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# Pharmacological, but not genetic, disruptions in 5-HT$_{2C}$ receptor function attenuate LPS anorexia in mice

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**Abstract**

Peripheral administration of bacterial lipopolysaccharide (LPS) elicits anorexia in several species, including rats and mice. There is strong evidence that antagonism of serotonergic activity at 2C receptors (5-HT$_{2C}$R) attenuates LPS anorexia in rats. Here we used pharmacological and genetic approaches to examine the role of the 5-HT$_{2C}$R in LPS anorexia in mice. In Experiment 1, SB 242084, a potent and selective 5-HT$_{2C}$ antagonist (0.3 mg/kg) was injected intraperitoneally 15 min before intraperitoneal LPS (2 μg/kg) injections just prior to dark onset in c57BL/6 mice. Food intake was recorded 1, 2 and 4 h after LPS administration. In Experiment 2, we recorded 2, 4 and 24 h food intake following dark onset intraperitoneal LPS (0.125, 0.25, 0.5, 1 and 2 μg/kg) injections in mice with a genetic deletion of 5-HT$_{2C}$R and their WT controls. Our pharmacological results suggest that at least part of the anorexia following peripheral LPS administration is mediated by an increase in 5-HT-ergic activity at the 5-HT$_{2C}$R. Our genetic data, in contrast, suggest that 5-HT$_{2C}$R is not a necessary part of LPS anorexia.


*Keywords:* Illness; Feeding; Brain; Antagonist; Knockout; Acute phase response

## 1. Introduction

The illness anorexia is a prominent component of the host defense response during bacterial infection. Evidence suggesting that anorexia can make a vital contribution comes from experiments showing that force-feeding mice infected with *L. monocytogenes* reduced survival time and increased mortality, whereas allowing the development of illness anorexia increased survival rates (Murray and Murray, 1979). Administration of bacterial lipopolysaccharide (LPS) is a standard model of bacterial infection that is widely used in studying the mechanisms mediating anorexia. Binding of LPS to its receptor complex activates intracellular responses leading to the release of immune, metabolic, endocrine and neural signaling molecules that participate in the development of anorexia. Serotonin (5-HT) is one of the neural signals that has received considerable attention (reviewed in Asarian and Langhans, 2005; Langhans, 2004).

The initial indication that 5-HT may play a role in the development of anorexia came from demonstrations that peripherally administered LPS activates central 5-HT-ergic neurons in rats, as indicated by neurochemical measures (Dunn, 1992; Dunn et al., 1999). Subsequent research was focused on the 5-HT receptor (5-HTR) mechanisms involved. Inhibition of 5-HT-ergic activity by administration of 5-HT$_{1A}$ autoreceptor agonist 8-OH-DPAT in the raphe nucleus attenuated the anorexia induced by intraperitoneally injected LPS in rats (von Meyenburg et al., 2003; Hrupka and Langhans, 2001). Similar results were observed following the administration of metergoline, an antagonist that blocks the function of both post-synaptic 5-HT$_1$ and 5-HT$_2$ receptors. Finally, pretreatment with SB 242084, the first reported selective, potent and brain-penetrant 5-HT$_{2C}$R antagonist (Kennett et al., 1997), selectively attenuated LPS anorexia in male rats without affecting food intake in non-LPS-treated rats (von Meyenburg et al., 2003), suggesting that activation of the 5-HT$_{2C}$R is a necessary part of the mechanism mediating the feeding-inhibitory effect of peripheral LPS.

The involvement of 5-HT$_{2C}$R in LPS anorexia has not yet been investigated in mice. To this end, we tested mice pretreated with the 5-HT$_{2C}$ receptor antagonist SB 242084 and mice

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lacking 5-$HT_{2C}$R. Our pharmacological data suggest that 5-$HT_{2C}$R participates in the development of LPS anorexia. In contrast, our genetic data suggest that they are not a necessary part.

