

Increased anxiety of mice lacking the serotonin_{1A} receptor

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ABSTRACT Brain serotonin (5-HT) has been implicated in a number of physiological processes and pathological conditions. These effects are mediated by at least 14 different 5-HT receptors. We have inactivated the gene encoding the 5-HT_{1A} receptor in mice and found that receptor-deficient animals have an increased tendency to avoid a novel and fearful environment and to escape a stressful situation, behaviors consistent with an increased anxiety and stress response. Based on the role of the 5-HT_{1A} receptor in the feedback regulation of the 5-HT system, we hypothesize that an increased serotonergic neurotransmission is responsible for the anxiety-like behavior of receptor-deficient animals. This view is consistent with earlier studies showing that pharmacological activation of the 5-HT system is anxiogenic in animal models and also in humans.

Brain serotonin (5-HT) is implicated in the control of a wide variety of physiological processes such as nociception, cardiovascular function, and thermoregulation, as well as in different behavioral processes including feeding, aggression, and response to stress (reviewed in refs. 1–5). The 5-HT system also appears to be involved in the etiology of neuropsychiatric disorders such as depression and anxiety (reviewed in refs. 6 and 7). In recent years, our understanding of the physiological and pathological aspects of the 5-HT system has benefited from the identification, classification, and more recently the cloning of the 5-HT receptor subtypes (8). Among the receptor subtypes that have received the most attention is the 5-HT_{1A} receptor (5-HT_{1A}R). This was because of the availability of 5-HT_{1A}R agonists and the implication of the 5-HT_{1A} receptor in anxiety (6, 9).

The 5-HT_{1A}R, like most of the 5-HT receptors, belongs to the superfamily of G-protein coupled receptors (10, 11). It is negatively coupled to adenylyl cyclase. Brain 5-HT_{1A}R is located both pre- and postsynaptically. Presynaptic 5-HT_{1A}R is found mainly in the dorsal and median raphe nuclei. Activation of these receptors by agonists causes a reduction in the firing rate of serotonergic neurons (12–14) and leads to the suppression of 5-HT synthesis, 5-HT turnover, and 5-HT release in the diverse projection areas (15, 16). Postsynaptic 5-HT_{1A}R is found in limbic regions (such as hippocampus and septum) and in some cortical layers. As in the case of presynaptic receptors, activation of postsynaptic 5-HT_{1A}R is generally believed to induce a decrease in the firing rate of the postsynaptic cell (14).

The 5-HT_{1A}R has been extensively studied by pharmacological methods. Activation of the receptor by agonists results in an anxiolytic effect (17, 18). Correlations were found among the time and dose dependency of the anxiolytic effect, the inhibition of serotonergic firing in the dorsal raphe nuclei, and the inhibition of 5-HT release after systemic administration of agonists (19, 20). The 5-HT_{1A}R partial agonist buspirone and a series of congeners also produce this neurochemical effect

and are used clinically for the treatment of anxiety (9). Based on these findings, it has been proposed that 5-HT_{1A}R agonists stimulate presynaptic receptors, which inhibit 5-HT release and consequently reduce 5-HT signaling at a multitude of diverse target receptors (18). These may include 5-HT_{2A}, 5-HT_{2C}, and 5-HT₃ receptors, because selective antagonists acting on these receptors have been suggested to be anxiolytics (18, 21). Because of the role of presynaptic 5-HT_{1A}R in regulating the 5-HT system, blockade of the receptor by antagonists is expected to increase central 5-HT function. Indeed, electrophysiological studies showed that the 5-HT_{1A}R antagonist WAY-100635 induced an increased firing of serotonergic neurons (22). However, microdialysis studies showed no detectable increase of 5-HT neurotransmission by 5-HT_{1A}R antagonists under normal conditions (23). When administered with selective 5-HT reuptake inhibitors, 5-HT_{1A}R antagonists augment 5-HT levels in terminal regions (24). At the behavioral level, the effect of 5-HT_{1A}R antagonists is controversial. Antagonists, such as pindolol, exhibit a biphasic dose-response curve with an initial anxiolytic effect that converts to an anxiogenic effect at high doses (25, 26). However, pindolol is also an adrenoceptor antagonist, and this activity may confound the drug's effect on the 5-HT_{1A}R (27). The more selective 5-HT_{1A}R antagonist, WAY-100635, induced anxiolytic-like effects in the light/dark box anxiety model with no anxiogenic effect at high doses (28).

Because buspirone is a clinically effective antidepressant, the 5-HT_{1A}R has also been associated with depression (9). Depressed patients show blunted HPA (hypothalamic-pituitary-adrenal)-axis responses following 5-HT_{1A}R agonist challenge, which can be interpreted as downregulation or hyporesponsiveness of postsynaptic 5-HT_{1A}R (29).

Because the 5-HT_{1A}R has been associated with anxiety and depression, receptor-deficient mutant mice could provide important information on predisposition toward these conditions. Here we show that mice lacking the 5-HT_{1A}R avoid a novel and fearful environment and vigorously attempt to escape stressful situations, behavior consistent with an increased anxiety and stress response.

