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Predator odor exposure in early adolescence influences the effects of the bacterial product, propionic acid, on anxiety, sensorimotor gating, and acoustic startle response in male rats in later adolescence and adulthood

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ABSTRACT

It is becoming evident than the adolescent period is a sensitive period in stress response programming. Stressors during this time may alter signaling from the gut microbiome, which has been shown to increase the risk for psychiatric disorders. It was hypothesized that adolescent stressors may potentiate the symptoms of anxiety and sensory abnormalities induced by a gut bacterial product, the short-chain fatty acid, propionic acid (PPA). The present study investigated the effects of repeated predator odor exposure during early adolescence on male rats administered PPA in late adolescence and adulthood on a behavioral test battery. Male adolescent Long-Evans rats were repeatedly exposed to a worn or unworn cat collar stimulus in early adolescence on postnatal days (P) 28, P30, P32, and P34. They were administered either PPA (500 mg/kg i.p.), or its vehicle in late adolescence on P40 and P43, and were subsequently tested on the light-dark anxiety task and acoustic startle task, respectively. In adulthood, the rats were again injected with PPA or its vehicle on P74 and P77, and subsequently tested on the light-dark apparatus and acoustic startle task, respectively. The repeated predator odor exposure was aversive and produced long-term anxiogenic effects as measured by the light-dark apparatus. PPA decreased activity and percent prepulse inhibition of the acoustic startle response, with its effects on vertical activity, a putative measure of escape behavior, being potentiated by prior predator stress. PPA's effects in adulthood were diminished in comparison to adolescence. These results suggest the importance of evaluating the effects of early adolescent stress on subsequent environmental insults on the development of behavioral abnormalities.

1. Introduction

Environmental stressors during early life increases the risk for clinical disorders including anxiety and depression [1]. Prior research has suggested that early life stressors may decrease an individual's threshold towards these psychopathologies, especially during prenatal and postnatal developmental periods. However, to date, there is relatively limited research that addresses the importance of the adolescent period on the development of behavioral and clinical disorders. The adolescent period has been hypothesized to be a “sensitive period” for stress response programming and social development [2,3]. Studies with rodents have indicated that the early adolescent period is important in developing adaptive coping strategies to stressors. In rats, the adolescent period begins on P28 and ends on P42, with some changes that may persist up to P55 in male rats [3]. Stressors during this period of development activates the hypothalamic-pituitary-adrenal (HPA) axis which can result in long term consequences [4,5].

Predator odors are ethologically relevant stressors for rodents [6,7]. Rats exposed to cat odors display anxiogenic and defensive behaviors, and suppress non-defensive behaviors [6,8,9]. Rats also show innate aversive and avoidance behaviors in response to the predator odor source [2,10]. In addition to the acute anxiogenic effects induced by predator odors, they can also induce sustained stress across the lifespan. When cat odor exposure is administered repeatedly, it induces long-lasting neuroadaptations against these stressful stimuli [11]. Cat odor exposure during adolescence elevates corticosterone levels and decreases activity in male and female adult rats in comparison to their controls [2]. Adolescent female rats repeatedly exposed to cat odor displays an elevated anxiety-like response in adulthood on an open field [2]. Hence, the adolescent period is a sensitive period for long-term

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stress response programming. This suggests that naturalistic adolescent stressors provided by cat odor may be a fruitful means of examining the impacts of subsequent environmental and physiological perturbations and in particular those associated with the gut microbiome.

There is accumulating evidence that short-term and long-term stress exposures can alter the gut microbiome by altering its bacterial profile [12]. Changes to the gut microbiome have been suggested to impact neurodevelopmental and psychological disorders because of the intricate and pervasive communication between the gut and the brain. This feedback system affects immune activation, emotional and cognitive processes, as well as gut signaling [13]. Bacterial metabolites including short-chain fatty acids (SCFAs) are important for energy metabolism at physiological levels [14]. However, at abnormally high levels, they can induce detrimental effects. One such SCFA that has been shown to induce behavioral abnormalities at relatively high doses is propionic acid (PPA). Prior studies on the effects of PPA have been mostly conducted with male rats, as PPA has been used in models of Autism Spectrum Disorder, a neurodevelopmental disorder that occurs disproportionately greater in males than females [15].

When administered intracerebroventricularly, acute PPA has been shown to induce increased locomotor activity [16,17], abnormal motor movements, stereotypic behavior [17], and impaired social interaction in adult male rats [18,19], similar to behaviors observed in children with Autism Spectrum Disorders [20]. Intraperitoneal (i.p.) peripheral administration of PPA has also been suggested to induce abnormal behaviors in rodents, which may stem from its' central effects. Ossenkopp et al. [21] found that systemic PPA induced aversive internal cues as measured with conditioned place avoidance and conditioned taste avoidance. There are also suggestions that systemic PPA may have effects on adolescent (P35–38) male rats by decreasing social behaviors [22]. The manner by which early stressors interact with PPA, however, to date are unclear.

The present study determined the effects of repeated early adolescence cat odor exposure in male rats on measures of anxiety, and responses to sensory stimuli as provided by prepulse inhibition (PPI) and acoustic startle response (ASR) in later adolescence and adulthood. The effects of PPA during adolescence and adulthood and its interactions with a prior stressor (predator odor) to alter behavior were also examined. It was hypothesized that predator odor would induce anxiety-like behaviors during the exposure, that persists into late adolescence and adulthood. It was further hypothesized that PPA would produce altered cognitive functioning as assessed by PPI. These effects of PPA were hypothesized to be augmented by prior exposure to the predator odor.

2. Material and methods

2.1. Subjects

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

2.2. Predator odor exposure

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

The cat odor exposure field was divided into the opposite perimeter zone (Fig. A.2A) and the cat odor exposure area (Fig. A.2B), as shown in Fig. A.2 in the light grey. The opposite perimeter zone was the furthest area away from the cat collar stimulus, while the exposure area contained the cat collar stimulus. The location of the cat collar stimulus is represented in black in Fig. A.2. Rat activity was not analyzed in the undefined areas of the field.

The VersaMax Analyzer collected the total time spent in each of the defined areas and the number of vertical movements produced in the cat odor exposure area (Table 1). The number of vertical movements were corrected for the rat's total time spent in the cat odor exposure area, such that corrected number of vertical movements was calculated as number of vertical movements per second spent in the cat odor exposure area.

2.3. Drugs

Sodium propionate (P1880, Sigma-Aldrich, St. Louis, MO, USA) at a dose of 500 mg/kg was dissolved in 0.1 M phosphate buffered saline (PBS) and buffered to pH off 7.5 by HCl. Sodium propionate and PBS were administered in late adolescence on P40 and P43, and in adulthood on P74 and P77. A vehicle control using an equivalent volume to body weight ratio of 0.1 M PBS was injected on the same days for the PBS treatment rats. All drug treatments were administered intraperitoneally (i.p.) at 2.0 ml/kg body weight. The dose of 500 mg/kg of sodium propionate was chosen because it reduced locomotion on the conditioned place avoidance task [21] and reduced social interaction

Table 1
Behavioral measures recorded in the cat odor exposure field.

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

Table 2
Behavioral measures recorded in the light-dark anxiety task.

