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Serotonergic involvement in the amelioration of behavioral abnormalities in dopamine transporter knockout mice by nicotine

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Abstract

Dopamine transporter knockout (DAT KO) mice exhibit elevated extracellular dopamine levels in brain regions that include the striatum and the nucleus accumbens, but not the prefrontal cortex. DAT KO mice model some aspects of psychiatric disorders, including schizophrenia. Smoking is more common in patients with schizophrenia, suggesting that nicotine might ameliorate aspects of the behavioral abnormalities and/or treatment side effects seen in these individuals. We report nicotine-induced normalization of effects on locomotion and prepulse inhibition of acoustic startle (PPI) in DAT KO mice that require intact serotonin 5-HT_{1A} systems. First, we observed that the marked hyperactivity displayed by DAT KO mice was reduced by administration of nicotine. This nicotine effect was blocked by pretreatment with the non-specific nicotinic acetylcholine (nACh) receptor antagonist mecamylamine, or the 5-HT_{1A} antagonist WAY100635. Secondly, we examined the effects of nicotine on PPI in DAT KO mice. Treatment with nicotine significantly ameliorated the PPI deficits observed in DAT KO mice. The ameliorating action of nicotine on PPI deficits in DAT KO mice was blocked by mecamylamine, the α_7 nACh receptor antagonist methyllycaconitine or WAY100635, while the $\alpha_4\beta_2$ nACh receptor antagonist dihydro- β -erythroidinehydrobromide (DH β E) produced only a non-significant trend toward attenuation of nicotine effects. Finally, we observed that administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT also ameliorated the deficit in PPI observed in DAT KO mice. This amelioration was antagonized by pretreatment with WAY100635. These data support the idea that nicotine might ameliorate some of the cognitive dysfunctions found in schizophrenia in a 5-HT_{1A}-dependent fashion.

Keywords

α_7 nACh receptor; $\alpha_4\beta_2$ nACh receptor; 5-HT_{1A} receptor; Schizophrenia; Locomotor activity; Prepulse inhibition

1. Introduction

Beneficial effects of nicotine and nicotinic acetylcholine (nACh) receptor agonists can be demonstrated in numerous behavioral and cognitive studies in both humans and animal

models (for review see (Hall et al., 2012)). There are apparent beneficial effects of nicotine and/or nicotinic acetylcholine receptor agonists in several psychiatric disorders, including schizophrenia. The high rates of smoking in schizophrenic patients (Conklin et al., 2008; Dursun and Kutcher, 1999; Picciotto et al., 2000), have led to the suggestion that nicotine may provide self-medication for these patients, perhaps for aspects of impaired behavior and cognition characteristic of this disease and/or for unwanted effects of antipsychotic medications (Dalack et al., 1998; Kumari and Postma, 2005; Ripoll et al., 2004). Nicotinic agonists do appear to improve deficits in cognitive functioning (Potter et al., 2006; Wilens and Decker, 2007), and sensory gating (George et al., 2006; Petrovsky et al., 2010) found in schizophrenia patients.

Nicotine affects function of nACh receptors that consist of combinations of the 12 nACh receptors subunits ($\alpha 2$ – $\alpha 10$ and $\beta 2$ – $\beta 4$) (Albuquerque et al., 2009; Mihailescu and Drucker-Colin, 2000). While homomeric $\alpha 7$ and heteromeric $\alpha 4\beta 2$ nACh receptors have been implicated in the pathophysiology of cognitive deficits in schizophrenia (Olincy et al., 2006), the exact nACh receptor subtype(s) responsible for nicotine-mediated improvement in these cognitive deficits remain to be elucidated.

Homozygous deletion of the dopamine transporter (DAT) gene in DAT knockout (DAT KO) mice produces a ten-fold increase of extracellular dopamine (DA) concentrations in the striatum measured by *in vivo* microdialysis (Shen et al., 2004). DAT KO mice exhibit behavioral alterations, many linked to these changes in dopaminergic function, that include hyperlocomotion (Sora et al., 1998; Gainetdinov et al., 1999b), cognitive deficits (Li et al., 2010; Morice et al., 2007), and impairments of prepulse inhibition (PPI) of the startle reflex (Arime et al., in press; Ralph et al., 2001; Yamashita et al., 2006). Pharmacological treatments that can ameliorate these deficits in DAT KO mice include psychostimulants, norepinephrine transporter (NET) blockers and DA antagonists.

Several lines of evidence suggest possible serotonergic influences on nicotine effects in DAT KO mice. In previous work examining the molecular basis of cocaine conditioned place preference, both DAT and the serotonin transporter (SERT) were found to be involved (Hall et al., 2002; Sora et al., 2001). Serotonergic mechanisms have been suggested to have locomotion-decreasing effects in DAT KO mice (Gainetdinov et al., 1999b). While the precise serotonin receptor subtypes that may underlie these differences between DAT KO and wild type (WT) mice have yet to be elucidated, treatment with 5-HT_{1A} receptor antagonists can attenuate cocaine-induced hyperactivity in rats (Muller et al., 2002). This result suggests that 5-HT_{1A} systems might play an important role in psychostimulant-mediated behavioral effects. Furthermore, post mortem brain tissue from schizophrenia patients displays increased numbers of prefrontal cortical 5-HT_{1A} receptor binding sites (Burnet et al., 1997). Treatment with 5-HT_{1A} receptor agonists can have beneficial effects on cognitive function in schizophrenia patients treated with atypical antipsychotic drugs (Sumiyoshi et al., 2007). However, we have no information about possible serotonergic involvement in the beneficial effects of nicotine or cognitive deficits in schizophrenia.

We now report effects of nicotine on hyperlocomotion and sensory gating deficits in DAT KO mice, as well as examination of the potential roles for 5-HT_{1A} receptors in these processes using 5-HT_{1A} receptor agonists and antagonists.

