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RESEARCH PAPER

Functional interactions between 5-HT$_{2A}$ and presynaptic 5-HT$_{1A}$ receptor-based responses in mice genetically deficient in the serotonin 5-HT transporter (SERT)

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Background and purpose: Despite decreased presynaptic 5-HT$_{1A}$ and altered 5-HT$_{2A}$ receptor function in genetically-deficient serotonin (5-HT) transporter (SERT) mice, the 5-HT$_{1A}$ receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt (WAY 100635) still induced head twitches in these mice, a well-established 5-HT$_{2A}$ receptor-mediated response.
Experimental approach: Interactions between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors were assessed using the head-twitch response following 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor agonists and antagonists in SERT wild-type (+/+), heterozygous (+/–), and knockout (–/–) mice. The role of brain 5-HT availability in WAY 100635 induced head twitches was also examined.
Key results: WAY 100635 induced head twitches in a SERT gene-dose dependent manner, inducing 5-fold more head twitches in SERT –/– versus SERT +/+ mice. In SERT –/– mice, inhibition of 5-HT synthesis with p-chlorophenylalanine (PCPA) markedly depleted tissue 5-HT in all five brain areas examined and abolished WAY 100635 induced head twitches. Further, the selective 5-HT reuptake inhibitor fluvoxamine increased WAY 100635 induced head twitches in SERT +/+ and +/– mice. Head twitches following the 5-HT$_{2A}$ receptor agonist (+/–)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) were robust in SERT +/+ and +/– mice but much reduced in SERT –/– mice. DOI-induced head twitches were decreased by the 5-HT$_{1A}$ agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in SERT +/+ and +/– mice. All drug-induced head twitches were blocked by the 5-HT$_{2A}$ receptor antagonist a-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol (MDL 11,939).
Conclusions and implications: These data show that indirect activation of 5-HT$_{2A}$ receptors via blockade of presynaptic 5-HT$_{1A}$ receptors potentiated head-twitch responses, suggesting functional interactions between these receptors, interactions affected by altered 5-HT availability. Our findings strongly support the correlation of WAY 100635 induced head twitches with increased 5-HT availability, induced genetically or pharmacologically.
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Keywords: head twitch response; 5-HT transporter (SERT) knockout mice; 5-HT$_{1A}$ receptors; 5-HT$_{2A}$ receptors; 8-OH-DPAT; DOI; fluvoxamine; L745870; MDL 11,939; WAY 100635; serotonin (5-HT)

Abbreviations: 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; DOI, (+/–)-2,5-dimethoxy-4-iodophenyl-2-aminopropane; L745870, 3-(4-[4-Chlorophenyl]piperazin-1-yl)-methyl-1H-pyrrolo[2,3-b]pyridine trihydrochloride; MDL 11,939, a-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol; PCPA, p-chlorophenylalanine; SSRI, selective 5-HT reuptake inhibitor; TCB-2, (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt

Introduction

In the central nervous system (CNS), 5-HT$_{2A}$ receptors (nomenclature follows Alexander *et al.*, 2008) are a target for drugs effective in the treatment of schizophrenia and other psychotic disorders and mediate the hallucinogenic effects of hallucinogenic 5-hydroxytryptaminergic drugs such as lysergic acid diethylamide (LSD). The head-twitch response in mice is mediated by 5-HT$_{2A}$ receptors, providing a measure of 5-HT$_{2A}$ function and possibly providing a 'behavioural proxy' in mice to assess hallucinogenic effects in humans (Willins and Meltzer, 1997; Gonzalez-Maeso *et al.*, 2003; 2007; Moya *et al.*, 2007).

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Recent studies show that altered 5-HT$_{2A}$ receptor-mediated responses are associated with alterations in serotonin 5-HT transporter (SERT) expression, the main mechanism for maintaining homeostatic levels of 5-HT. For example, SERT knockout (–/–) mice have marked decreases in head twitches induced by (+/–)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) and also by the recently described high-affinity 5-HT$_{2A}$ receptor agonist (4-bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide (TCB-2) (McLean *et al.*, 2006) compared with SERT wild-type (+/+) mice (Qu *et al.*, 2005; Basselin *et al.*, 2009; Fox *et al.*, in press) and demonstrate a decrease in the ability to establish stimulus control to LSD within a drug discrimination paradigm (Krall *et al.*, 2008). These functional alterations in SERT-deficient mice are associated with brain-area dependent alterations in 5-HT$_{2A}$ receptor density and diminished 5-HT$_{2A}$ receptor-mediated activation of the PLA$_2$-arachidonic acid (AA) signalling pathway (Rioux *et al.*, 1999; Li *et al.*, 2003; Qu *et al.*, 2005; Basselin *et al.*, 2009).

Despite these reports of decreased 5-HT$_{2A}$ receptor function in SERT-deficient mice, we recently reported in preliminary results that the selective 5-HT$_{1A}$ receptor antagonist N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt (WAY 100635) induced an enhanced head-twitch response in SERT –/– mice (Fox *et al.*, 2008), which has been demonstrated to be a response mediated by 5-HT$_{2A}$ receptors (Willins and Meltzer, 1997; Gonzalez-Maeso *et al.*, 2003; Moya *et al.*, 2007).

