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SPECIAL ISSUE

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Systemic treatment with the enteric bacterial metabolic product propionic acid results in reduction of social behavior in juvenile rats: Contribution to a rodent model of autism spectrum disorder

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Abstract

The role of the gut microbiome and its enteric metabolites, such as short-chain fatty acids (SCFAs), in the etiology of autism spectrum disorders (ASDs) has recently received increased attention. Of particular interest has been the SCFA, propionic acid (PPA). Several different rodent models have been developed using PPA treatment to examine behaviors of relevance to ASD. The effects of systemic (intraperitoneal, i.p.) administration of PPA on social behavior, anxiety-related behavior, and locomotor activity in juvenile male rats (age 35 days) were examined in this study. Rats received seven i.p. injections of buffered PPA (500 mg/kg) or phosphate-buffered saline. Behavior was video-recorded during social interaction in a large open field (first four injections) or assessed in an automated activity system (individual animals, last three injections). PPA treatment significantly reduced social interaction, increased anxiety-related behavior, and produced hypoactivity and increased abnormal motor movements. These findings suggest that PPA alters behaviors of relevance to ASD in juvenile rats. These results contribute to the behavioral validity of the rodent model of ASD with systemic PPA treatment.

KEYWORDS

abnormal motor behavior, anxiety, autism spectrum disorder, propionic acid, social behavior, systemic treatment

1 | INTRODUCTION

Autism spectrum disorders (ASDs) are characterized by social interaction impairment, disordered movements, hyperactivity, sensory disturbances, restrictive interests and repetitive behaviors, deficiencies of language, and cognitive deficits (Arndt, Stodgell, & Rodier, 2005; DiCicco-Bloom et al., 2006; Fombonne, 2009). Imaging and neuropathological studies of patients with ASD have noted increased brain size, white matter abnormalities, and increased neuronal density in neocortical, limbic, and cerebellar areas (Bauman & Kemper, 2005; Courchesne, Redcay, & Kennedy, 2004; Herbert, 2005). Reports show that ASD has a high comorbidity with other disorders, such as seizure disorder, anxiety disorders, and mental retardation (Campbell et al., 2009).

The etiology of ASD is poorly understood, but the disorder may be associated with a variety of other conditions and risk factors, including fetal alcohol syndrome, prenatal exposure to thalidomide or valproate (Arndt et al., 2005; Bromley, Mawer, Clayton-Smith, &

Baker, 2008), fragile X syndrome, and tuberous sclerosis (Herbert et al., 2006), amongst others. Autism usually manifests in early infancy, characterized by an absence of age-appropriate parental eye contact or social development as well as abnormal movement patterns (Zwaigenbaum et al., 2008, Zwaigenbaum et al., 2013) based on faulty development of some reflexes.

There is growing interest in the role that gastrointestinal factors may play in the behavioral, neuropathological, and neuroinflammatory sequelae observed in some ASD cases (Belkaid & Hand, 2014). Anecdotal reports have suggested that ASD symptomology may be induced or exacerbated following acute gastrointestinal abnormalities (Horvath & Perman, 2002), routine and antibiotic-resistant pediatric infections (Fallon, 2005), or ingestion of wheat- or dairy-containing foods (Jyonouchi, Sun, & Itokazu, 2002). A previous study (Adams, Johansen, Powell, Quig, & Rubin, 2011) also found that gastrointestinal symptoms were strongly and positively correlated with the severity of autism, possibly related to the fecal microflora of autistic children (Finegold et al., 2010; Parracho, Bingham, Gibson, & McCartney, 2005; Williams et al., 2011).

Animal models are useful to investigate brain–behavior relationships, which are difficult to examine in humans. They allow for examination of behavioral dysfunctions and their associated neurobiology. A number of animal models of ASD have been developed based on prenatal exposure to valproic acid (Dufour-Rainfray et al., 2010; Kim et al., 2011; Roullet, Wollaston, Decatanzaro, & Foster, 2010; Schneider & Przewlocki, 2005) or immune system activation (cf. Bilbo & Schwarz, 2012). Propionic acid (PPA) is a volatile short-chain fatty acid (SCFA) that is endogenous to the human body (Al-Lahham, Peppelenbosch, Roelofsen, Vonk, & Venema, 2010), being produced by fermentation of undigested food by the colonic microbiota (Cummings, 1981), especially dietary fiber and resistant starch, as well as from fatty acid metabolism (Al-Lahham et al., 2010; Villalba & Provenza, 1997). Propionic acid and the related SCFAs (e.g., acetate, butyrate) are capable of influencing central nervous system function (MacFabe, 2012; Wang et al., 2012), including neurotransmitter release and synthesis, gap junction communication, lipid metabolism, mitochondrial function, immune activation, and gene expression (see reviews by Koh, Vadder, Kovatcheva-Datchary, & Bäckhed, 2016; MacFabe, 2012).

Propionic acid has been investigated in a potential adult rodent model of ASD. Central (intracerebroventricular, ICV) administration of PPA has been shown to impair social behavior and some cognitive tasks, induce convulsions and seizures, and induce an innate neuroinflammatory response and oxidative stress in the brains of treated adult rats (MacFabe, 2012; MacFabe et al., 2007, 2008; MacFabe, Cain, Boon, Ossenkopp, & Cain, 2011; Ossenkopp et al., 2012; Shultz et al., 2008, 2009). This adult model is based on the premise that continuous high levels of PPA could be responsible for some of the behavioral abnormalities seen in ASD. This premise is supported by previous studies, showing that propionic acidemia and ASD overlap in a number of patients. Propionic acidemia, a neurodevelopmental metabolic disorder characterized by elevated levels of the SCFA (propionic acid), clinically resembles some aspects of autism (Feliz, Witt, & Harris, 2003), and case studies of comorbidity of propionic acidemia and ASD have been presented (Al-Owain et al., 2012; Witters et al., 2016).

