A preliminary investigation into the effects of antipsychotics on sub-chronic phencyclidine-induced deficits in attentional set-shifting in female rats.

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Abstract

Rationale: The NMDA receptor antagonist, phencyclidine (PCP), has been shown to induce symptoms characteristic of schizophrenia. A loss in executive function and the ability to shift attention between stimulus dimensions is impaired in schizophrenia; this can be assessed in rodents by the perceptual attentional set-shifting task. *Objective*: The aim of this study was to investigate whether the deficits induced by sub-chronic PCP in attentional set-shifting could be reversed by sub-chronic administration of clozapine, risperidone or haloperidol. *Methods*: Adult female hooded-Lister rats received sub-chronic PCP (2 mg/kg) or vehicle (1 ml/kg) i.p. twice daily for seven days, followed by a seven-day washout period. PCP-treated rats then received clozapine, risperidone, haloperidol or vehicle once daily for seven days and were then tested in the perceptual set-shifting task. *Results*: PCP significantly ($p<0.01$) increased the number of trials to reach criterion in the EDS phase when compared to vehicle and this deficit was significantly ($p<0.01$) attenuated by sub-chronic clozapine (2.5 mg/kg) and risperidone (0.2 mg/kg), but not by sub-chronic haloperidol treatment (0.05 mg/kg). *Conclusions*: These data show that sub-chronic PCP produced a robust deficit within the EDS phase in the attentional set-shifting task, in female rats. Atypical antipsychotics, clozapine and risperidone, but not the classical agent, haloperidol, significantly improved the PCP-induced cognitive deficit.

Keywords Schizophrenia; Set-shifting; Phencyclidine; Rat; Atypical antipsychotics; Haloperidol

Introduction

Impairments in executive function have long been considered a core feature of schizophrenia [17, 43, 54] as highlighted by the MATRICS initiative [44]. Novel antipsychotics are required to improve cognition as the CATIE study revealed that atypical antipsychotics, such as clozapine, show little efficacy against cognitive symptoms [38]. One aspect of executive function is the ability to modify behaviour in response to the changing relevance of a stimulus; this is frequently assessed in patients using the Wisconsin Card Sorting Test (WCST) [7] with impaired performance seen in patients with schizophrenia [27, 36, 47]. The perceptual attentional set-shifting task represents a rat analogue of the human WCST [8]. It investigates the ability to learn a rule and form an attentional set within the same sorting category (intra-dimensional shift (IDS), as well as the ability to shift attentional set between different sorting categories (extra-dimensional shift (EDS). Analysis of the ID/ED task (from the CANTAB computerised test battery; [15]) in patients with schizophrenia has revealed significant impairments in the EDS phase [57].

The non-competitive NMDA receptor antagonist, phencyclidine (PCP), is a psychomotor stimulant drug that has been shown to induce symptoms characteristic of schizophrenia [30, 31]. PCP and its structural analogue ketamine produce inactivation of inhibitory control by decreasing GABA release [58]. This disinhibition of excitatory neurotransmission is referred to as NMDA receptor hypofunction [32, 45, 46]. Acute administration of PCP in rats has been shown to produce deficits in the novel object recognition-NOR-task [21], an operant reversal learning paradigm [1, 28] and attentional set-shifting [16].

