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Evaluation of Antidepressant-related Behavioral Responses in Mice Lacking the Serotonin Transporter

Andrew Holmes, Rebecca J. Yang, Dennis L. Murphy, and Jacqueline N. Crawley

Inhibition of the serotonin transporter (5-HTT) is a principal initial target of many antidepressants. However, the contribution of the 5-HTT to their therapeutic efficacy is incompletely understood. We utilized a targeted gene mutation approach to examine the role of the 5-HTT in the behavioral actions of antidepressants. The 5-HTT mutation was bred onto two separate genetic backgrounds, C57BL/6J and 129S6. On a preliminary screen for gross physical, neurological and behavioral functions, all measures were normal with the exception that 5-HTT −/− mice on the C57BL/6J background showed increased body weight and poor rotarod performance, and 5-HTT −/− mice on the 129S6 background showed reduced neuromuscular strength. On the tail suspension test, 5-HTT −/− mice on the 129S6 background showed a baseline antidepressant-like reduction in immobility. In contrast, the same mice showed increased immobility in the forced swim test, possibly due to compromised neuromuscular strength. 5-HTT −/− mice on the C57BL/6J background showed no baseline antidepressant-related phenotype on either test. The behavioral effects of three antidepressants were tested in 5-HTT mutant mice (C57BL/6J background) in the tail suspension test. The anti-immobility effects of the serotonin reuptake inhibitor, fluoxetine (30 mg/kg), were abolished in 5-HTT −/− mice, confirming that the 5-HTT gene is required for the behavioral effects of fluoxetine. In contrast, 5-HTT −/− mice retained sensitivity to the anti-immobility effects of the norepinephrine reuptake inhibitor, desipramine (20 mg/kg), and the mixed serotonin/norepinephrine reuptake inhibitor, imipramine (25 mg/kg). 5-HTT knockout mice provide a valuable tool for delineating the neuropsychopharmacological actions of antidepressants.
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KEY WORDS: *Serotonin transporter; Fluoxetine; Desipramine; Antidepressant; Gene knockout mouse; Depression*

Antidepressants are thought to normalize the disturbances in monoamine function that occur in affective disorders (Feighner and Boyer 1996; Frazer 1997; Montgomery and Den Boer 2001). While dysfunctions in noradrenergic and dopaminergic systems are putative etiological factors in depression, there is considerable evidence indicating that perturbation of central serotonergic activity is a major etiological component of depression (Willner 1985; Murphy 1990; Maes and Meltzer 1995; Charney 1998). Cerebrospinal fluid of depressed patients contains lower levels of 5-HT metabolites, and depressed patients show reduced hormone responses to challenge with a serotonin agonist such as fenfluramine

From the Section on Behavioral Genomics (AH, RJY, JNC), and Laboratory of Clinical Science (DLM), National Institute of Mental Health, NIH, Bethesda, MD 20892, USA.

Address correspondence to: Andrew Holmes, Ph.D., Section on Behavioral Genomics, National Institute of Mental Health, Building 10, Room 4D11, Bethesda, MD 20892-1375. Tel.: (301) 496-4838; Fax: (301) 480-1164; E-mail: aholmes@intra.nimh.nih.gov

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The serotonin transporter (5-HTT) acts as a key regulator of serotonin signaling. By regulating reuptake of released serotonin, the 5-HTT controls the duration and intensity of serotoninergic activity at the synapse. The 5-HTT has been directly implicated in depression by the finding that brain 5-HTT binding density is reduced in brains and platelets of depressed patients (e.g., Nemeroff et al. 1994; Malison et al. 1998; Mann et al. 2000). Moreover, a number of studies have found an association between genetic variation in the regulatory region of the *5-HTT* gene and depression (e.g., Battersby et al. 1996; Collier et al. 1996a,b; Furlong et al. 1998; Rees et al. 1997; Menza et al. 1999; but see Seretti et al. 1999). The 5-HTT is also a major target for many antidepressant drug treatments (Blakely et al. 1991; Ramamoorthy et al. 1993). It is well known that the serotonin reuptake inhibitor (SRI) class of antidepressants increase concentrations of serotonin in the synapse by blocking the serotonin transporter (5-HTT) (Blakely et al. 1991; Ramamoorthy et al. 1993). However, given the time lag between the initial inhibitory effects of antidepressants on the 5-HTT and an observable improvement in symptoms of depression, the mechanisms underlying the therapeutic effects of these drugs are likely to occur downstream of 5-HTT inhibition (Blier et al. 1990; Chaput et al. 1991; Duman 1998; Manji et al. 2001). Another unresolved issue stems from the fact that antidepressants have differing affinity for the 5-HTT and even SRIs exert activity at other monoamine receptors and transporters (Stahl 1998). In this context, the relative contribution of the 5-HTT to the therapeutic effects of antidepressants is still not fully understood.

