Effects of Propionic Acid on Social Odour in Adult Male Rats: Implications for an Animal Model of Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a neurocognitive disorder characterized by sensorimotor and developmental impairments. Studies indicate that propionic acid (PPA) injections can create both behavioral abnormalities and neuroinflammatory responses in rats that are parallel to the effects of ASD. Both rats and humans bear the unique ability to discriminate others on the basis of olfactory signatures. Odours of conspecifics carry vital information such as whether an individual is safe to approach (i.e. individuals in altered physical states, individuals that carry illnesses). This ability has been demonstrated in rats using the sickness-inducing drug, lipopolysaccharide (LPS). Rats treated with LPS are avoided by control animals. There is limited research exploring the relationship between social odour and PPA. The present study seeks to investigate this relationship by examining social odour preferences in rats. The sample consisted of 16 adult rats assigned randomly to one of two groups: no-injections (used for odour response testing; n = 8) or injections with one of four compounds: PPA (500 mg/kg), LPS (200 μg/kg), PBS (50 μg/kg), or NaCl (2.0mL/kg). Bedding from the injected rats provided the odour cues to be presented to non-injected rats. Results revealed that rats can distinguish the odours of other rats treated with a sickness-inducing compound (LPS), and prefer the odours of conspecifics. No significant differences in activity were detected for PPA-comparisons.

Keywords: autism spectrum disorder, propionic acid, neurodevelopment, gut microbiology, social odour

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extensively (Brock et al., 2002). These bacteria have been said to arise from their role in regulating the gastrointestinal function as well as the immune system by stimulating the release of pro-inflammatory cytokines (Krajmalnik-Brown et al., 2015). Despite the abundance of research conducted in this field, the implications of these compounds are not understood in their entirety.

**Symptoms of ASD**

ASD is associated with a broad set of symptoms. While there are underlying neurological commonalities among individuals with ASD, symptoms vary greatly from one another and the disorder manifests itself differently in each case. The presentation of symptoms may be clear in one case but come across very subtly in another. Typical symptoms of ASD include decreased social and play behaviour, deficits in both verbal and nonverbal communication, problems integrating information from the senses, as well as displays of repetitive and dystonic behaviour (Walker, 2012). Progression of ASD yields distinctive and irregular thinking patterns, movements, as well as sensory and cognitive processes. Most individuals with autism show tremendous difficulty engaging in everyday human interaction. This difficulty is demonstrated even as early as infancy, the stage in which most babies tend to want to touch and explore others. It is within the realm of social interaction that individuals with autism seem to be consistently impaired (Walker, 2012). For this reason, they lack the most basic and vital social skills, such as verbal and nonverbal communication, that are necessary when forming bonds with others (Rapin & Tuchman, 2008).

Typically, when clinicians are referring to ASD, the three conditions most frequently diagnosed are: Autism, Asperger’s disorder, and Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS). The idea of autism as a spectrum disorder is now more recognized, and people note this diversity among affected individuals (Carl & Hardan, 2011). Putative risk factors include heavy metals, viruses, allergens as well as gastric inflammation (Newschaffer, Fallin, & Lee, 2002). Several dietary regimes have been experimented with, eliminating certain dietary factors, including refined wheat and dairy products (MacFabe et al., 2007). Inconclusive evidence points to the possibility that environmental factors influence ASD by interacting with genetic vulnerabilities (Benvenuto et al., 2009). Prevalence estimates have increased dramatically since the 1960’s, where diagnosis rates were around 4 per 10,000 and are now currently near 22 per 1,000 in children ages 3 through 17 (Zablotsky, Black, Maenner, Schieve, & Blumberg, 2015). The need for early diagnosis and behavioral intervention is paramount.

**Propionic Acid and ASD**

Research from clinical studies have found that various gut and dietary factors may be transiently worsening symptoms in children with ASD. A group of compounds known as short chain fatty acids are derived from the host gut microbiome (MacFabe et al., 2007). A connection between short chain fatty acids and gut and brain health has recently been made (MacFabe et al., 2007). It is partly because of this connection that they have been implicated in neurodevelopmental disorders such as autism (El-Ansary & Al-Ayadhi, 2014).

