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Selectively enhanced contextual fear conditioning in mice lacking the transcriptional regulator CCAAT/enhancer binding protein δ

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ABSTRACT **CCAAT/enhancer binding protein δ (C/EBPδ) is a transcriptional regulator implicated in the hepatic acute phase response and in adipogenic and myeloid cell differentiation. We found that C/EBPδ is widely expressed in the peripheral and central nervous systems, including neurons of the hippocampal formation, indicating a role in neural functions. To examine the role of C/EBPδ *in vivo*, we generated mice with a targeted deletion of the C/EBPδ gene. This mutation does not interfere with normal embryonic and postnatal development. Performance in a battery of behavioral tests indicates that basic neurological functions are normal. Furthermore, performance in a Morris water maze task suggests that C/EBPδ mutant mice have normal spatial learning. However, in the contextual and auditory-cue-conditioned fear task, C/EBPδ null mice displayed significantly more conditioned freezing to the test context than did wild-type controls, but equivalent conditioning to the auditory cue. These data demonstrate a selectively enhanced contextual fear response in mice carrying a targeted genomic mutation and implicate C/EBPδ in the regulation of a specific type of learning and memory.**

The CCAAT/enhancer binding protein (C/EBP) family of transcriptional regulators is composed of five related basic-leucine zipper DNA-binding proteins (C/EBPα, C/EBPβ, C/EBPδ, C/EBPε, and Ig/EBP) that recognize a common DNA sequence and exhibit similar leucine zipper dimerization specificities (1). Several observations suggest that, in addition to many other regulatory functions, C/EBPs are involved in learning and memory. For example, a C/EBP in *Aplysia* (ApC/EBP) plays an essential role in synaptic plasticity associated with long-term facilitation in sensory neurons (2). Furthermore, C/EBPβ and C/EBPδ expression is induced by pituitary adenylate cyclase-activating peptide in astrocytes (3) and *amnesia*, its *Drosophila* homolog, is known to modulate memory storage (4). Additionally, glutamate, which is implicated in synaptic mechanisms of learning and memory (5), modulates C/EBPβ and C/EBPδ expression in astrocytes (6).

In the present report, we have examined the role of C/EBPδ (a.k.a. CRP3, NF-IL6β, and CELF) in mice. Previous studies showed that C/EBPδ functions as a transcriptional activator in transactivation assays. Although low levels of C/EBPδ RNA are detectable in several organs of adult mice, expression is dramatically induced by bacterial lipopolysaccharide and inflammatory cytokines, suggesting a role in the acute phase and inflammatory responses. Furthermore, C/EBPδ expression is induced during differentiation of specific cell lines to adipocytes or granulocytes (1). C/EBPδ is also widely expressed in the murine nervous system (this report), similar to C/EBPβ (7). To address the role of C/EBPδ *in vivo*, we generated mice with a targeted deletion of the C/EBPδ gene. The mutant animals are viable and healthy and perform normally on several behavioral tasks, but exhibit enhanced contextual fear conditioning. These data demonstrate that a C/EBP gene is involved in learning and memory in mammals.

MATERIALS AND METHODS

Generation of C/EBPδ-Deficient Mice. The replacement-type targeting vector was constructed as indicated in Fig. 2 by using 129/Sv mouse genomic DNA (Stratagene) and the pGKneobpA and pGK-thymidine kinase cassettes (8). Electroporation and selection were performed as described by using the CJ7 embryonic stem (ES) cell line (9) isolated from a Sl^+ derivative of the 129/SvJR2448 strain (10). Two independent ES cell clones with the predicted rearrangements (nos. 433 and 466) were injected into C57BL/6 blastocysts to generate chimeras, which were mated to C57BL/6 females.

Histology and *in Situ* Hybridization. Tissues were prepared and analyzed by *in situ* hybridization as described (11). The C/EBPδ-specific 540-bp antisense rat cRNA probe corresponds to amino acids 1–181 of the coding region.

RNA Analysis. RNA was prepared and analyzed by Northern blotting as described (12). DNA probes were a cDNA clone for cyclophilin (13), the coding region of the rat C/EBPδ gene without the basic-leucine zipper domain, and the 3′ untranslated regions of the rat C/EBPβ and C/EBPα genes (14).

Measurement of Monoamine Levels. HPLC with electrochemical detection was used to measure brain levels of monoamines and their metabolites as previously described (15). For norepinephrine and 3-methoxy-4-hydroxy-phenylglycol, methanol content in the mobile phase was decreased to 2.5%.