We have employed a targeted gene mutation approach to begin to investigate the significance of the *5-HTT* gene in the behavioral effects of antidepressants. Previous studies have demonstrated neurochemical alterations in 5-HTT knockout (KO) mice, including increased extracellular 5-HT levels (Mathews et al. 2000; Daws et al. 2001) and reduced 5-HT neuronal firing (Gobbi et al. 2001), that mimic the effects of chronic SRI treatment. In the present studies, a multitiered strategy for behavioral phenotyping was employed to first assess these mice on measures of gross physical, neurological and behavioral abnormalities that might compromise performance on tests for antidepressant activity (Crawley and Paylor 1997; Crawley 2000; Holmes et al. 2001, 2002). 5-HTT KO mice were then tested in two behavioral tasks that are sensitive to clinically efficacious antidepressants, the tail suspension test and the forced swim test (Porsolt et al. 1977; Steru et al. 1985; Borsini 1995). Genetic differences across mouse strains have been demonstrated for these tests (van der Heyden et al. 1987; Trullas et al. 1989; Montkowski et al. 1997; Vaugeois et al. 1997; Liu and Gershenfeld 2001; Lucki et al. 2001). Therefore, antidepressant-related phenotypes in 5-HTT KO mice were evaluated with the mutation placed on two separate genetic backgrounds, C57BL/6J and 129SvEvTac. Finally, we assessed the role of the 5-HTT in the pharmacological actions of three antidepressants with differing affinity for the 5-HTT, fluoxetine, desipramine and imipramine. Fluoxetine has high affinity for the 5-HTT, desipramine has high affinity for the norepinephrine transporter, while imipramine has high affinity for both sites (Frazer 1997; Tatsumi et al. 1997; Stahl 1998).

METHODS

Subjects

5-HT transporter knockout mice (5-HTT KO) were generated by replacing the second exon of the 5-HTT gene with a phosphoglycerine kinase-neo gene cassette, as previously described (Bengel et al. 1998). 5-HTT KO are viable, and develop and reproduce normally. For behavioral studies, the 5-HTT mutation was separately backcrossed onto two genetic backgrounds: C57BL/6J (seven generations) and 129S6/SvEvTac (129S6) (six generations). Homozygous knockout (5-HTT −/−), heterozygous knockout (5-HTT +/−) and wild type littermate controls (+/+) were group-housed (5/cage) by gender and background strain in a temperature and humidity controlled vivarium, under a 12-h light/dark cycle (lights on 6:00 A.M.). Behavioral testing was conducted in adult mice, aged at least five months. Mice on the C57BL/6J background comprised 29 5-HTT −/− (15 male, 14 female), 34 5-HTT +/− (16 male, 18 female), and 28 +/+ (14 male, 14 female) mice. Mice on the 129S6 background were 29 5-HTT −/− (13 male, 16 female), 58 5-HTT +/− (29 male, 29 female), and 29 +/+ (15 male, 14 female) mice. Food and water were provided ad libitum in the home cage. All mice were first evaluated for general health, neurological reflexes, and motor functions. Mice were then tested in the forced swim test and tail suspension test, with an interval of eight weeks between tests. Because mice on the C57BL/6J background showed no genotype differences in the tail suspension test, these mice were further evaluated on a pharmacological challenge with fluoxetine (10 weeks after baseline testing). A separate group of 24 5-HTT −/−, 27 5-HTT +/−, and 20 +/+ mice on the C57BL/6J background were used for a desipramine-challenge experiment. A third group of 21 5-HTT −/−, 20 5-HTT +/−, and 18 +/+ mice on the C57BL/6J background were used for an imipramine-challenge experiment. All testing was conducted during the light phase of the light/dark cycle (9:00 A.M.–5:00 P.M.). Mice were 12-16 weeks old at the beginning of testing. All experimental procedures were approved by the National Institute of Mental Health Animal Care and Use Com-

mittee, and followed the NIH guidelines outlined in "Using Animals in Intramural Research."

