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Perturbed dentate gyrus function in serotonin 5-HT_{2C} receptor mutant mice

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ABSTRACT **Serotonin systems have been implicated in the regulation of hippocampal function. Serotonin 5-HT_{2C} receptors are widely expressed throughout the hippocampal formation, and these receptors have been proposed to modulate synaptic plasticity in the visual cortex. To assess the contribution of 5-HT_{2C} receptors to the serotonergic regulation of hippocampal function, mice with a targeted 5-HT_{2C}-receptor gene mutation were examined. An examination of long-term potentiation at each of four principal regions of the hippocampal formation revealed a selective impairment restricted to medial perforant path–dentate gyrus synapses of mutant mice. This deficit was accompanied by abnormal performance in behavioral assays associated with dentate gyrus function. 5-HT_{2C} receptor mutants exhibited abnormal performance in the Morris water maze assay of spatial learning and reduced aversion to a novel environment. These deficits were selective and were not associated with a generalized learning deficit or with an impairment in the discrimination of spatial context. These results indicate that a genetic perturbation of serotonin receptor function can modulate dentate gyrus plasticity and that plasticity in this structure may contribute to neural mechanisms underlying hippocampus-dependent behaviors.**

Serotonergic neurotransmission exerts a considerable influence on hippocampal function. This structure is influenced powerfully by serotonergic projections from midbrain raphe nuclei (1–4), which modulate hippocampal electrical activity, hippocampal-dependent behaviors, and long-term potentiation (LTP), a form of hippocampal plasticity that has been implicated in memory formation (5–7). Studies of the serotonergic modulation of hippocampal function have been complicated by the marked heterogeneity of 5-HT (5-hydroxytryptamine, or serotonin) receptor subtypes, with at least 14 distinct subtypes expressed in the central nervous system. Determining the contributions of individual 5-HT receptor subtypes to the serotonergic regulation of hippocampal function is hindered by a paucity of subtype-selective drugs (8–12).

In these studies, we focus on the 5-HT_{2C} receptor, which is abundantly expressed throughout the hippocampal formation and the subiculum (13). An involvement of this receptor subtype in the regulation of neuronal plasticity is suggested by recent evidence that 5-HT_{2C} receptor stimulation facilitates the induction of LTP in the visual cortex (14, 15). However, interpretations of such pharmacologic studies have been complicated by a lack of available 5-HT_{2C} receptor-selective agonists and antagonists. To investigate the functional roles of 5-HT_{2C} receptors, we have generated a line of mice bearing a targeted null mutation of the gene encoding this receptor (16). In these studies, we examine the effects of this mutation on hippocampal physiology and associated behaviors.

MATERIALS AND METHODS

5-HT_{2C} Receptor Mutant Mice. These animals were originally generated from a 129-derived embryonic stem (ES) cell line (16), and have been since backcrossed for six generations to a C57BL/6 background. Wild-type male mice were mated with heterozygous females; because the 5-HT_{2C} receptor gene is X linked (17), 50% of the resulting males were hemizygous mutants, and 50% were wild type. This approach was chosen (rather than the separate maintenance of mutant and wild-type strains to minimize interlitter variability) to control for potential phenotypic differences between mutant and wild-type mothers. Genotyping was performed by using Southern blot analysis with a genomic probe corresponding to a region located 3′ to the site of construct integration (16). Animals used were between the ages of 3 and 6 mo and were age-matched for individual experiments. Animals were group housed (3–5 mice per standard cage) with free access to food and water under a 12-hr light/dark cycle (lights on at 0700). Behavioral experiments were conducted between 1200 and 1600. Experimenters were blind to the genotypes of the mice.

Histology. Animals were deeply anesthetized with avertin and transcardially perfused with 4% paraformaldehyde in PBS. After perfusion, brains were dissected and immersion-fixed overnight in fresh fixative. The brains were transferred to 30% sucrose in PBS for an additional 24 hr before freezing in powdered dry ice. Cryostat sections (14 μm for coronal, 20 μm for sagittal) were slide-mounted and stained with cresyl violet and Timm's stain.

