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Spatial Memory Deficits Induced by Perinatal Treatment of Rats with PCP and Reversal Effect of D-Serine

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It has been suggested that perinatal treatment with the noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonist phencyclidine (PCP) induces transient neurodegeneration in the limbic and cortical structures of rats. Since dysfunction of these structures is associated with cognitive deficits in patients with schizophrenia, we studied the effects of subchronic treatment with PCP in perinatal rats with respect to spatial reference, reversal, and spatial working memories using the Morris water maze task in adulthood. In addition, we investigated the effect of D-serine, which has clinical relevance for the treatment of cognitive deficits in patients with schizophrenia. Our goal was to develop a neurodevelopmental model with predictive validity for the cognitive dysfunction described in patients with schizophrenia. Male and female Sprague–Dawley rats were treated with either saline or PCP (8.7 mg/kg s.c.) on days 7, 9, and 11, postnatal, and the long-term behavioral effects were investigated in adulthood. Male PCP-treated rats were slightly impaired during the spatial reference memory task, but strongly impaired during the reversal and spatial working memory tasks. Female rats were not significantly affected by this treatment. This cognitive deficit was reversed by chronic treatment with D-serine. We suggest that this model mimics some of the cognitive deficits of patients with schizophrenia and might be appropriate for the screening of putative antipsychotic agents for the treatment of these cognitive deficits.
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INTRODUCTION

It is hypothesized that the etiology of schizophrenia could, to some extent, originate during the second trimester of pregnancy (Green *et al*, 1994). The neurodevelopmental theory is based on several abnormal findings in patients with schizophrenia: abnormal architectural arrangements of individual nerve cells, cell clusters, or cortical layers (Bogerts, 1993), neurons that are misplaced, mis-sized, and disorganized (Harrison, 1997), absence of normal cerebral structural asymmetry (Crow *et al*, 1989), relative stability of cognitive dysfunction (Rund, 1998), absence of gliosis (Falkai *et al*, 1999), and premorbid behavioral abnormalities (Jones *et al*, 1994). All these observations are consistent with a disorder of prenatal brain development, and argue against the notion that schizophrenia is a progressive degenerative brain disorder (Bogerts, 1993; Mjellem and Kringlen, 2001; Weinberger, 1995; Harrison, 1995, 1997). There is, consequently, a robust clinical rationale to support the validation of a neurodevelopmental model of schizophrenia (Lillrank *et al*, 1995).

The neurodevelopmental theory of schizophrenia predicts that the late second trimester of pregnancy is the period of fetal central nervous system (CNS) development during which exposure to viral or environmental insult increases the probability of subsequently developing schizophrenia as an adult (Beckmann, 1999; Bunney *et al*, 1995). This period corresponds to the first 2 weeks of postnatal life in the rat, in terms of similar neurodevelopmental changes (Bayer *et al*, 1993; Clancy *et al*, 2001). This means that treatment with drugs that inhibit the neurodevelopmental process should occur during these weeks in rats. One way to induce such a neurodevelopmental abnormality is the systemic administration of *N*-methyl-D-aspartate (NMDA) receptor antagonists. The use of different treatment regimens with NMDA receptor antagonists during the development of CNS structures in animals has previously been shown to induce long-term effects on NMDA receptor activity (Gorter *et al*, 1992; Sircar and Li, 1994), and on the development of the CNS and synaptogenesis (Bellinger *et al*, 2002; McDonald and Johnston, 1990). These results demonstrate how crucial NMDA receptor activation is in synaptogenesis, and emphasizes the importance of NMDA receptors in synaptic plasticity (Brooks *et al*, 1997). In the immature rat brain, blockade of NMDA receptors for only a few hours during late fetal or early neonatal life increases the normal apoptotic neurodegeneration process (Ikonomidou *et al*,

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1999). The 7–10 day window appears to be critical, with even brief exposure to NMDA receptor antagonists resulting in deleterious effects on CNS development and function (Haberny *et al*, 2002). Perinatal treatment with NMDA receptor antagonists suggests that neonatal abnormalities in specific brain substrates lead to a delayed, postpubertal emergence of significant (Gorter and De Bruin, 1992) or mild (Németh *et al*, 2002) spatial learning dysfunction. Wang and co-workers (2001) showed that the subchronic administration of PCP in perinatal rats induced a post-pubertal emergence of motor and sensory gating abnormalities related to schizophrenia, and impaired acquisition of delayed spatial learning tasks. We have chosen the dose regimen for PCP (8.7 mg/kg on days 7, 9, and 11) described by Wang *et al* (2001) as it is a mild treatment, but inducing an apoptotic effect in cortical structures involved in cognitive processes (prefrontal cortex), and was shown to induce significant long-term behavioral effects (Wang *et al*, 2001).

Based on the high level of expression of the NMDA receptor in cortico-limbic structures (Monaghan and Cotman, 1985), we hypothesized that perinatal injection of PCP would impair learning and memory in rats (Wang *et al*, 2001). Consequently, we initially investigated the effect of PCP administration in perinatal rats on their performance in three different Morris (1984) water maze tasks in adulthood. Firstly, animals were tested on a reference spatial memory task in order to investigate their long-term spatial memory, and secondly on a reference reversal memory task in order to test their ability to inhibit a long-term acquisition, and finally on a spatial working memory task (Aggleton *et al*, 1986; Pouzet *et al*, 1999, 2002) in order to test the ability of rats to inhibit short-term memory acquisition (Eichenbaum and Cohen, 2001; Olton *et al*, 1979). Female rats have being previously described as sensitive to this PCP treatment (Wang *et al*, 2001), so we tested both males and females. In order to establish a relevant pharmacological validation of this model of cognitive dysfunction in patients with schizophrenia, we tested, during a second experiment, the reversal effect of D-serine on the observed disruption. D-Serine was selected due to its well-described clinical add-on effect on cognitive dysfunction in patients with schizophrenia (Tsai *et al*, 1998), and was expected to be superior to the effect of marketed antipsychotics for the treatment of this symptom (Sharma and Antonova, 2003). Finally, we tested the rats from the first experiment in the ultrasound vocalization (USV) model (Tonoue *et al*, 1986; Branchi *et al*, 2001), validated for the screening of anxiolytic drugs (Sánchez, 1993), to exclude the possibility that cognitive deficits might be associated with an anxiogenic effect of perinatal treatment with PCP.

