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Title Page

Effects of repeated ropinirole treatment on phencyclidine-induced hyperlocomotion, prepulse inhibition deficits, and social avoidance in rats*

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Running Title Page

Running title: Repeated dopamine agonist alleviates PCP-induced behaviors

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Abstract

Phencyclidine (PCP), a noncompetitive N-methyl D-aspartate (NMDA) receptor antagonist, provides the most complete pharmacological model of schizophrenia in humans and animals. Acute PCP causes hyperlocomotion, disrupts prepulse inhibition (PPI), and increases social avoidance in rats. We previously showed that repeated treatment with the dopamine (DA) D_2-like receptor agonists, quinpirole or ropinirole, prevents agonist-induced PPI disruption. The present study examines whether repeated ropinirole treatment similarly attenuates the effects of PCP in a more complete model of schizophrenia symptoms. This study examines the effect of repeated D_2-like agonist treatment on locomotion, PPI, and social interaction following acute PCP challenge. The acute effect of PCP (3.0 or 6.0 mg/kg) on locomotor activity was examined to establish a minimum effective dose. Thereafter, the effect of PCP challenge (3.0 mg/kg) on locomotor activity, PPI, and social interaction was assessed in adult male rats before or seven to ten days after termination of repeated daily treatment with ropinirole (0.1 mg/kg) or saline vehicle (0.1 ml/kg) for 28 days. Repeated ropinirole treatment attenuates PCP-induced hyperlocomotion, PPI deficits, and social avoidance. These findings suggest that repeated ropinirole treatment might affect a final common pathway that is vulnerable to both PCP- and dopamine agonist-induced behavioral disruption, thereby providing an alternative approach to block the effects of PCP.

Introduction

Phencyclidine (PCP), a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, is widely used in experimental animal models to study the underlying neurobiology of schizophrenia. PCP was first identified as a possible pharmacological model in rodents after it was noted that the drug exacerbated symptoms in patients with schizophrenia (Itil et al., 1967). Compared to other pharmacological animal models of schizophrenia, PCP is considered to be more complete because of its ability to induce positive, negative, and cognitive symptoms (Angrist and Gershon, 1970; Goldmann et al., 1999). In rodents, acute PCP treatment reduces cortical functioning and impairs behavioral tasks associated with schizophrenia symptoms, such as social behavior and prepulse inhibition of the acoustic startle response (PPI) (Rosenbaum et al., 1959; Hoehn-Saric et al., 1991; Aguado et al., 1994; Pallares et al., 1995; Sams-Dodd, 1996). PCP also increases locomotion in a dose-dependent manner (Sams-Dodd, 1995), which has been used as an indicator of its ability to induce or exacerbate psychotic symptoms (Ogren and Goldstein, 1994; Steinpreis, 1996).

Acute administration of dopamine D_2-like receptor agonists is also used to model symptoms of schizophrenia in rodents. Specifically, PPI deficits are observed after acute infusion of D_2-like agonists either systemically (Chen et al., 1991) or directly into the nucleus accumbens (NAc) (Wan and Swerdlow, 1993). In contrast to acute treatment, repeated treatment with the indirect dopamine agonist, cocaine (Collins et al., 2000) or the direct dopamine agonists, quinpirole or ropinirole (Culm and Hammer, 2004) alleviates prior PPI deficits. Such behavioral tolerance, which we have termed PPI recovery, is observable immediately after repeated ropinirole treatment, and even 28 days later in the absence of further ropinirole treatment (Berger

et al., 2011). This effect of repeated D_2-like agonist treatment on PPI, however, has never been assessed following an acute PCP challenge.

In the present study, we examined the hypothesis that repeated ropinirole treatment would block PCP-induced PPI deficits representing a cognitive symptom associated with schizophrenia. We also investigated the effect of repeated ropinirole on PCP-induced social avoidance to establish whether repeated ropinirole treatment could affect a behavior representing a negative symptom of schizophrenia. Finally, we measured PCP-induced hyperlocomotion after repeated ropinirole, which provides additional evidence of repeated ropinirole effects on PCP-induced behavior.

Materials and Methods

Subjects

Adult male naïve Sprague-Dawley rats (approximately 250 g at the start of the experiment, Harlan Labs, Indianapolis, IN) were triple-housed for the duration of the experiment and were provided with *ad libitum* food and water. Rats were housed with the same cage mates in their home cage (L, W, H: 19 x 10.25 x 8 inches) throughout the experiment regardless of group assignment. Behavioral testing occurred from 1000 to 1400 h during the dark phase of a 12 h reversed light-dark cycle (lights off at 0900 h). All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health, and were approved by the Institution's Animal Care and use Committee.

