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CREB$^{\alpha\delta-}$ deficient mice show inhibition and low activity in novel environments without changes in stress reactivity

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Abstract

The ability to respond to unexpected or novel stimuli is critical for survival. Determining that a stimulus is indeed novel requires memory to ascertain its lack of familiarity. As the long-term synaptic changes involved in memory formation require the cAMP response element binding protein (CREB), we examined the extent to which CREB is involved in responses to novel environments. These environments typically trigger an endocrine stress response. Thus, we measured behavioural and stress hormone responses to three novel and one familiar environment in mice with a targeted disruption of the alpha and delta isoforms of the CREB gene (CREB$^{\alpha\delta-}$ deficient mice). We found CREB$^{\alpha\delta-}$ deficient mice to be less active and more inhibited in the elevated plus maze, open field, and light/dark box, without showing differences in anxiety-like behaviour. This inhibition is unique to novel environments because these mice display a normal phenotype in the home cage, a familiar environment. Although CREB$^{\alpha\delta-}$ deficient mice exhibit altered behaviour in novel environments, they show normal reactivity to mild and moderate stress as both basal and stress levels of corticosterone are similar to those of wild-type controls. This is the first report of CREB$^{\alpha\delta-}$ deficient mice to: (i) show altered behaviour, not related to learning and memory-associated behaviours, upon initial exposure to environments and (ii) serve as an animal model that can dissociate locomotor activity from anxiety-like behaviour in novel environments.

Introduction

Novel environments elicit competition between curiosity (e.g. exploration) and fear (e.g. withdrawal or immobility) (O'Keefe & Nadel, 1978). Anxiety-like behaviour results from the conflict inherent in such approach-avoidance situations (Crawley, 2000). The degree of 'stress' organisms experience in new environments depends more upon how they assess and react to novelty than on the nature of novel stimuli (Akil & Morano, 1996). Animals' reactions to novel environments fall into two main emotional dimensions: anxiety-like (stress) behaviours and locomotion (Trullas & Skolnick, 1993; Rodgers & Johnson, 1995; Chaouloff *et al*., 1997; Ramos & Mormede, 1998; Grailhe *et al*., 1999; Griebel *et al*., 2000). These reactions to novelty are difficult to dissociate into two dimensions because they are intricately interconnected.

The hippocampus (HPC) helps detect novelty by assessing the nature of external stimuli and their degree of familiarity, thereby guiding an animal's behavioural responses (O'Keefe & Nadel, 1978). Classifying objects as novel or familiar requires comparison with memory. The HPC is part of a key circuit responding to unexpected or novel stimuli and is critical for survival and memory formation (Lemaire *et al*., 1999).

A correlate of novelty is activation of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. During stress, such as when encountering a novel environment, the LHPA axis activates synthesis and release of adrenal steroids (e.g. corticosterone in rodents) into the peripheral circulation. The HPC, rich in glucocorticoid receptors to which corticosterone binds, is also a key regulator of the LHPA (Morimoto *et al*., 1996). Thus, disrupted hippocampal function may alter reactions to novelty along one or both emotional dimensions (i.e. anxiety-like behaviour and locomotion) and modify the associated stress responses.

CREB activates transcription of genes required for long-term synaptic changes involved in memory formation (Dash *et al*., 1990; Bourtchuladze *et al*., 1994; Yin *et al*., 1994; Guzowski & McGaugh, 1997). CREB$^{\alpha\delta-}$ deficient mice have a targeted disruption of the alpha and delta isoforms of the CREB gene (Hummler *et al*., 1994) and exhibit disrupted hippocampal function. They have impaired long-term-potentiation (Bourtchuladze *et al*., 1994) and altered ability to code space, as demonstrated by decreased spatial selectivity and stability of hippocampal place cells (Cho *et al*., 1998). CREB$^{\alpha\delta-}$ deficient mice exhibit impaired long-term memory in at least three distinct tasks: contextual fear conditioning, Morris water maze, and socially transmitted food preferences (Bourtchuladze *et al*., 1994; Kogan *et al*., 1997). This impairment, however, is not evident under all learning/testing conditions (Kogan *et al*., 1997) (E. K. Hebda-Bauer, S. J. Watson and H. Akil, unpublished results). These mice also show increased anxiety in the elevated plus maze (Graves *et al*., 2002). As CREB mRNA and protein are abundant in the HPC (Hummler *et al*., 1994; Blendy *et al*., 1996), and assessment of novel stimuli is important for forming and updating memories, we examined the extent to which CREB is involved in responses to novelty. We measured behavioural and stress hormone responses to three novel and one familiar environment in CREB$^{\alpha\delta-}$ deficient mice, and asked whether these mice show disruptions in the neuroendocrine stress response or the emotional dimensions: anxiety-like behaviour and locomotion.

