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ORIGINAL INVESTIGATION

# The serotonin 5-HT$_{2A}$ receptor agonist TCB-2: a behavioral and neurophysiological analysis

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**Abstract**
*Rationale* There are few reports on the high-affinity 5-HT$_{2A}$ agonist (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl) methylamine hydrobromide (TCB-2).
*Objectives* Here we provide the first behavioral and neurophysiological profile of TCB-2 in C57BL/6J mice, with direct comparisons to the 5-HT$_{2A/2C}$ agonist (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI), in addition to determinations of 5-HT$_{2A}$ mediation via pretreatment with the selective 5-HT$_{2A}$ antagonist MDL 11,939.
*Results* In a dose-dependent manner, TCB-2 induced head twitches, decreased food consumption in food-deprived mice, induced hypothermia, and increased corticosterone levels, with no effects on locomotor activity or anxiety-like behaviors in the open field. Similar effects were observed in side-by-side dose–response comparisons with DOI; although at the highest dose tested (5.0 mg/kg), TCB-2 induced significantly fewer head twitches, and a significantly enhanced hypothermic response, versus DOI. Pretreatment with MDL 11,939 blocked head twitches and temperature change following TCB-2 and DOI, confirming 5-HT$_{2A}$ mediation of these responses. Although MDL 11,939 pretreatment blocked DOI-induced suppression of feeding, MDL 11,939 had no effect on TCB-2-induced suppression of feeding. Previous studies show that 5-HT$_{2A}$ function is altered by changes in serotonin transporter (SERT) expression and function. In SERT knockout (−/−) mice, TCB-2-induced head twitches and hypothermia were greatly diminished compared to SERT wild-type (+/+) mice.
*Conclusions* The current studies are important, as they are the first to assess the effects of TCB-2 in mice, and are among the first to report the behavioral and neurophysiological effects of this conformationally restricted phenethylamine analog compound, which has 65-fold greater effects on signaling via the phosphoinositide versus arachidonic acid pathways.

**Keywords** TCB-2 · DOI · MDL 11,939 · 5-HT$_{2A}$ receptors · Serotonin transporter knockout mice · Head twitch response · Feeding · Hypothermia · Corticosterone · Anxiety

## Introduction

Serotonin 5-HT$_{2A}$ receptors are involved in the hallucinogenic effects of serotonergic drugs including lysergic acid diethylamide (LSD), and are also a target for neuroleptics used in the treatment of schizophrenia. Typically, the phenethylamine (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) is used to assess 5-HT$_{2A}$ receptor function, although this compound is also an agonist at 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors. The $K_i$ values for DOI at 5-HT$_{2A}$ versus 5-HT$_{2C}$ receptors have been reported on average as quite similar (0.68 and 1.65 nM at 5-HT$_{2A}$ receptors, and 2.38 and 3.01 nM at 5-HT$_{2C}$ receptors, in human and rat clones, respectively) (http://pdsp.med.unc.edu/). DOI induces several 5-HT$_{2A}$-mediated responses, including activation of the hypothalamic–pituitary–adrenal

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(HPA) axis which stimulates corticosterone, adrenocorticotropic hormone (ACTH), and oxytocin release (Hemrick-Luecke and Evans 2002; Van de Kar et al. 2001; Zhang et al. 2002), and several 5-$HT_{2A}$-mediated behaviors, including the head twitch response (Gonzalez-Maeso et al. 2007; Moya et al. 2007; Willins and Meltzer 1997), a response thought to serve as a proxy for hallucinogenic effects in humans (Gonzalez-Maeso et al. 2007).

Recently, the high-affinity 5-$HT_{2A}$ agonist (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide (TCB-2) was described (McLean et al. 2006), although to date there are few reports on this compound. The chemical structures of TCB-2, DOI, and serotonin are depicted in Fig. 1. TCB-2 is a phenethylamine with high-affinity for rat ($K_i$=0.73 nM) and for human ($K_i$=0.75 nM) 5-$HT_{2A}$ receptors (McLean et al. 2006). In cell lines expressing the rat 5-$HT_{2A}$ receptor, TCB-2 had 65-fold higher potency in stimulating phosphoinositide turnover compared to producing arachidonic acid release (McLean et al. 2006), and activated the MAP kinase pathway, as evidenced by ERK phosphorylation, an effect which was blocked by the 5-$HT_2$ antagonist ketanserin (Chang et al. 2009). Recently, it was shown that activation of 5-$HT_{2A}$ receptors in rat aortic smooth muscle cells inhibits tumor necrosis factor (TNF)-α-mediated inflammation; in this report, TCB-2 (2C-BCB) inhibited proinflammatory marker expression, as did other 5-$HT_{2A}$ agonists including LSD and DOI (Yu et al. 2008). In vivo, in a drug discrimination task in which rats were trained to discriminate LSD from saline, TCB-2 was of equal potency to LSD, and was 11-fold more potent than DOI (McLean et al. 2006). Furthermore, in rats trained to discriminate DOI from saline, TCB-2 was 13-fold more potent than was DOI.

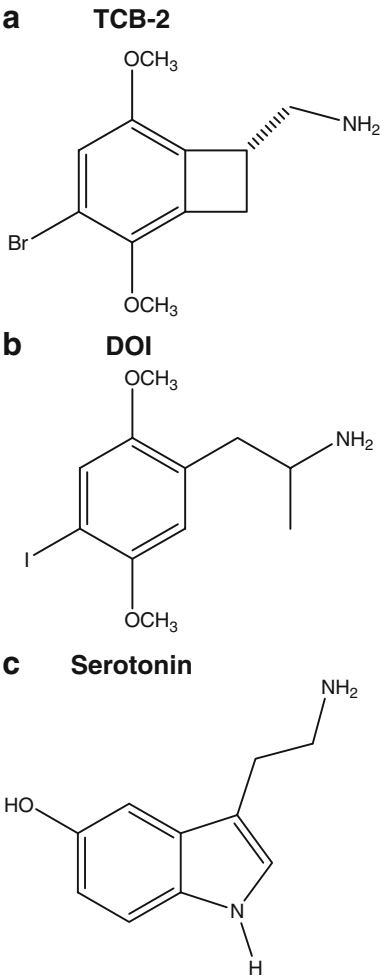


**Fig. 1** Chemical structures of TCB-2 ((4-Bromo-3,6-dimethoxy benzocyclobuten-1-yl)methylamine) (**a**), DOI ((±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane) (**b**), and serotonin (5-hydroxytryptamine) (**c**)

