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Repeated effects of the neurotensin receptor agonist PD149163 in three animal tests of antipsychotic activity: Assessing for tolerance and cross-tolerance to clozapine

Shinnyi Chou
University of Nebraska-Lincoln

Collin Davis
University of Nebraska-Lincoln, s-cdavis26@unl.edu

Sean Jones
University of Nebraska-Lincoln

Ming Li
University of Nebraska-Lincoln, mli2@unl.edu

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Repeated effects of the neurotensin receptor agonist PD149163 in three animal tests of antipsychotic activity: assessing for tolerance and cross-tolerance to clozapine

Shinnyi Chou, **Collin Davis**, **Sean Jones**, and **Ming Li***
Department of Psychology, University of Nebraska-Lincoln, USA

Abstract

Neurotensin is an endogenous neuropeptide closely associated with the mesolimbic dopaminergic system and shown to possess antipsychotic-like effects. In particular, acute neurotensin receptor activation can inhibit conditioned avoidance response (CAR), attenuate phencyclidine (PCP)-induced prepulse inhibition (PPI) disruptions, and reverse PCP-induced hyperlocomotion. However, few studies have examined the long term effects of repeated neurotensin receptor activation and results are inconsistent. Since clinical administration of antipsychotic therapy often requires a prolonged treatment schedule, here we assessed the effects of repeated activation of neurotensin receptors using an NTS1 receptor selective agonist, PD149163, in 3 behavioral tests of antipsychotic activity. We also investigated whether reactivity to the atypical antipsychotic clozapine was altered following prior PD149163 treatment. Using both normal and prenatally immune activated rats generated through maternal immune activation with polyinosinic:polycytidilic acid, we tested PD149163 in CAR, PCP (1.5 mg/kg)-induced PPI disruption, and PCP (3.2 mg/kg)-induced hyperlocomotion. For each paradigm, rats were first repeatedly tested with vehicle or PD149163 (1.0, 4.0, 8.0 mg/kg, sc) along with vehicle or PCP for PPI and hyperlocomotion tests, then challenged with PD149163 after 2 drug-free days. All rats were then challenged with clozapine (5.0 mg/kg, sc). During the repeated test period, PD149163 exhibited antipsychotic-like effects in all three models. On the PD149163 challenge day, prior drug treatment only caused a tolerance effect in CAR. This tolerance in CAR was transferrable to clozapine, as it enhanced clozapine tolerance in the same group of animals. Although no tolerance effect was seen in the PD149163 challenge for the PCP-induced hyperlocomotion test, the clozapine challenge showed increased sensitivity in groups previously exposed to repeated PD149163 treatment. Our findings suggest repeated exposure to NTS1 receptor agonists can induce a dose-dependent tolerance and cross-tolerance to clozapine to some of its behavioral effects but not others.

*Corresponding author: Ming Li, PhD, Associate Professor, Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE 68588-0308, USA. Telephone: 402-472-3144. mli2@unl.edu.

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Keywords

Neurotensin; Clozapine; Conditioned avoidance response; Phencyclidine; Locomotor activity; Prepulse inhibition; Maternal immune activation; Sensitization; Tolerance

INTRODUCTION

Neurotensin (NT) is an endogenous 13-amino acid neuropeptide ubiquitous in the central nervous system (CNS) (Carraway and Leeman, 1973, 1975). The neurotransmitter and its two main receptor subtypes, the NTS1 and NTS2 G protein-coupled receptors, are especially highly distributed in the hypothalamus, amygdala, and the nucleus accumbens, and are associated with the mesolimbic dopaminergic system (Boudin *et al*, 1996; Chalon *et al*, 1993; Cooper *et al*, 1981; Mazella *et al*, 1996; Tanaka *et al*, 1990; Vita *et al*, 1996). Within the CNS, NT is involved in an array of processes, including the activation of intracellular signaling pathways (Hermans *et al*, 1993), modulation of cytokine expression (Wang *et al*, 2006), stress-induced analgesia (Dobner *et al*, 2001; Gully *et al*, 1993, 1997; Maeno *et al*, 2004; Pettibone *et al*, 2002; Remaury *et al*, 2002), and the sensitization to psychostimulant drugs (Betancur *et al*, 1998; Costa *et al*, 2001; Horger *et al*, 1994; Panayi *et al*, 2002, 2005). NT has also been implicated in both the etiology and treatment of schizophrenia. Clinical studies have shown that some schizophrenic patients have reduced levels of NT in the cerebrospinal fluid, and treatment with antipsychotic drugs (APDs) is able to restore NT levels (Breslin *et al*, 1994; Garver *et al*, 1991; Lindström *et al*, n.d.; Nemeroff *et al*, 1989; Sharma *et al*, 1997; Widerlöv *et al*, 1982). In addition, NT signaling seems to play a crucial role in mediating the central actions of available APDs. Treatment of patients with APDs has been shown to increase NT levels in specific brain regions (Govoni *et al*, 1980; Kinkead *et al*, 2000), and both NT- and NT receptor-null mouse models have demonstrated defects in APD response compared to controls (Kinkead *et al*, 2005). Numerous studies have further examined the possibility of NT as an exogenous atypical APD (Boules *et al*, 2001; Feifel *et al*, 1997, 1999; Hertel *et al*, 2002; Li *et al*, 2010b; Shilling and Feifel, 2008; Shilling *et al*, 2003), a classification generally given to therapeutics effective in alleviating psychotic symptoms without causing severe extrapyramidal side effects (Wadenberg and Hicks, 1999). Receptor stimulations with NT or agonists have produced APD-like effects in models with high predictive validity of efficacy (Gleason and Shannon, 1997; Natesan *et al*, 2006; Wadenberg, 2010), including the attenuation of phencyclidine (PCP) and *d*-amphetamine induced hyperactivity (Boules *et al*, 2001; Li *et al*, 2010b), disruptions in the conditioned avoidance response (CAR) (Hertel *et al*, 2001), and the reversal of prepulse inhibition (PPI) deficits induced by *d*-amphetamine (Shilling *et al*, 2003), as well as dopamine, serotonin, and α-1 adrenoreceptor agonists (Feifel *et al*, 1999, 2003; Shilling *et al*, 2003, 2004).

PD149163 (PD) is an NTS1 receptor-selective agonist that crosses the blood-brain barrier and is shown to have APD-like properties in animal studies (Petrie *et al*, 2004). Acute systemic injections of PD exhibit diminished CAR without generating catalepsy (Holly *et al*, 2011), and improve the reduction of PPI in Brattleboro rats and by amphetamine, dizocilpine, and serotonin 2A and α-1 adrenoreceptor agonists (Feifel *et al*, 2009). However, as most APD regimens require a repeated and chronic administration, it is clinically relevant

to investigate the chronic effects of PD. Previous research using the NT analogue NT69L suggests the emergence of a tolerance phenomenon in both CAR and amphetamine-induced hyperactivity (Feifel *et al*, 2007; Hertel *et al*, 2001, 2002; Norman *et al*, 2008a), while more recent studies of repeated PD exposure seem to contradict the tolerance effect using amphetamine-induced hyperactivity and Brattleboro PPI models (Feifel *et al*, 2008). These discrepancies may be due to the choice of dosage, as earlier work has observed dose-dependent effects of NT infusion (Feifel *et al*, 1997), or perhaps the chosen time points. In order to resolve these discrepancies, in the present study, we assessed the long-term repeated effects of PD in three distinct animal tests of antipsychotic activity: the repeated CAR, PCP-induced hyperlocomotion, and PCP-induced PPI models. For each model, we employed a behavioral paradigm similar to those used for the study of psychomotor sensitization (Robinson *et al*, 1998; Stewart and Badiani, 1993). The procedure involves inducing changes in behavioral sensitivity to APDs over days through repeated drug administration, then assessing the expression of the long term behavioral alterations in a subsequent challenge test. We have shown that in the CAR test, the typical APD haloperidol and the atypical APD olanzapine (OLZ) both cause long term sensitization effects (Li *et al*, 2007, 2010a; Mead and Li, 2010; Qiao *et al*, 2013b; Qin *et al*, 2013; Sun *et al*, 2009; Zhang and Li, 2012; Zhao *et al*, 2012), while clozapine (CLZ) induces a tolerance effect during the challenge trial following repeated drug exposure (Feng *et al*, 2013; Li *et al*, 2011; Qiao *et al*, 2013a, 2013b). Furthermore, we have recently established that the long term modulation of behavioral sensitivity induced by one APD can affect the behavioral sensitivity to another APD not previously exposed to the animal (Qin *et al*, 2013). In light of these findings, we also examined whether prior PD treatment would alter drug sensitivity to CLZ.

