Enhanced contextual fear memory in central serotonin-deficient mice

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Central serotonin (5-HT) dysregulation contributes to the susceptibility for mental disorders, including depression, anxiety, and posttraumatic stress disorder, and learning and memory deficits. We report that the formation of hippocampus-dependent spatial memory is compromised, but the acquisition and retrieval of contextual fear memory are enhanced, in central 5-HT-deficient mice. Genetic deletion of serotonin in the brain was achieved by inactivating *Lmx1b* selectively in the raphe nuclei of the brainstem, resulting in a near-complete loss of 5-HT throughout the brain. These 5-HT-deficient mice exhibited no gross abnormality in brain structures and had normal locomotor activity. Spatial learning in the Morris water maze was unaffected, but the retrieval of spatial memory was impaired. In contrast, contextual fear learning and memory induced by foot-shock conditioning was markedly enhanced, but this enhancement could be prevented by intracerebroventricular administration of 5-HT. Foot shock impaired long-term potentiation and facilitated long-term depression in hippocampal slices in WT mice but had no effect in 5-HT-deficient mice. Furthermore, bath application of 5-HT in 5-HT-deficient mice restored foot shock-induced alterations of hippocampal synaptic plasticity. Thus, central 5-HT regulates hippocampus-dependent contextual fear memory, and 5-HT modulation of hippocampal synaptic plasticity may be the underlying mechanism. The enhanced fear memory in 5-HT-deficient mice supports the notion that 5-HT deficiency confers susceptibility to posttraumatic stress disorder in humans.

hippocampus | long-term depression | long-term potentiation | anxiety

The neurotransmitter serotonin (5-HT) exerts a wide spectrum of actions in the nervous system by modulating neural development, synaptic plasticity, pain sensation, rhythm, food intake, and a variety of behaviors (1–4). It has been proposed that perturbation of the 5-HT level in the brain contributes to depression and anxiety, and posttraumatic stress disorder (PTSD) (5–8), which are often accompanied by learning and memory deficits (9–11). The hippocampus is known to be critical for the formation of spatial and contextual fear memories (12–15), and the retrieval of hippocampus-dependent memories was found to be impaired in patients with depression and PTSD (16–18). Recent studies have also implicated the hippocampus as one of the primary sites for antidepressants (6, 19–23). Aversive stimuli such as foot shock that led to anxiety, depression, and fear memory in rodents also altered activity-dependent hippocampal synaptic plasticity (24–29). Because the modulation of 5-HT activity altered hippocampal long-term potentiation (LTP) and long-term depression (LTD) (30–32), it is possible that perturbation of 5-HT level in the brain may affect hippocampus-dependent learning and memory, and changes in hippocampal synaptic plasticity may also contribute to mental disorders, including anxiety and fear memory.

Inhibitors of 5-HT biosynthesis that deplete central 5-HT have been used in animal studies to delineate the role of 5-HT in mental disorders, but many of these agents also affect the synthesis of other neurotransmitters (33, 34). Removal of 5-HT neurons by chemical or electrical ablation suffers from the lack of specificity of the treatment. In the present study, we generated central 5-HT-deficient mice by a region-specific conditional knockout (CKO) of a transcriptional factor *Lmx1b* using a method reported (35). We found that these 5-HT-deficient mice exhibited normal acquisition but impaired retrieval of spatial memory. Surprisingly, anxiety-like behaviors were reduced, rather than enhanced, in these 5-HT-deficient mice. In contrast, fear behavior was exaggerated and the retrieval of contextual fear memory was greatly enhanced in these mice. Moreover, we show that foot shock impaired LTP induction but facilitated LTD induction in the hippocampus of WT mice, whereas it affected neither LTP nor LTD in the CKO mice. Furthermore, the altered fear memory and hippocampal synaptic plasticity in CKO mice were completely reversed to that of the WT mice by administration of 5-HT and were partially reversed by a 5-HT1A receptor agonist. These results indicate that the 5-HT level in the brain is critical for hippocampus-dependent memory, and these changes in hippocampal synaptic plasticity may contribute to the learning and memory deficits accompanying 5-HT-related mental disorders.

