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The Effects of Early Adolescent Lipopolysaccharide or Cat Odour Exposure Followed by Propionic Acid Administration on Anxiety, Startle Response, and Sensorimotor Gating in Adolescence and Adulthood

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Abstract

Autism spectrum disorders (ASD) are caused by genetic factors and a complex set of risk factors including early stressors and alterations in the gut microbiome. The present thesis investigated whether environmental stressors during early adolescence predisposes rats administered another stressor in later adolescence and adulthood to produce altered anxiety-like behaviours, startle response, and percent prepulse inhibition. The early adolescent stressors used in this study included repeated administration of a bacterial endotoxin, lipopolysaccharide (LPS), or a stressful predator cat odour exposure. In later adolescence and adulthood, rats were administered with a gut microbial by-product, propionic acid (PPA), and subsequently tested on behaviour. Repeated early adolescent LPS exposure induced long-term anxiety-like behaviours and elevated startle response in adulthood. Repeated early adolescent cat odour exposure induced long-term anxiogenic effects. PPA exposure decreased activity and percent prepulse inhibition. Combined LPS and PPA exposure induced additive effects on activity, while prior cat odour exposure potentiated PPA's reduction of vertical activity. The results of this thesis are important in evaluating the complex set of risk factors involved in the development of ASD.

Keywords

immune activation; predator odour; short chain fatty acid; locomotor activity; sensory sensitivity; development

Statement of Co-Authorship

All experimental work was conducted by Deanne Wah, with the exception of some data collection carried out by Indra Bishnoi. This thesis was written with the guidance of Dr. Klaus-Peter Ossenkopp and Dr. Martin Kavaliers, whom contributed to the study design and data analyses, and provided comments and revisions to the final manuscript.

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Abbreviations

ASD – autism spectrum disorders

ASR – acoustic startle response

BBB – blood brain barrier

CNS – central nervous system

dB – decibel

FFAR – free fatty acid receptor

GI – gastrointestinal

HDAC – histone deacetylase

HPA – hypothalamic-pituitary-adrenal axis

i.c.v. – intracerebroventricular

IL – interleukin

i.p. – intraperitoneal

ISI – inter-stimulus interval

ITI – inter-trial interval

LD – light dark task

LPS – lipopolysaccharide

NaCl – sodium chloride

No Tx – no treatment

P – postnatal day

PBS – phosphate buffered saline

PPA – propionic acid

PPI – prepulse inhibition

PTSD – post-traumatic stress disorder

SCFA – short-chain fatty acid

TNF – tumor necrosis factor

Chapter 1

1 Endotoxin and predator odour stress in adolescence and the effects of propionic acid on subsequent behaviour

1.1 Introduction

Early life stressors can be single or multiple aversive and/or threatening events that lead to phases of prolonged stress response activation (Pechtel & Pizzagalli, 2011). Stressors during early life are risk factors for developing a number of clinical disorders including anxiety and depression (Lupien et al., 2009). Acute stress responses are adaptive by allowing the body to mobilize its resources and to utilize its coping strategies to promote survival. However, chronic stress conditions, induced by repeated stimulation, can impair cognitive functioning, brain development, and emotional well-being later into adulthood (Pechtel & Pizzagalli, 2011; Lupien et al., 2009). Chronic stress during sensitive developmental periods can also induce physiological, neurobiological, and immunological responses (Lupien et al., 2009). Overall, given that specific brain regions undergo growth at different time points in development, the timing of stressors can impair specific developing brain regions (Pechtel & Pizzagalli, 2011; Andersen et al., 2008). These stressors can also predispose individuals to enhanced sensitivity to further environmental insults, especially during vulnerable periods such as in adolescence (Andersen & Teicher, 2008).

Early life stressors can also predispose individuals to a variety of developmental disorders. Developmental disorders that have been associated with early life stress and immune activation include autism spectrum disorders (ASD). They are a group of neurodevelopmental disorders that include the diagnosis of Autistic Disorder, Asperger Syndrome, and Pervasive Developmental Disorder-Not Otherwise Specified (APA, 2013). There is a 4:1 male to female ratio for the prevalence of this disorder (CDC, 2012), which underlies the importance of studying males. Individuals with ASD show deficits in social communication, restricted interests, and altered sensitivity to sensory stimuli (APA, 2013). Although a strong genetic component can explain around 64-91% heritability of ASD (Tick et al., 2016), its development cannot be fully explained by

genetics. There are many cases of idiopathic regression of ASD that can occur later in development. While some researchers point to environmental triggers, including infection or autoimmune dysfunction (Stefanatos, 2008), more research is still necessary to understand these cases.

Many studies using animal models of ASD examine prenatal and perinatal stressors as risk factors for the development of this disorder (as reviewed by Gardener, Spiegelman & Buka, 2009; Glasson et al., 2004). Most commonly, research on maternal immune activation shows that offspring have an enhanced sensitivity to later environmental insults to develop aberrant behaviour and regression of symptoms similar to ASD (Picci & Scherf, 2015). Although these early developmental periods are important to study, the adolescent period has been widely overlooked in the literature.

Adolescence is an important time-point in the developmental trajectory of humans. During this period, adolescents face increased expectations for social and cognitive functioning within society. These same expectations are also demanded of children with ASD, whom must face many social and cognitive challenges as peer relationships and social competence become increasingly important (Rosenthal et al., 2013). Unfortunately, this period is also a time in which many psychological problems tend to emerge (McCormick, Green & Simone, 2017). Therefore, this developmental stage is a sensitive period to consider when assessing the impact of various stressors on subsequent maladaptive behavioural responses.

The adolescent period in rats ranges from approximately postnatal day (P) 28 – 42, with some changes occurring in males up to P55 (Spear, 2000). During this period, microglia, a type of immune cell in the brain, are still relatively immature in their morphology and may be particularly sensitive to immune insults (as reviewed by Bilbo, Smith & Schwarz, 2012). This period is also key in stress response programming and social development (Wright, Muir & Perrot, 2012; Spear, 2000). When these developmental milestones are impaired by stress, long-term consequences are likely to occur.

The following sections describe the effects of two types of early-life repeated stressors, immune activation by lipopolysaccharide (LPS), and stress induced by predator odour

(cat odour). This will be followed by a discussion on the administration of another environmental insult, the short-chain fatty acid, propionic acid (PPA), a gut bacterial metabolite that has been implicated in the etiology of ASD (MacFabe, 2012).

1.2 Immune activation by lipopolysaccharide (LPS)

Results of previous research suggests that individuals with ASD show elevated levels of neuroinflammation (Pardo et al., 2005; Vargas et al., 2005; Rossignol & Frye, 2012). In a post-mortem study of individuals with ASD, activated microglia and astroglia were observed in the cerebellum, cortical regions, and white matter (Vargas et al., 2005). In addition to the proinflammatory profile observed in the brain tissue, Vargas et al. (2005) also found elevated proinflammatory cytokines in these patients' cerebral spinal fluid.

When ASD patients were given immune system stimulation, they responded with elevated levels of proinflammatory cytokines (TNF-α, IL-1β, IL-6) in comparison to their controls, suggesting an abnormal innate immune response (Jyonouchi, Sun & Le, 2001). More recently, Gupta et al. (2014) found dysregulated microglial responses with exaggerated M2 microglial activation responsible for an anti-inflammatory response observed in brain tissue from patients with ASD. Overall, these results may indicate an aberrant immune response in ASD.

LPS is a bacterial endotoxin component of the cell wall of Gram-negative bacteria that elicits inflammatory responses. LPS is also a by-product of gut bacteria, *Desulfovibrio*, that is found to be elevated in individuals with ASD (Finegold et al., 2010). Peripheral exposure to acute LPS produced chronic neuroinflammation in mice (Qin et al., 2007) that results in long-term behavioural effects. Prenatal LPS has been shown to produce a number of behavioural effects in adolescent rats (Foley et al., 2015). It also reduces adult male rats' social interaction and locomotor activity (Kirsten et al., 2010). Additionally, prenatal LPS decreases the ability of male and female adult rats to filter out sensory information as measured by decreased % prepulse inhibition (PPI; see Figure 1.1 for a description of PPI) (Borrell et al., 2002). Interestingly, when treated with the antipsychotic, haloperidol, this deficit in PPI was abolished.

Aside from the abundant literature on prenatal LPS administration, there is limited research on LPS administration during the sensitive period of adolescence on subsequent behavioural measures. When acute LPS is administered in mice on P14 during the pre-weaning period, prolonged anxiety-like behaviours are observed when later tested in adolescence on P30, as assessed by suppressed feeding behaviours in the center of an open field (Dinel et al., 2014). This same acute LPS exposure on P14 also resulted in increased depressive-like behaviours assessed in adulthood on P90 using the forced swim task (Dinel et al., 2014). Although there is some research on repeated adolescent LPS exposure that suggests behavioural and physiological tolerance following repeated exposure (Wickens et al., 2017; Roth et al., 1994), there is limited research on the long-term behavioural changes induced by repeated LPS administration beyond a 72-hour window.

Further, the immune system is tightly linked with the hypothalamic-pituitary-adrenal (HPA) axis, such that cytokines released in response to immune stimulation activates the HPA axis by releasing glucocorticoids from the adrenal glands (as reviewed by Silverman et al., 2005). These glucocorticoids act as a negative feedback regulator for the production of cytokines (Silverman et al., 2005). Peripheral administration of LPS on P14 in mice alters phosphorylation of the glucocorticoid receptors in the prefrontal cortex, which may contribute to altered cognitive performance in adulthood and impairment of neurogenesis (Dinel et al., 2014). As such, in addition to studying the impact of immune insults on long-term behavioural changes, it is also imperative to study the impact of HPA axis activation.

1.3 Predator stress (cat odour)

Aside from neuronal changes induced by a chronic state of inflammation, environmental stressors such as predator exposure can also result in deficient neuronal development and decreased cognitive functioning (Lupien et al., 2009). Predator odours are salient and innate environmental stressors that have been shown to reliably elicit anxiogenic responses and enhanced defensive behaviours (McGregor et al., 2002; Wright, Muir & Perrot, 2013; Blanchard et al., 1998). Repeated predator odours produce a psychological

stress response that has been used in animal models of post-traumatic stress disorder (PTSD) (Whitaker, Gilpin & Edwards, 2014).

Predator stress can induce behavioural and endocrine changes across the lifespan. A single acute cat odour exposure in adolescence can create anxiogenic responses on the elevated plus maze up to 21 days following exposure (Adamec & Shallow, 1993). When repeatedly exposed to cat odour in adolescence, rats also showed an elevated anxiety-like response on an open field in both adolescence and adulthood (Wright, Muir & Perrot, 2013). Additionally, repeated fox feces odour exposure on P23 – P27 resulted in elevated anxiety-like behaviour in rats as assessed by elevated plus maze in later adolescence and decreased pain sensitivity in adulthood (Post et al., 2014). These long-term behavioural outcomes following repeated adolescent stress indicates the importance of this sensitive period on stress response programming.

In addition to the behavioural changes elicited by rodents in response to predator odour, activation of the HPA axis occurs and can impact cognitive functioning and neuronal development (Lupien et al., 2009). Cat odour exposure increases basal levels of plasma corticosterone, indicating an activated HPA axis in rodents (Blanchard et al., 1998; Wright, Muir & Perrot, 2013). When assessing information processing deficits that may result from predator stress, the ability to filter out sensory information, as measured by PPI, was decreased up to 9 days following predator stress (Bakshi et al., 2012).

Cat odour exposure may alter neural circuitry involved in PPI of an acoustic startle response (ASR) by altering the excitatory and inhibitory balance. When rats are exposed to cat odour, GABA levels are increased in the synapses in the hippocampus and cortex (File, Zangrossi & Andrews, 1993). Cat odour exposure also elevates excitatory glutamate levels that activates NMDA receptors in the amygdala to aid in the establishment of fearful memories (Davis, Rainnie & Cassell, 1994; Ganella & Kim, 2014). This is a mechanism that has been proposed to be involved in establishing the PTSD phenotype in rodents.

HPA axis activation by predator odour, can interact with the gut microbiome and immune function. Corticotropin-releasing factor (CRF) can increase the gut's permeability to

macromolecules (Kelly et al., 2015) to be absorbed into the bloodstream. CRF can also increase the permeability of the blood-brain barrier (BBB) (Esposito et al., 2002). Given that HPA axis activation increases the permeability of critical barriers, it is also important to study the interactions between environmental stressors and the gut-brain axis.

1.4 Short-chain fatty acid: propionic acid (PPA)

A subset of individuals with ASD show elevated levels of *Clostridium* bacteria in their feces (Finegold et al., 2002; Song, Liu & Finegold, 2004; Parracho et al., 2005), which produce fermentation products including PPA and other short-chain fatty acids (SCFA). PPA has been speculated to be a likely link between the gut microbiome and ASD-like behaviour (as reviewed by MacFabe, 2012). When administered intracerebroventricularly (i.c.v.), PPA induces abnormal motor movements, stereotypic behaviour, restricted interests, and impaired social interaction in adult rats (MacFabe et al., 2008; Shultz et al., 2009) and adolescent rats (MacFabe et al., 2011). When administered peripherally in adulthood, PPA induces aversive internal cues as measured through conditioned place avoidance and conditioned taste avoidance (Ossenkopp et al., 2012). During the juvenile developmental period, peripheral PPA impairs social interaction (Shams et al., submitted). Aside from these studies, there is currently limited research on the impact of peripheral PPA on behaviour, and much less on adolescent behaviour.

Peripheral PPA may induce central effects by directly entering the brain via a monocarboxylate transporter (Pierre & Pellerin, 2005) and altering cell signaling by intracellular acidification of glia and neurons (Rörig, Klausa & Sutor, 1996). PPA, like other SCFAs, has been shown to produce epigenetic effects by its histone deactylase (HDAC) inhibiting activity (Bora-Tatar et al., 2009), which increases gene expression by unraveling chromatin to allow for binding of transcription factors (Leoni et al., 2005). HDAC inhibitors are capable of suppressing LPS-induced pro-inflammatory cytokine production (Leoni et al., 2005). In addition, PPA has been recently shown to impair histone decrotonylation (Fellows et al., 2018). Crotonylation is a histone post-translational modification mechanism that alters gene expression, and occurs at high levels in the brain (Fellows et al., 2018), which can contribute to cognitive dysfunction.

Since PPA's actions are tightly linked to immunity and the brain, it is conceivable that PPA's effects on behaviour may be impacted by prior immune or physical stress.

1.5 Objectives

The studies outlined in this thesis examined the effects of early adolescent stressors on late adolescent and adulthood behaviour along with their interactions with other environment insults. The objectives of the first study (Chapter 2) were to determine the effects of early adolescent exposure of LPS on P28, P30, P32, and P34 in male Long-Evans rats on anxiety-like behaviours, acoustic startle response (ASR), and prepulse inhibition (PPI; see Figure 1.1) in later adolescence (P40 and P43) and adulthood (P74 and P77). This study also examined the effects of subsequent acute and repeated PPA in adolescence and adulthood on behaviour. The study aimed to determine whether an interaction effect resulted from prior repeated immune activation followed by PPA in adolescence and adulthood.

In Chapter 3, the effects of an early adolescent repeated cat odour exposure in male Long-Evans rats were examined on anxiety-like behaviour, ASR, and PPI in late adolescence and adulthood. The study also examined the effects of subsequent acute and repeated PPA in adolescence and adulthood on behaviour. The study aimed to determine whether prior repeated stress exposure interacted with the effect of PPA to impact rodent behaviour. Together, these studies allow us to assess the effects of early adolescent immune stress followed by propionic acid and compare them to the effects of early adolescent psychological stress and propionic acid on long-term behavioural outcomes.

It was hypothesized that early immune activation and predator stress would induce long-term behavioural effects on the rats by elevating anxiety-like behaviours and ASR. Acute and repeated PPA administration was hypothesized to produce an ASD-like phenotype by decreasing PPI. Finally, it was hypothesized that early immune activation and predator stress would prime the rats to produce an exacerbated behavioural response to PPA.

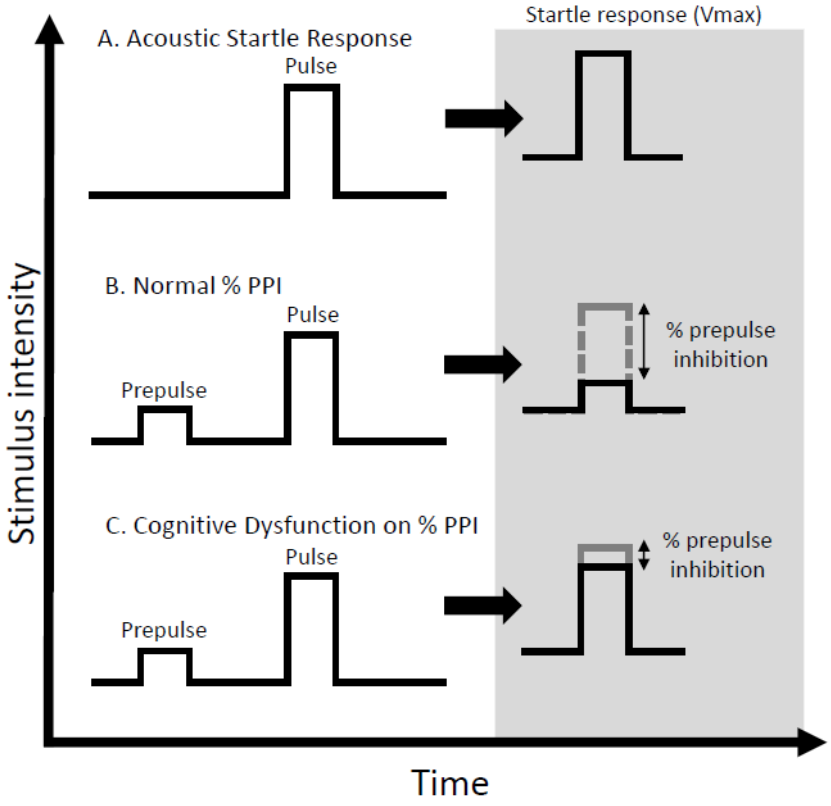

Figure 1.1 Acoustic startle response and % prepulse inhibition. A. Acoustic startle response following a startle pulse. B. Normal startle response to a prepulse. C. Abnormal startle response to a non-startling prepulse indicative of cognitive dysfunction.

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Chapter 2

2 Repeated early adolescent lipopolysaccharide exposure followed by propionic acid treatment later in adolescence and adulthood: effects on anxiety-like behaviours and acoustic startle reactivity in adolescent and adult male rats

2.1 Introduction

According to the Diagnostic and Statistical Manual Fifth Edition, Autism Spectrum Disorders (ASD) are a group of neurodevelopmental disorders that include autism, Asperger's syndrome, and pervasive developmental disorder not otherwise specified (APA, 2013). ASD is characterized by deficits in social behaviours, cognitive inflexibility, stereotyped behaviours, and hyper- or hyporeactivity to sensory input (Carpenter, 2013). In the recent years, there has been a marked increase in the prevalence of ASD in children from 1 out of 150 to 1 out of 68 children diagnosed with the disorder, with approximately four males diagnosed per female (CDC, 2000; CDC, 2012), which underlies the importance for studying males with the disorder.

Although there is no single known cause of ASD, there are several lines of research that support genetic, neurobiological, and immunological contributions to this disorder. The concordance rate of heritability for monozygotic twins with ASD are between 60 – 70% (Sutcliffe et al., 2005), suggesting that environmental factors must also be involved in the etiology of the disorder. An environmental factor that has been linked to ASD is early infection (Sweeten et al., 2004). Prenatal immune activation has been widely used in animal models of this disorder (Borrell et al., 2002; Kirsten et al., 2010).

Prenatal immune activation by lipopolysaccharide (LPS), a bacterial mimetic and endotoxin found as part of the cell wall of Gram negative bacteria, has been shown to reduce social interaction and decrease activity in adult rats (Kirsten et al., 2010). It also disrupts prepulse inhibition (PPI) of the acoustic startle response (ASR) in adulthood (Borrell et al., 2002). However, there is a lack of research on the impact of LPS administration during early adolescence on adulthood.

In humans, mental health problems tend to emerge during the developmental period of adolescence (McCormick, Green & Simone, 2017). Peer relationships and social competence are increasingly important during this time as adolescents navigate their social world.

In rats, the adolescent period ranges from postnatal day (P) 28 – 42, with adolescent changes in males that last up to P55 (Spear, 2000). During this time, immune cells in the brain are relatively immature and may be particularly sensitive to immune insults (as reviewed by Bilbo, Smith & Schwarz, 2012). Environmental stressors during this sensitive period can also greatly increase the risk of psychological disorders such as anxiety and depression (Lupien et al., 2009). Therefore, immune stress during adolescence is likely to induce neurodevelopmental and behavioural changes in later life.

LPS can induce central effects by crossing the blood brain barrier (BBB) via a lipoprotein transporter (Vargas-Caraveo et al., 2017), thereby activating microglia and elevating proinflammatory cytokines, IL-1β, IL-6, IL-12, and TNF-α in the brain (Qin et al., 2007). LPS administered peripherally can also stimulate the release of pro-inflammatory cytokines in the periphery, which can gain access to the central nervous system (CNS) via several routes. Cytokines may pass through leaky regions at the circumventricular organs that bypasses the BBB. Cytokine signals can also be transmitted via the vagus nerve into the brain. Finally, cytokines can bind to carrier proteins that are expressed on the brain endothelium, which generates prostaglandins and nitric oxide in the brain (Raison et al., 2006; Dantzer, 2004). Proinflammatory cytokines can be produced by macrophage cells in the brain in response to pathogens or peripheral cytokines (Dantzer, 2004). Systemic administration of LPS can result in chronic neuroinflammation and neurodegeneration by elevating levels of proinflammatory cytokines for up to 10 months in rodents (Qin et al., 2007). Hence, an LPS-induced increase in proinflammatory cytokines can directly impact brain regions involved in cognition, memory, and emotional processing, and thereby alter behaviour.