MATERIALS AND METHODS

Generation of 5-HT_{1A}R-Deficient Animals. The gene for the 5-HT_{1A}R was cloned from a BALB/c mouse genomic library (30). Construction of the targeting vector (Fig. 1A), electroporation of the targeting vector into E14 embryonic stem (ES) cells, and selection of the targeted clones were carried out by standard procedures (31). Correctly targeted cell clones were identified by a nested PCR assay (32) that could amplify a fragment from the substituted allele but not a fragment from the wild-type allele or targeting vector. The PCR primers were

Abbreviations: 5-HT, serotonin (5-hydroxytryptamine); 5-HT_{1A}R, 5-HT_{1A} receptor; ES, embryonic stem; 8-OH-DPAT, 8-hydroxy-*N*,*N*-dipropyl-2-amino-1,2,3,4-tetrahydronaphthalene.

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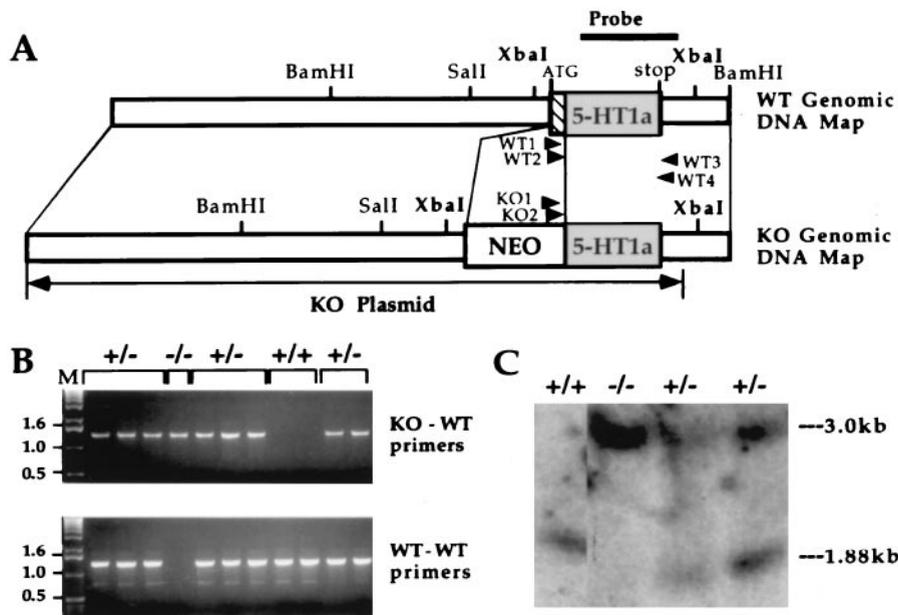


FIG. 1. Genetic inactivation of the 5-HT_{1A}R gene in ES cells and mice. (A) Genomic structure and restriction map of the 5-HT_{1A}R locus (WT Genomic) and the targeting vector (KO Genomic). Primers used for PCR (wild-type 1–4 and KO 1, 2) and a probe employed in Southern experiments are depicted by arrowheads and a bar, respectively. (B) PCR screening of progeny derived from matings of heterozygote animals. (Upper) The presence of an amplified product derived from the substituted allele in heterozygote (+/–) and homozygote (–/–) mice. (Lower) PCR products derived from the wild-type allele in wild-type (+/+) and heterozygote animals. Size markers are in lane M. (C) Southern blot analysis of the wild-type receptor-specific *Xba*I fragment. The size of this fragment is increased from 1.88 to 3.0 kb by the substitution mutation.

located in the 3' noncoding regions of the heterologous neomycin resistance gene (KO primers 1 and 2) and the endogenous receptor gene (wild-type primers 3 and 4). The first 20 cycles of PCR were performed with KO primer 1 (5'-GACCGCTATCAGGACATAGCG-3') and wild-type primer 3 (5'-TCTTAGGTGTTGCTTCCAGGG-3'). KO primer 2 (5'-ACGGTATCGCCGCTCCCGATTC-3) and wild-type primer 4 (5'-CCCTGTAAGCCCTACACTCTT-3') were used for the next 30 cycles. The wild-type allele was detected by a similar procedure except that KO primers 1 and 2 were replaced by wild-type primers 1 (5'-ATGGATATGTTCAGTCTTGGC-3') and 2 (5'-CAGGGCAACAACCAACAACG-3'), both corresponding to the 5' coding region of the receptor gene absent in the knockout allele. The wild-type primers could amplify a fragment only from the wild-type allele. Chimeras were generated by aggregation (33) from two ES cell lines (designated C9W and D8M). Two independent knockout (5-HT_{1A}R⁻) mouse lines were established from these clones. Because the 129^{SV} genetic background (the standard background for knockouts) is not particularly suitable for behavioral testing, chimeras were backcrossed to Swiss-Webster mice to obtain heterozygotes. These F₁ animals were crossbred to produce homozygous F₂ mutants. Control non-chimeric littermates were similarly bred to control for a disequilibrium of genes that are linked to the mutation (34). F₂ progeny with two wild-type 129^{SV} 5-HT_{1A}R alleles were selected by single-strand length polymorphism. Single-strand length polymorphism analysis was based on the closest marker (D13MIT193, located 5.1 centimorgans from the 5-HT_{1A}R locus) (35) that showed a difference between the two genotypes. D13MIT193-specific primers (Research Genetics, Huntsville, AL) were used in standard PCR reactions and separated on 4% agarose gels. Amplified bands were approximately 130 and 110 bp long in Swiss-Webster and 129^{SV} mice, respectively.