## 2. Methods

### 2.1. Subjects and housing

Homozygote male mice bearing a null mutation of the X-linked *htr2c* gene (5-$HT_{2C}$ KO; congenic on a C57BL/6J background) and age-matched wild-type (WT) mice were obtained by breeding heterozygote female mice (kindly provided by Dr. Lawrence Tecott, University of California, San Francisco, USA) with C57BL/6J male mice (Jackson Laboratories, Sulzfeld, Germany) in our animal facility. The phenotype of the 5-$HT_{2C}$ KO mice was originally described by Tecott et al. (1995). Prior to entering the experimental protocol, the mice were adapted to individual housing in solid bottom plastic cages with bedding in a room maintained at 22±0.5 °C on a 12:12 h LD cycle (lights off at 1200 h). Mice were maintained on standard pelleted laboratory chow (Nafag, Gossau, Switzerland) and tap water. All procedures were approved by the Veterinary Office of the Canton of Zurich.

### 2.2. PCR verification of genotype

The genotypes of all mice were assessed with standard PCR techniques. Genomic DNA was extracted from tail biopsies using proteinase K digestion followed by isopropanol precipitation. DNA was then amplified by PCR using a primer set that selectively amplifies a 114 bp fragment on the WT *htr2c* DNA and a 600 bp fragment on the mutant *htr2c* DNA. PCR was performed in a 25-µl reaction mixture containing the following components: 11.875 µl $H_2O$, 5 µl Flexi-PCR-Buffer (5×), 2 µl $MgCl_2$ (25 mM), 1 µl dNTP's (10 mM each), 1 µl *htr2c* Forward (10 µM, 5′ gcT cAg AAT TcT ggA AAT gTg T 3′), 1 µl *htr2c* Reverse (10 µM, 5′ cgg AcT gcT AAA TTg ggT c 3′), 2 µl Neo D Forward (10 µM, 5′ cAc cTT gcT ccT gcc gag AAA 3′) and 0.125 µl Go-Taq Flexi Polymerase. The PCR amplification conditions were: 95 °C (4 min), 95 °C (30 s), 57 °C (45 s), 72 °C (30 s) for 40 cycles, 72 °C (1 min), 4 °C (hold). All reagents were purchased from Catalys AG (Wallisellen, Switzerland). The primers were synthesized by Mycrosynth (Balgach, Switzerland).

### 2.3. Experiment 1: effect of 5-$HT_{2C}$ antagonism on LPS anorexia in mice

Thirteen WT mice (body weight, 26±1 g) were used. 20 min prior to dark onset, the food left from the previous day was removed and weighed, and the body weights of the mice were recorded. Just prior to dark onset, saline was intraperitoneally injected (0.9% NaCl, B. Braun Medical AG, Emmenbrücke, Switzerland) and food intake was measured 1, 2 and 4 later. Due to the short effect of the antagonist, 24 h data were not collected. Mice were adapted to this protocol for 1 wk. Because repeated injections of LPS result in the development of tolerance (Langhans et al., 1991), the experiment was designed in two cross-over test trials. During the first trial, all mice received saline and the selective 5-$HT_{2C}$R antagonist SB 242084 (1′-methyl-5-([2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-biphenyl-4-yl]carbonyl)-2,3,6,7-tetrahydrospiro(furol[2,3-*f*]indole-3,4′-piperidine, Sigma, 0.3 mg/kg). Two days later, during