It has been suggested that selective 5-HT$_{1A}$ receptor antagonists such as S-(-)UH 301 and WAY 100635 might induce 5-HT$_{2A}$ receptor -mediated head twitches in an indirect manner (Darmani *et al.*, 1990; Darmani and Reeves, 1996; Darmani, 1998). This effect might be dependent upon the presence of elevated 5-HT levels, as the effects of WAY 100635 were observed only when 5-HT levels were enhanced. For example, although WAY 100635 alone does not affect 5-HT levels, when a selective 5-HT reuptake inhibitor (SSRI), which increases 5-HT levels, was administered, WAY 100635 enhanced 5-HT levels and decreased firing rates in mice and rats (Hjorth, 1993; Gartside *et al.*, 1995; Romero *et al.*, 1996; Gundlah *et al.*, 1997; Hjorth *et al.*, 1997; Gobert *et al.*, 2000). Further, WAY 100635 induced head twitches in mice during the light phase and not the dark phase, which correlates with altered 5-HT levels during these time periods (Darmani, 1998). These effects are likely to be due to inhibition of presynaptic 5-HT$_{1A}$ receptors, which when activated serve as a negative feedback loop for 5-HT synthesis and release (Hoyer *et al.*, 1994; Sharp *et al.*, 2007).

SERT-deficient mice have elevated extracellular 5-HT levels and thus enhanced 5-HT availability (Fabre *et al.*, 2000; Mathews *et al.*, 2004; Shen *et al.*, 2004). However, life-long exposure to these enhanced levels of 5-HT have resulted in significant alterations in the density and function of several 5-HT receptor subtypes in SERT –/– mice (see Fox *et al.*, 2007b). In addition to these changes in 5-HT$_{2A}$ receptors and receptor function, SERT-deficient mice have more marked decreases in presynaptic 5-HT$_{1A}$ receptor density and function, including decreased or absent temperature, neural firing, and hormonal responses following 5-HT$_{1A}$ receptor agonists (Li *et al.*, 1999; 2000; Gobbi *et al.*, 2001; Bouali *et al.*, 2003; Holmes *et al.*, 2003b; Fox *et al.*, 2008).

The current studies were performed to examine functional pharmacological interactions between 5-HT$_{2A}$ and presynaptic 5-HT$_{1A}$ receptors in SERT +/+, heterozygous (+/–), and –/– mice. First, to replicate our preliminary findings for the present series of more detailed studies and to evaluate baseline head twitches, SERT +/+, +/–, and –/– mice were treated with vehicle or WAY 100635, and head twitches were recorded. On the basis of recent reports that suggest alterations in SERT expression and the consequent changes in availability of 5-HT affect DOI-induced head twitches (Jennings *et al.*, 2008; Basselin *et al.*, 2009), we next examined the role of 5-HT availability in this response in SERT –/– mice via pretreatment with p-chlorophenylalanine (PCPA), which depletes 5-HT via tryptophan hydroxylase inhibition (Cesana *et al.*, 1993; O'Leary *et al.*, 2007). Next, we determined the effects of pretreatment with the SSRI fluvoxamine, which blocks SERT and increases extracellular 5-HT levels in wild-type mice and in rats (Gobert *et al.*, 2000; Kobayashi *et al.*, 2008), on head twitches induced by WAY 100635 in SERT +/+, +/–, and –/– mice. To further examine pharmacological interactions between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors in SERT +/+, +/– and, –/– mice, we examined head twitches following administration of the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), the 5-HT$_{2A/2C}$ receptor agonist DOI, and again the 5-HT$_{1A}$ receptor antagonist WAY 100635, either alone or in combination. As previous reports have shown that WAY 100635 might also have agonist properties at dopamine D$_4$ receptors (Chemel *et al.*, 2006; Marona-Lewicka and Nichols, 2009; but see Martel *et al.*, 2007), we assessed the effects of pretreatment with the selective dopamine D$_4$ receptor antagonist 3-(4-[4-Chlorophenyl]piperazin-1-yl)-methyl-1H-pyrrolo[2 ,3-b]pyridine trihydrochloride (L745870) or vehicle on head twitches induced by WAY 100635 in SERT –/– mice. Finally, to confirm 5-HT$_{2A}$ receptor mediation of the head-twitch responses described above, mice were treated with a-Phenyl-1-(2-phenylethyl)-4-piperidineme thanol (MDL 11,939), a selective 5-HT$_{2A}$ receptor antagonist (Pehek *et al.*, 2006), or vehicle prior to the test drug(s). Despite earlier findings of altered 5-HT$_{2A}$ and presynaptic 5-HT$_{1A}$ receptor function in SERT-deficient mice, the current studies show a functional interaction between these receptors in these mice, an interaction that appears directly associated with 5-HT availability.

Methods

Animals

All animal care and experimental procedures adhered to the guidelines of the National Institutes of Health and were approved by the National Institute of Mental Health Animal Care and Use Committee. Mice were SERT +/+, +/– and –/– strains, originally produced by homologous recombination in ES cells (Bengel *et al.*, 1998) and currently the product of 19–23 heterozygous backcrosses on a C57BL/6J genetic background. Mice were approximately 20–35 g in weight at the beginning of the experiments and were housed in groups of three to five animals per cage with food and water available *ad libitum*. Animals were maintained on a 12 h light : 12 h dark cycle (lights on at 0600 h).

Behavioural assessments
Mice were moved to the testing room in their home cage 1 h prior to testing. Following 15 min of habituation in a plexiglass container, mice were administered the test drug(s). Head twitches were recorded for five 1 min periods (once every 5 min starting 5 min after drug administration) over a 30 min period; the scores from the five 1-min periods were summed together. When 5-HT$_{2A}$ receptor mediation was assessed, the selective 5-HT$_{2A}$ receptor antagonist MDL 11,939 (5 mg·kg^{-1}) was administered 30 min prior to administration of drug(s) (test drugs at the same doses as in initial experiments, administered at the same time). All experiments were conducted between 1000 h and 1300 h.