Alterations in the functions of the gut-brain axis has been implicated in the development of ASD and other neuropsychiatric disorders. The gut microbiome is a complex array of

commensal bacteria and other microorganisms that affect stress and neuroinflammation (as reviewed by Rea, Dinan & Cryan, 2016). Researchers have found that the gut microbial profile in children with ASD differs widely from those of typically developing children. Many of these children with ASD tend to have comorbid gastrointestinal symptoms of constipation, diarrhea, and abdominal distension (Mannion & Leader, 2014), and show elevated levels of antibiotic-resistant bacteria including *Desulfovibrio, Bacteroidetes,* and *Clostridium* in their stool (Finegold, et al., 2010). This is concerning since *Desulfovibrio* produces LPS, and *Bacteroidetes* and *Clostridium* produce a number of metabolites including the short-chain fatty acid (SCFA), propionate (PPA), which has been implicated in the etiology of ASD.

Acute intracerebroventricular administration of PPA in adulthood has been shown to increase locomotor activity (MacFabe et al., 2008; Thomas et al., 2010) and induce stereotypic movements in rats (Thomas et al., 2010). MacFabe et al., (2011) found that these rats also show impaired social behaviour as they spend less time in the presence of a novel rat than a novel object compared to controls. They also tended to show restricted interests to their preferred objects. Peripheral administration of PPA can enter the CNS from the blood via monocarboxylate transporters (Pierre & Pellerin, 2005), such that PPA peaks in the brain after 60 minutes (Brusque et al., 1999). Behaviourally, intraperitoneal (i.p.) administration of PPA produces aversive internal cues as measured by conditioned place avoidance and conditioned taste avoidance (Ossenkopp et al., 2012), suggesting that peripheral PPA may induce behavioural and cognitive changes. Repeated peripheral PPA also impairs social interaction in juvenile rats immediately after exposure (Shams et al., submitted). However, there is limited research on PPA administration given during adolescence, and subsequent repeated PPA exposure on behaviour in adulthood.

The present study aimed to better understand the effects of environmental stressors on the adolescent developmental period, as it is a sensitive period for stress response programming and social development (Spear, 2000). The present study examined the effect of early adolescent repeated LPS exposure on later adolescent and adulthood behaviour. In addition, it investigated the effects of acute and repeated PPA exposure on subsequent behaviour in adolescence and adulthood. This study examined whether prior

LPS exposure led to an interaction with PPA on behaviour as assessed by the light-dark anxiety task, ASR, and PPI. It was hypothesized that LPS would induce anxiety-like behaviour that persists into late adolescence and adulthood. It was further hypothesized that PPA would produce altered cognitive functioning as assessed by PPI. These effects of PPA were hypothesized to be augmented by prior exposure to immune activation by LPS.

2.2 Materials and Methods

2.2.1 Subjects

Fifty-six male Long Evans rats (Charles River, Quebec, Canada) were obtained at 51 - 75 g, which corresponds to the age of postnatal day (P) 22. All animals were pair-housed with another rat of the same treatment group in standard polypropylene cages (45 x 22 x 20 cm) and maintained in a temperature-controlled room at 20 ± 1°C on a 12:12 light-dark cycle (lights on at 0700 to 1900 h). All animals had ad libitum access to food (RHM Prolab 3000 rat chow) and tap water. Rats were identified using a numeric system and marked with permanent marker on their tails. When markings faded, the rats were re-marked. All experimental procedures complied with the Canadian Council for Animal Care and the Institutional Animal Care Committee.

2.2.2 Drugs

Lipopolysaccharide (LPS; from *E. coli* serotype 0111:B4, L-2630, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 0.2 mg/kg was dissolved in 0.9% NaCl and injected in early adolescence on P28, P30, P32, and P34. A vehicle control using 0.9% NaCl was injected on these same days. All treatments were administered intraperitoneally (i.p.) at 2.0 ml/kg body weight. A no treatment control (No Tx) group was lightly handled on these days. The dose of 0.2 mg/kg of LPS was chosen as it produces a significant dose-dependent reduction of ASR (Lockey, Kavaliers & Ossenkopp, 2009). To ensure that the behaviours detected by the behavioural assays were not just products of the sickness behaviours induced by LPS within a 24 hour post-injection period (Custódio, et al., 2013;

Dantzer et al., 2008), animals were behaviourally tested at least six days after the last LPS injection.

Sodium propionate (P1880, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 500 mg/kg was dissolved in 0.1 M phosphate buffered saline (PBS) and buffered to pH of 7.5 by HCl. This dose was chosen as it produces reduced locomotion on the conditioned place avoidance task (Ossenkopp, et al., 2012). Sodium propionate and PBS were administered in late adolescence on P40 and P43, and in adulthood on P74 and P77.

2.2.3 % Body Weight Change

Body weight decrease (O'Connor et al., 2009) was used as an indicator of an LPS-induced sickness response. It was calculated using the following formula:

$$\%\ Body\ Weight\ Change = 100 \times \frac{24h\ post\ test\ injection\ day\ body\ weight - injection\ day\ body\ weight}{injection\ day\ body\ weight}$$

2.2.4 Light-Dark Apparatus & Behavioural Measures

Anxiety-like behavioural variables from the light-dark apparatus were collected using eight VersaMax Animal Activity Monitors (Accuscan Model RXYZCM-16, Columbus, OH). Monitors were made of clear Plexiglas (42 x 42 x 30 cm) enclosed by a Plexiglas lid containing air-holes. The box was equipped with 16 infrared sensors located every 2.54 cm along the perimeter and 7 cm above the floor. Another 16 sensors were located 18 cm above the floor on opposite sides. This apparatus was divided into a light or dark chamber using a black opaque Plexiglas box insert (40 x 20 x 23 cm). The light chamber was illuminated by 3 fluorescent light sources located above the monitors. The dark chamber has small holes located on the sides to permit infrared beams to pass through. A hole in the partition between the light and dark chambers at floor level allowed for the rat to freely pass between chambers. Data were collected and analyzed by a VersaMax Analyzer (Accuscan Model CDA-8, Columbus, OH) and sent to a computer where it was recorded and retrieved. Procedures were adapted from Banasikowski et al. (2015).

The VersaMax Analyzer collected two types of behavioural variables: chamber-choice and activity variables (Table 2.1). The chamber-choice variables included the duration of time spent in the light chamber and the number of transitions to the light chamber. These variables provide measures for risk assessment and anxiety-like behaviours demonstrated by avoidance of the light chamber (Ossenkopp et al., 2005; Banasikowski et al., 2015).

The other type of behavioural variables collected by the VersaMax Analyzer were activity variables in both the light and dark chambers. These included the total cumulative distance travelled and time spent in a vertical position. All activity variables were corrected for the total time spent in their respective chambers. For example, the corrected total distance travelled in the light chamber was calculated as the total distance travelled per second spent in the light chamber.

Table 2.1 Behavioural measures recorded on the light-dark anxiety task

Behavioural Measures	Definition
Chamber Choice Variables	
Light Duration	Time spent in the light chamber.
Chamber Transitions	Number of transitions between the light and dark chambers.
Activity Variables	
Total Distance	Cumulative horizontal distance travelled.
Vertical Time	Time spent in rearing position. This measure does not consider the time when animal goes below the vertical sensor.

2.2.5 Acoustic Startle Response & Prepulse Inhibition

Acoustic startle response (ASR) and pre-pulse inhibition (PPI) testing were conducted in three startle devices (SRLAB, San Diego Instruments, San Diego, CA). Each apparatus consisted of a cylindrical, clear acrylic rat enclosure (8.89 cm inside diameter and 20.32 cm length) mounted on an acrylic platform. A piezoelectric accelerometer that lies underneath the platform detected the force of the rat's non-voluntary movement. This apparatus was placed inside a ventilated sound attenuated box containing fluorescent lighting and a speaker which was 11 cm from the top back of the box. Data were recorded

and stored by a computer attached to the accelerometer. Procedures were adapted from Lockey, Kavaliers, and Ossenkopp (2009).

The startle responses were measured as the magnitude of the maximum voltage detected by the startle device. The average peak voltage response for Startle, 76 dB PPI and 82 dB PPI trials were computed. Percent PPI was calculated as a percent difference from the startle-only startle response for the 76 dB and 82 dB pre-pulse level. This was calculated using the following formula:

$$\%\, PPI = 100 \times \frac{startle\ only\ startle\ magnitude - PPI\ startle\ magnitude}{startle\ only\ startle\ magnitude}$$

Habituation to the startle stimulus was measured using percent habituation to the startle stimuli. This was calculated using the following formula:

$$\%\, Habituation = 100 \times \frac{avg\ startle\ response\ on\ trials\ 6\ to\ 10 - avg\ startle\ response\ on\ last\ 5\ trials}{avg\ startle\ response\ on\ trials\ 6\ to\ 10}$$

2.2.6 Experimental Procedure

Rats were assigned to five weight-matched manipulation groups: 1. LPS-PPA, 2. LPS-PBS, 3. NaCl-PPA, 4. NaCl-PBS, and 5. No Tx. On the day of arrival, rats were pair-housed without manipulation for three days to habituate them to the facility. They were handled on their fourth and fifth day, and weighed on their sixth day (see **Error! Reference source not found.**).

2.2.6.1 Phase 1: Adolescence

According to Spear (2000), the adolescent period in rats occurs from approximately P28 - P42. In males, the adolescent phase has been suggested to last until P55. Repeated administration of the bacterial endotoxin, LPS, its vehicle, or No Tx, occurred in early adolescence on P28, P30, P32, and P34. Rats were weighed every day at approximately the same time per day throughout the LPS treatments.

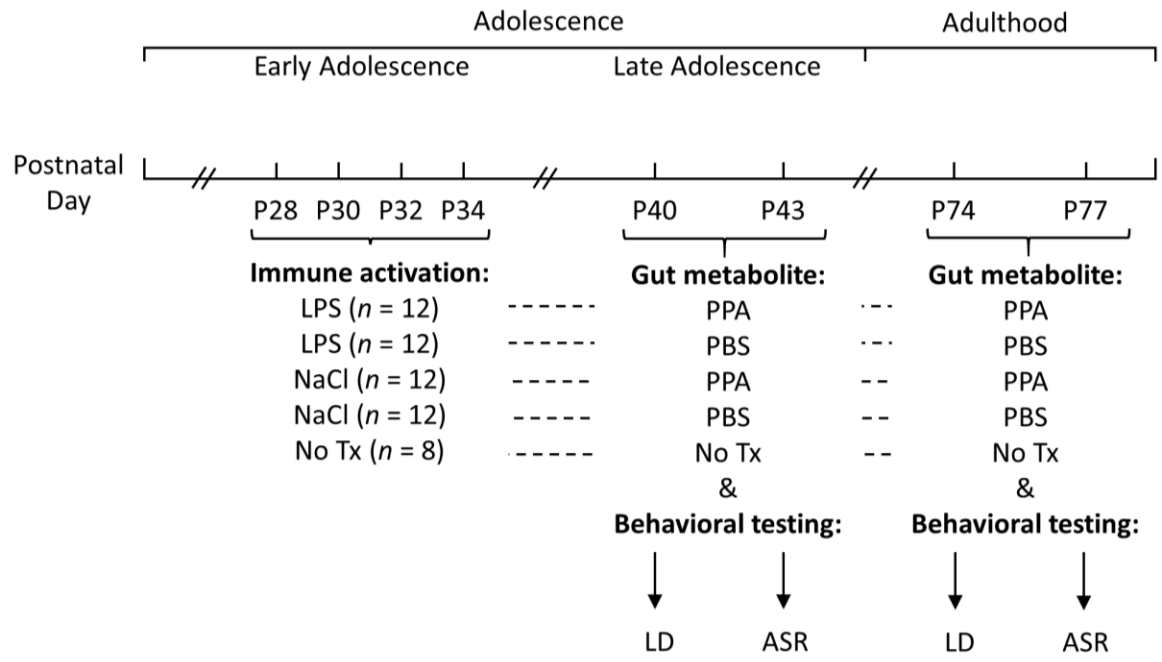


Figure 2.1 Experimental timeline.

The rats were given six days to recover from the LPS insult before they were injected with PPA, its vehicle, or received No Tx on P40. After waiting 15 minutes for PPA to take effect, rats were tested in the light-dark apparatus for 15 minutes. Between each testing session, the apparatus was cleaned with detergent and water, followed by baking soda in water to remove odours, and dried with paper towel to remove remaining stains.

On P43, the rats were again weighed and injected with PPA, PBS, or received No Tx. After waiting 10 minutes, the rats were placed in the startle apparatus. Each testing session began with a 5 min acclimation period with continuous background noise at 70 dB. The 11 min testing phase consisted of 45 trials starting with 10 startle-only trials at 120 dB presented for 40 ms. The subsequent 30 trials were presented in pseudo-randomized order: 10 startle-only (120 dB for 40 ms) and 20 PPI trials (ten 76 dB and ten 82 dB non-startling pulses presented for 20 ms and a 120 ms wait until the 120 dB startle pulse). The session ended with 5 startle-only trials (120 dB for 40 ms). The startle response was recorded by a computer for 100 ms following the onset of the startle stimulus. All trials were separated by an inter-trial interval (ITI) of 8-23 seconds in length (average ITI = 15 seconds). The apparatus was calibrated every four testing sessions to ensure consistency in recording. Between each testing session, the animal enclosure was cleaned with detergent and water, and dried with paper towel to remove remaining stains.

2.2.6.2 Phase 2: Adulthood

The same procedure used in late adolescence on P40 and P43 were conducted in adulthood on P74 and P77, respectively, with injections of PPA, PBS, or No Tx on those days followed by either light-dark testing or startle testing.

2.2.7 Statistical Analyses

All statistical analyses were completed using IBM SPSS Statistics 24. On the percent body weight change measure, repeated measures analyses of variances (ANOVA) was conducted comparing the interaction effect between postnatal day and LPS treatment. Greenhouse-Geisser corrections were used for repeated measures analyses that violates Mauchly's Test for Sphericity.

Repeated measures ANOVA for the behavioural measures from the light-dark and startle tasks compared the treatment effects between adolescence and adulthood. The between-subject factors were the treatment groups and the within-subject factors were the adolescent and adulthood time-points. LSD post-hoc pairwise comparisons were performed with a significance criteria set to $\alpha = 0.05$ for all hypotheses tested.

2.3 Results

2.3.1 % Body Weight Change

There was a postnatal day by LPS treatment interaction effect on % body weight change 24-hours after LPS injection $F(3.106, 82.317) = 8.433$, $p < .001$ (Figure 2.2). The body weight decrease observed on P28 in the LPS administered rats was significantly different from both the NaCl and No Tx group ($ps < .001$). On P30, the LPS administered rats showed a lower body weight increase in comparison to both control groups (NaCl: $p = .001$, No Tx: $p = .043$). No significant differences were observed on P32.

2.3.2 Light-Dark: Anxiety & Activity

2.3.2.1 Chamber-Choice Variables

There were no significant age by treatment effects on the duration spent in the light chamber (Figure 2.3) However, there was a main effect of PPA when combining the rats in adolescence and adulthood, whereby PPA-treated rats spent less time in the light chamber than their PBS controls ($p = .040$; Figure 2.3).

There were no significant age by treatment effects on the number of transitions into the light chamber (Figure 2.4). There was a main effect of LPS when combining the rats in adolescence and adulthood, such that LPS-treated rats showed fewer transitions into the light chamber compared to their NaCl controls ($p = .020$). There was a main effect of PPA, whereby PPA-treated rats showed fewer number of transitions into the light chamber compared to their PBS controls when adolescence and adulthood were combined ($p < .001$) (Figure 2.4).

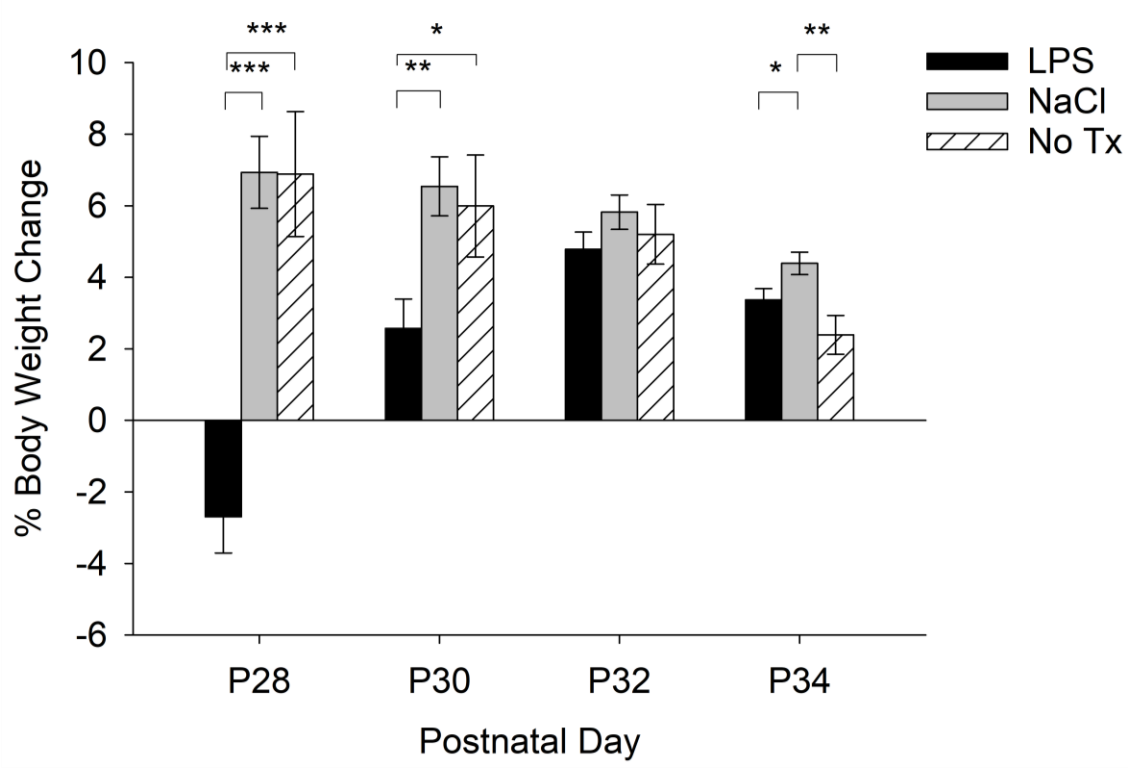


Figure 2.2 Percent body weight change 24-hours post-injection. Rats were injected i.p. with LPS (*n* = 24), NaCl (*n* = 24), or had No Tx (*n* = 8) on P28, 30, 32, and 34 depending on their treatment condition. The LPS-treated rats showed a decrease in body weight in comparison to the control groups 24 hours following the first and second injections. This effect was absent on P32. **p* < .05, ***p* < .01, ****p* < .001. Data is represented as means ± SEM.

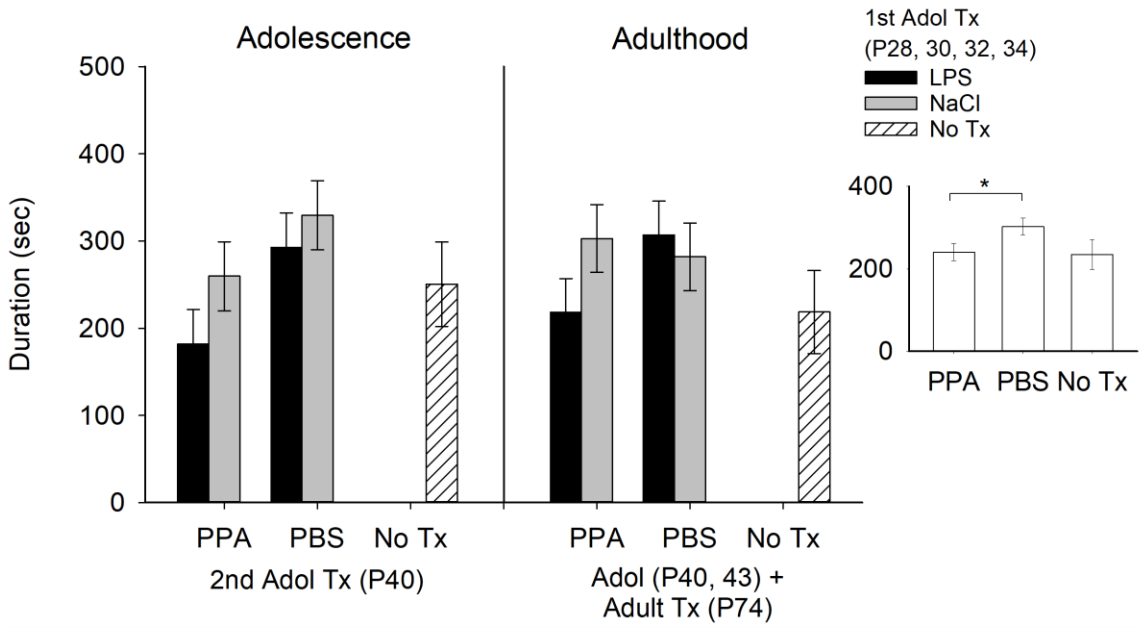


Figure 2.3 Duration spent in the light chamber of the light-dark apparatus on P40 and P74. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, 30, 32, and 34 and PPA, PBS, or had No Tx on P40, 43, and 74 (*n* = 12/injected group, *n* = 8/No Tx group). There were no significant age by treatment effects. There was a main effect of PPA treatment as seen in the graph on the right, such that PPA-treated rats showed decreased duration into the light chamber compared to their PBS controls. *$p < .05$. Data is represented as means ± SEM.