Acute dosing with PCP has limitations in modelling the chronic psychotic illness or the persistent cognitive deficits of schizophrenic patients. Alternatively, repeated sub-chronic exposure to psychomotor stimulant drugs such as PCP, and d-amphetamine, produce enduring behavioural, molecular, structural and cellular changes, which are believed to mimic schizophrenia more accurately than acute dosing [18, 32, 56]. Post-mortem analysis of brain tissue from schizophrenic patients has shown a reduction in GABAergic interneurons in the frontal cortex [5] and hippocampus [6]. We have recently demonstrated reduced density of parvalbumin immuno reactive neurons in the hippocampus and M1 (motor area 1) region of the frontal cortex following sub-chronic PCP treatment in the rat [3]. It has also been shown that chronic intermittent administration of PCP decreases parvalbumin mRNA expression in the prefrontal cortex of rats, and that this reduction can be reversed by clozapine but not haloperidol [11]. These findings provide further support for the theory that sub-chronic PCP treatment in the rat provides a good correlate of the clinical condition. Indeed, in our laboratory, administration of sub-chronic PCP to female rats (2 mg/kg twice daily for 7 days followed by 7 days drug free) produces deficits in reversal learning, attenuated by atypical but not classical antipsychotics [2]. Recently we have found that sub-chronic PCP-induced deficits in NOR are reversed by acute clozapine and risperidone, but not haloperidol [22]. Rodefer and colleagues combined the attentional set-shifting test with a sub-chronic dosage regimen (5 mg/kg twice daily for seven days followed by a ten-day drug washout period) and found a selective deficit in the EDS phase [49].

The aim of this study was to investigate whether the deficits induced by our dosage regimen of sub-chronic PCP in attentional set-shifting could be reversed by sub-chronic administration of clozapine, risperidone or haloperidol.

Materials and methods

Subjects

Fifty female hooded-Lister rats (Harlan, UK), weighing 200-220 g at the start of the study, were used. Animals were housed in groups of five, in a temperature-controlled room (21 ± 2 °C) at humidity of 45-55%, and maintained on a 12:12 h light/dark cycle (lights on at 0700h). Experimental procedures were performed in the light phase. All rats had free access to food (Special Diet Services, UK) and water until 1 week before testing began, at which point a minimal food restriction was imposed upon them (90% of free feeding bodyweight) but *ad libitum* access to water continued. 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.

Locomotor activity

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. Counts were recorded by means of photo beam interruptions within the chamber. Activity was

monitored every 5min over a 60-min period and summed to give a total count. 10 vehicle and 10 PCP-treated rats were tested in these chambers.

Set-shifting apparatus

The set-shifting apparatus was a modified version of that described originally by Birrell and Brown [8]. The test box was essentially a modified home cage measuring 59 x 35 x 24 cm made of transparent plastic (Fig. 1). A fixed 1 cm thick Plexiglas panel permanently divided 1/3 of the length of the box into two separate compartments (choice areas). These two choice areas could be further isolated from each other with the aid of two removable Plexiglas panels that were lowered into place along wall mounted vertical sliders. The digging bowls were placed in each choice area, and the rat was given access by lifting the divider(s) which could also be used to deny access to the bowls, such as when an incorrect choice was made. A third removable panel divided the remaining 2/3 length of the larger compartment into two sections when in place, along the horizontal (short) axis of the test box.

Habituation and training

Habituation to specific aspects of the testing paradigm commenced immediately following the end of the seven-day drug washout period. Habituation to the testing box occurred for 1 hour on 3 consecutive days prior to training. Two smooth ceramic circular digging pots (measuring 9cm in diameter by 5cm internal depth) identical to those intended for use during the testing phases were introduced to each home cage. Each bowl was filled with grade 5 sawdust (also covering the home cage floor) and baited with food

reward in the form of several 1/4 honey nut loops (Kellogs, UK). Digging bowls were continuously re-baited during this habituation period and remained in the home cage for the duration of the study. Following habituation, all rats had to successfully complete the entire training regime in order to proceed to testing. Rats were first trained to dig quickly and reliably in both bowls by progressively covering a single food reward per trial with incrementally thicker layers of digging media. Once a rat had repeatedly demonstrated that it had acquired the training procedure, the dividing panels were introduced. Digging itself was defined as a vigorous movement of front paws to displace digging media and obtain food reinforcement buried 2-2.5cm below surface level.

The second phase of training introduced the concept of simple discrimination (SD) between first, medium and then, odour. Rats were presented with identical digging bowls that had been anointed (smearing a few drops of oil around the rim of the bowl using a tissue) with two aromatic oils (Bodyshop, UK), only one of which was baited with food reward. Placement of bowls in either the left or right compartment was randomised with the aid of an adapted pseudorandom Gellerman schedule. Rats were permitted to explore both bowls for the first four trials irrespective of which bowl they dug in first, thereby acting as an opportunity to associate food reward with the positive predictor. Subsequent incorrect selections ended a trial without the opportunity to explore the correct bowl. A criterion for successful learning of each discrimination was set at 6 consecutive correct trials. All exemplars used in training were not used during testing or at any other point in the study.