Behavioral measures	Definition
Chamber choice variables	
Light duration	Time spent in the light chamber.
Light transitions	Number of transitions from the dark to the light chamber.
Nosepokes	Number of investigative postures extending into the light chamber.
Activity Variables	
Total distance	Cumulative horizontal distance travelled.
Vertical movement number	Number of times animal rears up and goes below the vertical sensor for at least 1 s before the next rear up.
Vertical time	Time spent in rearing position. This measure does not consider the time when animal goes below the vertical sensor.

[22] when administered i.p. This dose also reduced exploration and increased aggression when administered subcutaneously [23].

2.4. Light-dark apparatus & behavioral measures

Behavioral variables in the light-dark apparatus were collected using the same eight automated VersaMax Animal Activity Monitors (Accuscan Model RXYZCM-16, Columbus, OH), used in the predator odor exposure. This apparatus was divided into an illuminated light chamber or dark chamber by a black opaque Plexiglas box insert (40 × 20 × 23 cm). The addition of this insert modified the context to reduce contextual cues from the predator odor exposure. The dark chamber had small holes located so as to permit the infrared beams to pass through in order to detect movement. The rats were permitted to freely pass between the chambers by a hole in the partition between the light and dark chambers at floor level. Data were collected and analyzed by a VersaMax Analyzer (Accuscan Model CDA-8, Columbus, OH) and sent to a computer where it was recorded and retrieved. Procedures were adapted from Banasikowski et al. [24] and Ossenkopp et al. [25].

The VersaMax Analyzer collected two types of behavioral variables: chamber-choice variables and activity variables (Table 2). The chamber choice variables included the duration spent in the light chamber, number of transitions into the light chamber, and the number of nosepokes. Nosepokes were observed as investigative stretch-attend postures extending into the light chamber. These variables provide measures of anxiety-like behaviors, including avoidance of the light chamber and risk-assessment (nosepokes and transitions between the chambers) [24]. This apparatus assessed the conflicting drives of the rodent to explore a novel environment and to avoid the brightly-lit chamber [26].

The second type of behavioral variable recorded by the VersaMax Analyzer were the activity variables. These included the horizontal variable of total cumulative distance travelled, and the vertical variables of the number of vertical movements made (rearing) and time spent in vertical position. The horizontal variables of activity were used as measures of general activity and motivation to move. The vertical variables of activity were used as measures for escape and exploratory behavior [26], which are suggested to be elevated when the rodents are anxious [27]. All of the activity variables were recorded in the light and dark chambers, and were corrected for the total time spent in these respective chambers. For example, corrected total distance in the light chamber was calculated for the total distance in the light chamber per second spent in this chamber.

2.5. Acoustic startle response & prepulse inhibition

Acoustic startle response (ASR) and pre-pulse inhibition (PPI) were assessed in three startle devices (SRLAB, San Diego Instruments, San Diego, CA). Each apparatus consisted of a cylindrical, clear acrylic rat enclosure (8.89 cm inside diameter and 20.32 cm length) mounted on an acrylic platform. A piezoelectric accelerometer underneath the platform detected the force of the rat's movement in response to the startle stimulus. This apparatus was placed inside a ventilated sound attenuated box containing fluorescent lighting and an audio speaker 11 cm from the top back of the box. Data were recorded and stored by a computer attached to the accelerometer. Procedures were adapted from Lockey, Kavaliers, and Ossenkopp [28].

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

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

2.6. Experimental timeline

After arrival of the rats on P22, they were pair-housed without manipulation for three days to habituate them to the facility. The animals were handled on their fourth and fifth day, and weighed and handled on their sixth day. On P27, the rats were assigned to four weight-matched treatment groups: 1. CAT ODOR-PPA (n = 8), 2. CAT ODOR-PBS (n = 8), 3. NO CAT ODOR-PPA (n = 8), and 4. NO CAT ODOR-PBS (n = 8). The experimental procedure is outlined in Fig. 1.

2.6.1. Phase 1: adolescence

In early adolescence, the rats were exposed to either a cat odor or no cat odor collar stimulus for 30 min on days P28, P30, P32, and P34. Between each exposure session, the apparatus was cleaned with detergent and water, followed by baking soda in water to remove odors, and dried with paper towel to remove remaining stains. The rats were weighed the day of, and the day after each exposure day.

Six days after cat odor exposure in late adolescence on P40, the rats were weighed and injected i.p. with PPA (500 mg/kg) its vehicle, PBS and returned to their home cages. After 15 min, rats were individually placed in the light-dark apparatus for testing duration of 15 min. This wait period was based on the results of prior studies [21,22].The same cleaning procedure was conducted as for the predator odor exposure days.

On P43, the rats were again weighed and injected i.p. with PPA or PBS. After 10 min, the rats were placed into the acoustic startle apparatus. The order of behavioral testing used in this study is consistent across the literature [29,30]. A 5-min background noise of 70 dB was played to acclimate the rats to the apparatus, after which they were exposed to the startle task. The testing phase lasted for 11 min and consisted of 45 trials starting with 10 startle-only trials at 120 dB presented for 40 ms. The subsequent 30 trials were presented in a pseudo-randomized order: 10 startle-only (120 dB for 40 ms) and 20 PPI trials (ten 76 dB and ten 82 dB non-startling pulses presented for 20 ms and a 120 ms wait until the 120 dB startle pulse). The session ended with 5 startle-only trials (120 dB for 40 ms). The startle responses were recorded by a computer for 100 ms following the onset of the startle stimulus. All trials were separated by an inter-trial interval (ITI) varying

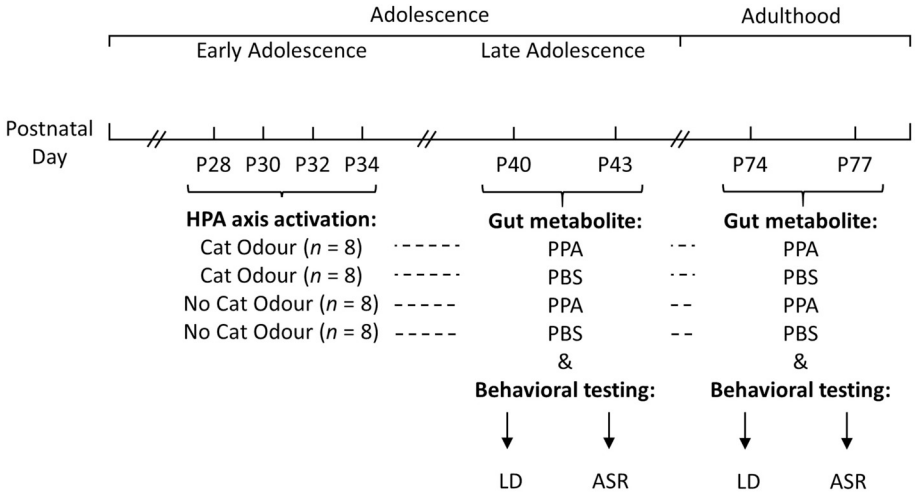

Fig. 1. Experimental timeline. PPA = propionic acid, PBS = phosphate buffered saline, LD = light-dark task, ASR = acoustic startle response.

from 8 to 23 s in length (average ITI = 15 s). The startle apparatus force detection hardware was calibrated at least once every four testing sessions to ensure consistent recording of startle reactivity. Between each testing session, the animal enclosure was cleaned with detergent and water, and dried with paper towel to remove remaining stains.