Behavioral testing and drug treatment

Experiment 1: Acute PCP dose-response locomotor testing

Each rat was placed into a locomotor testing cage, which was the same size as the home cage and contained clean bedding, and was injected with saline (1.0 ml/kg, ip) prior to a 30 min baseline locomotion period. Locomotor activity was assessed after saline and subsequent drug exposure using VideoTrack (Viewpoint Life Sciences, Montreal, Quebec). Following baseline testing, acute PCP (0, 3.0 or 6.0 mg/kg, ip) was administered, and the software characterized and recorded total movements for each animal within 10 min time intervals for 60 min. For each time interval, total distance traveled measured in cm was recorded.

Experiment 2: Locomotor testing after repeated ropinirole treatment and PCP challenge

Rats were injected for 28 days with repeated ropinirole HCl (0.1 mg/kg, sc) or saline vehicle (1.0 ml/kg, sc; Sigma-Aldrich, St. Louis, MO) (Fig. 1). Seven to ten days after termination of repeated treatment, rats were injected with saline (ip), and locomotor activity was recorded in a locomotor testing cage for 30 min to establish their baseline activity. Rats then received PCP (3.0 mg/kg, ip) or saline vehicle (1.0 ml/kg, ip), and were immediately returned to the testing cage for an additional 60 min of testing. PCP was graciously provided by the NIDA Drug Supply Program, Bethesda, MD.

Experiment 3: Prepulse inhibition testing

Startle amplitude was measured using the Startle Monitor behavioral testing system (Kinder Scientific, Poway, CA). After two days of saline injections (1.0 ml/kg, ip) followed by 5 min of acclimation to the PPI chamber each day, baseline PPI was determined by averaging the results of testing on both days. PPI testing included the rat being placed into a PPI chamber and exposed to a 70 dB ambient sound for 5 min, followed by a PPI baseline or test session. A **PPI baseline session** consisted of four consecutive pulse trials (120 dB, 40-msec pulses), a randomized presentation of 16 pulse, 15 prepulse (10 each of 73, 76, and 82 dB, 20 msec

prepulses, followed 100 msec later by a pulse), and eight trials without stimulation, and ending with four pulse trials. Based on the average of their PPI baseline tests, rats were ranked from highest to lowest, then each of four rats within clusters having the same average PPI response were randomly assigned to one of the four treatment groups. Thus, each treatment group contained subjects with the same average PPI response before experimental intervention.

The first **PPI test session** occurred one day after the PPI baseline sessions (Fig.1), when rats were given an acute PCP (3.0 mg/kg, ip) or saline vehicle (1.0 ml/kg, ip) challenge, then immediately placed into PPI chambers and exposed to 5 min of chamber acclimation (65 dB white noise), followed by four consecutive pulse trials, a randomized presentation of 16 pulse, 30 prepulse (10 each of 73, 76, and 82 dB, 20 msec prepulses, followed 100 msec later by a pulse), and 10 trials without stimulation, and ending with four pulse trials. The final PPI test session took place seven to ten days after termination of 28 daily ropinirole HCl (0.1 mg/kg, sc) or saline vehicle (1.0 ml/kg, sc) treatments, when rats received PCP (3.0 mg/kg, ip) or saline vehicle (1.0 ml/kg, ip) challenge, and were immediately placed into the PPI chambers for testing as described above. This dose of ropinirole was previously demonstrated to reliably induce PPI tolerance upon repeated treatment (Culm and Hammer, 2004; Culm et al., 2004; Berger et al., 2011).

Mean startle amplitude was measured over 100 ms following the presentation of the pulse stimulus in units of Newton (N). Percent PPI was calculated using the following equation: 1 - [(mean prepulse response/mean pulse response) x 100]; a higher percent PPI implies greater inhibition of startle response due to presentation of the prepulse. Intertrial intervals ranged from 5 to 30 sec, and averaged 15 sec for both PPI baseline and test sessions.

