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Modulatory Role of Postsynaptic 5-Hydroxytryptamine Type 1A Receptors in (±)-8-Hydroxy-*N*,*N*-dipropyl-2-aminotetralin-Induced Hyperphagia in Mice

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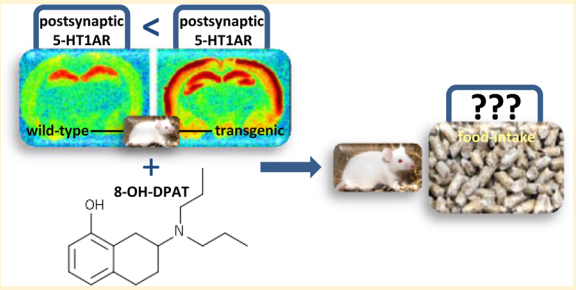
Supporting Information

ABSTRACT: Brain serotonin (5-HT) is involved in the control of food intake. The ingestive effects of 5-HT are mediated by various receptor subtypes, among others the 5-HT_{1A} receptor. While the involvement of presynaptic 5-HT_{1A} receptors is regarded as certain, the role of postsynaptic 5-HT_{1A} receptors is rather vague. Here, we studied the role of the 5-HT_{1A} receptor on feeding in non-food-deprived and food-deprived (young adult and adult, both sexes) wild-type NMRI mice as well as transgenic NMRI mice, which are characterized by a distinct overexpression of postsynaptic 5-HT_{1A} receptors. The known hyperphagic effect of the 5-HT_{1A} receptor full agonist 8-OH-DPAT ((±)-8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin) in non-food-deprived animals was demonstrated in male NMRI wild-type mice and could be antagonized by the selective 5-HT_{1A} receptor antagonist WAY100635. In transgenic mice, this hyperphagic response was induced at lower doses, with an earlier onset and even in females. However, in adult male transgenic mice, the hyperphagic effect did not occur. In food-deprived NMRI wild-type as well as transgenic mice, 8-OH-DPAT first induced a hypophagic and subsequently a hyperphagic effect. Again, in transgenic animals most responses occurred at lower doses and with an earlier onset. The results indicate that postsynaptic 5-HT_{1A} receptors exert a modulatory function in food intake in free-feeding and fasted mice, which for the first time shows an involvement of postsynaptic 5-HT_{1A} receptors in feeding behavior. Understanding the function of pre- and postsynaptic 5-HT_{1A} receptors may help to achieve new insights into the regulation of food intake and foster prospective treatment strategies for eating disorders.



KEYWORDS: *5-HT_{1A} receptor, 8-OH-DPAT, hyperphagia, overexpression, postsynaptic, transgenic mice*

The central serotonin (5-hydroxytryptamine, 5-HT) system exhibits a significant role in the regulation of food intake.[1−5] Among the various 5-HT receptor subtypes,[6] 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2C} receptors have been implicated in the modulation of ingestive behavior. While activation of 5-HT_{1B} and 5-HT_{2C} receptors exerts an inhibitory action on feeding behavior,[7−10] 5-HT_{1A} receptor agonists can cause both hyperphagia[11,12] and hypophagia,[11,13,14] depending on animals' feeding regime. Current data also suggest a role of 5-HT_6 receptors in food intake and body weight.[15−17]

The 5-HT_{1A} receptor subtype is found on both pre- and postsynaptic neuronal sites. Activation of presynaptic 5-HT_{1A} autoreceptors, which are located somatodendritically in the raphe nuclei, leads to a decreased 5-HT release into the synaptic cleft of 5-HT terminal structures.[18−20] Postsynaptic 5-HT_{1A} heteroreceptors are widely expressed on glutamatergic pyramidal cells and GABAergic interneurons in the hippocampus, cortical areas, hypothalamus, amygdala, and septum,[21−24] where they trigger neuronal hyperpolarization.[20,25,26]

Previous studies have demonstrated that the prototypic 5-HT_{1A} receptor agonist (±)-8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin (8-OH-DPAT), as well as 5-HT_{1A} receptor partial agonists (buspirone, gepirone, ipsapirone) elicit hyperphagia in satiated rats without affecting locomotion and other kinds of movements.[11,27,28] Because intra-raphe microinjections of 5-HT_{1A} receptor agonists induce the hyperphagic effect in the rat, it is suggested that inhibition of serotonergic raphe cell firing due to activation of 5-HT_{1A} autoreceptors leads to stimulation of food intake.[29−33] In contrast, hyperphagia does not occur in free-feeding female rats, possibly due to the influence of ovarian hormones,[34,35] or in food-deprived animals. In the latter, 8-OH-DPAT was not only ineffective[11] but even decreased food intake,[14,36] possibly by a modified sensitivity of 5-HT_{1A} autoreceptors or brain 5-HT metabolism as a consequence of food deprivation (refs 37−39, but see ref 40). In free-feeding mice, systemic injection of 8-OH-DPAT enhanced the basal feeding duration[41] and the total amount of consumed food,[12,42] which was antagonized by pretreatment with the selective 5-HT_{1A} receptor antagonist WAY100635.[12] Interestingly, Blanchard and colleagues demonstrated that a

