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Research report

A schizophrenia rat model induced by early postnatal phencyclidine treatment and characterized by Magnetic Resonance Imaging

Brian V. Broberg[a,b,d,*], Kristoffer H. Madsen[a], Niels Plath[b], Christina K. Olsen[e], Birte Y. Glenthøj[d], Olaf B. Paulson[a,c], Börje Bjelke[a,f,g], Lise V. Søgaard[a]

[a] Danish Research Centre for Magnetic Resonance, Copenhagen University Hospital Hvidovre, Kettegaard Allé 30, DK-2650 Hvidovre, Denmark
[b] Synaptic Transmission I, H.Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark
[c] Neurobiology Research Unit, Department of Neurology, The Neuroscience Centre, Rigshospitalet, Building 9201, Copenhagen University Hospital, Blegdamsvej 9, DK-2100 Copenhagen, Denmark
[d] Center for Neuropsychiatric Schizophrenia Research, Psychiatric Centre Glosturp, Copenhagen University Hospital Glostrup, Nordre Ringvej 29-67, DK-2600 Glostrup, Denmark
[e] International Clinical Research, H.Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark
[f] Department of Neurology, Akershus University Hospital, Lørenskog, Norway
[g] Division of Medicine and Laboratory Sciences, University of Oslo, Norway

HIGHLIGHTS

- Early postnatal phencyclidine (neoPCP) dosing is evaluated as an animal model of schizophrenia.
- Adult animals are tested using relative cerebral blood volume, a proxy measure of brain activity.
- Acute PCP injection produce higher brain activity in neoPCP compared to vehicle control animals.
- The altered sensitivity to NMDAR blockade observed in neoPCP animals further validates the model.
- The observed changes in the medial prefrontal cortex are interesting in relation to cognition.

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ABSTRACT

Better animal models are needed to aid the development of new medications to alleviate the cognitive deficits associated with schizophrenia. Growing evidence suggests neurodevelopmental insults and disturbances in NMDA receptor (NMDAR) signaling to be involved in the schizophrenia etiology. Acute administration of phencyclidine (PCP) induces schizophrenia-like symptoms in healthy volunteers and exacerbates symptoms in patients with schizophrenia. In this study, pharmacological Magnetic Resonance Imaging (phMRI) was used to evaluate if rats treated with 20 mg/kg PCP on postnatal days 7, 9, and 11 (neoPCP), compared to saline (neoVeh), were hypersensitive to acute PCP administration in adulthood (acutePCP). Intravenous administration of 0.5 mg/kg acutePCP produced robust and sustained relative cerebral blood volume (rCBV) increase in discrete frontal, neocortical, hippocampal, thalamic, and limbic brain structures in both neoPCP:acutePCP and neoVeh:acutePCP rats compared to acute saline treatment (Vehicle control group). AcutePCP injection significantly increased the rCBV response in the medial prefrontal cortex and nucleus accumbens compared to the Vehicle control group, without distinguishing neoPCP and neoVeh animals. However, at late time points (25–33 min post acutePCP injection), neoPCP animals showed significantly higher rCBV values compared to the Vehicle control group, suggesting an altered sensitivity toward NMDAR blockade in adult rats subjected to this neurodevelopmental procedure. In combination with the observed cognitive deficits revealed in this animal model, the present findings indicate that altered NMDAR signaling might underlie the symptomatic changes seen in schizophrenia, adding to the construct and face validity of this model.

1. Introduction

Schizophrenia is a severe chronic disorder affecting approximately 0.7% of the human population [1]. The disease is characterized by three main symptom clusters namely positive and negative symptoms, as well as cognitive deficits [2]. The

* Corresponding author at: Center for Neuropsychiatric Schizophrenia Research, CNSR, Psychiatric Center Glostrup, Nordre Ringvej 29-67, 2600 Glostrup, Denmark. Tel.: +45 3632 6455; fax: +45 3635 1630.
E-mail address: brian@cnsr.dk (B.V. Broberg).

