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12. A variable delay (up to several hundred milliseconds) was usually observed between the onset of the current pulse triggering the odor delivery and the onset of the physiological response in the antennal lobe or mushroom body. This delay is explained by the lag time separating the openings of the electrical and pneumatic valves and by the distance separating the odor delivery tubes from the antenna (up to 5 cm), which caused long transport times. Such long distances were chosen to minimize stimulation of antennal mechanoreceptors.
13. Field potential and intracellular data were digitized at 5 kHz with a TL1-DMA board (Axon Instruments). To produce the cross-correlograms, the electrophysiological traces typically were broken up into about 240 (or 120) windows of 1024 points. Each window overlapped the next one by 896 (or 768) points. The data were digitally filtered with a fourth-degree Savitsky-Golay smoothing algorithm (using 257 or 513 points) (W. H. Press *et al.*, *Numerical Recipes in C*, Cambridge Univ. Press, Cambridge, ed. 2, 1992). The filtering and cross-correlation algorithms were written in MATHEMATICA and run on NeXTstation and Silicon Graphics Indigo platforms.
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Enhanced Aggressive Behavior in Mice Lacking 5-HT_{1B} Receptor

Frédéric Saudou, Djamel Aït Amara, Andrée Dierich, Marianne LeMeur, Sylvie Ramboz,* Louis Segu, Marie-Christine Buhot, René Hen*

The neuromodulator serotonin (5-hydroxytryptamine, 5-HT) has been associated with mood disorders such as depression, anxiety, and impulsive violence. To define the contribution of 5-HT receptor subtypes to behavior, mutant mice lacking the 5-HT_{1B} receptor were generated by homologous recombination. These mice did not exhibit any obvious developmental or behavioral defects. However, the hyperlocomotor effect of the 5-HT_{1A}/$_{1B}$ agonist RU24969 was absent in mutant mice, indicating that this effect is mediated by 5-HT_{1B} receptors. Moreover, when confronted with an intruder, mutant mice attacked the intruder faster and more intensely than did wild-type mice, suggesting the participation of 5-HT_{1B} receptors in aggressive behavior.

Serotonergic drugs are used to treat migraine, depression, and anxiety, and a serotonin deficit has been associated with behaviors such as suicide, impulsive violence, depression, and alcoholism (*1*). The multiple actions of serotonin are mediated by the interaction of this amine with at least 14 receptors (*2*), most of which belong to the GTP-binding protein (G protein)–coupled receptor family.

The 5-HT_{1B} receptor, which is the rodent homolog of the human 5-$HT_{1D\beta}$ receptor, is expressed in a variety of brain regions, including the basal ganglia, central gray, hippocampus, and raphe nuclei (*3*, *4*). Pharmacological studies with weak specific agonists have suggested that activation of 5-HT_{1B} receptors might lead to an increase in anxiety and locomotion and to a decrease in food intake, sexual activity, and aggressive behavior (*5*). The consequences of a blockade of 5-HT_{1B} receptors or of their human counterpart are unknown because there are no specific antagonists for these receptors.

To study the function of the 5-HT_{1B} receptor, we have generated by homologous recombination in embryonic stem (ES) cells homozygous mutant mice lacking both copies of the gene encoding the 5-HT_{1B} receptor (*6*, *7*). Four positive ES cell clones were obtained with both the JA and the JB targeting vectors (Fig. 1 and Table 1). Southern (DNA) blot analyses with Xba I digests and the E2A1 probe or the *neo* probe confirmed that accurate targeting occurred and that no additional integration took place. Cells from the positive clones JA7 and JB13 were microinjected into 3.5-day C57BL/6 mouse blastocysts. The two clones gave rise to highly chimeric mice, which were bred with C57BL/6 females to test for germline transmission of the mutated 5-HT_{1B} receptor gene. The positive chimeras were bred with females from the 129/Sv-ter inbred strain to obtain heterozygotes on the 129/Sv-ter genetic background. Homozygous animals were generated by heterozygote crossings, and the expected 1:2:1 ratio of wild-type (WT), heterozygous, and

F. Saudou, A. Dierich, M. LeMeur, S. Ramboz, R. Hen, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, U184 de l'INSERM, Faculté de Médecine, 11 rue Humann, 67085 Strasbourg Cédex, France.
D. Aït Amara, L. Segu, M.-C. Buhot, CNRS, Groupe de Recherche Neurosciences, Equipe Mémoire et Récepteurs Sérotonine, BP71, 13402 Marseille Cédex 20, France.

