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Deconstructing Antiobesity Compound Action: Requirement of Serotonin 5-HT_{2B} Receptors for Dexfenfluramine Anorectic Effects

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The now-banned anorectic molecule, dexfenfluramine, promotes serotonin release through a serotonin transporter-dependent mechanism, and it has been widely prescribed for the treatment of obesity. Previous studies have identified that 5-HT_{2B} receptors have important roles in dexfenfluramine side effects, that is, pulmonary hypertension, plasma serotonin level regulation, and valvulopathy. We thus investigated a putative contribution of 5-HT_{2B} receptors in dexfenfluramine-dependent feeding behavior in mice. Interestingly, the hypophagic response to dexfenfluramine (3–10 mg/kg) observed in wild-type mice (1–4 h) was eliminated in mice lacking 5-HT_{2B} receptors (5-$HT_{2B}^{-/-}$). These findings were further validated by the lack of hypophagic response to dexfenfluramine in wild-type mice treated with RS127445, a highly selective and potent antagonist (pKi = 8.22 ± 0.24). Using microdialysis, we observed that in 5-$HT_{2B}^{-/-}$ awake mice, the dexfenfluramine-induced hypothalamic peak of serotonin release (1 h) was strongly reduced (fourfold) compared with wild type. Moreover, using hypothalamic synaptosomes, we established the serotonergic neuron autonomous properties of this effect: a strong serotonin release was observed upon dexfenfluramine stimulation of synaptosome preparation from wild type but not from mice lacking active 5-HT_{2B} receptors. These findings strongly suggest that activation of presynaptic 5-HT_{2B} receptors is a limiting step in the serotonin transporter dependant-releasing effect of dexfenfluramine, whereas other serotonin receptors act downstream with respect to feeding behavior.

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INTRODUCTION

Eating disorders are an important health problem in developed countries (Leibowitz and Alexander, 1998; Vickers and Dourish, 2004). It is now well established that in the central nervous system, serotonin (5-hydroxytryptamine (5-HT)) is one major neurotransmitter that controls numerous physiological processes affecting food intake. Dexfenfluramine (*d*-fenfluramine (DF)) is an amphetamine congener that has been utilized therapeutically as a highly efficient anorectic molecule for the treatment of obesity (Garfield and Heisler, 2009). Until recently, DF was used in the treatment of obesity as well as had potential for the treatment of bulimia. However, clinical use of DF is associated with several unacceptable side effects, including primary pulmonary hypertension and valvular heart disease (Fitzgerald *et al*, 2000; Rothman *et al*, 2000; Launay *et al*, 2002) and this anorexigen was withdrawn from the market in 1997.

Mennini and colleagues (Garattini *et al*, 1986), in early 1980s, have performed a pioneering work describing initially the effect of DF and its derivatives on the release of 5-HT into nerve terminals by targeting the serotonin transporter (SERT). However, the impact of 5-HT receptors in the *in vivo* mechanism of action of this compound began only to be identified in the 1990s. Many studies have suggested that among the numerous 5-HT receptors, 5-HT_{1B} and 5-HT_{2C} receptors are of particular importance in mediating satiety signals in the hypothalamus (Heisler *et al*, 2006; Lam *et al*, 2008; Nonogaki *et al*, 2007): 5-HT_{1B} receptors inhibit neurons that promote hunger, whereas 5-HT_{2C} receptors activate neurons that promote satiety in the hypothalamic nuclei. However, activation of these

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receptors is not sufficient to fully explain the modulatory effects of 5-HT in feeding behavior. Other 5-HT receptors, such as 5-HT$_4$ or 5-HT$_6$, have already been suggested to participate in the control of energy intake (Conductier *et al*, 2005; Jean *et al*, 2007; Vickers and Dourish, 2004).

Administration of DF suppresses food intake in both animals and humans. Animal studies have reported either a complete or partial blockade of DF-induced hypophagia by the 5-HT$_2$ antagonist ritanserin (Goodall *et al*, 1993; Neill and Cooper, 1989), the 5-HT$_{2B/2C}$ antagonist SB-200646 (Bourson *et al*, 1996), or the 5-HT$_{2C}$ antagonist SB242084 (Clifton *et al*, 2000). Thus, the anorectic effect of DF has been proposed to be mediated by activation of 5-HT$_{2C}$ receptors (Vickers *et al*, 1999, 2001), whereas 5-HT$_{2B}$ receptors participate in the DF-induced pulmonary hypertension (Launay *et al*, 2002) and valvulopathy (Setola *et al*, 2005). As the hypophagic effect of DF (10 mg/kg) persisted in 5-HT$_{2C}$ receptor knockout (5-HT$_{2C}^{-/-}$) mice (Vickers *et al*, 1999), other 5-HT receptor subtypes must be involved in DF-induced hypophagia. The 5-HT$_{2B}$ receptor had been proposed to have a role in the regulation of food intake (De Vry and Schreiber, 2000), and a study showed an orexigenic effect of agonist of this receptor (Kennett *et al*, 1997). Furthermore, it has been recently reported that 5-HT regulates appetite, possibly via 5-HT$_{2B}$ receptors on hypothalamic neurons (Yadav *et al*, 2009). We had previously shown that another 5-HT releaser, the 'club drug' 3,4-methylene dioxy methamphetamine (MDMA, Ecstasy)-induced 5-HT release was dependent on presynaptic 5-HT$_{2B}$ receptors in raphe neurons (Doly *et al*, 2008).

However, in the absence of highly selective ligands, the precise set of 5-HT receptor subtypes mediating DF-induced effects remained uncertain. Furthermore, the role of 5-HT$_{2B}$ receptors in food intake has never been directly documented. In this study, we investigated a putative contribution of 5-HT$_{2B}$ receptors in DF-dependent feeding behavior. We performed *in vivo* experiments to compare acute and chronic effects of DF on 5-HT levels in the hypothalamus and food intake, using mutant mice lacking 5-HT$_{2B}$ receptors (knockout, 5-HT$_{2B}^{-/-}$), and validated these findings pharmacologically using a selective antagonist, RS127445.

MATERIALS AND METHODS

Animals

Male mice (8–12 weeks old) used in these experiments are in 129/SvPAS background for the 5-HT$_{2B}^{-/-}$ mice and C57/BL6J for the SERT$^{-/-}$ mice (Bengel *et al*, 1998). All animals were raised from heterozygous crosses. The 5-HT$_{2B}^{-/-}$ mice were reported (Nebigil *et al*, 2000) to display midgestation and postnatal partial lethality due to cardiac defects. The 5-HT$_{2B}$ receptor-mutant mice that survived the first postnatal week (about 50%) developed to adulthood with cardiac problems, although these become compensated with age (Jaffre *et al*, 2009), and 10–20 weeks old mice have no more detectable defects. The 5-HT$_{2B}$ receptor-mutant mice display no basal pulmonary phenotype; instead, they are resistant to inducers of pulmonary hypertension (Launay *et al*, 2002). Hence, the cardiovascular and pulmonary phenotypes are undetectable in living adult mice. Animals were individually housed in cages designed to allow the recording of feeding behavior. The temperature was maintained at 21 ± 1 °C, under 12/12 h light/dark. Food for laboratory mice (SAFE A03, France; 3200 kcal/kg, moisture 12%, proteins 21%, lipids 5%, carbohydrates 52%, fibers 4%, and mineral ash 6%) and water were available *ad libitum* except during experiments. Behavioral tests and animal care were conducted in accordance with the standard ethical guidelines (National Institutes of Health's 'Guide for the care and use of Laboratory animals,' and the European Communities Council European Communities Directive 86/609 EEC). All experiments involving mice were approved by the Ile de France regional ethics committee for animal experiments.

Mice Feeding Behavior After Starvation

Groups of 10 male mice starved for 18 h received an acute injection of 5-HT receptor antagonist, 60 min before dark cycle onset. At 30 min after the injection of antagonist drugs, mice received a single injection of anorectic drugs or their vehicle. Food was returned at the onset of the dark cycle, and food intake was recorded over the subsequent 4 h.

