Amelioration of PCP-induced learning and memory deficits in the Morris' water maze by selective α7 nAChR agonists.

Engel M[a], [b], Sandager-Nielsen K[a], Mirza NR[a]

[a] Dept In Vivo Pharmacology, NeuroSearch A/S, 93 Pederstrupvej, DK-2750 Ballerup, Denmark (ksn@neurosearch.dk; max@neurosearch.dk)

[b] School of Biosciences, Cardiff University, Cardiff, United Kingdom **(engelm@cardiff.ac.uk)**

INTRODUCTION

•The N-methyl-D-aspartate antagonist phencyclidine (PCP) engenders schizophrenia-like symptoms in healthy subjects and exacerbates existing symptoms (positive, negative and cognitive) in schizophrenic patients (Murray 2002). Therefore PCP has been extensively studied as an animal model of schizophrenia (Morris et al. 2005).

•Treating cognitive dysfunction in schizophrenia is a high unmet patient need, and extensive Discovery and Development efforts are currently being made to address this symptom domain.

•Based on preclinical studies the α7 nicotinic acetylcholine receptor (nAChR) is a target which holds promise in potentially ameliorating cognitive deficits in disorders such as schizophrenia and Alzheimer's disease. Further, polymorphism of the α7 nAChR gene has been related to the sensory gating dysfunction (Leonard et al. 2002) in schizophrenic patients.

•The selective α7 nAChR agonist SSR180711 has been shown to reverse/prevent various PCP induced behavioural (Hashimoto et al. 2008) and molecular (Thomsen et al. 2009) changes in rodents; and MK-801 induced deficits in the water maze (Pichat et al. 2007). Another selective α7 nAChR agonist , PNU-282987, reverses amphetamine-induced deficits in sensory gating in the rat (Hajós et al. 2005).

Objective:

•Here we assess whether these two α7 nAChR agonists reverse subchronic PCP induced deficits in rat spatial water maze performance using a method by Didriksen et al. (2007)

MATERIALS AND METHODS

Subjects

•32 male Wistar rats (Harlan, Netherlands), with a start weight of 200g. The animals were kept under normal 12:12h dark-light cycle (light on at 6:00AM) in groups of four per Cage under controlled conditions (temperature 21C, humidity at 60%). No food or drink restrictions have been applied. Subjects were divided into 4 groups (n=8): control, PCP, SSR180711 and PNU-282987.

Drugs and administration

•Vehicle (0.9% Saline, control group) or PCP (1.3mg/kg/day, s.c., PCP and drug groups) were administered for 3 consecutive days prior to the first swim trial. Forty-five min. before the start of each swim session, vehicle (i.p.), SSR180711 (10mg/kg, i.p.) or PNU-282987 (10mg/kg, i.p.) were administered followed by vehicle or PCP (1.3mg/kg, s.c.) 15 min. later. Doses of the two α7 agonists was based on internal ex-vivo [^{3}H]bungaratoxing binding to the hippocampus.

Apparatus

•The Morris' water maze consisted of a circular black pool with a radius of 80cm and a height of 60cm. Tap water with a temperature of 20C was filled to a level of 28cm and changed every 2nd test day. A round platform (ø 8cm) was placed at location P (Fig. 1), 1cm below water surface. Four start positions have been set as north east, south east, south west and north west (see Fig. 1).
•A video camera two meter above the pool monitored the rats behaviour and transmitted the signal to a PC running Viewpoint (Viewpoint Life Sciences, France).
•The walls surrounding the pool were decorated with permanent cues, like coloured paper sheets and laboratory apparatus, but no cues were placed inside the maze.

Behavioural procedure

•The rats were placed individually into the water at one of the 4 start positions to locate the platform within 60 sec. At the end of each swim (either by time out or remaining on the platform for > 3sec.), the rat was placed on the platform for 15sec. Once the 15 sec. had passed, the rat was immediately released into the water maze from the next starting point until all four trials were performed. Each rat was tested 4 times a day, on four consecutive days

Statistical analysis

•Data was analysed by 2-way Repeated Measures ANOVA (test day as repeated factor), followed by Fisher LSD post hoc analysis where appropriate. The main dependent measures were: (i) Latency (average sec. until platform reached per test day), (ii) Distance (average cm swum per test day), (iii) Speed (average cm/sec. per test day) and (iv) Thigmotaxis (average sec. spend "wall-hugging" i.e. not searching per test day).

All experiments were conducted in compliance with the Danish Committee for Experiments on Animals.

