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Stereochemical and neuroanatomical selectivity of pramipexole effects on sensorimotor gating in rats

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Abstract

Background—In rats, prepulse inhibition (PPI) of acoustic startle is disrupted by systemic administration of dopaminergic agonists, such as the dopamine D3 receptor (D3R)-preferential agonist pramipexole (PPX). PPX has D3R-active (S) and -inactive (R) stereoisomers. Here, we tested the neuroanatomical and stereochemical selectivity of PPX effects on PPI.

Methods—(S)-PRA or (R)-PRA (0, 0.47, 1.42, 4.73 $\mu\text{mol/kg}$) was injected sc 15 min prior to PPI testing in adult male Sprague Dawley rats. In separate rats, (S)-PPX (0, 3, 10 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$, ic) was infused into the nucleus accumbens (NAc), caudodorsal striatum (CS), or olfactory tubercle / Islands of Calleja (ICj) 15 min prior to PPI testing. D3R expression in these brain regions was assessed using quantitative rt-PCR. The PPI-disruptive effects of systemic (S)-PPX were also tested after pretreatment with the D3R-selective antagonist, U99194 (10 mg/kg).

Results—Systemic administration of PPX stereoisomers demonstrated a dose-dependent effect of (S)-PPX on PPI, while (R)-PPX had no effect on PPI. PPX decreased PPI when infused into the NAc and ICj, but not the CS. Quantitative rt-PCR revealed D3R expression in ICj > NAc > CS. The PPI-disruptive effects of PPX were prevented by U99194.

Conclusion—The PPI-reducing effects of PPX are stereospecific for the D3R-active (S)-isomer, neuroanatomically preferential for the D3R-rich ventral vs. D3R poor caudodorsal striatum, and prevented by pharmacologic D3R blockade. These findings are consistent with the conclusion that PPX disrupts PPI via stimulation of mesolimbic D3Rs.

Keywords

dopamine receptor; Islands of Calleja; nucleus accumbens; pramipexole; prepulse inhibition; stereoisomer

1. Introduction

Prepulse inhibition of acoustic startle (PPI) is the automatic suppression of startle reflex magnitude that occurs when a weak lead stimulus precedes the startling noise. In rats, PPI is disrupted by systemic treatment with dopamine (DA) agonists such as amphetamine and apomorphine; this DA-mediated loss of PPI has been used to model PPI deficits in

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neuropsychiatric disorders, such as schizophrenia (Braff et al., 1978) and Tourette Syndrome (Castellanos et al., 1996), and to predict the clinical efficacy of antipsychotic compounds (Swerdlow et al., 1994; cf. Swerdlow et al., 2008). Several studies have assessed the regulation of PPI by specific DA receptor subtypes (Doherty et al., 2008; Peng et al., 1990; Stevenson and Gratton, 2004; Wan and Swerdlow, 1996), and the role of D1, D2 and D3 DA receptors in the regulation of PPI differs substantially across rodent strains and species, as well as across different brain regions. We and others have shown that PPI is disrupted by systemic treatment with D3-preferential agonists such as pramipexole (PPX) and PD128907 (Chang et al., 2010b; Weber et al., 2008, 2009; Zhang et al., 2007). PPX is among the most D3-selective agonists that are commercially available, with selectivity for D3 over D2 receptors reported to be between 7:1 and 160:1 (Millan et al., 2002; Piercey et al., 1996; Svensson et al., 1994). We previously reported the sensitivity of PPX-induced PPI deficits to several experimental parameters, including sex, species, stimulus modality, inter-stimulus interval, and startle-eliciting pulse magnitude, and demonstrated that PPX effects on PPI are dissociable from changes in startle magnitude or prepulse-elicited motor responses (Chang et al., 2010b; Swerdlow et al., 2009).

Despite its D3-preferential profile, it is unclear precisely how PPX disrupts PPI in rats. For example, a proposed role of D3 autoreceptor stimulation in suppressing presynaptic DA release (Carlsson, 1975; Chen et al., 2003; Diaz et al., 2000) would not easily account for the PPI-disruptive effects of PPX, since PPI is reduced by higher rather than lower levels of forebrain presynaptic DA release (Swerdlow et al. 1990, 2007). PPX has many other biological properties, beyond its effects on D3 receptors, some of which may have clinical utility. For example, PPX exists as both DA-active (S) and DA-inert (R) stereoisomers, both of which display neuroprotective properties in models of DA neurodegenerative disorders, purportedly acting as an antioxidant in neurons (Ferrari-Toninelli et al., 2010; Gu et al., 2004; Joyce et al., 2004; Le et al., 2000; Ramirez et al., 2003). Interestingly, the DA-inactive dextramipexole has already advanced through early phase clinical trials for neurodegenerative disorders (cf. Cheah and Kiernan, 2010). Here, we first assessed whether D3 activity is necessary for the observed PPX-disruption of PPI, by comparing the effects of (S)-PPX vs. (R)-PPX on this measure.

