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Sex Differences in the Regulation of Serotonergic Transmission and Behavior in 5-HT Receptor Knockout Mice

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Few studies have examined the relationship between genetics, stress, and sex-linked differences in neurotransmitter systems. Examining serotonin (5-HT) receptor knockout mice on stress-induced behavioral depression, female 5-HT_{1B} receptor knockout mice demonstrated significantly reduced immobility than either male 5-HT_{1B} receptor knockout mice or male and female wild-type mice on the tail suspension test (TST) and forced swimming test. The behavioral phenotype was identified as likely due to a disinhibition of 5-HT release, because depletion of 5-HT with parachlorophenylalanine selectively reduced immobility of female 5-HT_{1B} receptor knockout mice in the TST. In contrast, male and female 5-HT_{1A} receptor knockout mice demonstrated reduced immobility compared with control mice, but the depletion of 5-HT with PCPA did not reverse the antidepressant-like phenotype. Microdialysis studies confirmed significantly higher baseline levels of hippocampal 5-HT in female, but not male, 5-HT_{1B} receptor knockout mice. Both male and female 5-HT_{1B} receptor knockout mice demonstrated augmented dialysate responses to fluoxetine. Also, both male and female 5-HT_{1B} receptor knockout mice demonstrated reductions of immobility in the TST after treatment with fluoxetine. Therefore, female 5-HT_{1B} receptor knockout mice demonstrate a sex-linked disinhibition of 5-HT release that sustained higher baseline levels of hippocampal 5-HT and behavioral vulnerability to 5-HT depletion.

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INTRODUCTION

Depression is the most significant mental health risk for women, with women twice as likely to develop major depression as men (Kessler *et al*, 1994; Kessler, 2003; Murray and Lopez, 1997; Weissman and Olfson, 1995). Alterations in the tone of serotonin (5-HT) transmission may be a major contributor to gender disparity (Akiskal, 1989; Pfaff, 1997; Bagdy, 1998; Joffe and Cohen, 1998), and women have shown decreased whole brain 5-HT synthesis (Nishizawa *et al*, 1997), increased cerebrospinal fluid levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA), and decreased 5-HT_2 receptor binding in specific brain regions (Biver *et al*, 1996) when compared to men. The impact of 5-HT-related gene polymorphisms of serotonergic function also varies between sexes. Among women, the short-repeat promotor variant of the 5-HT transporter gene is associated with higher 5-HIAA levels, but in males with lower levels (Williams *et al*, 2003). Currently, the most common class of effective antidepressant drugs are selective 5-HT reuptake inhibitors (SSRIs) that enhance serotonergic neurotransmission by blocking reuptake (Delgado *et al*, 1999). Approximately 70% of prescribed SSRIs are given to women (Kessler *et al*, 1994; Kessler, 2003). While some studies indicate that sex may moderate the response to antidepressants, with women exhibiting a preferential response to SSRIs compared to tricyclic antidepressants (Kornstein *et al*, 2000; Martenyi *et al*, 2001), others have found no difference in treatment efficacy (Parker *et al*, 2003). Studies have also suggested a different pharmacokinetic disposition of antidepressants between men and women, and that women taking antidepressants may exhibit a different adverse event profile (Frackiewicz *et al*, 2000). However, a clear and consistent sex link between 5-HT and depression remains to be shown in women.

Few experiments have been conducted on the relationship between sex-linked differences and neurotransmitter systems. One reason why an interaction between 5-HT levels and sex could be difficult to establish consistently is that 5-HT transmission is ordinarily tightly regulated by somatodendritic and terminal autoreceptors. At least two

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types of 5-HT autoreceptors provide negative feedback to 5-HT transmission. 5-HT_{1A} receptors located on serotonergic cell bodies are presynaptic autoreceptors, although 5-HT_{1A} receptors are also located postsynaptically in forebrain terminal fields that receive serotonergic innervation from the dorsal and median raphe nuclei. 5-HT_{1B} receptors are located on terminals presynaptically and postsynaptically relative to 5-HT neurons. Presynaptic 5-HT_{1B} receptors are autoreceptors that regulate terminal release, whereas postsynaptic 5-HT_{1B} receptors are heteroreceptors located at nerve terminals that regulate the release of other neurotransmitters (Engel *et al*, 1986; Adell *et al*, 2001). The 5-HT_{1B} receptor has been localized to a variety of brain regions including the basal ganglia and hippocampus (Boschert *et al*, 1994). Both 5-HT_{1A} and 5-HT_{1B} autoreceptors are present in the CNS across species and maintain their respective distribution patterns, although 5-HT_{1D} receptors in humans appear to function analogously to 5-HT_{1B} receptors in rodents (Waeber *et al*, 1989). The constraint on extracellular levels of 5-HT by 5-HT autoreceptors can be demonstrated by their blockade or deletion during periods of stimulated activity. For example, blockade of 5-HT_{1A} autoreceptors by selective 5-HT_{1A} receptor antagonists, or in mice with 5-HT_{1A} receptor deletion, has been shown through *in vivo* microdialysis studies to facilitate the effect of SSRIs on extracellular 5-HT levels in various terminal regions of rat brain (Hjorth, 1993; Hjorth and Auerbach, 1994; Knobelman *et al*, 2001b; Parsons *et al*, 2001). SSRIs given in combination with 5-HT_{1B} receptor antagonists or in 5-HT_{1B} receptor knockout mice also augment 5-HT outflow compared to SSRI treatment alone (Knobelman *et al*, 2001b; Maswood *et al*, 1999; Malagie *et al*, 2001, 2002). Thus, both 5-HT autoreceptors, the somatodendritic 5-HT_{1A} autoreceptor and the terminal 5-HT_{1B} autoreceptor, can be involved in regulating the release of 5-HT during pharmacological or behavioral activation.