Mice Feeding Behavior Without Starvation

We used the procedure described in Rowland *et al* (2008). In brief, the palatable dessert comprised small (190 mg) fruit-flavored pellets of similar composition to chow (Fruit Crunchies; Bio-Serv-Phymep, Paris, France). Ten pellets were placed in glass beakers and were presented inside the home cage. During a familiarization period (7 days), the pellets were presented for 24 h, but thereafter for only 60 min per day (at midday around 1400 h) and 5 days per week for 2 weeks. The number of pellets consumed was counted daily. Anorectic drugs (3 or 10 mg/kg) or their vehicle were injected intraperitoneally (i.p.) 30 min before presentation of the dessert. Food intake was recorded for 60 min.

Drugs

Dexfenfluramine hydrochloride (Tocris, UK) and (+)-norfenfluramine hydrochloride (Sigma-Aldrich, France) were dissolved in 0.9% saline. RS127445 (Tocris), SB242084 (Sigma-Aldrich), and WAY161503 (Tocris) were dissolved in 0.9% saline containing 2.5% DMSO. The vehicle injections were 0.9% saline or 0.9% saline *plus* 2.5% DMSO according to the experiments. All injections (10 ml/kg) were i.p. administered. RS-127445 was found to have nanomolar affinity for the 5-HT$_{2B}$ receptor (pKi = 8.22 ± 0.24) and at least 1000-fold selectivity for this receptor when compared with numerous other receptors and monoamine uptake sites (Bonhaus *et al*, 1999). Based on the initial study showing that RS127445 completely (1, 0.5 and 0.1 mg/kg, i.p.) or partially (0.05 mg/kg) blocked MDMA-induced locomotion in wild-type (WT) mice and had no effect on basal locomotor activity or anxiety (Doly *et al*, 2008), we used the 0.5 mg/kg dose (i.p.) for this study. The acute pharmacological inhibition and genetic deletion of the 5-HT$_{2B}$ receptor gives rise to an identical phenotype *vis-à-vis*

DF-induced behavioral effect. For chronic DF treatments, the vehicle, or DF at the 'therapeutic' dose of 2.5 mg/kg/day, was delivered by miniosmotic pump (Alzet) implanted subcutaneously at the beginning of the experiment.

Cloning of Mouse 5-HT$_{2C}$ Receptor cDNA

A reverse transcriptase-polymerase chain reaction (RT-PCR)-based cloning strategy was used to obtain a full-length cDNA sequence of 5-HT$_{2C}$ receptor from mice whole brain, including 5′- and 3′-untranslated regions. Primer pairs (5′-AACTTCCCAATTCTCAGTTTGA; 3′-AAG AGATTTCCTGTAGGAAAGC) were selected from the published murine sequence (Accession: BC141085) and used in RT-PCR with total brain RNA. After electrophoretic separation of PCR mixture, a DNA band of approximately 1.4 kb size was excised from the gel. The resulting cDNA band was cloned into a pSG-5 plasmid (Invitrogen, San Diego, CA) and sequenced (Beckman Coulter, Takeley, UK).

Radioligand Binding Assays

Total brain or hypothalamus was homogenized with 25 ml of ice-cold buffer per g of wet tissue containing 50 mM Tris, 120 mM NaCl, and 5 mM $MgCl_2$ (pH 7.4). The homogenate was centrifuged for 20 min at 15 000 *g*. The pellet was resuspended and centrifuged under the same condition three times. For tissue culture, cells transfected with 10 μg of receptor plasmid using nanofectin kit (PAA Laboratories, France), exactly as specified by the manufacturer, were incubated overnight in serum-free DMEM. The next day, cells were harvested by scraping, pelleted, and resuspended in lysis buffer (50 mM Tris-HCl (pH 7.4)). Membranes were then pelleted by centrifugation and, after removal of supernatant, frozen at −80 °C (if not used immediately). Radioligand binding assays were set up in a 96-well plate (1 ml/well capacity) using 5 nM [^{3}H]mesulergine, [^{125}I]-GTI, or [^{3}H]citalopram (PerkinElmer Life and Analytical Sciences, Boston, MA) for 5-HT$_{2C}$ or 5-HT$_{2B}$, 5-HT$_{1B}$, and SERT, respectively, as previously described (Doly *et al*, 2008).

Microdialysis

Anesthetized animals were placed in a stereotaxic frame (D Kopf, Tujunga, CA), and microdialysis cannulas were implanted in the medial hypothalamus. Dialysis probes were equipped with a Cuprophan membrane (membrane length: 2 mm and diameter: 0.24 mm, 5000 Da cutoff; Microdialysis AB, Sweden). According to Paxinos and Franklin (2001), stereotaxic coordinates in mm were for medial hypothalamus-arcuate nuclei AP −1.5, L +0.3, DV −4.5 from bregma and top of the skull. Animals were kept in individual cages for a 7-day recovery. Microdialysis experiment was performed using awake mice as previously described (Doly *et al*, 2008). The 5-HT was quantified by HPLC and coulometric detection (see below).

Synaptosomes

Crude synaptosomes were prepared as described previously (Gray and Whittaker, 1962). Crude synaptosomes (0.20 mg of protein) were distributed onto cellulose mixed ester filters (0.65 μm pore size; Millipore, France) in a superfusion apparatus (four chambers) held thermostatically at 37 °C. The synaptosomes were layered onto the filters by aspiration from the bottom under moderate vacuum. Synaptosomes were preincubated at 37 °C for 5 min with Krebs-Henseleit buffer with the composition of (all in mM) NaCl (125), KCl (3), $CaCl_2$ (1.2), $MgSO_4$ (1.2), NaH_2PO_4 (1), $NaHCO_3$ (22), and glucose (10), and gassed with 95% O_2 and 5% CO_2 (pH 7.40). Then, DF or buffer was added, incubated at 37 °C and collected every 5 min for 45 min, and pooled and quantified by HPLC and coulometric detection.

5-HT Quantification

Samples were injected without any purification into an HPLC system that consists of a pump linked to an automatic injector (Agilent 1100, Palo Alto, CA), a reverse-phase column (Zorbax SB C18, 3.5 lm, 150 × 4.6 mm; Agilent Technologies), and a coulometric detector (Coulochem II; ESA, Chelmsford, MA) with a 5011 analytical cell to quantify DA or 5-HT. The first electrode was fixed at −100 mV and the second electrode at +300 mV. The gain of the detector was set at 50 nA. The signal of the second electrode was connected to an HP Chemstation for HPLC. The composition of the mobile phase was 50 mM NaH_2PO_4, 0.1 mM Na_2EDTA, 0.65 mM octyl sodium sulfate, and 14% (vv) methanol (pH 3.5). The flow rate was set at 1 ml/min.

Data Analysis

Data were analyzed by unpaired *t*-test using Welch's correction, and by one-way or two-way ANOVA. Bonferroni tests were used for *post hoc* comparisons.

