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The effects of phencyclidine (PCP) on anxiety-like behavior in the elevated plus maze and the light–dark exploration test are age dependent, sexually dimorphic, and task dependent

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ABSTRACT

Previous research in our laboratory revealed sexually dimorphic effects of prior exposure to phencyclidine (PCP) on elevated plus maze behavior. In an attempt to examine the developmental time course of this effect and determine the extent to which it generalizes to other anxiety paradigms, young adult (61–64 days old) and adult (96–107 days old) male and female rats were treated with PCP (15 mg/kg) or saline. Following a two week withdrawal period, animals were tested in either the elevated plus maze (EPM) or a light–dark exploration (LD) test. In adults, both tests revealed a sexually dimorphic effect driven by PCP-induced decreases in anxiety in females as indicated by increased time spent in the open arms of the EPM and in the lit compartment of the LD test and increased anxiety in males as indicated by decreased time spent in the lit compartment of the LD. In young animals, PCP pretreatment decreased open arm exploration in the elevated plus maze, indicating increased anxiety. However, PCP increased time spent in the light compartment in the light–dark exploration test, indicating decreased anxiety. Corticosterone levels measured 15 min after the onset of the EPM failed to reveal an association between the behavioral effects of PCP and corticosterone levels. The results in adults substantiate the previously observed sexually dimorphic effect of PCP on elevated plus maze behavior in adults and indicate that the effect generalizes to another anxiety paradigm. The results in the younger animals suggest an age dependent effect of PCP on anxiety in general and indicate that behaviors in the elevated plus maze and the light–dark exploration test reflect dissociable psychobiological states.

1. Introduction

We have previously shown that prior exposure to a single dose of PCP (15 mg/kg; 1–2 months before testing) produced sexually dimorphic effects on elevated plus maze (EPM) behavior, appearing to increase anxiety in males and decrease anxiety in females (Turgeon et al., 2010). Withdrawal from PCP has been investigated as a pharmacological model for schizophrenia, producing a range of schizophrenia-like behaviors (Jentsch and Roth, 1999), including increased anxiety in males (Audet et al., 2007). Anxiety measures have only infrequently been assessed in animal models of schizophrenia as anxiety has generally been thought of as a separate diagnosis comorbid with schizophrenia rather than a defining feature of the disease. However, the high prevalence of anxiety and associated syndromes in schizophrenic patients has led to the argument that anxiety might be better considered a component of the disease (Bermanzohn et al., 2000; Lysaker and Salyers, 2007). Such observations in humans have lead to the argument that animal models should exhibit distortions in emotional responses such as anxiety (Audet et al., 2007).

A variety of PCP administration paradigms have been used to model schizophrenia. The acute effects of PCP produce a number of behaviors argued to model schizophrenia such as cognitive deficits (Javitt and Zukin, 1991; Ogawa et al., 1994) and deficits in pre-pulse inhibition (Mansbach and Geyer, 1989); however, withdrawal from subchronic PCP has been argued to produce a more consistent model (Jentsch and Roth, 1999). In addition, withdrawal from a single injection of PCP has also been found to produce behavioral changes argued to model schizophrenia in animals. In comparison to controls, prior exposure to a single injection of PCP (15 mg/kg) increased the behavioral response to amphetamine (Turgeon and Roche, 1999), increased escape latency in a water maze (Okuyama et al., 1995) and produced decreases in voluntary sucrose consumption (Turgeon and Hoge, 2003) as well as increases in self-stimulation thresholds (5 and 10 mg/kg; Spieleway and Markou, 2003), suggesting decreased reward function.

While most research on PCP-induced animal models of schizophrenia has utilized males, PCP has a sexually dimorphic effect on sucrose consumption, thought to model the deficit symptom of anhedonia.

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This effect is age dependent; females did not show the characteristic PCP-induced decrease in sucrose consumption until later in adulthood than males (Turgeon et al., 2010). These results seem to parallel evidence from human studies suggesting that females have a later age of onset and a more favorable outcome than males following a diagnosis of schizophrenia (Grossman et al., 2006; Seeman and Lang, 1990; Tang et al., 2007). In this same study, animals were tested 1–2 months later in the EPM which unexpectedly revealed a sexually-dimorphic effect of PCP on EPM behavior as well. However, animals were injected as young adults and tested as adults so the presence of an age dependent change in PCP-induced behavior could not be assessed. Thus the first goal of the current research was to determine whether the effects of PCP on EPM behavior are age dependent. In order to keep drug exposure and testing times within a closer age range, the withdrawal period was reduced to 2 weeks.

