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# Role of 5-HT$_{1B}$ receptors in the regulation of extracellular serotonin and dopamine in the dorsal striatum of mice

Lotte De Groote[a,b], Berend Olivier[b,c], Herman G.M. Westenberg[a,b,*]

[a] *Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands*
[b] *Department of Psychiatry, Yale University School of Medicine, New Haven, CT, USA*
[c] *Department of Psychopharmacology, Utrecht University, Utrecht, The Netherlands*

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**Abstract**

To test the hypothesis that 5-HT$_{1B}$ receptors modulate serotonin (5-hydroxytryptamine, 5-HT) and dopamine release in the striatum, we used in vivo microdialysis in mice lacking 5-HT$_{1B}$ receptors. Local administration by reversed microdialysis of the selective 5-HT reuptake inhibitor, fluvoxamine (0.1–10 μM), concentration dependently increased 5-HT to the same extent in wildtype and in 5-HT$_{1B}$ knockout (KO) mice. Fluvoxamine (10 μM) increased dopamine levels similarly in both genotypes. The 5-HT releaser, fenfluramine (50 μM), increased both 5-HT and dopamine levels, but no difference was found between the genotypes. The 5-HT$_{1B}$ receptor agonist, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-*b*]pyridin-5-one (CP-93,129), reduced 5-HT levels in the wildtype, but not in 5-HT$_{1B}$ KO mice. CP-93,129 at a concentration of 0.5 μM did not affect striatal dopamine outflow in either genotype, whereas dopamine outflow was increased 5-fold by 50 μM CP-93,129 in both genotypes. The CP-93,129-induced dopamine increase was not attenuated by ritanserin, a 5-HT$_{2A/2C}$ receptor antagonist, but was completely blocked by tetrodotoxin, demonstrating that the dopamine release was of neuronal origin. In conclusion, 5-HT$_{1B}$ autoreceptors are functionally present in the mouse striatum, but do not appear to play a significant role in the effects of a selective 5-HT reuptake inhibitor on extracellular 5-HT. In addition, the results in 5-HT$_{1B}$ knockout mice do not support a role of 5-HT$_{1B}$ heteroreceptors in the striatum on dopamine outflow in this brain area of mice.


*Keywords:* CP-93,129; 5-HT (5-hydroxytryptamine, serotonin); Selective reuptake inhibitor; Fluvoxamine; Caudate putamen; Autoreceptor; Heteroreceptor

## 1. Introduction

It has been well established that serotonin (5-hydroxytryptamine, 5-HT) modulates dopamine neurotransmission (Soubrie et al., 1984; Bonhomme et al., 1995). The striatonigral pathway is innervated by serotonergic afferents both at dopaminergic nerve terminals in the striatum and at dopaminergic cell bodies in the substantia nigra (Steinbusch, 1981) and neurochemical data reveal that striatal dopamine release is modulated by 5-HT neurons (Benloucif and Galloway, 1991; Yadid et al., 1994; Bonhomme et al., 1995; De Deurwaerdere et al., 1995).

Anatomical data have shown that 5-HT$_{1B}$ receptors are abundantly present in the caudate putamen and in the substantia nigra (Bruinvels et al., 1994; Sari et al., 1999). The distribution of 5-HT$_{1B}$ receptor mRNA suggests that this receptor is expressed predominantly on nerve terminals (Boschert et al., 1994). This is consistent with the notion that 5-HT$_{1B}$ receptors function as autoreceptors on 5-HT neurons and as heteroreceptors on non-5-HT neurons to control neurotransmitter release (Johnson et al., 1992; Cameron and Williams, 1994; Morikawa et al., 2000). A role for 5-HT$_{1B}$ receptors in the modulation of dopamine release is supported by microdialysis studies in rats (Benloucif et al., 1993; Galloway et al., 1993; Bentue-Ferrer et al., 1998; Ng et al., 1999), showing that 5-HT$_{1B}$ receptor agonists enhance dopamine release. Studying dopamine

* Corresponding author. Department of Biological Psychiatry, Academic Hospital Utrecht, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, The Netherlands. Tel.: +31-30-2509019; fax: +31-30-2129205.
*E-mail address:* h.g.m.westenberg@azu.nl (H.G.M. Westenberg).

