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Title: Effects of sub-chronic phencyclidine on behaviour of female rats on the elevated plus maze and open field.

Running title: Effects of sub-chronic PCP on anxiety in rats

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Abstract

Female hooded-Lister rats received either sub-chronic phencyclidine (PCP) (2 mg/kg, n=20) or vehicle (1 ml/kg, n=20) i.p. twice daily for seven days, followed by a seven-day washout period. Rats were challenged with acute PCP or vehicle and tested for locomotor activity to ensure hyperactivity was observed in the sub-chronic PCP treated rats. Rats were then tested on the elevated plus maze and in an open field for 10 minutes. Sub-chronic PCP did not significantly affect behaviour on the elevated plus maze or in the open field. In conclusion, sub-chronic PCP does not induce anxiety-like behaviour.

Keywords: Anxiety; Phencyclidine; Female Rat

Introduction

The non-competitive NMDA receptor antagonist, phencyclidine (PCP), has been shown to produce enduring cognitive deficits similar to those observed in schizophrenia (Javitt and Zukin, 1991) particularly when administered sub-chronically rather than acutely (Jentsch and Roth, 1999). PCP produces inactivation of inhibitory control by decreasing GABA release (Yonezawa *et al.*, 1998). This disinhibition of excitatory neurotransmission is referred to as NMDA receptor hypofunction (Olney *et al.*, 1999). In our laboratory sub-chronic PCP administration to adult female rats has produced cognitive deficits in attentional set-shifting (McLean *et al.*, 2008), reversal learning (Abdul-Monim *et al.*, 2006) and novel object recognition (Grayson *et al.*, 2007). Furthermore, we have found females to be better at certain cognitive tasks compared with males, for example novel object recognition, and that this performance is not significantly affected by stage of the oestrous cycle (Sutcliffe *et al.*, 2007). This regimen has also produced social interaction deficits in our laboratory in female rats (Snigdha *et al.*, 2008). In addition, it has been demonstrated that sub-chronic PCP reduces the density of parvalbumin-immunoreactive neurons in the hippocampus (Abdul-Monim *et al.*, 2007), and brain-derived neurotrophic factor (BDNF) levels in cortical regions (Snigdha *et al.*, 2007) in female rats. Similar cognitive impairments (Rodefer *et al.*, 2005), neurochemical deficits (Cochran *et al.*, 2003) as well as metabolic hypofunction (Reynolds *et al.*, 2005) have previously been reported in male rats.

The aim of this study was to investigate whether sub-chronic PCP administration to adult female rats produces anxiety-like behaviour in two tests, namely the elevated plus maze (Pellow *et al.*, 1985) and open field (Walsh and

Cummins, 1976) tests which could confound results in novel object recognition and other cognitive tests.

Materials and methods

Forty adult female hooded-Lister rats weighing 200-250 g were housed in groups of five in standard laboratory conditions with free access to food and water. Light intensity in the holding and behavioural testing rooms was 400-500 lx. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act, UK (1986) and were approved by the University of Bradford ethical review process. At adulthood 20 rats received sub-chronic PCP (2 mg/kg) and 20 rats received vehicle (0.9% saline, 1 ml/kg) i.p. twice daily for seven days, followed by a seven-day washout period. PCP HCl (Sigma, UK) was dissolved in 0.9% saline and injected via the intraperitoneal (i.p.) route in a volume of 1 ml/kg.

The locomotor activity (LMA) response to a novel environment was monitored using automated photocell cages. The movement of each animal was monitored in a Plexiglas chamber (16 x 26 x 19 cm) covered with a compatible Plexiglas lid using AM1052 Activity Monitor (Linton Instrumentation). Rats were habituated to the cages for 2 hours on the day prior testing. On the test day, rats were either given an acute dose of PCP (2 mg/kg, i.p.) or vehicle (0.9% saline, i.p.) and were placed in the LMA boxes immediately after dosing. The acute dose of PCP was chosen based on a previous study showing that 2 mg/kg can produce cognitive deficits without affecting locomotor activity (Grayson, unpublished findings). Therefore, there were 4 test groups (all n=10) sub-chronic vehicle-vehicle, sub-chronic vehicle-PCP, sub-chronic PCP-vehicle, sub-chronic PCP-PCP. Counts were recorded by AmLogger software

(supplied by GSK, Harlow, UK) by means of photo beam interruptions within the chamber. Activity was monitored every 5min over a 120-min period. Rats were challenged to ensure that the sub-chronic PCP regimen was effective. The rats that did not receive the PCP challenge (veh-veh (n=10) and PCP-veh (n=10)) then went on to be tested in the elevated plus maze and open field paradigms.