As ASD is a developmental disorder, it is important to also examine the effects of PPA at earlier stages of development. In rats, the juvenile period is marked by major changes in the brain, such as prefrontal cortical pruning, changes in the glia, and a leaky blood–brain–barrier, amongst others (cf. Ono, Sakamoto, & Sakura, 2001). Developmental changes in behavior are also manifest as hypersensitivity to their environment and a display of more social behavior, amongst others (File & Seth, 2003). Previous studies have also demonstrated that subcutaneous injections of PPA from day 6 to day 28 in rats (Brusque et al., 1999) delay the appearance of hair coat, eye-opening, and free-fall righting reflex in the pups. As adults, these rats also failed to show habituation in an open-field task. Using a similar paradigm, Pettenuzzo et al. (2002) reported impaired spatial learning performance in the Morris water maze in the adult rats. However, neither study reported on impairments of measures such as social behavior and locomotor activity. These previous findings on behavioral effects of systemic PPA administration in rats suggested that a systemic PPA rat model of ASD could be useful addition to the ICV administration of PPA model. The systemic route would be more similar to an enteric source of PPA influencing behavior. The present experiment examined the putative effects of systemic administration of PPA in juvenile rats in order to provide further support for the PPA rodent model of ASD. On the basis of our previous study with ICV administration of PPA, we hypothesized that systemic administration of PPA in juvenile rats would impair social behavior and increase anxiety-related behaviors.

2 | METHODS AND MATERIALS

2.1 | Subjects

Subjects were 48 juvenile male Long Evans hooded rats obtained from a supplier (Charles River Laboratories, Quebec, Canada), which arrived on postnatal day (PND) 21. Rats were pair-housed in standard polypropylene shoe-box cages (26 cm × 48 cm × 21 cm) with Beta Chip bedding and at a controlled temperature (21 ± 1°C) in a 12:12-hr light/dark cycle with lights on from 7:00 to 19:00 hr. Animals were allowed access to food (ProLab RHM3000 rat chow) and tap water ad libitum. All behavioral experimentation was completed during the light phase. All experimental procedures were in accordance with guidelines of the Canadian Council on Animal Care and approved by the University of Western Ontario Animal Use Committee.

2.2 | Treatment groups

Rats were randomly assigned to one of the two treatment groups, PPA (500 mg/kg, 0.26 M solution buffered to a pH of 7.5) or vehicle (phosphate-buffered saline) (PBS; 0.1 M solution of phosphate), and were paired with a partner treated with the same dose of

either PPA or PBS during social interaction testing. This resulted in four treatment combinations: (a) PPA-PPA, a rat was injected with PPA and paired with a PPA-treated partner (n = 16); (b) PBS-PBS, a rat was injected with PBS and paired with a PBS-treated partner (n = 12); (c) PPA-PBS, a rat was injected with vehicle and tested with a partner treated with PPA (n = 10); and (d) PBS-PPA, a rat was injected with PPA and tested with a partner treated with PBS (n = 10).

Doses were chosen based on past dose–response findings that used repeated ICV infusions of 0.26 M solution of PPA (MacFabe, et al., 2011, 2007) and systemic treatment (Choi et al., 2018; Ossenkopp et al., 2012). Rats were injected i.p. on consecutive 4 days, then left undisturbed for 3 days, and then injected for another three consecutive days, for a total of seven injections (timeline for the experiment is shown in Supporting Information Figure S1). All behavioral testing occurred immediately after each injection.

2.3 | Apparatus

2.3.1 | Paired social interaction test

Social behavior (cf. Pellis, Field, Smith, & Pellis, 1997; Thor & Holloway, 1984) was evaluated in a circular open field (90 cm diameter, 40 cm high) with Beta Chip bedding covering the floor of the arena. A CD camera (WV-CP470; Panasonic) connected to a computer was mounted above the arena. Each animal's behavior was video-recorded for later analysis.

2.3.2 | Individual locomotor activity

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

2.4 | Experimental procedures

2.4.1 | Habituation and paired social interaction

Untreated pairs of cage-mate rats were habituated to experimental procedures and the apparatus on three consecutive days (PND 28–30) for a period of thirty minutes daily, prior to social testing. This included coloring the dorsal surface of one rat from each pair (to make video identification easier) with a black nontoxic permanent marker, ensuring the scent of the marker would not be novel to any of the rats on subsequent test days.

Testing occurred on four consecutive days, commencing at 08:00 hr at age PND 35–38 (injection days 1–4). Immediately following injection, a cage-mate pair was placed into the open field and behavioral data were collected for 1 hr.

2.4.2 | Individual locomotor activity

Following a 3-day holiday from injections and behavioral testing, rats were tested in the automated locomotor activity apparatus (VersaMax monitors) for three consecutive days at PND 42–44. Immediately following injection, rats were placed individually in the apparatus and behavior was recorded for 60 min.

2.5 | Behavioral measures

2.5.1 | Paired social interaction—video analysis

Video recordings of the animals' behavior were converted into media files and given random inconspicuous labels, allowing the experimenter coding the behavior to be blind to the treatment group of any particular rat. The first 10 min of the videotapes was visually scored using the Observer (Noldus Information Technology, Sterling, VA) event recording software.

The time, or frequency of various specific behaviors, was scored visually. These specific behaviors were aggregated into social (approach, avoidance, investigative sniffing, allogrooming/social grooming) and nonsocial (self-grooming, rearing, digging, and abnormal motor behaviors) categories (cf. Ossenkopp & Mazmanian, 1985). Definitions for these behaviors are as follows:

Time spent in social contact: amount of time (s) spent in direct physical contact with the partner for each rat pair.

Time spent moving: amount of time spent moving for each animal.

Frequency of allogrooming: the number of times each rat pair engaged in allogrooming.