Test Animals. Subjects were derived from crosses between $C/EBP\delta^{+/-}$ animals. Experiment (Exp.) 1 and Exp. 2 represent independent experimental groups evaluated for open-field activity, acoustic startle response, and contextual fear conditioning. In Exp. 1, there were 13 wild-type (wt) mice [eight male (M) and five female (F)] and 21 mutant mice (13 M, eight F) derived from both independent ES cell lines and representing either F_2 or F_4 generations of strain intercrosses ($433/F_2$: six wt, eight mutants; $466/F_4$: seven wt, 13 mutants). In Exp. 2, there were seven wt (five M, two F) and eight mutant

Abbreviations: C/EBP, CCAAT/enhancer binding protein; CREB, cAMP response element binding protein; ES, embryonic stem; wt, wild type; CS, conditioned stimulus; Exp., experiment; M, male; F, female.

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mice (five M, three F) of line 466/F_4. In Exp. 3 there were 14 mutant and 12 wt male mice of 466/F_2. Mice from Exps. 1 and 2 were tested on the rotarod, and in the light↔dark exploration and Morris water maze tasks, respectively. All behavioral testing was performed by experimenters blind to the genotype of the mice.

Gross Neurological Exam. Gross neurological function was assessed as described (16). The following behavioral responses were evaluated in six wt (three M, three F) and eight mutant mice (five M, three F): spontaneous behaviors in an empty cage and on an elevated platform; limb-extension reflexes in a moving cage; righting, eye-blink, and ear-twitch reflexes; whisker-orienting response; wire-suspension test, and vertical pole test (16).

Open-Field Activity. Locomotor activity was quantitated by using an open-field arena (RXYZCM, Omnitech Electronics, Columbus, OH). The ratio of center distance to total distance is considered a partially specific measure of anxiety-like behavior (17).

Light↔Dark Exploration Test. The light↔dark exploration test using a lighted open chamber and a dark closed chamber connected by a small opening with photocells was performed as described (18).

Motor Coordination and Balance. Motor coordination was tested by using an accelerating (4 to 40 rpm) Ugo Basile (Varese, Italy) rotarod. The time each mouse stayed on the rod was recorded in two trials.

Sensorimotor Behaviors. The acoustic startle response was measured by using SR-Lab Systems (San Diego Instruments) as previously described (19). In Exp. 1, the maximum response to stimuli ranging from 70 to 118 dB was recorded. In Exp. 2 the sounds ranged from 90 to 118 dB. Prepulse inhibition (PPI) of the acoustic startle response was measured as previously described (19). The acoustic startle stimulus was a 40-ms, 120-dB sound burst. Five different 20-ms acoustic prepulse stimuli (74–90 dB) were presented 100 ms before the startle stimulus. Three to 5 days later, PPI of a tactile startle response was measured. A 40-ms, 12-psi air puff was used as the tactile startle stimulus.

Contextual and Auditory-Cue-Conditioned Fear. Each mouse was placed in a test chamber inside a sound-attenuated chamber and allowed to explore freely for 2 min. A white noise (80 dB), which served as the conditioned stimulus (CS), was presented for 30 s followed by a mild (2 s, 0.5 mA) foot shock. The mouse was removed from the chamber 30 s later and returned to its home cage. Twenty-four hours (Exps. 1 and 2) or 30 min (Exp. 3) later, the mouse was placed back into the test chamber for 5 min, and the presence of freezing behavior was recorded every 10 s (context test). Two hours later, the mouse was tested for its freezing to the auditory CS. For the auditory CS test, the test chamber was modified (20), and freezing was recorded for 3 min without the auditory CS (pre-CS). Then, the auditory CS was turned on, and freezing was recorded for another 3 min. The dependent variable was the number of 10-s intervals when freezing was observed. For the auditory CS test, the number of freezing intervals obtained during the pre-CS period was subtracted from the number of freezing intervals obtained when the auditory CS was present (20).

Morris Water Maze Task. Mice were trained on the hidden platform version of the Morris water maze task as previously described (21). Each mouse was given 12 trials/day in blocks of four trials (30- to 60-min interblock intervals) for 4 consecutive days. The time taken to locate the escape platform was determined (escape latency). A 60-s probe trial was given after trials 36 and 48. Quadrant search time and platform crossings were obtained as previously described (22). The data for the two probe trials were averaged. Two days later, mice were given another block of four trials with the platform in its original location. The platform then was repositioned to a place in the opposite quadrant (reversal training). Mice then were given two blocks of four trials with the platform in its "reversed" place and two additional days of reversal-trial training (12 trials/day). After the last training trial, mice were given a 60-s probe trial.