Receptor Autoradiography. Brains were removed and kept at -80°C until assayed. Coronal sections were cut with a microtome cryostat at four neuroanatomical levels through the raphe, amygdala, septal nuclei, and, most anteriorly, through

the nucleus accumbens and prefrontal cortex. Consecutive superimposable sections were used to determine total and nonspecific binding. The 5-HT_{1A}R was labeled in the presence of 2 nM ³H-labeled 8-hydroxy-*N,N*-dipropyl-2-amino-1,2,3,4-tetrahydronaphthalene ([³H]-8-OH-DPAT; NEN catalog no. NET-929) as described (36). Nonspecific binding was determined in the presence of 1 μM 5-HT. Both C9W and D8M cell-derived mice were investigated by autoradiography. Sections were exposed to Hyperfilm (Amersham) for 2 weeks.

Behavioral Studies. The open field test (37) used a black box (38.1 cm × 53.3 cm), divided into 12 even-sized rectangles (7.6 cm × 10.2 cm). The total number of crosses in the open field was recorded for 10 min to measure the locomotor activity. The time spent in and number of entries into the two rectangles at the center of the field were recorded to evaluate anxiety. In the forced swim test, mice were forced to swim in a clear, water-filled cylinder (diameter, 20.3 cm; depth, 10 cm), essentially as described by Porsolt *et al.* (38). In this test, mobility of the mice is measured in blocks of 2 min for a total of 6 min. For the rotarod test, animals were placed on a rotating bar (five turns per minute) and the time spent on the rod without falling was recorded (39). The best time in three trials was used for each mouse. Statistical significance was calculated by the independent *t* test. In all experiments, data for males and females were analyzed separately. Mice derived from C9W ES cells were used in all behavioral tests, but both 5-HT_{1A}R⁻ mouse lines were evaluated in the open field test. Mice were cared for in accordance with institutional guidelines.

RESULTS

Analysis of the Knockout Genotype. A gene targeting vector was constructed by substituting a transcriptionally active neomycin resistance gene cassette for the 5'-coding region of the 5-HT_{1A}R gene (Fig. 1A). Homologous recombination between the targeting construct and the receptor allele resulted in a deletion that included the initiation codon and the first 123 bp of the coding sequence, inactivating the 5-HT_{1A}R gene. Initially, a PCR assay that was specific for the knockout allele was

used to identify animals carrying the disrupted receptor allele (Fig. 1*B Upper*). A wild-type-specific PCR assay differentiated between the homozygous and heterozygous mutant animals (Fig. 1*B Lower*). The disruption of the receptor allele was confirmed by Southern blot (Fig. 1*C*). In wild-type animals, the receptor-specific probe (Fig. 1*A*) recognized an *Xba*I fragment (1.88 kb) that contained the entire coding region (1.27 kb), a section of the 5' untranslated region (0.12 kb), and a portion of the 3' untranslated region (0.49 kb). The *Xba*I fragment in the homozygous 5-HT_{1A}R⁻ animals migrated relative to markers at about 3 kb, the sum of the wild-type allele (1.88 kb) and the neomycin resistance gene cassette (1.10 kb). As expected, heterozygote animals contained both the wild-type and the disrupted alleles.

The loss of the receptor at the protein level was demonstrated by receptor autoradiography using [³H]-8-OH-DPAT, a selective 5-HT_{1A}R ligand (Fig. 2). In wild-type animals, a strong signal was detected in the hippocampus, and somewhat less activity was found in the cortex (Fig. 2*A Upper Left*). The receptor was also detected in the raphe nuclei (Fig. 2*B Upper Left*). No significant binding was measured in the presence of 1 μM 5-HT in these regions, demonstrating a low nonspecific binding of [³H]-8-OH-DPAT (Fig. 2*A and B Lower Left*). Homozygotes showed no specific binding in either region confirming the inactivation of the 5-HT_{1A}R gene (Fig. 2*A and B Upper Right*), and heterozygotes showed an intermediate level of binding (Fig. 2*A and B Upper Middle*).