5-HT depletion
To deplete 5-HT levels, SERT –/– mice were given PCPA (300 mg·kg^{-1}) or its vehicle twice daily for 3 days (Cesana *et al.*, 1993; O'Leary *et al.*, 2007; Basselin *et al.*, 2009). On the fourth day, 18 h following the final PCPA treatment, half of the vehicle-treated and half of the PCPA-treated SERT –/– mice were administered WAY 100635 (1 mg·kg^{-1}) and head twitches were counted as described above. The remaining mice were killed via cervical dislocation for examination of 5-HT and other monoamine levels (see below).

Analysis of brain region monoamines and metabolites
Following the regimen with vehicle or PCPA described above, brains were removed 18 h following the last PCPA treatment and were immediately dissected on a glass plate set in ice. After the removal of the hypothalamus and the frontal cortex, brains were bisected sagitally to expose and dissect the hippocampus and the striatum from each hemisphere, followed by isolation of the brainstem containing pons and medulla (Bengel *et al.*, 1998). Brain samples were stored at –80°C before HPLC analysis using electrochemical detection, as previously described (Andrews and Murphy, 1993; Kim *et al.*, 2005; Fox *et al.*, 2008). Briefly, brain tissue samples were homogenized in 200–250 µL of 0.1 N $HC1O_4$ using sonication and centrifuged at 7200× *g* for 10 min. For quantification of monoamines in the supernatant, an Axxichrom ODS C18 (5 µm, 25 cm × 0.46 cm) analytical column, an ESA Coulochem II detector with analytical cell (*E*1 = 100 mV, *E*2 = 300 mV; Model 5014), and a guard cell (100 mV; Model 5020) were set up with an ESA solvent pump (Model 582) and a Gibson autosampler (Model 231) fitted with a 50 µL sample loop. The mobile phase was composed of 8.6 mM heptane sulfonic acid, 0.3% phosphoric acid, 0.27% triethylamine, 0.34 mM EDTA and 12% acetonitrile delivered at a flow rate of 0.6 mL·min^{-1}. Samples were prepared using 100 µL of the supernatant with the addition of an internal standard (50 µL of 1 µM *N*-methyl 5-HT for monoamines). A 55 µL aliquot of this mixture was injected onto the analytical column for HPLC-EC analysis.

Statistical analysis
The number of head twitches was analysed using two-way (genotype × drug condition) ANOVA or Student's *t*-tests when only two groups were compared, and 5-HT and other monoamines and metabolite concentrations were compared using *t*-tests. *Post hoc* analyses were performed using Tukey HSD tests or *t*-tests, as appropriate, to evaluate differences between the genotypes and between the drug conditions. Significance was based on $P < 0.05$.

Materials
WAY 100635, 8-OH-DPAT, DOI, PCPA and fluvoxamine were obtained from Sigma Chemical Company (St. Louis, MO, USA), and L745870 and MDL 11,939 were obtained from Tocris Bioscience (Ellisville, MO, USA). WAY 100635 (1 mg·kg^{-1}), 8-OH-DPAT (1 mg·kg^{-1}), DOI (2.5 mg·kg^{-1}), fluvoxamine (10 mg·kg^{-1}) and L745870 (1 or 3 mg·kg^{-1}) were dissolved in saline; PCPA (300 mg·kg^{-1}) was dissolved in distilled deionized water; MDL 11,939 (5 mg·kg^{-1}) was dissolved in a few drops of acetic acid and diluted in distilled water and adjusted to a pH of 6.5 ± 0.1. Doses were selected based on previous work performed in our laboratory in addition to existing literature confirmed by pilot studies in our laboratory [WAY 100635 (Fox *et al.*, 2007a; 2008; 2009), 8-OH-DPAT (Fox *et al.*, 2007a; 2008), DOI (Basselin *et al.*, 2009), PCPA (Cesana *et al.*, 1993; O'Leary *et al.*, 2007; Basselin *et al.*, 2009), fluvoxamine (Daws *et al.*, 2006), L745870 (Iasevoli *et al.*, 2009; Milstein *et al.*, 2010) and MDL 11,939 (Fox *et al.*, 2007a)]. All drugs were administered by i.p. injection at a volume of 10 mL·kg^{-1}.

Results

WAY 100635 induced head twitches
There were significant main effects for genotype ($F_{2,45} = 6.36$, $P = 0.004$) and drug condition ($F_{1,45} = 32.21$, $P < 0.0001$), and a significant genotype × drug condition interaction ($F_{2,45} = 6.24$, $P = 0.004$). Mice of all three SERT genotypes given WAY 100635 (1 mg·kg^{-1}) displayed significantly more head twitches than their respective counterparts receiving vehicle (*P*'s < 0.05). Compared with SERT +/+ mice, this effect of WAY 100635 was increased ~5-fold in SERT –/– mice ($P = 0.007$), with an intermediate, ~3-fold increase in SERT +/– mice (Figure 1). We evaluated the effects of WAY 100635 over a range of doses (0, 0.1, 0.25, 0.5 and 1.0 mg·kg^{-1}) in SERT –/– mice. There was a significant effect for dose ($F_{4,26} = 10.42$, $P < 0.0001$); WAY 100635 induced head twitches in SERT –/– mice in a dose-dependent manner, with doses of 0.25, 0.5 and 1.0 mg·kg^{-1} inducing more head twitches than vehicle (*P*'s < 0.001), with no difference between these three doses, and an intermediate response following 0.1 mg·kg^{-1} (data not shown).