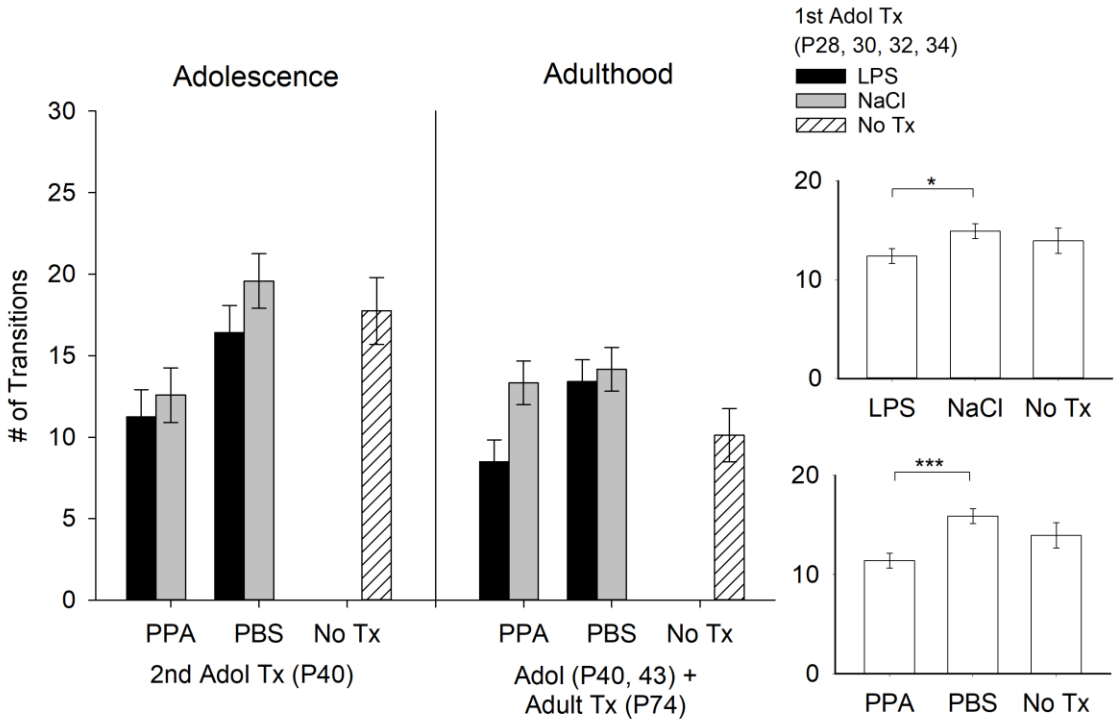


Figure 2.4 Number of transitions into the light chamber of the light-dark apparatus on P40 and P74. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, 30, 32, and 34 and PPA, PBS, or had No Tx on P40, 43, and 74 (*n* = 12/injected group, *n* = 8/No Tx group). There were no significant age by treatment effects. There was a main effect of LPS treatment as seen in the graph on the top right, whereby LPS-treated rats showed decreased transitions into the light chamber compared to their NaCl controls. There was a main effect of PPA treatment as seen in the graph on the bottom right, such that PPA-treated rats showed decreased transitions into the light chamber compared to their PBS controls. *$p < .05$, ***$p < .001$. Data is represented as means ± SEM.

2.3.2.2 Activity Variables: Corrected Horizontal Locomotion

In the light chamber, there were no significant age by treatment effects on the corrected horizontal locomotor measures in the light chamber.

In the dark chamber, corrected total distance travelled showed an age by treatment interaction effect, $F(4, 51) = 3.944$, $p = .007$, whereby in adolescence, the LPS-PPA group showed a lower corrected dark total distance travelled in comparison to the LPS-PBS, NaCl-PBS, and No Tx control groups (*ps* < .05; Figure 2.5). The LPS-PBS group showed a lower corrected dark total distance travelled in comparison to the NaCl-PBS and No Tx groups (*ps* < .05). Finally, the NaCl-PPA group showed a significantly lower corrected dark total distance travelled in comparison to the NaCl-PBS and No Tx control group (*ps* < .05). In adulthood, the LPS-PPA group maintained a lower corrected dark total distance than the LPS-PBS and NaCl-PBS group (*ps* < .05). When analyzing the main effects of LPS when combining adolescence and adulthood, the LPS-treated rats showed decreased corrected dark total distance than their NaCl and No Tx controls (*ps* < .05). There was also a main effect of PPA, whereby PPA-treated rats showed decreased corrected dark total distance compared to their PBS and No Tx controls (*ps* < .05).

2.3.2.3 Activity Variables: Corrected Vertical Locomotion

On corrected vertical time in the light chamber, there was a trending age by treatment effect, $F(4, 51) = 2.552$, $p = .050$. There was an age by LPS effect, $F(1, 51) = 7.263$, $p = .010$ (Figure 2.6B), such that LPS-treated rats showed an increased corrected light vertical time in adulthood compared to adolescence ($p < .001$). Further, the No Tx group showed a similar increase in corrected light vertical time in adulthood ($p = .001$). However, this was not seen in the NaCl control group. There was a main effect of PPA when adolescence and adulthood were combined, whereby PPA-treated rats exhibited less vertical movement time in the light chamber than their PBS controls (Figure 2.6A).

In the dark chamber, fewer treatment effects were found. There was a main effect of PPA treatment, such that PPA-treated rats showed decreased vertical movement time compared to their PBS controls ($p = .009$; Figure 2.7).

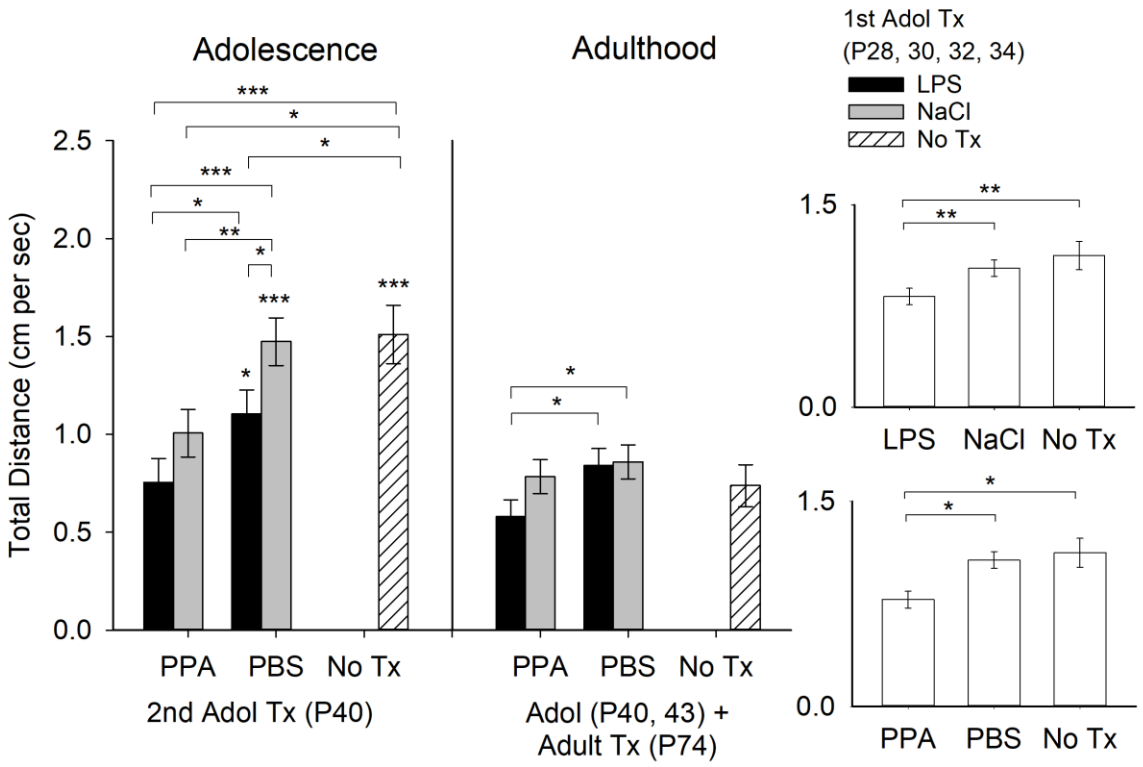
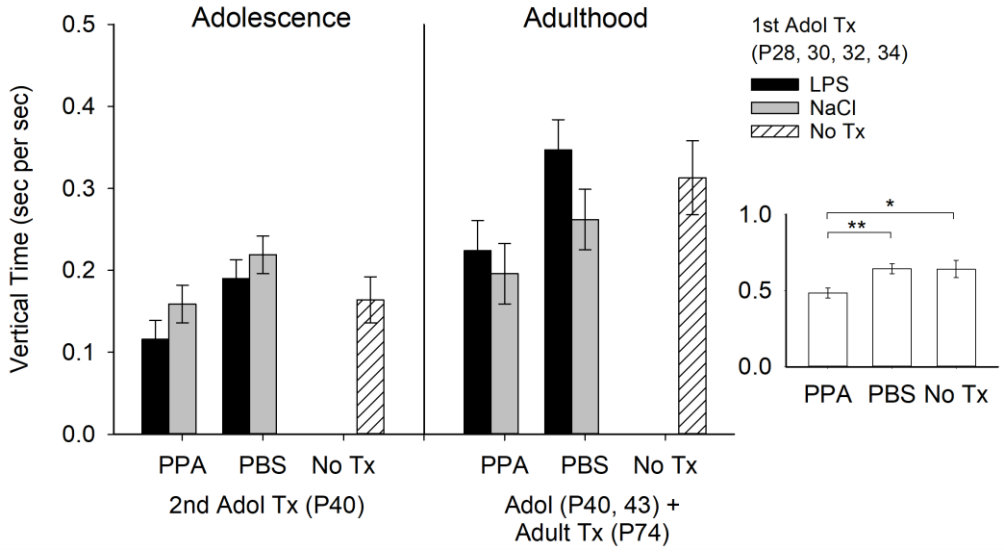


Figure 2.5 Corrected total distance travelled in the dark chamber of the light-dark apparatus on P40 and P74. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, P30, P32, and P34 and PPA, PBS, or had No Tx on P40, P43, and P74 (*n* = 12/injected group or *n* = 8/No Tx group). Corrections were made by dividing by the duration spent in the dark chamber. In both adolescence and adulthood, the LPS-PPA rats travelled less distance than their NaCl-PBS controls. There was a main effect of LPS as seen in the graph on the top right, such that LPS-treated rats also showed decreased total distance travelled in the dark chamber than the NaCl and No Tx controls when adolescence and adulthood were combined. There was a main effect of PPA as seen in the graph on the bottom right, whereby PPA-treated rats showed decreased total distance travelled in the dark chamber than PBS and No Tx controls when adolescence and adulthood were combined. **p* < .05, ***p* < .01, ****p* < .001. Data is represented as means ± SEM.

A)



B)

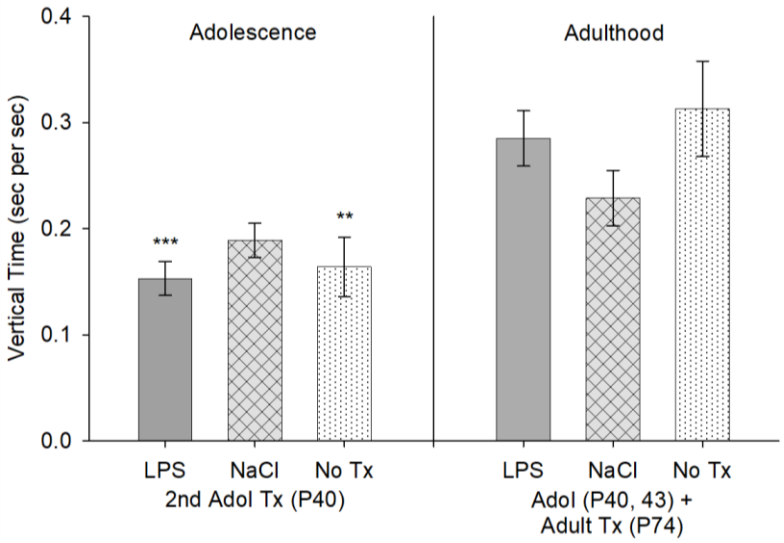


Figure 2.6 Corrected vertical time in the light chamber of the light-dark apparatus on P40 and P74. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, P30, P32, and P34 and PPA, PBS, or had No Tx on P40, P43, and P74 (*n* = 12/injected group or *n* = 8/No Tx group). Corrections were made by dividing by the duration spent in the light chamber. A) There was a main effect of PPA as seen in the graph on the right, such that PPA-treated rats spent less time in vertical position than PBS and No Tx controls. B) In adolescence, the LPS-treated rats spent less time in vertical position than in adulthood. The No Tx controls showed the same increase in vertical time in adulthood compared to adolescence. **p* < .05, ***p* < .01, ****p* < .001. Data is represented as means ± SEM.

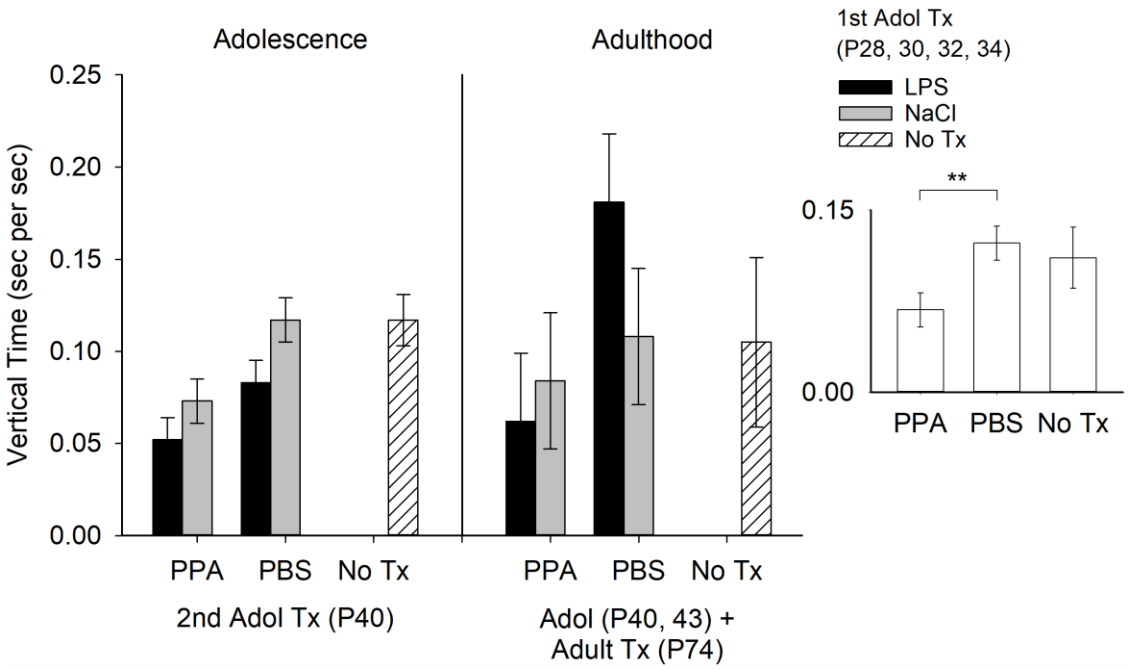


Figure 2.7 Corrected vertical time in the dark chamber of the light-dark apparatus on P40 and P74. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, P30, P32, and P34 and PPA, PBS, or had No Tx on P40, P43, and P74 (n = 12/injected group or n = 8/No Tx group). Corrections were made by dividing by the duration spent in the dark chamber. There was a main effect of PPA as seen in the graph on the right, such that PPA-treated rats spent less time in vertical position in the dark chamber than their PBS controls. $^{**}p < .01$. Data is represented as means ± SEM.

2.3.3 Acoustic Startle Response (ASR) & Percent Prepulse Inhibition (%PPI)

The average ASR also showed an age by LPS interaction effect, such that the LPS-treated rats showed an increased ASR in adulthood compared to adolescence, $F(1, 51) = 8.030$, $p = .007$ (Figure 2.8B), such that LPS increased average ASR to levels greater than their controls in adulthood ($p = .018$).

On average ASR, there was an age by treatment group effect, $F(4, 51) = 3.362$, $p = .016$ (Figure 2.8A). In adolescence, the LPS-PPA group showed a lower ASR than the LPS-PBS ($p = .044$) and No Tx control group ($p = .014$). The NaCl-PPA group showed a lower ASR than the No Tx group ($p = .025$). In adulthood, the LPS-PBS group showed a steep increase in startle response in comparison to adolescence ($p < .001$), where the response was greater than that presented by all other treatment groups, except the No Tx control group. The average ASR also showed an age by LPS interaction effect, such that the LPS-treated rats showed an increased ASR in adulthood compared to adolescence, $F(1, 51) = 8.030$, $p = .007$ (Figure 2.8B), such that LPS increased average ASR to levels greater than their controls in adulthood ($p = .018$). There was a main effect of LPS when both adolescence and adulthood were combined (Figure 2.8A), observed by LPS-treated rats showing greater ASR than the NaCl controls ($p = .048$). There was also a main effect of PPA combining both adolescence and adulthood, whereby PPA-treated rats showed a decreased ASR in comparison to their PBS controls ($p = .043$).

When given a 76 dB pre-pulse, 6 dB above background noise, an age by treatment interaction effect was observed, $F(4, 51) = 2.649$, $p = .044$ (Figure 2.9A), such that in adolescence, the NaCl-PPA group had a lower %PPI than the NaCl-PBS controls ($p = .046$). In addition, the 76 dB %PPI of LPS-PPA ($p = .001$), NaCl-PPA ($p < .001$), and the No Tx controls ($p = .001$) was lower in adolescence than in adulthood, such that in adulthood, their %PPI levels were normalized across all treatment groups. There was also an age by PPA interaction effect, $F(1, 51) = 7.253$, $p = .010$, whereby PPA decreased the 76 dB %PPI in adolescence compared to their controls ($p = .023$; Figure 2.9B). This decreased %PPI observed in PPA rats normalized in adulthood, at which point their %PPI

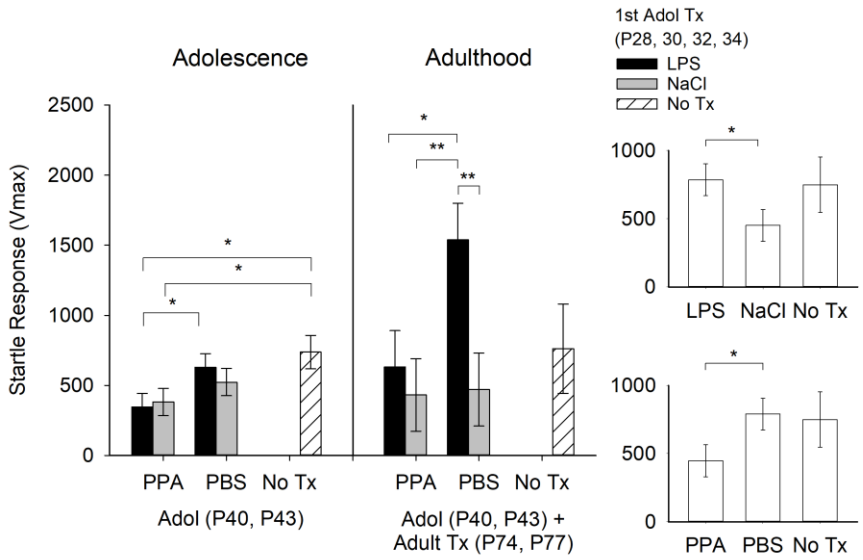
became similar to all other treatment groups. When given an 82 dB pre-pulse, 12 dB above background noise, an age by treatment interaction effect was observed, $F(4, 51) = 2.688$, $p = .041$ (see Appendix A.1), such that in adolescence, the LPS-PPA group showed a decreased %PPI in comparison to the NaCl-PBS group ($p = .038$).

On % habituation, there was an age by treatment interaction effect, $F(4, 51) = 2.873$, $p = .032$ (Figure 2.10). In adulthood, the No Tx group showed greater % habituation to the startle response than the injection groups ($ps < .05$), except LPS-PBS. The No Tx group showed an increase in % habituation to the startle response across age ($p = .002$).

2.3.4 Summary of Results

This study showed that early adolescent repeated LPS exposure reduced activity long after LPS administration when tested in later adolescence and adulthood. Rats exposed to LPS produced less transitions into the light chamber, which in conjunction with the former results indicates elevated anxiety levels in these rats. Additionally, early adolescent repeated LPS exposure produced a delay in increase in ASR that occurred in adulthood. PPA decreased activity on both horizontal and vertical measures, and decreased duration in the light chamber, which taken together indicates an aversive internal state which presented itself as elevated levels of anxiety and a decreased motivation to move. In adolescence, PPA administration produced decreased %PPI as a measure of sensorimotor gating. PPA showed no effect on %PPI in adulthood compared to their controls. Further, PPA decreased overall startle response when adolescence and adulthood were combined. On the activity behavioural measures, the effects of LPS and PPA occur in an additive fashion. However, the effects of LPS and PPA oppose each other on ASR, the measure of sensory sensitivity, whereby LPS increases ASR in a delayed fashion, while PPA depresses ASR.