*Present address: Center for Neurobiology and Behavior, Columbia University, 722 West 168 Street, New York, NY 10032, USA.

homozygous mutant progeny was observed (Table 1).

To verify the disruption of the 5-HT_{1B} receptor gene, we performed autoradiography (8) on brains of WT, heterozygous, and homozygous mutants with ^{125}I-labeled cyanopindolol (^{125}I-CYP). When used in the presence of appropriate masking agents, this radioligand binds specifically to the 5-HT_{1B} receptor (3, 4). In WT mice, ^{125}I-CYP binding sites were found in the globus pallidus, substantia nigra, cerebellar nuclei, subiculum, lateral geniculate nucleus, central gray, and colliculi (Fig. 2, A and B), whereas no specific binding was observed in homozygous mutants (Fig. 2, C to E). Heterozygous mice displayed the same number of binding sites as WT mice (Fig. 2E). The same results were obtained with another 5-HT_{1B} radioligand (9). Thus, we effectively disrupted the 5-HT_{1B} gene.

The activity of the mice was analyzed in an open field (10). No significant differences were detected between the mutant mice and their WT littermates (Fig. 3). Administration of the 5-HT_1 agonist RU24969 stimulated locomotor activity in the WT mice but had no effect in the mutants (Fig. 3). These results suggest that the hyperlocomotor effect of RU24969 is mediated by 5-HT_{1B} receptors, which is in good agreement with pharmacological studies (11). However, we cannot rule out the possibility that compensatory mechanisms are responsible for the lack of effect of RU24969 in the mutants. For example, the 5-HT_{1A} receptor, which has a high affinity for RU24969 and a hypolocomotor activity (5), might have been down-regulated. However, preliminary results indicate that the number of 5-HT_{1A} receptors is the same in mutant and WT mice (9).

A class of 5-HT_1 agonists, including eltoprazine and fluprazine, have been termed serenics because of their antiaggressive properties (12), and their effects have been suggested to be mediated by 5-HT_{1B} receptors. We therefore investigated the aggressiveness of mice lacking 5-HT_{1B} receptors (5-HT_{1B}^{-} mice). After an isolation period of 4 weeks, test mice (resident) were analyzed for intermale aggression after exposure to a WT mouse that had been reared in a group (intruder) (13). In this test, the latency of attack and the number of attacks performed by the resident during a 3-min period were used as indices of aggression. The mutant residents attacked the intruder faster than the WT or heterozygous residents (Fig. 4A). Furthermore, the number of attacks in the mutant group was significantly higher than in the WT or heterozygote groups (Fig. 4B). In addition, the intensity of attacks of the mutant residents was higher, as well as the number of tail rattlings preceding the attacks. Similar re-