Initial Evaluation

To avoid false positive interpretations of phenotypes in tests for antidepressant activity, 5-HTT KO mice were first evaluated for general health, neurological reflexes and motor functions (Crawley and Paylor 1997; Crawley 2000; Holmes et al. 2001, 2002). Physical characteristics measured were body weight, coat condition, barbered hair, missing whiskers, and piloerection. Basic neurological reflexes measured were the righting reflex from the supine position, and corneal, pinna, and vibrissae responses to an approaching cotton swab. Trunk curl was assessed by suspending the mouse by the tail. Neuromuscular strength and stamina were tested using the wire hang test (e.g., Caston et al. 1999; Gerlai et al. 2000). For this test, the mouse gripped onto ~5 mm round metal bars. The latency for the mouse to lose its grip and fall onto a foam pad below was timed with a stopwatch over a 60 s period. Motor coordination was assayed using an accelerating rotarod (Ugo Basile, Stoelting, Wood Dale, IL). For this test, mice were placed on a slowly rotating drum, which gradually accelerated from 4 to 40 rpm over a 5 min period. The latency to fall onto a platform ~8 cm below was timed using a stopwatch.

Tail Suspension Test

The tail suspension test was conducted as previously described (Steru et al. 1985; Mayorga et al. 2001). Mice were securely fastened by the distal end of the tail to a flat metallic surface and suspended in a visually isolated area (40 × 40 × 40 cm white Plexiglas box). The presence or absence of immobility, defined as the absence of limb movement, was sampled every 5 s over a 6-min test session by a highly trained observer who remained blind to genotype (Wong et al. 2000; Mayorga et al. 2001). An identical procedure was employed for drug challenge experiments.

Forced Swim Test

The Porsolt forced swim test was conducted as previously described (Porsolt et al. 1977, 2000; Lucki et al. 2001). Mice were gently placed in a transparent Plexiglas cylinder (20 cm in diameter) filled with water (25 ± 2°C). Filling the cylinder to a depth of 12 cm prevented mice from using their tails to support themselves in the water. Immobility was defined as the cessation of limb movements except minor movement necessary to keep the mouse afloat. Immobility was sampled every 5 s during the last 4 min of a 6-min test session by a highly experienced observer who remained blind to genotype (Redrobe and Bourin 1997; O'Neill and Conway 2001; Lucki et al. 2001).

Drugs

Fluoxetine hydrochloride, desipramine hydrochloride and imipramine hydrochloride were obtained from Research Biochemicals Incorporated (RBI, Natick, MA). Drugs were dissolved in a 0.9% physiological saline vehicle. Injections were given intraperitoneally in a volume of 10 ml/kg body weight 30 min prior to testing. Drug doses were 30 mg/kg fluoxetine, 20 mg/kg desipramine and 25 mg/kg imipramine. Doses were chosen on the basis of previous reports in mice of the anti-immobility effects of fluoxetine (Perrault et al. 1992; Cesana et al. 1993; Redrobe et al. 1996; Eckeli et al. 2000; Clenet et al. 2001; Mayorga et al. 2001; Conti et al. 2002), desipramine (Redrobe et al. 1996; Vaugeois et al. 1997; Srivastava and Nath 2000; Wong et al. 2000; Clenet et al. 2001; Cryan et al. 2001; Lucki et al. 2001; Mayorga et al. 2001; Conti et al. 2002), and imipramine (Redrobe and Bourin 1997; Vaugeois et al. 1997; Wong et al. 2000; David et al. 2001; Liu and Gershenfeld 2001; Do-Rego et al. 2002).

Statistical Analysis

Genotype, gender, and drug effects were analyzed using between subjects analysis of variance (ANOVA) and Newman-Keuls post-hoc comparisons where appropriate, using StatView (SAS Institute Inc., Cary, NC). After confirming the absence of gender × genotype interactions, gender was removed as a factor from all analyses in order to increase the statistical power of genotype and drug comparisons.

RESULTS

Initial Evaluation

Table 1 summarizes the results of the preliminary screen. 5-HTT −/−, +/− and +/+ mice were similar on measures of coat condition, missing whiskers, and piloerection. 11% of 5-HTT −/− mice on the 129S6 background displayed barbered hair, as compared with 0% of +/+. The righting, corneal, pinna, and vibrissae reflexes and trunk curl were all normal in 5-HTT KO mice. There was a significant effect of genotype on body weight for male ($F_{2,43} = 7.12$, $p = .002$) and female ($F_{2,42} = 6.35$, $p = .004$) mice on the C57BL/6J background. At six months of age, 5-HTT −/− mice had significantly higher body weights than their 5-HTT +/− or +/+ controls, in both males and females ($p < .01$). There was no significant effect of genotype on body weight in mice on the 129S6 background, for either males or females ($p > .09$), although there was a trend for male