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release in striatal synaptosomes of wildtype and in mice 5-$HT_{1B}$ knockout (KO) mice, Sarhan et al. (2000) reported that CP-93,129, a 5-$HT_{1B}$ receptor agonist, affected dopamine outflow in the wildtype but not in the 5-$HT_{1B}$ KO mice. Involvement of 5-$HT_{1B}$ receptors in dopamine release is also supported by other studies in 5-$HT_{1B}$ KO mice, showing altered dopamine neurotransmission (Scearce-Levie et al., 1999; Ase et al., 2000) and increased vulnerability to dopaminergic drugs such as cocaine (Lucas et al., 1997; Rocha et al., 1998).

Because 5-$HT_{1B}$ receptors function both as autoreceptors on 5-HT nerve terminals to reduce 5-HT levels in the striatum (Abellan et al., 2000, Knobelman et al., 2000) and as postsynaptic receptors, studies on the effects of a 5-$HT_{1B}$ receptor agonist on dopamine should take in account the effects of both receptor subtypes. In mice lacking 5-$HT_{1B}$ receptors, augmented 5-HT levels can be expected when 5-HT levels are increased by a 5-HT reuptake inhibitor. However, in 5-$HT_{1B}$ KO mice, no changes in striatal 5-HT levels were observed upon systemic administration of a 5-HT reuptake inhibitor (Knobelman et al., 2001), although activation of 5-$HT_{1A}$ autoreceptors may have limited the contribution of 5-$HT_{1B}$ autoreceptors.

The aim of the present study was to further explore the role of serotonergic intervention on striatal 5-HT and dopamine release. To this end, 5-HT and dopamine release was evaluated in wildtype mice with mice lacking 5-$HT_{1B}$ receptors using in vivo microdialysis.

## 2. Material and methods

### 2.1. Animals

In this study, male wildtype and 5-$HT_{1B}$ KO mice on a 129/SV genetic background were tested. The mice were housed eight per cage, kept on a 12-h light–dark cycle (6 a.m. on, 6 p.m. off) at constant room temperature (22±2 °C), controlled humidity (40–60%) and free access to food and water. At the time of the experiments, the mice were aged between 12 and 16 weeks with body weights between 25 and 35 g. The mice were bred in separate homozygous lines in the animal facilities, GDL, Utrecht, The Netherlands. Offspring from the two breeding lines were screened regularly to confirm their genotype. Due to the unexpected findings with 50 μM CP-93,129, wildtype and knockout mice from these groups were additionally screened and their genotypes were confirmed. The original wildtype and knockout mice were obtained from Dr. René Hen, Columbia University, New York, USA. For a detailed description of the generation of the 5-$HT_{1B}$ KO mice, see the study by Saudou et al. (1994). The studies were carried out in accordance with the European Community guidelines for the use of experimental animals and were approved by the ethical committee for animal research of the University Medical Center, Utrecht, The Netherlands.

### 2.2. Microdialysis procedure

Microdialysis probes were implanted in the dorsal striatum under chloral hydrate anaesthesia (400 mg/kg, i.p.) and lidocaine (2%) applied on the skull. For surgery, the mice were placed in a stereotaxic frame using a mouse adaptor (Kopf, Germany) with modified earbars. During surgery, mice were kept warm on a heating pad. A concentric microdialysis probe constructed in our lab with an AN Filtral 69 membrane, outer diameter 310 μm (Hospal, Uden, The Netherlands), was placed in the dorsal striatum according to the stereotaxic atlas of the mouse brain (Franklin and Paxinos, 1997). The coordinates were: AP +0.80, ML −1.7 mm from the bregma, DV −4.0 mm from the dura, with the tooth bar set at 0 mm. The active dialysis surface length of the membrane was 2 mm. The probe was secured in place with dental cement and two anchor screws in the skull. After surgery, the mice were injected with saline (0.5 ml, i.p.) to prevent dehydration and were housed individually in Plexiglas cages. Microdialysis experiments started 16–20 h after surgery. Ringer solution (147 mM NaCl, 2.3 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$) was perfused through the microdialysis probe at a flow rate of 1.13 μl/min using a high precision pump (Harvard PHD2000, Harvard Scientific, USA). Mouse swivels (Type 375/25, Instech Laboratories, USA) connected to PEEK-tubing (ID 0.005″, OD 0.020″) were used to allow unrestrained movements of the mice. Perfusion of the dialysis probes started after the lights were on and 3 h later samples were collected to obtain stable baseline values for the neurotransmitters. Samples were collected every 20 min in vials containing 7.5 μl acetic acid and stored at −80 °C until high-performance liquid chromatography (HPLC) analysis was done. At the end of the experiment, the mice were killed by cervical dislocation, the brains were removed and fixed in 4% formaldehyde solution. To verify the position of the probe, the brains were cut into 50 μm slices on a vibratome. In cases of improper probe placement, the data were excluded.

