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# Subchronic phencyclidine treatment in adult mice increases GABAergic transmission and LTP threshold in the hippocampus

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ABSTRACT

Repeated administration of non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists such as phencyclidine (PCP) to rodents causes long-lasting deficits in cognition and memory, and has effects on behaviors that have been suggested to be models of the cognitive impairment associated with schizophrenia (CIAS). Despite this being a widely studied animal model, little is known about the long lasting changes in synapses and circuits that underlie the altered behaviors. Here we examined synaptic transmission ex-vivo in the hippocampus of mice after a subchronic PCP (scPCP) administration regime. We found that after at least one week of drug free washout period when mice have impaired cognitive function, the threshold for long term potentiation (LTP) of CA1 excitatory synapses was elevated. This elevated LTP threshold was directly related to increased inhibitory input to CA1 pyramidal cells through increased activity of GABAergic neurons.

These results suggest repeated PCP administration causes a long-lasting metaplastic change in the inhibitory circuits in the hippocampus that results in impaired LTP, and could contribute to the deficits in hippocampal-dependent memory in PCP-treated mice. Changes in GABA signaling have been described in patients with schizophrenia, therefore our results support using scPCP as a model of CIAS.



## 1. Introduction

### 1.1. Subchronic NMDA receptor antagonism by PCP as a model of cognitive impairment in schizophrenia (CIAS)

Schizophrenia is one of the most common chronic and devastating psychiatric disorders affecting approximately 1% of the population (Sawa and Snyder, 2002). The symptoms of schizophrenia include delusions and hallucinations (positive symptoms), anhedonia, affective flattening, and avolition (negative symptoms), abnormalities in mood, and importantly, because of their impact on outcome, deficits in cognitive functions (Green, 1996; Sawa and Snyder, 2002). The multifaceted clinical syndrome and the complex pathophysiology of schizophrenia including multiple genes, epigenetic and environmental factors are not easily translatable to animals, making the study of the disorder in model organisms difficult (Hall et al., 2014; Jaaro-Peled et al., 2010; Siegel et al., 2013). In particular, attempts to model the cognitive impairment associated with schizophrenia (CIAS) has, of late, been of great interest because the treatment options for this domain are limited (Meltzer et al., 2013; Young et al., 2009). Several lines of evidence have demonstrated that N-methyl-D-aspartate (NMDA) receptor hypofunction may contribute to CIAS, including the observation that non-competitive NMDA receptor antagonists such as phencyclidine (PCP) produce some aspects of CIAS in healthy human subjects and exacerbate symptoms in individuals with schizophrenia (Coyle et al., 2012; Meltzer et al., 2013). While the acute effects of blocking NMDA receptors are noteworthy, repeated administration of NMDA receptor antagonists produce behavioral changes in rodents that persist for many weeks after wash out of the drug (Meltzer et al., 2013; Neill et al., 2010). The post-

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withdrawal effects include both the positive and negative symptoms of schizophrenia, as well as a pronounced deficit in cognitive function providing the model's face validity (Meltzer et al., 2013; Young et al., 2012). Remodeling of circuits and disruption in glutamatergic and GABAergic signaling are found not only in neonates but also in adolescent (Thomases et al., 2014, 2013) and adult rodents (Meltzer et al., 2013). Additionally, a variety of atypical antipsychotic drugs are effective in reversing the behavioral alterations in this pharmacologically-induced disease model, including cognitive deficits, demonstrating its predictive validity (Snigdha et al., 2010; Young et al., 2012). Therefore, the chronic administration of NMDA receptor antagonists has become a commonly used paradigm for understanding the basis of cognitive impairment and for preclinical drug discovery for the development of treatments for CIAS and psychosis (Wiescholleck and Manahan-Vaughan, 2013b).