5-HTT −/− to be heavier than +/+. For mice on the C57BL/6J background there was a significant effect of genotype on latency to fall in the accelerating rotarod test for motor coordination ($F_{2,80} = 6.13$, $p = .03$). Higher body weights in 5-HTT −/− mice on the C57BL/6J background may have impaired performance in this test, as body weight was negatively correlated with rotarod latencies in these mice ($r = -0.75$, $p < .001$). With body weight included as a covariate, analysis of covariance found no significant effect of genotype on rotarod latencies ($F_{2,75} = 2.27$, $p = .11$). There was no significant effect of genotype on latency to fall for mice on the 129S6 background ($F_{2,110} = 1.02$, $p = .36$). There was no significant effect of genotype on latency to fall for mice on the C57BL/6J background in the wire hang test of neuromuscular strength ($F_{2,88} = 0.45$, $p = .64$). There was a significant effect of genotype on wire hang latencies for mice on the 129S6 background ($F_{2,103} = 14.28$, $p < .001$), with significantly lower latencies in 5-HTT −/− mice as compared with +/+ controls ($p < .01$).

Antidepressant-related Behaviors; C57BL/6J Background

As shown in Figure 1, panel A, there was no significant effect of genotype on % immobility time in the tail suspension test for mice on the C57BL/6J background ($F_{2,77} = 1.36$, $p = .26$). Problems with mice on the C57BL/6J background climbing up their tails in the early stages of testing, as reported by other laboratories (Mayorga and Lucki 2001), were observed only in a very small percentage of subjects (<3%), which were excluded from further statistical analysis. As shown in Figure 1, panel B, there was no significant effect of genotype on % immobility time in the forced swim test for mice on the C57BL/6J background ($F_{2,83} = 0.21$, $p = .81$).

Antidepressant-related Behaviors; 129S6 Background

As shown in Figure 1, panel C, there was a significant effect of genotype on % immobility time in the tail suspension test for mice on the 129S6 background, ($F_{2,79} = 22.56$, $p < .001$), with significantly lower % immobility time in 5-HTT −/− mice as compared with 5-HTT +/− and +/+ controls ($p = .01$). As shown in Figure 1, panel D, there was a significant effect of genotype on % immobility time in the forced swim test for mice on the 129S6 background ($F_{2,104} = 20.91$, $p < .001$). % Immobility time was significantly higher in both 5-HT −/− and 5-HTT +/− mice, as compared with +/+ controls ($p = .01$).

Antidepressant Effects of Fluoxetine, Desipramine and Imipramine (C57BL/6J Background)

For the effects of 30 mg/kg fluoxetine in the tail suspension test, there was a significant effect of genotype ($F_{1,56} =$

Table 1. 5-HTT KO mice separately backcrossed onto C57BL/6J and 129S6 genetic backgrounds were normal for physical characteristics and neurological reflexes, with the exception that 6-month-old 5-HTT −/− mice on the C57BL/6J genetic background showed higher body weights than 5-HTT +/− and +/+ controls. 5-HTT −/− mice on the C57BL/6J, but not 129S6, background showed poor performance on the rotarod test, as compared to +/+ controls. 5-HTT −/− mice on the 129S6, but not C57BL/6J, background showed reduced neuromuscular strength on the wire hang test, as compared to +/+ controls. Data are expressed as the percentage of individuals showing a response, except where indicated in parentheses. **$p < .01$; *$p < .05$ vs. +/+.

	C57BL/6J Background			129S6 Background		
	+/+	+/−	−/−	+/+	+/−	−/−
Physical characteristics						
Body weight (g)						
males	31.1 ±0.8	32.5 ±0.8	*35.4 ±0.9	34.2 ±1.4	36.3 ±1.1	38.2 ±1.6
females	25.5 ±0.8	24.4 ±0.7	*29.4 ±1.5	29.5 ±0.9	28.4 ±0.7	30.4 ±1.4
Poor coat condition	3	3	0	0	0	4
Barbered hair	14	21	7	0	0	11
Missing whiskers	0	0	0	20	33	18
Piloerection	0	0	0	0	0	0
Motor/muscular abilities						
Rotarod latency (sec)	110.9 ±14.7	123.6 ±15.9	**57.3 ±8.4	47.5 ±6.6	44.8 ±4.9	34.3 ±8.4
Wire hang latency (sec)	50.6 ±2.5	46.9 ±2.4	41.2 ±2.8	57.5 ±1.5	50.5 ±2.4	**36.0 ±3.6
Neurological reflexes						
Righting reflex	100	100	100	100	100	100
Corneal reflex	100	100	100	100	100	100
Pinna reflex	100	100	100	100	100	100
Vibrissae orientating	93	97	100	93	97	100
Trunk curl	100	100	100	100	100	100