To date, a comprehensive profile of TCB-2 has not yet been reported. Additionally, there are no reports on the effects of TCB-2 in mice, and to our knowledge, only a single report in rats in vivo (McLean et al. 2006). Here, we conducted a thorough set of studies to determine the behavioral and neurophysiological profile of TCB-2 in C57BL/6J mice. We performed dose–response assessments of the effects of TCB-2 on several behaviors and responses previously shown to involve 5-$HT_{2A}$ receptor activation, and side-by-side dose–response comparisons were made to DOI. Specifically, we assessed the effects of TCB-2 on head twitches, on anxiety measures and locomotor activity in the open field, on food consumption in food-deprived mice, on body temperature, and on corticosterone levels. 5-$HT_{2A}$ mediation of these effects was examined via pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939. As 5-$HT_{2A}$ functional changes have been associated with genetically altered serotonin transporter (SERT) expression and function (Basselin et al. 2009; Jennings et al. 2008; Li et al. 2003; Qu et al. 2005; Rioux et al. 1999), we also assessed the effects of TCB-2 in mice lacking one or two copies of SERT.

The current studies are of importance, as they are the first to assess the effects of TCB-2 in mice, and are among the first to report the behavioral and physiological effects of this compound in any species. While TCB-2 and DOI are both agonists at 5-$HT_{2A}$ receptors, in cell lines TCB-2 preferentially activates phosphoinositide turnover versus arachidonic acid release (McLean et al. 2006), whereas DOI preferentially activates arachidonic acid release (Moya et al. 2007), although it is unclear if these downstream effects are cell or tissue specific. As such, the current studies are also of importance as by providing direct comparisons of the behavioral and physiological effects of these two compounds, the current findings help to elucidate the roles of these different second-messenger pathways in such responses.

## Materials and methods

### Animals

Mice were male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) and male SERT +/+, +/−, and −/− mice, which were originally produced by homologous recombination in ES cells (Bengel et al. 1998), and are currently the product of 19–23 heterozygous backcrosses on a C57BL/6J genetic background. Mice were approximately 20–35 g in weight at the beginning of the experiments, and were housed in groups of 3–5 animals per cage (except where noted) with food and water available ad libitum (except where noted). Animals were maintained on a 12-h light: 12-h dark cycle (lights on at 0600 hours). On test days, mice were moved to the testing room in their home cage 1 h prior to testing, and all testing occurred between 1000 h and 1300 h. All experiments adhered to the guidelines of the National Institutes of Health, and were approved by the National Institute of Mental Health Animal Care and Use Committee.

### Drugs

The 5-$HT_{2A}$ agonist (4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl)methylamine hydrobromide (TCB-2) and the selective 5-$HT_{2A}$ antagonist MDL 11,939 were obtained from Tocris Bioscience (Ellisville, MO, USA), and the 5-$HT_{2A/2C}$ agonist (±)-2,5-dimethoxy-4-iodophenyl-2-aminopropane (DOI) was obtained from Sigma Chemical Company (St. Louis, MO, USA). TCB-2 was dissolved in distilled water, MDL 11,939 was dissolved in a few drops of acetic acid and prepared in distilled water (adjusted to a pH of ~6.5), and DOI was dissolved in saline. All drugs and their vehicles were administered via intraperitoneal injection at a volume of 10 ml/kg.

### Head twitches

Following 15 min of habituation in a plexiglass container, mice were administered the test drug. Head twitches were recorded for five 1-min periods (once every 5 min starting 5 min after drug administration) over a 30-min period; the scores from the five 1-min periods were summed together. In an initial dose–response experiment, C57BL/6J mice were administered vehicle, TCB-2 (0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg) or DOI (0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg), and head twitches were counted as described above. Next, we assessed 5-$HT_{2A}$ mediation of the head twitch response via pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939. First, we confirmed that MDL 11,939 (1.0 mg/kg) administered alone had no effect on head twitches compared to vehicle (NS, data not shown). In two separate experiments, mice were pretreated with the selective 5-$HT_{2A}$ antagonist MDL 11,939 (1.0 mg/kg) or its vehicle 30 min prior to administration of either DOI (2.5 mg/kg) or TCB-2 (2.5 mg/kg). Finally, head twitches were assessed in SERT +/+, +/−, and −/− mice administered TCB-2 (2.5 mg/kg).

### Open field

C57BL/6J mice were injected with vehicle, TCB-2 (1.0 or 2.5 mg/kg) or DOI (1.0 or 2.5 mg/kg). Immediately following injection, mice were placed in the corner of a novel plexiglass open field arena (40×40×30 cm) in a room dimly lit with indirect red lighting and were allowed to explore for 30 min. In a separate study, using the parameters of the feedings study (see below), mice were administered TCB-2 (5.0 mg/kg) 30 min prior to placement in the open field, where they were allowed to explore for 60 min to verify that changes in food consumption were not due to changes in locomotor activity. Behavior (distance traveled, rears, and duration in center (20×20 cm)) was recorded using the Noldus Ethovision Video Tracking System (Noldus Information Technology, Leesburg, VA, USA).