Testing paradigm

In all cases rats were tested in the attentional set-shifting procedure 24 hours after training. A trial was initiated by raising both dividers to give access to both digging bowls, only one of which was baited. The first stage was the simple discrimination (SD), which was identical to the simple discrimination in the training session on the previous day, except new exemplars were used. Testing continued until the rat reached a criterion of six consecutive correct responses. In a test session, rats performed a series of discriminations (see Table 1). For the compound discrimination (CD) a second dimension was introduced (odour), but the correct and incorrect exemplars remained the same (digging medium). For the reversals the exemplars and relevant dimensions remained the same (medium) but the rats had to learn that the previously baited odour was now incorrect and the other odour was now the correct one. New exemplars were used for the ID and ED shifts. The specific exemplars used are shown in Table 2. For the ED shift the previously irrelevant parameter (i.e. odour) was now relevant. It has been shown that rats find the difficulty of each discrimination change, i.e. medium to odour or odour to medium, equivalent [8]. Therefore in simple discrimination digging medium was the relevant parameter for all rats.

Drugs

The study design involved five groups (n = 10 per group initially); rats were given either 2 mg/kg PCP (n = 40) or vehicle (0.9% saline; n = 10) by the intraperitoneal (i.p.) route twice daily for seven days. Dosing with sub-chronic PCP or vehicle was followed by a washout period of a further seven days. Following the washout period, PCP-treated rats

were sub-chronically dosed for seven days (daily, i.p. route) with vehicle (0.9% saline), clozapine (2.5 mg/kg), haloperidol (0.05 mg/kg) or risperidone (0.2 mg/kg). Rats were trained 30 minutes after dosing on the seventh day, and they were tested on day 8 30 minutes after drug administration.

The dose of haloperidol was chosen on the basis of a previous study showing that 0.05 mg/kg haloperidol significantly attenuated a d-amphetamine-induced reversal learning impairment in female hooded-Lister rats [28]. Furthermore, this dose of haloperidol has been shown to occupy 50% of dopamine D_2 receptors [34, 35]. The doses of clozapine and risperidone were chosen on the basis of our previous work showing efficacy against sub-chronic PCP in the reversal learning paradigm [2, 42] and NOR task [22]. PCP HCl (Sigma, UK) was dissolved in 0.9% saline. Haloperidol (Serenace liquid, 2 mg/kg, Baker, UK) was diluted with distilled water. Clozapine (Tocris, UK) and risperidone (provided by GSK, UK) were dissolved in a minimum volume of acetic acid, made up to volume with distilled water and pH adjusted to 6 with 0.1M NaOH.

Antipsychotics were given sub-chronically as a pilot study showed that acute dosing with clozapine and haloperidol prevented rats from being able to attend to the task due to slight sedation. We used female rats in this experiment as a recent study in our laboratory showed that PCP produced a more robust effect in females in this task when compared to males [39] and more pronounced reductions in brain derived neurotrophic factor-BDNF [53]. This may be due to increased sensitivity of female rats to PCP, potentially caused by differences in pharmacokinetics [41, 51]. We have also shown that there is no effect of the oestrous cycle on cognitive performance in other tasks, also of

relevance to schizophrenia, such as NOR and that females perform better in the NOR task compared with males [55].

Data and statistical analysis

LMA data were analysed by Student's unpaired t-test and are expressed as total counts in a 60-min period. Data for total trials to criterion (TTC) are expressed as mean ± SEM. Data were analysed by a two-way ANOVA (set-shifting phase x treatment group), followed by post-hoc analysis using Dunnett's t-test. All analysis was performed using SPSS for Windows software (SPSS Inc., Version 13).

