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Propionic acid induced behavioural effects of relevance to autism spectrum disorder evaluated in the hole board test with rats

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ABSTRACT

Autism spectrum disorders (ASD) are a set of neurodevelopmental disorders characterized by abnormal social interactions, impaired language, and stereotypic and repetitive behaviours. Among genetically susceptible subpopulations, gut and dietary influences may play a role in etiology. Propionic acid (PPA), produced by enteric gut bacteria, crosses both the gut-blood and the blood-brain barrier. Previous research has demonstrated that repeated intracerebroventricular (ICV) infusions of PPA in adult rats produce behavioural and neuropathological changes similar to those seen in ASD patients, including hyperactivity, stereotypy, and repetitive movements. The current study examined dose and time related changes of exploratory and repetitive behaviours with the use of the hole-board task. Adult male Long-Evans rats received ICV infusions twice a day, 4 h apart, of either buffered PPA (low dose 0.052 M or high dose 0.26 M, pH 7.5, 4 μL/infusion) or phosphate buffered saline (PBS, 0.1 M) for 7 consecutive days. Locomotor activity and hole-poke behaviour were recorded daily in an automated open field apparatus (Versamax), equipped with 16 open wells, for 30 min immediately after the second infusion. In a dose dependent manner PPA infused rats displayed significantly more locomotor activity, stereotypic behaviour and nose-pokes than PBS infused rats. Low-dose PPA animals showed locomotor activity levels similar to those of PBS animals at the start of the infusion schedule, but gradually increased to levels comparable to those of high-dose PPA animals by the end of the infusion schedule, demonstrating a dose and time dependent effect of the PPA treatments.

1. Introduction

Autism spectrum disorders (ASD) are a cluster of neurodevelopmental disorders characterized by impaired social interaction, communication deficits, abnormal motor movements, and restricted/repetitive interests and behaviour (Arndt et al., 2005; DiCicco-Bloom et al., 2006). Other symptoms associated with autism include abnormal sensitivity to sensory stimuli, hyperactivity, resistance to change, cognitive deficits, and seizures (Frye, 2015; Kootz et al., 1982; Markram et al., 2007; Murray, 2010; Sasson et al., 2008).

The gastrointestinal tract (GI) is home to over a trillion commensal bacteria, known as the microbiome, that have a bidirectional relationship with the central nervous system and contribute to normal immune system development and homeostasis in both humans and rodents. There has been an increasing interest in the role of the microbiome in communicating with the central nervous system and influencing gastrointestinal, immune, and neuropsychiatric health (Al-Asmakh et al., 2012; Collins et al., 2012; Cryan and Dinan, 2012; Forsythe et al., 2012; Nicholson et al., 2012; Stilling et al., 2014). Results of recent studies with germ-free mice have demonstrated that alterations in the GI microbiome are associated with changes in early gene expression, neurotransmitter turnover, stress response, immune function, as well as reduced social behavior (e.g., Desbonnet et al., 2014; Diaz Heijtz et al., 2011; Foster and Neufeld, 2013). There also is mounting evidence that alterations in the composition of the microbiome and its metabolic products may contribute to the development and/or maintenance of ASD in children (El-Ansary et al., 2013; Hsiao et al., 2013; Rosenfeld, 2015; Wang and Kasper, 2014; Williams et al., 2011).

Abbreviations: ANOVA, Analysis of variance; ANCOVA, Analysis of covariance; ASD, Autism spectrum disorders; CNS, central nervous system; ICV, intracerebroventricular; PBS, Phosphate buffered saline; PPA, Propionic acid; SCFA, Short chain fatty-acid

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Although there is a strong multigenetic basis for ASD susceptibility (e.g., Bailey et al., 1995; DiCicco-Bloom et al., 2006; Hallmayer et al., 2011), recent research suggests that environmental, dietary, and gastrointestinal factors may play a significant role in the etiology and pathogenesis of autism (Frye et al., 2015; Horvath and Perman, 2002; London and Etzel, 2000; MacFabe, 2015; MacFabe, 2012; Ratajczak, 2011; Williams et al., 2011). Several findings suggest that exposure to propionic acid (PPA), a short-chain fatty acid (SCFA) that is endogenous to the human body, may be associated with ASD (Al-Owain Kaya et al., 2013; MacFabe, 2015; MacFabe, 2012). PPA is an intermediary of fatty acid metabolism and is a metabolic end-product of microbial fermentation in the gut (Al-Lahham et al., 2010; Thompson et al., 1990). Parents frequently report an increase in behavioural symptoms when their autistic children ingest refined wheat and dairy products (Jyonouchi, 2009), which contain PPA, either as a result of the manufacturing process (e.g., dairy) or as an added food preservative (Brock and Buckel, 2004). Furthermore, consumption of these products can result in increased production of PPA via bacterial fermentation of undigested food within the gut (Cummings et al., 1987). In support of these anecdotal reports from parents, a recent study demonstrated that systemic treatment of rats with PPA induced aversive internal cues (Ossenkopp et al., 2012), a finding consistent with reports of nausea in people consuming foods containing PPA (Frost et al., 2003). In addition, a randomized controlled trial showed improvement in attention and reduced hyperactivity following implementation of a casein- and gluten-free diet in children with ASD (Whiteley et al., 2010).

A subset of autistic children with co-morbid gastrointestinal symptoms have abnormal gut microflora (Finegold, 2011; Finegold, 2010; Finegold et al., 2012; Finegold et al., 2002; Kang et al., 2018), including elevated levels of *Clostridium* and *Desulfovibrio*, both of which are known to produce short chain fatty acids, such as PPA (Finegold, 2011; Finegold et al., 2002; Parracho et al., 2005). Exposure to valproic acid early in development, which can increase levels of PPA and other SCFAs, increases the likelihood of ASD (Ornoy, 2009). Furthermore, serum analysis in ASD patients has shown metabolic impairment of glutathione, carnitine, and fatty acids consistent with the physiological effects of PPA (Bell et al., 2004; Filipek et al., 2004; Frye et al., 2013; James et al., 2006).

PPA is a weak organic acid and can readily cross the gut-blood barrier and gain access to the central nervous system (CNS), either passively across the blood-brain barrier or via monocarboxylate transporters (Bergersen et al., 2002). PPA and related SCFAs (e.g., acetate, butyrate) are capable of influencing central nervous system function. PPA has been implicated in inhibition of Na^+/K^+ ATPase, increased NMDA receptor sensitivity, alteration of mitochondrial and fatty acid metabolism, immune activation, and changes in gene expression (Brass and Beyerinck, 1988; de Mattos-Dutra et al., 2000; Parab et al., 2007; Wajner et al., 2004; Wyse et al., 1998). In addition, PPA can accumulate within cells, resulting in intracellular acidification which can alter neurotransmitter release, inhibit gap junctions, and promote intracellular calcium release, all of which can potentially affect neuronal communication and behaviour (Remblier et al., 1999; Rorig et al., 1996).

Repeated, central (intracerebroventricular; ICV) infusions of PPA in adult rats have been shown to induce hyperactivity, repetitive behaviours, turning behavior, retropulsion, kindled seizures, social impairments, cognitive deficits, altered brain phospholipid profiles, increased oxidative stress, and an innate neuroinflammatory response (MacFabe et al., 2007; MacFabe et al., 2011; MacFabe et al., 2008; Mepham et al., 2019; Shultz et al., 2009; Shultz et al., 2008; Thomas et al., 2010), consistent with findings from ASD patients (Bauman and Kemper, 2005; Vargas et al., 2005; Wiest et al., 2009). The ICV-PPA adult model is based on the premise that continuous high levels of PPA could be responsible for some of the phenotypic behavioural abnormalities seen in ASD. This premise is supported by studies which examined overlap of ASD with propionic acidemia. Propionic acidemia is a neurodevelopmental metabolic disorder characterized by elevated levels of PPA and clinically resembles some aspects of autism (Feliz et al., 2003), and case studies of comorbidity of propionic acidemia and ASD have been presented (Al-Owain Kaya et al., 2013; de la Batie et al., 2018; Witters et al., 2016). It is also consistent with studies using systemic treatment of rats with PPA (Kamen et al., 2019; Ossenkopp et al., 2012; Shams et al., 2019).

The present study examined the central (ICV) effects of two doses of PPA administered twice a day for 7 days. This infusion schedule was used to allow for comparison to findings from previous work which used this procedure in the adult PPA rodent model (MacFabe et al., 2007; Mepham et al., 2019; Thomas et al., 2012). The dependent variables examined were locomotor and repetitive behaviours, as well as nose-poking behaviours in a hole-board task. It was hypothesized that PPA treatment would produce increased locomotor and repetitive behaviour and do so in a dose-dependent manner.