RESULTS

Pharmacology Parameters

The lack of published pharmacological profile for 5-HT receptors in the mouse species has impaired the pharmacological approach and limited the understanding of the precise role of 5-HT receptor subtypes in mediating DF-induced hypophagia. After cloning the mouse 5-HT$_{2C}$ receptor cDNAs, we investigated the respective pharmacological profile of mouse 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. We selected the VNV and VNI edited isoforms of 5-HT$_{2C}$ receptor, as VNV has been shown to be the most prevalent in the hypothalamus (Wang *et al*, 2000) and in C57BL/6J and 129S1/SvImJ mice (Du *et al*, 2006). Table 1 summarizes the respective affinity (Ki) for 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors of commercially available compounds as determined by pharmacological characterization of membrane fraction of transiently transfected Cos7 cells. This study revealed that, among these compounds, many of the so-called selective 5-HT$_{2C}$ compounds have similar (if not higher) affinity for 5-HT$_{2B}$ receptors in mice. It is clear that a determination of the EC50 for particular coupling is needed to accurately assess the agonist selectivity for mouse 5-HT$_{2C}$ receptors. Only the antagonist RS-127445 is highly selective for 5-HT$_{2B}$ receptors. We took into account these data to define the

Table 1 Affinity Constants (pKi) for Different Binding Compounds to Mice 5-HT$_{2C}$-VNV, 5-HT$_{2C}$-VNI, and 5-HT$_{2B}$ Receptors Compared with Human 5-HT$_{2C}$-INI and 5-HT$_{2B}$ Receptors

Compounds	Mouse			Human	
	5-HT$_{2C}$ VNV	5-HT$_{2C}$VNI	5-HT$_{2B}$	5-HT$_{2C}$INI	5-HT$_{2B}$
Antagonists					
Mesulergine	8.84 ± 0.31	8.53 ± 0.21	7.81 ± 0.15	9.01 ± 0.01	8.39 ± 0.2
RS127445	5.44 ± 0.23	5.33 ± 0.45	8.22 ± 0.24	5.63 ± 0.05	8.51 ± 0.07
SB242084	6.88 ± 0.09	5.93 ± 0.27	6.07 ± 0.01	8.19 ± 0.22	6.36 ± 0.02
RS-102221	8.41 ± 0.45	7.72 ± 0.22	6.52 ± 0.08	8.01 ± 0.30	6.47 ± 0.02
SB215505	7.83 ± 0.60	7.24 ± 0.26	7.61 ± 0.21	7.40 ± 0.02	8.12 ± 0.01
SB206553	8.45 ± 0.19	8.21 ± 0.24	7.06 ± 0.41	8.24 ± 0.01	8.29 ± 0.04
Agonists					
WAY161503	7.18 ± 0.23	6.92 ± 0.11	7.84 ± 0.12	7.46 ± 0.05	7.28 ± 0.19
BW723C86	6.70 ± 0.15	6.78 ± 0.05	8.04 ± 0.15	6.90 ± 0.01	7.89 ± 0.01
Ro-600175	7.22 ± 0.15	7.35 ± 0.29	8.64 ± 0.14	7.72 ± 0.22	9.01 ± 0.13
CP809101	8.19 ± 0.07	7.92 ± 0.15	8.41 ± 0.18	8.35 ± 0.02	7.86 ± 0.18
DOI	7.46 ± 0.07	7.41 ± 0.24	7.87 ± 0.06	7.60 ± 0.02	8.29 ± 0.18
Norfenflu	6.39 ± 0.13	6.21 ± 0.07	6.76 ± 0.23	7.09 ± 0.62	8.02 ± 0.19
Dexfenflu	5.10 ± 0.10	5.06 ± 0.25	6.06 ± 0.17	5.66 ± 0.22	6.16 ± 0.17

Pharmacological determinations were performed, on transiently transfected cells expressing the relevant receptor, by heterologous competition using test compounds at concentrations spanning 6 orders of magnitude (in duplicate) and a final [^{3}H]mesulergine concentration of 5 nM. Values are presented as mean ± SEM, $n = 4$–9.

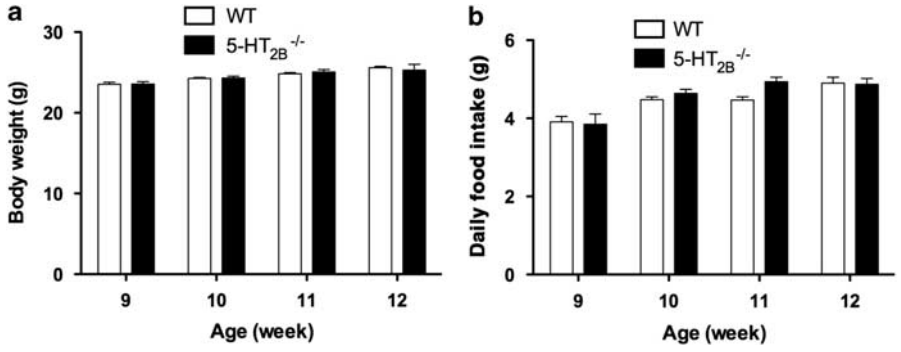

Figure 1 Basal body weight and daily food consumption in 5-HT$_{2B}^{-/-}$ mice. (a) Body weight and (b) daily food intake were recorded every day for 4 weeks. No significant differences were found between 5-HT$_{2B}^{-/-}$ and WT mice ($n = 6$–18, $p > 0.05$). Food intake was measured during 4 h in WT (WT, open bars) and 5-HT$_{2B}$ receptor impaired mice (5-HT$_{2B}^{-/-}$, black bars).

selectivity of the drugs used in this study and assess 5-HT$_{2C}$ agonists in 5-HT$_{2B}$ knockout mice for confirmation.

Basal Feeding Parameters in Mice Lacking 5-HT$_{2B}$ Receptors

To investigate a putative 5-HT$_{2B}$ receptor-mediated *in vivo* effect on feeding behavior, we first assessed basal feeding behavior in mice by recording the food intake every day over a 4-week period. Compared with WT mice, the 5-HT$_{2B}^{-/-}$ mice at 9–12 weeks of age showed no significant difference either in body weight (WT: 24.51 ± 0.47 g; 5-HT$_{2B}^{-/-}$: 24.93 ± 0.51 g; $n = 18$, $p > 0.05$; Figure 1a) or in daily food intake (WT: 4.14 ± 0.11 g; 5-HT$_{2B}^{-/-}$: 4.42 ± 0.14 g; $n = 6$–12, $p > 0.05$; Figure 1b).

Effect of Dexfenfluramine on Food Consumption in Mice with Inactive 5-HT$_{2B}$ Receptors

In a first series of experiments, mice were deprived for 18 h from food, but not water, the day before experiment. Then, they received a single injection of anorectic drug DF or its vehicle at 30 min before the onset of the dark cycle. Food was returned at the onset of the dark cycle, and food intake was recorded over the subsequent 4 h. Treatment with DF led to a dose-dependent decrease in food intake in WT mice: 3 and 10 mg/kg DF led to a 30 and 80% reduction after 1 h, respectively, that lasted 4 h. In contrast, the same dose totally failed to reduce significantly food ingested by 5-HT$_{2B}^{-/-}$ mice (Figure 2a). Similarly, a prior injection of a selective 5-HT$_{2B}$ receptor antagonist (RS127445; 0.5 mg/kg; pKi = 8.22 ± 0.24, see Table 1) in WT mice blocked completely