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Table 1. Food Intake of Saline-Treated NMRI and Tg NMRI Control Groups[a]

young adult	0−30 min	30−60 min	60−120 min	adult	0−30 min	30−60 min	60−120 min
			non-food-deprived				
♂ NMRI	5.86 ± 0.76	3.89 ± 1.28	1.92 ± 0.79	♂ NMRI	6.93 ± 1.65	5.65 ± 0.83	3.07 ± 0.76
♂ Tg NMRI	5.56 ± 1.55	0.82 ± 0.41*	1.03 ± 0.66	♂ Tg NMRI	6.56 ± 2.21	0.99 ± 0.55*	2.30 ± 1.26
♀ NMRI	2.25 ± 0.97	3.27 ± 1.31	2.09 ± 0.83	♀ NMRI	4.48 ± 1.37	4.60 ± 1.36	1.77 ± 0.55
♀ Tg NMRI	2.81 ± 1.07	0.71 ± 0.53*	2.74 ± 1.07	♀ Tg NMRI	3.54 ± 1.55	0.96 ± 0.67*	4.62 ± 1.81
			food-deprived				
♂ NMRI	26.02 ± 1.97	15.72 ± 1.70	13.40 ± 2.71	♂ NMRI	21.20 ± 1.75	11.29 ± 1.47	14.82 ± 2.97
♂ Tg NMRI	20.84 ± 2.04	3.64 ± 1.16*	6.79 ± 2.19	♂ Tg NMRI	17.18 ± 2.01	1.99 ± 0.74*	5.81 ± 2.14*
♀ NMRI	24.59 ± 3.21	10.05 ± 1.71	7.44 ± 2.52	♀ NMRI	15.57 ± 2.23	4.94 ± 1.44	8.43 ± 2.93
♀ Tg NMRI	22.46 ± 2.78	6.33 ± 1.87	10.01 ± 2.09	♀ Tg NMRI	21.02 ± 1.84	6.18 ± 1.27	8.56 ± 2.20

[a]Presented is the food intake [mg] in relation to the body weight [g] during three measurements intervals. All data are means ± SEM, *$p \leq 0.05$ NMRI vs. Tg NMRI (Mann−Whitney rank sum test). Tg: transgenic.

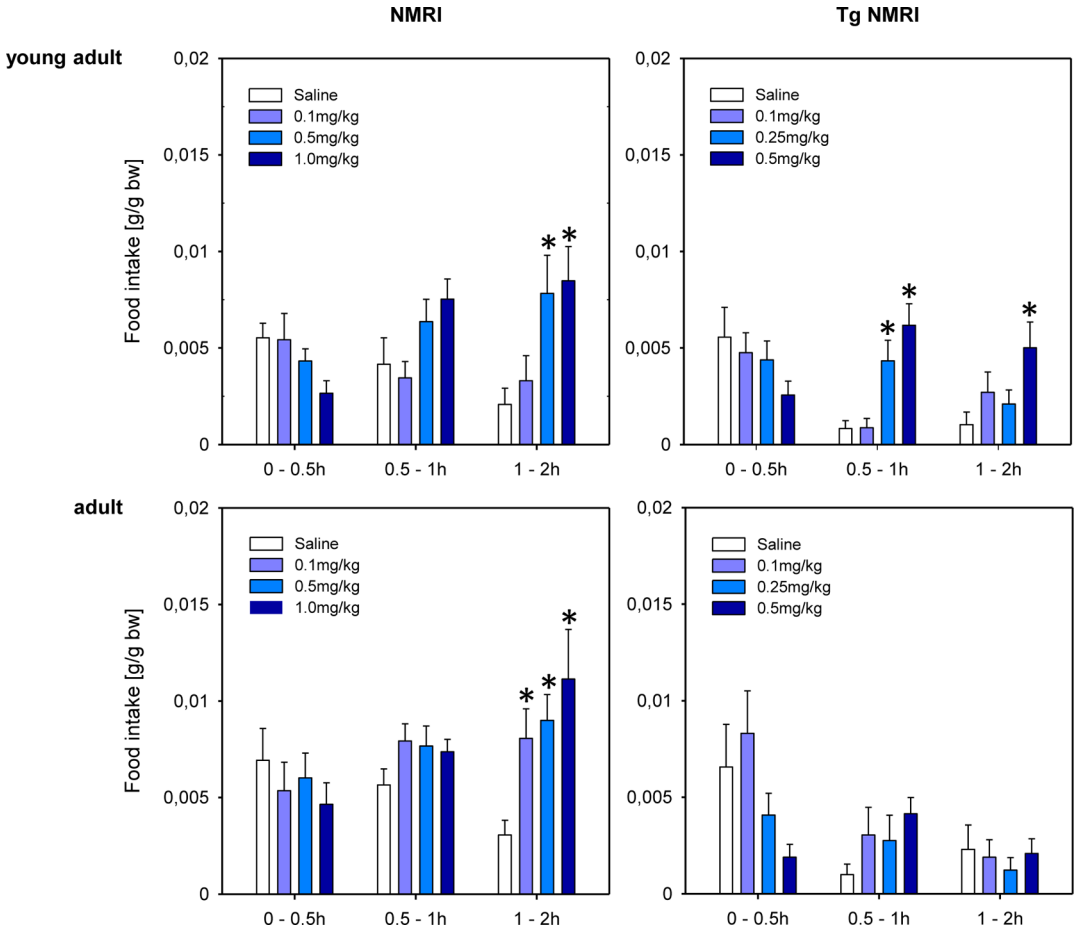

Figure 1. Effect of 8-OH-DPAT or saline administration on food intake in male young adult (upper graphs) and adult (lower graphs) non-food-deprived NMRI and Tg NMRI mice. Data are means + SEM, *$p \leq 0.05$ vs control (Kruskal−Wallis one-way ANOVA followed by Dunn's test). Tg: transgenic.

hyperphagic effect in mice was obtained only after the administration of a high dose of 8-OH-DPAT (10 mg/kg) in association with symptoms of the 5-HT-syndrome,[43] suggesting an involvement of postsynaptic 5-HT_{1A} receptors.