Most sources of PPA are produced by carbohydrate fermentation of amino acid by enteric bacteria (MacFabe et al., 2007). PPA readily crosses the gut-blood and blood-brain barrier and can therefore obtain access to the central nervous system by both active and passive means (Bergersen, Rafiki, & Ottersen, 2002), where it is then able to cross cellular membranes and accumulate within cells (Shultz et al., 2009). PPA is also used as a food preservative, and studies have shown that eating food containing this additive exacerbates the symptoms in children with autism (MacFabe et
MacFabe and colleagues (2007) investigated the wide range of effects of propionic acid at the behavioural, electrophysiological, neuropathological, and biochemical levels of analysis. Results of this investigation suggested that PPA produces dystonic behaviours, hyperactivity, turning behaviors, retropulsion, caudate spiking, and the progressive development of limbic kindled seizures, all of which are consistent with human symptoms of ASD. Neurological examinations revealed increased levels of neuroinflammation, greater levels of oxidative stress markers, and reduced immunoreactivity. These results led to their proposal that forms of autism may be an acquired disorder that involves altered PPA metabolism.

Shultz and colleagues (2008) examined social behavior and brain tissue of adult rats given intraventricular injections of PPA or a control compound. Pairs of adult male Long-Evans rats were placed in an open-field for observation. Compared to controls, rats treated with PPA displayed numerous social behaviour impairments. For example, PPA-injected rats demonstrated less playful interactions, altered responses to playful initiations, and less time spent in close proximity to each other. A brain tissue analysis of PPA-treated rats was also conducted. This analysis revealed an increase in the number of astrocytes due to the destruction of nearby neurons and inflammation. Their findings suggested that PPA can change both brain and behavior in a way that is consistent with symptoms of human ASD (Shultz et al., 2008).

Whether or not PPA induces the same effects in humans has not been studied to the same extent as it has been in animals. However, Jyonouchi, Sun, and Itokazu (2002) reviewed information that was collected by parents of children with ASD that noted that symptoms got noticeably worse when children ate wheat and dairy products that are filled with preservative, and an improvement when these products were removed from the diet. Similar findings were also reported in a review conducted by Horvath and colleagues (1999) with parents of affected children provided evidence that ASD behavioural symptoms substantially increased when the children ate refined foods that contained PPA as a food preservative. Further research about the phenomenon of environmental, dietary, and gastrointestinal factors contributing to autism is required; understanding the underlying mechanisms has various implications for several treatments and interventions.

**Social Odour**

The way that humans communicate with one another is controlled largely by various forms of auditory and visual information. Different species of animals have been found to use olfactory signals to communicate with another animal of the same species (Weller, 1998). Social recognition memory in both rats and mice has been found to rely on the acquisition of an individual’s olfactory signature. Sensory neurons will stimulate the hypothalamus via the amygdala to elicit neuroendocrine and behavioural effects (Noack et al., 2010). Several peptides within the brain have been found to exert powerful influences on complex social behaviours, such as social recognition (Tobin et al., 2010). Most notably of these proteins are two hormones, vasopressin and oxytocin (Hammock & Young, 2006). A study conducted by Tobin and colleagues (2010) reported that the rat olfactory bulb contains a large group of neurons that express the vasopressin hormone, and that blocking the expression of this hormone can impair social recognition memory by the olfactory system in the rat brain. These neuropeptides have several effects on an animal’s capacity to recognize and appropriately respond to an animal of the same species (Wacker & Ludwig, 2012). Behaviour is
activated by the detection of social cues, such as odour, for subsequent strategies such as avoidance or approach of conspecifics (Arakawa, Cruz, & Deak, 2011).

Research has shown that there exists a wide array of peptides and proteins in rodent urine that operate as chemosignals that can be detected (chemical signals the body gives off), encouraging either approach or avoidance behaviours by other animals (Stowers & Kuo, 2015). Two polymorphic gene complexes, the major histocompatibility complex (MHC) and the major urinary protein cluster (MUP) are involved in this process (Stowers & Kuo, 2015). Changes in the MHC gene complex due to infection is related to immune function and the odour constituents that are found in urine (Stowers & Kuo, 2015). Polymorphisms of MUPs contribute to the diversity of constituents of rodent urine that have been found to be related to social recognition and appraisal of physical condition (Hurst, 2009).