MATERIALS AND METHODS

Animals

Subject rats were mixed groups of female Sprague-Dawley adult offspring (> P75 days old) of pregnant dams from Charles River (Portage, MI; gestation day [G] 6 on delivery date) that were injected with either the immunostimulant polyinosinic:polycytidilic acid (PolyI:C, 4.0 mg/kg, intravenous [iv]) (Sigma-Aldrich, St. Louis, MO) or vehicle (VEH, 0.9% saline). Previous work suggests that maternal immune activation (MIA) with gestational PolyI:C treatment reproduces many behavioral abnormalities resembling symptoms of schizophrenia (Piontkewitz *et al*, 2009; Zuckerman and Weiner, 2005). Thus far only a few studies have investigated the effects of APDs using prenatally immune activated animals. We postulated that we would obtain more clinically relevant behavioral effects of PD by testing it in this model. PolyI:C 4.0 mg/kg was dissolved in 0.9% saline. This dose of PolyI:C was chosen based on previous studies using the same dose and route of administration to generate schizophrenia-like phenotypes (Piontkewitz *et al*, 2009, 2012; Wolff and Bilkey, 2008, 2010; Yee *et al*, 2012; Zuckerman and Weiner, 2005; Zuckerman *et al*, 2003). Prenatal PolyI:C treatment was performed on G13-15, when pregnant dams were anesthetized with 3% isoflurane (Fisher Scientific, Denver, CO) in 98% O2 and given a single iv injection at the tail vein. Siblings of pups born from these mothers and not used in the current study displayed abnormally low maternal separation-induced pup ultrasonic vocalizations (USVs, unpublished observation), indicating that these MIA animals do exhibit schizophrenia-like

phenotypes. For all experiments, rats were assigned such that the MIA and control offspring were distributed across each drug testing condition, and offspring from the same litter were assigned to different groups to minimize litter effects as a confound (Zorrilla, 1997). Table 1 summarizes the experimental groups and treatment conditions in each experiment.

Subjects were housed two per cage upon weaning (postnatal day [P] 21), in 182 × 50 × 188.1cm transparent polysulfone individually ventilated cages under 12h light/dark conditions (light on between 0630 and 1830 h). Room temperature was maintained at 22 ± 1°C with a relative humidity of 45–60%. Food and water was available *ad libitum*. Animals remained in their home cages until the time of the experiments (~P 75–95). All experiments were run during the light cycle. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln.

Drugs and choice of doses

PD (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% saline. Three doses of PD were tested: 1.0, 4.0 and 8.0mg/kg (subcutaneous [sc]). These doses of PD were chosen based on previous studies related to systemic injections of PD in CAR, PPI and hyperlocomotion tests (Feifel *et al*, 1999, 2003, 2004, 2007, 2008, 2010, 2011; Holly *et al*, 2011; Shilling and Feifel, 2008; Shilling *et al*, 2004). CLZ (5.0 mg/kg, sc) was dissolved in distilled sterile water with 1.0–1.5% glacial acetic acid. The dose of CLZ acutely inhibits CAR, PCP-induced disruption of PPI, and PCP-induced hyperlocomotion based on our previous work (Feng *et al*, 2013; Li *et al*, 2004, 2007, 2009, 2010a; Mead and Li, 2010; Sun *et al*, 2009; Zhang and Li, 2012). The injection solution of PCP (gift from National Institute on Drug Abuse Chemical Synthesis and Drug Supply Program) was obtained by mixing the drug with 0.9% saline. The doses of PCP (3.2 mg/kg for experiment 2; 1.5 mg/kg for experiment 3, sc) were chosen based on our previous work (Feng *et al*, 2013; Li *et al*, 2012a; Sun *et al*, 2009; Zhang and Li, 2012; Zhao and Li, 2010). These doses of PCP are shown to induce robust hyperlocomotion and disruptions in PPI without causing severe stereotypy (Gleason and Shannon, 1997; Kalinichev *et al*, 2008, 2009). All drugs were administrated at 1.0 ml/kg.

Two-way avoidance conditioning apparatus

Eight identical two-way shuttle boxes custom designed and manufactured by Med Associates (St. Albans, VT) were used. Each box was housed in a ventilated, sound-insulated isolation cubicle (96.52 cm W × 35.56 cm D × 63.5 cm H). Each box was 64 cm long, 30 cm high (from grid floor), and 24 cm wide, and was divided into two equal-sized compartments by a partition with an arch style doorway (15 cm high × 9 cm wide at base). A barrier (4 cm high) was placed between the two compartments, so the rats had to jump from one compartment to the other. The grid floor consisted of 40 stainless-steel rods with a diameter of 0.48 cm, spaced 1.6 cm apart center to center, through which a scrambled footshock (unconditioned stimulus [US], 0.8mA, maximum duration: 5 s) was delivered by a constant current shock generator (Model ENV-410B) and scrambler (Model ENV-412). The rat location and crossings between compartments were monitored by a set of 16 photobeams (ENV-256-8P) affixed at the bottom of the box (3.5 cm above the grid floor). Illumination was provided by two houselights mounted at the top of each compartment. The conditioned

stimulus (CS, i.e. 76 dB white noise) was produced by a speaker (ENV 224 AMX) mounted on the ceiling of the cubicle, centered above the shuttle box. Background noise (approximately 74 dB) was provided by a ventilation fan affixed at the top corner of each isolation cubicle. All training and testing procedures were controlled by Med Associates programs running on a computer. In each CAR box, an USV microphone (P 48/Emkay Microphone, Avisoft Bioacoustics, Berlin, Germany) was mounted on the ceiling of the two-compartment chamber. The microphone was connected via an E-MU 0404 USB Audio device to a computer. Acoustic data were displayed in real time by the Avisoft RECORDER, a multi-channel triggering hard-disk recording software (version 3.4; Avisoft Bioacoustics), and were recorded at a sampling rate of 192 kHz in 16 bit format and analyzed by Avisoft SASLab Pro (version 4.51; Avisoft Bioacoustics).

Prepulse inhibition of acoustic startle reflex apparatus

The PPI test was performed using six Startle Monitor Systems (Kinder Scientific, Julian, CA). Each system, controlled by a PC, was housed in a compact sound attenuation cabinet (36 cm wide × 28 cm deep × 50 cm high). A speaker (diameter: 11 cm) mounted on the cabinet's ceiling was used to generate acoustic stimuli (70 dB–120 dB). The startle response was measured by a piezoelectric sensing platform on the floor, which was calibrated daily. During testing, rats were placed in a rectangular box made of transparent Plexiglas (19 cm wide × 9.8 cm deep × 14.6 high) with an adjustable ceiling positioned atop the box, providing only limited restraint while prohibiting ambulation.

Locomotor activity monitoring apparatus

This apparatus has been described before (Feng *et al*, 2013; Sun *et al*, 2009; Zhao and Li, 2010). Sixteen activity boxes were housed in a quiet room. The boxes were 48.3 cm × 26.7 cm × 20.3 cm transparent polycarbonate cages, each equipped with a row of 6 photocell beams (7.8 cm between two adjacent photobeams) placed 3.2 cm above the floor of the cage. A computer with recording software (Aero Apparatus Sixbeam Locomotor System v1.4) was used to detect the disruption of the photocell beams and recorded the number of beam breaks.