Results

Locomotor activity (LMA)

Student's unpaired t-test showed that PCP-treated rats were not significantly different in total LMA over the 60-min test period compared to vehicle-treated rats [$p = 0.46$; Fig. 2].

Attentional set-shifting performance

A total of 42 rats were successfully trained and tested, rats were excluded from the study due to incompletion of the training within a single session as rats were required to complete the simple discriminations in training within 30 trials. Extra trials were not given to ensure all rats had the same level of training before testing. Treatment groups were vehicle + vehicle ($n = 8$), PCP + vehicle ($n = 9$), PCP + clozapine ($n = 9$), PCP +

haloperidol (n = 7) and PCP + risperidone (n = 9). Drug treatment did not have any effect on performance during habituation or training (data not shown).

A two-way ANOVA [drug treatment (vehicle, PCP, clozapine, haloperidol, risperidone)] x [set-shifting phase (SD, CD, Rev1, IDS, Rev2, EDS, Rev3)] showed a significant interaction [$F_{4,37} = 5.4$; $p<0.01$]. Total trials to criterion were observed to be lowest during the simple and compound discriminations and IDS phases with all rats requiring less than 7 trials [Fig. 3]. An increase in trials to criterion in reversal 1 was observed in all treatment groups, although these were not significant. Haloperidol-treated rats required the most trials in reversal 1 to reach criterion [11.4 ± 1.4] compared to vehicle-treated rats [7.3 ± 0.6], however this increase was not significant [$p = 0.08$]. Rats required fewer trials to reach criterion in the IDS phase (6.0 ± 0) than the EDS phase (6.9 ± 0.6) and also completed the IDS trials more rapidly than the EDS trials (data not shown). Post-hoc analysis within the EDS phase [Fig. 3] revealed that PCP significantly [$p<0.01$] increased the number of trials to reach criterion when compared to vehicle [from 6.9 ± 0.6 to 15.7 ± 2.8]. This deficit was significantly [$p<0.01$] attenuated equally by sub-chronic clozapine [7.3 ± 0.6] and risperidone [7.3 ± 0.6], but not by sub-chronic haloperidol [14.3 ± 1.3].

Discussion

The aim of the present study was to examine the effects of a sub-chronic PCP treatment regimen on attentional set-shifting ability in female rats and to investigate the effects of sub-chronic administration of antipsychotics. The rodent version of this test was adapted by [8] from a previous version used in primates [48] and is a useful analogue of the

WCST [7]. This task requires rats to initially learn a rule, and then switch their attention to a new stimulus dimension, demonstrating cognitive flexibility.

The present results show that in female rats, sub-chronic PCP produced a deficit in the ability to switch from one attentional set to another, indicated by a poor performance in the EDS stage of the task. These deficits were reversed by sub-chronic administration of clozapine and risperidone, but not haloperidol. There was no significant difference in locomotor activity observed between vehicle and sub-chronic PCP-treated groups; therefore it may be assumed that the cognitive deficit produced by sub-chronic PCP was independent of effects on locomotor activity.

In this study rats readily learned to discriminate food bowls based on the digging medium and odour, and also refrained from digging in the un-baited bowls, therefore making few incorrect decisions. In both vehicle and PCP-treated rats the first reversal (R1) required more trials to reach criterion compared to earlier stages of the task. This is supported by data showing a significant increase in trials to criterion in control rats [26]; however, other studies have shown more modest increases [8, 16, 18].

Vehicle-treated rats required fewer trials to reach criterion in the IDS phase than the EDS phase and completed the IDS trials faster, suggesting that an attentional set was formed. However, lack of a significant increase in trials to criterion from IDS to EDS does not support the idea that an attentional set had been formed in our control rats. One aspect of our study that may have influenced our data set is that, before each new discrimination begins, all rats have 4 discovery trials to learn the new rule; it is therefore possible that vehicle rats learned the EDS rule within these 4 trials thus improving their performance; this may also explain the occurrence of minimum trials to criterion without

