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Research report

Disturbances in social interaction occur along with pathophysiological deficits following sub-chronic phencyclidine administration in the rat

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ABSTRACT

A sub-chronic administration of phencyclidine to the rat brings about enduring pathophysiological and cognitive changes that resemble some features of schizophrenia. The present study aimed to determine whether the behavioural consequence of this phencyclidine regime extends to a long-term disruption of social interaction that might provide a parallel with some negative symptoms of the disease. Rats were treated with phencyclidine (2 mg/kg bi-daily for 1 week) or vehicle followed by a drug-free period. Social interaction was assessed 24 h, 1 week, 3 weeks and 6 weeks post-treatment. A long-lasting disturbance of social behaviour was observed in the phencyclidine group, namely more contact and non-contact interaction with an unfamiliar target rat at all time points. Six weeks post-phencyclidine, analysis of brains showed a reduction in expression of parvalbumin immunoreactive neurons in the hippocampus with significant reductions localised to the CA1 and dentate gyrus regions. These results show that sub-chronic phencyclidine produces long-lasting disruptions in social interaction that, however, do not model the social withdrawal seen in patients with schizophrenia. These disturbances of social behaviour may be associated with concurrent pathophysiological brain changes.

1. Introduction

While positive psychotic symptoms are the most distinctive feature of schizophrenia, negative symptoms such as emotional withdrawal and apathy, and cognitive and attentional deficits also severely diminish a patient's quality of life. Cognitive and negative features often precede the onset of positive symptoms [2,3,41,44], and can persist in periods of otherwise successful treatment [22,25] making them an important target for pharmaceutical therapies. The positive symptoms of schizophrenia respond relatively well to antipsychotic drug treatment in a large proportion of patients. However, treatment is substantially less effective in ameliorating the negative features [12,36] and cognitive deficits [8,24,40] of the disease. Relief from these symptoms, which pose a greater restriction than positive symptoms on the patient's functional outcome and quality of life [7,21], are a major unmet need in the treatment of schizophrenia.

Owing to its multi-faceted nature, animal models of schizophrenia are used to deconstruct the disorder into individual components, whether they are behavioural or pathophysiological. Much recent discussion has centred around the validity of animal models of schizophrenia as targets for studying negative symptoms [17,42], which are arguably the most difficult to model in animals due to their 'human qualities' [42].

The *N*-methyl-D-aspartate (NMDA) receptor antagonist, phencyclidine (PCP), has been investigated as a putative substance of interest due its inducement of a schizophrenic-like psychotic state in humans [32]. PCP has been shown to produce schizophrenic-like behaviours in animals with a general dysfunction that accounts for a variety of symptoms related to schizophrenia [27,35], including social withdrawal [48,53,54], thereby modelling an aspect of negative symptoms. From these and other studies pertaining to the neurotoxic and psychomimetic effects of PCP, Olney et al. produced the NMDA receptor hypofunction model of schizophrenia [38] through which it was surmised that the disease mechanism of schizophrenia might involve dysfunction of the NMDA receptor, or downstream effects that are modelled by blocking NMDA receptors.

Persistent blockade of NMDA receptor function by repeated PCP dosing is thought to produce pathophysiological changes that model both the behavioural and pathophysiological dysfunction observed in schizophrenia [27]. Sub-chronic PCP induces neurobiological changes: a reduction in parvalbumin containing GABAergic neurons in the hippocampus is seen 6 weeks after sub-chronic PCP administration to female rats [1]. These findings mirror the pathophysiology observed in schizophrenic patients [5,46,59]. There is subtle neuronal pathology in schizophrenia, involving

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deficits in subtypes of GABAergic neurons and dysfunction of glutamatergic systems in cortical and hippocampal GABA-containing neurons [31]. Moreover, investigations using a variety of different immunochemical markers of these interneurons, including co-existing neuropeptides, calcium-binding proteins [45] and the GABA-synthesizing enzyme glutamate decarboxylase [15], have all indicated selective deficits of subtypes of GABAergic neurons in cortex and hippocampus. A recent analysis of 100 neurochemical investigations on the Stanley series of post-mortem brain tissues demonstrated that the strongest findings in schizophrenia (and also, interestingly, in bipolar disorder) were frontal cortical or hippocampal deficits in parvalbumin, reelin and GAD, all associated with GABAergic neurons [57].