Experiment 1: Assessment of PD149163 tolerance using the conditioned avoidance response test

This experiment examined whether repeated PD treatment could induce a tolerance effect in adult female rats in the CAR model as previously found with CLZ in adult male rats (Feng *et al*, 2013; Li *et al*, 2010a; Qiao *et al*, 2013a). It consisted of four phases: *avoidance training*, *repeated PD testing*, *PD challenge test* and *CLZ challenge test*.

Avoidance training—35 rats (~P 75) were first habituated to the CAR boxes for 2 days (30 min/day) and then trained for conditioned avoidance responding for 10 consecutive days/sessions. Each session consisted of 30 trials. Every trial started by presenting a white noise (CS) for 10 s, followed by a continuous scrambled foot shock (0.8 mA, US, maximum duration = 5 s) on the grid floor. If a subject moved from one compartment into the other within the 10 s of CS presentation, it avoided the shock and this shuttling response was recorded as *avoidance*. If the rat remained in the same compartment for more than 10s and

made a crossing upon receiving the footshock, this response was considered as *escape*. If the rat did not respond during the entire 5 s presentation of the shock, the trial was terminated and *escape failure* was recorded. The total number of avoidance responses was recorded for each session. Intertrial intervals varied randomly between 30 and 60 s.

Repeated PD testing—At the end of the training session (~P 87), rats were assigned to one of four groups: VEH (0.9% saline, n = 9), PD 1.0 mg/kg (PD 1.0, n = 9), PD 4.0 mg/kg (PD 4.0, n = 9), and PD 8.0 mg/kg (PD 8.0, n=8) and tested daily for avoidance response for 5 consecutive days. The CS-only (no shock, 30 trials/daily session) condition was used to eliminate any relearning effect caused by the presence of the US. During each drug test, rats were first injected with PD or VEH. Thirty min later, they were placed in the CAR boxes and tested. USV at the 22 kHz range (20–32 kHz) – an established assay for fear and anxiety (Mead *et al*, 2008; Sun *et al*, 2010a) – were also recorded for the first 10 min of the testing using Avisoft Recorder software (Version 3.4). Settings included sampling rate at 192 kHz, format 16 bit. For acoustical analysis, recordings were transferred to Avisoft SASLab Pro (Version 4.51) and a fast Fourier transformation (FFT) was conducted. Spectrograms were generated with an FFT-length of 256 points and a time window overlap of 50% (100% Frame, FlatTop window). The spectrogram was produced at a frequency resolution of 750 Hz and a time resolution of 0.6667 ms. Call detection was provided by an automatic single threshold-based algorithm (threshold: −20 dB) and a hold-time mechanism (hold time: 0.02 s).

PD challenge test—One day after the last drug test day, all rats were retrained drug-free for one day under the CS-only (no shock) (~P 92) condition and one day under the CS-US condition (~P 93) to ensure all groups had a comparable level of avoidance responding before the tolerance assessment. PD tolerance was assessed 1 day later (~P 94), when all rats were injected with a challenge dose of PD 1.0 mg/kg and tested for avoidance performance, 22 kHz USV and intertrial crossing in the CS-only condition (30 trials) 30min later. This procedure of using a lower challenge dose of the same drug has been successfully used in our previous studies with many APDs (Feng *et al*, 2013; Li *et al*, 2010a, 2012b; Qiao *et al*, 2013a; Sparkman and Li, 2012; Swalve and Li, 2012; Zhang and Li, 2012). It also avoided the floor effect (i.e. a high dose may cause a maximal avoidance disruption, leaving no room to show a sensitization or tolerance effect).

CLZ challenge test—After the PD challenge test, all rats were once again retrained for 2 drug-free sessions, the first under the CS-only condition and the second under the CS-US condition, then challenged with CLZ 5.0 mg/kg 1 day later, when they were injected with CLZ and tested 1 h later for avoidance performance in the CS-only condition (30 trials).

Experiment 2: Assessment of PD149163 tolerance using the phencyclidine-induced hyperlocomotion test

This experiment examined whether repeated PD treatment could induce a tolerance effect in adult female rats in PCP-induced hyperlocomotion. It consisted of three phases: *repeated PD testing*, *PD challenge test* and *CLZ challenge test*.

Repeated PD testing—Forty six rats were first habituated to the locomotor activity apparatus for 2 days (~ P95, 30min/day). Following habituation rats were assigned to one of six groups: VEH+VEH (n=7), PCP+VEH (n=8), PD 1.0+PCP (n=8), PD 4.0+PCP (n=8), PD 8.0+PCP (n=8), or VEH+PD 4.0 (n=7), and tested daily for locomotor activity for 5 consecutive days. During each session, rats were first injected with PD or VEH and immediately placed in the boxes for 30 min. At the end of the 30 min period, they were taken out and injected with PCP 3.2 mg/kg or VEH and placed back in the boxes for another 60 min. Locomotor activity (number of photobeam breaks) was measured in 5min intervals throughout the entire 90-min testing session.

PD challenge test—Two days after the last drug test day, all rats were rehabituated to the locomotor activity boxes (~ P103, 30 min) and PD tolerance was assessed 1 day later (~ P104), when all rats were injected with both PD 1.0 mg/kg and PCP 3.2 mg/kg and tested for locomotor activity.

CLZ challenge test—After the PD challenge test, all rats were once again rehabituated to the locomotor activity boxes then challenged with CLZ 5.0 mg/kg + PCP 3.2 mg/kg 1 day later.

Experiment 3: Assessment of PD149163 tolerance using the phencyclidine-induced disruptions of prepulse inhibition test

This experiment examined whether repeated PD treatment induces a tolerance effect in adult female rats in PCP-induced disruption of PPI. It consisted of three phases: *repeated PD testing*, *PD challenge test* and *CLZ challenge test*.

Repeated PD testing—Forty six rats were first habituated to the prepulse inhibition apparatus for 2 days (~ P75, 10min). Following habituation rats were assigned to one of five groups: VEH+VEH (n=10), PCP+VEH (n=9), PD 1.0+PCP (n=9), PD 4.0+PCP (n=9), or PD 8.0+PCP (n=9), and tested daily for PPI for 3 consecutive days. During each session, rats were first injected with PD or VEH, followed 20 min later with an injection of PCP 1.5 mg/kg or VEH, then placed in the PPI boxes and tested 10 min later. PPI test procedures were adapted from Culm and Hammer (2004). The PPI session lasted approximately 18 min and began with a 5 min period of 70 dB background noise (which continued throughout the duration of the session) followed by four different trial types: PULSE ALONE trials and three types of PREPULSE+PULSE trials, which consisted of a 20 ms 73, 76, or 82 dB prepulse (3, 6, and 12 dB above background) followed 100 ms later by a 120 dB pulse. Each session was divided into 4 blocks. Blocks 1 and 4 were identical, each consisting of 4 PULSE ALONE trials. Blocks 2 and 3 were also identical and each consisted of 8 PULSE ALONE trials and 5 of each PREPULSE+PULSE trial type. A total of 54 trials were presented during each test session. Trials within each block were presented in a pseudorandom order and were separated by a variable intertrial interval averaging 15 s (ranging from 9 to 21 s). Startle magnitude was defined as the maximum force (measured in Newtons) applied by the rat to the startle apparatus recorded over a period of 100 ms beginning at the onset of the pulse stimulus. Between each stimulus trial, 100 ms of activity was recorded when no stimulus was present. These trials were called NOSTIM trials and

were not included in the calculation of intertrial intervals. Responses recorded during NOSTIM trials are considered a measure of gross motor activity within the PPI boxes. Startle responses from testing blocks 2 and 3 were used to calculate percent prepulse inhibition (%PPI) for each acoustic prepulse trial type:

$$\%\mathrm{PPI}=100-\left[\left(\frac{\text{average startle response to PREPULSE+PULSE trials}}{\text{average startle response to PULSE ALONE trials}}\right)\times 100\right]$$

PD challenge test—Two days after the last drug test day, all rats were rehabituated to the PPI apparatus (~P81) and PD tolerance was assessed 1 day later (~ P82), when all rats were injected with both the intermediate dose of PD 4.0 mg/kg and PCP 1.5 mg/kg and tested for PPI. Due to animal illness, only 9 rats were used for the VEH+VEH group during the PD +PCP challenge.

