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Dual actions of 5-MeO-DIPT at the serotonin transporter and serotonin 5-HT$_{1A}$ receptor in the mouse striatum and prefrontal cortex

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Abstract

Aims: 5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) is a synthetic orally active hallucinogenic tryptamine analogue. The present study examined whether the effects of 5-MeO-DIPT involve the serotonin transporter (SERT) and serotonin 5-hydroxytryptamine-1A (5-HT$_{1A}$) receptor in the striatum and prefrontal cortex (PFC).

Methods: We investigated the effects of 5-MeO-DIPT on extracellular 5-HT (5-HT$_{ex}$) and dopamine (DA$_{ex}$) levels in the striatum and PFC in wildtype and SERT knockout (KO) mice using in vivo microdialysis, and for comparison the effects of the 5-HT$_{1A}$ receptor antagonist WAY100635 and the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT on 5-HT$_{ex}$.

Results: 5-MeO-DIPT decreased 5-HT$_{ex}$ levels in the striatum, but not PFC. In SERT-KO mice, 5-MeO-DIPT did not affect 5-HT$_{ex}$ levels in the striatum or PFC. In the presence of WAY100635, 5-MeO-DIPT substantially increased 5-HT$_{ex}$ levels, suggesting that 5-MeO-DIPT acts on SERT and these effects are masked by its 5-HT$_{1A}$ actions in the absence of WAY100635. 8-OH-DPAT decreased 5-HT$_{ex}$ levels in the striatum and PFC in wildtype mice. WAY100635 antagonized the 8-OH-DPAT-induced decrease in 5-HT$_{ex}$ levels. In SERT-KO mice, 8-OH-DPAT did not decrease 5-HT$_{ex}$ levels in the striatum and PFC. 5-MeO-DIPT dose-dependently increased DA$_{ex}$ levels in the PFC, but not striatum, in wildtype and SERT-KO mice. The increase in DA$_{ex}$ levels that was induced by 5-MeO-DIPT was not antagonized by WAY100635.

Conclusion: 5-MeO-DIPT influences both 5-HT$_{ex}$ and DA$_{ex}$ levels in the striatum and PFC. 5-MeO-DIPT dually acts on SERT and 5-HT$_{1A}$ receptors so that elevations in 5-HT$_{ex}$ levels produced by reuptake inhibition are limited by actions of the drug on 5-HT$_{1A}$ receptors.

KEYWORDS

5-HT$_{1A}$ serotonin receptor, 5-MeO-DIPT, prefrontal cortex, serotonin transporter, striatum

1 | INTRODUCTON

5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) is a synthetic tryptaminergic hallucinogen that is known as a designer drug, with the street name "foxy" or "foxy methoxy." 5-MeO-DIPT has been controlled as a schedule I substance under the Controlled Substances Act in the US.[1,2] In Japan, 5-MeO-DIPT has been controlled as a Narcotic since 2005. 5-MeO-DIPT users exhibit euphoria, disinhibition, visual and auditory hallucinations, amnesia, catalepsy, mydriasis, tachypnea, hypertension, and tachycardia.[3–5] 5-MeO-DIPT use has been associated with hallucinogen-persisting perception disorder and was proposed to play a role in the development of prolonged delusions.[6,7]

Many tryptamines are serotonergic hallucinogens.[8–10] 5-MeO-DIPT appears to interact with the serotonergic system.[11–15] 5-MeO-DIPT has high affinity for serotonin 5-hydroxytryptamine-2A (5-HT_{2A}) and 5-HT_{2C} receptors, and an even higher affinity for 5-HT_{1A} receptors.[11] 5-MeO-DIPT has been shown to bind to the 5-HT transporter (SERT) and block 5-HT reuptake.[12–14] Surprisingly, given these last findings, 5-MeO-DIPT reportedly does not stimulate the release of 5-HT in vitro.[13–15]

Despite this discrepancy between in vivo and in vitro findings on 5-HT release, several pharmacological studies suggest that 5-MeO-DIPT stimulates serotonin function in some manner. Previous behavioral studies demonstrated that 5-MeO-DIPT induces the head-twitch response in mice, characteristic of 5-HT activation, and these effects are antagonized by 5-HT_{2A} antagonist M100907, despite its additional action at the 5-HT_{1A} receptor in vitro.[11] 5-MeO-DIPT was also shown to potentiate forepaw treading that was induced by the 5-HT_{1A} receptor agonist 8-OH-DPAT.[16] Animals treated with 5-MeO-DIPT also show hypoactivity.[17] Additional behavioral studies demonstrated that 5-MeO-DIPT given repeatedly to adolescent rats produced deleterious effects on learning and memory in adulthood.[18,19] These pharmacological results suggest that 5-MeO-DIPT stimulates not only serotonin function but also dopamine function.

To examine the action of 5-MeO-DIPT on the SERT and 5-HT_{1A} receptors, we investigated the effects of 5-MeO-DIPT on extracellular levels of 5-HT (5-HT_{ex}), as well as effects on extracellular DA levels (DA_{ex}), in the striatum and prefrontal cortex (PFC) in wildtype and SERT knockout (KO) mice using in vivo microdialysis. We also examined the role of 5-HT_{1A} receptors in the effects of 5-MeO-DIPT using the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT.

2 | METHODS

2.1 | Animals

Serotonin transporter KO mice and their wildtype littermates that were used in these experiments were from a line that was maintained on a C57BL/6J genetic background. The late Dr Dennis Murphy (National Institute of Mental Health, Bethesda, MD, USA) provided the founder mice. The experimental procedures and housing conditions were approved by the Animal Use and Care Committee of the Tokyo Metropolitan Institute of Medical Science. All of the mice were treated humanely in accordance with our institutional animal experimentation guidelines. Naive adult mice were housed in an animal facility at 23°C ± 1°C and 55% ± 5% relative humidity under a 12-hour/12-hour light/dark cycle (lights on at 8:00 AM and off at 8:00 PM). Food and water were available ad libitum. Male and female mice, 10-24 weeks old, were used.