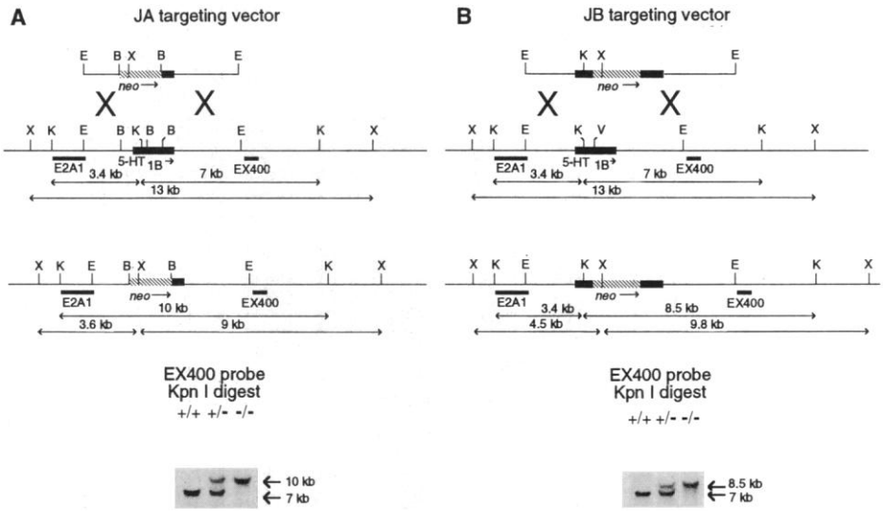

Fig. 1. Homologous recombination at the 5-HT_{1B} locus: targeting event with JA (**A**) and JB (**B**) constructs (7). (**Upper panels**) Targeting vectors, genomic structure of the 5-HT_{1B} gene, and predicted structures of the mutated alleles after homologous recombination. Black box, coding sequence of the 5-HT_{1B} receptor, which consists of a single exon; hatched box, *neo* cassette; arrows, direction of transcription. E2A1 and EX400 probes were used to screen neomycin-resistant clones after Xba I and Kpn I digests, respectively. (**Bottom panels**) Southern blot analysis. DNA from the tails of WT, heterozygous, and homozygous JA7 (A) and JB13 (B) mutant mice were digested with Kpn I and hybridized with the EX400 probe. E, Eco RI; B, Bal I; X, Xba I; K, Kpn I; V, Eco RV; +/+, WT; +/−, heterozygote; and −/−, homozygote.

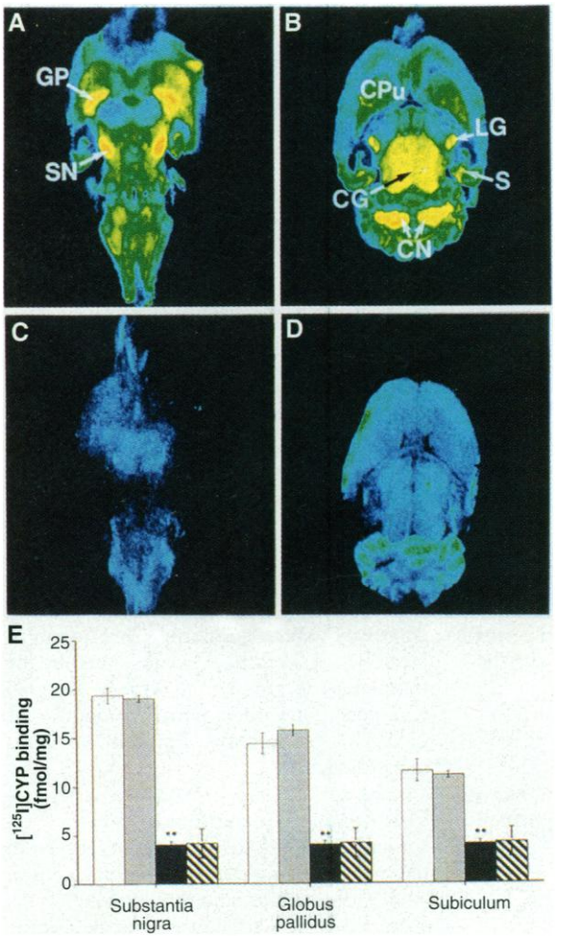

Fig. 2. 5-HT_{1B} receptor autoradiography in WT and mutant mice. ^{125}I-cyanopindolol (^{125}I-CYP) was used to label 5-HT_{1B} receptors in horizontal brain sections of 12-week-old male mice (8). (**A**) and (**B**) correspond to successively more dorsal brain sections of WT mice, and (**C**) and (**D**) are the corresponding sections from homozygous mutant mice. CG, central gray; CN, cerebellar nuclei; CPu, caudate putamen; GP, globus pallidus; LG, lateral geniculate nucleus; S, subiculum; SN, substantia nigra. (**E**) Density of ^{125}I-CYP binding sites (mean ± SEM; n = 3) in different brain regions for WT (open bars), heterozygous (stippled bars), and homozygous mutant mice (solid bars). A t test revealed no difference between WT and heterozygous mice, and between mutant mice and nonspecific binding (hatched bars). The differences were significant between mutant and WT and between mutant and heterozygous mice in all brain regions tested (**P < 0.001). The mice used in this experiment were derived from the JA7 cells, but the same results were obtained with mice derived from the JB13 cells.