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Materials and methods

Animals

CREB$^{\alpha\delta-}$ deficient mice were originally generated in the laboratory of Gunther Schutz (Hummler *et al*., 1994). They were initially obtained for our laboratory from Alcino Silva as F2 progeny derived from a cross between CREB$^{\alpha\delta-}$ deficient heterozygotes in the C57BL/6 background (> 87%) and wild-type 129SvJ mice. Thus, the genetic background of the wild-type and mutant mice subsequently bred and used for all the experiments described below consists of approximately a 50% contribution of genes from each of the C57BL/6 and 129SvJ strains. Approximately 15% of the newborn pups are homozygous for the CREB$^{\alpha\delta-}$ mutation, consistent with that of Silva's laboratory (J. Kogan, UCLA, personal communication, 1998).

All the mice used in the experiments were 3–6 months of age. The wild-type (WT, +/+) mice were age and sex-matched to the CREB$^{\alpha\delta-}$ heterozygous (+/–) and homozygous (–/–) deficient mice. Mice were group-housed in a temperature and humidity-controlled room with free access to food and water. They were maintained on a 14-h light : 10-h dark cycle (lights on at 06:00 h, lights off at 20:00 h). All behavioural testing, except for the 24-h home cage activity monitoring, was conducted between 08:00 h and 12:00 h. Thus, behavioural responses were obtained before the normal diurnal rise in corticosterone. As the purpose of the study was to assess behaviour in novel environments, different groups of mice were used for each behavioural test. The experiments were conducted in accordance with the guidelines of the University Committee on the Use and Care of Animals at the University of Michigan.

CREB$^{\alpha\delta-}$ polymerase chain reaction (PCR) genotyping

Mice were genotyped by PCR analysis. Tail biopsies were obtained at weaning and digested in 600 μL of TNES (10 mM Tris, pH 7.5, 400 mM NaCl, 100 mM EDTA, and 0.6% SDS) and 35 μL of Proteinase K (10 mg/mL) overnight at 57 °C. The next day, 166.7 μL of saturated NaCl was added and mixed. After centrifugation (20,800 ***g*** for 5 min) and recovery of the supernatant were performed twice, an equal volume of 100% EtOH was added and the DNA was spooled, dipped briefly in 70% EtOH, allowed to dry, and then resuspended in TE (10 mM Tris, 1 mM EDTA). One microlitre of the DNA was used directly in a PCR reaction. For genotyping CREB$^{\alpha\delta-}$ deficient mice, the following PCR primers were used:
CREB1 5′-CCATATTATTGTAGGTAACTAAATGA-3′, CREB2, 5′-ATGTATTTTTATACCTGGGC-3′, and
NEO, 5′-ATGATGGATACTTTCTCGGCAAGG-3′.
The following PCR conditions were used in a Peltier Thermal Cycler (PTC-2000, MJ Research): 4 °C for 180 s; 94 °C for 90 s; 40 cycles of 93 °C for 45 s, 47 °C for 45 s, and 72 °C for 90 s; then 72 °C for 600 s.

Behavioural testing

A videotracking system (Ethovision, Noldus Technology) was used to collect behavioural data during the following tests.

Elevated plus maze

Thirty-six mice (n = 12 +/+, 12 +/– , and 12 –/–) were tested in the elevated plus maze (EPM). The apparatus has four arms that are elevated 51 cm from the floor. Each arm is 27 cm in length and 6 cm in width. The arms are arranged in a cross, with two opposite arms being enclosed by 14 cm high clear acrylic walls. The other two arms are open. At the intersection of the four arms is a central 8 × 8 cm square platform giving access to all arms. Mice were gently placed in the centre area and their behaviour monitored for 5 min. Dependent measures included: (i) time spent in the open arms, closed arms, and middle area; (ii) number of entries into the open arms, closed arms, and middle area, and (iii) distance travelled in the whole maze. Testing occurred under dim lighting (97 lux).