2. Materials and methods

2.1. Animals

DAT KO mice (Sora et al., 1998) were bred at the Animal Laboratory Institute of Tohoku University Graduate School of Medicine and maintained on a mixed genetic background

combining C57BL/6 and 129Sv mouse strains. Offspring from heterozygote crosses were weaned at 28 days postnatal and housed in groups of two to five (segregated by sex), in a temperature- and light-controlled colony (lights on at 0800 h, lights off at 2000 h), with food and water available *ad libitum*. Mice were genotyped using multiplex polymerase chain reaction methods on DNA extracted from tail biopsies, as previously described (Shen et al., 2004). Behavioral testing was conducted in mice of both sexes, between 8 and 14 weeks of age. All animal experiments were performed in accordance with the Guidelines for the Care of Laboratory Animals of Tohoku University Graduate School of Medicine.

2.2. Drugs

For behavioral testing, all drugs were dissolved in physiological saline (0.9% sodium chloride), and were administered intraperitoneally in a volume of 10 ml/kg: (–)-nicotine tartrate (nicotine, Sigma-Aldrich, Japan), the non-specific nACh receptor antagonist mecamylamine hydrochloride (MCA, Sigma-Aldrich, Japan), the $\alpha_4\beta_2$ nACh receptor antagonist dihydro- β -erythroidinehydrobromide (DH β E, Sigma-Aldrich, Japan), the α_7 nACh receptor antagonist methyllycaconitine citrate hydrate (MLA, Sigma-Aldrich, Japan), the 5-HT1A agonist ($\pm$)-8-Hydroxy-2-(dipropylamino) tetralin hydrobromide (DPAT, Sigma-Aldrich, Japan), and the specific 5-HT1A antagonist WAY100635 maleate salt (WAY100635, Sigma-Aldrich, Japan). The pH of the nicotine solution was neutralized with sodium hydroxide. No published studies have documented the effects of acute nicotine on PPI in DAT KO mice. Hence, to determine dose ranges, we primarily consulted previous data on locomotor activity effects of nicotine in WT and DAT KO mice (Weiss et al., 2007). Since effective doses may differ between locomotion and prepulse inhibition, preliminary studies were performed. Based on the preliminary results, effective doses of each drug (described below) or dose intervals were determined.

2.3. Experimental procedures

2.3.1. Measurement of locomotor activity of WT and DAT KO mice after nicotine and/or receptor antagonist treatment—Mice were placed individually in clear plastic cages (40 × 30 × 26 cm), and locomotor activity was measured in using digital counters with passive infrared sensors (Supermex system, Muromachi Kikai, Tokyo, Japan). In nicotine-induced locomotor activity tests, WT and DAT KO mice were first habituated in the test chamber to the apparatus for 20 min and then injected with nicotine (0.3, 1 and 3 mg/kg) or saline intraperitoneally. Locomotor activity was then assessed for 60 min post-injection ($N = 8$ –12 per treatment condition per genotype).

In experiments examining the effect of nicotine in DAT KO mice after receptor antagonists, WT and DAT KO mice were first habituated for 20 min after pretreatment with 2 mg/kg of non-specific nACh receptor antagonist mecamylamine or 1 mg/kg of specific 5-HT1A antagonist WAY100635, and then injected with nicotine (1 mg/kg) or saline. Locomotor activity was assessed for 60 min post-nicotine or saline injection ($N = 8$ –12 per treatment condition per genotype).

2.3.2. Measurement of startle response and prepulse inhibition—Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used to measure the startle response. Each chamber consisted of a nonrestrictive Plexiglas cylinder mounted on a frame inside a lighted, ventilated box (35 × 35 × 47.5 cm³). Movement within the cylinder was detected by piezoelectric accelerometers attached to the cylinder's bottom. Force detected by the accelerometer was converted into analog signals that were digitized and stored electronically. In all experiments, 65 readings were recorded at 1 ms intervals beginning at stimulus onset; the average amplitude was used to describe the acoustic startle response. A high-frequency loudspeaker inside the chamber, mounted above the cylinder,

generated broadband background noise and acoustic stimuli, which were controlled by the SR-LAB software system and interface. Sound levels (dB (A) scale) and accelerometer sensitivity were calibrated routinely, as described previously (Geyer and Dulawa, 2003; Geyer and Swerdlow, 2001).

Experiments were conducted using previously reported methods (e.g., (Yamashita et al., 2006)). For acoustic startle experiments, mice were tested initially for baseline PPI and pseudo-randomly assigned to drug treatment groups based on these measurements. Experimental sessions consisted of a 5 min acclimatization period with 65 dB broadband background noise followed by PPI sessions. Sessions consisted of five different trial types: no stimulus trials (nostim); startle pulse alone, 40 ms duration at 120 dB (p120); and three prepulse + pulse trials, 20 ms duration prepulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms delay. The nostim trial consisted of only background broadband noise. All test sessions started and concluded with six presentations of the p120 trial, while the remainder of the session consisted of 12 presentations of the p120 trial type, 10 presentations of the nostim, the pp3, pp6, and pp12 trial types, in a pseudorandom order, with varying intertrial intervals (mean 15 s, range 8–23 s). Each PPI session lasted 21 min and consisted of a total of 64 trials.