MATERIALS AND METHODS

Subjects

For the water maze tasks and the USV test, 30 pregnant Sprague–Dawley rats (Møllegård Breeding Laboratories, Denmark) were used to produce 125 pups. For the screening of D-serine, 30 pregnant Sprague–Dawley rats were delivered from Charles River (Germany). The dams were mated over a period of 6 h in the facilities of the suppliers and delivered at gestational day 14. Following arrival, each animal was given a general physical examination to assess its health status. The dams were housed individually in plexiglas boxes ($42.5 \times 26.5 \times 18.5$ cm Macrolon type III), with a metal top and sawdust bedding. The animals were housed in an air-conditioned room ($21 \pm 2^{\circ}$C, relative humidity $60 \pm 10\%$) with white light on from 11:00 to 23:00. Regular rat pellet food and water were provided *ad libitum*. On day 7 after birth, the pups were separated from the dams and randomly assigned to new litters of 8–12 male or female pups. In each litter, the pups were randomly assigned to treatment with either PCP or saline. On day 21, the pups were weaned from the dams and kept together, two of the same gender and treatment per box. Behavioral experiments began when the animals were 56 days old. Rats were randomly assigned to treatment with D-serine or saline, with rats from the same box being given the same treatment. Animals were always tested during the light period of the light/dark cycle. All animal experiments performed in this study were in accordance with Danish legislation relating to animal use for scientific procedures as described in the 'Animal Testing Act' (Consolidation Act No. 726 of 9 September 1993 as amended by Act No. 1081 of 20 December 1995).

PCP Perinatal Treatment

The pups were treated with PCP (8.7 mg/kg) or saline (s.c.) on days 7, 9, and 11, the day of birth counted as day 0.

D-Serine Treatment

Drug or saline was injected each day 30 min before the animals were tested in the water maze. Two doses of D-serine were tested: 640 and 1280 mg/kg (s.c.). Drug administration was maintained between the two learning tasks performed.

Apparatus

Morris water maze. The Morris water maze was a black circular tank 1.2 m in diameter and 45 cm in height placed 40 cm above the floor. The maze was placed in a well-lit room enriched with distal visual stimuli. The tank was filled every day with water to a depth of 28 cm, and the temperature was maintained at $21 \pm 1^{\circ}$C. A black platform 8.3 cm in diameter was submerged 1.5 cm below the surface of the water (hidden from the rat's view), 25 cm from the wall. Above the maze, a black and white video camera was connected to a PC running Ethovision software (Noldus, Netherlands). The swim path of the rats was tracked, digitized, and stored for later behavioral analyses using this software. Each trial was started and ended manually by the experimenter, who operated a remote switch connected to the PC.

The arena of the water maze was divided into four different zones: north (N), south (S), east (E), and west (W), which were subdivided into two zones: inner and outer (sidewall), 10 cm from the edge of the wall. The platform could be placed in the middle of one of the four quadrants: N, S, E, and W, which also served as the starting position for

the rats. The escape platform could be made 'visible' by mounting a visual cue on top of it (a circular white disk 10 cm above the water surface).

Ultrasound vocalization (USV) test. The animals were placed in one of four gray plexiglas boxes (21.0 × 22.5 × 23.0 cm) with plexiglas lids, which were placed in a larger soundproof box. Foot-shocks were generated by a shock generator (ENV-413, Microcontroller Constant Current Shock Source) and scrambler and supplied via the grid floor. USV in the range of 22–28 kHz was recorded using a microphone in the lid. The number of calls in each session was calculated as the total number of calls minus the calls during the administration of shocks. The recordings were stored for later analyses using Ellegaard software (Ellegaard Systems A/S, Faaborg, Denmark). The boxes were cleaned after each session with water and soap. Before starting the experiment, a rat was placed in each box and exposed to two foot-shocks (0.50 mA × 4 s) in a short session to avoid any differences relating to urination and defecation.

Procedure

Morris water maze.

Reference memory water maze task: The reference memory task consisted of 4 days of acquisition. Acquisition consisted of reaching a submerged platform that the rats could use to escape from the water. The location of the escape platform was fixed at north (N). Four trials were carried out per day, with a different starting point used for the first three trials and then with the same starting point used in the fourth trial as in the first (E, S, or W). A new order of starting points was chosen randomly each day. For each trial, the rats were allowed to swim for max 60 s or until they located the platform, which ended the trial. After each trial, the rats were allowed to stay 15 s on the platform, and a further 10 s elapsed before starting a new trial. A rat that could not find the platform within 60 s was gently moved onto the platform and allowed to stay there for 15 s. At the end of each session, rats were dried before being returned to their home cage.

Reversal learning water maze task. The reversal learning task was performed 2 days after ending the reference memory task. Rats were given four trials per day for 4 consecutive days, similar to those described for the acquisition in the reference memory task, with starting points E, N, or W. The escape platform was located at S, opposite the location used for the reference memory task.

Spatial working memory water maze task. The spatial working memory was run 2 days after the reversal memory task. Rats were given four trials per day for 4 days, similar to those described for the acquisition of the reference memory task, but a new platform location was fixed everyday with a different starting point for each trial.

Visible platform task. During the visible platform task performed 6 days after the spatial working memory task, rats were given 1 day with four trials similar to those described above for the acquisition, but with a visible cue on top of the platform. The position of the escape platform was the same as used during the reference memory task (N).

Ultrasound vocalization test. Shocks of an intensity of 0.50 mA were delivered in each of the four boxes for 4 s. Ten foot-shocks were delivered, 1.0, 1.4, 2.4, 4.0, 4.4, 5.4, 7.0, 7.4, 8.4, and 9.3 min after starting the session, which lasted 10 min. A control experiment was carried out where no shocks were delivered during this 10-min session. The protocol was adapted from Van Den Berg *et al* (1998).

Effect of D-serine on memory dysfunction. Rats were tested for reversal of cognitive dysfunction in the reference memory task and the reversal memory task. The same protocols were used for the test of D-serine, as described above for the acquisition in the reference and the reversal memory tasks. Rats were weighed every second day in order to calculate the amount of drug administrated to each animal.