sults were obtained in two tests performed 1 week apart. The level of aggressiveness was higher in the second test with both the WT and the mutant animals, consistent with reports showing that aggression increases with fighting experience (*14*). A qualitative analysis of the attacks during the 3-min test revealed additional marked differences between WT and mutant mice (Fig. 5). In the first test, 29% of the mutant residents attacked the intruder within less than 10 s after introduction of the intruder in the cage, whereas no WT or heterozygous mice attacked the intruder during that time interval. Conversely, 75% of the WT mice and only 21% of the mutants did not attack during the 3-min test. In the second test, the percentage of mutants displaying short-latency attacks was even higher (46%), whereas no WT animals performed such attacks. These results indicate that the 5-HT_{1B}^- mice are more aggressive and possibly more impulsive than their WT or heterozygous littermates.

We analyzed two behaviors that have been postulated to be modulated by 5-HT_{1B} receptors—locomotion and aggression (*11*, *12*). No differences were detected in basal locomotor activity between the WT and mutant mice, although the hyperlocomotor effect of the 5-HT_1 agonist RU24969 was absent in the mutants. The absence of an alteration in locomotor activity suggests either that compensatory mechanisms occurred during development or, alternatively, that in normal, "baseline" conditions, the 5-HT_{1B} receptor is not activated. The results of the aggression test are consistent with the notion that 5-HT_{1B} receptors may be activated in response to environmental changes, such as stressful situations. When the mutants are housed as a group they are not more aggressive than WT mice. However, after a month of isolation and in the presence of an intruder, the mutants are significantly more aggressive than the WT mice.

Several studies have revealed an association between aggressive behavior and a reduction in the activity of the serotonergic system. In rodents and primates, aggressiveness is increased after inhibition of serotonin synthesis (*15*) or destruction of serotonergic neurons (*16*). Mouse strains that display increased aggressiveness have low brain serotonin concentrations (*17*). In humans, impulsive aggressive behaviors have been associated with a deficit in central serotonin (*18*). Low serotonergic activity might result in a decreased activation of certain serotonin receptors, including the 5-HT_{1B} receptors, and might therefore produce an effect that is similar to the phenotype of the 5-HT_{1B}^- mice.

The increased aggressiveness of 5-HT_{1B}^- mice might also be related to the fact that a family of 5-HT_{1B} agonists termed serenics have antiaggressive properties (*12*). These compounds decrease aggressive behavior in several animal models, including isolation-induced aggression in mice, resident-intruder aggression in rats, and maternal aggression in rats. Our results suggest

Table 1. Homologous recombination of 5-HT_{1B} gene. Analysis of the recombination events and generation of homozygote mutant mice. GLT, germline transmission.

Targeting vector	JA	JB
Length of homology (kb)	4.5	6
Neomycin-resistant colonies	62	49
Positive colonies	4	4
Positive colony used	JA7	JB13
Chimeric males	23	2
Chimeric males analyzed	8	2
GLT chimeric males	5	2
Offspring from heterozygote crosses		
Total	243	213
+/+	61	54
+/−	130	99
−/−	52	60

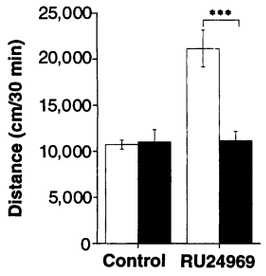

Fig. 3. Locomotor activity of the mutant mice in an open field (*10*). The bars on the left correspond to the locomotor activity (mean ± SEM) in control conditions of WT (n = 12) and mutant mice (n = 10), and the bars on the right to the effect of RU24969 injected in the same mice 10 days after the first test. There was no significant difference between the WT and the mutant mice in control conditions [t test; [$t_{(20)}$ = 0.22; not significant (NS)]]. After RU24969 treatment there was a significant difference between the two groups: [$t_{(20)}$ = 4.18; ***P < 0.001]. Open bars, WT mice; solid bars, homozygous mutant mice.