Results

Raphe Nuclei-Specific Deletion of *Lmx1b* Results in a Loss of 5-HT in the Brain. Using the methods reported recently (35), we generated *Lmx1b* CKO mice with nearly complete loss of 5-HT in the brain. Region-specific deletion of *Lmx1b* in the raphe nuclei was achieved by crossing *Pet1-cre*,*Lmx1b*$^{+/-}$ mice with *Lmx1b*$^{flox/flox}$ mice (35, 36). Raphe nuclei-specific Cre expression was shown by X-Gal staining and Cre immunostaining in *Pet1-cre*,*Rosa26* mice [supporting information (SI) Fig. S1 *A–D*]. Accordingly, expression of *Lmx1b* in *Pet1-cre*,*Lmx1b*$^{flox/-}$ mice (i.e., CKO mice) was eliminated only in the raphe nuclei (data not shown). Adult *Lmx1b* CKO mice lacked essentially all central 5-HT neurons, as shown by the nearly complete absence of immunostaining for 5-HT and 5-HT reuptake transporter, a marker of 5-HTergic terminals throughout the brain (Fig. S2 *A–F*). HPLC revealed that the 5-HT level of these mice was 10-fold lower than that of WT mice in the hippocampus, cortex, and striatum, and the level of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, was similarly reduced in these brain regions (Fig. S2*G*). Because

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the number of 5-HT-positive cells in *Lmx1b* CKO mice was only 1% of that in the WT mice, the remaining 5-HT detected by HPLC may be derived mostly from the blood supply.

Despite the loss of 5-HT neurons, the distribution and density of tyrosine hydroxylase-positive neurons were unchanged throughout the brain of CKO mice (Fig. S3 *A–D*). Consistently, CKO and WT brains exhibited no difference in the concentration of norepinephrine, dopamine, or its metabolites, dihydroxyphenylacetic acid and homovanillic acid (Fig. S3 *E* and *F*). In addition, the CKO mice showed no gross morphological defect in the brain and had normal locomotor activity (Figs. S4 and S5 *A* and *B*). Thus, raphe nuclei-specific deletion of *Lmx1b* eliminates expression of 5-HT in the brain without affecting that of other monoamines and has no apparent effect on the gross brain structure and locomotion activity.

CKO Mice Show Normal Acquisition but Impaired Retrieval of Spatial Memory. The Morris water maze is widely used for behavioral testing of hippocampus-dependent spatial learning and memory (37). We found that CKO and WT mice performed the spatial learning task equally well, as indicated by similar escape latencies in finding the platform throughout the 9-d training period (Fig. 1*A*). This suggests that the central 5-HT deficiency did not affect spatial learning. One day after completing the 9-day training period, however, the CKO mice spent less time in the target quadrant took a longer time to cross the platform location for the first time, and crossed the target location less frequently compared with the WT mice (Fig. 1 *B–D*). These deficiencies were more pronounced 10 d after training (Fig. 1 *B–D*). Because the CKO and WT mice showed similar swim speed during the period of memory acquisition and retrieval (Fig. S5*C*), these results indicate that CKO mice exhibit normal acquisition but impaired retrieval of spatial memory.

Reduced Anxiety-Like Behavior in CKO Mice. Perturbations of the central 5-HT level have been associated with anxiety/depression in humans and animal models (5–8). We therefore examined an anxiety-like behavior in CKO and WT mice, using both the elevated-plus maze test (38, 39) and the novelty suppressed feeding test (40). To our surprise, although CKO mice moved between closed and open arms with similar frequency as the WT controls in the elevated-plus maze, they spent more time in the open arm than the WT mice, reflecting reduced anxiety (Fig. 1*E*). In addition, compared with the WT mice, CKO mice exhibited a reduced latency between novelty presentation and feeding (Fig. 1*F*), another indication that anxiety was reduced in these animals, although both types of mice had similar food consumption (Fig. S5*D*). Therefore, both the elevated-plus maze and novelty-suppressed feeding tests reveal that central 5-HT deficiency in mice reduces, rather than enhances, anxiety-like behavior.