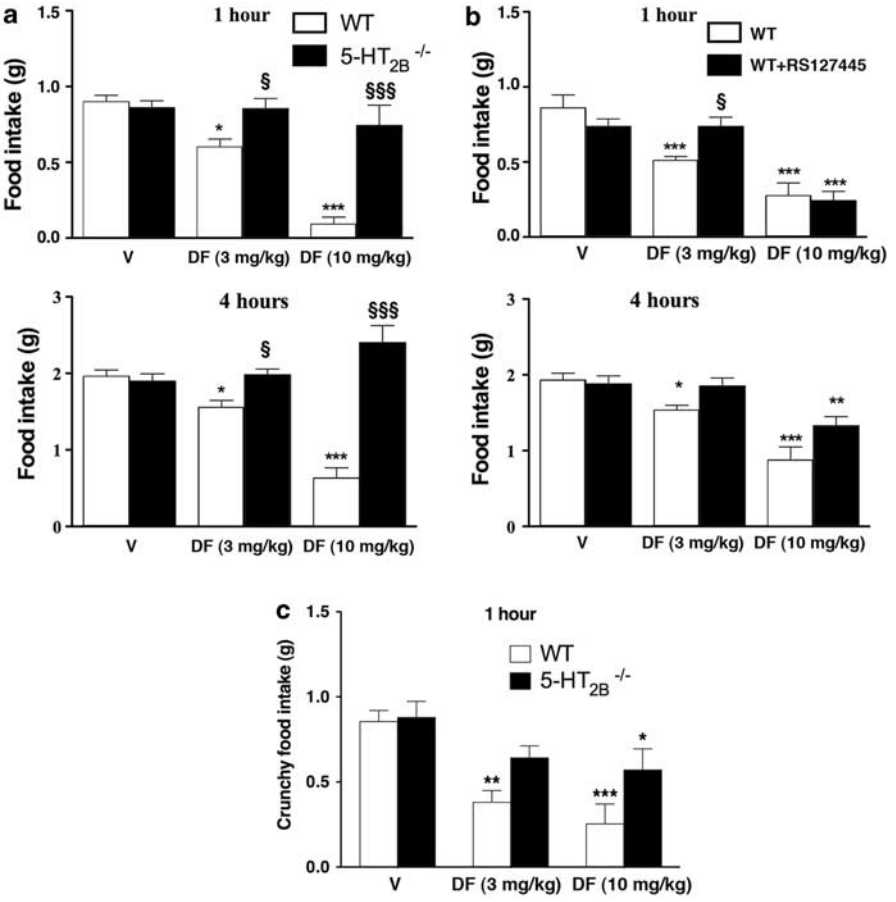

Figure 2 Dexfenfluramine and food consumption in 5-HT$_{2B}$ receptor impaired mice. (a) Cumulative food intake of 18 h starved WT and 5-HT$_{2B}^{-/-}$ mice. At 1 h, treatment with DF led to a dose-dependent decrease in food intake in WT mice. In contrast, DF failed to reduce food ingested by 5-HT$_{2B}^{-/-}$ mice at 3 and 10 mg/kg. Data were analyzed by two-way ANOVA with genotype and DF treatment as main factors. A significant interaction was observed ($F(2, 49) = 13.48$ $p < 0.0001$). The main effect of genotype ($F(1, 49) = 27.67$, $p < 0.0001$) and DF treatment ($F(2, 49) = 24.89$, $p < 0.0001$) was observed for food intake. Bonferroni tests were used for *post hoc* comparisons. Significant differences were found between DF (3 mg/kg) and vehicle-treated WT mice ($n = 7$ to 8, $p < 0.05$) but not 5-HT$_{2B}^{-/-}$ mice ($n = 10$ to 11, $p > 0.05$), and between DF (10 mg/kg) and vehicle-treated WT mice ($n = 8$–12, $p < 0.001$) but not 5-HT$_{2B}^{-/-}$ mice ($n = 7$–10, $p > 0.05$). At 4 h, treatment with DF led to a dose-dependent decrease in food intake in WT mice. In contrast, DF failed to reduce food ingested by 5-HT$_{2B}^{-/-}$ mice at 3 and 10 mg/kg. A significant interaction was observed ($F(2, 49) = 29.59$ $p < 0.0001$). The main effect of genotype ($F(1, 49) = 52.16$, $p < 0.0001$) and DF treatment ($F(2, 49) = 12.30$, $p < 0.0001$) was observed for food intake. Significant differences were found between DF (3 mg/kg) and vehicle-treated WT mice ($n = 7$ to 8, $p < 0.05$) but not 5-HT$_{2B}^{-/-}$ mice ($n = 10$ to 11, $p > 0.05$), and between DF (10 mg/kg) and vehicle-treated WT mice ($n = 8$–12, $p < 0.001$) but not 5-HT$_{2B}^{-/-}$ mice ($n = 7$–10, $p > 0.05$). (b) Cumulative food intake of 18 h starved WT vehicle and RS12445-treated mice. At 1 h, treatment with DF led to a dose-dependent decrease in food intake in WT mice. In contrast, DF failed to reduce significantly food ingested by RS12445-treated mice at 3 mg/kg. Data were analyzed by two-way ANOVA with RS and DF treatments as main factors. A significant interaction was observed ($F(2, 46) = 3.34$ $p < 0.05$). The main effect of DF treatment ($F(2, 46) = 27.14$, $p < 0.0001$) was observed for food intake. Bonferroni tests were used for *post hoc* comparisons. Significant differences were found between DF (3 mg/kg) and vehicle-treated WT mice ($n = 9$ to 10, $p < 0.001$) but not RS12445-treated mice ($n = 11$, $p > 0.05$), and between DF (10 mg/kg) and vehicle-treated WT mice ($n = 4$–10, $p < 0.001$) and RS12445-treated mice ($n = 7$–11, $p < 0.001$). At 4 h, treatment with DF led to a dose-dependent decrease in food intake in WT mice. DF failed to reduce significantly food ingested by RS12445-treated mice at 3 mg/kg and reduced at 10 mg/kg. The main effect of RS ($F(1, 46) = 6.99$, $p < 0.05$) and DF treatment ($F(2, 46) = 23.19$, $p < 0.0001$) was observed for food intake. Significant differences were found between DF (3 mg/kg) and vehicle-treated WT mice ($n = 7$–11, $p < 0.05$) but not RS12445-treated mice ($n = 11$, $p > 0.05$). Significant differences were found between DF (10 mg/kg) and vehicle-treated WT mice ($n = 4$–10, $p < 0.001$) and RS12445-treated mice ($n = 7$–11, $p < 0.01$). (c) Cumulative food intake of non-starved WT and 5-HT$_{2B}^{-/-}$ mice. After habituation to palatable dessert, the number of pellets consumed 30 min after injection was recorded for 60 min in freely fed mice. Again, treatment with DF led to a dose-dependent decrease in food intake in WT mice. In contrast, DF failed to reduce significantly food ingested by 5-HT$_{2B}^{-/-}$ mice at 3 mg/kg and reduced at 10 mg/kg to a lower extent. Data were analyzed by two-way ANOVA with genotype and DF treatment as main factors. The main effect of genotype ($F(1, 36) = 6.39$, $p < 0.05$) and DF treatment ($F(2, 36) = 12.14$, $p < 0.0001$) was observed for food intake. Bonferroni tests were used for *post hoc* comparisons. Significant differences were only found between DF (3 mg/kg) and vehicle-treated WT mice ($n = 6$, $p < 0.01$) but not vehicle-treated 5-HT$_{2B}^{-/-}$ ($n = 8$, $p > 0.05$), and between DF (10 mg/kg) and vehicle-treated WT mice ($n = 6$, $p < 0.001$) and vehicle-treated 5-HT$_{2B}^{-/-}$ ($n = 8$, $p < 0.05$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with vehicle groups; $^{\S}p < 0.05$, $^{\S\S\S}p < 0.001$ compared with the WT group. All results are expressed as mean ± SEM.

the anorectic effect of 3 mg/kg DF, and partially the effect of 10 mg/kg DF (Figure 2b).

In order to eliminate putative bias because of animal starvation, we used mice habituated to eat palatable dessert that comprised small (190 mg) fruit-flavored pellets. Mice adapted in about 1 week to avidly eat the dessert, averaging 5 crunchies (0.95 g) in 60 min of free access. Again, treatment with DF led to a dose-dependent decrease in food intake in WT mice: 3 and 10 mg/kg DF led to a 55 and 71% reduction after 1 h, respectively. In contrast, DF reduced,

