# The 5-HT$_7$ receptor as a mediator and modulator of antidepressant-like behavior

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## Abstract

The 5-HT$_7$ receptor has been suggested as a target for treating depression since inactivation or blockade of the receptor has an antidepressant-like behavioral effect. The present study investigated possible interactions between various classes of drugs with antidepressant properties and blockade or inactivation of the 5-HT$_7$ receptor. Immobility despair in the tail suspension test and the forced swim test was evaluated in mice lacking the 5-HT$_7$ receptor (5-HT$_7^{-/-}$) and in wild-type controls (5-HT$_7^{+/+}$) following acute drug treatments. Citalopram, a selective serotonin reuptake inhibitor and widely used antidepressant, dose-dependently reduced immobility in the tail suspension test in both 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice. Combining doses of citalopram and the 5-HT$_7$ receptor antagonist SB-269970 that by themselves did not affect behavior, reduced immobility in 5-HT$_7^{+/+}$ mice in both the tail suspension test and the forced swim test. No effect was seen in 5-HT$_7^{-/-}$ mice. Desipramine and reboxetine, two norepinephrine reuptake inhibitors, dose-dependently reduced immobility in the tail suspension test in 5-HT$_7^{+/+}$ mice, but had no effect in 5-HT$_7^{-/-}$ mice. A synergistic effect between desipramine and SB-269970 was found in both behavioral tests in 5-HT$_7^{+/+}$ mice. Reboxetine combined with SB-269970 had effect only in the forced swim test. GBR 12909, a dopamine reuptake inhibitor, dose-dependently reduced tail suspension test immobility in both genotypes. There was no interaction between GBR 12909 and SB-269970. Aripiprazole, an antipsychotic, reduced immobility in both tests in 5-HT$_7^{+/+}$ mice, but not in 5-HT$_7^{-/-}$ mice. The results show that the 5-HT$_7$ receptor is required for the observed interaction between this receptor and antidepressants such as citalopram. The data furthermore support the hypothesis that the 5-HT$_7$ receptor might be a suitable target for treating depression.

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## 1. Introduction

The 5-HT$_7$ receptor, a member of a more recently discovered group of 5-HT receptors [1–6], has been found to modulate behaviors affected by antidepressant drugs. Behavioral models of significant value for evaluating putative antidepressants and for characterizing transgenic mice [7,8] include the forced swim test [9] and the tail suspension test [10]. In both of these tests, inactivation or blockade of the 5-HT$_7$ receptor has been shown to lead to antidepressant-like behavior [11–13]. For inhibition of the 5-HT$_7$ receptor, these studies used the selective 5-HT$_7$ receptor antagonist SB-269970 [14,15]. Furthermore, inactivation or pharmacological blockade of the 5-HT$_7$ receptor lead to changes in rapid eye movement sleep that are opposite to those seen in depression [12,15].

These findings have led to the hypothesis that the 5-HT$_7$ receptor might be suitable new target for the treatment of depression [16–19]. It has been shown that certain antidepressants might exert some of their effects by acting directly at the 5-HT$_7$ receptor [20]. More interestingly, recent studies have found a synergistic interaction between SB-269970 and various antidepressants [21,22]. One of the studies found that mice given an ineffective dose of citalopram, a selective serotonin reuptake inhibitor (SSRI), in conjunction with an ineffective dose of SB-269970 exhibited antidepressant-like behavior in the tail suspension test [21]. At a higher dose, it has been demonstrated that SB-269970 alone reduces immobility in the mouse tail suspension test [12,21]. A similar synergistic interaction between SB-269970 and citalopram has also been described in the mouse forced swim test [22]. This study also reported that interactions occur also between SB-269970 and other classes of antidepressants. Thus, ineffective doses of imipramine (a tricyclic antidepressant), desipramine (a tricyclic antidepressant acting mainly as a norepinephrine reuptake inhibitor), and moclobemide (a monoamine oxidase inhibitor) all reduced immobility in the mouse forced swim test when given in combination with SB-269970 [22]. The interaction between SB-269970 and imipramine has also been shown in the forced swim test using Wistar rats [23]. The prefrontal cortex has been suggested as an important region for these interactions [21,22], but the hippocampus has also been implicated in the effects of SB-269970 and imipramine on the rat forced swim test [24].

Although the monoamine hypothesis of depression with its focus on 5-HT and norepinephrine has been very important for the understanding of this disorder, there are remaining issues, suggesting that other mechanisms might also be involved [25]. One significant pharmacological issue is that reuptake inhibition following treatment with an antidepressant occur rapidly, but its clinical effects require much longer time [26]. The present study aims to further investigate the relationships between the 5-HT$_7$ receptor and monoamine reuptake inhibition, and also to investigate possible relationships with other factors deemed to be relevant for the understanding of depression. Specifically, we have evaluated possible modulations of the 5-HT$_7$ receptor on dopamine reuptake, on the 5-HT$_{1A}$ receptor, on the antidepressant properties of the antipsychotic aripiprazole, and on corticosterone in models of depression.

Dopamine has been implicated in depression as it has been suggested that reduced availability of dopamine, in a similar way as for 5-HT and norepinephrine, might contribute to the pathophysiology of depression. In fact, dopaminergic mechanisms have gained increased

interest with the development of combined norepinephrine and dopamine reuptake inhibitors and even triple reuptake inhibitors [27–30]. Selective dopamine reuptake inhibitors have been shown to reduce immobility in the forced swim test in rats. Nomifensine at low doses, which significantly reduced the immobility time, had no significant effect on open field locomotor activity. At higher doses nomifensine increased overall locomotor activity as well as decreased immobility in the forced swim test [31]. GBR 12909 is another dopamine reuptake inhibitor that has recently been shown to reduce immobility in both the tail suspension test and the forced swim test, but also to increase overall locomotor activity [32].

Interestingly, it has recently been found that the dopamine $D_2/D_3$ antagonist amisulpride, an antipsychotic with known antidepressant properties [33,34], most likely exerts its antidepressant effect through the $5\text{-HT}_7$ receptor and not, as previously thought, through dopaminergic mechanisms [35]. Thus, amisulpride was shown to have high affinity for the human $5\text{-HT}_7$ receptor and to reduce immobility in both the tail suspension test and the forced swim test in $5\text{-HT}_7{}^{+/+}$ mice, but not in $5\text{-HT}_7{}^{-/-}$ mice. To extend these findings we have in the present study investigated aripiprazole, an atypical antipsychotic with high affinity for the $5\text{-HT}_7$ receptor [36] that is approved as augmentation therapy to antidepressants [37] and that has recently has been shown to potentiate the effect of fluoxetine [38].

The $5\text{-HT}_{1A}$ receptor and the $5\text{-HT}_7$ receptor have been shown to modulate the action of each other. After the discovery of the $5\text{-HT}_7$ receptor it was found that 8-OH-DPAT (8-hydroxy-2 (di-n-propylamino)tetralin), originally thought to be a selective agonist for the $5\text{-HT}_{1A}$ receptor, had high affinity for the $5\text{-HT}_7$ receptor [1]. It has subsequently been demonstrated that $5\text{-HT}_{1A}$ and $5\text{-HT}_7$ receptors both are involved in mechanisms such as thermoregulation [39]. Mice lacking the $5\text{-HT}_{1A}$ receptor show reduced immobility in the forced swim test [40], but it has not been possible to induce such an effect with antagonists for this receptor [41]. Nevertheless, $5\text{-HT}_{1A}$ receptor antagonists such as pindolol have been successfully used to reduce the time needed until clinical effect is achieved with antidepressants [42].