After establishing that D3-active properties are required for the PPI-disruptive effects of PPX, we next examined whether the PPI-disruptive effects of systemically-administered PPX could be reproduced via intracerebral infusion into discrete forebrain DA terminal regions. Previous studies have demonstrated that infusion of the D2/D3 agonist quinpirole into the nucleus accumbens (NAc) core and shell regions dose-dependently reduces PPI (Wan et al., 1994). D3 receptors are localized within mesolimbic and limbic regions; highest densities are found in the NAc and Islands of Calleja (ICj) in rats and primates, including humans (Landwehrmeyer et al., 1993; Levesque et al., 1992; Sokoloff et al., 2006). In addition to being implicated in the regulation of PPI, many of these brain regions are also of particular relevance to schizophrenia pathology and/or therapeutics. Here, we assessed changes in PPI in Sprague Dawley rats after infusion of PPX into 3 brain regions: 1) the NAc, based on the prominent role of the NAc in mediating DAergic PPI deficits, and the high D3 receptor expression levels in this brain region; 2) the ICj, based on its reported high levels of D3 receptor expression; and 3) the caudodorsal striatum (CS), a region known to regulate PPI, but which has little or no D3 receptor expression. Levels of D3 expression in these three target regions were confirmed via quantitative RT-PCR of tissue collected from drug-naïve rats. Finally, the PPI-disruptive effects of PPX were assessed in rats after blockade of D3Rs using the D3R-selective antagonist, U99194, which has a 13-fold selectivity for D3 over D2 receptors (Audinot et al., 1998).

2. Results

(S)-PPX vs. (R)-PPX effects

Repeated measures ANOVA of %PPI showed significant main effects of stereoisomer ($F=14.27$, df 1,22; $p<0.002$), PPX dose ($F=7.68$, df 3,66; $p<0.0003$), trial block ($F=5.89$, df 1,22; $p<0.03$), and prepulse intensity ($F=108.07$, df 2,44; $p<0.0001$), and a significant PPX dose $\times$ stereoisomer interaction ($F=5.91$, df 3,66; $p<0.002$). There were no other significant two- three- or four-way interactions involving stereoisomer, except for stereoisomer $\times$ prepulse intensity ($F=4.85$, df 2,44; $p<0.02$) and stereoisomer $\times$ prepulse intensity $\times$ PPX dose ($F=3.26$, df 6,132; $p<0.006$). Both of these interaction effects appeared to reflect a lack of any PPX effect on %PPI in the (R)-PPX treated group. Post-hoc tests revealed that %PPI was significantly reduced by the middle and high doses of (S)-PPX ($p<0.009$, $p<0.0001$, respectively), but were not reduced by any doses of (R)-PPX (Fig 2A).

Startle magnitude to PULSE-ALONE trials also demonstrated significant main effects of PPX dose ($F=3.48$, df 3,66; $p<0.03$) and block ($F=8.30$, df 1, 22; $p<0.009$), but interestingly, no effect of stereoisomer ($F<1$), and no significant interactions. Thus, statistically, both isomers reduce startle magnitude. However, inspection of the data suggests an orderly effect of PPX dose on startle magnitude in the DA-active, but not DA-inactive drug groups (Fig. 2B). As in previous studies (Chang et al., 2010b; Swerdlow et al., 2009; Weber et al. 2008, 2009), to understand the relationship between the startle- and PPI-reducing effects of (S)-PPX, difference scores were calculated between startle magnitude after vehicle and after active drug doses, and an ANOVA was conducted with a median split of these difference scores in each stereoisomer group. The PPI-disruptive effects of PPX did not differ among groups that exhibited high vs. low PPX-induced startle reduction ($F<1$), nor was there a median split $\times$ stereoisomer interaction ($F<1$).

Quantitative rt-PCR

As expected, highest levels of DRD3 expression were observed in the ICj, and expression levels in other brain regions were calculated as a percentage of ICj expression. NAc expressed 28% and CS expressed 8% as much DRD3 as the ICj.

Intracerebral infusion of (S)-PPX

Repeated measures ANOVA of %PPI showed significant main effects of PPX dose in the NAc ($F=5.39$, df 2,13; $p<0.02$) and ICj ($F=10.63$, df 2,17; $p<0.0005$) but not the CS ($F=2.85$, df 2,12; NS) (Fig. 3A). In all groups, there was a significant main effect of intensity ($p<0.0001$), but no other main effects and no significant interactions. Post-hoc tests revealed that, within the NAc and ICj infusion groups, PPI was significantly decreased at both active PPX doses (p 's $< 0.05 - 0.01$). PPX sensitivity appeared very comparable after infusion into the NAc and ICj; for example, the PPI-reducing effects of the low dose of PPX (3.0 μ g) were at least half-maximal ($\geq 50\%$ of the 10 μ g dose effects) in 44% of ICj group rats, and 43% of NAc group rats. There was no significant effect of PPX infusion on startle magnitude for any site-group (F 's = 1.61, < 1 and < 1 for NAc, ICj and CS, respectively; all p 's > 0.20), but inspection of the data suggested a trend towards startle magnitude reduction with PPX infusion in the NAc (Fig. 3B). Following a similar strategy as that used above for the stereoisomer experiment, difference scores were calculated between startle magnitude after intra-NAc infusion of vehicle and high dose PPX (10 μ g). Again, the PPI-disruptive effects of PPX did not differ among groups that exhibited high vs. low PPX-induced startle reduction ($F<1$).