Data Analysis. Data were analyzed with two-way or three-way ANOVA. Post-hoc comparisons were made with Newman-Keuls and simple effects tests. Where appropriate, student's *t* tests were used.

RESULTS

C/EBPδ Expression in Neural Tissues. Fig. 1*A* shows a coronal section of the brain stained with cresyl violet for the neuron-specific Nissl substance. *In situ* hybridization analysis of an adjacent section (Fig. 1*B*) revealed a pattern of C/EBPδ expression that largely overlaps with the staining seen in Fig. 1*A*, indicating neuronal expression in many regions of the brain. Similar analysis of the spinal region (Fig. 1 *C* and *D*) showed that C/EBPδ is highly expressed in the neurons of dorsal root and sympathetic ganglia, as well as in the ventral and dorsal horns of the spinal cord. The sections also demonstrate expression of C/EBPδ in the bone marrow. A sense control probe did not generate significant hybridization signals (data not shown).

Cultured astrocytes express C/EBPδ (6) and norepinephrine or certain neuropeptides further induce its expression in these cells (3). Thus, some of the hybridization signal seen in Fig. 1 may be caused by astrocytes. However, expression in distinct neuronal populations was observed, including the granule neurons of the dentate gyrus, the pyramidal neurons of the hippocampus, sensory and sympathetic neurons of the peripheral nervous system (PNS), and motoneurons. We conclude that C/EBPδ is widely expressed in the central nervous

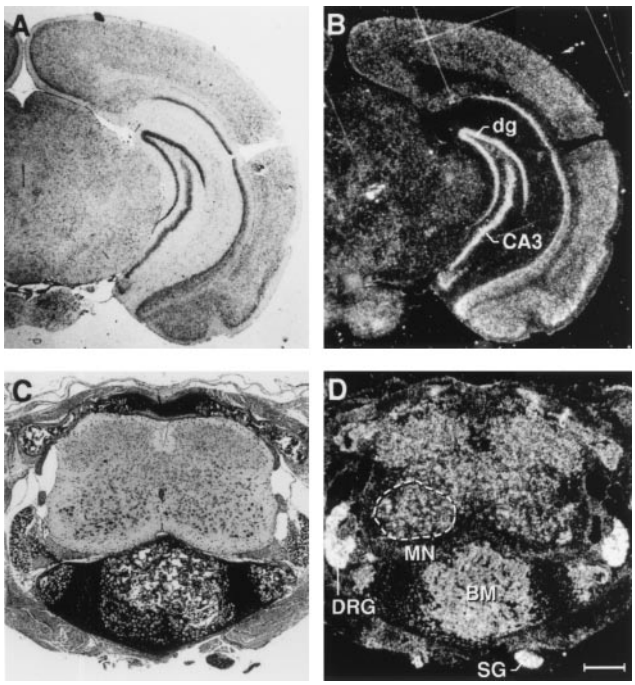

FIG. 1. Expression of C/EBPδ in the nervous system. Photomicrographs of coronal sections of adult mouse brain (*A* and *B*; ×12.5) and cross sections of lumbar spinal cord of a 1-week-old mouse (*C* and *D*; ×50) stained for Nissl substance (*A* and *C*) or after *in situ* hybridization to a C/EBPδ-specific antisense cRNA probe (*B* and *D*). dg, dentate gyrus; CA3, pyramidal layer of the hippocampus; DRG, dorsal root ganglion; SG, sympathetic ganglion; MN, motoneurons; BM, bone marrow. (The scale bar in *D* represents 20 μm.)

system and PNS, suggesting a role in the development or function of neurons.

Generation of C/EBPδ Null Mice. To address the role of C/EBPδ in the brain, we generated mice with a targeted deletion of the C/EBPδ gene (Fig. 2 *A* and *B*). Northern analysis of tissue RNA confirmed that C/EBPδ-specific mRNA is not expressed in mutant mice (Fig. 2*C*). Mice homozygous for the targeted deletion were obtained at Mendelian frequency, indicating normal embryonic development. Female and male C/EBPδ null mice were fertile, and gross histological analysis of adult animals suggested normal postnatal development. Differential cell counts of peripheral blood, blood smear analysis, and measurements of core body temperature and body weights of mutant mice were normal (data not shown). Thus, C/EBPδ$^{-/-}$ animals do not display overt developmental or physiological defects.