The effect of nicotine on PPI was examined in WT and DAT KO mice. Nicotine (0.3 mg/kg, 1 mg/kg, or 3 mg/kg) was administered 5 min before testing to WT and DAT KO mice ($N=8-16$ per treatment condition). The effects of nicotinic antagonists on the amelioration of PPI deficits in DAT KO mice by nicotine were examined by injecting DAT KO mice with the nicotinic receptor antagonists MCA (5 mg/kg or 10 mg/kg), DH β E (1 mg/kg or 2 mg/kg), or MLA (2 mg/kg or 5 mg/kg), 20 min before the nicotine treatment. Nicotine was administered 5 min before PPI testing ($N=10-15$ per treatment condition). The effects of 5-HT1A antagonism on the amelioration of impaired PPI in DAT KO mice by nicotine was examined by administering the 5-HT1A antagonist WAY100635 (1 mg/kg or 3 mg/kg), 20 min before the nicotine treatment. Nicotine was administered 5 min before PPI testing ($N=14-15$ per treatment condition). The effect of the 5-HT1A agonist DPAT and/or 5-HT1A antagonist WAY100635 on PPI was examined in WT and DAT KO mice. Mice of each genotype were administered saline or 3 mg/kg of WAY100635 20 min before DPAT treatment (0.3 mg/kg or 1 mg/kg DPAT, or saline), 10 min before testing ($N=10-14$ per treatment condition).

2.4. Data and statistical analysis

2.4.1. Locomotor activity—Locomotor activity data were submitted to ANOVA with GENOTYPE (WT versus DAT KO), SEX and nicotine dose (NICOTINE; nicotine versus saline) as between subjects factors. In studies examining the effects of the nACh receptor antagonist, mecamylamine, or 5-HT1A receptor antagonist WAY100635, in DAT KO mice. The data were first submitted to ANOVA with GENOTYPE $\times$ SEX as between subjects factors. Subsequently, data from DAT KO mice were submitted to ANOVA with saline, mecamylamine, or WAY100635 (PRETREATMENT) and SEX as between subjects factors.

2.4.2. Prepulse inhibition—Prepulse inhibition was calculated as a percentage score for each prepulse intensity using the following equation: % PPI = $100 - \{(\text{startle response for prepulse + pulse trials (pp3, pp6, or pp12)}) / (\text{startle response for pulse alone trials (p120)}) \times 100\}$. The startle magnitude was calculated as the average of all pulse alone (p120) trials, excluding the first six and last six p120 trials in each session. The effects of nicotine on PPI and startle data were submitted to ANOVA with the between-subjects factors of nicotine dose (NICOTINE; nicotine versus saline), SEX and GENOTYPE (WT versus DAT KO), and the within-subjects factor of prepulse intensity (INTENSITY; pp3, pp6, and pp12). In

the antagonist study, the effects of pretreatments with antagonists and NICOTINE on PPI and startle data were first submitted to ANOVA with SEX and GENOTYPE (WT versus DAT KO mice after saline treatment) as between subject factors, with the within-subjects factor of INTENSITY to confirm baseline genotypic effects. The PPI and startle data for DAT KO mice were then submitted to ANOVA with SEX and TREATMENT (saline, nicotine or nicotine in combination with one of the nACh receptor antagonists, mecamylamine, methyllycaconitine, or dihydro- β -erythroidinehydrobromide) as between subjects factors, and the within-subjects factor of INTENSITY. The effects of the 5-HT1A receptor antagonist WAY100635 were examined by ANOVA in a similar fashion. The effects of DPAT treatments on PPI and startle data were submitted to ANOVA with the between-subjects factors of DPAT treatment (DPAT), SEX and GENOTYPE, and the within-subjects factor of INTENSITY. For brevity, main effects of prepulse intensity will not be discussed because they were always significant. Where ANOVA indicated significant effects of one or more factors, Bonferroni tests of individual mean differences were conducted for *post hoc* analysis.

All data were presented as the mean $\pm$ SEM. Data were analyzed using the SPSS statistical package (SPSS Ver11.5J for Windows, SPSS Inc., Tokyo, Japan).

3. Results

3.1. Locomotor activity studies

As is quite clear in Fig. 1, DAT KO mice were profoundly hyperactive compared to WT littermates, an effect that was greatly reduced by nicotine. The ANOVA revealed a significant GENOTYPE effect on locomotor activity ($F(1, 73) = 108.7$; $p < 0.001$), and a significant GENOTYPE $\times$ NICOTINE interaction on locomotor activity ($F(3, 73) = 28.6$; $p < 0.001$). Furthermore, the usual pattern of response was altered in DAT KO mice compared to WT mice. There was no significant effect of SEX nor any significant interactions with SEX. Therefore, data are presented discounting SEX. In *post hoc* comparisons, at the 0.3 mg/kg nicotine dose, locomotor activity of WT mice was higher than the saline treatment group ($p < 0.05$), but this was not observed in DAT KO mice. Treatment with only the highest dose of nicotine, 3 mg/kg nicotine, caused significant locomotor decreases in WT mice while both the 1 and 3 mg/kg doses of nicotine reduced locomotor activity ($p < 0.05$) in DAT KO mice. Thus, DAT KO mice were profoundly hyperactive after treatment with saline or the low dose of nicotine compared to WT mice ($p < 0.001$) but at the higher doses of nicotine, 1 mg/kg and 3 mg/kg nicotine treatments, activity was significantly attenuated in DAT KO mice, nearly to WT levels.

The interaction of nicotine-induced hypolocomotion in DAT KO mice with the non-specific nACh receptor antagonist mecamylamine hydrochloride (MCA) and the specific 5-HT1A antagonist WAY100635 (PRETREATMENT) were examined. Neither pretreatment with 2 mg/kg of MCA (MCA2) nor WAY100635 had significant effects on baseline DAT KO hyperactivity. However, pretreatment with either antagonist almost completely eliminated the locomotor decreasing effects of nicotine on DAT KO induced hyperlocomotion (MCA, $p < 0.001$ compared to DAT KO nicotine after saline pretreatment; WAY100635, $p < 0.001$ compared to DAT KO nicotine after saline pretreatment; Fig. 2). Again, no significant effects of SEX or significant interactions with SEX were observed, so data is presented ignoring this factor.