Studies have proposed that with time, some diseases will develop a specific and unique odour which will emanate from the body of the ill individual (Olsson et al., 2014). This ability has been observed in human subjects as well. Research has suggested that injecting human participants with certain compounds followed by odour collection will result in aversive responses from others compared to those who were not injected (Olsson et al., 2014). One example of such compounds is lipopolysaccharide (LPS), an endotoxin found in the outer membrane of certain bacteria which is known to evoke substantial immune responses in animals (Yirmiya et al., 1994).

In 2014, Olsson and colleagues explored human’s sensitivity to the odours of sick individuals. They activated the immune system of healthy participants with LPS. A few hours after endotoxin exposure, injected individuals produced a more aversive body odour relative to when they were injected with a placebo compound, as measured by proximity time spent with either individual. This research was the first line of evidence to demonstrate that diseases have a characteristic smell, as well as support for the notion of the “behavioral immune response” (Olsson et al., 2014). This serves to protect healthy individuals from pathogen-infected conspecifics.

Similar findings were obtained in a study conducted by Arakawa, Cruz, and Deak (2011) exploring the social odour cues conveying a lot of information. Their research focused on the idea that sensing the odour of an individual determines the extent and nature of contact that ensues. For example, detecting an odour associated with a disease or pathogen will motivate one to avoid the source of this odour. Their results revealed that the inflammatory process has a dual impact, impacting both the healthy and sick individual. The sick individual showed reduced levels of motivation to engage in social behaviours, while the healthy individual showed reduced levels of social investigation of sick conspecifics. This study offers support for the idea that olfactory signals play an important role in animal communication and can be a crucial source of information.

**Social Investigation in Rats**

Under laboratory conditions, social recognition memory in rats and mice is investigated based on the idea that rats have an innate drive to investigate unfamiliar individuals (Thor & Holloway, 1982). Thor and Holloway (1982) used duration of social-investigation behavior in male Long Evan’s rats to assess social memory. Their results supported the idea that there is a need in rodents to investigate the identity of a novel conspecific and that social investigation increases familiarity by decreasing novelty and diminishing attractiveness.

**The Present Study**

The relationship between autism spectrum disorder and propionic acid has been extensively studied (MacFabe et al., 2007). The
importance of odours acting as guides for communication has been explored; however, whether rodents will demonstrate the ability to distinguish the odour of conspecifics with elevated levels of PPA has yet to be studied. Findings demonstrate that rodents are very receptive to changes in alterations in behaviour and will react to these changes, and these changes are detectable by respective changes in odour (Gregory & Pfaff, 1971). The various general effects of PPA have been established experimentally and have been found to produce several alterations in behavior and state (Fontella et al., 2000; Shultz et al., 2008).

The present study explores the following question: does propionic acid impact adult rat social odour preferences? Three hypotheses are made based on previous research. Firstly, rats show a preference for the odours of conspecifics (Thor & Holloway, 1982). Secondly, rats can detect and show a reduced interest of the odours of rats with elevated levels of PPA. Finally, rats can distinguish the odours of other rats treated with LPS and show an aversion of those odours (Olsson et al., 2014). LPS acts as a positive control and NaCl and Phosphate Buffered Saline (PBS) as both control comparisons to demonstrate that non-injected rats will avoid the LPS-associated odour as found by previous investigations, and that there will be a preference for saline-associated odours. The present study will then compare the LPS effect to the effect obtained by PPA.

Methods

Subjects

The sample consisted of 16 adult male Long Evans rats (Charles River, Quebec) that weighed between 300-350 g. Rats were housed individually in polypropylene cages (45 cm x 22 cm x 20 cm) in a colony room that was 21 ± 1 °C and in a 12:12 light to dark cycle, with lights on at 7:00 am Each cage was provided with ProLab (RMH3000) rat chow and water ad libitum. All testing took place during the light phase of the light:dark cycle. All procedures were approved by the University of Western Ontario Animal Care Committee and were in accordance with the Canadian Council of Animal Care (CCAC) Guidelines. The sample was randomly divided into two groups of eight. Rats numbered one to eight were used for social odour sources to be injected with PPA (500 mg/kg), LPS (200 μg/kg), PBS (0.1mol/L), or 0.9% NaCl. Rats numbered nine to 16 were used for behavioural observation and testing to examine social odour preferences of the eight rats that were injected.