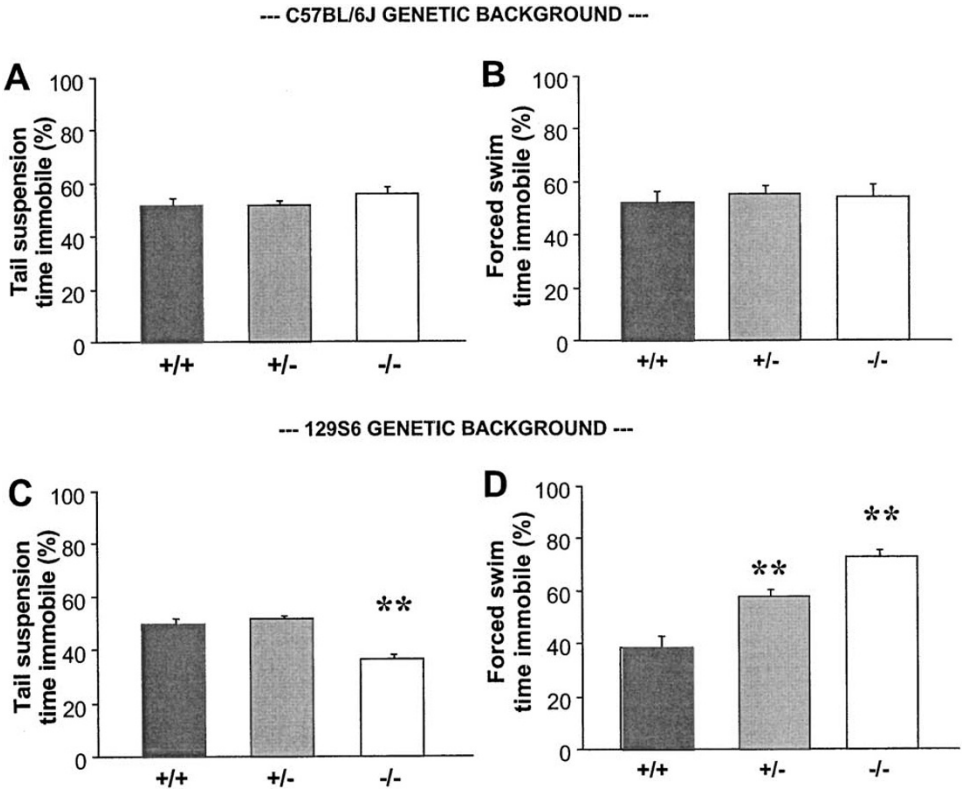

Figure 1. Baseline phenotypes in 5-HTT KO mice on the tail suspension and forced swim tests. 5-HTT KO mice on the C57BL/6J background showed no alteration in % time immobile in the tail suspension **(A)** or forced swim **(B)** tests. 5-HTT −/− mice on the 129S6 background showed less % time immobile than +/+ controls in the tail suspension test **(C)**, and more % time immobile than +/+ controls in the forced swim test **(D)**. For the tail suspension test, immobility was sampled every 5 s over a 6 min test session. For the forced swim test, immobility was sampled every 5 s over the last 4 min of a 6-min test session. $^{**}p < .01$ vs. +/+.

4.80, $p = .01$), drug ($F_{1,56} = 19.66$, $p < .001$), and a genotype × drug interaction ($F_{2,56} = 9.42$, $p < .001$) on % immobility time. As shown in Figure 2, panel A, fluoxetine significantly reduced immobility in 5-HT +/− and +/+ mice ($p < .01$), but had no effect on % immobility time in 5-HT −/− mice ($p = .12$).

For the effects of 20 mg/kg desipramine in the tail suspension test, there was significant effect of genotype ($F_{1,65} = 15.19$, $p < .001$), drug ($F_{1,65} = 37.00$, $p < .001$), but no genotype × drug interaction ($F_{2,65} = 1.71$, $p = .19$) on % immobility time. As shown in Figure 2, panel B, desipramine significantly reduced % immobility time in 5-HTT +/− mice and +/+ controls ($p < .05$) and in 5-HTT −/− mice ($p < .01$). In this experiment, baseline % immobility time in vehicle-treated 5-HT −/− mice was significantly lower than in +/+ controls ($p < .05$). Percent immobility time following desipramine treatment was significantly lower in 5HTT−/− then in either 5HTT +/− or +/+ controls ($p < .01$).