### Feeding

Mice were singly housed for at least 48 h prior to food deprivation. Food was removed from the home cage overnight (18 h deprivation). Following administration of vehicle, TCB-2 (1.0, 2.5, or 5.0 mg/kg) or DOI (1.0, 2.5, or 5.0 mg/kg), mice were placed in the test cage. Thirty minutes later, mice were presented with two pre-weighed pellets of their normal chow (PMI Nutrition International, Brentwood, MD, USA) placed in the hopper, the same food delivery method under day-to-day conditions. Mice had access to this food for a 1-h period. The food remaining at the end of this test period was re-weighed. Animals had ad libitum access to water throughout the food deprivation period and during the test phase. To assess 5-$HT_{2A}$-mediation, we first confirmed that the selective 5-$HT_{2A}$ antagonist MDL 11,939 (1.0, 2.5, or 5.0) administered alone did not affect feeding compared to vehicle [$F_{3,16}$=0.60, NS]. In a first antagonism study, mice were pretreated with vehicle or MDL 11,939 (1.0 mg/kg) followed 30 min later by DOI (5.0 mg/kg), and feeding was assessed. In a separate study, mice were pretreated with vehicle or MDL 11,939 (1.0, 2.5, or 5.0 mg/kg) followed 30 min later by TCB-2 (5.0 mg/kg), and feeding was assessed as described. In a final feeding study, mice were pretreated with vehicle or MDL 11,939 (1.0 mg/kg) followed 30 min later by an intermediate dose of TCB-2 (3.75 mg/kg).

### Temperature

Temperature was assessed using a digital thermometer (Model BAT 12, RET-3 probe, Physitemp Instruments, Clifton, NJ, USA). The probe was inserted approximately 2 cm into the rectum of the mouse, using mild tail restraint to hold the mouse in place when necessary. Following the first temperature assessment, animals were placed individually into plexiglass containers in a room with an ambient temperature of 20±1°C. Rectal temperature was measured every 15 min beginning 15 min prior to drug administration, over a 45-min period. In an initial dose–response study, C57BL/6 J mice were administered vehicle, TCB-2 (0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg) or DOI (0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg). Next, in two separate studies to assess 5-$HT_{2A}$ mediation of temperature change, mice were pretreated with vehicle or MDL 11,939 (1.0 mg/kg), a dose which did not affect temperature when administered alone, 30 min prior to administration of either DOI (5.0 mg/kg) or TCB-2 (5.0 mg/kg). Finally, temperature change was assessed in SERT +/+, +/−, and −/− mice administered TCB-2 (2.5 mg/kg).

### Corticosterone levels

C57BL/6J mice were administered vehicle, TCB-2 (2.5, 5.0, or 10.0 mg/kg) or DOI (2.5, 5.0, or 10.0 mg/kg). Thirty minutes later, mice were sacrificed by cervical dislocation and were decapitated to collect trunk blood. The trunk blood was collected in a 1.5 ml microcentrifuge tube containing 50 µl of 300 mM EDTA (pH 7.4) on ice. After collection, the tube was inverted several times and centrifuged (3,800×*g*, 20 min, 4°C) to separate plasma from red blood cells. Plasma samples were stored at −80°C until assayed for corticosterone. Corticosterone levels were measured using commercially available enzyme-linked immunosorbent assay kits obtained from Immunodiagnostic Systems Inc. (Fountain Hills, AZ, USA). Assays were performed following manufacturer's protocol. Plasma samples were diluted 1:20 using manufacturer supplied dilution buffer. A range of doses of MDL 11,939 (0.01 to 2.5 mg/kg) were tested to determine the highest dose that did not affect corticosterone levels (0.1 mg/kg); however, the added stress of double injections increased corticosterone levels significantly over levels following vehicle. As such, we were unable to perform any further antagonism studies to assess specificity.

### Statistical analysis

Data was analyzed using one- or two-way analyses of variance or *t* tests where appropriate. Post hoc analyses were performed using Tukey HSD tests or *t* tests as appropriate. Significance was based on $p<0.05$.

## Results

### Head twitches

In an initial dose–response study, TCB-2 (0.5, 1.0, and 2.5 mg/kg) and DOI (0.5, 1.0, 2.5, and 5.0 mg/kg) induced head twitches in C57BL/6J mice in a dose-dependent manner compared to vehicle ($p$'s<0.05; Fig. 2a) [$F_{10,60}$= 11.74, $p<0.0001$]. Head twitches induced by TCB-2 and DOI were similar across the doses, with the exception that at the highest dose (5.0 mg/kg), TCB-2 induced half as many head twitches as did DOI ($p=0.021$). Confirming 5-$HT_{2A}$ mediation of the head twitch response, in two separate studies, pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939 (1.0 mg/kg) blocked head twitches induced by DOI (2.5 mg/kg) [$F_{2,18}=102.93$, $p<0.0001$] (vehicle + vehicle vs. vehicle + DOI, $p<0.0001$; vehicle + DOI vs. MDL 11,939 + DOI, $p<0.0001$; vehicle + vehicle vs. MDL 11,939 + DOI, NS; data not shown), and by TCB-2 (2.5 mg/kg; Fig. 2b) [$F_{2,19}=27.30$, $p<0.0001$].

### Open field

There were significant main effects of drug group for the distance traveled [$F_{4,39}=2.88$, $p=0.035$], the number of rears [$F_{4,39}=5.02$, $p=0.002$], and the duration of time spent in the center of the open field [$F_{4,39}=2.93$, $p=0.033$] (Table 1). Compared to vehicle, TCB-2 and DOI did not affect the total distance traveled, although there was a difference between mice administered 2.5 mg/kg TCB-2 versus mice administered 1.0 mg/kg DOI ($p=0.019$). Rears were decreased in mice administered 2.5 mg/kg TCB-2 compared to vehicle ($p=0.004$), and there was a trend toward an increase in the duration in the center of the open field in mice administered 1.0 mg/kg TCB-2 compared to vehicle ($p=0.115$; Table 1).