Apparatus

The apparatus consisted of eight modified Versamax Animal Activity Monitors (Accusan Model EXXYZCM-16, Columbus, OH). Each monitor consisted of a clear Plexiglas open-field (40 cm x 40 cm x 30.5 cm) covered by a Plexiglas lid with air holes. Each monitor was divided into two chambers (20 cm × 40 cm × 30.5 cm each) by a Plexiglas partition. The partition contained a 10 cm x 15 cm doorway for unrestricted access to either side of the chamber that contained the different odours. Infrared photobeams were located 2.54 cm apart and 5.7 cm above the floor and along the perimeter of the apparatus with 16 photobeams per side. All activity monitors were connected to a Versamax data analyzer (Accusan Model DCM-8, Columbus, OH), which transmitted data to an IBM Pentium II computer. Versamon Software was used to ensure that there were no materials blocking the infrared sensors in the activity chamber.

Procedure

Handling. Three days of handling the rats took place prior to testing to minimize anxiety. Each day of handling took place for approximately 2 hr. The first day of handling consisted of labelling the tails of each rat and were kept and handled inside of their own cages. The second day of handling, rats were taken outside of their home cages and allowed to explore a cart filled with clean bedding. On the
third and last day of handling, injection holds were done on the eight rats that were to be injected to familiarize them with this position.

**Determination of odour preferences.**
The first day of testing consisted of a habituation task to the chambers for the eight rats used to observe social odour preferences. The purpose of this session was to familiarize the rats with the setting in which they were to be eventually tested. This session consisted of 30 min of observation, with two 15 min bouts. The social odour preference task required eight chambers connected to the VersaMax data analyzer to transmit data to a receiving computer for later analysis. Each chamber contained one rat with two petri dishes containing bedding with different odours. Before beginning each trial, Versamon software was checked to ensure that there was nothing blocking the infrared sensors. During the 30 min (2 x 15 min bouts) session, number of entries into quadrant and chamber side (left vs. right), horizontal activity, movement time, duration, and total distance were recorded on the computer program associated with the VersaMax activity chambers.

**Injections.** All injections were given intraperitoneally (i.p.) with a twenty-three-gauge needle and took place approximately 17 hr prior to testing, giving the odours time to emanate onto the bedding. To collect the odours, rats were injected with either PPA (500 mg/kg), LPS (200 μg/kg), PBS at a volume of 0.1 mol/L, or 0.9% NaCl. PBS was the vehicle for PPA and NaCl was the vehicle for LPS. Doses of PPA and LPS were selected on the basis of concentrations used in prior studies, and all injections were given at a rate of 2.0 mL/kg. Rats injected with PPA and PBS were given two injections that were administered 30 min apart.

**Odour sources.** Approximately 2 hr before testing, 8 g of bedding acted as odour sources and was collected from the respective animal cages to be put into sixteen 10 mm x 15 mm petri dishes and used to observe social odour preferences.

**Trial procedure.** For all six days of testing, injections took place at approximately 5:30 p.m. and took 30 min to complete. The following day, rats that were injected the night before were taken out from where they were kept and then brought to the testing area. Equal amounts of bedding were taken from each cage and replaced with clean bedding. Bedding was removed with an odourless material and weighed to be exactly 8 g in the petri dish. This was done 16 times on each trial day so that each of the eight chambers contained two petri dishes. Once the bedding was collected, injected rats were brought back to their home cage. All time spent outside of their cages was carefully recorded. Each petri dish was then covered with a black mesh material to prevent the rats from moving around the petri dish or removing any bedding from the petri dish during the testing session. Two petri dishes were placed in each chamber, one on either side of the partition and diagonal from each other. Placement of the petri dishes were measured using a centimeter ruler. A square piece of Velcro tape was placed in the center of the bottom of the petri dish for placement into the apparatus. Once all chambers were prepared and ready for testing, rats numbered nine through 16 were taken out from the room where they were kept and brought to the testing room in their individual cages. All rats were placed into the chambers facing the same way, and upon entering the VersaMax activity chamber, their activity began to be recorded. After each session was complete, the rats were carefully removed from their respective chambers and brought back to their home cages. Upon completion, the apparatus was cleaned using Alconox cleaning solution and baking soda. The eight rats used for social odour testing were put in the same chambers for all six days of testing.
Testing trials. The social odour preference task was conducted six times in total. The first day of testing looked at the animals’ preference for the odour of clean bedding versus the bedding of a non-injected rat. The second day of testing compared PBS versus PBS as a control trial. The following day, preference for the PBS odour versus the PPA odour was tested, followed by PPA versus clean bedding, LPS versus NaCl, and lastly, LPS versus clean bedding. The order of testing trials was random. Only one social odour preference task was conducted on a given day, and testing trials took place over the course of a two-week period.