CLZ challenge test—After the PD challenge test, all rats were once again rehabituated to the PPI chambers (~P 84) then challenged with CLZ 5.0 mg/kg 1 day later, when they were injected first with CLZ, followed 50 min later with PCP 1.5 mg/kg, and tested 10min later for PPI. Due to procedural errors, only data from 8 rats in the VEH+VEH group were used for the CLZ challenge.

Statistical analysis

All data are expressed as mean ± SEM. In the case where no group differences were found between control and MIA animals, the data are combined. Avoidance data in experiment 1 and motor activity data in experiment 2 from the five drug test sessions were analyzed using a factorial repeated measures analysis of variance (ANOVA) with the between-subjects factor being drug group and the within-subjects factor being test day, followed by *post hoc* LSD tests. Data from the predrug and challenge test days were analyzed by one-way ANOVA followed by *post hoc* LSD tests. As there was no significant interaction between the three prepulse intensities and PD treatment, percent PPI data for the three drug days in experiment 3 were reported as the average of the three prepulse intensities (e.g. 73, 76 and 82 dB). The magnitude of the acoustic startle reflex (ASR) was calculated as the average response on the PULSE ALONE trials, excluding the first and last block of 4 PULSE ALONE trials. The general activity was calculated as the average response on the NOSTIM trials. Percent PPI, ASR and activity data from the drug test period were first analyzed using repeated measures ANOVAs with the drug treatment group as a between subjects factor and test day as a within-subjects factor, followed by *post hoc* LSD tests. For all analyses, $p<0.05$ was considered statistically significant.

RESULTS

Experiment 1: Assessment of PD149163 tolerance using the conditioned avoidance response test

Avoidance training and repeated PD testing—Figure 1a shows the percentage of avoidance responses on the last training (predrug) day and the five drug test days. There was no group difference on the last training day. Throughout the drug test phase, PD treatment

consistently disrupted avoidance response. Repeated measures ANOVA revealed a main effect of group, $F(3,31)=144.654$, $p<0.001$ and a significant group × day interaction, $F(12,90)=2.655$, $p=0.004$. *Post hoc* LSD tests revealed that the three PD groups all made significantly less avoidances than the VEH group, all $p<0.001$, although they did not differ significantly from each other.

22 kHz USV—Figure 1b shows the number of 22 kHz USVs on the last training (predrug) day and the five drug test days. There was no group difference on the last training day. Throughout the drug test phase, PD treatment persistently suppressed the number of 22 kHz USVs. Repeated measures ANOVA revealed a main effect of group, $F(3,30)=9.244$, $p<0.001$. *Post hoc* LSD tests revealed that the three PD groups all emitted significantly less vocalizations than the VEH group, all $p<0.001$, although they did not differ significantly from each other.

PD challenge test—Figure 1c shows the percentage of avoidance responses on the second retraining (predrug) day and the PD tolerance challenge day. There was no group difference on the predrug day. When all rats were given PD 1.0 mg/kg on the challenge day, one-way ANOVA revealed a significant effect of group, $F(3,30)=7.011$, $p=0.001$. *Post hoc* LSD tests showed that the PD 4.0 and 8.0 groups made significantly more avoidances than the VEH group, $p=0.005$ and $p<0.001$, respectively. The PD 8.0 group also showed significantly higher levels of avoidance than the PD 1.0 group, $p=0.005$. No significant group difference was detected on the number of 22 kHz USVs on the predrug or challenge day.

CLZ challenge test—Figure 1d shows the percentage of avoidance responses on the predrug day and the CLZ challenge test day. Before the CLZ challenge, there was no significant group difference. On the challenge day when all rats were given CLZ 5.0 mg/kg, one-way ANOVA revealed a significant effect of group, $F(3,27) = 4.365$, $p=0.012$. *Post hoc* LSD tests showed that the PD 4.0 and 8.0 groups made significantly fewer avoidances than the VEH group, $p=0.003$ and 0.004, respectively, as well as fewer avoidances than the PD 1.0 group, $p=0.036$ and 0.042, respectively. Statistical analysis also revealed a significant effect of prenatal treatment, $F(1,27)=6.195$, $p=0.019$, as well as a significant group × prenatal treatment interaction, $F(3,27)=3.890$, $p=0.020$ (figure 1e). *Post hoc LSD* analysis showed that prenatally immune activated animals previously exposed to VEH or PD 1.0 showed significantly higher numbers of avoidances compared to the non-prenatally challenge animals that were also exposed to VEH and PD, $p=0.001$ and 0.043, respectively. No significant group difference was detected on the number of 22 kHz USVs on the predrug or challenge day.

Experiment 2: Assessment of PD149163 tolerance using the phencyclidine-induced hyperlocomotion test

Repeated PD test: 30 min before PCP—Figure 2a shows the mean locomotor activity during the 30-min test period after the PD or VEH injection throughout the five days of drug testing. Repeated measures ANOVA revealed a main effect of group, $F(5,40)=24.952$, $p<0.001$ and day, $F(4,37)=5.652$, $p=0.001$, and a significant group × day interaction, F(20,

160)=2.701, $p<0.001$. *Post hoc* LSD tests showed that, except on day 1, the PCP+VEH group has significantly lower locomotor activity than the VEH+VEH group, $p=0.007$. The PD 1.0, 4.0, and 8.0+PCP groups also showed significantly lower activities than the PCP +VEH group, all $p<0.001$. Additionally, except on day 1, the VEH+PD 4.0 group showed significantly higher levels of activity than the PD 1.0 and 4.0+PCP groups, both $p<0.01$.

Repeated PD test: 60 min after PCP—Figure 2b shows the mean locomotor activity during the 60-min test period after the PCP or VEH injection throughout the five days of drug testing. Repeated measures ANOVA revealed a main effect of group, $F(5,40)=17.506$, $p<0.001$ and day, $F(4,37)=6.788$, $p<0.001$. *Post hoc* LSD tests showed that the PCP+VEH group had significantly higher locomotor activities than all other groups, all $p<0.001$, while the PD 4.0+VEH group showed significantly lower levels of activity than the VEH+VEH group, $p=0.016$.

PD challenge test—Figure 2c shows the mean locomotor activity during the rehabituation (predrug) and PD challenge test day. One-way ANOVA revealed a significant main effect of group during the predrug day, $F(5,40)=6.193$, $p<0.001$. *Post* hoc LSD tests showed that the PD 1.0, 4.0 and 8.0+PCP groups all displayed significantly lower levels of activity than the VEH+VEH group, all $p<0.002$. The PD 1.0 and 8.0+PCP groups also showed significantly lower activity levels than the PCP+VEH group, $p=0.013$ and 0.007, respectively. There was no significant difference between groups throughout the duration of the challenge test, when all groups were given PCP and PD 1.0 mg/kg.