For decades, it has been known that individuals under sustained, elevated stress levels are at a higher risk of developing mental illnesses, especially depression. Stressors induce the secretion of glucocorticoids such as corticosterone in rodents and cortisol in humans through the hypothalamic-pituitary-adrenal axis. The serotonergic system has been shown to modulate the effects of glucocorticoids in mood disorders as well as regulate the expression of glucocorticoid and mineralocorticoid receptors [43,44]. An increase in $5\text{-HT}_7$ mRNA in the hippocampus was observed following blockade of corticosterone synthesis in male Wistar rats likely due to a reduction in glucocorticoid receptor transmission in the hippocampus [45].

In the present study we have further tested the hypothesis that the $5\text{-HT}_7$ receptor is a putative new target for the treatment of depressions [16–19]. We have performed an expanded series of experiments on the interaction between reuptake inhibitors for 5-HT, norepinephrine, and dopamine, and antagonists for the $5\text{-HT}_{1A}$ and $5\text{-HT}_7$ receptors, as well as the antipsychotic aripiprazole. Furthermore, we have investigated possible influences on serum corticosterone levels. In all experiments, comparisons were made between wild-type mice ($5\text{-HT}_7{}^{+/+}$) and mice lacking the $5\text{-HT}_7$ receptor ($5\text{-HT}_7{}^{-/-}$) in order to test the hypothesis that this receptor is required for the observed interactions.

## 2. Materials and methods

### 2.1. Animals

Ten-to-twelve week old male $5\text{-HT}_7{}^{-/-}$ mice and their male $5\text{-HT}_7{}^{+/+}$ sibling controls were used. The generation of the $5\text{-HT}_7{}^{-/-}$ mouse strain has been described previously [46]. The mice used in this study had been back-crossed on a C57BL/6J background for at least 16

generations. As the forced swim and tail suspension tests involve learning, the same animal was never used twice for the same behavioral method. A larger number of mice were exposed to the tail suspension test than the forced swim test. Thus, all mice tested in the forced swim test were also used for the tail suspension test, but not vice versa. A minimum of one week separated the two tests. A separate cohort of mice was used for evaluation of locomotor activity. All behavioral experiments were started at 09.00 h. The mice were housed in a 12-hour light/ dark cycle (lights on at 06.00 and off at 18.00) and had free access to water and food pellets. All the experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health, and were approved by the Animal Care and Use Committee at The Scripps Research Institute. Every effort was made to reduce the number of animals used and to minimize potential suffering.

## 2.2. Tail suspension test

The tail suspension test was performed as previously described [12]. Briefly, mice were suspended from a metal rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. The duration of the test was 6 minutes and immobility was measured during the last 4 minutes to facilitate comparison with the forced swim test. Immobility was defined as the absence of any limb or body movements, except those caused by respiration.

## 2.3. Forced swim test

The forced swim test was performed as previously described [12]. Briefly, mice were gently placed in a clear plastic cylinder, diameter of 16 cm, height 25 cm, filled with 10 cm of clear water at 25°C. Test duration was 6 minutes and immobility was measured during the last 4 minutes. Immobility was defined as the absence of any horizontal or vertical movement in the water, but excluded minor movements required for the mouse to keep its head above the surface. The water was replaced before each animal.

## 2.4. Locomotor activity

Locomotor activity was measured in Plexiglas cages ($42 \times 22 \times 20$ cm) placed into frames ($25.5 \times 47$ cm) mounted with two levels of photocell beams at 2 and 7 cm above the bottom of the cage (San Diego Instruments, San Diego, CA, USA). The two sets of beams allowed for the recording of both horizontal (locomotion) and vertical (rearing) behavior. A thin layer of bedding material was applied to the bottom of the cage. Mice were habituated to the activity boxes during three once daily 30-min sessions. The mice were then tested for drug for possible drug effects. For each treatment evaluated a mouse first had a 15-min session in the activity box followed by the injection. The mouse was then tested again 30 min later for 15 min.

## 2.5. Serum corticosterone

Blood was collected in heparinized capillaries through eye bleeds. Approximately 150 µl was collected from each animal sampled. The blood was transferred to 1.5 ml plastic tubes and allowed to coagulate. The samples were then centrifuged at 3000 rpm for 15 min. The serum was transferred to a new tube and stored at −80°C until analyzed. Serum corticosterone was measured using a radio-immuno assay kit following the instructions provided by the manufacturer (MP Biomedicals, Solon, OH).

## 2.6. Drug treatments

For the forced swim test, tail suspension test, and locomotor activity test, single intra-peritoneal injections were given 30 minutes prior to the test. Citalopram, desipramine, and WAY 100135 were obtained from Sigma (St. Louis, MO). GBR 12909 and SB-269970 were purchased from Tocris (Ellisville, MO). Reboxetine and aripiprazole was bought from Toronto Research

Chemicals (North York, ON). All drugs were dissolved in 0.9% NaCl and administered in the doses indicated in a total volume of 8 ml/kg. Although long-term treatment with drugs like citalopram is required in the clinic to obtain therapeutic effect, acute single injections are sufficient when using animal models [7]. The vehicle 0.9% NaCl alone was used as control.

### 2.7. Data analysis

All values are expressed as means ± standard errors of the mean (S.E.M.). Possible differences between genotypes and/or drug treatments were analyzed using Student's t-test or two-way analysis of variance (ANOVA) with genotype as one factor and drug treatment as the other factor. The ANOVA was followed by an appropriate Bonferroni posttest. All analyzes were performed using the GraphPad Prism (http://www.graphpad.com) software package. Differences were considered significant at $P < 0.05$.

## 3. Results

### 3.1. Tail suspension test and forced swim test

As previously reported [12], $5\text{-HT}_7^{-/-}$ mice showed lower immobility than $5\text{-HT}_7^{+/+}$ mice in both the tail suspension test and the forced swim test when given vehicle only.

**3.1.1. Serotonin reuptake inhibition**—Citalopram dose-dependently reduced immobility in $5\text{-HT}_7^{+/+}$ mice (Fig. 1A) in the tail suspension test. Citalopram also reduced immobility in $5\text{-HT}_7^{-/-}$ mice. A two-way ANOVA revealed significant effects for treatment ($F(4, 58) = 35.56$, $P < 0.001$), genotype ($F(1, 58) = 40.80$, $P < 0.001$), and interaction ($F(4, 58) = 4.66$, $P < 0.05$). A maximal effect (reduction) for the doses tested of approximately 20 s of immobility was reached at 10 mg/kg for $5\text{-HT}_7^{+/+}$ mice and at 3 mg/kg for $5\text{-HT}_7^{-/-}$ mice. When an ineffective dose of citalopram (1 mg/kg) was combined with an ineffective dose of SB-269970 (1 mg/kg), immobility was reduced in $5\text{-HT}_7^{+/+}$ mice ($F_{interaction}(3, 34) = 3.48$, $P < 0.05$; Fig. 1B). The dose of SB-269970 was chosen based on initial testing and earlier demonstrations that doses of 3 mg/kg and higher reduce immobility in wild-type mice [12,21]. This combination had no additional effect in $5\text{-HT}_7^{-/-}$ mice. A similar synergistic interaction between citalopram and SB-269970 was observed for $5\text{-HT}_7^{+/+}$ mice in the forced swim test ($F_{interaction}(3, 64) = 8.41$, $P < 0.001$; Fig. 1C). Interestingly, in this test, the dose 1 mg/kg of citalopram had an effect of its own in $5\text{-HT}_7^{-/-}$ mice (Fig. 1C).