2.2 | Surgery

The mice were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and stereotaxically implanted with microdialysis probes in the striatum (anterior/posterior, +0.6 mm; medial/lateral, +1.8 mm; dorsal/ventral, −4.0 mm from bregma) or PFC (anterior/posterior, +2.0 mm; medial/lateral, +0.5 mm; dorsal/ventral, −3.0 mm from bregma) according to the atlas of Franklin and Paxinos.[20] The probe tips had a regenerated cellulose membrane (50 kDa molecular weight cut-off, 0.22 mm outer diameter and 2 mm membrane length; Eicom, Kyoto, Japan). The dialysis probe placements were verified histologically at the end of the experiments.

2.3 | Microdialysis and analytical procedure

Twenty-four hours after probe implantation, the dialysis experiments were performed in freely moving animals. Ringer's solution (145 mmol/L NaCl, 3 mmol/L KCl, 1.26 mmol/L $CaCl_2$, and 1 mmol/L $MgCl_2$, pH 6.5) was perfused at a constant flow rate of 1 μL/min. Perfusates were directly injected in the high-performance liquid chromatography system every 10 minutes using an autoinjector (EAS-20; Eicom). Serotonin and DA in the dialysate were separated using a reverse-phase ODS column (PP-ODS, Eicom) and detected with a graphite electrode (HTEC-500, Eicom). The mobile phase consisted of 0.1 mol/L phosphate buffer (pH 5.5) that contained sodium decanesulfonate (500 mg/L), ethylenediaminetetraacetic acid (50 mg/L), and 1% methanol. Perfusion was initiated 180 minutes before collecting baseline samples. Basal levels of DA_{ex} and 5-HT_{ex} were calculated as average concentrations of three consecutive samples when they were stable.

2.4 | Drugs

5-MeO-DIPT was synthesized by Dr T. Iwamura (Gifu Pharmaceutical University). *R* (+)-8-hydroxy-DPAT (8-OH-DPAT; Sigma-Aldrich, St. Louis, MO, USA) and WAY100635 (Sigma-Aldrich) were dissolved in saline and administered subcutaneously (s.c.) in a volume of 10 mL/kg. 5-MeO-DIPT (10 or 20 mg/kg) or 8-OH-DPAT (0.1 or 1 mg/kg) was administered after a stable baseline was established, and the dialysate was continuously collected for 120 minutes. In separate