Light/dark box

The light/dark (LD) box is 46 cm long with two-thirds of the length comprising the light compartment (made of white acrylic) and one-third comprising the dark compartment (made of black acrylic with a lid). A small (10 cm wide × 4 cm long) middle area by the door connecting the light and dark compartments was identified so the videotracking system (Ethovision, Noldus Technology) could determine when all four paws of a mouse entered either the light or dark compartment. This area could not be observed by the naked eye. Mice were placed in either the light (n: 12 +/+; 12 +/–; and 12 –/–) or dark (n: 20 +/+; 20 +/–; and 17 –/–) compartment under either normal (150 lux) or low lighting, depending upon the test condition, and their behaviour was observed for 5 min. Independent groups of mice were used for each test condition. Dependent measures collected were: (i) latency to enter either the dark or light compartment (depending upon the test condition); (ii) time spent in the light, middle, and dark compartments; (iii) number of transitions between compartments, and (iv) distance travelled in the light and middle compartments. Distance travelled was adjusted for the time spent in the light and middle compartments to avoid the confound of mice who spend less time in a compartment are going to travel less distance in that compartment. This adjustment resulted in a more reliable indicator of exploration and made the comparison between test conditions easier. We were unable to measure distance travelled in the dark because our LD apparatus contains a cover over the dark compartment, thus, making that area not viewable by the overhead camera.

Open field

Fifty-seven mice (n: 20 +/+; 20 +/–; and 17 –/–) were tested in the open field (OF). The OF is 71 cm^2 and made of white acrylic. Mice were placed in the centre of the OF and their behaviour monitored for the 5- and 30-min tests. The 5- and 30-min tests were administered 2 weeks apart. Dependent measures included: (i) the latency to first enter the periphery (ii) the amount of time spent and (iii) the distance travelled in the centre (30.5 cm^2) and the periphery.

Home cage activity

Activity of 72 mice (n: 36 +/+ and 36 –/–) in their home cages (a familiar environment) was monitored for 48 h with the first 24 h considered adjustment to the experimental setup and the second 24 h considered home cage activity. As the videotracking system cannot track the activity of several animals in a home cage, a clear acrylic wall with holes was inserted in the centre of the home cage and a mouse placed on each side. Water bottles were inserted on both sides of the cage. This setup minimizes social isolation stress as two mice are able to smell and see each other, but not touch each other, within the same cage. The videotracking system collected three samples per second during the 24 h monitoring of activity. An infrared filter in the camera allowed uninterrupted tracking of the mice in the light and the dark.

Corticosterone levels

In the 5- and 30-min OF tests, blood was collected from different mice, with equal representation by genotype, at the following time points: baseline and 0, 15, 45, and 90 min after OF exposure. In the LD box test, blood was collected from each mouse 15 min after the test.

For plasma corticosterone (CORT) measurement, aliquoted samples (5 μL) from each animal were suspended in radioimmunoassay buffer and heated for 30 min at 70 °C to separate CORT from CORT binding globulin. Total CORT was assayed by radioimmunoassay using a rabbit antiserum (Ab 195) raised against B 21-hemisuccinate:BSA. Ab 195 cross-reacts 8% with cortisol, 1% with deoxycorticosterone and progesterone, and less than 0.1% with aldosterone, testosterone, and estradiol. ^{3}H CORT was used as tracer. The radioimmunoassay method used was developed in our laboratory. The detection limit of the radioimmunoassay was 1 pg of CORT, and the intraassay and interassay coefficients of variation were 2% and 3%, respectively.

Statistical analysis

Data were analysed using SAS, Statview, and Kaleidagraph statistical software. Two-way analysis of variance was used to analyse the EPM, LD box, and 5-min OF data. Thirty-minute OF data and home cage activity were analysed in 5-min and 60-min epochs, respectively, by two methods: (i) using two-way analysis of variance with repeated measures, and a Bonferroni correction for multiple tests, in the General Linear Model procedure in SAS and (ii) calculating the area under the curve (time vs. distance) using the trapezoid method as defined by Kaleidagraph's area integration macro. CORT levels were analysed using two-way analysis of variance in the General Linear Model procedure of SAS, using genotype and time point as the independent variables. Tukey's *posthoc* test was used to compare specific groups.