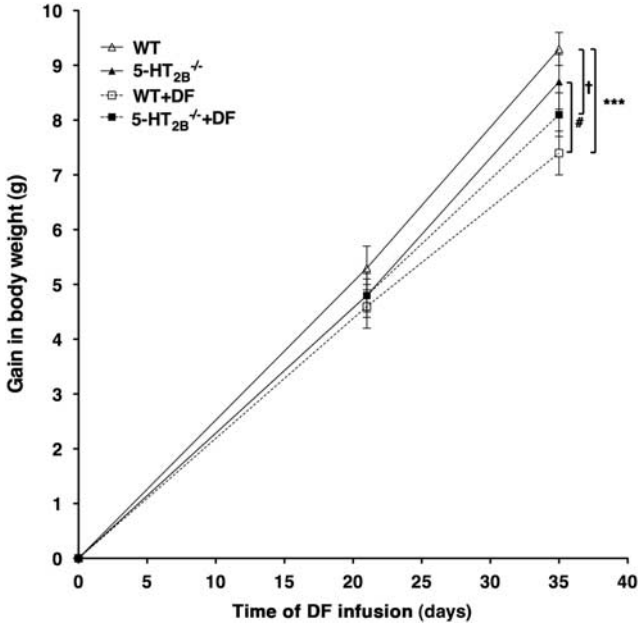

Figure 3 Chronic dexfenfluramine and body weight in 5-HT$_{2B}^{-/-}$ mice. Body weight was recorded every day for 5 weeks in 8 week-old mice exposed to 2.5 mg/kg/day of DF infused via miniosmotic pumps. The weight of the mice at the beginning of treatment was: WT 25.77 ± 1.3 g; WT-DF 24.7 ± 1.2 g; 5-HT$_{2B}^{-/-}$ 23.9 ± 1.1 g; 5-HT$_{2B}^{-/-}$-DF 23.1 ± 1.6 g. DF had a significant effect in reducing the weight gain in WT mice after 5 weeks of treatment, but not in 5-HT$_{2B}^{-/-}$ mice. Data were analyzed by two-way ANOVA (repeated measures) with genotype or DF treatment and time as main factors. The main effect of genotype or DF treatment ($F(3, 108) = 3.87$, $p < 0.05$) and time ($F(2, 108) = 690.4$, $p < 0.0001$) was observed for gain in body weight. Bonferroni tests were used for *post hoc* comparisons. Significant differences were found between DF and vehicle-treated WT mice ($n = 10$, $p < 0.001$) but not between DF and vehicle-treated 5-HT$_{2B}^{-/-}$ ($n = 10$, $p > 0.05$). ***$p < 0.001$ DF-treated WT mice compared with the WT saline group, $^{\dagger}p < 0.05$ DF-treated 5-HT$_{2B}^{-/-}$ mice compared with the WT saline group, and $^{\#}p < 0.05$ DF-treated WT mice compared with the 5-HT$_{2B}^{-/-}$ saline group. All results are expressed as mean ± SEM.

but non-significantly, food ingested by 5-HT$_{2B}^{-/-}$ mice at 3 mg/kg and reduced only by 35% food ingested by 5-HT$_{2B}^{-/-}$ mice at 10 mg/kg after 1 h (Figure 2c).

Chronic Effect of Dexfenfluramine on Food Consumption in Mice Lacking 5-HT$_{2B}$ Receptors

In order to further document the anorectic effects of DF, we evaluated the result of chronic administration of DF (2.5 mg/kg/day) using a mini osmotic pump for 5 weeks in 8 week-old mice. Interestingly, DF had a significant effect in reducing the weight gain only in WT mice (6%) after 5 weeks of treatment, but not in 5-HT$_{2B}^{-/-}$ mice (Figure 3). Again, this experiment confirmed the absence of response to anorectic effect of DF in the absence of 5-HT$_{2B}$ receptors.

Presynaptic Effects of Dexfenfluramine

We had previously shown that another 5-HT releaser, MDMA, induced 5-HT release in a 5-HT$_{2B}$ receptor-dependent manner (Doly *et al*, 2008). In order to investigate the putative contribution of 5-HT$_{2B}$ receptors in brain responses to DF, we first compared DF-induced changes in 5-HT extracellular concentrations in medial nuclei of hypothalamus by microdialysis in awake WT and 5-HT$_{2B}^{-/-}$ mice (Figure 4a and b). In WT mice, DF (10 mg/kg) induced a 35-fold increase in extracellular 5-HT levels in the hypothalamus within 160 min, an effect that was strongly reduced (about fourfold) in 5-HT$_{2B}^{-/-}$ mice (Figure 4a). In spite of this striking effect, basal levels of extracellular 5-HT were not significantly different among genotypes (Figure 4b).

Using a superfused mouse hypothalamus synaptosome preparation, we then asked whether DF-induced 5-HT release *in vivo* (ie, microdialysis studies) was 5-HT$_{2B}$ receptor dependent in a cell-autonomous fashion. As shown in Figure 4c, DF (0.5 and 1 μM) caused a dose-dependent (2.4- and 5-fold, respectively) increase in synaptosomal 5-HT release compared with saline in WT synaptosomes. In contrast, DF did not significantly increase 5-HT release over baseline levels in synaptosomes from either 5-HT$_{2B}^{-/-}$ or WT mice in the presence of 100 nM RS-127445. Notably, basal synaptosomal 5-HT release was similar for WT and 5-HT$_{2B}^{-/-}$ synaptosomes (7.1 ± 0.6 *vs* 7.7 ± 1.0 fmoles/sample). These data show that activation of 5-HT$_{2B}$ receptors in serotonergic nerve-ending particles is required for DF-induced SERT-dependent 5-HT release, supporting a cell autonomous effect.

SERT Contribution to Anorexic Effects of Dexfenfluramine

Knowing that the effects of DF on extracellular 5-HT are controlled by SERT, we tested food intake in SERT$^{-/-}$ mice after DF injection. Treatment with 10 mg/kg DF led to a decrease in food intake in WT but not in SERT$^{-/-}$ mice (Figure 5a). This result, similar to that observed in 5-HT$_{2B}^{-/-}$ mice, confirmed the strict dependence of DF-induced 5-HT release on both 5-HT$_{2B}$ receptor and SERT. In addition, we verified that the SERT expression was not modified in the hypothalamus of 5-HT$_{2B}^{-/-}$ mice (Table 2 and Figure 5b).

Effect of 5-HT$_{2C}$ Receptor Agonists on Food Consumption in 5-HT$_{2B}^{-/-}$ Mice

As many studies have suggested that among 5-HT receptors, the 5-HT$_{2C}$ receptors are of particular importance in DF-mediated satiety signals in the hypothalamus, we wondered if 5-HT$_{2C}$ receptor signaling pathway was affected in 5-HT$_{2B}^{-/-}$ mice. Treatment with the 5-HT$_{2C}$ agonist, WAY161503 (10 mg/kg; pKi = 7.18 ± 0.23 for 5-HT$_{2C}$ receptors, see Table 1), led to a milder, but significant, decrease in food intake compared with DF (Figure 6a) in both WT and 5-HT$_{2B}^{-/-}$ mice (50 and 60% after 1 h, respectively). It thus appears that the anorectic effects of 5-HT$_{2C}$ receptor agonist remain in mice lacking 5-HT$_{2B}$ receptor. In addition, no change in 5-HT$_{2C}$ receptor expression in hypothalamus was revealed by radioligand binding assay in 5-HT$_{2B}^{-/-}$ mice compared with WT mice (Table 2 and Figure 6b).

Nordexfenfluramine and Food Consumption in 5-HT$_{2B}^{-/-}$, SERT$^{-/-}$, and SB242084-Treated WT Mice

As the main DF metabolite, NDF, can stimulate the release of 5-HT or norepinephrine and is also a potent 5-HT$_{2B/2C}$ agonist (see Table 1 and Rothman and Baumann, 2002),

we compared its effect on WT and 5-HT$_{2B}^{-/-}$ mice. Strikingly, treatment with 5 mg/kg NDF also led to a significant decrease in food intake in both WT and 5-HT$_{2B}^{-/-}$ mice, but at a significantly lower efficacy for 5-HT$_{2B}^{-/-}$ mice (65 and 30% after 1 h, respectively, Figure 7a). This result suggested that NDF-induced hypophagia may not solely be mediated by the 5-HT releaser effect of NDF. In order to confirm this result, we compared NDF effect on WT and SERT$^{-/-}$ mice. Treatment with 5 mg/kg NDF also led to a milder decrease in food intake in SERT$^{-/-}$ than in WT mice (73 and 40% after 1 h, respectively), similar to the results obtained in 5-HT$_{2B}^{-/-}$ mice (Figure 7b). These results strongly suggested that NDF could directly stimulate 5-HT$_{2C}$ receptor. However, pretreatment of WT mice with the 5-HT$_{2C}$ receptor antagonist, SB242084 (0.5 mg/kg), failed to block the NDF-induced hypophagia (Figure 7c). Together, these data suggest that NDF not only acts as a 5-HT releaser, but also as a direct potent agonist or antagonist at unidentified pharmacological target, which may include 5-HT$_7$ or adrenergic α_{2C} receptors (Setola *et al*, 2003).