2.6.2. Phase 2: adulthood

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

2.7. Statistical analyses

All statistical analyses were completed using IBM SPSS Statistics 25. Repeated measures analyses of variances (ANOVA) were conducted to compare the treatment effects across the four cat odor exposure days. The within-subjects factor was the treatment days, and the between-subjects factor was cat odor treatment. Additionally, the behavioral measures obtained from the light-dark and acoustic startle response tasks were analyzed using repeated measures ANOVAs to compare behavior across adolescence and adulthood. The within-subjects factor was age, and the between-subjects factor were the cat odor and PPA treatment conditions. The significance criteria was set to $\alpha = 0.05$ for all hypotheses tested.

3. Results

3.1. Cat odor exposure

Rats that were exposed to the cat odor spent significant a greater amount of time in the opposite perimeter along the wall than rats exposed to no cat odor via an unworn cat collar, $F(1, 30) = 11.646$, $p = .002$, $\eta^2 = 0.280$, OP = 0.910 (Fig. 2A). This provided measures of both avoidance and thigmotaxis, an anxiety-related behavior [24,25].

The cat odor exposure area was the immediate and surrounding area of the cat collar stimulus. Since the cat collar was placed between the lower infrared sensors and the upper infrared sensors, vertical movements in this area would indicate exploration and sniffing of the cat collars. The rats exposed to the cat odor spent less time in the cat exposure area in comparison to the control rats, $F(1, 30) = 7.163$, $p = .012$, $\eta^2 = 0.193$, OP = 0.736 (Fig. 2B). In a separate analysis of each exposure day, it was found that the rats exposed to cat odor via worn cat collar stimulus showed no significant difference in the duration spent in the cat exposure area in comparison to their controls on P28. However, this diverged in the later exposure days to produce the effect shown in Fig. 2B.

On the measure of risk assessment, corrected vertical time within the cat odor exposure area was used. There was an postnatal day by cat odor interaction effect, $F(3, 90) = 7.004$, $p < .001$, $\eta^2 = 0.189$, OP = 0.976. On P34, habituation occurred, such that the cat odor exposed rats exhibited more time spent in vertical position than their controls ($p = .004$) (Fig. 2C). There was also a significant main effect of postnatal day, $F(3, 90) = 4.437$, $p = .006$, $\eta^2 = 0.129$, OP = 0.863, such that at subsequent cat odor exposure days, the rats spent more time in vertical position than on P28 ($ps < 0.005$). This effect was driven by the increase in vertical time exhibited by the cat odor exposed rats.

3.2. Light-dark: anxiety & activity

3.2.1. Chamber-choice variables

Following PPA or PBS treatment, the rats were tested on the light-dark apparatus where their anxiety-related behaviors and activity levels were measured. Anxiety and risk assessment behaviors were addressed with the chamber-choice variables. There was no significant difference in the duration spent in the light chamber by cat odor treatment, $F(1, 28) = 3.082$, $p = .090$, $\eta^2 = 0.099$, OP = 0.396, or PPA treatment, $F(1, 28) = 1.420$, $p = .243$, $\eta^2 = 0.048$, OP = 0.210 (Table 3).

On the risk assessment measure using nosepokes into the light chamber, a main effect of cat odor was observed for the combined adolescence and adulthood data, such that prior cat odor exposure resulted in a decreased number of nosepokes into the light chamber relative to the control odors, $F(1, 28) = 4.350$, $p = .046$, $\eta^2 = 0.134$, OP = 0.522 (Fig. 3).

For the number of transitions into the light chamber, there was a main effect of PPA based on decreased number of transitions into the light chamber compared to rats treated with PBS, $F(1, 28) = 22.774$, $p < .001$, $\eta^2 = 0.449$, OP = 0.996 (Fig. 4). There was no significant main effect of cat odor, $F(1, 28) = 1.497$, $p = .231$, $\eta^2 = 0.051$, OP = 0.219.

3.2.2. Activity variables: corrected horizontal locomotion

There were no significant cat odor treatment, $F(1, 28) = 3.434$, $p = .074$, $\eta^2 = 0.109$, OP = 0.432, or PPA treatment effects, $F(1, 28) = 0.000$, $p = .991$, $\eta^2 = 0.000$, OP = 0.050, on corrected total distance travelled in the light chamber. There was a main effect of PPA for the combined adolescence and adulthood data in the dark chamber evidenced by PPA-treated rats showing decreased corrected total distance travelled relative to the PBS controls, $F(1, 28) = 21.521$,