3.2. Prepulse inhibition studies

3.2.1. The effects of nicotine on PPI in WT and DAT KO mice—ANOVA revealed a significant GENOTYPE effect on PPI ($F(1, 90) = 11.3$; $p < 0.005$) and a significant

GENOTYPE $\times$ NICOTINE interaction on PPI ($F(3, 90) = 2.8$; $p < 0.05$). There was significant main effect of NICOTINE alone on PPI ($F(3, 90) = 4.1$; $p < 0.01$). DAT KO mice displayed significantly reduced levels of PPI compared to WT mice at baseline, e.g. after saline treatment (Fig. 3). Nicotine (1 mg/kg) significantly increased PPI in DAT KO mice compared to saline treatment. *Post hoc* testing showed a significant difference between the saline (sal) group and the nicotine 1 mg/kg treatment (Nic1 mg) group at 12 dB prepulse intensities, for example ($p < 0.005$; Fig. 3). Table 1 shows the effect on startle amplitude. Startle reactivity was not significantly affected by either GENOTYPE or NICOTINE treatment (Table 1). Again, no significant effects of SEX or significant interactions with SEX were observed, so data is presented ignoring this factor.

3.2.2. nACh receptor and 5-HT_{1A} receptor antagonism of the effects of nicotine on PPI in DAT KO mice—In the nicotine antagonist studies, the effects of DAT KO on PPI and the effects of nicotine on these impairments were assessed. ANOVA revealed a significant GENOTYPE effect on PPI in saline-tested wild type and DAT KO mice ($F(1, 24) = 23.5$, $p < 0.001$; Fig. 4). Again, saline-treated DAT KO mice displayed significantly reduced levels of PPI compared to similarly treated WT mice. Fig. 4 shows the effects of nicotinic receptor antagonists on the nicotine-induced amelioration of PPI deficits in DAT KO mice. ANOVA revealed a significant PRETREATMENT effect on the nicotine-induced amelioration of PPI deficits in DAT KO mice ($F(7, 90) = 2.8$; $p < 0.05$), with all antagonists reducing the effect of nicotine, although significant reductions were only observed for mecamylamine and methyllycaconitine. *Post hoc* tests of PRETREATMENT data showed a significant difference ($p < 0.05$) between the sal–Nic1 group and the MCA10–Nic1 group in DAT KO mice which at the 12 dB prepulse intensity. The selective α_7 nACh receptor antagonist methyllycaconitine (MLA; 2 and 5 mg/kg) also dose-dependently antagonized the nicotine-induced PPI amelioration in DAT KO mice. *Post hoc* testing showed a significant difference between the nicotine 1 mg/kg/saline pretreatment (sal–Nic1) group and the nicotine 1 mg/kg methyllycaconitine pretreatment (MLA5–Nic1) group at the 12 dB prepulse intensity ($p < 0.05$; Fig. 4). Three of five DAT KO mice died after receiving 5 mg/kg doses of the selective $\alpha_4\beta_2$ nACh receptor antagonist Dh β E, therefore analysis was restricted to lower doses. 1 and 2 mg/kg DH β E treatments were tested for their antagonism of nicotine effects on PPI deficits in DAT KO mice. DH β E treatments at 1 or 2 mg/kg dose showed modest trends toward attenuation of the effects of nicotine on PPI deficits in DAT KO mice. Table 1 shows the effect on startle amplitude. Again, no significant effects of SEX or significant interactions with SEX were observed, so data is presented ignoring this factor.

Fig. 5 shows the effects of WAY100635 on the nicotine-induced amelioration of PPI deficits in DAT KO mice. ANOVA revealed a significant PRETREATMENT (5-HT_{1A} receptor antagonist WAY100635) effect on PPI ($F(3, 54) = 6.0$; $p < 0.005$). There was thus a reduced effect of nicotine amelioration of PPI deficits after pretreatment with the 5-HT_{1A} receptor antagonist WAY100635 in DAT KO mice. This effect of WAY100635 (1 and 3 mg/kg) was dose-dependent. *Post hoc* testing showed a significant difference between the nicotine 1 mg/kg saline pretreatment (sal–Nic1) group and the nicotine 1 mg/kg WAY100635 3 mg/kg pretreatment (WAY3–Nic1) group at the 12 dB prepulse intensity ($p < 0.05$; Fig. 5). Table 1 shows the effect on startle amplitude, which was not significantly affected by nicotine or co-administered antagonists. Again, no significant effects of SEX or significant interactions with SEX were observed, so data is presented ignoring this factor.

3.2.3. The effects of DPAT on PPI in WT and DAT KO mice—In the DPAT experiment ANOVA again revealed a significant GENOTYPE effect on PPI at baseline ($F(1, 89) = 27.0$; $p < 0.001$). DAT KO mice displayed significantly reduced levels of PPI compared to WT mice after saline treatment (Fig. 6). DPAT (1 mg/kg) significantly

ameliorated impairments of PPI in DAT KO mice compared to sal-sal treatment (Fig. 6). Pretreatment with WAY100635 antagonized the amelioration of PPI deficits by DPAT in DAT KO mice. ANOVA revealed a significant effect of DPAT ($F(3, 89) = 3.2$; $p < 0.05$), and a significant GENOTYPE $\times$ DPAT interaction on PPI ($F(3, 89) = 3.2$; $p < 0.05$), reflecting normalization of PPI in DAT KO mice. *Post hoc* testing showed a significant difference between the DPAT1 mg/kg and saline pretreatment group (sal-DPAT1) and the DPAT1 mg/kg WAY100635 3 mg/kg pretreatment group (WAY3-DPAT1) at a prepulse intensity of 12 dB ($P < 0.005$; Fig. 6). Again, no significant effects of SEX or significant interactions with SEX were observed, so data is presented ignoring this factor.