CKO Mice Show Fast Acquisition and Enhanced Fear Memory. Serotonin selective reuptake inhibitors (SSRIs) are effective at mitigating the retrieval of fear memory in patients with PTSD (6), and chronic treatment with SSRIs reduces fear behavior in fear-conditioned rats (41). We therefore examined whether the acquisition and retrieval of contextual fear memory are altered in 5-HT-deficient mice. Contextual fear memories were provoked in WT and CKO mice by associating a novel environment (i.e., conditioned stimulus) with a discrete aversive stimulus: the foot shock. Mice were conditioned with five foot shocks at 2-min intervals, and the freezing behavior was measured as the percentage of time spent in freezing during the 2-min interval between shocks. We found that the freezing behavior in CKO mice was not different during the first three postshock intervals compared with WT controls, but was significantly increased during the fourth and fifth intervals (Fig. 2*A*), indicating an increase of acquisition of fear memory. Of note, CKO and WT mice displayed similar freezing behavior before the conditioning (Fig. 2*A*), indicating that the CKO mice were not more prone to freezing behavior when introduced to a novel environment. It should be also noted that the CKO mice showed normal nociceptive responses (42), and therefore the enhanced freezing behaviors are less likely to be caused by the increased nociceptive responsivity to foot shock. When placed back into the conditioned environment 30 min after conditioning, CKO mice froze for a much longer duration than WT mice (Fig. 2*B*). The enhanced fear memory persisted even 10 d after fear conditioning (Fig. 2*B*), at a time when WT mice have largely ceased to freeze in response to the conditioned environment (Fig. 2*B*). Thus, intensity and persistence of contextual fear memory were increased in CKO mice.

To study whether increased contextual fear memory in 5-HT CKO mice is a direct consequence of central 5-HT deficiency, we injected 5-HT into the lateral ventricle and examined the

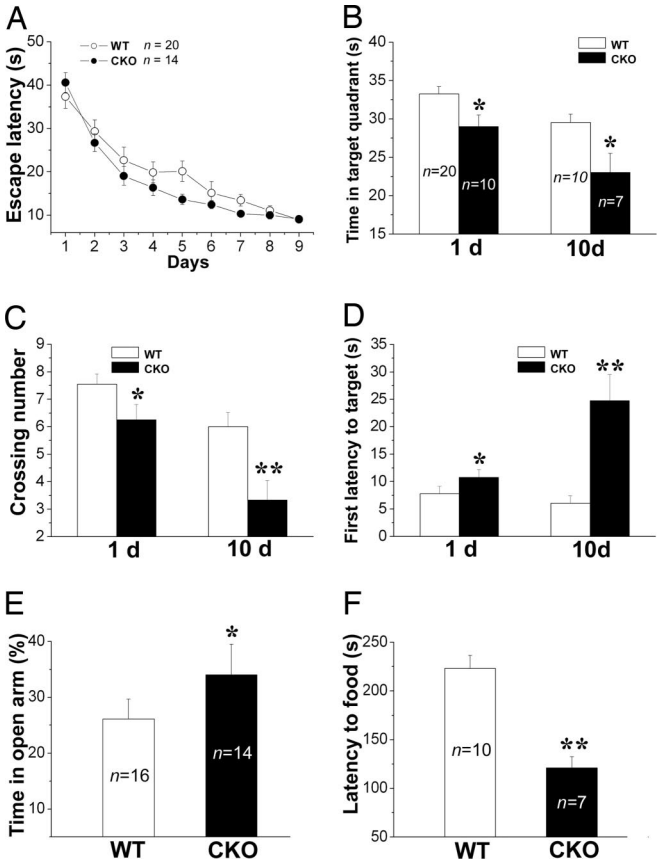

Fig. 1. Retrieval of spatial memory is impaired and anxiety-like behavior is reduced in CKO mice. (*A–D*) Normal spatial learning but impaired spatial memory is seen in CKO mice. (*A*) WT and CKO mice had comparable mean escape latencies throughout the 9-d Morris water maze spatial learning trials (WT vs. CKO; $F_{8,288} = 0.813$, $P = 0.591$). (*B*) One day and 10 d after training, WT mice spent significantly more time in the target quadrant than CKO mice (1-d comparison, $F_{1,32} = 4.442$, *, $P = 0.046$; 10-d comparison, $F_{1,15} = 5.050$, *, $P = 0.044$). (*C*) WT mice also made more frequent crossings onto the target site than CKO mice (1-d comparison, $F_{1,32} = 4.825$, *, $P = 0.037$; 10-d comparison, $F_{1,15} = 16.946$, **, $P < 0.001$). (*D*) WT mice exhibited shorter mean latency for the first crossing of the location of the platform than CKO mice (1-d comparison, $F_{1,32} = 7.073$, *, $P = 0.012$; 10-d comparison, $F_{1,15} = 16.567$, **, $P < 0.001$). (*E*) These CKO mice spent more time in the open arm than WT mice in the elevated-plus maze test ($F_{1,28} = 5.336$, *, $P = 0.028$). (*F*) These CKO mice exhibited shorter mean feeding latency after exposure to a novel context than WT mice ($F_{1,15} = 26.782$, **, $P < 0.001$).