Statistical analysis. Social odour preferences were analyzed using a One-Way Analysis of Variance (ANOVA). The dependent measures analyzed consisted of number of entries into quadrant and side of chamber (left vs. right), duration, horizontal activity, movement time, and total distance. All of the dependent variables were corrected for the duration of time spent in the relevant quadrant. All statistical tests were calculated using SPSS 12.0 for Windows. Hypothesis tests were completed using $\alpha = 0.05$ and Greenhouse–Geisser $F$-values were used as the criterion for significant effects. Post hoc comparison consisted of the Least Significant Difference (LSD) test. Only the results from the first 15 min bout were analyzed due to the lack of activity detected in the second bout.

Results
Clean Bedding versus Non-Injected
To determine if rats show a preference for social odours (odours of another rat vs. clean odour), a one-way ANOVA was conducted to compare activity in the quadrants containing the clean bedding odour cue and the non-injected rat odour cue. The one-way ANOVA comparing number of entries in the left versus right side of the chamber did not show results that were statistically significant, $F(1, 30) = .00, p = 1.000$. There was no significant difference found between the quadrants containing the odour of clean bedding in comparison to the quadrant containing the bedding of the non-injected rat with respect to number of entries ($F(3, 28) = 7.45, p = .274$), horizontal activity ($F(3, 28) = 1.47, p = .243$), movement time ($F(3, 28) = 4.28, p = .587$), duration ($F(3, 28) = 8.12, p = .638$), or total distance ($F(3, 28) = 1.98, p = .140$). Results were not in support of the first hypothesis, as rats did not demonstrate a preference for social odours.

PBS versus PBS
As a control trial, a one-way ANOVA was conducted to compare activity in the quadrants containing the PBS-associated odour cues, anticipating that rats would not show a preference for either side of the chamber as they are both social odours. The one-way ANOVA comparing number of entries in the left versus right side of the chamber did not show results that were statistically significant, $F(1, 30) = .31, p = .580$. As hypothesized, rats did not show a preference for either side of the chamber.

PPA versus PBS
To determine if rats show a reduced preference for PPA-associated odours in comparison to social odours, a one-way ANOVA was conducted to compare activity in the quadrants containing the PPA odour cue and the PBS odour cue. The one-way ANOVA comparing number of entries in the left versus right side of the chamber did not show results that were statistically significant, $F(1, 30) = .02,$
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$p = 0.90$ (see Figure 1). There was no significant difference in activity found between the quadrant containing the odour of rats treated with PBS in comparison to the quadrant containing the odour of rats with elevated levels of PPA with respect to number of entries ($F(3, 28) = 2.09, p = .124$), horizontal activity ($F(3, 28) = .33, p = .801$), movement time ($F(3, 28) = 1.91, p = .151$; see Figure 2), duration ($F(3, 28) = 3.57, p = .289$), or total distance ($F(3, 28) = .32, p = .809$). Results were not in support of the second hypothesis, as rats did not exhibit a reduced preference for PPA-associated odours in comparison to social odours.

**PPA versus Clean Bedding**

To determine if rats show a reduced preference for PPA-associated odours, a one-way ANOVA was conducted to compare activity in the quadrants containing the clean bedding odour cue and the PPA odour cue. Rats did not demonstrate a preference for either side of the chamber, which contained the odour of a PPA-injected rat and the odour of clean bedding. The one-way ANOVA comparing number of entries in the left versus right side of the chamber did not show results that were statistically significant, $F(1, 30) = 0.27, p = .607$. There was no significant difference found between the quadrant containing the odour of clean bedding in comparison to the quadrant containing the odour of rats with elevated levels of PPA with respect to number of entries ($F(3, 28) = 4.38, p = .987$), horizontal activity ($F(3, 28) = 0.44, p = .723$), movement time ($F(3, 28) = 1.80, p = .170$), duration ($F(3, 28) = 6.73, p = .925$; see Figure 3), or total distance ($F(3, 28) = .39, p = .758$). Results were not in support of the second hypothesis, as rats did not exhibit a reduced preference for PPA-associated odours.