Frequency of approach: the number of times the animal approached their partner.

Frequency of avoidance: number of times the animal avoided the social initiation when approached by the partner.

Frequency of sniffing: number of times each rat explored its partner by sniffing.

Frequency of self-grooming: number of times the animal engaged in grooming itself.

Frequency of abnormal motor behavior: number of times each rat displayed any abnormal motor behaviors such as retropulsion (dorsiflexion of spine with repeated forelimb extension, pushing the body backward), snake-like posture (hyperextension of body parallel to floor, usually with paddling motions of the limbs), and limb dystonia (dystonic movement of forelimb or hindlimb, usually repeated adduction and extension).

2.6 | Individual locomotor activity testing (VersaMax)

The VersaMax Animal Activity Monitors automatically collected data using a grid of infrared beams. Variables reflecting locomotor and thigmotaxis activity (Ossenkopp & Kavaliers, 1996) were collected and are described below. For the thigmotaxis measure, the center square region consisted of the inner 25 cm square area of the open-field floor.

Total distance (cm): the total horizontal distance (cm) traveled.

Rearing: the number of individual vertical movements made with a minimum stop time of 1 s to separate movements.

Frequency of center entries: the number of entries to the center region of the open field. This is a measure of anxiety, as rats with increased levels of thigmotaxis (anxiety) avoid the center area.

2.7 | Statistical analysis

Data were analyzed for main effects using a mixed design ANOVA with treatment as the between-subject factor and test days as the within-subject factor. If a main effect of treatment or day or an interaction was found, post hoc one-way ANOVAs were carried out for group differences on individual days. Post hoc pairwise comparisons consisted of Tukey's HSD test. All statistical tests were calculated using SPSS 16.0 (SPSS, Inc.) for Windows. Hypothesis tests used $\alpha = 0.05$ as the criterion for significant effects.

3 | RESULTS

3.1 | Body weight

All of the rats had similar body weight gains throughout the experiment. There was no significant difference in mean body weight gains between the PBS- and PPA-treated groups ($F(1, 46) = 0.05$, $p = 0.82$).

3.2 | Paired social interaction

3.2.1 | Time spent in social contact

Compared to the PBS-PBS pairs, rats in the PPA-PPA and mixed pairs spent less time in contact with the partner (Figure 1a). The ANOVA revealed a significant main effect of Group (treatment pair) ($F(2, 21) = 17.35$, $p < 0.0001$) and a significant Group-by-Day interaction ($F(6, 36) = 2.66$, $p = 0.034$). Post hoc analyses revealed that compared to rats in the PBS-PBS pair, rats in the PPA-PPA pairs spent significantly less time in contact with the partner on days 2, 3, and 4 ($ps < 0.05$). Similarly, compared to rats in the PBS-PBS group, rats in the mixed treatment pairs spent significantly less time in contact with the partner on days 2, 3, and 4 ($ps < 0.05$).

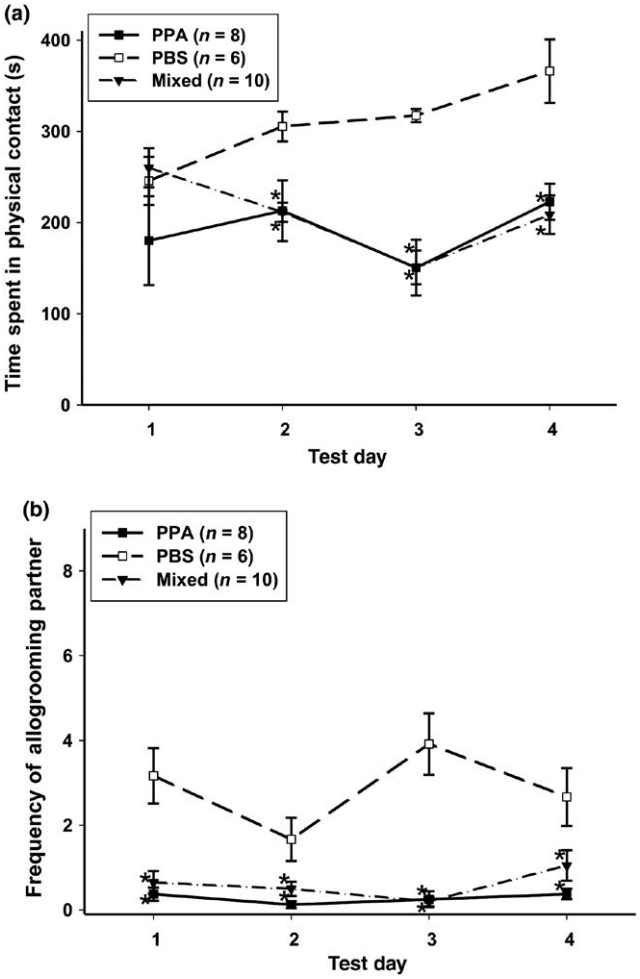

FIGURE 1 Mean time spent in physical contact with the partner (a) and mean frequency of allogrooming (b), by pairs of juvenile rats systemically (i.p.) treated with either PPA or PBS (vehicle) and placed in an open field. Means are for the first 10 min of testing on four consecutive days Error bars are standard errors of the mean (*SEM*). Significant differences ($p < 0.05$) are denoted with * relative to the control group (PBS-PBS). PBS: phosphate-buffered saline; PPA: propionic acid

3.2.2 | Time spent moving

Propionic acid-treated rats from both the PPA-PPA and the PPA-PBS pairs spent less time moving regardless of their partner type (see Supporting Information Figure S2). The ANOVA revealed a significant main effect of Treatment ($F(1, 44) = 80.56$, $p < 0.0001$) but no main effect of Partner type ($F(1, 44) = 0.01$, $p = 0.92$). A significant Day-by-Treatment interaction was also obtained ($F(3, 132) = 5.15$, $p = 0.003$). Post hoc comparisons indicated that rats in the PPA-PPA pairs were significantly less active than in PBS-PBS pairs ($ps < 0.01$).