Hippocampal LTP. All hippocampal slice data were acquired and analyzed by an experimenter blind to the genotype of each mouse. Hippocampal slices were cut 400 μm from the ventral hippocampus by using a vibratome as described (18). For CA1 and dentate gyrus LTP experiments, coronal slices posterior to the midhippocampus were used. For subiculum and CA3 LTP experiments, horizontal slices were cut from the ventral aspect of the brain; sections taken at this orientation gave superior field potentials in these subfields. Slices were cut into ice-cold artificial cerebrospinal fluid (ACSF) (in mM): NaCl, 119; KCl, 2.5; $MgSO_4$, 1.3; $CaCl_2$, 2.5; $NaHPO_4$, 1; $NaHCO_3$, 26; dextrose, 11, continuously bubbled with O_2/CO_2. Slices for CA3 LTP were cut into sucrose-containing ACSF and transferred into regular ACSF 40 min later (19). Slices were maintained in an interface chamber at room temperature for 1–4 hr before use.

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Abbreviations: LTP, long-term potentiation; MWM, Morris water maze; DL-APV, DL-2-amino-5-phosphonovaleric acid; GABA, γ-aminobutyric acid.
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A single slice was submerged in a recording chamber with oxygenated ACSF flowing at 2–3 ml/min at 28–30°C. Stimulation was at 0.05 Hz except in CA3, for which stimulation was delivered at 0.1 Hz. Standard recording methods were used to identify and isolate field potentials in CA1 (20), CA3 (21), and dentate gyrus (22). Briefly, glass electrodes filled with 2 M NaCl were used to record extracellular potentials stimulated with bipolar stainless steel stimulating electrodes. Signals were amplified by using an Axoclamp 2B amplifier and stored on a personal computer. Recordings in CA1 and dentate gyrus always included an unstimulated control pathway to ensure stability of the preparation. For recordings in subiculum, the stimulating electrode was placed at the CA1–subiculum border in stratum pyramidale while recording in the dendritic (molecular) layer of subiculum. The location of each electrode was confirmed post hoc after fixing and staining with cresyl violet. A polysynaptically evoked potential was evoked by using a second stimulating electrode placed in stratum radiatum of the CA3 region; this field potential was used as a control pathway and remained stable throughout the recordings. Subiculum field potentials generally demonstrated paired-pulse facilitation at a 50-msec interpulse interval. LTP has not been reported previously at this synapse, but we found it to be robust and easily induced by using theta-burst stimulation (see below).

In each hippocampal subfield, at least 20 min of recording preceded LTP induction. Field potentials in all slices appeared grossly similar, and no differences in current intensity were noted between wild-type and mutant slices in any of the four regions. LTP was induced in each hippocampal region by using protocols that elicited maximal LTP in adult C57BL/6 mice, as determined empirically. Dentate perforant path LTP was initiated by using 0.5-sec stimulation at 100 Hz at 1.5× test intensity repeated four times, 20 sec apart (22). LTP was induced at the dentate mossy fiber-to-CA3 synapse by using 1-sec stimulation at 100 Hz at test intensity repeated four times, 20 sec apart. DL-2-Amino-5-phosphonovaleric acid (DL-APV; 50 μM) was present for 10 min before tetanus and 20 min thereafter (21). LTP was induced at the CA1-to-CA3 synapse by using either 1-sec stimulation at 100 Hz at 1.5× test intensity repeated twice, 20 sec apart (20), or by using theta stimulation at test intensity (see below). The 100-Hz tetanus and theta-burst stimulation produced indistinguishable LTP in area CA1, and the results have been pooled. LTP was triggered at the CA1-to-subiculum synapse by using theta stimulation at test intensity: four 100 Hz pulses were delivered 10 times at 200-msec intervals; this entire train was repeated four times, 20 sec apart. For experiments in CA1, CA3, and subiculum, standard ACSF was used; for dentate gyrus experiments, 0.1 mM picrotoxin was added to block γ-aminobutyric acid type A ($GABA_A$) receptors; Mg^{2+} and Ca^{2+} levels were raised to 4 mM each to prevent epileptiform bursting.