### 2.3. Drugs

The following drugs were used; CP-93,129 dihydrochloride (1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-*b*]pyridin-5-one, obtained from Tocris, UK), fluvoxamine maleate (donated by Solvay Pharmaceuticals, The Netherlands), ritanserin (Janssen Life Sciences Products, Belgium), *S*(+)-fenfluramine hydrochloride (RBI, Natick, MA, USA) and tetrodotoxin (Sigma, St. Louis, USA). All drugs except ritanserin were dissolved in distilled water and further diluted in Ringer solution to the final concentration on the day of the experiment. Ritanserin was first dissolved in a drop of acetic acid, further diluted in Ringer solution to the final concentration and the pH was adjusted to that of the Ringer solution.

### 2.4. HPLC-ECD analysis

5-HT and dopamine were analysed by HPLC with electrochemical detection (ECD). Samples (25 μl) were injected onto an Inertsil ODS-3 column (3 μM, 2.1×100 mm, Aurora Borealis, The Netherlands) using a Gilson pump and autosampler (Separations, The Netherlands). Detection was performed at 40 °C with an electrochemical detector (Intro, ANTEC Leyden, The Netherlands) set at a potential of 600 mV against an Ag/AgCl reference electrode. The signal was analysed using Gynkotek software. The mobile phase consisted of 5 g/l $(NH_4)_2SO_4$, 150 mg/l heptane sulphonic acid sodium salt, 0.5 g/l EDTA, 5% methanol, 30 μl/l triethylamine, 30 μl/l acetic acid, pH 4.6. Flow rate was 0.3 ml/min. The detection limit for 5-HT was 0.5 fmol/25 μl sample (signal to noise ratio 2). In the presence of 50 μM CP-93,129 in the dialysates, 5-HT levels could not be analysed due to interference of this concentration of CP-93,129 with the chromatographic separation of 5-HT.

### 2.5. Data analysis and statistics

The values for the last four samples before drug administration were averaged to calculate the basal levels of extracellular 5-HT and dopamine, uncorrected for probe recovery. Student's *t*-tests were used to compare basal 5-HT and dopamine values between the two genotypes. In the figures, all 5-HT or dopamine levels are expressed as percentages of basal levels ±S.E.M. Effects of drug treatment on these parameters were analysed using a repeated multivariate analysis of variance (ANOVA) with time as 'within' and treatment (or concentration) and genotype as 'between' factors. When appropriate, the data were broken down and paired comparisons were made. To test the concentration effects of fluvoxamine, area under the curve (AUC) values were calculated for the 200-min infusion period of vehicle (Ringer solution) and the different fluvoxamine concentration groups. AUC values were analysed using an ANOVA with concentration and genotype as 'between' subject factors. When appropriate, the data were broken down on genotype and post-hoc comparisons were made, using Bonferroni corrections. The significance level for all analyses was set at 5%. In the figures, the start of local infusion (time point zero) of drugs has been corrected for the dead volume of the dialysis system.

## 3. Results

### 3.1. Basal levels

Basal levels of extracellular 5-HT and dopamine levels in the dorsal striatum were not different between wildtype and 5-$HT_{1B}$ KO mice. Basal 5-HT levels were 4.0±0.3 fmol/sample in wildtype ($n$=51) and 5.0±0.6 fmol/sample in 5-$HT_{1B}$ KO mice ($n$=45). Basal dopamine levels were 181.3±14.6 fmol/sample in wildtype ($n$=51) and 183.1±15.9 fmol/sample in 5-$HT_{1B}$ KO mice ($n$=45).

### 3.2. Effects of fluvoxamine and CP-93,129 on 5-HT outflow

Local administration of fluvoxamine into the striatum increased 5-HT levels concentration dependently as shown in Fig. 1. Three concentrations of fluvoxamine were administered by reversed microdialysis. A repeated measures ANOVA indicated an effect of time×concentration [$F(27,333)=18.4$, $P<0.001$] of concentration [$F(1,37)=72.6$, $P<0.001$], but not of genotype [$F(1,37)=0.14$, $P=0.9$]. Fluvoxamine at concentrations of 0.1, 1.0 and 10 μM increased 5-HT about three-, six- and eight-fold, respectively. The concentration–response curve for fluvoxamine was similar in wildtype and 5-$HT_{1B}$ KO mice. Similar results were obtained by taking the AUC values as outcome variable (not shown in the figure). Post-hoc comparison (within genotype) showed that fluvoxamine con-