Experiment 4: Social interaction

Rats received 28 days of repeated ropinirole HCl (0.1 mg/kg, sc) or saline vehicle (1.0 ml/kg, sc) treatment; seven to ten days later, they received a PCP (3.0 mg/kg, ip) or saline vehicle (1.0 ml/kg, ip) challenge as described above (Fig.1). Treatment groups were randomly, assigned and two rats taken from different home cages and naïve to any prior behavioral testing (i.e., novel conspecific exposure) were placed simultaneously at opposite corners of a clean open arena (3' X 3') 10 min after either PCP or vehicle treatment. These rats received the same acute challenge with the only criteria were that they were not cage mates. One rat of the pair was identified by a black stripe placed on their back with permanent marker and social activity for each rat was recorded individually. Interaction between rats was recorded for 5 min using Top Scan (Clever Sys, Reston, VA) under red light using a camera (Panasonic WV-CP284 Camera-540 TVL- Day/Night (Uemura et al.)), that detected the location of each subject within either a center interaction zone (2' X 2') or the remaining peripheral interaction zone. Social contact was detected when subjects were within 2 cm of each other. Additionally, the velocity of approach before contact determined active or passive contact. For example, if one rat was stationary and the other rat moved towards it, the activity of the animal in motion was labeled active contact, while the stationary animal's activity was labeled passive contact. Identification of contacts between rats were defined by the velocity of the animal's approach; Active contacts required greater than 80 mm/sec per 15 frames approach speed, while passive contacts were less than 20 mm/sec per 15 frames.

Statistical analysis

Changes in locomotor activity between trials were analyzed using two-way repeated measures analyses of variance (ANOVA) followed by Tukey's post hoc test for the cumulative post-injection activity to identify between-group differences at specific time intervals. Percent

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PPI data were calculated for each prepulse, then combined to determine the mean pulse response on the first and the last day of PCP challenge, which were analyzed using a repeated measures ANOVA with drug treatment as the between-subject factor and Tukey's post hoc test. Startle (pulse only) and no stimulation responses were analyzed on last day of PCP challenge. Differences in social interaction between drug treatments were analyzed using between subjects ANOVA followed by Tukey's post hoc test. Statistical significance was determined using GraphPadPrism (GraphPad Software Inc., San Diego, CA), and the researcher was blinded to the animal's prior experimental condition.

Results

Experiment 1: Locomotion following acute PCP challenge

PCP (3.0 or 6.0 mg/kg, ip) increased distance traveled (cm) in a dose-dependent manner at 50 and 60 min after PCP challenge over the observation period, as indicated by a significant interaction between PCP and time ($F_{16,232} = 4.545$, $P < 0.0001$; Fig. 2A). The higher PCP dose produced extended hyperlocomotion beyond the end of the observation period ($P < 0.001$), while the lower PCP dose increased locomotion for 30 min after the challenge injection ($P = 0.018$) as compared to rats that received saline treatment. Distance traveled collapsed over time revealed a significant effect of PCP ($F_{2,15} = 36.82$, $P < 0.0001$; Fig. 2B) when the higher PCP dose ($P = 0.02$) and the lower PCP dose ($P < 0.0001$) were compared to saline treatment. The lower dose of PCP (3.0 mg/kg) was used for all subsequent challenge studies because distance traveled had normalized to baseline levels by the end of the test.

Experiment 2: Locomotion following acute PCP challenge after repeated ropinirole treatment

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PCP challenge after repeated saline treatment significantly increased distance traveled over time as indicated by a significant interaction between PCP and time ($F_{24,232} = 1.741$, $P = 0.026$; Fig. 3A). This acute PCP challenge significantly ($P = 0.037$) increased total distance traveled compared to all other drug treatment groups 10-40 min after injection. Distance traveled collapsed over time revealed a significant main effect of PCP ($F_{3,32} = 5.091$, $P = 0.005$; Fig. 3B) wherein acute PCP induced greater locomotion compared to acute saline challenge ($P = 0.038$, post-hoc comparison). By contrast, PCP did not increase locomotion in rats that were treated with repeated ropinirole, as there was no significant effect of PCP challenge in those rats. Neither distance travelled nor the number of ambulations was affected by saline challenge after either repeated saline or repeated ropinirole treatment (Fig. 3, A and B).

Experiment 3: Prepulse inhibition following acute PCP challenge after repeated ropinirole treatment

Independent analyses of individual prepulse levels revealed a similar trend as combined PPI prepulse intensities. At 73 dB prepulse PPI, a main effect of drug treatment ($F_{3,124} = 16.84$, $P < 0.0001$; Fig. 4A) was detected and PCP reduced PPI on both first ($P = 0.025$) and final ($P = 0.014$) PPI tests. At 76 dB prepulse PPI, a main effect of drug treatment ($F_{3, 124} = 23.88$, $P < 0.0001$; Fig. 4B) was detected, and PCP reduced PPI in rats repeatedly treated with saline on the first ($P = 0.015$) and final ($P < 0.0001$) PPI tests. By contrast, PCP failed to have the same effect on PPI 7 days after 28 days of repeated ropinirole compared to repeated vehicle treatment ($P = 0.0058$). At 82 dB prepulse PPI, main effects of drug treatment ($F_{3, 124} = 14.43$, $P < 0.0001$; Fig. 4C) and day of testing ($F_{3, 124} = 8.81$, $P < 0.001$; Fig. 4C) were detected, and PCP reduced PPI on Day 35 ($P < 0.0001$). Thus, repeated ropinirole treatment attenuated the effect of acute PCP-induced PPI deficits.