Whether sub-chronic PCP administration is a valid model for the induction of enduring negative symptoms is uncertain. Sams-Dodd [49,51,52] and Bruins Slot et al. [11] investigate sociality in rats 45 min after the last dose of a sub-chronic PCP regime and observe social withdrawal, although there appear to be no deficits in social interaction after sub-chronic PCP with a drug-free period [16,47], except in female rats at 6 weeks after PCP [55]. Interestingly, Lee et al. reported differential changes in the active component of social interaction within 3 days of sub-chronic PCP administration to rats suggesting disrupted social behaviour [30].

The aim of the present study was to assess the long-term changes in social interaction after sub-chronic PCP treatment, investigating the active component of social behaviour. Long-term brain changes after a sub-chronic PCP regime was also investigated.

2. Methods

2.1. Animals

Male Lister-hooded rats ($n = 18$, Harlan, UK), weighing 242–282 g at time of first drug administration, were used for the time course of experimental behavioural testing and subsequent brain analysis. A further 18 male Lister-hooded rats (Harlan, UK), weighing 232–263 g at time of first saline administration, were used as target animals for the social interaction task. All animals were housed in groups of three same-treated rats, under a 12 h/12 h light/dark cycle (lights on 08:00 h) and food and water available *ad libutum* in the home cage. Room temperature ($21 \pm 2\,^{\circ}C$) and humidity (45–55%) were kept constant throughout. The experiments were performed in accordance with the Animals Scientific Procedures Act (1986).

2.2. Drugs and drug administration

Animals were assigned to two weight-matched groups ($n = 9$/group). Phencyclidine HCl (Sigma, USA) was dissolved in 0.9% saline and administered bi-daily (0900 h and 1630 h) for 7 days at a dose of 2 mg/kg i.p. Control animals received vehicle solution (0.9% saline) with the same dosage regime.

2.3. Experimental procedures

Behavioural testing began 24 h after the last PCP or saline injection where rats were tested in the social interaction paradigm. Then at 7, 21 and 42 days post-injection rats were tested again using a test battery over a 2-day period of social interaction and locomotor activity, with each test performed on a separate day and always in the same order. It should be noted that a cognitive test (novel object recognition) was performed at each of the latter time points however no significant effects were observed and are thus not included in this publication.

2.4. Social interaction test

The social interaction procedure and analysis method was adapted from Lee et al. [30]. Testing took place in a room (185 cm × 305 cm × 285 cm) under 360 lux lighting in a black plastic box (63 cm × 63 cm × 45 cm), with sawdust on the floor. Between each pairing the sawdust was replaced, and the arena cleaned with 70% alcohol.

On each social interaction test day a PCP-treated or saline-treated rat was placed together with a weight-matched (within 20 g) target rat (all bi-daily saline-treated to mirror handling and experience) for 10 min. All target rats were sprayed the previous day with sheep spray, a commercial dye used by farmers to spray their sheep for identification (Ritchey Tagg Ltd., UK), to distinguish them from their experimental pair. All sessions were videotaped for later analysis. In each social interaction pairing (a) the rat was paired with an animal from a different home-cage and (b) no rat was ever paired with the same rat twice.

Videos were scored blind, determining the time the experimental rat (PCP-treated or saline-treated) actively engaged in social interaction behaviours (e.g. sniffing, grooming, following, crawling over/under, boxing (pushing or pawing the target rat with front legs and paws, usually around the head, neck, shoulders and front legs) or wrestling (rats wrapping around each other into a ball, rolling around together)) with the target rat. Further analysis assessed how much of this active interaction was contact or non-contact, where contact was defined as physical contact including sniffing with direct contact, crawling, grooming, anogenital exploration and play behaviour and non-contact was considered to be following and non-contact sniffing (sniffing within 10 cm of the target rat but without contact).