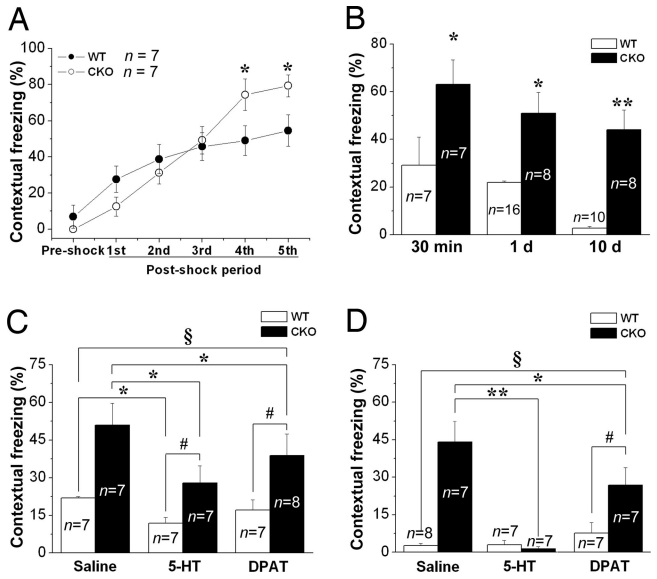

Fig. 2. Intensity and persistence of foot shock-induced fear memory are enhanced in 5-HT-deficient mice, but this enhancement is reversed to the level of WT mice after intracerebroventricular administration of 5-HT. (*A*) Foot shock provokes a significant increase of freezing behavior in CKO mice after the fourth and fifth shock compared with WT mice (after fourth shock, *, $P = 0.036$; after fifth shock, *, $P = 0.025$). (*B*) Thirty minutes, 1 d, and 10 d after fear conditioning, exposure to the conditioned environment evokes much stronger freezing behavior in CKO mice than in WT mice (30 min, *, $P = 0.033$; 1 d, *, $P = 0.002$; 10 d, **, $P = 0.001$). (*C*) Enhanced contextual fear memory 1 d after conditioning in CKO mice (CKO 5-HT vs. CKO saline, *, $P = 0.032$) is converted to the level of WT mice after intracerebroventricular administration of 5-HT (CKO 5-HT vs. WT saline, $P = 0.468$; CKO 5-HT vs. WT 5-HT, #, $P = 0.045$). Enhanced contextual fear memory in CKO mice is reduced by i.p. injection of DPAT (*, $P = 0.008$; *t* test), but it does not reach the level of WT mice (CKO DPAT vs. WT saline; §$P = 0.030$; CKO DPAT vs. WT DPAT, #, $P = 0.025$). Administration of 5-HT but not DPAT in WT mice also reduces contextual fear memory compared with WT mice treated with saline solution (*, $P = 0.002$). (*D*) Enhanced contextual fear memory in CKO mice 10 d after conditioning is also converted by intracerebroventricular administration of 5-HT (CKO 5-HT vs. CKO saline, **, $P < 0.001$) to a level similar to WT mice (CKO 5-HT vs. WT saline, $P = 0.250$; CKO 5-HT vs. WT 5-HT, $P = 0.385$). DPAT administration via i.p. route also reduced contextual fear memory in CKO mice ($n = 7$ for each; CKO DPAT vs. CKO saline, *, $P = 0.040$), but freezing time was still higher than in WT mice (CKO DPAT vs. WT saline, §, $P = 0.008$; CKO DPAT vs. WT DPAT, #, $P = 0.040$). Administration of either 5-HT or DPAT in WT mice did not reduce freezing behavior compared with WT mice treated with saline solution at this time point.