Sex differences in the 5-HT system have been demonstrated in rodents and humans (for reviews, see Nishizawa *et al*, 1997; McEwen *et al*, 1998). The current behavioral and microdialysis studies demonstrate how 5-HT regulation in mice with a targeted genetic dysfunction can lead to sex-specific outcomes. Both sexes of 5-HT_{1A} and 5-HT_{1B} receptor knockout mice were examined in behavioral stress-inducing paradigms, the forced swimming test (FST) and the tail suspension test (TST). These behavioral tests are considered pharmacologically valid tests of acute antidepressant responses in rodents. Performance at baseline without drug could model a phenotype of a kind of behavioral response to stress that may be associated with clinical depression (for discussion, see Lucki, 2001; Cryan *et al*, in press). As differences in baseline performance could reflect the endogenous differences in 5-HT transmission and prior studies have not systematically compared neurochemical responses between male and female mice with genetic deletion of 5-HT_{1A} and 5-HT_{1B} receptors, baseline levels of 5-HT and the response to fluoxetine were examined using *in vivo* microdialysis. Mice with genetic deletion of 5-HT autoreceptors provide an opportune model to examine sex-linked neurobiological consequences that may arise from the genetic dysfunction of critical regulatory components of neuronal release.

MATERIALS AND METHODS

Subjects

Both sexes of 5-HT_{1A} receptor knockout and 5-HT_{1B} receptor knockout and wild-type mice with a 129/Sv-ter background were bred and housed in a colony at the University of Pennsylvania. Mice were generated by breeding homozygote mutant or wild-type mice as described previously (Knobelman *et al*, 2001a, b). Mice were housed four per cage, given free access to standard rodent chow and water, and maintained on a 12-h light–dark schedule, with lights on at 0700 h. They were 12–15 weeks of age when used in these studies. All studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health and were reviewed by the University of Pennsylvania Institutional Animal Care and Use Committee.

Surgery

Mice were anesthetized with chloral hydrate (400 mg/kg, i.p.). The probe was positioned in the ventral hippocampus using the following coordinates: AP -2.8, ML ± 3.2, and DV -5.0 mm from bregma, according to the mouse brain atlas of Franklin and Paxinos (1997). A drop of cyanoacrylate was spread thinly over the exposed skull and the probe was then cemented in place. Following surgery, the mice were placed into a 21.5 cm high, clear polycarbonate cylindrical *in vivo* microdialysis apparatus with a counterbalance arm holding a liquid swivel (Instech Laboratories) and allowed to recover overnight. Microdialysis experiments started 17–20 h after surgery.

Dialysis Procedure

Microdialysis procedures were performed with custom-made microdialysis probes, as previously described (Knobelman *et al*, 2000). The probes were continuously perfused with filtered artificial cerebrospinal fluid (ACSF; 147 mM NaCl, 1.7 mM $CaCl_2$, 0.9 mM $MgCl_2$, and 4 mM KCl, pH 6.3–6.5) at a rate of 0.8 µl/min using a Harvard Apparatus syringe pump (Instech Laboratories). Dialysate samples were collected into polypropylene microcentrifuge vials at 20-min intervals. Four fractions were collected to measure baseline values before systemic administration of the SSRI, fluoxetine (20 mg/kg). Samples were collected for three additional hours after drug challenge to compare the effects of fluoxetine on extracellular 5-HT levels between wild-type and 5-HT_{1B} receptor knockout male and female mice. After drug injection, sample collection was discontinued for 5 min to correct for the dead space in the perfusion tubes. Upon completion of the experiment, samples were stored at -80°C until analysis.

Samples were automatically injected into a Bioanalytical Systems 460 High Pressure Liquid Chromatograph by a BAS Sample Sentinel Refrigerated Microsampler set to a 12 µl injection volume. The HPLC mobile phase (1.25 mM 1-octanesulfonic acid sodium salt, 100 mM sodium acetate, 0.50 mM EDTA, 10.0 mM sodium chloride, 10–11% Acetonitrile, pH 5.0) is pumped through a reverse phase 1×100 mm ODS 3 µm microbore column (C18; BAS) and a 10 µl sample loop at a flow rate of 70 µl/min (see Kreiss

et al, 1993). The 5-HT from chromatographs of dialysate samples were identified by comparing their elution times with those of reference standards. The amount of 5-HT in each dialysate sample was quantified from their respective peak heights using a linear regression analysis of the peak heights obtained from a series of reference standards.