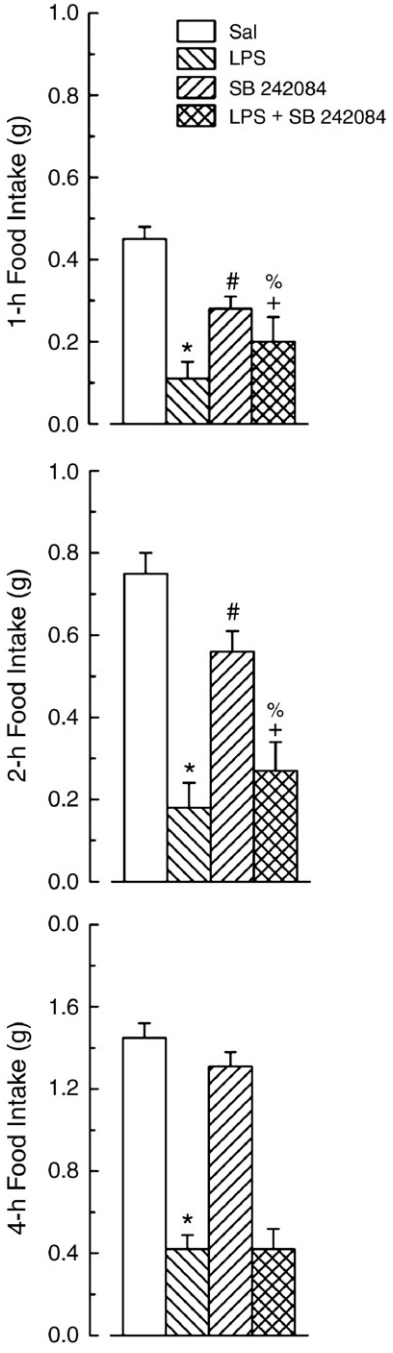


Fig. 1. Pre-treatment with the 5-$HT_{2C}$R antagonist SB 242084 attenuates LPS anorexia in male mice at 1 h (top) and 2 h (middle) post-LPS, but not at 4 h (bottom) post-LPS administration. *LPS significantly different than saline (Sal), $P<0.05$; % SB 242084+LPS significantly different than SB 242084; # SB 242084 significantly different than Sal, $P<0.05$; +(Sal-LPS) significantly different than (SB 242084-(LPS+SB 242084)), $P<0.05$.

the second trial, one group ($n=6$) of mice received saline and LPS (2 μg/kg, from Escherichia coli, serotype 0111:B4, Sigma) and another group ($n=7$) of mice received saline and SB 242084+LPS. Thus, each mouse received both saline and antagonist treatments alone and received LPS treatment only once, either alone or in combination with SB 242084. Injections were intraperitoneal, 0.01 ml/g volume, and done just prior to dark onset: first, saline or SB 242084 injections at about 11:45 h and then saline or LPS at about 11:55 h.

### *2.4. Experiment 2: effect of 5-HT$_{2C}$ mutation on LPS anorexia in mice*

Forty 5-HT$_{2C}$ KO and 43 WT mice (body weight, 27±1 g) underwent the same adaptation procedure as in Experiment 1, except that food intake was measured only 2, 4 and 24 h after IP treatment. Five doses of LPS (0.125, 0.25, 0.5, 1 and 2 μg/kg) were then tested in separate groups of animals, in cross-over trials with saline injections as control.

