autistic patients. Clinical guidelines released in Pediatrics and Neurology (Johnson, Myers, & Disabilities, 2007; Filipek, et al., 2000) give recommendations to doctors for treating autistic patients who present with these additional symptoms. Zwaigenbaum, et al. (2005) investigated the behavioural manifestations of autism in the first year of life for infants considered high risk for developing ASD (have an older sibling

with ASD) and for those considered low risk (do not have a 1st or 2nd degree relative with ASD). They found high risk infants, who were later diagnosed with autism, presented with *decreased* activity level at six months of age. Furthermore, studies have highlighted certain genetic abnormalities in a subset of autistic patients who also present with seizures and/or mitochondrial dysfunction. In these cases children often present with hypotonia and periods of lethargy (Filipek, et al., 2003; Zecavati & Spence, 2009). Also, initial presentation in a variety of metabolic disorders, which result in an increase of PPA, includes hypotonia and autistic features (Knerr, Gibson, Jakobs, & Pearl, 2008; Pearl, et al., 2003; Ching, et al., 2010; Filiano, Goldenthal, Harker Rhodes, & Marin-Garcia, 2002). Thus, perhaps the amount of PPA gaining access to the brain following IP injections of PPA more accurately represent the early stages of ASD, where patients are found to be hypoactive compared to controls. ICV studies, where PPA is being infused directly into the brain, may in turn represent the later hyperactive manifestation of the disorder.

2.4.2 Anxiety

An increase in thigmotaxis, wall hugging behaviour, reflects an increase in anxiety (Treit & Fundytus, 1989). In Versamax, an effect of drug treatment was found for distance travelled in the centre and time in the centre, with PPA treated animals travelling less and spending less time in the centre. Thus, in Versamax, PPA treated animals were found to be more anxious than controls. In EthoVision, no effect of treatment was found for time in the centre, nor was an effect found for number of centre entries in either Versamax or EthoVision. The discrepancy in the findings

between the two types of apparatus could be due to the differing open-field sizes or the fact that animals were tested individually in Versamax and in pairs in EthoVision.

The results of the current study are consistent with past studies using IP injections of PPA. In adolescent male rats, Shams (2009) also found that PPA treated rats displayed more anxiety-like behaviour than controls as PPA treated rats travelled less in the centre and spent less time moving in the centre while in Versamax. Consistent with the current study, this effect was not found in EthoVision. Additionally, Hanstock, et al. (2004) found a fermentable-carbohydrate based diet given to adult rats to result in an increase in propionic acid in the gut as well as increased anxiety-related behaviour, as measured by a light/dark emergence test. In subsequent studies it will be important to look at additional measures of anxiety to get a better indication of what effect PPA may be having.

A study by Skokauskas & Gallagher (2010) investigated the co-morbidity of autism and Asperger's syndrome with psychotic, anxiety and/or mood disorders. They found anxiety disorders to be the most common psychiatric co-morbidity in this population. Thus, the finding that PPA administration can increase anxiety in rats is consistent with what is seen in the human manifestation of autism. Furthermore, Oblak, et al. (2010) found individuals with autism to have abnormalities in their GABAergic (gamma aminobutyric acid) system in that they were found to have significant reductions in $GABA_B$ receptor density (Oblak, Gibbs, & Blatt, 2010). $GABA_B$ receptors play an important role in maintaining the balance of excitation-inhibition in the brain (Oblak, Gibbs, & Blatt, 2010). As an imbalance in this system has been implicated in anxiety

disorders (Bergink, van Megan, & Westenberg, 2004), this may explain increased anxiety seen in autism. However, before drawing any definitive conclusions, other measures of anxiety should be looked at in both the light and dark (i.e. active) period.

2.4.3 Social Interaction

Variables for social behaviour between unfamiliar, same-treatment, same-sex pairs were analyzed by the automated system EthoVision, as well as through the behavioural scoring of videos. Automated variables were mean distance apart and percentage of time spent in proximity, while visually scored variables included the percentage of time in physical contact, frequency of approach and avoidance, as well as frequency of facing and evasive defense. A facing defense occurs when an animal withdraws its nape from a partner's snout by turning to face the partner. An evasive defense occurs when an animal withdraws its nape from the partner's snout by either leaping, running, or turning away from the partner. Both types of defense involve withdrawing the nape area following an 'attack' (when an animal contacts its partner with its snout), but the facing defense blocks access to the animal's nape while allowing it to use its limbs to ward off the partner and engage in play-fighting (Pellis & Pellis, 1997). For the automated analysis in EthoVision, a main effect of sex was found for both mean distance apart and percentage of time in proximity, with female animals spending, on average, a greater distance apart than male animals. Post-hoc analyses, however, revealed no group differences for percentage of time in proximity. Animals were considered to be in proximity to one another when within 5 cm of each other. Thus, proximity does not represent time animals spend in physical contact with one another,

as animals could be in proximity to one another but not interacting in any way. Therefore, a video analysis was conducted to get a more accurate measure of physical contact. The measure of percentage of time spent in physical contact thus ensured that only instances where the animals were interacting would be counted. This explains why percentages for this variable are essentially the same, or slightly lower, than the values for proximity. Analysis of the scored variables revealed no main effect for frequency of approach or evasive defense. A main effect of drug and sex was found for frequency of rearing, frequency of facing defense, and percentage of time in physical contact. Post-hoc analyses revealed no group differences for rearing. However, for facing defense, post-hoc analyses revealed that on both days male and female PPA animals, as well as female PBS animals, made fewer displays of facing defense than the PBS treated male rats. Also, there was a drug by sex interaction for facing defense. That is, male PPA treated rats exhibited this facing behaviour much less than male vehicle treated rats. This difference was not found in the females, with both groups exhibiting little facing behaviour. For time in physical duration, post-hoc analyses revealed that on both test days, male and female PPA animals, as well as female PBS animals, spent less time in physical contact than male PBS treated animals. There was a non-significant trend towards a drug by sex interaction. That is, PPA treated males spent significantly less time in physical contact than PBS treated males. This difference was not seen in females, as the two female groups (PPA and PBS treated) did not significantly differ in time spent in physical contact. This suggests that PPA may possibly affect social behaviour in a sex specific manner. Since this variable is for pairs of animals, there is a

low n-value of four. Further studies should be conducted in order to establish if a drug by sex interaction exists.

An effect of sex for mean distance apart and time in proximity, with females averaging a greater distance apart, spending less time in proximity and physical contact, is an expected finding, as literature shows females engage in social play less often than males (Meaney & Stewart, 1981; Poole & Fish, 1976; Olioff & Stewart, 1978). In turn, this decrease in social play means females may average a greater distance apart and spend less time in proximity and physical contact compared to males. Play-fighting is often considered to be male-typical, as in most species investigated, males engage in play-fighting more often than females (Pellis S. M., Field, Smith, & Pellis, 1997). This type of play is thought to reflect agonistic behavior, with one partner attempting to gain an 'advantage' over the other (Pellis S. M., Field, Smith, & Pellis, 1997). Males initiate play fighting more than females, and females respond less to this initiation, especially if this initiation is by another female (Pellis & Pellis, 1990; Thor & Hollowat, 1983). The current study also demonstrated that control females were less likely than control males to exhibit a facing defense. This too is consistent with prior results, with males being more likely to respond to play initiation by turning supine. Females, however, are more likely to respond, if they respond, by evasion (Pellis & Pellis, 1990). It should also be noted, however, that female rats tend to have better social recognition than males (Choleris, Clipperton-Allen, Phan, & Kavaliers, 2009). Although these were unfamiliar pairs on the first day of testing, by the second test day female rats may have more readily recognized their partner from the previous day, and thus did not spend equal

time investigating and interacting with their partner compared to male animals, which are less adept at social recognition.

The finding that PPA administration alters social interaction in male rats is consistent with past studies. However, this is the first study to look at the effects of PPA administration in female rats. As previously mentioned, Shultz, et al. (2008) looked at the effect of ICV infusions of PPA on social behaviour in adult male rats immediately following infusion. Rats received two infusions, separated by one week, and demonstrated reduced social behaviour as measured by distance apart, proximity, as well as facing and evasive defense. PPA treated rats spent, on average, a greater mean distance apart, less time in proximity to each other, and exhibited less facing defense responses and more evasive defense responses. These findings were the same in both the light and dark phase. Shams (2009) investigated the effect of once daily IP injections of PPA on social behaviour in adult and adolescent male rats. This study also found impaired social behaviour as measured by time spent in physical contact, frequency of approach and frequency of avoidance. PPA treated animals spent less time in physical contact, approached their partner less, and avoided their partner more. Thus, the finding that PPA treatment affects social behaviour in males is consistent with past studies.

The finding that PPA affects social behaviour in a sex-specific manner is a novel finding as the effect of PPA on female behaviour has never been investigated in either adolescent or adult rats. However, in an animal model of autism using valproic acid (VPA), Schneider, et al. (2008) evaluated both male and female rats in a variety of

behavioural and immunological tests following prenatal exposure to VPA. Males behaved abnormally on a variety of tests looking at pain sensitivity, repetitive/stereotypic activity, as well as anxiety and social behaviour. Social behaviour was investigated by pairing a VPA or control animal that had been socially isolated for one week with a non-isolated, untreated, same-sex conspecific. Measures of social behavior for the VPA or control rat were scored, and included frequency of sniffing or licking the conspecific, crawling or mounting (standing on hind legs and putting one or two forepaws on the back of conspecific or climbing over the conspecific), and approaching or following the conspecific. Except for repetitive/stereotypic behaviour, females showed no behavioural alterations compared to controls (Schneider, et al., 2008). Thus, the finding that PPA affects social behaviour in a sex-specific manner is not unexpected based on the valproic acid model, and the fact that autism is four times more prevalent in males.

Abnormal social interaction is one of the hallmark symptoms of autism spectrum disorders (Diagnostic and Statistical Manual of Mental Disorders, 2000). In the current study, time in physical contact, along with other variables, sought to investigate the effect PPA had on social interaction. These variables are consistent with those used in past studies. For example, animal experiments looking at social behaviour have used measures such as number of playful initiations, probability of facing defense, and physical contact (Field, Whishaw, & Pellis, 2006; Witt, Winslow, & Insel, 1992). Furthermore, in studies investigating ASD in humans, researchers have used similar measurements as a measure of social behaviour (Nikopoulos & Keenan, 2003). For

instance, Nikopoulos & Keenan (2003) evaluated the effectiveness of a treatment on social behaviour by monitoring the number of social initiations a child with ASD made. Thus, the way in which social interaction was measured is consistent with other experimental work in animals and humans.

2.4.4 Mechanisms

The mechanism behind PPA's ability to decrease locomotor activity is unknown. One possibility is that PPA is inducing aversive internal cues, whether this is nausea, irritation or pain, or an acute sickness response, etc. Additionally, hypoactivity may be due to decreased energy production as in this animal model PPA administration has been found to alter the expression of genes involved in mitochondrial function, which is where PPA and short chain fatty acids are metabolized, and where energy is produced (Nankova, Tichenoff, Taylor, Mohammad-Asef, La Gamma, & MacFabe, 2008). PPA has also been found to alter acylcarnitine profiles (Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010). Carnitine is required to transport short chain fatty acids from the cytosol into the mitochondria. Studies of individuals with chronic fatigue syndrome (CFS) found those with CFS have low levels of serum acetylcarnitine which well correlated with the rating score of fatigue (Kuratsune, et al., 2002). Thus, one mechanism behind this hypoactivity may be decreased energy production. Lastly, PPA is known to alter cytokine levels and the immune response. Studies looking at the exact nature of these effects are inconsistent. The literature suggests that the effect PPA has on cytokines and the immune response are dependent on a variety of factors including; the concentration of PPA being used, where in the body PPA is being investigated (i.e.

the gut, mouth, brain), if it is being investigated *in vitro* or *in vivo*, or if other short-chain fatty acids (SCFAs) are present (Kashket, Zhang, & Van Houte, 1998; Kurita-Ochiai, Fukushima, & Ochiai, 1995; Cavaglieri, Nishiyama, Fernandes, Curi, Miles, & Calder, 2003). This is important as pro-inflammatory cytokines, for example, are known to elicit the sickness response. This includes behaviours such as; fever, reduction in food and water intake, reduced social and sexual behaviours, and hypoactivity (Aubert, Goodall, & Dantzer, 1995; Avitsur, Donchin, Barak, Cohen, & Yirmiya, 1997; Bluthe R. , Michaud, Poli, & Dantzer, 2000; Engeland, Kavaliers, & Ossenkopp, 2003). It should also be noted that in this PPA animal model of autism, PPA has been found to alter gene expression of immune system related genes and has been found to increase IL-6 in the CNS (Nankova, Tichenoff, Taylor, Mohammad-Asef, La Gamma, & MacFabe, 2008; Foley, et al., 2008). Thus, based on the fact that PPA can cause changes to the immune response and cytokines, it can be speculated that the hypoactivity seen in PPA treated animals may reflect a sickness response.

PPA's capacity to alter cytokine levels may also explain its ability to increase anxiety. Literature suggests that cytokines may be involved in the pathophysiology of anxiety and depression (Konsman, Parnet, & Dantzer, 2002). Some evidence for this comes from the use of immunotherapy. 30-45% of individuals being chronically treated with interleukin-2 (IL-2) or interferon-α (IF-α) for cancer or viral diseases (e.g. Hepatitis C) develop depressed mood, anxiety, anhedonia and cognitive impairments (Valentine, Meyers, Kling, Richelson, & Hauser, 1998; Capuron, Ravaud, & Dantzer, 2000). Furthermore, a study by Maes, et al. (1998) found students with high anxiety responses

before an exam had significantly higher production of IFN-γ and lower production of IL-10 and IL-4 than students without anxiety. These findings, and others, seem to illustrate an association between cytokines and anxiety. However, it has not been determined whether cytokines are causally involved, or simply a biological marker of anxiety. Nonetheless, it is possible that PPA may affect anxiety via its ability to alter cytokine levels.

A second possible mechanism behind PPA's ability to increase anxiety-related behaviour may be its ability to decrease gene expression involved in gamma-aminobutyric acid (GABA) receptor genes (Nankova, Tichenoff, Taylor, Mohammad-Asef, La Gamma, & MacFabe, 2008) and cause seizures (MacFabe, et al., 2007). Indeed, a decrease in inhibition and increase in excitation has been found in anxiety disorders (Bergink, van Megan, & Westenberg, 2004). Anxiolytic drugs function to either decrease excitatory/glutamatergic output or increase inhibitory/GABAergic output in the amygdala (Bergink, van Megan, & Westenberg, 2004). Thus, perhaps PPA is influencing these systems to create increased anxiety behaviour in the rat.

This alteration of cytokines and the inflammatory response may also help to explain PPA's ability to alter social interaction. A variety of studies by Dantzer and colleagues have investigated the effects of sickness inducing agents on social exploration. They found that LPS, IL-1β, and TNF affect social exploration in a time- and dose- dependent manner (Bluthe, Dantzer, & Kelley, 1992; Bluthe, et al., 1994; Bluthe R. M., Michaud, Poli, Bernay, Parnet, & Dantzer, 1998). Furthermore, animals will avoid other sick animals (Kavaliers, Choleris, & Pfaff, 2005). Although not all cytokines have this effect

on activity, perhaps PPA's effects on cytokines can account for the alterations in social behaviour seen here. This is relevant to ASD as many studies have found not only an inflammatory response in the brain, but systemic inflammation (Croonenberghs, Bosmans, Deboutte, Kenis, & Maes, 2002a; Croonenberghs, et al., 2002b; Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004). For instance, Croonenberghs, et al. (2002a) found significantly increased production of IFN-γ and IL-1RA, as well as a trend toward significantly increased levels of IL-6 and TNF-α in children with autism. Researchers concluded that autism may be associated with a specific activation of the immune response, and hypothesized that increased production of these proinflammatory cytokines may play a role in the pathophysiology of autism (Croonenberghs, Bosmans, Deboutte, Kenis, & Maes, 2002a).

A second possible mechanism behind PPA's ability to alter social behaviour comes from findings by Shultz, et al. (2008). They found that PPA and sodium acetate produced similar changes in social behaviour. Although treatments were buffered to a pH of 7.5, this suggests that perhaps the acidic properties of these treatments are important in the effect of PPA on social behaviour.