Behavioral Analysis. The 5-HT_{1A}R gene was disrupted in two 129^{sv}-derived ES cell clones (C9W and D8M). Chimeric animals were generated from each of the two mutant ES cell lines. For behavioral analysis, the chimeras were crossed to Swiss-Webster mice to produce F₁ heterozygotes that were then crossbred to produce homozygous F₂ mutants. To control for disequilibrium of genes linked to the mutation (34, 40), wild-type 129^{sv} mice were similarly bred to produce F₂ animals

with the 129^{sv}-derived 5-HT_{1A}R locus at both alleles and a distribution of linked genes similar to that in the mutant F₂ population. The two independent lines of homozygous mutant animals exhibited normal weight gain, fertility, and survival (data not shown).

Pharmacological data indicating that the 5-HT_{1A}R plays a role in anxiety (6) prompted us to compare the homozygous mutant and wild-type animals in an anxiety-related behavioral paradigm. Mice fear open space and avoid the center of the open field apparatus. The extent of anxiety can be determined by counting the number of entries by a test animal into the open field and by measuring the time spent in the center of the open field. Fig. 3*A* shows that 5-HT_{1A}R⁻ males entered into the center approximately three times less frequently ($P < 0.05$) than wild-type males (Fig. 3*A*). The overall locomotor activities of mutant and wild-type males were not statistically different in the open field test [143.7 ± 16.9 crosses (mean \pm SD) and 162.1 ± 9.6 crosses, respectively]. In addition to the reduced number of entries into the center, receptor-deficient males spent approximately 4-fold less time in the center of the open field ($P < 0.05$) as compared with wild-type males (Fig. 3*A*). When normalized to the number of entries into the center of the open field, time spent in the center (time per cross) was still less in the mutant group (Fig. 3*A*). These changes in open field behavior were also observed in similar experiments with another 5-HT_{1A}R⁻ mouse line derived from an independent ES cell clone (data not shown). Lack of the 5-HT_{1A}R also resulted in anxiety in females, but the effect was less pronounced than in males. 5-HT_{1A}R⁻ females entered into the center of the open field, determined as the percent of total crosses, two times less frequently ($P < 0.05$) than wild-type females (Fig. 3*B*). However, this difference was found only after normalization to total crosses, because mutant females showed an increased locomotor activity compared with wild-type females (239.5 ± 28.8 and 151.4 ± 18.4 crosses, respec-

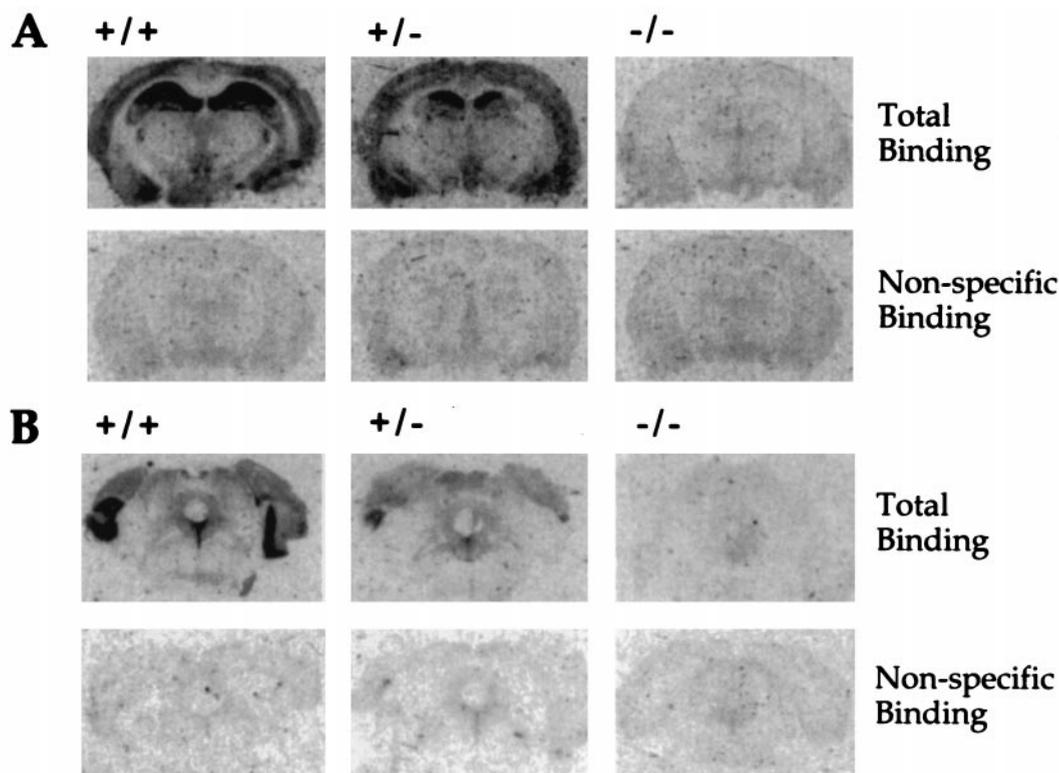


FIG. 2. 5-HT_{1A}R density of wild-type (+/+), heterozygote (+/-), and homozygote (-/-) animals in coronal sections of the hippocampus and cortex (*A*) and sections of the dorsal raphe nuclei (*B*). Receptors were labeled by the binding of [³H]-8-OH-DPAT. Nonspecific binding was determined in the presence of 5-HT. Three mice from each group were studied in this experiment, and sections from one is displayed.