Levels of C/EBPα or C/EBPβ transcripts in total brain (data not shown) and the hippocampal formation (Fig. 2*D*) were equivalent in wt and mutant mice, demonstrating that gross changes in expression of other C/EBP genes did not occur as a result of C/EBPδ deficiency. We also analyzed expression of the cAMP response element binding protein (CREB). Preliminary data suggest similar levels of CREB mRNA in wt and mutant brain and hippocampus (data not shown).

Neurochemical Assessment of Mutant Mice. Considering the abundant expression of C/EBPδ in the brain (Fig. 1*B*), its potential to be regulated by norepinephrine (3), and the established interaction of the noradrenergic and serotonergic systems in the brain (23), we measured brain monoamine levels in C/EBPδ null mice. Selected brain regions from six mutant and six wt 3-month-old male littermates were analyzed for serotonin and 5-hydroxyindoleacetic acid (striatum, frontal cortex, and hippocampus), dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid (striatum and frontal cortex), and norepinephrine and 3-methoxy-4-hydroxy-phenylglycol (frontal cortex). Regional levels of monoamines and their major metabolites were consistent with our previous measurements in mice using similar techniques (24) and did not differ significantly between wt and mutant mice (data not shown). These results indicate that C/EBPδ deficiency does not cause gross brain neurochemical abnormalities, at least with respect to total tissue monoamine content.

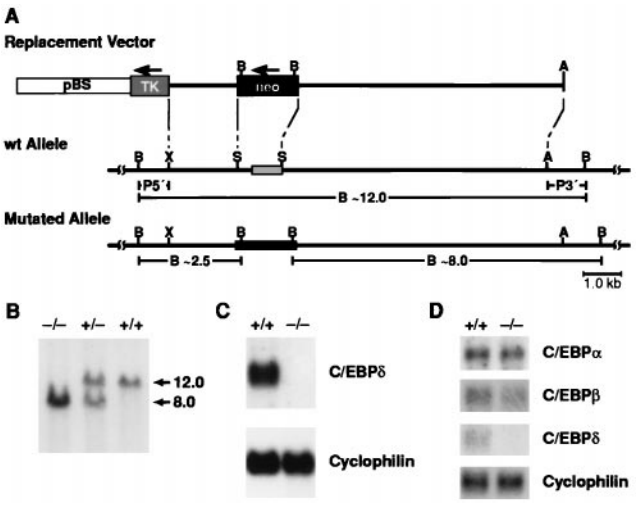

FIG. 2. Targeted mutation of the C/EBPδ gene. (*A*) Diagram of the targeting vector, wt allele, and mutated allele. The coding region was replaced by a neomycin-resistance gene. Homologous recombination at the 5′ side and the 3′ side of the gene was screened by probes (P5′ and P3′) that detect the conversion of a 12.0-kb *Bam*HI fragment into 2.5 and 8.0 kb, respectively. B, *Bam*HI; X, *Xba*I; S, *Sma*I; A, *Apa*I. (*B*) Southern blot analysis of *Bam*HI-digested genomic mouse DNA probed with P3′ (see *A*). The 12.0-kb fragment diagnostic of the wt allele (+/+) and the 8.0-kb fragment diagnostic of the mutated allele (−/−) are indicated. (*C*) Northern blot analysis of total RNA extracted from kidney tissue of adult wt and mutant animals hybridized sequentially to probes for C/EBPδ and cyclophilin. (*D*) Expression of C/EBP genes in the hippocampal formation. Northern blot analysis of 10 μg of total RNA isolated from the hippocampal formation of 3-month-old wt (+/+) and C/EBPδ-deficient (−/−) female mice. The blot was hybridized sequentially with probes for the indicated genes.

Evaluation of Basic Neural Functions of C/EBPδ Null Mice. When compared on a battery of behavioral tests (see *Materials and Methods*) C/EBPδ$^{-/-}$ mice behaved similarly to wt mice. In the following tests, the performance of wt and mutant mice was not significantly different ($P \geq 0.09$): locomotor activity and rearing (Fig. 3 *A* and *B*); rotarod performance (Fig. 3*C*); light↔dark transitions (Fig. 3*D*); center/total distance ratio in the open field (Fig. 3 *E* and *F*); acoustic startle responses (Fig. 4 *A* and *B*); and prepulse inhibition of the acoustic and tactile startle response (Fig. 4 *C* and *D*). These data demonstrate that C/EBPδ null mice exhibit normal sensory and motor functions, locomotor activity, and anxiety-related responses.