Histology

At the completion of the experiment, brains were removed, placed in cold isopentane and frozen at −80°C. The brains were then sectioned (35-μm) with a refrigerated cryostat, stained with Neutral Red, and the tissue examined for the location of the dialysis probe. In cases of improper probe placement, data were excluded.

Behavior

The FST and TST tests were used as animal models of stress-induced depressive behavior. Female mice were monitored for the phase of estrus cycle by vaginal smears prior to behavioral testing. The TST was a modified version of previously validated procedures (Steru *et al*, 1985; Mayorga *et al*, 2001). Mice were transported a short distance from the holding facility to the testing room and left there undisturbed for at least 3 h. Subjects were randomly allocated to treatment conditions and tested in counterbalanced order. Mice were individually suspended by the tail to a horizontal ring-stand bar (distance from floor = 35 cm) using adhesive tape (distance from tip of tail = 2 cm). Typically, mice demonstrated several escape-oriented behaviors interspersed with temporally increasing bouts of immobility. A 6-min test session was employed, which was videotaped. Videotapes were subsequently scored by a highly trained observer who was unaware of the treatment. The parameter recorded was the number of seconds spent immobile.

The FST involved forcing the animals to swim in an apparatus with no possibility of escape (Porsolt *et al*, 1978; Lucki *et al*, 2001). The mice were placed into individual glass cylinders (46 cm tall × 21 cm diameter) filled with water (temperature = 23–25°C) to a depth of 15 cm. A 6-min test session was employed, which was videotaped. Videotapes were scored by a trained observer who was unaware of the treatment conditions for the individual mice. The parameter recorded was the number of seconds spent immobile.

Mice ($N = 6$ mice per group) were placed in clear polycarbonate boxes (MED Associates, $28.5 \times 17.5 \times 13.0\,cm^3$) equipped with photoelectric beams for the automated measurement of locomotor activity. The total amount of locomotor activity for a 2-h period was recorded. Ambulation was defined as the total number of beam breaks as the animal explores the home cage. The quantity of crosses describes the number of times the mouse traversed or crossed the entire length of the cage.

Drugs

All drugs were prepared just prior to use and doses were calculated as the weight of the base. Fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN) was dissolved in deionized water and administered in a volume of 10 ml/kg (s.c.).

PCPA methylester hydrochloride (250 mg/kg, i.p.) was administered twice daily for 3 days, with the last dose given 18 h prior to behavioral testing (Cesana *et al*, 1993). Depletion of 5-HT was confirmed by killing mice 1 h after behavioral testing and dissecting the hippocampus from mouse brain. Tissue samples were then homogenized in 0.1 N perchloric acid with 100 μM EDTA using a Tissuemizer (Tekmar, Cleveland, OH). Samples were centrifuged at 15 000 rpm for 15 min at 2–8°C. The supernatant was filtered through a 0.45-μm nylon filter in microcentrifuge spin-tubes. 5-HT values were expressed as pg/sample.

Data Analysis

The first four samples were averaged to derive the baseline value against which the remaining sample values were compared. Baseline values were expressed as fentomoles/10 μl sample. Probes were calibrated and tested for *in vitro* recovery prior to surgical placement. Experiments were standardized by only including probes in a confined range (17–21%) of *in vitro* 5-HT recovery. Baseline values were compared using a two-way analysis of variance (ANOVA) with genotype and sex as factors. A two-factor repeated-measures ANOVA was used to analyze the effects of acute administration of fluoxetine on dialysate concentrations. Follow-up comparisons were conducted using Fisher's PLSD test.

The duration of immobility in the TST or FST was compared between groups by ANOVA. Pairwise comparisons were conducted using Fisher's PLSD test. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Sex Differences in 5-HT_{1B} Mice in the TST and FST

The TST revealed significant differences in performance for both male and female 5-HT_{1A} receptor knockout mice as a function of genotype ($F_{(1,57)} = 33.14$, $p < 0.0001$), but no significant sex differences or genotype × sex interaction (sex, $F_{(1,57)} = 0.09$, $p = 0.77$; genotype × sex interaction, $F_{(1,57)} = 3.53$, $p = 0.07$). As shown in Figure 1, both male ($p < 0.001$) and female 5-HT_{1A} receptor mutants ($p < 0.05$) were less immobile than the corresponding wild-type mice of the same sex. Due to abnormal motor responses of hind limb rigidity during the FST challenge (Lucki *et al*, 2001; Mayorga *et al*, 2001), data from 5-HT_{1A} receptor knockout mice were confounded and not included in the assessment.

