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ORIGINAL PAPER

Effects of Mild Early Life Stress on Abnormal Emotion-related Behaviors in 5-HTT Knockout Mice

Jenna C. Carroll · Janel M. Boyce-Rustay · Rachel Millstein · Rebecca Yang · Lisa M. Wiedholz · Dennis L. Murphy · Andrew Holmes

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Abstract A low-expressing polymorphic variant of the serotonin transporter (5-HTT) gene has been associated with emotional disorders in humans and non-human primates following exposure to early life trauma. 5-HTT gene knockout (KO) mice exhibit increased anxiety- and depression-related behaviors, and provide a model to study interactions between 5-HTT gene variation and early life stress. The present study assessed the effects of postnatal footshock stress on the development of emotion-related behaviors in 5-HTT KO mice. Results showed that 5-HTT KO mice displayed a profile of suppressed exploratory behavior and increased anxiety-like behavior in the light/dark, elevated plus-maze and open field tests, as well as increased depression-related behavior in the forced swim test following repeated exposure to the test. Postnatal exposure to footshock stress did not affect emotion-related behaviors in non-mutant C57BL/6J mice or modify phenotypic abnormalities in 5-HTT KO. Data provide further evidence of emotional abnormalities following genetic disruption of the 5-HTT.

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J. C. Carroll · D. L. Murphy
Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892, USA

J. M. Boyce-Rustay · R. Millstein · R. Yang · L. M. Wiedholz · A. Holmes (✉)
Section on Behavioral Science and Genetics, Laboratory for Integrative Neuroscience, National Institute on Alcoholism and Alcohol Abuse, National Institutes of Health, Bethesda, Rockville, MD 20852, USA
e-mail: holmesan@mail.nih.gov

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Introduction

There is growing evidence that emotional experiences during early life exert a pervasive influence on later risk for a range of neuropsychiatric disease states. The prevalence of affective illnesses, such as anxiety disorders and depression, is significantly greater in individuals who were abused or neglected during childhood (de Wilde et al. 1992; Heim and Nemeroff 2002). Interestingly, there is also evidence that certain mild forms of stress may protect or 'inoculate' against the deleterious consequences of later adversity across a range of species (Solomon et al. 1968; Boyce and Chesterman 1990; Parker et al. 2004). Currently, however, there remains little understanding of how early experiences affect the development of neural systems subserving emotional behavior, or why certain individuals appear to be susceptible to the effects of adverse childhood experiences while others are resilient (Rutter 1985; Charney 2004).

Recent studies demonstrate that genetic variation between individuals can profoundly influence how childhood exposure to stressful environments affects later risk for various psychopathologies (Kendler et al. 1995a, b). For example, a functional polymorphism in the gene encoding for the major catabolizing enzyme for brain monoamines, monoamine oxidase A (*MAOA*), is associated with higher rates of anti-social behavior in people that were abused as children (Caspi et al. 2002). A major interaction between variation in another major regulatory component of the brain 5-HT

system, the 5-HT transporter (5-HTT) and childhood trauma has also been recently documented. The 5-HTT belongs to a family of Na^+/Cl^--dependent transporters that translocate biogenic amines through the plasma membrane (Blakely et al. 1991) and functions to regulate synaptic levels of 5-HT. Individuals with a low-functioning variant of the 5-HTT gene (5-HTT-linked polymorphic region, *HTTLPR*, (Lesch et al. 1996; Little et al. 1998) are at significantly greater risk for major depressive disorder following repeated adult stress or childhood trauma (Caspi et al. 2003). Comparable findings have been obtained in rhesus monkey, in which the low-functioning orthologue of the 5-HTT gene (*rh-httlpr*) is associated with greater stress-reactivity in monkeys reared under impoverished, peer-only, environmental conditions (Champoux et al. 2002; Barr et al. 2004). This emerging literature is consistent with recent findings in rodents demonstrating a critical role of the 5-HT system in mediating the depression-related, 'despair-like' behaviors and abnormal fear conditioning caused by repeated, uncontrollable stress (Grahn et al. 1999; Pernar et al. 2004; Amat et al. 2005).