Evaluation of Learning and Memory in C/EBPδ Null Mice. Conditioned fear and spatial learning were assessed to evaluate learning and memory functions in the mutant mice. During the 24-hr context test the number of freezing intervals

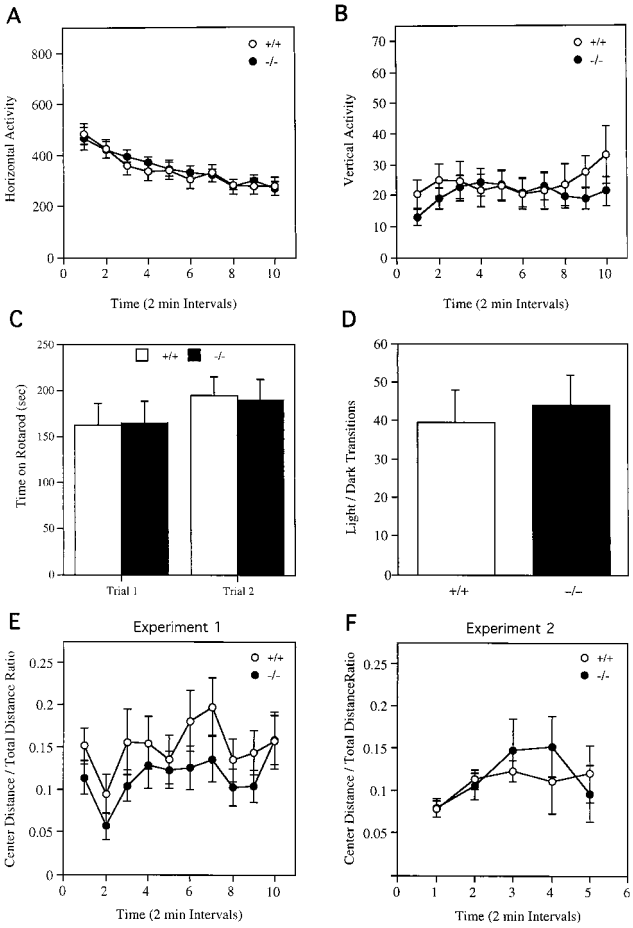

FIG. 3. Behavioral responses of C/EBPδ mutant and wt mice in open-field, rotarod, and light↔dark exploration tests. Horizontal activity (*A*) and vertical activity (*B*) in the open-field test. (*C*) The time spent on the accelerating rotarod across two trials. (*D*) The number of light↔dark transitions in the light↔dark exploration test for anxiety-related behaviors. Two sets of mice (*E*, Exp. 1 and *F*, Exp. 2) were used to assess anxiety-related behaviors in the open-field test by calculating the ratio of center distance to total distance. Data represent the mean ± SEM.

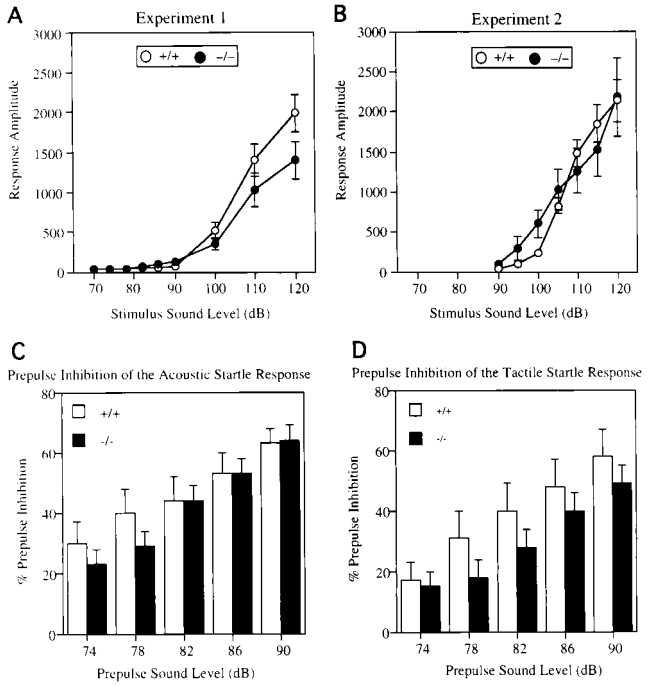

FIG. 4. Acoustic startle and prepulse inhibition in C/EBPδ-deficient and wt mice. The response amplitude to various sound stimuli in Exp. 1 (*A*) and Exp. 2 (*B*). Prepulse inhibition (%) of the acoustic startle response (*C*) and the tactile startle response (*D*). Data represent the mean ± SEM.

for mutant mice were 45.4% (Exp. 1) and 142.4% (Exp. 2) greater than the number of freezing intervals for wt mice (Fig. 5 *A* and *B*). These differences were significant in the two independent experiments (Exp. 1, $P < 0.02$; Exp. 2, $P < 0.047$). In contrast, mutant and wt mice displayed similar levels of freezing ($P > 0.35$) during the 30-min context test (Fig. 5*C*) and similar levels of auditory-cue-conditioned freezing ($P > 0.25$) when tested 24 hr after training (Fig. 5 *D* and *E*). These results show that mutant mice display a selective increase in contextual fear conditioning between 30 min and 24 hr of training.