Results

Behaviour in novel and familiar environments

Elevated plus maze

Figure 1 illustrates the results of this test, which demonstrate significantly diminished locomotion in the CREB$^{\alpha\delta-}$ homozygous deficient mice but no significant changes in the proportion of time spent in the various components of the maze. All mice, WT and CREB$^{\alpha\delta-}$ heterozygous and homozygous deficient mice, spent very little time in the open arms of the elevated plus maze (Fig. 1A) and entered the closed arms much more frequently than the open arms (Closed Arms: 15.25 ± 1.27 times for WT, 12.42 ± 2.15 times for heterozygotes, 11.09 ± 2.26 times for homozygotes. Open Arms: 1.75 ± 0.59 times for WTs, 1.92 ± 0.73 times for heterozygotes, 2.27 ± 0.79 times for homozygotes; Fig. 1B). This strong preference for the closed arms was unexpected as the test took place under dim lighting conditions (97 lux), which should make the open arms less aversive. There were no significant differences, however, in the pattern of behaviour observed among the three genotypes (Time in Arms: Open $F_{2,33} = 0.49$, $P > 0.05$; Closed $F_{2,33} = 0.86$, $P > 0.05$; Middle $F_{2,33} = 0.74$, $P > 0.05$. Number of Arm Entries: Open $F_{2,33} = 0.09$, $P > 0.05$, Closed $F_{2,33} = 1.63$, $P > 0.05$; Middle $F_{2,33} = 0.90$, $P > 0.05$). By contrast, the CREB$^{\alpha\delta-}$ homozygous deficient mice exhibited significantly less locomotor activity than the WT and CREB$^{\alpha\delta-}$ heterozygous deficient mice during the 5-min test (homozygous 724.74 ± 57.41 cm vs. heterozygous 877.38 ± 84.04 cm and WT 999.47 ± 96.89 cm; $F_{2,33} = 3.58$, $P < 0.05$; Fig. 1C).

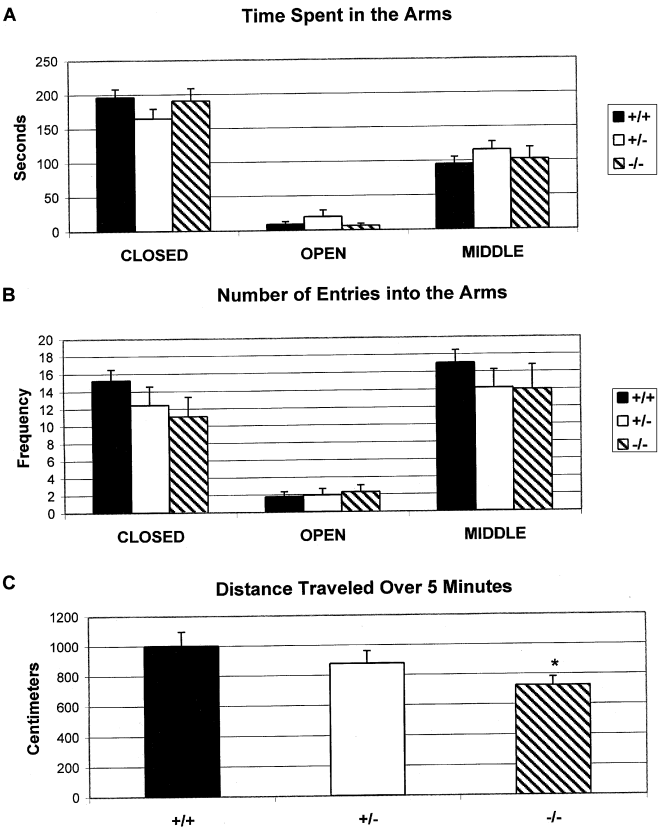

FIG. 1. Performance on the EPM. Data show the mean ± SEM. Wild-type, CREB$^{\alpha\delta-}$ heterozygous and homozygous deficient mice are designated as +/+, +/– and –/–, respectively. Behavioural measures are time spent in the arms (A), number of entries into the arms (B), and distance travelled during the 5-min test (C). Middle 'arm' was the middle area of the EPM where the mice were initially placed. CREB$^{\alpha\delta-}$ homozygous deficient mice travelled significantly less distances over the whole maze than WT and CREB$^{\alpha\delta-}$ heterozygous deficient mice. *$P < 0.05$.