2. Apparatus and procedures

2.1. Subjects

Thirty-five naive male Long-Evans rats were used, weighing 200–225 g (approximately 47–49 days old) at the time of arrival to the facility. Animals were housed individually in standard rat polypropylene cages (W 26 x L 48 x H 21 cm) with ad libitum access to food (LabDiet RMH 3000) and tap water in a temperature-controlled colony room (21 ± °C) on a 12:12 h light-dark cycle (lights on at 07:00 h). Behavioural testing occurred during the light phase of the cycle. Animals were left undisturbed for one week prior to the cannulation surgery. Fig. 1 presents a timeline of the experimental procedures. All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the University of Western Ontario Animal Use Subcommittee.

2.2. Cannula implantation

To induce anaesthesia, animals were placed in a sealed plastic box into which 4% isoflurane at 2 L/min oxygen was introduced. The animal was then placed into a Kopf stereotaxic device equipped with a gas flow mask delivering 2.5% isoflurane at 500 mL/min of oxygen to maintain anaesthesia during surgery. Right lateral ventricular cannulation surgery was performed under aseptic conditions. The tip of the guide cannula was placed immediately below the border of the corpus callosum into the lateral ventricle (AP -1.4 mm, ML −1.8 mm, DV -3.0 mm; (see Paxinos and Watson, 1998). The tip of the 30 gauge injection cannula protruded 0.5 mm beyond the tip of the guide cannula to allow compound infusion into the lateral ventricle. The indwelling cannula was secured chronically using dental acrylic anchored in place with small stainless steel screws inserted into the skull. A removable obturator sealed the guide cannula and was only removed for infusions during the experiment. Animals received a subcutaneous injection of analgesic (ketoprofen, 1 mL/kg) immediately post-operatively. After surgery, animals were kept warm under a heating lamp until righting

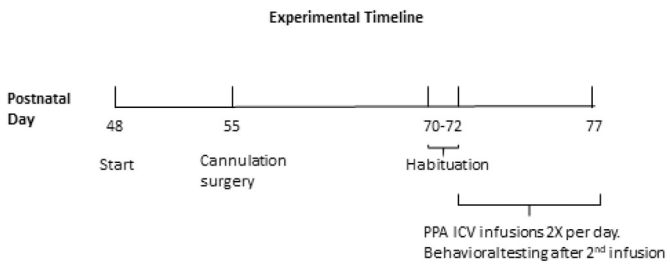

Fig. 1. Experimental timeline showing ages at which various manipulations occurred. PPA – propionic acid; ICV – intracerebroventricular.

responses and locomotion returned. Animals were housed individually and allowed two weeks recovery prior to testing.

2.3. Hole-board apparatus

Locomotor activity (see Ossenkopp and Kavaliers, 1996; Ossenkopp and Mazmanian, 1985) was monitored using three Versamax Animal Activity Monitors (AccuScan Model DCM-8, Columbus, OH, USA). Each monitor consisted of a clear Plexiglas open field chamber (W 40 cm x L 40 cm x H 30.5 cm) and a clear plastic lid with air holes. Movement was recorded via a grid of infrared beams located on all four sides of the chamber for horizontal activity (16 equally spaced beams 2.54 cm apart and 4.5 cm from the floor) and a second grid of infrared beams located on two sides of the chamber for vertical activity (16 beams) located 15 cm above the box floor. The automated activity monitors were equipped with a hole-board on the floor of the chamber to measure nose poke responses (see Supplementary Fig. 1 A-D). The hole-board consisted of an elevated platform with 16 equally spaced holes (2.54 cm diameter) with small plastic cups (5.08 cm diameter) underneath each hole. A set of infrared beam sensors, separate from those recording locomotor activity, were located between the cup and the platform, allowing for nose poke counts for each hole to be recorded via beam breaks. VersaMax Analyzer software (Accuscan Model VSA-16, Columbus, OH) recorded data from each automated activity monitor and relayed it to a computer that stored the data for subsequent analysis. All sessions within the automated activity monitors were video-recorded and later reviewed to ensure accuracy of the computer generated nose poke data.

2.4. Drug treatment

Following 2 weeks of recovery after surgery, animals were randomly assigned to one of three groups: high-dose PPA (0.26 M, $n = 11$), low-dose PPA (0.052 M, $n = 9$), or phosphate buffered saline vehicle (PBS, $n = 15$). Doses were based on the results of prior studies (MacFabe et al., 2007; MacFabe et al., 2008). Propionic acid was dissolved in PBS vehicle, and all solutions were buffered to pH 7.5 using concentrated HCl or NaOH. Each animal received ICV infusions twice daily (separated by 4 h) for seven consecutive days. The first infusion occurred during the light phase at 09:00 h. Solutions were infused using a 30 gauge injection cannula that was connected to a Sage syringe pump with sterile PE10 tubing. The tip of the injection cannula protruded 0.5 mm beyond the tip of the guide cannula. The syringe pump dispensed 4.0 μL of solution over a 60 s interval, and the injection cannula remained in place for an additional 60 s before being removed.

2.5. Behavioural testing procedure

Rats were handled and habituated to the automated activity monitors for two days (30 min per day). On the third day, baseline levels of activity and nose poke responses were recorded in the absence of infusion. During the seven treatment days, animals were placed in the automated monitors following the second infusion of the day for 30 min to record locomotor activity and nose poke counts (six 5 min time bins). All cups within the hole-board platform remained empty for the entire duration of behavioural testing (including habituation, baseline, and testing days). Rats were weighed daily to monitor health.

2.6. Behavioural measures

Locomotor activity was analyzed using eight distinct measures. The horizontal activity measures analyzed were: **total distance** – total horizontal distance (cm) traveled; **horizontal movement time** – amount of time (s) an animal was engaged in horizontal movement; and **number of horizontal movements** – the number of horizontal movements separated by a 1 s stop time. The vertical activity measures analyzed were: **vertical movement time** – amount of time (s) an animal spent in a vertical position; and **number of vertical movements** – number of vertical movements (rearing responses) separated by a 1 s stop time. The repetitive locomotor measures were: **clockwise revolutions** – the numbers of times an animal moved around in a clockwise circle of at least 5.04 cm in diameter; **counterclockwise revolutions** – the number of times an animal moved around in a counterclockwise circle of at least 5.04 cm. in diameter; and the **number of stereotypic movements** – repeated breaking of the same infrared beam separated by 1 s or more. A previous study demonstrated that the dependent variables obtained in the automated animal activity apparatus exhibit substantial reliability across test sessions (Ossenkopp et al., 1987). More importantly, validity of a number of the variables was previously verified by correlating visual scoring of videotapes of the behavior with the values of the automated measures obtained in the activity monitors (Sanberg et al., 1985; Sanberg et al., 1987). In particular, the automated stereotypy (repetitive movement) measure in rats was shown to correlate highly (Sanberg et al., 1987) with visual observations of amphetamine-induced stereotypic movements indexed with a stereotypy rating scale. The automated measure was also superior to the visual rating scale in detecting a drug dose relationship.

Nose poke behaviour was analyzed using **total nose poke counts** and nose poke counts at different hole location categories. Total nose poke counts consisted of the total number of nose pokes across an entire testing session (for all 16 holes). Hole location categories were used to examine the patterns of hole preferences. The three hole categories included **corner holes** (1, 4, 13, and 16); **centre holes** (6, 7, 10, and 11), and **wall holes** (2, 3, 5, 8, 9, 12, 14, and 15) (see Supplementary Fig. 2). Hole poke frequencies at these different locations were then converted to percent of total nose pokes for each rat (e.g., centre hole nose pokes divided by total number of nose pokes multiplied by 100) to control for overall rate of nose poking. Nose poke behavior has been previously shown to have good reliability (File and Wardill, 1975a).

2.7. Brain tissue preparation and histological verification of cannula placements

After the last test session rats were deeply anesthetized and perfused transcardially with ice cold 0.1 M phosphate buffered saline (pH 7.5, PBS) followed by 4% paraformaldehyde in PBS. The brain was removed from the skull and cryoprotected in 18% sucrose in PBS. Serial coronal 40 μm thick brain sections were cut with a cryostat along the cannula track, then mounted on glass slides, dehydrated with increasing concentrations of ethanol and xylenes using standard histological procedures, and stained with cresyl violet for Nissl substance to allow confirmation of cannula placement. All cannula tips were confirmed to lie in the lateral ventricle.