Experimental Design

Morris water maze. Rats were tested in the Morris water maze between days 56 and 77. A total of 10 rats were used in each of the four groups: males saline-treated (M/Veh) and PCP-treated (M/PCP), females saline-treated (F/Veh) and PCP-treated (F/PCP). The same rats were used in three different water maze tasks, as well as in the visual task.

Ultrasound vocalization test. The ultrasound vocalization test was conducted with the rats previously used in the cognitive test, 15 days after finishing this test. Five rats from each treatment group received foot-shocks and five other rats from each treatment group were tested without administration of foot-shock.

Effect of D-serine on spatial memory deficits. Naïve male rats, from age 68 to 80 days, were used. Eight rats were used in each group: vehicle–vehicle (Veh/Veh), PCP–vehicle (PCP/Veh), vehicle–640 mg/kg (Veh/640), PCP–640 mg/kg (PCP/640), vehicle–1280 mg/kg (Veh/1280), and PCP–1280 mg/kg (PCP/1280). The same animals were used for both the reference memory task and the reversal memory task.

Data Analysis

The data were analyzed by analysis of variance (ANOVA) using StatView software (SAS Institute Inc.). Three-way repeated measures ANOVA was performed on data generated during the reference memory, reversal memory, and spatial working memory of the Morris water maze tasks. This permitted analysis of the interactions between factors of Treatment (two levels: Vehicle and PCP), Days (four levels: D1, D2, D3, and D4), and Trials (four levels: T1, T2, T3, and T4) for each gender. Two-way repeated measures ANOVA assessed significant differences of Treatment and Trials on data generated during the visual test. Four-way repeated measures ANOVA was used for data generated in the test of D-serine. Interactions between factors of Treatment (two levels: Vehicle and PCP), Drug

(D-Serine) (three levels: Vehicle, Low, High), Days (four levels: D1, D2, D3, and D4), and Trials (four levels: T1, T2, T3, and T4) were analyzed. Only significant interactions between factors Treatment × Drug × Days were assessed with the Student–Newman–Keuls *post hoc* test, as statistically significant full interactions including the factor Trial never occurred. We present the performance of rats and differences between groups based on latency to reach the escape platform, and the 'sidewall' behavior. 'Sidewall' is defined as the percent distance traveled in the outer zone of all of the four different zones (N, E, W, S), ⩽10 cm from the edge of the pool and permits to highlight an anxiogenic effect of treatment or the use of a particular strategy to locate the hidden platform. Parallel analyses were conducted to compare swim speed, path length, and nonfinder (number of trials for which the rat did not locate the platform), but are not presented here, because these results were similar to those described for analyses of latency. Data generated during the two USV tests were calculated with SigmaStat software (SPSS Inc.) and analyzed by one-way ANOVA assessing significant differences of Treatment (two levels: Vehicle and PCP). Data are presented as the mean ± SEM. $P<0.05$ was necessary to reject the null hypothesis.

Formulations

Phencyclidine (PCP) hydrochloride was synthesized at H. Lundbeck A/S and dissolved in 0.1 M methanesulfonic acid dissolved in 0.9% NaCl. D-Serine was purchased from Sigma (Denmark) and dissolved in H_2O. Vehicle was 0.9% NaCl in all cases. For perinatal treatment, PCP or vehicle were given in a final volume of 10 ml/kg and D-serine or vehicle was given in a final volume of 5.0 ml/kg. All doses are expressed in mg/kg, based on the free base. All compounds were administrated s.c. Fresh solutions of PCP were prepared for each of the 3 days, and fresh solutions of D-serine were prepared for each day of testing.

RESULTS

First Experiment

Effect of perinatal PCP on spatial reference memory. Three-way repeated measurement ANOVA demonstrated that perinatal PCP treatment did not disrupt the latency to reach the platform (Figure 1a), for male (P's > 0.080) or female (P's > 0.182) rats. There was a significant effect of Days and Trials in both males (P's < 0.001) and in females (P's < 0.001), showing that the animals improved with time. Treatment with PCP had a significant effect on the percentage of the swimming distance in the 'sidewall' zone in males ($F_{(1,18)} = 7.15$; $P<0.016$), showing that PCP-treated male rats, contrary to the vehicle ones, were swimming preferentially along the edge of the pool during the four days of training (Figure 1b). Male rats reduced the percent distance swum in the 'sidewall' zone over days ($F_{(3,54)} = 9.84$; $P<0.001$). None of the other main factors or interactions with the factor Treatment reached significance (P's > 0.145).

Effect of perinatal PCP on reversal memory task. As shown in Figure 1c, the male vehicle-treated group performed significantly better than the PCP-treated group during all 4 days of acquisition. This was confirmed by a significant main effect of Treatment ($F_{(1,18)} = 5.02$;