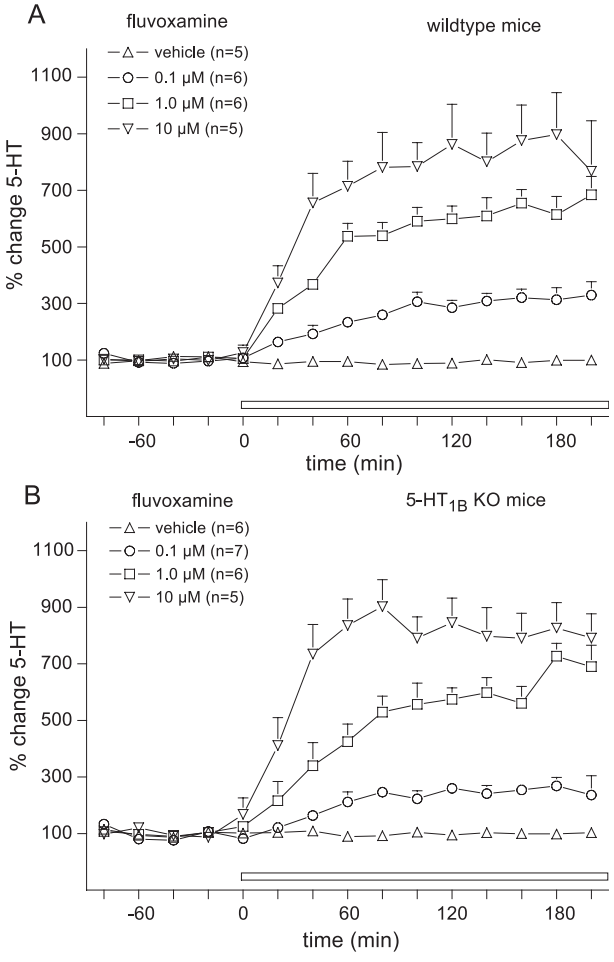


Fig. 1. Effects of local administration of fluvoxamine into the dorsal striatum on extracellular 5-HT. Data are expressed as the mean percent change from basal 5-HT levels ±S.E.M. Time course of the effects of fluvoxamine at concentrations of 0.1, 1.0 and 10 μM in wildtype mice (A) and 5-$HT_{1B}$ KO mice (B).

centration dependently increased 5-HT levels as indicated by significant effects of concentration between 1.0 and 10 μM fluvoxamine relative to vehicle ($P<0.001$) and between 0.1, 1.0 and 10 μM fluvoxamine ($P<0.01$), except for the 1.0 vs. 10 μM group in 5-$HT_{1B}$ KO mice ($P=0.062$).

Local administration of the 5-$HT_{1B}$ receptor agonist, CP-93,129, decreased 5-HT levels in wildtype, but not in 5-$HT_{1B}$ KO mice as shown in Fig. 2. Repeated measures ANOVA indicated an effect of treatment [$F(1,17)=6.2$, $P<0.05$] and of genotype [$F(1,17)=13.5$, $P<0.01$]. CP-93,129 (0.5 μM) reduced 5-HT levels in wildtype mice, but not in 5-$HT_{1B}$ KO mice ($P<0.05$). In wildtype mice, CP-93,129 (0.5 μM) reduced 5-HT to 51±9% of baseline as compared to vehicle [$F(1,10)=11.2$, $P<0.01$].

### 3.3. Effects of 5-HT on dopamine outflow

Local administration of the 5-HT releaser, fenfluramine, increased 5-HT and dopamine levels as shown in Fig. 3. For the effects of fenfluramine on 5-HT outflow, a repeated measures ANOVA indicated an effect of treatment [$F(1,18)=62.6$, $P<0.001$], but not of genotype [$F(1,18)=0.01$, $P=0.9$]. Fenfluramine (50 μM) as compared to vehicle, significantly increased 5-HT levels to the same extent in both genotypes ($P<0.001$). The mean maximum increases in 5-HT following fenfluramine were 1047±134% and 1122±167% in wildtype and 5-$HT_{1B}$ KO mice, respectively.