Furthermore, previous research indicates that PPA and other fatty acids increase 5-HT (serotonin) release in the gut (Mitsui, Ono, Karaki, & Kuwahara, 2005). In the brain, PPA can accumulate intracellularly, thereby directly increasing 5-HT release by the reduction of intracellular pH (Bonnet, Bingmann, & Wiemann, 2000; Karuri, Dobrowsky, & Tannock, 1993; Severson, Wang, Pieribone, Dohle, & Richerson, 2003). This is relevant as work has shown that an increase of 5-HT levels can reduce social interaction.

Gonzalez, et al. (1996) demonstrated that the administration of a 5-HT receptor agonist into the amygdala reduced levels of social interaction, while the administration of a 5-HT antagonist reversed this effect. Thus, by increasing 5-HT levels, PPA can reduce social interaction. Furthermore, this is associated with ASD as studies have found elevated levels of plasma 5-HT in patients (Chugani, 2004). Additionally, medications that act on 5-HT levels have been found to improve a number of autistic symptoms, such as repetitive and social behaviour (McDougle, Naylor, Cohen, Volkmar, Heninger, & Price, 1996).

Of course the fact that PPA is decreasing activity in general, as well as slightly increasing anxiety, could also potentially account for the decrease in social interaction. Indeed, in all the studies by Dantzer's group mentioned above, administration of sickness inducing agents and cytokines resulted in decreased social exploration as well as decreased locomotor activity in general. This may not be the case, however, as Shultz, et al. (2008) found a decrease in social behaviour in the absence of any significant differences in locomotor activity between PPA and control groups.

It is unclear exactly how the effects of PPA on social behaviour are mitigated in females. One possibility is again related to PPA's ability to alter cytokine levels and the immune response. Sex differences, in favour of females, have been found in adult immune function as well the neonatal response to immune activation (Lahita, 2000; Martin, 2000; Shanks, McCormick, & Meaney, 1994). For example, a sex difference, in favour of females, was found for pattern and rate of behavioural tolerance development to repeated administration of bacterial agents muramyl dipeptide (MDP) and LPS

(Engeland, Kavaliers, & Ossenkopp, 2003). Also, immune activation with LPS in the first week of life results in increased disease severity following an immune challenge for males, but not females. For example, LPS administration on postnatal days 1, 3, 5, and 7 resulted in decreased resistance to tumor colonization and lower natural killer cell activity in adult males, but not females (Hodgson & Knott, 2002). Thus, it is possible that the sex difference seen in regard to how PPA affects social behavior is due to the fact that PPA is altering cytokines and the immune response more substantially in males than females.

A second possible mechanism may be the protective effects of estrogen and progesterone. Experimental models have demonstrated females to be less susceptible to postischemic and posttraumatic brain injury (Hall, Braughler, & Yonkers, 1991; Roof, Duvdevani, & Stein, 1993; Roof & Hall, 1999). Studies suggest that this is due to circulating estrogens and progestins (Roof & Hall, 2000). Indeed, exogenous administration of these hormones has led to improved outcome for males from posttraumatic brain injury. Furthermore, estrogen has been found to have considerable antioxidant properties, and to also prevent the degeneration of neurons due to oxidative stress (Behl, et al., 1997). This is significant as repeated ICV infusions of PPA is known to cause increased markers of oxidative stress as well as induce an innate neuroinflammatory response in the brain (MacFabe, et al., 2007), findings consistent with human autopsy studies (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2004; Chauhan & Chauhan, 2006). Thus, perhaps it is estrogen and progesterone that are playing a key role in mitigating PPA induced effects on social behaviour. Estrogen

and progesterone may be attenuating PPA's effects in a variety of ways, as they have been found to enhance anti-oxidant mechanisms, reduce innate neuroinflammation, and decrease excitotoxicity by altering glutamate receptor activity (Roof & Hall, 2000; Garcia-Segura, Azcoitia, & DonCarlos, 2001; Stein, 2001). Furthermore, in a model of Hungtington's using 3-NP, a mitochondrial toxin and derivative of PPA, 17 β-estradiol was found to have many protective effects (Tunez, et al., 2006). In ovariectomized rats, 3-NP was found to exacerbate oxidative stress induced by ovariectomy, while administration of 17 β-estradiol decreased oxidative stress and cell death induced by ovariectomy and 3-NP (Tunez, et al., 2006).

Total cholesterol levels have been found to be low in those with ASDs (Tierney, et al., 2006). Additionally, a rare genetic condition of impaired cholesterol biosynthesis, Smith-Lemli-Opitz syndrome, is associated with autism. Furthermore, colonic short-chain fatty acids, such as PPA, decreases cholesterol in males but not in females (Wolever, Fernandes, & Rao, 1996). In fact, work in this PPA model demonstrated a decrease in plasmologens, which have been suggested to play a role in cholesterol effux (Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010). Thus, perhaps the sex differences in systemic lipid metabolism are responsible for the sexually dimorphic effects of PPA on social behaviour.

2.4.5 Summary

It is important to note some limitations of this study. To begin with, the sample size was limited. Specifically, percentage of time in physical contact, which is perhaps the most informative variable, was a measure using pairs of animals, thus leading to a small

n value of four per group. It is important that this variable be looked at again with a larger sample size. Secondly, only one dose of PPA was used. Further studies using IP injections of PPA should be conducted to gain a better understanding of the effects PPA may have at varying systemic doses. Thirdly, since animals were not behaviourally tested until the 8th day of injections, the initial effects of IP injections on male and female adolescent rats were not characterized. It would be interesting to examine the initial effects of PPA on locomotor activity and determine if hypoactivity is an immediate response, or one that occurs over time. Also, only same-treatment pairs were used in the investigation of social behaviour. Although some work by Shams, et al. (2009) has been done in adolescents looking at both same- and different- treatment pairs, this was only done in males. Thus, it would be interesting to investigate the effects of PPA administration on social behaviour using females in different-treatment pairs as well. Additionally, more measures of social behaviour should be investigated, as the current study focused largely on play-fighting behaviour, which is considered to reflect more agonistic behaviour.

The finding that intraperitoneal injections of PPA elicit hypoactivity, rather than hyperactivity, was unexpected. One possible reason as to why this was found is that systemic administration of PPA induces aversive internal cues. To further examine this, a second experiment using adolescent male rats was conducted and investigated whether conditioning with PPA can establish a conditioned place and taste avoidance for a context or taste previously paired with PPA. This study also attempted to establish if IP injections of PPA elicit hypoactivity from the initial injection onward.

In conclusion, this first study provides information on the effects of IP injections in adolescent male and female rats in terms of locomotor activity, anxiety-related behaviour, and social interaction. Results show that such injections result in hypoactivity, slight anxiety, and sex-specific impairments in social interaction. The findings of this study are consistent with ASD, and offer further support of PPA administration as an animal model of autism.

2.5 References

Arndt, T. L., Stodgell, C. J., & Rodier, P. M. (2005). The teratology of autism. International Journal of Developmental Neuroscience , 23, 189-199.

Aubert, A., Goodall, G., & Dantzer, R. (1995). Compared effects of cold ambient temperature and cytokines on macronutrient intake in rats. Physiology and Behaviour , 57, 869-873.

Avitsur, R., Donchin, O., Barak, O., Cohen, E., & Yirmiya, R. (1997). Different receptor mechanisms mediate the effects of endotoxin and interleukin-1 on female sexual behaviour. Brain Research , 773, 149-161.

Beal, M. F., Brouillet, E., Jenkins, B. G., Ferrante, R. J., Kowall, N. W., Miller, J. M., et al. (1993). Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitopropionic acid. The Journal of Neuroscience , 12 (10), 4181-4192.

Behl, C., Skutella, T., Lezoualc'h, F., Post, A., Widmann, M., Newton, C. J., et al. (1997). Neuroprotection against oxidative stress by estrogens; Structure-activity relationship. Molecular Pharmacology , 51, 535-541.

Bergink, V., van Megan, H. J., & Westenberg, H. G. (2004). Glutamate and Anxiety. European Neuropsychopharmacology , 14, 175-183.

Bluthe, R. M., Dantzer, R., & Kelley, K. W. (1992). Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat. Brain Research , 573, 318-320.

Bluthe, R. M., Michaud, B., Poli, V., Bernay, F., Parnet, P., & Dantzer, R. (1998). Interleukin-6 is active only in presence of other proinflammatory cytokines to induce sickness behavior. Neuroimmunomodulation , 5, 67.

Bluthe, R. M., Pawlowski, M., Suarez, S., Parnet, P., Pittman, Q., Kelley, K. W., et al. (1994). Synergy between tumor necrosis factor a and interleukin-1 in the induction of sickness behavior. Psychoneuroendocrinology , 19, 197-207.

Bluthe, R., Michaud, B., Poli, V., & Dantzer, R. (2000). Role of IL-6 in cytokine-induced sickness behaviour: a study with IL-6 deficient mice. Physiology and Behaviour , 70, 367-373.

Bonnet, U., Bingmann, D., & Wiemann, M. (2000). Intracellular pH modulates spontaneous and epileptiform bioelectric cell coupling of hippocampal CA3-neurons. European Neuropsychophamacology , 10, 97-103.

Borlongan, C. V., Koutouzis, T. K., Freeman, T. B., Hauser, R. A., Cahil, D. W., & Sanberg, P. R. (1997). Hyperactivity and hypoactivity in a rat model of Huntington's disease: the systemic 3-nitropropionc acid model. Brain Research Protocols , 1, 253-257.

Brusque, A. M., Mello, C. F., Buchanan, D. N., Terracciano, S. T., Rocha, M. P., Vargas, C. R., et al. (1999). Effect of chemically induced propionic acidemia on neurobehavioral development of rats. Pharmacology Biochemistry and Behavior , 34 (3), 529-534.

Capuron, L., Ravaud, A., & Dantzer, R. (2000). Early depressive symptoms in cancer patients receiving interleukin 2 and /or interferon alpha. Journal of Clinical Oncology , 18, 2143-2151.

Cavaglieri, C. R., Nishiyama, A., Fernandes, L. C., Curi, R., Miles, E. A., & Calder, P. C. (2003). Differential effects of short-chain fatty acids on proliferation and production of pro- and anti-inflammatroy cytokines by cultured lymphocytes. Life Sciences , 73, 1683-1690.

Centers for Disease Control & Prevention. (2009). Prevalence of Autism Spectrum Disorders - Autism & Developmental Disabilities Monitoring Network. MMWR Surveillance Summaries , 58 (SS-10), 1-20.

Chauhan, A., & Chauhan, V. (2006). Oxidative stress in autism. Pathophysiology , 13, 171-181.

Ching, M. S., Shen, Y., Tan, W. H., Jeste, S. S., Morrow, E. M., Chen, X., et al. (2010). Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics , 153B (4), 937-47.

Choleris, E., Clipperton-Allen, A. E., Phan, A., & Kavaliers, M. (2009). Neuroendocrinology of social information processing in rats and mice. Frontiers in Neuroendocrinology , 30, 442-459.

Chugani, D. C. (2004). Serotonin in autism and pediatric epilepsies. Mental Retardation and Developmental Disabilities Research Review , 10, 112-116.

Croonenberghs, J., Bosmans, E., Deboutte, D., Kenis, G., & Maes, M. (2002a). Activation of the inflammatory response system in autism. Neuropsychobiology , 45, 1-6.

Croonenberghs, J., Wauters, A., Devreese, K., Verkerk, R., Scharpe, S., Bosmans, E., et al. (2002b). Increased serum albumin, y globulin, immunoglobulin IgG, and IgG2 and IgG4 in autism. Psychological Medicine , 32 (8), 1457-1463.

Diagnostic and Statistical Manual of Mental Disorders (4th ed.). (2000). Washington DC: American Psychiatric Association.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S. R., Schmitz, C., et al. (2006). The developmental neurobiology of autism spectrum disorder. The Journal of Neuroscience , 62, 6897-6906.

Engeland, C., Kavaliers, M., & Ossenkopp, K.-P. (2003). Sex differences in the effects of muramyl dipeptide and lipopolysaccharide on locomotor activity and the development of behavioural tolerance in rats. Pharmacology Biochemistry and Behaviour , 74, 433-447.

Fallon, J. (2005). Could one of the most widely prescribed antibiotics amoxicillin/clavulanate "augmentin" be a risk factor for autism? Medical Hypotheses , 64, 312-315.

Fatemi, S. H., Pearce, D. A., Brooks, A. I., & Sidwell, R. W. (2005). Prenatal viral infection in mouse causes differential expression of genes in brains of mouse progeny: a potential animal model for schizophrenia and autism. Synapse , 11, 91-99.

Field, E. F., Whishaw, I. Q., & Pellis, S. M. (2006). Play fighting in androgen-insensitive tfm rats: Evidence that androgen receptors are necessary for the development of adult playful attack and defense. Developmental Psychobiology , 48, 111-120.

Filiano, J. J., Goldenthal, M. J., Harker Rhodes, C., & Marin-Garcia, J. (2002). Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. Journal of Child Neurology , 17 (6), 435-439.

Filipek, P. A., Juranek, J., Smith, M., Mays, L. Z., Ramos, E. R., Bocian, M., et al. (2003). Mitochondrial Dysfunction in Autistic Patients with 15q Inverted Duplication. Annals of Neurology , 53 (6), 801-804.

Filipek, P., Accardo, P., Ashwal, S., Ashwal, G., Baranek, G., Cook, E., et al. (2000). Practice parameter: Screening and diagnosis of autism: Report of the Quality

Standards Subcommittee of the American Academy of Neuroloy and the Child Neurology Society. Neurology , 468-479.

Finegold, S. M., Molitoris, D., Song, Y., Liu, C., Vaisanen, M.-L., Bolte, E., et al. (2002). Gastrointestinal microflora studies in late-onset autism. Clinical Infectious Diseases , 35, S6-S16.

Finegold, S. M., Song, Y., & Liu, C. (2002). Taxonomy-general comments and update on taxonomy of Clostridia and Anaerobic cocci. Anaerobe , 8, 283-285.

Foley, K. A., Gordon, M. M., Taylor, A. R., Boon, F., Tichenoff, L. J., Ossenkopp, K.-P., et al. (2008). Intraventricular infusions of propionic acid increases locomotor activity, neuroinflammation, and monocarboxylate transporter immunoreactivity in rats: spaced vs chronic administration. 2008 International Meeting for Autism Research Program (p. Program No. 157.4). London, UK: International Society for Autism Research, Online.

Garcia-Segura, L. M., Azcoitia, I., & DonCarlos, L. L. (2001). Neuroprotection by estradiol. Progress in Neurobiology , 63, 29-60.

Gonzalez, L. E., Andrews, N., & File, S. E. (1996). 5-HT1A and benzodiazepine receptors in the basolateral amygdala modulate anxiety in the social interaction text, but not in the elevated plus-maze. Brain Research , 732, 145-153.

Hall, E. D., Braughler, J. M., & Yonkers, P. A. (1991). U-78517F: a potent inhibitor of lipid peroxidation with activity in experimental brain injury and ischemia. Journal of Pharmacology and Experimental Therapeutics , 258, 688-694.

Hodgson, D. M., & Knott, B. (2002). Potentiation of tumor metastasis in adulthood by neonatal endotoxin exposure: sex differences. Psychoneuroendocrinology , 27, 791-804.

Hoffmann, G. F., Meier-Augenstein, W., Stocker, S., Surtees, R., Rating, D., & Nyhan, W. L. (1993). Physiology and pathophysiology of organic acids in cerebrospinal fluid. Journal of Inherited Metabolic Disease , 16, 648-669.

Horvath, K., Papadimitriou, J. C., Rabsztyn, A., Drachenberg, C., & Tildon, J. T. (1999). Gastrointestinal abnormalities in children with autistic disorder. Journal of Pediatrics , 135, 559-563.

Hu, V. W., Frank, B. C., Heine, S., Lee, N. H., & Quackenbush, J. (2006). Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in

severity of autism reveals differential regulation of neurologically relevant genes. BMC Genomics , 7, 118.

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.