3.2.3 | Frequency of allogrooming

The frequency of allogrooming was significantly lower in both the PPA-PPA group and the mixed group pairs (Figure 1b). The ANOVA showed a significant main effect of Group ($F(2, 21) = 54.18$,

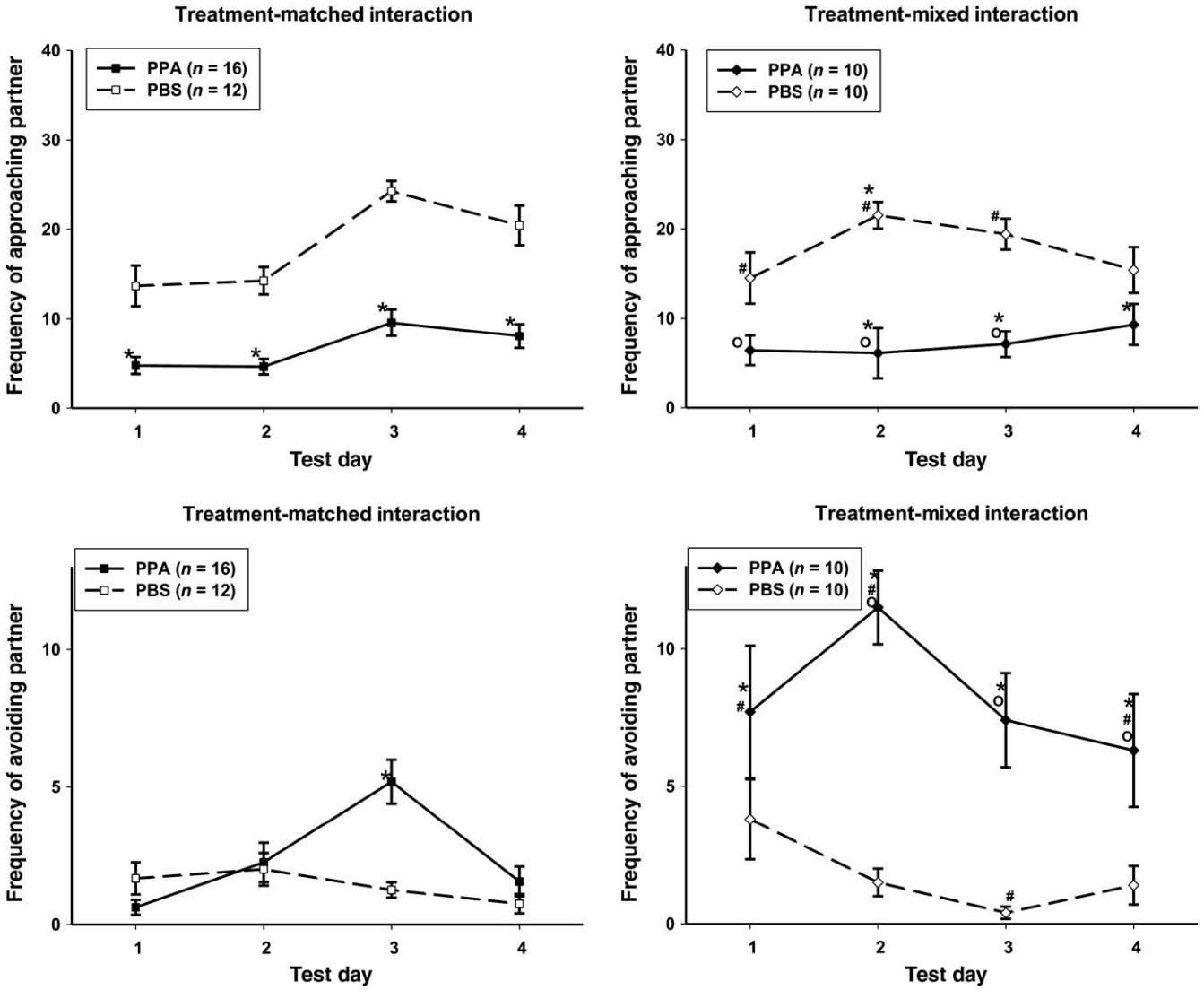

FIGURE 2 Mean (±*SEM*) frequency of approaching (a) and avoiding (b) the partner by individual juvenile rats, treated with PPA or PBS (control) and placed in the open field. Means are for the first 10 min of testing on four consecutive days. The left panel represents rats treated in treatment-matched combinations (PBS-PBS and PPA-PPA). The right panel represents rats tested in treatment-mixed combinations. Significant differences ($p < 0.05$) are denoted with * relative to the PBS-PBS group and with # relative to the PPA-PPA group. Significant differences ($p < 0.05$) between partners in the mixed group are denoted with "o." PBS: phosphate-buffered saline; PPA: propionic acid

$p < 0.0001$) and a significant Group-by-Day interaction ($F(6, 36) = 2.82$, $p = 0.025$) for the allogrooming measure. Post hoc comparisons revealed that PPA-treated rats from both the PPA-PPA and mix pair group had significantly reduced allogrooming on all days in comparison with the control group ($ps < 0.05$). These results indicate that PPA-treated rats did not engage in normal allogrooming behavior, regardless of their partner's treatment.