Open field

In the 5-min OF test, all three genotypes entered the periphery within 13 ± 2.84 s and spent the majority of the 5 min (89%) in the periphery (Fig. 2A and B). At first glance, no differences among genotypes are found in the distance travelled in the OF (Center, $F_{2,46} = 1.29$, $P > 0.05$; Periphery, $F_{2,46} = 0.74$, $P > 0.05$; Arena (centre + periphery), $F_{2,46} = 1.02$, $P > 0.05$). When the data are analysed by genotype and sex, however, two-way ANOVAs are significant for distance travelled in the centre, periphery, and the whole OF (Center, $F_{5,43} = 2.70$, $P < 0.05$; Periphery, $F_{5,43} = 5.12$, $P < 0.001$; Arena, $F_{5,43}$ 5.67, $P < 0.001$). In all three ANOVAs, there are significant effects for sex (Center, $F_{1,43} = 8.28$, $P < 0.01$; Periphery, $F_{1,43} = 10.52$, $P < 0.01$; Arena, $F_{1,43} = 12.92$, $P < 0.001$), but not genotype (Center, $F_{2,43} = 1.50$, $P > 0.05$; Periphery, $F_{2,43} = 1.07$, $P > 0.05$; Arena, $F_{2,43} = 1.51$, $P > 0.05$). A genotype–sex interaction was found for distance travelled in the periphery and the whole arena, but not the centre (Periphery, $F_{2,43} = 6.48$, $P < 0.01$; Arena, $F_{2,43} = 6.20$, $P < 0.01$; Center, $F_{2,43} = 1.12$, $P > 0.05$). Thus, all male mice travelled less distance in the centre than did the female mice. Tukey *posthoc* comparisons show that male CREB$^{\alpha\delta-}$ homozygous deficient mice exhibited less locomotor activity in the periphery and the whole OF than WT and female CREB$^{\alpha\delta-}$ homozygous deficient mice ($F_{5,43} = 4.22$, $P < 0.05$; Fig. 2C).