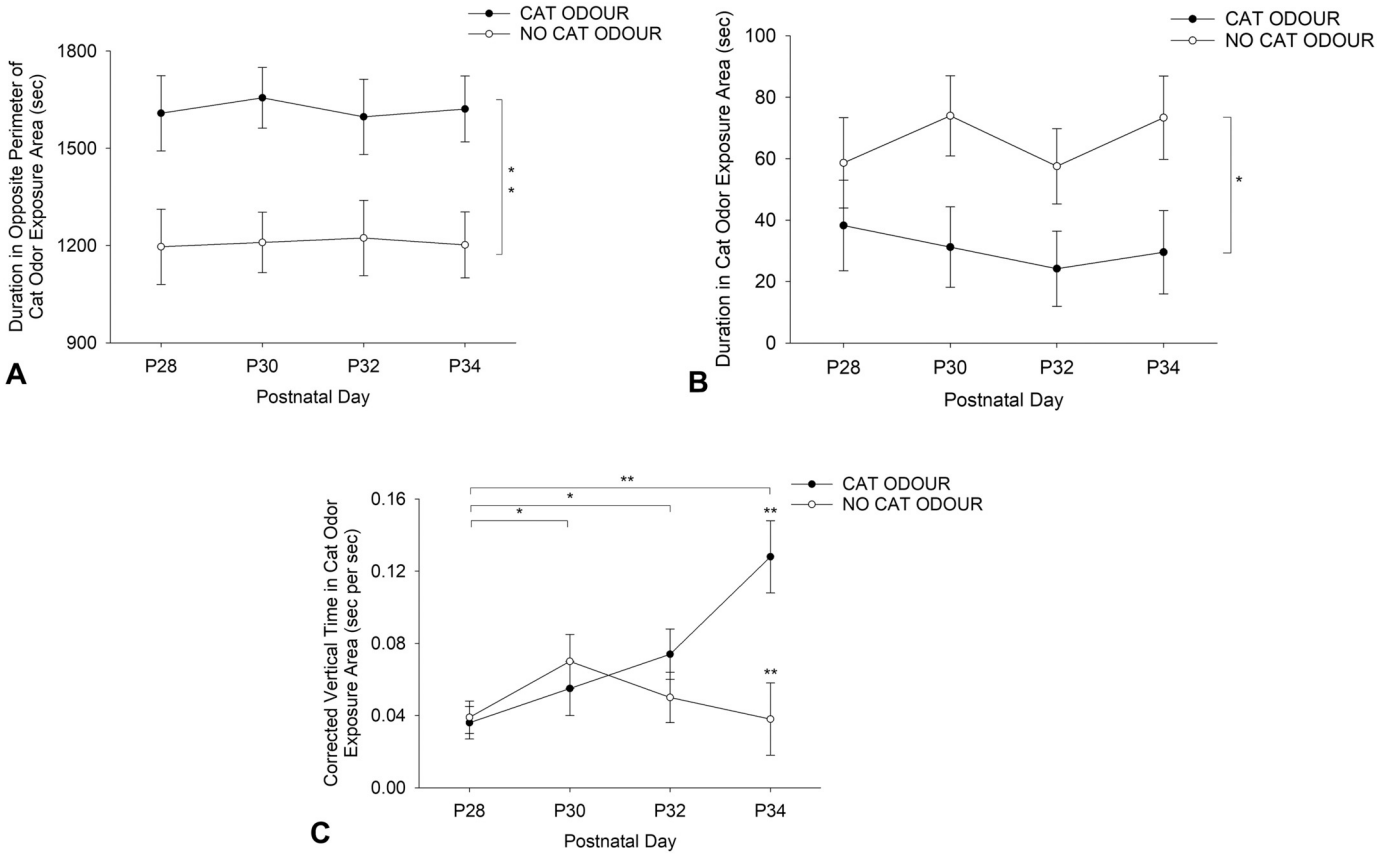

Fig. 2. (A-C). Duration of time spent during a 15 min exposure period in the (A) opposite perimeter, (B) cat odor exposure area, and (C) corrected vertical time spent in the cat odor exposure area. Rats were exposed to a cat odor via a worn cat collar (n = 16) or no cat odor via an unworn (n = 16) cat collar stimulus on P28, 30, 32 and 34. (A) The cat odor exposed rats spent more time in the opposite perimeter than the unworn cat collar exposed rats. (B) The cat odor exposed rats spent less time in the cat odor exposure area than the unworn cat collar exposed rats. (C) The cat odor exposed rats spent greater time in vertical position on P34 than the controls. There is a postnatal day effect, such that repeated cat odor exposure results in increased time spent in vertical position compared to P28, driven by the cat odor exposed rats.*p < .05, **p < .01. Data is represented as mean ± SEM.

Table 3
Duration spent in the light chamber of the light-dark apparatus.

		Duration spent in the light chamber (s)	
Treatment		Adolescence	Adulthood
Cat Odour	PPA	85.538 ± 56.020	116.675 ± 64.663
Cat Odour	PBS	220.050 ± 56.020	252.075 ± 64.663
No Cat Odour	PPA	235.700 ± 56.020	299.213 ± 64.663
No Cat Odour	PBS	285.863 ± 56.020	244.363 ± 64.663

N.S. differences were observed. Data is represented as means ± SEM.

p < .001, η^2 = 0.435, OP = 0.994 (Fig. 5).

3.2.3. Activity variables: corrected vertical locomotion

There was a main effect of cat odor on the measure of corrected vertical time in the light chamber when adolescence and adulthood data were combined. Cat odor exposed rats showed more time in vertical position in the light chamber compared to the controls, F(1, 28) = 4.342, p = .046, η^2 = 0.134, OP = 0.521 (Fig. 6A). A main effect of PPA was also observed for corrected vertical time in the light chamber, with the PPA-treated rats showing decreased corrected vertical time relative to the PBS controls, F(1, 28) = 9.621, p = .004, η^2 = 0.256, OP = 0.849 (Fig. 6A). Additionally, in adulthood, the rats administered PPA showed decreased corrected vertical time in comparison to the controls (p = .002; Fig. 6B).

As in the light chamber, a main effect of PPA administration was seen in the dark chamber, where PPA decreased the corrected vertical time in comparison to PBS controls when adolescence and adulthood data were combined, F(1, 28) = 36.970, p < .001, η^2 = 0.569, OP = 1.000 (Fig. 7A). This effect was observed for PPA treatment in both adolescence and adulthood producing less time in vertical

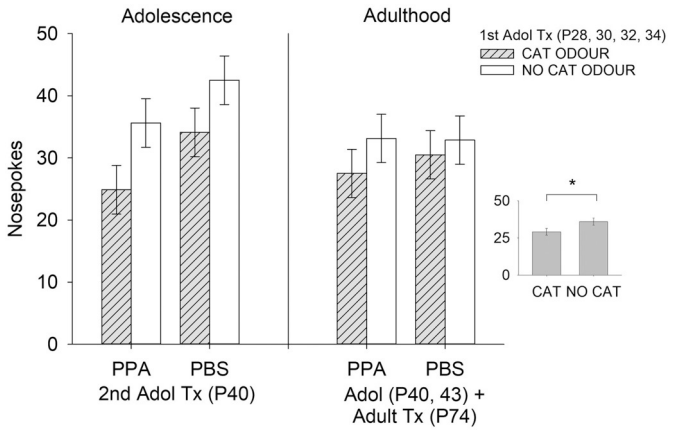

Fig. 3. Nosepokes into the light chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). A main effect of cat odor exposure when combining adolescent and adulthood was observed, such that the cat odor exposed rats produced fewer nosepokes into the light chamber than the unworn cat collar exposed rats. *p < .05. Data is represented as mean ± SEM.

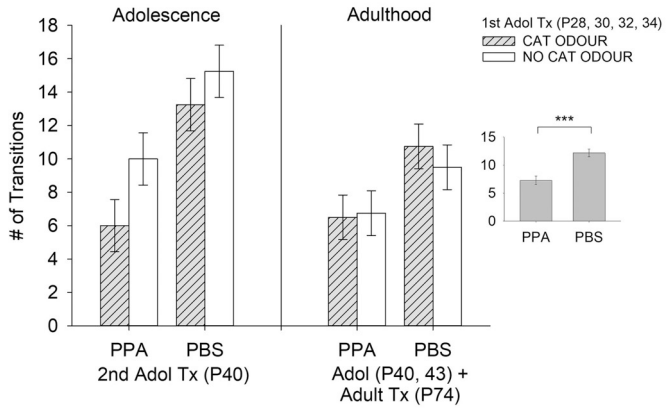

Fig. 4. Number of transitions into the light chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). A main effect of PPA when combining adolescent and adulthood was observed, such that the PPA-treated rats produced fewer transitions into the light chamber than PBS controls. ***p < .001. Data is represented as mean ± SEM.

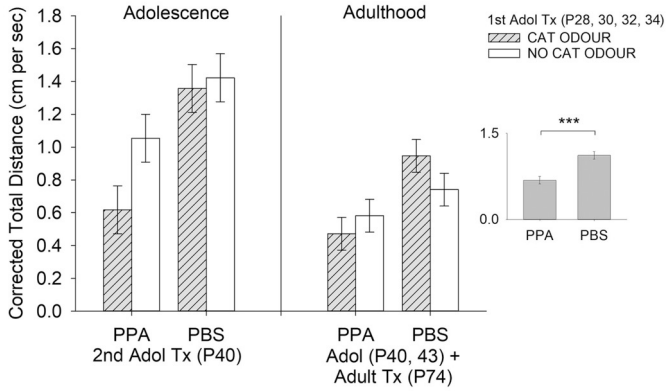

Fig. 5. Corrected total distance travelled in the dark chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the dark chamber. A main effect of PPA when combining adolescence and adulthood was found, such that PPA-treated rats produced less corrected total distance travelled than the PBS controls. ***p < .001. Data is represented as mean ± SEM.

exploration than the PBS controls in adolescence and adulthood, respectively (Fig. 7B).