Mutant mice provide a singular tool with which to explore gene x early life stress interactions; permitting control over both gene function and environmental experience during specific periods of development (Gross and Hen 2004; Cryan and Holmes 2005; Holmes et al. 2005). A 5-HTT 'knockout' (KO) mouse has been employed as a model to study the effects of constitutive genetic deficiency in 5-HTT function on emotionality. Previous studies have found that 5-HTT KO mice exhibit phenotypic abnormalities that provide interesting parallels with the human and non-human primate data, including heightened levels of anxiety-like and depression-related behaviors and increased stress-reactivity (Hariri and Holmes 2006). In the present study, we examined whether 5-HTT KO mice were vulnerable to the effects of exposure to early life stress. To this end, we first examined the effects of a postnatal stressor (repeated mild footshock during the second postnatal week) in non-mutant C57BL/6J mice. Next, 5-HTT KO mice were exposed to this postnatal stressor and then tested in adulthood for anxiety-related behavior (via the elevated plus-maze, light-dark test, and open field) and depression-related behavior (via repeated testing in the forced swim test).

Methods

Subjects

5-HTT KO mice were generated as previously described by replacing a 1.1-kbp fragment of the *htt* gene containing exon 2 with a 1.8-kbp pPNT-neo replacement targeting vector (Bengel et al. 1998). For the present study, mice originally from a 129P1 (129P1/ReJ) × C57BL/6J hybrid genetic background were repeatedly backcrossed onto a C57BL/6J for >15 generations to produce a congenic background. To control for possible influences on behavior resulting from genotypic differences in maternal behavior, all mice were bred from 5-HTT heterozygous (HET) parents. 5-HTT KO, 5-HTT HET and wild type (WT) littermates were housed together from weaning (at 21 days of age) in same-sex groups of 1–5 mice/cage until testing. A total of 34 litters were bred to obtain the no stress condition, and 32 litters bred to obtain the stress condition.

In order to examine the effects of postnatal stress in non-mutant C57BL/6J mice, parental mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and housed in breeding dyads. Their offspring were subjected to postnatal stress using the same procedure as used for 5-HTT KO mice and housed from weaning in same-sex groups of 1–5 mice/cage until testing. A total of 5 litters were bred to obtain the no stress condition, and 5 litters bred to obtain the stress condition. All mice were housed in a temperature- and humidity-controlled vivarium under a 12 h light/dark cycle (lights on 0600 h). Experimental procedures were performed in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the local Animal Care and Use Committee.

Postnatal stress

Whole litters containing at least 3 pups were pseudorandomly assigned to either postnatal stress or no stress control groups. Postnatal stress entailed removing the whole litter from the home cage and placing the pups on the grid floor of a shock generator apparatus (San Diego Instruments, San Diego, CA). Pups were subjected to 3 mild scrambled footshocks (0.4 mA) over a 150 s period (30 s inter-shock interval) and then returned immediately to the home cage nest. The stress procedure was repeated daily for 7 days from postnatal days P7 through P13 (birth designated as P0). No stress controls underwent an identical procedure with the exception that they did not receive footshock. The second postnatal week corresponds to a developmental period during which disruptions of 5-HT function have been shown to affect later emotional behavior in mice (Gross et al. 2002; Ansorge et al. 2004).

Effects of postnatal stress in non-mutant C57BL/6J mice

Twenty-seven mice (12 males, 15 females) were subject to postnatal stress and 28 mice (17 males, 11 females) served as no stress controls. In order to test whether the stressor affected anxiety-like behavior in these non-mutant mice, they were tested at 8 weeks of age on the novel open field test and, 1 week later, the elevated plus-maze.

Effects of postnatal stress in 5-HTT KO mice

Thirty-three WT (15 males, 18 females), 43 5-HTT HET (29 males, 14 females) and 19 5-HTT KO (12 males, 7 females) mice were subject to postnatal stress, and 35 WT (14 males, 21 females), 48 HET (23 males, 25 females) and 29 KO (21 males, 14 females) served as no stress controls. Beginning at 8 weeks of age, mice were tested on the novel open field test, elevated plus-maze, light/dark exploration test, and forced swim test, in that order, with at least 1 week between tests.

For all testing in C57BL/6J and 5-HTT KO mice, subjects were transported to the test room at least 1 hr before testing and the experimenter was blind to genotype and postnatal stress condition. All apparatuses were washed with 30% water/70% ethanol solution and dried between subjects.