Johnson, C., Myers, S., & Disabilities, C. o. (2007). Identification and Evaluation of Children with Autism Spectrum Disorders. Pediatrics , 120 (5), 1183-1215.

Jyonouchi, H., Sun, S., & Itokazu, N. (2002). Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. Neuropsychobiology , 46, 76-84.

Kamitani, W., Ono, E., & Yoshino, S. (2003). Glial expression of Borna disease virus phosphoprotein induces behavioral and neurological abnormalities in transgenic mice. Proceedings of the National Academy of Sciences USA , 100, 8969-8974.

Karuri, A. R., Dobrowsky, E., & Tannock, I. F. (1993). Selective cellular acidification and toxicity of weak organic acids in an acidic microenvironment. British Journal of Cancer , 68, 1080-1087.

Kashket, S., Zhang, J., & Van Houte, J. (1998). Gingival inflammation induced by food and short-chain carboxylic acids. Journal of Dental Research , 77 (2), 412-417.

Kavaliers, M., Choleris, E., & Pfaff, D. W. (2005). Genes, odours and the recognition of parasitized individuals by rodents. Trends in Parasitology , 21 (9), 423-429.

King, M., & Bearman, P. (2009). Diagnostic change and the increased prevalence of autism. International Journal of Epidemiology , 38 (5), 1224-1234.

Knerr, I., Gibson, K. M., Jakobs, C., & Pearl, P. L. (2008). Neuropsychiatric morbidity in adolescent and adult succinic semialdehyde dehydrogenase deficiency patients. CNS Spectrums , 13 (7), 598-605.

Konsman, J. P., Parnet, P., & Dantzer, R. (2002). Cytokine-induced sickness behaviour: mechanisms and implications. Trends in Neuroscience , 25 (3), 154-159.

Kuratsune, H., Yamaguti, K., Lindh, G., Evengard, B., Hagberg, G., Matsumura, K., et al. (2002). Brain regions involved in fatigue senstation; Reduced acetylcarnitine uptake into the brain. NeuroImage , 17, 1256-1265.

Kurita-Ochiai, T., Fukushima, K., & Ochiai, K. (1995). Volatile fatty acids, metabolic by-products of periodontopathic bacteria, inhibit lymphocyte proliferation and cytokine production. Journal of Dental Research , 74 (7), 1367-1373.

Lahita, R. G. (2000). Gender and the immune system. Journal of Gender-Specific Medicine , 3, 19-22.

Lazar, N., Rajakumar, N., & Cain, D. P. (2008). Injections of NFG into neonatal frontal cortex decreases social interaction as adults: a rat model of schizophrenia. Schizophrenia Bulletin , 34, 127-136.

Leng, R. A., Annison, E. F. (1963). Metabolism of acetate, propionate and butyrate by sheep-liver slices. Biochemical Journal, 86, 319-327.

MacFabe, D., Cain, D., Rodriguez-Capote, K., Franklin, A., Hoffman, J., Boon, F., et al. (2007). Neurobiological effects of intraventicular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behavioural Brain Research , 176, 149-169.

MacFabe, D., Rodriguez-Capote, K., Hoffman, J., Franklin, A., Mohammad-Asef, Y., Taylor, R., et al. (2008). A novel rodent model of autism: intraventricular infusions of propionic acid increase locomotor activity and induce neuroinflammation and oxidative stress in discrete regions of adult rat brain. American Journal of Biochemistry and Biotechnology , 4 (2), 146-166.

Maes, M., Song, C., Lin, A., De Jongh, R., Van Gastel, A., Kenis, G., et al. (1998). The effects of psychological stress on humans: Increased production of pro-inflammatory cyokines and a Th1-like response in stress-induced anxiety. Cytokine , 10 (4), 313-318.

Martin, J. T. (2000). Sexual dimorphism in immune function: the role of prenatal exposure to androgens and estrogens. European Journal of Pharmacology , 405, 251-261.

Maurer, M. H., Canis, M., Kuschinsky, W., & Duelli, R. (2004). Correlation between local monocarboxylate transporter 1 (MCT1) and glucose transporter 1 (GLUT1) densities in the adult rat brain. Neuroscience Letters , 355, 105-108.

McDougle, C. J., Naylor, S. T., Cohen, D. J., Volkmar, F. R., Heninger, G. R., & Price, L. H. (1996). A double-blind, placebo-controlled study of fluvoxamine in adults with autistic disorder. Archives of General Psychiatry , 53, 1001-1008.

Meaney, M. J., & Stewart, J. (1981). A descriptive study of social decelopment in the rat (Rattus norvegicus). Animal Behavior , 29, 34-45.

Mitsui, R., Ono, S., Karaki, S., & Kuwahara, A. (2005). Neural and non-neural mediation of propionate-induced contractile responses in rat distal colon. Neurogastroenterology and Motility , 17, 585-594.

Nankova, B. B., Tichenoff, L., Taylor, A. R., Mohammad-Asef, Y., La Gamma, E. F., & MacFabe, D. F. (2008). Intracerebroventricular infusions of propionic and butyric acid induce behavioural, neuropathological and epigenetic changes in rats - possible relevance to autism spectrum disorders. International Meeting for Autism Research. London, UK: International Society for Autism Research.

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.

Newschaffer, C. J., Falb, M. D., & Gurney, J. G. (2005). National autism prevalence trends from United States special education data. Pediatrics , 115 (3), e277-282.

Niederman, R., Zhang, J., & Kashket, S. (1997). Short-chain carboxylic-acid-stimulated, PMN-mediated gingival inflammation. Critical Reviews in Oral Biology & Medicine , 8, 269-290.

Nikopoulos, C. K., & Keenan, M. (2003). Promoting social initiation in children with autism using video monitoring. Behavioural Interventions , 18, 87-108.

Oblak, A. L., Gibbs, T. T., & Blatt, G. J. (2010). Decreased GABAb receptors in the cingulate cortex and fusiform gyrus in autism. Journal of Neurochemistry , Online.

Olioff, M., & Stewart, J. (1978). Sex differences in the play behavior of prepubescent rats. Pysiology and Behavior , 20, 113-115.

Ossenkopp, K. P., & Kavaliers, M. (1996). Measuring spontaneous locomotor activity in small mammals. In K. P. Ossenkopp, M. Kavaliers, & R. R. Sanberg, Measuring movement & locomotion: from invertebrates to humans (pp. 33-59). Austin, Texas: RG Landes Company.

Pearl, P. L., gibson, K. M., Acosta, M. T., Vezina, L. G., Theodore, W. H., Rogawski, M. A., et al. (2003). CLinical spectrum of succinic semialdehyde dehydrogenase deficiency. Neurology , 60 (9), 1413-1417.

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.

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

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 Behavioral Reviews , 21 (1), 105-120.

Pletnikov, M. V., Rubin, S. A., Vasudevan, K., Moran, T. H., & Carbone, K. M. (1999). Developmental brain injury associated with abnormal play behavior in neonatally Borna disease virus-infected Lewis rats: a model of autism. Behavioral Brain Research , 100, 43-50.

Poole, T., & Fish, J. (1976). Investigation of individual, age and sexual differences in play of Rattus norvigicus (Mammalia: Rodentia). Journal of Zoology , 176, 249-260.

Roof, R. L., & Hall, E. D. (1999). Gender difference in survival and cerebral blood flow following impact-acceleration head injury in rats. Journal of Neurotrauma , 16, 988.

Roof, R. L., & Hall, E. D. (2000). Gender differences in acute CNS trauma and stoke: neuroprotective effects of estrogen and progesterone. Journal of Neurotrauma , 17, 367-388.

Roof, R. L., Duvdevani, R., & Stein, D. G. (1993). Gender influences outcome of brain injury: progesterone plays a protective role. Brain Research , 607, 333-336.

Rutter, M. (2005). Incidence of autism spectrum disorders: changes over time and their meaning. Acta Paediatrica , 94 (1), 2-15.

Schneider, T., Roman, A., Basta-Kaim, A., Kulbera, M., Budziszewska, B., Schneider, K., et al. (2008). Gender-specific behavioural and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. Psychoeuroendocrinology , 33, 728-740.

Severson, C. A., Wang, W., Pieribone, V. A., Dohle, C. I., & Richerson, G. B. (2003). Midbrain serotonergic receptor neurons are central pH chemoreceptors. Nature Neuroscience , 6, 1139-1140.

Shams, S. (2009). The effects of intraperitoneal injections of propionic acid on social interaction, anxiety-related behavior and locomotor activity of male rats; Support for an animal model of autism. Ontario, London: University of Western Ontario.

Shanks, N., McCormick, C. M., & Meaney, M. J. (1994). Sex differences in hypothalamic-pituitary-adrenal responding to endotocin challenge in the neonate: reversal by gonadectomy. Developmental Brain Research , 79, 260-266.

Shultz, S. R., MacFabe, D. F., Ossenkopp, K.-P., Scratch, S., Whelan, J., Taylor, R., et al. (2008). Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behaviour in the rat: Implications for an animal model of autism. Neuropharmacology , 54, 901-911.

Shultz, S. R., MacFabe, D. K., Martin, S., Jackson, J., Taylor, R., Boon, F., et al. (2009). Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. Behavioral Brain Research , 200, 33-41.

Skokauskas, N., & Gallagher, L. (2010). Psychosis, affective disorders and anxiety in autism spectrum disorder: prevalence and nosological considerations. Psychopathology , 43 (1), 8-16.

Stein, D. (2001). Brain damage, sex hormones and recovery: a new role for progesterone and estrogen? Trends in Neuroscience , 24, 386-391.

Thomas, R. H., Foley, K. A., Mepham, J. R., Tichenoff, L. J., Possmayer, F., & MacFabe, D. F. (2010). Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders. Journal of Neurochemistry , 113 (2), 515-529.

Thor, D. H., & Hollowat, W. R. (1983). Play solicitation behavior in juvenile male and female rats. Animal Learning and Behavior , 11, 173-178.

Tierney, E., Bukelis, I., Thompson, R. E., Ahmed, K., Aneja, A., Kratz, L., et al. (2006). Abnormalities of cholesterol metabolism in autism spectrum disorders. American Journal of Medical Genetics Part B (Neuropsychiatric Genetics) , 141B, 666-668.

Treit, D., & Fundytus, M. (1989). Thigmotaxis as a test for anxiolytic activity in rats. Pharmacology Biochemistry and Behavior , 31, 959-962.

Tunez, I., Collado, J. A., Medina, F. J., Pena, J., Munoz, M. d., Jimena, I., et al. (2006). 17 B-Estradiol may affect vulnerability of striatum in a 3-nitropropionic acid-induced experimental model of Huntington's disease in ovariectomized rats. Neurochemistry International , 48, 363-373.

Valentine, A. D., Meyers, C. A., Kling, M. A., Richelson, E., & Hauser, P. (1998). Mood and cognitive side effects of interferon alpha therapy. Seminars in Oncology , 25 (1), 39-47.

Vargas, D. L., Nascimbene, C., Krishnan, C., Zimmerman, A. W., & Pardo, C. A. (2004). Neuroglial activation and neuroinflammation in the brain of patients with autism. Annals of Neurology , 57, 67-81.

Witt, D. M., Winslow, J. T., & Insel, T. R. (1992). Enhanced social interactions in rats following chronic, centrally infused oxytocin. Pharmacology Biochemistry and Behavior , 43, 855-861.

Wolever, T. M., Fernandes, J., & Rao, A. V. (1996). Serum acetate: propionate ratio is related to serum cholesterol in men but not in women. Journal of Nutrition , 126, 2790-2797.

Zecavati, N., & Spence, S. (2009). Neurometabolic Disorders and Dysfunction in Autism Spectrum Disorders. Current Neurology and Neuroscience Reports , 9, 129-136.

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Bejavioral manifestations of autism in the first year of life. International Journal of Developmental Neuroscience , 23, 143-152.

Chapter 3:

Using Place & Taste Avoidance to Investigate Aversive Internal Cues Produced by Intraperitoneal Injections of Propionic Acid in Male Adolescent Rats

3.1 Introduction

Autism spectrum disorder is a family of neurodevelopmental lifelong disorders characterized by social impairment , altered motor activity, restricted interests, and language problems (Arndt, Stodgell, & Rodier, 2005; DiCicco-Bloom, et al., 2006).

Incomplete concordance rates amongst monozygotic twins, as well as variance in the severity of autism in monozygotic twins, suggest environmental factors may play a role in ASD (Hu, Frank, Heine, Lee, & Quackenbush, 2006). Furthermore, research has established a variety of environmental agents that may contribute to the development of autism including postnatal exposure to valproic acid (Ingram, Peckham, Tisdale, & Rodier, 2000), ethanol (Arndt, Stodgell, & Rodier, 2005), and thalidomide (Narita, Kato, Tazoe, Miyazaki, Narita, & Okado, 2002). Thus, many animal models now aim to study how these environmental agents play a role in the development of autism (Ingram, Peckham, Tisdale, & Rodier, 2000; Narita, Kato, Tazoe, Miyazaki, Narita, & Okado, 2002; Kamitani, Ono, & Yoshino, 2003; Fatemi, Pearce, Brooks, & Sidwell, 2005).

One such model involves the administration of propionic acid, a short-chain fatty acid, in the rat (MacFabe, et al., 2007; MacFabe, et al., 2008; Shultz S. R., et al., 2008; Shultz S. R., et al., 2009). This model hypothesizes that due to PPA's ability to affect a variety of physiological processes in the body, it may provide a link among the disparate behavioural, dietary, gut, metabolic and immune factors in autism. ICV infusions of PPA directly into the lateral cerebral ventricles of adult male rats have induced behavioural, biochemical, neuropathological and electrophysiological effects consistent with those seen in ASD (MacFabe, et al., 2007; MacFabe, et al., 2008; Shultz S. R., et al., 2008;

Shultz S. R., et al., 2009; Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010).

In a previous study, Benzaquen, et al. (2010), investigated the effects of systemic IP injections of PPA on locomotor activity and social behaviour in adolescent male and female rats. One significant finding of that study was hypoactivity in some measures of locomotor behaviour. This was interesting as all studies that had been done using ICV infusions of PPA had found hyperactivity. Benzaquen, et al. (2010) speculated that animals were hypoactive due to PPA inducing aversive internal cues, whether this is nausea, irritation or pain, or an acute sickness response. The aim of the current study was to determine if IP injections of PPA were in fact inducing aversive internal cues using a place and taste avoidance paradigm. Conditioned place avoidance is a widely accepted measure of aversive states in rodents (Cunningham, Gremel, & Groblewski, 2006). Place avoidance paradigms typically involve conditioning trials where on alternating days, either the drug in question is paired with a distinct context or a vehicle is paired with a distinct context on alternating days. During this time the animal is conditioned to form an association between the effects of the injected substance (i.e. drug or vehicle) and the context. The animal is then assessed in a drug-free state to determine if a conditioned place avoidance (CPA) or preference (CPP) has formed. If the animal avoids the context that was previously paired with the drug, then it is reasoned that the drug in question induced a CPA and thus has some kind of aversive property. In the same vein, a conditioned taste avoidance paradigm traditionally involves conditioning trials where animals drink from a bottle containing a novel taste or flavor

shortly before receiving an injection of the drug in question. Once again, in a drug-free state, avoidance of this flavour in a two-bottle taste test implies that the drug in question has aversive properties. In the current studies, however, rats received an injection of PPA and saccharin (to provide a novel taste) simultaneously, allowing place and taste conditioning to occur simultaneously using the concept of 'intravascular taste'. Lithium chloride (LiCl) was used as a positive control, as it is a well known toxin, and has been repeatedly shown to elicit conditioned place and taste avoidance (Tenk, Kavaliers, & Ossenkopp, 2005). Thus, it was used to demonstrate that a conditioned place and taste avoidance (CPA/CTA) can, in fact, be simultaneously produced in the same animal using intravascular taste. Establishing if PPA does induce aversive internal cues when injected intraperitoneally is important as finding a systemic mode of administration is one key aspect of this animal model. Furthermore, by monitoring locomotor activity across conditioning days, the time course of PPA's effects can be determined.

The present experiment investigated whether IP injection of PPA induces aversive internal cues using a place and taste avoidance paradigm. Hence, animals were investigated immediately following an injection of PPA or vehicle to identify abnormalities in locomotor behaviour, as well as in a drug-free state to elucidate the presence of a conditioned place or taste avoidance. This also allowed for the examination of unconditioned versus conditioned effects of PPA.