any apparent errors in certain discriminations. There was a significant increase in trials required to reach criterion in PCP-treated rats only in the EDS stage, suggesting that sub-chronic PCP causes a selective deficit in attentional set-shifting ability. These results are supported by a previous study where PCP at 5 mg/kg twice daily for seven days followed by a ten-day drug washout period, in male Long-Evans rats, selectively impaired the EDS without affecting other discrimination tasks or reversals [49]. Acute PCP (2.58 mg/kg) also produces a deficit in set-shifting ability in rats [16]. However, repeated exposure i.e. sub-chronic administration of PCP is believed to mimic schizophrenia more closely than acute administration [32]. The success of the treatment regime used in this investigation is further supported by a comparative study of sub-chronic PCP dosing regimes by Gartlon *et al.* [19] who reported that a bi-daily (7-day) regime, but not a 26-day intermittent regime, produces cognitive deficits in the NOR task. Furthermore, recent studies have shown that long-term administration of PCP (3 mg/kg once per day administered Monday, Wednesday and Friday for 5 weeks, and 10 mg/kg daily for 14 days) did not impair set-shifting ability in male rats [13, 18]. It is important to note that PCP is not the only pharmacological means of producing schizophrenia-like impairments, for example amphetamine sensitised rats show deficits in sensorimotor gating, in addition to cognitive impairment in tasks such as attentional set-shifting [18, 56].

It is clear that PCP given at various dosing schedules and in both sexes induces cognitive deficits. However, there is evidence that the dosing schedule used here produces robust deficits in female rats as we have demonstrated long-term deficits in reversal learning [2, 3], NOR performance [22] and now attentional set-shifting in addition to neurobiological changes of relevance to schizophrenia such as reduced

parvalbumin and BDNF [3, 53]. These PCP-induced behavioural deficits in reversal learning and NOR are improved by atypical but not classical antipsychotics [2, 22, 42]. Although beyond the scope of this manuscript, a full examination of sex and dosing schedule differences in the effects of PCP in different paradigms is required.

Our study is not without limitations; e.g. the lack of a significant impairment in performance of vehicle treated animals from the IDS to the EDS phase. The use of 4 discovery trials, as previously mentioned, before each new phase of the task may be an explanation for this. The use of the discovery trials could explain this and also the apparent lack of errors as rats could learn the rule within these trials, and therefore not make any errors during the test. A further limitation of the current experimental design was the decision not to counterbalance the dimension shifts, i.e. all the rats were switched from medium to odour. Indeed, it would have improved our study if half the rats were switched from odour to medium at the EDS phase, although it has previously been shown that rats find the difficulty of each discrimination change, i.e. medium to odour or odour to medium, equivalent (Birrell and Brown, 2000). The use of only one dose of antipsychotics is another limitation, a dose response should ideally have been conducted; however, in order to limit the number of animals used, and length of testing, we carefully selected one active dose of each antipsychotic. The doses of clozapine and risperidone were chosen on the basis of our previous work showing efficacy against sub-chronic PCP-induced deficits in reversal learning [2, 42] and NOR [22]. The dose of haloperidol was chosen on the basis of our previous work showing that 0.05 mg/kg haloperidol significantly attenuated a d-amphetamine-induced reversal learning impairment in female hooded-Lister rats [28]. We believe a higher dose of haloperidol would not have been

any more effective as a dose of 0.075 mg/kg haloperidol was less efficacious to reverse the amphetamine deficit in reversal learning when compared to the dose of 0.05 mg/kg [28] and a dose of 0.1 mg/kg was shown to impair performance [1]. Furthermore 0.05 mg/kg haloperidol has been shown to occupy 50% of dopamine D_2 receptors [34, 35].

It has been widely reported that atypical antipsychotics have some beneficial effect on cognitive deficits [9, 23, 24, 40, 50]. However, it is important to appreciate the limitations of models such as this one as, although clozapine and risperidone effectively reversed the cognitive deficits observed in this experiment, the CATIE study highlights the fact that these antipsychotics do not provide consistent improvement of cognitive symptoms in patients [38]. Clearly, there is a need to develop more robust cognitive deficit models which are only partially improved by currently available antipsychotics, in order to correspond more closely with the clinical situation and allow for assessment of improved therapies.