(S)-PPX vs. U99194

Repeated measures ANOVA of PPI data revealed no significant main or interaction effects of pretreatment interval (30 vs. 60 min), so all rats ($n=20$) were combined into a single analysis. ANOVA revealed significant main effects of (S)-PPX ($F=43.95$, df 1,18; $p<0.0001$) and U99194 ($F=5.05$, df 1,18; $p<0.04$), and a significant interaction of (S)-PPX $\times$ U99194 ($F=4.55$, df 1,18; $p<0.05$). There was also a significant 3-way interaction of (S)-PPX $\times$ U99194 $\times$ trial block ($F=3.38$, df 2,36; $p<0.05$). Post-hoc tests confirmed that U99194 opposed the PPI-disruptive effects of (S)-PPX: among rats treated with 1.0 mg/kg (S)-PPX, PPI was significantly greater after pretreatment with 10 mg/kg vs. 0 mg/kg of U99194 ($p=0.02$). Analysis of the interaction of trial block with this effect revealed that blockade of the (S)-PPX effect developed across the test session, with minimal, intermediate and complete blockade evident in the initial, middle and final PPI blocks, respectively (Fig. 4); this did not reflect a waning of the (S)-PPX effect, which - in rats pretreated with 0 mg/kg U99194 (vehicle) - was unrelated to trial block ($F<1$). This block-dependent pattern was evident with both 30- and 60-minute pretreatment intervals, indicating that it did not reflect the pharmacokinetics of U99194 per se, but rather a true interaction of (S)-PPX, U99194 and startle testing. ANOVA of startle magnitude on PULSE-ALONE trials confirmed the expected startle-suppressing effects of (S)-PPX ($F=14.58$, df 1,18; $p<0.002$) and startle reduction across trial blocks ($F=4.20$, df 2,36; $p<0.025$), but no main or 2- or 3-way interaction effects of U99194 dose (F 's 0.06 – 1.05, ns) (data not shown).

3. Discussion

Our previous reports of the ability of PPX to disrupt PPI in rats (Weber et al., 2008, 2009; Chang et al., 2010b, 2011; Swerdlow et al., 2009) were based on the assumption that these effects were mediated by interactions between PPX and DA receptors in the forebrain. However, PPX is bioactive in other ways; it has been shown to be neuroprotective in neurodegenerative models using MPTP, apparently through DA-dependent and DA-independent mechanisms (cf. Albrecht and Buerger, 2009), and the DA-inactive dexamipexole has demonstrated clinical evidence of neuroprotection in patients with amyotrophic lateral sclerosis (cf. Cheah and Kiernan, 2010). Indeed, in preclinical models, we reported that PPX alters locomotor activity in mice and rats through presumably DA-dependent and DA-independent mechanisms (Chang et al., 2010a, 2011, Richtand et al., 2011). In the present studies, we compared the effects of molar equivalent doses of (S)- and (R)-PPX on startle and PPI in rats. As has been shown previously, (S)-PPX dose-dependently decreased PPI; in contrast, (R)-PPX had no significant effect on PPI, confirming that active DA receptor-stimulating properties of PPX are necessary for its effects on PPI. The dissociation of these isomers was less evident in measures of startle magnitude: both stereoisomers reduced startle magnitude, though the effects of (S)-PPX were more clearly dose-dependent.

Once the stereochemical selectivity of PPX-induced PPI effects was established, we next tested the anatomical selectivity of these effects by infusing PPX directly into brain areas with different levels of DA D3 receptor (DRD3) expression, which were compared using quantitative rt-PCR. In keeping with what has been shown through radiolabeling, *in situ* hybridization, and immunohistochemistry studies (Bouthenet et al., 1991; Diaz et al., 1995, 2000; Herroelen et al., 1994; Schwartz et al., 2000; Suzuki et al., 1998), we found the highest levels of DRD3 in the ICj -- roughly 4 times higher than in the NAc, and 12–13 times higher than the CS. Based on the differential DRD3 expression levels, as well as varying degrees of involvement of these regions in startle circuitry (Swerdlow et al., 2000, 2001), we hypothesized that: 1) the PPI-disruptive effects of PPX after systemic drug infusion could be reproduced by direct infusion into forebrain DA terminal regions that regulate PPI; and 2) the different infusion sites would demonstrate different sensitivities to

the PPI-disruptive effects of PPX. Indeed, direct infusion of PPX was sufficient to significantly reduce PPI. Moreover, regions with higher DRD3 expression -- the ICj and NAc -- appeared to be sensitive to these PPX effects, while PPX infusion into the CS did not disrupt PPI. This differential sensitivity to the PPI-disruptive effects of PPX might certainly reflect a more prominent role of the ventral striatum vs. the CS in the regulation of PPI; on the other hand, the CS also regulates PPI, since lesions of the CS and pharmacological manipulations of its efferent projections are known to significantly disrupt PPI in rats (Kodsi and Swerdlow, 1995) and mice (Takahashi et al., 2007).

Other nonselective D2/D3 agonists can induce PPI deficits with localized infusion in the brain, with the strongest effect being observed in regions of the NAc (e.g. Wan et al. 1994). To the best of our knowledge, the present data provide the first suggestion for a role of the ICj in the DAergic regulation of PPI. Interestingly, despite the fact that the ICj has a 4-fold higher level of DRD3 expression compared to the NAc, the PPI-disruptive effects of the D3 agonist, PPX, were not greater - and, in fact, arithmetically diminished - compared to the NAc. Thus, it appears that the relative levels of DRD3 expression cannot fully account for the sensitivity to the PPI-disruptive effects after intracerebral infusion of PPX.