In the Morris spatial learning task, the time to find the platform (Fig. 6*A*) was not different between mutant and wt mice ($P > 0.34$). During the probe trials (Fig. 6*B*), mice of both genotypes spent significantly more time in the training quadrant than in the other quadrants ($P > 0.0002$). Analysis of the escape latency data from the reversal trials (Fig. 6*C*) revealed a significant genotype X trial block interaction ($P < 0.04$). Simple-effects analysis revealed that the time to find the platform during reversal training did not change for mutant mice ($P > 0.14$), but did change for wt mice ($P < 0.001$). In addition, the escape latency for mutant mice was significantly ($P < 0.03$) faster than wt mice during the first block of four trials, but the escape latencies were similar on the remaining seven trial blocks ($P > 0.3$). During the reversal probe trial (Fig. 6*D*), both mutant and wt mice selectively searched the place in the pool where the platform had been located during the reversal training as measured by quadrant search time ($P < 0.01$). The data for the platform crossing measure were consistent with the quadrant search time data (data not shown). Therefore, spatial learning and memory appears to be similar in C/EBPδ$^{-/-}$ and wt mice.

DISCUSSION

To discern the specific regulatory functions of the C/EBP family members, we and others have introduced targeted mutations of C/EBP genes into the germ line of mice. Disruption of C/EBPα results in perinatal lethality caused by impaired energy homeostasis (25) as well as deficiencies of the hematopoietic system (26). Deletion of C/EBPβ results in female infertility (27) and multiple impairments of immune functions (28, 29), and the health of C/EBPβ$^{-/-}$ mice deteriorates within weeks to months after birth. In contrast, we have demonstrated that prenatal and postnatal development of C/EBPδ null mice is normal and the animals are healthy, although it is possible that defects would become apparent if the animals were challenged appropriately.

The observation that C/EBPδ mRNA is expressed in specific areas of the brain, including the hippocampal formation, prompted us to investigate the behavior of C/EBPδ null mice. Our results indicate that the C/EBPδ null mutation does not have a generally deleterious effect on sensory and motor functions or complex behaviors. However, C/EBPδ$^{-/-}$ mice displayed significantly more contextual-conditioned fear after a 24-hr retention interval. Because two independent experiments were performed with mice of F_2 and F_4 generations of strain intercrosses, derived from two independent ES cell clones, and with different training histories, it is unlikely that this difference between mutant and wt mice is the result of type I errors. This increase in conditioned fear is specific to the context, because mutant and wt mice showed similar levels of freezing in the training phase and in the auditory CS test. In addition, increased conditioned fear was not observed when mice were tested after a 30-min retention interval. Further, fear-like and anxiety-like behaviors appear normal in C/EBPδ$^{-/-}$ mice, as measured by freezing during the training and auditory cue test, and in the light↔dark anxiety test. In summary, our data show that C/EBPδ deficiency does not