In addition to being used as a measure of anxiety, exposure to the EPM serves as a stressor and elevates corticosterone levels (File et al., 1994). Altered behavioral and HPA axis responses to stress have been noted in schizophrenia (Yeap and Thakore, 2005), suggesting that alterations in the stress response in animals may be a valid model of the disease state. Withdrawal from subchronic PCP has been shown to alter the behavioral response to a number of stressors including novel environment (Jentsch et al., 1998; Turgeon et al., 2007) and forced swim (Abdel-Naby Sayed et al., 2001; Noda et al., 1995, 1997, 2000; Turgeon et al., 2007). Studies in humans have found higher baseline levels of cortisol in schizophrenics and have demonstrated a positive correlation between cortisol levels and symptom severity (Walder et al., 2000) as well as neuroleptic-induced decreases in cortisol levels (Meier et al., 2005; Meltzer, 1989). Thus one mechanism by which PCP might be affecting the behavioral response to stressors could involve altered HPA axis regulation. Both acute and chronic PCP elevate CORT levels with 1 h of the final injection (Pechnick et al., 1990); however, the effects of withdrawal from PCP on stress-induced CORT levels have not been reported. Thus the second goal of these experiments was to determine whether PCP-induced changes in EPM behavior are accompanied by changes in CORT levels.

While the EPM is considered a standard measure of anxiety in rodents which has been shown to have pharmacological validity in rats (Pellow et al., 1985), behavior in the EPM does not always predict behavior in other anxiety paradigms. For example, Trullas and Skolnick (1993) found no relationship between EPM behavior and open-field behavior in mice. Another study found that while behavior of Spontaneously Hypertensive Rats (SHR) was consistent between the EPM and the light/dark exploration test, behavior of female Lewis rats was not (Ramos and Mormede, 1998). In addition, Johnston and File (1991) examined sex differences in the EPM, punished drinking and social interaction tests and reported that the three tests did not lead to similar conclusions regarding anxiety. Differences have also been noted in the effects of fluoxetine on elevated X-maze behavior and punished drinking (Handley and McBlane, 1993). Given these inconsistencies across paradigms, the third goal of these experiments was to determine whether the effects of PCP on anxiety as assessed by the EPM generalize to another measure of anxiety, the light–dark exploration test (LD).

2. Methods

2.1. Animals

Fifty-six Sprague–Dawley rats (Charles River, Wilmington, MA) were used for the EPM/CORT experiments and 48 animals were used for the light–dark experiment. Animals arrived in the facility at least 1 week prior to the onset of experiments, were individually housed in hanging wire cages and maintained on a reverse 12-hour light–dark cycle (lights on at 6 pm and lights off at 6 am). They were allowed access to food and water *ad libitum*. Animals were handled daily for a minimum of three days prior to both injection and testing. All experiments were approved by the Institutional Animal Care and Use Committee at Amherst College.

For the EPM/CORT study, animals were either PND 61 (young adult; 20 males and 12 females) or PND 96–106 (adult; 12 males and 12 females) at the time of injection. For the LD study, animals were either PND 62–64 (young adult; 12 males and 12 females) or PND 105–107 (adult; 12 males and 12 females) at the time of injection.

2.2. Drug treatment

Animals were injected with either PCP (15 mg/kg in 2 ml/kg saline, i.p.; Sigma) or saline (2 ml/kg). Half of the males and females in each age group received PCP. Rats were inspected for symptoms of ataxia five minutes following PCP injection to confirm successful injections. Two weeks following saline or PCP injection, animals underwent EPM testing followed by CORT analysis, or LD testing.

2.3. Elevated plus maze

EPM testing took place in a room with black walls illuminated by red fluorescent lighting. The maze was elevated 55 cm above the floor and consisted of a central platform (10 cm × 10 cm) and four arms (50 cm × 10 cm): two closed arms with side walls (40 cm high) two open arms. Two 40 W light bulbs were affixed to the wall 55 cm above the maze at a 45° angle from the outer edge of each open arm.

On the testing day, animals were placed in the center of the maze facing an open arm. Behavior was videotaped and the first five minutes in the EPM were analyzed. Tapes were scored by an experimenter blind to the treatment condition for the number of entries and number of seconds spent in the open arms, closed arms, and on the center platform. Behavioral data from two young adult saline-treated males were lost due to technical difficulties. Based on the literature (Lister, 1987; Pellow et al., 1985), an arm entry was defined as entering the arm with all four paws. Half-entries into different parts of the maze (entries involving only the head and two forepaws) were also assessed as a measure of risk-assessment (Cruz et al., 1994). Locomotion was assessed as total number of full entries into different parts of the maze (open + closed + center). Open entries were analyzed as the percentage of total arm entries (open + closed). In order to account for differences in locomotion, half entries were analyzed with respect to total full entries (half entries/total entries).

2.4. Blood serum collection and corticosterone analysis

Animals remained in the EPM for a total of 15 min and were then removed and anesthetized with a 0.7 ml i.p. injection of sodium pentobarbital (100 mg/ml), decapitated and trunk blood was collected. The blood was put on ice and allowed to clot for 10 min. The remaining liquid was centrifuged at 5000 rpm for 10 min at room temperature. The supernatant was collected and stored at −80 °C. Serum CORT concentrations were measured in duplicate by a rat CORT ELISA kit (Kamiya Biomedical, Seattle) according to the manufacturer's instructions.