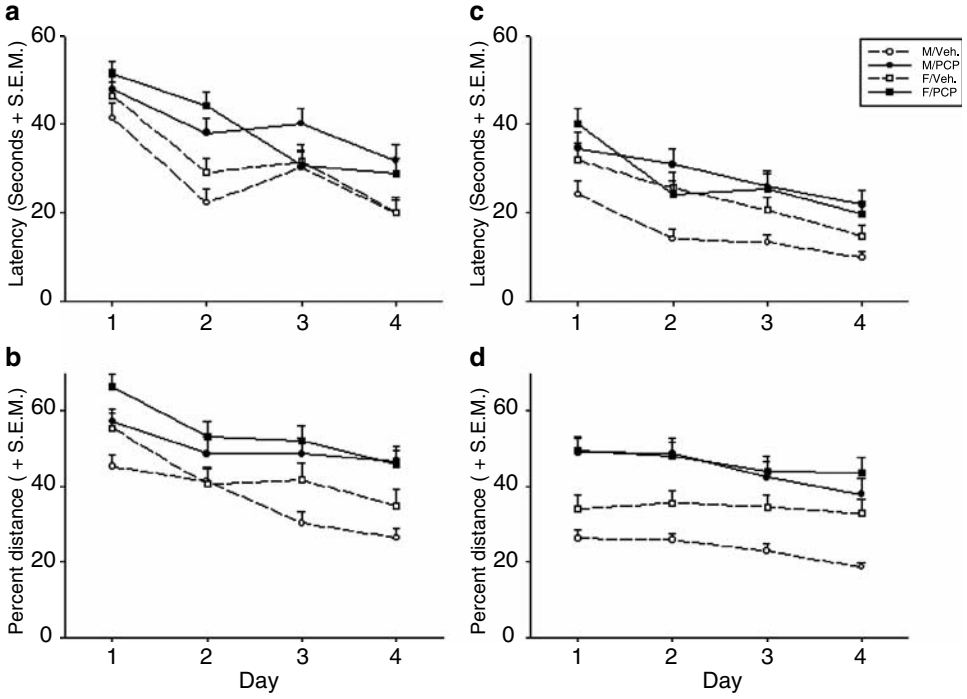

Figure 1 Performance of adult males (M) and females (F) PCP- (PCP) *vs* vehicle-treated (Veh) rats during the spatial reference memory task of the first experiment. (a) Mean escape latency to locate the hidden platform during the 4 days of acquisition training. (b) Mean percentage of the distance swum in the 'sidewall' zone of the pool during the 4 days of acquisition training. (c) Mean escape latency in locating the hidden platform during the 4 days of the reversal memory training. (d) Mean percentage of the distance swum in the 'sidewall' zone of the pool during the 4 days of the reversal memory training. $N = 10$ per group.

$P<0.038$), although Treatment had no significant effect in females ($P>0.512$). ANOVA yielded a significant Day and Trial effect in both males and females (P's <0.001). As shown in Figure 1d, male PCP-treated rats swam significantly longer in the 'sidewall' zone for all 4 days than the vehicle group ($F_{(1,18)}=7.81$; $P<0.012$). In females, only the Trial effect was significant ($F_{(3,54)}=9.03$; $P<0.001$) on this measurement. No other main factors or interactions with the factor Treatment reached significance (P's >0.147).

Effect of perinatal PCP on the spatial working memory task. Over the four daily trials, the male vehicle group reduced their latency to locate the platform as training progressed, but the PCP-treated rats did not show an equivalent overall improvement (Figure 2a). This was supported by the three-way repeated measurement ANOVA, which yielded a significant effect of Treatment ($F_{(1,18)}=4.79$; $P<0.042$) and Trial ($F_{(3,54)}=37.62$; $P<0.001$). The treatment had no effect on the spatial working memory in females ($P>0.855$), but there was a significant effect of the Trial factor ($F_{(3,54)}=35.78$; $P<0.001$), which showed that the female rats improved over the four daily trials. The percentage of the swimming distance in the 'sidewall' zone (Figure 2b) was significantly higher in the male PCP-treated group ($F_{(1,18)}=7.35$; $P<0.014$) compared to the male vehicle-treated group. The treatment had no significant effect on behavior in female rats ($P>0.463$), and only a Trial effect reached significance ($F_{(3,54)}=3.67$; $P<0.018$). No other interactions with the factor Treatment were significant in the male or female groups (P's >0.071).

Effect of perinatal PCP on the visual task. The visual task showed that treatment with PCP had no effect on sensorimotor performance or on motivation of the rats (Figure 3a). There was no difference between treatment groups, in males ($P>0.062$) or in females ($P>0.127$). Again, the percent distance swum by the PCP-treated male rats in the 'sidewall' zone was significantly higher than the percentage for the vehicle-treated male rats (Figure 3b) ($F_{(1,18)}=5.83$; $P<0.027$), but not females ($P>0.364$).

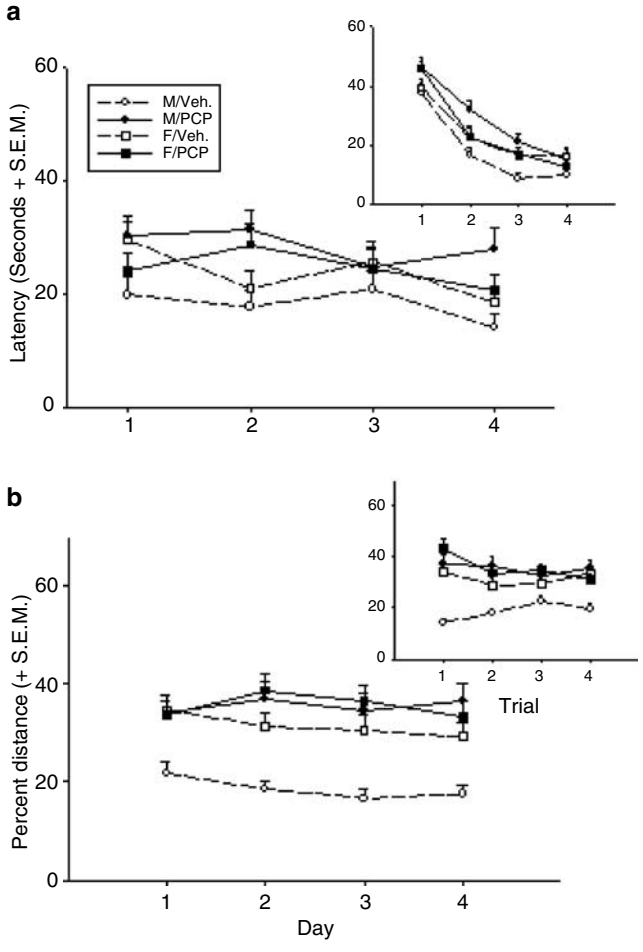

Figure 2 Performance of adult males (M) and females (F) PCP-treated (PCP) *vs* vehicle-treated (Veh) rats during the spatial working memory task of the first experiment. (a) Mean escape latency in locating the hidden platform. (b) Mean percentage of the distance swum in the 'sidewall' zone of the pool. The inset shows the mean percentage of the distance during the four trials taken as a mean of the 4 days. $N=10$ per group.