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After combining all PPI intensities and days of testing, repeated measures ANOVA with PPI intensities (73, 76, and 82 dB) and day of testing (Day 0 and Day 35) as within subject factors and drug treatment (saline-saline, ropinirole-saline, saline-PCP, ropinirole-PCP) as the between subject factor detected an interaction between day of testing and decibel level ($F_{3,62}$ = 4.02, P = 0.019), main effect of day of testing ($F_{1,62}$ = 4.853, P = 0.031), decibel level ($F_{1,62}$ = 296.661, P < 0.0001), and drug treatment ($F_{1,62}$ = 10.36, P < 0.0001; Fig. 5). Acute PCP challenge prior to repeated ropinirole treatment induced a PPI deficit on Day 0 (P < 0.0001), but rats that had received 28 days of repeated ropinirole treatment attenuated the PCP-induced PPI deficit, as observed by a significant difference between rats repeatedly treated with ropinirole versus saline (P < 0.0001). Neither repeated treatment nor PCP challenge affected behavior during the no stimulation trials before and after repeated ropinirole treatment; there was a significant effect of PCP on startle response during the final PPI test (P = 0.05), but this parameter was unaffected by repeated treatment (Table 1).

Experiment 4: Social interaction following acute PCP challenge after repeated ropinirole treatment

The amount of time rats engaged in active social interaction was reduced by acute PCP challenge 10 days after repeated saline treatment ($F_{3,61}$ = 12.27, P < 0.001; Fig. 5), however, this effect did not occur in rats treated repeatedly with ropinirole prior to PCP challenge (P < 0.0001). There was no significant effect of repeated treatment or PCP challenge on amount of time in passive contact (P = 0.90; Table 2). Locomotion was unaffected by repeated treatment or drug challenge as no significant difference in total distance traveled was detected ($F_{3,61}$ = 1.389, P = 0.254). Active contact, as determined by the velocity of the approach before social

interaction, was also assessed and replicated the ropinirole-induced reduction of PCP's effect on social contact (data not shown).

Discussion

The focus of the present study was to determine whether repeated ropinirole treatment could block the effects of PCP on various behaviors associated with symptoms of schizophrenia. After determining that acute PCP dose-dependently increased locomotion, we then utilized in subsequent experiments the minimal PCP dose whose effect normalized within the test period (3.0 mg/kg). We observed that repeated ropinirole treatment blocked hyperlocomotion caused by acute PCP challenge without affecting locomotion following saline challenge. Furthermore, acute PCP challenge produced PPI deficits before and after repeated saline treatment, while repeated ropinirole treatment attenuated PCP-induced PPI deficits and significantly increased PPI. Similarly, acute PCP challenge after repeated saline treatment increased social avoidance, while repeated ropinirole treatment led to recovery of social interaction after PCP challenge.

Acute PCP challenge dose-dependently increases regional local cerebral glucose utilization in the NAc and pallidum (Weissman et al., 1987), defining a brain circuit which may be responsible for its effects on locomotion and PPI (Ogren and Goldstein, 1994; Swerdlow et al., 2001). The observed attenuation of these behavioral effects suggests that the effect of PCP on this circuitry may be reduced following repeated ropinirole treatment. We have shown that repeated treatment with quinpirole or ropinirole produced recovery of agonist-induced PPI deficits which requires activation of cAMP response element binding protein (CREB) in NAc neurons, as overexpression of mutant CREB in this region prevents repeated ropinirole-induced PPI recovery (Culm and Hammer, 2004; Culm et al., 2004; Berger et al., 2011). The reversal of

both PCP- and D_2-like agonist-induced deficits suggests that repeated ropinirole treatment might have altered a final common pathway for both PCP and D_2-like agonist effects.