Table 1 shows the effect on startle amplitude. Startle reactivity was not affected by either GENOTYPE or DPAT treatment (Table 1). Again, no significant effects of SEX or significant interactions with SEX were observed, so data is presented ignoring this factor.

4. Discussion

The results from our experiments demonstrate that both of the behavioral abnormalities in DAT KO mice that are the focus of the current studies can be normalized by treatment with nicotine. We observed that both the hyperactivity and PPI deficits displayed by DAT KO mice were reduced by administration of nicotine. These effects of nicotine were attenuated by pretreatment with a α_7 nACh receptor antagonist, but not a $\alpha_4\beta_2$ nACh receptor antagonist, consistent with involvement of a selective subset of nACh receptors. Attenuation of nicotine effects by pretreatment with a 5-HT1A receptor antagonist, normalization of DAT KO PPI deficits following and reversal of this 5-HT1A agonist effect by pretreatment with a 5-HT1A receptor antagonist all support roles for specific serotonin systems in nicotine's effects in DAT KO mice.

Nicotine can activate midbrain dopaminergic neurons and alter function of presynaptic striatal dopaminergic terminals (Zhou et al., 2001), altering DA release and dopaminergic control of reward and motor activity. Locomotor activity in WT mice increased significantly after a low dose of nicotine (0.3 mg/kg), while a higher dose of nicotine (1 mg/kg) did not increase locomotion, and locomotion was decreased at the highest nicotine dose. By contrast, the profound hyperactivity displayed by DAT KO mice was significantly, and substantially, reduced by administration of either 1 or 3 mg/kg nicotine. These data confirm previous observations that DAT KO mice are more sensitive than WT mice to the locomotor decreasing effects of nicotine (Weiss et al., 2007). However, it must be noted that WT mice in that study were found to have a greater reduction in locomotor activity than in the present study, which may suggest that genetic background (or other factors) alter the effects of nicotine in these two DAT mutant strains. Nonetheless, the main finding of that study was replicated in another DAT KO strain.

Nicotine, as well as the psychostimulant monoamine transporter blockers cocaine and amphetamine induced increases extracellular DA concentrations in the prefrontal cortex and nucleus accumbens of WT mice as assessed by *in vivo* microdialysis (Budygin et al., 2004; Carboni et al., 2001). While DAT KO mice display vastly increased basal DA levels in striatal microdialysates (Weiss et al., 2007), basal prefrontal DA levels are unchanged, as are responses to psychostimulants in the cortex and striatum (Shen et al., 2004). Monoamine transporter blockers can attenuate hyperlocomotion in DAT KO mice in ways that interact with level of habituation (e.g. compare (Gainetdinov et al., 1999a; Giros et al., 1996; Spieleswoy et al., 2001)). The locomotor decreasing effects of psychostimulants were initially suggested to be associated with influences of those drugs on serotonin release (Gainetdinov et al., 1999a). The failure of DAT deletion to affect prefrontal DA function can be attributed to low DAT expression in this region, allowing much released DA to be taken

up into norepinephrine terminals (Bymaster et al., 2002; Moron et al., 2002) (Fig. 7). We have thus hypothesized that attenuated hyperlocomotion and normalization of PPI deficits in DAT KO mice treated with monoamine transporter blockers could be explained by alterations in dopaminergic neurotransmission produced by NET blockade in the prefrontal cortex and consequent alterations in prefrontal–accumbens activity (Arime et al., 2011). This hypothesis was confirmed by the demonstration that direct injections of NET blockers into the prefrontal cortex normalizes PPI deficits in DAT KO mice (Arime et al., in press). Nicotine treatment also regulates DA neurotransmission in the prefrontal cortex of rats (Shearman et al., 2008). Thus, it would appear that dopaminergic neurotransmission in the prefrontal cortex regulated by nicotine treatment may contribute to the attenuated hyperlocomotion in DAT KO mice treated with nicotine.

To elucidate the possible mechanisms underlying the effects of nicotine on hyperlocomotion in DAT KO mice, we examined locomotion after pretreatment with nACh or serotonin receptor antagonists. The reduction of hyperlocomotion by nicotine treatment in DAT KO mice was blocked by pretreatment with 2 mg/kg of the non-specific nACh antagonist mecamylamine, confirming the involvement of nACh receptors in these effects. This finding confirmed the previous work of Weiss et al. who reported that co-administration of the $\alpha_4\beta_2$ and α_7 nACh receptor antagonists, DH β E and methyllycaconitine, respectively, reversed the effects of 1 mg/kg nicotine treatment in DAT KO mice (Weiss et al., 2007). Furthermore, based on the present series of experiments the effects of nicotine in DAT KO mice appear to depend on serotonergic mechanisms. Pretreatment with the selective 5-HT_{1A} antagonist WAY100635 blocked the reduction of hyperlocomotion produced by nicotine treatment in DAT KO mice. Pretreatment with a 5-HT_{1A} antagonist also attenuated cocaine-induced hyperactivity in rats (Muller et al., 2002). These results suggest that serotonergic mechanisms involving 5-HT_{1A} receptors may play an important role in the reduction of locomotor activity induced by nicotine treatment in DAT KO mice. Electrophysiology studies have revealed the influence of nicotine on serotonin release from the dorsal raphe nucleus (DRN). Serotonergic DRN cells express postsynaptic functional nACh receptors (Galindo-Charles et al., 2008). Presynaptic α_7 nACh receptors may modulate function of serotonergic neurons that innervate to the prefrontal cortex (Soria-Fregozo et al., in press) (Fig. 7). The majority of serotonergic DRN neurons increase their action potential firing in response to nicotine, leading to increased serotonin release (Mihailescu et al., 2002). Thus, increased release of serotonin by nicotine treatment might reduce locomotor hyperactivity and PPI deficits observed in DAT KO mice.