2.5. Locomotor activity

Locomotor activity was performed under 320 lux light conditions in a room (185 cm × 305 cm × 285 cm) adjacent to that of the room where social interaction was performed using a Med Associates (USA) open field test chamber (44.5 cm × 44.5 cm × 30.5 cm) with four pairs of photocells spaced evenly along the length of the cage. Rats were placed in the box for 10 min and analysis performed by Med Associates (USA) activity monitor software, version 4.

2.6. Parvalbumin immunohistochemistry

Immediately following the behavioural testing (6 weeks post-PCP), rats were sacrificed by CO_2 inhalation, brains were removed and fixed in 10% phosphate-buffered formalin for 3 days, before being embedded in wax. Three sections (10 μm) from the hippocampus (bregma −3.3 mm), determined using a rat brain atlas [39] were mounted onto slides coated with 3-aminopropyltriethoxysilane (APES) (Sigma, UK) and stained for parvalbumin [4,23]. Briefly, following clearing in xylene and rehydration in graded alcohol, sections were incubated in 0.6% hydrogen peroxide solution to inhibit endogenous peroxidase activity and a microwave treatment protocol was employed to aid antigen retrieval. Sections were then blocked with 5% normal horse serum diluted in 0.01 M phosphate-buffered saline (PBS) containing 0.1% Triton X-100. After incubation at 4 °C, with a mouse monoclonal antibody against parvalbumin (Swant, Switzerland) at a dilution of 1:10,000 in PBS for 36 h, biotinylated secondary antibody was added. Sections were processed by the avidin–biotin method using a Vectastain ABC kit (Vector Laboratories, UK) and peroxidase was visualised using 3′,3′-diaminobenzidine (DAB) intensified with nickel chloride. No immunoreactivity could be detected in control sections, in which the primary antibody was omitted from the staining protocol. All slides were coded and analysed blind to treatment.

2.7. Image analysis

Hippocampal sections (bregma −3.3 mm) were scanned at 4× magnification using an Olympus microscope interfaced to an Image ProPlus analysis system (USA) via a JVC 3-CCD video camera. Estimations of neuronal density (cells/mm^2) were carried out, in three coronal sections (60 μm apart), using composite images of total left and right hippocampus. Immunostained neurons were counted at a higher magnification in defined complete sub-fields of each region according to the Atlas of Paxinos and Watson [39]. Within the hippocampal formation, three regions were examined, the dentate gyrus (DG), CA2 plus CA3, and CA1 plus subiculum.

2.8. Statistical analysis

All behavioural data was analysed by repeated measure ANOVA, with time point and group as variables, using SPSS V14 for windows (USA). Post hoc analysis was performed on CLRANOVA. Parvalbumin data was analysed by multivariant ANOVA using SPSS with post hoc statistical analysis.

3. Results

3.1. Social interaction

PCP treatment significantly increased the amount of time rats actively engaged in social interaction compared to control rats over the 6-week time course ($F_{(1,16)} = 18.0$; $p = 0.001$). There was also an observed time effect ($F_{(1,16)} = 14.2$; $p = 0.01$), though no group × time interaction ($F < 1$). Post hoc analysis show a difference in active social interaction between groups at all time points (24 h: $F_{(1,64)} = 15.4$, $p < 0.001$; 1 week: $F_{(1,64)} = 4.0$, $p = 0.05$; 3 weeks: $F_{(1,64)} = 4.7$, $p < 0.05$; 6 weeks: $F_{(1,64)} = 5.2$, $p < 0.05$) (Table 1).

A breakdown of the scored behaviours showed that PCP-treated animals exhibited more contact social interaction than