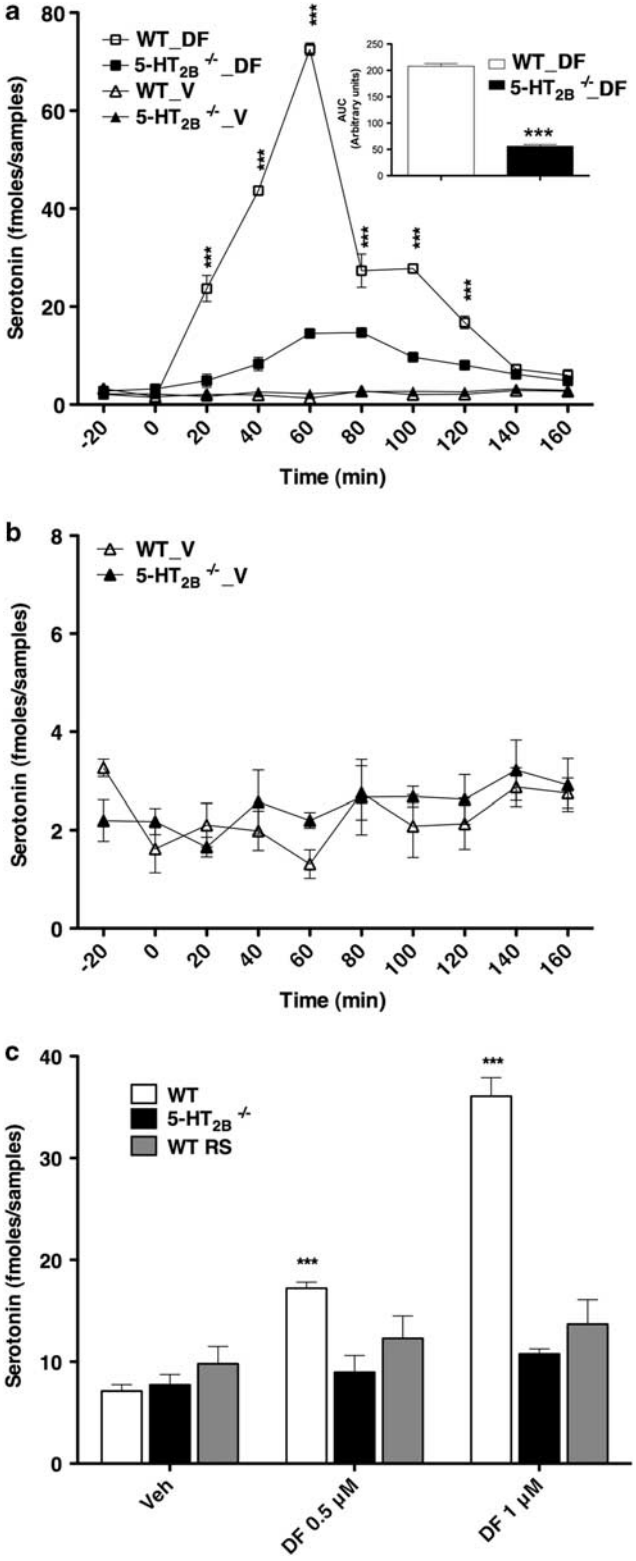

Figure 4 Releasing properties of DF. (a) Effect of DF (10 mg/kg, i.p.) or saline (V) injection on 5-HT concentrations in dialysates from the medial hypothalamic nuclei of WT or 5-HT$_{2B}^{-/-}$ awake mice. After 3 h of equilibration, DF or saline solutions were injected at zero time (0). Data ($n=3$–5 per group) were analyzed by two-way ANOVA (repeated measures) with genotype and treatment as main factors. A significant interaction was observed ($F(27,108)=80.7$, $p<0.0001$). The main effect of genotype and treatment was observed for 5-HT levels ($F(3,108)=1116$, $p<0.0001$). (b) Higher scaling of (a) showing the absence of difference in basal 5-HT levels between WT and 5-HT$_{2B}^{-/-}$ mice. (c) DF (0.5 or 1 µM) induced 5-HT release from superfused midbrain synaptosome preparation of WT mice, whereas it had no effect on 5-HT$_{2B}^{-/-}$ mice synaptosomes. Data ($n=4$–5 per group) were analyzed by two-way ANOVA with genotype or RS and DF dose as main factors. A significant interaction was observed ($F(4,33)=118.1$ $p<0.0001$). The main effect of genotype ($F(2,33)=204.4$, $p<0.0001$) and dose ($F(2,33)=227.9$, $p<0.0001$) was observed for 5-HT levels. Bonferroni tests were used for *post hoc* comparisons. ***$p<0.001$ compared with the respective saline groups. All results are expressed as mean ± SEM.

DISCUSSION

The present data show that although 5-HT$_{2B}^{-/-}$ mice display no apparent difference in the regulation of energy homeostasis as revealed by daily food intake or body weight, these mice can help to understand the basic mechanisms involved in feeding behavior and their relation to the mode of action of antiobesity compounds, that is, the presynaptic mechanisms controlling their releasing properties.

In this study we identified, for the first time, that anorectic responses to DF are strictly dependent on 5-HT$_{2B}$ receptor activity: in 5-HT$_{2B}^{-/-}$ mice, both acute and chronic DF anorectic effects are abolished. It has to be noticed that the acute data were obtained in either food-deprived or palatable food-habituated animals, and that each model may introduce some specific bias in the results. Furthermore, the observation that the 5-HT$_{2B}$ receptor selective antagonist RS127445 similarly inhibits the acute action of DF in at least a low dose of DF in WT mice excludes strong compensatory or developmental events in these mice. Previous studies have identified 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors in DF action (Heisler *et al*, 2002; Vickers *et al*, 1999, 2001). Nevertheless, DF had only a blunted effect on food intake in 5-HT$_{2C}^{-/-}$ mice at 3 mg/kg and identical effect at higher doses (10 mg/kg) compared with WT mice (Vickers *et al*, 1999). The action of either DF or Ro 60-0175 (a 5-HT$_{2B/2C}$ agonist, see Table 1), challenged with the

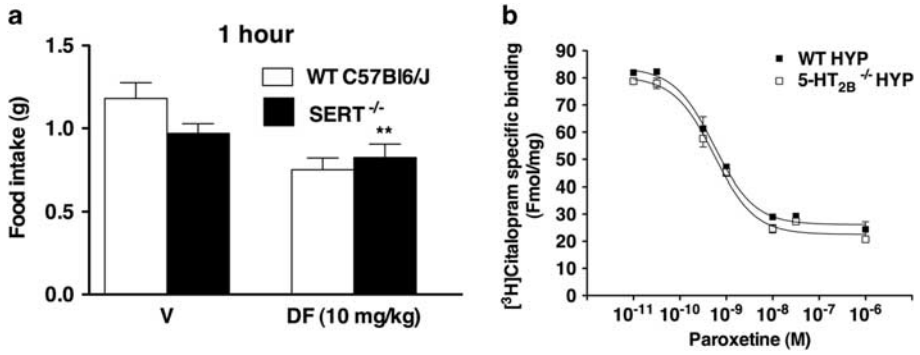

Figure 5 Dexfenfluramine and food consumption in SERT$^{-/-}$ mice. (a) Food intake was measured in C57Bl6/J WT (WT, *open bars*) and SERT$^{-/-}$ (black bars) mice treated with DF at 10 mg/kg. There was a significant effect of DF (DF simple effect, $F(1, 33) = 13.49$, $p = 0.0008$; $n = 8$–10). Bonferroni tests were used for *post hoc* comparisons. $^{**}p < 0.01$, compared with the vehicle group. All results are expressed as mean ± SEM. (b) Putative modifications in SERT expression in hypothalamus (HYP) was evaluated by [^{3}H]citalopram binding and competition with Paroxetine. The graph is representative of three independent experiments performed in triplicates.