For the effects of fenfluramine on dopamine outflow, a repeated measures ANOVA indicated an effect of treatment [$F(1,17)=19.5$, $P<0.001$], but not of genotype [$F(1,17)=0.04$, $P=0.8$]. Fenfluramine (50 μM) as compared to vehicle, significantly increased dopamine levels to the same extent in wildtype ($P<0.01$) and 5-$HT_{1B}$ KO mice ($P<0.5$). After 80 min infusion, the dopamine outflow in response to fenflur-

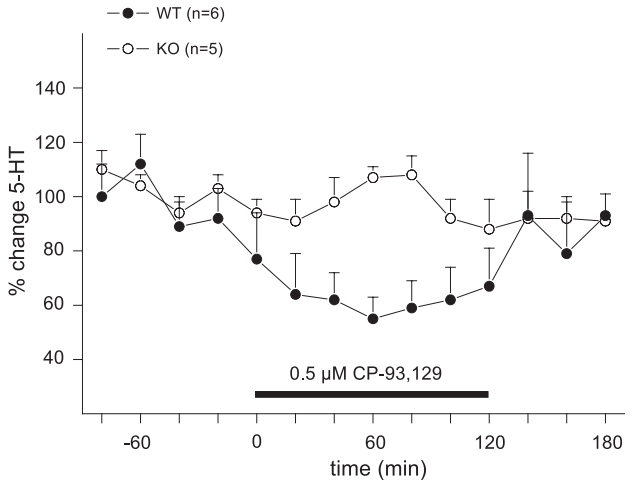


Fig. 2. Effects of local administration of the 5-$HT_{1B}$ receptor agonist, CP-93,129, into the dorsal striatum on 5-HT outflow in wildtype (WT) and 5-$HT_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level ±S.E.M. Time course of the effect of 0.5 μM CP-93,129 (black bar) administered for 120 min. For clarity, vehicle groups are not shown in the figure.

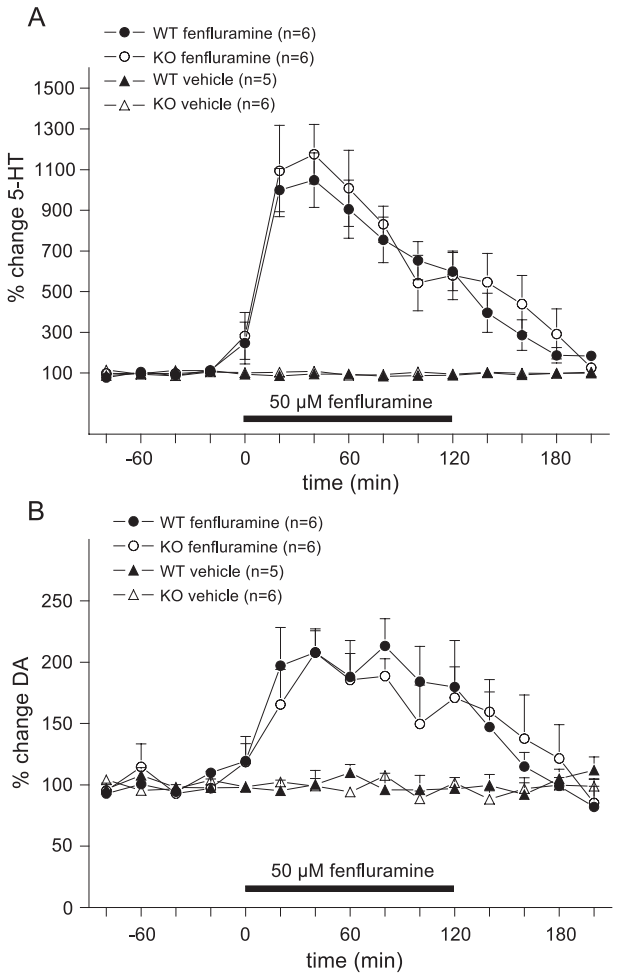


Fig. 3. Effects of the 5-HT releaser, fenfluramine, on striatal 5-HT and dopamine outflow in wildtype (WT) and 5-$HT_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level ±S.E.M. Time course of the effects of 50 μM fenfluramine applied locally into the striatum for 120 min (black bar) on 5-HT outflow (A) and dopamine outflow (B).

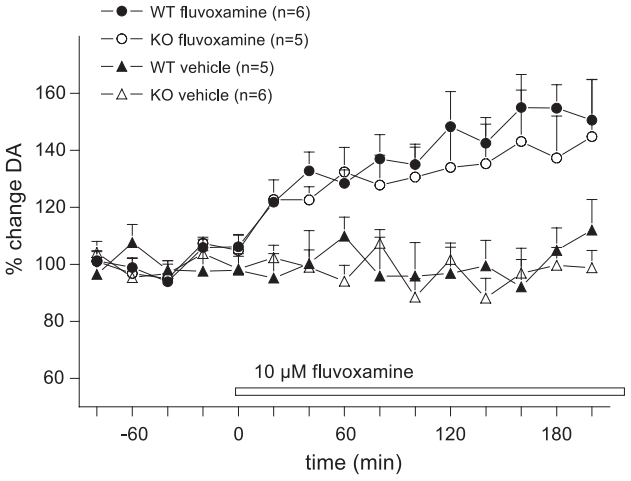


Fig. 4. Effects of fluvoxamine on dopamine outflow in wildtype (WT) and 5-$HT_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level ±S.E.M. Time course of the effects of 10 μM fluvoxamine (open bar) on striatal dopamine outflow.

amine was increased to a mean maximum of 213±15% and 188±14% in wildtype and 5-HT$_{1B}$ KO mice, respectively.