Novel open field test

The novel open field test was conducted as previously described (Boyce-Rustay and Holmes 2006). The apparatus was a square arena (40 × 40 × 35 cm) with clear Plexiglas walls and floor that was evenly-illuminated to ~95 lx. Mice were individually placed in a corner and allowed to freely explore for 10 min. Horizontal activity and time spent in the inner 20 × 20 cm center area was automatically measured via photocell beams positioned around the walls of the arena via the Digiscan Optical Activity System (Accuscan Instruments, Columbus, OH).

Elevated plus-maze

The elevated plus-maze test was conducted as previously described (Handley and Mithani 1984; Boyce-Rustay and Holmes 2006). The maze (San Diego Instruments, San Diego, CA) comprised two open arms (30 × 5 cm) and two closed arms (30 × 5 × 15 cm) that extended from a common central platform (5 × 5 cm) and was evenly-illuminated to ~55 lx. A small raised lip (0.5 cm) around the edges of the open arms prevented animals from slipping off. The apparatus was constructed from black acrylonitrile butadiene styrene plastic and elevated to a height of 38 cm above floor level. The mouse was placed in the center square, facing a closed arm, and allowed to freely explore the apparatus for 5 min. Behavior was video recorded and scored for time spent in open arms and the number of entries into the open and closed arms (an arm entry was defined as all 4 paws in an arm) by an experienced observer using the 'Hindsight' behavioral observation system (Scientific Programming Services, Wokingham, UK). Open and closed entries were summed to give total arm entries.

Light/dark exploration test

The light/dark exploration test was conducted using methods based on those previously described (Crawley 1981; Boyce-Rustay and Holmes 2006). The apparatus consisted of a white-walled ('light') compartment (16.8 × 12.7 × 12.7 cm; ~35 lx) and a black-walled ('dark') compartment of the same size (~0 lx), that were interconnected by a small grey-walled compartment (7.2 × 12.7 × 12.7 cm) (Mouse Place Preference System, Med Associates, St. Albans, VT). The mouse was placed in the dark compartment and allowed to freely explore for 10 min. The time spent in the light compartment and the number of whole-body transitions into the light and dark compartments was automatically measured via photocell beams spaced 1.5 cm apart throughout the length of the apparatus.

Forced swim test

The forced swim test (FST) was conducted using methods based on those previously described mice (Cryan et al. 2001; Boyce-Rustay and Holmes 2006) with modifications to test the effects of repeated exposure (Porsolt et al. 1977). The mouse was gently placed in a 20-cm-diameter cylinder filled to ~13 cm with 24 ± 1.0°C water for 15 min, and then returned to the home cage (first exposure). Twenty-four hours later, the mouse was re-tested in the same manner for a 6 min trial (second exposure). Behavior was video recorded and scored for 'immobility' (cessation of limb movements except minor involuntary hind limb movements) every 5 s during min 2–6 on each day. Immobility was expressed as the percent number of instances of immobility ([number of immobility observations/total number of observations [=72]] × 100).

Statistics

Gender did not significantly interact with either genotype or postnatal stress; therefore, data were collapsed across gender for all analyses. The effects of genotype and postnatal stress were analyzed using 2-factor ANOVA. In cases of a significant main effect of 1 factor but no significant 2-way interaction, data were collapsed across the second factor and analyzed using Bonferroni post-hoc tests.

Results

Effects of postnatal stress in C57BL/6J mice

In the elevated plus-maze, there was no effect of postnatal stress on time spent in the open arms, or on the number of open arm entries, closed arm entries, or total arm entries (Table 1). In the novel open field test, there was no effect of postnatal stress on locomotor activity or percent time spent in the center of the open field (Table 1).

Effects of 5-HTT KO genotype and postnatal stress

Increased anxiety-like behavior in 5-HTT KO mice in the light/dark exploration test

There was a significant effect of genotype ($F_{2,201} = 8.06$, $P < 0.01$), but not of postnatal stress and no genotype × stress interaction on time spent in the light compartment. Collapsing across stress condition, post-hoc analysis showed that 5-HTT KO mice spent significantly less time in the light compartment than WT controls ($P < 0.01$) (Fig. 1A). There was a significant effect of genotype ($F_{2,201} = 7.99$, $P < 0.01$) and postnatal stress ($F_{1,201} = 5.65$, $P < 0.05$), but no genotype × stress interaction, on the number of light/dark transitions. Collapsing across stress condition, post-hoc analysis showed that 5-HTT KO mice made significantly fewer transitions than WT controls, and collapsing across genotype, stressed mice made significantly fewer transitions than no-stress mice ($P < 0.01$) (Fig. 1B).