A)



B)

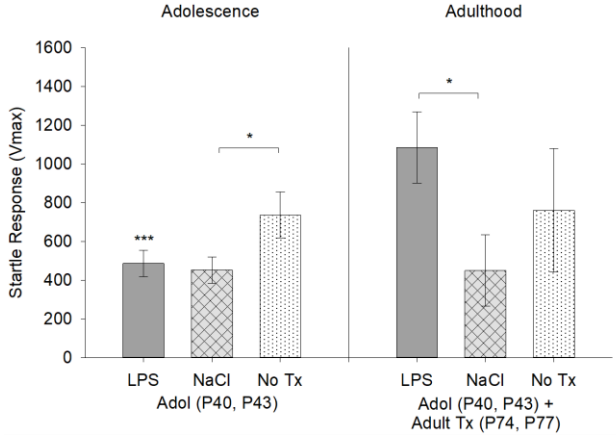
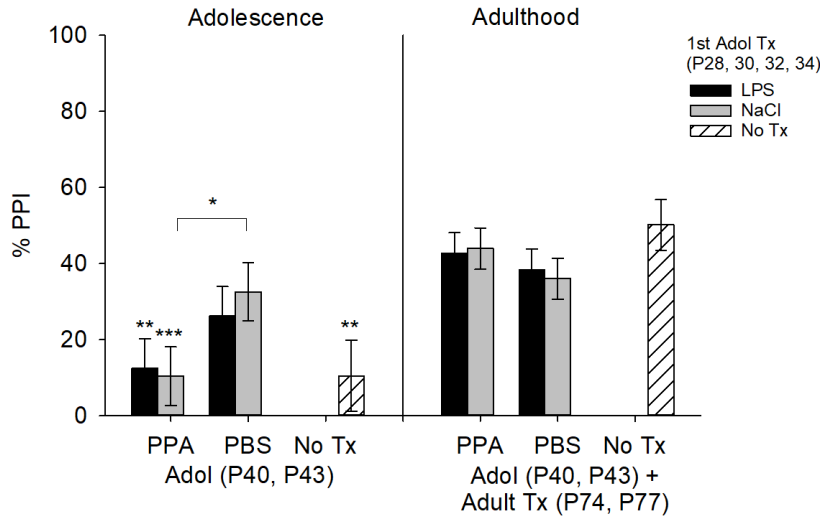


Figure 2.8 Average acoustic startle response for a 120 dB pulse on P43 and P77. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, 30, 32, and 34 and PPA, PBS, or had No Tx on P40, 43, 74, and 77 (n = 12/injected group or n = 8/No Tx group). A) In adolescence, the LPS-PPA group had a lower ASR than the No Tx group. In adulthood, the LPS-treated rats had a greater ASR in comparison to their controls. There was a main effect of LPS as seen in the graph on the top right, such that LPS-treated rats exhibited a greater ASR than their NaCl controls. There was a main effect of PPA as seen in the graph on the bottom right, such that PPA-treated rats exhibited a lower ASR than their PBS controls. B) LPS treatment elevated average ASR in adulthood compared to adolescence. In adulthood, the LPS-treated rats showed a greater ASR than their NaCl controls. *p < .05, **p < .01, ***p < .001. Data is represented as means ± SEM.

A)



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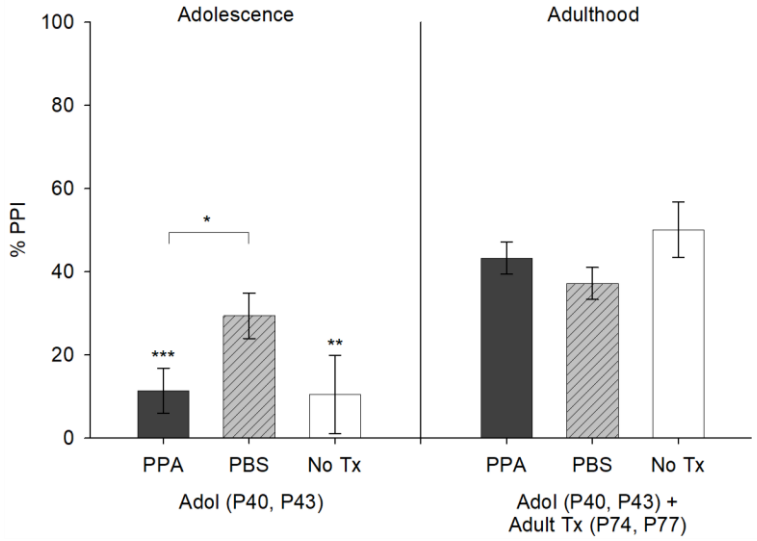

Figure 2.9 % Prepulse Inhibition for a 76 dB prepulse on P43 and P77. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, 30, 32, and 34 and PPA, PBS, or had No Tx on P40, 43, 74, and 77 (*n* = 12/injected group or *n* = 8/No Tx group). A) In adolescence, the NaCl-PPA group showed a decreased %PPI than the NaCl-PBS group for a 76 dB prepulse. The LPS-PPA, NaCl-PPA, and No Tx groups experienced an age increase in %PPI. B) PPA-treated rats showed a decreased %PPI for a 76 dB in adolescence compared to adulthood. In adolescence, the PPA group showed a lower %PPI for a 76 dB prepulse compared to PBS controls. In adulthood, there were no significant differences between treatment groups. *$p < .05$, **$p < .01$, ***$p < .001$. Data is represented as means ± SEM.

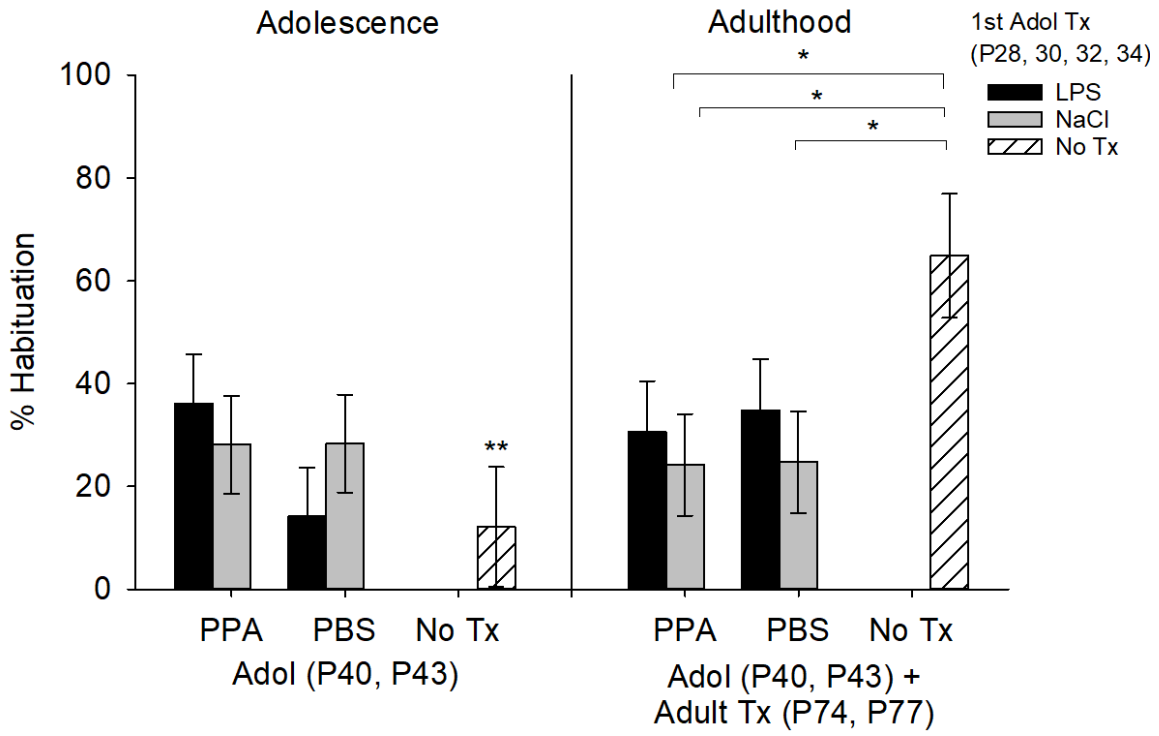

Figure 2.10 % Habituation of the startle response from the first 6 to 10 trials to the last 5 trials on P43 and P77. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, 30, 32, and 34 and PPA, PBS, or had No Tx on P40, 43, 74, and 77 (n = 12/injected group or n = 8/No Tx group). In adulthood, the No Tx group showed greater % habituation to the startle response than the injection groups, except LPS-PBS. The No Tx group showed an increase in % habituation to the startle response across age. *p < .05, **p < .01. Data is represented as mean ± SEM.

2.4 Discussion

2.4.1 Early adolescent repeated LPS exposure increased anxiety-like behaviour, escape behaviour, and produced long-term effects on startle response

In many species, the early adolescent period is a vulnerable period, where there are prominent changes in the prefrontal cortex and limbic regions (Spear, 2000). Exposure to immune challenges during this period are critical to development and were explored in the present study. When rats were administered LPS repeatedly in early adolescence, long-term behavioural changes were induced when tested later in adolescence and in adulthood. LPS induced anxiety-like behaviours in adolescence and adulthood as demonstrated by a decreased number of transitions into the light chamber and decreased horizontal locomotion in the dark chamber when adolescence and adulthood were combined. In the light-dark task, it was advantageous to present normal locomotor activity in the risky brightly lit environment to avoid potential predators. However, in the dark chamber that was considered safe, the rats resumed their abnormal behaviours. Hence, fewer treatment differences were observed on activity measures in the light chamber compared to the dark chamber.

The results of this study taken together indicate that the LPS-treated rats display aversiveness to the brightly lit chamber. This is consistent with previous research on neonatal LPS-treated rats, such that LPS treatment resulted in more time spent hiding in a hide box on an open field in adulthood (Walker et al., 2009). In the present study, prior LPS treatment induces decreased activity in the dark chamber after injection stress, consistent with double-hit models of psychopathology. When LPS is administered neonatally followed by stress exposure in adulthood, a decreased general activity in male rats is observed to a greater extent than the rats only exposed to neonatal LPS without the stress exposure (Walker et al., 2009). The present study used early adolescent repeated LPS exposure followed by PPA and stressful behavioural testing resembling multiple stress hits, which are predictive of behavioural dysfunction and psychopathology (Maynard et al., 2001; Picci & Scherf, 2015).

In adolescence, the rats administered repeated LPS six days prior spent less time in vertical position in the light chamber compared with adulthood. In adulthood, these vertical locomotor levels of the LPS-treated rats returned to levels comparable to their controls. This provides an approximate timeline by which depressive-like symptoms dissipate following repeated early adolescence LPS administration. The decrease in vertical locomotion observed in adolescence was not surprising as previous research on neonatal LPS treatment also induced decreased exploration on the open arms of the elevated plus maze in adulthood (Walker et al., 2004). This decreased exploration and escape behaviour observed in rats administered LPS resemble depressive-like behaviours, as these rats show less motivation to escape the aversive stimuli.

Early adolescent repeated LPS-treated rats produced an increased ASR in adulthood in comparison to adolescence. This delay in increased ASR is consistent with prior research where neonatal exposure to LPS predisposed animals to an elevated ASR when given restraint stress in adulthood (Walker et al., 2008). This elevated ASR may be driven by increased HPA axis activity in response to stressors as indicated by elevated corticosterone following LPS exposure (Shanks et al., 2000; Nilsson et al., 2002; Walker et al., 2008). Further, early LPS induces a long-term proinflammatory state into adulthood by elevating proinflammatory cytokines: IL-2, IL-6 (Borrell et al., 2002) and TNF-α (Qin et al., 2007; Tedelind et al., 2007), which can explain why the effects of LPS are sustained 45 days after the rats' last exposure.

During the LPS treatment days, it was imperative to ensure that LPS induced an immune challenge to the rat. As such, the body weight decrease observed 24 hours after the first two LPS injections were indicative of an immediate sickness response. By the third injection, a tolerance effect was observed, such that body weight change became no different from the controls, as expected from prior research (Chan et al., 2013; Cross-Mellor et al., 2009). Since repeated constant doses of LPS exposure induces a tolerance effect, future studies using repeated LPS to induce a chronic inflammatory state may choose to use increasing doses of LPS. According to Wickens et al. (2017), increasing repeated doses of LPS produced a more sustained behavioural response on the forced swim test, measured by increased immobility time than produced by a constant dose of

LPS. Perhaps, the repeated immune activation in adolescence produced by increasing LPS doses may result in greater behavioural changes.

2.4.2 PPA administration increased anxiety-like behaviour, decreased activity, and decreased sensorimotor gating

Intracerebroventricular (i.c.v.) administration of PPA has been shown to elicit bouts of abnormal behaviours including hyperextension of the body to the surface, rotation, and contraction of limb muscles (MacFabe et al., 2007). These behaviours observed using a central route of administration are also observed anecdotally through the peripheral route of administration used in the present study. This transient behaviour returned to normal over a couple of minutes, after which behavioural testing occurred. In the present study, PPA increased anxiety-like behaviours in rodents and decreased activity localized in the dark chamber of the light-dark apparatus. Similar to the effect of LPS, PPA-treated rats showed greater aversiveness to the brightly lit chamber than their PBS controls. The PPA-administered rats showed decreased horizontal locomotor levels in the dark chamber of the light-dark apparatus as well as decreased vertical locomotor variables in both the light and dark chambers when adolescence and adulthood were combined, indicating a decreased motivation to move. The decrease in vertical locomotion was not surprising as this result confirms prior findings that PPA produces an unconditioned reduction of vertical movement numbers on the first trial of a conditioned place aversion task (Ossenkopp et al., 2012). This evidence for decreased activity using the peripheral route of PPA administration is contrary to the hyperlocomotion observed using i.c.v. administration. It was also interesting that PPA altered behaviours even in the brightly lit chamber where the rats should be presenting as normal locomotor activity as possible. These observable behavioural abnormalities indicate an aversive internal state as described by Ossenkopp et al. (2012) which presented itself as a decreased interest in exploration and altered ability to avoid predators.

In addition to the decreased activity and exploration, PPA administration also decreased that rats' sensorimotor gating ability. They showed a decreased ability to modulate the startle response when given a prepulse, which is indicative of a maladapted ability to filter out redundant auditory stimuli. The proposed neural circuit involved in PPI is

composed of the auditory system (cochlear nuclei, superior olivary complex and nuclei of the lateral lemniscus), the inferior and superior colliculus, and pedunculopontine tegmentum, which conveys information to the pontine reticular nucleus (Koch, 1999). This is all modulated by input from the prefrontal cortex, hippocampus, amygdala, ventral tegmental area, and nucleus accumbens (Koch, 1999). PPI is not only affected by sensorimotor integration, but also requires attentional systems that allow for perception of the prepulse stimulus (Dawson et al., 1993). Therefore, altered PPI as evidenced by PPA administration is a result of cognitive dysfunctioning.

Some putative modes of action for peripheral PPA administration are as follows. PPA is a known ligand for the short-chain fatty acid G-protein coupled receptors: FFAR2 and FFAR3. FFAR2 is expressed on a number of immune cells including leukocytes (Nøhr et al., 2013), monocytes, and neutrophils (Ang, Er & Ding, 2015). Peripheral PPA may also take central effects by passing the blood-brain barrier via the monocarboxylate transporter (Pierre & Pellerin, 2005). Peripheral PPA has been shown to enter the brain and peak at 60 minutes following administration (Brusque et al., 1999). Given that PPA has been observed to activate microglia of the hippocampus, white matter, cingulate, and neocortex (MacFabe et al., 2011), it is surprising that FFAR2 are not expressed on microglia, which are the resident immune cells of the central nervous system (Sampson et al., 2016; Erny et al., 2015). However, once PPA enters the brain, it can alter the excitatory and inhibitory balance in neural circuitry via increased glutamatergic and decreased GABAergic transmission (MacFabe, 2012). PPA can also induce intracellular acidification of neurons and glia, leading to disrupted synaptic transmission in the prefrontal cortex and sensorimotor cortex (Rörig et al., 1996), which are key brain regions involved in sensorimotor gating.

The PPA administration regimen occurred on two days in adolescence, and two days in adulthood. Their cumulative action may have produced central effects through epigenetic action. This may explain why PPA's effects on sensorimotor gating were so prominent in adolescence following PPA's second injection. SCFAs including propionate act epigenetically via its histone deacetylase (HDAC) inhibiting activity and impairment of histone decrotonylation (Fellows et al., 2018). Crotonylation is a histone post-

translational modification mechanism that alters gene expression. It was found that high levels of histone crotonylation occur in the brain (Fellows et al., 2018). Therefore, it is likely that propionate, an HDAC inhibitor, may alter cognitive functioning through this manner. However, further research is warranted as the present study found that PPA administration in adulthood showed little behavioural effects.

Although research on repeated PPA administration is scarce, there is some research on a double-hit model of PPA exposure that occurs prenatally and postnatally. This model induced elevated behavioural changes in adolescence measured through increased ASR (Foley et al., 2015). Although this study suggests that a double-hit of PPA induces altered sensory sensitivity, the present study did not show these same results. The results of the present study did not show an increased ASR in adulthood compared to adolescence when PPA was administered in both time points. These findings may be a result of methodological differences particularly in the timing of PPA administration. Perhaps prenatal administration may result in exacerbated behavioural changes in adulthood that cannot be achieved by adolescent exposure. Further, the diminished behavioural changes observed in adulthood PPA administration perhaps indicates a behavioural tolerance to the drug. Further research is necessary to better understand the behavioural tolerance produced by PPA following repeated exposures in adolescence and adulthood.

2.4.3 Prior repeated LPS exposure and PPA together decreased activity in additive fashion, but also produced opposing effects on startle response

Prior repeated exposure to the immune agent, LPS, followed by acute exposure to PPA decreases activity in an additive fashion. Although no interaction effects were found in behaviour, there may be physiological interactions which cannot be assayed by this study. For instance, LPS i.p. has been found to increase intestinal permeability by increasing enterocyte membrane toll-like receptor 4 expression (Guo et al., 2013). The gut barrier plays an important role of preventing the systemic circulation of bacterial antigens from the gut. Hence, gut permeability could lead to faster absorption of endogenous PPA.

Interestingly, LPS and PPA produced opposing effects on ASR, where LPS increased the magnitude of the ASR, while PPA decreased the magnitude of the ASR compared to their vehicle controls. This is consistent with a prior study conducted by Foley et al. (2015), where prenatal LPS-induced increases in ASR that were attenuated in postnatal PPA treated rats. This effect may result from PPA's anti-inflammatory properties, as it has previously been shown to reduce proinflammatory cytokines (Tedelind et al., 2007). Early LPS exposure induces a proinflammatory state into adulthood demonstrated by elevated levels of IL-2, IL-6 (Borrell et al., 2002) and TNF-α (Qin et al., 2007; Tedelind et al., 2007). PPA can suppress this LPS-induced TNF-α release from neutrophils (Vinolo et al., 2011; Tedelind et al., 2007) and can decrease IL-6 mRNA levels (Tedelind et al., 2007). Aside from PPA's anti-inflammatory properties which counteracts the effects of LPS, PPA can also act as a histone deacetylase (HDAC) inhibitor (Nguyen et al., 2007). By increasing histone H4 acetylation, histone deacetylase inhibitors are capable of suppressing microglial over-activation produced by LPS (Chen et al., 2007).

PPA interacts with the immune system as it is a ligand for FFAR2 (Brown et al., 2003), a G-protein coupled receptor found on immune cells. When administered LPS, FFAR2 expression in monocytes increased (Senga et al., 2003), suggesting that FFAR2 is involved in innate immunity. This provides another link between LPS and PPA activity. FFAR2 activation has been associated with anti-inflammatory effects, such that propionate treatment decreased the production of LPS-induced elevation of proinflammatory cytokine, IL-6, to levels comparable to controls not administered LPS (Li et al., 2018). In the same study, propionate also decreased the TNF-α induced elevation of IL-6. Although no behavioural interaction effects were found in the present study, it is important to consider the possible physiological and immunological interactions that may have occurred and gone undetected.

Aside from the main findings, this study also wanted to determine the behavioural differences between injected and non-injected controls. Although most behavioural measures showed no difference between the NaCl-PBS and No Tx control groups, the measure of percent habituation to the startle response was an exception. The No Tx group showed greater habituation to the startle response than the injected treatment groups in

adulthood. This suggests that on certain behavioural measures, it is important to have a control group that receives no injection stress. This is corroborated by previous research that suggests that intraperitoneally injected saline mice show greater anxiety than sham-injected and lightly handled animals on the elevated plus maze (Lapin, 1995). Therefore, it may be wise to include a no treatment control group for measures sensitive to anxiety.

2.4.4 Conclusions

Although animals share many physiological and behavioural characteristics with humans, it is unlikely that they will perfectly mirror human pathological diseases. However, these models are useful in examining physiological changes and elucidating therapeutic mechanisms that cannot be assessed in humans (Nestler & Hyman, 2010). Modeling of these human neuropsychiatric disorders such as ASD is extremely challenging as symptoms of these disorders are complex and may not map perfectly onto human symptoms. Crawley (2004) has provided a list of symptoms and hypothesized analogous tests to model ASD behaviour. The present study hoped to establish face validity and construct validity of the immune and PPA model of ASD by assessing anxiety behaviours and responses to sensory stimuli. Future studies should aim to evaluate this model's effect on social interaction and restricted interests through social approach and novel object exploration tasks.