Effects of PCPA pretreatment on WAY 100635 induced head twitches and brain region monoamines and metabolites in SERT –/– mice
PCPA pretreatment (300 mg·kg^{-1} twice daily for 3 days) completely abolished head twitches induced by WAY 100635 (1 mg·kg^{-1}) in SERT –/– mice ($P < 0.0001$) (Figure 2) and decreased 5-HT levels by 94% in the frontal cortex, 93% in the

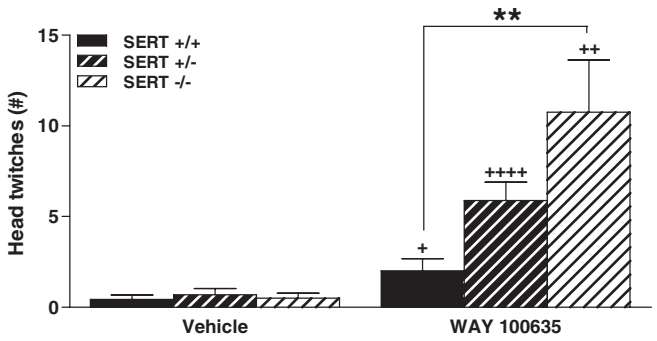

Figure 1 Head twitches in SERT +/+, +/– and –/– mice following vehicle or the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 (1 mg·kg^{-1}) (n = 8–10). Data represent the mean ± SEM. $+P < 0.05$, $++P < 0.01$, $++++P < 0.0001$ compared with vehicle-treated mice of the same genotype; $**P < 0.01$ compared with SERT +/+ mice in the same drug condition. SERT, 5-HT transporter; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt.

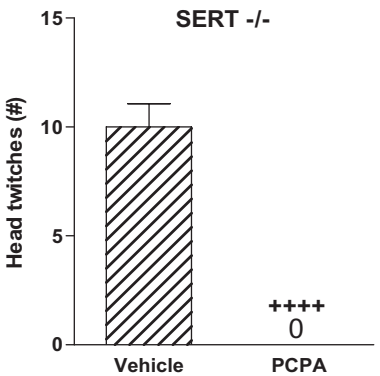

Figure 2 Effects of PCPA pretreatment (300 mg·kg^{-1} twice daily for 3 days) on head twitches induced by WAY 100635 (1 mg·kg^{-1}) in SERT –/– mice (n = 4–5). Data represent the mean ± SEM. $++++P < 0.0001$ compared with vehicle-pretreated mice. SERT, 5-HT transporter; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt; PCPA, p-chlorophenylalanine.

hippocampus, 88% in the hypothalamus, 94% in the striatum and 67% in the brainstem compared with levels in vehicle-pretreated SERT –/– mice (all P's < 0.0001) (Table 1). Tissue levels of the major 5-HT metabolite, 5-hydroxyindole acetic acid (5-HIAA), were also decreased in all five brain areas examined in PCPA-pretreated mice compared with vehicle pretreated mice (all P's < 0.0003). 5-HT turnover (as measured by the 5-HIAA : 5-HT ratio) was not altered by PCPA pretreatment (NS; data not shown). Brain tissue levels of 5-HT, 5-HIAA, noradrenaline, dopamine and 3,4-dihydroxyph enylacetic acid (DOPAC), a metabolite of dopamine, are presented in Table 1.

Effects of pretreatment with the SSRI fluvoxamine on WAY 100635 induced head twitches

Administered alone, fluvoxamine (10 mg·kg^{-1}) did not induce head twitches (data not shown). There were significant main effects for genotype ($F_{2,29}$ = 6.61, P = 0.004) and drug condition

Table 1 Tissue levels of 5-HT, 5-HIAA, noradrenaline, dopamine and DOPAC in SERT –/– mice treated with vehicle or PCPA (300 mg·kg^{-1} twice daily for 3 days)

Measurement (pg·mg^{-1} tissue)	*Drug condition*	
Brain area	*Vehicle*	*PCPA*
5-HT		
Frontal cortex	233 ± 61	14 ± 4^{++++}
Brain stem	193 ± 29	63 ± 14^{++++}
Hippocampus	152 ± 28	10 ± 4^{++++}
Hypothalamus	469 ± 99	59 ± 49^{++++}
Striatum	216 ± 57	13 ± 6^{++++}
5-HIAA		
Frontal cortex	128 ± 49	8 ± 5^{++}
Brain stem	510 ± 131	151 ± 58^{++}
Hippocampus	260 ± 90	13 ± 4^{++++}
Hypothalamus	423 ± 124	37 ± 13^{++++}
Striatum	252 ± 15	15 ± 9^{++++}
Noradrenaline		
Frontal cortex	366 ± 78	290 ± 12
Brain stem	398 ± 35	364 ± 42
Hippocampus	297 ± 18	236 ± 49
Hypothalamus	1627 ± 166	1228 ± 266^{+}
Dopamine		
Striatum	11 422 ± 639	10 660 ± 689
DOPAC		
Striatum	516 ± 61	428 ± 89

Data represent the mean ± SEM; n = 4–5 per group.
$^{+}P < 0.05$, $^{++}P < 0.01$, $^{++++}P < 0.0001$ compared with SERT –/– mice administered vehicle (t-test).
SERT, 5-HT transporter; PCPA, p-chlorophenylalanine; 5-HIAA, 5-hydroxyindole acetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid.

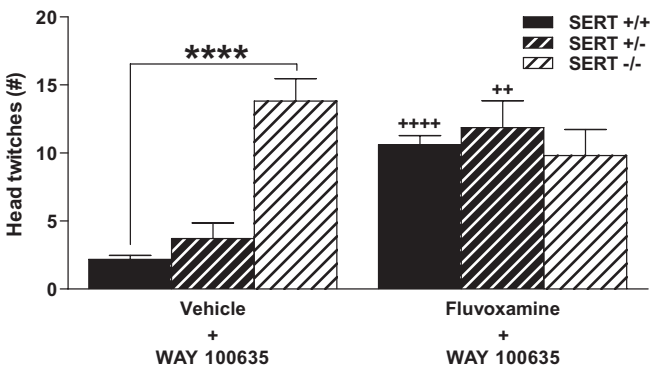

Figure 3 Effects of pretreatment with vehicle or the SSRI fluvoxamine (10 mg·kg^{-1}) 30 min earlier on head twitches induced by WAY 100635 (1 mg·kg^{-1}) in SERT +/+, +/– and –/– mice (n = 5–7). Data represent the mean ± SEM. $++P < 0.01$, $++++P < 0.0001$ compared with vehicle-pretreated mice of the same genotype; $****P < 0.0001$ compared with SERT +/+ mice in the same drug condition. SERT, 5-HT transporter;
WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt; SSRI, selective 5-HT reuptake inhibitor.