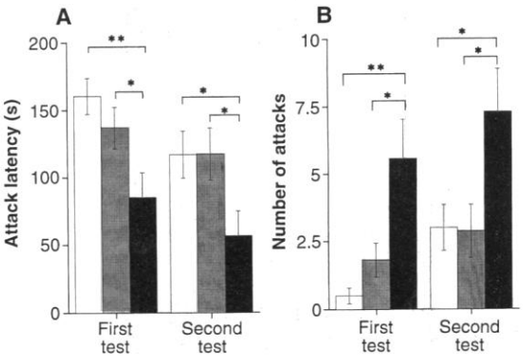

Fig. 4. Resident-intruder aggression test (*13*). Resident mice were WT (n = 12), heterozygotes (n = 16), and mutant mice (n = 14). (**A**) Attack latency (mean ± SEM): time between the introduction of the intruder and the first attack by the resident. Analysis of variance (ANOVA) revealed significant differences for the attack latency both in the first test [$F_{(2,39)}$ = 5.38, P < 0.01] and in the second test [$F_{(2,37)}$ = 3.49, P < 0.05]. Further statistical analyses revealed significant differences between WT and mutant mice (first test, [$t_{(24)}$ = 3.19, P < 0.01]; second test, [$t_{(23)}$ = 2.38, P < 0.05]), heterozygotes and mutant mice (first test, [$t_{(28)}$ = 2.17, P < 0.05]; second test, [$t_{(26)}$ = 2.26, P < 0.05]), but not between WT and heterozygous mice (first test, [$t_{(26)}$ = 1.10, NS]; second test, [$t_{(25)}$ = 0.01, NS]). (**B**) Number of attacks (mean ± SEM) during the session. ANOVA: (first test, [$F_{(2,39)}$ = 7.39, P < 0.01]; second test, [$F_{(2,37)}$ = 4.48, P < 0.02]). t tests: WT versus mutants (first test, [$t_{(24)}$ = 3.19, P < 0.01]; second test, [t_{23} = 2.32, P < 0.05]), heterozygotes versus mutants (first test, [$t_{(28)}$ = 2.16, P < 0.05]; second test, [$t_{(26)}$ = 2.44, P < 0.05]), WT versus heterozygotes (first test, [$t_{(26)}$ = 1.72, NS]; second test, [$t_{(25)}$ = 0.99, NS]). *P < 0.05; **P < 0.01. Open bars, WT mice; stippled bars, heterozygote mice; solid bars, homozygous mutant mice.

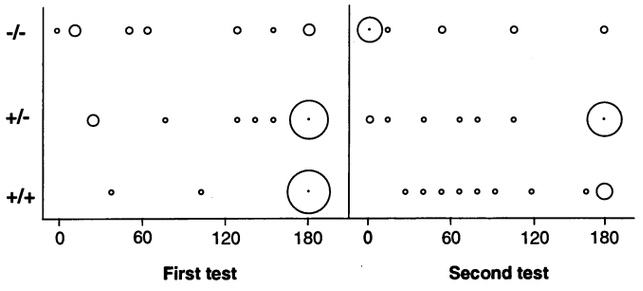

Fig. 5. Resident-intruder aggression test: scatter plot of attack latencies. The data correspond to the experiment presented in Fig. 4. −/−, homozygous mutant mice; −/+, heterozygous mice; +/+, WT mice. Attack latencies are expressed in seconds (0 to 180). Each small circle corresponds to one animal. Circle sizes are proportional to the number of animals displaying the same attack latency.

that the 5-HT_{1B} receptor is at least in part responsible for the antiaggressive properties of the serenics, but do not rule out a participation of other receptors with a high affinity for these compounds, such as the 5-HT_{1A} receptor. The antiaggressive properties of the serenics are not affected by serotonin depletions and are therefore most likely mediated by postsynaptic receptors (*19*). 5-HT_{1B} receptors are expressed in a variety of brain structures, but predominantly on the terminals of projecting neurons where they often inhibit transmitter release (*3*). Candidate structures could be the amygdala and the central gray, which express 5-HT_{1B} mRNA and 5-HT_{1B} binding sites, respectively (*3*, *4*), and which are two regions involved in the response to fear and defensive behaviors (*20*).