The present findings demonstrate that the PPI-disruptive effects of PPX are selective to its D3R-active S-enantiomer, and are mediated predominantly within the D3R-rich ventral striatum more so than the D3R-poor caudodorsal striatum. These findings do not, however, definitively resolve whether these PPX effects are mediated by its predominant D3-stimulatory properties, vs. its activity at other D2-family receptors. We have reported that, compared to the selective D2 agonist sumanirole, the mixed D1/D2 agonist apomorphine, and even the relatively D3-favoring agonists 7OH-DPAT and PD-128907, the PPI-disruptive effects of PPX are relatively insensitive to "rescue" by selective D2 receptor blockade (Breier et al., 2011; Weber et al., 2009, 2010). However, a clear demonstration that the PPI-disruptive effects of PPX reflect its action at D3 receptors has been hindered by the relative paucity of highly selective and potent D3 receptors antagonists, and by apparent species differences in the DA receptor-specific regulation of PPI (Chang et al. 2010a). Our present findings with the D3R-preferential antagonist, U99194 (Audinot et al. 1998), directly support a role of D3R's in the PPI-disruptive effects of (S)-PPX. While the present agonist-antagonist study does not include comprehensive dose-response analyses, we recently determined that the active dose of U99194 studied here (10 mg/kg sc) prevents the (S)-PPX-induced suppression of c-Fos expression within the NAc core and shell subregions (Chang et al. 2011), and has been shown by others to inhibit other behaviors related to D3R activity (Carr et al., 2002; Collins et al., 2005; Millan et al., 2004). Thus, converging lines of stereochemical, anatomical and pharmacologic evidence support the conclusion that (S)-PPX disrupts PPI in rats via stimulation of mesolimbic D3Rs. This evidence helps clarify some of the mechanisms of action of PPX, which is being advanced in humans as a putative procognitive agent, with potential applications in the treatment of schizophrenia and other neuropsychiatric disorders (Ersche et al., 2011; Swerdlow, 2011).

4. Methods and Materials

Experimental Animals

Adult male Sprague-Dawley (n = 74, 225–250 g; Harlan Laboratories, Livermore, CA) rats were housed 2–3 animals per cage, and maintained on a reversed light/dark schedule with water and food available *ad libitum*. Rats were handled within 2 d of arrival. For drug infusion studies, surgery occurred between 7–10 days after arrival. Testing occurred during the dark phase. All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 85–23,

revised 1985) and were approved by the UCSD Animal Subjects Committee (protocol #S01221).

Drugs

Pramipexole (PPX), (S)-pramipexole dihydrochloride [(S)-PPX], and (R)-pramipexole dihydrochloride [(R)-PPX] were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Drug doses for systemic PPX stereoisomer salts (0, 0.47, 1.42, 4.73 $\mu\text{mol/kg}$) are molar equivalents of milligram/kilogram doses used in previous systemic studies with (S)-PPX base (Chang et al., 2010b; Swerdlow et al., 2009; Weber et al., 2008, 2009), namely 0.1, 0.3, 1.0 mg/kg, respectively. Preliminary studies tested the behavioral effects of (S)-PPX base doses compared to molar equivalents of the (S)-PPX salt and found nearly identical effect sizes on PPI and startle magnitude (data not shown). For the PPX stereoisomer studies, drug was injected subcutaneously (sc) in a volume of 1 ml/kg body weight 15 min prior to behavioral testing. Drug doses for intracerebral infusions were based on $\mu\text{g}/\mu\text{l}$ base. (S)-PPX (0, 3 or 10 $\mu\text{g}/0.5 \mu\text{l}$ saline/side) was infused bilaterally at a rate of 0.5 μl over 42 s. Injectors remained in place for 30 s post-infusion, and then were replaced with a stylet. Rats were placed in the startle chambers after 15 min. For agonist-antagonist studies, U99194 (0 or 10 mg/kg sc; Tocris Pharmaceuticals) was dissolved in saline and injected in a volume of 1 ml/kg body weight 30 or 60 min injection prior to injection of (S)-PPX (0 or 1.0 mg/kg sc, as above), followed 15 min later by testing. This dose of U99194 was selected because it prevented a PPX-induced suppression of c-Fos expression in the NAc (Chang et al. 2011); two pretreatment intervals were used due to the unknown time-course of U99194 effects in, this paradigm.

Surgery/Histology

Rats received 0.1 ml atropine sulfate (Vedco, 0.054 mg/ml sc) 15–30 min before being anesthetized with sodium pentobarbital (Abbott, 60.0 mg/kg/ml ip). Fully anesthetized rats were placed in a Kopf stereotaxic instrument (tooth bar at -3.3 mm). Cannulae (10 mm pieces of 23 ga stainless steel tubing) were lowered 3 mm above specific target sites in the NAc core (AP +1.2, L ± 1.7 , DV -4.3 (Bregma)), olfactory tubercle containing the ICj (AP +0.7, L ± 4.0 , DV -5.5 (Bregma)), or CS (AP +0.2, L ± 3.5 , DV -2.5 (Bregma)) (Fig. 1A). ICj cannulae were placed at a 10° angle from vertical. Cannulae were anchored to the skull via dental cement and skull screws, and filled with a wire stylet. Rats were monitored daily postoperatively. For i.c. infusions, stylets were removed and 30 ga needles attached to PE 10 tubing were inserted through the cannulae 3 mm beyond their tips.

Histological localization was performed after completion of behavioral testing (Fig. 1A). Rats were anesthetized and transcardially perfused with saline followed by 10% buffered formalin. Brains were cryoprotected, then 50 μm coronal sections were cut on a frozen-stage sliding microtome, mounted on glass slides and Nissl-stained. Injector placements were plotted based on the ventral-most extent of the injector tract, and categorized as a "hit" or "miss," blind to behavioral data. One rat from the NAc group was excluded from analysis on this basis.