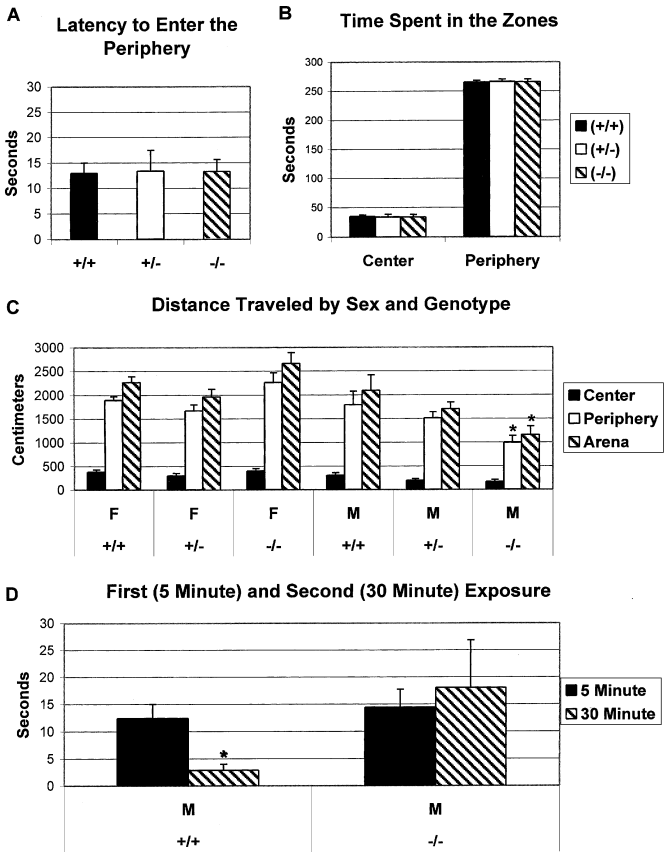

FIG. 2. Performance in the OF test. Data show the mean ± SEM. Wild-type, CREB$^{\alpha\delta-}$ heterozygous and homozygous deficient mice are designated as +/+, +/– and –/–, respectively. Female and male mice are designated as F and M, respectively. All mice exhibited a similar latency to first enter the periphery of the OF (A) and spent the majority of the 5-min test in the periphery (B). Male CREB$^{\alpha\delta-}$ homozygous deficient mice travelled significantly less distances in the OF across the 5-min test than all other mice (C). During the second exposure to the OF (30-min test), male CREB$^{\alpha\delta-}$ homozygous deficient mice took as much time to initially enter the periphery as they did during the 5-min test (D). In contrast, their WT counterparts entered the periphery significantly quicker during the second exposure (D). *$P < 0.05$.

Two weeks later when the mice were placed in the centre of the OF for a 30-min test, male CREB$^{\alpha\delta-}$ homozygous deficient mice remained in the centre longer, before entering the periphery, than all other mice. Comparison of the latency to first enter the periphery between the 5- and 30-min tests reveals that the male CREB$^{\alpha\delta-}$ homozygous deficient mice had a similar latency for both tests (14.37 ± 3.39 s and 18.07 ± 8.80 s, respectively; $t_5 = -0.479$, $P > 0.05$), while their male WT counterparts entered the periphery much earlier during the second exposure to the OF (first 12.35 ± 2.68 s vs. second 2.91 ± 1.12 s, $t_6 = 3.41$, $P = 0.01$; Fig. 2D). Once the mice entered the periphery, however, male CREB$^{\alpha\delta-}$ homozygous deficient mice exhibited similar locomotor activity to that of WT and CREB$^{\alpha\delta-}$ heterozygous deficient mice during the 30 min ($F_{2,19} = 1.34$, $P > 0.05$). Thus, male CREB$^{\alpha\delta-}$ homozygous deficient mice were inhibited when initially placed in the OF, appearing not to recognize the environment. After they became familiar with the environment, however, they behaved like WT mice.

CORT levels were measured at baseline and 0, 15, 45, and 90 min after removal from the OF in both the 5- and 30-min tests. Both OF tests produced a stress response in all mice, as demonstrated by significant effects of time for CORT levels (5-min test, $F_{4,42} = 7.39$, $P < 0.001$; 30-min test, $F_{4,42} = 24.17$, $P < 0.001$). As expected, the magnitude of the CORT response immediately after removal from the OF was greater in the 30-min vs. the 5-min test. This magnitude and the duration of the stress response were similar for WT and CREB$^{\alpha\delta-}$ deficient mice as baseline and stress levels of CORT did not differ among the genotypes at any time point in either test (5-min test: $F_{4,42} = 0.47$, $P > 0.05$; 30-min test: $F_{4,42} = 0.63$, $P > 0.05$; Fig. 3A and B). As different mice had to be used for each time point, the resulting sample size for each time point was not large enough to examine the effect of sex or the possibility of a sex–genotype interaction.

Light/dark box

When the mice were first placed in the light compartment, CREB$^{\alpha\delta-}$ homozygous deficient mice (males and females) took significantly longer to enter the dark compartment than the WT and CREB$^{\alpha\delta-}$ heterozygous deficient mice (homozygous 92.08 ± 32.31 s; WT 33.03 ± 13.47 s; heterozygous 25.10 ± 8.18 s; $F_{2,33} = 3.11$, $P < 0.05$; Fig. 4A). This result could have been construed as a decrease in anxiety-like behaviour. None of the other tests, however, suggested this phenotype. Thus, we reversed the starting position. When another group of mice was first placed in the dark compartment, all mice spent the majority of the 5-min test in the dark (data not shown). This experiment therefore was repeated with altered conditions. A third group of mice was tested under low lighting to make the light compartment less aversive and first placed in the dark compartment. Under dim lighting, CREB$^{\alpha\delta-}$ homozygous deficient mice remained in the initial starting compartment (i.e. dark compartment) longer than mice of the other two genotypes (Fig. 4B). This increased latency to enter the light compartment is due to the male CREB$^{\alpha\delta-}$ homozygous deficient mice that took significantly longer to enter the light compartment than all other mice ($F_{3,48} = 2.86$, $P < 0.05$; Fig. 4C).