### 1.2. Involvement of hippocampal function in CIAS

Amongst the neural circuits that are affected in CIAS, there is strong evidence for the involvement of the hippocampus. Consistent with gross morphological changes and functional alterations in the hippocampus (Heckers, 2001; Kraguljac et al., 2014; Rasetti et al., 2014), there are well-established deficits in hippocampal-dependent learning and memory in schizophrenic patients (Perry et al., 2000; Saykin et al., 1991). Similarly, in animal models, chronic administration of NMDA receptor antagonists cause clear deficits in hippocampal-dependent behaviors, such as spatial reference memory tasks as assessed by the Morris Water Maze (Andersen and Pouzet, 2004) and novel object recognition (Horiguchi et al., 2011b; McLean et al., 2009; Snigdha et al., 2010). There has been detailed biochemical, histological (Javitt et al., 2004; Reynolds et al., 2004) and behavioral (Abdul-Monim et al., 2007; Horiguchi et al., 2011a,b; Jenkins et al., 2008) characterization of these pharmacological models of cognitive impairment. However, to date, there have been no published studies that have examined potential functional synaptic alterations in the hippocampus that are correlated with the deficit in hippocampal memory performance.

### 1.3. Alterations in hippocampal synaptic properties by subchronic PCP (scPCP) relevant to CIAS

Here, we report adaptive changes in the physiology of hippocampal synapses in mice after subchronic PCP (scPCP) injection. After induction of cognitive impairment by repeated administration of PCP to animals and following at least one week of drug washout (Rajagopal et al., 2013), hippocampal sections were made from mice and synaptic plasticity tested in the CA1 region of the hippocampus. We found that long-term potentiation (LTP) at CA3-CA1 synapses was impaired in scPCP treated mice in comparison to vehicle treated controls. We did not observe any alterations in basal excitatory synaptic transmission. However, we found that GABAergic inhibitory synaptic input to the CA1 pyramidal cells was enhanced. The increased GABA transmission was directly responsible for increasing the threshold of LTP induction, because LTP could be induced normally in a disinhibited slice. These results suggest that scPCP causes a long-lasting adaptive enhancement of GABA synapses in the CA1 region of the hippocampus that increases the plasticity threshold of excitatory synapses, and is correlated with alterations in hippocampal-dependent learning. These results suggest a novel and previously unknown elevation in GABA signaling in the hippocampus that could contribute to the hippocampal cognitive dysfunction in rodent models of CIAS and possibly underlies the cognitive disruption in patients with schizophrenia.

## 2. Methods and materials

### 2.1. Animals

All procedures related to the care and treatments of animals were approved by the Northwestern University IAUCUC. Mice on a congenic C57Bl/6 background strain (2–3 months old) were purchased from The Jackson Laboratory. Phencyclidine (PCP) (provided by the National Institute on Drug Abuse) was administered (10 mg/kg i.p.) twice daily with a 6–8 h interval for a total of 7 days. Control animals were handled in exactly the same manner and were injected with vehicle for the same period. After PCP or vehicle treatment, animals underwent greater than one week of washout period before being used for subsequent ex-vivo electrophysiological analysis of hippocampal synaptic transmission.