The maximal initial slope of each extracellularly recorded excitatory postsynaptic potential was measured by using custom software donated by D. Madison (Stanford University, Palo Alto, CA). Each experiment was normalized to the values taken for the 5-min period before LTP induction.

Morris Water Maze (MWM). The MWM procedure was performed as described (23). Briefly, animals were placed in a circular pool (112 cm in diameter) containing opacified water. In the hidden-platform phase, animals were allowed 60 sec to locate a square platform (10.8-cm sides) submerged 0.64 cm below the water surface. Whereas the platform remained in a fixed location during training, the start positions were varied among four sites. Animals received one block of four trials per day. On the ninth day of hidden-platform training, a probe trial was performed in which animals were allowed to swim for 60 sec in the pool after removal of the platform. Swim paths were recorded and analyzed by using a Polytrack video tracking system (San Diego Instruments). Animals subsequently received training in a visible-platform task in which animals located a platform that was made visible by the addition of a white plastic flag. Each mouse received three blocks of four trials on 1 day.

Contextual Fear Conditioning. This assay was performed by using a modification of a prior procedure (24). On day 1, animals were exposed for 10 min to a conditioning chamber (54 cm × 27 cm × 30 cm high) with a metal grid floor and a clear Plexiglas rectangular cover (26.1 cm × 18.5 cm) with a wire mesh top. On day 2, animals were returned to the same chamber and allowed to explore for 2 min, during which time freezing behavior (the absence of all but respiratory movement) was monitored at 10-sec intervals. Animals were then exposed to an 80-dB, 6 clicks per sec auditory clicker for 30 sec, followed immediately by a 0.35-mA foot shock (2-sec duration). This pairing was repeated with a 2-min intertrial interval. Animals were returned to their home cages 30 sec after the second foot shock. On day 3, animals were returned to the chamber and during a 5-min period, freezing behavior was monitored every 10 sec (context condition). Approximately 1 hr later, animals were returned to the chamber after it had been altered by the replacement of the grid floor with Plexiglas, the addition of a divider to restrict the animals to a small triangular area (234.5 cm^2), and the introduction of orange extract to provide an olfactory cue. Once placed in the altered context, freezing was observed for 3 min in the absence of the tone and then during a 3-min exposure to the tone. A percent freezing score was calculated for each animal during the four periods tested: baseline, context, altered context, and tone. Baseline freezing was negligible for both mutant and wild-type mice.

Context Discrimination. This protocol was adapted from described procedures (25). On day 1, animals received a 5-min preexposure to two different chambers (located in two separate rooms with altered lighting, odors, and enclosure shapes). On days 2–4, each mouse received 3 min, separated by about 1 hr, in each chamber. Half of the animals of each genotype were shocked in the first chamber that they experienced, and the other half were shocked in the second chamber. The order of chamber exposure was constant across days. In the chamber in which they were shocked, the 2-sec shock (delivered as described above) occurred 2.5 min into the session, and the mice were removed at 3 min. On days 2–4, animals shocked in the first context displayed elevated freezing rates in the second box. Therefore, the fifth day was a test day in which no shocks were given during the 3-min exposure to each chamber. Freezing rates were monitored as described above during all chamber exposures.

Emergence Neophobia. Mutant and wild-type mice ($n = 10$ per genotype) were placed in a small opaque box (12 × 12 × 7.5 cm high), open at one side, within a brightly lit open field (square, 50 cm per side, white surfaces) to which they had not been previously exposed. The emergence latency was defined as the time required for all four paws to emerge from the container.