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8. Preparation of the brains, horizontal sections, preincubation of the sections, washing, and exposure to film were as described (*3*). Incubations were done with 82 pM ^{125}I-CYP in the presence of 30 μM isoproterenol and 100 nM (±)-8-hydroxydipropylaminotetralin (8-OH-DPAT) (RBI) to mask β-adrenergic and 5-HT_{1A} receptors, respectively. Nonspecific binding was determined with 10 μM serotonin. Quantitative analysis of the autoradiograms was carried out for the different anatomical structures with a computer device for image analysis (Biolab); [L. Segu, P. Rage, P. Boulenguez, *J. Neurosci. Methods* **31**, 197 (1990)].
9. Serotonin-*O*-carboxy-methylglycol[^{125}I]tyrosinamide (S-CM-G[^{125}I]TNH_2) was synthesized by Immunotech S.A. and was used as described in (*3*); [^{3}H]8-OH-DPAT was used to analyze 5-HT_{1A} receptor levels (D. Aït Amara and L. Segu, unpublished data).
10. Twelve-week-old male mice were housed alone in standard cages and kept on a 12:12-hour light-dark cycle with light onset at 0700 hours. The mice were tested between 1000 hours and 1600 hours during the light phase. Mice were placed in a circular open field (70 cm in diameter), and the distance traveled by the animal was recorded by a videotracking apparatus (Videotrack, Viewpoint; Lyon, France). The locomotor activity was scored over a 30-min period divided into 1-min intervals and was analyzed both in the central part of the open field and in the periphery. Ten days after the first test, mice were injected with RU24969 before a second test. RU24969 [5-methoxy-3-(1,2,5,6-tetrahydropyrid-4-yl)-1H-indole] was dissolved in saline and administered intraperitoneally, 40 min before testing, at a concentration of 5 mg per kilogram of body weight in a volume of 10 ml/kg.
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Mediation of Hippocampal Mossy Fiber Long-Term Potentiation by Cyclic AMP

Marc G. Weisskopf, Pablo E. Castillo, Robert A. Zalutsky, Roger A. Nicoll*

Repetitive activation of hippocampal mossy fibers evokes a long-term potentiation (LTP) of synaptic responses in pyramidal cells in the CA3 region that is independent of *N*-methyl-D-aspartate receptor activation. Previous results suggest that the site for both the induction and expression of this form of LTP is presynaptic. Experimental elevation of cyclic adenosine 3′,5′-monophosphate (cAMP) both mimics and interferes with tetanus-induced mossy fiber LTP, and blockers of the cAMP cascade block mossy fiber LTP. It is proposed that calcium entry into the presynaptic terminal may activate Ca^{2+}-calmodulin–sensitive adenylyl cyclase I which, through protein kinase A, causes a persistent enhancement of evoked glutamate release.

Long-lasting modifications of the strength of synaptic signals after repetitive stimulation of synapses is a common property of excitatory synapses in the central nervous system. This use-dependent synaptic plasticity provides a possible cellular basis for many types of learning and memory. One of the most studied forms of synaptic plasticity is LTP, which in most cases requires the activation of postsynaptic *N*-methyl-D-aspartate (NMDA) glutamate receptors (*1*). In contrast to this pattern, the LTP evoked at mossy fiber synapses of CA3 hippocampal pyramidal cells is entirely independent of NMDA receptors (*2*). Most evidence favors a presynaptic site for both the induction (*3–5*) [but see (*6*)] and expression (*3*,

M. G. Weisskopf, Neuroscience Graduate Program, University of California, San Francisco, San Francisco, CA 94143–0450, USA.
P. E. Castillo, R. A. Zalutsky, R. A. Nicoll, Departments of Pharmacology and Physiology, University of California, San Francisco, San Francisco, CA 94143–0450, USA.

*To whom correspondence should be addressed at Department of Pharmacology, University of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143–0450, USA.