3.2.4 | Approach behavior

As seen in Figure 2a, the number of times each rat approached its partner was lower in PPA-treated rats, regardless of their partner's treatment. This was supported by the ANOVA, which indicated a significant main effect of Group ($F(1, 44) = 151.41$, $p < 0.0001$) and Day ($F(3, 132) = 4.52$, $p = 0.007$) but no main effect of Partner type ($F(1, 44) = 0.01$, $p = 0.92$). There was also a significant Day-by-Partner interaction ($F(3, 132) = 6.14$, $p = 0.001$). Post hoc comparisons indicated that rats in the PPA-PPA pairs exhibited significantly reduced frequency of approach on all days ($ps < 0.01$) when compared to control pairs and significantly lower frequency of approach on days 1–3 ($ps < 0.01$) in comparison with PBS-PPA pairs. In addition, PPA-PBS pairs exhibited lower approach frequency on days 2–4 ($ps < 0.01$) in comparison with PBS-PBS pairs.

3.2.5 | Avoidance behavior

The number of times a rat avoided social initiation when approached by the partner was greatest in PPA-PBS pairs (see Figure 2b).

The ANOVA revealed significant main effects of Treatment ($F(1, 44) = 47.15$, $p < 0.0001$) and Partner type ($F(1, 44) = 21.09$, $p < 0.0001$) and a significant Treatment-by-Partner type interaction (F ($F(1, 44) = 42.36$, $p < 0.0001$). Post hoc analyses indicated that PPA-PPA rats avoided their partners significantly more often than PBS-PBS rats ($ps < 0.01$) and PBS-PPA rats ($p < 0.01$) on day 3. Rats in PPA-PBS pairs showed more avoidance than in PPA-PPA pairs ($p < 0.05$).

3.2.6 | Frequency of sniffing partner

Overall, PPA-treated rats sniffed their partners less than PBS-treated rats (see Figure 3a). This was confirmed by the ANOVAs, which indicated a significant main effect of Treatment ($F(1, 44) = 97.18$, $p < 0.0001$) and Partner type ($F(1, 44) = 9.54$, $p = 0.003$). There was also a significant Treatment-by-Partner type interaction ($F(1, 44) = 21.71$, $p < 0.0001$) and a Treatment by Partner type by Day interaction ($F(3, 132) = 2.85$, $p = 0.049$). PPA-PPA rats and PPA-PBS rats sniffed their partners less than did PBS-PBS rats ($p < 0.05$).

3.2.7 | Frequency of self-grooming

As can be seen in Figure 3b, self-grooming was reduced in PPA-treated rats regardless of whether they were tested in treatment-matched or treatment-mixed pairs. This was confirmed by the ANOVA results, which revealed significant main effects of Treatment ($F(1, 44) = 43.65$, $p < 0.0001$) and Day ($F(3, 132) = 6.36$, $p = 0.0005$) but no main effect of Partner type ($F(1, 44) = 0.04$, $p = 0.842$). Post