**3.1.2. Norepinephrine reuptake inhibition**—Desipramine dose-dependently reduced immobility in the tail suspension test for $5\text{-HT}_7^{+/+}$ mice (Fig. 2A). For the doses tested desipramine did not further affect immobility in $5\text{-HT}_7^{-/-}$ mice. A two-way ANOVA showed significant effects for treatment ($F(4, 71) = 7.75$, $P < 0.001$), genotype ($F(1, 71) = 7.21$, $P < 0.01$), and interaction ($F(4, 71) = 4.05$, $P < 0.01$). When an ineffective dose of desipramine (1 mg/kg) was combined with an ineffective dose of SB-269970 (1 mg/kg), immobility was reduced in $5\text{-HT}_7^{+/+}$ mice ($F_{interaction}(3, 70) = 4.02$, $P < 0.05$; Fig. 2B). Such an effect was also seen in the forced swim test where the combination of desipramine (1 mg/kg) and SB-269970 (1 mg/kg) reduced immobility in the $5\text{-HT}_7^{+/+}$ mice ($F_{interaction}(3, 56) = 3.00$, $P < 0.05$; Fig. 2C). The dose 1 mg/kg of desipramine had no effect on immobility in the $5\text{-HT}_7^{-/-}$ mice.

In view of the results obtained with desipramine, especially its inability to reduce immobility in the $5\text{-HT}_7^{-/-}$ mice, the norepinephrine reuptake inhibitor reboxetine was also evaluated. The results for reboxetine were similar to those obtained with desipramine. Thus, reboxetine also dose-dependently reduced immobility in the tail suspension test for $5\text{-HT}_7^{+/+}$ mice (Fig. 2D), but the doses tested did not further affect immobility in $5\text{-HT}_7^{-/-}$ mice. A two-way ANOVA showed significant effects for treatment ($F(3, 49) = 10.09$, $P < 0.001$), genotype ($F(1, 49) = 6.39$, $P < 0.05$), and interaction ($F(3, 49) = 6.68$, $P < 0.001$). When an ineffective dose of

reboxetine (0.3 mg/kg) was combined with an ineffective dose of SB-269970 (1 mg/kg), immobility was not altered in the tail suspension test in any of the genotypes ($F_{interaction}$(3, 56) = 0.52, P = 0.67; Fig. 2E). However, in the forced swim test an interaction was observed where the combination of reboxetine (0.3 mg/kg) and SB-269970 (1 mg/kg) reduced immobility in the 5-HT$_7^{+/+}$ mice ($F_{interaction}$(3, 54) = 2.99, P < 0.05; Fig. 2F).

**3.1.3. Dopamine reuptake inhibition—**GBR 12909 dose-dependently reduced immobility in 5-HT$_7^{+/+}$ mice (Fig. 3A) in the tail suspension test. GBR 12909 also reduced immobility in 5-HT$_7^{-/-}$ mice. A two-way ANOVA revealed significant effects for treatment (F(3, 54) = 46.76, P < 0.001), genotype (F(1, 54) = 16.25, P < 0.001), and interaction (F(3, 54) = 6.38, P < 0.001). A maximal effect (reduction) for the doses tested of approximately 15 s of immobility was reached at 10 mg/kg for both genotypes. The dose 1 mg/kg of GBR 12909 had no effect on immobility in either genotype. Interestingly, an intermediate dose (3 mg/kg) reduced immobility in 5-HT$_7^{+/+}$ mice, but had no effect in 5-HT$_7^{-/-}$ mice (Fig. 3A). Combining the dose 1 mg/kg of GBR 12909 with 1 mg/kg of SB-269970 did not influence immobility ($F_{interaction}$(3, 59) = 1.33, P = 0.27; Fig. 3B). Comparable results were obtained in the forced swim test where 10 mg/kg of GBR 12909 reduced immobility in both genotypes (37.0 ± 14.1 s immobility, n = 9, for 5-HT$_7^{+/+}$ and 9.3 ± 6.2 s, n = 6 for 5-HT$_7^{-/-}$), but 1 mg/kg did not and there was no interaction with SB-269970 ($F_{interaction}$(3, 74) = 0.24, P = 0.87; Fig. 3C).

**3.1.4. 5-HT$_{1A}$ receptor inhibition—**WAY 100135 (10 mg/kg) did not influence immobility in the tail suspension test for 5-HT$_7^{+/+}$ or 5-HT$_7^{-/-}$ mice. The dose of WAY 100135 was chosen based on its ability to inhibit 8-OH-DPAT induced hypothermia [39]. As expected, there was an effect for genotype ($F_{(1,1)}$ = 30.12, P < 0.001), but no effect for treatment ($F_{(1, 1)}$ = 0.31, P = 0.58). The observed immobility values (s) were 127.70 ± 7.81 (5-HT$_7^{+/+}$, vehicle, n = 10), 75.60 ± 5.16 (5-HT$_7^{-/-}$, vehicle, n = 10), 114.00 ± 12.72 (5-HT$_7^{+/+}$, WAY 100135, n = 8), and 80.56 ± 4.45 (5-HT$_7^{-/-}$, WAY 100135, n = 9).

**3.1.5. Aripiprazole—**In 5-HT$_7^{+/+}$ mice aripiprazole showed a U-shaped dose-response curve (Fig. 4). In the tail suspension test immobility was reduced at 0.1 mg/kg, but then increased at higher doses to reach durations longer than for vehicle (Fig. 4A). In the forced swim test immobility was reduced at 0.03 mg/kg (Fig. 4B). In 5-HT$_7^{-/-}$ mice aripiprazole dose-dependently increased immobility in the tail suspension test (Fig. 4A). Within the doses tested aripiprazole did not alter immobility in the 5-HT$_7^{-/-}$ mice in the forced swim test (Fig. 4B). The two-way ANOVA values were for the tail suspension test for treatment (F(4, 58) = 27.53, P < 0.001), genotype (F(1, 58) = 11.17, P < 0.01), and interaction (F(4, 58) = 7.86, P < 0.001), and for the forced swim test for treatment (F(4, 69) = 3.51, P < 0.05), genotype (F(1, 69) = 9.8, P < 0.01), and interaction (F(4, 69) = 1.32, P = 0.27).

## 3.2. Locomotor activity

Citalopram, SB-269970, and their combination were assessed for any possible effect on locomotor activity as such an effect could influence behavior in the tail suspension or forced swim tests. The 1 mg/kg doses of citalopram or SB-269970 or their combination did not affect horizontal locomotor activity (Fig. 5). Rearing was also not affected (data not shown). As the limited availability of 5-HT$_7^{-/-}$ mice required that the same animals were tested for all the different treatments it was noted that repeated testing increased locomotor activity in the 5-HT$_7^{-/-}$ mice but not the 5-HT$_7^{+/+}$ (Fig. 5). The effect was however identical for vehicle treated animals and thus not a drug effect. The two-way ANOVA effects for genotype were F(1, 36) = 21.23, P < 0.001 for citalopram, F(1, 36) = 4.65, P < 0.05 for SB-269970, and F(1, 36) = 10.48, P < 0.01 for citalopram + SB-269970.

### 3.3 Serum corticosterone

Serum levels of corticosterone showed an expected circadian pattern in both $5\text{-HT}_7^{+/+}$ and $5\text{-HT}_7^{-/-}$ mice that had not been exposed to any behavioral tests (Fig. 4A). Serum corticosterone was also measured in vehicle-treated mice exposed to the tail suspension test and the forced swim test (Fig. 4B). These measurements were taken at approximately 10.30 am during the light cycle. Thus, there appears to be an increase in serum corticosterone due to the stress of the behavioral tests, but this was not evaluated statistically as the aim was to determine any difference between the genotypes. As no such differences were observed, serum corticosterone levels were not studied further.