Acute PCP challenge (5.0 mg/kg, ip) is known to increase extracellular levels of dopamine and glutamate in both NAc and medial prefrontal cortex (Adams and Moghaddam, 1998), which may be due to disinhibition of VTA dopamine neurons projecting to the forebrain and ventral striatum due to reduced local GABAergic function (Deutch et al., 1987; Moghaddam et al., 1997; Yonezawa et al., 1998; Goldmann et al., 1999). While PCP targets its receptors on the NMDA channel complex, which are present on both NAc and cortical pyramidal neurons, there is also evidence that it may bind directly to D_2 receptors in their high affinity state (Seeman and Guan, 2008). Despite these potential relationships of PCP with D_2 receptors, haloperidol did not block the effect of PCP (5.0 mg/kg) on either PPI or social interaction (Keith et al., 1991; Steinpreis et al., 1994), although haloperidol did block the effects of PCP (3.0 mg/kg) on locomotor activity (Ogren and Goldstein, 1994) even though PCP was observed to induce locomotion in dopamine-deficient mice (Chartoff et al., 2005). Thus, dopamine binding to dopamine receptors after PCP challenge might not underlie ropinirole-induced recovery of PCP effects. Instead, repeated ropinirole-induced alteration of function in a final common neuronal pathway from the NAc might be responsible for attenuation of both PCP and dopamine agonist effects on locomotion, PPI and social behavior.

An interesting corollary of our findings on repeated ropinirole treatment is the PPI recovery is present up to 30 days after termination of ropinirole treatment (Berger et al., 2011). In the present study, repeated ropinirole attenuated PCP effects on PPI, locomotion and social behavior 7-10 days after termination of ropinirole treatment, demonstrating that long-lasting effects on the final common pathway underlying certain symptoms of schizophrenia may be

present. The mechanism of such long-lasting effects is unknown. We showed previously that acute quinpirole reduces, while repeated treatment increases NAc CREB phosphorylation (Culm et al., 2004). Similarly, we have observed that acute quinpirole reduces, while repeated treatment increases expression of the long-lasting transcription factor, ΔFosB, in NAc neurons (Maple, submitted). We speculate that transcriptional regulation by ΔFosB within NAc circuits may underlie the long-lasting behavioral response to repeated D_2-like agonist treatment, which is opposite to that caused by acute treatment.

It should be noted that chronic ropinirole treatment as utilized herein could exacerbate existing sensorimotor gating deficits in patients with schizophrenia (Braff et al., 2001). However, dopamine agonist effects on sensorimotor gating are dose-dependent, suggesting that an escalating treatment paradigm starting with minimal doses might avoid initial disruption, while ultimately reversing PCP- or schizophrenia-induced sensorimotor gating deficits. However, the present study did not examine effects on working memory, which may be more closely related to glutamatergic effects of PCP in frontal cortex (Adams and Moghaddam, 1998). Future studies are needed to determine the effect of repeated dopamine agonist treatment on the frontal cortical function and dysfunction.

Here we showed that repeated D_2-like agonist treatment blocked PCP-induced hyperlocomotion, PPI deficits, and social avoidance seven days after termination of treatment. This is the first study to examine the effects of repeated D_2-like agonist treatment on acute PCP deficits. These findings suggest that changes induced by repeated ropinirole treatment may alter the function of the neuronal circuitry that regulates locomotion, PPI, and social interaction, perhaps stemming from the NAc. The use of PCP challenge providing a more complete model of

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schizophrenia symptoms may be a more reasonable alternative to assess the behavioral effects of repeated dopamine agonists.

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Authorship Contributions

Developed the concept and the designed experiments: (Maple and Hammer)

Completed the drug administrations and behavioral testing: (Maple, Call, and Kimmel)

Conducted statistical analysis: (Maple)

Interpreted the results and prepared the manuscript: (Maple, Call, and Hammer)

References

Adams B and Moghaddam B (1998) Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine. *J Neurosci* **18**:5545-5554.

Aguado L, San Antonio A, Perez L, del Valle R and Gomez J (1994) Effects of the NMDA receptor antagonist ketamine on flavor memory: conditioned aversion, latent inhibition, and habituation of neophobia. *Behav Neural Biol* **61**:271-281.

Angrist BM and Gershon S (1970) The phenomenology of experimentally induced amphetamine psychosis--preliminary observations. *Biol Psychiatry* **2**:95-107.

Berger AK, Green T, Siegel SJ, Nestler EJ and Hammer RP, Jr. (2011) cAMP response element binding protein phosphorylation in nucleus accumbens underlies sustained recovery of sensorimotor gating following repeated D-like receptor agonist treatment in rats. *Biol Psychiatry* **69**:288-294.

Braff DL, Geyer MA and Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* **156**:234-258.