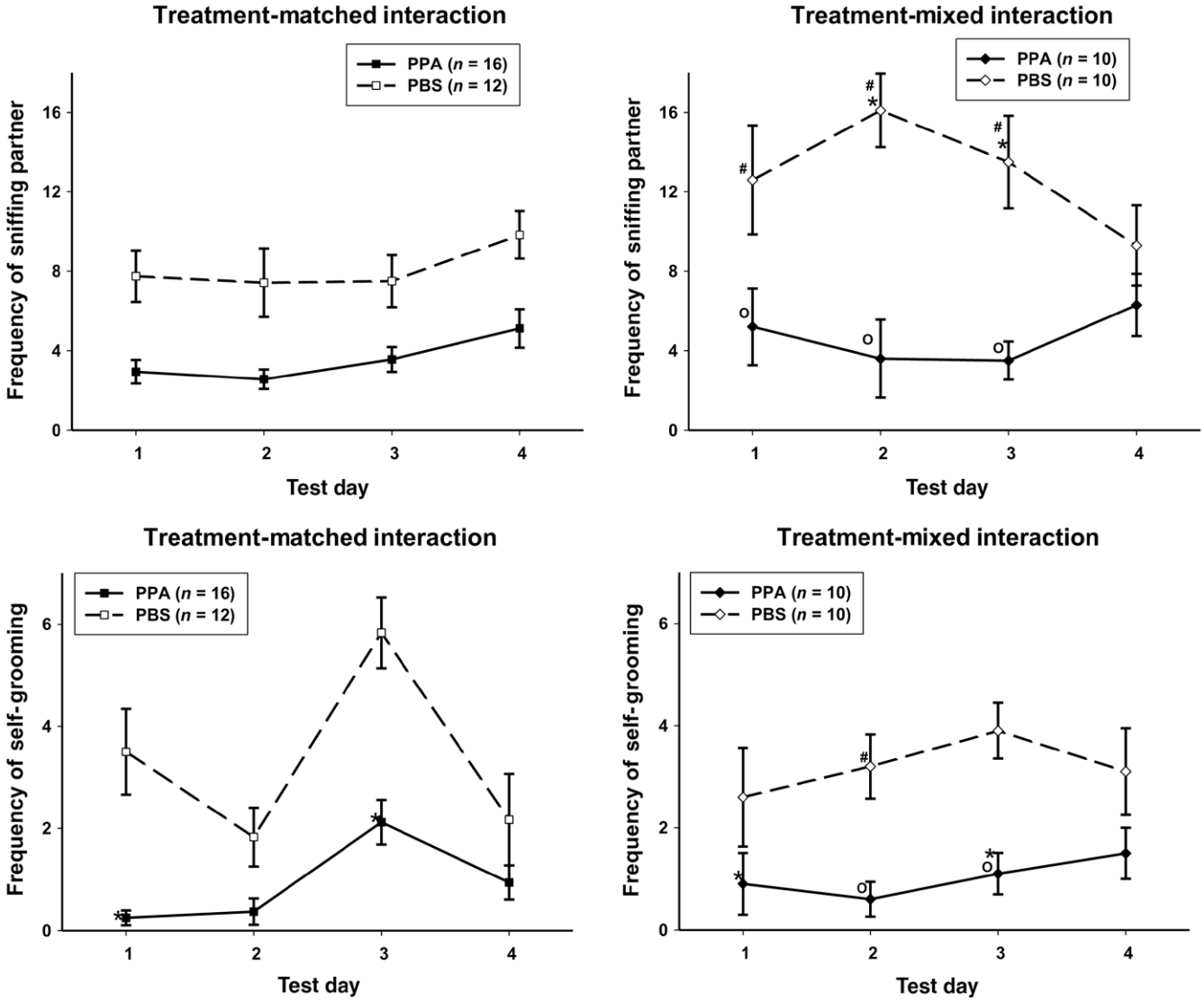

FIGURE 3 Mean (±*SEM*) frequency of sniffing the partner (a) and self-grooming (b) by individual juvenile rats treated with PPA or PBS and placed in an open field. Means are for the first 10 min of testing on four consecutive days. The left panel represents rats treated in treatment-matched combinations (PBS-PBS and PPA-PPA). The right panel represents rats tested in treatment-mixed combinations. Significant differences ($p < 0.05$) are denoted with * relative to the PBS-PBS group and with # relative to the PPA-PPA group. Significant differences ($p < 0.05$) between partners in the mixed group are denoted with "o." PBS: phosphate-buffered saline; PPA: propionic acid

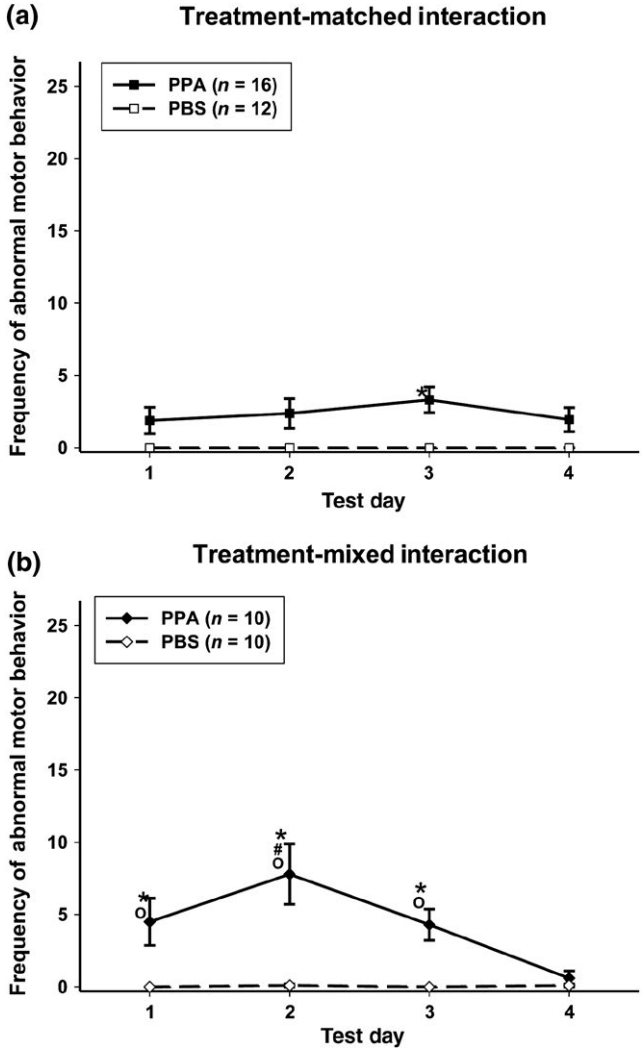

FIGURE 4 Mean (±*SEM*) frequency of abnormal motor behavior in treatment-matched pairs (a) and treatment-mixed pairs (b) treated with PPA or PBS (control). Means are for the first 10 min of testing on four consecutive days. Significant differences ($p < 0.05$) are denoted with * relative to the PBS-PBS group and with # relative to the PPA-PPA group. Significant differences between partners in the mixed group are denoted with "o." PBS: phosphate-buffered saline; PPA: propionic acid

hoc comparisons showed that PPA-PPA rats groomed themselves significantly less than PBS-PBS rats ($p < 0.05$).

3.2.8 | Frequency of abnormal motor behavior

As can be seen in Figure 4a,b, the frequency of abnormal motor behavior increased in PPA-treated rats tested in both the PPA-PPA pairs and the PPA-PBS pairs. The ANOVA confirmed this, showing significant main effects of Treatment ($F(1, 44) = 44.28$, $p < 0.0001$) and Day ($F(3, 132) = 2.64$, $p = 0.034$) and a Treatment-by-Day interaction ($F(3, 132) = 3.54$, $p = 0.026$). Post hoc analyses revealed a significant increase in occurrences of abnormal motor behavior in PPA-treated rats relative to PBS-treated rats ($ps < 0.05$).