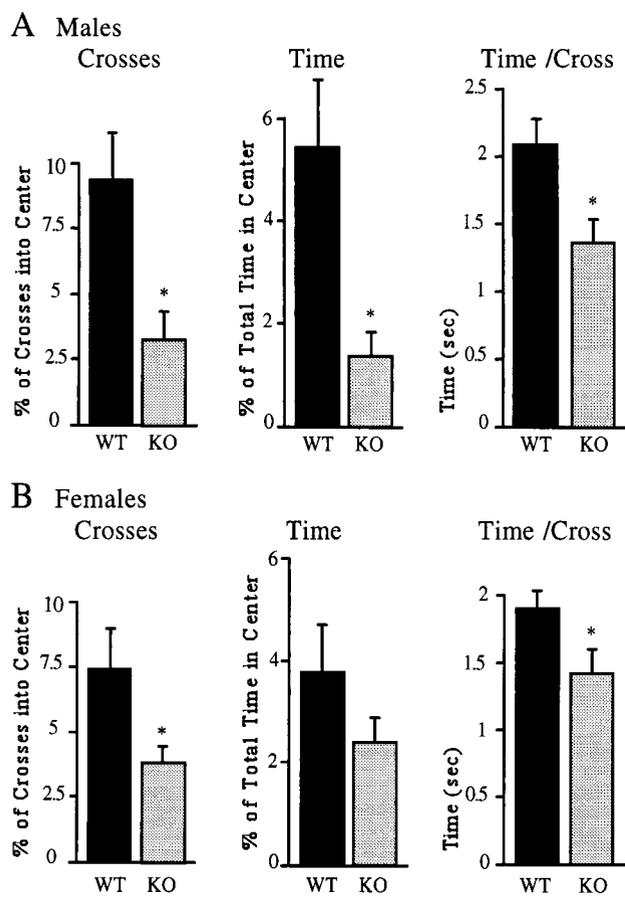


FIG. 3. Open field test of wild-type and 5-HT_{1A}R^{-/-} males (A) and females (B). Fifteen wild-type and homozygous males, 10 wild-type females, and 12 heterozygous females were studied. The number of crosses into the center was normalized to locomotor activity and expressed as the percent of total crosses. Time spent in the center of the test apparatus is expressed as the percent of total time (10 min). Time per cross indicates the average time spent in the center of the open field for each entry. Asterisks designate statistically significant differences ($P < 0.05$) for the mutant as compared with the wild-type group.

tively). When normalized to the number of entries into the center, the time spent in the center (time per cross) was significantly reduced in the group of mutant females as compared with wild-type female mice (Fig. 3B).

In addition to anxiety, the 5-HT_{1A}R also appears to play a role in the stress response (41). Stress can be elicited by a number of stimuli including forced swim (42, 43). Mice, when placed into a water-filled tank, show a stress-induced increase in mobility that can be quantified by measuring swimming and climbing activity. With habituation, mice become progressively less mobile during the 6 min test (Fig. 4). The stressful nature of forced swim is demonstrated by the activation of the hypothalamic-pituitary-adrenal axis (44) and induction of the immediate-early gene *c-fos* in subcortical nuclei (lateral septal nucleus, bed nucleus of the stria terminalis, and hypothalamic and thalamic paraventricular nuclei) (45). As shown in Fig. 4, we tested whether receptor-deficient animals exhibit an altered response to swim stress. Interestingly, both 5-HT_{1A}R^{-/-} males and females were significantly more mobile as compared with wild-type controls. No gender difference was observed in this test.

Performance in the rotarod apparatus, a test for motor and spatial coordination (46), showed no difference between mutant and wild-type animals. Fall latencies in wild-type and 5-HT_{1A}R^{-/-} males were 16.9 ± 5.8 and 16.9 ± 5.0 sec, respec-

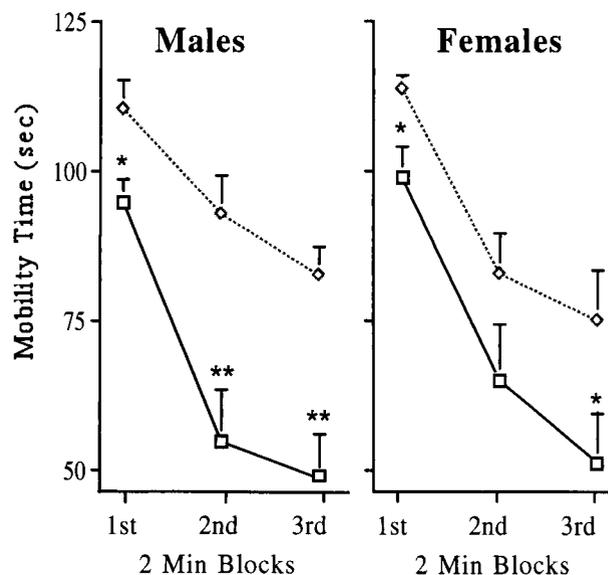


FIG. 4. Mobility in the forced swim test of wild-type and 5-HT_{1A}R^{-/-} mice. Each group consisted of 8–10 mice. The mobility of animals was measured in seconds between 0–2 min (1st block), 2–4 min (2nd block), and 4–6 min (3rd block) of the test. (□), Wild-type animals; (◇), 5-HT_{1A}R^{-/-} mice. Asterisks designate statistically significant differences (*, $P < 0.05$ and **, $P < 0.005$) for the mutant as compared with wild-type group.