Apparatus

Startle chambers for rats (San Diego Instruments, San Diego, CA, USA) were housed in a sound-attenuated room, and consisted of a Plexiglas cylinder 8.2 cm in diameter resting on a $12.5 \times 25.5 \text{ cm}$ Plexiglas frame within a ventilated enclosure. Noise bursts were presented via a speaker mounted 24 cm above the cylinder. A piezoelectric accelerometer mounted below the Plexiglas frame detected and transduced motion from within the cylinder. Stimulus delivery was controlled by the SR-LAB microcomputer and interface assembly, which also digitized (0–4095), rectified, and recorded stabilimeter readings. One hundred 1-

ms readings were collected beginning at stimulus onset. Startle amplitude was defined as the average of the 100 readings.

Startle testing procedure

Approximately 7–9 d after shipment arrival for the systemically treated group or 5 d after cannula placement for the intracerebral infusion group, rats were placed in the startle chambers for a 5 min acclimation period with a 70 dB(A) background noise, and then exposed to a total of 17 PULSE-ALONE trials (40 ms – 120 dB(A) noise bursts) that were interspersed with 3 PREPULSE+PULSE trials in which PULSE-ALONE was preceded 100 ms (onset-to-onset) by a 20 ms noise burst, 12 dB above background. Based on this "matching session", rats were assigned to drug dose groups based on average %PPI to ensure similar baseline PPI levels across groups. Starting 2–5 d after the matching session, drug testing began. Inter-test interval was 5–7 d. For both systemic and intracerebral administration studies, drug testing was done on multiple days with PPX dose as a within-subject factor and pseudo-randomized dose order. For stereoisomer studies, (S)-PPX vs. (R)-PPX was a between-subject factor. For drug infusions studies, targeted infusion site was a between-subject factor. For antagonist studies, U99194 dose was a between-subject factor.

Test sessions began with a 5 min acclimation period with a 70 dB(A) background noise and then a series of trial types presented in pseudorandom order: (1) PULSE-ALONE trials consisting of 40 ms 120 dB(A) noise bursts, (2) PREPULSE+PULSE trials where PULSE-ALONE was preceded 100 ms (onset-to-onset) by a 20-ms noise burst that was either 5, 10, or 15 dB(A) above background; (3) NOSTIM trials, in which no stimulus was presented but motor activity was measured. The session began with 4 consecutive PULSE-ALONE trials and ended with 3 consecutive PULSE-ALONE trials; between these trials were either two (stereoisomer and intracerebral infusion studies) or three (agonist-antagonist study) blocks, each consisting of eight PULSE-ALONE trials, five 5, 10, and 15 dB(A) PREPULSE +PULSE trials. NOSTIM trials were interspersed between active trials. Average ITI between active trials was 15 s, and NOSTIM trials were not included in the calculation of intertrial intervals.

Quantitative rt-PCR

Two drug- and testing-naïve rats were decapitated and brains rapidly dissected and chilled in ice-cold saline. NAc samples were obtained with a 2 mm diameter punch from 1 mm thick coronal slabs (Bregma +1.2 to +2.2 mm; Fig. 1B). The remaining half-moon shaped brain region containing the Islands of Calleja dissected off from this same slab. Caudodorsal striatum was collected with a 2.5 mm diameter punch from the adjacent 1 mm thick coronal slab (Bregma +0.2 to +1.2). All tissue was placed immediately into RNALater (Ambion) and onto dry ice. Samples were stored at –80°C until RNA extraction with QIAGEN RNeasy Mini Kit. Quantitative rt-PCR was performed by UCSD Center for Aids Research Genomic Core. On-column DNase digestion was performed with the QIAGEN RNase-free DNase set, and cDNA was made with qScript cDNA SuperMix (Quanta Biosciences). qPCR was conducted on Applied Biosystems 7900HT Fast Real-Time PCR System. Standard curves for DRD3 (Applied Biosystems primer) expression were made with one ICj sample; all samples were run in duplicate and normalized to GAPDH (Applied Biosystems primer) expression levels. Data are expressed as a fold change from average DRD3 expression in the ICj samples and are an average between duplicates of two brains.

Data Analysis

PPI was defined as $100 - [(startle\ amplitude\ on\ PREPULSE+PULSE\ trials / startle\ amplitude\ on\ PULSE-ALONE\ trials) \times 100]$, and was analyzed by mixed design ANOVAs. All data were inspected for the presence of "non-responders" defined by a mean startle response to

PULSE-ALONE trials of < 10 units (vs. group means of approximately 266 units); none met this criteria. One rat from the ICj infusion group had startle magnitude >5 standard deviations above the group mean and was eliminated from further analyses. Other ANOVAs were used to assess responses on PULSE-ALONE, PREPULSE-ALONE, or NOSTIM trials. Based on differing DRD3 expression levels found with rt-PCR of infusion sites, our *a priori* design was to conduct ANOVAs for PPX effects on PPI within each infusion site group. Where different prepulse intensities were present, data were collapsed across all prepulse intensities, unless otherwise noted based on significant interaction effects. Post-hoc comparisons were conducted using Fisher's protected least significance difference (PLSD). Difference scores for startle magnitude were calculated by averaging the response to PULSE-ALONE across blocks on vehicle test day and subtracting the average PULSE-ALONE response across blocks on high dose (10 µg/0.5 µl PPX) test day. The relationship between startle suppression and PPI effects were tested via comparison of PPI changes after a median split for startle magnitude difference scores. Alpha was set at 0.05.

Highlights

- Pramipexole (PPX) effects on prepulse inhibition (PPI) are stereospecific to the (S) isomer
- PPI is disrupted by (S)-PPX infusion into the nucleus accumbens and Islands of Calleja, but not the dorsal posterior striatum.
- DA D3 receptor expression in Islands of Calleja > nucleus accumbens > posterior striatum by rt-PCR
- The PPI-disruptive effects of (S)-PPX are significantly opposed by the D3 receptor-preferential antagonist, U99194.