For the effects of 25 mg/kg imipramine in the tail suspension test, there was significant effect of drug type ($F_{1,53} = 46.92$, $p < .001$), but not genotype ($F_{1,53} = 0.94$, $p = .40$) and no genotype × drug interaction ($F_{2,53} = 0.81$, $p = .45$) on % immobility time. As shown in Figure 2, panel C, imipramine significantly reduced % immobility time in all 5-HTT +/− mice and +/+ controls ($p < .01$) and in 5-HTT −/− mice ($p < .01$). Reductions in % immobility time showed a trend to be lesser in 5-HTT −/− mice than 5-HTT +/− or +/+ controls.

DISCUSSION

Present findings demonstrate that 5-HTT KO mice exhibit alterations in antidepressant-like behaviors that are dependent upon the genetic background on which the mutation is placed. 5-HTT−/− mice on a 129S6 genetic background showed less immobility in the tail suspension test, as compared with +/+ controls. A baseline phenotype of reduced immobility in the tail suspension test mimics the effects of antidepressants. This finding is intriguing given that 5-HTT KO mice show increased extracellular levels of 5-HT (Mathews et al. 2000; Daws et al. 2001) similar to that seen following chronic treatment with antidepressants, and reduced 5-HT neuronal firing (Gobbi et al. 2001), analogous to the effects of antidepressants on dorsal raphe neurons (de Montigny et al. 1991). Thus, 5-HTT KO mice appear to model several of the neurochemical and behavioral effects of prolonged exposure to SRIs. However, certain caveats preclude making generalizations from a behavioral phenotype in a rodent test to the complex etiology and symptomatology of depression, and the complex mechanisms underlying antidepressant drug effects. While rodent behavioral models have good predictive validity for antidepressants, they are sensitive to acute administration of these compounds, whereas symptoms of depression are only ameliorated after chronic drug treatment. In the case of 5-HTT KO

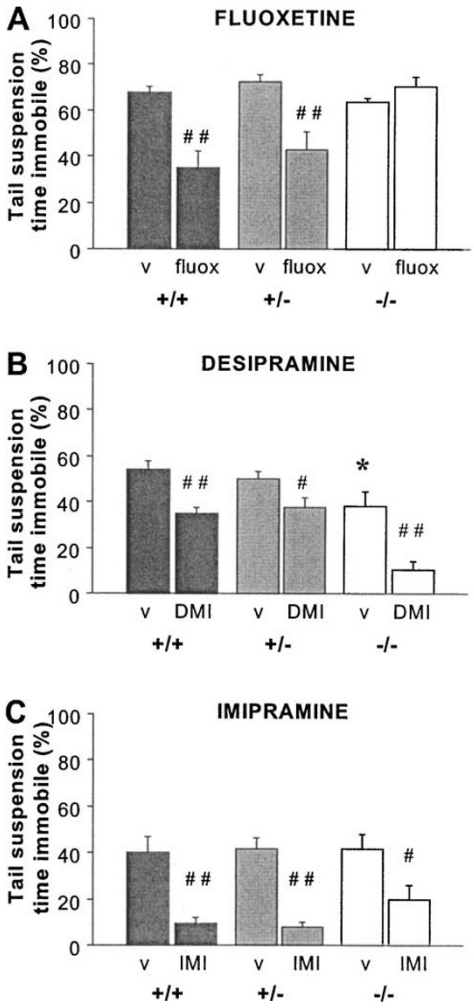

Figure 2. Behavioral effects of antidepressants in 5-HTT KO (C57BL/6J background) mice in the tail suspension test. 5-HTT KO mice were insensitive to the anti-immobility effects of fluoxetine **(A)**; acute treatment with 30 mg/kg fluoxetine (fluox) significantly reduced % time immobile in 5-HTT +/− and +/+ mice, but had no effect in 5-HTT −/− mice. 5-HTT KO mice were sensitive to the anti-immobility effects of desipramine **(B)**; acute treatment with 20 mg/kg desipramine (DMI) significantly reduced % time immobile in all genotypes, with the strongest effects seen in 5-HTT −/− mice. In this experiment, 5-HTT −/− mice showed a significantly lower baseline level of % time immobile than +/+ controls. 5-HTT KO mice were sensitive to the anti-immobility effects of imipramine **(C)**; acute treatment with 25 mg/kg imipramine (IMI) significantly reduced % time immobile in all genotypes, with the weakest effects seen in 5-HTT −/− mice. For each experiment, immobility was sampled every 5 s over a 6-min test session. ##$p < .01$, #$p < .05$ vs. vehicle; *$p < .05$ vs. +/+.

mice, the 5-HTT is absent throughout development as well as in adulthood, as opposed to a temporally limited treatment regimen with SRIs in adult patients. For this reason in particular, 5-HTT KO mice are unlikely to represent a simple model of the effects of SRIs.