RESULTS

Histology. Examination of cresyl violet-stained sections throughout the neuraxis revealed no apparent cytoarchitectural abnormalities (Fig. 1 *A–D*) in 5-HT_{2C} receptor mutant mice. In addition, Timm's staining (which stains zinc-containing axon terminals) revealed no differences in the organization of hippocampal terminal fields (Fig. 1 *E* and *F*). HPLC analysis of monoamines from dissected hippocampi and [^{3}H]ketanserin autoradiography revealed no evidence for compensatory alterations of monoaminergic content or 5-HT_{2A} receptor number in the mutants (data not shown).

Hippocampal LTP. To determine whether the absence of 5-HT_{2C} receptors perturbs hippocampal physiology in mutant

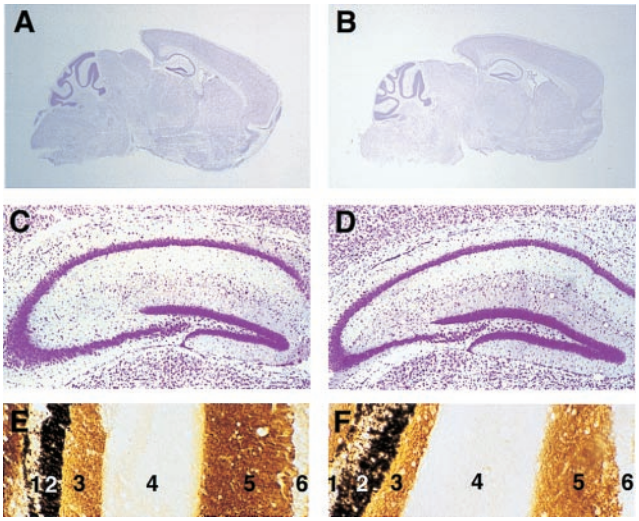

FIG. 1. Brain anatomy of 5-HT_{2C} receptor mutant mice. No phenotypic differences were detected in cytoarchitecture. Cresyl violet staining of sagittal brain sections from wild-type (*A*) and hemizygous mutant (*B*) mice. Cresyl violet staining of hippocampal coronal sections from wild-type (*C*) and mutant (*D*) mice. Timm's staining of hippocampal coronal sections revealed no differences in the laminar organization of terminal fields between wild-type (*E*) and mutant (*F*) animals. The layers correspond to 1, dentate gyrus granule cells; 2, dentate inner molecular layer; 3, dentate mid/outer molecular layer; 4, stratum lacunosum-moleculare; 5, stratum radiatum; and 6, CA1 pyramidal cells.

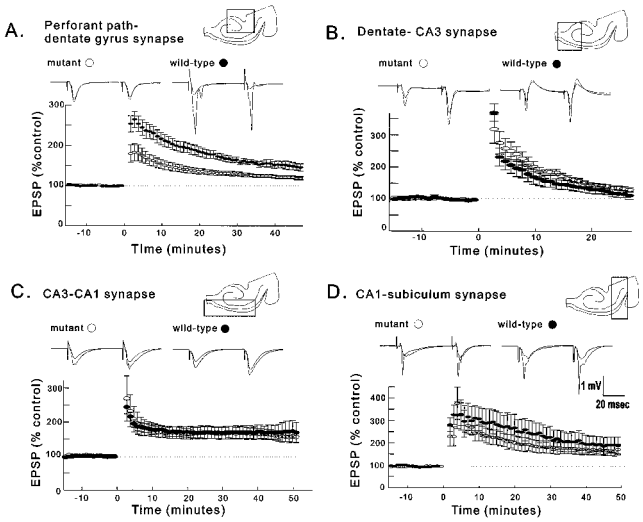

FIG. 2. LTP in hippocampal slices from wild-type and mutant mice. LTP was induced in each of four hippocampal regions (*A–D*). ● represent data from wild-type slices, whereas ○ represent data from mutant slices. Each graph plots the percent increase in extracellular excitatory postsynaptic transmissions recorded before and after LTP induction; error bars represent the SEM. For each animal, 2–4 slices were examined; *n* refers to the number of animals per group. (*A*) Attenuated LTP in slices from mutant animals is observed only in the medial perforant path-to-dentate granule cell synapse ($n = 9$ wild type, 8 mutant). (*B*) LTP at mossy fiber-CA3 synapse ($n = 7$ wild type, 7 mutant). (*C*) CA3-to-CA1 LTP ($n = 5$ wild type, 5 mutant). (*D*) CA1-to-subiculum LTP ($n = 5$ wild type, 5 mutant). Traces above each graph show representative examples of field potentials from mutant and wild-type slices before and 30 min after LTP induction. Paired pulses were delivered 50 msec apart. Calibrations apply to all EPSPs.