## 4. Discussion

The major finding of the present study was that although drugs representing various classes of antidepressants seem to act independently of the $5\text{-HT}_7$ receptor in reducing immobility in the tail suspension and forced swim tests, the interaction experiments clearly show a synergistic effect between certain monoamine uptake inhibitors, but not others, and a $5\text{-HT}_7$ receptor antagonist. Renewed evidence that atypical antipsychotics with high affinity for the $5\text{-HT}_7$ receptor mediate their clinically established antidepressant effect through the $5\text{-HT}_7$ receptor is also presented.

Citalopram represents the selective serotonin reuptake inhibitors and is widely used to treat depression [25]. It has been shown to reduce immobility in the forced swim test [47] and the tail suspension test [48]. At least in the tail suspension test the effect is dose-dependent [48]. Thus, the present results confirm and extend previous findings by showing a dose-dependent effect of citalopram in reducing immobility and that the effect is present in both $5\text{-HT}_7^{+/+}$ and $5\text{-HT}_7^{-/-}$ mice. We have previously demonstrated that the selective $5\text{-HT}_7$ receptor antagonist SB-269970 reduces immobility in these test [12]. This has been confirmed for the tail suspension test by others [21]. The present results revealed a synergistic interaction between citalopram and SB-269970 when both compounds were given in ineffective doses in $5\text{-HT}_7^{+/+}$ mice. The data also showed that the interaction affects both the tail suspension test and the forced swim test. The specificity of the synergism to $5\text{-HT}_7$ receptors and their requirement for this interaction was confirmed by the lack of such an effect in $5\text{-HT}_7^{-/-}$ mice. A synergistic interaction between citalopram and SB-269970 has previously been demonstrated for the forced swim test [22] and the tail suspension test [21]. The first group also showed similar synergism with imipramine (serotonin and norepinephrine reuptake inhibitor) where an ineffective dose of imipramine concurrently administered with SB-269970 resulted in significant reduction in immobility in the forced swim test.

Using microdialysis, attempts have been made to find correlates between observed behavioral changes in response to antidepressants and possible changes in brain 5-HT concentrations [21,23,49]. A straight forward interpretation of the results has proved to be difficult, most likely due to different experimental designs. One study showed no effect of SB-269970 on 5-HT concentration in the frontal cortex [21]. Another study showed increases in 5-HT concentration in response to SB-269970 treatment in the prefrontal cortex, but this study was done already 24 h after surgery [23]. One possible explanation for the differing results is different stress levels at different post-surgical intervals. It has been shown that $5\text{-HT}_7$ receptor mRNA expression is upregulated in response to stress [50]. Possibly contradicting results were obtained in a study showing that a selective $5\text{-HT}_7$ receptor antagonist, SB-258741, could inhibit the increase in 5-HT levels induced by citalopram in the ventral hippocampus, but only if $5\text{-HT}_{1A}$ receptors were simultaneously blocked by WAY 100635 [49].

Desipramine represents a different class of anti-depressants in two ways. It belongs to the so called tricyclic antidepressants and it acts mainly as a norepinephrine reuptake inhibitor [51].

In contrast to citalopram, desipramine only reduced immobility in 5-HT$_7^{+/+}$ mice even at higher doses. The highest dose of desipramine used in the current study has previously been shown to induce a maximal reduction in immobility in C57BL/6J mice [48,52]. A difference in action between citalopram and desipramine in 5-HT$_7^{-/-}$ mice has previously been described in a sleep study [53]. This study showed that citalopram had a greater ability than desipramine to suppress rapid eye movement sleep in these mice. Nevertheless, a similar interaction as with citalopram was seen with ineffective doses of desipramine in the tail suspension test and the forced swim test. Again, that 5-HT$_7$ receptors are required for this interaction was confirmed by the lack of such an effect in 5-HT$_7^{-/-}$ mice. A synergistic interaction between desipramine and SB-269970 has previously been demonstrated for the forced swim test [22].

To verify and extend the results obtained with desipramine we also tested the selective norepinephrine reuptake inhibitor reboxetine, a clinically used antidepressant [54]. Reboxetine has been shown to reduce immobility in the tail suspension and forced swim tests including in mice on a C57BL/6J background [54,55]. The results were very similar compared with desipramine since both drugs reduced immobility in wild-type mice but not in 5-HT$_7^{-/-}$ mice in the tail suspension test. A weak or absent interaction with SB-269970 was seen in the tail suspension test, whereas a more distinct interaction was observed for both drugs in the forced swim test. Thus, although both serotonin and norepinephrine reuptake are modulated by the 5-HT$_7$ receptor there appears to be differences in how the different classes of drugs interact.

GBR 12909 represents a selective dopamine reuptake inhibitor. It has several hundred times higher affinity for the dopamine transporter than for the 5-HT transporter and no affinity for the norepinephrine transporter [56]. Thus, GBR 12909 should not affect synaptic 5-HT levels, at least not in animals with intact 5-HT transporters [57]. GBR 12909 has recently been shown to reduce immobility in both the tail suspension test and the forced swim test, but it is possible that this, at least in part, is due to an increase in overall locomotor activity [32]. The possible impact of changes in locomotor activity is, however, reduced by the fact that there is no difference in ambulatory or rearing activity over a 24-h period between 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice [58]. The present data confirm the previous findings and show that the effects on immobility are dose-dependent. GBR 12909 (10 mg/kg) had an effect in both 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice. Interestingly, however, the effect was not additive in 5-HT$_7^{-/-}$ mice as 3 mg/kg of GBR 12909 reduced immobility in 5-HT$_7^{+/+}$ mice but had no additional effect in 5-HT$_7^{-/-}$ mice. It is noteworthy that GBR 12909 did not interact with SB-269970 to modulate the immobility response in either test. Thus, even though to our knowledge the affinity of GBR 12909 for the 5-HT$_7$ receptor is not known, the most probable interpretation of the present results is that GBR 12909 acts independently of the 5-HT$_7$ receptor, and that as yet undescribed mechanisms in the 5-HT$_7^{-/-}$ mice are responsible for the dose related differences seen between the genotypes. Even though there is evidence supporting the hypothesis that dopamine is involved in depression, the recent finding that the antidepressant effect of amisulpride is most likely mediated by the 5-HT$_7$ receptor [35] might suggest the need for a reevaluation of some studies, especially those using antipsychotics with high affinity for the 5-HT$_7$ receptor [59].

In a recent study it was found that the atypical antipsychotic amisulpride had high affinity for the 5-HT$_7$ receptor and that it was able to reduce immobility in both the tail suspension and forced swim tests in 5-HT$_7^{+/+}$, but not 5-HT$_7^{-/-}$ mice [35]. These findings provided the first rational explanation for the antidepressant properties of amisulpride [60–62]. Aripiprazole is another atypical antipsychotic with high affinity for the 5-HT$_7$ receptor [63]. Aripiprazole is approved as augmentation therapy in depression [37,64]. Interestingly it was recently shown that aripiprazole potentiates the effect of fluoxetine in the mouse tail suspension test, an effect the authors were not able to fully explain [38]. With the present findings that aripiprazole reduced immobility in both the tail suspension and forced swim tests in 5-HT$_7^{+/+}$ mice but not

in 5-HT$_7{}^{-/-}$ mice it appears that also the antidepressant properties of aripiprazole are mediated by the 5-HT$_7$ receptor.