2.5. Light–dark testing

Light–dark testing was conducted in the same room as EPM testing, illuminated by an overhead fluorescent light and a 75 W light bulb attached to the wall of the room, 115 cm from the center of the floor of the lit compartment. The box consisted of a dark Plexiglas compartment (30 × 15 × 15 cm) with a black lid and a small door (6 × 8 cm) connecting to a larger, transparent Plexiglas box (46 × 42 × 44 cm) with no lid. Animals were placed in the dark box, the lid was shut, and their

behavior was videotaped for 5 min. Videotapes were scored by an experimenter blind to treatment condition for the amount of time spent in each compartment, the number of transitions between compartments, and the time spent with one, two, or three paws extending from the dark compartment into the light compartment (risk assessment time).

2.6. Statistical analysis

Behavior and CORT levels were analyzed separately in young adult and adult animals using two-way ANOVAs to examine the effects of drug (SAL vs. PCP) and sex (male vs. female). Post-hoc *t*-tests were performed to examine the effects of PCP separately in males and females. In order to avoid the possibility of type I error given the 2 comparisons performed for each behavior in each age group (male-SAL vs male-PCP and female-SAL vs female-PCP), the criterion for significance was adjusted to $p<0.025$ for the post-hoc tests. In addition, in order to enable comparisons with prior studies using EPM and LD analyses, separate analyses of behavior in saline treated controls were performed using two-way ANOVAs with age and sex as variables. Post-hoc *t*-tests were performed to compare males versus females at each age and young adult versus adult animals within sex. Given the four comparisons performed, the criterion for acceptance was adjusted to $p<0.0125$. In should be noted that given the small n's used in these experiments, the possibility of type II errors cannot be excluded.

3. Results

3.1. EPM

Analyses of EPM behavior support an anxiogenic effect of PCP at PND 75 and a sexually dimorphic effect at PND 110–120 (Fig. 1).

3.1.1. Arm time

For open arm time, a 2-way ANOVA in young adult animals supports an anxiogenic effect of PCP as indicated by a drug-induced decrease in open arm time ($F(1,26)=14.92$, $p<0.001$; Fig. 1A) and

