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Dose-response effect of acute phencyclidine on functional connectivity and dopamine levels, and their association with schizophrenia-like symptom classes in rat

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ABSTRACT

Current drug treatments for schizophrenia (SCZ) can alleviate positive symptoms, but have little effect on the negative symptoms and cognitive deficits that are difficult to translate into preclinical models for drug development. Therefore, we aimed to determine the dose-response effects of acute phencyclidine (PCP, 1.0–5.0 mg/kg) on rat brain connectivity and detect markers for different SCZ-like symptoms. Pharmacological functional magnetic resonance imaging (phMRI) and microdialysis were used to investigate PCP-induced effects on functional connectivity (FC) and dopamine levels, respectively. Next, we evaluated the association between PCP-induced changes in imaging parameters and behavior. PCP at doses of 3.0–5.0 mg/kg induced fMRI signal changes in several brain regions associated with SCZ. Additionally, the FC was globally disturbed, dopamine levels increased, and locomotor activity increased, reflecting the manifestation of SCZ-like positive symptoms. A distinct pattern in the measures was observed at lower PCP doses (1.0–2.0 mg/kg); PCP induced fMRI signal changes in the fronto-cortical regions, and increased dopamine levels in the medial prefrontal cortex. In addition to the dysconnectivity of these regions, the hippocampal FC was disrupted. These observations are consistent with the induction of SCZ-like cognitive deficits and negative symptoms, which were observed as impaired novel object recognition and decreased social interaction. No indicators for positive symptoms were observed at lower PCP doses. We conclude that acute PCP induces SCZ-like symptom classes in a dose-dependent manner; PCP doses of 1.0–2.0 mg/kg are more suitable for modeling SCZ-like negative symptoms and cognitive deficits, while SCZ-like positive symptoms dominate at doses of 3.0–5.0 mg/kg.

1. Introduction

Schizophrenia (SCZ) is a severe neuropsychiatric disorder affecting 1% of the global population (Pratt et al., 2012). Although SCZ was described already a century ago, the neurobiology underlying this multifactorial disease remains poorly understood (Kahn and Keefe, 2013). The introduction of modern neuroimaging techniques, however, has significantly improved our understanding of the etiology and pathophysiology of central nervous system (CNS) disorders; the use of functional imaging methods, such as blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) (Ogawa et al., 1992) and resting-state fMRI (rs-fMRI) (Biswal et al., 1995), has revealed characteristic changes in brain organization and networking in patients diagnosed with various CNS diseases, including SCZ (Fox and Raichle, 2007; Lu and Stein, 2014; Smucny et al., 2014).

Symptoms of SCZ are commonly categorized into three classes:

Abbreviations: AUC, area under curve; BOLD, blood oxygenation level dependent; CNS, central nervous system; DA, dopamine; FC, functional connectivity; fMRI, functional magnetic resonance imaging; mPFC, medial prefrontal cortex; NMDA, *N*-methyl-D-aspartate; PCP, phencyclidine; phMRI, pharmacological functional magnetic resonance imaging; ROI, region of interest; rs-fMRI, resting-state fMRI; SCZ, schizophrenia.

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positive (hallucinations, delusions, conceptual disorganization), negative (emotional flattening, social withdrawal, anhedonia, avolition), and cognitive (impaired executive function, working memory, and attention) (Pratt et al., 2012). According to current knowledge, the expression of different SCZ symptom domains originates, at least partly, from the disrupted dopaminergic and glutamatergic systems (Lin et al., 2012; Mura et al., 2012; Pratt et al., 2012; Sendt et al., 2012), which are thought to lead – either directly or via modulatory pathways – to the functional dysconnectivity and disintegration of the schizophrenic brain (Stephan et al., 2006; Stephan et al., 2009). Indeed, rs-fMRI studies have demonstrated disrupted functional connectivity (FC), involving regions such frontal cortex, hippocampus, and thalamus, in patients diagnosed with SCZ (Dawson et al., 2015). As stated by Dawson et al. (2015), the relevance of these network-level changes to specific symptom domains of SCZ is an essential topic in current and forthcoming SCZ connectivity research aimed at characterizing the disease.

In addition to elucidating the mechanisms, modern imaging approaches have enabled the exploration of new biomarkers for diagnostic purposes and the search of targets for novel treatments. Currently available drugs to treat the symptoms of SCZ can relieve only the positive symptoms, while negative symptoms and particularly cognitive deficits remain resistant to drug treatment. One of the challenges in discovering novel drug candidates to treat the cognitive deficits and negative symptoms is their translation into animal models (Pratt et al., 2012). As similar FC structures can be observed across species (Lu et al., 2012), modern imaging methods provide novel translational tools to facilitate SCZ drug development; rapidly increasing amounts of clinical imaging data obtained from patients with SCZ can be exploited for the development of more accurate preclinical disease models.

Currently, the most common approach to modeling SCZ in animals is based on the use of non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists (Gilmour et al., 2012), because compounds such as phencyclidine (PCP) and ketamine induce SCZ-like symptoms in both humans and animals, and exacerbate symptoms in patients with SCZ (Pratt et al., 2012). PCP is the most frequently used NMDA antagonist to induce SCZ-like symptoms in rodents; in addition to its psychotomimetic properties, PCP induces sustained SCZ-like negative symptoms and cognitive deficits across species (Jentsch and Roth, 1999). In contrast, ketamine is used extensively in human studies to induce a variety of SCZ-like cognitive deficits; it is less commonly used in rodent studies, however, possibly due to relatively short half-life of the compound after acute administration (Gilmour et al., 2012).