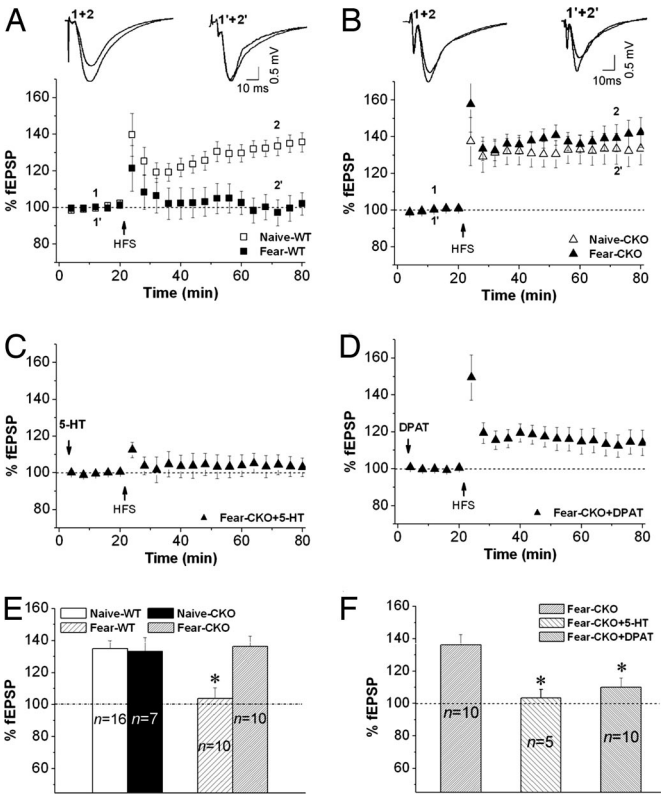

Fig. 3. Foot shock impairs hippocampal CA1 LTP measured by fEPSP amplitude (% baseline) at the striatum radiatum in WT, but has no effect in CKO mice. However, this inability is restored after bath application of 5-HT and DPAT (an agonist of 5HT1A receptor) in CKO slices. (*A*, *B*, and *E*) Foot shock (Fear) impairs HFS-evoked LTP in hippocampal slices from WT mice ($F_{1,24} = 8.440$, *, $P = 0.008$), but has no effect on LTP in CKO mice ($F_{1,15} = 0.358$, $P = 0.558$). (*B*, *C*, and *F*) When 5-HT is applied in the hippocampal slices of CKO mice, foot shock is able to impair LTP (Fear CKO 5-HT vs. Fear CKO, $F_{1,13} = 10.472$, *, $P = 0.006$), as it does in WT mice. (*B*, *D*, and *F*) Foot shock-induced impairment of LTP is partially restored after bath application of DPAT in CKO mice (Fear CKO DPAT vs. Fear CKO, $F_{1,18} = 5.443$, *, $P = 0.031$).

freezing behavior of these mice 1 d and 10 d after contextual fear conditioning. Intracerebroventricular administration of 5-HT fully restored the freezing behavior of CKO mice to that of WT mice (Fig. 2 *C* and *D*) and significantly reduced that of WT mice 1 d after conditioning, indicating that the intensity and persistence of fear memory is regulated by the 5-HT level in the brain. Furthermore, the persistence of fear memory was also significantly reduced in CKO mice by i.p. administration of the specific 5-HT1A receptor agonist 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin], although it remained exaggerated compared with WT controls (Fig. 2 *C* and *D*). Taken together, we conclude that eliminating 5-HT from the brain enhances the contextual fear memory in mice.

Foot Shock Alters LTP/LTD Induction in WT but Not CKO Mice. Hippocampal LTP/LTD is critical for the formation of spatial and contextual fear memories (43–45). Therefore, we examined LTP/LTD induction in the CA1 region of hippocampal slices from CKO and WT mice that had experienced the contextual foot shock conditioning described earlier. In the absence of conditioning, we found that high-frequency stimulation (HFS; 100 Hz for 1 s) induced LTP in both WT and CKO mice (Fig. 3 *A*, *B*, and *E*). Although prolonged low-frequency stimulation (LFS; 1 Hz for 15 min) does not induce LTP or LTD in adult rats (26, 31, 45, 46), we found that LFS induced a small LTP in WT mice but not in CKO mice (Fig. 4 *A*, *B*, and *E*). When hippocampal slices were prepared after foot shock conditioning of WT mice, HFS failed to induce LTP (Fig. 3 *A* and *E*), whereas LFS induced LTD rather than LTP (Fig. 4 *A* and *E*), which is consistent with previous reports in rats (26, 29, 47–49). Surprisingly, synaptic plasticity in CKO mice was not altered by foot shocks, as shown by the presence of LTP (Fig. 3 *B* and *E*) and the absence of LTD (Fig. 4 *B* and *E*). Thus, hippocampal synaptic plasticity is altered in CKO mice, and foot shocks fail to provoke changes in hippocampal LTP/LTD.