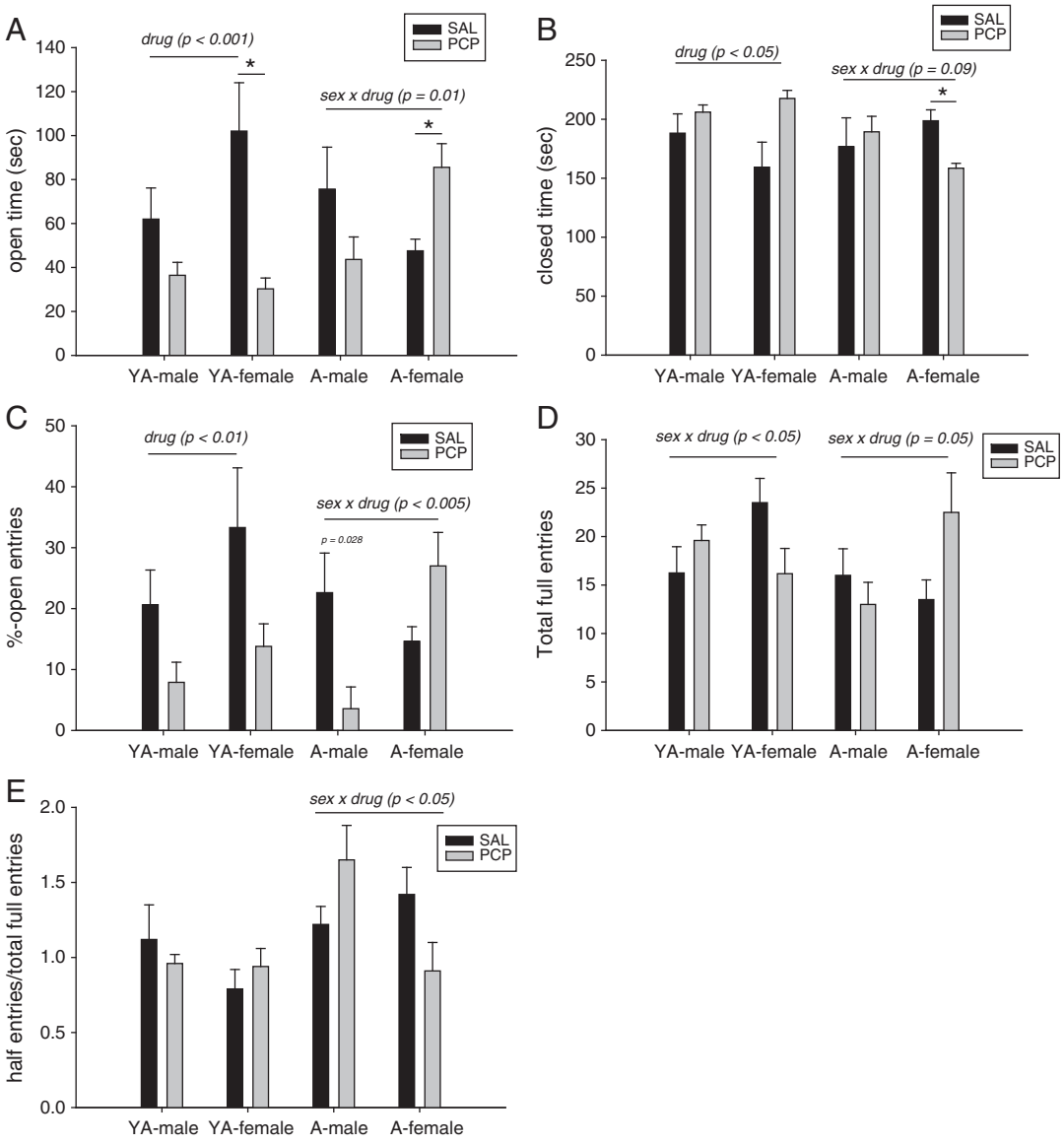

Fig. 1. PCP produced an anxiogenic effect in young adult as evidenced by PCP-induced decreases in time spent in the open arms (A), increases in time spent in the closed arms (B), and increases in the percentage of open arm entries (C) in the EPM. In adults, the effect of PCP was sexually dimorphic as indicated by sex × drug interaction effects. Group differences in locomotion mirrored open arm exploration in adults (D) whereas differences in half entries/total entries were in the opposite direction (E). All values are presented as mean ± SEM. p-Values indicate results of 2-way ANOVAs (sex × drug) in young and adult animals (see Results). *$p<0.025$, post-hoc *t*-test.

increase in closed arm time ($F(1,26) = 8.14$, $p<0.05$; Fig. 1B). Post-hoc *t*-tests revealed the decrease in open arm time to be significant in females ($t(10) = 3.18$, $p<0.025$). In adult animals, a 2-way ANOVA revealed a significant sex by drug interaction effect on open arm time ($F(1,20) = 8.00$, $p = 0.01$), with a post-hoc *t*-test revealing a significant PCP-induced increase in open arm time in females ($t(10) = 3.94$, $p<0.025$). In addition, there was a trend toward a sex by drug interaction effect for closed time ($F(1.20) = 3.18$, $p = 0.09$) and a post-hoc *t*-test revealed a significant decrease in closed arm time in adult females ($t(10) = 3.94$, $p<0.01$).

An additional analysis of open arm time in saline-treated animals was performed in order to compare our data with prior sex difference data from the literature. A 2-way ANOVA revealed a significant sex by age effect in saline-treated animals ($F(1,22) = 4.36$, $p<0.001$) driven by adult females spending marginally less time in the open arms than younger females (Fig. 3A).

3.1.2. Percentage of open arm entries

A 2-way ANOVA in young adult animals supports an anxiogenic effect of PCP as indicated by a drug-induced decrease in the percentage of open arm entries ($F(1,26) = 8.00$, $p<0.01$; Fig. 1C). In adult animals, there was a significant sex by drug interaction effect on the percentage of open arm entries ($F(1,20) = 10.81$, $p<0.005$) accompanied by a marginal PCP-induced decrease in males as indicated by a post-hoc *t*-test.

It should be noted that the PCP-induced increase in open arm time in adult females could reflect an increase in activity in this group as there was an insignificant increase in total entries in PCP-treated adult females. In order to examine this question more closely, the raw number of open arm entries was also assessed. The results were similar to those seen with %-open arm entries: significant effects of drug in young adults ($F(1,26) = 6.64$, $p<0.05$) and a sex by drug interaction in the adults ($F(1,26) = 9.47$, $p<0.01$) were observed.

3.1.3. Locomotion

Two-way ANOVAs in the two age groups revealed significant sex by drug interaction effects in both young adult ($F(1,26) = 5.15$, $p<0.05$) and adult ($F(1,20) = 4.29$, $p = 0.05$) animals. There were no significant post-hoc effects.

3.1.4. Risk assessment

In addition to full entries, half entries between sections of the maze were assessed as a measure of risk assessment. When the absolute number of half entries was considered, there were no significant effects. However, in order to account for differences in overall locomotion, the number of half entries was analyzed with respect to the total number of full entries into different parts of the maze. Two-way ANOVAs in young adult and adult animals revealed no significant effects of drug or sex in the young animals; however, there was a significant sex by drug interaction in the adults ($F(1,20) = 6.49$, $p<0.05$). There were no significant post-hoc effects.

3.2. Corticosterone

PCP had only marginal effects on EPM-induced CORT levels (Table 1). Two-way ANOVAs revealed a main effects of sex in young adults ($F(1,28) = 62.9$, $p<0.001$) and adults ($F(1,20) = 31.7$, $p<0.001$) on CORT as well as a marginal sex by drug interaction in adults ($F(1,28) = 4.0$, $p = 0.059$).