A role of postsynaptic 5-HT_{1A} receptors in the 8-OH-DPAT-mediated effect on food intake in free-feeding and fasted animals is generally not taken into account, although several studies do not exclude an involvement of the heteroreceptors.[11,31,44] In an immunohistochemical study, Collin and colleagues suggested that the activation of hypothalamic 5-HT_{1A} receptors may control food intake by affecting the expression of orexigenic and anorexigenic peptides.[45] 5-HT_{1A} receptors in the arcuate nucleus (ARC) and lateral hypothalamic area (LH) seem to participate in the serotonergic control of feeding,[46,47] and microinjections of 8-OH-DPAT into the hypothalamic paraventricular nucleus (PVN) enhanced the behavioral satiety sequence, possibly by acting at postsynaptic sites.[48] Interestingly, selective inhibition of noradrenaline transporters in combination with activation of pre- and postsynaptic 5-HT_{1A} receptors is discussed as an antiobesity treatment option with fewer cardiovascular side effects than sibutramine.[49,50]

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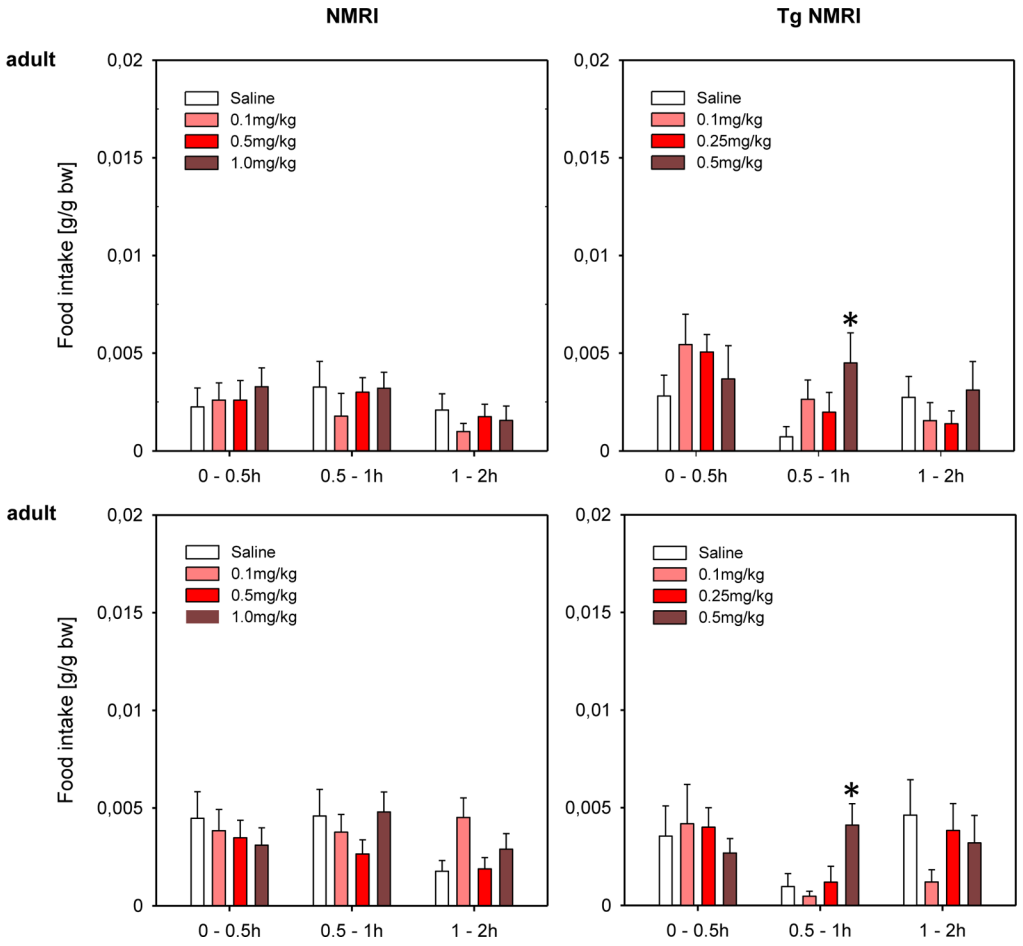

Figure 2. Effect of 8-OH-DPAT or saline administration on food intake in female young adult (upper graphs) and adult (lower graphs) non-food-deprived NMRI and Tg NMRI mice. Data are means + SEM, $*p \leq 0.05$ vs control (Kruskal–Wallis one-way ANOVA followed by Dunn's test). Tg: transgenic.

The primary aim of the study at hand was to assess whether murine postsynaptic 5-HT_{1A} receptors are involved in feeding control. As a prerequisite to achieve this goal, it was necessary to comprehensively study the effect of 8-OH-DPAT on feeding behavior in mice. Generally, in rats, the impact of 5-HT_{1A} receptors on the mechanisms of food intake is mainly characterized with regard to procedural and organismic variables. For example, age- and sex-dependent differences in 8-OH-DPAT-elicited feeding behavior in non-food-deprived and food-deprived rats have been shown. By contrast, in mice, the acute effect of 8-OH-DPAT on food intake was only investigated in male, young adult animals housed under free-feeding conditions. Therefore, we used male and female young adult and adult NMRI mice to study the effect of systemic administration of 8-OH-DPAT on the feeding behavior in non-food-deprived and food-deprived animals. In order to specify the role of postsynaptic 5-HT_{1A} receptors on food intake, we investigated the drug effect on ingestive behavior in transgenic (Tg) mice, characterized by a stable overexpression of postsynaptic 5-HT_{1A} receptors in various brain regions, that is, hypothalamus, cortex, hippocampus, amygdala, lateral septum, claustrum, and tenia tecta.[51,52]

RESULTS

Body Weight and Basal Food Intake. Genotypes were of comparable body weight at the onset of experiments. Yet, some groups showed small but significant differences (young adult ♂ NMRI = 35.8 ± 0.3 g vs Tg NMRI = 34.2 ± 0.3 g*, ♀ NMRI = 29.7 ± 0.3 g vs Tg NMRI = 28.5 ± 0.3 g*; adult ♂ NMRI = 37.9 ± 0.4 g vs Tg NMRI = 37.8 ± 0.4 g; ♀ NMRI = 31.8 ± 0.3 g vs Tg NMRI = 30.3 ± 0.3 g*; asterisk indicates significant difference between NMRI and Tg NMRI mice). Therefore, food intake was calculated in g in relation to body weight.

While the basal food intake between saline-treated NMRI and Tg NMRI control groups did not differ at the onset of measurements (first 30 min), transgenic groups showed a decline in food consumption at the subsequent measurement interval (0.5–1 h; $p \leq 0.05$). Exceptions to this were female controls under deprived feeding conditions, showing no variations in food intake behavior between genotypes (Table 1).