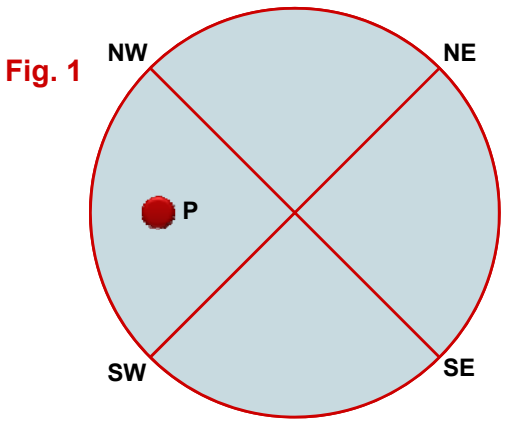
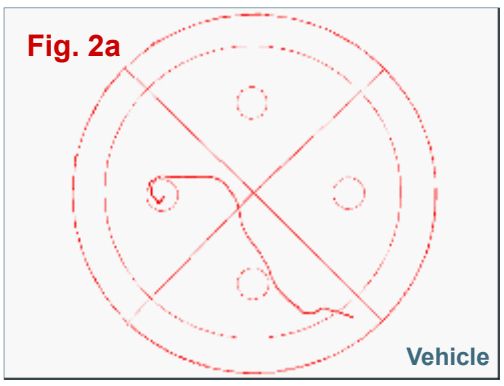
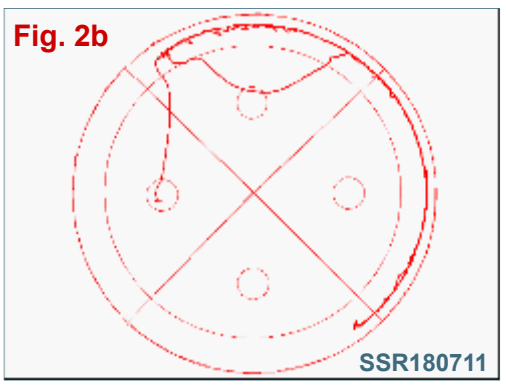


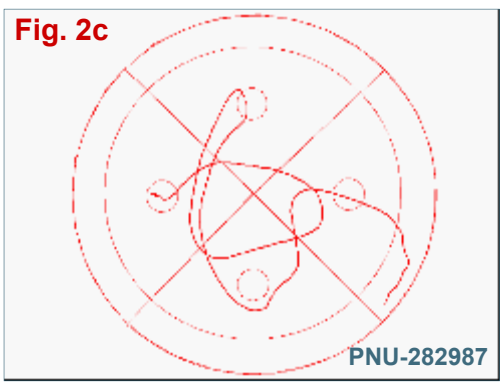
Fig. 1

Fig.1: Morris' water maze setup,
P: platform; NE, SE, SW, NW: start positions after cardinal directions

RESULTS







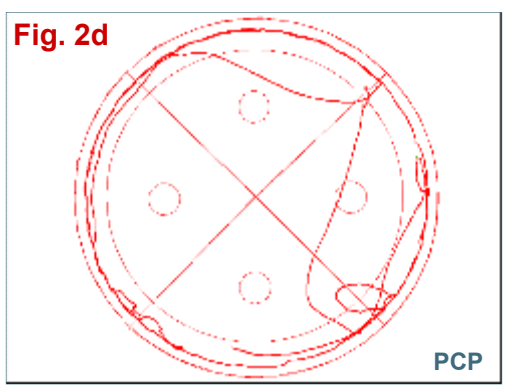


Fig.2 a-d: Example of swim routes for one representative rat from each treatment group on the 3rd swim of day 3 (south east start position)