2.8. Statistical analyses

Data were analyzed for main effects and interactions using a repeated measures split-plot analysis of variance (ANOVA) with drug treatment (PBS, low-dose PPA, and high-dose-PPA) as the between-subjects factor and infusion day (7 infusions days) and time block (six 5 min time blocks) as the within subjects factors (with the exception of hole category, which was analyzed with only infusion day as the within subjects factor). The dependent variables were the various locomotor variables and two nose poke response variables. Separate statistical analyses were conducted for each variable. A one-way ANOVA was conducted on the baseline data to ensure that there were no group differences prior to treatment days. For any variable in which the baseline ANOVA indicated significant group differences, a repeated measures ANCOVA was performed, using the baseline data as a covariate. Where appropriate, post-hoc pair-wise comparisons were conducted using Tukey's HSD. Significance was set to $\alpha = 0.05$.

3. Results

There were no significant differences in group body weights, either at the time of cannulation, or across the 7 drug treatment days.

3.1. Locomotor activity variables

3.1.1. Baseline data

A one-way ANOVA was performed on the baseline data for each locomotor variable with group as a dummy variable. The one-way ANOVAs were not significant for total distance traveled, horizontal movement time, vertical movement time, or any of the repetitive activity measures (number of stereotypic movements, number of clockwise revolutions, and number of counterclockwise revolutions). Thus, there were no significant differences in locomotor activity among the treatment groups for these behavioural measures prior to the first infusion day. The ANOVAs were significant on baseline day for number of horizontal movements and number of vertical movements; therefore, baseline data were used as a covariate on infusion days for these variables.

3.1.2. Horizontal activity measures

Analysis revealed a significant day x treatment interaction for number of horizontal movements, $F(12, 192) = 3.25$, $p < .01$, and horizontal movement time, $F(12, 192) = 2.74$, $p < .05$. There was a significant main effect of treatment for total distance traveled, $F(2, 32) = 16.67$, $p < .001$. In general, PPA infusions produced an increase in all three horizontal activity measures that was dose dependent. High-dose PPA infused rats traveled further, made more horizontal movements, and spent more time traveling horizontally than PBS rats across infusion days. Low-dose PPA animals showed the same effects as high-dose PPA animals, but not until later into the infusion schedule.

Post-hoc analyses were conducted to determine differences among treatment groups across infusion days or across time. As seen in Fig. 2A, high-dose PPA animals made significantly more horizontal movements than both PBS animals (on infusion days 2, and 4–7, $ps < 0.05$), and low-dose PPA animals (on infusion day 2, $p < .05$) across infusion days. Low-dose PPA animals made significantly more horizontal movements than PBS animals on infusion days 6 and 7 ($ps < 0.05$). There were no significant differences among treatment groups for number of horizontal movements on infusion days 1 and 3.

Across the 30 min testing sessions, there was a significant time x treatment interaction for number of horizontal movements, $F(10, 155) = 8.35$, $p < .001$. Post-hoc analyses were conducted across the six 5 min time bins for number of horizontal movements. On infusion day 1, there were no significant differences among groups during the entire 30 min testing session (see Fig. 2B). On infusion day 4 both PPA groups made significantly more horizontal movements than PBS animals at 25 min ($p < .05$; see Fig. 2C). There were no significant differences among treatment groups for all other times during the testing session. On infusion day 7, both PPA groups made significantly more horizontal movements than PBS animals at 10 and 15 min and at 25 and 30 min ($ps < 0.05$; see Fig. 2D). In general (other than the first infusion day), both PPA groups showed a slight decrease in number of horizontal movements across time within testing sessions, whereas, PBS animals showed a sharp decline in number of horizontal movements across time within testing sessions.

A similar pattern was seen across infusion days for horizontal movement time as for number of horizontal movements (see Fig. 3). High-dose PPA animals spent significantly more time traveling horizontally than PBS animals on all but the first infusion day (infusion days 2–7, $ps < 0.05$). High-dose PPA animals also spent significantly more time traveling horizontally than low-dose PPA animals on infusion days 2–5 ($ps < 0.05$), but did not differ on days 6 and 7. Low-dose PPA animals spent significantly more time traveling horizontally than PBS animals on infusion days 3 to 7, ($ps < 0.05$).

Post-hoc analyses for total distance traveled across infusion days were similar to number of horizontal movements and horizontal movement time. High-dose PPA animals traveled significantly more than PBS animals on infusion days 2–7 ($ps < 0.05$; see Fig. 4). High-dose PPA animals also traveled significantly more than low-dose PPA animals on infusion days 2 and 3 ($ps < 0.05$). Low-dose PPA animals traveled significantly more than PBS animals on infusion days 4, 6, and 7 ($ps < 0.05$) and did not differ from the high-dose group on the last 2 days. There were no significant differences among treatment groups for total distance traveled on infusion day 1.

Overall, ICV administration of PPA resulted in increased horizontal locomotion across infusion days that was dose dependent, with the high dose of PPA showing effects by the second infusion day and the low dose on days 6 and 7 of the infusion schedule. There was also evidence of dose dependent effects of PPA within test sessions, especially toward the ends of test sessions, in the second half of the daily PPA infusion schedule (days 4–7).

3.1.3. Vertical activity measures

ANOVAs revealed a significant day x treatment interaction for number of vertical movements, $F(12, 192) = 2.40$, $p < .05$, but no significant time x treatment interaction, $F(10, 155) = 0.27$, *ns* (see Fig. 5). Post-hoc analysis across infusion days showed that low-dose PPA animals made significantly more vertical movements than PBS animals on infusion days 4, 6, and 7 ($ps < 0.05$). High-dose PPA animals also made significantly more vertical movements than PBS animals on infusion days 4, 6 and 7 ($ps < 0.05$). There were no significant differences among treatment groups on infusion days 1–3, and 5.

For vertical movement time, analyses showed a significant time x treatment interaction, $F(10, 160) = 6.49$, $p < .001$. There was no significant main effect of treatment, $F(2, 32) = 1.58$, *ns*, or day x treatment interaction, $F(12, 192) = 0.82$, *ns*. Post-hoc analyses across time and within a testing session revealed that on infusion day 1, high-dose PPA animals had significantly shorter vertical movement time than PBS animals during the first 5 min ($p < .05$, data not shown). For the remaining 25 min, there were no significant differences among treatment groups for vertical movement time. On infusion day 4, there were no significant differences among treatment groups for vertical movement time during the entire 30 min testing session (data not shown). On infusion day 7, there were no significant differences among treatment groups for vertical movement time for the first 20 min of the testing session. During the remainder of the testing session, low-dose PPA animals had significantly longer vertical movement time than the PBS (time 20–30, $ps < 0.05$) and high-dose PPA animals (time 25–30, $p < .05$, data not shown).

3.1.4. Repetitive activity measures

Analyses revealed a significant day x treatment interaction for number of stereotypic movements, $F(12, 192) = 3.15$, $p < .01$. Across days, the number of stereotypic movements differed among the treatment groups (see Fig. 6A). In general, both PPA groups showed an increase in the number of stereotypic movements across infusion days; whereas, PBS animals remained relatively constant in the number of stereotypic movements across infusion days. Post-hoc analyses revealed that high-dose PPA animals made significantly more stereotypic movements than the PBS group on infusion days 3–7 ($ps < 0.05$), and the low-dose PPA animals on infusion days 3–6, ($ps < 0.05$). In addition, low-dose PPA animals made significantly more stereotypic movements than PBS animals on infusion days 6 and 7 ($ps < 0.05$). There were no significant differences among treatment groups for number of stereotypic movements on infusion days 1 and 2.