Experiment 1: The Effect of 8-OH-DPAT on Food and Water Intake in Male and Female Non-Food-Deprived NMRI and Tg NMRI Mice. *Male.* Doses of 0.5 and 1.0 mg/kg 8-OH-DPAT increased food intake in young adult NMRI mice (1–2 h; $p < 0.01$). In young adult Tg NMRI mice, doses of 0.25 and 0.5 mg/kg (0.5–1 h; $p < 0.001$) and 0.5 mg/kg (1–2 h; $p = 0.023$) elevated feeding behavior. Whereas all doses of 8-OH-DPAT resulted in hyperphagia in adult NMRI mice (1–2 h; $p = 0.005$), no treatment effect was seen in adult Tg NMRI mice (Figure 1). Hyperphagia is more pronounced in Tg mice than in wild-type mice (Table S1, Supporting Information).

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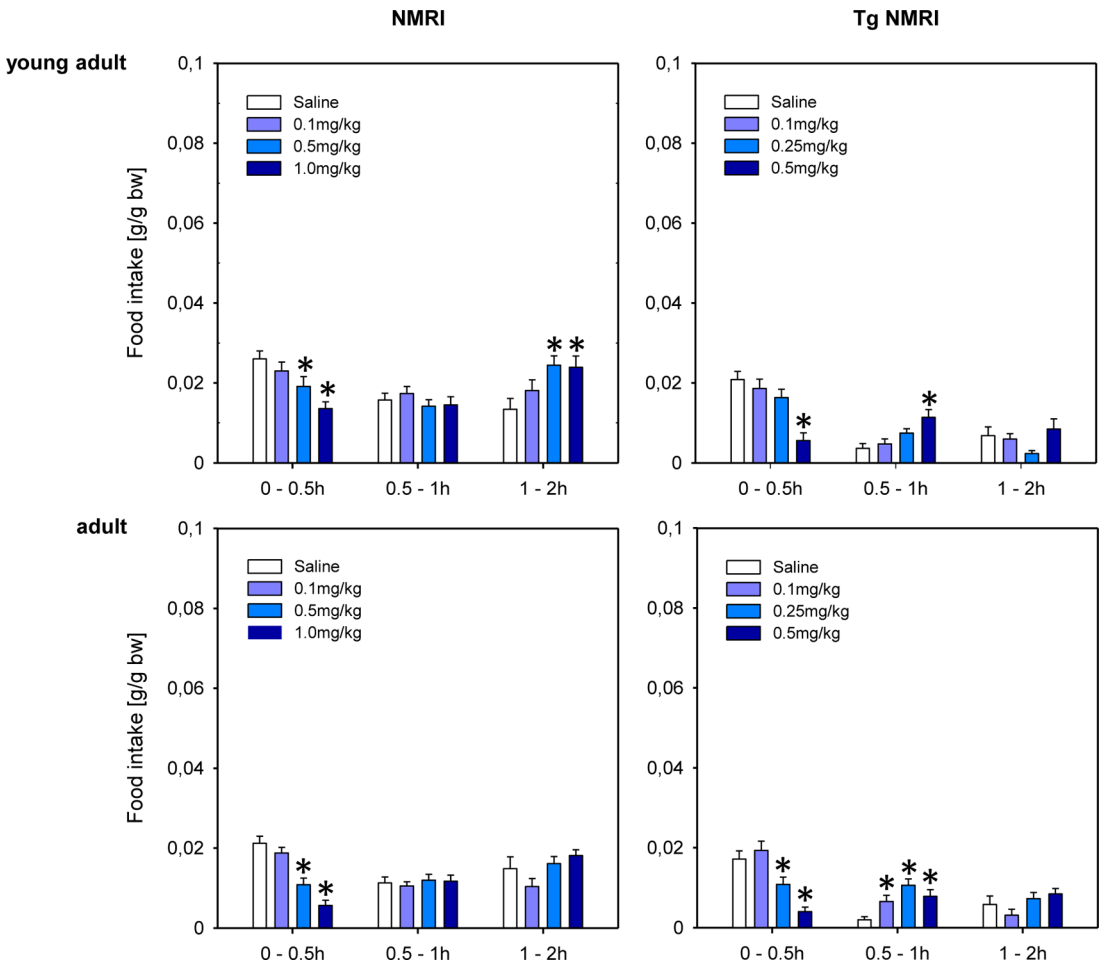

Figure 3. Effect of 8-OH-DPAT or saline administration on food intake in male young adult (upper graphs) and adult (lower graphs) food-deprived NMRI and Tg NMRI mice. Data are means + SEM, $*p \leq 0.05$ vs control (Kruskal–Wallis one-way ANOVA followed by Dunn's test). Tg: transgenic.

Female. In contrast to young adult and adult NMRI mice, transgenic animals showed hyperphagia (young adult 0.5 mg/kg, 0.5–1 h, $p = 0.044$; adult 0.5 mg/kg, 0.5–1 h, $p = 0.006$) (Figure 2).

Furthermore, no differences in drinking behavior, motor activity, or food intake after 24 h were observed in male and female animals (data not shown).

Experiment 2: The Effect of 8-OH-DPAT on Food and Water Intake in Male and Female Food-Deprived NMRI and Tg NMRI Mice. *Male.* During the first 30 min, 8-OH-DPAT led to a hypophagic effect in young adult NMRI (0.5 and 1.0 mg/kg; $p < 0.001$) and Tg NMRI mice (0.5 mg/kg; $p < 0.001$). Subsequent measurements revealed an increase in food intake (NMRI 0.5 and 1.0 mg/kg, 1–2 h, $p = 0.019$; Tg NMRI 0.5 mg/kg, 0.5–1 h, $p = 0.003$). In adult mice, the two high doses of 8-OH-DPAT induced an initial hypophagia in both genotypes ($p < 0.001$). A hyperphagic response to all doses of 8-OH-DPAT was only seen in Tg mice (0.5–1 h, $p < 0.001$) (Figure 3). Hypophagia is more pronounced in Tg mice than in wild-type mice (Table S1, Supporting Information).