To fully understand the mechanisms regulating the degree of immobility in the tail suspension test and the forced swim test has proven to be difficult. Recent studies have shown that there are pronounced strain differences in the behavioral response [52,65]. One notable finding is that C57BL/6J mice, the background strain for the 5-HT$_7{}^{-/-}$ mice, generally have a high baseline immobility and do not respond to certain selective serotonin reuptake inhibitors (e.g. fluoxetine) [65]. There is a linear correlation between the amount of 5-HT transporter binding and immobility, but no such correlation exists for the norepinephrine transporter [52]. Interesting differences in response are seen also in the present study. Citalopram and GBR 12909 dose-dependently reduced immobility in the tail suspension test in both 5-HT$_7{}^{+/+}$ and 5-HT$_7{}^{-/-}$ mice. In contrast, desipramine and reboxetine failed to further reduce immobility in the 5-HT$_7{}^{-/-}$ mice. Citalopram, desipramine and reboxetine, but not GBR 12909, interacted synergistically with the 5-HT$_7$ receptor antagonist SB-269970. Especially the interaction between citalopram and SB-269970 had an interesting profile that it might be possible to exploit therapeutically. With the norepinephrine reuptake inhibitors the interaction was most prominent in the forced swim test, whereas it was weak or absent in the tail suspension test. It should be noted that combining citalopram and SB-269970 synergistically increased prefrontal cortex levels of 5-HT [21], whereas the combination of imipramine and SB-269970 did not [23]. It even appears that blockade of the 5-HT$_7$ receptor might lead to decreased 5-HT levels [49]. Nevertheless, an explanation based on a mechanism resulting in increased synaptic 5-HT (and possibly norepinephrine) levels for the enhanced antidepressant-like responses observed by combining antidepressants with 5-HT$_7$ receptor blockade remains closest at hand. Inhibition of 5-HT$_7$ receptors located on axon terminals might cause such an effect [21,66,67].

One factor that can influence the results in the tail suspension and the forced swim tests is changes in locomotor activity induced by drugs or genetic manipulations. We verified that the interactions seen between low doses of citalopram and SB-269970 were not due to changes in locomotion. This is in agreement with other studies [21,22]. Nevertheless, it was of importance to exclude this possibility since higher doses of citalopram has been shown to increase locomotor activity [68,69]. SB-269970 has been found not to affect locomotion [13]. Due to the increased activity seen in the 5-HT$_7{}^{-/-}$ mice with repeated testing we did not test any of the other drugs used in this study. The cause and implications of this effect needs further more detailed studies as a previous 24 hour comparison between 5-HT$_7{}^{+/+}$ and 5-HT$_7{}^{-/-}$ mice did no reveal any differences in locomotor activity [58]. It should be noted that desipramine has been found to reduce locomotor activity [69] and that reboxetine does not affect locomotion [70,69]. Thus, the observations made in the present study for these drugs are likely not due to changes in locomotion. On the other hand, the effects seen with GBR 12909 might be due to increased locomotor activity induced by this compound [71].

Previous studies [11,12] and the present data show a strong correlation between effects seen in the tail suspension test and the forced swim test in 5-HT$_7{}^{-/-}$ mice and by using a selective 5-HT$_7$ receptor antagonists. Thus, one can be highly confident that the findings in 5-HT$_7{}^{-/-}$ mice are due to the lack of 5-HT$_7$ receptors. With the 5-HT$_{1A}$ receptor the situation is different. Mice lacking the 5-HT$_{1A}$ receptor exhibit reduced immobility in the forced swim test [40]. Interestingly, agonists for the 5-HT$_{1A}$ receptor, where 8-OH-DPAT is the most commonly used, also reduce immobility, an effect that can be blocked by relatively selective antagonists [72]. Selective antagonists for the 5-HT$_{1A}$ receptor by themselves do not influence immobility [41]. It has also been shown that there is no interaction between the 5-HT$_{1A}$ receptor and antidepressants in the forced swim test [41]. Although less studied, the results are similar for the 5-HT$_{1A}$ receptor and the tail suspension test [73]. Even though 8-OH-DPAT is also an agonist for 5-HT$_7$ receptors it is unlikely that its ability to reduce immobility is mediated by

this receptor since inactivation or blockade reduces immobility. In the present study, using the 5-HT$_{1A}$ receptor antagonist/partial agonist WAY 100135, no indication was found for a direct effect on immobility in the tail suspension test nor an interaction with 5-HT$_7$ receptors.

Glucocorticoids are believed to play a major role in depression [25]. Several studies have also shown that the 5-HT$_7$ receptor is involved in the central regulation of glucocorticoids. Serotonin-mediated upregulation of glucocorticoid receptors is mediated by 5-HT$_7$ receptors [74]. Furthermore, both adrenalectomy and restraint stress has been shown to upregulate 5-HT$_7$ receptor mRNA expression in the hippocampus [45,50]. Thus, we hypothesized that glucocorticoid homeostasis might be altered in 5-HT$_7^{-/-}$ mice. However, the present data show that serum corticosterone levels were not altered in 5-HT$_7^{-/-}$ mice, and that the serum levels had a normal circadian pattern. Serum corticosterone levels were also not differentially regulated in 5-HT$_7^{+/+}$ and 5-HT$_7^{-/-}$ mice following the tail suspension test or the forced swim test.

The present findings support the hypothesis that the 5-HT$_7$ receptor is a new target for disorders treated with antidepressants. Possibly a 5-HT$_7$ receptor antagonist would be beneficial by itself as inactivation or blockade of the 5-HT$_7$ receptor in itself is sufficient to induce antidepressant-like behavior. Alternatively, the combination of a 5-HT$_7$ receptor antagonist with an antidepressant might prove to be a treatment with improved efficacy and reduced side-effects as lower doses most likely could be used.

## Acknowledgments

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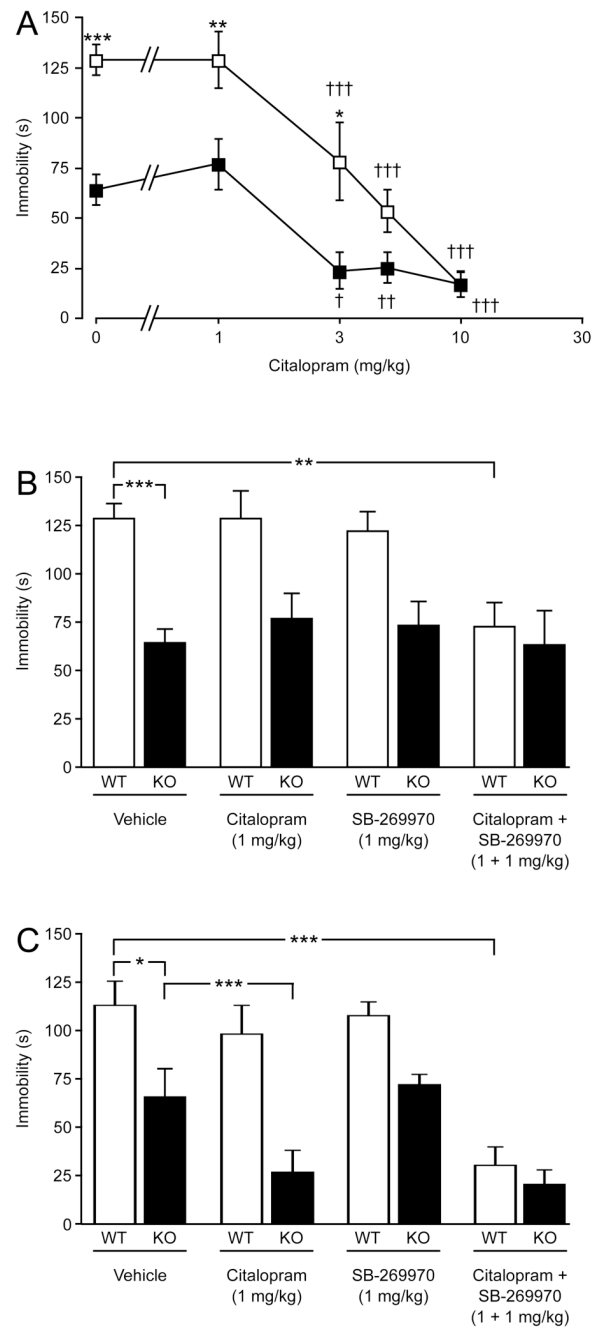
**Fig. 1.**
Effects of citalopram on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of citalopram on the immobility profile of $5\text{-}HT_7^{+/+}$ ($\square$) and $5\text{-}HT_7^{-/-}$ ($\blacksquare$) mice in the tail suspension test. (B) Effects of individual and concurrent injections of 1 mg/kg citalopram and 1mg/kg SB-269970 in the tail suspension test in $5\text{-}HT_7^{+/+}$ (WT) and $5\text{-}HT_7^{-/-}$ (KO) mice. (C) Effects of individual and concurrent injections of 1 mg/kg citalopram and 1 mg/kg SB-269970 in the forced swim test in $5\text{-}HT_7^{+/+}$ (WT) and $5\text{-}HT_7^{-/-}$ (KO) mice. Values are mean $\pm$ SEM. n = 8–10 animals per genotype per treatment group. *P < 0.05, **P < 0.01, ***P < 0.001 between the genotypes (A) or indicated groups (B, C); †P < 0.05, ††P <