There was also a significant time x treatment interaction for number of stereotypic movements, $F(10, 160) = 8.30$, $p < .001$. Across time, PBS animals showed a decrease in the number of stereotypic movements. Although both PPA groups exhibited a decrease in the number of stereotypic movements across time, this decline was more gradual than

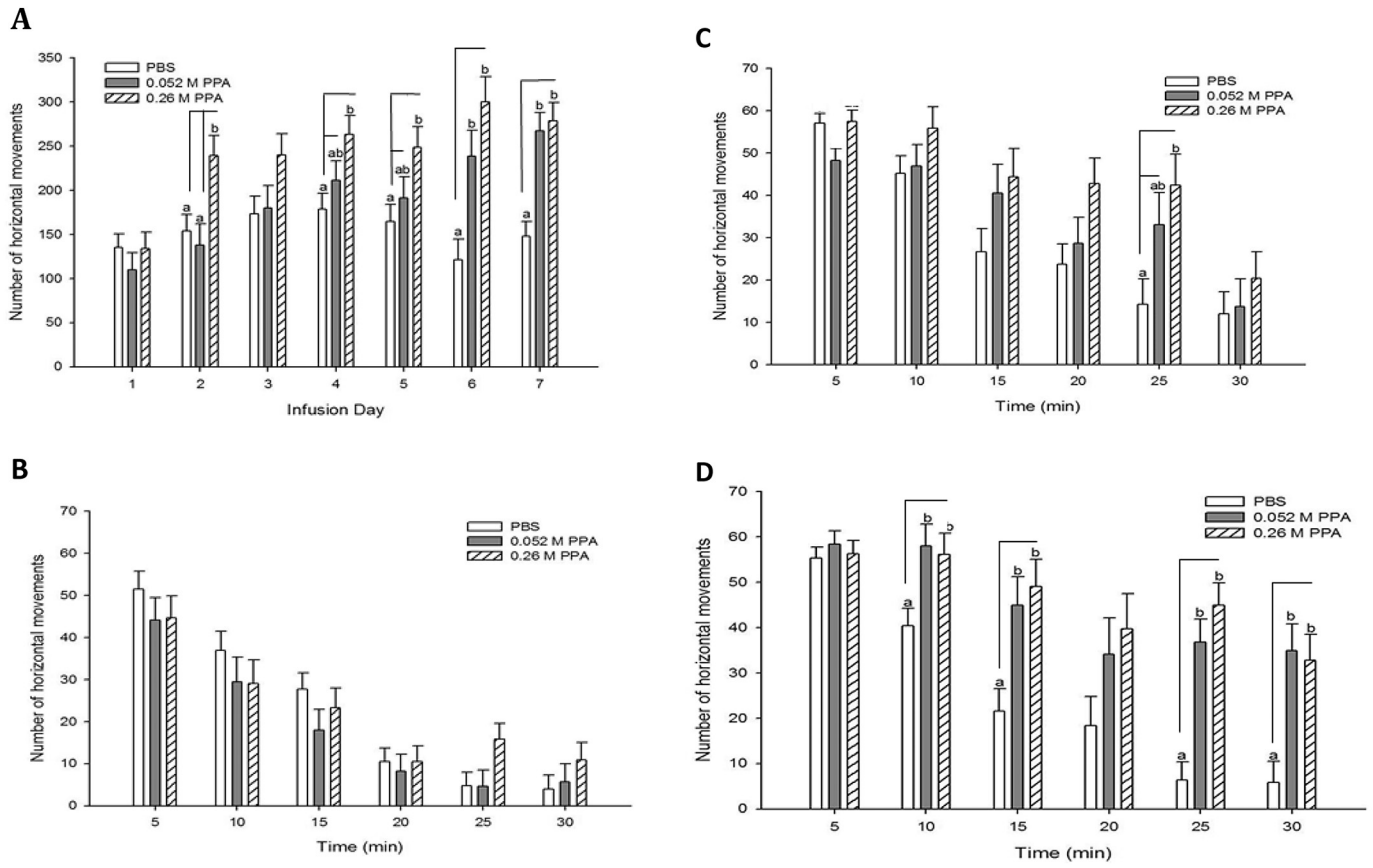

Fig. 2. Mean number of horizontal movements + *SEM*. A. across infusion days; B. across time on infusion Day 1; C. across time on infusion Day 4; D. across time on infusion Day 7. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. In Panel A there are significant group differences on Day 2 (high dose PPA group is significantly different from both other groups), Days 4 and 5 (all 3 groups differ significantly), and Days 6 and 7 (both PPA groups differ significantly from the PBS group). In panel C there are significant group differences at 25 min (all 3 groups differ significantly). In Panel D there are significant group differences at 10 min, 15 min, 25 min and 30 min (group PBS differs significantly from both PPA groups).

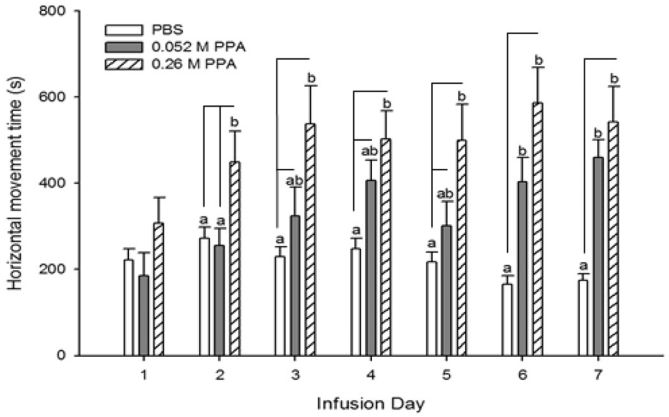

Fig. 3. Mean horizontal movement time (s) + *SEM* across infusion days. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. Significant group differences were found on Day 2 (high PPA group is significantly different from the other 2 groups), Days 3, 4 and 5 (all 3 groups differ significantly), and Days 6 and 7 (group PBS differs significantly from both PPA groups).

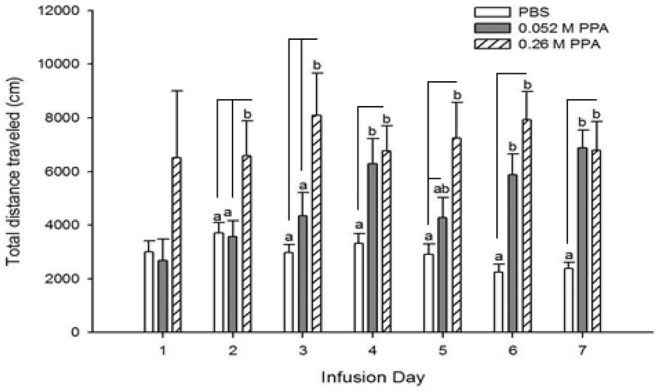

Fig. 4. Mean total distance traveled (cm) + *SEM* across infusion days. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. Significant group differences were found on Days 2 and 3 (high PPA group differed significantly from the other 2 groups), Days 4, 6 and 7 (the PBS group differed significantly from the 2 PPA groups), and Day 5 (all 3 groups differed significantly).

that exhibited by the PBS animals. Post-hoc analyses for infusion day 1 showed that low-dose PPA animals made significantly fewer stereotypic movements than both PBS and high-dose PPA animals at 20 min ($p < .05$; see Fig. 6B). There were no significant differences among treatment groups for number of stereotypic movements during the remainder of the testing session. On infusion day 4, high-dose and low-dose PPA animals made significantly more stereotypic movements than PBS animals during the first 5 min ($p < .05$) and at 25 min ($p < .05$; see Fig. 6C). There were no significant differences among treatment

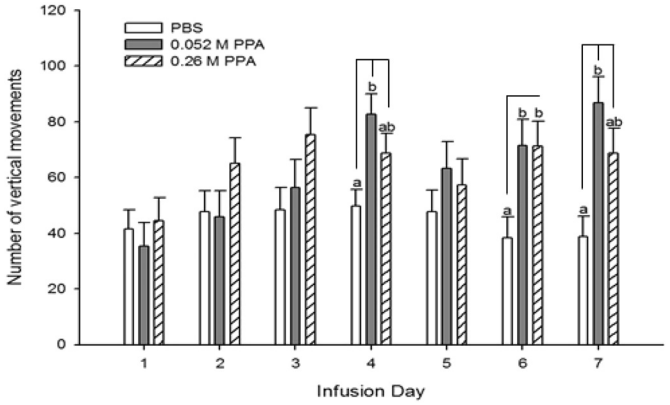

Fig. 5. Mean number of vertical movements + *SEM* across infusion days. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. Significant group differences were found on Days 4 and 7 (all 3 groups differed significantly), and Day 6 (group PBS differed significantly from the 2 PPA groups).