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References

- Albrecht S, Buerger E. Potential neuroprotection mechanisms in PD: focus on dopamine agonist pramipexole. *Curr. Med. Res. Opin.* 2009; 25:2977–2987. [PubMed: 19842998]
- Audinot V, Newman-Tancredi A, Gobert A, Rivet JM, Brocco M, Lejeune F, Gluck L, Desposte I, Bervoets K, Dekeyne A, Millan MJ. A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D3 receptor antagonists (+)-S 14297, nafadotride, GR 103,691 and U 99194. *J. Pharmacol. Exp. Ther.* 1998; 287:187–197. [PubMed: 9765337]
- Bouthenet ML, Souil E, Martres MP, Sokoloff P, Giros B, Schwartz JC. Localization of dopamine D3 receptor messenger RNA in the rat brain using in situ hybridization histochemistry – comparison with dopamine D2 receptor messenger. *Brain Res.* 1991; 564:203–219. [PubMed: 1839781]
- Braff DL, Stone C, Callaway E, Geyer M, Glick I, Bali L. Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology.* 1978; 15:339–343. [PubMed: 693742]
- Breier MR, Chang WL, Weber M, Mizera M, Swerdlow NR. Pharmacological parsing of D2- vs. D3-mediated regulation of sensorimotor gating in rats. Abstract, Society for Neuroscience. 2011
- Carlsson A. Drugs acting through dopamine release. *Pharmacol. Ther. B.* 1975; 1:401–405. [PubMed: 772710]

- Carr KD, Yamamoto N, Omura M, Cabeza de Vaca S, Krahne L. Effects of the D(3) dopamine receptor antagonist, U99194A, on brain stimulation and d-amphetamine reward, motor activity, and c-fos expression in ad libitum fed and food-restricted rats. *Psychopharmacology (Berl)*. 2002; 163:76–84. [PubMed: 12185403]
- Castellanos FX, Fine EJ, Kaysen D, Marsh WL, Rapoport JL, Hallett M. Sensorimotor gating in boys with Tourette's syndrome and ADHD: preliminary results. *Biol. Psychiatry*. 1996; 39:33–41. [PubMed: 8719124]
- Chang WL, Breier MR, Yang A, Swerdlow NR. Disparate effects of pramipexole on locomotor activity and sensorimotor gating in Sprague Dawley rats. *Pharmacol. Biochem. Behav*. 2011; 99:634–638. [PubMed: 21683731]
- Chang WL, Geyer MA, Buell MR, Weber M, Swerdlow NR. The effects of pramipexole on prepulse inhibition and locomotor activity in C57BL/6J mice. *Behav. Pharmacol*. 2010a; 21:135–143. [PubMed: 20215963]
- Chang WL, Swerdlow NR, Breier MR, Thangaraj N, Weber M. Parametric approaches towards understanding the effects of the preferential D3 receptor agonist pramipexole on prepulse inhibition in rats. *Pharmacol. Biochem. Behav*. 2010b; 95:473–478. [PubMed: 20385162]
- Chang WL, Breier MR, Saint Marie RL, Yang A, Hines SR, Swerdlow NR. Blockade of pramipexole effects on prepulse inhibition and accumbens c-Fos expression by U99194. *Proc American College of Neuropsychopharmacology*. 2011 (in press) 2011.
- Cheah BC, Kiernan MC. Dextramipexole, the R(+) enantiomer of pramipexole, for the potential treatment of amyotrophic lateral sclerosis. *IDrugs*. 2010; 13:911–920. [PubMed: 21154151]
- Chen PC, Lao CL, Chen JC. The D₃ dopamine receptor inhibits dopamine release in PC-12/hD₃ cells by autoreceptor signaling via PP-2B, CK1, and Cdk-5. *J. Neurochem*. 2003; 110:1180–1190. [PubMed: 19522735]
- Collins GT, Witkin JM, Newman AH, Svensson KA, Grundt P, Cao J, Woods JH. Dopamine agonist-induced yawning in rats: a dopamine D3 receptor-mediated behavior. *J. Pharmacol. Exp. Ther*. 2005; 314:310–319. [PubMed: 15833897]
- Diaz L, Pilon C, Le Foll B, Gross C, Triller A, Schwartz JC, Sokoloff P. Dopamine D₃ receptors expressed by all mesencephalic dopamine neurons. *J. Neurosci*. 2000; 20:8677–8684. [PubMed: 11102473]
- Diaz J, Levesque D, Lammers CH, Griffon N, Martres MP, Schwartz JC, Sokoloff P. Phenotypical characterization of neurons expressing the dopamine D3 receptor in the rat brain. *Neuroscience*. 1995; 65:731–745. [PubMed: 7609872]
- Doherty JM, Masten VL, Powell SB, Ralph RJ, Klamer D, Low MJ, Geyer MA. Contributions of dopamine D1, D2, and D3 receptor subtypes to the disruptive effects of cocaine on prepulse inhibition in mice. *Neuropsychopharmacology*. 2008; 33:2648–2656. [PubMed: 18075489]
- Ersche KD, Roiser JP, Lucas M, Domenici E, Robbins TW, Bullmore ET. Peripheral biomarkers of cognitive response to dopamine receptor agonist treatment. *Psychopharmacology (Berl)*. 2011; 214:779–789. [PubMed: 21088959]
- Ferrari-Toninelli G, Maccarinelli G, Uberti D, Buerger E, Memo M. Mitochondria-targeted antioxidant effects of S(–) and R(+) pramipexole. *BMC Pharmacol*. 2010; 10:2. [PubMed: 20137065]
- Gu M, Iravani MM, Cooper JM, King D, Jenner P, Schapira AH. Pramipexole protects against apoptotic cell death by non-dopaminergic mechanisms. *J. Neurochem*. 2004; 91:1075–1081. [PubMed: 15569251]
- Herroelen L, De Backer JP, Wilczak N, Flamez A, Vauquelin G, De Keyser J. Autoradiographic distribution of D-3-type dopamine receptors in human brain using [H-3] 7-hydroxy-N,N-di-N-propyl-2-aminotetralin. *Brain Res*. 1994; 648:222–228. [PubMed: 7922537]
- Joyce JN, Woolsey C, Ryoo H, Borwege S, Hagner D. Low dose pramipexole is neuroprotective in the MPTP mouse model of Parkinson's disease, and downregulates the dopamine transporter via the D3 receptor. *BMC Biol*. 2004; 2:22. [PubMed: 15473914]
- Kodsi MH, Swerdlow NR. Prepulse inhibition in the rat is regulated by ventral and caudodorsal striato-pallidal circuitry. *Behav. Neurosci*. 1995; 109:912–928. [PubMed: 8554715]
- Landwehrmeyer B, Mengod G, Palacios JM. Dopamine D3 receptor mRNA and binding sites in human brain. *Brain Res. Mol. Brain Res*. 1993; 18:187–192. [PubMed: 8097550]