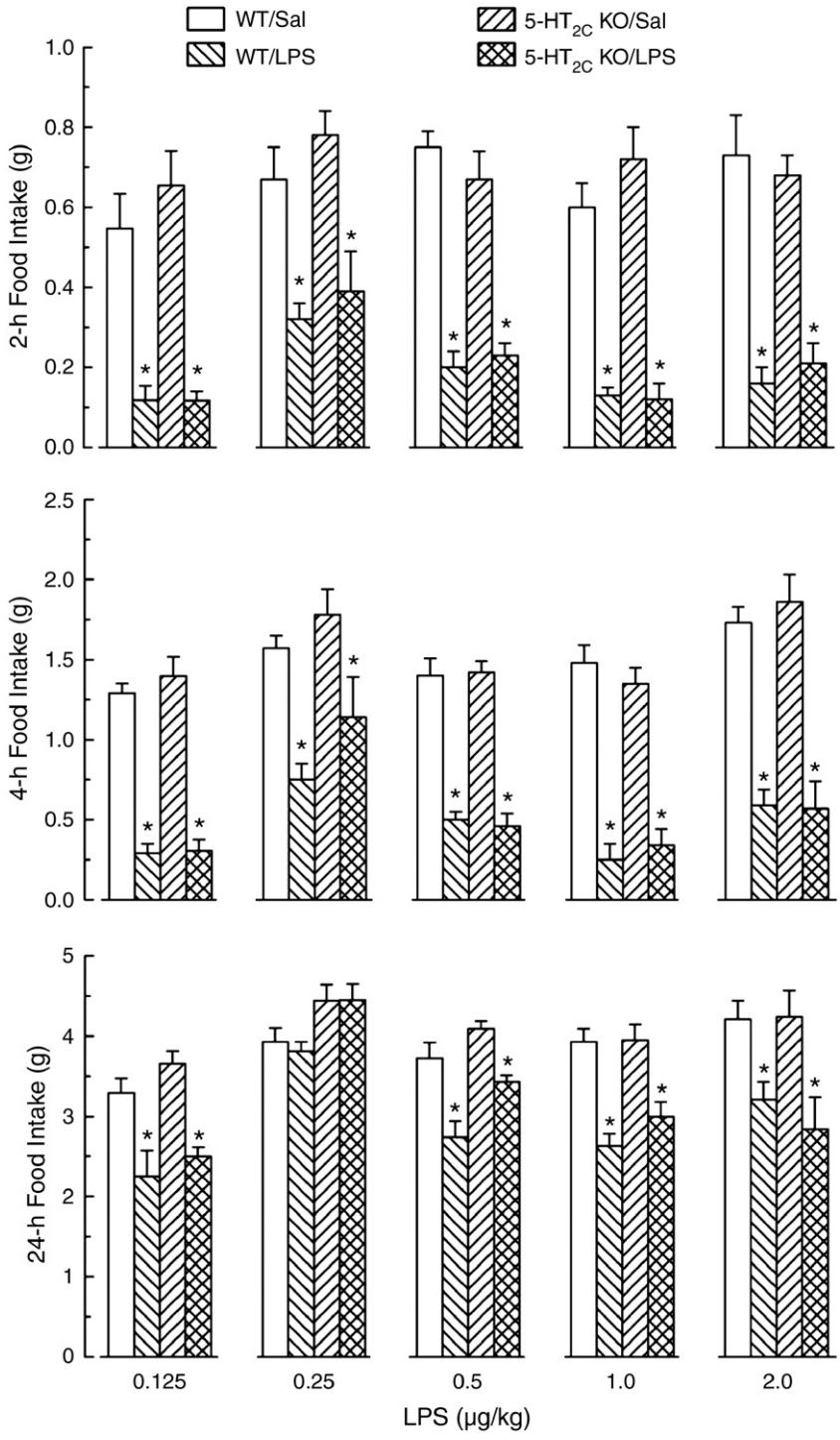


Fig. 2. LPS has similar anorectic potencies in WT and 5-HT$_{2C}$ KO mice across all doses tested. *LPS significantly different than saline (Sal), $P<0.001$.

### 2.5. Statistical analysis

Data were analyzed with ANOVA followed up with Bonferroni–Holm post-hoc tests between individual means. In Experiment 1, four post-hoc comparisons were of interest: (1) LPS vs. Sal, (2) LPS+SB 242084 vs. SB 242084, (3) Sal vs. SB 242084 and (4) (Sal-LPS) vs. (SB 242084 vs. LPS+SB 242084). Data for each time point (1, 2 and 4 h post treatment) were analyzed separately. Because the design of Experiment 1 was unbalanced (each animal received only 3 of the 4 treatments), comparisons were based on the following ANOVAs: for comparisons (1) and (2), separate within-subject ANOVA were done using the mice receiving LPS and the mice receiving LPS+SB 242084, each together with their matched saline and SB 242084 data. A one-way, within subjects ANOVA of data from all mice was used for comparison (3), between Sal and SB 242084, and a one-way, between subjects ANOVA of data (difference scores) from all mice was used for comparison (4), between the effect of LPS with and without SB 242084. Data from Experiment 2 were analyzed with two-way ANOVA (genotype × treatment), with repeated measures on one factor (treatment), for each LPS dose and time point tested. The standard error of the difference (SED) for each comparison is reported as an index of experiment-wide residual variability.