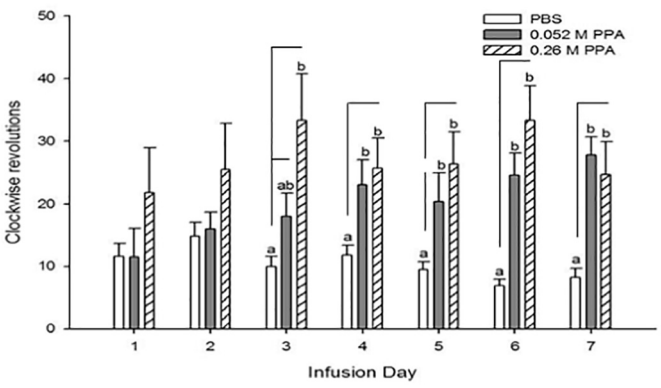

Fig. 7. Mean number of clockwise revolutions + *SEM* across infusion days. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. Significant group differences occurred on Day 3 (all 3 groups differed significantly) and on Days 4, 5, 6, and 7 (group PBS differed significantly from the 2 PPA groups).

groups at any other times during the testing session. On infusion day 7, high-dose PPA animals made significantly more stereotypic movements than PBS animals during the entire 30 min testing session (time 5–30, *ps* < 0.05; see Fig. 6D). Low-dose PPA animals also made significantly more stereotypic movements than PBS animals in 3 time bins (time 5 min and time 25 and 30 min, *ps* < 0.05).

Significant main effects of treatment for clockwise revolutions, $F(2, 32) = 12.87$, $p < .001$, and counterclockwise revolutions, $F(2, 32) = 12.15$, $p < .001$, were obtained in the ANOVA. There was no significant day x treatment interaction for either clockwise, $F(12, 192) = 1.87$, *ns*, or counterclockwise revolutions, $F(12, 192) = 1.97$, *ns*. In general, PPA infusions produced an increase in revolutions that was dose dependant. High-dose PPA animals made more clockwise and counterclockwise revolutions than PBS animals across infusion days. Low-dose PPA animals showed similar effects, but not until later into

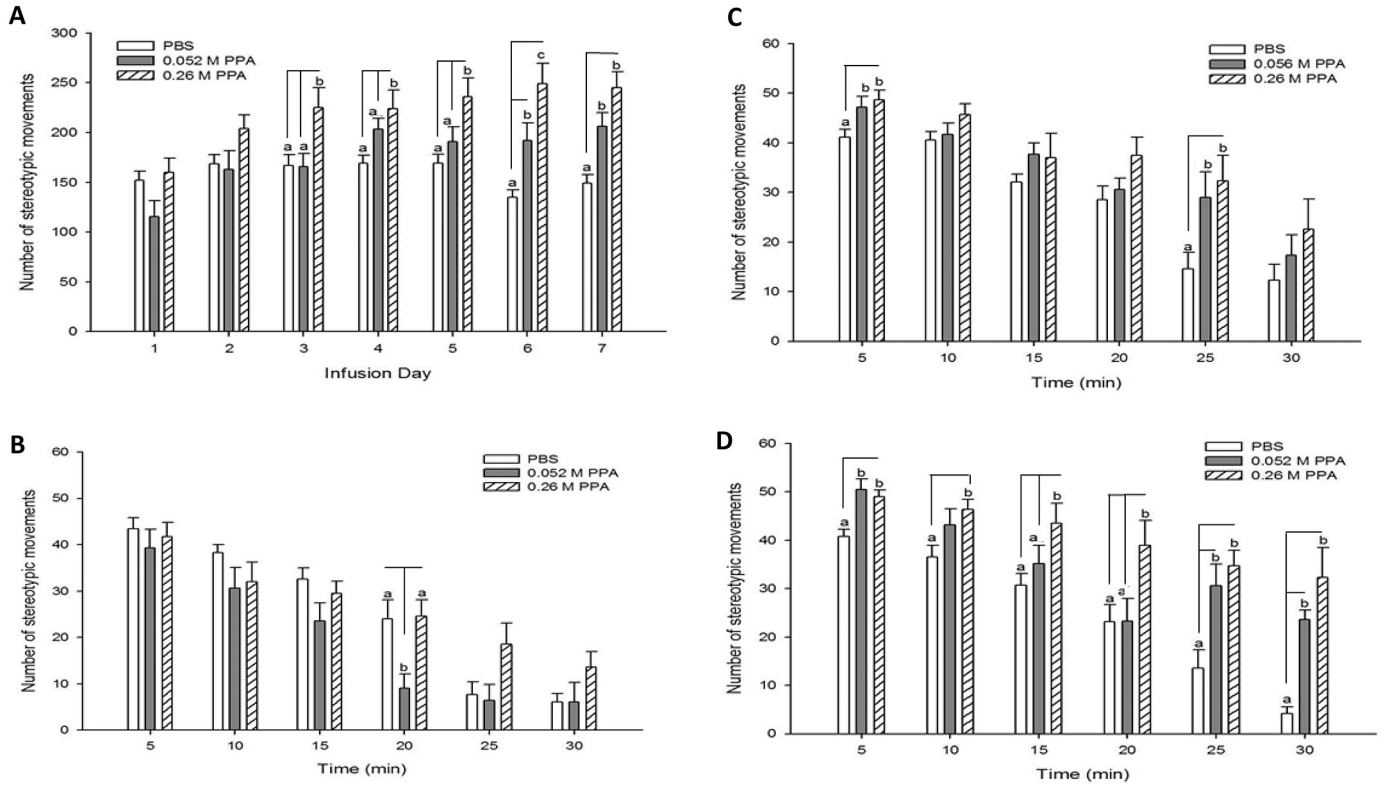

Fig. 6. Mean number of stereotypic movements + *SEM*. A. across infusion days; B. across time on infusion Day 1; C. across time on infusion Day 4; D. across time on infusion Day 7. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. In Panel A there are significant group differences on Days 3, 4, and 5 (high PPA group significantly different from the other 2 groups), Day 6 (all 3 groups differed significantly) and Day 7 (group PBS differed significantly from the 2 PPA groups). In Panel B there are significant group differences at 20 min (low PPA group differed significantly from the other 2 groups). In Panel C there are significant group differences at 5 min and 25 min (the PBS group differed significantly from the 2 PPA groups). In Panel D there are significant group differences at all times. At 5 min, 25 min and 30 min (group PBS significantly different from both PPA groups), at 10 min (group PBS is significantly different from the high PPA group), at 15 min and 20 min (high PPA group is significantly different from the other 2 groups).

the infusion schedule. Post-hoc analyses revealed an identical pattern across infusion days for clockwise and counterclockwise revolutions. High-dose and low-dose PPA animals made significantly more clockwise and counterclockwise revolutions than PBS animals on infusion days 3 to 7 ($ps < 0.05$; see Fig. 7 for clockwise revolutions). High-dose PPA animals made significantly more clockwise and counterclockwise revolutions than low-dose PPA animals on infusion day 3 ($p < .05$). There were no significant differences among treatment groups for clockwise or counterclockwise revolutions on infusion days 1 or 2.

Across the 30 min testing session, there was a significant time x treatment interaction for clockwise revolutions, $F(10, 160) = 2.87$, $p < .05$, but not for counterclockwise revolutions, $F(10, 160) = 1.81$, *ns*. In general, PBS animals showed a sharp decline in number of clockwise revolutions across time within a testing session. In contrast, high-dose PPA animals showed a more gradual decline in clockwise revolutions within the first half of the testing session, and during the second half of the testing session, the number of clockwise revolutions remained relatively stable. Low-dose PPA animals showed a pattern more consistent with PBS animals across time within the first 3 infusion days, and then exhibited a pattern more similar to high-dose PPA across time for the remainder of the infusion schedule. On infusion day 1, post-hoc analyses showed that there were no significant differences among treatment groups for number of clockwise revolutions during the first 20 min of the testing session. For the remaining 10 min, high-dose PPA made significantly more clockwise revolutions than PBS animals ($ps < 0.05$; data not shown). On infusion day 4, high-dose PPA animals made significantly more clockwise revolutions than PBS animals, but only between 15 and 20 min ($p < .05$; data not shown). During the last infusion day, high-dose PPA animals made significantly more clockwise revolutions than PBS animals for 2 time bins (time 5–10 and time 15–20, $ps < 0.05$; data not shown). Low-dose PPA animals made significantly more clockwise revolutions than PBS animals during the first 5 min and the last 15 min ($ps < 0.05$).

Overall, daily ICV infusions of PPA resulted in dose dependent increases in stereotypic and repetitive behaviours, with the high dose of PPA showing effects on these variables by the 3rd day and the low dose by the 6th and 7th days of the infusion schedule. There was also evidence of dose dependent effects of PPA within test sessions, especially toward the ends of test sessions, in the second half of the daily PPA infusion schedule (days 4–7).