Both acute and subchronic PCP administrations are utilized for modeling SCZ. Although subchronic PCP exposure is thought to better model the psychopathology of SCZ (Jentsch and Roth, 1999), recent behavioral studies measuring cognitive performance demonstrated that acute PCP effects are necessary to induce SCZ-like cognitive deficits (e.g., Fellini et al., 2014; Ihalainen et al., 2016; Janhunen et al., 2015; Podhorna and Didriksen, 2005; Savage et al., 2011). In addition, acute PCP exposure impairs social interaction (e.g., Bruins Slot et al., 2005; Corbett et al., 1995; Sallinen et al., 2013; Sams-Dodd, 1996), and thus acute PCP administration may offer a promising means to model both SCZ-like cognitive deficits and negative symptoms. On the other hand, acute PCP induces undesired motor and motivational side effects in rodents (e.g., Dix et al., 2010; Gilmour et al., 2009; Smith et al., 2011), which can heavily affect cognitive performance and social behavior, and interfere with their assessment.

The dose-dependent manifestation of PCP-induced SCZ-like symptoms in animals has not been thoroughly characterized, which limits the feasibility of acute PCP as a SCZ model in the assessment of novel drug candidates. At different doses, PCP may have primary effects in specific brain regions (Large, 2007) or functional networks, and highlight different SCZ-like symptoms. Characterization of the dose-response effects of acute PCP on brain function, and comparison with clinical observations obtained with neuroimaging methods, could reveal a dose range for inducing SCZ-like negative and cognitive symptoms with minimal confounding effects, and improve the validity of the animal model for testing novel drug candidates for SCZ-like negative and cognitive symptoms.

Therefore, the aim of the present study was to detect possible imaging markers for the three SCZ-like symptom classes in rat within a PCP dose range of 1.0–5.0 mg/kg by using pharmacological fMRI (phMRI) and microdialysis approaches, and corroborate the findings with selected behavioral tests. The effects of acute PCP were characterized by measuring changes in BOLD signals, FC, dopamine (DA) and PCP concentrations, locomotor activity, novel object recognition, and social interaction. We hypothesized that disruption of the brain activity and connectivity associated with SCZ-like cognitive deficits and/or negative symptoms would be induced specifically at low acute PCP doses (<3 mg/kg, s.c.), while more global changes associated with SCZ-like positive symptoms would be induced at higher doses (≥3 mg/kg, s.c.).

2. Materials and methods

All animal procedures were approved by the National Animal Experiment Board, and conducted in accordance with the European Commission Directive 2010/63/EEC. Experiments were performed using male Wistar rats (RccHan:WIST, Kuopio Laboratory Animal Center, University of Eastern Finland, Kuopio, Finland; age: 10–13 weeks, weight: 240–385 g), which were group-housed in cages, maintained on a 12/12 h light-dark cycle at 22 ± 2 °C with humidity of 50%–60%, and allowed food and water *ad libitum*. PCP (Tocris Bioscience, Bristol, UK) was dissolved in physiologic saline (doses refer to hydrochloride), and injected into the neck of the rat. The subcutaneous route of administration was used instead of the intraperitoneal, as it yields higher PCP concentrations in the brain with equivalent doses (Kalinichev et al., 2008). In control experiments, physiologic saline was administered in an equivalent volume. The PCP doses used in behavioral tests were selected on the basis of our present phMRI and *in vivo* microdialysis experiments. Statistical tests were performed either with GraphPad Prism (Version 5.03, GraphPad Software Inc. La Jolla, CA, USA) or Matlab (Version 2014a; The Mathworks Inc. Natick, MA, USA). All values are represented as mean ± standard error of the mean.

2.1. Functional magnetic resonance imaging

The phMRI protocol was similar to that described in our previous study (Paasonen et al., 2016b), and additional details are given in the appendix. Briefly, mechanically ventilated rats were imaged with 7 T Bruker Pharmascan (Bruker Biospin, Ettlingen, Germany) using spin-echo echo-planar imaging sequence (repetition time 2 s, echo time 45 ms, field-of-view 2.5 × 2.5 cm, matrix size 64 × 64, and 11 slices with a thickness of 1.5 mm) under urethane (Sigma-Aldrich, Helsinki, Finland, 1000 mg/kg, i.v.) anesthesia. Spin-echo sequences have higher specificity, especially at high magnetic fields (Lee et al., 1999), and fewer susceptibility-induced artefacts compared with traditional gradient-echo sequences, which has promoted the use of spin-echo sequences in FC investigations (Khatamian et al., 2016). A rat brain quadrature surface coil was used for signal reception, and a quadrature resonator volume coil (89 mm outer diameter, 72 mm inner diameter) for transmission (both coils from Bruker Biospin). Anatomic images were acquired with TurboRARE-T2 sequence (repetition time 4.68 s, echo time 16.1 ms, effective echo time 48.4 ms, RARE factor 8, field-of-view