0.01, †††P < 0.001 within a genotype compared to control; two-way ANOVA followed by Bonferroni's post-hoc test.
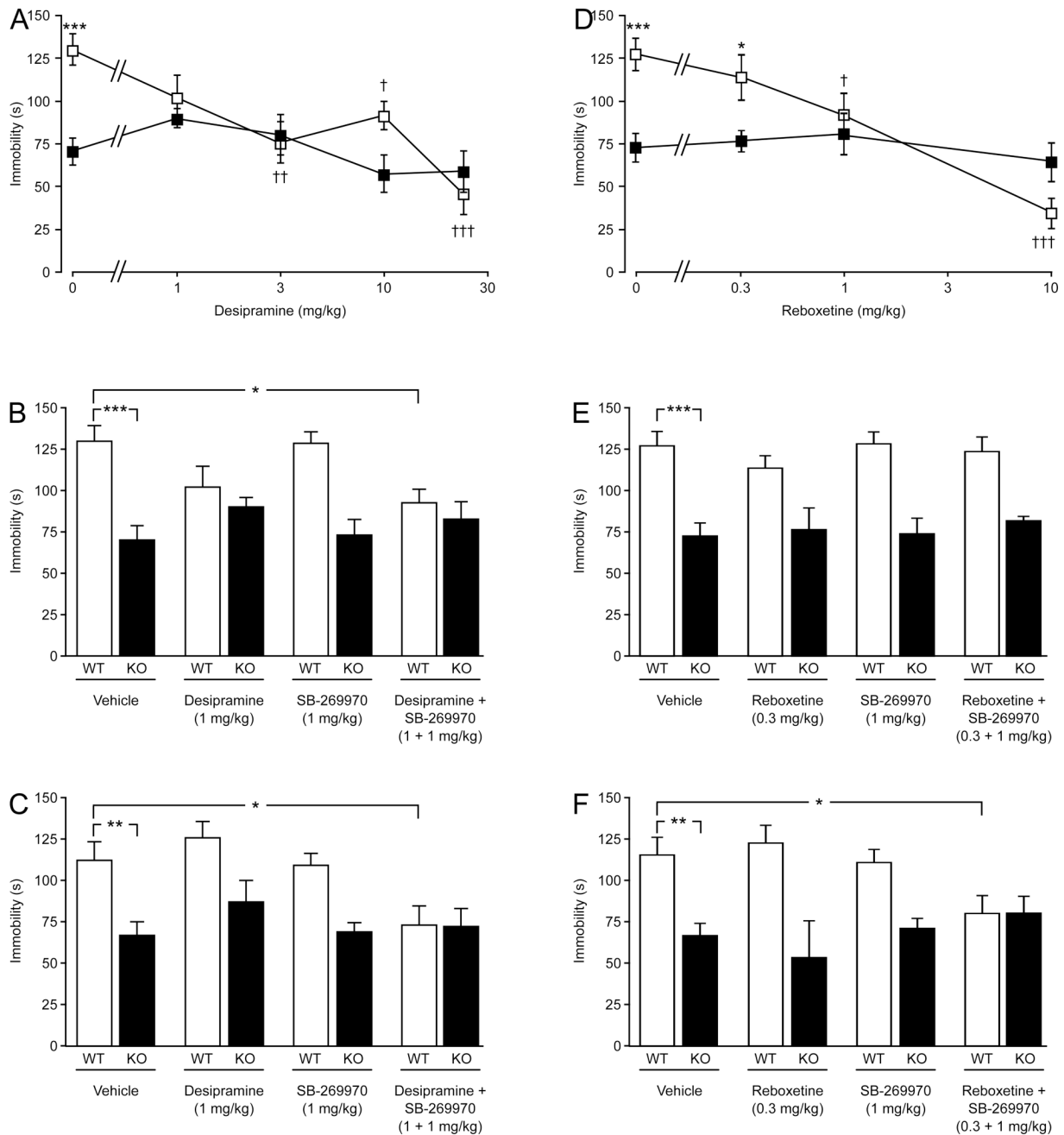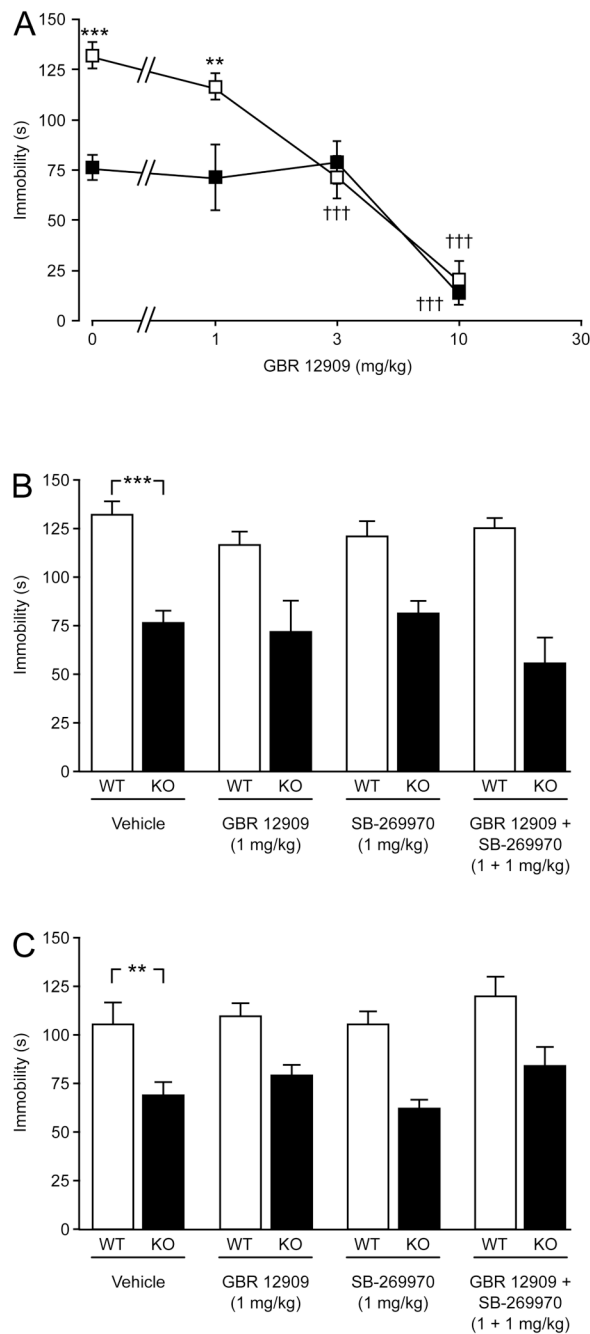
**Fig. 2.**
Effects of desipramine and reboxetine on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of desipramine on the immobility profile of 5-HT$_7^{+/+}$ (□) and 5-HT$_7^{-/-}$ (■) mice in the tail suspension test. (B) Effects of individual and concurrent injections of 1 mg/kg desipramine and 1mg/kg SB-269970 in the tail suspension test in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. (C) Effects of individual and concurrent injections of 1 mg/kg desipramine and 1mg/kg SB-269970 in the forced swim test in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. (D) Dose-response effect of reboxetine on the immobility profile of 5-HT$_7^{+/+}$ (□) and 5-HT$_7^{-/-}$ (■) mice in the tail suspension test. (E) Effects of individual and concurrent injections of 0.3 mg/kg reboxetine and 1mg/kg SB-269970 in the tail suspension