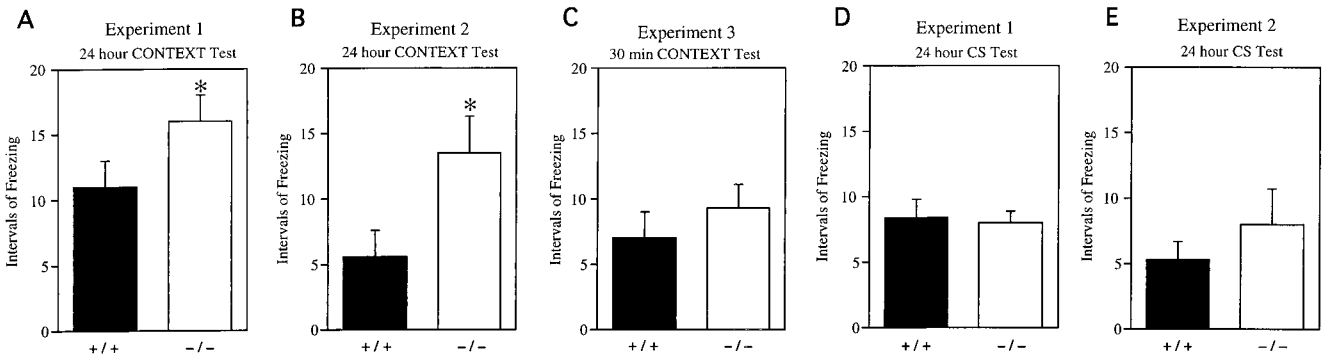

FIG. 5. Pavlovian conditioned fear in C/EBPδ-deficient (empty bars) and wt mice (filled bars). The mean (± SEM) number of freezing intervals during the 24-hr context test from Exp. 1 (*A*) and Exp. 2 (*B*). The mean (± SEM) number of freezing intervals during the 30-min context test from Exp. 3 (*C*). The mean (± SEM) number of freezing intervals during the auditory CS test from Exp. 1 (*D*) and Exp. 2 (*E*). *, indicate that the C/EBPδ$^{-/-}$ mice have significantly higher levels of freezing than wt mice during the 24-hr context test ($P < 0.05$).

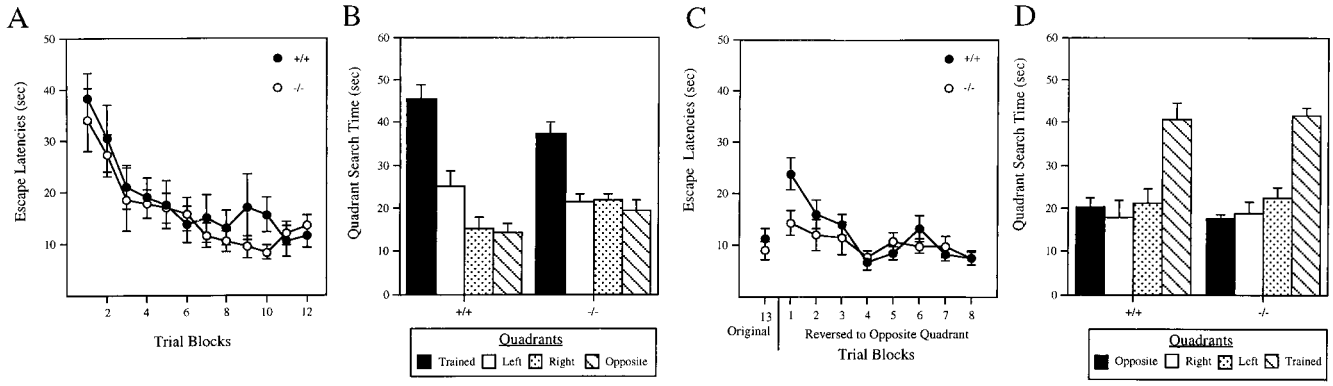

FIG. 6. Spatial learning performance of C/EBPδ mutant and wt mice in the Morris water maze task. (*A*) Escape latency (sec) during the 48 training trials presented in blocks of four trials. (*B*) Quadrant search time data for two probe trials. (*C*) Escape latencies during reversal training. (*D*) Quadrant search time for the probe trial after reversal training. Data represent the mean ± SEM.

impair simple associative learning (auditory CS test), spatial learning (Morris task), or the processes necessary for forming an association between contextual information and the foot shock and remembering this association over shorter (e.g., 30 min) delay intervals. In contrast, C/EBPδ deficiency affects the memory of contextual information over longer (e.g., 24 hr) delay intervals.

More extensive studies are required to better understand the role of C/EBPδ in the different phases (e.g., short term through long term) of memory. Future studies also will determine whether there are particular processes (e.g., formation, consolidation, and retrieval) of the 24-hr context memory effect that are altered by differences in C/EBPδ. However, the current data indicate that C/EBPδ may play a role subsequent to the memory formation processes induced during training, because mutant mice displayed similar levels of freezing after 30 min. Abel *et al.* (41) showed that the expression of 24 hr, but not 60 min, contextual fear is sensitive to protein synthesis inhibition. To confirm that C/EBPδ is important for "long-term", but not "short-term," contextual memory, future studies will evaluate additional time points, as well as the effects of protein synthesis inhibitors.

In reversal training in the Morris task, C/EBPδ$^{-/-}$ mice found the escape platform faster than wt mice during the first block of four trials and showed no significant decrease in the time to locate the platform during reversal training. Considering that mutant and wt mice showed similar selective search patterns during the reversal probe trial, the small difference during the first block of training may not reflect a reliable difference between reversal learning processes of wt and mutant mice. Further detailed investigations of reversal learning in C/EBPδ mutant mice will be necessary to delineate the relevant components of reversal learning.