*Figure 1. Group mean (± S.E.M.) corrected number of entries into the PPA-odour side of the chamber and the PBS-odour side of the chamber. Bars represent means and vertical lines represent SEM. No significant difference was detected in the number of entries made into either side of the chamber, $F(1, 30) = 0.02, p = .900$. *$p < .05$, **$p < .01$, ***$p < .001$. 

*Figure 2. Group mean (± S.E.M.) corrected movement time into each quadrant for the PBS vs. PPA odour trial. The suffix “Blank” refers to the side of chamber that does not contain an odour cue. No significant difference was detected for movement time between any of the four quadrants, $F(3, 28) = 1.91, p = .151$. *$p < .05$, **$p < .01$, ***$p < .001$. 


LPS versus NaCl
To determine if rats show a preference for social odours and an aversion to LPS odours, a one-way ANOVA was conducted to compare activity in the quadrants containing the LPS odour cue and NaCl odour cue. The one-way ANOVA comparing number of entries in the left versus right side of the chamber did not show results that were statistically significant, $F(1, 30) = 0.08, p = .779$. As shown by Figure 4, rats exhibited significantly greater movement time in the quadrant containing the bedding of NaCl-injected rats in comparison to the quadrant containing the bedding of LPS-injected rats, $F(3, 28) = 7.93, p = .002$. Rats travelled a significantly greater total distance in the quadrant containing the bedding of NaCl-injected rats in comparison to the quadrant containing the bedding of LPS-injected rats, $F(3, 28) = 4.21, p = .041$. No significant differences were detected in number of entries ($F(3, 28) = 13.53, p = .272$), horizontal activity ($F(3, 28) = 2.82, p = .057$), or duration ($F(3, 28) = 14.66, p = .387$). These results partially supported the second hypothesis, as rats did show significantly reduced preference for LPS-associated odours.

LPS versus Clean Bedding
To determine if rats show an aversion to LPS odours, a one-way ANOVA was conducted to compare activity in the quadrants containing the LPS odour cue and clean bedding odour cue. The one-way ANOVA comparing number of entries in the left versus right side of the chamber did not show results that were statistically significant, $F(1, 30) = 0.00, p = .956$. There was no significant difference found between the quadrant containing the odour of rats treated with LPS in comparison to the quadrant with clean bedding with respect to duration ($F(3, 28) = 17.91, p = .322$) or movement time ($F(3, 28) = 6.61, p = .092$). Test rats made a significantly greater number of...
entries into the quadrant containing the odour of LPS-treated rats in comparison to the quadrant containing the clean bedding, $F(3, 28) = 13.23, p = .004$ (see Figure 5). Rats exhibited significantly greater horizontal activity in the quadrant containing the LPS-associated odour in comparison to the quadrant containing the clean bedding, $F(3, 28) = 4.26, p = .013$. Finally, rats travelled a significantly greater total distance in the quadrant containing the LPS-associated odour in comparison to the quadrant containing clean bedding, $F(3, 28) = 3.60, p = .026$. These results were not in support of the third hypothesis, as rats did not show significantly reduced preference for LPS-associated odours in comparison to the odour of clean bedding.

Figure 5. Group mean (± S.E.M.) corrected number of entries made in each quadrant for the LPS v. Clean Bedding trial. The suffix “Blank” refers to the side of chamber that does not contain an odour cue. A significant difference ($p < .01$) in number of entries was detected between the quadrant containing the odour of clean bedding and the LPS-associated odour, $F(3, 28) = 13.23, p = .004$. *$p < .05$, **$p < .01$, ***$p < .001$.

**Discussion**

The present research sought to explore whether adult male rats can distinguish the odours of conspecifics treated with either LPS or PPA and was the first investigation to explore the effects of PPA on social odour preferences in rodents. The findings from this study suggest that exposure to the odour of a conspecific treated with a sickness-inducing compound (e.g. LPS) will show a degree of aversion to that odour. However, the same findings were not discovered for those exposed to the odour of conspecifics with elevated levels of PPA.