Chartoff EH, Heusner CL and Palmiter RD (2005) Dopamine is not required for the hyperlocomotor response to NMDA receptor antagonists. *Neuropsychopharmacology* **30**:1324-1333.

Chen DY, Swerdlow HP, Harke HR, Zhang JZ and Dovichi NJ (1991) Low-cost, high-sensitivity laser-induced fluorescence detection for DNA sequencing by capillary gel electrophoresis. *J Chromatogr* **559**:237-246.

Collins JJ, Byrnes ME, Dunkel IJ, Lapin J, Nadel T, Thaler HT, Polyak T, Rapkin B and Portenoy RK (2000) The measurement of symptoms in children with cancer. *J Pain Symptom Manage* **19**:363-377.

Culm KE and Hammer RP, Jr. (2004) Recovery of sensorimotor gating without G protein adaptation after repeated D2-like dopamine receptor agonist treatment in rats. *J Pharmacol Exp Ther* **308**:487-494.

Culm KE, Lugo-Escobar N, Hope BT and Hammer RP, Jr. (2004) Repeated quinpirole treatment increases cAMP-dependent protein kinase activity and CREB phosphorylation in nucleus accumbens and reverses quinpirole-induced sensorimotor gating deficits in rats. *Neuropsychopharmacology* **29**:1823-1830.

Deutch AY, Tam SY, Freeman AS, Bowers MB, Jr. and Roth RH (1987) Mesolimbic and mesocortical dopamine activation induced by phencyclidine: contrasting pattern to striatal response. *Eur J Pharmacol* **134**:257-264.

Goldmann C, Petry H, Frye S, Ast O, Ebitsch S, Jentsch KD, Kaup FJ, Weber F, Trebst C, Nisslein T, Hunsmann G, Weber T and Luke W (1999) Molecular cloning and expression of major structural protein VP1 of the human polyomavirus JC virus: formation of virus-like particles useful for immunological and therapeutic studies. *J Virol* **73**:4465-4469.

Hoehn-Saric R, McLeod DR and Glowa JR (1991) The effects of NMDA receptor blockade on the acquisition of a conditioned emotional response. *Biol Psychiatry* **30**:170-176.

Itil TM, Rizzo AE and Shapiro DM (1967) Study of behavior and EEG correlation during treatment of disturbed children. *Diseases of the nervous system* **28**:731-736.

Keith VA, Mansbach RS and Geyer MA (1991) Failure of haloperidol to block the effects of phencyclidine and dizocilpine on prepulse inhibition of startle. *Biol Psychiatry* **30**:557-566.

Maple AMH, R.; Britton, M. J.; Nikulina, E. M.; Hammer, R. P, Jr. (submitted) Repeated quinpirole treatment induces tolerance of prepulse inhibition and conditioned avoidance responding, with concurrent FosB expression in the rat nucleus accumbens

. *Pharmacol Biochem Behav*.

Moghaddam PH, Zwinderman AH, de Knijff P, Roep BO, Schipper RF, Van der Auwera B, Naipal A, Gorus F, Schuit F and Giphart MJ (1997) TNFa microsatellite polymorphism modulates the risk of IDDM in Caucasians with the high-risk genotype HLA DQA1*0501-DQB1*0201/DQA1*0301-DQB1*0302. Belgian Diabetes Registry. *Diabetes* **46**:1514-1515.

Ogren SO and Goldstein M (1994) Phencyclidine- and dizocilpine-induced hyperlocomotion are differentially mediated. *Neuropsychopharmacology* **11**:167-177.

Pallares MA, Nadal RA, Silvestre JS and Ferre NS (1995) Effects of ketamine, a noncompetitive NMDA antagonist, on the acquisition of the lever-press response in rats. *Physiol Behav* **57**:389-392.

Rosenbaum G, Cohen BD, Luby ED, Gottlieb JS and Yelen D (1959) Comparison of sernyl with other drugs: simulation of schizophrenic performance with sernyl, LSD-25, and amobarbital (amytal) sodium; I. Attention, motor function, and proprioception. *AMA Arch Gen Psychiatry* **1**:651-656.

Sams-Dodd F (1995) Distinct effects of d-amphetamine and phencyclidine on the social behaviour of rats. *Behav Pharmacol* **6**:55-65.

Sams-Dodd F (1996) Phencyclidine-induced stereotyped behaviour and social isolation in rats: a possible animal model of schizophrenia. *Behav Pharmacol* **7**:3-23.