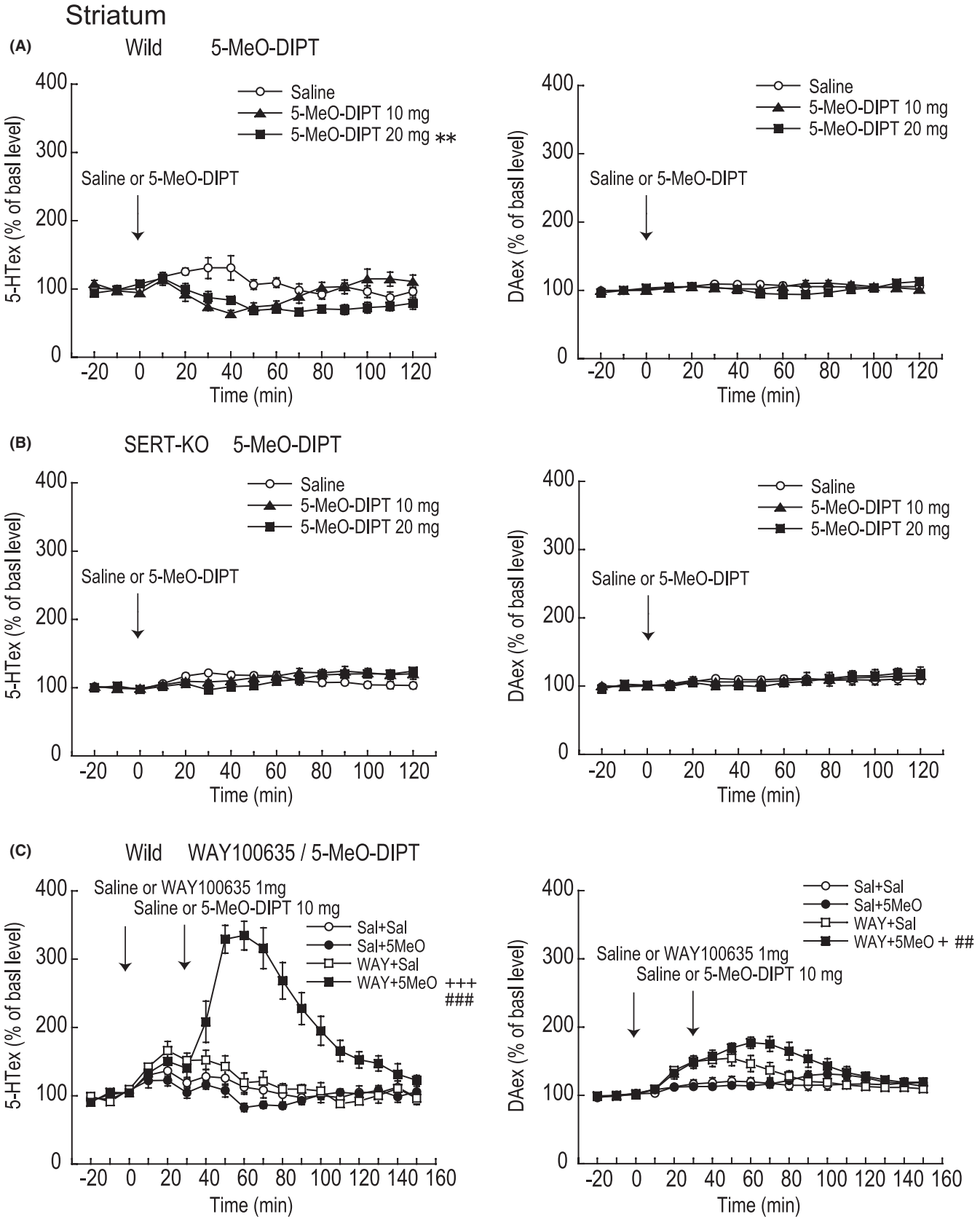

FIGURE 1 Effects of 5-MeO-DIPT on 5-HT_{ex} and DA_{ex} levels in the striatum in wildtype (A), SERT-KO (B), and wildtype (C) mice. The arrows indicate the drug injection times. The data are expressed as the mean ± SEM (n = 7-12/group) of the percentage of 5-HT_{ex} and DA_{ex} baselines. (A) $^{**}P < .01$, vs saline group. (C) $^{+}P < .05$, $^{+++}P < .001$, vs WAY100635/saline group. $^{\#\#}P < .01$, $^{\#\#\#}P < .001$, vs saline/5-MeO-DIPT group (repeated-measures ANOVA followed by Fisher's PLSD *post hoc* test)

experiments, the mice were pretreated with WAY100635 (1 mg/kg) 30 minutes before 5-MeO-DIPT (10 mg/kg) or 8-OH-DPAT (0.1 mg/kg) administration.

2.5 | Statistical analysis

5-HT_{ex} and DA_{ex} responses to drug treatment are expressed as a percentage of basal levels. The statistical analyses were performed using one- or two-way repeated-measures analysis of variance (ANOVA). Individual *post hoc* comparisons among groups were performed using Fisher's Protected Least Significant Difference (PLSD) test. Values of $P < .05$ were considered statistically significant.

3 | RESULTS

3.1 | Basal 5-HT_{ex} and DA_{ex} levels in the striatum and PFC

Basal levels of 5-HT_{ex} and DA_{ex} in striatal dialysates from each test group were as follows: wildtype (5-HT_{ex}: 1.20 ± 0.12 fmol/10 μL; DA_{ex}: 42.49 ± 4.82 fmol/10 μL, n = 28), SERT-KO (5-HT_{ex}: 9.63 ± 1.27 fmol/10 μL; DA_{ex}: 52.40 ± 7.59 fmol/10 μL, n = 21). Basal levels of 5-HT_{ex} and DA_{ex} in dialysates from the PFC were as follows: wildtype (5-HT_{ex}: 1.36 ± 0.08 fmol/10 μL; DA_{ex}: 0.73 ± 0.05 fmol/10 μL, n = 32, n = 32), SERT-KO (5-HT_{ex}: 11.19 ± 0.73 fmol/10 μL; DA_{ex}: 0.70 ± 0.07 fmol/10 μL, n = 30, n = 30). As previously reported,[21] basal levels of 5-HT_{ex} were significantly higher in SERT-KO mice than in wildtype mice in both the striatum ($F_{1,47} = 64.851$, $P < .001$) and PFC ($F_{1,60} = 191.006$, $P < .001$). Basal levels of DA_{ex} were not different between wildtype and SERT-KO mice in either the striatum ($F_{1,47} = 1.451$, $P = .234$) or PFC ($F_{1,60} = 0.0953$, $P = .7587$).

3.2 | Effects of 5-MeO-DIPT on 5-HT_{ex} and DA_{ex} levels in the striatum and PFC

5-MeO-DIPT (10 and 20 mg/kg) dose-dependently decreased 5-HT_{ex} levels in the striatum, without altering DA_{ex} levels in the striatum in wildtype mice (Figure 1A). The two-way ANOVA of 5-HT_{ex} levels revealed significant effects of treatment ($F_{2,20} = 6.870$, $P < .01$) and time ($F_{11,220} = 5.114$, $P < .001$) and a significant treatment × time interaction ($F_{22,220} = 5.572$, $P < .001$). 5-MeO-DIPT (10 and 20 mg/kg) did not affect 5-HT_{ex} or DA_{ex} levels in the striatum in SERT-KO mice (Figure 1B).

To investigate the role of 5-HT_{1A} receptor activation in the effect of 5-MeO-DIPT on 5-HT_{ex} and DA_{ex} levels, mice were pretreated with the selective 5-HT_{1A} antagonist WAY100635 (1 mg/kg) 30 minutes before 10 mg/kg 5-MeO-DIPT administration. WAY100635 (1 mg/kg) administered alone slightly increased DA_{ex} levels but not 5-HT_{ex} levels. In the presence of WAY100635, 5-MeO-DIPT (10 mg/kg) significantly increased 5-HT_{ex} and DA_{ex} levels (Figure 1C). The two-way ANOVA of 5-HT_{ex} levels revealed significant effects of treatment ($F_{3,33} = 40.298$, $P < .001$) and time ($F_{11,363} = 28.221$, $P < .001$) and a significant treatment × time interaction ($F_{33,363} = 17.632$, $P < .001$). The two-way ANOVA of DA_{ex} levels revealed significant effects of treatment ($F_{3,33} = 4.299$, $P < .05$) and time ($F_{11,363} = 22.173$, $P < .001$) and a significant treatment × time interaction ($F_{33,363} = 11.437$, $P < .001$).