Contrary to the reduced immobility shown in the tail suspension test, 5-HTT KO mice on the 129S6 background showed markedly increased immobility in the forced swim test. These data show behavioral phenotypes in 5-HTT KO mice on the 129S6 background that are opposite in two putatively similar behavioral tests. Behavioral profiles in the tail suspension test and forced swim test are known to be affected by non-specific drug effects on motor function (Steru et al. 1985; van der Heyden et al. 1987; Perrault et al. 1992; O'Neill and Conway 2001). A parsimonious explanation for increased forced swim test immobility in 129S6-background 5-HTT KO mice is that this effect resulted from a physical defect. While 5-HTT KO mice on the 129S6 background were normal on measures of general health, neurological reflexes and rotarod motor coordination, they exhibited reduced neuromuscular strength and stamina on the wire hang test. This neuromuscular impairment may have seriously compromised swimming in the forced swim test, leading to a false positive increase in immobility.

In contrast to the clear phenotypes seen in 5-HTT KO mice on the 129S6 background, 5-HTT KO mice on a C57BL/6J background showed no consistent baseline phenotype on either the tail suspension test or the forced swim test. The absence of antidepressant-like phenotypes in these mice was not related to any gross abnormality in general health or neurological reflexes and, unlike mutant mice on the 129S6 background, 5-HTT KO mice on the C57BL/6J background were normal on the wire hang test of neuromuscular strength. 5-HTT −/− mice on the C57BL/6J background did display increased body weights relative to +/+ controls, and shorter latencies to fall in the accelerating rotarod test for motor coordination. High negative correlations between body weights and rotarod latencies suggest that poor rotarod performance in 5-HTT KO mice on the C57BL/6J background may have been the result of their higher body weights, rather than impaired motor coordination.

The observation that antidepressant-related phenotypes in 5-HTT KO mice were present on the 129S6 background but absent on the C57BL/6J background suggests that the manifestation of these phenotypes was strongly affected by background genes. This finding adds to previous evidence that genetic background is a major influence on both baseline performance and responses to antidepressants in the tail suspension and forced swim tests (van der Heyden et al. 1987; Trullas et al. 1989; Montkowski et al. 1997; Vaugeois et al. 1997; Liu and Gershenfeld 2001; Lucki et al. 2001). It would be interesting to investigate the identity of background genes that differ between these two strains that may interact with the *5-HTT* mutation (Murphy et al. 1999, 2001). This could provide insight into genes that modify the effects of 5-HTT disruption.

The present study demonstrates that mice lacking the serotonin transporter (5-HTT−/−) are insensitive to the

behavioral effects of fluoxetine, but not desipramine or imipramine. 5-HTT KO mice on the C57BL/6J background were used for these studies because the absence of baseline genotype differences in the tail suspension test facilitated interpretation of pharmacological effects. Consistent with previous reports, time spent immobile in the tail suspension test in +/+ control mice was significantly reduced by acute administration of fluoxetine (Perrault et al. 1992; Mayorga et al. 2001; Conti et al. 2002). In marked contrast, 5-HTT −/− mice were completely insensitive to the anti-immobility effects of fluoxetine in this test.

These findings demonstrate that the 5-HTT is essential for the behavioral actions of fluoxetine in this behavioral assay, and support prior evidence that the acute anti-immobility effects of this compound occur, at least initially, via increased availability of 5-HT following 5-HTT blockade (Ranganathan et al. 2001). Interestingly, the effects of fluoxetine were unaltered in heterozygous 5-HTT knockout mice, indicating that a 50% loss of 5-HTT is sufficient to retain the anti-immobility effects of fluoxetine. These data also show that fluoxetine's direct actions on 5-HT_{2C} receptors (Jenck et al. 1993; Pinder and Wieringa 1993; Pälvimäki et al. 1996) do not mediate the anti-immmobility effects of the drug (Borsini et al. 1991; Bourin et al. 1996; Redrobe and Bourin 1997; Cryan and Lucki 2000; Clenet et al. 2001). However, although the loss of fluoxetine's anti-immobility effects in 5-HTT −/− mice was clear and unequivocal at the single dose tested, it will be important to conduct a full dose-response curve to determine whether 5-HTT KO mice are differentially sensitive to other doses of this compound. Notwithstanding, the present data are salient to recent reports that depressed individuals with the lesser expressing form of the *5-HTT* gene promoter polymorphism show reduced antidepressant responses to SRIs (Smeraldi et al. 1998; Zanardi et al. 2000, 2001).