CLZ challenge test—Figure 2d shows the mean locomotor activity during the rehabituation (predrug) and CLZ challenge test day. One-way ANOVA revealed a significant main effect of group only for the 60-min test period after the PCP or VEH injection, $F(5,40)=2.644$, $p=0.037$. *Post hoc* LSD test for the 60-min test period on the CLZ challenge test day showed that the PD 1.0 and 4.0+PCP groups showed significantly suppressed activities compared to the VEH+VEH group, $p=0.020$ and 0.028, respectively. The VEH+PD 4.0 group also saw a significantly lower level of locomotor activity than both the VEH+VEH and PCP+VEH groups, $p=0.006$ and 0.019, respectively. There was no significant difference between the groups for the predrug day or the 30-min test period after the CLZ 5.0 mg/kg or VEH injection during the CLZ challenge test day. There was also no significant difference between prenatal treatments across all phases of the PCP-induced hyperlocomotion testing.

Experiment 3: Assessment of PD149163 tolerance using the phencyclidine-induced disruptions of prepulse inhibition test

Repeated PD testing: PPI—Figures 3a shows the percentage of PPI on the three drug test days. Statistical analysis revealed a main effect of day, $F(2,40)=3.308$. $p=0.047$, and a main effect of group, $F(4,41)=14.092$, $p<0.001$. *Post hoc* LSD tests showed that the PD 1.0, 4.0 and 8.0+PCP groups all displayed higher PPI than the VEH+VEH and the PCP+VEH groups, $p=0.002$ for PD 1.0+PCP and $p<0.001$ for 4.0 and 8.0. While we observed a significantly lower PPI in the PCP+VEH group compared to VEH+VEH on the first day of PCP administration (multivariate ANOVA of PCP × PD: $F(4,45)=9.044$, $p<0.001$, *post hoc*

LSD of VEH+VEH versus PCP+VEH, p=0.033), this effect was abolished following the second and third day of PCP administration.

Repeated PD testing: ASR—Figure 3b shows the startle magnitudes on the three drug test days. Repeated measures ANOVA revealed a main effect of group, F(4,41)=7.788, p<0.001 and day, F(2,40)=3.343, p=0.045. *Post hoc* LSD tests showed that the PD 1.0, 4.0, and 8.0+PCP groups all had significantly lower startle responses than the PCP+VEH group, all p=0.001. PD 4.0 and 8.0+PCP groups also showed significantly lower startles than the VEH+VEH group, p=0.012 and 0.003, respectively.

Repeated PD testing: General motor activity—Figure 3c shows the general motor activity recorded during the NOSTIM trials on the three drug test days. Repeated measures ANOVA revealed a main effect of group, F(4,41)=5.064, p=0.002 and a significant group × day interaction, F(8,82)=2.629, p=0.013. *Post hoc* LSD tests showed that the PD 4.0 and 8.0+PCP groups had significantly lower activity than the VEH+VEH and PCP+VEH groups, p<0.01 for all groups.

PD challenge test—Figure 4a shows the PPI percentage on the rehabituation (predrug) and PD challenge test day. There was no significant difference in PPI or ASR on the predrug day. However, one-way ANOVA of general motor activity on the predrug day revealed a main effect of group, F(4,35)=4.176, p=0.007 (figure 4b). *Post* hoc LSD tests showed that the PD 4.0 and 8.0+PCP groups generated significantly lower magnitudes than the VEH +VEH group, p=0.005 and 0.001, respectively, and additionally the PD 8.0+PCP group's general motor activity was significantly lower than the PCP+VEH group as well, p=0.030. No significant difference was found between the groups in PPI, ASR or general motor activity on the PD challenge day, when all rats were given PD 4.0+PCP.

CLZ challenge test—Figure 4c shows the percentage of PPI on the rehabituation (predrug) and CLZ challenge test day. There was no significant difference in PPI or ASR on the predrug day. However, one-way ANOVA of general motor activity during the predrug day again showed a main effect of group, F(4,34)=2.882, p=0.037 (figure 4d). *Post hoc* LSD tests showed that the PD 8.0+PCP group displayed significantly reduced general motor activity compared to the VEH+VEH, PCP+VEH, and PD 1.0+PCP groups, p=0.010, 0.011, and 0.013, respectively. No significant difference was found between the groups in PPI, ASR or general motor activity on the CLZ challenge day, when all rats were given CLZ 5.0 mg/kg+PCP. There was no significant difference between prenatal treatments across all phases of the PCP-induced PPI deficits testing.

DISCUSSION

This study investigated the acute and chronic effects of the NT analogue PD149163 (PD) using three validated preclinical assays of antipsychotic (APD) efficacy, conditioned avoidance response (CAR), phencyclidine (PCP)-induced hyperlocomotion, and PCP-induced disruptions in prepulse inhibition (PPI). PD (Lys(CH2NH)Lys-Pro-Trp-tLe-Leu-OET) is a NT analogue selective for the NTS1 receptor (Petrie *et al*, 2004) with structural modifications that makes it less prone to enzymatic degradations when injected peripherally

(Banks *et al*, 1995). Using the two-phase paradigm that our group has established for assessing APD sensitization and tolerance (i.e. increased and decreased behavioral response to a drug due to past drug exposure), we confirmed the tolerance effect of PD in the CAR test in the higher doses tested, and saw APD-like effects in other well established tests without tolerance development over days of treatment. The discrepancy among different tests suggested that differences in sensitivities for assessing effects of chronic APD treatments exist across different behavioral measurements.

In accordance with previous studies on NT receptor activation using both central (Norman *et al*, 2008a) and systemic (Feifel *et al*, 2003; Shilling *et al*, 2003) administrations, our results showed that acute PD treatment at three different doses (1.0, 4.0, and 8.0 mg/kg) were able to inhibit CAR, reverse the PPI disruptions produced by PCP, and suppress PCP-induced hyperlocomotion. These acute effects were most likely due to central mechanisms of NT receptor activation after peripheral injection. As the inhibition of CAR by APDs has been suggested to be mediated by blockade of dopamine (DA) D_2 receptors in the mesocorticolimbic DA pathway (Wadenberg and Hicks, 1999), the therapeutic mechanism of NT receptor stimulation might be related to its close association with the mesolimbic DA system (Binder *et al*, 2001). NTS1 is found to co-localize with DA D_2 receptors, and activation of these receptors on DAergic neurons in the ventral tegmental area (VTA) has been shown to increase DAergic neuronal activity (Mercuri *et al*, 1993; Nalivaiko *et al*, 1998; Wu and Wang, 1995). In addition, NT in the prefrontal cortex (PFC) can also increase VTA DAergic neuronal activity (Fatigati *et al*, 2000; Rompré *et al*, 1998), and peripheral NT can enhance DA efflux in both the nucleus accumbens and the medial PFC (mPFC), similar to atypical APDs (Prus *et al*, 2007). Furthermore, it has been suggested that the mechanism of PCP-induced hyperactivity involves increased DA output in the mesolimbic area as well as a rise in extracellular DA concentration in the mPFC, and systemic pre-treatment of animals with the NT analogue NT69L prevented the DA increase (Li *et al*, 2010b).

Previous work only reports PD's tolerance effect in the induction phase (the repeated drug testing phase) in which PD gradually loses its ability to inhibit CAR. In the present study, we were able to demonstrate it in the expression phase, when the previously drug treated groups were compared directly with the vehicle (VEH) control group. This finding provided a better and cleaner demonstration of tolerance as it was supported by a between-group comparison – that the PD groups and VEH group were not different on the predrug days, but only showed differences under the PD challenge. We showed PD 4.0 and 8.0 mg/kg were able to induce a partial tolerance effect in the CAR model. After five days of PD treatment, the effect of PD in the challenge test using the lowest dose of PD (1.0 mg/kg) tested during the repeated drug days was severely reduced, resulting in a high level of avoidance response in rats previously exposed to PD compared to drug naïve rats. It is important to note that many previous studies investigating PD saw APD-like effects in doses lower than 1.0 mg/kg, suggesting the higher doses of 4.0 and 8.0 mg/kg may have activated other non-NT systems within the brain. However, since there is no guidance currently on the clinically relevant doses of PD, our dosing choices were based on previous work by Holly *et al*, 2011 showing that while doses below 1.0 mg/kg were able to rescue drug-induced PPI deficits in

other literature, PD was unable to induce acute CAR disruption at doses below 1.0 mg/kg, and even at 8.0 mg/kg was only able to produce a 60% disruption of CAR.