### Feeding

Compared to vehicle-treated mice, food consumption was decreased in mice administered TCB-2 (5.0 mg/kg, $p<0.0001$) or DOI (2.5 mg/kg, $p=0.01$; 5.0 mg/kg, $p<0.0001$; Fig. 3a) [$F_{6,57}=12.86$, $p<0.0001$]. There were no differences in food consumption between mice treated with the same dose of TCB-2 versus DOI. Pretreatment with MDL 11,939 (1.0 mg/kg) blocked the suppression of feeding following administration of DOI (5.0 mg/kg), to levels observed in vehicle-treated mice [$F_{2,33}=8.54$, $p=0.001$] (vehicle + vehicle vs. vehicle + DOI, $p=0.007$; vehicle + DOI vs. MDL 11,939 + DOI, $p=0.002$; vehicle + vehicle vs. MDL 11,939 + DOI, NS; data not shown). In a separate study, TCB-2 (5.0 mg/kg) again decreased feeding compared to vehicle ($p<0.0001$); however, pretreatment with a range of

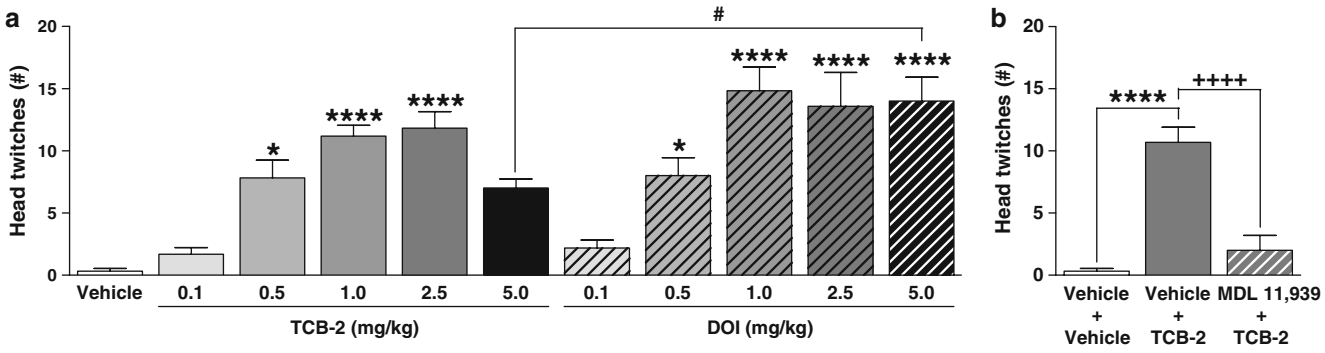


**Fig. 2** Dose–response curves for head twitches induced by TCB-2 and DOI (0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg) (**a**) and 5-$HT_{2A}$ mediation of TCB-2-induced head twitches (**b**) in C57BL/6J mice. TCB-2 (0.5, 1.0, and 2.5 mg/kg) and DOI (0.5, 1.0, 2.5, and 5.0 mg/kg) induced head twitches compared to vehicle ($n$=6–8). At a dose of 5.0 mg/kg, TCB-2 induced significantly fewer head twitches than DOI (**a**). Pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939 (1.0 mg/kg) blocked head twitches induced by TCB-2 (2.5 mg/kg; $n$=6–10) (**b**). Data represent the mean ± SEM. *$p$<0.05, ****$p$<0.0001 compared to vehicle-treated mice; #$p$<0.05 compared to mice treated with the same dose of DOI; ++++$p$<0.0001 compared to mice treated with vehicle + TCB-2

doses of MDL 11,939 (1.0, 2.5, or 5.0 mg/kg) did not affect this suppression of feeding ($p$'s<0.012 compared to vehicle + vehicle; NS compared to vehicle + TCB-2; Fig. 3b) [$F_{4,31}$= 11.91, $p$<0.0001]. To ensure that this TCB-2-induced suppression of feeding was not due to differences in locomotor activity, we performed another open field experiment using the same dosing and timing as in the feeding studies (i.e. 5.0 mg/kg TCB-2 administered 30 min prior to 60 min in the open field). There were no differences between mice administered vehicle and mice administered TCB-2 in the total distance traveled (17,479±3,690 cm vs. 19,978±7,305 cm, NS), in the percent of time moving (70±7% vs. 71±11%, NS) or in the number of rears (245±54 vs. 194±122, NS). Finally, we tested the effects of pretreatment with MDL 11,939 (1.0 mg/kg) on an intermediate dose of TCB-2 (3.75 mg/kg), in order to rule out the possibility that the reduction in feeding induced by 5.0 mg/kg TCB-2 was insurmountable. There was a significant effect of drug condition [$F_{2,12}$=10.61, $p$=0.002]; this intermediate dose of TCB-2 decreased feeding compared to vehicle ($p$=0.003), and pretreatment with MDL 11,939 was again without effect (NS vs. vehicle + TCB-2; $p$=0.011 vs. vehicle + vehicle; data not shown).

**Table 1** Effects of vehicle, TCB-2 (1.0 or 2.5 mg/kg) or DOI (1.0 or 2.5 mg/kg) on total distance traveled, rears, and duration in the center of the open field (30 min)

| Drug condition | Total distance (cm) | Rears (number) | Duration in center (s) |
|---|---|---|---|
| Vehicle | 10,271±1,770 | 202±68 | 219±67 |
| 1.0 mg/kg TCB-2 | 10,114±1,662 | 156±61 | 313±120 |
| 2.5 mg/kg TCB-2 | 8,571±1,767$^{+}$ | 96±33** | 291±88 |
| 1.0 mg/kg DOI | 11,176±1,216 | 206±71 | 233±49 |
| 2.5 mg/kg DOI | 10,472±1,445 | 144±49 | 201±74 |

Data represent the mean ± SEM; $n$=8–10 per group

$^{+}p$<0.05 compared to 1.0 mg/kg DOI; **$p$<0.001 compared to vehicle

## Temperature

TCB-2 decreased temperature in a dose-dependent manner, with a significant difference from vehicle at a dose of 5.0 mg/kg ($p$<0.0001), whereas DOI induced only a moderate temperature change across doses (Fig. 4a) [$F_{10,56}$=6.26, $p$<0.0001]. At a dose of 5.0 mg/kg, TCB-2-induced temperature change was greater than that induced by 5.0 mg/kg DOI ($p$=0.002). In a second study to assess 5-$HT_{2A}$ mediation of temperature change, DOI (5.0 mg/kg) decreased temperature compared to vehicle ($p$< 0.0001), and pretreatment with MDL 11,939 (1.0 mg/kg) blocked this temperature change induced by DOI ($p$=0.001) [$F_{3,32}$=10.94, $p$<0.0001] (data not shown). In a third study, TCB-2 (5.0 mg/kg) again decreased temperature compared to vehicle ($p$<0.0001), and pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939 (1.0 mg/kg) blocked this effect ($p$<0.0001; Fig. 4b) [$F_{3,32}$=40.25, $p$<0.0001].