($F_{1,29}$ = 12.04, P = 0.002) and a significant genotype × drug condition interaction ($F_{2,29}$ = 10.77, $P < 0.0001$). In mice pretreated with vehicle, WAY 100635 (1 mg·kg^{-1}) again induced an enhanced head-twitch response in SERT –/– mice compared with SERT +/+ mice ($P < 0.0001$) (Figure 3). Pretreatment with fluvoxamine (10 mg·kg^{-1}) 30 min earlier increased WAY 100635 induced head twitches in SERT +/+ and +/– mice

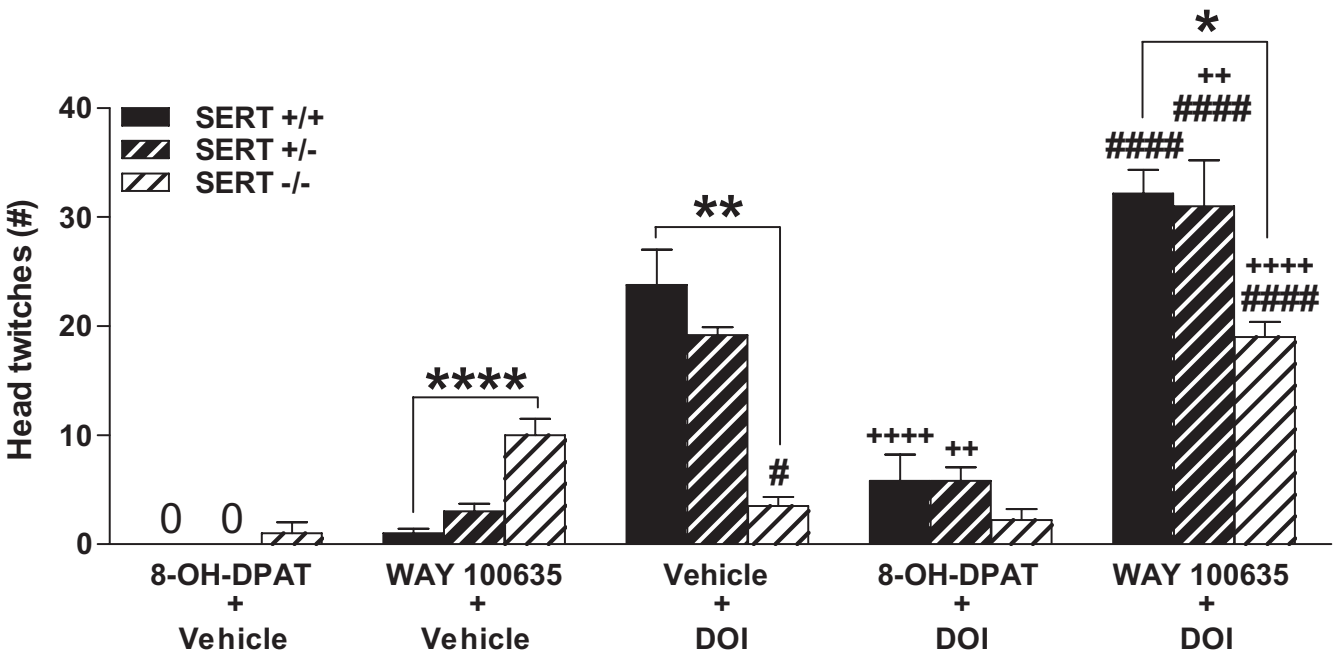

Figure 4 Head twitches in SERT +/+, +/–, and –/– mice following 8-OH-DPAT (1 mg·kg^{-1}), WAY 100635 (1 mg·kg^{-1}) and DOI (2.5 mg·kg^{-1}) administered alone or in combination (administered at the same time) (n = 4–9). Data represent the mean ± SEM. #P < 0.05, ####P < 0.0001 compared with WAY 100635 alone; ++P < 0.01, ++++P < 0.0001 compared with DOI alone; *P < 0.05, **P < 0.01, ****P < 0.0001 compared with SERT +/+ mice in the same drug condition. SERT, 5-HT transporter; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; DOI, (+/–)-2,5-dimethoxy-4-iodophenyl-2-aminopropane.

compared with their respective vehicle-pretreated counterparts (P's < 0.004). In mice pretreated with fluvoxamine and then given WAY 100635, there were no differences between the three SERT genotypes. In fact, following pretreatment with fluvoxamine, WAY 100635 induced a similar number of head twitches in SERT +/+ and +/– mice compared with those observed in SERT –/– mice pretreated with vehicle and given WAY 100635.

