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# Effects of withdrawal from repeated phencyclidine administration on behavioural function and brain arginine metabolism in rats

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ABSTRACT

Phencyclidine (PCP) induces behavioural changes in humans and laboratory animals that resemble positive and negative symptoms, and cognitive impairments in schizophrenia. It has been shown repeated treatment of PCP leading to persistent symptoms even after the drug discontinuation, and there is a growing body of evidence implicating altered arginine metabolism in the pathogenesis of schizophrenia. The present study investigated the effects of withdrawal from repeated daily injection of PCP (2 mg/kg) for 12 consecutive days on animals'behavioural performance and arginine metabolism in the hippocampus and prefrontal cortex in male young adult rats. Repeated PCP treatment reduced spontaneous alternations in the Y-maze and exploratory and locomotor activities in the open field under the condition of a washout period of 24 h, but not 4 days. Interestingly, the PCP treated rats also displayed spatial working memory deficits when tested 8–10 days after withdrawal from PCP and showed altered levels of arginase activities and eight out of ten L-arginine metabolites in neurochemical- and region-specific manner. Cluster analyses showed altered relationships among L-arginine and its three main metabolites as a function of withdrawal from repeated PCP treatment in a duration-specific manner. Multiple regression analysis revealed significant neurochemical-behavioural correlations. Collectively, the results suggest both the residual and long-term effects of withdrawal from repeated PCP treatment on behavioural function and brain arginine metabolism. These findings demonstrate, for the first time, the influence of the withdrawal duration on animals' behaviour and brain arginine metabolism.



## 1. Introduction

Patients with schizophrenia show positive symptoms (e.g., hallucinations, delusions and thought disorder), negative symptoms (e.g., deficits in social interaction, emotion and motivation) and cognitive dysfunction (e.g., impairments of attention and working memory), with prominent prefrontal and hippocampal dysfunction (Goldman-Rakic and Selemon, 1997; Harrison, 2004). While the exact cause of schizophrenia is poorly understood, glutamatergic hypofunction has been linked to the aetiology and/or pathophysiology of the disease (Paz et al., 2008; Tsai and Coyle, 2002). It has been well documented that a single dose of phencyclidine (PCP), a non-competitive *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist, induces schizophrenia-like symptoms (including psychotic and negative symptoms and cognitive impairments) in healthy individuals, and repeated treatment leads to persistent symptoms over weeks, even after the drug discontinuation (Luby et al., 1959; Javitt and Zukin, 1991; Murray, 2002). Altered NMDA receptor expression and phosphorylation have been found in the postmortem prefrontal cortex of schizophrenic patients, and the NMDA receptor subunit gene polymorphisms increase susceptibility to schizophrenia (Akbarian et al., 1996; Dracheva et al., 2001; Ohtsuki et al., 2001; Rice et al., 2001; Itokawa et al., 2003; Emamian et al., 2004).

A growing body of evidence implicates altered metabolism of L-arginine, a versatile semi-essential amino acid with a number of bioactive molecules, in the pathogenesis of schizophrenia (Perez-Neri et al., 2006; Fiori and Turecki, 2008; Liu et al., 2016). L-arginine can be metabolized by nitric oxide (NO) synthase (NOS) to form NO and L-citrulline, by arginase to generate L-ornithine and urea, and by arginine decarboxylase (ADC) to produce agmatine and carbon dioxide (Wu and Morris, 1998). The gaseous molecule NO is a double-edged sword – it maintains normal function of the nervous system at physiological levels (Feil and Kleppisch, 2008; Steinert et al., 2010), but can be neurotoxic when present in excessive amounts (Calabrese et al., 2007). L-ornithine is metabolized to form polyamines putrescine, spermidine

*Abbreviations:* ADC, arginine decarboxylase; CN, cued navigation; DG, dentate gyrus; FST, forced swim test; GABA, γ-aminobutyric acid; HPLC, high performance liquid chromatography; LC/MS, liquid chromatography/mass spectrometry; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; OB, object recognition; OF, open field; PFC, prefrontal cortex; PCP, phencyclidine; s.c., subcutaneous; SD, Sprague-Dawley; WM, water maze.

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and spermine (that are essential in maintaining normal cellular function; Williams, 1997; Wallace et al., 2003), and to produce glutamate, glutamine and γ-aminobutyric acid (GABA) through an alternative pathway (Wu and Morris, 1998). Agmatine is a novel putative neurotransmitter, interacts with the NMDA receptors and regulates the production of NO and polyamines (Reis and Regunathan, 2000; Halaris and Piletz, 2007). Human research has shown schizophrenia risk genes encoding neuronal NOS (Shinkai et al., 2002; Reif et al., 2006; Cui et al., 2010), elevated brain and/or plasma levels of NO and NOS activity and expression (Baba et al., 2004; Yao et al., 2004; Djordjevic et al., 2010), decreased plasma arginase activity (Yanik et al., 2003) and positive correlation between serum L-ornithine and the disease duration (Tomiya et al., 2007), and increased plasma level of agmatine (Uzbay et al., 2013), in schizophrenic patients. Given the parallel pathways involved in arginine metabolism, we determined how its metabolic profile changed in the frontal cortex (Brodmann's area 8) from individuals with schizophrenia (Liu et al., 2016). There were significantly increased arginase activity and agmatine level, reduced GABA level, and altered cluster relationships between L-arginine and its main metabolites in the schizophrenia group. Regression analysis indicated significant positive correlations between arginase activity and the age of disease onset, and between L-ornithine level and the duration of illness. While the findings described here demonstrate altered arginine metabolism in schizophrenia, the functional significance and the mechanisms underlying these changes are unclear at present.