Recently, another targeted mutation, disruption of the monoamine oxidase A (MAO-A) gene, was reported to cause enhanced contextual fear conditioning (30). This mutant also displayed more auditory-cue-conditioned fear, but not eyeblink conditioning, suggesting that MAO-A deficiency results in enhancement of emotional, but not motor, learning. The MAO-A mutant mice displayed elevated levels of total serotonin and norepinephrine in the cortex and hippocampus (30). The mechanisms underlying the improved performance in MAO-A and C/EBPδ mutant mice may be different, because C/EBPδ mutant mice did not display more auditory-cue-conditioned fear, and serotonin and norepinephrine levels were normal in C/EBPδ mutant cortex.

Our finding that C/EBPδ$^{-/-}$ mice display an enhancement in contextual fear conditioning but not spatial learning in the Morris task was unexpected, because most targeted mutations have affected performance in both tasks (31, 32). However, it is possible that wt and C/EBPδ mutant mice could be distinguished on the Morris task if different training protocols were used. For example, Fyn$^{-/-}$ mice and CREB mutant mice display differential spatial learning performance that depends on the nature of the training protocol (33–35). The specific training protocol used in this study was chosen because it is used routinely for mutant and inbred mice (36, 37). Previous studies have shown that different genetic mechanisms can contribute to contextual fear conditioning and spatial learning. For example, comparison of 13 inbred strains of mice (37) and of BXD RI strains tested on both the Morris task (38) and contextual fear-conditioning test (39) showed that performance in one task does not correlate with performance in the other task. Therefore, even though hippocampal damage can impair both spatial learning and contextual fear conditioning, the cellular and molecular events contributing to these types of learning can clearly be dissociated.

In species as distant as *Aplysia*, *Drosophila,* and mice, cAMP-mediated signaling has been linked to the formation of long-term memory, in particular of spatial information (40). Protein kinase A (41), cAMP phosphodiesterase (42), adenylyl cyclase (43), and CREB (34) are among the genes found to be essential for normal performance in learning and memory tasks. Interestingly, several reports link C/EBP proteins to cAMP signaling pathways. In astrocytes, expression of C/EBPβ and C/EBPδ is induced by cAMP (3), and the C/EBPδ promoter contains a potential CREB binding site (44). CREB and C/EBP can compete for binding to a CRE, resulting in differential regulation of the somatostatin promoter (45). Furthermore, the ATF/CREB family member C/ATF heterodimerizes with C/EBPs and directs their binding to specific CRE-like sites (46). Lastly, cAMP signaling induces ApC/EBP expression, which is an essential step in establishing long-term facilitation in *Aplysia* sensory neurons (2). Thus, it is tempting to speculate that an intersection of C/EBP- and CREB-mediated signaling underlies the enhanced contextual fear conditioning of C/EBPδ mutant mice.

How might deletion of the C/EBPδ gene cause an enhancement in learning and memory? We cannot rule out that the behavioral phenotype of the mutants is caused by developmental defects that are not detected at the gross anatomical level. However, three recent reports offer potential molecular mechanisms. Hegde *et al.* (47) found that the ubiquitin C-terminal hydrolase is essential for long-term facilitation in *Aplysia.* Thus, regulated proteolysis may remove proteins that exert inhibitory effects on memory processes. Second, long-term facilitation in *Aplysia* sensory neurons can be achieved with stimuli that normally are not sufficient provided that ApCREB2, an inhibitor of CREB, is inactivated by antibodies (48). Thus, memory suppressor genes may exert inhibitory

constraints on memory storage (49). Third, glutamate, a mediator of synaptic plasticity correlated with learning and memory (5), triggers transient repression of C/EBPδ expression in astrocytes (6). Based on these results, one can hypothesize that C/EBPδ is a selective memory suppressor gene whose product must be removed for long-term memory to occur. A negative influence of C/EBPδ could result either from direct opposition of CREB function, similar to ApCREB2, or from activation of target genes whose products inhibit memory formation. Targeted deletion of the C/EBPδ gene thus would eliminate this inhibitor permanently and result in enhanced learning of the mutant mice. Future studies using C/EBPδ null mice may allow the dissection of distinct classes of memory at the level of genetics and reveal insights into the molecular events underlying learning and memory in complex organisms.

Note Added in Proof. During review of this manuscript, Tanaka *et al.* (50) also reported that C/EBPδ-deficient mice display no overt abnormalities.

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