Fig. 3a: Mean Latency

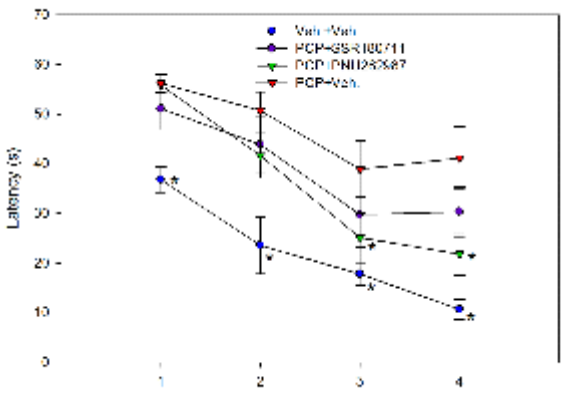


Fig. 3b: Swim Distance

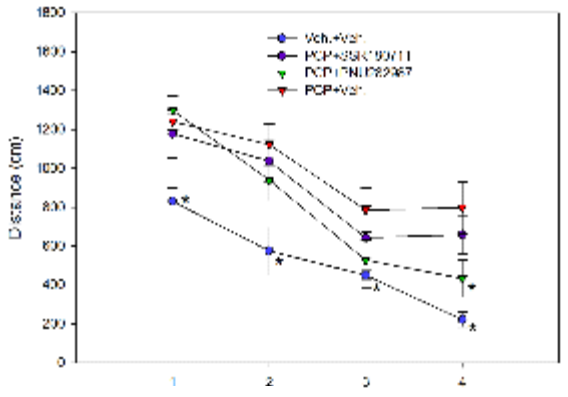


Fig. 3c: Thigmotaxis

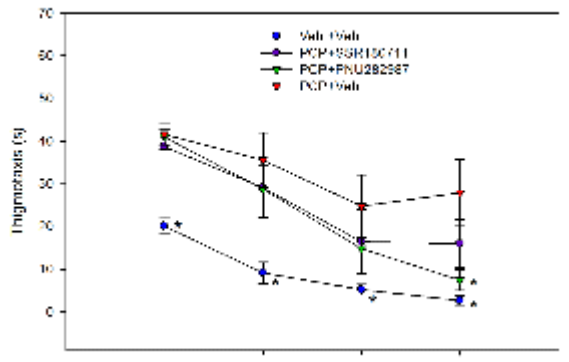
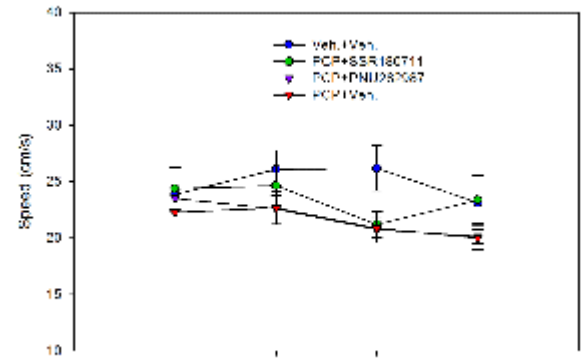


Fig. 3d: Swim Speed



Data are presented as means + SEMs. *P<0.05 compared to the PCP+Veh. group

DISCUSSION

The present study shows that subchronic PCP administration induces significant impairment in spatial water maze acquisition and that this is reversed by α7 agonists.

•Both latency and distance to locate the platform clearly showed that PCP treatment resulted in a shallow acquisition curve compared to control (Fig. 3a,b)

•PNU-282987 significantly reversed all of the PCP induced impairments, most pronounced on days 3-4.

•As highlighted in Fig. 2a-d, rats treated with α7 agonists clearly reduced marked thigmotaxis on day 3, compared to PCP-treated animals (Fig. 2a-d).

•Swimming speed was not altered by any compound, indicating that motor function was unaffected (fig. 3d).

•Despite these overall conclusions, our data suggest that the α7 agonist PNU-282987 more reliably reverses the PCP deficit compared to SSR180711.

•The reason(s) for this are unclear, but might simply be related to the limited number of doses employed in our study. Alternatively, it might be that these and other α7 agonists would differ in their profiles in various in-vivo models due to differences in in-vitro affinity, efficacy, and additional off-target activity (e.g., 5-HT$_3$ receptors).

References

Didriksen, M., Skarsfeldt, T. & Arnt, J., 2007. Psychopharmacology, 193(2), 225-233; **Hajós, M. et al., 2005**. The Journal of Pharmacology and Experimental Therapeutics, 312(3), 1213-1222; **Hashimoto, K. et al., 2008**. Biological Psychiatry, 63(1), 92-97; **Leonard, S. et al., 2002**. Archives of General Psychiatry, 59(12), 1085-1096; **Morris, B.J., Cochran, S.M. & Pratt, J.A., 2005**. Current Opinion in Pharmacology, 5(1), 101-106; **Murray, J.B., 2002**. The Journal of Psychology, 136(3), 319-327; **Pichat, P. et al., 2007**. Neuropsychopharmacology, Neuropsychopharmacology, 32(1), 17-34; **Thomsen, M.S. et al., 2009**. Neuropharmacology, 56(6-7), 1001-1009.