As shown in Fig. 1, 10 μM fluvoxamine increased 5-HT levels to ~800% of baseline. Fig. 4 shows the effect of 10 μM fluvoxamine on dopamine outflow. A repeated measures ANOVA indicated an effect of treatment [$F(1,18)=31.0$, $P<0.001$], but not of genotype [$F(1,18)=0.5$, $P=0.5$]. Fluvoxamine (10 μM) significantly increased dopamine outflow as compared to vehicle to the same extent in both genotypes, to a mean maximum of 148±13% in wildtype ($P<0.001$) and to 139±18% in 5-HT$_{1B}$ KO mice ($P<0.01$).

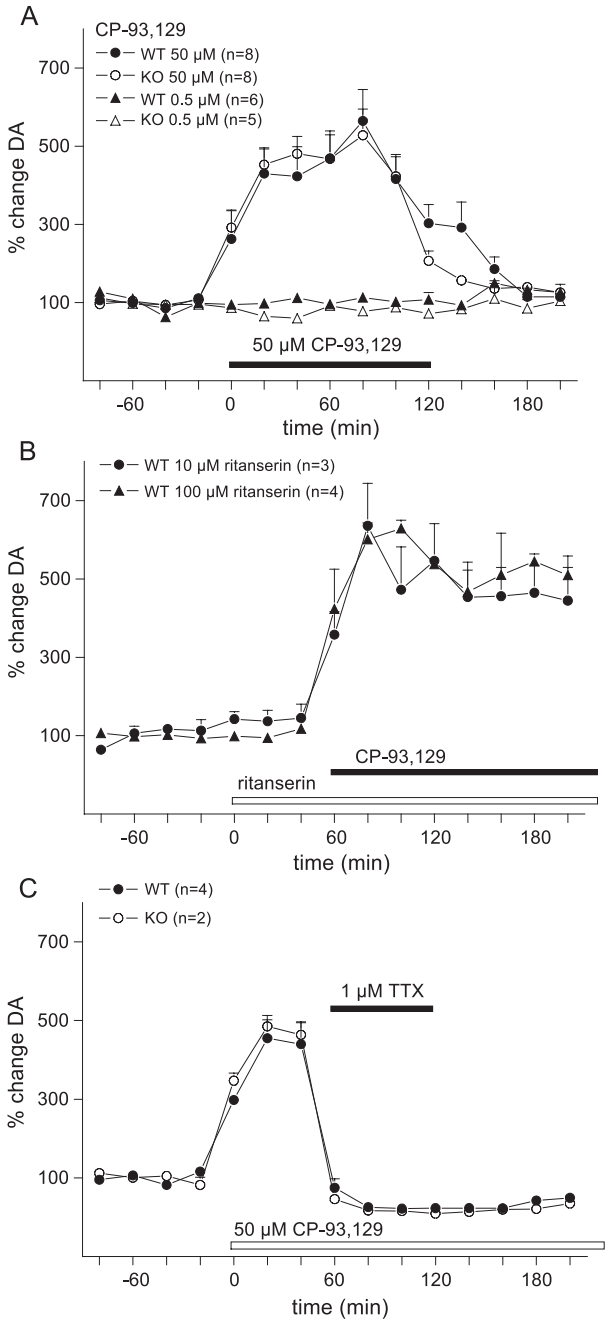


Fig. 5. Effects of local administration of the 5-HT$_{1B}$ receptor agonist, CP-93,129, to the dorsal striatum on dopamine outflow in wildtype (WT) and 5-HT$_{1B}$ KO mice (KO). Data are expressed as the mean percent change from basal level ±S.E.M. Time course of the effect of 0.5 and 50 μM CP-93,129 (black bar) administered for 120 min in wildtype and 5-HT$_{1B}$ KO mice (A). Time course of the effect of local infusion of 10 and 100 μM of the 5-HT$_{2A/2C}$ receptor antagonist, ritanserin (white bar), for 60 min followed by co-perfusion with 50 μM CP-93,129 (black bar) in wildtype mice (B). Time course of the effect of local infusion of 50 μM CP-93,129 co-perfused with tetrodotoxin (TTX, striped bar) for 60 min (C).