Cat odor exposure and PPA treatment interaction effects were found for dark corrected vertical movement numbers and dark corrected vertical time. Cat odor and PBS treated rats showed greater dark vertical time in comparison to the no cat odor and PBS group, $F(1, 28) = 8.217$, $p = .008$, $\eta^2 = 0.227$, OP = 0.790, suggesting that cat odor increased vertical time. There was a trend in which the no cat odor and PPA group showed fewer corrected vertical movements than the no cat odor and PBS controls ($p = .094$), suggesting that PPA administration may have led to decreased vertical movements. Given that cat odor increases vertical time and that PPA reduces vertical movements, their interaction effect on vertical activity was determined to be antagonistic. Additionally, the rats treated with cat odor and PPA showed fewer dark corrected vertical movement numbers than those treated with cat odor and PBS, $F(1, 28) = 4.638$, $p = .040$, $\eta^2 = 0.438$, OP = 0.995 (Fig. 8). These results together suggest that cat odor potentiates PPA's reduction of vertical movements, although cat odor by itself increases vertical time.

3.3. Acoustic startle response (ASR) & percent prepulse inhibition (%PPI)

The average ASR for a 120 dB startle pulse also produced no significant differences between the cat odor, $F(1, 28) = 0.347$, $p = .561$, $\eta^2 = 0.012$, OP = 0.088, and PPA treatment groups, $F(1, 28) = 2.370$, $p = .135$, $\eta^2 = 0.078$, OP = 0.318 (Table 4). On the measure of sensorimotor gating using a 76 dB prepulse, a main effect of PPA was observed such that rats treated with PPA showed a depressed %PPI compared to the PBS controls when adolescence and adulthood were combined, $F(1, 28) = 5.440$, $p = .027$, $\eta^2 = 0.163$, OP = 0.615 (Fig. 9). Additionally, no significant treatment differences was observed for the 82 dB prepulse trials. There was a trending main effect of PPA, although not significant, $F(1, 28) = 3.863$, $p = .059$, $\eta^2 = 0.121$, OP = 0.475 (Table 4).

3.4. Summary of results

This study found that repeated cat odor exposure in early adolescence was aversive to the rats and induced anxiety-like behaviors during the exposure period. When the adolescent and adulthood periods were combined, early adolescent repeated cat odor exposure resulted in elevated anxiety-like behaviors. A main effect of PPA was observed, such that PPA decreased activity levels as assessed through horizontal and vertical locomotor variables when adolescent and adulthood were combined. PPA administered rats also showed a

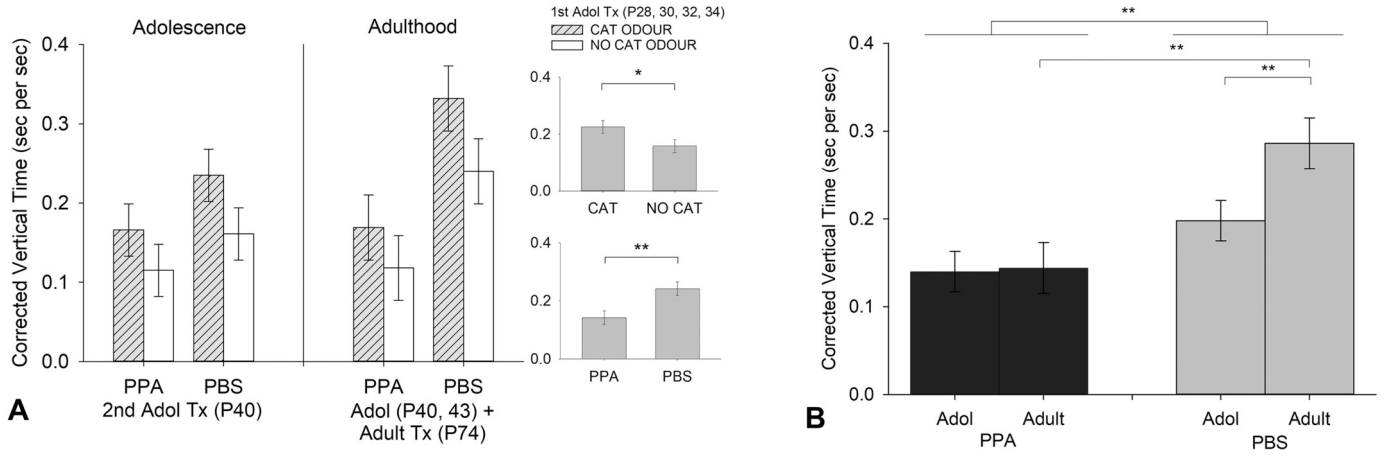

Fig. 6. (A-B). Corrected vertical time in the light chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the light chamber. (A) A main effect of cat odor was found.

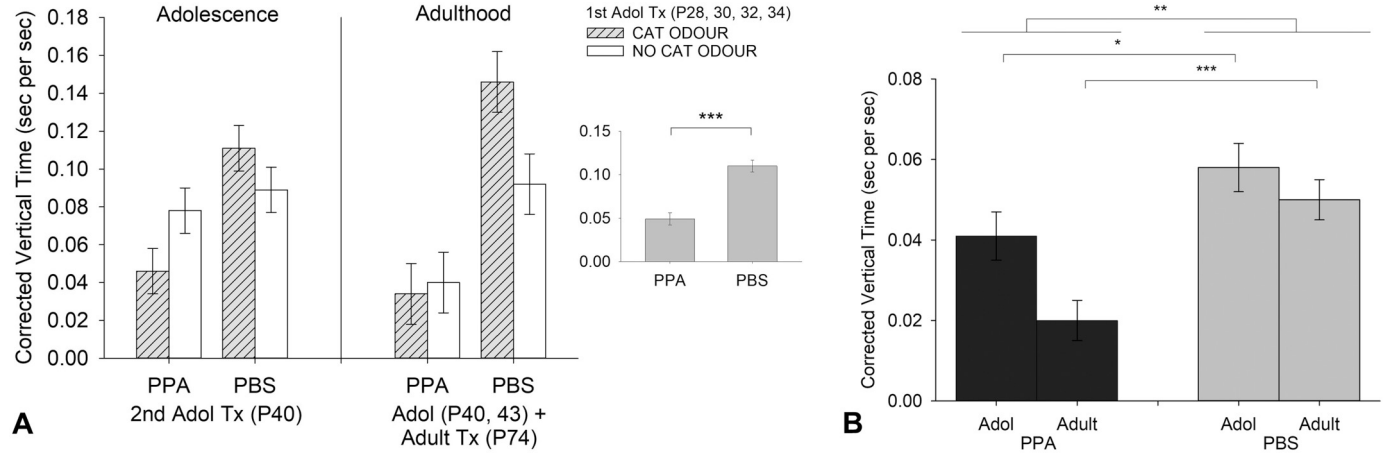

Fig. 7. (A-B). Corrected vertical time in the dark chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections were made by dividing the duration spent in the dark chamber. (A) A main effect of PPA when combining adolescence and adulthood was found, such that PPA-treated rats produced less corrected vertical time than the PBS controls in the dark chamber. (B) This effect was observed because both PPA in adolescence and adulthood spent less time in vertical exploration than the PBS controls in adolescence and adulthood, respectively. *$p < .05$, **$p < .01$, ***$p < .001$. Data is represented as mean ± SEM.