Effects of 8-OH-DPAT and WAY 100635 on DOI-induced head twitches

There were significant main effects for genotype ($F_{2,61}$ = 7.70, P = 0.001) and drug condition ($F_{4,61}$ = 67.50, P < 0.0001), in addition to a significant genotype × drug condition interaction ($F_{8,61}$ = 6.84, P < 0.0001). DOI (2.5 mg·kg^{-1}) induced ~85% fewer head twitches in SERT –/– mice compared with SERT +/+ mice (P = 0.001) (Figure 4). Administration of WAY 100635 (1 mg·kg^{-1}) alone again induced more head twitches in SERT –/– mice compared with SERT +/+ mice (P < 0.0001), and of note in SERT –/– mice, WAY 100635 induced more head twitches than did DOI (P = 0.014). The combination of WAY 100635 plus DOI induced more head twitches compared with DOI alone in SERT +/– mice (P = 0.004), with additive or super-additive effects in SERT –/– mice (the combination of WAY 100635 plus DOI compared with either drug alone, P's < 0.0001). Further, 8-OH-DPAT (1 mg·kg^{-1}) decreased DOI-induced head twitches in SERT +/+ and +/– mice (P's < 0.001), with no effect when administered alone and no effects in SERT –/– mice.

Effects of pretreatment with the selective dopamine D_4 receptor antagonist L745870 on WAY 100635 induced head twitches in SERT –/– mice

Administered alone, the selective dopamnine D_4 receptor antagonist L745870 (1 or 3 mg·kg^{-1}) did not alter head twitches compared with vehicle (data not shown). Further, pretreatment with L745870 did not affect head twitches induced by WAY 100635 (1 mg·kg^{-1}) in SERT –/– mice ($F_{2,15}$ = 0.86, NS) (mean ± SEM; vehicle + WAY 100635, 8.33 ± 2.58; 1 mg·kg^{-1} L745870 + WAY 100635, 9.67 ± 2.07; 3 mg·kg^{-1} L745870 + WAY 100635, 9.67 ± 1.21; n = 6 per group), thus ruling out a role for dopamine D_4 receptors in this response.

Assessments of 5-HT$_{2A}$ receptor mediation of head twitches

MDL 11,939 administered alone did not affect head twitches (data not shown). In SERT –/– mice, pretreatment with MDL 11,939 completely blocked WAY 100635 induced head twitches (P < 0.0001) (Figure 5A). Similarly, MDL 11,939 completely blocked head twitches induced by the combination of WAY 100635 plus DOI in mice of all three SERT genotypes (P's < 0.05) (Figure 5B) [main effect of genotype ($F_{2,20}$ = 7.05, P = 0.005); main effect of drug condition ($F_{1,20}$ = 160.89, P < 0.0001); genotype × drug condition interaction ($F_{2,20}$ = 7.05, P = 0.005)]. Additionally, regardless of genotype, MDL 11,939 pretreatment completely blocked the head twitches induced by fluvoxamine plus WAY 100635 in SERT +/+ and +/– mice (P < 0.0001) (Figure 5C) [main effect of genotype ($F_{1,14}$ = 0.67, NS); main effect of drug condition ($F_{1,14}$ = 45.08, P < 0.0001); genotype × drug condition interaction ($F_{1,14}$ = 0.67, NS)].