5-MeO-DIPT (10 and 20 mg/kg) did not decrease 5-HT_{ex} levels in the PFC in wildtype mice (Figure 2A). 5-MeO-DIPT (10 and 20 mg/kg) dose-dependently increased DA_{ex} levels in wildtype mice in the PFC (Figure 2A). The two-way ANOVA of DA_{ex} levels revealed significant effects of treatment ($F_{2,15} = 16.967$, $P < .001$) and time ($F_{11,165} = 16.153$, $P < .001$) and a significant treatment × time interaction ($F_{22,165} = 6.519$, $P < .001$). 5-MeO-DIPT (10 and 20 mg/kg) dose-dependently increased DA_{ex} levels in the PFC in SERT-KO mice (Figure 2B). The two-way ANOVA of DA_{ex} levels revealed significant effects of treatment ($F_{2,16} = 4.993$, $P < .05$) and time ($F_{11,176} = 6.382$, $P < .001$) and a significant treatment × time interaction ($F_{22,176} = 1.832$, $P < .05$).

To investigate the role of 5-HT_{1A} receptor activation in the effects of 5-MeO-DIPT on 5-HT_{ex} and DA_{ex} levels, mice were pretreated with the selective 5-HT_{1A} antagonist WAY100635 (1 mg/kg) 30 minutes before 10 mg/kg 5-MeO-DIPT or 0.1 mg/kg 8-OH-DPAT administration. WAY100635 (1 mg/kg) alone did not affect 5-HT_{ex} and DA_{ex} levels. In the presence of WAY100635, the influence of 5-MeO-DIPT (10 mg/kg) on 5-HT_{ex} levels, but not DA_{ex} levels, was markedly enhanced in the PFC (Figure 2C). The two-way ANOVA of 5-HT_{ex} levels revealed significant effects of treatment ($F_{3,33} = 12.679$, $P < .001$) and time ($F_{11,352} = 18.469$, $P < .001$), and a significant treatment × time interaction ($F_{33,352} = 10.005$, $P < .001$). The two-way ANOVA of DA_{ex} levels revealed significant effects of treatment ($F_{3,33} = 4.839$, $P < .01$) and time ($F_{11,352} = 11.926$, $P < .001$), and a significant treatment × time interaction ($F_{33,352} = 3.846$, $P < .001$).

3.3 | Effects of 8-OH-DPAT on 5-HT_{ex} and DA_{ex} levels in the striatum and PFC

8-OH-DPAT (0.1 mg/kg) decreased 5-HT_{ex} levels in the striatum in wildtype mice but not in SERT-KO mice (Figure 3A,B). 8-OH-DPAT (0.1 mg/kg) did not affect DA_{ex} levels in the striatum in wildtype or SERT-KO mice (Figure 3A,B). The two-way ANOVA of 5-HT_{ex} levels revealed a significant effect of treatment ($F_{1,10} = 14.048$, $P < .01$) and a significant treatment × time interaction ($F_{11,110} = 5.433$, $P < .001$). WAY100635 blocked the effects of 8-OH-DPAT on 5-HT_{ex} levels in the striatum (Figure 3C). The two-way ANOVA of 5-HT_{ex} levels revealed significant effects of treatment ($F_{3,33} = 3.623$, $P < .05$) and time ($F_{11,308} = 6.137$, $P < .001$), and a significant treatment × time interaction ($F_{33,308} = 4.270$, $P < .001$).