## Corticosterone levels

Compared to vehicle, corticosterone levels were increased following TCB-2 (5.0 mg/kg, $p$=0.01; 10.0, $p$=0.012; with a trend following 2.5 mg/kg, $p$=0.079) and DOI (10.0 mg/kg, $p$=0.035; Fig. 5) [$F_{6,43}$=3.33, $p$=0.009]. There were no differences in corticosterone levels between mice administered the same dose of TCB-2 versus DOI.

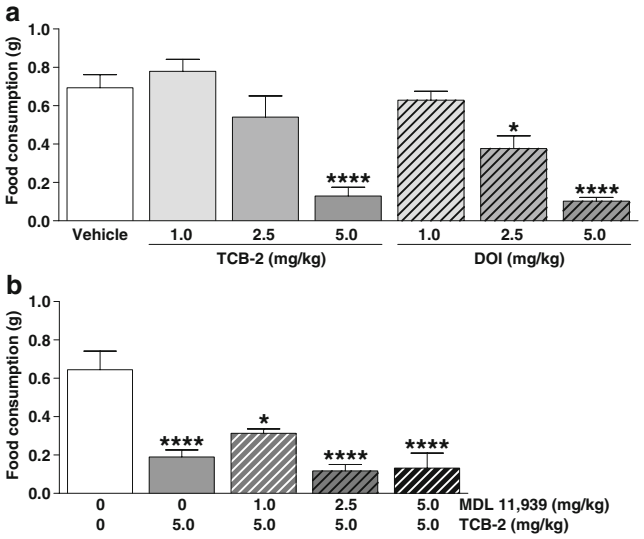


**Fig. 3** Dose–response effects of TCB-2 and DOI (1.0, 2.5, or 5.0 mg/kg) versus vehicle on food consumption (**a**) and 5-$HT_{2A}$ mediation of TCB-2-induced suppression of feeding (**b**) in food-deprived C57BL/6J mice. TCB-2 (5.0 mg/kg) and DOI (2.5 and 5.0 mg/kg) decreased food consumption in a dose-dependent manner compared to vehicle, with no significant differences between the same doses of TCB-2 and DOI ($n$=7–17) (**a**). Pretreatment with MDL 11,939 (1.0, 2.5, or 5.0 mg/kg) did not affect the suppression of feeding induced by TCB-2 (5.0 mg/kg; $n$=6–9) (**b**). Data represent the mean ± SEM. *$p$<0.05, ****$p$<0.0001 compared to vehicle-treated mice

Head twitches and temperature change in SERT +/+, +/−, and −/− mice

TCB-2 (2.5 mg/kg) induced ~51% fewer head twitches in SERT −/− mice compared to SERT +/+ mice ($p$=0.029), and head twitches were slightly but not significantly increased (~20%) in SERT +/− mice compared to SERT +/+ mice (Fig. 6a) [$F_{2,36}$=8.48, $p$=0.001]. In a separate study in SERT +/+, +/−, and −/− mice pretreated with MDL 11,939 (5.0 mg/kg) ($n$=10–12), there were no head twitches following TCB-2 (2.5 mg/kg; data not shown). Furthermore, TCB-2 (2.5 mg/kg) decreased temperature to a greater degree in SERT +/+ mice compared to SERT −/− mice ($p$=0.004), with no difference between SERT +/+ and SERT +/− mice (Fig. 6b) [$F_{2,34}$=6.94, $p$=0.003].

## Discussion

The current studies are the first to report a behavioral and neurophysiological profile of the high-affinity 5-$HT_{2A}$ agonist TCB-2 in mice, specifically the C57BL/6J strain. Overall, we show that in a dose-dependent manner, TCB-2 induced the head twitch response, increased corticosterone levels, suppressed feeding in food-deprived mice, and decreased body temperature, with no effects on locomotor activity compared to vehicle. In side-by-side dose-response comparisons, we show that TCB-2 induces similar responses to DOI, with a significantly decreased head twitch response following the highest dose of TCB-2 (5.0 mg/kg) compared to DOI, and conversely, a significantly increased temperature change following this same dose of TCB-2 compared to DOI. Furthermore, we show that the head twitch and temperature changes induced by TCB-2 and DOI are blocked by pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939, suggesting mediation by 5-$HT_{2A}$ receptors. However, whereas DOI-induced suppression of feeding was blocked by MDL 11,939, this selective 5-$HT_{2A}$ antagonist was without effect on TCB-2-induced suppression of feeding. Together, the current studies show that TCB-2 induces several robust 5-$HT_{2A}$-mediated behaviors and responses, in keeping with prior in vitro studies showing that TCB-2 acts as a 5-$HT_{2A}$