3.3. Light–dark behavior

Analyses of light–dark behavior suggest an anxiolytic effect of PCP in young adults and a sexually dimorphic effect in adults with PCP-pretreated males displaying increased anxiety and PCP-pretreated females displaying decreased anxiety (Fig. 2). Two-way ANOVAs in young animals revealed significant effects of drug on lit time ($F(1,20) = 9.73$, $p<0.01$; Fig. 2A), dark time ($F(1,20) = 5.34$, $p<0.05$; Fig. 2B), and risk time ($F(1,20) = 7.42$, $p<0.05$; Fig. 2D) but no interaction effects. In adult animals, the 2-way ANOVAs revealed significant sex by drug effects on lit time ($F(1,20) = 19.24$, $p<0.001$), dark time ($F(1,20) = 14.30$, $p<0.005$), and risk time ($F(1,20) = 7.07$, $p<0.05$). Post hoc *t*-tests revealed PCP-induced increases in lit time in adult females ($t(10) = 3.35$, $p<0.01$) and decreases in adult males ($t(10) = 2.90$, $p<0.025$) as well as a PCP-induced decrease in dark time in adult females ($t(10) = 3.15$, $p<0.025$). There were no significant effects on total number of transitions (Fig. 2C).

An additional analysis of light–dark behavior in saline-treated animals was performed in order to compare our data with prior sex difference data. A 2-way ANOVA revealed significant sex by age interaction effect on lit time ($F(1,20) = 7.43$, $p<0.05$) with a post-hoc *t*-test revealing that adult males spent more time in lit area than younger males ($t(10) = 3.51$, $p<0.005$; Fig. 3B) and a marginal difference between adult males and females.

Table 1
Effects of PCP serum corticosterone levels (ng/ml) in young adult and adult males and females following exposure to the EPM. p Values indicate results of 2-way ANOVAs (sex by drug) at each age.

	Males	Females
Young adult (P75)	Sex ($p \leq 0.001$)	
Saline	281.1 ± 22.0	489.2 ± 26.8
PCP	308.9 ± 17.2	516.7 ± 42.0
Adult (P110–120)	Sex ($p \leq 0.001$); sex × drug ($p = 0.059$)	
Saline	238.5 ± 18.9	385.6 ± 24.3
PCP	273.1 ± 17.8	353.0 ± 28.9

4. Discussion

The EPM data demonstrate that prior exposure to a single injection of PCP followed by a two week withdrawal produces sexually dimorphic changes in EPM behavior in adults. These data substantiate prior results from this laboratory reporting similar results 1–2 months following PCP exposure (Turgeon et al., 2010). In addition, the effect of PCP in the EPM is age dependent in females as PCP produced anxiogenic effects in young adults but anxiolytic effects in adults. The effects of PCP on adults in the LD experiment mimicked the results in the EPM, indicating that the effects of PCP in adults generalize to another test of anxiety. Interestingly, while the LD data from the young adults also revealed an age dependent shift in the effect of PCP, the direction of the effect appears to be opposite; PCP-treated young adult animals spent more time in the light, indicating an anxiolytic effect of the drug.

Analyses of risk assessment and locomotion in the two paradigms revealed that these behaviors seemed to parallel increased anxiety and decreased anxiety, respectively. With regard to risk assessment, a sex by drug interaction effect was seen for half entries in the EPM which had a pattern opposite that seen for open time and %-open entries. While post-hoc tests were not significant, the interaction effects were driven by PCP-treated females having an increase in open arm exploration and a decrease in half-entries and vice versa for males. Similarly, in the LD test, the drug effect in young adults and the sex by drug effect in adults on risk time mirrored the effects on time in the dark compartment and were opposite the effects on time in the lit compartment. With regard to locomotion, the sex by drug interaction on the number of total entries in the EPM mirrored the pattern of open time and open entries in the adults. Again, post-hoc tests were not significant, but the interaction effects were driven by PCP-treated females tending to have more total entries as well as more open arm exploration and PCP-treated males tending to have less.