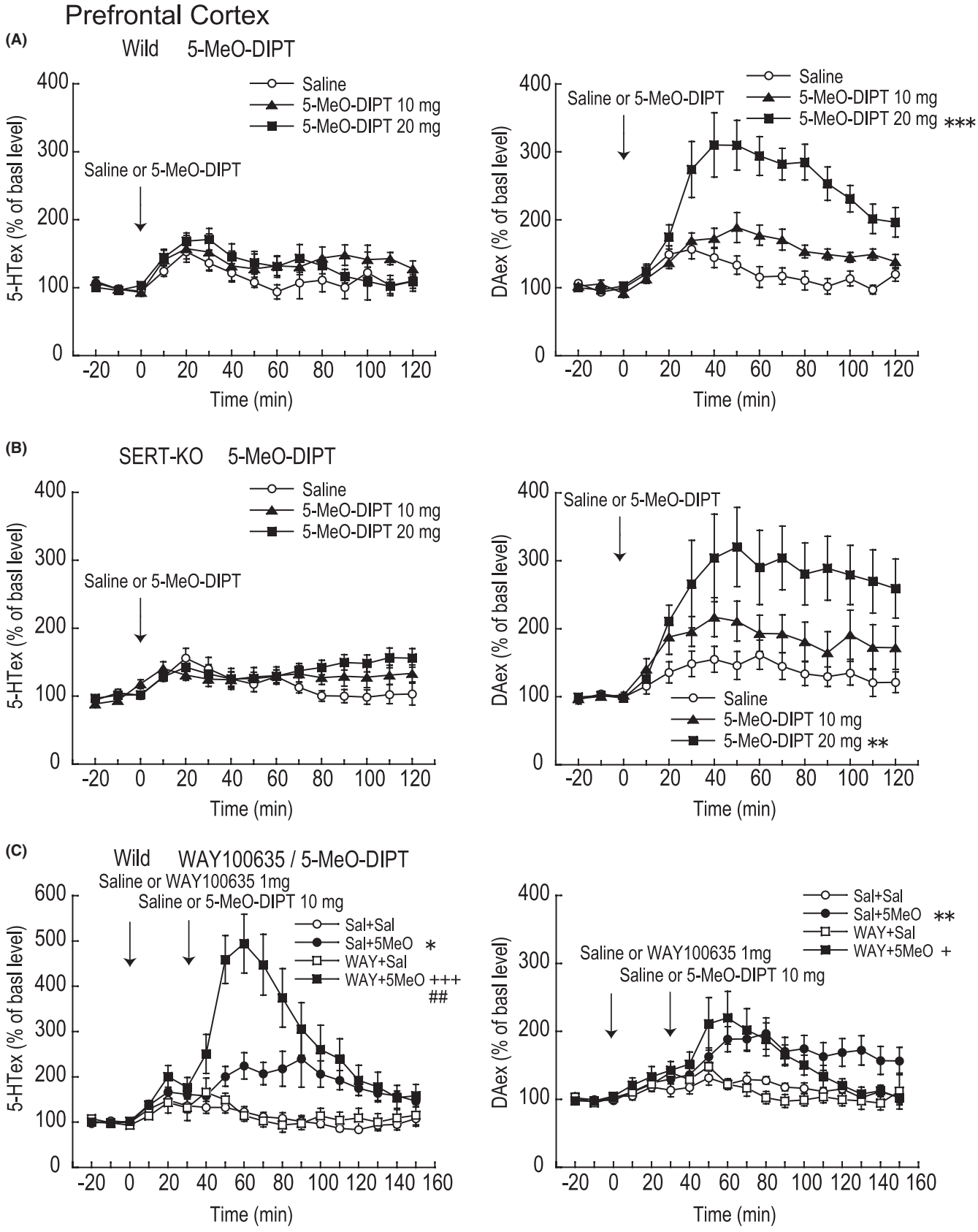

FIGURE 2 Effects of 5-MeO-DIPT on 5-HT_{ex} and DA_{ex} levels in the PFC in wildtype (A), SERT-KO (B), and wildtype (C) mice. The arrows indicate the drug injection times. The data are expressed as the mean ± SEM (n = 6-11/group) of the percentage of 5-HT_{ex} and DA_{ex} baselines. (A, B) $^{**}P < .01$, $^{***}P < .001$, vs saline group. (C) $^{*}P < .05$, $^{**}P < .01$, vs saline/saline group; $^{+}P < .05$, $^{+++}P < .001$, vs WAY100635/saline group; $^{\#\#}P < .01$, vs saline/5-MeO-DIPT group (repeated-measures ANOVA followed by Fisher's PLSD *post hoc* test)

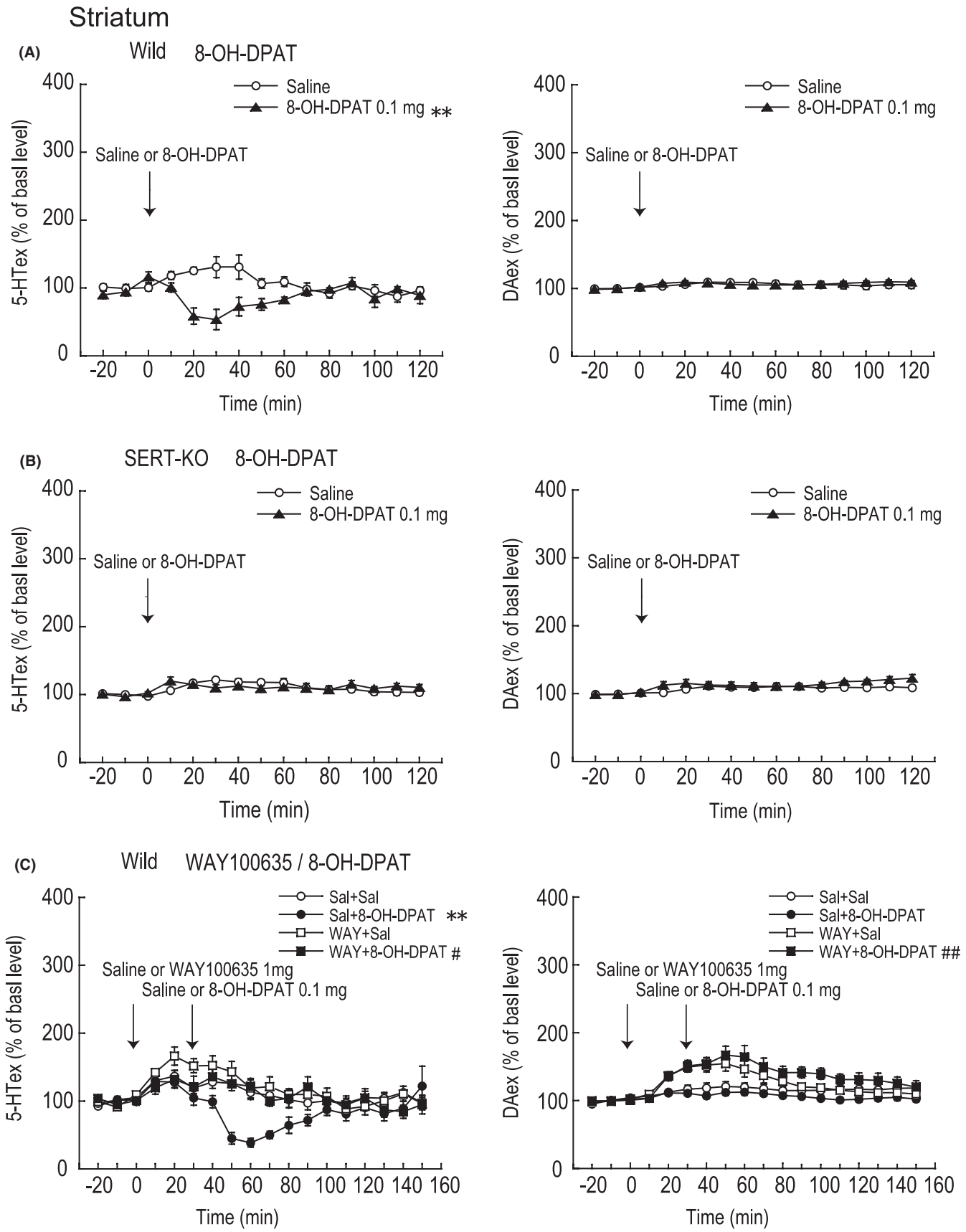

FIGURE 3 Effects of 8-OH-DPAT on 5-HT_{ex} and DA_{ex} levels in the striatum in wildtype (A), SERT-KO (B), and wildtype (C) mice. The arrows indicate the drug injection times. The data are expressed as the mean ± SEM (n = 5-11/group) of the percentage of 5-HT_{ex} and DA_{ex} baselines. (A) $^{**}P < .01$, vs saline group. (C) $^{**}P < .01$, vs saline/saline group; $^{\#}P < .05$, $^{\#\#}P < .01$, vs saline/8-OH-DPAT group (repeated-measures ANOVA followed by Fisher's PLSD *post hoc* test)