Table 1 Repeated footshock during the second postnatal week did not significant affect anxiety-related behaviors in non-mutant C57BL/6J mice

	No stress	Postnatal stress
Elevated plus-maze		
Time in open arms (sec)	25.9 ± 4.1	36.2 ± 5.0
Entries into open arms	3.6 ± 0.6	5.0 ± 0.5
Entries into closed arms	14.5 ± 0.8	15.0 ± 0.9
Total arm entries	18.1 ± 1.1	20.0 ± 1.1
Novel open field		
Locomotor activity (beam breaks)	2994 ± 128	3057 ± 131
Percent time spent in center	9.5 ± 1.2	11.2 ± 1.3

Postnatal footshock had no effect on the behavior of C57BL/6J mice in the elevated plus-maze or novel open field test. n = 23–28/stress condition. Data are Mean ± SEM

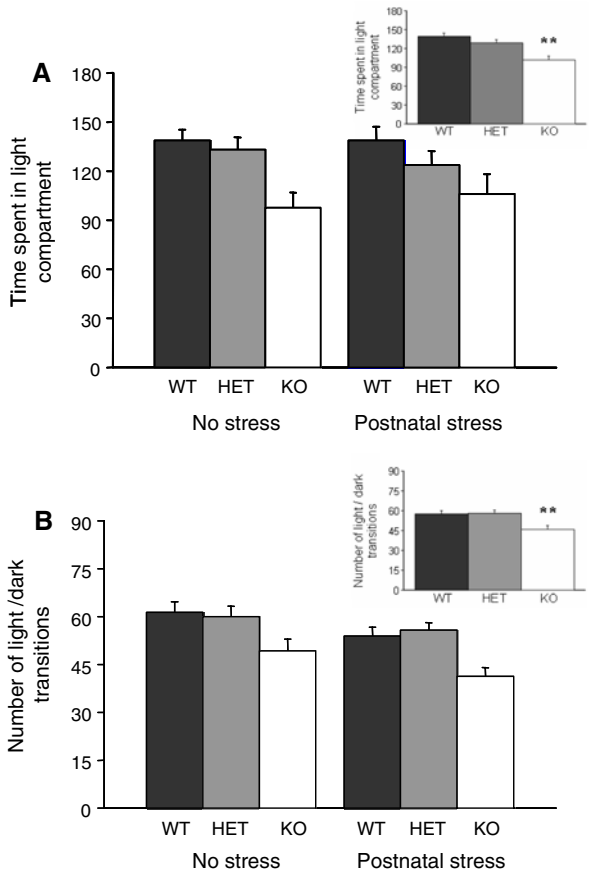

Fig. 1 5-HTT KO mice showed increased anxiety-related behavior in the light/dark exploration test regardless of postnatal stress. 5-HTT KO mice generally spent significantly less time in the light compartment (**A** and inset) and made significantly fewer light/dark transitions (**B** and inset) than WT controls. Postnatal-stressed mice made fewer light/dark transitions than no-stress controls. n = 15–44/genotype/stress condition. Data in Figs. 1–4 are Mean ± SEM

Increased anxiety-like behavior in 5-HTT KO mice in the elevated plus-maze test

There was a significant effect of genotype ($F_{2,193} = 3.34$, $P < 0.05$), but not of postnatal stress and no genotype × stress interaction on time spent in the open arms. Collapsing across genotype, post-hoc analysis showed that 5-HTT KO mice spent less time in the open arms than WT controls ($P < 0.05$) (Fig. 2A). There was a marginally significant effect of genotype ($F_{2,193} = 2.94$, $P = 0.0554$) but not of postnatal stress and no genotype