3.2 Methods

3.2.1 Subjects

Subjects were 48 male Long-Evans hooded rats (Charles River Laboratories, Quebec, Canada) who arrived on postnatal day (PND) 23. Rats were housed in groups of four until PND 26 when groups were split and housed in pairs. Rats were single housed on PND 41 for three days. This coincided with the conclusion of injections and the drug-free place avoidance test day, and lasted until the end of the taste aversion test day, which concluded the study. Animals were housed in standard acrylic cages (26 cm x 48 cam x 21 cm) with wood shavings at a controlled temperature (21 ± 1°C) in a 12:12 light/dark cycle with lights on from 7:00 to 19:00h. Animals were allowed access to food (Rat Chow) and water *ad libitum*. All experimental procedures were carried out in accordance with guidelines of the Canadian Council on Animal Care and approved by the University of Western Ontario Animal Use Committee.

3.2.2 Treatment Groups

Rats were randomly assigned to five treatment groups;

- *PBS* (phosphate buffered saline, 0.1M solution of phosphate, n = 10)
- *PPA* (propionic acid; 500mg/kg, 0.26M, n = 10)
- *PPAsac* (propionic acid with saccharin; 500mg/kg, 0.26M PPA with 2% saccharin, n = 10)
- *PBSsac* (phosphate buffered saline with saccharin; 0.1M solution of phosphate with 2% saccharin, n = 10)
- *LiClsac* (lithium chloride with saccharin; 127mg/kg, 0.15M, n = 8).

The vehicle group, PBS, acted as a control for all groups. The PPA group permitted testing of a place avoidance due to conditioning with PPA. PPAsac enabled testing for a place and taste avoidance in the same animal by pairing the unconditioned stimulus (US; PPA) with a taste conditioned stimulus (CS; saccharin) based on the concept of "intravascular taste" (Bradley & Mistretta, 1971; Rinaman, Saboury, & Litvina, 2009). Thus, the US was delivered IP in a solution containing 2% saccharin (sac). The reasoning behind intravascular taste is that saccharin is absorbed into the bloodstream following an IP injection, and thus accesses and stimulates oral taste receptors. Thus the animal is experiencing the systemic effects of the US (PPA) while tasting saccharin. Holland, Zampighi and Simon (1991) demonstrated that small molecules that leave the bloodstream can diffuse into the taste pore and interact with receptors in the microvilli of taste cells, resulting in intravascular taste (Holland, Zampighi, & Simon, 1991). PBSsac acted as a control. Lastly, there was a lithium chloride with saccharin group as a positive control, to demonstrate that intravascular taste can in fact elicit a place and taste avoidance in the same animal (LiClsac, 127mg/kg, 0.15M, n = 8).

PPA was buffered to physiological pH 7.5 before injection using hydrochloric acid or sodium hydroxide. The PPA dose was based on past dose-response findings (MacFabe, et al., 2007). The 2% saccharin injection concentration was chosen as it results in a saccharin concentration of approximately 0.2% after absorption and dilution within a total blood volume of about 17mL (Lee & Blaufox, 1985) and is consistent with other studies using intravascular taste (Bradley & Mistretta, 1971; Rinaman, Saboury, &

Litvina, 2009). The LiCl dose has been established as a dose that can produce both a place and taste avoidance in past studies (Tenk, Kavaliers, & Ossenkopp, 2005) .

Rats were injected intraperitoneally daily for 12 conditioning days. Injections alternated for the 12 days between treatment and vehicle. That is, an animal may receive a treatment injection on the first conditioning day, and a vehicle injection on the second conditioning day. Injections alternated in this manner throughout the study. Thus, each animal received in total six injections of treatment and six injections of vehicle. On these conditioning days, behavioural data were collected for the 30 minutes immediately following injection.

3.3.3 Apparatus

3.3.3.1 Locomotor Activity – Conditioning Days. The two-chamber place conditioning apparatus (Tenk, Kavaliers, & Ossenkopp, 2005) allowed measurement of locomotor activity in eight modified *VersaMax Animal Activity Monitors* (Model NVMA16TT/W, Accuscan Instruments Inc., Columbus, OH). Each monitor is a Plexiglas open field (40 cm x 40 cm x 30.5 cm) with a Plexiglas lid with air holes. Horizontal activity was measured by a set of infrared beams located every 2.54 cm for a total of 16 beams on each side of the monitor, creating a grid at a height of 2.5 cm. To measure vertical activity, two additional sets of beams were located 14 cm above the floor of the open-field. A *VersaMax Analyser* (Accuscan Model VSA-16, Columbus, OH) recorded data from each monitor to a computer in an adjacent room.

Each monitor was divided into two equal sized chambers (20 x 40 x 30.5 cm) by a clear Plexiglas partition located parallel to the elevated photobeams ensuring sensors

were not blocked. Each of the two chamber contexts for conditioning differed both in visual and tactile floor cues. One context contained black and white stripes (of 2cm width) on the walls with a removable wire grid floor. The second context contained solid grey walls with a removable clear rough Plexiglas floor. During a conditioning trial, the animal was confined to one chamber of the monitor, and thus one context, by a solid Plexiglas partition. This apparatus is unbiased, in that animals do not show a natural preference for either context.

3.3.3.2 Locomotor Activity – Place Avoidance Test Day. The apparatus used on the test day was identical to that during conditioning days (see above) except for one key factor. Instead of using a solid Plexiglas partition and confining animals to one chamber within the monitor, a Plexiglas partition with a doorway (10 x 15 cm) was used. This allowed the free passage of the animal between the two chambers with different contexts (striped walls and wire grid floor or grey walls and rough Plexiglas floor).

3.3.4 Experimental Procedures (Fig. 3.1)

3.3.4.1 Locomotor Activity – Conditioning Days. Rats were handled for an hour on three consecutive days. Six days after arrival (PND 29) conditioning was started, with rats receiving daily intraperitoneal injections for 12 days. Prior to each conditioning trial, rats were weighed and received an IP injection immediately before being placed in the apparatus. Conditioning trials were 30 minutes in length as pilot work and past studies show the majority of behavioural effects occur in the first 30 minutes following infusion or injection of PPA (MacFabe, et al., 2007; Shams, 2009). Furthermore, LiCl has been shown to exert its maximal effects on behaviour in the 15-30 minutes following

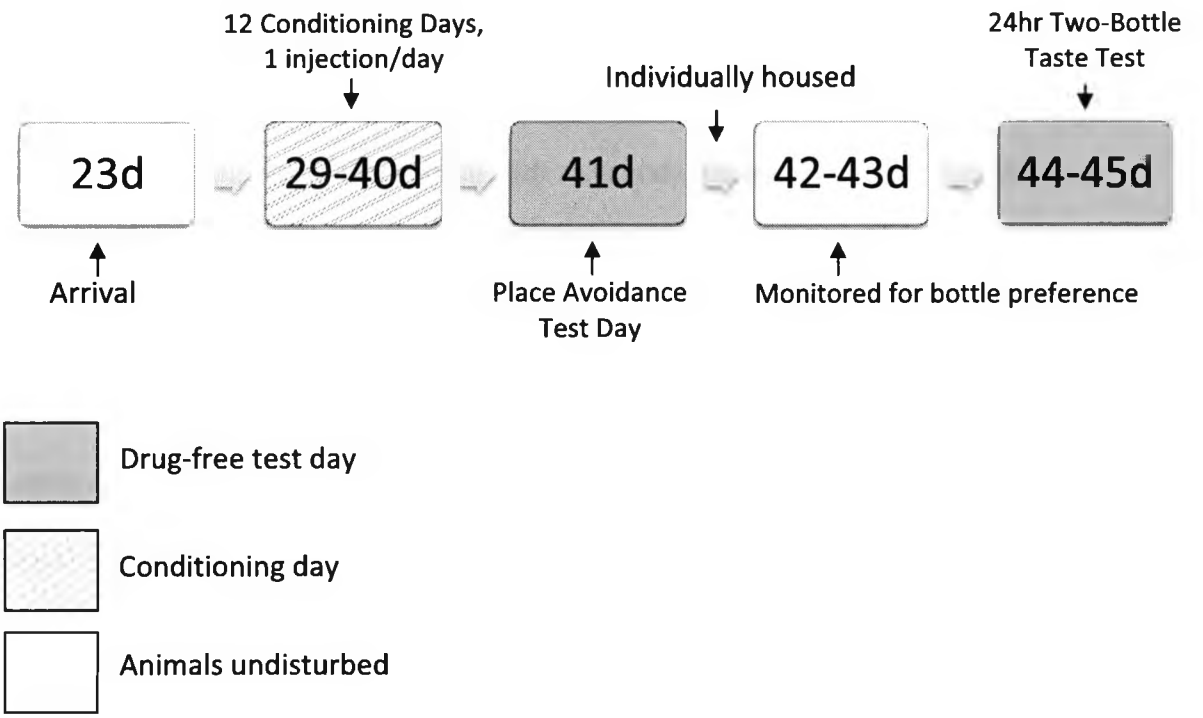

Figure 3.1 Methods timeline, where d = days old. Conditioning days alternated between administration of one IP injection of either drug treatment in one context, or vehicle treatment in another. Place avoidance test day occurred in a drug-free state, as did the two-bottle taste test, which evaluated the presence of a conditioned place and taste avoidance, respectively.

administration (Parker, Hills, & Jensen, 1984). Each monitor and all removable parts were cleaned with a mild detergent solution (Alconox Inc., New York) and rinsed with baking soda solution after each conditioning trial.

Conditioning trials occurred once every 24 hours and alternated between treatment and vehicle trials. Thus, animals received 6 pairings of the treatment with a context and 6 pairings of the vehicle with a second context. The experimental design was balanced so that half of the rats received drug treatment on odd conditioning days, and half received vehicle on odd conditioning days. Of the half that received drug treatment on odd conditioning days, half received treatment with the striped context while the other half received treatment with the grey context. All five treatment groups were balanced in this way. Locomotor activity during conditioning was assessed.

3.3.4.2 Locomotor Activity – Place Avoidance Test Day. 24 hours following the last conditioning trial the place avoidance test took place. Animals were placed into the chamber at the far outer corner, facing the wall. Half the animals started in their drug-paired chamber, while the other half started in their vehicle-paired chamber. Animals were placed into the chamber in a drug-free state and were able to explore both chambers via an opening in the centre partition. Locomotor activity, along with time spent in each chamber, was assessed.

3.3.4.3 Taste Avoidance 24 hour Taste Test. Following the place avoidance test, animals were placed into individual home cages with two water bottles. One water bottle was placed in the original position, where bottles had been placed throughout the study. A second water bottle was placed next to it. Rats were left undisturbed in

their home cages for about 60 hours before starting the 24 hour taste avoidance test. Before commencing the test, bottle side preference was determined by noting from which bottle the animal had consumed the most water.

The 24 hour taste test commenced at 9:00h on PND 44 with a 0.2% saccharin water bottle being placed on the preferred side of the cage (which for most animals was the original water bottle site). Next to the preferred bottle was a bottle filled with water. Animals were assessed for their voluntary intake of saccharin (0.2% in water) and water at 0.5 hrs, 1 hr, 2 hrs, 4 hrs, and 24 hrs. This was done by weighing bottles before commencing testing, and at each subsequent time period.

3.3.5 Behavioural Measures

3.3.5.1 Locomotor Activity – Conditioning Days. As described, VersaMax Animal Activity Monitors automatically collected data using a grid of infrared beams. Variables reflecting locomotor activity were collected and are described below.

- Total distance (cm) – the total horizontal distance (cm) travelled.
- Frequency of stereotypy – the number of times a rat breaks the same beam without breaking an adjacent beam, with a minimum stop time of 1 s to separate movements.
- Frequency of rearing – the number of individual vertical movements made with a minimum stop time of 1 s to separate movements.

3.3.5.2 Locomotor Activity – Place Avoidance Test Day. As described, VersaMax Animal Activity Monitors automatically collected data using a grid of infrared beams.

Variables reflecting locomotor activity in each of the two chambers were collected and are described below.

- Total distance (cm) – the total horizontal distance (cm) travelled.
- Frequency of stereotypy – the number of stereotypic movements with a minimum stop time of 1 s to separate movements.
- Frequency of rearing –the number of individual vertical movements made with a minimum stop time of 1 s to separate movements.
- Duration of time (s) spent in context – the time (s) spent in each the treatment-paired and vehicle-paired context.
- Percentage of time rearing – the time (s) spent rearing in a particular context divided by the total time spent in that context, multiplied by 100.
- Percentage of time in horizontal movement – the time (s) spent in horizontal movement in a particular context divided by the total time spent in that context, multiplied by 100.

3.3.5.3 Fluid Intake – Taste Avoidance 24 hour Taste Test. As described, saccharin-water and water volumes were taken over a 24 hour period to monitor voluntary saccharin intake. The variable used to quantify this data is defined below.

- Saccharin Ratio – the amount of saccharin water consumed divided by the total amount of fluid (saccharin water + water) consumed.

3.3.6 Statistical Analysis of Behaviours

All statistical tests were calculated using SPSS 17.0 (SPSS, Inc.) for Windows. Tests were completed using $\alpha = .05$ as the criterion for significant effects.

3.3.6.1 Locomotor Activity – Conditioning Days. Data was analyzed for main effects and interactions using a mixed design ANOVA with PPA treatment and saccharin treatment as the between-subjects factors and drug OR vehicle conditioning day as the within-subject factor. This allowed overall effects of PPA or saccharin to be revealed for every group except the LiCl saccharin group. Student-Newman-Keuls (S-N-K) post-hoc pair-wise comparisons were carried out to examine group differences (including the LiCl saccharin group) on individual days.

Averaging each variable's values across the six drug OR vehicle conditioning days (ex. average total distance travelled across drug exposure days 1-6) allowed bar graphs to show trends in locomotor variables across days. A univariate ANOVA was performed on these averaged values, with PPA treatment and saccharin treatment as the between-subjects factors and drug or vehicle conditioning day as the within-subject factor. If an effect of PPA or saccharin treatment was found, an S-N-K post-hoc revealed group differences (including the LiCl saccharin group).

3.3.6.2 Locomotor Activity- Place Avoidance Test Day. Data were analyzed with a mixed design ANOVA with PPA treatment and saccharin treatment as the between-subjects factors with the within-subject factor being the context (drug or vehicle-paired). To further explore treatment differences from controls, a paired samples t-test was performed to reveal differences in behaviour in each context.

3.3.6.3 Fluid Intake – 24 hour Taste Test. Data was analyzed for main effects using a mixed design ANOVA with PPA treatment and saccharin treatment as the

between-subjects factors and time as the within-subject factor. An S-N-K post-hoc revealed group differences.

3.3 Results

3.3.1 Locomotor Activity – Conditioning Days

There was no significant main effect of PPA or saccharin exposure for total distance or frequency of stereotypy during *vehicle* exposure days (a dummy variable).

During drug exposure days, an overall main effect of PPA exposure was found for total distance travelled, $F(1, 24) = 13.310$, $p<.005$. Post-hoc analyses revealed PPA and PPA saccharin animals travelled a shorter distance compared to PBS saccharin animals on drug exposure days 2 and 4 ($p < .05$ and .01, respectively). When total distance was averaged across drug exposure days for each treatment group, a main effect of PPA treatment was present, $F(1,44) = 11.491$, $p<.005$ with post-hoc analyses revealing that on average, the PPA and PPA saccharin groups travelled a shorter distance than the PBS saccharin group (*ps*<.01; Fig. 3.2A).