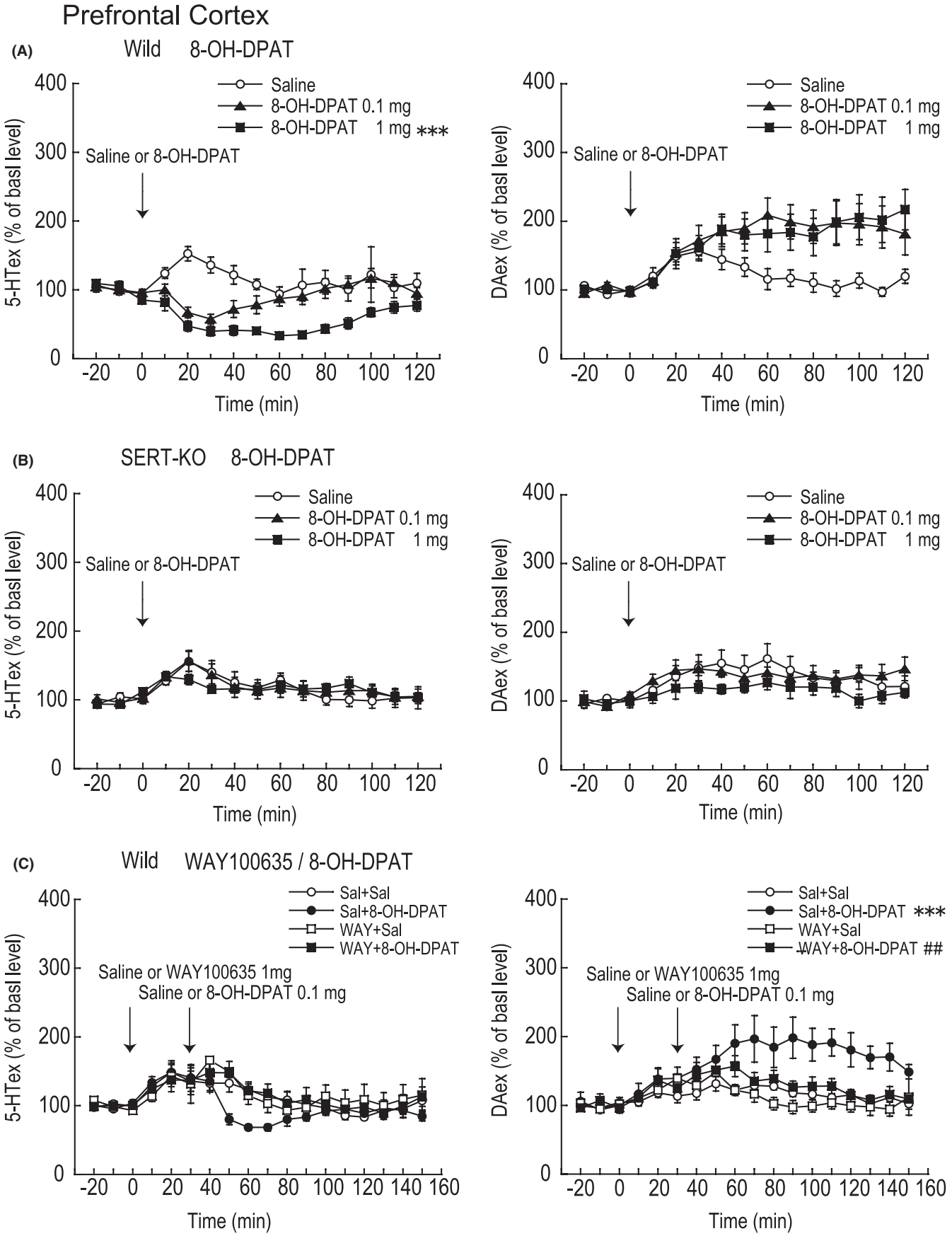

FIGURE 4 Effects of 8-OH-DPAT on 5-HT_{ex} and DA_{ex} levels in the PFC in wildtype (A), SERT-KO (B), and wildtype (C) mice. The arrows indicate the drug injection times. The data are expressed as the mean ± SEM (n = 6-11/group) of the percentage of 5-HT_{ex} and DA_{ex} baselines. (A) ***$P < .001$, vs saline group. (C) ***$P < .001$, vs saline/saline group; $^{\#\#}P < .01$, vs saline/8-OH-DPAT group (repeated-measures ANOVA followed by Fisher's PLSD *post hoc* test)