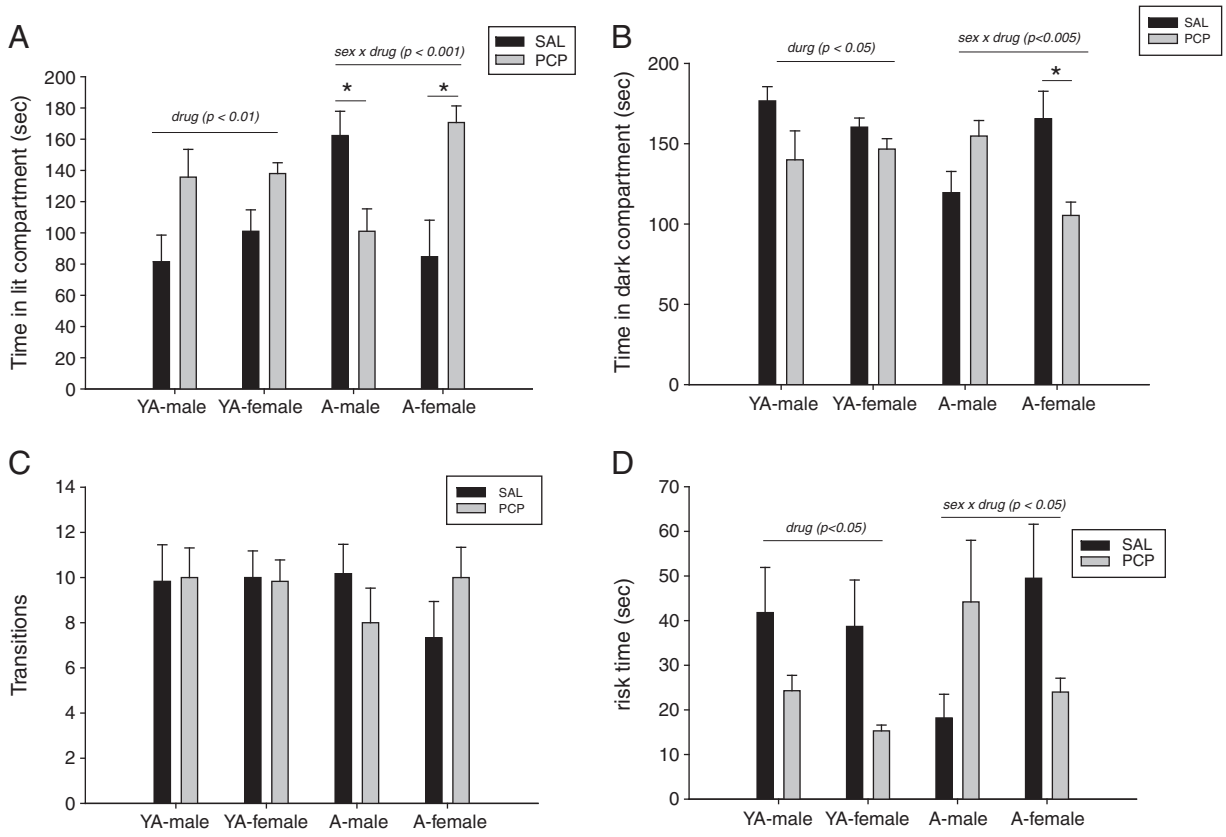

Fig. 2. PCP produced an anxiolytic effect in young animals as evidenced by PCP-induced increases in time spent in the lit compartment of the light/dark test (A) and decreases in time spent in the dark compartment (B). In adults, the effect of PCP was sexually dimorphic; PCP increased time in the lit compartment in females and decreased it in males. There were no group differences in the number of transitions (C). The effects of sex and drug on risk time (D) were opposite the effects on lit time. All values are presented as mean ± SEM. p-Values indicate results of 2-way ANOVAs (drug × sex) in young and adult animals (see Results). *p<0.025, post-hoc *t*-test.

There were no significant overall effects on transitions in the LD; however, the pattern in adults mirrored the sex by drug interaction in lit time.

The observation of a sexually dimorphic effect of PCP in adults parallels the effects of prenatal stress (PNS), another animal model for schizophrenia. Zuena et al. (2008) reported that PNS decreased open arm exploration in Sprague–Dawley males and increased it in females when animals were tested at 3 months. In a similar study, Brunton and Russell (2010) reported that PNS increased anxiety in the EPM in Sprague–Dawley males tested between 12 and 13 weeks but had no effect on females tested at 12 weeks. These results could be argued to be consistent with the present results as the testing age in females was between the ages tested in the present study and may reflect the developmental shift in the effect of PCP on EPM behavior from anxiogenic in young adult females to anxiolytic in adult females. Neonatal lesions of either the mPFC in Wistar males (Schwabe et al., 2006) or the ventral hippocampus in Sprague–Dawley males and females (Beninger et al., 2009), both neurodevelopmental models of schizophrenia, produce decreased anxiety in the EPM around P70. These results are consistent with a non-sexually dimorphic effect of PCP on anxiety in young adults; however, the direction of the effect in the EPM following neurodevelopmental manipulation is opposite that seen following PCP. Consistent with our young adult data, exposure to MK-801 during development increased anxiety in the EPM at P60 in Wistar males (Wedzony et al., 2008). However, the same treatment has been shown to decrease anxiety in Wistar males at P22 (Latyshevea and Rayevsky, 2003) and has no effect on anxiety in Sprague–Dawley males at P73 (Simpson et al., 2010).