Our present result of PD tolerance in CAR is similar to our previous work showing that prior repeated clozapine (CLZ) administration causes a decreased response to the APD in a challenge test compared to control (Feng *et al*, 2013; Li *et al*, 2011; Qiao *et al*, 2013a). It also agrees with results using another NT analogue, NT69L, by Hertel *et al*, 2002, although in that study tolerance was assessed at only one time point, at the end of twice daily treatments for seven days. Using our unique CS-only test procedure allowed us to identify true avoidance responses that are not contaminated by US-induced learning that might occur throughout the test sessions. This paradigm allowed us to note that with both CLZ and PD, there was no gradual development of tolerance throughout the five repeated test days in CAR under the drug condition. Instead, tolerance was only expressed during the challenge test after two days of CAR retraining under the drug free condition. The mechanism of this phenomenon possibly involves the down-regulation of NTS1 receptors. Cell and tissue culture studies have shown that NT receptor stimulation with agonists causes receptor desensitization and internalization (Hermans *et al*, 1997; Vanisberg *et al*, 1991), as well as a decrease in cyclic GMP formation (Gilbert *et al*, 1988; Hermans *et al*, 1996), although no studies have looked at the time course of receptor down-regulation *in vivo*. It is thus possible that a time-dependent delay of onset accompanies the development of this behavioral tolerance.

PD has also been implicated as a potential anxiolytic (Shilling and Feifel, 2008), hence we also compared the 22 kHz ultrasonic vocalizations (USVs) – a measure of negative affect (Brudzynski and Chiu, 1995; Tonoue *et al*, 1986) – in CAR. Although repeated PD treatment reduced 22 kHz USVs, no tolerance was seen during challenge. These results contradicted those by Prus *et al*, 2013, where repeated PD administration decreased the number of conditioned footshock-induced 22 kHz USVs. Since the mentioned study administered PD 1.0 mg/kg for ten consecutive days, it is possible that the discrepancy is due to the longer time period required for tolerance to develop in 22 kHz USV-related behaviors.

The drug-induced hyperlocomotion model has been extensively used in reporting the chronic effects of NT receptor activation (Boules *et al*, 2003; Feifel *et al*, 2008; Hertel *et al*, 2001; Meisenberg and Simmons, 1985; Norman *et al*, 2008a; Rompré, 1997). Using our previously established model (Feng *et al*, 2013; Li *et al*, 2011; Qin *et al*, 2013; Sun *et al*, 2009, 2010b; Zhang and Li, 2012; Zhao *et al*, 2012), after five days of repeated combined administration of PD and PCP, we saw no tolerance in the subsequent challenge test, as low dose PD (1.0 mg/kg) challenge resulted in the same level of PCP hyperlocomotion reduction between drug naïve animals and those with prior PD exposure at all doses. However, we did see a decrease in spontaneous locomotor activity under the drug free condition for groups that received repeated PD treatment in conjuction with PCP, suggesting that the drug treatment did induce lasting behavioral changes that persisted in the drug free system. In terms of PD's tolerance phenomenon in the drug-induced hyperactivity model, discrepancies exist among current literature. This could largely be attributed to variations in experimental designs. For example, repeated intracerebroventricular (ICV) NT infusions for four and 21

days sensitized rats to amphetamine-induced hyperactivity using an amphetamine-only challenge (Rompré, 1997), while ICV PD infusions for seven days exhibited tolerance under the amphetamine-only challenge, with the acute PD attenuation of amphetamine-induced hyperlocomotion abolished after seven days of PD infusion (Norman *et al*, 2008b). Other studies saw tolerance development in NT agonism's suppression of spontaneous motor activity (Meisenberg and Simmons, 1985). Finally, Boules *et al*, 2003 and Feifel *et al*, 2008 both concluded no change in behavioral sensitivity using the two commonly studied NT analogues, NT69L and PD, respectively. Our model included daily testing of PD's suppression of PCP-induced hyperlocomotion, which more accurately captured the time course of the drug effect. In addition, most of the previous studies only tested one dose of the drug, while here we compared three different doses and saw no dose-dependent response.

In the hyperlocomotion experiment, it is also important to note that while the PD4+VEH group exhibited low baseline locomotion, suggesting a sedating effect of this dose of PD, it is indisputable that general sedating effects can occur with any drugs suggested to induce APD-like effects, especially atypical drugs such as CLZ and olanzapine (OLZ). Our previous work showed that the sedative effect of an APD drug could not account for its avoidance disruptive effect. In our CAR experiment, we saw a general decrease in intertrial crossing (a measurement of gross motor activity) under the influence of currently established APDs (Qiao *et al.* 2013). However, this does not imply a lack of APD efficacy. Therefore, while the sedating effect of PD may exist, we do not believe that it diminished the significance of the results presented here. In our opinion, the sedative effect and putative APD effect of PD all contributed to its avoidance disruptive effect, and both effects may undergo a tolerance development with different time courses. Future research is needed to tease out their individual contributions in various behavioral tests.

Unlike CAR and PCP-induced hyperlocomotion, as a preclinical model of APD efficacy and schizophrenia pathology, few studies have utilized PPI to capture the time course effects of APD actions. Feifel *et al*, 2007 have attempted to evaluate the tolerance development of PD using PPI and found that 16 days of continuous PD 1.0 mg/kg administration in the naturally PPI-deficient Brattleboro rats resulted in the same level of PPI enhancement as the first day of PD treatment, indicating a lack of tolerance or sensitization over time. However, as the study only examined the drug's effect on PPI at the beginning and end of the treatment period, it was unable to assess the changes that might have occurred throughout the treatment duration. In our experiment, we gave daily injections of PD at three different doses (1.0, 4.0, and 8.0 mg/kg), and tested the animals' PPI each day. Although our results also saw an absence of development over days in PPI, similar to our hyperlocomotion results, we again saw a decrease in the gross motor activity under the drug free condition for groups that received repeated PD treatment in conjunction with PCP. The results from drug free days in the PPI and hyperlocomotion experiments pointed to the likelihood that this long lasting change requires the combined action of PD with PCP. In support of this result, previous NT agonism studies have shown that there is a possible dose dissociation amongst various behaviors (Feifel *et al*, 1997; Prus *et al*, 2007), and the optimal effective dose may differ between behaviors.

Our group has previously investigated the phenomenon of sensitization cross-transfer (Li *et al*, 2007; Mead and Li, 2010; Qiao *et al*, 2013b). In a recent study on the repeated treatment effects of the atypical APD asenapine (ASE) (Qin *et al*, 2013), our group evaluated whether sensitization induced by ASE could be transferred to other atypical APDs such as OLZ and CLZ. We showed that the behavioral sensitization induced by ASE did in fact transfer to a subsequent OLZ challenge. Similarly, here we saw a transfer of the tolerance induced by PD in CAR in a subsequent CLZ challenge. In addition to our previous work showing CLZ to be the only atypical APD that produces a tolerance effect in our repeated CAR assessment paradigm (Feng *et al*, 2013; Qiao *et al*, 2013a, 2013b; Zhao *et al*, 2012), the results here of PD's tolerance in CAR and its tolerance cross-transferring to CLZ suggest the possibility that the therapeutic mechanisms of PD might be similar to those of the atypical APD CLZ. It is also interesting to note that while no tolerance was seen in the PD challenge for PCP-induced hyperlocomotion, the subsequent CLZ challenge actually showed an increased sensitivity to CLZ in groups previously repeatedly treated with PD and PCP. Though the reason for this finding is currently unclear, it adds strength to the idea that PD and CLZ might share unique similarities, and supports the notion that NTS1 receptor agonists might be therapeutically efficacious due to the production of similar behavioral response as CLZ in a preclinical test of high predictive validity for APD efficacy.