For stereotypy frequency on drug exposure days, a large main effect for PPA exposure was found, $F(1, 24) = 61.275$, $p<.001$. Post-hoc analyses revealed significant group differences on all drug exposure days. On day 1, PPA and PPA saccharin animals displayed less stereotypic movements than PBS and PBS saccharin animals ($p < .005$). On days 2 and 5, PPA and PPA saccharin animals displayed less stereotypy than all other groups ($p < .005$ and 001, respectively) while on day 3 only PPA animals exhibited less stereotypic movements than all other groups ($p < .05$). On days 4 and 6 PPA and PPA

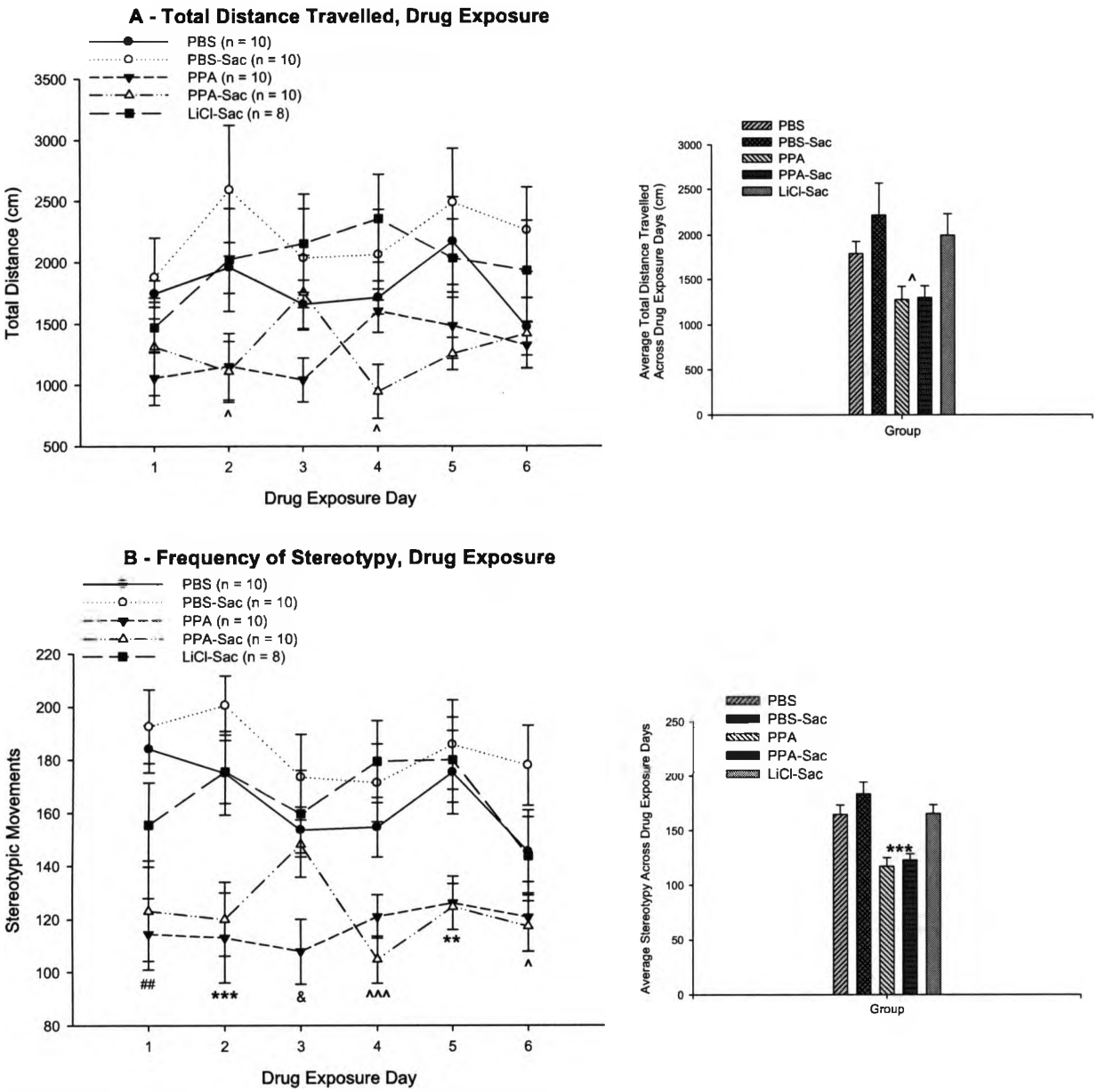

Figure 3.2 Total distance (cm) travelled **(A)** and frequency of stereotypy **(B)** in a chamber across drug exposure days during conditioning for male adolescent rats, injected (IP) with PBS (vehicle), PBS saccharin (2% sac), PPA (0.26M), PPA saccharin (0.26M with 2% sac) or LiCl saccharin (0.15M with 2% sac). Each bar represents mean data across all six drug exposure days. Error bars represent ± SEM. ^ $p < .05$, ^^^ $p < .001$, PPA and PPA saccharin less than PBS saccharin. ## $p < .005$, PPA and PPA saccharin less than PBS and PBS saccharin. ** $p < .005$, *** $p < .001$, PPA and PPA saccharin less than all other groups. & $p < .05$, PPA less than all other groups.

saccharin animals made less stereotypic movements than PBS saccharin animals ($p <$ .001 and .05, respectively). When stereotypy frequency was averaged across drug exposure days, an overall effect of PPA exposure was still present, $F(1, 44) = 39.909$, $p<.001$. Post-hoc analyses revealed PPA and PPA saccharin groups exhibited less stereotypy than all other groups (Fig. 3.2B).

On drug exposure days an overall effect of PPA exposure was found for frequency of rearing, $F(1, 24) = 16.889$, $p<.001$. Post-hoc analyses showed significant group differences on days 1, 2, and 4. On day 1 PPA animals exhibited less rearing than PBS animals ($p<.05$). On day 2 PPA and PPA saccharin animals displayed less rearing than PBS saccharin animals ($p<.01$) and on day 4 PPA saccharin animals showed less rearing than PBS saccharin animals ($p<.05$). When rearing frequency was averaged across days for each group, and overall main effect of PPA treatment was still present, $F(1, 44) = 17.634$, $p<.001$. Post-hoc analyses revealed that the PPA and PPA saccharin groups were exhibiting less rearing than the PBS saccharin group (Fig. 3.3 A1). During vehicle exposure days, a significant main effect of PPA exposure was still present, $F(1, 24) = 5.743$, $p<.05$. However, post-hoc analyses revealed no significant group differences. Averaging rearing frequency across vehicle exposure days also showed a main effect of PPA exposure, $F(1, 44) = 6.762$, $p<.05$, but once again post-hoc analyses did not reveal any significant group differences (Fig. 3.3 A2).

3.3.2 Locomotor Activity – Place Avoidance Test Day

No effect of treatment was found for total distance travelled (Fig. 3.4 A1 & A2).

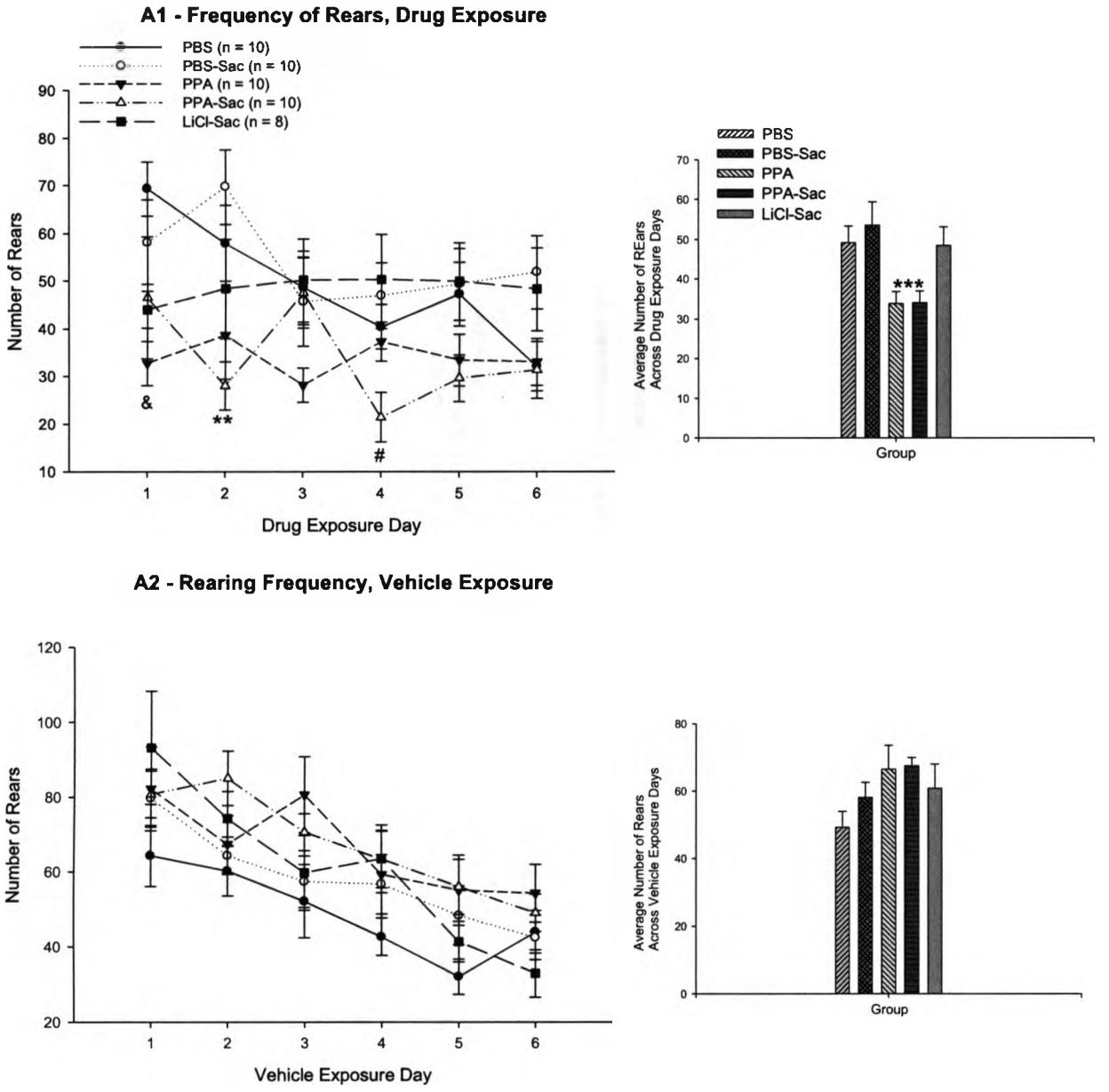

Figure 3.3 Rearing frequency in a chamber across drug exposure days **(A1)** and vehicle exposure days **(A2)** during condition trials for male juvenile rats, injected (IP) with PBS (vehicle), PBS saccharin (2% sac), PPA (0.26M), PPA saccharin (0.26M with 2% sac) or LiCl saccharin (0.15M with 2% sac). Each bar represents mean data for each group across all drug **(A1)** or vehicle **(A2)** exposure days. Error bars represent ± SEM. & $p < .05$ PPA less than PBS. ** $p < .01$, *** $p < .005$, PPA and PPA sac less than PBS sac. #, $p < .05$, PPA sac less than PBS sac & LiCl sac.

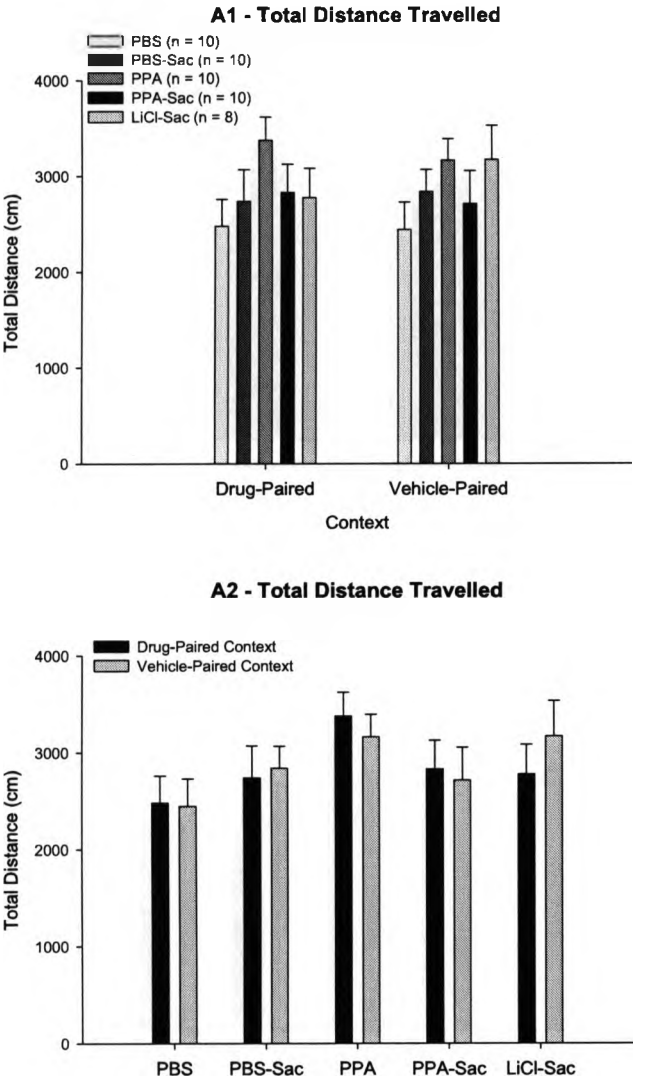

Figure 3.4 Total distance (cm) travelled in each context, between groups **(A1)** and within group **(A2)**, during the place avoidance drug free test day. Each bar represents group mean of data for the 30 minutes in the two-chambered monitor. Error bars represent + SEM. No between groups or within group differences.

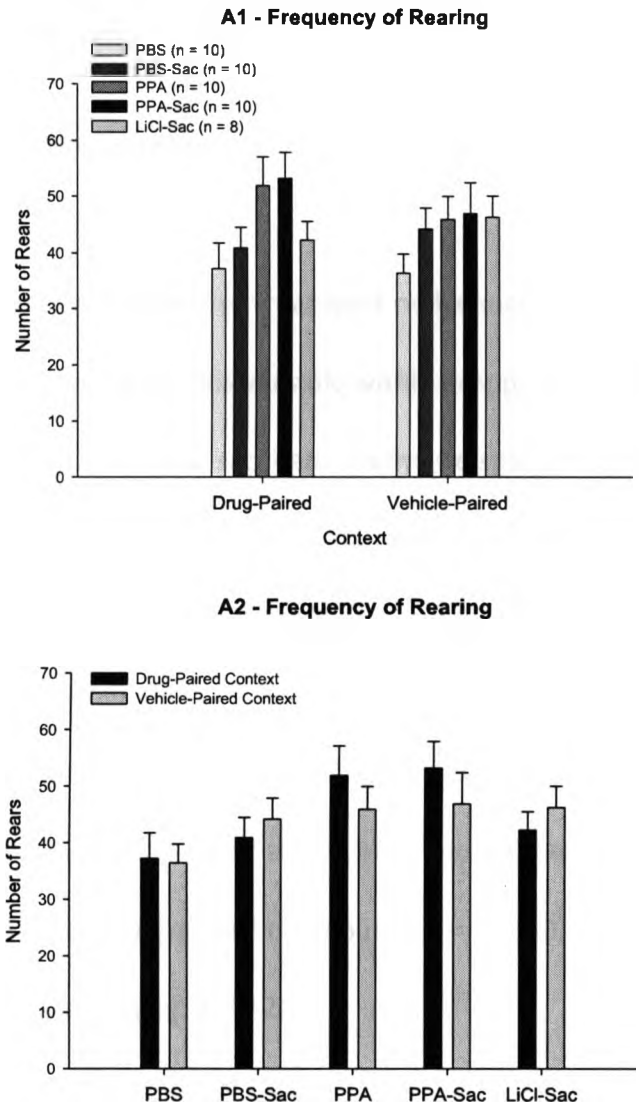

Figure 3.5 Frequency of rearing in each context, between groups **(A1)** and within group **(A2)**, during the place avoidance drug free test day. Each bar represents group mean of data for the 30 minutes in the two-chambered monitor. Error bars represent + SEM. No between groups or within group differences.

An overall effect of PPA exposure was found for frequency of rearing, $F(1, 25) = 5.140$, $p < .05$, however, post-hoc analysis revealed no group differences (Fig. 3.4 a1). Furthermore, none of the treatment groups exhibited significantly greater rearing frequency in one context compared to the other (drug-paired or vehicle-paired) (Fig. 3.5 A2).