### 2.2. Slice preparation and electrophysiology

Horizontal slices containing the ventral hippocampus were prepared using standard techniques. Briefly, animals were deeply anesthetized (xylazine 10 mg/kg and ketamine 100 mg/kg i.p.) before undergoing cardiac perfusion with an ice-cold sucrose artificial cerebrospinal fluid (ACSF) solution containing (in mM): 85 NaCl, 2.5 KCl, 1.25 $NaH_2PO_4$, 25 $NaHCO_3$, 25 glucose, 75 sucrose, 0.5 $CaCl_2$ and 4 $MgCl_2$, equilibrated with 95% $O_2$ and 5% $CO_2$. In a control group of experiments to exclude any potential effects of acute ketamine, animals were anesthetized with isoflurane and directly decapitated. The brain was removed quickly and mounted on the stage of vibratome (Leica Microsystems, Inc). 350 μm thick sections were made in the same ice-cold sucrose ACSF. Slices were transferred to a recovery chamber containing the sucrose slicing ACSF solution, which was gradually exchanged for a normal ACSF containing (in mM): 125 NaCl, 2.4 KCl, 1.2 $Na_2PO_4$, 25 $NaHCO_3$, 25 glucose, 1 $CaCl_2$ and 2 $MgCl_2$, while the slices were maintained at 30 °C. Individual slices were transferred to a recording chamber and visualized using Dodt contrast optics. For extracellular recordings, slices were perfused with normal ACSF containing (in mM): 125 NaCl, 2.4 KCl, 1.2 $Na_2PO_4$, 25 $NaHCO_3$, 25 glucose, 2 $CaCl_2$ and 1 $MgCl_2$. Recording electrodes were manufactured from borosilicate glass pipettes and had resistances of 3–5 MΩ when filled with regular ACSF. Extracellular field postsynaptic potentials (fPSPs) were evoked using a monopolar electrode filled with ACSF placed in the stratum radiatum. LTP was induced by 100 Hz tetanic stimulation (1 or 3 trains of 100 Hz for 1 s with an inter-train interval of 20 s). Data were collected and analyzed using pClamp 10 software (Molecular Devices, Sunnyvale, CA). For whole-cell patch clamp experiments, recording electrodes were filled with internal solution containing (in mM) 95 CsF, 25 CsCl, 10 Cs-HEPES, 10 Cs-EGTA, 2 NaCl, 2 Mg-ATP, 10 QX-314, 5 TEA-Cl, 5 4-AP for recording of EPSCs, or 75 $CsCH_3SO_3$, 60 CsCl, 1 $MgCl_2$, 0.2 EGTA, 10 HEPES, 2 Mg-ATP 0.3 GTP-$Na_2$, 10 $Na_2$-phosphocreatine, 10 TEA, 5 QX-314 for IPSCs. Pyramidal cells were voltage clamped at +40 mV for measurement of NMDAR currents or at −70 mV for measurement of AMPA receptor mediated EPSCs and $GABA_A$ mediated IPSCs. EPSCs were isolated by the inclusion of the $GABA_A$ antagonist bicuculline (10 μM), and IPSCs were isolated by the inclusion of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM) and D-(−)-2-Amino-5-phosphonopentanoic acid (D-APV) (50 μM) in the extracellular solution. Miniature IPSCs (mIPSCs) were recorded in the presence of tetrodotoxin (TTX) (1 μM) and analyzed using MiniAnalysis (Synaptosoft Inc.).

### 2.3. Data analysis

Statistical analyses were conducted with Microsoft Excel, Graphpad Prism, and OriginPro9.0 software. Two sample comparisons were made using an unpaired two-tailed Student's *t*-test and non-parametric data were compared using the Kolmogorov–Smirnov test. For multiple comparisons, repeated two-way analysis of variance (ANOVA) followed by post-hoc Sidak's correction was employed. Differences were considered significant when $p < 0.05$. Data are shown as mean ± SEM.

## 3. Results

### 3.1. Hippocampal LTP is impaired after scPCP treatment

To determine what changes in hippocampal synapses are caused by scPCP treatment, we administered repeated doses of PCP for seven days and then examined mice at least one week post withdrawal (see methods). During this period, animals display elevated locomotor activity and impairments in tests of hippocampal-dependent declarative memory including impaired novel object recognition (Pyndt Jorgensen et al., 2014; Rajagopal et al., 2013). Hippocampal sections were prepared from scPCP-treated mice and vehicle-treated control mice and extracellular field potential recordings were made of postsynaptic potentials (fPSPs). Standard 100 Hz tetanic stimulation (single train of 100 Hz for 1 s) induced robust LTP of Schaffer collateral-CA1 synapses in vehicle-treated mice; however, in interleaved experiments in slices from mice

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