In contrast, there were significant differences for genotype, sex, and an interaction between genotype and sex in immobility between male and female wild-type and 5-HT_{1B} receptor knockout mice in the TST (genotype, $F_{(1,48)} = 14.51$, $p < 0.001$; sex, $F_{(1,48)} = 11.42$, $p < 0.001$; sex × genotype interaction, $F_{(1,1,48)} = 4.82$, $p < 0.05$), as shown in Figure 2a. The female 5-HT_{1B} receptor knockout mice were significantly less immobile than their female or male wild-type counterparts ($p < 0.0001$). In contrast, male 5-HT_{1B} receptor knockout mice did not differ from the corresponding wild-type mice of the same sex.

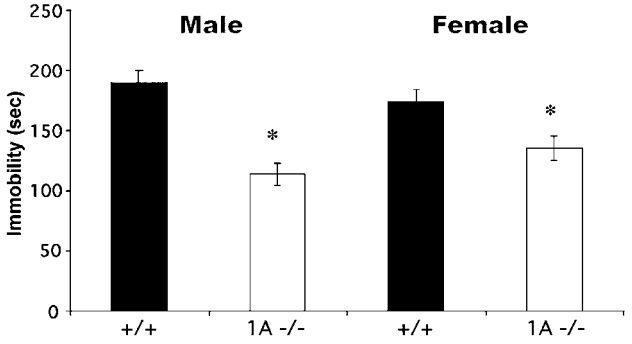

Figure 1 Duration of immobility behavior of 5-HT_{1A} receptor knockout and wild-type mice as measured in the TST. Data are presented as mean ± SEM ($n = 14$–16 for female and male mice of both genotypes); *$p < 0.0001$ *vs* genotype of the same sex.

Similar to the findings in the TST, there were significant differences for genotype and an interaction between genotype and sex in the total time spent immobile in the FST (genotype, $F_{(1,61)} = 13.46$, $p < 0.001$; sex, $F_{(1.61)} = 2.33$, $p = 0.13$; sex × genotype interaction, $F_{(1,1,61)} = 4.55$, $p < 0.05$), as shown in Figure 2b. Although male 5-HT_{1B} receptor mutant mice demonstrated immobility times comparable to wild-type animals of both sexes, female 5-HT_{1B} receptor knockout mice were less immobile than male 5-HT_{1B} receptor knockout mice ($p < 0.05$). They also were less immobile than male and female wild-type mice ($p < 0.001$). This sex-specific behavioral response did not vary by the stage of the estrus cycle (data not shown), which was routinely examined by consecutive vaginal smears. In addition, the reduction of immobility of female 5-HT_{1B} receptor knockout mice from female wild-type mice during the TST and FST was not based on differences in locomotor activity, as confirmed by measuring motor activity in automated cages (Table 1) ($t(10) = 0.88$, $p = 0.40$ for ambulation; $t(10) = 1.11$, $p = 0.29$ for crosses). The findings in both TST and FST illustrate that the female 5-HT_{1B} receptor knockout mice exhibit a distinct behavioral response to stressful challenges that can be consistently measured and is reproducible across different experimental paradigms.

Effect of 5-HT Depletion in Female 5-HT_{1A} and 5-HT_{1B} Mice

An increase in extracellular 5-HT levels produced by the deletion of one of the 5-HT autoreceptors could account for the antidepressant-like phenotype in either 5-HT_{1A} or female 5-HT_{1B} receptor knockout mice. In order to demonstrate a *direct* relationship between 5-HT transmission and the TST performance, female 5-HT_{1A} and 5-HT_{1B} receptor knockout mice and controls were administered the tryptophan hydroxylase inhibitor para-chlorophenylalanine (PCPA) to deplete 5-HT. Pretreatment with PCPA produced an approximate 70% reduction in 5-HT hippocampal content in female wild-type (74.8%: vehicle = 192.32 ± 17.25; 17.25; PCPA = 48.10 ± 4.08) and 5-HT_{1B} receptor knockout (71.2%: vehicle = 185.96 ± 19.43; PCPA = 53.42 ± 4.36) mice.

Depletion of 5-HT resulted in a restoration of normal immobility scores in female 5-HT_{1B} receptor knockout mice (Figure 3). A two-way ANOVA showed a significant interaction between genotype and treatment ($F_{(1,1,30)} = 4.90$, $p < 0.01$). Pretreatment with PCPA significantly increased TST immobility in the female 5-HT_{1B} receptor knockout mice ($p < 0.0001$). In contrast, depletion of 5-HT in either female wild-type or 5-HT_{1A} receptor knockout mice failed to alter immobility in the TST. In a previous study (Mayorga *et al*, 2001), PCPA treatment also did not reverse the antidepressant-like phenotype in male 5-HT_{1A} mutant mice. Thus, the normalization of TST performance by 5-HT depletion suggests that the antidepressant-like phenotype in female 5-HT_{1B} receptor knockout mice could be due to a disinhibition of 5-HT transmission caused by the deletion of 5-HT_{1B} receptors. In contrast, the lack of effect of PCPA in either male or female 5-HT_{1A} receptor knockout mice