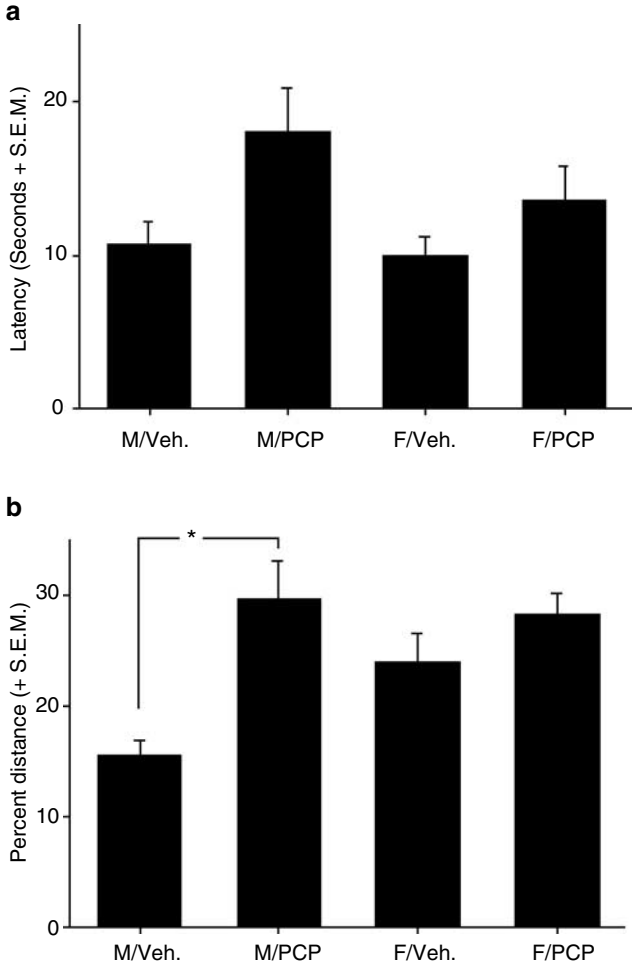

Figure 3 Performance of adult males (M) and females (F) PCP-treated (PCP) and vehicle (Veh) rats during the visual task of the first experiment. (a) Mean escape latency to climb onto the platform. (b) Mean percentage of the distance swum in the 'sidewall' zone of the pool. $N=10$ per group. *: $P<0.05$ *vs* the respective vehicle-treated group.

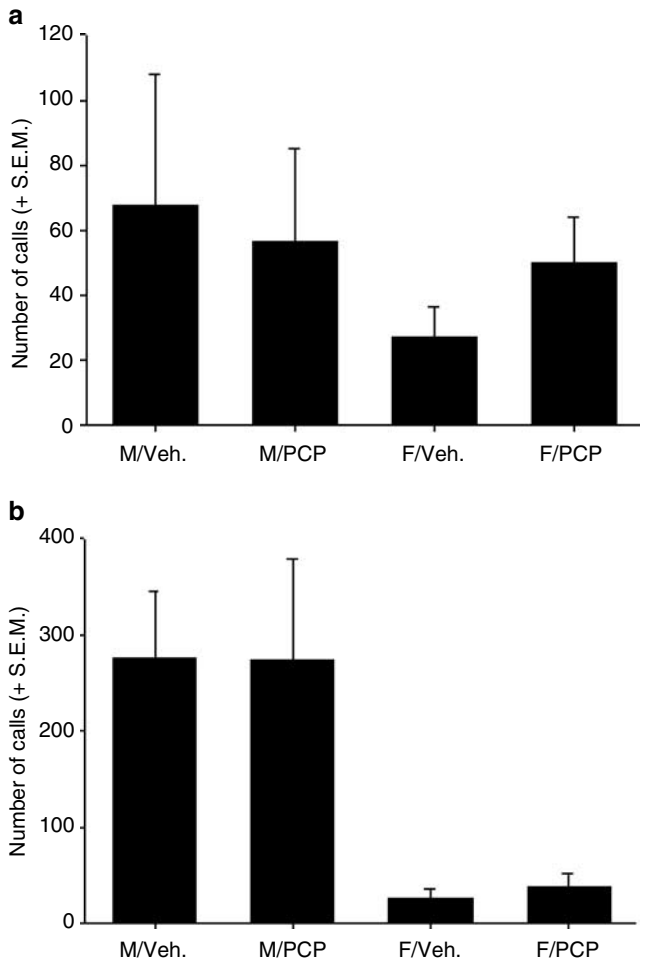

Figure 4 Performance of adult males (M) and females (F) PCP-treated (PCP) *vs* vehicle-treated (Veh) rats in the ultrasound vocalisation test. (a) Mean number of calls during the control condition. (b) Mean number of calls during the foot-shock (0.5 mA or 4.0 s) condition. $N = 10$ per group.

Effect of perinatal PCP on the ultrasound vocalization test. One-way ANOVA analyses yielded no significant effect of PCP treatment on USV in the control test (Figure 4a) or in the shock test (Figure 4b), for males ($P > 0.823$) or females ($P > 0.208$). Induction of foot-shocks induced a strong USV effect in male but not in female controls.

Second Experiment

Effect of D-serine on perinatal PCP-disrupted spatial reference memory. Four-way repeated measurement ANOVA showed a significant effect of PCP treatment on the latency of rats to locate the platform ($F_{(1,42)} = 7.04$; $P < 0.011$), and D-serine caused a significant improvement in the PCP-treated groups. There was a significant interaction of Treatment and Drug ($F_{(2,42)} = 8.08$; $P < 0.001$) (Figure 5a). *Post hoc* analysis showed that there was a significant difference between groups, with Veh/Veh performing slightly better than Veh/640. The two doses of D-serine tested reversed the disruptive effect of PCP on latency (P's < 0.05).There was a significant effect of Days and Trials (P's < 0.001), which showed that the animals progressed both over days and trials. The increase of the percent distance swum along the sidewall induced by the PCP-treatment was also reversed at both doses of D-serine tested (Figure 5b). ANOVA yielded a significant interaction between Treatment and Drug ($F_{(2,42)} = 7.26$; $P < 0.002$), and *post hoc* analysis demonstrated that the Veh/1280 treatment increased this percentage in comparison to the Veh/Veh group ($P < 0.05$). However, both doses of PCP/D-serine normalized the increase of percent distance relative to the PCP/Veh group (P's < 0.05). The interaction between factors Days, Treatment, and Drug yielded a significant effect ($F_{(6,126)} = 3.04$; $P < 0.008$), demonstrating that both doses of D-serine reduced the percent distance swum by the sidewall compared to the PCP/Veh-treated group on days 2, 3, and 4. There was also a significant effect of factors Days and Trials (P's < 0.001), which showed that the animals reduced the 'sidewall' effect both over days and trials. No other interactions with factors Treatment or Drug caused a significant effect (P's > 0.075).