test in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. (F) Effects of individual and concurrent injections of 0.3 mg/kg reboxetine and 1mg/kg SB-269970 in the forced swim test in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. Values are mean ± SEM. n = 8–10 animals per genotype per treatment group. *P < 0.05, **P < 0.01, ***P < 0.001 between the genotypes (A, D) or indicated groups (B, C, E, F); †P < 0.05, ††P < 0.01, †††P < 0.001 within a genotype compared to control; two-way ANOVA followed by Bonferroni's post-hoc test.

**Fig. 3.**
Effects of GBR 12909 on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of GBR 12909 on the immobility profile of 5-HT$_7^{+/+}$ (□) and 5-HT$_7^{-/-}$ (■) mice in the tail suspension test. (B) Effects of individual and concurrent injections of 1 mg/kg GBR 12909 and 1mg/kg SB-269970 in the tail suspension test in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. (C) Effects of individual and concurrent injections of 1 mg/kg GBR 12909 and 1mg/kg SB-269970 in the forced swim test in 5-HT$_7^{+/+}$ (WT) and 5-HT$_7^{-/-}$ (KO) mice. Values are mean ± SEM. n = 8–10 animals per genotype per treatment group. **P < 0.01, ***P < 0.001 between the genotypes (A) or indicated groups (B, C); †††P < 0.001 within a genotype compared to control; two-way ANOVA followed by Bonferroni's post-hoc test.
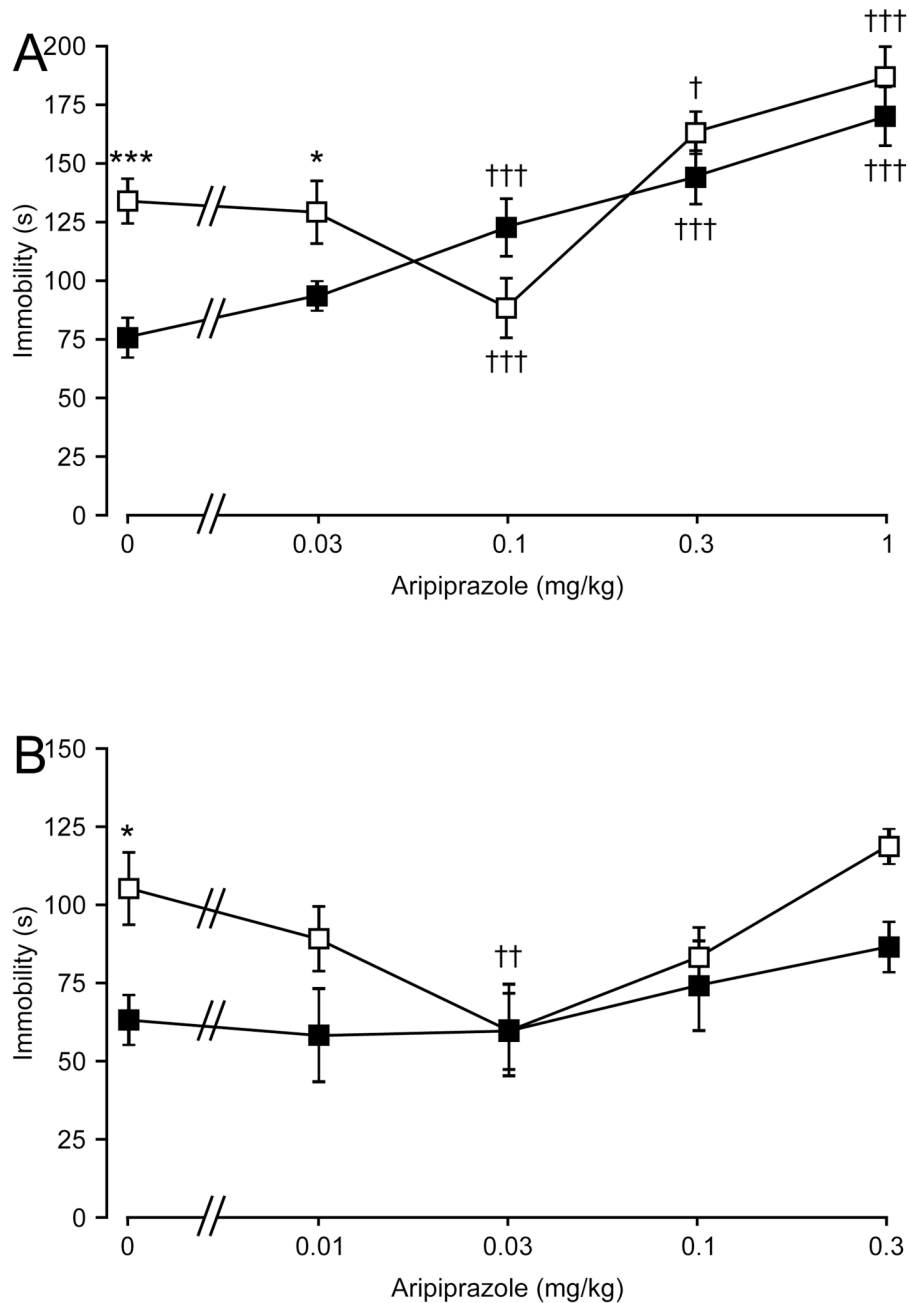
**Fig. 4.**
Effects of aripiprazole on mouse behavior in the tail suspension and forced swim tests. (A) Dose-response effect of aripiprazole on the immobility profile of 5-HT$_7^{+/+}$ (□) and 5-HT$_7^{-/-}$ (■) mice in the tail suspension test. (B) Dose-response effect of aripiprazole on the immobility profile of 5-HT$_7^{+/+}$ (□) and 5-HT$_7^{-/-}$ (■) mice in the forced swim. Values are mean ± SEM. n = 8–10 animals per genotype per treatment group. *P < 0.05, ***P < 0.001 between the genotypes; †P < 0.05, ††P < 0.01, †††P < 0.001 within a genotype compared to control; two-way ANOVA followed by Bonferroni's post-hoc test.