tively; fall latencies in wild-type and 5-HT_{1A}R^{-/-} females were 16.3 ± 3.1 and 19.9 ± 4.3 sec, respectively.

DISCUSSION

The major finding of this study is that mice lacking the 5-HT_{1A}R display enhanced anxiety and an increased response to stress. These mutant animals grow and reproduce normally, so we assume that their somatic and sexual development is normal. The absence of major developmental abnormalities in the receptor-deficient animals suggests that either the receptor does not play an essential role during development or that the receptor loss is compensated for by redundant functions during development. In either case, pharmacological studies support the view that the receptor deficiency in adult animals is responsible for the behavioral abnormalities that we have observed (Figs. 3 and 4). The anxiety level of test subjects can be modified by both 5-HT_{1A}R agonists and antagonists. Agonists are generally anxiolytics, whereas antagonists can have an anxiogenic effect (18, 26). However, there are exceptions, and not all antagonists induce anxiety (25). The difference between antagonists is likely because of variations in selectivity, distribution, and potency. Based on the fact that many antagonists are anxiogenic and that the mutant mice exhibit increased anxiety, we favor the hypothesis that the behavior of the mutant animals is because of the absence of the 5-HT_{1A}R in the adult animals. However, it will be necessary to produce conditionally deficient animals to exclude conclusively the possibility that a developmental defect contributes to the behavioral abnormalities that we have observed.

How can loss of the 5-HT_{1A}R lead to increased anxiety? It is known that the 5-HT_{1A}R is involved in the regulation of 5-HT release, either directly as is the case for presynaptic autoreceptors in the midbrain serotonergic nuclei or indirectly as for postsynaptic receptors in the hippocampus (18). Indeed, stimulation of the presynaptic receptors by an agonist results in an inhibition of the firing rate of the serotonergic neurons and suppression of 5-HT synthesis, as well as a reduction in 5-HT turnover and 5-HT release (12–16). Pharmacological inhibition of the 5-HT_{1A}R, presumably by disrupting the

inhibitory feedback, results in an increased activation of serotonergic neurons in the dorsal raphe nuclei that leads to an increased 5-HT turnover (22). Mutant animals are predicted to lack the inhibitory feedback because of the loss of the 5-HT_{1A}R in the dorsal raphe nuclei (Fig. 2B), and the altered behavioral responses of these mice could be caused by the absence of critical presynaptic regulation of 5-HT release, causing 5-HT to be augmented in stressful situations. The absence of postsynaptic 5-HT_{1A}R may also contribute to the altered behavior. Finally, the 5-HT_{1A}R likely interacts with other neurotransmitters, such as dopamine and norepinephrine. The absence of 5-HT_{1A}R could contribute to the altered behavioral responses of mutant mice, especially if compensation develops in the function of these neurotransmitter systems. The hypothesis that a hyperactive serotonergic system causes the anxiety-like phenotype in the mutant animals is supported further by the notion that increased serotonergic neurotransmission is anxiogenic in animal models as well as in humans (47–51). This hypothesis can be tested by measuring 5-HT turnover and extracellular 5-HT levels at baseline and after stress in the receptor-deficient animals.

Although 5-HT_{1A}R⁻ males and females showed a similar phenotype, anxiety was less prominent in female animals (Fig. 3). Also, mutant females but not males showed an increased locomotor activity in the open field test. The cause of this gender difference is not known, but differences in response to serotonergic drugs of males and females have been observed (52).

In addition to anxiety, 5-HT_{1A}R⁻ animals demonstrated an increased mobility in the forced swim test (Fig. 4). This might reflect the involvement of increased anxiety and emotional reactivity when mutant mice are exposed to inescapable stress. Alternatively, the behavior might reflect increased resistance to the development of behaviors associated with depression and antidepressants. Elevated serotonergic neurotransmission in the mutant animals could explain the changes in response in the forced swim test because stimulation of the 5-HT system increases the mobility of mice. For example, injection of the 5-HT precursor tryptophan increased the time that mice spent swimming in the forced swim test (53).