It was of considerable interest to test whether the behavioral actions of antidepressants with a more mixed pharmacological profile than fluoxetine would be altered in 5-HTT KO mice. Previous research has reliably demonstrated anti-immobility effects of both acute desipramine (Srivastava and Nath 2000; Wong et al. 2000; Clenet et al. 2001; Cryan et al. 2001; Mayorga et al. 2001) and imipramine (Vaugeois et al. 1997; Wong et al. 2000; David et al. 2001; Liu and Gershenfeld 2001; Do-Rego et al. 2002) treatment in mice. Desipramine has a much higher affinity for the norepinephrine transporter (NET) than the 5-HTT, while imipramine has high affinity for both sites (Frazer 1997; Tatsumi et al. 1997). Consistent with these pharmacological profiles, the anti-immobility effects of imipramine were retained but slightly blunted in 5-HTT −/− mice, while the effects of desipramine were retained and even augmented in 5-HTT −/− mice. These data indicate that activity at the 5-HTT is not essential for the anti-immobility effects of either compound, but that genetic deletion of the 5-HTT alters the behavioral effects of these antidepressants in subtle ways.

Desipramine and imipramine have relatively higher affinity for H_1 histamine, α_1-adrenergic and cholinergic receptors than fluoxetine (Frazer 1997), but these actions are unlikely to be related to their antidepressant effects. Rather, the finding that 5-HTT KO mice retained sensitivity to the behavioral effects of desipramine and imipramine can be explained by the affinityof these drugs for the NET. In support of this interpretation, Cryan et al. (2001) have recently shown that dopamine-β-hydroxylase knockout mice, which are unable to synthesize norepinephrine or epinephrine, are insensitive to the anti-immobility effects of desipramine in the forced swim test. In an interesting parallel in humans, depressed patients treated with serotonin reuptake inhibitors are prone to relapse if serotonin levels are pharmacologically depleted, but not if catecholamines are depleted. Conversely, catecholamine, but not serotonin, depletion produces relapse in patients that have been treated with norepinephrine reuptake inhibitors (Delgado et al. 1990, 1991; Heninger et al. 1996). On the basis of such findings, some authors have suggested that antidepressants that have varying affinity for noradrenergic versus serotonergic systems produce their behavioral effects via separate mechanisms (Page et al. 1999). This hypothesis is supported by present data demonstrating that fluoxetine's behavioral effects are lost, while desipramine's effects are retained, in 5-HTT KO mice. However, the observation that imipramine's effects were retained but somewhat blunted in 5-HTT KO mice provide tentative evidence that activity at both serotonergic and noradrenergic system can contribute to the anti-immobility effects of this drug.

Lifelong absence of the 5-HTT in 5-HTT KO mice may have led to development changes that altered the normal effects of antidepressants. Specifically, it is possible that while the 5-HTT may normally mediate the anti-immobility effects of imipramine and even desipramine, the importance of these actions were masked by a compensatory upregulation of noradrenergic mechanisms in 5-HTT KO mice. An example of compensatory changes in another 5-HT mutant mouse was recently provided by Mayorga et al. (2001). These authors found that an antidepressant-related phenotype in 5-HT_{1A} receptor KO mice was reversed by depletion of catecholamines but not forebrain serotonin, suggesting that the behavioral alterations in these mice were caused by compensatory alterations in dopamine and/or norepinephrine neurotransmission. In the context of 5-HTT KO mice, there is evidence that serotonin can be taken up by the NET under conditions of extreme 5-HTT blockade (Bel and Artigas 1996). While we cannot fully exclude the possibility that changes in NET function contribute to antidepressant-related phenotypes in 5-HTT KO mice, there is no evidence to date of alterations in norepinephrine reuptake mechanisms in these mice (Daws et al. 2001).

In conclusion, mice lacking the 5-HTT showed baseline behavioral phenotypes in tests for antidepressant activity that were strongly influenced by the genetic background onto which the 5-HTT null mutation was placed and the behavioral test employed. 5-HTT−/− mice on a C57BL/6J background showed normal baseline performance on both the tail suspension and forced swim tests. In contrast, 5-HTT−/− mice on a 129S6 background showed an antidepressant-like decrease in immobility in the tail suspension test, but an increase in immobility in the forced swim test. 5-HTT−/− mice on the C57BL/6J background were insensitive to the effects of fluoxetine, but not desipramine or imipramine, in the tail suspension test. These data from a genetic model support the extensive pharmacological evidence that activity at the 5-HTT is essential for the behavioral effects of 5-HTT-selective antidepressants, but not for the behavioral actions of drugs which have affinity for both the 5-HTT and NET. 5-HTT−/− mice will provide a useful tool for further delineating the pharmacological actions of antidepressants and the pharmacogenetics of treating depression.

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