Seeman P and Guan HC (2008) Phencyclidine and glutamate agonist LY379268 stimulate dopamine D2High receptors: D2 basis for schizophrenia. *Synapse* **62**:819-828.

Steinpreis RE (1996) The behavioral and neurochemical effects of phencyclidine in humans and animals: some implications for modeling psychosis. *Behav Brain Res* **74**:45-55.

Steinpreis RE, Sokolowski JD, Papanikolaou A and Salamone JD (1994) The effects of haloperidol and clozapine on PCP- and amphetamine-induced suppression of social behavior in the rat. *Pharmacol Biochem Behav* **47**:579-585.

Swerdlow NR, Geyer MA and Braff DL (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology (Berl)* **156**:194-215.

Uemura H, Katsuura-Kamano S, Yamaguchi M, Arisawa K, Hamajima N, Hishida A, Kawai S, Oze I, Shinchi K, Takashima N, Suzuki S, Nakahata N, Mikami H, Ohnaka K, Kuriyama N, Kubo M, Tanaka H and Japan Multi-institutional Collaborative Cohort Study G (2015) A variant of the CLOCK gene and related haplotypes are associated with the prevalence of type 2 diabetes in the Japanese population. *J Diabetes*.

Wan FJ and Swerdlow NR (1993) Intra-accumbens infusion of quinpirole impairs sensorimotor gating of acoustic startle in rats. *Psychopharmacology (Berl)* **113**:103-109.

Weissman AD, Dam M and London ED (1987) Alterations in local cerebral glucose utilization induced by phencyclidine. *Brain Res* **435**:29-40.

Yonezawa Y, Kuroki T, Kawahara T, Tashiro N and Uchimura H (1998) Involvement of gamma-aminobutyric acid neurotransmission in phencyclidine-induced dopamine release in the medial prefrontal cortex. *Eur J Pharmacol* **341**:45-56.

Footnotes

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Legends for Figures

Figure 1. Timeline and design of chronic treatment experiments. In each experiment, rats received 28 days of repeated daily ropinirole (0.0 or 0.1 mg/kg, sc) treatment followed by an acute PCP (3.0 mg/kg, ip) challenge 7-10 days after termination of treatment.

Figure 2. (A) Distance traveled over time before and after acute PCP (3.0 and 6.0 mg/kg) or saline injection. Injection time indicated by the vertical arrow. **(B)** Total distance traveled during 60 min after PCP or saline injection. *$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$ versus saline treatment by two-way ANOVA followed by Tukey's test. Data are expressed as amount of distance traveled (mean ± SEM). $n = 11$ rats/group.

Figure 3. (A) Distance traveled over time before and after PCP (3.0 mg/kg) or saline challenge ten days after repeated saline or ropinirole (0.1 mg/kg) treatment. Injection time indicated by the vertical arrow. **(B)** Total distance traveled during 60 min after PCP or saline injection. **$P <$ 0.01 versus repeated saline treatment and challenge by two-way ANOVA followed by Tukey's test. Data are expressed as amount of distance traveled (mean ± SEM). $n = 8$ rats/group.

Figure 4. Percent PPI data determined at prepulse levels 3 **(A)**, 6 **(B)**, or 12 **(C)** dB above ambient noise (70 dB); PCP challenge one day before repeated treatment (Day 0) and seven days after repeated treatment (Day 35). *$P < 0.05$; **$P < 0.01$ ****$P < 0.0001$ on Day 0 or Day 35. PCP challenge after repeated saline treatment by two-way ANOVA followed by Tukey's test. Data are expressed as percentage of PPI (mean ± SEM). $n = 16$ rats/group.

Figure 5. Percent PPI data collapsed across prepulse levels 3, 6, or 12 dB above ambient noise (70 dB); PCP challenge one day before repeated treatment (Day 0) and seven days after repeated treatment (Day 35). ****$P < 0.0001$ on Day 0, versus saline acute challenges, ****$P < 0.0001$ on Day 35 versus repeated saline treatment and challenge, also PCP challenge after repeated

ropinirole treatment versus PCP challenge after repeated saline treatment by two-way ANOVA followed by Tukey's test. Data are expressed as percentage of PPI (mean ± SEM). $n = 16$ rats/group.

Figure 6. Time (mean ± SEM) spent engaged in social interaction after acute PCP challenge seven days after repeated treatment. ****$P < 0.0001$ compared to saline challenge by two-way ANOVA followed by Tukey's test. Data are expressed as average of seconds (mean ± SEM). $n = 16$ rats/group.