## 3. Results

### 3.1. Experiment 1

SB 242084 significantly attenuated the effect of LPS (Fig. 1). LPS significantly decreased food intake in the absence of SB 242084 at 1, 2 and 4 h ($F(2,17)=16.81$, 21.27 and 49.33, $P$-values $<0.001$, and SEDs = 0.06, 0.09, and 0.15 g for 1, 2 and 4 h, respectively). When administered in combination with SB 242084, LPS significantly decreased food intake when compared to SB 242084 alone ($F(2, 20)=20.37$, 32.02 and 57.06, $P$-values $<0.001$, and SEDs = 0.04, 0.06, and 0.1 g, for 1, 2 and 4 h respectively). At 1 and 2 h, but not at 4 h post administration, SB 242084 significantly decreased food intake when compared to saline ($F(1, 25)=24.50$, 12.02 and 2.54, $P<0.0003$, $P<0.004$, and $P>0.05$, and SEDs = 0.03, 0.05 and 0.08 g for 1, 2 and 4 h, respectively). Finally, and most importantly, SB 242084 decreased the anorectic effect of LPS in comparison to the appropriate control. That is, the difference between Sal and LPS was significantly larger than the difference between SB 242084 and LPS+SB 242084. This occurred at 1 and 2, but not at 4 h, post administration ($F(1, 12)=24.24$, 13.01 and 2.34, $P<0.0005$, $P<0.004$, and $P>0.05$, and SEDs = 0.04, 0.06, and 0.24 g for 1, 2 and 4 h, respectively).

### 3.2. Experiment 2

LPS (0.125, 0.5, 1 and 2 μg/kg) decreased food intake similarly in WT and 5-$HT_{2C}$ KO mice at all times tested (Fig. 2). The effect of each LPS dose was significant in both genotypes at every time point ($F(1,10)=30.72$, 55.07 and 18.6 for 2, 4 and 24 h following 0.125 μg/kg LPS; $F(1, 18)=152.73$ and 147.54, SEDs = 0.03 and 0.09, respectively, for 2 and 4 h following 0.25 μg/kg LPS; $F(1, 11)=44.96$, 259.06, 955.07, SEDs = 0.1, 0.15 and 0.21, respectively for 2, 4 and 24 h following 0.5 μg/kg LPS; $F(1, 13)=448.96$, 235.02, 371.8, SEDs = 0.17, 0.21 and 0.25, respectively for 2, 4 and 24 h following 1 μg/kg LPS; $F(1, 10)=106.28$, 223.69 and 210.6, SEDs = 0.13, 0.08 and 0.2 respectively for 2, 4 and 24 h following 2 μg/kg LPS; all $P$-values $<0.001$), except the 0.25 μg/kg LPS dose failed to reduce 24 h food intake in either genotype ($F(1,10)<0.01$, $P>0.05$, SED = 0.18 g). No significant main effects of genotype or interaction effects of LPS and genotype were detected for any of the 5 doses or three time points, $F$-values not shown, except for a main effect of genotype 24 h following 0.5 μg/kg LPS ($F(1, 19)=8.31$, $P<0.01$, SED = 0.25 g). Because there was no significant interaction ($F(1,17)=1.77$, $P>0.05$, SED = 0.19 g), however, this genotype effect probably reflects the overall higher food intake in 5-$HT_{2C}$ KO vs. WT mice in this condition (KO Sal: KO LPS: 4.08: 3.42 g; WT Sal: WT LPS: 3.61: 2.74 g).

There were significant treatment and genotype factors 4 h after 0.25 μg/kg LPS administration, however, post-hoc analysis did not reveal differential effects of LPS between genotypes (KO (Sal-LPS) = 0.51 g; WT (Sal-LPS) = 0.82 g). Finally, 0.25 μg/kg LPS did not decrease food intake 24 h after administration, although 0.125 μg/kg LPS did. There was also a significant genotype effect 24 h after 0.25 μg/kg LPS, reflecting the higher overall food intake in 5-$HT_{2C}$ KO vs. WT mice (KO Sal: KO LPS = 4.44: 4.45 g; WT Sal: WT LPS = 3.92: 3.81 g).