The findings of human research have led to the use of PCP as a pharmacological tool to induce NMDA receptor hypofunction in experimental animals. Acute administration of PCP is often used to resemble first-episode schizophrenia, whereas repeated use of PCP results in a more persistent schizophrenic symptomatology that better model the chronic disease symptoms, such as the negative symptoms and cognitive deficits (for reviews see Jentsch and Roth, 1999; Mouri et al., 2007; Adell et al., 2012). A number of studies have used PCP to investigate how glutamatergic hypofunction affects arginine metabolism in the brain. Earlier research has indicated the involvement of NO in PCP-induced behavioural impairments (Wass et al., 2006a,b, 2009; Fejgin et al., 2008). Knox et al. (2014), for the first time, reported that a single subcutaneous (s.c.) injection of PCP (2, 5 or 10 mg/kg) dose-dependently reduced exploratory activity in the open field, and the highest dose led to severe stereotype behaviour and ataxia in male young adult Sprague-Dawley (SD) rats. Moreover, acute administration of PCP significantly altered the levels of NOS activity, L-arginine, agmatine, spermine, glutamate and GABA, and the cluster relationships between L-arginine and its main metabolites in the hippocampus and prefrontal cortex in a dose-dependent and/or region-specific manner. However, there is no previous research on how repeated PCP administration affects brain arginine metabolism.

Repeated PCP administration results in an enduring effect even after withdrawal of the treatment (Jentsch and Roth, 1999; Jentsch et al., 1998; Enomoto et al., 2007; Mouri et al., 2007). This model allows assessing behavioural alterations, as well as neurochemical and/or molecular changes, under drug-free conditions. However, it is unclear at present how the duration of withdrawal from repeated PCP treatment would affect animals' behavioural performance, as well as arginine metabolism in the brain. The present study was therefore designed to address these two issues by using repeated administration of low dose PCP at 2 mg/kg (s.c., once daily for consecutive 12 days) under the condition of a washout period of 4 days (Experiment 1) or 24 h (Experiment 2), as high dose of PCP (>5 mg/kg) significantly reduced weight gain in a dose-dependent manner (Pechnick and George, 1989). Because of the functional dissociation across the CA1, CA3 and dentate gyrus (DG) sub-region of the hippocampus (Kesner et al., 2004), neurochemical changes in this region were examined at the sub-regional level.

## 2. Materials and methods

### 2.1. Subjects

Male Sprague-Dawley rats, weighing between 290 and 370 g, were housed five to six per cage (53 × 33 × 26 $cm^3$) with free access to water and food, and maintained on a 12-h light/dark cycle (lights on 8 am). Treatments, behavioural assessment and brain tissue collections were conducted during the light period of the light-dark cycle. All experimental procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Every attempt was made to limit the number of animals used and to minimise their suffering.

### 2.2. Drug and treatment

The rats were randomly allocated to the saline and PCP groups ($n = 18$ in each group). PCP was synthesized by the BDG Synthesis Limited (New Zealand) with a purity of 99.7%, and was freshly dissolved in saline to a concentration of 1 mg/ml. Animals received a subcutaneous (s.c.) injection of saline (2 ml/kg) or PCP at a dose of 2 mg/kg once daily for 12 consecutive days. The variation in animal's body weight was considered and counterbalanced between groups.

### 2.3. Behavioural procedures

After a 4-day washout period (Experiment 1), nine rats in each group were tested in the Y-maze and open field (day 16), object recognition memory task (day 17), forced swimming test (days 18 and 19) and water maze (days 20 to 23), and sacrificed 3 days after completion of behavioural testing (Fig. 1A). In Experiment 2, the remaining nine rats in each group were tested in the Y-maze and open field after a 24-h washout period and sacrificed 3 days after the last treatment (Fig. 1B). The two experiments were conducted at different times, and the experimenters were blind to the grouping information.

All behavioural testing was conducted in a windowless room with four clear 75 W bulbs mounted on the ceiling. A video camera was mounted at ceiling height in the centre of the room and used for recording animals' behaviour during the testing period. A radio speaker was located adjacent to the video camera at ceiling height to provide

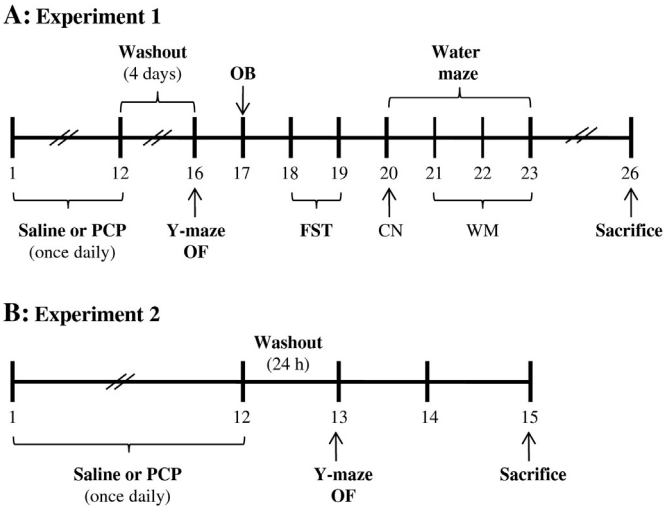


**Fig. 1.** Experimental timeline. All animals received either saline or PCP (2 mg/kg, s.c.) once daily for 12 consecutive days. In Experiment 1, rats were tested in the Y-maze and open field (OF; day 16), object recognition memory task (OB; day 17), forced swimming test (FST; days 18 and 19), and the cued navigation (CN; day 20) and the working memory version (WM, days 21–23) of the water maze, and were sacrificed on day 26. In Experiment 2, animals were tested in the Y-maze and OF on day 13 and sacrificed on day 15.