Thus, CREB$^{\alpha\delta-}$ homozygous deficient mice, especially males, tended to remain in the initial starting compartment no matter whether it was in the light or the dark. The number of transitions among compartments and the distance travelled, further highlight the low activity level of the male CREB$^{\alpha\delta-}$ homozygous deficient mice. Male CREB$^{\alpha\delta-}$ homozygous deficient mice exhibited significantly fewer transitions among the LD compartments than WT controls under both test conditions (Begin in the light: $t_{11} = -3.173$, $P < 0.01$ and Begin in the dark: $t_{10} = -2.886$, $P < 0.05$; Fig. 4D). The males also travelled less distance than WT controls during the 5-min test when first placed in the light, but not the dark, compartment (Begin in the Light: light compartment $t_{11} = -2.189$, $P < 0.05$ and middle compartment $t_{11} = -2.354$, $P < 0.05$; Begin in the Dark: light compartment $t_{10} = -1.703$, $P > 0.05$ and middle compartment $t_{10} = -0.558$, $p > 0.05$; Fig. 4E).

Fifteen minutes after removal from the LD box, CREB$^{\alpha\delta-}$ deficient mice had similar CORT levels to those of WT mice ($F_{2,43} = 1.28$, $P > 0.05$; Fig. 5A). As expected, there was a sex difference in stress levels of CORT; females of all three genotypes exhibited higher CORT levels than that of males ($F_{1,43} = 24.04$, $P < 0.001$; Fig. 5B). No sex–genotype interaction in CORT levels was found ($F_{2,43} = 2.47$, $P > 0.05$).

Home cage activity

To determine whether alterations in locomotor activity are due to an overall alteration in motor function, the activity of CREB$^{\alpha\delta-}$ homozygous and WT mice was observed in home-like cages (a familiar

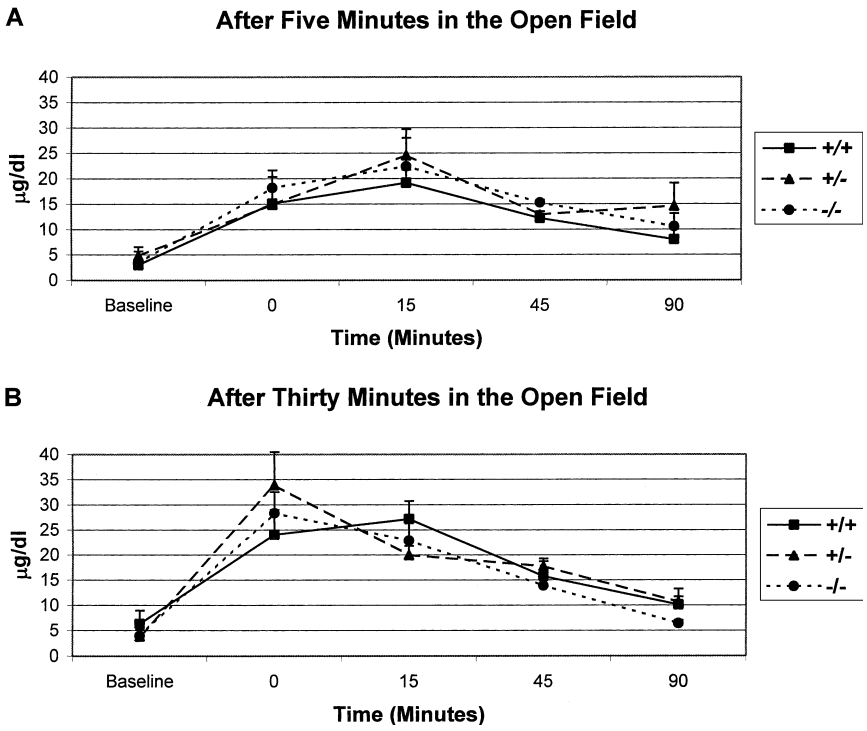

FIG. 3. CORT levels before and after the 5- and 30-min open field tests. CORT levels were measured at baseline and 0, 15, 45, and 90 min after removal from the open field in both tests. Data show the mean ± SEM. WT, CREB$^{\alpha\delta-}$ heterozygous and homozygous deficient mice are designated as +/+, +/– , and –/–, respectively. Baseline CORT levels did not differ among the genotypes (A and B). Stress levels of CORT did not differ among the genotypes at any time-point, but, as expected, the magnitude of the stress response was greater in the 30-min test (B) vs. the 5-min test (A).