mice, we examined LTP at four synaptic sites in the *in vitro* slice preparation. Field potentials recorded during afferent stimulation were indistinguishable in slices from wild-type and mutant animals in all regions. The synapses between medial perforant path afferents from entorhinal cortex and dentate granule cells exhibited characteristic paired-pulse depression; paired-pulse depression was similar in mutant and wild-type animals, indicating that a basic aspect of excitatory synaptic transmission was normal [paired-pulse depression (26), ratio of first-response amplitude to second-response amplitude: wild-type, 66 ± 4%; mutant, 73 ± 3%, $P = 0.22$, paired Student's *t* test]. However, we found that LTP in this pathway was strongly attenuated in slices from mutant animals (Fig. 2*A*). LTP levels were significantly diminished following the induction protocol (repeated measures ANOVA, $F_{1,15} = 9.0$, $P = 0.01$). These experiments were performed with $GABA_A$ receptors blocked, so these results cannot be accounted for by phenotypic differences in inhibitory synaptic transmission.

We next examined LTP at synapses between dentate mossy fibers and CA3, between CA3 and CA1 pyramidal cells, and between CA1 pyramidal cells and subiculum neurons [because the 5-HT_{2C} receptor is highly expressed in the subiculum (13)]. Because serotonergic fibers heavily innervate all of these hippocampal regions and because the 5-HT_{2C} receptor is expressed throughout the hippocampus, we expected an attenuation of LTP similar to that seen at the perforant path–dentate synapse. To our surprise, LTP was normal at these three glutamatergic synapses (Fig. 2 *B–D*). Thus, 5-HT_{2C} receptor mutant animals have a selective impairment of perforant path LTP within the dentate gyrus.

Spatial Learning in 5-HT_{2C} Receptor Mutant Mice. Mutant and wild-type mice were evaluated in the MWM assay of spatial learning. During the hidden platform phase, animals learned to locate the position of a submerged (hidden) platform in a pool filled with opaque water. To find the platform, animals typically learn to navigate by using spatial cues located around the pool. The development of a spatially selective search strategy in this task requires intact hippocampal function (27–30). No significant differences were detected in the escape latencies of mutant and wild-type mice during training with the hidden platform (Fig. 3*A*). However, clear differences were observed in the subsequent probe trial. In this test, the platform was removed before placement of the animal in the pool for 60 sec. We then counted the number of times that the animals crossed the trained-platform position relative to the corresponding positions in the other three quadrants of the pool. Although wild-type mice clearly favored the trained-platform location (Fig. 3*B*), mutant mice failed to demonstrate a preference for the trained site. These results raise the possibility that the strategy used by mutant animals to locate the hidden platform differs from that used by wild-type mice. Mutant and wild-type groups did not differ in their performance when the platform position was indicated by a visible flag (Fig. 3*C*). This and the normal escape latencies in the hidden platform phase indicated that no gross differences existed between the groups in swimming ability or motivation to locate the platform.