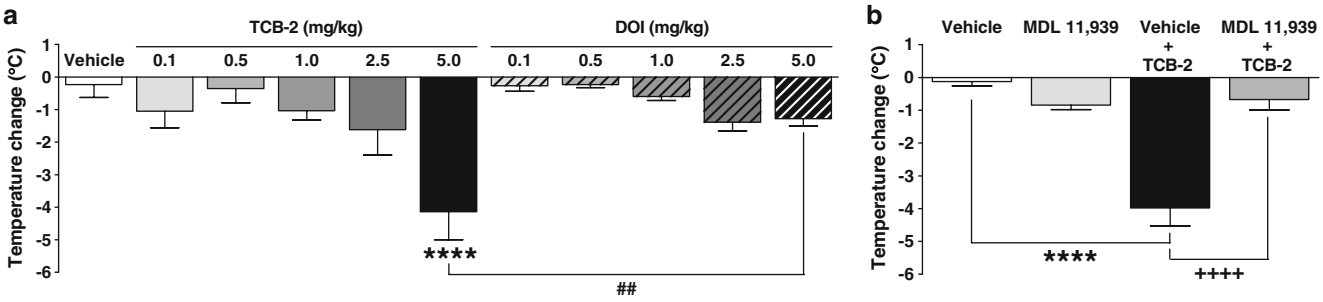


**Fig. 4** Dose–response curves for temperature change following TCB-2 or DOI (0.1, 0.5, 1.0, 2.5, or 5.0 mg/kg) (**a**) and 5-$HT_{2A}$ mediation of TCB-2-induced temperature change (**b**) in C57BL/6J mice. TCB-2 (5.0 mg/kg) significantly decreased temperature compared to vehicle, and did so to a greater degree than the same dose of DOI ($n$=6–7) (**a**). Pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939 (1.0 mg/kg) blocked the temperature change following TCB-2 (5.0 mg/kg; $n$=6–12) (**b**). There was a small difference in baseline temperature between mice administered 0.5 mg/kg TCB-2 (37.36± 0.45°C) and mice administered 0.5 (38.56±0.08°C), 2.5 (38.44±0.20°C), or 5.0 mg/kg DOI (38.46±0.10°C) (**a**), with no differences in baseline temperature in the assessment of 5-$HT_{2A}$ mediation (**b**). Data represent the mean ± SEM. ****$p$<0.0001 compared to mice treated with vehicle; ##$p$<0.01 compared to mice treated with the same dose of DOI; ++++$p$<0.0001 compared to mice treated with vehicle + TCB-2

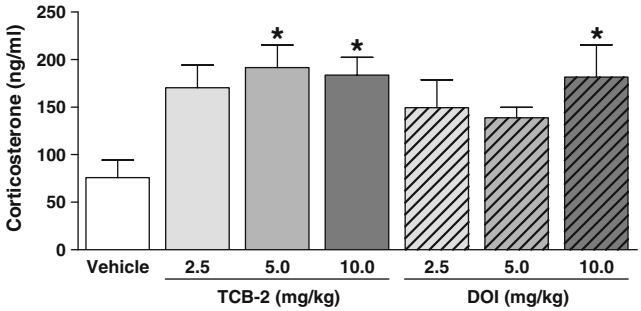


**Fig. 5** Dose–response curves for corticosterone levels following TCB-2 or DOI (2.5, 5.0, or 10.0 mg/kg) in C57BL/6J mice. Corticosterone levels were increased following TCB-2 (5.0 and 10.0 mg/kg) and DOI (10.0 mg/kg) compared to vehicle. There were no differences in corticosterone levels between mice administered the same doses of TCB-2 and DOI. Data represent the mean ± SEM; $n$=6–9. *$p$<0.05 compared to mice treated with vehicle

agonist (Chang et al. 2009; McLean et al. 2006), whereas some of the effects of this compound might be mediated by other receptor subtypes.

Previous research strongly suggests that the head twitch response is mediated by cortical 5-$HT_{2A}$ receptors (Gonzalez-Maeso et al. 2007, 2003; Moya et al. 2007; Willins and Meltzer 1997). In the current studies, TCB-2 induced head twitches in a dose-dependent manner, and 5-$HT_{2A}$ mediation was confirmed by pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 11,939. Furthermore, in SERT-deficient mice, which have brain-area-dependent changes in 5-$HT_{2A}$ receptors (Li et al. 2003; Rioux et al. 1999), TCB-2-induced head twitches were decreased by ~51% in SERT −/− mice compared to SERT +/+ mice. This is consistent with previous reports showing that DOI-induced head twitches are decreased ~86% in SERT −/− mice compared to SERT +/+ mice (Basselin et al. 2009; Qu et al. 2005), whereas DOI-induced head twitches are increased in mice over-expressing SERT (Jennings et al. 2008). These changes in DOI-induced head twitches in mice with altered SERT expression have been associated with alterations in serotonin levels (Basselin et al. 2009; Jennings et al. 2008), and it will be interesting to determine if altering serotonin levels, for example via administration of the serotonin synthesis inhibitor para-chlorophenylalanine PCPA, also affects head twitches induced by TCB-2. As the head twitch response is often used to determine 5-$HT_{2A}$ function, TCB-2 might be a useful compound in such functional assessments in the future.

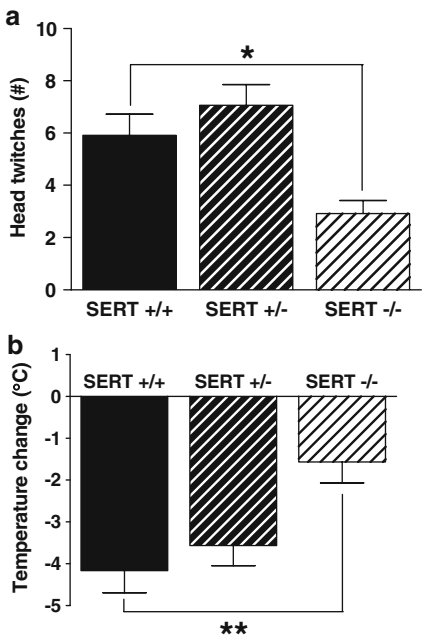


**Fig. 6** Head twitches (**a**) and temperature change (**b**) induced by TCB-2 in SERT +/+, +/−, and −/− mice. TCB-2 (2.5 mg/kg) induced head twitches in SERT +/+, +/−, and −/− mice, with significantly fewer head twitches in SERT −/− mice compared to SERT +/+ mice ($n$=11–16) (**a**). TCB-2 (2.5 mg/kg) decreased temperature to a greater degree in SERT +/+ and +/− mice compared to SERT −/− mice ($n$=11–14) (**b**). There were no differences in baseline temperature between the three SERT genotypes. Data represent the mean ± SEM. *$p$<0.05, **$p$<0.01 compared to SERT +/+ mice