The current study shows that the PCP-induced deficit is improved by sub-chronic administration of clozapine and risperidone, but not haloperidol. As mentioned above, these results are in agreement with previous studies in our laboratory. In addition, we have recently shown that sub-chronic administration of clozapine (5 mg/kg) but not haloperidol (0.05 mg/kg) can prevent the PCP-induced cognitive deficit when administered in conjunction with the daily sub-chronic PCP treatment regimen [29].

In this study, antipsychotics were given sub-chronically as a pilot study showed that acute dosing with clozapine and haloperidol prevented rats from being able to complete the task. In order to make a direct comparison, risperidone was also given sub-chronically. Didriksen and colleagues used doses of 0.63 and 1.3 mg/kg of clozapine for

their reversal learning study as their pilot study showed worsening of performance with higher doses of clozapine in combination with PCP [14]. It has also been shown that tolerance can develop to the sedative effects of clozapine after repeated dosing [10]. Our data are supported by a study by Hashimoto and colleagues who showed that acute administration of clozapine (5 mg/kg) and haloperidol (0.1 mg/kg) did not improve a sub-chronic PCP-induced cognitive deficit in the NOR task in mice; however, they found that sub-chronic administration of clozapine (5 mg/kg daily for 14 days) significantly improved the deficit, whereas sub-chronic haloperidol (0.1 mg/kg daily for 14 days) did not [25].

The current results show that sub-chronic administration of haloperidol did not significantly improve the deficit in set-shifting performance induced by PCP. This is supported by clinical data as patients chronically treated with haloperidol have shown impaired performance in tests of working memory and executive function [20]. The inability of haloperidol to reverse the PCP-induced deficit may be due to its high D_2 receptor affinity and minimal 5-HT_{2A} receptor affinity [52].

The current study does not provide direct evidence for specific receptor involvement in mediating the efficacy of the antipsychotics against the PCP effect, but data from receptor binding studies in other laboratories does allow us to suggest potential receptor mechanisms which may be involved. Risperidone was highly effective in reversing the PCP-induced deficit and has high 5-HT_{2A} and D_2 receptor affinity [37]. Risperidone is suggested to be the most effective atypical antipsychotic to improve working memory in the clinic [40], although, in this study in rats, clozapine was equally effective. Clozapine has a lower affinity for D_2 receptors than risperidone [4]. It is

believed that a 40-80% threshold of D_2 receptor occupancy is required to achieve an antipsychotic response [35]. Clozapine is able to reach its antipsychotic response at 45-65% D_2 receptor occupancy levels [35]. Risperidone is only effective at occupancy levels above the threshold of 65%, suggesting that risperidone is more reliant upon its D_2 receptor affinity than clozapine. Although risperidone and clozapine have high affinity for 5-HT_{2A} receptors, it is unlikely that this mechanism alone is responsible for their antipsychotic action, as both have been shown to saturate 5-HT_2 receptors at sub-therapeutic doses [33]. Risperidone also has high affinity for D_3, D_4 and α_2-adrenoceptors, which may add to its therapeutic effect [52]. Clozapine has affinity at a wide range of receptors including 5-HT_6 receptors. A selective 5-HT_6 receptor antagonist has been shown to improve set-shifting ability [26], this may be via facilitation of cortical and hippocampal glutamatergic activity [12].

In conclusion, sub-chronic PCP administration impairs attentional set-shifting ability in female rats as demonstrated by poor performance in the EDS stage of the task. This and previous data suggest that NMDA antagonists provide a reliable means of inducing impairments in set-shifting ability as well as other cognitive deficits of relevance to schizophrenia. To our knowledge this is the first study to demonstrate that sub-chronic treatment with the atypical antipsychotics, clozapine and risperidone, is effective in reversing the PCP-induced cognitive deficit in the attentional set-shifting task, and that, the typical antipsychotic, haloperidol, is ineffective. These data strengthen the predictive validity of the rat attentional set-shifting model.