## 4. Discussion

We characterized for the first time the role of the 5-$HT_{2C}$R in the anorectic effect of the gram-negative bacterial toxin LPS in male mice. LPS anorexia was decreased in genetically normal mice pretreated with the potent and selective 5-$HT_{2C}$R antagonist SB 242084, but was unaffected over a wide range of doses in mice lacking 5-$HT_{2C}$R function due to a genetic mutation of *htr2c* gene. These results extend similar pharmacological reports in rats (Hrupka and Langhans, 2001; von Meyenburg et al, 2003) and reveal an unusual discrepancy between the results of pharmacological and genetic analyses of 5-HTR function. The implications of the data and some potential causes of the lack of accord between the results generated by the two methodological approaches are discussed below.

### 4.1. Role of 5$HT_{2C}$R in LPS anorexia: pharmacological data

Previous work in rats established a role for 5-HT–5-$HT_{2C}$R neurotransmission in LPS anorexia (Langhans, 2004). The 5$HT_{2C}$R receptor antagonist SB 242084 blocked LPS anorexia, whereas pharmacological antagonism of other receptors (1A, 1B, 2A, 3) or CNS 5-HT depletion failed to do so (Hrupka and Langhans, 2001; von Meyenburg et al., 2003).

In contrast to these results in rats, data implicating 5-HT or 5-HTR in LPS anorexia in mice are scarce. LPS anorexia was not reversed in mice receiving chronic treatment with 5-HT agonist antidepressants (Dunn and Swiergiel, 2001). CNS 5-HT

depletion, however, caused an increase in LPS anorexia (Swiergiel and Dunn, 2000). A plausible explanation for these apparently asymmetric results is that the lack of 5-HT could have produced a compensatory increase in synthesis and release of other cytokines or immune mediators that control food intake after LPS treatment, such as IL-6. For example, the increase in the hypothalamic levels of tryptophan and 5-HIAA/5-HT that normally occurs between 1–3 h following LPS administration was blocked by administration of IL-6 antibodies prior to LPS injections (Wang and Dunn, 1999), suggesting that (1) IL-6 plays a role in controlling the rate of 5-HT synthesis during immune responses and (2) the lack of 5-HT could result in an up-regulation in levels of IL-6, and consequently increase LPS anorexia.

Further indirect evidence that 5-HT may play a role in LPS anorexia in mice is that 5-HT turnover is increased by LPS administration in the paraventricular and arcuate nuclei of the hypothalamus, brain sites that have been implicated in food intake regulation (Lacosta et al., 1999). To our knowledge, there are no other data implicating the 5-$HT_{2C}$R in LPS anorexia in mice.

SB 242084 is the first reported selective, potent and brain-penetrant 5-$HT_{2C}$R antagonist. It has very high affinity for the 5-$HT_{2C}$R (p$K_i$=9) and 150 fold selectivity for the 5-$HT_{2C}$R over 5-$HT_{2A}$ and 5-$HT_{2B}$R (Kennett et al., 1997; Wood et al., 2001). Under our conditions, pretreatment with SB 242084 resulted in an attenuation of anorexia 1 and 2 h, although not 4 h, post-LPS administration, thus implicating the 5-$HT_{2C}$R in the mechanism underlying LPS anorexia in mice. The short duration of the effect of SB 242084 is similar to those previously reported in rats (Hrupka and Langhans, 2001). This result could arise either if the role of 5-$HT_{2C}$R is limited to the first few hours of LPS anorexia or if SB 242084 is cleared rapidly. Neither of these possibilities has been tested so far. Interestingly, however, reports of the effects of SB 242084 on feeding, locomotion, grooming and anxiety in rats are all limited to intervals less than 2 h following the antagonist administration (Mosher et al., 2005; Graf et al., 2003), suggesting that longer term effects may not occur.