Recently, it was shown that head twitches are induced via two different signal transduction pathways at 5-$HT_{2A}$ receptors (Schmid et al. 2008). Specifically, Schmid and colleagues (2008) showed that serotonin, via administration of its precursor 5-L-hydroxytryptophan 5-HTP, induced head twitches via a β-arrestin-2-dependent pathway, whereas DOI induced this same response via a β-arrestin-2-independent pathway. It is of interest to determine the pathway by which TCB-2 induces the head twitch response in future studies, for example by assessing TCB-2-induced head twitches in β-arrestin-2 knockout mice.

As mentioned, the head twitch response has been suggested as a possible "proxy" for hallucinogenic effects of various compounds in humans (Gonzalez-Maeso et al. 2007). One line of evidence comes from studies showing that compounds considered to have hallucinogenic effects in humans, including DOI, its close analog 2,5-dimethoxy-4-methylphenylisopropylamine, and LSD (Hollister et al. 1969; Snyder et al. 1968; Weingartner et al. 1971; for reviews, see Geyer and Vollenweider 2008; Nichols 2004), induce the head twitch response in rodents (Gonzalez-Maeso et al. 2007, 2003; Moya et al. 2007), whereas other 5-$HT_{2A}$ agonists which are not hallucinogenic do not induce head twitches (Gonzalez-Maeso et al. 2007). As such, the current findings could suggest that TCB-2 might have hallucinogenic effects in humans, despite suggestions that TCB-2 might represent an example of a 5-$HT_{2A}$ agonist without hallucinogenic properties based on its preferential (65-fold) phosphoinositide turnover effects versus arachidonic acid release, the latter thought to be associated with hallucinogenic activity (McLean et al.

2006). As the current head twitch studies with TCB-2 were performed in mice, it will be of importance to examine phosphoinositide effects versus arachidonic acid release in mice and additional species, including other rodents, non-human primates, and, eventually, humans.

Further evidence that TCB-2 might have hallucinogenic effects in humans comes from previous data showing that TCB-2 substitutes for the hallucinogenic compounds LSD and DOI in LSD- and DOI-trained rats in a drug discrimination paradigm (McLean et al. 2006). Additionally, as head twitches induced by both TCB-2 (current studies) and DOI (Basselin et al. 2009; Qu et al. 2005) are markedly reduced in SERT −/− mice compared to SERT +/+ mice, and as SERT −/− mice have a marked decrease in the ability to establish stimulus control to LSD in a drug discrimination task (90% in SERT +/+ mice versus 31% in SERT −/− mice; Krall et al. 2008), together these findings might suggest that hallucinogenic effects of such serotonergic compounds, possibly including TCB-2, might be decreased in humans with lesser-expressing SERT polymorphisms (Hu et al. 2006; Lesch et al. 1996; Praschak-Rieder et al. 2007; Wendland et al. 2008).

The serotonin system has also been shown to be involved in feeding, and specifically, agonists at 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors have been shown to decrease feeding via separate mechanisms (Aulakh et al. 1995; Tecott et al. 1995; Vickers et al. 2001). Previous research using less selective 5-$HT_{2A}$ antagonists suggest that DOI-induced suppression of feeding is mediated by 5-$HT_{2A}$ receptors, and not by 5-$HT_{2C}$ receptors (Aulakh et al. 1995; Schechter and Simansky 1988), a finding confirmed in the current studies. Here, we show that both TCB-2 and DOI suppressed feeding in a dose-dependent manner in food-deprived C57BL/6J mice. Furthermore, although DOI-induced suppression of feeding was blocked by MDL 11,939, a range of doses of MDL 11,939 were without effect in blocking TCB-2-induced suppression of feeding, suggesting that another mechanism underlies this effect of TCB-2. To date, there are no reports showing the affinity of TCB-2 for receptors other than the 5-$HT_{2A}$ receptor (McLean et al. 2006), including that at other serotonin receptor subtypes. It is critical that future studies investigate the affinity of TCB-2 for other receptors, which will help to clarify the receptor mediation of this and other TCB-2-induced responses.

5-$HT_{2A}$ receptors have also been implicated in thermoregulation. Studies show that in rats, DOI induces a hyperthermic response via a 5-$HT_{2A}$-mediated mechanism (Mazzola-Pomietto et al. 1995; Salmi and Ahlenius 1998), whereas DOI induces a hypothermic response in mice (Yamada et al. 1995; although see Morishima and Shibano 1995). In the current studies, TCB-2 induced a hypothermic response in a dose-dependent manner in C57BL/6J mice, an effect which was ~3-fold greater than that induced by DOI at the highest dose (5.0 mg/kg). Furthermore, TCB-2-induced hypothermia was diminished in SERT −/− mice compared to SERT +/+ mice. Although previous studies in mice have also shown a role for presynaptic 5-$HT_{1A}$ (Bill et al. 1991; Goodwin et al. 1985) and 5-$HT_7$ (Faure et al. 2006; Fox et al. 2008; Guscott et al. 2003; Hedlund et al. 2003) receptors in murine temperature regulation, here we demonstrate that hypothermia following TCB-2 and DOI is mediated by 5-$HT_{2A}$ receptors.