### 3.4. Effects of CP-93,129 on striatal dopamine outflow

Local administration of 50 μM CP-93,129 into the striatum increased dopamine levels, whereas 0.5 μM did not affect dopamine outflow as shown in Fig. 5A. A repeated measures ANOVA indicated an effect time×concentration [$F(16, 256)=7.9$, $P<0.001$], of concentration [$F(2,32)=55.6$, $P<0.001$], but not of genotype [$F(2,32)=0.5$, $P=0.98$]. CP-93,129 (50 μM) increased dopamine levels in 5-HT$_{1B}$ KO mice to the same extent as in wildtype mice when compared to vehicle ($P<0.001$). The CP-93,129-induced increases were 525±79% and 527±67% in wildtype and 5-HT$_{1B}$ KO, respectively. To test the involvement of 5-HT$_2$ receptors in the effect of CP-93,129, the 5-HT$_{2A/2C}$ receptor antagonist, ritanserin, was administered starting 60 min before CP-93,129 was co-perfused. In wildtype mice, the increase in striatal dopamine induced by 50 μM CP-93,129 was not affected by ritanserin (10 or 100 μM) as shown in Fig. 5B. To test the neuronal origin of the CP-93,129-induced dopamine release, the sodium potassium channel blocker, tetrodotoxin, was co-perfused with CP-93,129. As shown in Fig. 5C, tetrodotoxin (1 μM), co-perfused for 60 min, blocked the effect of 50 μM CP-93,129 on dopamine outflow in both genotypes. Dopamine levels were decreased to −80% of baseline levels. Comparison of 50 μM CP-93,129 and co-perfusion with tetrodotoxin indicated a significant treatment effect [$F(1,10)=20.1$, $P<0.001$].

## 4. Discussion

Consistently with other studies, we found no difference in basal extracellular levels of 5-HT and dopamine in the dorsal striatum of wildtype and those in 5-HT$_{1B}$ KO mice (Knobelman et al., 2001; Shippenberg et al., 2000). This suggests that, either 5-HT$_{1B}$ receptors do not affect 5-HT and dopamine release in the striatum under basal conditions, or that compensatory effects may have taken place during neurodevelopment.

No genotype difference was found for the effects on 5-HT levels following local administration of fluvoxamine into the striatum, suggesting that 5-HT$_{1B}$ autoreceptors do not limit these effects of the selective 5-HT reuptake inhibitor. This is in line with results of a previous study that showed a similar striatal 5-HT increase in wildtype and 5-HT$_{1B}$ KO mice upon systemic administration of a 5-HT reuptake inhibitor, although involvement of somatodendritic 5-HT$_{1A}$ autorecep-

tors may account for a lack of difference in this effect between the genotypes (Knobelman et al., 2001). In contrast to the effects in the striatum, we previously reported augmented 5-HT responses after locally applied fluvoxamine (1 μM) into the hippocampus and prefrontal cortex of 5-$HT_{1B}$ KO mice (de Groote et al., 2002a,b). Densities of 5-HT transporters are twice as high in the striatum as in hippocampal or cortical areas of mice (Bengel et al., 1997). Possibly, extracellular 5-HT is more efficiently removed by 5-HT transporters in the striatum than in other brain areas. This might also explain why basal levels of 5-HT did not differ between 5-$HT_{1B}$ KO and wildtype mice. Interestingly, Tao et al. (2000) reported an augmented 5-HT release in the nucleus accumbens of rats by (−)-penbutolol, a non-selective 5-$HT_{1B}$ receptor antagonist, in the presence of a high concentration of the selective 5-HT reuptake inhibitor, citalopram (100 μM). Possibly, terminal 5-$HT_{1B}$ autoreceptors inhibit 5-HT release only when 5-HT reuptake sites are maximally blocked in striatal areas, although non-selective effects of (−)-penbutolol on 5-HT cannot be excluded. Whether the 10 μM fluvoxamine used in this study blocked striatal 5-HT reuptake sites maximally is unclear, but in view of the observed eight-fold 5-HT increase it is unlikely that the absence of an augmented 5-HT response in 5-$HT_{1B}$ KO mice can be accounted for by insufficient reuptake blockade. Nevertheless, infusion of CP-93,129 (0.5 μM) reduced 5-HT levels in wildtype mice, indicating that 5-$HT_{1B}$ autoreceptors are functionally present in the striatum in this genotype.