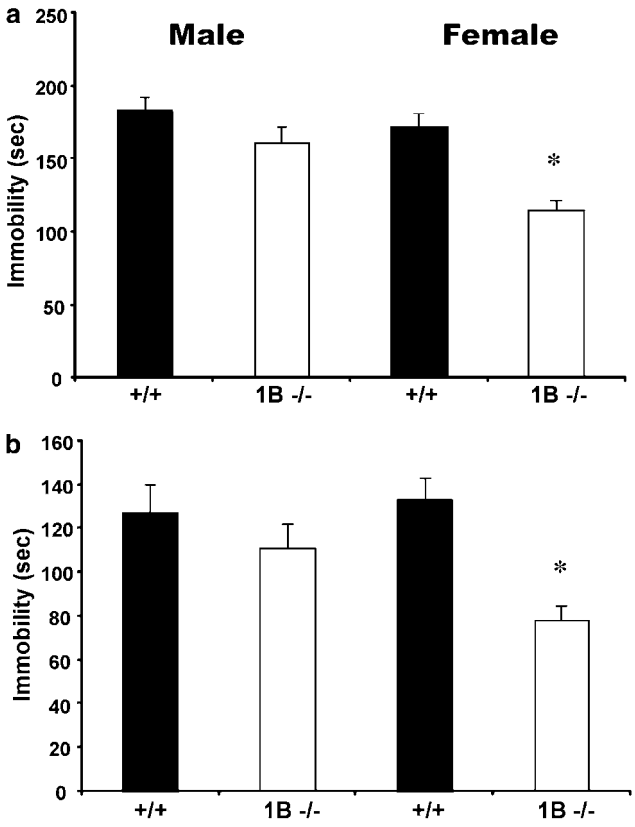

Figure 2 Behavioral effects of male and female 5-HT_{1B} receptor knockout and wild-type mice in different groups of animals tested in the tail suspension (panel a) and the forced swimming (panel b) tests. Data are presented as mean ± SEM ($n = 15$–22 for female mice; $n = 9$–12 for male mice). Female 5-HT_{1B} receptor knockout mice demonstrated significantly reduced immobility as compared to both female wild-type and male 5-HT_{1B} receptor knockout mice (*$p < 0.05$).

Table 1 Locomotor Activity of 5-HT_{1B} Receptor Knockout Female Mice

Genotype	Ambulation	Number of crosses
+/+	1488.33 ± 46.14	56.33 ± 2.62
1B−/−	1421.17 ± 69.74	60.67 ± 3.40

Values represent total amount of ambulation and crosses (mean ± 1 SEM) in a locomotor activity chamber during a 2-h session. $N = 6$ mice/group.

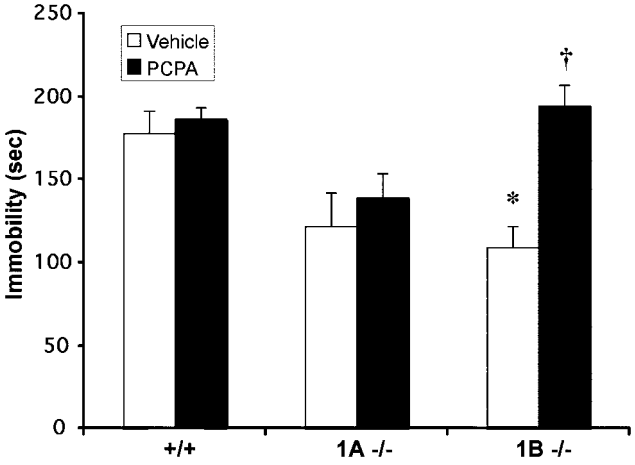

Figure 3 Effects of depletion of 5-HT by pretreatment with PCPA on the behavior of wild-type, 5-HT_{1A} receptor knockout, and 5-HT_{1B} receptor knockout mice in the TST. Female 5-HT_{1B} receptor knockout mice, but no other group, demonstrated a significant increase in immobility after PCPA. *$p<0.01$ *vs* wild-type vehicle, †$p<0.0001$ *vs* same genotype vehicle control. Data represent mean ± SEM, $n=8$–10 mice/group.

suggests that endogenous 5-HT is not involved in the behavioral phenotype of these mutant mice.

Extracellular Levels of 5-HT in 5-HT_{1B} Receptor Knockout Mice

Baseline levels. Although extracellular 5-HT levels have been previously examined in male 5-HT_{1B} receptor knockout mice, the effect of sex has not been reported. Baseline extracellular 5-HT (fmol/20 min sample, mean ± 1 SEM) was measured in the ventral hippocampus of male wild-type (4.06 ± 0.43), male 5-HT_{1B} receptor knockout (4.45 ± 0.49), female wild-type (3.36 ± 0.24), and female 5-HT_{1B} receptor knockout mice (7.07 ± 0.23). The mean baseline value from female 5-HT_{1B} receptor knockout mice was significantly greater than values from corresponding male knockout (63% increase, $p<0.001$) and female wild-type mice (141% increase, $p<0.001$). ANOVA indicated a significant genotype × sex interaction ($F_{1,19}=22.60$, $p<0.001$). Although the extracellular levels of 5-HT in wild-type males were 33% higher than females, the value for wild-type females did not differ significantly from male mice of either genotype.