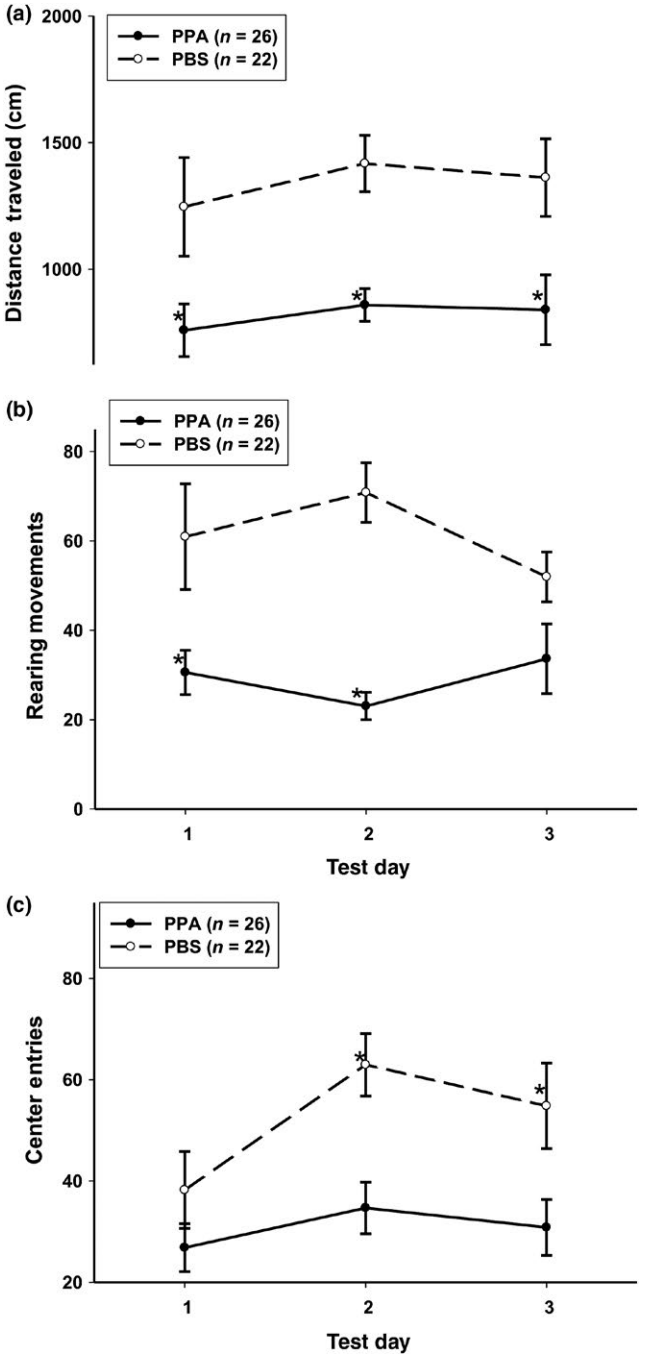

FIGURE 5 Mean (±*SEM*) total distance traveled (a), frequency of rearing movements (b), and number of center area entries (c) for individual juvenile rats treated with PPA or PBS (control) during 60 min of testing in the automated open-field apparatus on three consecutive days. Significant differences ($p < 0.05$) between the two groups are denoted with *. PBS: phosphate-buffered saline; PPA: propionic acid

3.3 | Locomotor activity in the automated activity monitor

3.3.1 | Total distance traveled

Figure 5a shows that PPA-treated rats exhibited significantly less distance traveled on all 3 test days. This was confirmed by the

ANOVA ($F(1, 46) = 13.85$, $p < 0.0001$) and the post hoc comparisons on all 3 days ($ps < 0.05$).

3.3.2 | Number of vertical movements (rearing)

Similar to the horizontal movement data, rearing movements were also significantly reduced in PPA-treated rats (see Figure 5b) relative to controls on all 3 test days ($F(1, 46) = 19.35$, $p < 0.0001$).

3.3.3 | Number of center area entries

As can be seen in Figure 5c, the number of center area entries was reduced in PPA-treated rats on days 2 and 3. This was confirmed by the ANOVA ($F(1, 46) = 10.68$, $p = 0.002$) and by post hoc comparisons ($ps < 0.05$).

4 | DISCUSSION

The focus of the present study was to examine, in detail, the effects of systemic treatment with PPA on social interaction in juvenile rats. This was accomplished via video-record analyses of dyadic behavioral interactions of rats in treatment-matched and treatment-mixed pairs. In general, juvenile rats treated with PPA showed reduced social interaction, increased anxiety-related behavior, and hypoactivity. Detailed behavioral analysis showed that, compared to control dyads (PBS-PBS), the PPA-PPA pairs exhibited significantly reduced social interaction assessed by frequency of approaching, sniffing, and allogrooming the partner. PBS-treated rats in mixed pairs sniffed their PPA-treated partner more than the partners reciprocated, and also more than in the PBS-PBS control rats. However, PBS-treated rats in the mixed group spent less time allogrooming than did the rats in the PBS-PBS pairs, possibly because their PPA-treated partner avoided social contact more than PBS-treated rats in the PBS-PBS group. The number of times each rat approached the partner was lower in the PPA-treated rats, regardless of their partner's treatment. PPA-treated rats in the mixed group avoided social initiation by their partners more than PPA-treated rats in the like-treated group; however, this effect could be the result of PBS-treated rats, making more approaches toward their partners than the PPA-treated rats.

Rats in treatment-mixed pairs spent significantly less time in contact with their partners than did rats in PPA-PPA pairs. This suggests that PPA treatment induces a reduction in physical contact with the partner and is consistent with previous reports of abnormal social behavior in rats given ICV infusions of PPA (Shultz et al., 2008). This previous study found that central administration of PPA resulted in reduced playful initiations, reduced probability of defense, and reduced facing but increased evasion of the partner. These effects were evident when tested in the light phase or the dark phase of the light/dark cycle (Shultz et al., 2008). The present study examined social interactions more extensively than did the Shultz et al. (2008) study, but for the behaviors that were similar (e.g., avoiding partner and evasion of partner), the findings were very similar. These findings of reduced social interaction are also consistent with observations in rats with high PPA concentrations in the cecum (Hanstock, Clayton, Li, & Mallet, 2004).

Propionic acid can cause changes in the metabolism of other SCFAs and result in intracellular acidosis, which in turn can induce changes in gut motility and alter synthesis and release of neurotransmitters such as serotonin (5-HT; Mitsui, Ono, Karaki, & Kuwahara, 2005a; Mitsui, Ono, Karaki, & Kuwahara, 2005b). Indeed, PPA is capable of altering a number of neurotransmitter systems, including dopamine, calcium, and, of particular interest to the current findings, serotonin (Cannizzaro, Monastero, Vacca, & Martire, 2003; Mitsui et al., 2005a, 2005b). A previous study showed that administration of a 5-HT receptor agonist reduced levels of social investigation and that a 5-HT antagonist reversed these effects (Gonzalez, Andrews, & File, 1996). In addition, Kalueff, Fox, Gallagher, and Murphy (2007) discovered that mice with enhanced 5-HT availability exhibited reduced social behavior.