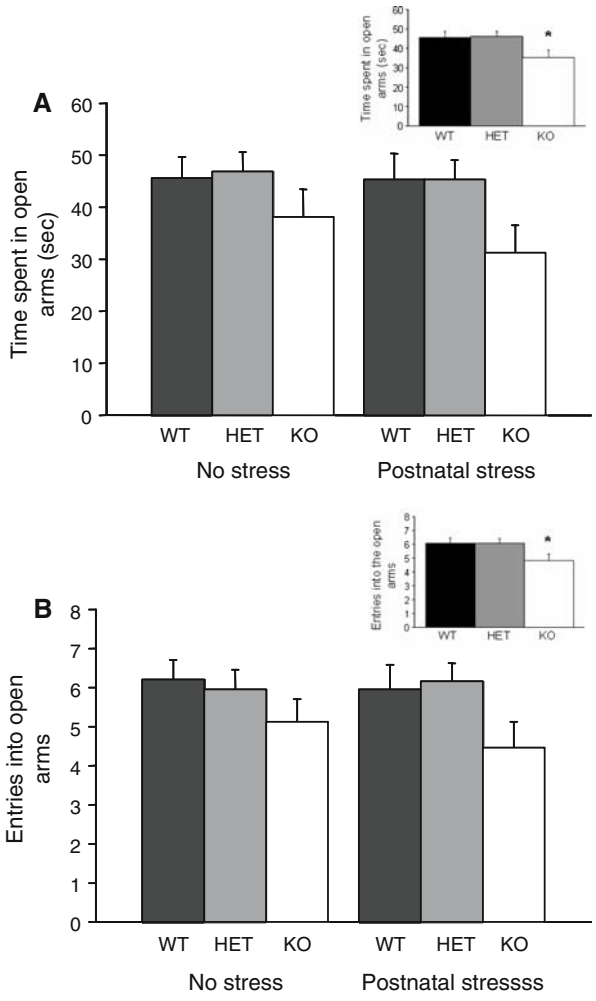

Fig. 2 5-HTT KO mice showed increased anxiety-related behavior in the elevated plus-maze regardless of postnatal stress. 5-HTT KO mice generally spent significantly less time (**A** and inset) and made significantly fewer open arm entries (**B** and inset) than WT controls. n = 15–44/genotype/stress condition

x stress interaction on open arm entries. Collapsing across stress condition, post-hoc analysis showed that 5-HTT KO mice made significantly fewer open arm entries than WT controls ($P < 0.05$) (Fig. 2B). There was a significant main effect of genotype on closed arm entries ($F_{2,193} = 5.83$, $P < 0.01$) and total arm entries ($F_{2,193} = 7.85$, $P < 0.01$) but no effect of postnatal stress and no genotype × stress interaction for either measure. Collapsing across stress condition, post-hoc analysis showed that 5-HTT KO mice made significantly fewer closed arm entries (WT = 15.1 ± 0.4, HET = 15.3 ± 0.6, KO = 13.8 ± 0.7; postnatal stress WT = 15.9 ± 0.7, HET = 13.6 ± 0.5, KO = 12.6 ± 1.0) and total arm entries (WT = 21.3 ± 0.5, HET = 21.2 ± 0.8, KO = 18.9 ± 1.0; postnatal stress WT = 21.9 ± 0.8, HET = 19.8 ± 0.7, KO = 17.1 ± 1.4) than WT controls.

Increased anxiety-like behavior in 5-HTT KO mice in the novel open field

There was a significant main effect of postnatal stress ($F_{2,151} = 4.48$, $P < 0.05$) but not genotype and no stress x genotype interaction on horizontal activity. Collapsing across genotype, post-hoc analysis found no significant difference between stressed and no stress mice (Fig. 3A). There was a significant effect of genotype ($F_{2,161} = 5.91$, $P < 0.01$) but not stress and no genotype x stress interaction on percent time spent in the center of the open field. Collapsing across stress condition, post-hoc analysis showed that 5-HTT KO mice spent less time in the center than WT controls ($P < 0.05$) (Fig. 3B).