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insufficient effect of current pharmacotherapy on negative symptoms and cognitive deficits reflects our poor understanding of the schizophrenia etiology [3–5]. However, a general consensus exists suggesting neurodevelopmental disturbances as a key element of the schizophrenia etiology [6,7]. The neurodevelopmental hypothesis of schizophrenia is supported by epidemiological and clinical lines of evidence, suggesting complications in brain development to be caused by genetic factors [8,9] and/or environmental influences. Despite suggestions of a strong genetic component, ~80% from heritability estimates, the identification of specific susceptibility genes for schizophrenia has proven difficult [5]. This suggests a polygenetic disorder [10] and emphasizes the contribution of environmental factors, such as maternal exposure to drugs [11–13], in the schizophrenia etiology. To address this, rats exposed to phencyclidine (PCP), a non-competitive N-Methyl-D-Aspartate receptor (NMDAR) antagonist, on post natal day (PND) 7, 9, and 11, were suggested as an animal model of schizophrenia [14]. This early postnatal PCP (neoPCP) treatment paradigm produces a range of schizophrenia-like behaviors, including hypersensitivity (locomotor activity) to acute PCP stimulation [14,15], impaired prepulse inhibition [14,16], and cognitive deficits [17–19]. Furthermore, alterations in NMDA-, $GABA_A$-receptor binding across several brain regions [20], in muscarinic M4/M1 receptor binding and neuregulin 1/erbB4 expression in the prefrontal cortex (PFC) [20,21], and reduction in cortical parvalbumin-containing interneurons have been established [19,22].

Translational medicine requires use of similar techniques for cross species comparison. PhMRI represents a technique that can be used to study central drug effects in both humans and rats [23–25]. PhMRI is a non-invasive technique measuring changes in the central hemodynamic response following an acute drug challenge and is considered a proxy measure of changes in the underlying brain activity [26]. The phMRI neuroimaging technique has been used to examine the regional effects of the NMDAR antagonist – ketamine – in the human brain. Ketamine increase regional cerebral blood flow, but with marked variation between subjects both with regard to average of cerebral blood flow and to regional changes, a phenomenon most likely was caused by regional neuronal activation [27]. Later studies demonstrated that ketamine robustly and dose-dependently increased brain activity in the anterior cingulate, thalamus, putamen and frontal cortex of healthy volunteers [24,25]. In rats, the activation in discrete cortico-limbo-thalamic structures observed following acute PCP administration can be antagonized by antipsychotics and glutamate-release modulators [28]. Furthermore, increased brain activity in the hippocampus, retrosplenial cortex and orbital cortex has been reported in rats challenged with ketamine [29].

To further validate the neoPCP model of schizophrenia, we applied phMRI to test if the animal model could mirror the observations from human studies showing that exposure to non-competitive NMDAR antagonists induces schizophrenia-like symptoms in healthy volunteers [30], and exacerbates symptoms in patients with schizophrenia [31,32]. We hypothesized that neoPCP, compared to neoVeh, rats would be hypersensitive to an acute PCP challenge during adulthood (acutePCP).

2. Methods

2.1. Animal preparation

All experimental procedures were conducted in strict adherence to the guidelines given by The Danish Animal Experiments Inspectorate and with the explicit approval of the local veterinary authorities. The early postnatal PCP treatment paradigm, previously described in [18], was applied as an animal disease model of schizophrenia. Timed pregnant Lister hooded rats were obtained at gestational days 14/15 from Charles River (Germany) and housed individually, in standard conditions as described below, including nesting material, until delivery. The day of parturition was counted as postnatal day (PND) 0. On PND 6, pups were cross fostered and randomly assigned to a lactating dam. Subsequently rats were dosed subcutaneously (s.c.) with vehicle (0.9% w/v saline) or PCP (20 mg/kg, molecular weight 243.4 g/mol, H. Lundbeck A/S) solution on PNDs 7, 9, and 11 in a 10-ml/kg dose volume. Early postnatal treatment groups were named neoVeh (saline treatment) and neoPCP (PCP treatment) for simplicity. As the first two weeks of the rats' postnatal life correspond to the late second trimester in human pregnancy in terms of neurodevelopmental changes [33,34], the time of early postnatal dosing fits well with the hypothesis stating that exposure to PCP during the late second trimester increases the probability for the progeny to develop schizophrenia [12,13]. Pups were weaned on PND 25 and henceforward animals were housed two rats per cage under controlled conditions (12 h of light starting at 06:00; 20 ± 2 °C; 30–70% humidity) in Macrolon (type III) cages with standard sawdust bedding and environmental enrichment (plastic house and wooden chew blocks).