Research has demonstrated that human odours convey rich information regarding a range of states such as emotion and sickness (De Groot, Semin, & Smeets, 2017). Odours give off information regarding sex, while other odours that are given off may vary depending on the social environment and the individual’s condition (Kavaliers & Choleris, 2011). The neuropeptides oxytocin and vasopressin play crucial roles in the mediation of various elements of social behavior, such as social recognition and responses to threats such as illness. Studies have shown that these two hormones are also involved in the recognition and avoidance of individuals using an olfactory-mediated mechanism (Bielsky & Young, 2004).

There is evidence that some animals prefer mates with a major histocompatibility complex that is different from their own and it has been suggested that these animals use urine as the olfactory signal for discrimination (Ehman & Scott, 2001). Rodents have been found to use urinary odours to guide social behaviours including recognition, avoidance and approach behaviours to conspecifics that have been infected with certain bacterial mimetics such as LPS (Kavaliers, Choleris, & Pfaff, 2005).

Arakawa, Arakawa, and Deak (2010) conducted a study with findings that suggested that the inflammatory response that results when rodents are injected with LPS may play a significant role in odor communication. Injection of adult male rats with the bacterial endotoxin, LPS, decreased investigation through a wire-mesh partition between healthy conspecifics. This avoidance reaction was detected in rodents’
responses to soiled bedding collected from LPS-treated rats, regardless of the method of injection used (intraperitoneally or centrally). These findings are not limited to rodent subjects but have also been found using human participants. Olsson and colleagues (2014) reported that after activating the innate immune system in healthy participants by injecting them with LPS, individuals had a more aversive body odour relative to when they were exposed to the odour of a placebo compound.

Social relationships are dependent on an individual’s ability to remember others. Social memory can be accurately investigated by analyzing the decrease in social exploration of a familiar partner relative to novel conspecifics (Ferguson, Young, & Insel, 2002). Social recognition memory in rodents is explored using the finding that rodents have an innate drive to investigate unfamiliar conspecifics (Ferguson et al., 2002). Results of a study conducted by Thor and Holloway (1982) used duration of social investigatory behavior to measure rat recognition and social memory. Repeated exposure to the same rat led to significantly decreased social investigation in comparison to a novel individual. These findings support the idea that rodents prefer to investigate the identity of novel rats and that social investigation increases familiarity by decreasing novelty and diminishing attractiveness.

The present study examined the effects of PPA on social odour in a sample of adult male, Long-Evans rats. Contrary to the first hypothesis, rats did not demonstrate a preference for the bedding containing the odours of conspecifics, as shown by the results of the first testing trial that was conducted: clean bedding versus bedding of a non-injected rat. There was no significant difference detected in the duration of time spent in the quadrant containing the non-injected bedding and the clean bedding. Rats also did not make a significantly different number of entries in either of these quadrants and no significant difference in horizontal activity, movement time, or total distance was detected. The lack of significance detected in activity for the clean bedding odour in comparison to the odour of a non-injected rat trial can be attributed to many factors. This may be due to the fact that this trial was the first social odour preference task that the testing rats participated in. Moreover, they may have had higher than normal levels of anxiety and may not have been given enough time to habituate to the chamber before social odour preference testing began. The absence of significance can also be attributed to a flaw in the design of the experiment, although this is unlikely due to the significance that was detected for LPS comparisons.

Studies have indicated that rats have the capacity to differentiate the condition of others on the basis of their smell and can tell a sick rat from a non-sick rat (Brown, 1995). Exposure to sickness-related odours in adult rats has been found to produce increased avoidance in both sexes (Arakawa, Arakawa, & Deak, 2010). There was support for the hypothesis which stated that rats could distinguish the odours of LPS-treated rats. Comparing the activity in quadrants containing the LPS odour cue and NaCl odour cue, rats showed increased preference for NaCl-associated odours (social odours) and reduced preference for LPS odours. These findings indicate that rats have a preference to investigate odours of conspecifics as shown by increased activity in the NaCl quadrant and less activity in the quadrant with the LPS-odour. The LPS and clean bedding comparison revealed that rats spent a greater amount of time in the LPS-odour quadrant in comparison to the clean bedding odour quadrant, as shown by number of entries, horizontal activity, and total distance. It is possible that rats associated the odours of LPS with odours of conspecifics, showing a significant preference.
for these odours in comparison to the odour of unused, clean bedding.