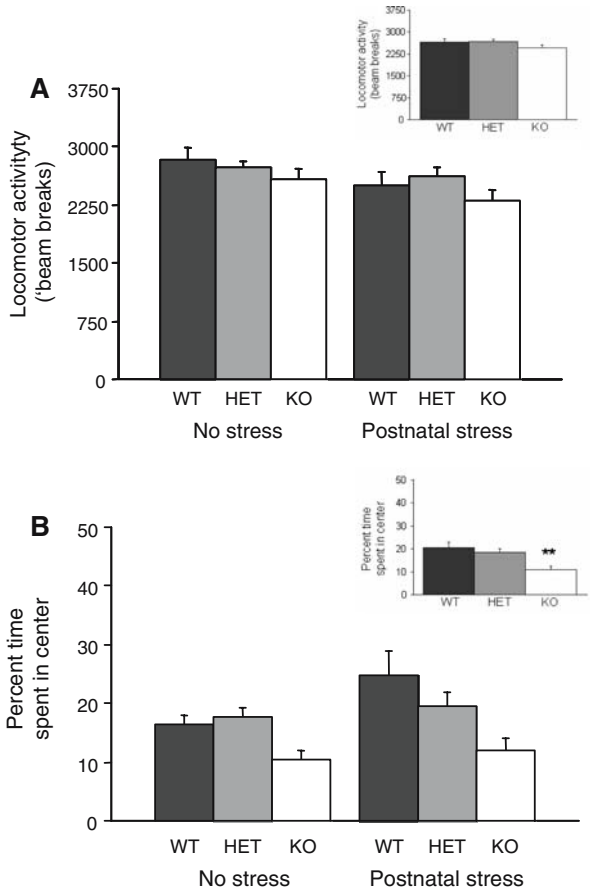

Fig. 3 5-HTT KO mice showed increased anxiety-related behavior in the novel open field test regardless of postnatal stress. 5-HTT KO mice showed similar levels of locomotor activity (**A** and inset) but generally spent significantly less time in the center (**B** and inset) than WT controls. Postnatal-stressed mice exhibited lesser locomotor activity than no-stress controls. n = 15–44/genotype/stress condition

Increased depression-like behavior in 5-HTT KO mice with repeated exposure to the FST

There was no effect of genotype or postnatal stress and no genotype x stress interaction on immobility during the first FST exposure (Fig. 4A). During the second FST exposure, there was a highly significant main effect of genotype ($F_{2,141} = 7.26$, $P < 0.01$), but not postnatal stress and no genotype × stress interaction, on immobility. Collapsing across stress conditions, post-hoc analysis showed significantly higher immobility in 5-HTT KO mice than WT controls during the second exposure ($P < 0.01$) (Fig. 4B).