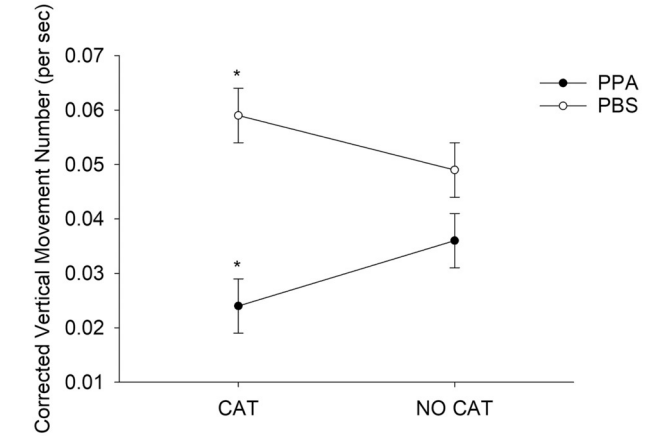

Fig. 8. Corrected vertical movement numbers in the dark chamber of the light-dark apparatus on P40 and P74. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, and P74 (n = 8/group). Corrections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

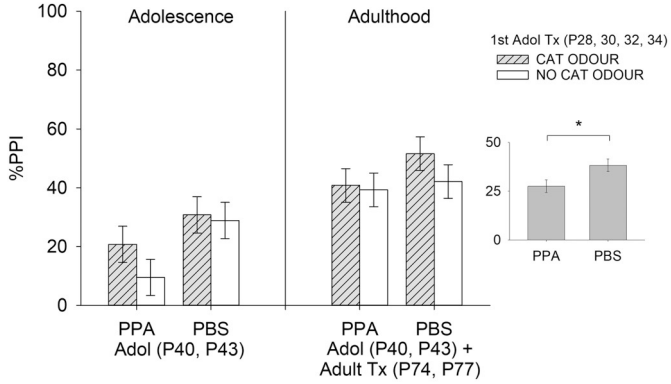

Fig. 9. % Prepulse inhibition for a 76 dB prepulse with a baseline of 70 dB followed by a 120 dB startle pulse on P43 and P77. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, P74, and P77 (n = 8/group). A main effect of PPA when combining adolescence and adulthood was found, such that PPA-treated rats showed a decreased %PPI to a 76 dB prepulse than the PBS controls. *$p < .05$. Data is represented as mean ± SEM.

decreased % PPI for a 76 dB prepulse when adolescent and adulthood were combined. Finally, prior repeated cat odor exposure induced an antagonistic effect of PPA on escape behaviors, such that cat odor increased vertical activity and prior cat odor exposure potentiated PPA's reduction of vertical movement numbers.

4. Discussion

The early adolescent repeated cat odor exposure elicited aversive and anxiogenic responses during the exposure period to the cat odor stimulus. These anxiogenic responses persisted when tested later in adolescence and in adulthood. Further, PPA administration decreased locomotor activity and sensorimotor gating as assessed by prepulse inhibition of the acoustic startle response. PPA's depression of escape behaviors were potentiated by prior predator stress experienced in early adolescence.

Table 4
Acoustic startle response and % prepulse inhibition of an 82 dB prepulse trial.

Treatment		Acoustic Startle Response		%PPI 82 dB	
		Adolescence	Adulthood	Adolescence	Adulthood
Cat Odour	PPA	524.829 ± 163.120	518.462 ± 220.579	24.234 ± 6.835	54.565 ± 4.267
Cat Odour	PBS	887.500 ± 163.120	864.325 ± 220.579	39.675 ± 6.835	62.233 ± 4.267
No Cat Odour	PPA	605.225 ± 163.120	429.763 ± 220.579	31.079 ± 6.835	60.602 ± 4.267
No Cat Odour	PBS	598.388 ± 163.120	765.138 ± 220.579	40.671 ± 6.835	62.741 ± 4.267

N.S. differences were observed. Data is represented as means ± SEM.

4.1. Early adolescent repeated cat odor exposure is aversive and anxiogenic

Early adolescent repeated cat odor exposure produced immediate aversive and anxiogenic behaviors indicative of stress and fearful responses in the rats. The anxiety behaviors displayed by the rat did not habituate throughout the cat odor exposures. During all of the exposure days, rats exposed to cat odor spent more time in the opposite perimeter of the cat odor exposure area. This is consistent with previous research showing that when rats are exposed to a predator odor, they spend greater amount of time in regions furthest away from the odor source [10]. Previous research also suggests that when rats are given a hide box in which they can hide from the predator odor, they tend to spend most of their time in this hide box and produce a "head out" stance of risk assessment in which they face the predator odor stimulus without turning their heads [6,31]. This is an adaptive response to potentially threatening stimuli. Additionally, rats exposed to the predator odor chose to spend less time in the area of the cat odor stimulus than their controls. This may arise from controls rats investigating a novel stimulus and/or cat odor exposed rats spending less time at the worn cat collar stimulus due to its aversive nature. The rats showed no significant difference between the cat odor and no cat odor groups when analyzing each exposure day separately for the time spent in the cat odor exposure area on the first day of exposure (P28). This suggests that the rats were stressed by the novel open field. They were likely more focused on the stress associated with the open field. However, on P30, P32, and P34, the aversion to the predator odor stimulus and/or the attractiveness to the unworn cat collar was evident.

In the present study, rats' avoidance behavior, demonstrated by the duration spent in the opposite perimeter of the cat odor exposure area and the odor exposure area itself, did not habituate. However, within the cat odor exposure area, the rats explored the cat collar stimulus to a greater extent on the fourth exposure day (P34) than on the first exposure day (P28) as shown by the vertical measures of activity corrected for the time spent in this region. This is not surprising as previous studies show increased stimulus investigation after 5 repeated cat odor exposure sessions [2], which suggests an increasing interest in the stimulus and/or decreased aversion to the stimulus. Results of studies on habituation to predator odor are inconclusive, with some investigation showing that avoidance behaviors do not habituate [8,32,33], while others have found that certain behaviors habituated over time to the stimulus [6,34]. Plasma corticosterone levels have also been shown to increase across chronic exposure days independent of changes in locomotor activity, which suggests that habituation did not occur [8]. Contrary to this, other researchers including Dielenberg and McGregor [34] found that hiding behavior did habituate in response to a worn cat collar over daily repeated exposures. Further, McGregor et al. [6] found that the immobility response decreased over time.