Female. In young adult mice, administration of the highest dose of 8-OH-DPAT both decreased food consumption during the first 30 min (NMRI, $p = 0.033$; Tg NMRI, $p = 0.002$) and increased food intake in Tg NMRI mice (0.5–1 h, $p = 0.008$). 8-OH-DPAT induced hypophagia in adult Tg NMRI mice (0–0.5 h, 0.5 mg/kg, $p = 0.022$), followed by hyperphagia in both NMRI (0.5–1 h, 1.0 mg/kg, $p = 0.045$) and Tg NMRI mice (0.5–1 h, 0.5 mg/kg, $p = 0.032$) (Figure 4). In Tg mice, hypophagia is more pronounced than in wild-type mice (Table S1, Supporting Information).

No differences in drinking behavior, motor activity, or food intake after 24 h were observed in male and female animals (data not shown).

Experiment 3: The Effect of WAY100635 and 8-OH-DPAT on Food Intake in Male Non-Food-Deprived NMRI Mice. Pretreatment with WAY100635 suppressed the hyperphagic effect of 8-OH-DPAT. We found a general effect of treatment on food intake [$F(3,40) = 5.7$, $p = 0.005$]. Holm–Sidak t test showed a significant increase of food consumption in the saline + 8-OH-DPAT group compared with the WAY100635 + 8-OH-DPAT group (0.5–1.0 h, $p = 0.005$). Furthermore, administration of saline + 8-OH-DPAT increased food intake during 1–2 h compared with other treatment groups ($p < 0.05$) (Figure 5).

DISCUSSION

5-HT_{1A} receptors play a vital role in the regulation of food intake. To the best of our knowledge, this is the first study in mice that investigates the effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT on feeding behavior in regard to sex, age, and feeding conditions with a special focus on postsynaptic 5-HT_{1A} receptors. In terms of developing efficacious drugs capable of

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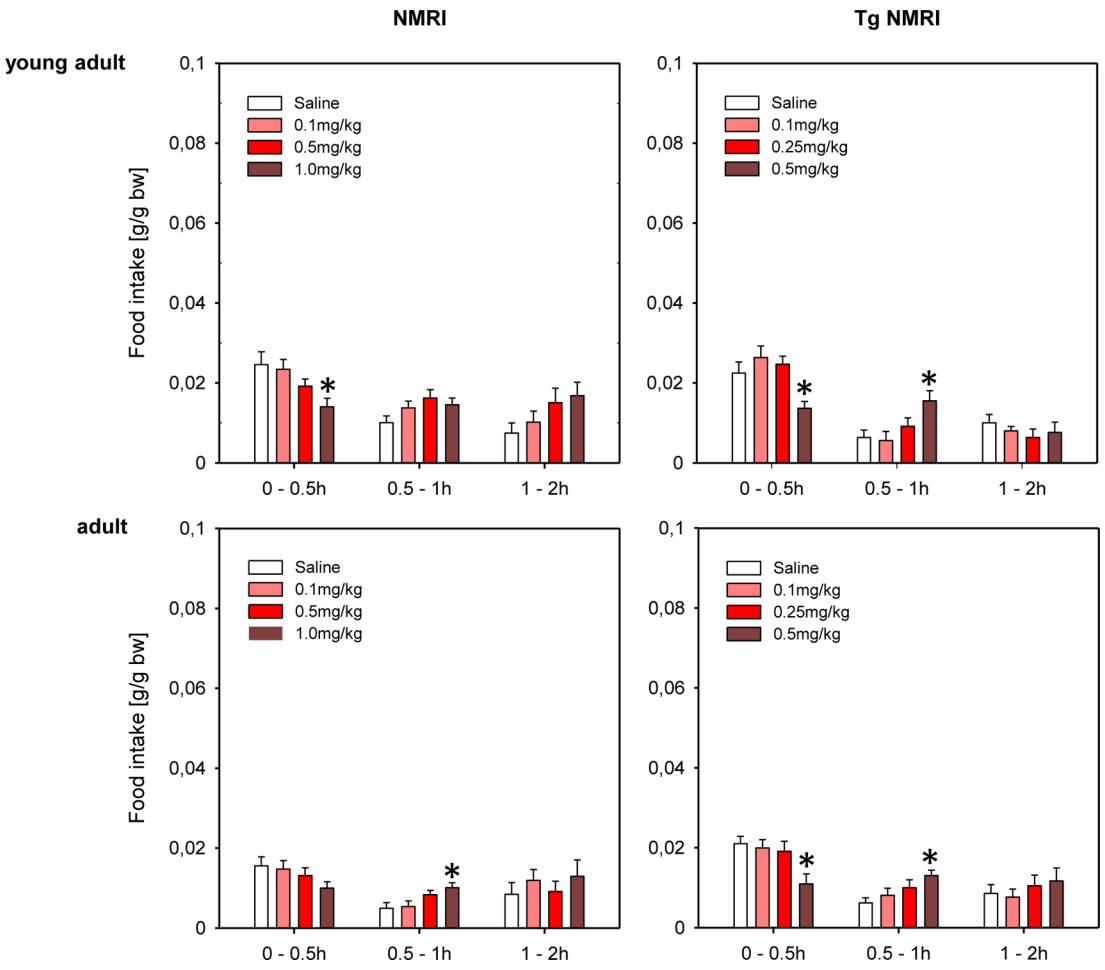

Figure 4. Effect of 8-OH-DPAT or saline administration on food intake in female young adult (upper graphs) and adult (lower graphs) food-deprived NMRI and Tg NMRI mice. Data are means + SEM, $^{*}p \leq 0.05$ vs control (Kruskal–Wallis one-way ANOVA followed by Dunn's test). Tg: transgenic.