8-OH-DPAT (0.1 and 1 mg/kg) dose-dependently decreased 5-HT_{ex} levels in the PFC in wildtype mice (Figure 4A). 8-OH-DPAT (0.1 and 1 mg/kg) induced a nonsignificant increase in DA_{ex} levels in the PFC in wildtype mice (Figure 4A). The two-way ANOVA of 5-HT_{ex} levels revealed significant effects of treatment ($F_{2, 17} = 10.302$, $P < .01$) and time ($F_{11, 187} = 4.153$, $P < .001$), and a significant treatment × time interaction ($F_{22, 187} = 3.812$, $P < .001$). The two-way ANOVA of DA_{ex} levels revealed a significant effect of time ($F_{11,187} = 6.764$, $P < .001$) and a significant treatment × time interaction ($F_{22,187} = 4.544$, $P < .001$), but no significant main effect of treatment ($F_{2,17} = 2.482$, $P = .1133$). 8-OH-DPAT (0.1 and 1 mg/kg) did not affect 5-HT_{ex} or DA_{ex} levels in the PFC in SERT-KO mice (Figure 4B). WAY100635 blocked the effects of 8-OH-DPAT on 5-HT_{ex} and DA_{ex} levels in the PFC (Figure 4C). The two-way ANOVA of 5-HT_{ex} levels revealed a significant effect of time ($F_{11, 308} = 8.594$, $P < .001$) and a treatment × time interaction ($F_{33, 308} = 1.251$, $P = .1687$), but no significant main effect of treatment ($F_{3, 33} = 1.240$, $P = .3137$). The two-way ANOVA of DA_{ex} levels revealed significant effects of treatment ($F_{3,33} = 7.295$, $P < .001$) and time ($F_{11,308} = 6.122$, $P < .001$), and a significant treatment × time interaction ($F_{33,308} = 1.879$, $P < .01$).

4 | DISCUSSION

The present study demonstrated that 5-MeO-DIPT decreased 5-HT_{ex} levels in the striatum, but not PFC, and increased DA_{ex} levels in the PFC, but not striatum. These regionally specific effects are likely the result of a balance of actions of 5-MeO-DIPT on SERT and serotonin receptors that subsequently also influence dopamine release. This was revealed by subsequent pharmacologic experiments and comparisons in SERT KO mice. The most revealing effects are summarized in Figure 5. These effects are consistent with many of the known actions of 5-MeO-DIPT, which has been shown to inhibit SERT[12–14] and to act on 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptors.[11]

Pronounced dopaminergic actions of 5-MeO-DIPT were observed in the PFC, but not the striatum. These effects were not affected by SERT KO, so likely involve other mechanisms. Serotonin-containing cell bodies of the raphe nuclei send projections to dopaminergic cells in both the ventral tegmental area and the substantia nigra, and to their terminal fields in the nucleus accumbens, PFC, and striatum.[22–25] The serotonergic system modulates the activity of dopaminergic neurons in both the nigrostriatal pathway and the

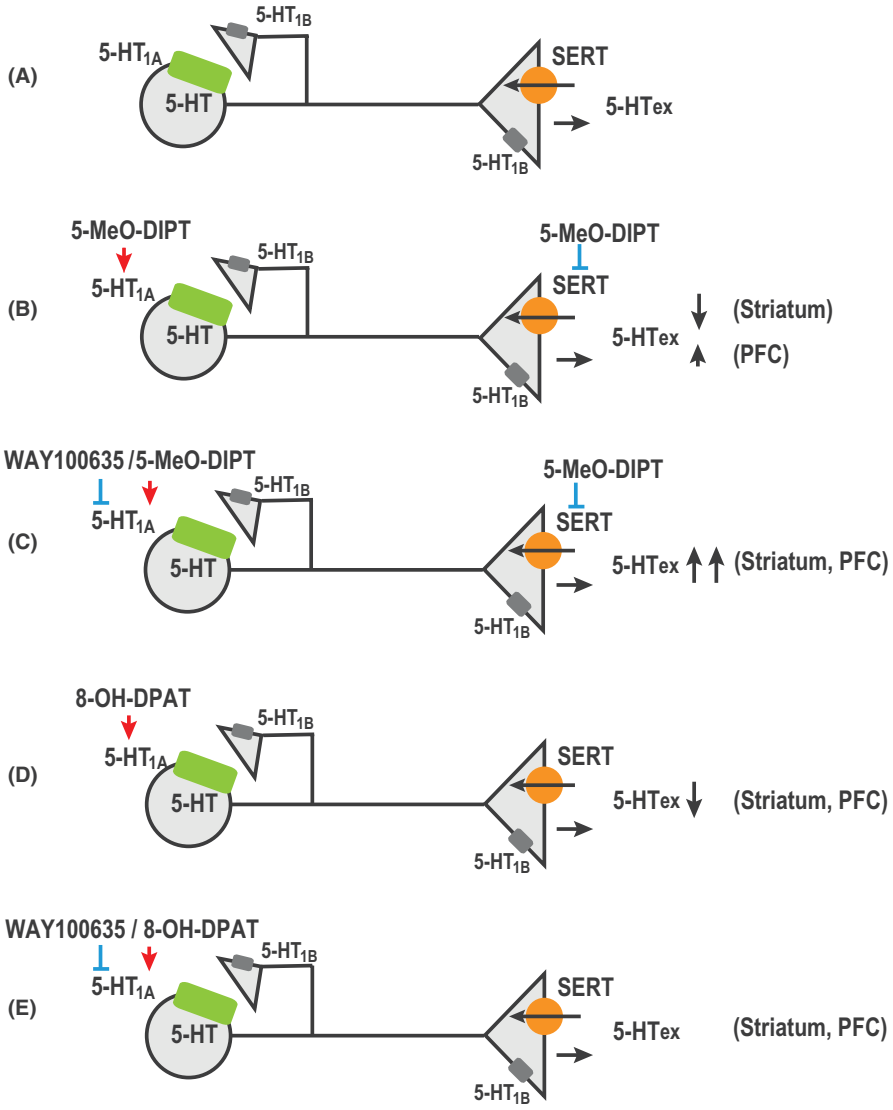

FIGURE 5 Schematic illustration of the effects of 5-MeO-DIPT and 8-OH-DPAT on 5-HT_{ex} levels. Serotonin neurons are regulated by 5-HT_{1A} and 5-HT_{1B} receptors and the SERT (A). 5-MeO-DIPT activates 5-HT_{1A} receptors and inhibits the SERT (B). WAY100635 antagonizes the actions of 5-MeO-DIPT on 5-HT_{1A} receptors (C). 8-OH-DPAT activates 5-HT_{1A} receptors (D). WAY100635 antagonizes the actions of 8-OH-DPAT at 5-HT_{1A} receptors (E)