Effect of D-serine on perinatal PCP-disrupted spatial reversal memory. As shown in Figure 5c, PCP treatment induced a significant disruption in the latency to locate the hidden platform during the reversal learning task ($F_{(1,42)}) = 4.13$; $P < 0.049$). There was also a significant interaction between factors of Treatment and Days ($F_{(3,126)} = 4.76$; $P < 0.004$), showing that the disruptive effect of PCP was only significant during the first day of testing. Four-way repeated measurement ANOVA yielded a significant effect for the factors Days and Trials (P's < 0.001), showing that the groups improved over trials and days. For the percent distance swum in the 'sidewall' zone (Figure 5d), the analysis yielded a significant effect for the interaction Treatment by Drug ($F_{(2,42)} = 4.07$; $P < 0.024$), and *post hoc* analysis showed that there was a significant difference ($P < 0.05$) between the Veh/Veh and Veh/1280 groups, and also between the Veh/640 and Veh/1280 groups. The highest dose combined with Veh increased the percent distance swum in the 'sidewall' zone compared to the Veh/Veh and Veh/640 groups. However, the combination of the low and the high doses of D-serine with PCP reduced the 'sidewall' effect induced by the treatment PCP/Veh (P's < 0.05). The significant main effects of Trials and Days (P's < 0.001) demonstrated that the 'sidewall' effect was also reduced for all groups over days and trials. No other main factors or interactions with the factors Treatment or Drug reached significance ($P > 0.068$).

DISCUSSION

The present results show that subchronic treatment with PCP in perinatal male rats leads to deficits in spatial memory in adulthood. Learning of the reference memory task was inconsistently impaired in the male PCP-treated group, but learning of the reversal memory task and spatial working memory was systematically impaired in the same group, suggesting that the inability of these rats to acquire new spatial information was the main cause of impairment. We explain this impairment by a loss of allocentric learning abilities of rats, which compensate by developing an appropriate, but time-consuming, idiocentric strategy. This impairment also affected the performance of male

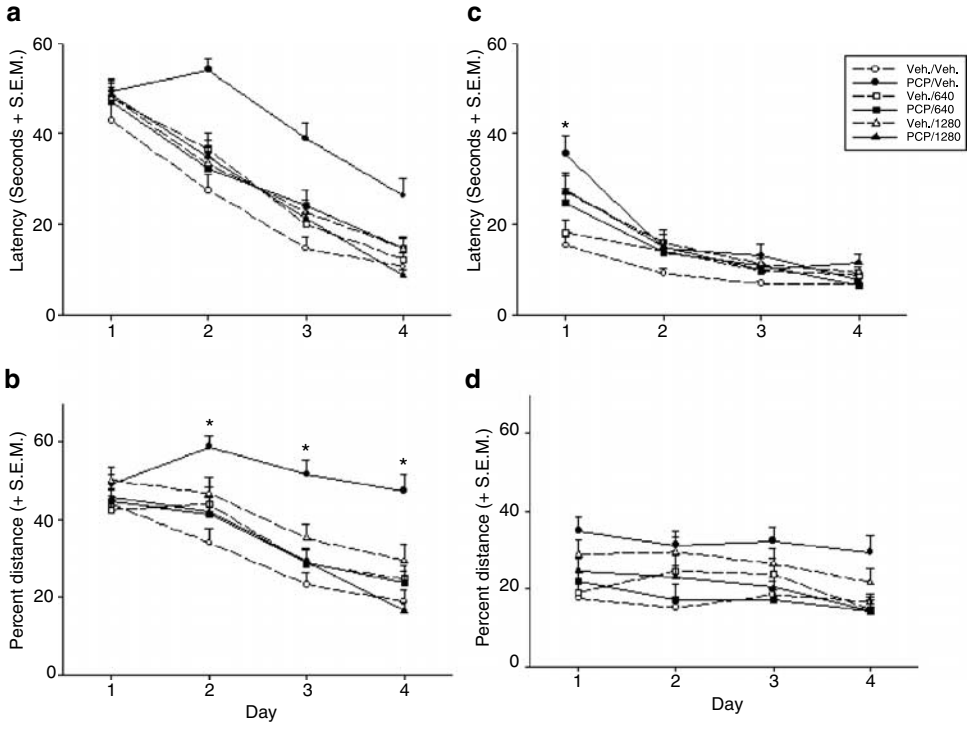

Figure 5 Performance of adult male PCP-treated (PCP) and vehicle (Veh) rats administrated D-serine at the low (640 mg/kg s.c.) and the high (1280 mg/kg s.c.) dose during the water maze task of the second experiment. (a) Mean escape latency to locate the hidden platform during the 4 days of acquisition training of the reference memory task. (b) Mean percentage of the distance swum in the 'sidewall' zone of the pool during the 4 days of acquisition of the reference memory task. *: $P<0.05$ *vs* the PCP/640 and PCP/1280 groups. (c) Mean escape latency to locate the hidden platform during the 4 days of acquisition training of the reversal memory task. *: $P<0.05$ *vs* the Veh/Veh group. (d) Mean percentage of the distance swum in the 'sidewall' zone of the pool during the 4 days of acquisition of the reversal memory task. $N=8$ per group.

PCP-treated rats in the spatial working memory task, which were not significantly impaired on latency to reach the platform in the visual learning task. This means that the very significant disruption observed with the PCP-treated male animals in the reversal and spatial working memory was due to specific spatial learning impairments, and was not secondary to locomotor, sensory, or motivational deficits. Finally, we demonstrate, for the first time in a preclinical study, that chronic treatment with an agonist of the glycine site of the NMDA receptor, D-serine, normalizes cognitive dysfunction in rats, similar to the clinical situation (Tsai *et al*, 1998), at least when using the model and doses described in this study.

In the first experiment using the spatial reference memory task, we showed that perinatal treatment with PCP did not significantly impair latency to reach the hidden platform for both male and female adult rats. On the other hand, PCP-treated male rats, contrary to the females for which the significant level was not reached, were preferentially swimming close to the edge of the pool during the 4 days of training. The second part of the first experiment, the reversal memory task, showed that male PCP-treated rats improved during training over the 4 days, but never reached the escape platform as fast as the vehicle-treated animals on the same day. As in the previous test, male PCP-treated rats swam preferentially along the edge of the pool during the 4 days of training, compared to their respective vehicle controls. Only a trend of effect occurred for female rats. These two consecutive tests showed that perinatal treatment with PCP induced cognitive deficits only in males, and that this impairment is directly related to a modification of swim path that did not occur in female PCP-treated rats.