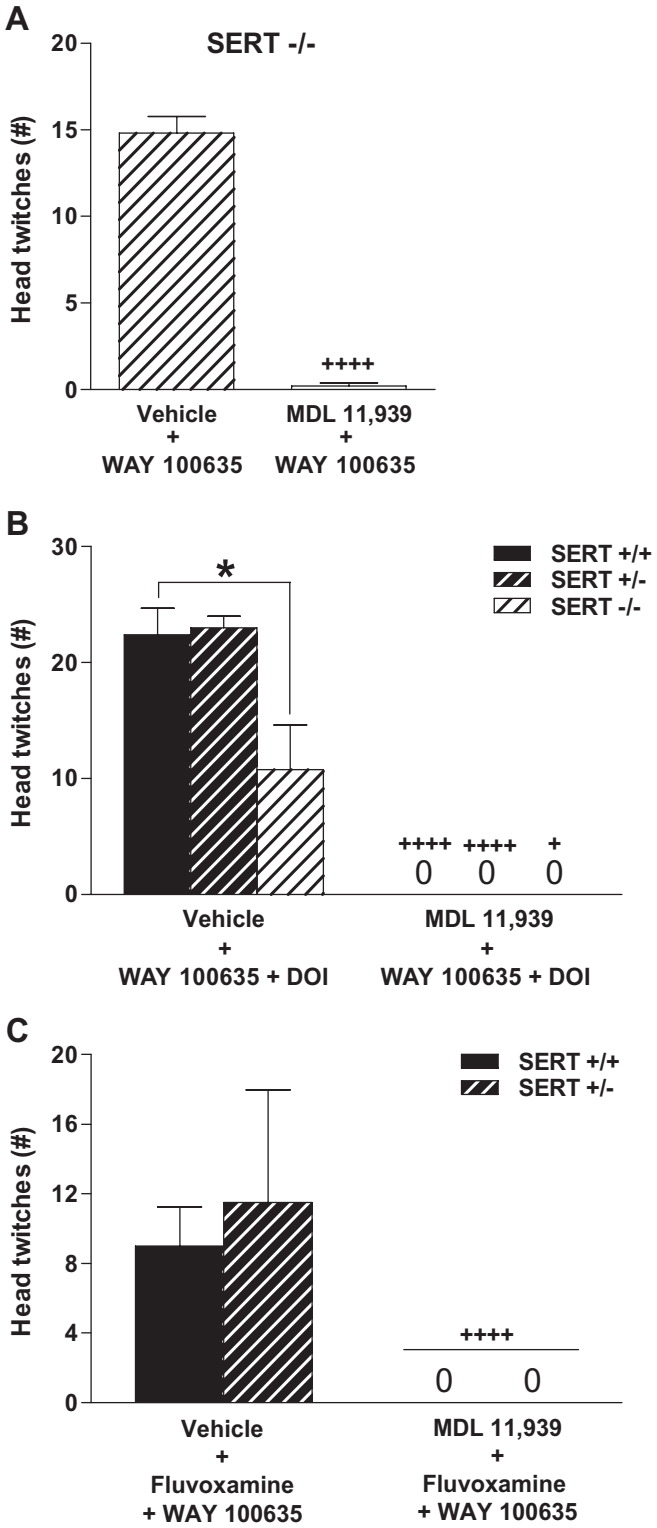

Figure 5 Effects of pretreatment 30 min earlier with vehicle or the selective 5-HT$_{2A}$ receptor antagonist MDL 11,939 (5 mg·kg^{-1}) on head twitches induced (A) by WAY 100635 (1 mg·kg^{-1}) in SERT –/– mice (n = 5), (B) by WAY 100635 (1 mg·kg^{-1}) plus DOI (2.5 mg·kg^{-1}) in SERT +/+, +/– and –/– mice (n = 4–5) or (C) by fluvoxamine (10 mg·kg^{-1}) plus WAY 100635 (1 mg·kg^{-1}) in SERT +/+ and +/– mice (n = 4–5). Data represent the mean ± SEM. +P < 0.05, ++++P < 0.0001 compared with vehicle-pretreated mice [regardless of genotype in panel (C)]; *P < 0.05 compared with SERT +/+ mice in the same drug condition. SERT, 5-HT transporter; WAY 100635, N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyridinylcyclohexanecarboxamide maleate salt; DOI, (+/–)-2,5-dimethoxy-4-iodophenyl-2-aminopropane; MDL 11,939, a-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol.

Discussion

In the current studies, the selective 5-HT$_{1A}$ receptor antagonist WAY 100635 induced an enhanced head-twitch response in SERT –/– mice. It is well established that the head-twitch response is mediated by 5-HT$_{2A}$ receptors (Darmani and Reeves, 1996; Willins and Meltzer, 1997; Darmani, 1998; Gonzalez-Maeso *et al.*, 2003; Moya *et al.*, 2007). For example, studies have established that direct injection of 5-HT$_{2A}$ receptor agonists such as DOI into the medial prefrontal cortex induce head twitches that are blocked by 5-HT$_{2A}$ receptor antagonists MDL 100907 and ketanserin, but not by the 5-HT$_{2C/2B}$ receptor antagonist SDZ SER 082 (Willins and Meltzer, 1997). Consistent with this, the selective 5-HT$_{2A}$ receptor antagonist MDL 11,939 blocked all head-twitch responses assessed in the current studies.

This enhanced 5-HT$_{2A}$ receptor-mediated response following the 5-HT$_{1A}$ receptor antagonist WAY 100635 in SERT –/– mice is of note as SERT-deficient mice have brain area-dependent alterations in 5-HT$_{2A}$ receptor density and function, in addition to markedly reduced post-receptor signalling by 5-HT$_{2A}$ receptors along the PLA$_2$-AA pathway in multiple brain regions (Rioux *et al.*, 1999; Li *et al.*, 2003; Qu *et al.*, 2005; Basselin *et al.*, 2009; Fox *et al.*, in press). Further, SERT-deficient mice have markedly reduced density and function of presynaptic 5-HT$_{1A}$ autoreceptors, including decreased temperature, neural firing, and hormonal responses following 5-HT$_{1A}$ receptor agonists (Li *et al.*, 1999; 2000; Gobbi *et al.*, 2001; Bouali *et al.*, 2003; Holmes *et al.*, 2003a; Fox *et al.*, 2008).

SERT +/– and –/– mice have ~3-fold and ~6-fold increases in baseline extracellular 5-HT respectively (Fabre *et al.*, 2000; Mathews *et al.*, 2004; Shen *et al.*, 2004). In SERT –/– mice, this elevated availability of 5-HT appears to play a role in WAY 100635 induced head twitches.

In the current studies in SERT –/– mice, pretreatment with the 5-HT synthesis inhibitor PCPA, using a regimen previously shown to decrease 5-HT levels by ~80% in wild-type mice (Cesana *et al.*, 1993; O'Leary *et al.*, 2007), decreased tissue 5-HT levels by ~67–94% compared with vehicle in all five brain areas examined, thus decreasing 5-HT availability. Further, this pretreatment with PCPA completely abolished WAY 100635 induced head twitches in SERT –/– mice, strongly suggesting a role for elevated 5-HT availability in this response.

We also report that pretreatment with the SSRI fluvoxamine, which blocks SERT and increases extracellular 5-HT levels (Gobert *et al.*, 2000; Kobayashi *et al.*, 2008), increased the number of head twitches induced by WAY 100635 in SERT +/+ and +/– mice, findings consistent with a previous report of

treatment with fluoxetine plus WAY 100635 in rats (Gobert *et al.*, 2000). As expected, pretreatment with fluvoxamine was without effect in SERT –/– mice. We had hypothesized that fluvoxamine might have an exaggerated effect in SERT +/– mice, as a previous report showed that fluvoxamine prolonged 5-HT clearance in an exaggerated manner in SERT +/– compared with SERT +/+ mice (Montanez *et al.*, 2003); however, the effects of fluvoxamine were similar in SERT +/+ and +/– mice.

Together, these findings from the genetically-deficient SERT mice and the pharmacological inhibition of SERT suggest that in the presence of the consequently elevated 5-HT availability, such as in SERT –/– mice at baseline and in SERT +/+ and +/– mice pretreated with an SSRI, WAY 100635 further enhances 5-HT levels, most likely by blocking presynaptic 5-HT$_{1A}$ receptors, which when activated serve as a negative feedback loop for 5-HT synthesis and release, as shown earlier (Hoyer *et al.*, 1994; Sharp *et al.*, 2007). Activation of presynaptic 5-HT$_{1A}$ autoreceptors by 5-HT or other 5-HT$_{1A}$ agonists decreases neural activity and inhibits 5-HT synthesis, thus decreasing 5-HT levels (Hoyer *et al.*, 1994). As mentioned, administration of a 5-HT$_{1A}$ receptor antagonist blocks this inhibition, in this case the inhibition induced by 5-HT, thus increasing neural activity. As such, administration of a 5-HT$_{1A}$ antagonist increases 5-HT availability by blocking 5-HT$_{1A}$ autoreceptors, and this extra 5-HT is then available to activate downstream postsynaptic 5-HT receptors including, in this case, 5-HT$_{2A}$ receptors.