Besides the 5-HT_{1A}R⁻ mice, there are only a few rodent strains that display increased anxiety. The Maudsley reactive and nonreactive inbred rat strains were created as an animal model of anxiety by selective breeding. The Maudsley reactive strain shows a stable and reproducible deficit in an open field compared with the Maudsley nonreactive strain (54). Similar differences in anxiety behavior have been described between recombinant inbred strains of mice (55). Transgenic mice are proving to be useful models for exploration of the pathogenesis of anxiety. For example, the neuronal mechanism involved in anxiety, induced by corticotropin-releasing factor, was studied with corticotropin-releasing factor-overproducing transgenic mice (56). The involvement of enkephalins in the modulation of anxiety has been discovered in a preproenkephalin knock-out mouse strain (57). The 5-HT_{1A}R⁻ mice might prove to be useful in studies probing the involvement of the 5-HT system in anxiety-like behavior and anxiety disorders.

Our results raise the possibility that disturbances in the 5-HT_{1A}R and/or its downstream signaling pathway could contribute to the development of certain forms of anxiety disorders. This notion is consistent with the well-established role of the 5-HT_{1A}R in controlling 5-HT levels, the association between high 5-HT levels and anxiety, and the anxiolytic properties of partial receptor agonists. An allelic variation in the human 5-HT_{1A}R has been recently identified (58), but it is not yet known whether this polymorphism is functionally relevant.

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- Jacobs, B. L., Wilkinson, L. O. & Fornal, C. A. (1990) *Neuropsychopharmacology* **3**, 473–479.
- Leysen, J. E. (1990) *Neuropsychopharmacology* **3**, 361–369.
- Fuller, R. W. (1990) *Neuropsychopharmacology* **3**, 495–502.
- Buhot, M. C. (1997) *Curr. Opin. Neurobiol.* **7**, 243–254.
- Weiger, W. A. (1997) *Biol. Rev. Camb. Philos. Soc.* **72**, 61–95.
- Murphy, D. L. (1990) *Neuropsychopharmacology* **3**, 457–471.
- Maes, M. & Meltzer, H. (1995) in *The Serotonin Hypothesis of Depression.*, eds. Bloom, F. & Kupfer, D. (Raven, New York), pp. 933–944.
- Martin, G. R. & Humphrey, P. P. A. (1994) *Neuropharmacology* **33**, 261–273.
- Coplan, J., Wolk, S. & Klein, D. (1995) in *Anxiety and the Serotonin_{1A} Receptor*, eds. Bloom, F. & Kupfer, D. (Raven, New York), pp. 1301–1310.
- Hoyer, D. & Schoeffter, P. (1991) *J. Recept. Res.* **11**, 197–214.
- Julius, D. (1991) *Annu. Rev. Neurosci.* **14**, 335–360.
- Blier, P., de Montigny, C. & Tardif, D. (1987) *Synapse* **1**, 225–232.
- Jolas, T., Haj-Dahmane, S., Lanfumey, L., Fattacini, C. M., Kidd, E. J., Adrien, J., Gozlan, H., Guardiola-Lemaitre, B. & Hamon, M. (1993) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **347**, 453–463.
- Sprouse, J. S. & Aghajanian, G. K. (1988) *Neuropharmacology* **27**, 707–715.
- Kennett, G. A., Marcou, M., Dourish, C. T. & Curzon, G. (1987) *Eur. J. Pharmacol.* **138**, 53–60.
- Bohmker, K., Eison, A. S., Yocca, F. D. & Meller, E. (1993) *Neuropharmacology* **32**, 527–534.
- Lucki, I., Singh, A. & Kreiss, D. S. (1994) *Neurosci. Biobehav. Rev.* **18**, 85–95.
- De Vry, J. (1995) *Psychopharmacology* **121**, 1–26.
- Jolas, T., Schreiber, R., Laporte, A. M., Chastanet, M., De Vry, J., Glaser, T., Adrien, J. & Hamon, M. (1995) *J. Pharmacol. Exp. Ther.* **272**, 920–929.
- Sommermeier, H., Schreiber, R., Greuel, J. M., De Vry, J. & Glaser, T. (1993) *Eur. J. Pharmacol.* **240**, 29–37.
- Keane, P. & Soubrie, P. (1997) in *Animal Models of Integrated Serotonergic Functions: Their Predictive Value for the Clinical Applicability of Drug Interfering with Serotonergic Transmission*, eds. Baumgarten, H. & Gothert, M. (Springer, New York), pp. 707–725.
- Fornal, C. A., Metzler, C. W., Gallegos, R. A., Veasey, S. C., McCreary, A. C. & Jacobs, B. L. (1996) *J. Pharmacol. Exp. Ther.* **278**, 752–762.
- Kreiss, D. S. & Lucki, I. (1994) *J. Pharmacol. Exp. Ther.* **269**, 1268–1279.
- Hjorth, S. (1993) *J. Neurochem.* **60**, 776–779.
- Fletcher, A., Forster, E. A., Bill, D. J., Brown, G., Cliffe, I. A., Hartley, J. E., Jones, D. E., McLenachan, A., Stanhope, K. J., Critchley, D. J., *et al.* (1996) *Behav. Brain Res.* **73**, 337–353.
- Critchley, M. A. & Handley, S. L. (1987) *Psychopharmacology* **93**, 502–506.
- Sanghera, M. K., McMillen, B. A. & German, D. C. (1982) *Eur. J. Pharmacol.* **86**, 107–110.
- Sanchez, C. (1995) *Pharmacol. Toxicol.* **77**, 71–78.
- Lesch, K., Mayer, S., Disselkamp-Tietze, J., Hoh, A., Wiesmann, M., Osterheider, M. & Schulte, H. (1990) *Biol. Psychiatry* **620**–628.
- Parks, C. L. & Shenk, T. (1996) *J. Biol. Chem.* **271**, 4417–4430.
- Hasty, P. & Bradley, A. (1993) in *Gene Targeting Vectors for Mammalian Cells*, ed. Joyner, A. L. (Oxford Univ. Press, New York), pp. 1–31.
- Nitschke, L., Kopf, M. & Lamers, M. C. (1993) *BioTechniques* **14**, 914–916.
- Wood, S. A., Pascoe, W. S., Schmidt, C., Kemler, R., Evans, M. J. & Allen, N. D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4582–4585.