In summary, the present study assessed the effects of repeated adolescent LPS and adolescent PPA, and adulthood PPA on anxiety and exploratory behaviour, ASR and %PPI. The results of this study suggest that repeated early adolescent exposure to immune stimuli can induce long-term effects as assessed through the increase in startle response long after sickness response has dissipated. LPS also produces organizational changes that alter behaviour in rats by decreasing activity and producing depressive-like symptoms. PPA acutely decreases activity and exploration behaviours, while decreasing ASR and %PPI. The complex interactions of these two compounds can mirror the hyper and hypo-sensitivity to sensory stimuli found in individuals with ASD. The present study will guide further research in understanding how environmental insults can contribute to changes in neurophysiology and the gut microbiome to result in the development of neuropsychological disorders including ASD.

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Chapter 3

3 Exposure to predator odour in early adolescence alters the behavioural reactions of male rats to propionic acid treatments later in adolescence and adulthood on measures of anxiety and sensory sensitivity

3.1 Introduction

Autism spectrum disorders (ASD) are a group of developmental disorders including Autistic Disorder, Asperger Syndrome, and Pervasive Developmental Disorder-Not Otherwise Specified, characterized by deficits in social communication, repetitive and restricted interests, and altered sensitivity to sensory stimuli (APA, 2013). There is a 4:1 male to female ratio in this disorder's prevalence (CDC, 2012), which underlies the importance of studying males with the disorder. Although ASD is so widespread, the causes for ASD are widely unknown but has been suggested to have both genetic and environmental origins.

A meta-analysis on ASD has estimated between 64 – 91% heritability of this disorder, which suggests a large genetic component (Tick et al., 2016). However, genes cannot account fully for the prevalence of ASD. Environmental stressors during early life increases the risk for clinical disorders including anxiety and depression (Lupien et al., 2009). Prior research has suggested that early life stressors may decrease an individual's threshold towards these psychopathologies, especially during prenatal and postnatal developmental periods. However, limited research has studied the importance of the adolescent period on developmental disorders such as ASD. This period has been hypothesized to be a "sensitive period" for stress response programming and social development (Wright, Muir & Perrot, 2012; Spear, 2000). Stressors during this period activate the hypothalamic-pituitary-adrenal (HPA) axis which is predicted to have long term consequences. As such, it is important to consider whether early adolescent social stressors, such as that provided by cat odours can predict later behavioural abnormalities.

Predator odours are ethologically relevant and aversive stimuli for rodents (McGregor et al., 2002). When rats are exposed to cat odours, they tend to produce anxiogenic and

defensive behaviours, and suppress non-defensive behaviours (Blanchard et al., 1998; McGregor et al., 2002; Papes, Logan & Stowers, 2010). They also show innate aversion to the predator odour source by spending less time investigating it in comparison to their unworn cat collar controls (Wright, Muir & Perrot, 2012).

In addition to the acute anxiogenic effects induced by predator odours, they can also induce sustained stress across the lifespan. Cat odour exposure during adolescence elevates corticosterone levels and decreases activity in male and female adult rats in comparison to their controls (Wright, Muir & Perrot, 2012). Adolescent female rats repeatedly exposed to cat odour displays an elevated anxiety-like response in adulthood on an open field (Wright, Muir & Perrot, 2012). This long-term outcome supports the significance of the adolescent period in stress response programming.

Short-term and long-term stress exposures can alter the gut microbiome by altering its bacterial profile (Galley et al., 2014). Changes to the gut microbiome have been suggested to impact neurodevelopmental and psychological disorders. Changes to the gut microbiome are important environmental risk factors in the development of ASD because of the intricate and pervasive communication between the gut and the brain. This feedback system affects immune activation, emotional and cognitive processes, as well as gut signaling (as reviewed by Foster, Rinaman & Cryan, 2017). Interestingly, a subset of children with ASD have elevated levels of *Clostridium* bacteria in their feces (Finegold et al., 2002). The fermentation products of these bacteria and other ASD-associated bacteria include propionic acid (PPA) and other short-chain fatty acids (SCFAs). At endogenous levels, SCFAs are important for energy metabolism (den Besten et al, 2013), however, at abnormally high levels, the effects can be detrimental.

When administered intracerebroventricularly, acute PPA has been shown to induce increased locomotor activity (MacFabe et al., 2008; Thomas et al., 2010), abnormal motor movements, stereotypic behaviour (Thomas et al., 2010), and impaired social interaction in rodents (MacFabe et al., 2011), similar to that found in the behaviour of children with ASD (as reviewed by MacFabe, 2012). However, peripheral administration of PPA has also been suggested to induce abnormal behaviours that may stem from its

central effects. Intraperitoneal (i.p.) injection of PPA allows for the systemic administration of this drug. This model has been used as a less invasive route of administration of PPA than the intracerebroventricular model. Using the i.p. injection model, Ossenkopp et al. (2012) found that PPA induced aversive internal cues as measured through conditioned place avoidance and conditioned taste avoidance. Repeated PPA i.p. has also been shown to reduce social interaction in juvenile male rats (Shams et al., submitted). In addition, PPA i.p. decreases prepulse inhibition (PPI) which is a measure of a decreased ability to filter out auditory stimuli (see Chapter 2).

Taking together the impact of early stressors on neurodevelopmental changes, the present study hoped to address the following questions. First, the present study determined the effects of repeated cat odour exposure in early adolescence on male rats on the measures of anxiety, prepulse inhibition (PPI), and acoustic startle response (ASR) in later adolescence. It then compared these behaviours with adulthood. This study then examined the effects of repeated PPA administration in adolescence and adulthood. Finally, the present study examined the interaction effect of prior adolescent predator odour exposure followed by later PPA exposure in late adolescence and adulthood. It was hypothesized that predator odour would induce anxiety-like behaviours during the exposure, that persists into late adolescence and adulthood. It was further hypothesized that PPA would produce altered cognitive functioning as assessed by PPI. These effects of PPA were hypothesized to be augmented by prior exposure to the predator odour.

3.2 Materials & Methods

3.2.1 Subjects

Thirty-two male Long Evans rats were obtained at 51 - 75 g from Charles River, Quebec, Canada. This weight class corresponds to postnatal day (P) 22. All animals were pair-housed with another rat of the same treatment group in standard polypropylene cages (45 x 22 x 20 cm). They were maintained in a temperature-controlled room at 20 ± 1°C on a 12:12 light-dark cycle (lights on at 0700 to 1900 h). All rats were given ad libitum access to food (RHM Prolab 3000 rat chow) and tap water. Rats were identified using a numeric system marked using a permanent marker on their tails. When the tail markings faded, the

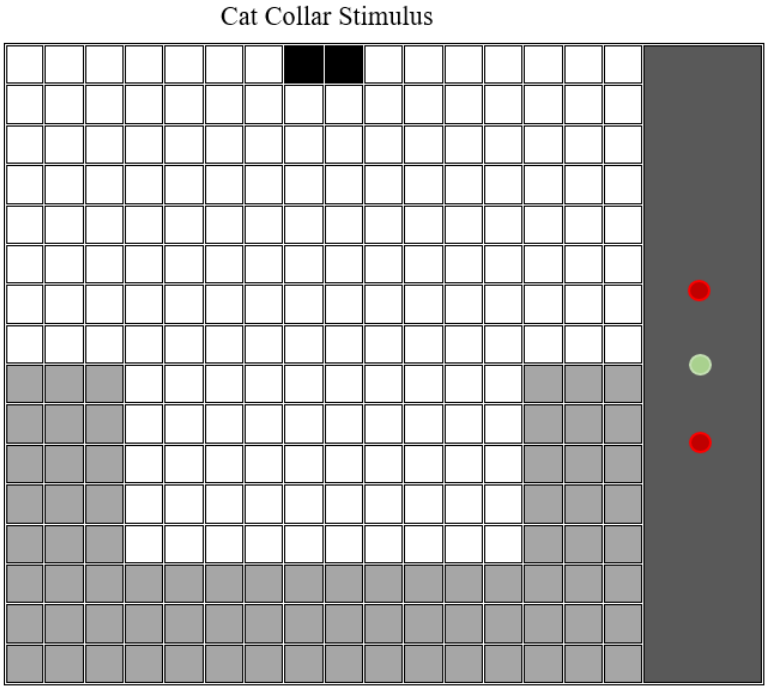
rats were re-marked. All experimental procedures complied with the Canadian Council for Animal Care guidelines and the Institutional Animal Care Committee.

3.2.2 Predator Odour Exposure

Animals were exposed to cat odours from collars that were worn by male and female cats for 2 weeks. Pieces of the collar that were in contact with the cat's neck were cut into 4 cm lengths and stored in an air-tight plastic bag at -10°C. When the collars were ready for use, they were handled with latex gloves and warmed to room temperature in a plastic bag placed in a warm bath. The control cat collars were the same length cat collars that were never worn by a cat. The piece of collar, whether worn or unworn, was taped to the VersaMax Animal Activity Monitors (42 x 42 x 30 cm; Accuscan Model RXYZCM-16, Columbus, OH) by 3 cm of black electric tape at 13 cm from the bottom of the apparatus and centered on one of the walls of the activity field (see Appendix B.1). Each monitor was made of clear Plexiglas enclosed by a Plexiglas lid with air-holes. The activity monitors were equipped with 16 infrared sensors located 7 cm above the floor located every 2.54 cm along the perimeter on opposing sides of the apparatus. Another 16 infrared sensors were located 18 cm above the floor to detect vertical activity. The cat collars were placed between the two sets of horizontal infrared sensors to ensure that the beams were not interfered with by the cat collar itself. Since eight activity chambers were available to be used simultaneously per session, to minimize odour contamination, each of these sessions comprised of rats either only in the worn cat collar condition or only the unworn cat collar condition. Data were collected and analyzed by a VersaMax Analyzer (Accuscan Model CDA-8, Columbus, OH) and sent to a computer where it was recorded.

The cat odour exposure field was divided into the opposite perimeter zone and the cat odour exposure area, as seen in Figure 3.1 in the light grey colour. The opposite perimeter zone was the furthest area away from the cat collar stimulus, while the exposure area contains the cat collar stimulus. The location of the cat collar stimulus was represented in black. Rat activity was not analyzed in the undefined areas of the field.

A)



B)

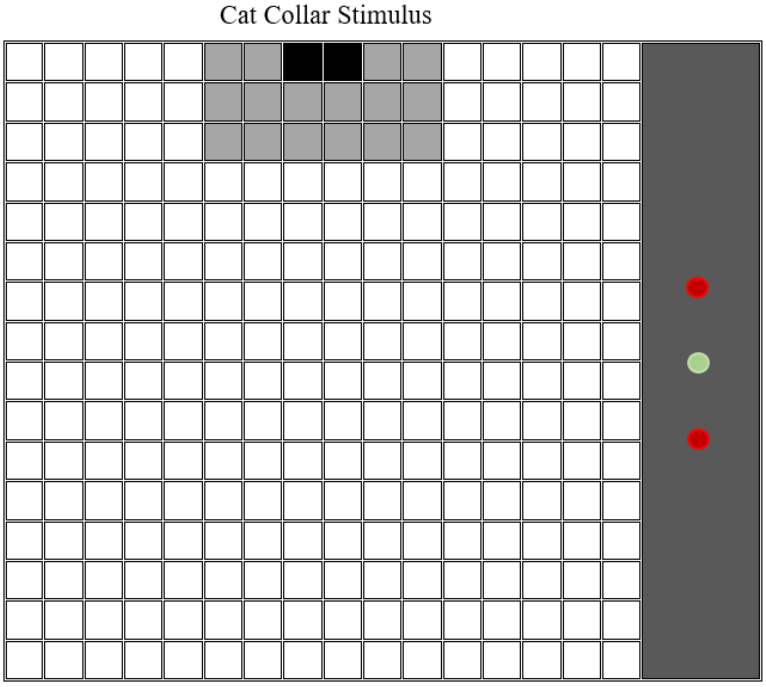


Figure 3.1 Map of the cat odour exposure field: A) opposite perimeter and B) cat odour exposure area. Light grey represents the zone analyzed. Black represents the location of the worn or unworn cat collar stimulus.

The VersaMax Analyzer collected the total time spent in each of the defined areas and the number of vertical movements produced in the cat odour exposure area (Table 3.1). The number of vertical movements were corrected for the rat's total time spent in the cat odour exposure area, such that corrected number of vertical movements was calculated as number of vertical movements per second spent in the cat odour exposure area.

Table 3.1 Behavioural measures recorded in the cat odour exposure field

Behavioural Measures	Definition
Duration	Time spent in the area.
Vertical Movement Numbers	Number of times animal rears up and goes below the vertical sensor for at least 1 second before the next rear up.

3.2.3 Drugs

Sodium propionate (P1880, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 500 mg/kg was dissolved in 0.1 M phosphate buffered saline (PBS) and buffered to pH off 7.5 by HCl. Sodium propionate and PBS were administered in late adolescence on P40 and P43, and in adulthood on P74 and P77. A vehicle control using an equivalent volume to body weight ratio of 0.1 M PBS was injected on the same days for the PBS treatment rats. All drug treatments were administered intraperitoneally (i.p.) at 2.0 ml/kg body weight. The dose of 500 mg/kg of sodium propionate i.p. was chosen because it produces reduced locomotion on the conditioned place avoidance task (Ossenkopp et al., 2012), reduced social interaction (Shams et al., submitted), and decreased %PPI (see Chapter 2).

3.2.4 Light-Dark Apparatus & Behavioural Measures

Behavioural variables in the light-dark apparatus were collected using eight automated VersaMax Animal Activity Monitors (Accuscan Model RXYZCM-16, Columbus, OH), that were the same used in the predator odour exposure. This apparatus was divided into an illuminated light chamber or dark chamber by a black opaque Plexiglas box insert (40 x 20 x 23 cm). The addition of this insert modifies the context to reduce contextual cues from the predator odour exposure. The dark chamber had small holes located on the sides to permit the infrared beams to pass through in order to detect movement. The rats were permitted to freely pass between the chambers by a hole in the partition between the light

and dark chambers at floor level. Data were collected and analyzed by a VersaMax Analyzer (Accuscan Model CDA-8, Columbus, OH) and sent to a computer where it was recorded and retrieved. Procedures were adapted from Banasikowski et al. (2015).

The VersaMax Analyzer collected two types of behavioural variables: chamber-choice variables and activity variables (Table 3.2). The chamber choice variables included the duration spent in the light chamber, number of transitions into the light chamber, and the number of nosepokes. Nosepokes were observed as investigative stretch-attend postures extending into the light chamber. These variables provide measures for anxiety-like behaviours including avoidance of the light chamber and risk-assessment (nosepokes and transitions between the chambers; Banasikowski et al., 2015).

The second type of behavioural variables recorded by the VersaMax Analyzer were the activity variables. These included the total cumulative distance travelled, the number of times the rat rears up, and time spent in vertical position, vertical. All of the activity variables were recorded in the light and dark chambers, and were corrected for the total time spent in these respective chambers. For example, corrected total distance in the light chamber was calculated for the total distance in the light chamber per second spent in this chamber.

Table 3.2 Behavioural measures recorded on the light-dark anxiety task

Behavioural Measures	Definition
Chamber Choice Variables	
Light Duration	Time spent in the light chamber.
Light Transitions	Number of transitions from the dark to the light chamber.
Nosepokes	Number of investigative postures extending into the light chamber.
Activity Variables	
Total Distance	Cumulative horizontal distance travelled.
Vertical Movement Number	Number of times animal rears up and goes below the vertical sensor for at least 1 second before the next rear up.
Vertical Time	Time spent in rearing position. This measure does not consider the time when animal goes below the vertical sensor.

3.2.5 Acoustic Startle Response & Prepulse Inhibition

Acoustic startle response (ASR) and pre-pulse inhibition (PPI) were conducted on three startle devices (SRLAB, San Diego Instruments, San Diego, CA). Each apparatus consisted of a cylindrical, clear acrylic rat enclosure (8.89 cm inside diameter and 20.32 cm length) mounted on an acrylic platform. A piezoelectric accelerometer that lies underneath the platform detects the force of the rat's movement in response to the startle stimulus. This apparatus was placed inside a ventilated sound attenuated box containing fluorescent lighting and an audio speaker 11 cm from the top back of the box. Data were recorded and stored by a computer attached to the accelerometer. Procedures were adapted from Lockey, Kavaliers, and Ossenkopp (2008).

The startle responses were measured as the magnitude of the peak voltage detected by the startle device during a 100 ms detection period after the onset of the startle stimulus. The average peak response of each trial type (Startle, 76 dB PPI, 82 dB PPI) was computed. Percent PPI was calculated as a percent difference from the startle-only startle response for the 76 dB and 82 dB pre-pulse level for each individual rat. This was calculated using the following formula:

$$\%\ PPI = 100 \times \frac{startle\ only\ startle\ magnitude - PPI\ startle\ magnitude}{startle\ only\ startle\ magnitude}$$

3.2.6 % Body Weight Change

Body weight change of rats tends to decrease in response to stressors compared to controls (Stankiewicz et al, 2014; Genné-Bacon, Trinko & DiLeone, 2016). Therefore, body weight change was used as an indicator of stress in the rats exposed to predator odours. Body weight change was calculated using the following formula:

$$\%\ Body\ Weight\ Change = 100 \times \frac{24h\ post\ test\ injection\ day\ body\ weight - injection\ day\ body\ weight}{injection\ day\ body\ weight}$$

3.2.7 Experimental Procedure

On the arrival of the rats on P22, they were pair-housed without manipulation for three days to habituate them to the facility. The animals were handled on their fourth and fifth day, and weighed and handled on their sixth day. On P27, the rats were assigned to four weight-matched manipulation groups: 1. CAT ODOUR-PPA, 2. CAT ODOUR-PBS, 3. NO CAT ODOUR-PPA, and 4. NO CAT ODOUR-PBS. The experimental procedure is outlined in Figure 3.2.

3.2.7.1 Phase 1: Adolescence

In early adolescence, the rats were exposed to either a cat odour via a worn or no cat odour via an unworn cat collar stimulus for 30 minutes on days P28, P30, P32, and P34. Between each exposure session, the apparatus was cleaned with detergent and water, followed by baking soda in water to remove odours, and dried with paper towel to remove remaining stains. The rats were weighed the day of, and the day after, each exposure day to assess body weight change.

Six days after cat odour exposure, the rats were weighed and injected with PPA i.p. or its vehicle, PBS, in late adolescence on P40. After 15 minutes of wait time, rats were placed in the light-dark apparatus for 15 minutes. The same cleaning procedure was conducted as for the predator odour exposure days.

On P43, the rats were again weighed and injected with PPA or PBS i.p. After 10 minutes, these rats were placed into the acoustic startle apparatus. A 5-minute background noise of 70 dB was played to acclimate the rats to the apparatus, after which they performed the startle task. The testing phase lasted for 11 minutes, consisting of 45 trials starting with 10 startle-only trials at 120 dB presented for 40 ms. The subsequent 30 trials were presented in a pseudo-randomized order: 10 startle-only (120 dB for 40 ms) and 20 PPI trials (ten 76 dB and ten 82 dB non-startling pulses presented for 20 ms and a 120 ms wait until the 120 dB startle pulse). The session ended with 5 startle-only trials (120 dB for 40 ms). The startle response was recorded by a computer for 100 ms following the

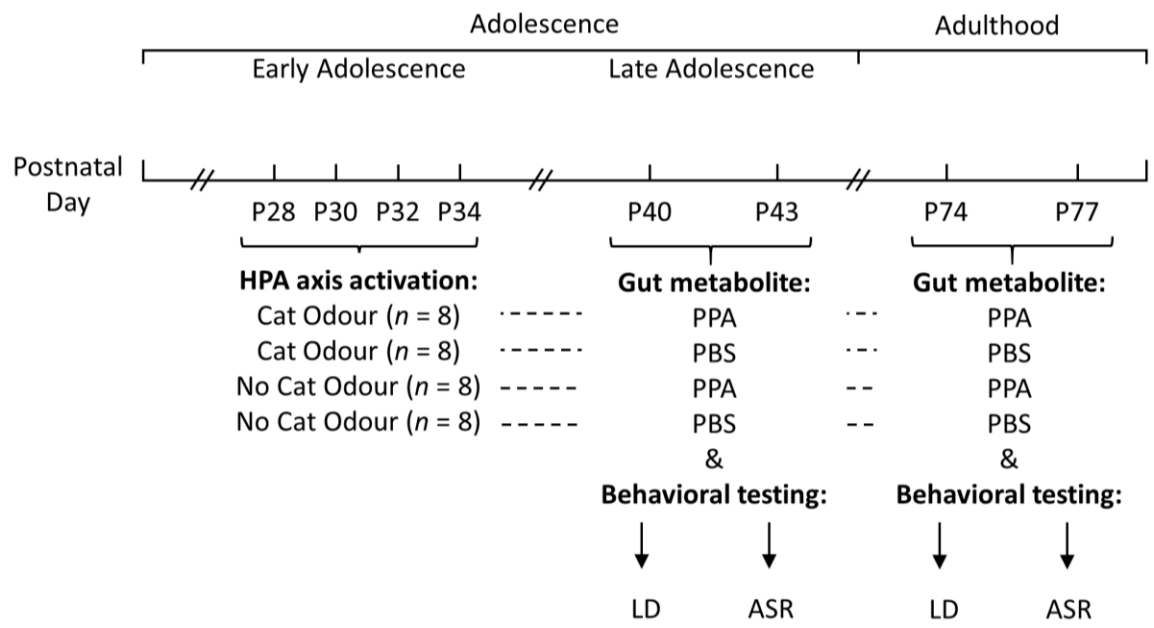


Figure 3.2 Experimental timeline.

onset of the startle stimulus. All trials were separated by an inter-trial interval (ITI) of 8-23 seconds in length (average ITI = 15 seconds). The startle apparatus force detection hardware was calibrated at least once every four testing sessions to ensure consistent recording of startle reactivity. Between each testing session, the animal enclosure was cleaned with detergent and water, and dried with paper towel to remove remaining stains.