In contrast to the lack of effect of SB 242084 on food intake observed in rats (von Meyenburg et al., 2003), under our conditions SB 242084 alone decreased food intake by 35 and 25% at 1 and 2 h post administration, respectively. Two points are worth noting in connection with this apparent discrepancy. First, whether this resulted from a species differences or from methodological differences is unclear. It may have been important that in the rat study, SB 242084 was injected 4 h after LPS injections, whereas here we administered it just prior to LPS injections. Second, that SB 242084 appeared to reverse LPS anorexia less in mice than previously reported in rats could be an artifact of the inhibitory effect of SB 242084 alone on food intake in mice.

Dalton et al. (2006) also reported that SB 242084 reduced 2-h wet mash intake by about 20%, although the statistical significance of the effect apparently was not tested. Postprandial behavioral observations indicated that SB 242084 specifically reduced the motivation to eat rather than inhibiting feeding by producing toxicity, illness or aversion (Hewitt et al., 2002; Dalton et al., 2006). The differential feeding effects of SB 242084 in mice and rats may represent a species difference. Perhaps as a consequence of this feeding-inhibitory effect of SB 242084 in mice, the absolute magnitude of the attenuation of LPS anorexia by SB 242084 after 1 and 2 h was less in mice than previously observed in rats (von Meyenburg et al., 2003).

### 4.2. Role of 5$HT_{2C}$ receptor in LPS anorexia: genetic data

Although 5-$HT_{2C}$ KO mice have been used extensively as metabolic and depression models, this is the first study using them in an illness anorexia model. Surprisingly, in contrast to the pharmacological results, none of the LPS doses tested produced a differential anorectic response in WT and 5-$HT_{2C}$ KO mice. One explanation for the lack of genotype effect comes from a major disadvantage associated with all null mutation genetic models: secondary effects, especially developmental changes, arising from indirect actions of the genetic deletion that may mask the primary cell- or tissue-specific intended effect of the knockout (Davey and MacLean, 2006). Among the secondary effects that occur in 5-$HT_{2C}$ KO mice are chronic hyperphagia, overweight, spontaneous epileptic seizure activity, and increased locomotor activity after 5-HT agonist (mCPP) treatment (Dalton et al., 2006; Tecott et al, 1995). How any of these might be related to the lack of effect of *htr2c* gene deletion on LPS anorexia, however, is unclear.

Another possible explanation comes from the distinction between 5-$HT_{2C}$ KO mice and mice treated with SB 242084. The antagonist does not elicit either proconvulsant or hyperphagic responses, both of which are characteristic of 5-$HT_{2C}$ KO mice (Kennett et al., 1997; Tecott et al., 1995). In a recent study investigating the effect of the genetic deletion of 5-$HT_{2C}$R vs. SB 242084 pretreatment on the anorectic response to 5-HT agonist mCPP, Dalton et al. (2006) report that the genetic and pharmacological blockade of the 5-$HT_{2C}$R cause a similar attenuation in mCPP anorexia, but had a different effect on the postprandial locomotor activity. That is, 5-$HT_{2C}$ KO mice increase their activity by about 50% from the baseline level, whereas the antagonist treated mice did not.

## 5. Summary

In the experiments described here we employed pharmacological and genetic tools to determine whether the 5-$HT_{2C}$R is part of the mechanism mediating LPS anorexia in mice. Our pharmacological data extend similar findings in rats and suggest that 5-$HT_{2C}$R activation is a necessary part of LPS anorexia. In contrast, our results in mice with genetic deletions of the 5-$HT_{2C}$R do not support this conclusion. Further studies, such as identifying whether LPS activates neurons expressing 5-$HT_{2C}$R or 5-HT-ergic neurons that synapse on 5-$HT_{2C}$R positive terminals, might strengthen the pharmacological finding that 5-$HT_{2C}$R are part of the mechanism underlying LPS anorexia in mice and illuminate the reason for the disparate outcomes of the pharmacological and transgenic genetic approaches. In any case, this disparity exemplifies the shortcomings of gene knockout models, which can

have unintended and difficult-to-detect consequences, so that findings from these experiments should be interpreted with caution.

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