In addition to assessing the repeated effects of PD using three distinct preclinical models, we also examined the generalizability of our findings by including both wild type animals and prenatally immune activated ones. Maternal immune activation (MIA) is widely accepted as a preclinical model for the neuroinflammatory theory of schizophrenia (Piontkewitz *et al*, 2009; Zuckerman and Weiner, 2005). In this study, we saw no difference in repeated PD treatments between the MIA and control animals. However, we did see some differences within the CLZ challenges. The cause for this observation is uncertain, but conceivably involves differences in receptor levels. Various work regarding MIA animals have implicated DA dysfunction as a possible cause of phenotypic abnormalities (Meyer *et al*, 2010; Vuillermot *et al*, 2010; Zuckerman and Weiner, 2003). For example, Zuckerman, et. al. showed that prenatally immune activated animals displayed increased sensitivity in the amphetamine-induced hyperlocomotion test and increased striatal DA release, while Ozawa *et al*, 2006 found increased striatal DA metabolites, 3,4-dihydroxyphenylacetic acid and homovanillic acid, in MIA animals using high-performance liquid chromatography. It seems likely that while APDs may still alter baseline functions in these animals as seen in the current work, the DAergic disruption results in differences of drug effects on this population relative to controls.

It is possible that our choices of experimental parameters produced results that are different compared to other previous work. Our choice of using female subjects provided novel findings of PD's APD-like effects in females. However, this also limited the ability for comparison with previous work in male rats. For example, previous studies have shown that there are significant sex differences in behavioral response to MK-801, an NMDA antagonists with similar behavioral disruptions to PCP (Zhao *et al*. 2013), and human studies have clearly shown a sex difference in PPI response (Aasen *et al*. 2005). Thus, the use of female subjects, inclusion of both control and prenatally immune activated animals, choice

of dosage, numbers of test days, and challenge dose might have all contributed to differences in our study. Regardless, in summary, the findings we present here provide novel observations of PD's tolerance properties and contribute data to the increasing body of knowledge supporting NT receptor agonists as possible novel APDs.

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Highlights

1. The NTS1 receptor agonist PD149163 produces tolerance in CAR in adult female rats.
2. PD149163 tolerance cross-transfers to clozapine challenge in CAR.
3. PD149163 acutely attenuates PCP-induced hyperlocomotion but shows no tolerance.
4. PD149163 acutely rescues PCP-induced PPI deficits in but shows no tolerance.

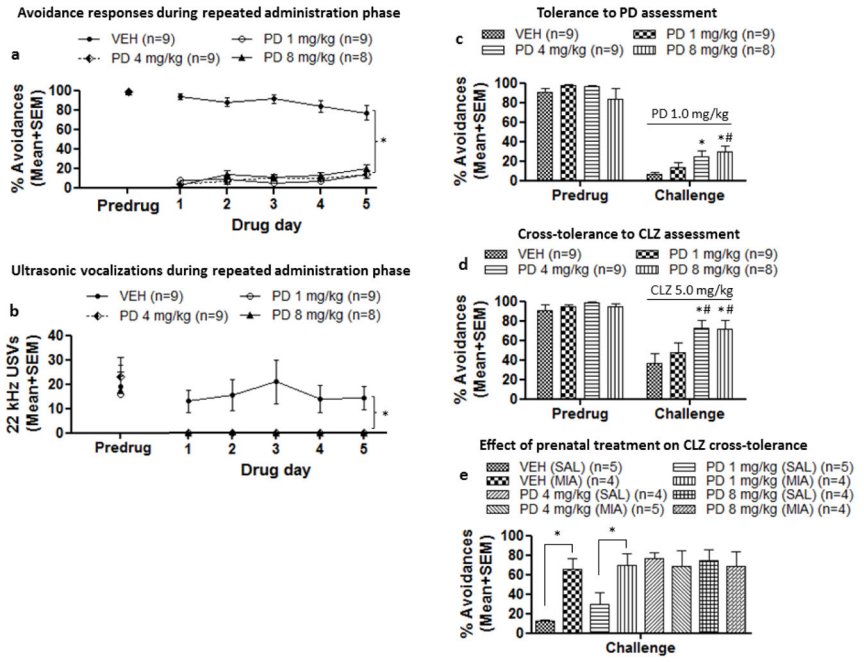

Figure 1.

Assessment of PD149163 (PD) tolerance in conditioned avoidance response: (a) The percentage of avoidance responses and (b) 22kHz ultrasonic vocalizations made by rats from vehicle (VEH), PD 1.0, 4.0, and 8.0 mg/kg groups on the last training day (predrug) and throughout the five drug test days. *p<0.01 relative to the VEH group. (c) The percentage of avoidance responses made by rats on the retraining day (predrug) and the PD tolerance assessment day. *p<0.01 relative to the VEH group; #p<0.01 relative to the PD 1.0 group. (d) The percentage of avoidance responses made by rats on the second retraining day (predrug) and the clozapine (CLZ) tolerance assessment day. *p<0.01 relative to the VEH group; #p<0.05 relative to the PD 1.0 group. (e) Effects of prenatal treatment on CLZ tolerance. Percentage avoidance responses made by rats from the two prenatal treatment groups. *p<0.01 for the prenatally immune-activated group relative to control in the VEH condition and #p<0.05 in the PD 1.0 condition. All values are expressed as mean + SEM.

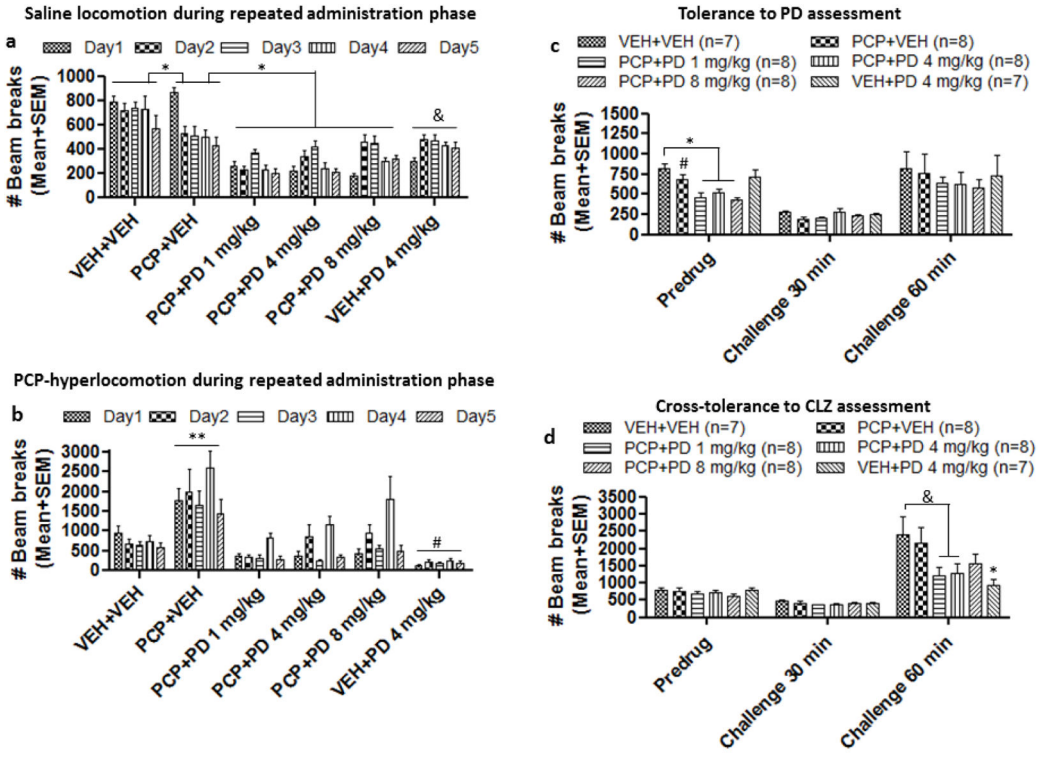

Figure 2.