During the spatial working memory task, male PCP-treated animals had a longer latency in locating the hidden platform over the four consecutive trials during the 4 days of testing. The PCP-treated group appeared particularly impaired during the first three trials, showing that these animals were partially amnesic in applying the strategy needed to resolve the task, or used other search strategies that were less efficient. However, they progressed over trials and had a latency on the fourth trial similar to that of the controls. Again, the male PCP-treated rats, compared to their respective vehicle controls, swam preferentially along the edge of the pool during the 4 days of this spatial working memory task. Consequently, the combined impairment observed on measurement of latency to climb onto the hidden platform, and the percent distance spent following the edge of the pool, confirmed the effect described during the reference memory task and its reversal: compared to the controls, PCP-treated animals used a different strategy, which required more trials to learn the new location of the platform each day. These results imply that the PCP-treated males could localize the escape platform, but used a strategy that was less efficient than that used by the vehicle-treated rats. The PCP-treated males took longer and more circuitous routes to find the platform.

There are two main strategies used by rats to learn the location of a hidden platform, and these are often complementary: an idiocentric strategy, based on the subject's

body position in space, and an allocentric strategy, which is dependent on the subject's ability to learn spatial cues (Long and Kesner, 1996; Whishaw, 1985). The second strategy has been shown to be hippocampal specific, while the first is striatal specific (Devan *et al*, 1996; Packard and McGaugh, 1996). We suggest that the PCP-treated male rats were impaired in their capacity to use efficiently spatial cues (allocentric strategy), and compensated this spatial learning deficit by using a striatal idiocentric strategy. As can be seen by comparison of the path of a representative Veh/Veh- *vs* PCP/Veh-treated male rat (see Figure 6), the idiocentric strategy of PCP/Veh-treated rats allowed them to locate the hidden platform, but with a longer latency and distance traveled than the vehicle group. As shown in trial 4 *vs* trial 3 of Figure 6a, the representative PCP/Veh-treated animal was still swimming along the sidewall, even after it learned the location of the hidden platform. Interestingly, this switch of spatial learning strategy is very similar to that described in rats receiving dorsal hippocampal (Pouzet *et al*, 2002) or fornix-fimbria lesions (Devan *et al*, 1996), limbic structures crucial for spatial learning (Morris and Frey, 1997; O'Keefe, 1976; O'Keefe and Nadel, 1978). On the days of PCP administration (days 7, 9, and 11), only the dentate gyrus of the hippocampus is still developing (Bayer *et al*, 1993). Since this structure is crucial for spatial learning (Bannerman *et al*, 1999; Moser *et al*, 1995) and allocentric orientation (Czéh *et al*, 2001), it seems possible that injury to the hippocampus by perinatal injection of NMDA antagonists leads to the same behavioral anomalies observed after surgical lesions of the dorsal hippocampus (Pouzet *et al*, 2002), and consequently after impairing processes that enable the rats to retain spatial information (Morris *et al*, 1982; Morris and Frey, 1997). Since the striatum develops earlier than the hippocampus (Bayer *et al*, 1993), and is consequently not altered by our perinatal treatment with PCP, the animals can still use idiocentric strategies, characterized by large loop-shaped swim paths, and following the edge of the pool. The period of synaptic plasticity and maturation of some parahippocampal structures, such as the entorhinal cortex (Ecx), is similar to that of the hippocampus (McDonald and Johnston, 1990), so the maturation of the Ecx could also be affected by our perinatal treatment with PCP. However, many published data demonstrate that the Ecx is not a crucial structure for spatial learning (Galani *et al*, 2002; Bannerman *et al*, 2001; Pouzet *et al*, 1999), and consequently that alteration of this structure cannot explain the behavioral effects observed in our study. Thus, we can hypothesize that the treatment with PCP in perinatal rats described in this study induces dorsal hippocampal dysfunction by alteration of the glutamatergic system.

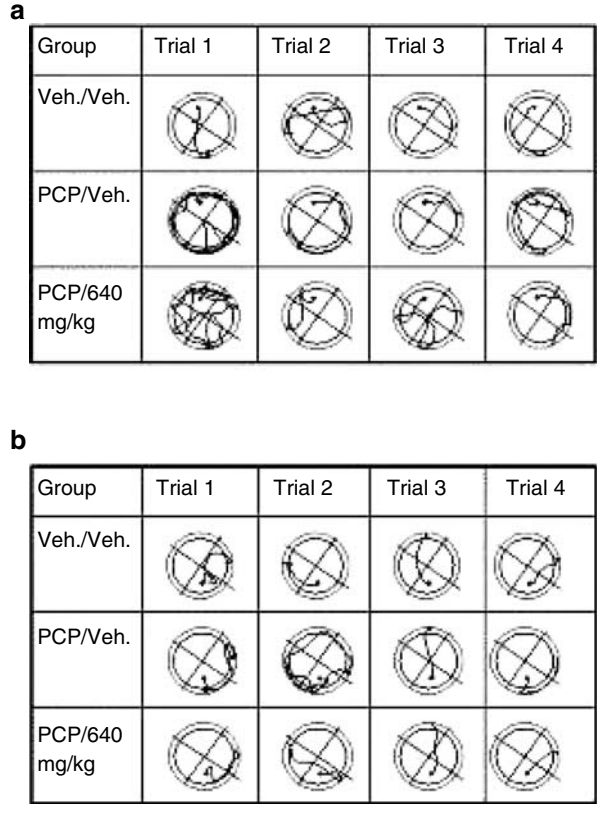

Figure 6 Representative search patterns of the male Veh/Veh-, PCP/Veh-, and PCP/D-serine (low dose: 640 mg/kg s.c.)-treated groups. (a) Search patterns during day 4 of the acquisition training of the reference memory task. (b) Search patterns during day 4 of acquisition training of the reversal memory task. All four trials are shown for these 2 days. A solid circle represents the location of the escape platform and the open circle represents the 'sidewall' area of the pool. $N = 8$ per group.

The lack of treatment effect observed when testing rats in the USV test, a model sensitive to anxiogenic treatments (Tonoue *et al*, 1986), confirmed that the male PCP-treated rats were not swimming along the sidewall because of an anxiogenic effect of PCP treatment, but because of a well-adapted search strategy.