The elevated plus maze (constructed in house) consisted of four arms elevated 50 cm above the floor. Each arm (12 cm wide, 46 cm long) was joined to the others by a central square (12 cm×12 cm) in a cross-like disposition. A wall (12 cm in height) enclosed two opposite arms, while the other two arms were open. Placing the rat on the platform facing an open arm started the test. The arms of the maze were cleaned between the tests. The experimenter monitored the movements of the rats via a video camera mounted above the maze, and recordings were scored using Hindsight version 1. During the 10 min test session, the number of entries into the open or closed-arms and the time spent in the open or closed-arms were recorded. The rats were considered to have entered an arm when all four limbs were located in an arm of the maze.

The open field arena (constructed in house) consisted of an open box (52 cm wide by 40 cm high by 52 cm long) with black Perspex sides, a white Perspex base and black grid lines dividing the arena into 9 equally sized squares. No additional illumination was placed on the box and the box was placed in the experimental room in such a position where no shadows fell. Placing the rat in the centre square started the test. The arena was cleaned in between tests. The experiment was recorded and scored as described previously for the elevated plus maze. During the 10 min test session, the number of entries into the centre square and the time spent in the centre

square were recorded. The rats were considered to have entered a square when all four limbs were located in that square of the arena.

Area under the curve was calculated for the LMA data using the trapezoid rule, and analysed using post-hoc Bonferroni's multiple comparison test. Independent Student's t-tests were carried out on the elevated plus maze and open field data between the vehicle and PCP treated groups.

Results

Bonferroni's multiple comparison test on the area under the curves (figure 1A) and the total LMA counts (figure 1B) showed that the PCP-PCP group was significantly more active compared to veh-veh ($P < 0.05$), veh-PCP ($P < 0.01$) and PCP-veh ($P < 0.01$) groups. There were no significant differences between the other groups. Rats which had received an acute dose of vehicle were then tested in the behavioural paradigms (vehicle and PCP $n=10$). Rats were excluded from the elevated plus maze study if they did not remain on the maze for the full test session, therefore groups for the elevated plus maze experiment were $n=8$. Behaviour in the open field and on the elevated plus maze was unaffected by sub-chronic PCP with no significant differences observed between groups in time spent in the open/closed arms and number of entries into the arms, or time spent and entries into the centre square of the open field (table 1). As the number of open arm entries appears low in the vehicle group, this parameter was also measured after 5 min, revealing that the number of open arm entries for vehicles was 4.4 ± 0.5 compared to 4.5 ± 0.4 in the PCP group; there was no significant difference between these groups ($P=0.844$).

Discussion

This study examined whether sub-chronic PCP would induce anxiety-like behaviour in the adult rat. The main findings were that sub-chronic PCP had no effect on behaviour in the open field or elevated plus maze. Following an acute challenge with PCP, we observed an increase in locomotor activity in the sub-chronic PCP treated rats, with no effect in vehicle treated animals. Previous studies in male rats, using similar treatment regimes at comparable doses have reported similar findings (Kalinichev *et al.*, 2008). This sensitisation in the sub-chronic PCP treated animals may be related to the disruption of GABAergic interneurons in the medial prefrontal cortex (Abekawa *et al.*, 2007). Taken together these results indicate that the sub-chronic PCP regimen was successful in producing behavioural changes in these animals thus validating the treatment prior to testing in the anxiety paradigms.

The elevated plus-maze (Pellow *et al.*, 1985) involves placing a naive rat in the centre of an elevated plus-maze with two open and two enclosed arms, and allowing it to freely explore (Rodgers and Cole, 1993). It has been suggested that the reluctance of rats to explore the open arms of the maze is caused by fear of open spaces, rather than the novelty of the maze or its height (Pellow *et al.*, 1985). The open field test is also a frequently used test of anxiety (Gray, 1979). Both tests have been pharmacologically validated with anxiolytic compounds increasing, and conversely anxiogenic compounds decreasing the percentage of time spent in the open areas (Pellow *et al.*, 1985; Gentsch *et al.*, 1987; Cole *et al.*, 1995).

In the present study there were no significant differences between vehicle and PCP-treated rats in time spent in each arm or number of entries on the elevated plus maze. There were also no differences in open field behaviour with rats spending a

similar duration in the centre square and making a similar number of entries into the centre. These data suggest that sub-chronic PCP does not have an anxiogenic effect as assessed by the elevated plus maze or open field test. Our findings are supported by studies using a perinatal (du Bois *et al.*, 2008) and sub-chronic pubertal (Schwabe *et al.*, 2006) regimen of PCP dosing which showed no difference in elevated plus maze behaviour in adult female and male rats respectively, suggesting that these PCP regimes do not produce anxiety-like behaviour. However, these data are in disagreement with a recent study by Audet and co-workers. They found that sub-chronic PCP followed by a week washout period resulted in male rats spending less time in the lit area of a lit/dark apparatus (Audet *et al.*, 2007). However, it should be noted that they used a different strain and sex of rat and a higher dosing regimen of 5 mg/kg twice daily for 7 days, compared to our regimen of 2 mg/kg twice daily for the same duration. It is important to note that results vary between laboratories and differences are observed between sexes (see Toufexis *et al.*, 2006) and strains (Hall *et al.*, 2000; Rex *et al.*, 2004).