Table 2 Maximum Binding Capacity (Bmax in fmoles/mg of Proteins) Determined for Different Molecules in Various Brain Regions in WT Mice Compared with 5-HT$_{2B}^{-/-}$ Mice

	WT	5-HT$_{2B}^{-/-}$
Total brain 5-HT$_{1B}$	25.5 ± 1.9	26.7 ± 2.1
Hypothalamus SERT	92.7 ± 3.1	93.1 ± 3.5
Hypothalamus 5-HT$_{2C}$	9.9 ± 0.9	11.9 ± 0.9

Pharmacological determinations were performed by heterologous competition in duplicate. Values are presented as mean ± SEM, $n = 4$.

5-HT$_{2C}$ receptor antagonist SB 242084, confirmed that only some of the alterations in feeding behavior are mediated by an action at 5-HT$_{2C}$ receptors (Hewitt *et al*, 2002). Stimulation of more than one postsynaptic 5-HT receptor subtype following DF treatment was proposed to be necessary for a reduction in feeding rate. The 5-HT$_{2C}^{-/-}$ mice are less sensitive to the hypophagic effects of the nonselective 5-HT receptor agonist m-chlorophenyl-piperazine (mCPP), but are more sensitive to the selective 5-HT$_{1B}$ receptor agonist CP-94 253 than WT controls (Dalton *et al*, 2006), supporting the presence of adaptive changes in these mice. Similarly, the hypophagic effects of DF are blocked in 5-HT$_{1B}^{-/-}$ mice (Lee *et al*, 2004; Lucas *et al*, 1998). However, pretreatment of WT mice with 5-HT$_{1B}$ receptor antagonists did not reproduce the effects of DF on feeding behavior observed in 5-HT$_{1B}^{-/-}$ mice (Lee *et al*, 2004). The current evidence suggests that adaptive modifications also occur in 5-HT$_{1B}^{-/-}$ mice that may contribute to their reduced response to DF (Lee *et al*, 2004), that is, 5-HT$_{2C}$ receptor effect reduction (Clifton *et al*, 2003). In contrast, in 5-HT$_{2B}^{-/-}$ mice, the DF (3 mg/kg) anorectic effect is totally abolished, and the selective 5-HT$_{2B}$ receptor antagonist, RS127445, also blocks the action of DF in WT mice. In addition, the absence of detectable changes in either global brain 5-HT$_{1B}$ receptor expression, in hypothalamic SERT, or in 5-HT$_{2C}$ receptor expression (Table 2), together with similar hypophagic response to WAY161503 in WT as in 5-HT$_{2B}^{-/-}$ mice (here selective 5-HT$_{2C}$ agonists), support a lack of major compensatory mechanisms in 5-HT$_{2B}^{-/-}$ mice. Our result using the 5-HT$_{2C}$ receptor agonist WAY-161503 in 5-HT$_{2B}^{-/-}$ mice confirm the participation of 5-HT$_{2C}$ receptors in feeding behavior, as observed in humans during the recent clinical trial for another 5-HT$_{2C}$ agonist lorcaserin (Smith *et al*, 2010).

The combined unaltered expression and uptake activity of SERT in 5-HT$_{2B}^{-/-}$ mice (Doly *et al*, 2008), including hypothalamus, together with the 5-HT$_{2B}$ receptor mRNA expression in both mouse raphe nucleus and primary culture of serotonergic neurons (Bonaventure *et al*, 2002; Doly *et al*, 2008; Launay *et al*, 2006; Regard *et al*, 2008), led us to hypothesize that 5-HT$_{2B}$ receptors regulate presynaptically DF-induced and SERT-dependent 5-HT release. Our microdialysis experiments show convincingly that DF induces a robust increase in extracellular 5-HT levels in WT hypothalamus, an effect that is strongly reduced in 5-HT$_{2B}^{-/-}$ mice, also supporting the need of this receptor for sustained 5-HT release. As hypothalamic ascending serotonergic projections arise from the dorsal raphe nuclei, and given that raphe neurons are the source of 5-HT release in the brain, this result supports the notion that the 5-HT$_{2B}$ receptor acts presynaptically in serotonergic raphe neurons.

The 5-HT release from serotonergic neurons is controlled through complex and multiple neural inputs to 5-HT cell bodies and nerve terminals (Adell *et al*, 2002; Sharp *et al*, 2007). Thus, indirect regulation of DF-induced 5-HT release, by other afferent inputs expressing 5-HT$_{2B}$ receptor, could also explain the lack of 5-HT release seen *in vivo* in 5-HT$_{2B}^{-/-}$ or RS127445-treated mice. Alternatively, 5-HT$_{2B}$ receptors were formerly known as 5-HT$_{2F}$ because of their abundant expression in the stomach fundus; a logical localization for their affect on hunger/satiety. Thus, DF could act at peripheral 5-HT release, via peripheral 5-HT$_{2B}$ receptors, which would influence the activity of satiety circuitry feeding into the brain, including raphe-hypothalamus 5-HT pathways. Although we cannot strictly rule out some peripheral or central postsynaptic contribution of 5-HT$_{2B}$ receptors on DF anorectic effects, our synaptosome experiment demonstrates a serotonergic raphe neurons autonomous contribution of 5-HT$_{2B}$ receptors for DF-dependent 5-HT release. The blockade of DF-induced 5-HT release by the 5-HT$_{2B}$ receptor antagonist, RS-127445, further establishes this finding. To our knowledge,

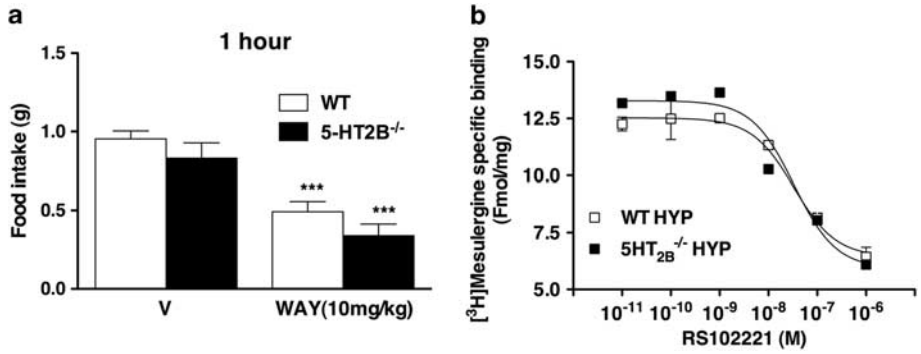

Figure 6 Effect of 5-HT$_{2C}$ receptor agonists on food consumption in 5-HT$_{2B}^{-/-}$ mice. (a) Food intake was measured in WT (*open bars*) and 5-HT$_{2B}^{-/-}$ (*black bars*) mice treated with WAY161503 at 10 mg/kg (WAY simple effect, $F(1, 42) = 45.03$, $p < 0.0001$; $n = 10$–13). Bonferroni tests were used for *post hoc* comparisons. ***$p < 0.001$ compared with the vehicle group. All results are expressed as mean ± SEM. (b) Putative modifications in 5-HT$_{2C}$ receptor expression in hypothalamus (HYP) was evaluated by [^{3}H]mesulergine binding and competition with RS102221. The graph is representative of three independent experiments performed in triplicates.