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Figures

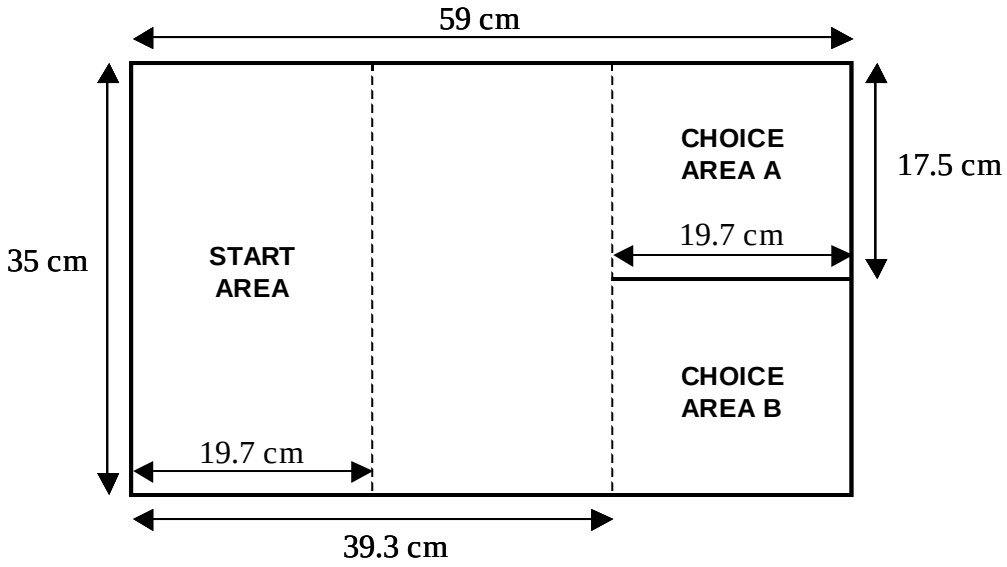


Fig. 1

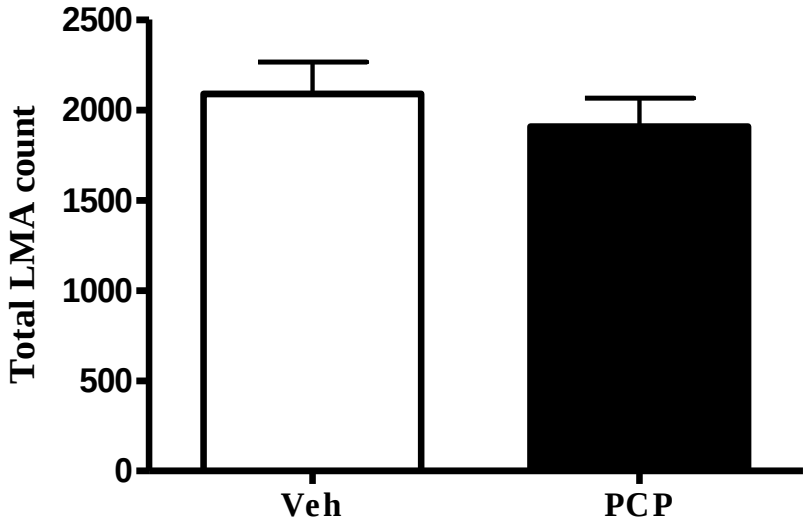
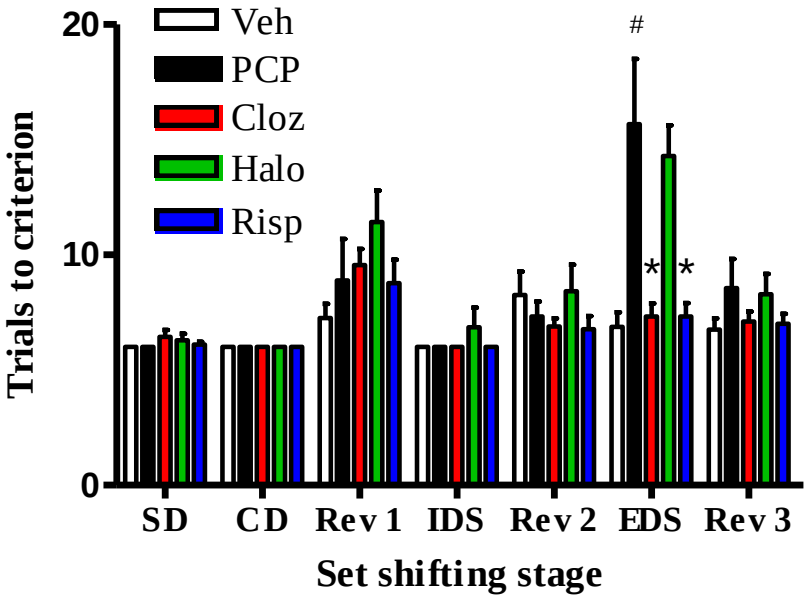