On the day of the phMRI experiment (PND 66–81) animals weighed 300 ± 7 g (neoVeh) 290 ± 8 g (neoPCP). Student's *t*-test analysis of weights did not reveal systematic differences between animal groups ($p > 0.05$). Anesthesia was induced with 3% isoflurane (Baxter A/S, Allerød, Denmark) and maintained at 2–2.2% during animal preparation in a mixture of oxygen and air (30% O_2/70% N_2). Two lateral tail veins were cannulated, one for administration of an iron oxide blood pool contrast agent – Endorem (Guerbet, France) – and one for drug administration. Pavulon (Organon Teknika, Sweden) was injected for muscle relaxation, 2 mg/kg induction and 0.6 mg/kg/h maintenance dose, to allow tracheotomization and artificial ventilation of rats (Small Animal Ventilator SAR-830 series, Stoelting Co., IL, USA). Throughout the MR image acquisition protocol isoflurane was administered at 0.8% and the breathing rate kept constant at 75 min^{-1}. To ensure that changes in rCBV could be attributed to functional brain activity rather than gross changes in physiological parameters, which may produce confounding effects in the rCBV measurements [35], all rats were continuously monitored during scanning. The level of oxygen and ventilation volumes were adjusted continuously to keep expiration levels of end expiratory CO_2 (OxiMax® NPB-75 capnograph) and blood O_2 saturation (Nonin 8600 V pulse oximeter) within the physiological range of 34–46 mmHg and >92%, respectively [36]. Animal temperature was kept at 38 °C (±0.5 °C) during all procedures.

2.2. Animal groups

Three treatment combinations were tested in the phMRI setting:

1. neoPCP and acute PCP (neoPCP:acutePCP, $n = 7$);
2. neoVeh and acute PCP (neoVeh:acutePCP, $n = 7$);
3. Vehicle control group ($n = 5$).

The Vehicle control group contains animals from the neoVeh:acuteVeh ($n = 3$) and neoPCP:acuteVeh ($n = 2$) groups which were averaged together since statistical analysis did not reveal significant changes in rCBV values over time following acute vehicle administration in any region of interest (ROI) studied here (p-values > 0.05). The acutePCP treatment was an intra-venous (i.v.) injection (0.5 mg/kg, 1 ml/kg). This dose has previously been shown to elicit robust behavioral and metabolic (2-Deoxy-D-glucose) effects in freely moving rats [37], as well as producing robust brain activation in a phMRI setting [28]. The effect of acute treatment with PCP on mean arterial blood pressure (MABP), pulse, and arterial blood values (pH and pCO_2) was investigated in a separate batch of animals using an identical time scheme and animal preparation to that described above plus a femoral artery catheter. Separate animals were used because withdrawal of blood from an animal might introduce a confounding factor in phMRI experiments [38,39]. Blood samples at three time points, one before injection of contrast agent, one before baseline recording, and one at the end of the experiment, were analyzed to get pH and pCO_2 using a GEM Premier 3000 (Instumentation Laboratory, Massachusetts, USA).

2.3. PCP exposure (acute dosing)

Plasma and brain exposure levels following acutePCP administration were collected in a separate experiment in order to investigate the relationship between PCP plasma-and brain levels. To evaluate the effect of pharmacokinetic differences on the collected rCBV values plasma and brain exposure levels were compared between neoPCP and neoVeh treated rats after acutePCP (0.5 mg/kg, i.v.) administration.

Plasma and brain contents were determined by Ultra Performance Liquid Chromatography (UPLC) using tandem Mass spectrometry on a Sciex API 4000 (Applied Biosystems). Parent and daughter mass-to-charge ratios were 244.16 > 91.1. Half of each rat brain was homogenized in four volumes of 70% MeCN/H2O followed by centrifugation and transfer of the supernatant to a vial. 25uL of plasma and brain samples were spiked with 150 μL MeCN + internal standard to precipitate. This was followed by centrifugation for 20 min and dilution of 100 μL sample with 100 μL water + 0.1% formic acid. Liquid chromatography was performed using a Waters Acquity UPLC with a gradient method running for 3 min. The calibration curves were between 1–1000 ng/mL and 5–5000 ng/g for plasma and brain, respectively.