It was hypothesized that testing rats would be able to distinguish the odours of PPA-treated rats and show a lesser preference for these odours. This hypothesis was not supported, as there was no significant difference in activity detected for PPA comparisons. This may suggest that rats are not able to detect the odours of a PPA-treated sample; the procedure was not sensitive enough to produce significant effects for the PPA comparisons, or that rats are indifferent to the odours of other rats injected with PPA and do not respond to them differently.

Limitations

The present study has several limitations that must be noted for the purposes of further investigations. The mesh material that was used to cover the petri dishes containing the odour cue had a fairly noticeable smell which may have made the odour of the bedding less detectable by the testing rats. While significant results were still found for several comparisons, the mesh material may have concealed the odours of propionic acid, had they not been as strong as the LPS-associated odours. Moreover, the time interval post-PPA injection may have not been sufficient time to allow for the PPA-associated odours to be emitted onto the bedding. Given the novel nature of the present study, it is equally possible that the time interval post-treatment with PPA was too long and that the odour was no longer detectable on the bedding by the time odour preferences were observed in the social odour preference task. Additionally, more time could have been allowed for the rats to be socialized to the chamber and the task. Rats were socialized for approximately 2 hr each day for three days prior to testing did a habituation task in the chamber one day prior to testing. On the first day of testing comparing the odour of a non-injected rat and the odour of clean bedding, rats appeared very anxious in the chamber and exhibited high levels of freezing behaviour. This may have led the rats to be less active than they may have been if they were less anxious in this environment.

Implications and Future Directions

Social recognition memory using the olfactory sense provides a framework for investigating the ways in which individuals choose to avoid or approach others. As outlined by the current study, there is accumulating evidence that detection of social odours is associated with a wide array of social behaviours and that certain odours can act as guides or predictors of how a rodent will react when they detect a certain odour. Rodent behavior is activated by the identification of social cues to gather information about conspecifics for subsequent strategies, such as avoidance of odours associated with pathogens and illnesses. However, as revealed by the present investigation, there is currently no evidence that suggests that rats distinguish the odours of conspecifics with levels of PPA that are seen in individuals with ASDs.

The present investigation has several implications for an animal model of ASD. While it has been found that individuals with ASD show a reduced ability to form social bonds due to various impairments in their ability to interact socially (Powers, 2000), this research explored the possibility that others might show a degree of reduced interest in affected individuals or whether their responses and behaviour towards individuals with ASDs are altered at all. However, testing rats did not show a significantly different response to the odours of rats with higher than normal PPA levels. This finding suggests that responses towards individuals with ASD are not different from others with normal PPA levels. However, due to the novelty of the present study, further research is required.
Future research should explore the possibility of having a longer or shorter time interval post-PPA injection before collecting the bedding to be used as odour cues. The present study injected the sample of rats with PPA approximately 17 hr before the bedding was collected. This may have not been enough time for the odours of PPA-treated rats to emanate onto the bedding and it is also possible that this was too great of a time interval between the time of injection and odour collection. Future studies looking to replicate the present study may also consider a design with more chronic exposure to PPA, as this may produce different results, possibly showing differences in preference for the PPA-associated odour. Future investigations should seek to contextualize the findings of the present study within the larger body of research regarding animal models of ASD. With replications of the present study and further investigations expanding on the present research, whether or not individuals can distinguish the odours of a PPA-treated sample using similar mechanisms that are used to detect sickness-related odours can be further explored.

**Concluding Remarks**

To conclude, the present study established that adult male rats exposed to the odours of rats treated with PPA did not show a reduced interest in these odours. This study was the first to explore how rats will respond to odours of conspecifics treated with an acute dose of PPA, exploring social odour in the context of an animal model of ASD. Results indicate that the current study design is appropriate in assessing the effects of LPS on social recognition memory and that rats show some preference for social odours. Rats do not seem to demonstrate altered activity in the presence of the odours of rats with elevated PPA levels. Expanding on this animal model can help complement the existing information on social behavior in rodents and assist future investigations on the neurobiology of irregular sociality, such as in ASDs.

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References


an animal model of autism. *Neuropsychopharmacology*, 54(6), 901-911.