We also show that TCB-2 increased corticosterone levels in C57BL/6J mice in a similar fashion to DOI. Previous research suggests that HPA activation, including corticosterone release, is mediated by 5-$HT_{2A}$ receptors (Hemrick-Luecke and Evans 2002; Van de Kar et al. 2001). These previous studies show that DOI-induced increases in hormones, including corticosterone, were blocked by pretreatment with the selective 5-$HT_{2A}$ antagonist MDL 100,907, with no effects on baseline hormone levels, whereas pretreatment with the selective 5-$HT_{2C}$ antagonist SB 242084 was without effect (Hemrick-Luecke and Evans 2002; Van de Kar et al. 2001). Similar findings of 5-$HT_{2A}$ mediation following administration of other serotonergic compounds, including mCPP and the selective 5-$HT_{2C}$ agonist RO 60-0175, have also been reported (Hemrick-Luecke and Evans 2002).

Although these and other studies point to 5-$HT_{2A}$ mediation of these HPA responses, these previous studies have all been performed in rats. To date, there is a lack of studies examining 5-$HT_{2A}$ versus 5-$HT_{2C}$ mediation of HPA activation in mice. In the current studies, in addition to our findings with TCB-2, we show that DOI increases corticosterone levels in C57BL/6J mice. This finding is in line with previous work from our lab, showing that DOI increases corticosterone, ACTH, and oxytocin in mice, including in SERT +/+, +/−, and −/− mice (Li et al. 2003). Other investigations of HPA mediation in knockout mice show that in both 5-$HT_{2A}$ (Weisstaub et al. 2006) and 5-$HT_{2C}$ (Heisler et al. 2007) knockout mice, baseline corticosterone levels are intact. Weisstaub and colleagues (2006) also showed that stress-induced increases in corticosterone, for example following exposure to the open field or forced swim tests, are intact in 5-$HT_{2A}$ knockout mice. However, in 5-$HT_{2C}$ knockout mice, increases in corticosterone are absent following mCPP or fenfluramine, suggesting a role for 5-$HT_{2C}$ receptors in this response (Heisler et al. 2007). Furthermore, corticotrophin-releasing hormone release following application of DOI to hippocampal brain slices from 5-$HT_{2C}$ knockout mice is absent (Heisler et al. 2007), although the effects of DOI on corticosterone levels in mice in vivo have not yet been reported. In the current studies, as MDL 11,939 alone and double injections affected corticosterone levels, we were

unable to assess 5-$HT_{2A}$ mediation of the corticosterone changes induced by TCB-2 and DOI in mice. As such, further studies are required to unravel the mechanism underlying these effects, and to clarify roles for these two serotonin receptor subtypes in HPA activation in mice, including investigations of the effects of TCB-2 and DOI on HPA activation in 5-$HT_{2A}$ and 5-$HT_{2C}$ knockout mice.

TCB-2, along with its analogs, were designed to investigate the possibility that altering the conformation of phenethylamines might result in the activation of different second messenger, post-receptor signaling pathways (McLean et al. 2006). Although DOI and TCB-2 are both agonists at 5-$HT_{2A}$ receptors, as mentioned, in cell lines TCB-2 preferentially activates phosphoinositide turnover versus arachidonic acid release (McLean et al. 2006), whereas DOI preferentially activates arachidonic acid release (Moya et al. 2007). The term "functional selectivity" has been coined to refer to the ability of different agonist compounds acting on the same receptor to activate different post-receptor signaling pathways (Urban et al. 2007). Comparisons of the behavioral and physiological effects of differentially functionally selective compounds are of importance in order to elucidate the role of different second-messenger pathways in such responses. Previous work comparing these two compounds shows that in a drug discrimination paradigm in rats, TCB-2 was 11- and 13-fold more potent than DOI in LSD- and DOI-trained rats, respectively (McLean et al. 2006), whereas DOI has been shown to be more potent than TCB-2 (2C-BCB) in inhibiting proinflammatory marker expression in rat aortic smooth muscle cells (Yu et al. 2008). In the current studies, TCB-2 and DOI induced similar responses in most of the assessments over the range of doses tested, with two exceptions; at a dose of 5.0 mg/kg, TCB-2 induced 50% fewer head twitches than this same dose of DOI, whereas temperature change following this same dose of TCB-2 was enhanced ~3-fold compared to DOI. It is possible that these different responses and differences in efficacy could be related to the different pathways activated, i.e., to their differences in functional selectivity. If the reported functional selectivity of TCB-2 observed in vitro is true in vivo, this might suggest that the current behavioral and physiological responses elicited by TCB-2 are mediated by phosphoinositide turnover versus arachidonic acid release. More research is required to confirm possible associations of behaviors and physiological responses with specific signaling pathways, research which will be of importance in creating new effective therapeutic compounds, possibly with fewer side effects.

Together, the current studies provide the first profile of TCB-2 in mice. We show that this compound induces many behaviors and physiological responses previously reported to involve 5-$HT_{2A}$ receptor activation, and here we confirm 5-$HT_{2A}$ mediation of hypothermic and head twitch responses induced by TCB-2. However, the current studies also show that 5-$HT_{2A}$ receptors do not likely mediate TCB-2-induced suppression of feeding. Further studies are required to elucidate the mechanism underlying the effect of TCB-2 on food consumption, in addition to TCB-2-induced increases in corticosterone levels. It is also important that future studies determine the selectivity of TCB-2 at serotonin receptors other than at the 5-$HT_{2A}$ receptor, as the only reported $K_i$ value for TCB-2 is at the 5-$HT_{2A}$ receptor (McLean et al. 2006). As DOI is typically used to assess 5-$HT_{2A}$ receptor function, and yet also has affinity for 5-$HT_{2B}$ and 5-$HT_{2C}$ receptors, it is of particular interest to evaluate the affinity of TCB-2 across the 5-$HT_2$ receptor subfamily. Studies of the effects of TCB-2 in 5-$HT_{2A}$ and 5-$HT_{2C}$ knockout mice will be of particular importance in further determining the profile of this compound. Once the selectivity of TCB-2 for other serotonin receptor subtypes has been further established, the utility of TCB-2 as a compound to assess 5-$HT_{2A}$ receptor function can be determined.

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