Fig. 2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SD
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Rev 1
IDS
Rev 2
EDS
Rev 3
Set shifting stage

Fig. 3

Tables

Table 1 Order of discriminations

Discriminations	Dimensions		Exemplar combinations	
	Relevant	Irrelevant	Rewarded	Unrewarded
SD	Medium	Odour	M1	M2
CD	Medium	Odour	M1/O1	M2/O1
			M1/O2	M2/O2
Rev1	Medium	Odour	M2/O1	M1/O1
			M2/O2	M1/O2
IDS	Medium	Odour	M3/O3	M4/O3
			M3/O4	M4/O4
Rev2	Medium	Odour	M4/O3	M3/O3
			M4/O4	M3/O4
EDS	Odour	Medium	O5/M5	O6/M5
			O5/M6	O6/M6
Rev3	Odour	Medium	O6/M5	O5/M5
			O6/M6	O5/M6

Table 2 Specific exemplars used (presented in pairs)

Dimension	**Pairing 1 (CD)**	**Pairing 2 (IDS)**	**Pairing 3 (EDS)**
Odour	Rose = O1 White flower = O2	Green Meadow = O3 Coconut = O4	Orange = O5 Almond = O6
Medium	Wood shavings = M1 Cat litter = M2	Small pebbles = M3 Aspen = M4	Fine sawdust = M5 Wood blocks = M6

Legends

Table 1 Overall order of discriminations presented to rats undertaking the attentional set-shifting task. Only rewarded stimuli were baited with food reward. [M1 = medium 1; O1 = odour 1]. Table adapted from Birrell and Brown (2000).

Table 2 Specific exemplars used in each phase of attentional set-shifting. Odours were applied around the rims of digging bowls which were filled with various digging media depending upon the phase being tested. The significance of pairing within a test phase ensures that for example rose is always accompanied with white flower within a test trial [O = Odour, M = Medium].

Fig. 1 Plan view of attentional set-shifting test box, showing gross dimensions, division into thirds and then into sixths to create two choice areas. Dotted lines indicate removable Plexiglas dividers and unbroken lines indicate fixed panels and walls.

Fig. 2 The effect of sub-chronic PCP (2 mg/kg) on locomotor activity in a novel environment. Data are expressed as mean ± SEM activity counts over a 60-min period (n = 10 per group). No significant difference between the groups.

Fig. 3 The effect of sub-chronic PCP (2 mg/kg; n = 9) against vehicle-treated (n = 8) female rats on trials to reach criterion in attentional set-shifting and effects of sub-chronic administration of clozapine (2.5 mg/kg; n = 9), haloperidol (0.05 mg/kg; n = 7),

risperidone (0.2 mg/kg; $n = 9$) in PCP-treated rats. Data are expressed as mean ± SEM and were analysed by two-way ANOVA and post-hoc Dunnett's t-test ($p < 0.01^{\#}$ compared to vehicle group in EDS; $p < 0.01$* compared to PCP group in EDS). Simple discrimination (SD), compound discrimination (CD), reversal 1 (Rev1), intra-dimensional shift (IDS), reversal 2 (Rev2), extra-dimensional shift (EDS), reversal 3 (Rev3).