There was no overall effect of treatment on frequency of stereotypy (Fig. 3.6 A1). However, when comparing this variable within groups and between contexts, a paired samples t-test revealed LiCl saccharin animals displayed significantly less stereotypic movements in the drug-paired chamber compared with the vehicle-paired chamber, $t(7) = -4.833$, $p < .005$ (Fig. 3.6 A2).

There was no overall effect of treatment on duration of time spent in each of the two contexts, drug or vehicle-paired (Fig. 3.7 A1). However, a paired samples t-test did show that LiCl saccharin animals spent significantly more time in the vehicle-paired chamber compared to the drug-paired chamber, $t(7) = -.4630$, $p < .005$. This effect was not found in the PPA groups (Fig. 3.7 A2).

There was an overall effect of treatment on the percentage of time spent rearing, $F(1, 25) = 5.312$, $p < .05$. Post-hoc analyses revealed that in the drug-paired chamber both the PPA and LiCl saccharin group spent a significantly greater percentage of time rearing compared to the PBS group ($p < .01$; Fig. 3.8 A1 & A2). A paired samples t-test showed that the LiCl saccharin group spent a significantly greater percentage of

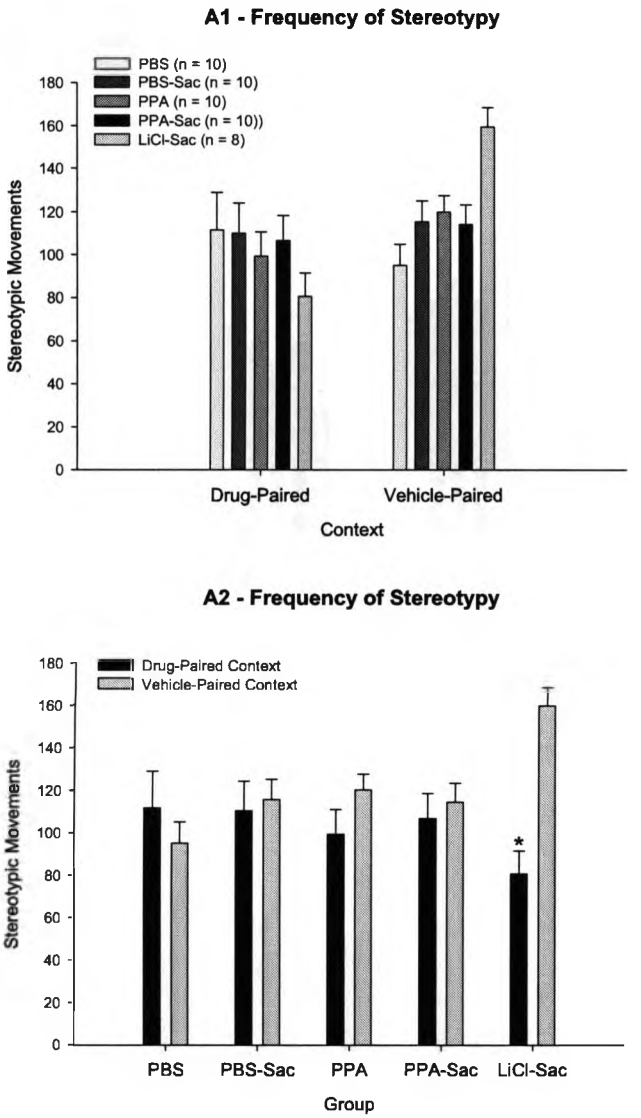

Figure 3.6 Frequency of stereotypy in each context, between groups **(A1)** and within group **(A2)**, during the place avoidance drug free test day. Each bar represents group mean of data for the 30 minutes in the two-chambered monitor. Error bars represent + SEM. No between group differences. * $p < .05$, LiCl-Sac less movements in drug-paired context than vehicle paired context.

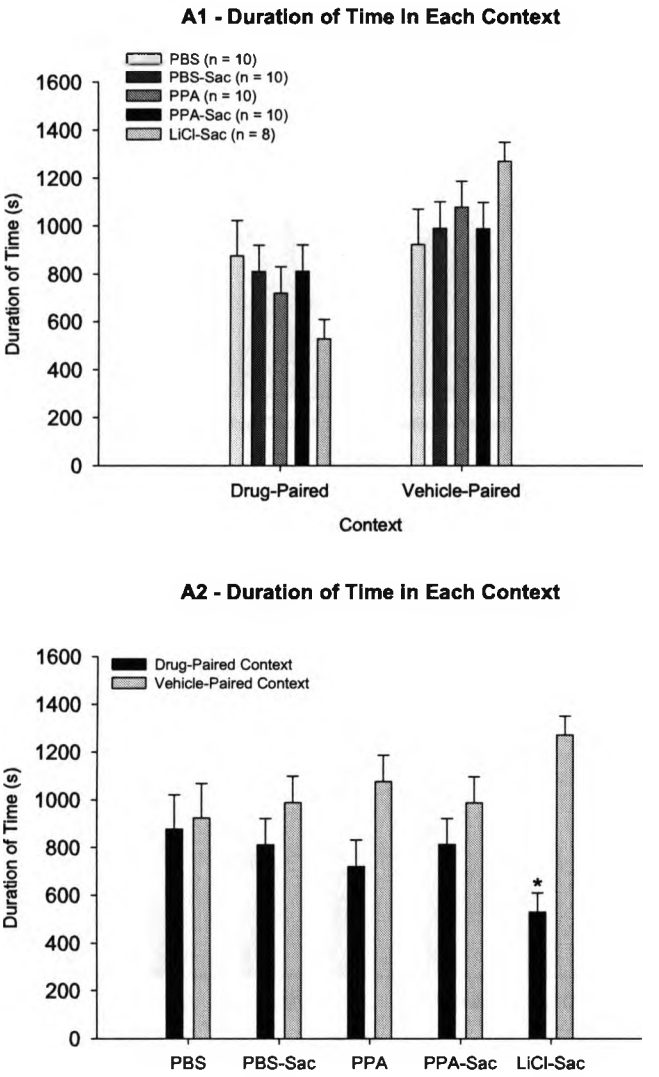

Figure 3.7 Duration of time in each context, between groups **(A1)** and within group **(A2)**, during the place avoidance drug free test day. Each bar represents group mean of data for the 30 minutes in the two-chambered monitor. Error bars represent + SEM. No between group differences. * $p < .05$, LiCl-Sac less time in drug-paired context than vehicle paired context.

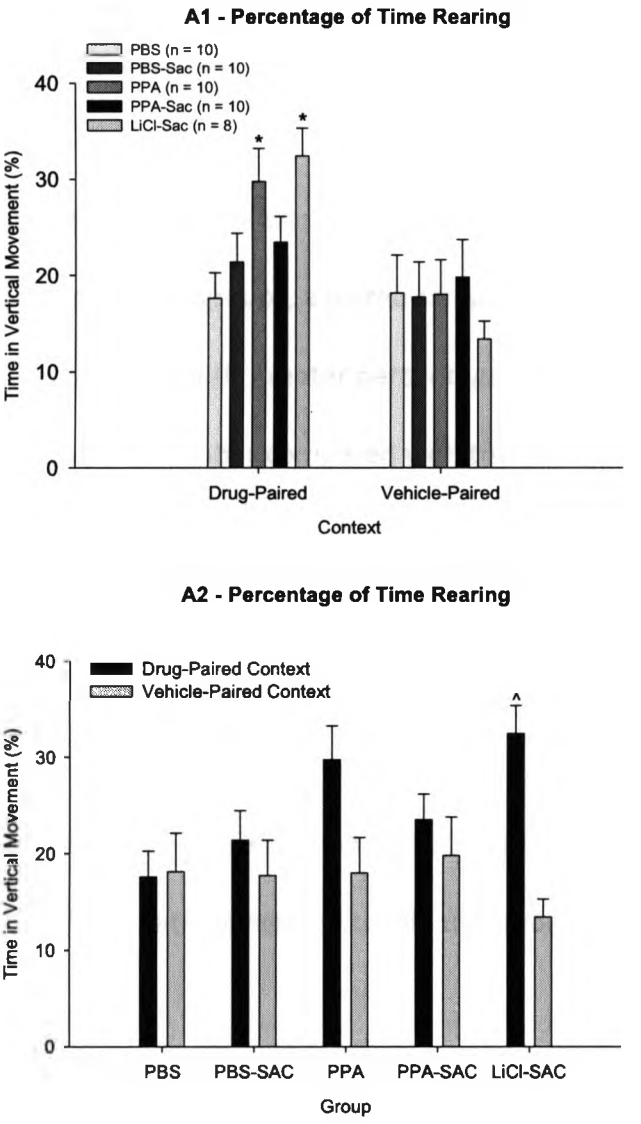

Figure 3.8 Percentage of time rearing, between groups **(A1)** and within group **(A2)**, during the place avoidance drug free test day. Each bar represents group mean of data for the 30 minutes in the two-chambered monitor. Error bars represent + SEM. * $p < .05$, PPA and LiCl saccharin more time rearing in the drug-paired context compared to all other groups. ^ $p < .05$, LiCl-Sac more time rearing in drug-paired context than vehicle paired context.

time rearing in the drug-paired chamber compared to the vehicle-paired chamber, t (7) =4.344, $p < .005$. This comparison did not reach significance for the PPA group (p=.085; Fig. 3.8 A2).

For horizontal movement there was no overall effect of treatment (Fig. 3.9 A1). However, when comparing within groups, a paired samples t-test revealed that LiCl saccharin animals spent a significantly greater percentage of time in horizontal movement in the drug-paired chamber compared with the vehicle-paired chamber, t(7) = 5.101, $p < .005$ (Fig. 3.9 A2).

3.3.3 *Fluid Intake – Taste Avoidance 24 hour Taste Test*

There were no significant group differences in the saccharin ratio at any of the time points before 24 hours (0.5, 1, 2, and 4 hours).

Univariate analysis of variance revealed the LiCl saccharin group had a significantly smaller saccharin ratio compared to all other groups at 24 hours, F (4, 43) = 4.702, $p < .005$ (Fig. 3.10).

3.4 Discussion

This study used a place and taste conditioning paradigm to investigate the presence of aversive internal cues induced by IP injections of PPA in adolescent male rats. A drug-free test day revealed that only the LiCl saccharin group demonstrated a conditioned place avoidance to the drug-paired side. However, in the drug-paired chamber the PPA group (but not the PPA saccharin group) exhibited more escape behaviour than controls, in the form of increased rearing. A conditioned taste avoidance to saccharin water was established for the LiCl saccharin group, but not the

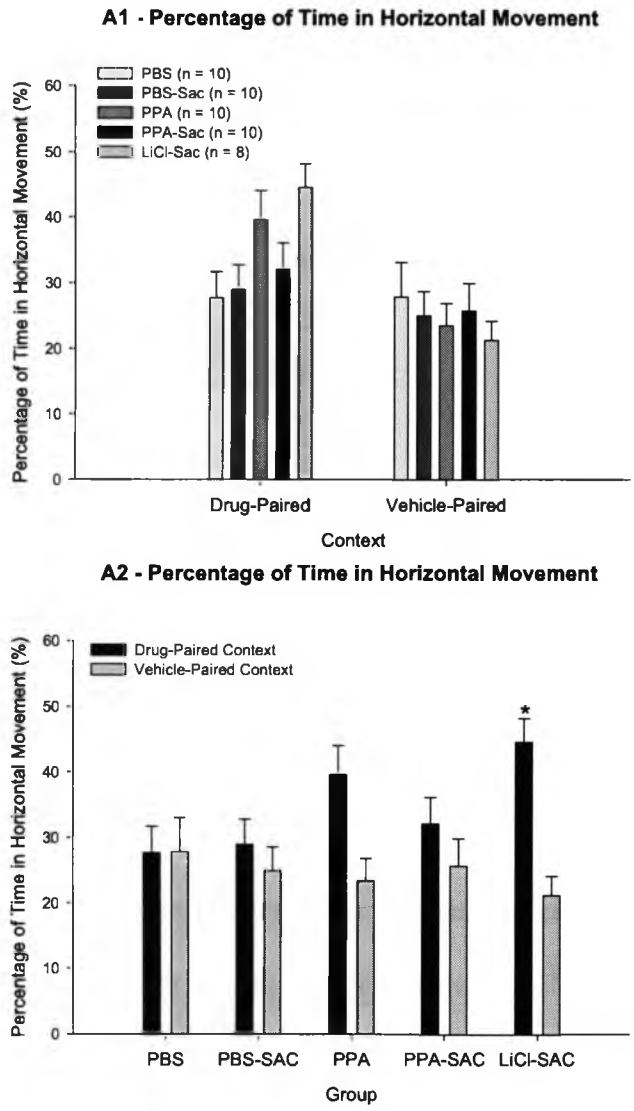

Figure 3.9 Percentage of time in horizontal movement in each context, between groups **(A1)** and within group **(A2)**, during the place avoidance drug free test day. Each bar represents group mean of data for the 30 minutes in the two-chambered monitor. Error bars represent + SEM. No between group differences. * $p < .05$, LiCl saccharin spent more time moving in drug-paired context than vehicle-paired context.

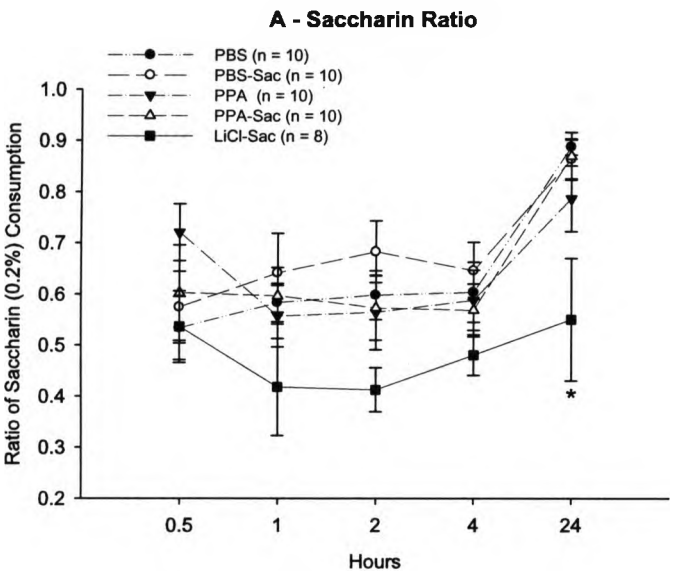

Figure 3.10 Saccharin ratio (amount of saccharin consumed/total consumption) during the taste avoidance 24 hour two-bottle taste test. Error bars represent ± SEM. * $p < .05$, LiCl less than all other groups.

PPA saccharin group. This was expected considering the PPA saccharin group did not show increased escape behaviour in the drug-paired chamber like the PPA group. During conditioning days, PPA groups exhibited hypoactivity, while the activity level of LiCl saccharin animals was the same as controls.

3.4.1 Conditioning Days

On conditioning days, variables were assessed on drug exposure trials and vehicle exposure trials. On drug exposure days, there was a main effect of PPA treatment for total distance and frequency of rearing and stereotypy. When values were averaged across drug exposure days, a main effect of PPA treatment remained. Post-hoc analyses showed PPA and PPA saccharin treated animals to travel a smaller total distance, rear less, and exhibit fewer stereotypic movements. The only variable to exhibit a main effect of treatment on vehicle exposure days was frequency of rearing. However, post-hoc analyses revealed no group differences. The LiCl saccharin treated group closely resembled the PBS group on all variables. These findings seem to reflect hypoactivity in rats treated with PPA, and no behavioural abnormalities in those treated with LiCl saccharin.