- Le WD, Jankovic J, Xie W, Appel SH. Antioxidant property of pramipexole independent of dopamine receptor activation in neuroprotection. *J. Neural. Transm.* 2000; 107:1165–1173. [PubMed: 11129106]
- Levesque D, Diaz J, Pilon C, Martres MP, Giros B, Souil E, Schott D, Morgat JL, Schwartz JC, Sokoloff P. Identification, characterization, and localization of the dopamine D3 receptor in rat brain using 7-[3H]hydroxy-N,N-di-n-propyl-2-aminotetralin. *Proc. Natl. Acad. Sci. U. S. A.* 1992; 89:8155–8159. [PubMed: 1518841]
- Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor I. A multivariate analysis of the binding profiles of 14 drugs at 21 native and cloned human receptor subtypes. *J. Pharmacol. Exp. Ther.* 2002; 303:791–804. [PubMed: 12388666]
- Millan MJ, Seguin L, Gobert A, Cussac D, Brocco M. The role of dopamine D3 compared with D2 receptors in the control of locomotor activity: a combined behavioural and neurochemical analysis with novel, selective antagonists in rats. *Psychopharmacology (Berl)*. 2004; 174:341–357. [PubMed: 14985929]
- Peng RY, Mansbach RS, Braff DL, Geyer MA. A D2 dopamine receptor agonist disrupts sensorimotor gating in rats. Implications for dopaminergic abnormalities in schizophrenia. *Neuropsychopharmacology*. 1990; 3:211–218. [PubMed: 2141986]
- Piercey MF, Hoffmann WE, Smith MW, Hyslop DK. Inhibition of dopamine neuron firing by pramipexole, a dopamine D3 receptor-preferring agonist: comparison to other dopamine receptor agonists. *Eur. J. Pharmacol.* 1996; 312:35–44. [PubMed: 8891576]
- Ramirez AD, Wong SK, Menniti FS. Pramipexole inhibits MPTP toxicity in mice by dopamine D3 receptor dependent and independent mechanisms. *Eur. J. Pharmacol.* 2003; 475:29–35. [PubMed: 12954356]
- Richtand NM, Chang W, Ahlbrand R, Swerdlow NR. The effects of pramipexole on locomotor activity in D3 mutant mice. Abstract, Society for Neuroscience. 2011
- Schwartz JC, Diaz J, Pilon C, Sokoloff P. Possible implications of the dopamine D-3 receptor in schizophrenia and in antipsychotic drug actions. *Brain Res. Rev.* 2000; 31:277–287. [PubMed: 10719154]
- Sokoloff P, Diaz J, Le Foll B, Guillin O, Leriche L, Bezard E, Gross C. The dopamine D3 receptor: a therapeutic target for the treatment of neuropsychiatric disorders. *CNS Neurol. Disord. Drug Targets*. 2006; 5:25–43. [PubMed: 16613552]
- Stevenson CW, Gratton A. Basolateral amygdala dopamine receptor antagonism modulates initial reactivity to but not habituation of the acoustic startle response. *Behav. Brain Res.* 2004; 153:383–387. [PubMed: 15265633]
- Suzuki M, Hurd YL, Sokoloff P, Schwartz JC, Schwartz JC, Sedvall G. D-3 dopamine receptor mRNA is widely expressed in the human brain. *Brain Res.* 1998; 779:58–74. [PubMed: 9473588]
- Svensson K, Carlsson A, Huff RM, Kling-Petersen T, Waters N. Behavioral and neurochemical data suggest functional differences between dopamine D2 and D3 receptors. *Eur. J. Pharmacol.* 1994; 263:235–243. [PubMed: 7843260]
- Swerdlow NR, Braff DL, Geyer MA. Animal models of deficient sensorimotor gating: what we know, what we think we know, and what we hope to know soon. *Behav. Pharmacol.* 2000; 11:185–204. [PubMed: 11103873]
- Swerdlow NR, Braff DL, Taaid N, Geyer MA. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. *Arch. Gen. Psychiatry*. 1994; 51:139–154. [PubMed: 8297213]
- Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacology (Berl)*. 2001; 156:194–215. [PubMed: 11549223]
- Swerdlow NR, Lelham SA, Sutherland Owens AN, Chang WL, Sassen SDT, Talledo JA. Pramipexole effects on startle gating in rats and normal men. *Psychopharmacology (Berl)*. 2009; 205:689–698. [PubMed: 19506839]