5-HT availability as examined in SERT-deficient and SERT over-expressing mice also affects DOI-induced head twitches (Jennings *et al.*, 2008; Basselin *et al.*, 2009), and appears to do so in a manner opposite to the effect on WAY 100635 induced head twitches. In SERT over-expressing mice, which have decreased 5-HT levels, and in wild-type mice with decreased 5-HT levels (via PCPA administration), DOI-induced head twitches are increased (Jennings *et al.*, 2008). As mentioned, in SERT –/– mice which have increased extracellular 5-HT, DOI- and TCB-2-induced head twitches are decreased (this study) (Qu *et al.*, 2005; Basselin *et al.*, 2009; Fox *et al.*, in press). Further, we recently reported that PCPA pretreatment in SERT –/– mice essentially restored the DOI-induced head-twitch response to levels observed in SERT +/+ mice (Basselin *et al.*, 2009). Thus, it appears that 5-HT might compete with DOI for occupancy at 5-HT$_{2A}$ receptors, resulting in fewer DOI-induced head twitches in the presence of elevated levels of 5-HT in the synapse.

Recent research shows that 5-HT and DOI induce head twitches by different post-receptor signalling pathways. Specifically, 5-HT induces head twitches via a β-arrestin-2-dependent pathway, whereas DOI-induced head twitches occur independent of β-arrestin-2 (Schmid *et al.*, 2008). Evidence for this comes from studies in β-arrestin-2-knockout mice, which display an intact head-twitch response to DOI but no head-twitch response following 5-hydroxytryptophan (5-HTP), which increases 5-HT levels (Schmid *et al.*, 2008). If WAY 100635 is inducing head twitches in SERT –/– mice by further increasing 5-HT availability, it is likely that these head twitches are being induced via a β-arrestin-2-dependent pathway, although confirmation of this is required by further studies, for example in β-arrestin-2-knockout mice. Additionally, it is possible that there are differences in post-receptor signalling in SERT –/– mice, which might underlie the current report of increased head twitches following WAY 100635 in the presence of elevated 5-HT availability despite DOI-induced head twitches in SERT –/– mice being reduced.

It has been hypothesized that head twitches induced via a β-arrestin-2-independent pathway, for example by 5-HT$_{2A}$ receptor agonists such as DOI, are associated with hallucinogenic activity in humans, whereas head twitches induced by 5-HT (and 5-HT-enhancing agents such as 5-HTP) via a β-arrestin-2-dependent pathway are not associated with hallucinogenic activity (Abbas and Roth, 2008). As such, the current findings might suggest that if WAY 100635 does indeed induce head twitches in a β-arrestin-2-dependent manner, that WAY 100635 and other structurally related 5-HT$_{1A}$ receptor agents might not have hallucinogenic effects, even in the presence of increased 5-HT availability.

Consistent with previous findings (Qu *et al.*, 2005; Basselin *et al.*, 2009; Fox *et al.*, in press), the number of head twitches induced by the 5-HT$_{2A/2C}$ receptor agonist DOI in the current studies was decreased by ~85% in SERT –/– mice compared with SERT +/+ mice, with intermediate changes in SERT +/– mice compared with SERT +/+ mice. Further, 8-OH-DPAT decreased DOI-induced head twitches in SERT +/+ and +/– mice in the present study, consistent with previous reports (Darmani *et al.*, 1990; Bjork *et al.*, 1991; Willins and Meltzer, 1997), and we show that 8-OH-DPAT was without effect in SERT –/– mice. Interestingly, the combination of WAY 100635 and DOI induced more head twitches than either drug alone in SERT +/– and –/– mice. In SERT –/– mice, despite decreased responses following DOI, this effect following the combination of WAY 100635 and DOI was super-additive, confirming an important functional change in this genetic mouse model. We recently reported another super-additive receptor–receptor interaction in SERT –/– mice involving presynaptic 5-HT$_{1A}$ receptors (Fox *et al.*, 2008). Although in SERT +/+ and +/– mice, WAY 100635 blocked hypothermia following the 5-HT precursor 5-HTP, WAY 100635 had no effect on the exaggerated 5-HTP-induced hypothermic response in SERT –/– mice. Pretreatment with the selective 5-HT$_7$ receptor antagonist SB 269970 (Hagan *et al.*, 2000) did decrease 5-HTP-induced hypothermia in SERT –/– mice, and when administered in combination, WAY 100635 and SB 269970 had super-additive effects in these mice despite no effect of WAY 100635 administered alone. Together, these studies show that although presynaptic 5-HT$_{1A}$ receptors are downregulated in SERT –/– mice, interesting functional interactions exist between this 5-HT$_{1A}$ receptor and other 5-HT receptor subtypes.

The current studies used this unique animal model to further explore interactions between 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors. We show that despite numerous reports of decreased or absent 5-HT$_{2A}$ and presynaptic 5-HT$_{1A}$ receptor function in SERT-deficient mice, indirect activation of 5-HT$_{2A}$ receptors via inhibition of presynaptic 5-HT$_{1A}$ receptors results in an exaggerated response, showing that these receptors have functional interactive consequences that are strongly correlated with both genetically and pharmacologically induced alterations in the availability of 5-HT.

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Conflict of interest

None.

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