We next added 5-HT to the slices to determine whether 5-HT could restore normal WT hippocampal plasticity in CKO mice. We found that 5-HT application indeed resulted in the failure of LTP induction by HFS (Fig. 3 *C* and *F*) and the presence of LFS-induced LTD in foot-shocked CKO mice (Fig. 4 *C* and *F*). This supports the idea that central 5-HT deficiency is responsible for the lack of foot shock-induced changes in hippocampal LTP/LTD in CKO mice. To further validate this idea and to determine whether the 5-HT1A receptor is involved, we added

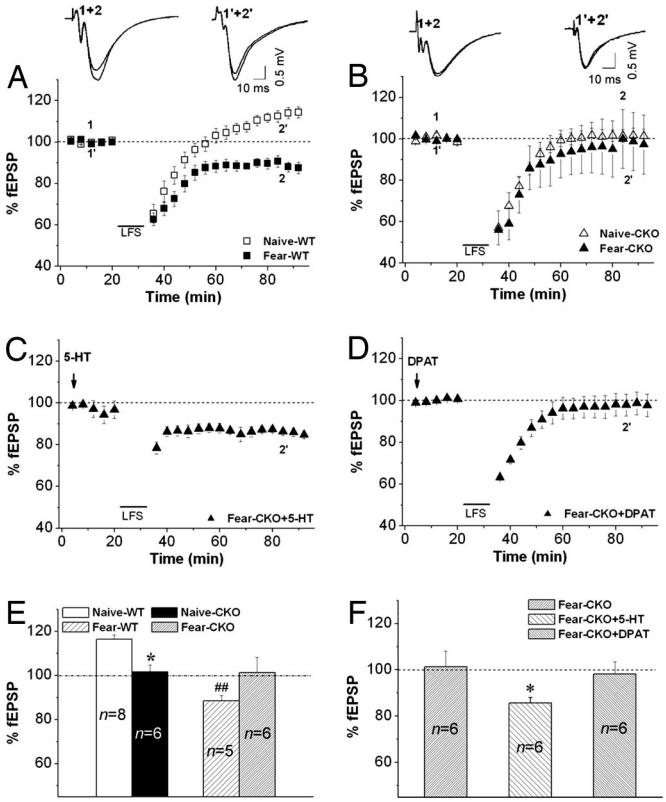

Fig. 4. Foot shock facilitates hippocampal CA1 LTD measured by fEPSP amplitude (% baseline) at the striatum radiatum in WT mice but has no effect in CKO mice. However, this inability is restored after bath application of 5-HT. (*A*, *B*, and *E*) LFS of the CA1 of hippocampal slice from WT mice induces small LTP, but evokes neither LTP nor LTD in CKO mice (naïve CKO vs. naïve WT, $F_{1,12} = 17.775$, *, $P = 0.002$). Foot shock (Fear) converts LFS-induced LTP to LTD in WT mice (Fear WT vs. naïve WT, $F_{1,11} = 33.562$, ##, $P < 0.001$; one-way ANOVA), whereas foot shock has no effect in CKO mice (Fear CKO vs. naïve CKO, $F_{1,10} = 0.225$, $P = 0.645$). (*C*, *D*, and *F*) The failure of LFS-induced LTD in CKO mice is rescued by bath application of 5-HT (Fear CKO 5-HT vs. Fear CKO, $F_{1,10} = 3.825$, *, $P = 0.039$), but not DPAT (Fear CKO DPAT vs. Fear CKO, $F_{1,10} = 0.183$, $P = 0.678$).

DPAT to the slices from foot-shocked CKO mice and found that this drug partially restored foot shock-induced alteration in LTP induction (Fig. 3 *D* and *F*) but not LTD induction (Fig. 4 *D* and *F*). Thus, 5-HT appears to be both necessary and sufficient for foot shock-induced alteration of hippocampal synaptic plasticity.

Discussion

It has been widely reported that the low 5-HT level in the brain is involved in depression and anxiety disorders in humans and rodent models. We found, surprisingly, that mice with <10% of the normal level of central 5-HT exhibited reduced rather than enhanced anxiety-like behaviors. Furthermore, contextual fear memory was greatly enhanced and became more persistent in 5-HT-deficient CKO mice. Interestingly, this enhanced fear memory was completely eliminated by intracerebroventricular injection of 5-HT. The fear-provoking foot shock was unable to alter hippocampal LTP/LTD induction in CKO mice, unlike that found for WT mice. This altered hippocampal plasticity of CKO mice was likewise completely converted to that of the WT mice by bath application of 5-HT. Thus, elimination of 5-HT not only reduced anxiety-like behavior but also enhanced contextual fear memories.