3.2. Nose poke variables

3.2.1. Nose poke counts

The one-way ANOVA performed on the baseline data for total nose poke counts revealed no significant group differences.

The ANOVA of the number of nose pokes revealed a significant day x treatment interaction across infusion days, $F(12, 192) = 2.96$, $p < .01$. As seen in Fig. 8A, PPA infusions resulted in a dose-dependent increase in number of nose pokes toward the end of the infusion schedule. Post-hoc analyses showed that high-dose PPA animals made significantly more nose pokes than PBS animals on infusion days 5, 6 and 7 ($ps < 0.05$). On infusion days 6 and 7 low-dose PPA animals made significantly more nose pokes than PBS animals ($ps < 0.05$). There were no significant differences among treatment groups for number of nose pokes on infusion days 1 through 4.

There was a significant time x treatment interaction for number of nose pokes, $F(10, 160) = 5.11$, $p < .01$. Across time within a testing session, PBS animals exhibited a sharp decrease in number of nose pokes. Low-dose PPA animals resembled PBS animals across time for most of the infusion days, with the exception of infusion days 6 and 7, where low-dose PPA animals showed a pattern more similar to high-dose PPA animals across time. Post-hoc analysis on infusion day 1 showed that there were no significant differences among treatment groups for number of nose pokes during the first 20 min of the testing session (see Fig. 8B). During the last 10 min, high-dose PPA animals made significantly more nose pokes than PBS animals ($ps < 0.05$) and the low-dose PPA group made more nose pokes than the PBS group at 30 min. On infusion day 4, high-dose and low-dose PPA animals made significantly more nose pokes than PBS animals during 2 time bins (time 15 and time 25, $ps < 0.05$; see Fig. 8C). During the last infusion day, high-dose and low-dose PPA animals made significantly more nose pokes than PBS animals during the last 4 time bins (15, 20, 25 and 30 min, $ps < 0.05$; see Fig. 8D).

In summary, daily ICV infusions of PPA resulted in dose dependent increases in nose poke behaviours, with the high dose of PPA showing effects by the 5th day and the low dose by the 6th and 7th days of the infusion schedule. There was also evidence of dose dependent effects of PPA within test sessions, starting by the middle of test sessions, in the second half of the daily PPA infusion schedule (days 4–7).

3.2.2. Hole category

A one-way ANOVA was performed on the baseline data for each hole category variable (i.e., corner, wall, and centre nose pokes). The ANOVAs failed to reveal any significant group differences for any of the hole category variables.

Further analyses revealed a significant main effect of treatment for number of corner nose pokes, $F(2, 32) = 4.13$, $p < .05$, but not for number of wall nose pokes, $F(2, 32) = 2.99$, *ns*, or for number of centre nose pokes, $F(2, 32) = 2.15$, *ns*. In general, all treatment groups made a similar proportion of corner nose pokes, wall nose pokes, and centre nose pokes (see Fig. 9A, B, and C, respectively). Post-hoc analysis for number of corner nose pokes showed that PBS animals made significantly more corner nose pokes (in proportion to total nose pokes) than high-dose PPA animals on infusion days 4 and 6 ($ps < 0.05$). On all other infusion days, there were no significant differences among treatment groups for number of corner nose pokes.

4. Discussion

The present study demonstrates that centrally (ICV) infused PPA in adult male rats produces increased locomotor behavior, stereotypy, and nose poking behaviour, and does so in a dose-dependent manner, with the higher dose producing greater effects. In general, high-dose PPA animals were more hyperactive, made more vertical movements, displayed more stereotypic and repetitive movements, and nose poked more often than PBS animals across infusion days and across time. Low-dose PPA animals showed similar effects to high-dose PPA animals, but not until later into the infusion schedule. In particular, low-dose PPA animals showed locomotor activity levels similar to those of PBS animals at the start of the infusion schedule, but gradually increased to levels comparable to those of high-dose PPA animals by the end of the infusion schedule (i.e., infusion days 6 and 7). As well, analyses of the hole category data indicated that all treatment groups made a proportionately similar number of wall and centre area nose pokes. For corner nose pokes, PBS animals made proportionately more corner nose pokes than both PPA groups, but only on infusion days 4 and 6.

The increase in locomotor activity and stereotyped/repetitive behaviour following infusion of PPA in the current study is consistent with, but not identical, to previous work using the ICV-PPA rodent model (MacFabe et al., 2007; MacFabe et al., 2008). Both the current, and previous work, used the same chronic infusion schedule over seven days (two infusions per day), as well as the same automated activity monitors to measure locomotor activity. The PPA dose used in these previous studies was identical to that of the high-dose PPA group in the current experiment. Despite the similarities in experimental design, the animals in the present investigation displayed much greater levels of horizontal activity than in previous work. This was true for both PBS control animals and high-dose PPA animals. This is particularly notable for total distance traveled. Unlike the horizontal movement measures, the vertical movement measures and number of stereotypic movements in PBS and high-dose PPA animals in the current study was similar to

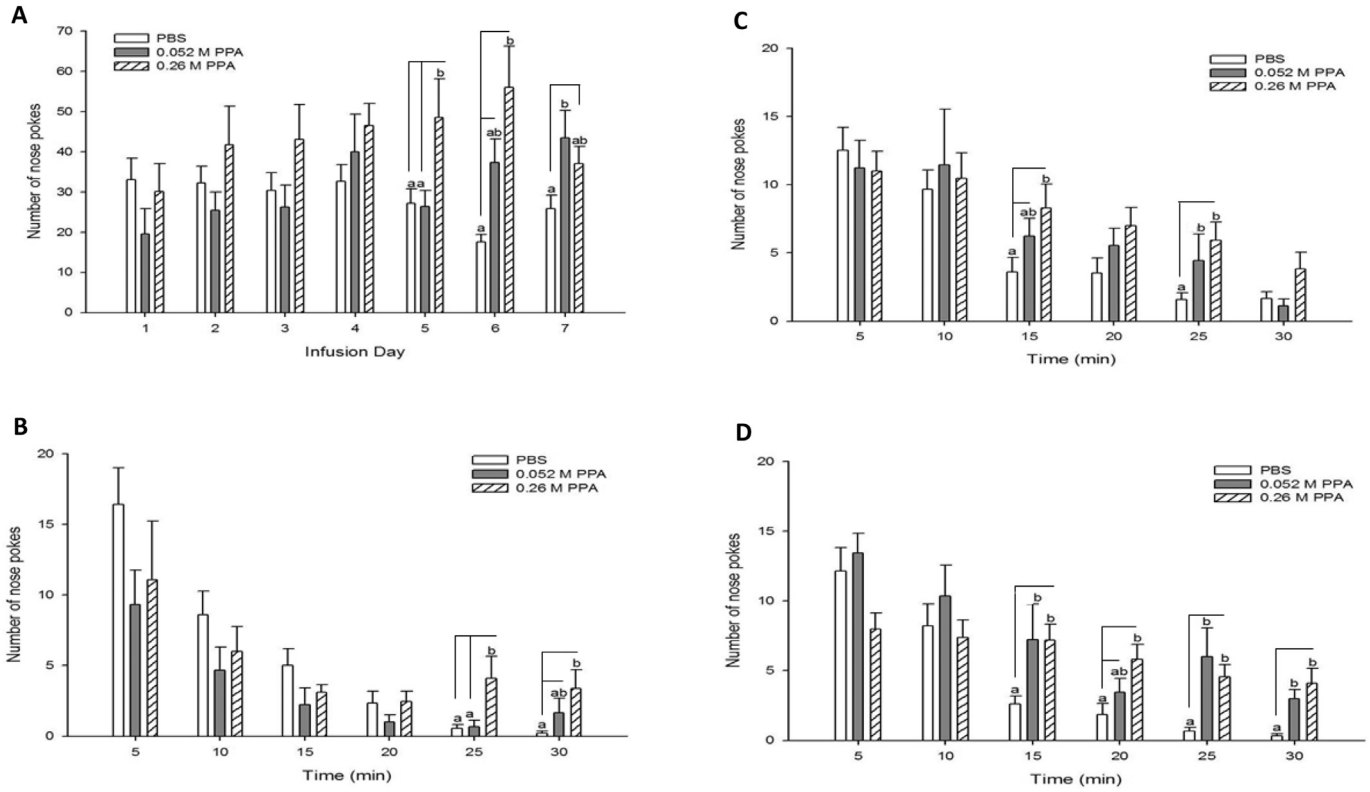

Fig. 8. Mean number of nose pokes + *SEM*. A. across infusion days; B. across time on infusion Day 1; C. across time on infusion Day 4; D. across time on infusion Day 7. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. In Panel A there are significant group differences on Day 5 (high PPA group is significantly different from the other 2 groups), Day 6 (all 3 groups differ significantly) and Day 7 (group PBS is significantly different from the 2 PPA groups). In Panel B there are significant group differences at 25 min (high PPA group differs significantly from the other 2 groups) and 30 min (all 3 groups differ significantly). In Panel C at 15 min (all 3 groups differ significantly) and at 25 min (group PBS differs significantly from the 2 PPA groups). In Panel D at 15 min, 25 min and 30 min (group PBS differs significantly from the 2 PPA groups) and at 20 min (all 3 groups differ significantly).

those observed in previous studies (MacFabe et al., 2008; Thomas et al., 2010; Thomas et al., 2012).