mesolimbic pathway.[26] Serotonin neurotransmission is regulated by SERT and serotonin autoreceptors through negative feedback inhibition at the somatodendritic level (5-HT_{1A} receptors) and axonal level (5-HT_{1B} receptors; Figure 5A).[27,28] 5-MeO-DIPT decreased 5-HT_{ex} levels in the striatum, but not PFC. Although 5-MeO-DIPT acts on SERT, it also activates somatodendritic 5-HT_{1A} receptors (Figure 5B). The mechanism underlying the regional differences in 5-HT_{ex} levels after 5-MeO-DIPT treatment is not known, but likely involves the relative balance of these effects. In SERT-KO mice, 5-MeO-DIPT did not affect 5-HT_{ex} levels in the striatum and PFC, probably because 5-HT_{1A} autoreceptors are strongly desensitized and their expression down-regulated in SERT-KO mice.[29–31] In the presence of WAY100635, 5-MeO-DIPT increased 5-HT_{ex} levels, suggesting that 5-MeO-DIPT acts on SERT, but these effects are masked by its 5-HT_{1A} actions (Figure 5C). Previous studies have shown that 5-HT_{1A} receptor agonists reduce 5-HT_{ex} levels in the striatum and frontal cortex.[32–36] In the present study, 8-OH-DPAT also decreased 5-HT_{ex} levels in the striatum and PFC in wildtype mice (Figure 5D). The 5-HT_{1A} receptor antagonist WAY100635 antagonized the 8-OH-DPAT-induced decrease in 5-HT_{ex} levels in the striatum and PFC (Figure 5E). In SERT-KO mice, 8-OH-DPAT did not decrease 5-HT_{ex} levels in the striatum and PFC. SERT-KO mice have reduced density and function of presynaptic 5-HT_{1A} autoreceptors, neural firing, and neuroendocrine and temperature responses to 8-OH-DPAT are reduced.[29–31,37–40]

The 5-HT_{1A} receptor agonist 8-OH-DPAT increased DA_{ex} levels in the PFC but not in the striatum. The 5-HT_{1A} receptor antagonist WAY100635 antagonized the 8-OH-DPAT-induced increase in DA_{ex} levels in the PFC. This is consistent with the finding that the selective 5-HT_{1A} receptor agonist 8-OH-DPAT increased DA_{ex} levels in the PFC, without affecting striatal DA_{ex} levels.[41,42] 5-HT_{1A} receptor agonists increase DA_{ex} levels in a brain region-specific manner via postsynaptic 5-HT_{1A} receptor activation.[36,41,43] 5-MeO-DIPT dose-dependently increased DA_{ex} levels in the PFC, but not striatum, in wildtype and SERT-KO mice. The increase in DA_{ex} levels that was induced by 5-MeO-DIPT was not antagonized by the 5-HT_{1A} receptor antagonist WAY100635. The dose of WAY100635 that was tested in the present study has been shown to completely antagonize the 8-OH-DPAT-induced increase in DA_{ex} levels. The increase in DA_{ex} levels induced 5-MeO-DIPT in the PFC is substantially 5-HT_{1A} receptor independent. 5-MeO-DIPT has an affinity for 5-HT_{2A} and 5-HT_{2C} in addition to 5-HT_{1A} receptors,[11] so these receptors may be involved in these effects. The 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) has been reported to increase DA_{ex} but not 5-HT_{ex} levels. This action was abolished by the 5-HT_{2A} receptor antagonist M100907.[44,45] 5-MeO-DIPT-induced head-twitch responses have been used as a behavioral correlate to assess 5-HT_{2A} receptor agonist activity.[11,16] Furthermore, the head-twitch response that was induced by 5-MeO-DIPT was blocked by the 5-HT_{2A} receptor antagonist M100907.[11] Therefore, the augmenting effect of 5-MeO-DIPT on DA_{ex} levels may be mediated by 5-HT_{2A} receptors. 5-MeO-DIPT was shown to inhibit the reuptake of norepinephrine, whereas its inhibitory effects on DA transporter were weak.[13,14] Norepinephrine transporter inhibitors were reported to increase DA_{ex} levels in the PFC.[46,47] Thus, 5-MeO-DIPT may also act at the norepinephrine transporter to increase DA_{ex} levels in the PFC. Although the mechanism of the augmenting effect of 5-MeO-DIPT on DA_{ex} levels is not clear and needs further study, differential effects of 5-MeO-DIPT on DA_{ex} levels in the striatum and PFC may underlie some of the characteristic behaviors induced by 5-MeO-DIPT.

In conclusion, 5-MeO-DIPT influenced both 5-HT_{ex} and DA_{ex} levels in the striatum and PFC. 5-MeO-DIPT dually acts on SERT and 5-HT_{1A} receptors, and the balance of actions at these targets determines the effect of 5-MeO-DIPT on 5-HT_{ex} in a regionally dependent manner. This would also suggest that other factors that inhibit 5-HT_{1A} receptor functions might lead to greater effects of 5-MeO-DIPT on 5-HT_{ex}.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

YH and KI conceived and designed the study. SH, GU, and IS provided the mice. YH performed the experiments and analyzed the data. YH and KI wrote the manuscript. All authors read and approved the final manuscript.

ANIMAL STUDIES

The experimental procedures and housing conditions were approved by the Institutional Animal Care and Use Committee (permission number: 20-019), and all of the animals were cared for and treated humanely in accordance with our institutional animal experimentation guidelines.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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