Contextual Fear Conditioning. Hippocampal lesions alter conditioned fear responses to environmental contexts in which aversive stimuli had been delivered (31–33). Therefore, conditioned fear responses to a discrete auditory cue and to an aversive context were examined in mutant and wild-type mice in a contextual fear conditioning paradigm. Animals were placed in a conditioning chamber in which they received two pairings of a tone with a mild footshock. The next day, animals were returned to the conditioning chamber. In this situation, wild-type mice displayed freezing, a rodent fear response indicating the association of the shock with the context in which it occurred (34). Mice were then moved into a chamber with contextual cues (shape, size, odor) distinct from the shock chamber. Wild-type animals placed in such an altered chamber exhibited significantly less freezing than in the shock chamber (Fig. 4*A*). As a measure of conditioning to a discrete cue, freezing responses in the altered chamber also were observed

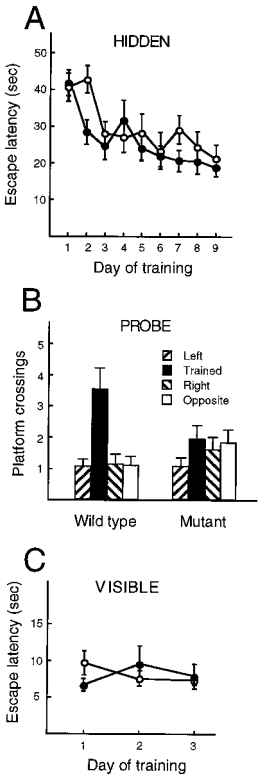

FIG. 3. MWM task. In the hidden-platform phase of the task (*A*), escape latencies between mutant ($n = 10$, ○) and wild-type ($n = 10$, ●) animals did not differ significantly. In contrast, mutant animals differed significantly from wild-type animals in the probe test (*B*). The number of times animals crossed the trained-platform location or its untrained equivalent in the other three quadrants was assessed. Whereas wild-type animals displayed significantly fewer ($P < 0.05$) crossings in the three untrained sites relative to the trained site, mutants did not exhibit a preference for the trained site ($P > 0.05$ for differences between the trained and each untrained site). In the visible-platform phase (*C*), mutants did not differ from wild types in escape latency ($F_{1,17} = 0.1$, $P > 0.05$).

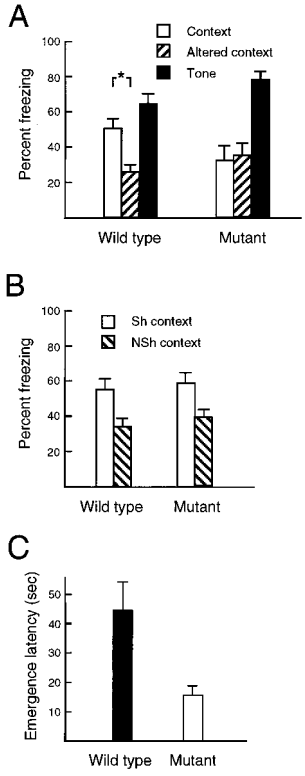

FIG. 4. Contextual fear conditioning, contextual discrimination, and emergence neophobia in 5-HT_{2C} receptor mutant and wild-type mice. (*A*) After preexposure and two pairings of the tone and footshock in the conditioning chamber, reexposure to this chamber produced freezing responses that appeared to be moderately diminished in mutants; however, this effect was not statistically significant ($P = 0.11$). On subsequent placement in the altered chamber, freezing rates were significantly decreased in wild-type mice relative to rates in the shock context (∗ paired *t* test, $P < 0.001$). In contrast, mutants exhibited equivalent rates of freezing in both enclosures (paired *t* test, $P > 0.05$). During subsequent exposure to the tone, freezing rates increased to a similar extent in both groups ($n = 13$ mutant, $n = 11$ wild-type mice). Error bars represent the SEM. (*B*) After three days of discrimination training, mutant and wild-type animals did not differ in freezing rates in the shock context or the nonshock context ($n = 12$ mutant, $n = 12$ wild-type mice). (*C*) Reduced emergence latencies (latency for all four paws to emerge) in mutant mice ($P < 0.05$), indicative of diminished emergence neophobia ($n = 12$ mutant, $n = 12$ wild-type mice). Error bars represent the SEM.

during delivery of the tone. Wild-type mice responded to the tone by dramatically increasing the amount of time spent frozen, indicating conditioning to this discrete cue. Mutants also were effectively conditioned to the tone, however, their freezing rates in the shock chamber appeared to be diminished, although this phenotype difference did not achieve statistical significance ($P = 0.11$). Unlike wild-type mice, the freezing rates of mutant animals did not differ between the shock chamber and the altered chamber.