3.2.7.2 Phase 2: Adulthood

The same procedure used in late adolescence on P40 and P43 were conducted in adulthood on P74 and P77, respectively, whereby the rats received PPA or PBS i.p. on both P74 and P77 followed by light-dark testing and startle testing.

3.2.8 Statistical Analyses

All statistical analyses were completed using IBM SPSS Statistics 25. Missing values were replaced by the average value obtained within their respective treatment groups. Repeated measures analyses of variances (ANOVA) were conducted to compare the treatment effects across the four cat odour exposure days. Greenhouse-Geisser corrections were used for repeated measures analyses that violates Mauchly's Test for Sphericity. Additionally, the behavioural measures obtained from the light-dark and acoustic startle response tasks were analyzed using repeated measures ANOVAs to compare behaviour across adolescence and adulthood. The between-subject factors were the treatment groups, while the within-subject factors were the adolescent and adult time-points. The significance criteria was set to $\alpha = 0.05$ for all hypotheses tested.

3.3 Results

3.3.1 % Body Weight Change

There were no significant differences between treatments on % body weight change 24 hours following each cat odour exposure day (see Appendix B.2).

3.3.2 Cat Odour Exposure

3.3.2.1 Cat Odour Exposure: Opposite Perimeter

Rats that were exposed to the cat odour (worn cat collar stimulus) spent a greater amount of time in the opposite perimeter than rats exposed to no cat odour (unworn cat collar stimulus), $F(1, 30) = 11.646$, $p = .002$ (Figure 3.3). This was used as a measure of thigmotaxis, an anxiety-like behaviour exhibited by rodents in which they spend greater amounts of time in the outside perimeter of an open field.

3.3.2.2 Cat Odour Exposure: Exposure Area

The cat odour exposure area was the immediate and surrounding area of the cat collar stimulus. Since the cat collar was placed between the lower infrared sensors and the upper infrared sensors, vertical movements in this area would indicate exploration and sniffing of the cat collars. The rats exposed to the cat odour spent less time in the cat exposure area in comparison to the control rats, $F(1, 30) = 7.163$, $p = .012$ (Figure 3.4).

In a separate analysis of each exposure day, it was found that the rats exposed to cat odour via worn cat collar stimulus showed no significant difference in the duration spent in the cat exposure area in comparison to their controls on P28. However, this diverged in the later exposure days to drive the effect shown in Figure 3.4Figure 3.4.

On the measure of corrected vertical time there was an age by treatment interaction effect, $F(3, 90) = 7.004$, $p < .001$, where on P34, the cat odour exposed rats exhibited more time spent in vertical position than their controls ($p = .004$; Figure 3.5). There was also a significant main effect of postnatal day, $F(3, 90) = 4.437$, $p = .006$, such that at subsequent cat odour exposure days, the rats spent more time in vertical position than on P28 (ps $< .005$). This effect was driven by the increase in vertical time by the cat odour exposed rats.

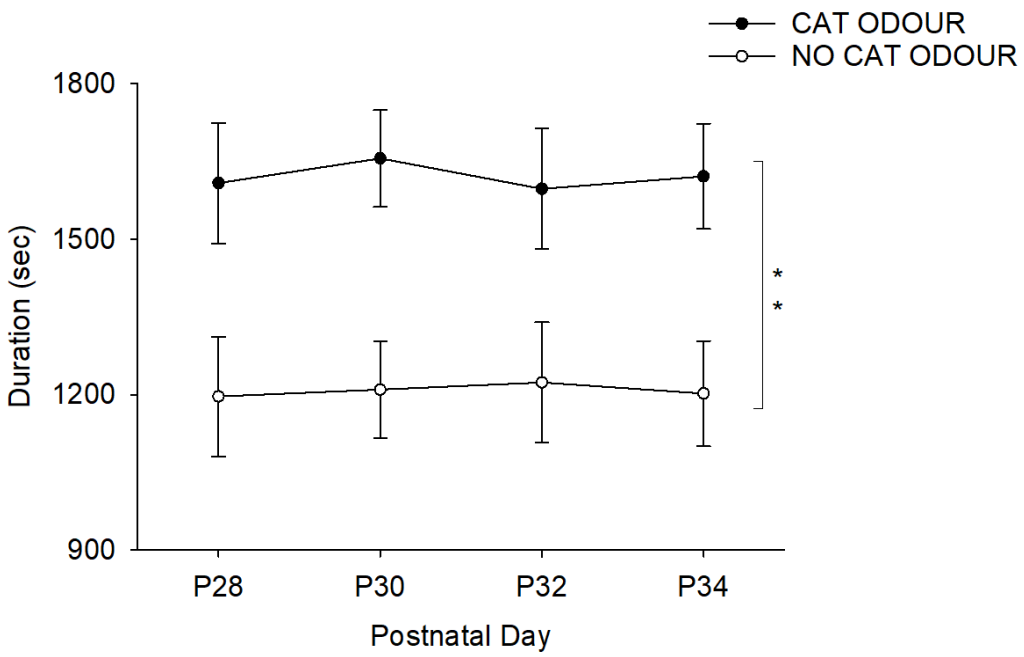


Figure 3.3 Duration spent in the opposite perimeter of the cat odour exposure area on P28, 30, 32 and 34. Rats were exposed to a cat odour via a worn cat collar (*n* = 16) or no cat odour via an unworn (*n* = 16) cat collar stimulus. The cat odour exposed rats spent more time in the opposite perimeter than the unworn cat collar exposed rats. ***p* < .01. Data is represented as mean ± SEM.

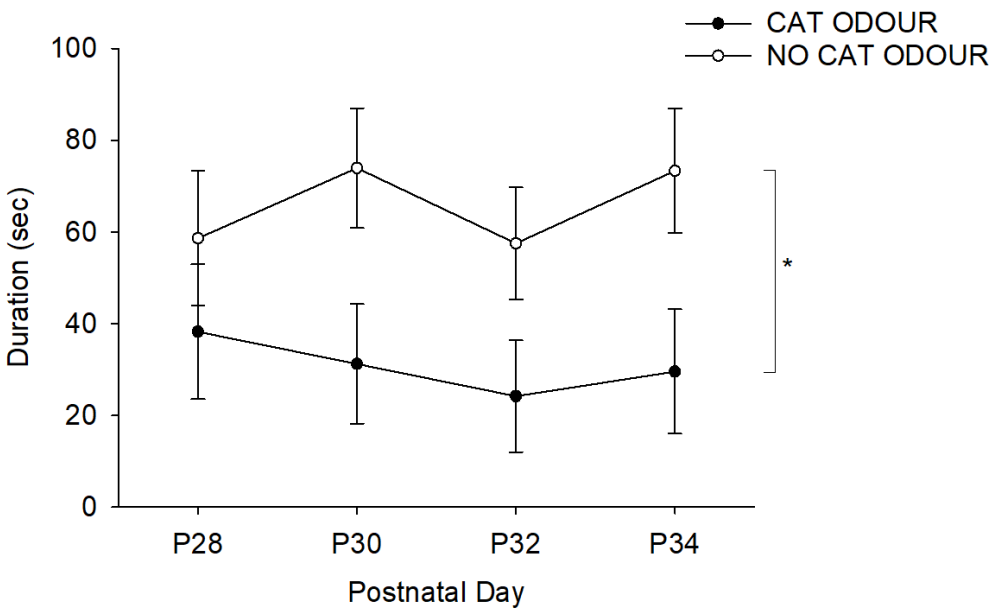


Figure 3.4 Duration spent in the cat odour exposure area on P28, 30, 32, and 34. Rats were exposed to a cat odour via a worn cat collar (n = 16) or no cat odour via an unworn (n = 16) cat collar stimulus. The cat odour exposed rats spent less time in the cat odour exposure area than the unworn cat collar exposed rats. *p < .05. Data is represented as mean ± SEM.

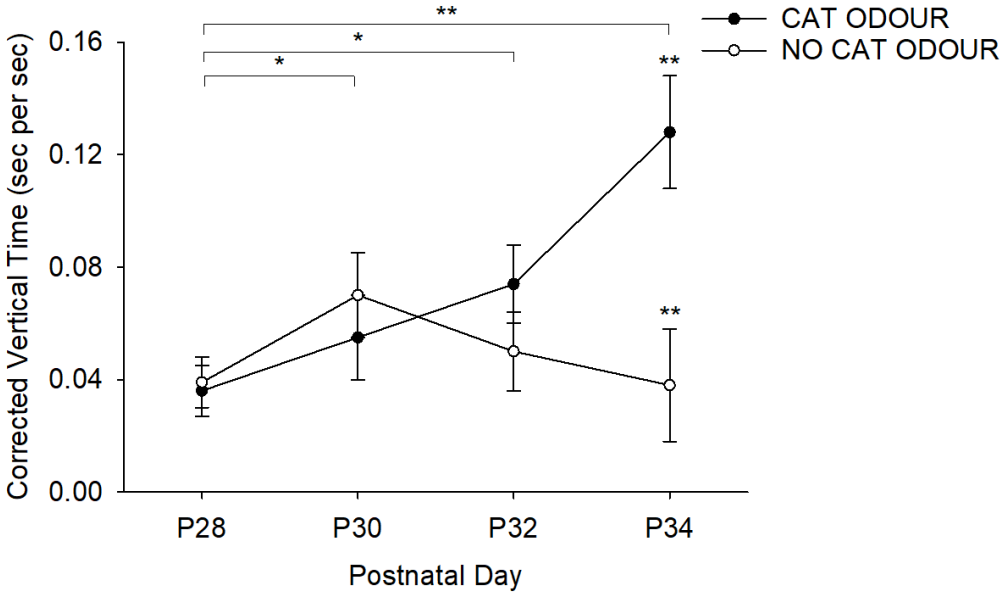


Figure 3.5 Corrected vertical time in the cat odour exposure area on P28, 30, 32, and 34. Rats were exposed to a cat odour via a worn cat collar (n = 16) or no cat odour via an unworn (n = 16) cat collar stimulus. Corrections were made by dividing the duration spent in the cat odour exposure area. The cat odour exposed rats spent greater time in vertical position on P34 than their controls. There is a postnatal day effect, such that repeated cat odour exposure results in increased time spent in vertical position compared to P28, driven by the cat odour exposed rats. *p < .05, **p < .01. Data is represented as mean ± SEM.

3.3.3 Light-Dark: Anxiety & Activity

3.3.3.1 Chamber-Choice Variables

Following PPA or PBS treatment, the rats were tested on the light-dark apparatus where their anxiety-like behaviours and activity levels were measured. Anxiety and risk assessment behaviours were addressed through chamber-choice variables. There was no significant difference in the duration spent in the light chamber across treatment groups. However, there was a trend for the main effect of cat odour when adolescent and adulthood were combined to show a decreased amount of time spent in the light chamber than the no cat odour exposed rats, $F(1, 28) = 3.082$, $p = .090$.

On the risk assessment measure using nosepokes into the light chamber, a main effect of cat odour when combining adolescence and adulthood was observed, such that prior cat odour exposure resulted in a decreased number of nosepokes into the light chamber compared to control odours, $F(1, 28) = 4.350$, $p = .046$ (Figure 3.6).

On the number of transitions into the light chamber, there was a main effect of observed by a decreased number of transitions into the light chamber compared to rats treated with PBS, $F(1, 28) = 22.774$, $p < .001$ (Figure 3.7).

3.3.3.2 Activity Variables: Corrected Horizontal Locomotion

There were no treatment effects on corrected total distance travelled in the light chamber. The dark chamber gave rise to greater observable differences in activity. A main effect of PPA when combining adolescence and adulthood was found, such that PPA-treated rats showed decreased corrected total distance travelled than their PBS controls, $F(1, 28) = 21.521$, $p < .001$ (Figure 3.8).

3.3.3.3 Activity Variables: Corrected Vertical Locomotion

In the light chamber, on the measure of corrected vertical time, there was a main effect of cat odour when adolescence and adulthood were combined, observed by cat odour exposed rats showing elevated time in vertical position in the light chamber compared to their controls, $F(1, 28) = 9.621$, $p = .004$ (Figure 3.8A). A main effect of PPA was also

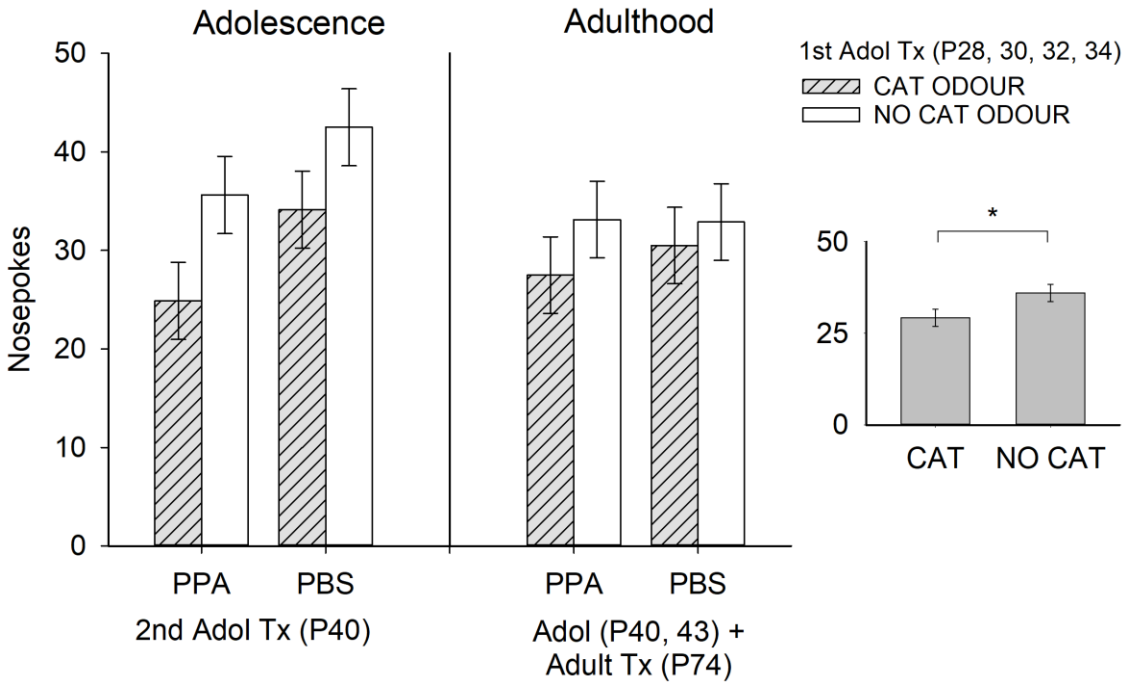


Figure 3.6 Nosepokes into the light chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). A main effect of cat odour exposure when combining adolescent and adulthood was observed as seen in the graph on the right, such that the cat odour exposed rats produced fewer nosepokes into the light chamber than the unworn cat collar exposed rats. *p < .05. Data is represented as mean ± SEM.

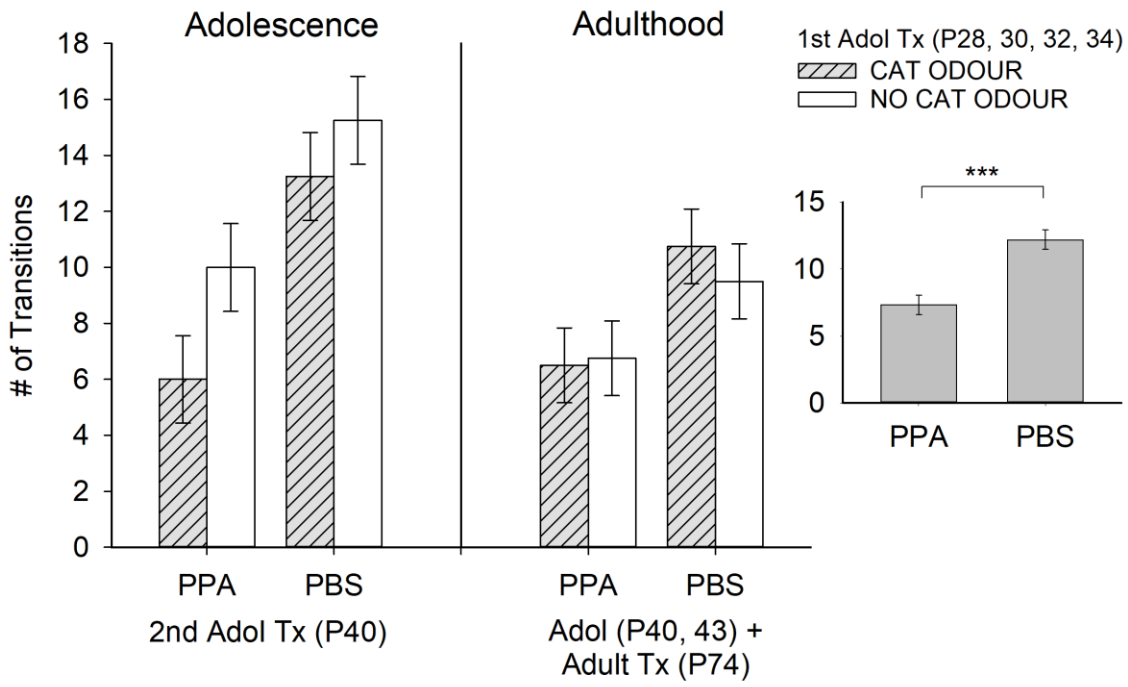


Figure 3.7 Number of transitions into the light chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). A main effect of PPA when combining adolescent and adulthood was observed as seen in the graph on the right, such that the PPA-treated rats produced fewer transitions into the light chamber than PBS controls. ***$p < .001$. Data is represented as mean ± SEM.

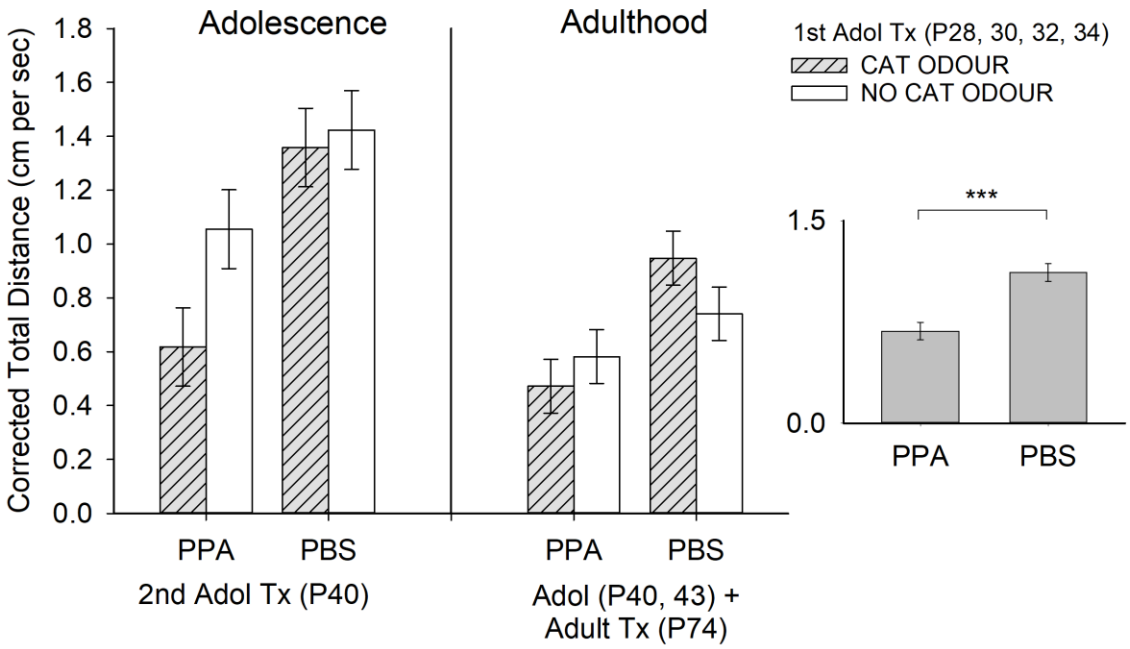


Figure 3.8 Corrected total distance travelled in the dark chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the dark chamber. A main effect of PPA when combining adolescence and adulthood was found as seen in the graph on the right, such that PPA-treated rats produced less corrected total distance travelled than their PBS controls. ***$p < .001$. Data is represented as mean ± SEM.

observed for corrected vertical time in the light chamber, whereby PPA-treated rats showed decreased corrected vertical time than their PBS controls, $F(1, 28) = 4.342$, $p = .046$ (Figure 3.9A). Additionally in adulthood, the rats administered PPA showed a decreased corrected vertical time in comparison to the controls ($p = .002$; Figure 3.9Figure 3.9B).

As in the light chamber, the same main effect of PPA administration was seen in the dark chamber, where PPA decreases the corrected vertical time in comparison to PBS controls when adolescence and adulthood were combined, $F(1, 28) = 36.970$, $p < .001$ (Figure 3.10A). This effect was observed because both PPA in adolescence and adulthood spent less time in vertical exploration than their PBS controls in adolescence and adulthood, respectively (Figure 3.10B).