When the rats were later tested on an anxiety measure, the light-dark test, cat odor exposure produced an anxiogenic effect in later adolescence and adulthood. The cat odor exposed rats showed fewer nosepokes into the light chamber than their controls, they showed hyperactivity in the light chamber, as well as increased time in vertical position in the light chamber, indicating an elevated motivation to escape. Given that these light-dark testing sessions occurred six days and forty days following the cat odor exposure, this study supports prior research indicating that predator stress can induce long-term anxiogenic effects as assessed by anxiety behavioral assays [2,35,36]. This sustained psychological stress response exhibited by the rodent is adaptive in preventing similar life-threatening circumstances in the future.

Although anxiogenic effects were observed, it is also important to consider whether the anxiety response was generalized, since the predator odor exposure and the light-dark testing occurred in the same testing arena. It is then possible that the anxiogenic response observed in the light-dark task could have been conditioned. Conditioned fear would not be surprising as repeated adolescent cat fur exposure elicits conditioned fear by reducing distance travelled and increasing time spent inside of a hide box when rats are tested in early adulthood on P58 [37].

Another possible mechanism by which predatory stress induces long-term anxiogenic effects is through its ability to alter the excitatory and inhibitory balance in neural circuitry. When exposed to a fearful stimulus, excitatory NMDA mechanisms are activated [38]. When NMDA receptors are blocked by an antagonist, anxiety behaviors and risk assessment following exposure to cat odors are reduced when assessed one week later [39]. This suggests that NMDA mechanisms are involved in long-term anxiety-like behavior produced by cat odor stress. These processes are thought to be important in establishing fear-related memories in models of post-traumatic stress disorder and can in part also explain the long-term anxiogenic effects observed.

In addition to the excitatory neurotransmission induced by activation of NMDA receptors, cat odor exposure also increases the release of the inhibitory neurotransmitter, GABA, and decreases its uptake in the hippocampus and cortex [40]. GABA is important in regulating PPI of an acoustic startle response [41]. However, in the present study, no significant treatment effects were observed on the measure of PPI. This was not surprising as previous literature has shown that predator exposure decreases PPI 24 h after exposure, but not after 48 h or after 9 days [42]. Since the present study assessed PPI 9 days and 43 days after cat odor exposure, it is likely the PPI deficits produced by predator odors were not detected.

Although previous studies on early stressors [36] and predator stress show an increased mean startle amplitude [35], the present study found no effect of the stressor on startle response. There likely are methodological differences, for instance, testing in Bazak et al. [35] occurred on P60, while the present study tested the rats on P43 and P77. The cat odor exposure methodology also differed between the studies such that Bazak et al. [35] used acute cat litter exposure on P28 instead of a repeated cat collar stimulus exposure across four days as in the present study.

4.2. PPA administration decreases activity and sensorimotor gating

The results of this study suggest that PPA affects the behavior of both adolescent and adult rats. PPA administration occurred at four separate time points, twice in adolescence and twice in adulthood. The first dose produced acute effects, while subsequent PPA exposures produced both repeated and acute effects. PPA administration reduced activity localized to the dark chamber of the light-dark apparatus, which is considered to be the "safer" chamber of the light and dark chambers of this apparatus. In the present study, the rats administered PPA showed a decreased number of transitions into the light chamber, decreased total distance travelled in the dark chamber, decreased vertical time in the light and dark chambers. This reduction in activity is likely due to the aversive internal state produced by PPA causing a reluctance to move [21].

Although PPA had no effect on startle response magnitude, it did decrease % PPI which is a measure of sensorimotor gating, the ability to filter out redundant auditory information. Sensorimotor gating is an important measure of cognitive dysfunction as it is a symptom of many neurological disorders including schizophrenia, and a subset of individuals with Autism Spectrum Disorder. This sensory filtration of information occurs through a feedforward inhibitory pathway involving the cochlear nucleus, inferior colliculus, superior colliculus, and tegmentum which sends information to the reticular formation [43,44]. When changes are made to this neural circuitry, sensorimotor gating deficits occur.

Given that peripheral administration of PPA has also been shown to induce conditioned place avoidance and conditioned taste avoidance [21], while direct intracerebroventricular administration of PPA has been shown to induce stereotypic behavior, hyperactivity, social impairment, restricted preference for objects, and behavioral rigidity [18,19], there is support for the hypothesis that PPA has central effect [20]. There are a number of manners in which peripheral administration of PPA can elicit central effects. Peripheral PPA has been shown to peak in the brain at 60 min following its administration [45]. PPA can activate microglia of the hippocampus and white matter which release proinflammatory cytokines [18,20]. Peripheral PPA may enter the brain by passing the gut-blood barrier and the blood-brain barrier via a monocarboxylate transporter [46].

Once in the brain, PPA may alter neural circuitry by potentiating glutamatergic and inhibiting GABAergic transmission which may impact the excitatory and inhibitory balance in neural circuitry [20]. GABA is an important neurotransmitter for prepulse inhibition of the acoustic startle response, such that when $GABA_A$ receptors are disrupted by an antagonist, PPI is reduced [43]. As such, inhibiting GABAergic transmission can result in a decreased PPI as observed with PPA administration. Further, PPA can also lead to intracellular acidification by uncoupling gap junctions between neurons and disrupting synaptic transmission in the prefrontal cortex and sensorimotor cortex [47]. Together, these effects of PPA on neural circuitry can explain the deficits in sensorimotor gating.

In addition to the short-lived effects of PPA on behavior and neuronal functioning, PPA's effects in adulthood seemed to decrease in comparison to adolescence. Although PPA was administered at four separate time points, the effect of PPA in adulthood decreased. This was not expected as previous literature has suggested a double-hit model of PPA exposure towards increased dysfunction [48]. Foley et al. [48] administered PPA prenatally and postnatally, and found that PPA induced an increased ASR when tested in adolescence, suggesting elevated behavioral changes produced by repeated PPA administration. The present study did not obtain these same results, and instead found decreased behavioral changes in adulthood when repeatedly exposed to PPA in both adolescence and adulthood. Methodological differences may explain the differences in results, particularly in the timing of PPA administrations. As well, behavioral changes were diminished in adulthood, which suggests that behavioral tolerance may have been produced by repeated PPA exposure. Further research is warranted as this area of research is limited and is necessary to understand the effects of repeated PPA exposure in adolescence and adulthood.

4.3. Prior repeated cat odor exposure potentiates the reduction of escape behavior induced by PPA

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

This antagonistic effect of cat odor exposure on PPA as measured through behavior can be explained by both treatments' effects on excitatory and inhibitory neural circuitry. Cat odor exposure can activate excitatory NMDA receptors [39] to produce long-term anxiogenic behavioral effects. It can also increase the release of the inhibitory neurotransmitter GABA, and decrease GABA's uptake [40]. PPA can also alter the excitatory and inhibitory balance by potentiating glutamatergic excitatory neurotransmission and inhibiting GABAergic inhibitory transmission [20]. These two treatments appear to have slightly different effects, such that cat odor exposure increases the availability of GABA, while PPA decreases the availability of GABA, which may explain their antagonistic behavioral effect on vertical locomotor variables. However, further research is warranted.

Stress is capable of increasing the permeability of critical barriers including the gut blood barrier [49–51] and the blood brain barrier [52,53]. Barrier dysfunction may allow for macromolecules including SCFAs like PPA to bypass the barriers to gain access to the central nervous system. Although the present study uses i.p. injection of PPA, which bypasses the gut-blood barrier, the systemic PPA administered may enter the brain with greater ease than without cat odor exposure.