There are some limitations to the present study. 1 mg/kg nicotine treatment induces hypothermia in mice (Sack et al., 2005). Lower body temperature with nicotine treatment may reduce locomotion. It is conceivable that nicotine-reduced body temperature might contribute to its effects on the locomotor activity in DAT KO mice. In this study, we used mice whose genetic background combined C57BL/6 and 129Sv/J mouse strains. Although 129Sv/J mouse strain had lower locomotor activity compared with C57BL/6, mixed genetic background of C57BL/6 and 129Sv/J shows locomotor activity similar to that of C57BL/6 strains (Miner, 1997). While we regard effects of overall genetic background upon locomotion as unlikely to have substantially affected the results, a more important issue may be that WT mice do express lower locomotor activity than DAT KO mice, possibly reducing the sensitivity with which we could detect motor-suppressive effects of nicotine in WT mice.

Attempts to correlate PPI deficits with cognitive function in human studies have yielded mixed results (Powell et al., 2009). Some studies did not report positive correlations between PPI and cognitive function as measured by Wisconsin Card Sorting Task (Swerdlow et al., 2006). However, there was positive relationship between PPI and

Independent Living scales (Swerdlow et al., 2006) or Cambridge Neuropsychological Test Automated Battery (CANTAB) (Giakoumaki et al., 2006). The CNTRICS (Cognitive Neuroscience measures of Treatment Response of Impaired Cognition in Schizophrenia) program also considered PPI to provide a measure of cognitive function as a specific aspect of the perceptual abnormalities seen in patients with schizophrenia (Green et al., 2009).

Nicotine and subtype specific nACh receptor agonists affect auditory gating. Reports of the magnitude and direction of these effects have been highly variable however. This variability may be due to differences in the tested dose range, selectivity of the test compound, species and strain. Nicotine has mixed effects on PPI in rat models, producing an increase in PPI at low doses. The effects of nicotine are notoriously dose-dependent for virtually all nicotine effects. Furthermore, the present results may suggest that effects of nicotine on PPI might only be observed in subjects that have impaired PPI to begin with. Nicotine had a biphasic dose effect on startle amplitude, with increases at lower doses (0.01 mg/kg) and decreases at higher doses (0.5–5.0 mg/kg SC) in rat models (Acri et al., 1994). Nicotinic acetylcholine receptor subtypes are differentially involved in the control of auditory gating. PPI was disrupted at higher doses that were sensitive to antagonism of $\alpha_4\beta_2$, but not α_7 nACh receptors (Schreiber et al., 2002). However, opposite effects of 5-HT_{1A} receptor activation on PPI in rats and mice have been previously reported (Dulawa et al., 1997).

Considerable animal research has been performed with PPI. PPI can be measured by similar means in human and animals, providing translational validity to this model of sensorimotor gating deficits (Castagne et al., 2009). Although there are no humans that have a complete deficit of DAT expression, DAT KO mice nonetheless appear to model some aspects of neuropsychiatric disorders that depend on DA function. The model has predictive validity for these conditions and may produce a state of altered fronto-striatal activity that models important aspects of schizophrenia. Our experiments have further demonstrated that nicotine ameliorates PPI deficits in DAT KO mice. DAT KO mice displayed significant deficits of PPI compared to WT mice, consistent with previous reports (Ralph et al., 2001; Yamashita et al., 2006). Nicotine treatment normalized these PPI deficits in DAT KO mice. This recovery of PPI produced by nicotine was blocked by non-selective and α_7 selective nACh receptor antagonists, mecamylamine, methyllycaconitine, respectively, while $\alpha_4\beta_2$ nACh receptor antagonist, DH β E produced only a slight trend toward attenuation of these effects (although it must be noted that toxicity prevented testing of higher doses of $\alpha_4\beta_2$ nACh receptor antagonist). The blockade of the recovery of PPI deficits by nicotine in animals treated with the α_7 selective nACh receptor antagonist methyllycaconitine suggests that the recovery of PPI deficits in DAT KO mice was mediated by the α_7 nACh receptor. By contrast, nACh receptor antagonists MCA (Gould et al., 2005; Schreiber et al., 2002), DH β E and MLA (Mizoguchi et al., 2009) did not affect PPI in WT mice.

The α_7 nACh receptor has been previously associated with the pathophysiology of attentional deficits of schizophrenia (Olincy et al., 2006). Nicotinic $\alpha_4\beta_2$ receptors also have been implicated in cognitive function, and in improvement of attentional deficits (Sarter et al., 2009). Treatments with either α_7 or $\alpha_4\beta_2$ nACh receptor antagonists in the ventral hippocampus impair working memory (Levin et al., 2002). Patients with schizophrenia display a P50 auditory-evoked potential gating deficit that may be related to these hippocampal α_7 nACh receptors (Adler et al., 1998; Leonard et al., 1996). Furthermore, Leonard et al. reported that nicotine treatment normalizes P50 gating deficits in patients with schizophrenia (Leonard et al., 1996). An α_7 nACh receptor agonist, 3-2,4 dimethoxybenzylidene anabaseine (DMXB-A) can also improve the P50 auditory-evoked potential in schizophrenia patients (Olincy et al., 2006). Our results that the recovery of PPI deficits in DAT KO mice involves the α_7 nACh receptor adds to support for development of α_7 nACh receptor agonists to improve cognitive function in schizophrenia and/or other

attentional or cognitive disorders. A recent study reported that the atypical antipsychotics clozapine and quetiapine reversed PPI deficits in DAT KO mice (Powell et al., 2008), however these treatments do not sufficiently improve cognition in schizophrenia patients. Therapeutic studies have reported that the beneficial effects on cognitive function in schizophrenia patients treated with the 5-HT_{1A} receptor agonist buspirone in combination with atypical antipsychotic drugs but not treatment with buspirone alone (Sumiyoshi et al., 2007). The high rate of smoking in schizophrenia patients might also be beneficial in a similar way, augmenting the effects of antipsychotic drugs co-administration.