We have generated central 5-HT-deficient mice by a specific genetic deletion of *Lmx1b* in the raphe nuclei. Although it has been reported that 5-HT modulates early embryogenesis in sea urchin and the development of somatosensory cortex (1, 50), we found no gross anatomical changes in the brain, and the appearance of the barrel structure was normal in adult somatosensory cortex in these 5-HT-deficient mice (J.-X.D. and Y.-Q.D., unpublished data). Thus, 5-HT deficiency did not result in a serious deleterious effect on brain development and patterning. In addition, the expression of 5-HT receptors in the spinal cord (42) and 5-HT1A receptor in the hippocampus (Fig. S6), and the expression of other monoamines in the brain, was unchanged in CKO mice. Furthermore, swimming and other locomotor activities were apparently normal in CKO mice. Importantly, the foot shock-induced contextual fear memory in CKO mice was completely converted to that of WT mice by intracerebroventricular injection of 5-HT. Therefore, although undetected defects and possible compensatory changes may exist in CKO mice, behavior phenotypes we observed in CKO mice are most likely to result from the deficiency of 5-HT in the brain.

SSRIs that increase synaptic 5-HT level are effective in treating depression/anxiety, and thus it is generally accepted that central 5-HT dysregulation is associated with depression/anxiety. To our surprise, 5-HT-deficient mice show reduced rather than enhanced anxiety-like behavior. These results suggest that central 5-HT deficiency is not sufficient to cause some anxiety-like behaviors in mice. Conversely, 5-HT-deficient mice exhibited enhanced intensity and persistence of conditioned contextual fear memory. Foot shock used in provoking contextual fear memory in rodents can be regarded as equivalent to the traumatic event that leads to PTSD in susceptible people, and thus studies of contextual fear behavior in rodents could provide insights into the etiology of PTSD (25). We found that central 5-HT deficiency enhanced foot shock-induced contextual fear memory in mice and these phenotypes were rescued by intracerebroventricular administration of 5-HT. In light of the fact that SSRIs are effective drugs for treating PTSD (6), our findings strongly support the hypothesis that central 5-HT deficiency is a susceptibility factor for the onset of PTSD in people who experience intense traumatic events.

The hippocampus is highly sensitive to stressful stimuli (e.g., foot shock in rodents) and plays a critical role in regulating stressful event-associated learning and memory (51). The underlying mechanism is believed to be changes of hippocampal synaptic plasticity in response to the stimuli (28, 52), because foot shock impairs hippocampal LTP (48) and facilitates hippocampal LTD (26, 47). It has been shown that human susceptibility to PTSD is associated with the small volume of the hippocampus and with the stress-induced alteration of the hippocampal functions (16, 17). Typical syndromes of PTSD, such as persistent nightmares, flashbacks, and intrusive recollections of fear memories, may be attributed to the dysfunction of the hippocampus in the acquisition and retrieval of fear memory. Hippocampal synaptic plasticity is altered in the formation and retrieval of contextual fear memory evoked by foot shock in rats (24, 25), and we found similar changes of hippocampal synaptic plasticity after foot shock in WT mice. However, such changes were undetectable in 5-HT-deficient mice, but could be restored by adding 5-HT. Therefore, it is possible that, in the absence of 5-HT during or after stressful events, adaptation of hippocampal synaptic plasticity fails to take place, and this facilitates the acquisition and the persistence of contextual fear memory. In other words, central 5-HT may be necessary for protecting the hippocampus from the stressful events and for reducing the recollection of fear memory.

Materials and Methods

Generation of Central 5-HT-Deficient Mice. We generated *Pet1-cre* mice by using BAC-based transgenesis. The *Pet1* BAC clone was obtained from Children's Hospital Oakland Research Institute, and the Cre coding sequence and

3′-splice/transcription termination signals was inserted into the *Pet1* locus at the initiation codon of the first exon by homologous recombination. To inactivate *Lmx1b* in the raphe nuclei, the *Pet1-cre* mice were then crossed with heterozygous *Lmx1b* mice, and their *Pet1-cre*,*Lmx1b*$^{+/-}$ offspring were mated with *Lmx1b*$^{flox/flox}$ mice (35, 36).