Effect of fluoxetine during microdialysis. The absolute values of 5-HT in the ventral hippocampus after an acute systemic challenge dose of fluoxetine (20 mg/kg i.p.) are shown in Figure 4 for males (panel a) and for females (panel b). Fluoxetine significantly increased extracellular 5-HT levels in wild-type and 5-HT_{1B} receptor knockout mice of both sexes (Figure 4a). Fluoxetine increased hippocampal 5-HT in male 5-HT_{1B} receptor knockout mice more than wild-type mice (genotype, $F_{(1,9)}=7.11$, $p<0.05$; genotype × time interaction, $F_{(9,72)}=2.53$, $p<0.01$). Fluoxetine also increased hippocampal 5-HT in female 5-HT_{1B} receptor knockout mice more than wild-type mice (genotype, $F_{(1,12)}=9.90$, $p<0.01$; genotype × time interaction, $F_{(9,108)}=4.58$, $p<0.001$). Follow-up analysis, comparing difference scores from baseline to accommodate the different baseline values between groups, indicated that a significantly augmented value was sustained only in the first 20-min collection period ($p<0.01$).

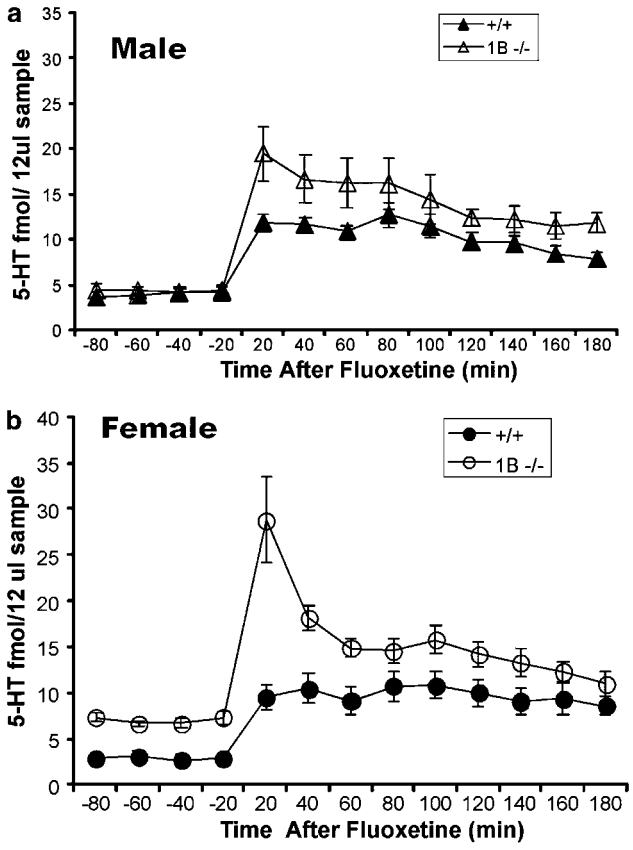

Figure 4 Effects of acute systemic administration of 20 mg/kg fluoxetine on extracellular 5-HT levels in the ventral hippocampus using *in vivo* microdialysis. Panel a shows that baseline 5-HT levels for male 5-HT_{1B}−/− mice (4.45 ± 0.49) did not differ from male WT mice (4.06 ± 0.43), $n=5$ mice/group. Panel b shows that baseline 5-HT levels for female 5-HT_{1B}−/− mice (7.34 ± 0.34) were at least two-fold higher than female WT mice (3.06 ± 0.36), $n=7$–8 mice/group.

Effects of Fluoxetine on TST Immobility

The effect of fluoxetine (20 mg/kg) was examined in female wild-type and 5-HT_{1B} receptor knockout mice on the TST. As shown for previous groups of mice (Figures 2 and 3), female mutant mice demonstrated lower immobility values compared with the corresponding female wild-type mice (Figure 5). The differences at baseline (without drug) were further enhanced after administration of the SSRI fluoxetine (Figure 5). ANOVA indicated a significant effect of both genotype and treatment in the TST ($F_{(1,1,27)}=47.08$, $p<0.0001$ for genotype; $F_{(1,1,27)}=43.16$, $p<0.0001$ for treatment), but no significant interaction.

DISCUSSION

Few studies have evaluated the ways in which stress might interact with genetic vulnerabilities that could be sex-specific. The behavioral outcomes for 5-HT_{1B} knockout female mice differed dramatically from males of the same

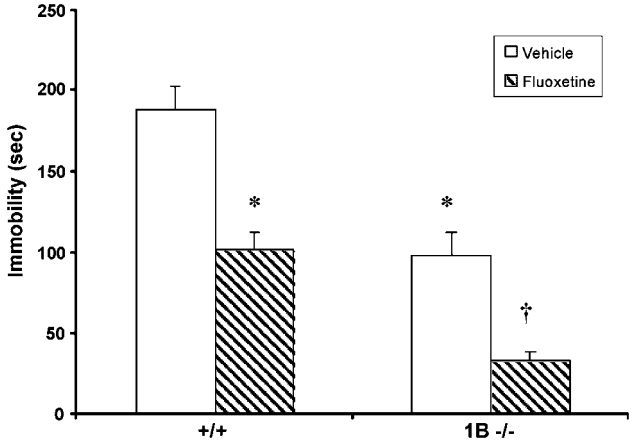

Figure 5 Effects of fluoxetine (20 mg/kg) on the behavior of female wild-type and 5-HT_{1B} receptor knockout mice in the TST. For both groups, fluoxetine produced a significant decrease in immobility ($p<0.01$), $n=$ 8–10 mice/group. Data represent mean ± SEM.