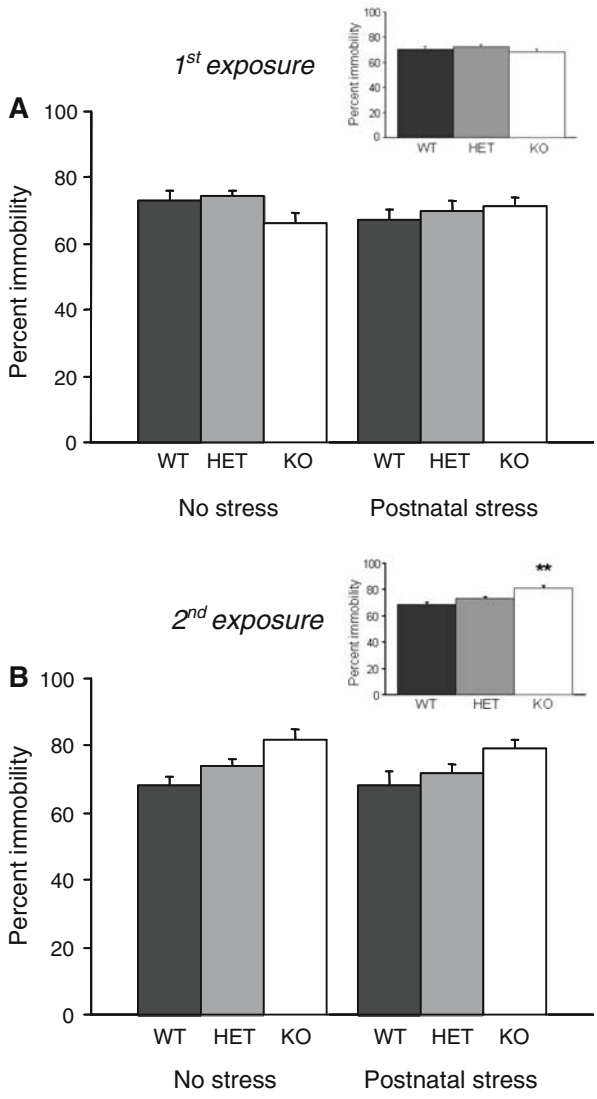

Fig. 4 5-HTT KO mice showed increased depression-related behavior with repeated exposure to the forced swim test (FST) regardless of postnatal stress. 5-HTT KO mice showed similar levels of immobility as WT controls during first exposure to the FST (**A** and inset). Generally, 5-HTT KO mice showed significantly higher levels of immobility than WT controls during the second exposure to the FST (**B** and inset). n = 14–33/genotype/stress condition

Discussion

Abnormal stress-coping and increased anxiety-like behaviors in 5-HTT KO mice

Previous studies have found phenotypic abnormalities in some 5-HTT KO lines that are consistent with increased 'depression-related' behavior and exaggerated neuroendocrine and catecholamine responses to stress (Holmes et al. 2002b; Tjurmina et al. 2002; Lira et al. 2003; Li et al. 2004). Recent findings have extended these data by showing that 5-HTT KO mice display a selective increase in depression-related behaviors in the tail suspension test (TST). Thus, while 5-HTT KO mice showed normal levels of immobility on first exposure, depression-related immobility was significantly elevated as compared to WT controls during subsequent exposures to the test (Zhao et al. 2006). Present findings provide a further replication of these data. 5-HTT KO mice exhibited significantly elevated levels of immobility during a second, but not first, exposure to the FST relative to WT controls. Increased immobility in the FST is hypothesized to represent a 'despair-like' response, in which the animal disengages from active forms of coping in manner that may have presumed relevance to symptoms of hopelessness and entrapment observed in depressed individuals (Porsolt 2000; Cryan and Holmes 2005). In this context, the profile in 5-HTT KO mice would be consistent with impaired stress-coping or 'learned helplessness' behavior following repeated, but not acute, exposure to stress.

Interestingly, previous studies from our laboratory and others have shown that 5-HTT KO mice exhibit altered behaviors consistent with increased depression-related behavior on *acute* exposure to the FST or TST (Holmes et al. 2002b; Lira et al. 2003; Zhao et al. 2006). There are numerous examples of behavioral phenotypes in mutant mice that are influenced by genetic background (Crabbe 2001; Nadeau 2001). In this context, in studies reporting increased immobility in 5-HTT KO mice during acute testing, the mutation was on a 129S6 genetic background, whereas increased depression-related behavior following repeated exposure, as in the present study, has been observed in C57BL/6J-background 5-HTT KO mice. These epistatic interactions between the 5-HTT mutation and genetic background related effects have been observed under the same test conditions, excluding the possibility that they

stem from methodological differences between studies (Holmes et al. 2002b). Thus, genetic background appears to determine sensitivity to the effects of stress in 5-HTT KO mice, with C57BL/6J alleles conferring relative protection against the 'depression-related' effects of the 5-HTT null mutation.

5-HTT KO mice exhibited robust and consistent behavioral abnormalities on three separate tests for anxiety-like behaviors; light/dark exploration test, elevated plus-maze and novel open field test. These findings replicate previous studies showing increased anxiety-like behavior in four independent lines of 5-HTT KO mice assessed on this and other tests (Hariri and Holmes 2006) and add to recent data showing abnormally high anxiety-like responses in 5-HTT KO mice following exposure to predator stress (i.e., cat odor) (Adamec et al. 2006).

Notably, 5-HTT KO mice exhibited heightened avoidance of the open arms of the elevated plus-maze as well as a general suppression of exploratory behavior in the test (i.e., reduced closed and total arm entries), as compared to WT controls. Such a profile could result from either a profound anxiety-like phenotype that reduces exploratory behavior *per se* or a non-specific reduction in locomotor behavior (Holmes 2001). In this context, 5-HTT KO mice exhibit reduced locomotor activity in the non-aversive context of the home cage (Holmes et al. 2002a). While a potentially confounding influence of this phenotype on measurement of anxiety-related behaviors cannot be fully discounted, it is unlikely given that 5-HTT KO mice do not consistently show suppression of exploratory behavior in tests that are less anxiety-provoking than the elevated plus-maze (Holmes et al. 2003; Ansorge et al. 2004) (e.g., novel open field test in the present study), or under conditions where the stress of testing is reduced by repeated handling (Adamec et al. 2006). Moreover, 5-HTT KO mice exhibit a broad abnormal emotion-related phenotype, including impaired stress-coping and exaggerated neuroendocrine and catecholamine responses discussed above, that cannot be explained by a locomotor deficit. Notwithstanding, it would be informative for future studies to test 5-HTT KO mice on reliable tests for anxiety-like behavior that do not contain a major motor component.