Another important area of research that may explain the interaction effect observed with cat odor and PPA, is the stress produced by multiple environmental “hits” during sensitive periods of development. Research on many models of neural dysfunction stem from the double hit hypothesis or multiple hit hypothesis including research on schizophrenia [54] and Autism Spectrum Disorders [55].

5. Conclusions

Prior research on PPA has been primarily conducted on male rats, with robust findings indicating PPA's effect on social impairment, restricted preference for objects, and stereotypic behavior [18,19]. PPA has been associated with neurodevelopmental disorders including Autism Spectrum Disorders, which tends to occur more commonly in males than females [15]. As such, male rats were the primary focus in the present study, albeit a limitation, as there is a lack of research on the effect of PPA on female rats. Future research should investigate possible sex differences in the effects of repeated cat odor exposure in early adolescence on anxiety-like behavior, startle response, and PPI in adulthood.

Early adolescent repeated predator odor exposure was aversive to the male rats. When tested on an anxiety measure in later adolescence and adulthood, the prior repeated predator odor exposure produced long-term anxiety-like behavior. This further supports previous research that suggests that the adolescent period is a sensitive period in stress response programming [2]. In addition, PPA administration decreased the rats' motivation to move, while also decreasing the rats' ability to filter out redundant auditory information. Finally, the predator odor exposure condition predisposed rats that were later administered PPA to a more enhanced reduction in escape behavior as assessed on the light-dark apparatus. These results together may suggest that early adolescent repeated predator odor exposure induces stress which in addition to PPA administration may result in imbalances in the excitatory and inhibitory neural circuitry to induce behavioral abnormalities.

Acknowledgments

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Declaration of interest

None.

Appendix

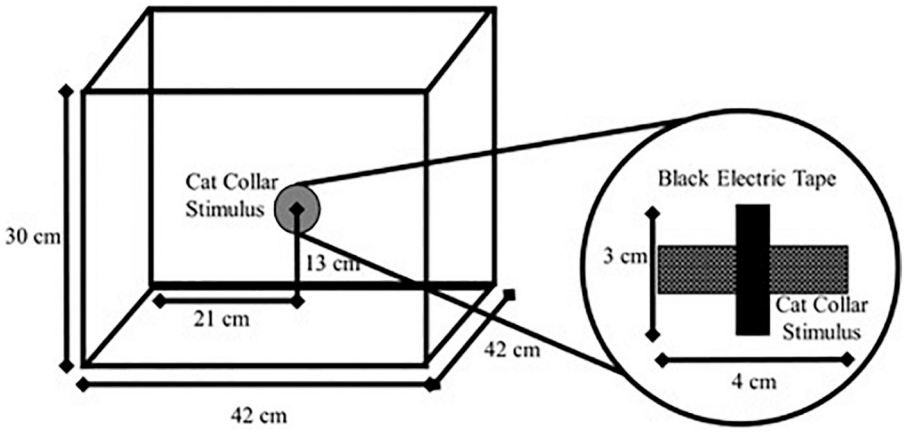

Fig. A.1. Cat odor exposure chamber. Activity monitors were 42 × 42 × 30 cm, with the cat collar stimulus placed 13 cm from the bottom of the apparatus centered on one of the walls of the activity field. Black electric tape was used to tape the 4 cm piece of cat collar onto the wall.

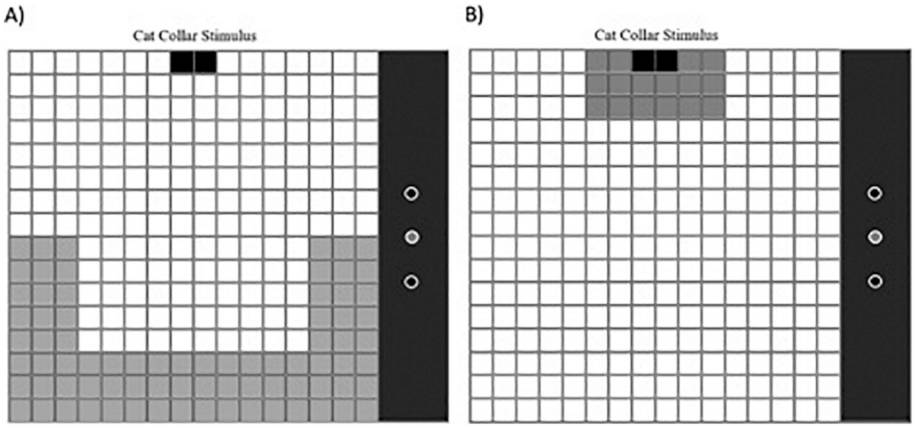

Fig. A.2. (A-B) Diagram of the cat odor exposure field (A) opposite perimeter and (B) cat odor exposure area. Light grey represents the zone analyzed. Black represents the location of the worn or unworn cat collar stimulus. Each activity square is 2.54 cm × 2.54 cm.

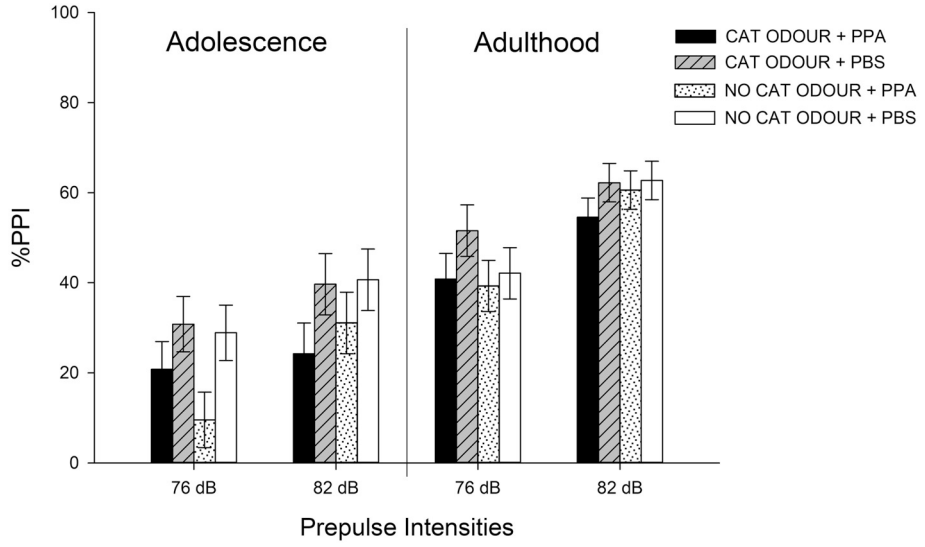

Fig. A.3. % Prepulse inhibition for a 76 dB and 82 dB prepulse with a baseline of 70 dB followed by a 120 dB startle pulse on P43 and P77. Rats were exposed to a cat odor via a worn or no cat odor via an unworn cat collar stimulus on P28, P30, P32, and P34 and injected i.p. with PPA or PBS on P40, P43, P74, and P77 (n = 8/group). Data is represented as mean ± SEM.

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