genotype on two tests for antidepressant efficacy, the tail suspension and forced swim tests. The likely mechanism for this underlying behavioral phenotype of decreased baseline immobility was identified as a disinhibition of serotonergic transmission, as evidenced by both microdialysis and serotonergic depletion studies.

5-HT Depletion

The 5-HT_{1B} receptor functions presynaptically as an inhibitory autoreceptor located on terminals of 5-HT neurons (Engel *et al*, 1986). 5-HT_{1B} receptors also function postsynaptically as inhibitory heteroreceptors to control the release of other neurotransmitters (Boschert *et al*, 1994; Moret and Briley, 1997; Adell *et al*, 2001). For example, 5-HT_{1B} receptors within the ventral tegmental area and nucleus accumbens modulate the release of dopamine in the terminal area of the mesolimbic system (Yan and Yan, 2001). As such, dysregulation of either 5-HT or other neurotransmitter targets could be responsible for the abnormal behavioral phenotype of female 5-HT_{1B} knockout mice.

The decreased level of immobility observed for female 5-HT_{1B} knockout mice in both TST and FST was restored to control levels after the selective depletion of 5-HT using PCPA, suggesting that disinhibition of 5-HT transmission was involved in producing the antidepressant-like phenotype of female 5-HT_{1B} mutant mice. In contrast, wild-type female mice exhibited no behavioral change after PCPA treatment, indicating that the higher baseline levels of 5-HT were required to observe this vulnerability in 5-HT_{1B} receptor knockout mice. This result corresponds with studies showing that the 5-HT system ordinarily maintains its adaptability even after a great reduction of endogenous 5-HT (Chaput *et al*, 1990), but may reveal vulnerability if baseline levels of 5-HT transmission have changed. The results with female 5-HT_{1B} receptor knockout mice contrasted with those for female 5-HT_{1A} receptor knockout mice, which maintained their reduced immobility after PCPA administration despite lower TST baseline immobility values. Similarly, the lower TST immobility of male 5-HT_{1A} receptor knockout mice was shown to not be reversed by 5-HT depletion in a previous study (Mayorga *et al*, 2001). Thus, although the behavioral phenotype between 5-HT_{1A} and 5-HT_{1B} receptor knockout mice appeared similar, it was caused for different reasons. The reduced immobility of male and female 5-HT_{1A} receptor knockout mice is caused by compensations due to the constitutive loss of 5-HT_{1A} receptors during development, because the conditional deletion of 5-HT_{1A} receptors during maturity fails to produce the same behavioral phenotype (Gross *et al*, 2002; Lucki, unpublished data). In contrast, the antidepressant-like phenotype of female 5-HT_{1B} receptor knockout mice was likely caused by higher baseline 5-HT levels in female 5-HT_{1B} knockout mice and this was a necessary requisite for the reversal of the behavioral phenotype by depletion of 5-HT produced by PCPA administration.

Antidepressant Response

Another contrasting behavior between 5-HT_{1A} and 5-HT_{1B} receptor knockout mice is their responses to antidepressant drugs. The current study showed that female 5-HT_{1B} receptor mutant mice demonstrated an enhanced response to fluoxetine administration. Even though the TST immobility of female 5-HT_{1B} receptor knockout mice started from a lower baseline, they showed further augmentation in their response to fluoxetine. The pharmacological response of females was similar to that of male 5-HT_{1B} receptor mutant mice shown in previous studies (Mayorga *et al*, 2001), that also showed an enhanced response to fluoxetine even though their TST immobility did not differ from wild-type controls. Also, female 5-HT_{1B} receptor knockout mice were unlike male 5-HT_{1A} receptor mutants, that also started from a lower baseline but blocked the response to SSRI treatment (Mayorga *et al*, 2001). Thus, 5-HT_{1A} receptors appear to be necessary for the effects of SSRI antidepressant drugs in both acute and chronic behavioral models and in both males and females (Mayorga *et al*, 2001; Santarelli *et al*, 2003). In summary, the response to SSRI antidepressants was enhanced in both male and female 5-HT_{1B} receptor mutant mice, supporting an association between the absence of 5-HT_{1B} receptors and augmented responses to antidepressant drugs.