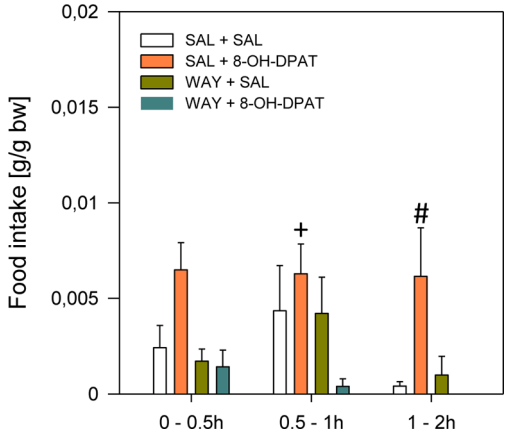

Figure 5. Effect of WAY100635 (0.3 mg/kg) administration on 8-OH-DPAT (0.5 mg/kg) induced hyperphagia in male young adult, non-food-deprived NMRI mice. Data are means + SEM, $^{+}p < 0.05$ saline + 8-OH-DPAT vs WAY100635 + 8-OH-DPAT, $^{\#}p < 0.05$ saline +8-OH-DPAT vs other treatment groups (two-way RM ANOVA followed by Holm–Sidak t test).

treating eating disorders, 5-HT_{1A} receptors are a rewarding research area, given the urgent medical need for these drugs.

Our results confirm and extend previous observations on the general participation of 5-HT_{1A} receptors in the control of feeding.[11,12] In our study, the 5-HT_{1A} receptor agonist 8-OH-DPAT elevated food consumption in non-food-deprived mice and induced a hypophagic followed by a hyperphagic effect in food-deprived animals, which had not yet been observed. These results occurred in dependence on age and sex. Interestingly, postsynaptic 5-HT_{1A} receptors appear to exert modulatory functions in food intake in free-feeding and fasted mice, which shows an involvement of the heteroreceptors in feeding behavior for the first time.

Freely fed and food-deprived NMRI and Tg NMRI control groups were of similar body weight and food intake after saline injection at the onset of experiments. However, most transgenic groups consumed a lower amount of food than the wild-type mice during the measurement interval of 0.5–1 h following saline injection. External stressors are known to influence food intake behavior and may have influenced mice of both genotypes to varying degrees. For example, mild stressors such as tail pinches induce elevated food intake in rats.[53,54] Because Tg NMRI mice are less sensitive to acute stress than the wild-type mice,[55] the elevated food intake in wild-type mice may be due to injection stress. In addition, genotypes revealed no differences in anxiety-related behavior[51] or overall motor activity (data not shown), factors which can interfere with food intake behavior.

Non-Food-Deprived Mice. 8-OH-DPAT induced hyperphagia in male NMRI mice. This is consistent with previous

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studies in male C57BL/6 mice[12] and rats[11,33,34,56] examining the role of 5-HT_{1A} receptor agonists on food intake. Interestingly, hyperphagia occurred rather late, with a latency of 1 h, compared with previous studies in mice and rats.[11,12] Beside these effects on ingestive behavior and as shown previously,[51] the dose range of 8-OH-DPAT used in this study (NMRI 0.1−1.0 mg/kg, Tg NMRI 0.1−0.5 mg/kg) did not produce changes of motor activity or any signs of murine 5-HT syndrome,[57] which would interfere with the process of eating. Beyond, 8-OH-DPAT is regarded as a prototypical 5-HT_{1A} receptor agonist with additional affinity to 5-HT_7 receptors.[6,58] Here, the selective 5-HT_{1A} receptor antagonist WAY100635 suppressed 8-OH-DPAT-induced hyperphagia without influencing ingestive and noningestive behavior on its own, indicating that the hyperphagic response to 8-OH-DPAT is mediated by 5-HT_{1A} receptors in our mice. These results are in line with previous studies, in which WAY100635 abolished the hyperphagic effect of 8-OH-DPAT.[12,59]

Because systemic as well as intra-raphe injections of 8-OH-DPAT induce hyperphagic responses, it is hypothesized that enhanced feeding is mediated by an activation of somatodendritic 5-HT_{1A} autoreceptors. A subsequent decrease of 5-HT tone in forebrain terminal areas is therefore believed to be responsible for increased food intake.[29−33] However, since no 5-HT_{1A} receptor ligands selective for either pre- or postsynaptic receptors are available, the role of 5-HT_{1A} postsynaptic receptors on ingestive behavior is vague. Collin and colleagues identified 5-HT_{1A} receptor immunoreactivity in hypothalamic nuclei (ARC and LH) and claimed a regulatory role of postsynaptic 5-HT_{1A} receptors in food intake.[45] The immunoreactivity was detected on postsynaptic sites in orexigenic and anorexigenic peptide (e.g., neuropeptide Y (NPY), agouti-related peptide, cocaine- and amphetamine-regulated transcript)-producing neurons that have been shown to affect feeding and body weight.[45] In addition, expression levels of various hypothalamic neuropeptides and all $GABA_A\beta$ subunits dropped significantly in response to prolonged 8-OH-DPAT administration (1.0 mg/kg for 3 weeks, sc) in adult female mice.[5] Apparently, 5-HT_{1A} heteroreceptors mediate the serotonergic input to peptidergic neurons. In addition, acute administration of the 5-HT_{1A} receptor agonist flesinoxan elevated food intake and concomitantly orexigenic NPY levels in ARC and paraventricular nucleus, therefore pointing to a regulatory role of NPY in the 5-HT_{1A} receptor-mediated effect on food intake.[60] In line with these results, a distinct influence of postsynaptic 5-HT_{1A} receptors on feeding was evident in our transgenic mice. Here, young adult Tg NMRI mice showed a hyperphagic response to 8-OH-DPAT already at a lower dose and an earlier onset compared with wild-type mice. Because Tg NMRI mice are characterized by an increased 5-HT_{1A} receptor binding in brain areas including the hypothalamus,[52] it is plausible that an activation of postsynaptic 5-HT_{1A} receptors by 8-OH-DPAT may influence hypothalamic peptidergic homeostasis by decreasing anorexigenic or increasing orexigenic peptides. However, so far we cannot explain the exact mechanism by which overexpressed 5-HT_{1A} receptors mediate the changes in food intake. Further investigations of hypothalamic neuropeptides relevant for ingestive behavior, for example, as conducted in female 5-HT_{1A} receptor knockout mice,[5] are required to determine the physiological basis of the present results and to exclude putative neuroadaptive changes, which may partly underlie the differences in feeding behavior of transgenic animals. Interestingly, 8-OH-DPAT did not promote hyperphagia in aged Tg NMRI. Likewise, Chaouloff and colleagues showed an 8-OH-DPAT (0.5 mg/kg, sc) induced hyperphagia in young rats, but the effect faded as the animals advanced in age.[37] Because 8-OH-DPAT did not alter 5-HT turnover in young and adult rats differently,[37] it is possible that the unequal effect is regulated via postsynaptic 5-HT_{1A} receptors. Assuming that the drug's effect on the 5-HT system is independent of age but its effect on other metabolic circuits or signal transduction systems is not, it appears plausible that an activation of a surplus of postsynaptic 5-HT_{1A} receptors reveals a potential age-dependent effect sooner than in the wild-type mice tested here.