Hypoactivity as a response to PPA treatment is consistent with results reported by Benzaquen, et al. (2010) and Shams (2009), both of which used IP injections of PPA. Benzaquen, et al. (2010) IP injected adolescent male and female rats with PPA twice daily for 14 days, with behavioural testing taking place on injection days 8-14. Hypoactivity was reported, as measured by decreased rearing and stereotypy, for both males and females. Shams (2009) IP injected PPA daily for 7 days, with behavioural

testing taking place in two sets; on injection days 1-4 and 5-7, with a three day break in between the two. Hypoactivity was also a finding of this study, as measured by a decreased total distance, less time spent moving, and less rearing. Unlike the study by Benzaquen, et al. (2010), the current study looked at locomotor activity beginning on the first injection day. Consistent with findings by Shams (2009) hypoactivity was present from the first drug exposure day and throughout the entirety of the study. This helps to validate the finding that IP injections of PPA cause hypoactivity. However, it should be noted that most studies using ICV infusions of PPA have found hyperactivity as a response to PPA treatment (MacFabe, et al., 2007; MacFabe, et al., 2008). These studies used a schedule of twice daily ICV infusions for seven days. Shultz, et al. (2008), also used ICV infusions; however, animals only received a total of two infusions, one week apart, with testing occurring immediately after each infusion. Shultz, et al. (2008) found little to no difference in activity levels between PPA and control animals. Thus, the effects of PPA on locomotor activity seem to vary based on the amount of PPA exposure and not the mode of administration. Furthermore, since IP administration of PPA requires PPA to gain access to the brain by first passing through the blood-brain barrier, the amount of PPA reaching the brain following IP injection of PPA is most likely different from the amount following ICV infusion directly into the ventricles of the brain. According to Brusque, et al. (1999) the BBB is differentially permeable to PPA dependent on age, with the barrier becoming less permeable to PPA as age increases. This also suggests the varying effects of PPA on locomotor activity could be due to differences in PPA exposure.

LiCl administration is known to suppress locomotor activity in rats (Tenk, Kavaliers, & Ossenkopp, 2005; Parker, Hills, & Jensen, 1984; Meachum & Bernstein, 1992). However, in the current study, LiCl saccharin rats demonstrated the same activity levels as control animals. Possible reasons for this will be discussed later in the discussion.

3.4.2 Place Avoidance Test Day

The primary findings on the test day were that the LiCl saccharin group spent a significantly greater amount of time in the vehicle-paired chamber compared to the drug-paired chamber. PPA treated animals showed a slight trend in this direction, while the PPA saccharin treated group did not demonstrate this trend at all. This suggests a place avoidance to the drug paired chamber for the LiCl saccharin treated animals. Furthermore, a main effect of PPA treatment was found for percentage of time spent rearing in each context, with LiCl saccharin and PPA treated animals spending a greater percentage of time rearing than control animals in the drug paired context. This effect was not seen in the vehicle paired context. Lastly, LiCl saccharin treated animals exhibited a smaller number of stereotypic movements, and a greater percentage of time moving, in the drug-paired chamber compared to the vehicle-paired chamber.

In the current study, a conditioned place avoidance was not found for animals conditioned with PPA or PPA saccharin, however one was established for those conditioned with LiCl saccharin. This is a novel finding as no published studies have looked at the effect of PPA administration on conditioning a place avoidance in adolescent rats. The ability for LiCl to elicit a CPA is consistent with past literature and

its widely accepted ability to induce aversive internal cues. Although most of this literature used injections of solely LiCl, one recent study by Rinaman, Saboury & Litvina (2009) used LiCl saccharin in a conditioned place avoidance paradigm. They found LiCl saccharin was able to elicit a CPA, and that there was no significant effect of intraperitoneal saccharin pairing on experimental outcomes (Rinaman, Saboury, & Litvina, 2009).

Although conditioning with PPA and PPA saccharin was not able to elicit a CPA, one notable finding of this study was that PPA animals, along with LiCl saccharin animals, spent a greater percentage of time rearing in the drug-paired context compared to controls. In the vehicle-paired chamber, however, there were no group differences. This is significant as a study by Tenk, Kavaliers, & Ossenkopp (2005) looking at dose-response effects of LiCl on locomotor activity and CPA found that dose had no differential effect on the amount of time spent in the drug-paired chamber. They did find, however, that on the drug-free test day, animals exhibited enhanced rearing in a dose-dependent manner (Tenk, Kavaliers, & Ossenkopp, 2005). Specifically, 32 mg/kg LiCl did not elicit increased rearing, while 95 mg/kg and 127 mg/kg did. This is consistent with work by Parker, et al. (1984) which found increased rearing during a test trial for animals conditioned with 254 mg/kg but not those conditioned wit 51 mg/kg and 13 mg/kg (Parker, Hills, & Jensen, 1984). These studies suggest that this increased rearing behaviour can be interpreted as escape behaviour. In fact, other studies have suggested rearing is a measure of escape, not exploratory, behaviour (Exner & Clark, 1993). Furthermore, some studies have demonstrated that increased rearing is present

with measures of active avoidance. For example, Roman High Avoidance (RHA/Verh) rats exhibit more rearing in response to stress than their Roman Low Avoidance (RLA/Verh) counterparts (Wiersma, Knollema, Konsman, Bohus, & Koolhass, 1997). So, although PPA animals did not spend significantly more time in the vehicle-paired context than the drug-paired context, the increased rearing that occurred only in the drug-paired context may signify escape behaviour due to the aversive effects of PPA. Of course, during this test day animals were free to explore both chambers within the monitor, and an animal only needed to walk through the opening in the centre partition to 'escape' the drug-paired context. However, as animals had spent twelve 30 minute conditioning sessions in this apparatus already, where there was no opening into the adjoining chamber, animals may not have been immediately aware of this fact and needed to learn of this new option.

Another notable finding from the test day is the fact that not only did the PPA saccharin group fail to show a trend towards a conditioned place avoidance like the PPA group, this group did not exhibit more rearing in the drug-paired chamber compared to controls. Although the data from the conditioning trials suggests PPA paired with saccharin still results in the same hypoactivity, as indicated by the similar decrease in total distance, rearing and stereotypy, the failure to elicit any kind of CPA or escape behaviour on the test day suggests that saccharin was able to somehow mitigate the effects of PPA. The possible mechanism behind this will be addressed later in the discussion. No other studies have looked at the effect of PPA saccharin administration on locomotor activity and the establishment of a conditioned place avoidance.

3.4.3 Taste Aversion Test Day

In the 24 hour two-bottle taste test, the LiCl saccharin treated group had a significantly smaller saccharin ratio than all other groups. This indicates that this was the sole group to demonstrate a taste avoidance for 0.2% saccharin water. PPA saccharin treated animals did not show an avoidance to 0.2% saccharin water, and thus did not demonstrate a conditioned taste avoidance. This is a novel finding as no published studies have looked at the effect of PPA administration on conditioning a taste avoidance in adolescent rats.

The ability for LiCl saccharin to elicit a conditioned taste avoidance is consistent with recent work by Rinaman, Saboury, & Litvina (2009). Using IP injection LiCl plus 2% saccharin, as in the current study, they established a CTA for 0.2% saccharin solution in a two-bottle choice test.

3.4.4 Mechanisms

On a drug-free test day PPA produced an increase in the percentage of time rearing, a putative index of escape behaviour, only when in the PPA-paired chamber. This suggests that PPA may be inducing some aversive internal cues. The exact nature of this aversive effect is unknown, but one possibility lies in PPA's ability to alter cytokine levels and the immune response. Inconsistent findings suggest PPA can both inhibit and cause inflammation dependent on a variety of factors such as; the concentration of PPA being used, where in the body PPA is being investigated (i.e. the gut, mouth, brain), and if other short-chain fatty acids are present. Kurita-Ochiai, et al. (1995) found PPA to significantly suppress T- and B-cell proliferation dose-dependently.

As PPA concentration increased, so too did proliferation inhibition. Cavaglieri, et al. (2003) however, did not find PPA to inhibit lymphocyte proliferation. They reasoned, based on other literature, that they used a dose of PPA too small to elicit this inhibition. It is at high concentrations that PPA is able to inhibit the lymphocyte response (Kurita-Ochiai, Fukushima, & Ochiai, 1995; Cavaglieri, Nishiyama, Fernandes, Curi, Miles, & Calder, 2003; Wajner, Santos, Schlottfeldt, Rocha, & Wannmacher, 1999). Cavaglieri, et al. (2003) did find that the effect PPA had on cytokines varied depending on if it was investigated alone, or with one or two additional short-chain fatty acids (butyrate, acetate). It is therefore difficult to speculate what kind of effects IP injections of PPA have on the immune response. Nonetheless, the established finding that PPA can in someway alter the immune response and cytokine levels is important as pro-inflammatory cytokines are known to elicit the sickness response. The sickness response is dictated by behaviours such as; fever, reduction in food and water intake, reduced social and sexual behaviours, and hypoactivity. Thus, based on the fact that PPA can alter cytokine levels, it can subsequently be speculated that this is the mechanism behind the decrease in activity seen after IP administration of PPA.

One interesting finding of this study was the fact that despite both PPA and PPA saccharin groups demonstrating hypoactivity during conditioning trials, during the drug-free test day only the PPA group showed increased escape behaviour in the drug-paired chamber. Literature shows that saccharin does not produce a CPP when administered on its own (Holman, 1975; Messier & White, 1984; White & Carr, 1984). Thus, PPA saccharin animals should show the same magnitude of avoidance as the PPA animals.

However, that was not the finding of the current study. Though literature does report that when saccharin is paired with glucose, it is able to elicit a CPP (Agmo & Marroquin, 1997). This is due to the fact that when administered alone, the positive gustatory experience produced by saccharin does not outlast its consumatory experience (the instance in which saccharin activates the taste receptors), as saccharin does not elicit postabsorptive actions (Agmo & Marroquin, 1997). It is reasoned that the postabsorptive actions induced by glucose, thought to include the activation of dopamine systems involved in food reward and motivation, enhance the impact of saccharin, and thus result in acquiring a conditioned place preference (Touzani, Bodnar, & Sclafani, 2008). Thus, it could be speculated that if PPA is inducing any nutritional post-ingestive effects, pairing it with a saccharin taste could result in a positive cue. This, in turn, would make PPA saccharin administration less aversive, resulting in the failure to establish the same level of avoidance as found in the PPA group.

Another interesting finding of this study was that LiCl saccharin animals performed at the same level as controls in various measures of locomotor activity during conditioning trials. As mentioned, previous literature reports that LiCl induces hypoactivity at the dose used in this study. However, no study has examined the effect of LiCl on locomotor activity in adolescent rats. It is possible that the effect LiCl had on locomotor activity in the current study is due to the fact that the animals were adolescents, and not adult rats. It is well known that adolescent rats are differentially susceptible to the aversive effects of psychostimulants. For example, adolescent male rats are more likely to acquire self-administration of nicotine and acetaldehyde than

adult male rats (Belluzzi, Wang, & Leslie, 2005). Also, conditioned taste aversion to amphetamine and nicotine is reduced in adolescent rats compared to adult rats (Infurna & Spear, 1979; Shram, Funk, Li, & Le, 2006). Furthermore, Schramm-Sapyta, et al (2007) found THC to be more aversive in adults than adolescents as measured by place and taste aversion tasks. It was also more anxiogenic, and had stronger locomotor decreasing effects in adults. One study did look at the difference in conditioned aversive properties for LiCl, a non-addictive substance, between adolescents and adults. Schramm-Sapyta, Morris & Kuhn (2006) reported that at the 19 mg/kg, but not 76 mg/kg, adolescents exhibited less of a conditioned taste avoidance in a saccharin choice test than adults. Thus, it is reasonable that the lack of hypoactivity seen in the current study is because LiCl does not act in the same manner in adolescent animals compared to adult rats. Further study comparing varying doses of LiCl induced locomotor activity in both adolescent and adult animals would help to get at this question.

3.4.5 Summary

A subset of autistic patients present initially with decreased activity, hypotonia and lethargy. Zwaigenbaum, et al. (2005) investigated the behavioural manifestations of autism in the first year of life for infants considered to be high risk for developing autism (have an older sibling with an ASD). They compared these infants with low risk controls (children without a 1st or 2nd degree relative with ASD). They found high risk infants, who were later diagnosed with autism, presented with *decreased* activity level at 6 month of age. Additionally, research has highlighted certain genetic abnormalities in a subset of autistic patients who present with seizures and/or mitochondrial

dysfunction. These children often present with hypotonia and periods of lethargy (Filipek, et al., 2003; Zecavati & Spence, 2009). Thus, PPA eliciting hypoactivity and initially inducing an aversive state can be considered consistent with what is seen in some human forms of ASD.

It is important to note the limitations of this study. The sample size was somewhat limited, with eight to ten animals per group. Also, only one dose of PPA was used. The LiCl saccharin group was initially used solely as a positive control, to demonstrate the ability to obtain a conditioned place and taste avoidance in the same animal using intravascular taste. However, considering the findings in terms of locomotor activity, a LiCl adolescent group, and/or a LiCl saccharin adult group, would be helpful in interpreting the results. This study succeeded in investigating if PPA induced aversive internal cues, a question resulting from Benzaquen, et al. (2010). It also revealed the effects of PPA on locomotor activity from the first day of injections, a secondary question resulting from that study.

In conclusion, this study provides information on the effects of IP injections on adolescent male rats in terms of locomotor activity, and aversive internal cues. Results provide evidence that such injections result in hypoactivity and a slight conditioned place avoidance, as indicated by increased time rearing in a drug-paired context. The findings of this study are consistent with some forms of ASD, and offer further support that PPA administration in rats may be a way to model autism.

3.5 References

Agmo, A., & Marroquin, E. (1997). Role of gustatory and postingestive actions of sweeteners in the generation of positive affect as evaluated by place preference conditioning. Appetite , 29, 269-289.

Arndt, T. L., Stodgell, C. J., & Rodier, P. M. (2005). The teratology of autism. International Journal of Developmental Neuroscience , 23, 189-199.

Belluzzi, J. D., Wang, R., & Leslie, F. M. (2005). Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. Neuropsychopharmacology , 30, 705-712.

Benzaquen, J., Foley, K., Tichenoff, L., MacFabe, D. F., Kavaliers, M., & Ossenkopp, K.-P. (2010). The effects of intraperitoneal injections of propionic acid on locomotor activity, anxiety-related behaviour and social interaction in adolescent male & female rats. In prep .

Bradley, R. M., & Mistretta, C. M. (1971). Intravascular taste in rats as demonstrated by conditioned aversion to sodium saccharin. Journal of Comparative and Physiological Psychology , 75, 186-189.

Cavaglieri, C. R., Nishiyama, A., Fernandes, L. C., Curi, R., Miles, E. A., & Calder, P. C. (2003). Differential effects of short-chain fatty acids on proliferation and production of pro- and anti-inflammatroy cytokines by cultured lymphocytes. Life Sciences , 73, 1683-1690.

Cunningham, C. L., Gremel, C. M., & Groblewski, P. A. (2006). Dug-induced conditioned place preference and aversion to sodium saccharin. Journal of Comparative and Physiological Psychology , 1 (4), 1662-1670.

DiCicco-Bloom, E., Lord, C., Zwaigenbaum, L., Courchesne, E., Dager, S. R., Schmitz, C., et al. (2006). The developmental neurobiology of autism spectrum disorder. The Journal of Neuroscience , 62, 6897-6906.

Exner, M., & Clark, D. (1993). Behaviour in the novel environment predicts responsiveness to d-amphetamine in the rat: a multivariate approach. Behavioural Pharmacology , 4, 47-56.

Fatemi, S. H., Pearce, D. A., Brooks, A. I., & Sidwell, R. W. (2005). Prenatal viral infection in mouse causes differential expression of genes in brains of mouse progeny: a potential animal model for schizophrenia and autism. Synapse , 11, 91-99.

Filipek, P. A., Juranek, J., Smith, M., Mays, L. Z., Ramos, E. R., Bocian, M., et al. (2003). Mitochondrial Dysfunction in Autistic Patients with 15q Inverted Duplication. Annals of Neurology , 53 (6), 801-804.

Holland, V. F., Zampighi, G. A., & Simon, S. A. (1991). Tight junctions in taste buds: possible role in perception of intravascular gustatory stimuli. Chemical Senses , 16 (1), 69-79.

Holman, E. W. (1975). Immediate and delayed reinforcers for flavor preferences in rats. Learning and Motivation , 6, 91-100.

Hu, V. W., Frank, B. C., Heine, S., Lee, N. H., & Quackenbush, J. (2006). Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes. BMC Genomics , 7, 118.

Infurna, R. N., & Spear, L. P. (1979). Developmental changes in amphetamine-induced taste aversions. Pharmacology Biochemistry and Behavior , 31-35.

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.