We found that local administration of fenfluramine, a 5-HT releaser, and fluvoxamine, 5-HT reuptake inhibitor, into the striatum increased both 5-HT and dopamine levels, confirming results of previous studies that 5-HT modulates striatal dopamine outflow. However, since the effect of fenfluramine was also shown in rats after lesioning of the serotonergic pathways, fenfluramine may not be an ideal tool to study 5-HT and dopamine interactions in the striatum (De Deurwaerdere et al., 1995).

Several in vivo microdialysis studies have indicated that the 5-$HT_{1B}$ receptor agonist, CP-93,129, in concentrations ranging from 10-500 μM, increases dopamine outflow in rat striatum (Benloucif et al., 1993; Galloway et al., 1993; Bentue-Ferrer et al., 1998) and other brain structures (Iyer and Bradberry, 1996; Yan and Yan, 2001). This effect of CP-93,129 on dopamine outflow has been attributed to stimulation of 5-$HT_{1B}$ heteroreceptors. Furthermore, Sarhan and Fillion (1999) proposed a differential sensitivity of 5-$HT_{1B}$ auto vs. 5-$HT_{1B}$ heteroreceptors. Based on these findings we chose 50 μM CP-93,129 to study the contribution of 5-$HT_{1B}$ heteroreceptors on dopamine outflow. Surprisingly, however, dopamine release did not differ between the genotypes, suggesting that in mice, the dopamine increase induced by 50 μM CP-93,129 is not mediated through 5-$HT_{1B}$ heteroreceptors. In view of the present findings, it may be necessary to reinterpret the results of previous studies that attribute the effects of similar concentrations of CP-93,129 on striatal dopamine outflow in the rat to stimulation of 5-$HT_{1B}$ heteroreceptors (Benloucif et al., 1993; Galloway et al., 1993; Bentue-Ferrer et al., 1998). CP-93,129 also has affinity for 5-$HT_{1D}$, 5-$HT_{1A}$ and 5-$HT_{2C}$ receptor subtypes (Macor et al., 1990). Based on the affinity profile of CP-93,129 and the expression of 5-$HT_{2A/2C}$ receptors in the striatum we used ritanserin, a 5-$HT_{2A/2C}$ receptor antagonist, to investigate the possibility of a role of this receptor in the effects of CP-93,129 on dopamine release in the striatum. Co-perfusion with ritanserin did not attenuate the effect of CP-93,129 on dopamine outflow, suggesting that 5-$HT_{2A/2C}$ receptor subtypes are not involved. The effect of CP-93,129 on dopamine outflow was blocked by co-perfusion with tetrodotoxin, indicating that the dopamine release induced by CP-93,129 was of neuronal origin. There is circumstantial evidence that 5-$HT_3$ and 5-$HT_4$ receptor subtypes can also modulate dopamine outflow (Bonhomme et al., 1995; Lucas et al., 2001). CP-93,129 may exert its effect on dopamine outflow through stimulation of 5-$HT_3$ or 5-$HT_4$ receptors, although to our knowledge, affinity of CP-93,129 for these receptor subtypes has not been reported.

Although no evidence was found for a role of striatal 5-$HT_{1B}$ receptors in the release of dopamine outflow, it cannot be excluded that 5-$HT_{1B}$ receptors in other brain regions are implicated in striatal dopamine release. Enriched binding densities for 5-$HT_{1B}$ receptors are found in the substantia nigra and these receptors are thought to play a role in the regulation of dopamine neuronal activity. Interestingly, a microdialysis study of the substantia nigra showed a 4770% increase of dopamine outflow following 1 μM CP-93,129, suggesting that low concentrations of CP-93,129 may activate 5-$HT_{1B}$ heteroreceptors in this brain structure (Thorre et al., 1998). Another possible explanation for the lack of effect of striatal 5-$HT_{1B}$ heteroreceptors on dopamine outflow in the striatum could be that interactions between 5-HT and dopamine neurotransmission are not elicited by activation of striatal 5-$HT_{1B}$ receptors under basal conditions. There is evidence to suggest that stimulation of striatal 5-HT induced by low concentrations of 5-HT reuptake inhibitors, increases dopamine outflow only when the striato-nigral pathway is activated (Lucas et al., 2000).

In conclusion, 5-$HT_{1B}$ autoreceptors are functionally present in the mouse dorsal striatum, but do not play a significant role in the effects of a locally applied selective 5-HT reuptake inhibitor on 5-HT levels. Our results do not support a role of 5-$HT_{1B}$ heteroreceptors in the striatum on dopamine neurotransmission in this brain area.

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