Propionic acid can also induce changes in immune response and cytokine production (Kurita-Ochiai, Fukushima, & Ochiai, 1995). Cytokines can affect exploratory behavior and social interaction (Bilbo & Schwarz, 2012), and systemic administration of cytokines has been reported to induce changes in the hypothalamus, hippocampus, and nucleus accumbens (Ashwood, Wills, & Water, 2006). In addition, enzymes that control the conversion of tryptophan into serotonin are influenced by cytokines (INF-γ and IL-1), while proinflammatory cytokines (IL-1β, IFN-γ, and TNF-α) are also capable of affecting gene expression regulating serotonin (Ashwood et al., 2006). Thus, PPA can affect social behavior in a variety of ways. These effects can be produced by changing intracellular pH and the resultant acidosis, by changing serotonin release and activity of serotonergic pathways and by changing the expression of cytokines and stimulation of immune responses.

The attenuation of social interaction was not specific to social pairing type and could be due to an increase in anxiety, a decrease in motor activity levels, or lack of reciprocity by the partner for social interaction. Using video scoring, social interaction was measured individually to determine whether the reduced social initiation in a pair was the result of decreased social reciprocity by the treated rat. A decrease in social interaction may be secondary to a decrease in social solicitation by the partner rat and by a lower willingness of the partner to engage in social interaction once this is solicited. PPA attenuated social activity in treated rats, but it did not diminish the treated rat's attractiveness as a potential social target to the other juvenile rat. PPA-treated rats are an effective social stimulus for the control rats. Control rats exhibited more solicitation than PPA-treated rats regardless of treatment of their partner. Thus, the unwillingness of the PPA-treated rats to engage in social behavior was not due to lack of solicitation by the partner.

Juvenile PPA-treated rats in the present study showed increased anxiety-related behaviors in both the social and nonsocial settings, during social interaction and in the locomotor activity

test. Increased anxiety was demonstrated by increased thigmotaxis, reduced grooming and rearing, and increased avoidance of the partner during social interaction. There is a previous report that increased concentrations of PPA in the cecum are associated with enhanced anxiety-related behavior and changes in feeding in horses (Willard, Willard, Wolfram, & Baker, 1977). It is also possible that systemic administration of PPA induces sickness behavior in rats. Rats develop aversion to a novel taste, odor, or place after pairing with the aversive consequences of foods that induce visceral malaise (Garcia, Hankins, & Rusiniak, 1974; Ossenkopp et al., 2012). Aversion to PPA-associated stimuli has been reported in rats and sheep (Ossenkopp et al., 2012; Villalba & Provenza, 1996). Villalba and Provenza (1996) reported that sheep are sensitive to PPA, even in low doses, and develop a strong aversion to straw conditioned with higher doses of PPA. Similarly, rats treated systemically with PPA showed conditioned taste and place avoidance (Ossenkopp et al., 2012). Reduced activity levels and increased anxiety are also induced by immune system stimulation (Foster & Neufeld, 2013; Tenk, Kavaliers, & Ossenkopp, 2013) or cytokine treatment (Goehler, Lyte, & Gaykema, 2007). Several recent studies on the effects of systemic PPA treatment in the prenatal developmental stage, or early neonatal stage, have found that PPA treatment resulted in impaired social behavior and increased anxiety levels in adolescent animals (Choi et al., 2018; El-Ansary, Al-Daihan, & El-Gezeery, 2011; Foley, MacFabe, Vaz, Ossenkopp, & Kavaliers, 2014; Foley, Ossenkopp, Kavaliers, & MacFabe, 2014), consistent with the present findings involving juvenile treatment with PPA. Systemic administration of PPA also has been shown to reduce startle reactivity in adult rats, in a dose-dependent fashion (Kamen et al., 2018), another behavioral effect consistent with ASD symptomology.

The cerebellum in patients with ASD has been reported to show an active and chronic neuroinflammatory process, and these cerebellar abnormalities may be responsible for the dysfunctions within the motor output system (Fatemi, Reutiman, Folsom, & Sidwell, 2008), similar to the abnormal motor behavior seen in the present study and in the PPA studies involving intraventricular infusions (MacFabe et al., 2011, 2007, 2008; Shultz et al., 2008). Along with a reduced number of cerebellar Purkinje cells, the cerebellum exhibits the most prominent neuroglial activation in ASD brain tissues with up-regulation of selective cytokines (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005).

5 | SUMMARY

The present study examined the effects of systemic (peripheral) administration of PPA on social interaction, anxiety, and abnormal motor behaviors. Findings showed significantly reduced social interaction, increased anxiety-related behavior and hypoactivity, and increased abnormal motor behaviors. These abnormalities in behavior may manifest directly due to the unique physiological properties of PPA or indirectly through metabolic, neurotoxic, and/or immunological effects of PPA. 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). Thus, PPA may alter preexisting neural circuits in the juvenile rat to impair complex behavior in a way that is consistent with a rat model of autism. The present findings show similarities with symptoms of ASD and provide support for the establishment of systemic administration of PPA in rats as a potential rodent model of ASD. The findings obtained with the present systemic administration of PPA model are also consistent with our previous observation in rats treated with ICV-PPA (MacFabe et al., 2011, 2007; Shultz et al., 2008). Examination of behavioral changes, neuroanatomical abnormalities, and immune responses of rats exposed to systemic PPA at different stages of development would provide a better understanding of the etiology and underlying mechanisms of ASD.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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SUPPORTING INFORMATION

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