5-HTT KO mice do not show increased vulnerability to a mild early life stressor

The present study tested the hypothesis that 5-HTT HET or KO mice would be vulnerable to the effects of early life stress. Repeated exposure to footshock during the second postnatal week did not lead to increases in adult anxiety-like behavior in the elevated plus-maze or novel open field test in non-mutant C57BL/6J mice. An advantage of using a mild stressor to test for increased vulnerability in 5-HTT KO mice is that it does not produce a high level of anxiety- or depression-related behavior that would preclude the detection of an effect in the mutants (i.e., 'a ceiling effect'). However, the consistent finding across four tests of anxiety- and depression-related behavior used was that postnatal stress did not further modify the phenotypes shown by no stress 5-HTT KO mice. Although, as discussed, 5-HTT KO mice exhibited abnormalities in these tests in the absence of stress, it did not appear that a ceiling level of anxiety- or depression-related behavior occluded the detection of further increases due to stress. For example, no stress 5-HTT KO mice spent ~90 s in the light compartment in the light/dark exploration test and ~40 s in the open arms of the elevated plus-maze; levels that are well above floor. 5-HTT HET mice, which were no different from WT controls under no stress conditions, also failed to show an effect of postnatal stress.

Taken together, these data indicate that 5-HTT mutant mice were not vulnerable to the effects of footshock stress. This does not exclude the possibility that significant interactions between 5-HTT genotype and postnatal footshock stress would be evident on other, perhaps more challenging, phenotypic measures. Alternatively, this form of stress may not have been sufficiently potent to exert lasting influences on behavior in these mice. While footshock stress produced flight-like locomotion and audible vocalizations indicative of distress in pups, measurement of hypothalamic-adrenal-pituitary axis activation would help determine the strength of this stressor. Notwithstanding, previous studies have shown that various forms of postnatal stress, including maternal separation and saline injection, can produce lasting changes in anxiety-related behaviors and stress-reactivity in rodents (although there is currently a larger literature in rats than mice) (Lehmann and Feldon 2000; de Kloet et al. 2005; Holmes et al. 2005), and it is possible that 5-HTT null mutant mice would be vulnerable to these or other forms of postnatal stress. A final consideration is whether there might be specific stress-hypersensitive developmental periods which were missed in the current study. Further studies would serve to delineate these various, potentially critical factors.

Conclusions

The results of the present study provide a further demonstration of anxiety- and depression-related

abnormalities in 5-HTT KO mice. 5-HTT KO mice exhibited increased anxiety-like behavior on three separate tests, as well as increased depression-like behavior following repeated, but not acute, testing on the forced swim test. However, exposure to repeated footshock during the second postnatal week failed to further modify these phenotypes and had little effect on anxiety- and depression-related behaviors.

As noted in the Introduction, heightened anxiety-like behavior and impaired stress-coping in 5-HTT KO mice have interesting parallels with emerging data in humans and non-human primates. A low-functioning polymorphic variant (*S* allele) of the human 5-HTT gene is associated with increased trait anxiety and increased risk for depression and suicide (Hariri and Holmes 2006). Importantly, these effects are most clearly manifested following adverse environmental conditions such as repeated stress. Similarly, the *Rhesus macaque* orthologue of the 5-HTT polymorphism also leads to abnormal emotional reactivity, but only in monkeys that were reared under stressful environmental conditions. These converging findings provide an interesting parallel with the increased depression-like behavior shown by 5-HTT KO mice following repeated, but not acute, swim stress, and support the hypothesis that, across species, genetic disruptions to 5-HTT function impairs the organism's ability to effectively deal with stress. 5-HTT mutant mice will provide a valuable model to further test this hypothesis and, ultimately, to delineate the neural basis of this genetic influence.

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