Contextual Discrimination. One possible explanation for the similarity in the freezing rates of mutant mice in the shock chamber and the altered chamber is that mutant animals are impaired in their ability to discriminate environmental contexts. Animals were exposed to two chambers for five consecutive days, receiving footshock in one of the chambers on days 2–4. On day five, freezing rates of mice were observed in both the shock chamber and the nonshock chamber. For both mutant and wild-type animals, freezing rates in the shock chamber were similarly elevated relative to the nonshock chamber, revealing no deficits in contextual discrimination (Fig. 4*B*).

Emergence Neophobia. In addition to spatial and contextual learning, hippocampal efferents modulate exploratory behavior. In particular, the magnitude of dentate gyrus LTP has been associated with the predisposition of animals to enter a brightly lit novel environment (emergence neophobia; ref. 35). Consistent with these findings, we observed that the latencies of 5-HT_{2C} receptor mutant mice to emerge from a small dark enclosure into a brightly lit open field were markedly reduced relative to wild-type animals (Fig. 4*C*).

DISCUSSION

Our findings reveal that a genetic perturbation of serotonin 5-HT_{2C} receptor function produces a dentate gyrus-specific deficit in hippocampal long term potentiation, and perturbations in assays of spatial learning and emergence neophobia. Because 5-HT_{2C} receptor transcripts are abundantly expressed in principal neurons throughout the hippocampal formation (13), it was surprising to find that LTP at three major excitatory synapses was normal in the mutant animals but was attenuated at the medial perforant path–dentate granule cell synapse. However, these findings are in accord with prior evidence that serotonergic afferents arising from the dorsal and median raphe nuclei exert a substantial influence on dentate gyrus function. For example, electrical stimulation of the mesencephalic raphe augments the perforant path-evoked population spike (36). These serotonergic afferents have been implicated in the facilitation of neuronal transmission through the dentate gyrus observed during slow-wave sleep (5). Con-

versely, reductions of serotonin levels by 5,7-dihydroxytryptamine lesions reduce dentate gyrus LTP *in vivo*, indicating a potential role for serotonin systems in the regulation of dentate gyrus plasticity (6).

The reduction of perforant-path LTP in 5-HT_{2C} receptor mutant mice indicates that stimulation of this receptor subtype may contribute to the effects of serotonin on neuronal plasticity in the dentate gyrus. Recently, 5-HT_2 receptors also have been implicated in the serotonergic facilitation of LTP within the visual cortex of kittens and rats (14, 15). 5-HT_2 receptor activation stimulates phospholipase C and phosphoinositol turnover (37), leading to the release of Ca^{2+} from intracellular stores. Increased intracellular Ca^{2+} activates protein kinases, which phosphorylate a number of substrates, including *N*-methyl-D-aspartate (NMDA) receptors. 5-HT_{2C} receptors also may modulate dentate gyrus-granule cell plasticity through the regulation of intracellular Ca^{2+} levels.

Although a role for CA1-CA3 LTP in spatial learning and normal place cell function is becoming established (38, 39), the relationship between LTP and dentate gyrus-dependent cognitive processes remains controversial (40). In two recent reports, mutant mice lacking the neuronal glycoprotein Thy-1 and the Ca^{2+}-binding protein calretinin had normal MWM performance despite partial deficits in perforant-path LTP, leading to the suggestion of a dissociation between LTP and MWM performance (41, 42). However, these deficits both were demonstrated to result from enhanced GABAergic inhibition in the dentate gyrus of these mutants; when inhibition was blocked by using bicuculline, normal LTP was observed, indicating that the LTP mechanism itself was intact in these animals. By contrast, the blockade of GABAergic inhibition failed to normalize dentate gyrus LTP in slices from 5-HT_{2C} receptor mutants, suggesting a direct impairment of perforant-path LTP in these animals. Perforant path input to the dentate gyrus represents a major route through which information is processed by the hippocampal formation. Our results indicate that plasticity in this pathway still warrants consideration among the neural mechanisms underlying hippocampal-dependent behaviors.