Tables

TABLE 1

Average raw Newtons (N) response to pulse (120 dB) and no stimulus trials (mean ± SEM) on challenge day (Day 0 or 35).

Drug Treatment Repeated-Challenge	120dB pulse Day 0	No Stimulus Day 0	120dB pulse Day 35	No Stimulus Day 35
Saline-Saline	0.466 ± 0.090	0.087 ± 0.049	0.549 ± 0.080	0.047 ± 0.005
Ropinirole-Saline	0.688 ± 0.148	0.081 ± 0.038	1.018 ± 0.214	0.049 ± 0.003
Saline-PCP	0.854 ± 0.136	0.047 ± 0.005	1.509 ± 0.346*	0.051 ± 0.005
Ropinirole-PCP	0.672 ± 0.152	0.041 ± 0.004	1.326 ± 0.238*	0.048 ± 0.006

* $P < 0.05$ as compared to Saline-Saline.

TABLE 2

Average amount of time (mean sec ± SEM) spent in passive interaction on challenge day.

Drug Treatment	Time (mean sec ± SEM)
Saline-Saline	32.74 ± 5.27
Ropinirole-Saline	37.04 ± 8.71
Saline-PCP	31.60 ± 6.59
Ropinirole-PCP	38.78 ± 10.71

No significant effect of repeated drug treatment or PCP challenge on time during passive interaction.

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Figure 1

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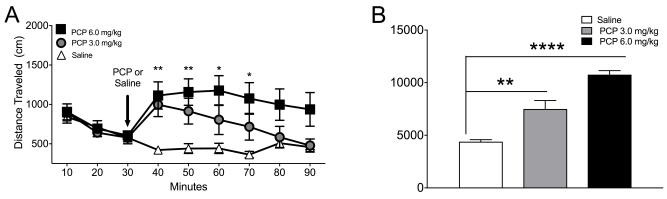

Figure 2

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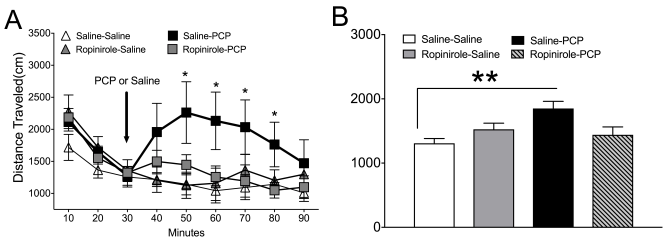

Figure 3

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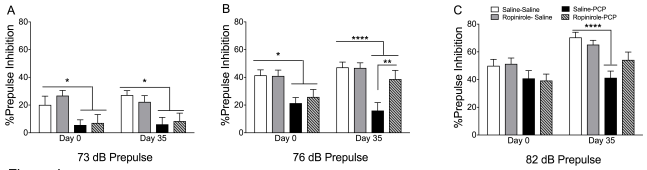

Figure 4

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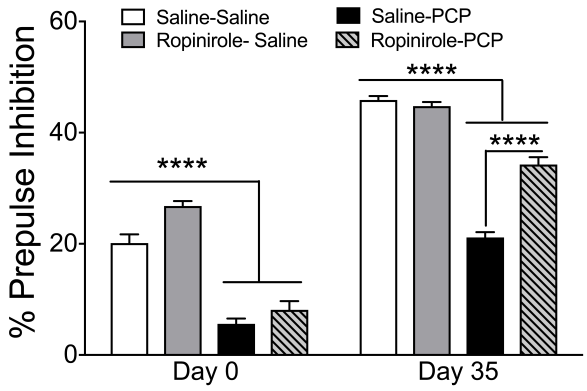

Figure 5

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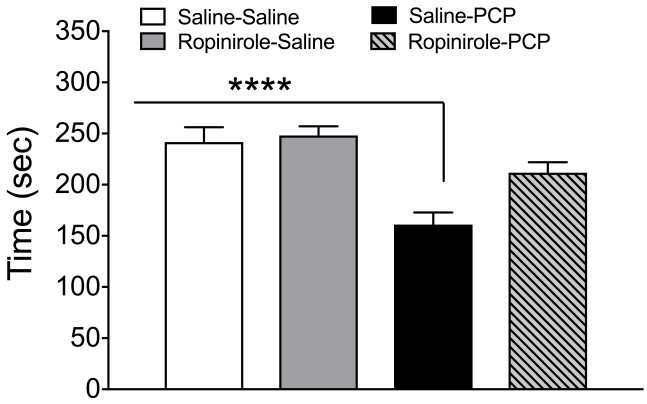

Figure 6