Assessment of PD149163 (PD) tolerance using phencyclidine-induced hyperlocomotion: (a) Mean locomotor activity during the 30-min test period after the PD or vehicle (VEH) injection and (b) the 60-min test period after the phencyclidine (PCP) or VEH injection made by rats from the VEH+VEH, PCP+VEH, PCP+PD 1.0, PCP+PD 4.0, PCP+PD 8.0, and VEH+PD 4.0 groups throughout the five drug test days. *p<0.01 relative to the PCP +VEH group; &p<0.01 relative to the PD 1.0+PCP and PD 4.0+PCP groups; **p<0.01 relative to all groups; #p<0.05 relative to the VEH+VEH group. (c) Mean locomotor activity during the rehabituation day (predrug), 30-min test period after the PD or VEH injection, and 60-min test period after the PCP or VEH injection made by rats on the PD tolerance assessment day and (d) clozapine tolerance assessment day are expressed as mean + SEM. *p<0.01 relative to the VEH+VEH group; #p<0.05 relative to the PCP+PD1.0 and PCP +PD8.0 groups; &p<0.05 relative to the VEH+VEH group. All values are expressed as mean + SEM.

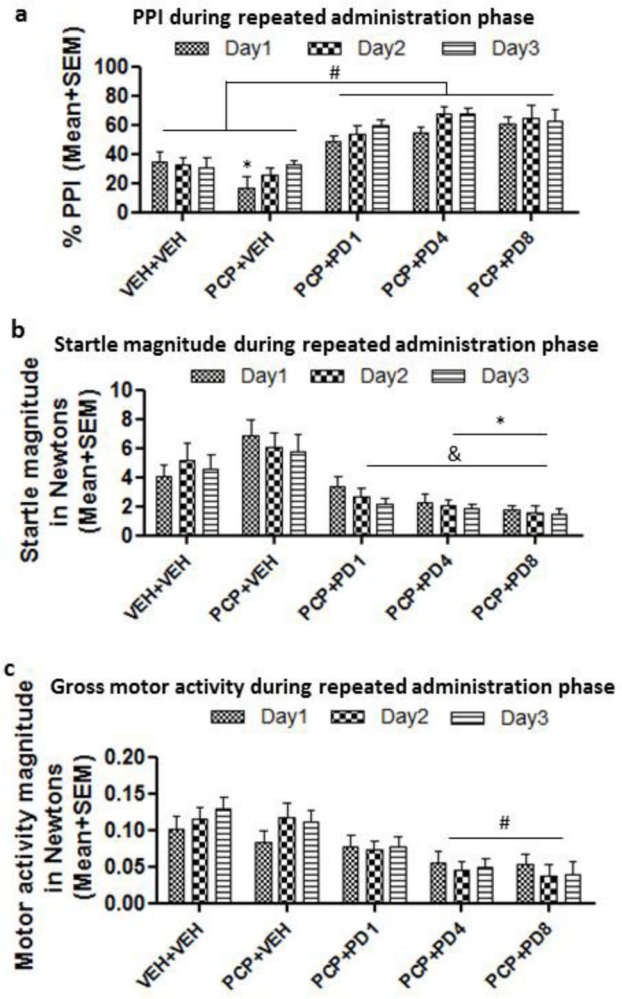

Figure 3.

Repeated PD149163 (PD) administration in phencyclidine (PCP)-induced disruption of prepulse inhibition (PPI): (a) Percentage of PPI, (b) startle magnitude in Newtons, and (c) general motor activity in Newtons made by rats from the vehicle (VEH)+VEH, PCP+ VEH, PCP+PD 1.0, PCP+PD 4.0, and PCP+PD 8.0 groups throughout the three drug test days are expressed as mean + SEM. #p<0.01 relative to both the VEH+VEH and PCP+VEH groups; *p<0.05 relative to the VEH+VEH group alone; &p<0.01 relative to the PCP+VEH group alone.

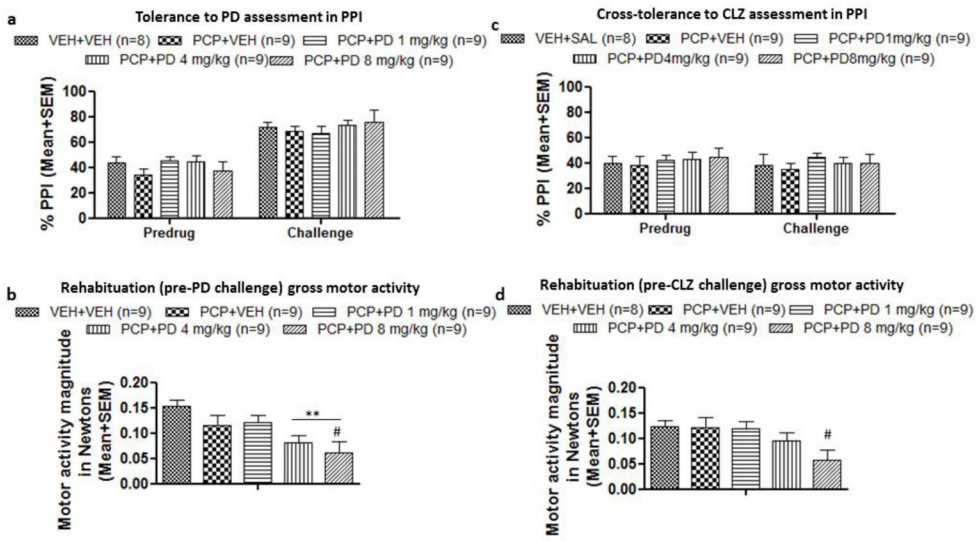

Figure 4.

Assessment of PD149163 (PD) tolerance in phencyclidine (PCP)-induced disruption of prepulse inhibition (PPI): (a) Percentage of PPI during the rehabituation day (predrug) and the PD tolerance assessment. (b) General motor activities in Newtons during the rehabituation day prior to the PD tolerance assessment. **$p<0.01$ relative to the VEH+VEH group; #$p<0.05$ relative to the PCP+VEH group. (c) Percentage of PPI during the rehabituation day (predrug) and the clozapine (CLZ) tolerance assessment day. (d) General motor activities in Newtons during the rehabituation day prior to the CLZ tolerance assessment. #$p<0.05$ relative to the VEH+VEH, PCP+VEH, and PCP+PD 1.0 groups. All values are expressed as mean + SEM.

Table 1

Summary of the experimental groups and treatment conditions in each experiment.

Experiment 1		Experiment 2		Experiment 3	
Condition	**n**	**Condition**	**n**	**Condition**	**n**
VEH (SAL)	5	VEH+VEH (SAL)	3	VEH+VEH (SAL)	6
VEH (MIA)	4	VEH+VEH (MIA)	4	VEH+VEH (MIA)	2
PD1 (SAL)	5	PCP+VEH (SAL)	3	PCP+VEH (SAL)	5
PD1 (MIA)	4	PCP+VEH (MIA)	5	PCP+VEH (MIA)	4
PD4 (SAL)	4	PCP+NT1 (SAL)	5	PCP+PD1 (SAL)	5
PD4 (MIA)	5	PCP+NT1 (MIA)	3	PCP+PD1 (MIA)	4
PD8 (SAL)	4	PCP+NT4 (SAL)	4	PCP+PD4 (SAL)	4
PD8 (MIA)	4	PCP+NT4 (MIA)	4	PCP+PD4 (MIA)	5
		PCP+NT8 (SAL)	4	PCP+PD8 (SAL)	5
		PCP+NT8 (MIA)	4	PCP+PD8 (MIA)	4
		VEH+NT4 (SAL)	4		
		VEH+NT4 (MIA)	3		