Examining anxiety in the PCP model of schizophrenia, McLean et al. (2010) found that a 7-day withdrawal from subchronic administration of a lower dose of PCP (2 mg/kg) did not affect EPM behavior in P80 Lister females. While this is a much lower dose of PCP, these results may also be consistent with the age dependent shift in the effect of PCP in the EPM in females. Audet et al. (2007) reported that a 7-day withdrawal from 7 days of 5 mg/kg PCP per day increased anxiety as evidenced by decreased time in the light in a light/dark emergence test and decreased the number of contacts with a cat collar in P70–80 Sprague–Dawley males. The age of the animals is in between the ages tested in the current study making comparison difficult; however, the results are consistent with our adult findings, but inconsistent with the anxiolytic effects of PCP in the LD test in young adults. Thus a number of manipulations argued to model schizophrenia in animals produce alterations in behavioral measures of anxiety; however, the qualitative nature of the effect varies and age at which animals are tested clearly needs to be taken into consideration. Consistent with the timing of the onset of schizophrenic symptoms in early adulthood, our data suggest that developmental changes in the effects of PCP continue post-puberty.

Given the qualitative differences in the effects of PCP in young adults on behavior in the EPM and the LD experiments, separate analyses of saline-treated animals were performed to enable comparisons with prior studies from other laboratories using these behavioral assays. Both EPM and LD data revealed significant age by sex interaction effects in the saline treated animals; however, the patterns driving the interaction effects differ for the two paradigms. In the EPM, the interaction effect appears to be driven by age-dependent changes in females with young adult females demonstrating less anxious behavior than adult females. In the LD test, the effect is driven by males with young adult males demonstrating more anxious behavior than adult males and adult females showing marginally more anxiety than adult males. It should be noted that while these effects were noted in Sprague–Dawley animals, differences between

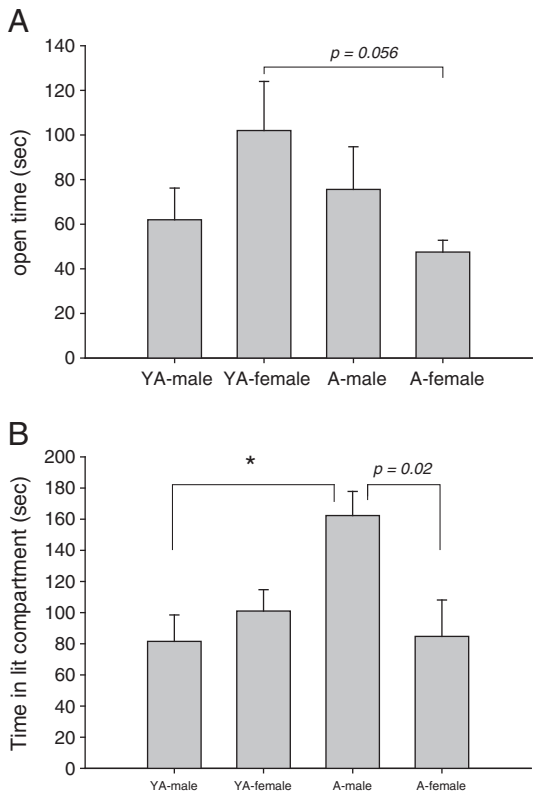

Fig. 3. Separate analyses of behavior in saline-treated control animals revealed significant sex by drug effects in the EPM (A) and the LD (B) test. Note: Data in these figures are re-presented from Figs. 1 and 2 for ease of comparison. *$p<0.0125$, post-hoc *t*-test.

strains in both the EPM and the light–dark test have been noted (Ramos et al., 1997; van den Buuse, 2003; Ferguson and Cada, 2004).

Very few studies have examined developmental shifts in anxiety behavior across an age range similar to that reported here. Torras-Garcia et al. (2005) showed a decrease in anxiety in Wistar males in the EPM from P90 to 17 months. However, Bessa et al. (2005) showed the opposite effect in similarly aged Wistar animals. Lynn and Brown (2009) reported that open arm time increased with age in Lister rats from P30 to P59 and was greater in females; however, adults were not tested. Similarly, Slawecki (2005) reported greater anxiety in Sprague–Dawley males in both the LD and the EPM at P28–32 than P60–70, but older animals were not tested. von Wilmsdorff et al. (2010) reported higher levels of anxiety in Fisher males than females as indicated by latency to enter a lit field from a dark box which is opposite the trend observed in our LD data; however, there were no age dependent changes from 8 weeks to 24 weeks. Imhof et al. (1993) reported a decrease in open arm time with age in Wistar rats from P60 to P120 with males demonstrating the age-dependent decrease by P90 but females not showing it until P120. This observation is consistent with our EPM data in which females showed a decrease from young adult to adult; however, we did not observe a similar decrease in males. Thus, the paucity of data on developmental shifts between young adult and adult makes comparison with prior studies difficult. However, it is clear that behavior in these anxiety paradigms continues to change with age beyond post-puberty and that age needs to be considered an important variable when assessing anxiety.