5-HT Microdialysis Measurement

The evaluation of baseline extracellular 5-HT corresponded with the behavioral findings of antidepressant-like baseline immobility in female 5-HT_{1B} receptor knockout mice. Female 5-HT_{1B} receptor knockout mice showed an approximately two-fold elevation of baseline values for extracellular 5-HT in the ventral hippocampus as compared to wild-type mice from either sex or from male 5-HT_{1B} receptor mutant mice. Extracellular levels of 5-HT were 18% lower levels in female *vs* male wild-type mice. Although 5-HT_{1B} receptors were also absent in male knockout mice, 5-HT transmission was not affected under baseline conditions.

The selective increase of TST immobility in 5-HT_{1B} receptor knockout mice by 5-HT depletion in the present study could reflect a more general greater vulnerability to alterations in precursor availability in females than males. For example, even though plasma tryptophan concentration

decreases progressively during pregnancy and ordinarily recovers normal values after delivery, a reduction of plasma tryptophan levels during the post-partum period has been suggested to contribute to the onset of symptoms of post-partum depression in some women (Handley *et al*, 1980; Maes *et al*, 2002; Schrocksnadel *et al*, 2003). Supporting this hypothesis, normal healthy women experienced a marked decrease in several parameters of mood following tryptophan depletion, whereas men did not (Ellenbogen *et al*, 1996).

A disparity in 5-HT baseline levels or 5-HT utilization could also create discordant regulation of 5-HT transmission with respect to sex. Lower rates of 5-HT synthesis have been reported in human females (Nishizawa *et al*, 1997). Female rats showed lower baseline hypothalamic 5-HT levels and a reduced response to paroxetine or fenfluramine during estrous than males (Gundlah *et al*, 1998). Dramatic sex differences were also shown in the regulation of 5-HT efflux in the hypothalamus of rats after local perfusion of the nonselective 5-$HT_{1B/1D}$ receptor antagonist methiothepin (Maswood *et al*, 1999). Female rats showed a significant increase in 5-HT efflux after methiothepin, whereas methiothepin did not change extracellular 5-HT levels of male rats. Thus, females appear to rely more than males on the regulation of 5-HT efflux by terminal autoreceptors, consistent with our microdialysis findings in 5-HT_{1B} receptor knockout mice.

Acute administration of fluoxetine caused a significant augmentation in the increase of extracellular 5-HT levels in the ventral hippocampus of female 5-HT_{1B} receptor mutant mice. This effect was similar to that shown by male 5-HT_{1B} receptor mutant mice, and similar to previous studies (Knobelman *et al*, 2001b; Malagie *et al*, 2001, 2002). Although the effects of fluoxetine were augmented initially following the injection of fluoxetine in the female 5-HT_{1B} mutant mice, the effect of fluoxetine returned rapidly to levels comparable to wild-type mice. This effect could be mediated by compensatory regulation by the activation of 5-HT_{1A} autoreceptors, as shown in 5-HT_{1B} receptor mutant mice (Knobelman *et al*, 2001a, b). The involvement of the somatodendritic 5-HT_{1A} autoreceptors in offsetting the increase in 5-HT output induced by SSRIs is well documented (see Stanford (1996) and Artigas *et al* (1996) for reviews). Although the relationship between 5-HT_{1A} receptor regulation and sex differences is well documented (Cidis Meltzer *et al*, 2001; Maswood *et al*, 1995; Palego *et al*, 1997; Serretti *et al*, 2000; Zhang *et al*, 1999), this hypothesis was not tested in the present study. Interestingly, female 5-HT transporter knockout mice have demonstrated more extensive reduction of 5-HT_{1A} receptors than male mutant mice (Bouali *et al*, 2003; Li *et al*, 2000). The regulation between presynaptic 5-HT_{1A} receptors, 5-HT_{1B} receptors, and the 5-HT transporter in regulating 5-HT transmission could be differentially controlled when superimposed on the drive produced by sex and gonadal hormones.

Conclusions

Sex differences in the 5-HT system have long been demonstrated in rodents and humans (Nishizawa *et al*, 1997; McEwen *et al*, 1998). If the increased baseline hippocampal 5-HT efflux in female 5-HT_{1B} receptor mutant mice were extended to other brain regions, this finding would suggest the existence of a broader range of phenotypic and functional differences that are sex-dependent. Polymorphisms of the human 5-HT_{1B} receptor gene have been reported with potential functional significance (Hasegawa *et al*, 2002; Sanders *et al*, 2001, 2002; Sun *et al*, 2002). The present results suggest that sex-dependent effects caused by differential 5-HT_{1B} function of the 5-HT_{1B} receptor gene should be considered.

In conclusion, the present study demonstrated that regulation of 5-HT_{1B} receptors produced different functional consequences in male and female mice. The deletion of 5-HT_{1B} receptors produced a sex-specific disinhibition of 5-HT efflux due to the loss of terminal autoreceptors. 5-HT_{1B} receptor deletion in female mice also leads to augmented behavioral drug responses to SSRIs and behavioral performance on tests of stress-induced behavioral depression. These effects differed from those produced by the deletion of 5-HT_{1A} receptors. This research presents a model for how sex differences in the normal development of the brain may interact with genetic influences to account for the sex differences in onset and prevalence observed in neuropsychiatric disorders such as depression or anxiety.

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