Our findings suggest that nicotine's actions *via* the α_7 subunit of the nACh receptor depend on intact 5-HT_{1A} receptor function indicates that serotonergic manipulations may provide additional avenues through which to ameliorate the behavioral impairments observed in DAT KO mice, and the pathophysiological conditions that these interesting animals may model. The effects of nicotine on PPI in DAT KO mice were blocked by WAY100635, a selective 5-HT_{1A} receptor antagonist that did not affect PPI in WT mice (Sakaue et al., 2003; Shanahan et al., 2009). Thus, we have demonstrated that administration of the 5-HT_{1A} receptor agonist DPAT also reversed deficits in PPI in DAT KO mice, and this effect was antagonized by pretreatment with a selective 5-HT_{1A} antagonist. Administration of a DA D₁ receptor agonist leads to normalization of prefrontal activity revealed by fMRI observations in schizophrenia patients (Mu et al., 2007). *In vivo* microdialysis studies reveal that 5-HT_{1A} receptor agonists increase DA efflux in the prefrontal cortex in experimental animals (Ago et al., 2002; Sakaue et al., 2000). In addition, the atypical antipsychotics clozapine and ziprasidone, also increase DA release in the cortex of rats, and the effects are blocked by treatment with selective 5-HT_{1A} antagonists (Rollema et al., 2000, 1997). In addition, 5-HT_{1A} receptors are expressed on pyramidal neurons of the rat medial prefrontal cortex (Wedzony et al., 2007), so that cortical glutamatergic neurotransmission *via* 5-HT_{1A} receptors may be involved in the regulation of DA release in the prefrontal cortex (Fig. 7). Our results may suggest the possibility that normalization of PPI in DAT KO mice by 5-HT_{1A} receptor agonists is caused by dopaminergic activation in the prefrontal cortex of DAT KO mice, and consequent restoration of the balance between prefrontal and striatal DA activity, in a manner similar to the effects of NET blockers.

The cognition enhancing effect of nicotine does not appear to diminish with continued treatment. Similar to acute nicotine, the beneficial effects of chronic nicotine administration were found to be specific to working memory function (Levin et al., 1996). A better mechanistic understanding of the neural systems involved in the nicotine-induced cognitive improvement is necessary for development of nicotinic treatments of cognitive dysfunction (Bancroft and Levin, 2000).

5. Conclusion

The results from our experiments demonstrate behavioral abnormalities in DAT KO mice that are normalized by treatment with nicotine. We observed that the hyperactivity and PPI deficits displayed by DAT KO mice were reduced by administration of nicotine. These nicotine effects were attenuated by pretreatment with α_7 nACh receptor antagonists, or a 5-HT_{1A} receptor antagonist, but not a $\alpha_4\beta_2$ nACh receptor antagonist. Furthermore, treatment with a 5-HT_{1A} receptor agonist also directly ameliorated PPI deficits in DAT KO mice, and effect which was also blocked by pretreatment with a 5-HT_{1A} receptor antagonist. These data support the idea that nicotine might ameliorate some of the cognitive dysfunctions found in schizophrenia *via* a mechanism that involves 5-HT_{1A} receptors. In addition, our present study suggests that examination of the effects of 5HT_{1A} agonists would also be potentially beneficial in the treatment of schizophrenia patients.

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Fig. 1.

Dose-response study of nicotine-induced locomotor activity in WT and DAT KO mice.

Total ambulatory activity for 60 min after administration of saline (sal), 0.3 mg/kg nicotine (Nic 0.3 mg), 1 mg/kg nicotine (Nic 1 mg), or nicotine (Nic 3 mg) were indicated. Values represent mean $\pm$ SEM. * p = 0.05, compared with WT sal; *** p < 0.001 compared with DAT KO sal; ### p < 0.001 compared with WT sal; ## p < 0.005 compared with WT nicotine 1 mg; # p < 0.05 compared with WT nicotine 3 mg. N = 8–12 per treatment condition per genotype (male N = 4–8, female N = 4–7).

Fig. 2.

Effects of antagonist pretreatments on nicotine-induced locomotor activity in DAT KO mice. Total ambulatory activity for 60 min after administration of saline and 1 mg/kg nicotine (sal-Nic1) treatments significantly reduced hyperactivity compared to DAT KO treated with saline and saline (sal-sal). Pretreatment with mecamylamine 2 mg/kg (MCA2) or WAY100635 1 mg/kg (WAY1) significantly reversed the normalization of DAT hyperactivity produced by treatment with saline and 1 mg/kg nicotine (sal-Nic1). Values represent mean $\pm$ SEM. # $p < 0.001$ compared with WT sal-sal; * $p < 0.005$ compared with DAT KO sal-sal; \$ $p < 0.001$ compared with DAT KO sal-Nic1. $N = 8-12$ per treatment condition (male $N = 4-6$, female $N = 4-6$).

Fig. 3.

Effects of nicotine on PPI in WT and DAT KO mice. PPI in WT and DAT KO mice after administration of saline (sal), 0.3 mg/kg nicotine (Nic 0.3 mg), 1 mg/kg nicotine (Nic 1 mg), or 3 mg/kg nicotine (Nic 3 mg); DAT KO mice treated with saline displayed significantly reduced PPI compared with WT mice. Nicotine (1 mg/kg) significantly ameliorated PPI deficits in DAT KO mice, which was significant for the pp12 dB condition. %PPI values represent mean $\pm$ SEM. * $p < 0.05$, compared with WT Nic 0.3 mg pp3 dB; *** $p < 0.001$ compared with WT sal; # $p < 0.005$ compared with DAT KO sal pp12 dB. $N = 8-16$ per treatment condition per genotype (male $N = 4-7$, female $N = 4-9$).