The speed of the PCP-treated rats, both males and females, was reduced in the three spatial learning tasks, but we did not observe a direct correlation between days for which speed was reduced and days for which spatial learning was impaired. Moreover, the speed of the female PCP-treated rats was reduced, but these animals were not impaired in the various spatial memory tests described in this study. It is consequently unlikely that inhibition of spatial learning in PCP-treated males was caused by a slower swim speed. Finally, we observed a similar impairment from measurement of the distance travelled and latency to reach the hidden platform, meaning that the possible reduction of speed does not explain the impairment for localization of the platform.

Animals receiving subchronic PCP perinatal treatment were not impaired in the latency to climb onto the platform during the visual task, demonstrating that vision, motivation, and motility of rats were not affected by this treatment. Analysis of the percent distance swum along the edge of the pool during this visual task showed that male PCP-treated rats spent more time than the controls in this area, but performed similar to both vehicle- and PCP-treated female rats, which were not impaired in general spatial learning or memory. This particular effect observed in the PCP-treated male rats during the visual task could be explained by the fact that this task was run after the other three learning tasks of the first experiment. Consequently, the swimming strategy was learned over 3 weeks and could not be reversed after only four trials.

A consistent gender difference was observed. Only male rats were significantly impaired in the water maze tasks

after perinatal treatment with PCP, as there was only a tendency of impairment in females. Moreover, a different response to shock occurred in male *vs* female rats in the USV model. These observations are different from those published by Wang *et al* (2001), who used only female rats and showed impairment using models for cognitive deficits and other dysfunctions. Different housing conditions and stress levels in facilities can be considered, but the relation with cognition is difficult to confirm (Andrews, 1996). As a consequence of our observations, we pursued the second part of this study, the pharmacological investigation, with male rats only, which showed a clear disruptive effect of the treatment on cognition.

In the second experiment, both chronic doses of D-serine administrated to the PCP-treated group enabled these impaired animals to perform at the level of the control groups during the reference memory task. The same reversal effect was observed in the percent distance swum in the 'sidewall' zone, which was reduced to control levels in the two groups receiving PCP and D-serine. In the reversal memory task, the PCP-treated animals were impaired in the latency to locate the platform, but to a lesser extent than the disruption obtained in the first experiment. The significant learning impairment observed on day 4 of the reference memory task could affect the reversal learning. In comparison to animals from the first experiment, which were not significantly impaired during the reference memory task, the PCP/Veh-treated rats had a lower baseline of reference learning that needed to be reversed, and consequently less reason to be disrupted on this reversal task. There was no significant effect of D-serine on the latency to locate the hidden platform during this reversal task; this could be because the window between the PCP-treated group and the vehicle-treated group was too small to show an effect of D-serine. Consistent with the first experiment, and with the reference memory task of this second experiment, PCP-treated animals swam most of the distance close to the edge of the pool. This abnormal behavior was reversed by both doses of D-serine tested. This beneficial effect is confirmed by the pattern of the swimming path of PCP- *vs* vehicle-treated animals. As shown in Figure 6, the typical Veh/Veh-treated rat never followed the edge of the pool, in contrast to the representative animal from the PCP/Veh group, which followed the edge of the pool during all trials except the third one. When animals received PCP/D-serine 640 mg/kg, they performed very similar to Veh/Veh-treated rats, immediately targeting the hidden platform. As previously suggested, chronic treatment with D-serine allowed normalization of both the path search and consequently the spatial learning of PCP/Veh-treated rats. D-Serine permitted the disrupted animals to use a proper allocentric strategy, instead of a compensatory idiocentric strategy, and permitted the restructuring of the proper spatial mapping process. Similar facilitation of the allocentric strategy has been described in adult rats receiving local intrahippocampal injection of glutamate (Packard, 1999), showing that increasing glutamatergic activity facilitates spatial mapping abilities of rats.

D-Serine selectively stimulates the glycine site on the NMDA receptor and facilitates NMDA receptor function in animals (Tanii *et al*, 1994). It has been demonstrated that treatment with agonists of the glycine site of the NMDA receptor improves negative symptoms of patients with schizophrenia (Goff *et al*, 1995; Javitt *et al*, 1994) and addition of D-serine to antipsychotics improves the cognitive deficits of these patients (Tsai *et al*, 1998). These clinical results support the hypothesis of NMDA receptor hypofunction in schizophrenia, and provide a new possibility of treatment for these cognitive and negative symptoms. In comparison, the *in vivo* pharmacological effect obtained with D-serine as mono-treatment suggests that the model used in this study might be a relevant tool for the screening of putative antipsychotics expected to treat cognitive dysfunction of patients with schizophrenia.

Our results complement and support data obtained by Wang *et al* (2001) using the delayed spatial alternation task, and those obtained by Gorter and De Bruin (1992) using the water maze task, showing that perinatal treatment with NMDA receptor antagonists induces severe cognitive deficits in rats. Wang *et al* (2001) described some cognitive dysfunction dependent on prefrontal structures, and we confirm the results of Gorter and De Bruin (1992) that hippocampal functions are also directly or indirectly impaired.

CONCLUSION

This study demonstrates that perinatal treatment with PCP significantly impairs the spatial mapping abilities of male, but not female rats, when subsequently tested as adults. This treatment impairs the hippocampal-dependent allocentric strategy for localization of the hidden platform in water maze tasks, which can only be compensated by a procedural striatal idiocentric strategy. This specific cognitive impairment is very interesting, considering that the spatial allocentric strategy is defined as part of declarative memory (O'Keefe and Nadel, 1978), which is the type of memory disrupted in patients with schizophrenia (Perry *et al*, 2000). We show for the first time that the loss of the allocentric strategy and the spatial memory deficit can be reversed by chronic treatment with D-serine, a compound known to cure some of the cognitive dysfunctions of patients with schizophrenia. PCP treatment of perinatal rats will need further pharmacological validation, but it appears to be an interesting model of the cognitive deficits of patients with schizophrenia. It might be a useful tool for screening of putative antipsychotic drugs aiming at treating cognitive dysfunction of patients with schizophrenia. These results also support the neurodevelopmental hypothesis of schizophrenia.

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