Given that the present experiment used the same strain and sex of rat (i.e., male Long-Evans) and the same dose and infusion regimen of PPA (i.e., two infusions per day for seven consecutive days), and that PBS animals also showed an increase in horizontal activity measures, it is highly unlikely that the increase was due to a fundamental difference in the ability of these particular rats to metabolize or compensate for PPA infusions, as compared to rats used in previous work using this model. The most plausible explanation for the increase in horizontal activity in the current experiment is the addition of the hole-board on the floor of the automated activity monitors suggesting that exploration in the hole-board apparatus increased overall locomotor activity.

Several studies have shown that locomotor activity changes independently of nose poking in rodents (Abel, 1995; Durcan and Lister, 1989; File and Wardill, 1975a; File and Wardill, 1975b), but others have found no such dissociation or mixed results depending on drug treatment (Kliethermes and Crabbe, 2006; Moy et al., 2008). Kliethermes and Crabbe (2006) showed that, although drug treatment resulted in similar effects on both locomotor and nose poking behaviours at a group level, analysis at the individual level in untreated mice showed that these behaviours could vary independently. Overall, there appears to be a complicated and equivocal relationship between nose poking and locomotion as measured in the hole-board apparatus.

Both PPA groups in the present study exhibited a decrement in locomotor behaviour over the 30 min testing session. This decrease likely reflects an habituation effect, but it could also be related to metabolic clearance of PPA which is known to have a half-life of 18 to 57 min when administered to rats (Brusque et al., 1999). Across infusion days, low-dose PPA animals gradually reached levels of locomotor activity and nose poking that were comparable to those of the high-dose PPA group, suggesting that putative compensatory mechanisms were unable to effectively counteract the effects of PPA. These compensatory mechanisms could include increased synthesis of certain enzymes, such as propionyl CoA decarboxylase which metabolizes PPA, or carbonic anhydrase which helps maintain acid-base balance (Nguyen et al., 2007; Schlue et al., 1991).

Propionic acid has several physiological/biochemical effects that alter neural function which could account for the increased locomotor behaviour seen in PPA-infused animals. PPA can inhibit Na^+/K^+ ATPase, increase NMDA receptor sensitivity, promote intracellular calcium release, and elevate nitric oxide, all of which can alter neurotransmission in brain regions relevant to locomotor behaviour (de Mattos-Dutra et al., 2000; Wajner et al., 2004; Wyse et al., 1998).

As a weak organic acid, PPA can passively accumulate in CNS cells resulting in a reduction in intracellular pH, which has many physiological consequences (Karuri et al., 1993). Previous work in our laboratory has shown that PPA and other short chain fatty acids (i.e., butyric acid and sodium acetate) produce similar behavioural impairments, but the non-acidic analogue of PPA, 1-propanol, did not produce behavioural impairments, suggesting that pH dependent mechanisms are an important component of the observed effects (MacFabe et al., 2007; Shultz et al., 2009; Shultz et al., 2008; Thomas et al., 2010). Intracellular acidification of neurons is known to increase the synthesis and release of several neurotransmitters that can influence locomotor activity, including glutamate, dopamine, norepinepherine, and serotonin (Cannizzaro et al., 2003; Remblier et al., 1999; Severson et al., 2003). Furthermore, 3-nitropropionic acid, a derivative of PPA, causes

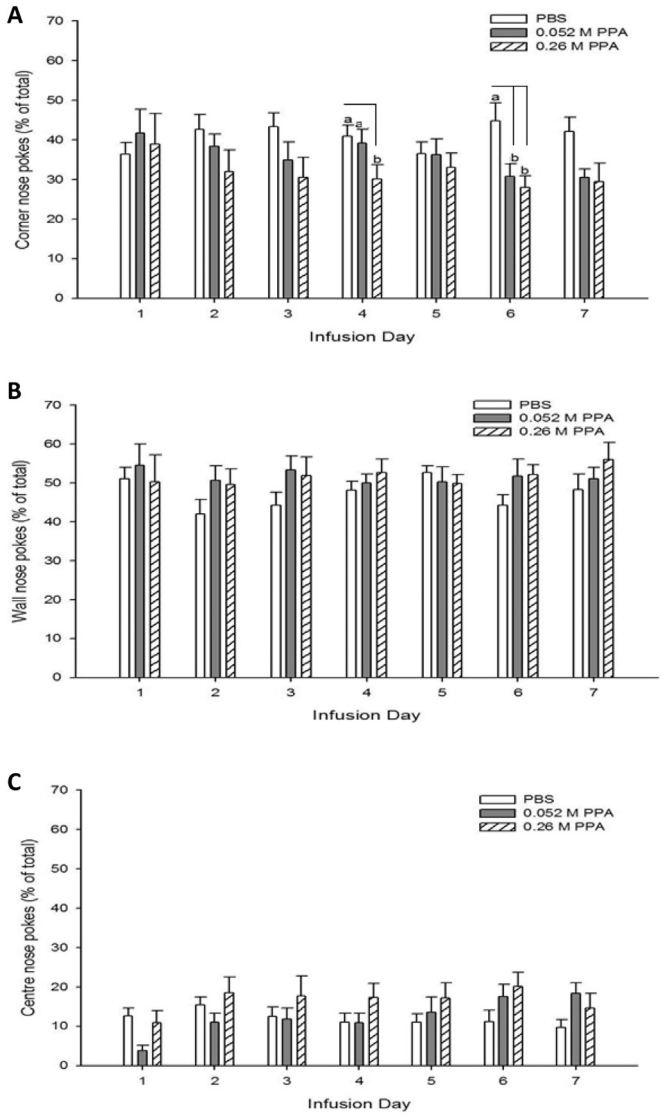

Fig. 9. Mean number of corner nose pokes (A), wall nose pokes (B), or centre nose pokes (C) expressed as a percent of total nose pokes + *SEM* across infusion days. Groups shown are PBS, low-dose PPA (0.052 M), and high-dose PPA (0.26 M) infused animals. Significant differences ($p < .05$) between treatment groups are indicated by lines and letters. In Panel A significant group differences occur on Day 4 (high dose PPA differs significantly from the other 2 groups), and Day 6 (group PBS differs significantly from the 2 PPA groups). There were no significant group differences in Panels B and C.

motor abnormalities and is used as a rodent model of Huntington's disease, emphasizing that intracellular pH reduction is linked to increased locomotor behaviour (Brouillet et al., 2005).

Another consequence of intracellular acidification is a reduction in intercellular coupling via the rapid and reversible closure of gap junctions (Rorig et al., 1996). Gap junctions play a vital role in electrotonic transmission in brain areas involved in locomotor activity, including the basal ganglia, prefrontal cortex, and hippocampal formation (O'Donnell and Grace, 1997; Velazquez et al., 1997). In addition, intrastriatal infusions of gap junction blockers produce movement stereotypies in rodents (Moore and Grace, 2002). Thus, the increased locomotor behaviour seen in PPA treated rats could be a consequence of the closure of gap junctions.

In addition to the abnormal locomotor behaviour observed in PPA treated animals, there was also an increase in nose poking compared to controls. PPA infusions led to a dose-dependent increase in nose poking toward the end of the infusion schedule. Nose poking, sometimes referred to as head-dipping, has been interpreted as representing exploratory behaviour or investigation of a novel environment (File and Wardill, 1975a; File and Wardill, 1975b). However, some researchers have argued that nose poking in the hole-board apparatus does not measure exploratory behaviour, but instead reflects the anxiety state of the animal (Takeda et al., 1998), an escape response (Brown and Nemes, 2008), or stereotyped behaviour (Makanjuola et al., 1977a). The wide spread in opinions regarding what the hole-board task is actually measuring in rodents are likely due, at least in part, to methodological differences.