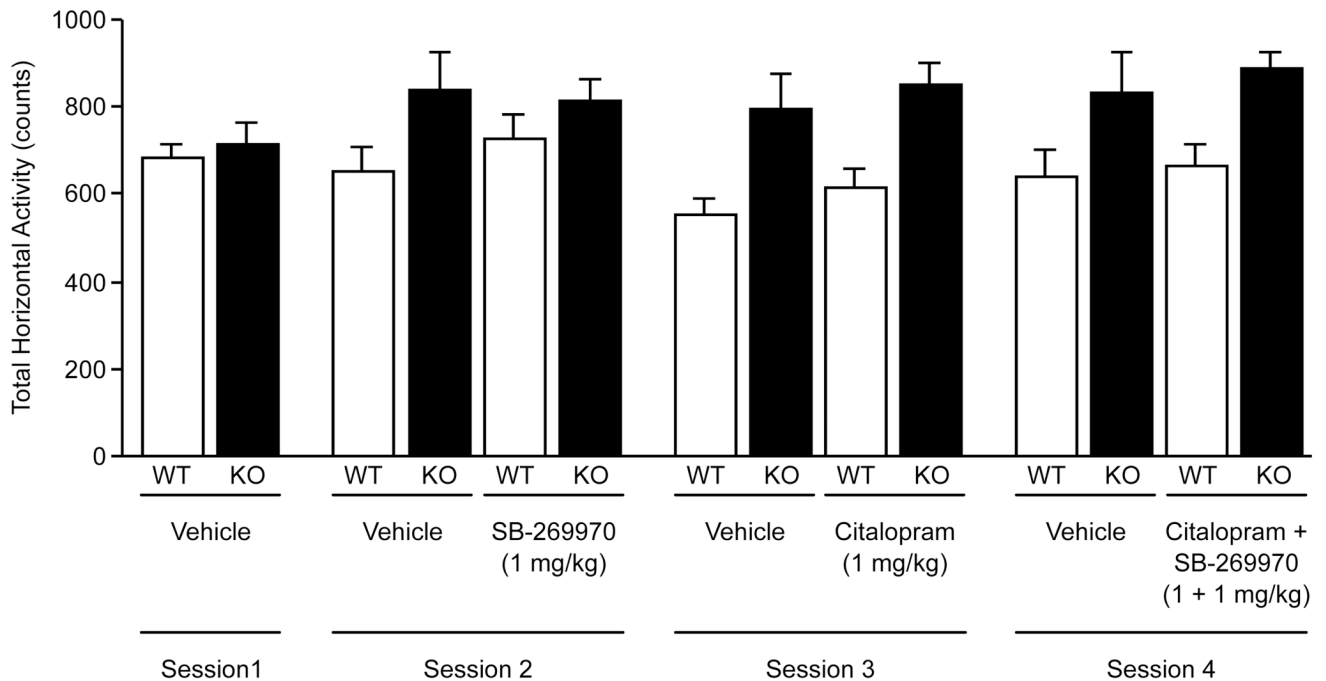
**Fig. 5.**
Effects of citalopram and SB-269970 on mouse locomotor activity. Mice were habituated to the test apparatus for three sessions (not shown). They were then tested in four separate sessions as indicated. In the first session all mice received vehicle. In the subsequent sessions half of the mice were randomly assigned to receive vehicle and the other half drug. Comparisons were made between $5\text{-}HT_7^{+/+}$ (WT) and $5\text{-}HT_7^{-/-}$ (KO) mice. There were no treatment-induced effects, but $5\text{-}HT_7^{-/-}$ mice increased their activity with repeated testing, see text for details. Values are mean $\pm$ SEM. n = 10 (20 in session 1) animals per genotype per treatment group.
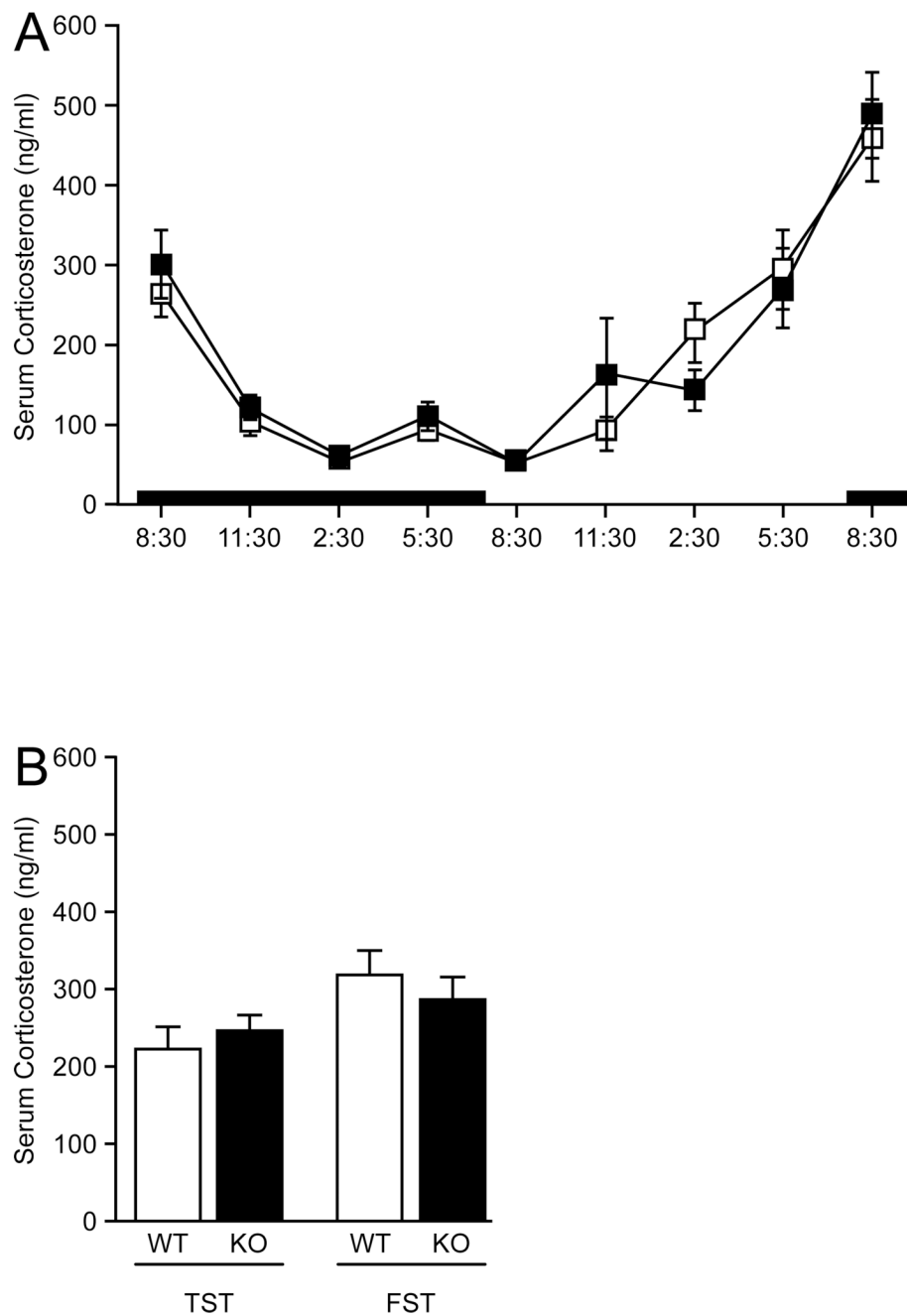
**Fig. 6.**
(A) Corticosterone levels measured in blood serum. Expected corticosterone concentrations changes were observed during a 24-hour cycle. There were no differences between $5\text{-}HT_7^{+/+}$ (□) and $5\text{-}HT_7^{-/-}$ (■) mice. The black bar represents the dark period of the day. (B) Serum corticosterone levels after the tail suspension test (TST) and the forced swim test (FST). These measurements were made at approximately 10:30 during the light phase and thus appear to be elevated as a result of the behavioral tests performed although this was not statistically evaluated as the objective was to determine possible changes between the genotypes. There were no differences in serum corticosterone levels between $5\text{-}HT_7^{+/+}$ (WT) and $5\text{-}HT_7^{-/-}$ (KO) mice following either test. Values are mean ± SEM. n = 8 animals per genotype for the time-

course data; n = 6 per genotype for the tail suspension test; n = 8 per genotype for the forced swim test.