The performance deficits of 5-HT_{2C} receptor mutants are not associated with a global impairment in the ability to form learned associations. These animals performed normally in the visible platform task of the MWM, which requires the association of the flag with escape from the water. In the fear-conditioning assay, mutants exhibited normal levels of freezing in response to the tone, which is believed to require intact amygdala function (43). In addition, the normal performance of mutants in the contextual-discrimination assay indicates that their behavior in spatial learning and exploration assays does not reflect an inability to discriminate between spatial contexts. Finally, preliminary experiments reveal that the 5-HT_{2C} receptor mutants exhibit normal acquisition of appetitively motivated operant lever-press behavior (G. Heyser, L. Koob, and L. Gold, personal communication).

In 5-HT_{2C} receptor mutant mice, the dentate gyrus-specific perturbation of LTP was associated with perturbations of behaviors linked to dentate gyrus function. The diminished spatial selectivity of the mutants in the MWM task is in accord with prior studies demonstrating impaired spatial learning with selective dentate granule cell lesions (44–49). The effects of dentate gyrus lesions on contextual fear conditioning have not been reported. Our results indicate a (nonsignificant) trend toward suppressed context-induced freezing in mutant mice that could not be accounted for by impaired discrimination of environmental context. The perturbation of spatial learning appears to be greater in 5-HT_{2C} receptor mutants, in accord with genetic and lesion studies indicating that spatial and contextual learning are mediated by different mechanisms (50, 51). Information processing within the dentate gyrus also has been associated with exploratory behavior, as evidenced by the enhancement of dentate field potentials induced by exposure to a novel environment (52). Moreover, individual differences in the magnitude of perforant-path LTP correlate positively with the predisposition of rats to avoid entering a novel environment (35). Consistent with this, we find that in 5-HT_{2C} receptor mutant mice, a defect in dentate gyrus LTP is associated with reduced avoidance of a novel environment.

Alterations of hippocampal physiology and behavior in these animals were not attributable to developmental defects producing overt cytoarchitectural abnormalities of the dentate gyrus or other brain regions, nor is it likely that spontaneous seizures in these mice account for the results reported here: such seizures are rare, and ambulatory electroencephalogram recordings revealed no subclinical epileptiform activity (J. Noebels, personal communication). Moreover, prior studies indicated that repeated seizure kindling does not impair MWM performance unless a seizure occurs within ≈24 hr of testing (53). Frequent seizures have been observed to produce nonspecific alterations of LTP magnitude at multiple hippocampal subregions, particularly in area CA1 (54–56). Because LTP is normal in our mutants in area CA1, CA3, and subiculum, it is unlikely that seizures produced the observed deficit in dentate gyrus LTP.

Serotonergic systems have been implicated strongly in human cognition (57, 58). Our studies indicate that the 5-HT_{2C} receptor contributes to the serotonergic modulation of hippocampal function. Such mechanisms may be relevant to cognitive impairments that characterize a number of clinical conditions. For example, the marked loss of serotonergic neurons and 5-HT_2 receptors in Alzheimer's disease may contribute to the cognitive decline that characterizes this disorder (59, 60). Interestingly, a common human allelic variant of the 5-HT_{2C} receptor (substituting cysteine for serine at the 23rd amino acid in the N-terminal extracellular domain) has been reported with an allele frequency of 13% (61). This change appears to be pharmacologically significant, modifying the binding affinity of exogenous agonists and serotonin (ref. 62; D. Goldman, personal communication). 5-HT_{2C} receptor mutant mice therefore provide a useful tool for elucidating potential associations between serotonergic function and cognitive impairments observed in humans.

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