Across studies, the hole-board apparatus often differs in total number of holes (usually 4 or 16), the location of the holes (centrally, peripherally, or equally dispersed within the floor of the apparatus), the diameter of the holes (allowing the rodent to either insert only its nose, or its entire head), the depth of the hole (ranging from 1 cm to 20 cm), and whether the hole-board is enclosed or open (occasionally the hole-board is used as an elevated platform without walls). In addition, the amount of time that rodents are observed within the hole-board apparatus ranges widely (e.g., 5 min, 10 min, 30 min, and 3 h). The variability of the hole-board apparatus has led to conflicting interpretations of nose poking behaviour, and this underscores the need for a standardized hole-board apparatus and procedure.

Another important consideration in the interpretation of the hole-board task is the complexity of exploratory behaviour. When rodents are exposed to a novel environment, they often exhibit exploratory behaviours, such as locomoting around the environment or orienting toward novelty (Ballaz, 2009; Berlyne, 1950). Presumably, exploratory behaviour allows the animal to gather relevant survival-related information about the unfamiliar place, such as food sources, mating opportunities, or the presence of predators. With continued exposure, the environment becomes familiar, and exploration decreases as animals habituate to it. Decreased exploratory behaviour in response to repeated exposure to a novel environment is one of the most common forms of habituation seen in rodents. However, there are a variety of factors influencing exploratory behaviour, including arousal level, attention, learning, memory, and fear of novelty (Berlyne, 1969; Bronson, 1968). The presence of these mitigating factors in exploratory behaviour makes the interpretation of any measure of exploration complex.

Given the complexity of interpreting nose poking behaviour in the hole-board apparatus, there are several possible explanations for the dose-dependent increase in nose pokes following ICV-PPA infusions. Assuming that nose poking represents exploratory behaviour, one possible explanation is that PPA infusions resulted in an increase in exploratory behaviour secondary to cognitive deficits in learning and memory. Intersession habituation, measured as a decrease in exploratory behaviour upon re-exposure to a novel environment, is considered to be an indicator of learning and memory (Leussis and Bolivar, 2006). Since PPA infusions resulted in an increase in exploratory behaviour across testing sessions, this may indicate that PPA animals failed to retain information about the novel environment. However, it is important to note that control animals maintained a relatively stable level of nose poking across infusion days, which would signify little or no habituation to the hole-board apparatus. In contrast Mirza and Sharma (2018) found that postnatal PPA-treated rats displayed low exploratory activity in a hole-board test. This difference may reflect a different route of administration of PPA (oral) and administration at an earlier age (24–48 days of age) and for a longer time period. Further examination of this discrepancy is warranted.

Another possibility is that nose poking may encompass both exploratory and repetitive behaviour. Makanjuola and colleagues (Makanjuola et al., 1977a; Makanjuola et al., 1977b) described exploratory behaviour in terms of the overall pattern of nose pokes, wherein repeated responses into one hole were considered a sign of stereotypy. The hole category data from the current study allows for the assessment of the pattern of nose pokes within the apparatus, and

therefore exploratory behaviour. Overall, animals made a similar proportion of corner, wall, and centre nose pokes. This suggests that PPA infusions did not alter exploratory behaviour. Although the current study did not measure repeated responses into one hole, the increase in nose poking seen in PPA animals could plausibly result from repetitive patterns of nose poking. Studies using a hole-board with 16 empty holes (similar to the current study), have shown that there is a gradual transition from exploratory to stereotyped nose poking observed across time, with most exploratory nose poking occurring within the first 10 min in the hole-board apparatus (Makanjuola et al., 1977a). This trend was seen in both control and drug-treated animals, with control animals displaying some stereotyped nose poking (as defined by repeated responses to one hole).

Results from the current study are consistent with this interpretation. PBS animals displayed a relatively stable level of nose poking across infusion days. Across time, PBS animals showed a marked decrease in nose poking after the first 10 min within the hole-board apparatus. This suggests that in control animals, nose poking represented mostly an exploratory response, as opposed to a repetitive response in the hole-board. In contrast, PPA infused animals showed an increase in nose poking behaviour across infusion days, which corresponded with a relatively consistent level of nose poking after the first 10 min within the apparatus, suggestive of a repetitive response. This interpretation is also consistent with the changes in repetitive movements (stereotypy) seen in the present study.

There are a number of ways in which PPA might affect brain function to cause the behavioural impairments reported here. Histological examination of brain tissue in rodents has shown that PPA can induce an innate neuroinflammatory response, characterized by reactive astrogliosis and activated microglia in the hippocampus and neocortical white matter (MacFabe et al., 2007; MacFabe et al., 2008). Neuroinflammation is also known to occur in other diseases, such as Parkinson's and Alzheimer's disease, suggesting that this response may impair normal cognitive processes (Ferretti and Cuello, 2011; Whitton, 2007). Activated microglia secrete cytokines and toxic substances (e.g., nitric oxide) that are potentially damaging to neurons, which may impair brain function (Barron, 1995). However, fast-acting mechanisms caused by PPA, such as closure of gap junctions and pH-dependent increases in serotonin, may also explain the behavioural changes seen in the current study. Connexin-36 knock-out mice show deficits in normal spatial coding and short-term spatial memory, suggesting that electrical coupling of interneurons in the hippocampus via gap junctions are important for spatial coding and cognition (Allen et al., 2011). Moreover, studies have shown that 5-HT(1A) receptor agonists produce learning and memory deficits in rodents (Ogren et al., 2008). It is possible that a fast-acting mechanism combined with neuroinflammation may have produced the behavioural impairments seen here.

The synthesis and release of dopamine and serotonin following intracellular acidification by PPA may explain the increased stereotypic and nose poking behaviour seen in these animals. Increases in both serotonin and dopamine in corticostriatal circuits of the brain have been linked to stereotyped and repetitive behaviour in rodents (Langen et al., 2011b) and humans (Langen et al., 2011a). Indeed, studies using the hole-board apparatus suggest that some aspects of the dopaminergic system are involved in nose poking behaviour (Kliethermes and Crabbe, 2006).

The findings from the current study indicate that ICV-PPA induces locomotor behaviour and stereotyped/repetitive behaviour, similar to the symptoms seen is ASD. Individuals with ASD often display hyperactivity, motor stereotypies, and repetitive behaviour (Matson et al., 2009; Murray, 2010). Importantly, the proposed mechanisms underlying the behavioural impairments seen is PPA-infused animals are also theoretically linked to ASD. Aberrations in dopamine and serotonin have been reported in autistic individuals (Chugani, 2004; Previc, 2007), and treatment of repetitive behaviours has been shown to be effective with serotonin reuptake inhibitors, and serotonin and dopamine antagonists (McDougle et al., 2000; McPheeters et al., 2011). An active neuroinflammatory process has also been observed in individuals with ASD (Depino, 2013; Morgan et al., 2010; Vargas et al., 2005).

5. Conclusion

In conclusion, central infusions of PPA in adult male rats resulted in locomotor abnormalities and increased repetitive behaviour compared to controls. These effects were dose and time dependent and consistent with the hypothesis that elevated levels of PPA are putatively responsible for manifestation of an autistic behavioural phenotype. The direct or indirect physiological properties of PPA, such as intracellular acidosis and neuroinflammatory changes, may be a plausible mechanism underlying these behavioural effects. The current findings are consistent with the symptoms seen in individuals with ASD and, taken together with previous findings from this rodent model, support the use of ICV-PPA in an animal model of ASD. Further research at critical developmental periods, as reported in recent studies (Al-Ghamdi et al., 2014; Choi et al., 2018; El-Ansary et al., 2013; El-Ansary et al., 2012; Foley et al., 2015; Foley et al., 2014a; Foley et al., 2014b; Shams et al., 2019; Wah et al., 2019), is needed to better understand the mechanisms responsible for the behaviours produced by PPA treatment and their potential involvement in human ASD.

Declaration of Competing Interest

There are no conflicts of interest for any of the authors.

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Ethical statement

Manuscript: Dose and time dependent increases in locomotor activity, repetitive movements, and nose poking behaviour in rats given repeated intracerebroventricular (ICV) infusions of the enteric bacterial product, propionic acid and tested in a hole-board task: Contributions to a rodent model of ASD.

All procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the University of Western Ontario Animal Use Subcommittee.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2019.109794.

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