To our knowledge this is the first study into the effects on anxiety of sub-chronic PCP in female adult hooded-Lister rats. In conclusion, our sub-chronic PCP regimen does not induce anxiety-like behaviour in the open field and elevated plus maze tests.

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Figure 1

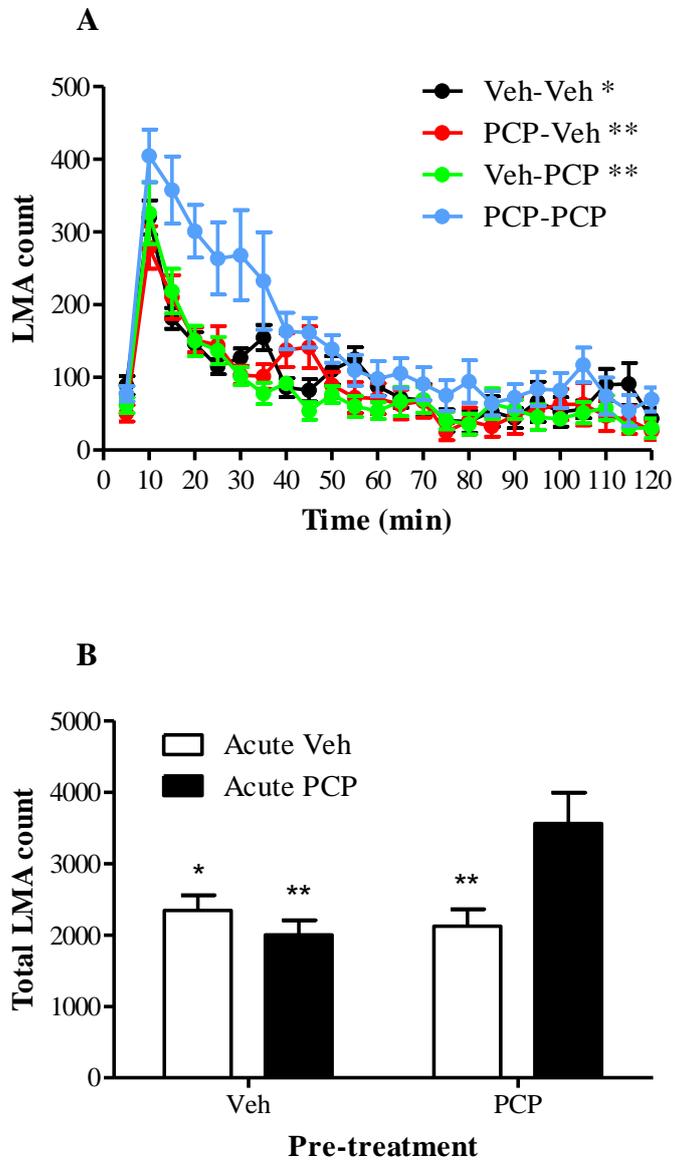


Table 1

Behaviour of vehicle and PCP-treated rats on the elevated plus maze and open field tests.

Behaviour	Vehicle	PCP	P value
Elevated plus maze			
Open arm duration (s)	109.8 ± 11.3	94.2 ± 10.0	0.31
Closed arm duration (s)	377.5 ± 18.0	368.9 ± 14.3	0.71
Centre duration (s)	112.7 ± 22.2	131.0 ± 7.9	0.45
Open arm entries	6.8 ± 0.7	6.6 ± 0.5	0.89
Closed arm entries	12.9 ± 0.7	15.5 ± 1.1	0.07
Centre entries	19.8 ± 1.3	22.3 ± 1.3	0.20
Open arm latency (s)	28.8 ± 6.8	17.4 ± 7.0	0.26
Open field test			
Centre square duration (s)	25.2 ± 5.9	27.1 ± 4.9	0.80
Centre square entries	9.1 ± 1.5	11.1 ± 1.8	0.32
Line crossings	111.4 ± 5.9	111.1 ± 8.1	0.98

Legends

Figure 1: Locomotor activity in vehicle or sub-chronic PCP-treated rats (2 mg/kg twice daily for seven days) following acute challenge with PCP (2 mg/kg) measured **(A)** over a 2-hour period at 5-min intervals and **(B)** as total LMA count after 2-hours (n=10). Post-hoc Bonferroni multiple comparison test on the area under the curves and total LMA showed that the PCP-PCP group was significantly more active compared to veh-veh (*P<0.05), veh-PCP (**P<0.01) and PCP-veh (**P<0.01) groups.

Table 1: Behaviour of vehicle and sub-chronic PCP-treated rats on the elevated plus maze (n=8) and open field tests (n=10). Data are means ± S.E.M. P values were obtained from Independent Student's t-test comparing PCP to vehicle-treated group.