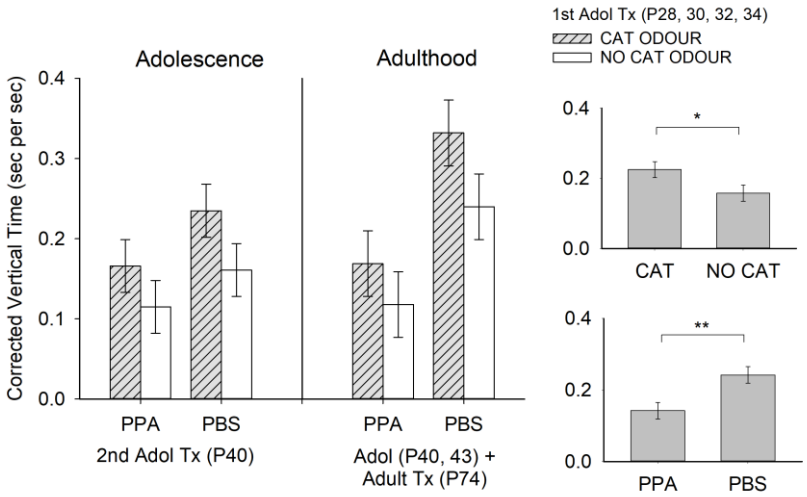
Cat odour exposure and PPA treatment interaction effects were found for dark corrected vertical movement numbers and dark corrected vertical time. Cat odour and PBS treated rats showed greater dark vertical time in comparison to the no cat odour and PBS group, $F(1, 28) = 8.217$, $p = .008$, suggesting that cat odour increased vertical time. Given that cat odour increases vertical time and that PPA reduces vertical time, their interaction effect on vertical activity was determined to be antagonistic. Additionally, the rats treated with cat odour and PPA showed fewer dark corrected vertical movement numbers than those treated with cat odour and PBS, $F(1, 28) = 4.639$, $p = .040$ (Figure 3.11). These results together suggests that cat odour potentiates PPA's reduction of vertical movements, although cat odour by itself increases vertical time.

3.3.4 Acoustic Startle Response (ASR) & Percent Prepulse Inhibition (%PPI)

The average ASR for a 120 dB startle pulse also produced no significant differences between the treatment groups. On the measure of sensorimotor gating using a 76 dB prepulse, a main effect of PPA was observed such that rats treated with PPA showed a depressed %PPI compared to the PBS controls when adolescence and adulthood were combined, $F(1, 28) = 5.440$, $p = .027$ (Figure 3.12). Using an 82 dB prepulse, the rats

administered PPA showed a trending decrease in %PPI compared to the PBS controls, $F(1, 28) = 3.863$, $p = .059$.

A)



B)

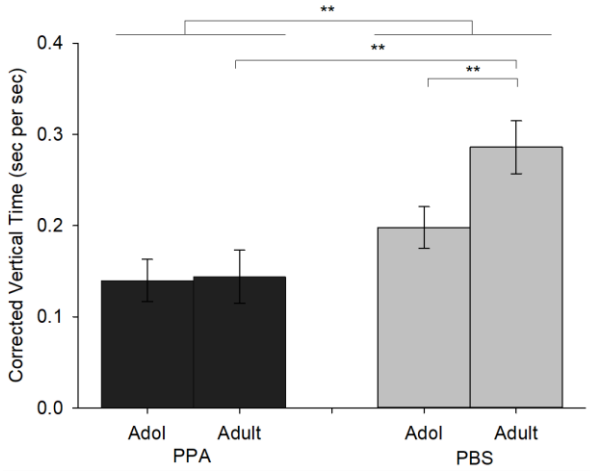
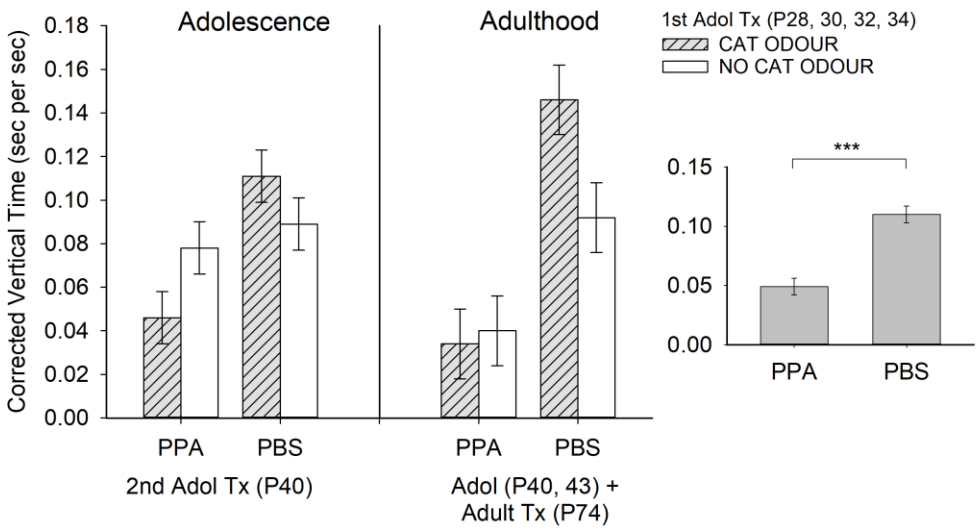


Figure 3.9 Corrected vertical time in the light chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the light chamber. A) A main effect of cat odour was found as seen in the graph on the top right, such that cat odour exposed rats spent more time in vertical exploration than their controls. A main effect of PPA was found as seen in the graph on the bottom right, whereby PPA-treated rats produced less corrected vertical time than their PBS controls. B) In adulthood, PBS controls showed greater vertical time than in adolescence,

and when compared to PPA-treated adults. *$p < .05$, **$p < .01$. Data is represented as mean ± SEM.

A)



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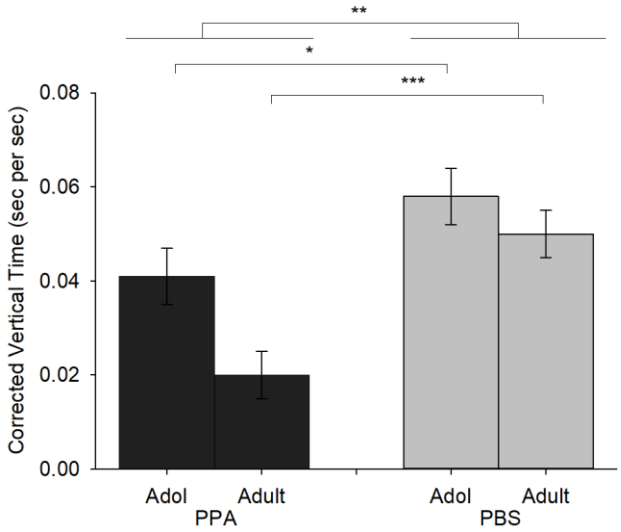

Figure 3.10 Corrected vertical time in the dark chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the dark chamber. A) A main effect of PPA when combining adolescence and adulthood was found as seen in the graph on the right, such that PPA-treated rats produced less corrected vertical time than their PBS controls in the dark chamber. B) This effect was observed because both PPA in adolescence and adulthood spent less time in vertical exploration than their PBS controls in adolescence and adulthood, respectively. *p < .05, **p < .01, ***p < .001. Data is represented as mean ± SEM.

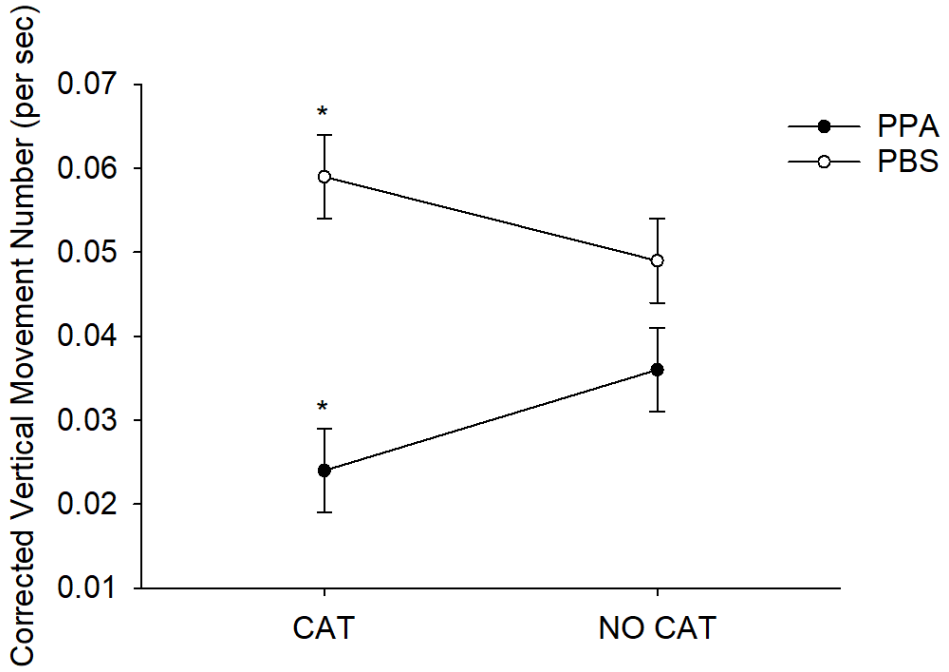

Figure 3.11 Corrected vertical movement numbers in the dark chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the dark chamber. There is cat odour by PPA treatment interaction effect, whereby cat odour exposed rats given PPA showed a decreased number of vertical movements in the dark chamber than the cat odour and PBS exposed rats. *p < .05. Data is represented as mean ± SEM

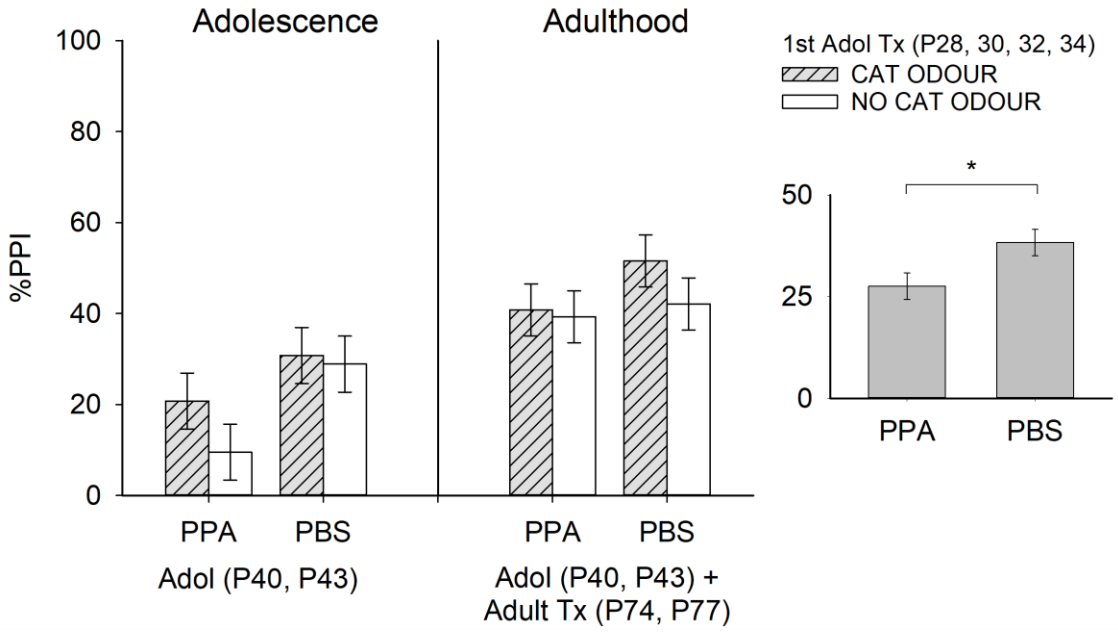

Figure 3.12 % Prepulse inhibition for a 76 dB prepulse with a baseline of 70 dB followed by a 120 dB startle pulse on P43 and P77. Rats were exposed to a cat odour via a worn or no cat odour via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, P74, and P77 (n = 8/group). A main effect of PPA when combining adolescence and adulthood was found as seen in the graph on the right, such that PPA-treated rats showed a decreased %PPI to a 76 dB prepulse than their PBS controls. *p < .05. Data is represented as mean ± SEM.

3.3.5 Summary of Results

This study showed that early adolescent repeated cat odour exposure was aversive to the rats and induced anxiety-like behaviours during the exposure period. When the adolescent and adulthood periods were combined, a main effect of early adolescent repeated cat odour exposure induced elevated anxiety-like behaviours. A main effect of PPA was observed, such that PPA decreased activity levels as assessed through horizontal and vertical locomotor variables when adolescent and adulthood were combined. PPA administered rats also showed a decreased % PPI for a 76 dB prepulse when adolescent and adulthood were combined. Finally, prior repeated cat odour exposure induced an antagonistic effect of PPA on escape behaviours, whereby prior cat odour exposure potentiated PPA's reduction of vertical movement numbers.

3.4 Discussion

3.4.1 Early adolescent repeated cat odour exposure was aversive and anxiogenic

Early adolescent repeated cat odour exposure produced aversive and anxiogenic responses in the rats. The rats were able to detect and display avoidance behaviour in response to the cat odour exposure, which indicates that the cat odour exposure was stressful. The anxiety behaviours presented by the rat did not habituate throughout the cat odour exposures. During the exposure days, they spent more time in the opposite perimeter of the cat odour exposure area with minimal horizontal movement, and a decreased number of vertical movements in comparison to their controls. The rats were motivated to avoid the potentially threatening stimulus as much as possible by spending time in the regions furthest away from the cat odour stimulus. This is consistent with previous research that showed that when rats are given a hide box in which they can hide from the predator odour, they tend to spend most of their time in this hide box and produce a "head out" stance in which they face the predator odour stimulus without turning their heads (McGregor et al., 2002; Dielenberg, Carrive & McGregor, 2001). By directly facing the odour stimulus, with their heads protruding out of the entrance of the hide box, these rats are engaging in risk assessment behaviour which is an adaptive

response to a potentially threatening stimulus. The deficit in horizontal movements observed in the rats repeatedly exposed to predator odour is not surprising as rats tend to elicit anxiety-like behavioural responses such as freezing and immobility in response to stressors (McGregor et al., 2002; Dielenberg, Carrive & McGregor, 2001).

After assessing the locomotor activity within the cat odour exposure area, it is clear that the rats exposed to the predator odour chose to spend much less time in the area than their controls. This may be explained either by the increased time spent at the cat collar stimulus by controls rats in investigating a novel stimulus and/or the decreased time spent at the worn cat collar stimulus because of its aversiveness. In future studies, this limitation can be resolved by having a control condition in the open field without a cat collar stimulus. This result, in addition to the previous findings of the increased anxiogenic behaviour in which the cat odour exposed rats spent more time in the opposite perimeter than their controls, suggests the cat odour stimulus has anxiogenic and aversive properties. When analyzing each exposure day separately for the time spent in the cat odour exposure area, on the first day of exposure (P28), the rats showed no significant difference between the cat odour and no cat odour groups. This could suggest that the rats were stressed by the novel open field. They were likely more focused on the stress associated with the open field. However, on P30, P32, and P34, the aversion to the predator odour stimulus and/or the attractiveness to the unworn cat collar was evident.

In the present study, rats' avoidance behaviour, demonstrated by the duration spent in the opposite perimeter of the cat odour exposure area and the odour exposure area itself, did not habituate. However, within the cat odour exposure area, the rats explored the cat collar stimulus to a greater extent on the fourth exposure day (P34) than on the first exposure day (P28) as shown by the vertical measures of activity corrected for the time spent in this region. This is not surprising as previous studies show increased stimulus investigation after 5 repeated cat odour exposure sessions (Wright, Muir & Perrot, 2012), which suggests an increasing interest in the stimulus. Taken together, these results suggest that certain behaviours occurred independent of habituation. Perhaps the cat odour stimulus became more interesting once the animals learned that the stimulus itself is not harmful in nature. The literature on habituation to predator odour is inconclusive.

Some researchers have shown that avoidance behaviours do not habituate (File et al., 1993; Masini et al., 2006; Blanchard et al., 1998), while others have found that certain behaviours habituated over time to the stimulus (Dielenberg & McGregor, 1999; McGregor et al., 2002). Since predatory fear is an innate and salient response in prey animals, it not surprising that predator cues can elicit behavioural and endocrine responses independent of habituation. Plasma corticosterone levels have been shown to increase across chronic exposure days, which suggests that habituation did not occur (Blanchard et al., 1998). Contrary to this, other researchers including Dielenberg and McGregor (1999) found that hiding behaviour did habituate in response to a worn cat collar over daily repeated exposures. Further, McGregor et al. (2002) found that the immobility response decreased over time. The present study continues to show that some behaviours habituate while others do not.

When the rats were later tested on an anxiety measure, the light-dark apparatus, a main effect of cat odour exposure produced an anxiogenic response in later adolescence and adulthood. The cat odour exposed rats showed fewer nosepokes into the light chamber than their controls, they showed hyperactivity in the light chamber, as well as increased time in vertical position in the light chamber, indicating an elevated desire to escape. Given that these light-dark testing sessions occurred six days and forty days following the cat odour exposure, this study supports prior research indicating that predator stress can induce long-term anxiogenic effects as assessed through anxiety behavioural assays (Bazak et al., 2009; Wright, Muir & Perrot, 2012; Avital et al., 2006). This sustained psychological stress response exhibited by the rodent is adaptive in preventing similar life-threatening circumstances in the future.

Although anxiogenic effects were observed, it is also important to consider the possible generalization of the anxiety response from the similar context presented in the predator odour exposure phase to the light-dark testing phase. Since both occur in the same testing arena, it is likely that an anxiogenic response observed in the light-dark task could have been conditioned. Conditioned fear would not be surprising as repeated adolescent cat fur exposure elicits conditioned fear by reducing distance travelled and increasing time spent inside of a hide box when rats are tested in early adulthood on P58 (Kendig et al., 2011).

Another possible mechanism by which predatory stress induces long-term anxiogenic effects is through its ability to alter the excitatory and inhibitory balance in neural circuitry. When exposed to a fearful stimulus, excitatory NMDA receptors are activated as the magnesium ion that blocks the channel is displaced, thereby opening the NMDA receptor channel (Davis, Rainnie & Cassell, 1994). When NMDA receptors are blocked by an antagonist, anxiety behaviours and risk assessment following exposure to cat odours are reduced when assessed one week later (Adamec et al., 1998). This suggests that NMDA receptors are involved in long-term anxiety-like behaviour produced by cat odour stress. These processes are thought to be important in establishing fear-related memories in models of post-traumatic stress disorder and can also explain the long-term anxiogenic effects observed.

In addition to the excitatory neurotransmission induced by activation of NMDA receptors, cat odour exposure also increases the release of the inhibitory neurotransmitter, GABA, and decreases its uptake in the hippocampus and cortex (File, Zangrossi & Andrews, 1993). GABA is important in regulating PPI of an acoustic startle response (Yeomans et al., 2010). However, in the present study, no significant treatment effects were observed on the measure of PPI. This was not surprising as previous literature has shown that predator exposure decreases PPI 24 hours after exposure, but not after 48 hours or after 9 days (Bakshi et al., 2012). Since the present study assessed PPI 9 days and 43 days after cat odour exposure, it is likely the PPI deficits produced by predator odours were not detected.

Although previous studies on early stressors (Avital et al., 2006) and predator stress show an increased mean startle amplitude (Bazak et al., 2009), the present study found no effect of the stressor on startle response. Perhaps there are methodological differences, for instance, testing in Bazak et al. (2009) occurred on P60, while the present study tested the rats on P43 and P77. The cat odour exposure methodology also differed between the studies such that Bazak et al. (2009) used an acute cat litter exposure on P28 instead of a repeated cat collar stimulus exposure across four days as in the present study. Differences in methodology may lead to differences in results, hence, there is room for further investigation.

In addition, prior research has suggested that males respond less to the predator odour stressor than females, perhaps explaining why ASR and PPI show no significant effects of cat odour treatment. Adult male rats exposed to cat odour stimuli in an open field containing a hide box show more risk assessment behaviour and spend more time in the proximity of the stimulus than their adult female counterparts (Jolles et al., 2015). Further, female rats repeatedly exposed to a cat odour stimulus in adolescence show elevated anxiety behaviours compared to male rats in adulthood by spending more time in a hide box in an open field than their male counterparts (Wright, Muir & Perrot, 2012). Future research should investigate the sex differences in early adolescent repeated cat odour exposure on anxiety-like behaviour, startle response, and PPI in adulthood.

3.4.2 PPA administration decreased activity and sensorimotor gating

PPA administration occurred at four separate time points, twice in adolescence and twice in adulthood. The first dose produced acute effects, while subsequent PPA exposures produced repeated effects. PPA administration reduced activity localized to the dark chamber of the light-dark apparatus, which is considered to be the "safer" chamber of the light and dark chambers of this apparatus. In the present study, the rats administered PPA showed a decreased number of transitions into the light chamber, decreased total distance travelled in the dark chamber, decreased vertical time in the light and dark chambers. These results support the findings from Chapter 2, which show that PPA reduced activity, likely due to the aversive internal state produced by PPA causing a reluctance to move.

Although PPA had no effect on startle response, it did decrease % PPI which is a measure of sensorimotor gating, the ability to filter out redundant auditory information. Sensorimotor gating is an important measure of cognitive dysfunction as it is a symptom of many neurological disorders including schizophrenia, and a subset of individuals with Autism Spectrum Disorder. This sensory filtration of information occurs through a feedforward inhibitory pathway involving the cochlear nucleus, inferior colliculus, superior colliculus, and tegmentum which sends information to the reticular formation (Swerdlow et al., 1992; Sinclair et al., 2017). When changes are made to this neural circuitry, sensorimotor gating deficits occur.