X-Gal Staining, Immunohistochemistry, Nissl Staining, and HPLC. *Pet1cre*, *Rosa26* mice were characterized by X-Gal staining and double immunostaining for Cre and 5-HT, and adult CKO mice were examined by immunostaining. The following antibodies were used: rabbit anti-5-HT (1:2000; Immunostar), rabbit anti-TH (1:2000; Chemicon), rabbit anti-Cre (1:1000; Babco), rabbit anti-Sert (1:2000; a gift from Randy D. Blakely, Vanderbilt University, Nashville, TN). Gross brain structure of adult CKO mice was examined by Nissl staining. Standard HPLC was performed to measure 5-HT and other monoamines in adult CKO and WT mice.

Behavioral Studies. ***Rotating rod test.*** Mice were placed on the rod and the latency fell from the rod was measured for each speed (5, 8, and 15 rpm). Each mouse was tested at each speed twice daily for 3 d and was placed on the rotating rod for a maximum of 15 min per trial.

Open field test. Mice were placed in a Plexiglas box enclosed in a sound-attenuated cage for a 5-min habituation period. Locomotor activity in the open field was then monitored for 30 min.

Morris water maze. Training and testing procedures were performed as described in ref. 13. Mice were trained for 9 days with four trials per day at intertrial intervals of 40–50 min. Performance in the spatial learning task was measured by timing escape latencies during each training sessions. The escape latencies of four trials per day were averaged for each animal. To test spatial memory, the hidden platform was removed and mice were subjected to retention tests at 1 d and 10 d after the spatial learning task. Memory retrieval was measured by quantifying the time spent in the target quadrant, the time taken to first cross the platform location, and the number of platform location crossings in a 1-min trial. Swim speed was also measured during both the spatial learning and memory retrieval tasks.

Elevated-plus maze test. The elevated-plus maze test was used to test anxiety-like behavior and has been described (38, 39). Mice were placed on the central platform facing a closed arm and allowed to freely explore the maze for 5 min. The percentage of time spent in the two open arms and the frequency of transitions made between open and closed arms were measured.

Novelty suppressed feeding test. The novelty suppressed feeding test procedures are similar to those described in ref. 40. After 1 day of food deprivation, mice were placed in a corner of a brightly lit arena for 6 min. Two pellets of food were attached by a wire to the circle of a Whatman paper at the center of the arena. The time taken to explore the novel environment to the point when the animal was sitting on its haunches and eating food with its forepaws was measured. After testing, mice were immediately returned to their home cage and allowed to feed for 5 min, and food consumption was measured.

Contextual fear conditioning. The procedures for contextual fear conditioning were similar to those described in ref. 24. Mice were placed in the box and allowed to freely explore for 2 min before receiving five foot shocks (1 mA, 2 s) with intershock intervals of 2 min (MED Associates). Mice were then placed back in their home cages 2 min after the final foot shock. Freezing behavior was measured as the amount of time exhibiting freezing behavior during each intershock interval. To study contextual fear memory, mice were placed in the conditioned fear context 30 min, 1 d, and 10 d after fear conditioning and their contextual freezing behavior was measured for 11 min without any foot shocks.

To study whether aberrant contextual fear memory in CKO mice is caused by the loss of central 5-HT, 0.5 μl of 2 mM 5-HT in saline solution (Sigma) was injected into the lateral ventricle under ether anesthesia or a specific 5-HT1A receptor agonist DPAT was administered i.p. in saline solution (1 mg/kg; Sigma). Contextual fear memory testing was performed 40 min after recovery from anesthesia or i.p. injection of DPAT.

Electrophysiology. Mice were subjected to electrophysiological studies immediately after five foot shocks. Field excitatory postsynaptic potentials (fEPSPs) in the CA1 area were recorded in coronal hippocampal slices as described in our previous study (53). LTP was induced by HFS (three trains of 100 stimuli at 100 Hz separated by 20-s inter-train intervals) and LTD was induced by LFS (LFS; 900 pulses at 1 Hz), with the same stimulation intensity used for baseline recordings, in which fEPSP amplitude was set at 50% of the maximal response. 5-HT (2 μM; Sigma) was added in artificial cerebrospinal fluid (ACSF) and 5-HT1A agonist DPAT (400 nM; Sigma) was added to ACSF as described in *Results*.

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