Fig. 4.

Effects of nACh receptor antagonist treatments on the amelioration of PPI deficits in DAT KO mice by nicotine. Although DAT KO mice treated with saline and saline (sal-sal) displayed significantly reduced PPI compared with WT mice treated with saline and saline (sal-sal), saline and nicotine 1 mg/kg (sal-Nic1) treatments significantly reversed PPI deficits in DAT KO mice at pp12 dB. Pretreatment with mecamylamine, 10 mg/kg (MCA10) or methyllycaconitine, 5 mg/kg (MLA5) significantly antagonized the recovery of PPI by nicotine in DAT KO mice. %PPI values represent mean $\pm$ SEM. *** $p < 0.001$ compared with WT sal-sal; # $p < 0.01$ compared with DAT KO sal-sal pp12 dB; \$ $p < 0.05$ compared with DAT KO sal-Nic1 + MCA10-Nic1 group, and DAT KO sal-Nic1 + MLA5-Nic1 group. $N = 11-15$ per treatment condition (male $N = 5-8$, female $N = 6-8$).

Fig. 5.

Effects of a 5-HT_{1A} receptor antagonist on the amelioration of PPI deficits in DAT KO mice by nicotine. Although DAT KO mice treated with saline and saline (sal-sal) displayed significantly reduced PPI compared with WT mice, saline and nicotine 1 mg/kg (sal-Nic1) treatments significantly reversed PPI deficits in DAT KO mice compared to saline and saline (sal-sal) treated mice at pp12 dB. Pretreatment with WAY100635 3 mg/kg (WAY3) significantly antagonized the recovery of PPI by nicotine in DAT KO mice. %PPI values represent mean $\pm$ SEM. *** p < 0.001 compared with WT sal-sal; # p < 0.01 compared with DAT KO sal-sal pp12 dB; \$ p < 0.05 compared with DAT KO sal-Nic1 + WAY3-Nic1 group. N = 11–15 per treatment condition (male N = 5–8, female N = 6–7).

Fig. 6.

The effects of a 5-HT_{1A} receptor agonist on PPI in WT and DAT KO mice. PPI in WT and DAT KO mice after administration of saline-saline (sal-sal), saline and 0.3 mg/kg DPAT (sal-DPAT0.3), saline and 1 mg/kg DPAT (sal-DPAT1), or WAY100635 3 mg/kg-1 mg/kg DPAT (WAY3-DPAT1). sal-DPAT treatments reversed PPI deficits in DAT KO mice (sal-sal group) at pp6 dB and pp12 dB. The ameliorating action of DPAT was antagonized by pretreatment with 3 mg/kg of WAY100635. %PPI values represent mean $\pm$ SEM. *** p < 0.001 compared with WT sal-sal; ** p < 0.005 compared with WT WAY3-DPAT1; # p < 0.05 compared with DAT KO sal-sal pp6 dB; ## p < 0.001 compared with DAT KO sal-sal pp12 dB; \$ p < 0.005 compared with DAT KO sal-DPAT1 + WAY3-DPAT1 group. N = 10-14 per treatment condition per genotype (male N = 5-8, female N = 5-7).

Fig. 7. Schematic representation of the structures and neurotransmitters involved in the regulation of the nicotine-serotonin interactive dopamine release in the prefrontal cortex (PFC). Serotonin (5-HT) from Raphe nuclei (Raphe), might be released by activation of presynaptic α_7 nicotinic acetylcholine (nACh) receptor in the prefrontal cortex (Soria-Fregozo et al., in press). Pyramidal neurons (Pyr) of the prefrontal cortex innervate glutamatergic neurotransmission to the ventral tegmental area (VTA) via 5-HT_{1A} receptor may be involved in the regulation of dopamine (DA) release (Wedzony et al., 2007). Norepinephrine transporter (NET) blockers also could be explained by alterations in dopaminergic neurotransmission produced by NET blockade in the prefrontal cortex activity (Arime et al., 2011). α_7 ; α_7 nACh receptor, 5-HT_{1A}; Serotonin_{1A} receptor, GluR; glutamate receptor, NE; norepinephrine, LC; locus coeruleus.

Table 1

Effects of drug treatment on acoustic startle reactivity.

Treatments	Wild type	DAT KO
Nicotine treatments		
Saline	101.8 ± 22.8	109.1 ± 17.3
Nicotine 0.3	101.8 ± 22.8	121.8 ± 25.0
Nicotine 1	106.6 ± 32.4	112.6 ± 24.4
Nicotine 3	77.5 ± 22.1	103.8 ± 18.8
Nicotine and nACh receptor antagonists treatments		
sal-sal	95.2 ± 18.2	78.7 ± 14.4
sal-nic1		108.7 ± 19.4
MCA5-nic1		109.8 ± 18.6
MCA10-nic1		83.6 ± 22.8
DHβE1-nic1		85.1 ± 20.5
DHβE2-nic1		102.0 ± 21.4
MLA2-nic1		77.2 ± 16.3
MLA5-nic1		130.5 ± 29.3
Nicotine and 5-HT receptor antagonist treatments		
sal-sal	95.2 ± 18.2	78.7 ± 14.4
sal-nic1		108.7 ± 19.4
WAY1-nic1		89.0 ± 20.3
WAY3-nic1		77.7 ± 15.5
5-HT receptor agonist and antagonist treatments		
sal-sal	94.5 ± 23.3	61.9 ± 9.7
sal-DPAT0.3	55.1 ± 12.2	51.4 ± 8.7
sal-DPAT1	87.9 ± 22.2	78.2 ± 13.3
WAY3-DPAT1	96.4 ± 21.1	60.3 ± 10.7

Values (arbitrary units) represent mean startle magnitude ± SEM. KO, dopamine transporter knockout mice. No significant main effect on acoustic startle reactivity in each treatments was found.