Feeding-related serotonergic circuits are affected by ovarian hormones.[46] Here, non-food-deprived female mice showed no hyperphagic response to 8-OH-DPAT, which is in agreement with data of previous studies in which female rats failed to show hyperphagia following 8-OH-DPAT or gepirone, whereas increased feeding was observed in males.[34,35] Autoradiography studies in NMRI mice described brain region-dependent sex differences in 5-HT_{1A} receptor binding.[52,61] Different expression patterns and densities of 5-HT_{1A} receptors may provide an explanation for observed sex-related differences, but no differences were spotted in the examined brain region primary for feeding (hypothalamus).[52] Therefore, we suggest that varying 5-HT_{1A} receptor densities are not crucial for different feeding behavior of male and female NMRI mice. Estrogen plasma levels seem rather to influence the serotonergic control of feeding behavior.[46,62] In addition, 8-OH-DPAT-induced hyperphagia was less pronounced in ovariectomized rats after pretreatment with estradiol.[5] In contrast to wild-type females, 8-OH-DPAT induces hyperphagia in Tg females. Interestingly, female Tg NMRI revealed a particularly high 5-HT_{1A} receptor density in the hypothalamus compared with wild-type or male Tg NMRI.[52] If ovarian hormones indeed mask the hyperphagic effect of 8-OH-DPAT, an activation of a substantial number of postsynaptic 5-HT_{1A} receptors may superpose the hormone effect. However, the exact mechanisms underlying the effect of estrogen on 5-HT_{1A} receptors remain unclear.

The present study confirms the hyperphagic effect of 8-OH-DPAT. The diverging effects of 8-OH-DPAT in mice of different age and sex point to a gender-related role of 5-HT_{1A} receptors in food intake. By comparing the effects of 8-OH-DPAT in Tg and wild-type NMRI, it can be concluded that due to overexpression of postsynaptic 5-HT_{1A} receptors the agonist effect emerged clearly in an age and sex dependent manner. The results suggest that postsynaptic 5-HT_{1A} receptors play a facilitating role in food intake. However, further studies are needed to reveal the neurobiological basis of the investigated differences in feeding behavior.

Food-Deprived Mice. Doses of 8-OH-DPAT that induce hyperphagia in non-food-deprived rats are known to suppress feeding in food-deprived animals via the 5-HT_{1A}-receptor.[14] Here, 8-OH-DPAT induced hypophagia or showed a trend to decreased food intake in fasted mice during the first 30 min after administration. As far as we know, this is the first study to report a hypophagic response to 8-OH-DPAT in this species. The anorexic action of 8-OH-DPAT was not secondary to changes of motor activity, was independent of age, sex or genotype, and is consistent with previous studies in which 8-OH-DPAT inhibited food intake in fasted rats,[11,14,36] pigs,[63] and chickens.[64]

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The mechanism by which 8-OH-DPAT decreases food intake remains to be established.[14] Because brain tryptophan level[65,66] and 5-HT turnover and release are elevated in fasted animals,[67−69] this may counteract the decrease of 5-HT release by 8-OH-DPAT. Present results do not uncover the underlying mechanisms in the anorexic action of 8-OH-DPAT but promote a role of heteroreceptors because in Tg mice hypophagia is more pronounced than in wild-type mice. Studies that focus on neuroendocrine markers involved in feeding behavior may help to elucidate the mediation of the anorectic action of 8-OH-DPAT by postsynaptic 5-HT_{1A} receptors.

Interestingly, all groups of fasted mice showed elevated food intake or a trend toward increased feeding within 30−120 min after drug administration. However, some of the orexigenic responses may be rebound phenomena due to previously induced hypophagia. In contrast, other hyperphagic responses to 8-OH-DPAT should not be interpreted as balancing effects (e.g., adult male Tg NMRI show hyperphagia at 0.1 mg/kg, a dose which was unable to induce hypophagia) or as a result of a previous decrease of motor activity but as an emphasis of the amplifying role of postsynaptic 5-HT_{1A} receptors in food intake. Again hyperphagia became present at lower doses and with an earlier onset in Tg mice. In general, the hyperphagic effect that is seen in food-deprived and non-food-deprived mice in response to 8-OH-DPAT is characterized by a late onset, whereas hypophagia shows an early onset in fasted animals. This is important to consider for upcoming studies, in order not to miss individual drug effects on food intake behavior.

Conclusion. The present data confirm and extend previous studies: the 5-HT_{1A} receptor agonist 8-OH-DPAT induced hyperphagia in free-feeding mice and an initial hypophagic response followed by increased food intake in fasted animals. Further, we demonstrated sex and age differences in 8-OH-DPAT-elicited feeding behavior. Due to overexpression of postsynaptic 5-HT_{1A} receptors, the responses to 5-HT_{1A} receptor stimulation are amplified. Postsynaptic 5-HT_{1A} receptors modulate food-intake behavior under freely feeding and fasting conditions. The results of this study not only highlight further aspects of the role of pre- and postsynaptic 5-HT_{1A} receptors in ingestive behavior but also provide useful information about possible ingestive side effects of antidepressants or anxiolytics. However, further investigations seem warranted to uncover the mechanisms by which overexpressed 5-HT_{1A} receptors influence feeding behavior.