34. Zimmer, A. (1996) *Trends Neurosci.* **19**, 470.
35. Beckers, M. C., Ernst, E., Diez, E., Morissette, C., Gervais, F., Hunter, K., Housman, D., Yoshida, S., Skamene, E. & Gros, P. (1997) *Genomics* **39**, 254–263.
36. Watanabe, Y., Sakai, R. R., McEwen, B. S. & Mendelson, S. (1993) *Brain Res.* **615**, 87–94.
37. Crawley, J. N. (1989) *Curr. Opin. Psychiatry* **2**, 773–776.
38. Porsolt, R. D., Bertin, A. & Jalfre, M. (1977) *Arch. Int. Pharmacodyn. Ther.* **229**, 327–336.
39. Baik, J. H., Picetti, R., Saiardi, A., Thiriet, G., Dierich, A., Depaulis, A., Le Meur, M. & Borrelli, E. (1995) *Nature (London)* **377**, 424–428.
40. Gerlai, R. (1996) *Trends Neurosci.* **19**, 177–181.
41. McKittrick, C. R., Blanchard, D. C., Blanchard, R. J., McEwen, B. S. & Sakai, R. R. (1995) *Biol. Psychiatry* **37**, 383–393.
42. Prince, C. R. & Anisman, H. (1990) *Pharmacol. Biochem. Behav.* **37**, 613–621.
43. Cullinan, W. E., Herman, J. P., Battaglia, D. F., Akil, H. & Watson, S. J. (1995) *Neuroscience* **64**, 477–505.
44. Kirby, L. G., Chou-Green, J. M., Davis, K. & Lucki, I. (1997) *Brain Res.* **760**, 218–230.
45. Duncan, G. E., Knapp, D. J. & Breese, G. R. (1996) *Brain Res.* **713**, 79–91.
46. Hamm, R. J., Pike, B. R., O'Dell, D. M., Lyeth, B. G. & Jenkins, L. W. (1994) *J. Neurotrauma* **11**, 187–196.
47. Cloninger, C. R. (1987) *Arch. Gen. Psychiatry* **44**, 573–588.
48. den Boer, J. A., Westenberg, H. G., De Leeuw, A. S. & van Vliet, I. M. (1995) *Int. Clin. Psychopharmacol.* **4**, 47–52.
49. Griebel, G. (1995) *Pharmacol. Ther.* **65**, 319–395.
50. Handley, S. L. (1995) *Pharmacol. Ther.* **66**, 103–148.
51. Plomin, R., Owen, M. J. & McGuffin, P. (1994) *Science* **264**, 1733–1739.
52. Kahn, R. S., Kling, M. A., Wetzler, S., Asnis, G. M. & van Praag, H. (1992) *Psychopharmacology* **108**, 225–228.
53. Hilakivi-Clarke, L. A., Durcan, M. J., Lister, R. G. & Linnoila, M. (1990) *Pharmacol. Biochem. Behav.* **37**, 273–276.
54. Berrettini, W. H., Harris, N., Ferraro, T. N. & Vogel, W. H. (1994) *Psychiatr. Genet.* **4**, 91–94.
55. Mathis, C., Neumann, P. E., Gershenfeld, H., Paul, S. M. & Crawley, J. N. (1995) *Behav. Genet.* **25**, 557–568.
56. Stenzel-Poore, M. P., Heinrichs, S. C., Rivest, S., Koob, G. F. & Vale, W. W. (1994) *J. Neurosci.* **14**, 2579–2584.
57. Konig, M., Zimmer, A. M., Steiner, H., Holmes, P. V., Crawley, J. N., Brownstein, M. J. & Zimmer, A. (1996) *Nature (London)* **383**, 535–538.
58. Bergen, A., Wang, C. Y., Nakhai, B. & Goldman, D. (1996) *Hum. Mutat.* **7**, 135–143.