- Swerdlow NR, Mansbach RS, Geyer MA, Pulvirenti L, Koob GF, Braff DL. Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine. *Psychopharmacology (Berl)*. 1990; 100:413–416. [PubMed: 2315438]
- Swerdlow NR, Shoemaker JM, Bongiovanni MJ, Neary AC, Tochen LS, Saint Marie RL. Strain differences in the disruption of prepulse inhibition of startle after systemic and intra-accumbens amphetamine administration. *Pharmacol. Biochem. Behav.* 2007; 87:1–10. [PubMed: 17475315]
- Swerdlow NR, Weber M, Qu Y, Light GA, Braff DL. Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology (Berl)*. 2008; 199:331–388. [PubMed: 18568339]
- Swerdlow NR. Are we studying and treating schizophrenia correctly? *Schizophr Res.* 2011; 130:1–10. [PubMed: 21645998]
- Takahashi K, Nagai T, Kamei H, Maeda K, Matsuya T, Arai S, Mizoguchi H, Yoneda Y, Nabeshima T, Takuma K, Yamada K. Neural circuits containing pallidotegmental GABAergic neurons are involved in the prepulse inhibition of the startle reflex in mice. *Biol. Psychiatry*. 2007; 62:148–157. [PubMed: 17027927]
- Wan FJ, Geyer MA, Swerdlow NR. Accumbens D2 modulation of sensorimotor gating in rats: assessing anatomical localization. *Pharmacol. Biochem. Behav.* 1994; 49:155–163. [PubMed: 7816867]
- Wan FJ, Swerdlow NR. Sensorimotor gating in rats is regulated by different dopamine-glutamate interactions in the nucleus accumbens core and shell subregions. *Brain Res.* 1996; 722:168–176. [PubMed: 8813362]
- Weber M, Chang WL, Breier M, Ko D, Swerdlow NR. Heritable strain differences in sensitivity to the startle gating-disruptive effects of D2 but not D3 receptor stimulation. *Behav. Pharmacol.* 2008; 19:786–795. [PubMed: 19020413]
- Weber M, Chang WL, Breier MR, Yang A, Millan MJ, Swerdlow NR. The effects of sumanirole on prepulse inhibition in rats. *Eur. Neuropsychopharmacol.* 2010; 20:421–425. [PubMed: 20346635]
- Weber M, Chang WL, Durbin JP, Park PE, Luedtke RR, Mach RH, Swerdlow NR. Using prepulse inhibition to detect functional D3 receptor antagonism: effects of WC10 and WC44. *Pharmacol. Biochem. Behav.* 2009; 93:141–147. [PubMed: 19426754]
- Zhang M, Ballard ME, Unger LV, Haupt A, Gross G, Decker MW, Drescher KU, Rueter LE. Effects of antipsychotics and selective D3 antagonists on PPI deficits induced by PD 128907 and apomorphine. *Behav. Brain. Res.* 2007; 182:1–11. [PubMed: 17570538]

Figure 1. Illustration of infusion sites and corresponding areas of rt-PCR analysis
 [A]: Diagram of injector placements confirmed by histology post-testing. [B]: Diagram of tissue collection sites for measurement of D3 receptor expression in the brain regions corresponding to PPX infusion sites. Samples were collected bilaterally for each region.

Figure 2. Effects of (S)- vs. (R)-PPX on PPI (A) and startle magnitude (B)

[A]: The DA-active (S)-PPX salt induced a dose-dependent reduction in PPI, while the DA-inactive (R)-PPX isomer had no effect on PPI. Drug doses represent molar equivalents of 0.1, 0.3, and 1.0 mg/kg of PPX base previously shown to disrupt PPI (Chang et al., 2010b; Swerdlow et al., 2009; Weber et al., 2008, 2009). [B]: While there was an overall main effect of PPX dose on startle magnitude and no effect of stereoisomer or stereoisomer $\times$ PPX dose interaction, the data shown here suggest a dose-dependent effect of (S)-PPX on startle suppression, while the (R)-PPX effect is less orderly. * $p < 0.009$, ** $p < 0.0001$.

Figure 3. (S)-PPX effects on PPI (A) and startle magnitude (B) after intracerebral infusion

A: Significant main effects of PPX infusion on PPI were observed in rats infused in the ICj and NAc, but not the CS. $*p < 0.05$; B: There were no significant effects of PPX infusion on startle magnitude, though there appears to be a trend towards dose-dependent startle reduction after PPX infusion in the NAc. NAc=nucleus accumbens, ICj=Islands of Calleja, CS=caudodorsal striatum.

Figure 4. (S)-PPX effects on PPI after pretreatment with U99194

PPI was significantly disrupted by (S)-PPX (1.0 mg/kg) across the entire test session (inset), and in each of the 3 trial blocks (main figure) (#: all values $p < 0.0001$). The D3R-preferential antagonist U99194 opposed these effects of (S)-PPX, as evidenced by the fact that it significantly increased PPI in (S)-PPX-treated rats across the entire test session (inset) and in trial blocks 2 and 3 (main figure) (*: all p 's < 0.05).