METHODS

Animals. A total of 437 naive male and female NMRI mice (n = 229) and homozygous transgenic (Tg) NMRI mice (n = 208) with a stable overexpression of postsynaptic 5-HT_{1A} receptors (the DNA fragment comprised the coding sequence together with 4.5 kb upstream and 1 kb downstream of the murine 5-HT_{1A} receptor gene sequences; generation of mice described in detail by Bert and colleagues)[51] at an age of 10 (young adult) and 20 weeks (adult) were used. The mice were housed in groups of 4−6 animals in Macrolon cages (type IV) under standard conditions (22 ± 2 °C room temperature, 55 ± 10% humidity) on a 12 h light−dark schedule (lights on at 06:00 a.m.). Occurrence of estrus cycle was not monitored. With the exception of experiment 2, in which the mice were food-deprived 16 h before the testing (see below), all animals received free access to standard lab chow (ssniff, Soest, Germany) and tap water during experiments.

All experiments were performed in accordance with the guidelines of the German Animal Protection Law and were approved by the Berlin State Authority ("Landesamt für Gesundheit und Soziales").

Drugs. 8-OH-DPAT ((±)-8-hydroxy-*N*,*N*-dipropyl-2-aminotetralin; Sigma-Aldrich, Schnelldorf, Germany) is a centrally acting[70] prototypical 5-HT_{1A} receptor agonist with additional affinity for 5-HT_7 receptors.[6,58] 8-OH-DPAT was dissolved in saline (0.9% NaCl) and injected intraperitoneally (ip) 5 min before testing. Preliminary behavioral studies revealed that transgenic mice were more sensitive to 8-OH-DPAT treatment than wild-type mice.[51,71] Therefore, they received 8-OH-DPAT at a lower dosage (see below, experiment 1). The selective 5-HT_{1A} receptor antagonist WAY100635 (Sigma-Aldrich, Schnelldorf, Germany) was dissolved in saline and injected ip 30 min before testing. All control animals received saline. Injection volume was 10 mL/kg, and drugs were freshly prepared before being used.

Experimental Procedure. Mice were weaned 21 days postnatally and their body weight was determined twice weekly including on the day of experiments. They were handled regularly and single-housed in experimental cages (Macrolon cages type III; habituation period) 2 days before testing. During experiments, the mice received five pellets of the standard lab chow to which they were accustomed (ssniff R/M-H: 19% protein, 3.3% fat, 4.9% fiber, 6.4% ash, trace elements, and vitamins), and motor activity was investigated by an InfraMot activity system (TSE-Systems, Bad Homburg, Germany). All experiments were conducted during the light cycle between 09:00 a.m. and 01:00 p.m.

Experiment 1: The Effect of 8-OH-DPAT on Food and Water Intake in Male and Female Non-Food-Deprived NMRI and Tg NMRI Mice. The animals had free access to food and water during habituation period and on test days. Mice received either 8-OH-DPAT (0.1, 0.5, or 1.0 mg/kg) or saline (n = 12 or 13). Due to increased sensitivity to 8-OH-DPAT,[51,71] Tg NMRI mice were injected with 0.1, 0.25, or 0.5 mg/kg (n = 12 or 13). Food pellets were presented 5 min after the injection. The food consumption during three time intervals (0−0.5 h, 0.5−1 h, and 1−2 h) was calculated in grams in relation to body weight. Basal food intake was estimated in NMRI and Tg NMRI saline-treated control groups. The amount of water consumed was calculated in milliliters in relation to body weight after 4 h in order to not disturb the determination of food intake. To rule out possible long-term effects, food and water intake were determined once more after 24 h. Each animal was tested twice at an age of 10 and 20 weeks.

Experiment 2: The Effect of 8-OH-DPAT on Food and Water Intake in Male and Female Food-Deprived NMRI and Tg NMRI Mice. The experiment was performed as described for experiment 1, except that the animals were food-deprived 16 h before testing (n = 12 or 13).

Experiment 3: The Effect of WAY100635 and 8-OH-DPAT on Food Intake in Male Non-Food-Deprived NMRI Mice. A total of 24 mice at an age of 10 weeks were divided into four treatment groups (n = 6). Groups 1 and 2 received saline 30 min before testing. Five minutes before food presentation, group 1 received a second saline injection, group 2 received 0.5 mg/kg 8-OH-DPAT. Groups 3 and 4 received 0.3 mg/kg WAY100635 30 min before testing and either saline or 0.5 mg/kg 8-OH-DPAT 5 min before the start of the experiment. The food intake was measured as described in experiment 1.

Data Presentation and Analysis. The descriptive statistics are based on means; the variance is indicated by the standard error of the mean (±SEM). Data were analyzed and presented using SigmaPlot 11 software (Systat Software, Erkrath, Germany). Statistical significance was set at $P \leq 0.05$. Data of experiments 1 and 2 were not normally distributed (tested with the Shapiro−Wilk method) and therefore were analyzed with nonparametric Kruskal−Wallis one-way analysis of variance (ANOVA). Dunn's tests were conducted for post-hoc comparison versus control group. Water intake was evaluated by one-way ANOVA. Data of experiment 3 were analyzed using a two-way repeated measures ANOVA (treatment × time). The significant main effect was analyzed using post-hoc Holm−Sidak t tests. Data of

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body weight and basal food intake were analyzed by *t* test and Mann−Whitney rank sum test, respectively.

■ ASSOCIATED CONTENT

Supporting Information

Food intake of NMRI and Tg NMRI 8-OH-DPAT treatment groups presented as delta values [%] in relation to saline treated control groups. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

Participated in research design: Bert, Brosda, Fink, and Müller. Conducted experiments: Brosda and Müller. Performed data analysis: Brosda and Müller. Wrote or contributed to the writing of the manuscript: Brosda and Fink.

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Notes

The authors declare no competing financial interest.

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