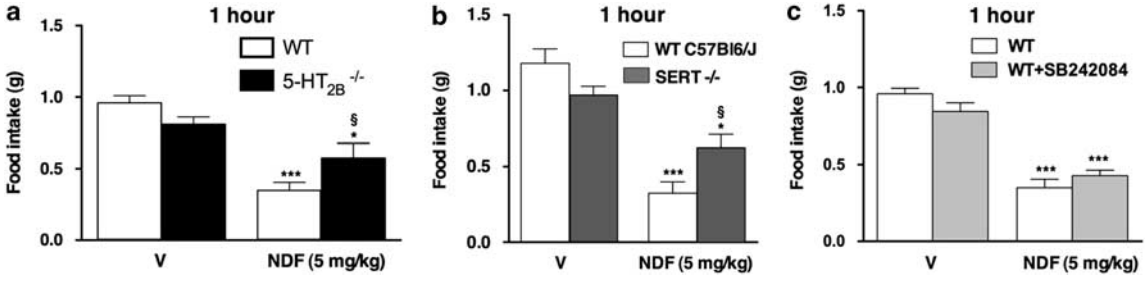

Figure 7 Nordexfenfluramine and food consumption in 5-HT$_{2B}^{-/-}$, SERT$^{-/-}$, and SB242084-treated WT mice. (a) Treatment with 5 mg/kg NDF led to a significant decrease in food intake after 1 h in WT and 5-HT$_{2B}^{-/-}$ mice, although to a lower extent (NDF simple effect, $F(1, 34) = 31.64$, $p < 0.0001$; $n = 9$ to 10). (b) Similarly, treatment with 5 mg/kg NDF also led to a significant decrease in food intake after 1 h in WT and SERT$^{-/-}$ mice to a lower extent (NDF simple effect, $F(1, 24) = 38.81$, $p < 0.0001$; $n = 5$–10). (c) In contrast, pretreatment of WT mice with the 5-HT$_{2C}$ receptor antagonist, SB242084 (0.5 mg/kg), failed to alter the NDF-induced hypophagia (NDF simple effect, $F(1, 39) = 76.48$, $p < 0.0001$; $n = 7$–15). Bonferroni tests were used for *post hoc* comparisons. *$p < 0.05$, ***$p < 0.001$ compared with the vehicle group; $^{\S}p < 0.05$ compared with the WT group. All results are expressed as mean ± SEM.

a contribution of presynaptic 5-HT autoreceptors in DF-induced behavioral effect or 5-HT release, such as the one revealed here for 5-HT$_{2B}$ receptors, has not yet been described.

Together, these observations suggest that DF acts presynaptically at SERT, which needs activation of 5-HT$_{2B}$ receptors to release efficiently 5-HT in a feed-forward loop, and that 5-HT$_{2B}$ receptors act upstream (presynaptic) of 5-HT$_{2C}$ receptors (Figure 8). A direct control of SERT uptake activity by 5-HT receptors has been recently documented: a 5-HT$_{2B}$ receptor coupling to PKG-dependent NO production can phosphorylate SERT to basal level and maximal 5-HT uptake (Launay *et al*, 2006). In the presence of 5-HT, however, the 5-HT$_{2B}$ receptor-PKC coupling promotes additional phosphorylations of both SERT and Na+, K+-ATPase α- subunit, impairing the electrochemical gradient necessary for 5-HT uptake (Launay *et al*, 2006). Whether SERT-releasing activity can also be modulated by 5-HT$_{2B}$ receptor-dependent activation remains to be documented. In this line, we recently showed that MDMA- or DF-induced synaptosomal 5-HT release requires PKC activation (data not shown). A presynaptic interplay between 5-HT$_{2B}$ and 5-HT$_{1B}$ receptors cannot be totally excluded as a role of 5-HT$_{2B}$ receptors in the cross-desensitization of 5-HT$_{1B}$ receptors has been previously documented (Janoshazi *et al*, 2007).

Nevertheless, postsynaptic receptors including 5-HT$_{1B}$ and 5-HT$_{2C}$ receptors seem to be indirectly activated via DF-induced 5-HT transporter- and 5-HT$_{2B}$ receptor-dependent 5-HT release. Previous studies have established that released serotonin (1) hyperpolarizes and inhibits AgRP/NPY neurons and decreases an inhibitory drive onto POMC cells by activation of 5-HT$_{1B}$ receptors and (2) activates POMC/CART neurons via activation of 5-HT$_{2C}$ receptors (Heisler *et al*, 2006). This leads to reciprocal increases in α-MSH release and decreases in AgRP release at Melanocortin4 receptors in target sites. Subsequent increased serotonin neurotransmission has also been shown to regulate the hypothalamic–pituitary–adrenal (HPA) axis upstream corticotropin-releasing hormone among others (Heisler *et al*, 2007). All these postsynaptic events are not illustrated in the scheme as they were not directly assessed in this study.

The main DF metabolite, NDF, stimulates the release of 5-HT and is a potent 5-HT$_{2B/2C}$ agonist. Our results show that treatment with 5 mg/kg NDF also led to a milder decrease in food intake in both SERT$^{-/-}$ and 5-HT$_{2B}^{-/-}$ mice than in WT mice, confirming their close relationships. Also, this result indicates that NDF-induced hypophagia is not only mediated by its 5-HT-releasing effect. As pretreatment of WT mice with the 5-HT$_{2C}$ receptor antagonist, SB242084, failed to block extensively the NDF-induced hypophagia, NDF should also act at other unidentified

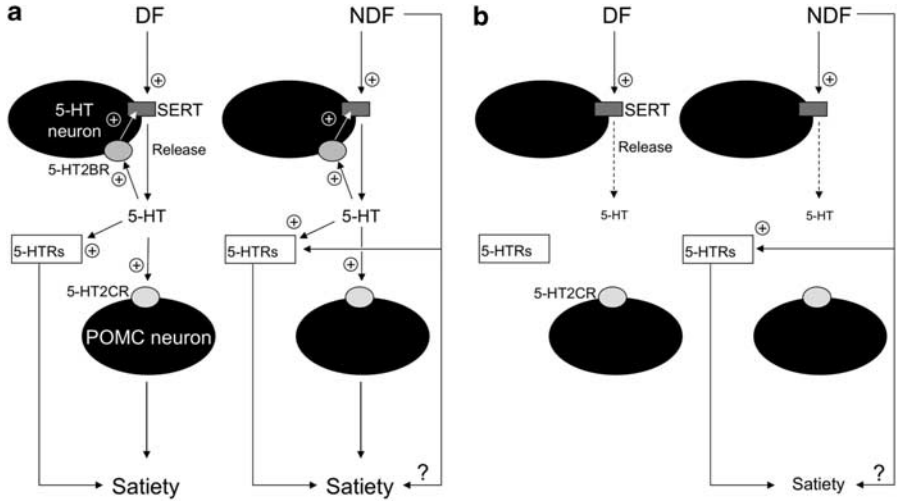

Figure 8 Working model summarizing the putative contribution of 5-HT$_{2B}$ receptors and SERT in the satiating action of DF and NDF as revealed by the present data. (a) In WT mice raphe neurons, DF activates a SERT-dependent release of 5-HT that requires 5-HT$_{2B}$ receptors to induce robust extracellular 5-HT levels in a feed-forward regulatory loop. Released 5-HT also stimulates postsynaptic 5-HT receptors (5-HTRs) including 5-HT$_{1B}$, 5-HT$_{2C}$, and others, the combination of which leads to hypophagia. In addition, NDF may act via 5-HT release by SERT or directly at 5-HT receptors including 5-HT$_{2C}$ and other unidentified receptors to induce satiety. (b) In 5-HT$_{2B}^{-/-}$, DF has lost any anorectic effect (as in SERT$^{-/-}$) because of the strong reduction of released 5-HT; NDF seems to act (as in SERT$^{-/-}$) only via a direct postsynaptic activation of unidentified pharmacological target that may include 5-HT$_7$ or adrenergic α_{2C} receptors, explaining the difference in satiating effects with DF action.

pharmacological targets that may include 5-HT$_7$ or adrenergic α_{2C} receptors (Setola *et al*, 2003) (Figure 8).

In summary, this work shows that the most effective of anorectic drugs, DF, acts at the release of 5-HT that requires 5-HT$_{2B}$ receptors to induce robust extracellular 5-HT levels and hypophagia in mice. Postsynaptic receptors including 5-HT$_{2C}$ receptors seem to be indirectly activated via DF-induced 5-HT transporter- and 5-HT$_{2B}$ receptor-dependent 5-HT release. This work supports the notion that 5-HT$_{2B}$ receptors have a limiting role in controlling the release of 5-HT via its transporter, and that central 5-HT$_{2B}$ receptors should participate in feeding behavior in mice.

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DISCLOSURE

The authors declare no conflict of interest.

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