The apparent disparity in the effects of PCP on behavior in the EPM versus the LD in the younger animals is consistent with a general consensus that different anxiety paradigms may reflect different aspects of emotionality. Ramos and Mormede (1998) argue that an examination of results from a variety of studies suggests that emotionality is likely a multidimentional construct involving a number of factors which may be differentially assayed by various behavioral measures. However, the observation that the EPM and the LD results are consistent in adults suggests that whatever behavioral factors underlie the apparently disparate effects of PCP on behavior in the young adults have changed by adulthood. The post-pubertal timing of this developmental shift in the effect of PCP on anxiety behavior points to possible involvement of the prefrontal cortex (PFC), which is one of the later structures to complete development (Markham et al., 2007) and undergoes shifts in NMDA receptor function between adolescence and adulthood (Wang and Gao, 2009). Lesions to the PFC have been shown to decrease anxiety in the EPM (Lacroix et al., 2000; Shah and Treit, 2003) as has pharmacological inactivation with cobalt (Stern et al., 2010) and the $GABA_A$ agonist muscimol (Shah et al., 2004). The relationship between the PFC and behavior in the LD test is less clear; however, another study involving temporary inactivation with cobalt revealed a decrease in anxiety in associative learning tasks, but an increase in anxiety as assessed by the EPM and the LD (Lisboa et al., 2010). The disparity between the results of these inactivation studies might be due to the age at which the animals were tested. In Stern et al. (2010), animals were tested at 14–16 weeks, corresponding to our adult animals. While the age is not specified in Lisboa et al.'s (2010) study, the weight of the animals was considerably less, suggesting that animals were much younger in that study. However, their observation that EPM and LD behaviors were similarly affected by the PFC manipulation is not consistent with the opposite effects of PCP on behavior in these two tests in young adults.

A number of studies have provided evidence that PFC glutamate activity is related to EPM behavior. Beninger et al. (2009) reported that the neonatal ventral hippocampal lesion-induced increase in open arm exploration in the EPM at P66 was accompanied by a decrease in potassium-evoked glutamate release in the prefrontal cortex in nVHL animals. This result suggests that decreased glutamate activity in the PFC might be associated with increased open arm exploration. This interpretation is consistent with the observation that acute PCP and competitive NMDA receptor antagonists increase open arm exploration (Wiley et al., 1995) as does intra-PFC injection of the $GABA_A$ receptor agonist muscimol (Shah et al., 2004).

The effects of PCP on PFC glutamate function have been examined in both rats and mice. Subchronic PCP (10 mg/kg for 14 days) impairs both presynaptic and postsynaptic glutamatergic functions in the prefrontal cortex and this effect is associated with increased immobility in a forced swim test (Nabeshima et al., 2006; Murai et al., 2007) when tested 24 h after the end of the treatment. Prior experiments in our lab replicated the effect of this PCP regimen on forced swim test behavior in adult male rats (Turgeon et al., 2007). This same regimen failed to produce a significant effect on EPM behavior (unpublished data); however, there was a trend toward increased percent open arm entries in PCP-treated rats which is consistent with the effects of decreased glutamate activity in the PFC (Beninger et al., 2009). The present results reveal a PCP-induced decrease in open arm activity in males and young adult females. Thus, in contrast to results obtained following a one day withdrawal from a subchronic PCP regimen, the apparent association between PFC glutamate function and EPM behavior suggests that a longer withdrawal from a single PCP injection may lead to increased PFC glutamate function. It should be noted that the bulk of these studies were done in males and whether an opposite effect of PCP on PFC glutamate function in females can be inferred from the anxiolytic effect of PCP in adult females depends on whether behavior in the EPM reflects similar underlying processes in females.

This study also investigated the possibility that the effect of PCP on EPM behavior might be related to an alteration in HPA axis function. The observation that CORT levels were higher in females is consistent with sex differences reported in the literature (Beiko et al., 2004; Kant et al., 1983). There was a trend toward a significant sex by drug interaction on corticosterone levels in adults which parallels the behavioral results; however, there were no drug effects in the younger animals.

Due to the absence of a convincing correlation between behavior and corticosterone in the EPM study, levels were not assessed in the LD animals. The lack of a strong association between behavior in the EPM and corticosterone levels suggests that any effect of PCP on HPA axis function is not tightly related to the behavioral effects seen in the EPM. This observation is consistent with Wilson et al. (2004) who reported that changes in EPM behavior and corticosterone levels following diazepam and alcohol were dissociated.

These results support the previously observed sexually dimorphic effect of PCP on anxiety in the EPM in adults and reveal that the effect generalizes to another measure of anxiety, the light–dark test. The observation that the effect of PCP in both of these behavioral assays shifts between young adulthood and adulthood supports the possibility that this model may access maturational processes associated with the postpubertal onset of schizophrenia. In addition, the observation of opposite effects of PCP on anxiety as assessed by the EPM and LD tests in young adults supports the hypothesis that these two paradigms are differentially sensitive to certain aspects of emotionality. Finally, these results underscore the importance of age and sex as variables in interpreting results from the EPM and the LD tests.

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