Given that peripheral administration of PPA has also been shown to induce conditioned place avoidance and conditioned taste avoidance (Ossenkopp et al., 2012), while direct intracerebroventricular administration of PPA has been shown to induce stereotypic behaviour, hyperactivity, social impairment, restricted preference for objects, and behavioural rigidity (MacFabe et al., 2011), there is support for the hypothesis that PPA has central effect. There are a number of manners in which peripheral administration of PPA can elicit central effects. PPA is able to bind to SCFA G-protein coupled receptors, primarily FFAR2, which is involved in immune function and is expressed on immune cells including enteric leukocytes (Nøhr et al., 2013), monocytes and neutrophils (Ang, Er & Ding, 2015). Centrally, PPA activates microglia of the hippocampus and white matter (MacFabe et al., 2011) that releases proinflammatory cytokines as evidenced in autism spectrum disorder (Vargas et al, 2005). Surprisingly, FFAR2 are not expressed on microglia, which are the resident immune cells of the central nervous system (Sampson et al., 2016; Erny et al., 2015). Peripheral PPA may enter the brain by passing the gut-blood barrier and the blood-brain barrier via a monocarboxylate transporter (Pierre & Pellerin, 2005). Peripheral PPA has been shown to peak in the brain at 60 min following its administration (Brusque et al., 1999).

Once in the brain, PPA may alter neural circuitry by potentiating glutamatergic and inhibiting GABAergic transmission which may impact the excitatory and inhibitory balance in neural circuitry (MacFabe, 2012). GABA is an important neurotransmitter for prepulse inhibition of the acoustic startle response, such that when $GABA_A$ receptors are disrupted by an antagonist, PPI is reduced (Yeomans et al., 2010). As such, inhibiting GABAergic transmission can result in a decreased PPI as observed with PPA administration. Further, PPA can also lead to intracellular acidification by uncoupling gap junctions between neurons and disrupting synaptic transmission in the prefrontal cortex and sensorimotor cortex (Rörig et al., 1996). Together, these effects of PPA on neural circuitry can explain the deficits in sensorimotor gating.

In addition to the short-lived effects of PPA on behaviour and neuronal functioning, PPA's effects in adulthood seemed to decrease in comparison to adolescence. Although PPA was administered at four separate time points, the effect of PPA in adulthood

decreased. This was not expected as previous literature has suggested a double-hit model of PPA exposure towards increased dysfunction (Foley et al., 2015). Foley et al. (2015) administered PPA prenatally and postnatally, and found that PPA induced an increased ASR when tested in adolescence, suggesting elevated behavioural changes produced by repeated PPA administration. The present study did not show these same results, and instead found decreased behavioural changes in adulthood when repeatedly exposed to PPA in both adolescence and adulthood. Perhaps methodological differences can explain the differences in results, particularly in the timing of PPA administrations. Further, behavioural changes were diminished in adulthood, which suggests that behavioural tolerance may have been produced by repeated PPA exposure. Further research is warranted as this area of research is limited and is necessary to understand the effects of repeated PPA exposure in adolescence and adulthood.

3.4.3 Prior repeated cat odour exposure potentiates the reduction of escape behaviour induced by PPA

There was a significant interaction effect of cat odour and PPA treatment, such that rats exposed to cat odour and PPA showed a decreased number of dark vertical movements compared to rats exposed to cat odour and PBS, suggesting that PPA reduces dark vertical movements. There were no significant differences between the cat odour and PBS and no cat odour and PBS groups such that cat odour by itself did not alter dark vertical movement numbers. This suggests that the prior cat odour exposure potentiates PPA's depression of exploratory and escape behaviours as assessed through vertical movements produced in the dark chamber.

This antagonistic effect of cat odour exposure on PPA as measured through behaviour can be explained by both treatments' effects on excitatory and inhibitory neural circuitry. Cat odour exposure can activate excitatory NMDA receptors (Adamec et al., 1998) to produce long-term anxiogenic behavioural effects. It can also increase the release of the inhibitory neurotransmitter GABA, and decrease GABA's uptake (File, Zangrossi & Andrews, 1993). PPA can also alter the excitatory and inhibitory balance by potentiating glutamatergic excitatory neurotransmission and inhibiting GABAergic inhibitory transmission (MacFabe, 2012). These two treatments appear to have slightly different

effects, such that cat odour exposure increases the availability of GABA, while PPA decreases the availability of GABA, which may explain their antagonistic behavioural effect on vertical locomotor variables. However, further research is warranted.

Stress is capable of increasing the permeability of critical barriers including the gut blood barrier (Kelly et al., 2015; Larauche, Kiank & Tache, 2009; Overman et al., 2012) and the blood brain barrier (Sharma, Cervós-Navarro & Dey, 1991; Esposito et al., 2002). Barrier dysfunction may allow for macromolecules including SCFAs like PPA to bypass the barriers to gain access to the central nervous system. Although the present study uses i.p. injection of PPA, which bypasses the gut-blood barrier, the systemic PPA administered may enter the brain with greater ease than without cat odour exposure, which has direct relevance to ASD.

Another important area of research that may explain the interaction effect observed with cat odour and PPA, is the stress produced by multiple environmental "hits" during sensitive periods of development. Research on many models of neural dysfunction stem from the double hit hypothesis or multiple hit hypothesis including research on schizophrenia (Maynard et al., 2001) and ASD (Picci & Scherf, 2015).

3.4.4 Conclusions

Despite animals sharing many physiological and behavioural characteristics with humans, it is unlikely that animal models can perfectly mirror human pathological diseases. Animal models are useful in that they can be used to examine physiological changes and to elucidate therapeutic mechanisms that cannot be assessed in humans (Nestler & Hyman, 2010). Neurodevelopmental disorders such as ASD are complex in its etiology and hence, also have complex symptomatology. The behaviours observed in individuals with ASD may not perfectly map onto behaviours observed in animal models. However, the present study attempted to establish face validity and construct validity of a stress and PPA model of ASD. It assessed this model by measuring anxiety-like behaviour, acoustic startle response, and prepulse inhibition, behaviours that are found to be abnormal in many cases of ASD.

In summary, early adolescent repeated predator odour exposure was aversive to the male rats. When tested on an anxiety measure in later adolescence and adulthood, the prior repeated predator odour exposure produced long-term anxiety-like behaviour, which further supports previous research that suggests that the adolescent period is a sensitive period in stress response programming (Wright, Muir & Perrot, 2012). In addition, PPA administration decreased the rats' motivation to move, while also decreasing the rats' ability to filter out redundant auditory information. The predator odour exposure condition predisposed rats that were later administered PPA to a more enhanced reduction in escape behaviour as assessed on the light-dark apparatus.

These results together may suggest that stress induced by early adolescent repeated predator odour exposure may induce imbalances in the excitatory and inhibitory neural circuitry that is also impacted by PPA administration to induce behavioural changes. This study also supports the multiple hit hypothesis towards neural dysfunction.

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4 General Discussion

This thesis examined the impact of early adolescent stressors, including immune stress and predator stress, followed by propionic acid (PPA), on anxiety-like behaviour, acoustic startle response (ASR), and prepulse inhibition (PPI) in later adolescence and adulthood. The results of the studies emphasize the importance of adolescent stress on long-term behavioural outcomes. In chapter 2, when the adolescent rats were repeatedly exposed to the immune activating agent, lipopolysaccharide (LPS), a delayed increase in ASR was observed in adulthood. LPS also increased the rats' anxiety-like behaviour and escape behaviours. When adolescence and adulthood were combined, administration of PPA resulted in decreased PPI, which is an indicator of cognitive dysfunction. Together, LPS and PPA decreased activity variables in an additive fashion as observed on the light-dark apparatus. However, they had opposing effects on ASR such that PPA reduced ASR, while LPS induced a delayed increase in ASR.

In chapter 3, the impact of PPA closely resembled that observed in chapter 2, such that it decreased activity and altered PPI. Early adolescent cat odour exposure increased anxiety-like behaviour in males during the exposure period. When adolescent and adulthood testing on the light-dark apparatus were combined, elevated anxiety levels were found for the rats previously exposed to cat odours. Further, prior exposure to cat odour stress potentiated PPA's effects such that escape behaviours decreased in the dark chamber of the light-dark apparatus when administered both cat odour and PPA.

These research findings together indicate a need for further research on the effects of various environmental and psychological stressors on the adolescent period of development. Currently, there is a lack of research using adolescent immune activation to study behavioural changes in adulthood. Many studies assessing the behavioural effects of LPS tend to administer LPS either prenatally or neonatally to assess their long-term behavioural effects. Although there are some studies on adolescent LPS administration, these tend to involve acute doses followed by testing within 24 hours of the immune insult, during which animals are displaying sickness responses (Doremus-Fitzwater et al., 2015; Goble et al., 2011). There is a clear lack of research on behavioural studies using

LPS during this time period. Although a study of LPS administration on P14 pups showed elevated anxiety-like behaviours in adolescence and adulthood along with spatial memory deficits (Dinel et al., 2014), this LPS administration occurred before the adolescent developmental period. The present thesis is the first to date to show that adolescent repeated LPS exposure results in a delayed increase in startle response in adulthood. Together, these studies pave the way for further research on adolescent stress response programming, social development, and cognitive dysfunction in adulthood, and the reduced resilience to future stressors.

The finding that repeated cat odour exposure in adolescence followed by PPA administration, decreased escape behaviours to a greater extent than either treatments themselves, could suggest a link between stress response and the gut-brain axis. These results support the hypothesis that HPA axis activation leads to increased BBB permeability (Esposito et al., 2002), thereby influencing PPA's ability to bypass the BBB. Another possible mechanism by which cat odour may interact with PPA is through their actions on altering the excitatory and inhibitory balance. Cat odour exposure has been shown to increase the release of the inhibitory neurotransmitter, GABA, and decrease its uptake, such that elevated levels of GABA are found in the synapses within the hippocampus and cortex (File, Zangrossi & Andrews, 1993). Cat odour exposure also elevates excitatory processes by elevating glutamate levels that can bind to NMDA receptors (as reviewed by Davis, Rainnie & Cassell, 1994). PPA on the other hand can cause excitotoxicity by increasing glutamatergic transmission and decreasing GABAergic transmission (Brusque et al., 2001).

While the present studies were limited in assessing the behavioural effects of PPA, it is possible that future studies may use antibiotic-induced gut depletion to assess the effects of PPA without disruption from other bacterial metabolites. This type of manipulation, using gut depletion followed by SCFA administration seems to be gaining interest in literature as endogenous levels of SCFA administration has been shown to reduce behavioural abnormalities observed in gut depleted animals (Kiraly et al., 2016).

4.1 Relation to Autism Spectrum Disorders

Since the purpose of this thesis was to model ASD in rats, it is imperative to take the results of the present study and relate it back to ASD. The ASD phenotype is quite variable and consists of many different behavioural alterations. The two behaviours that were assessed were sensory processing alterations and anxiety-like behaviours.

Children with ASD tend to have deficient inhibitory control of sensory information such that they may be overloaded with sensory information and external stimulation (Sinclair et al., 2017). Adults and children with ASD tend to show greater startle response, which indicates an elevated sensitivity to auditory stimuli (Takahashi et al., 2016, Kohl et al., 2014). Many studies with ASD in adults and children show no changes in sensorimotor gating as assessed by % prepulse inhibition (PPI), however, the severity of autistic traits have been correlated with deficits in PPI. In addition, other psychiatric disorders such as schizophrenia have been characterized by deficits in PPI (Braff et al., 2001). Hence, PPI is an information measure of cognitive deficits observed in clinical disorders. This thesis showed that LPS induced an increased startle response in adulthood, while PPA induced deficits in PPI, suggesting that these rodents experienced cognitive dysfunction.

Anxiety-like behaviour was the other behavioural assay used in this study. Although anxiety is not a core symptom of ASD, 11 to 84% of individuals with ASD have a comorbid diagnosis with a form of anxiety disorder (White et al., 2009). Therefore, it is important to study anxiety-like behaviour, in addition to activity and escape behaviour that can be measured through the light-dark apparatus. Cat odour and LPS both produced long-term anxiogenic effects, which are indicative of behavioural abnormalities.

4.2 Conclusions

Overall these studies examined putative risk factors that may result in behaviours that resemble ASD in humans. It aids in the development of an ASD-like phenotype observed in rodents, as well as other models of neuropsychopathology. These research findings will inform future research on the impact of early adolescent stressors and their impact on behaviour, and the underlying processes that may contribute to dysfunctional behaviours.

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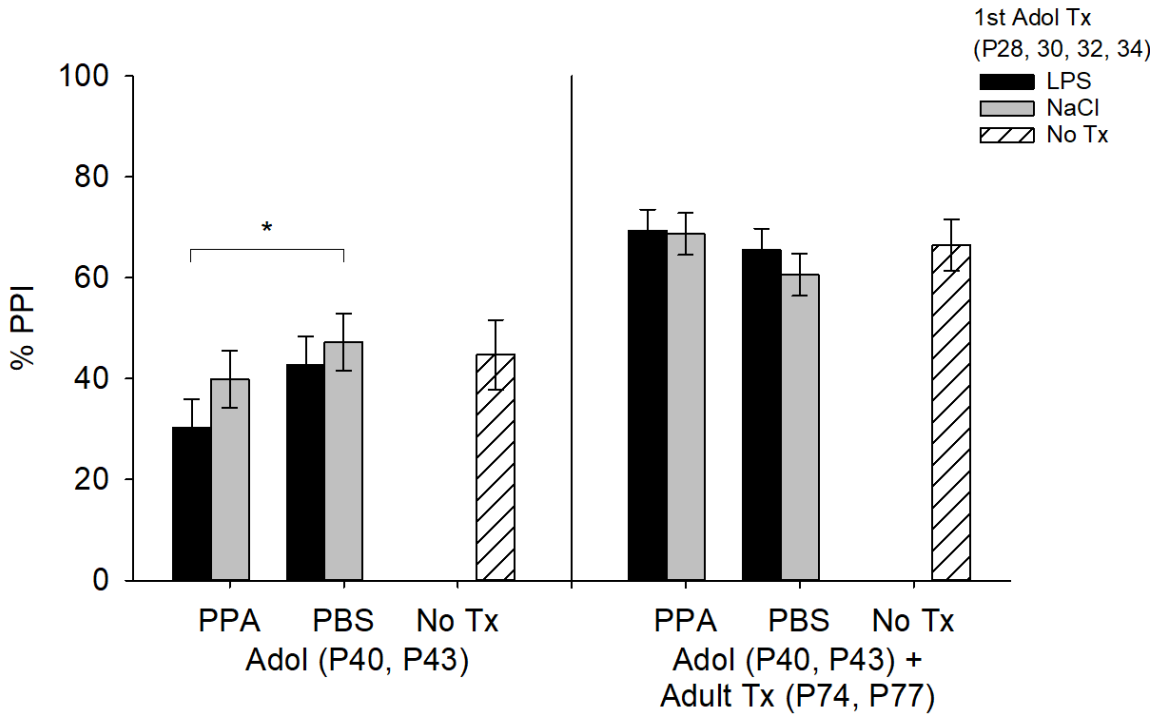
Appendix A.

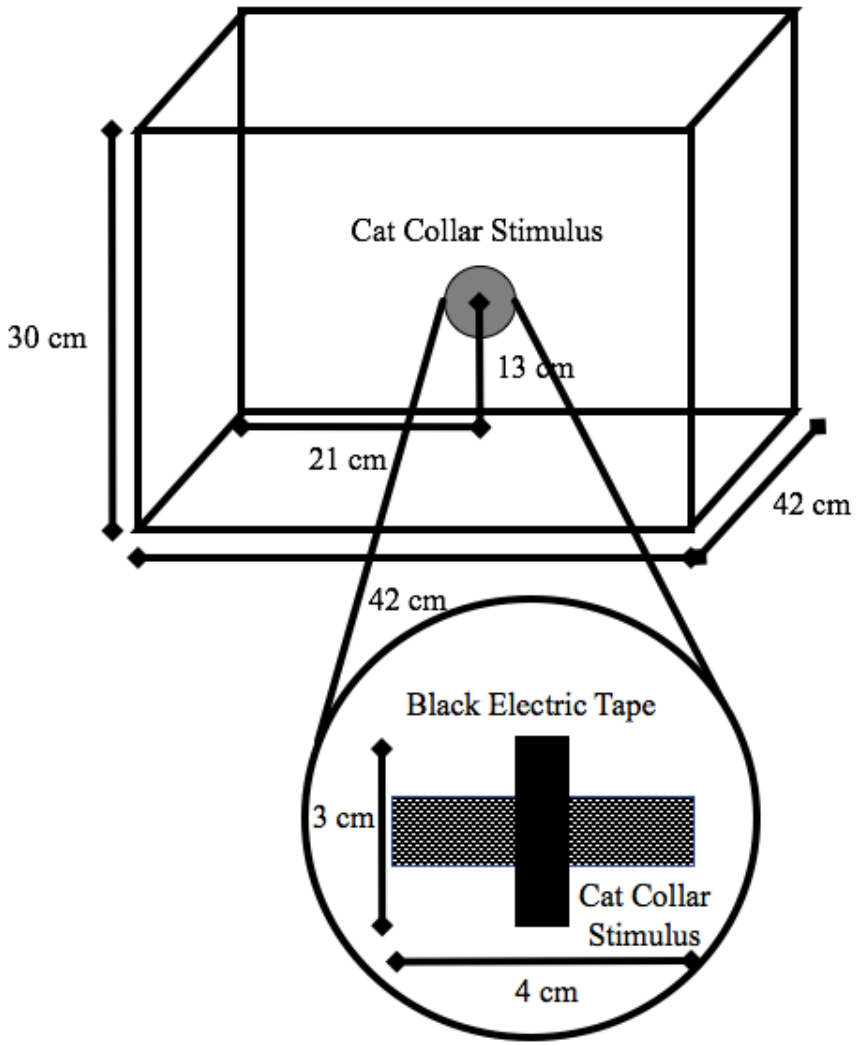
Additional Figures from Chapter 2



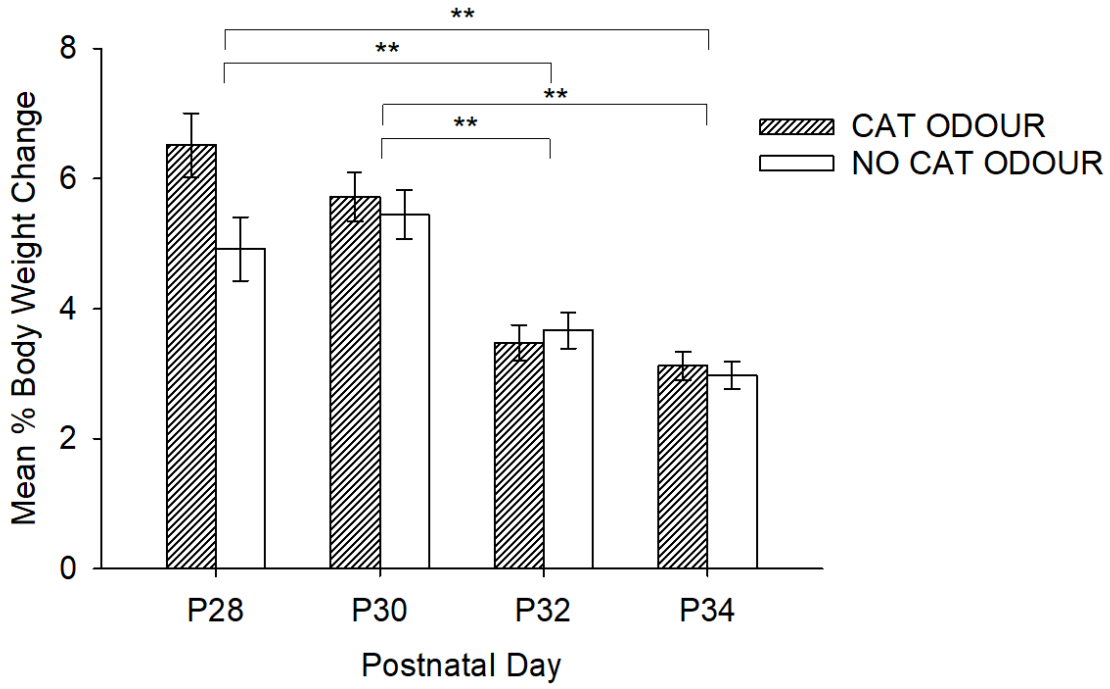
Appendix A.1 % Prepulse Inhibition for an 82 dB prepulse on P43 and P77. Rats were injected i.p. with LPS, NaCl, or had No Tx on P28, 30, 32, and 34 and PPA, PBS, or had No Tx on P40, 43, 74, and 77 (n = 12/injected group or n = 8/No Tx group). The LPS-PPA treated rats showed decreased %PPI for an 82 dB prepulse compared to their NaCl-PBS controls in adolescence. *$p < .05$. Data is represented as means ± SEM.

Appendix B.

Additional Figures from Chapter 3



Appendix B.1 Cat odour exposure chamber. Activity monitors were 42 x 42 x 30 cm, with the cat collar stimulus placed 13 cm from the bottom of the apparatus centered on one of the walls of the activity field. Black electric tape was used to tape the 4 cm piece of cat collar onto the wall.



Appendix B.2 Mean percent body weight change 24-hours post cat odour exposure. Rats exposed to cat odour via a worn cat collar or no cat odour via an unworn cat collar stimulus on P28, 30, 32, and 34 depending on their treatment condition. There were no significant differences between treatment group. Mean % body weight change decreased across age. $^{**}p < .01$. Data is represented as mean ± SEM.

Curriculum Vitae

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