Kamitani, W., Ono, E., & Yoshino, S. (2003). Glial expression of Borna disease virus phosphoprotein induces behavioral and neurological abnormalities in transgenic mice. Proceedings of the National Academy of Sciences USA , 100, 8969-8974.

Kurita-Ochiai, T., Fukushima, K., & Ochiai, K. (1995). Volatile fatty acids, metabolic by-products of periodontopathic bacteria, inhibit lymphocyte proliferation and cytokine production. Journal of Dental Research , 74 (7), 1367-1373.

Lee, H. B., & Blaufox, M. D. (1985). Blood volume in the rat. Journal of Nuclear Medicine , 26 (1), 72-76.

MacFabe, D., Cain, D., Rodriguez-Capote, K., Franklin, A., Hoffman, J., Boon, F., et al. (2007). Neurobiological effects of intraventicular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behavioural Brain Research , 176, 149-169.

MacFabe, D., Rodriguez-Capote, K., Hoffman, J., Franklin, A., Mohammad-Asef, Y., Taylor, R., et al. (2008). A novel rodent model of autism: intraventricular infusions of propionic acid increase locomotor activity and induce

neuroinflammation and oxidative stress in discrete regions of adult rat brain. American Journal of Biochemistry and Biotechnology , 4 (2), 146-166.

Meachum, C. L., & Bernstein, I. L. (1992). Behavioral conditioned responses to contextual and odor stimuli paired with LiCl administration. Physiology and Behavior , 52, 895-899.

Messier, C., & White, N. M. (1984). Contingent and non-contingent actions of sucrose and saccharin reinforcers; Effects on taste preference and memory. Physiology and Behavior , 32, 195-203.

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.

Parker, L. A., Hills, K., & Jensen, K. (1984). Behavioral CRs elicited by a lithium- or an amphetamine-paired contextual test chamber. Animal Learning & Behavior , 12, 307-315.

Rinaman, L., Saboury, M., & Litvina, E. (2009). Ondansetron blocks LiCl-induced conditioned place avoidance but not conditioned taste/flavor avoidance in rats. Physiology and Behavior , 98 (4), 381-385.

Schramm-Sapyta, N. L., Cha, Y. M., Chaudhry, S., Wilson, W. A., Swartzwelder, H. S., & Kuhn, C. M. (2007). Differential anxiogenic, aversive, and locomotor effects of THC in adolescent and adult rats. Psychopharmacology , 191, 867-877.

Schramm-Sapyta, N. L., Morris, R. W., & Kuhn, C. M. (2006). Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride. Pharmacology, Biochemistry and Behavior , 84 (2), 344-352.

Shams, S. (2009). The effects of intraperitoneal injections of propionic acid on social interaction, anxiety-related behavior and locomotor activity of male rats; Support for an animal model of autism. Ontario, London: University of Western Ontario.

Shram, M. J., Funk, D., Li, Z., & Le, A. D. (2006). Periadolescent and adult rats response differently in tests measuring the rewarding and aversive effects of nicotine. Psychopharmacology , 186, 201-208.

Shultz, S. R., MacFabe, D. F., Ossenkopp, K.-P., Scratch, S., Whelan, J., Taylor, R., et al. (2008). Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behaviour in the rat: Implications for an animal model of autism. Neuropharmacology , 54, 901-911.

Shultz, S. R., MacFabe, D. K., Martin, S., Jackson, J., Taylor, R., Boon, F., et al. (2009). Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. Behavioral Brain Research , 200, 33-41.

Tenk, C., Kavaliers, M., & Ossenkopp, K.-P. (2005). Dose response effects of lithium chloride on conditioned place aversions and locomotor activity in rats. European Journal of Pharmacology , 515, 117-127.

Thomas, R. H., Foley, K. A., Mepham, J. R., Tichenoff, L. J., Possmayer, F., & MacFabe, D. F. (2010). Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders. Journal of Neurochemistry , 113 (2), 515-529.

Touzani, K., Bodnar, R., & Sclafani, A. (2008). Activation of dopamine D1-like receptors in nucleus accumbens is critical for the acquisition, but not the expression, of nutrient-conditioned flavor preferences in rats. European Journal of Neuroscience , 27, 1525-1533.

Wajner, M., Santos, K. D., Schlottfeldt, J. L., Rocha, M. P., & Wannmacher, C. M. (1999). Inhibition of mitogen-activated proliferation of human peripheral lymphocytes in vitro by propionic acid. Clinical Science , 96, 99-103.

White, N. M., & Carr, D. (1984). The conditioned place preference is affected by two independent reinforcement processess. Pharmacology, Biochemistry and Behavior , 23, 37-42.

Wiersma, A., Knollema, S., Konsman, J. P., Bohus, B., & Koolhass, J. M. (1997). Corticotropin-releasing hormone modulation of a conditioned stress response in the central amygdala of Roman high (RHA/Verh)- avoidance and low (RLA/Verh)-avoidance rats. Behavioural Genetics , 27 (6), 547-555.

Zecavati, N., & Spence, S. (2009). Neurometabolic Disorders and Dysfunction in Autism Spectrum Disorders. Current Neurology and Neuroscience Reports , 9, 129-136.

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Bejavioral manifestations of autism in the first year of life. International Journal of Developmental Neuroscience , 23, 143-152.

Chapter 4

General Discussion

4.1 Discussion

The present studies investigated the effects of systemic, or intraperitoneal, administration of PPA on locomotor activity, anxiety-related behaviour, and social interaction in adolescent male and female rats. They also examined the ability for this type of administration to induce aversive internal cues in a place and taste avoidance paradigm. Chapter 2 reported a significant decrease in locomotor activity, inconsistent with past work using central ICV infusions of PPA, which found hyperactivity. It also reported increased anxiety-related behaviour as indexed by increased thigmotaxis, as well as reduced social interaction in males, but not females, treated with PPA. Chapter 3 examined whether the decrease in locomotor activity might have been due to an induction of aversive internal cues by PPA when administered systemically. Chapter 3 reported that PPA conditioning did not induce a place avoidance of the PPA-paired chamber, though it did result in an increase of rearing in the PPA-paired chamber, a putative index of escape behaviour. The taste avoidance paradigm in this experiment used simultaneous intraperitoneal injection of PPA and saccharin in order for animals to experience the aversive effects of PPA and the taste of saccharin concurrently. However, the place avoidance test day revealed that saccharin had somehow mitigated the effects of PPA during conditioning, and thus PPA saccharin animals did not demonstrate a place or taste avoidance. Yet it was established that it is possible to elicit a place and taste avoidance in the same animal using this method of intravascular taste, as in a drug-free state LiCl saccharin animals exhibited an avoidance of a LiCl-paired chamber, as well as an avoidance of saccharin water. Though interestingly, LiCl

saccharin animals did not exhibit hypoactivity during conditioning days as is typically seen in animals under the influence of LiCl. In sum, these two studies established that systemic injections of PPA in adolescent rats elicit hypoactivity, anxiety, affect social interaction in a sex-specific manner, and induce aversive internal cues.

There are a number of limitations to consider with regard to these findings, which in turn help to highlight some future directions that may be taken in order to further validate this animal model. To begin with, both experiments had a reasonably small sample size of 8-10 for each group. For percentage of time in physical duration in chapter 2, the sample size was four for each group, due to the fact that this is a variable representing a pair of animals. Since this is quite an important variable with regard to social interaction, and the drug by sex interaction was very close to significance, it is imperative that this experiment be conducted with a larger sample size. This would allow a more definitive conclusion of whether or not PPA affects social behaviour in a sex-specific manner. Secondly, only one dose of PPA was used. It would be interesting, and necessary in terms of validating the model, to investigate the effects of PPA in varying doses, especially in terms of social behaviour, as abnormalities in social behaviour are a hallmark symptom of autism (Diagnostic and Statistical Manual of Mental Disorders, 2000). Furthermore, with respect to investigating social behaviour in chapter 2, more measures of social behaviour should be explored as that study focused largely on play behaviour which is traditionally considered to be more agonistic in nature. Lastly, although many mechanisms were proposed, none were validated, as this is beyond the scope of this paper. However, one consistently suggested mechanism,

although speculative at this point, is the possibility that PPA is altering cytokine levels or the immune response. If PPA is in fact having this effect, it would provide a more substantiated mechanism behind PPA inducing hypoactivity, anxiety, social abnormalities, and aversive internal cues. Thus, future studies should prioritize the investigation of cytokine levels in order to get at this question of if, and how, PPA is altering the immune response in these rodents.

In terms of implications for this animal model of autism, no published study in this rodent model has investigated the effect of systemic or intraperitoneal injection of PPA on rats. All published studies to date have used direct administration of PPA into the cerebral ventricles of the brain via intracerebroventricular infusion of PPA (MacFabe, et al., 2007; MacFabe, et al., 2008; Shultz S. R., et al., 2008; Shultz S. R., et al., 2009; Thomas, Foley, Mepham, Tichenoff, Possmayer, & MacFabe, 2010). Together, the current studies looked at the effect of this systemic administration on locomotor activity during different stages of PPA exposure. In chapter 2, rats received twice daily injections of PPA for 14 days, with behavioural testing taking place on injection days 8-14. Chapter 3, on the other hand, investigated locomotor activity from the first injection day, over a period of 12 days of injections, alternating between drug and vehicle. Thus, these studies helped to confirm that PPA injections can, in fact, elicit hypoactivity when injected intraperitoneally starting with the initial injection. This also helps to further validate this rodent model of autism, as initially those with ASD may present with hypoactivity or periods of lethargy. As discussed in chapter 2 and 3, Zwaigenbaum, et al. (2005) investigated the behavioural manifestations of autism in the

first year of life for infants considered high risk for developing ASD (have an older sibling with ASD) and for those considered low risk (do not have a 1st or 2nd degree relative with ASD). They found high risk infants, who were later diagnosed with autism, presented with *decreased* activity level at six months of age (Zwaigenbaum, Bryson, Rogers, Roberts, Brian, & Szatmari, 2005). Furthermore, the association of autism with metabolic or mitochondrial dysfunction was discussed in depth in chapter 1. In fact, some of these cases of dysfunction result in an increase of PPA and other short-chain fatty acids (Knerr, Gibson, Jakobs, & Pearl, 2008; Pearl, et al., 2003; Ching, et al., 2010; Filiano, Goldenthal, Harker Rhodes, & Marin-Garcia, 2002). In these cases where children present with autism (or autistic features) and metabolic dysfunction, they often initially present with hypoactivity and lethargy (Filipek, et al., 2003; Zecavati & Spence, 2009; Knerr, Gibson, Jakobs, & Pearl, 2008; Pearl, et al., 2003; Ching, et al., 2010; Filiano, Goldenthal, Harker Rhodes, & Marin-Garcia, 2002).

The finding that PPA can cause an increase in anxiety-related behaviour, specifically an increase in thigmotaxis, also helps to further validate this animal model. Skokauskas & Gallagher (2010) investigated the co-morbidity of autism and Asperger's syndrome with psychotic, anxiety and/or mood disorders. They found anxiety disorders to be the most common psychiatric co-morbidity in this population. Thus, this finding offers another point of face validity for this rodent model, as it is consistent with what is seen in the human manifestation of autism.

Lastly, these were the first studies to explore the effects of PPA on female rats. The finding that PPA affects social behaviour in a sex-specific manner helps to further

validate this animal model of autism as ASDs are four times more prevalent in males than females (Centers for Disease Control & Prevention, 2009). Also, as discussed in chapter 2, other animal models of autism, such as the valproic acid model, demonstrated that females exposed to valproic acid showed no evident behavioural alterations compared to controls, except for repetitive/stereotypic behaviour, where males performed abnormally on a variety of measures (Schneider, et al., 2008). As mentioned, more work needs to be done on the investigation of this sex difference in the effect of PPA on social behaviour, nonetheless this study has helped to further validate this model by offering another aspect of face validity.

Further development of this model may help aid in understanding the disparate aspects of autism spectrum disorders and will enable further understanding of the manifestation of this disorder. An animal model allows investigation of this disorder in a safe, effective and efficient way, and may one day provide a method in which prevention and treatment options can be explored.

4.2 References

Centers for Disease Control & Prevention. (2009). Prevalence of Autism Spectrum Disorders - Autism & Developmental Disabilities Monitoring Network. MMWR Surveillance Summaries , 58 (SS-10), 1-20.

Ching, M. S., Shen, Y., Tan, W. H., Jeste, S. S., Morrow, E. M., Chen, X., et al. (2010). Deletions of NRXN1 (neurexin-1) predispose to a wide spectrum of developmental disorders. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics , 153B (4), 937-47.

Diagnostic and Statistical Manual of Mental Disorders (4th ed.). (2000). Washington DC: American Psychiatric Association.

Filiano, J. J., Goldenthal, M. J., Harker Rhodes, C., & Marin-Garcia, J. (2002). Mitochondrial dysfunction in patients with hypotonia, epilepsy, autism, and developmental delay: HEADD syndrome. Journal of Child Neurology , 17 (6), 435-439.

Filipek, P. A., Juranek, J., Smith, M., Mays, L. Z., Ramos, E. R., Bocian, M., et al. (2003). Mitochondrial Dysfunction in Autistic Patients with 15q Inverted Duplication. Annals of Neurology , 53 (6), 801-804.

Knerr, I., Gibson, K. M., Jakobs, C., & Pearl, P. L. (2008). Neuropsychiatric morbidity in adolescent and adult succinic semialdehyde dehydrogenase deficiency patients. CNS Spectrums , 13 (7), 598-605.

MacFabe, D., Cain, D., Rodriguez-Capote, K., Franklin, A., Hoffman, J., Boon, F., et al. (2007). Neurobiological effects of intraventicular propionic acid in rats: Possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behavioural Brain Research , 176, 149-169.

MacFabe, D., Rodriguez-Capote, K., Hoffman, J., Franklin, A., Mohammad-Asef, Y., Taylor, R., et al. (2008). A novel rodent model of autism: intraventricular infusions of propionic acid increase locomotor activity and induce neuroinflammation and oxidative stress in discrete regions of adult rat brain. American Journal of Biochemistry and Biotechnology , 4 (2), 146-166.

Pearl, P. L., gibson, K. M., Acosta, M. T., Vezina, L. G., Theodore, W. H., Rogawski, M. A., et al. (2003). CLinical spectrum of succinic semialdehyde dehydrogenase deficiency. Neurology , 60 (9), 1413-1417.

Schneider, T., Roman, A., Basta-Kaim, A., Kulbera, M., Budziszewska, B., Schneider, K., et al. (2008). Gender-specific behavioural and immunological alterations in an animal model of autism induced by prenatal exposure to valproic acid. Psychoeuroendocrinology , 33, 728-740.

Shultz, S. R., MacFabe, D. F., Ossenkopp, K.-P., Scratch, S., Whelan, J., Taylor, R., et al. (2008). Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behaviour in the rat: Implications for an animal model of autism. Neuropharmacology , 54, 901-911.

Shultz, S. R., MacFabe, D. K., Martin, S., Jackson, J., Taylor, R., Boon, F., et al. (2009). Intracerebroventricular injections of the enteric bacterial metabolic product propionic acid impair cognition and sensorimotor ability in the Long-Evans rat: further development of a rodent model of autism. Behavioral Brain Research , 200, 33-41.

Skokauskas, N., & Gallagher, L. (2010). Psychosis, affective disorders and anxiety in autism spectrum disorder: prevalence and nosological considerations. Psychopathology , 43 (1), 8-16.

Thomas, R. H., Foley, K. A., Mepham, J. R., Tichenoff, L. J., Possmayer, F., & MacFabe, D. F. (2010). Altered brain phospholipid and acylcarnitine profiles in propionic acid infused rodents: further development of a potential model of autism spectrum disorders. Journal of Neurochemistry , 113 (2), 515-529.

Zecavati, N., & Spence, S. (2009). Neurometabolic Disorders and Dysfunction in Autism Spectrum Disorders. Current Neurology and Neuroscience Reports , 9, 129-136.

Zwaigenbaum, L., Bryson, S., Rogers, T., Roberts, W., Brian, J., & Szatmari, P. (2005). Bejavioral manifestations of autism in the first year of life. International Journal of Developmental Neuroscience , 23, 143-152.