environment), the least stressful of the four tested environments. Figure 6 shows that CREB$^{\alpha\delta-}$ homozygous mice displayed a normal light-dark circadian rhythm, which was similar to that of the WT mice. As expected, all mice were most active during the lights off period (from 20:00 h to 06:00 h), reflected in the significant effect of time ($F_{23,1586} = 65.28$, $P < 0.001$). Statistical analysis of home cage activity, with mean activity taken per hour, also revealed a significant effect of genotype and a time–sex interaction ($F_{1,69} = 4.09$, $P < 0.05$ and $F_{23,1586} = 3.18$, $P < 0.0001$, respectively). *Posthoc* analyses, however, reveal that mice of the two genotypes only showed a significant difference in activity level during one of the 24 h ($F_{1,1586} = 11.60$, $P < 0.002$), which was mainly due to the female WT mice. Figure 6 shows that the female WT mice displayed a more active pattern of locomotion during a small portion of the lights off period, than did all other mice. Importantly, both male and female CREB$^{\alpha\delta-}$ homozygous deficient mice showed similar locomotor activity levels to that of male WT mice throughout all of the 24 h tested. Further, area under the curve values for the distance travelled over the 24-h period do not show significant sex or genotype differences (female +/+ 52918.12 ± 2959.37; female –/– 45996.07 ± 2716.24; male +/+ 48406.28 ± 4209.72; male –/– 42060.69 ± 3580.73; Sex $F_{1,68} = 1.53$, $P > 0.05$; Genotype $F_{1,68} = 3.77$, $P > 0.05$).

Discussion

Findings from the present study show that CREB$^{\alpha\delta-}$ deficient mice, particularly males, are less active and more inhibited when placed in novel, mildly stressful environments. This inhibition is unique to novel environments because they display a normal phenotype in a home-like cage, a familiar environment. This inhibition is also not due to anxiety because CREB$^{\alpha\delta-}$ deficient mice do not differ from WT mice on traditional behavioural measures of anxiety. Although CREB$^{\alpha\delta-}$ deficient mice exhibit altered behaviour in novel environments, they show normal reactivity to mild and moderate stress as both basal and stress levels of CORT are similar to that of WT controls.

Some investigators, including our laboratory, have adopted the multiple test approach of dissecting out types of emotional behaviour. Various results have been reported suggesting that emotionality is a highly complex trait with distinct forms exhibited under different conditions (Ramos & Mormede, 1998). Nevertheless, several investigators have extracted two main emotional dimensions of novel environments, anxiety-related and locomotion factors (Trullas & Skolnick, 1993; Rodgers & Johnson, 1995; Chaouloff *et al*., 1997; Ramos & Mormede, 1998; Grailhe *et al*., 1999; Griebel *et al*., 2000). The first dimension relates to behaviours dealing with approach/avoidance conflicts (i.e. anxiety-like or fear-related behaviours). The second dimension most clearly describes locomotor activity. The independence of these two factors suggests that approach/avoidance towards aversive stimuli (i.e. anxiety-like behaviours) and locomotion in novel environments represent two dimensions of the emotional response. For the anxiety-related factor in the study of Ramos *et al*. (1997), animals tending to approach the centre of the OF, also approach the open arms of the EPM and the white compartment of the LD box. For the locomotion factor, animals that are highly active in the OF periphery are also highly active in the EPM, as measured by the number of total and closed arm entries. Accordingly, CREB$^{\alpha\delta-}$ homozygous deficient mice are inhibited and exhibit low activity, without anxiety-like behaviour, in the EPM, OF, and LD box. The behavioural phenotype of CREB$^{\alpha\delta-}$ deficient mice in novel environments suggests a distinction between anxiety-like behaviour and locomotor activity. The independence and consistency

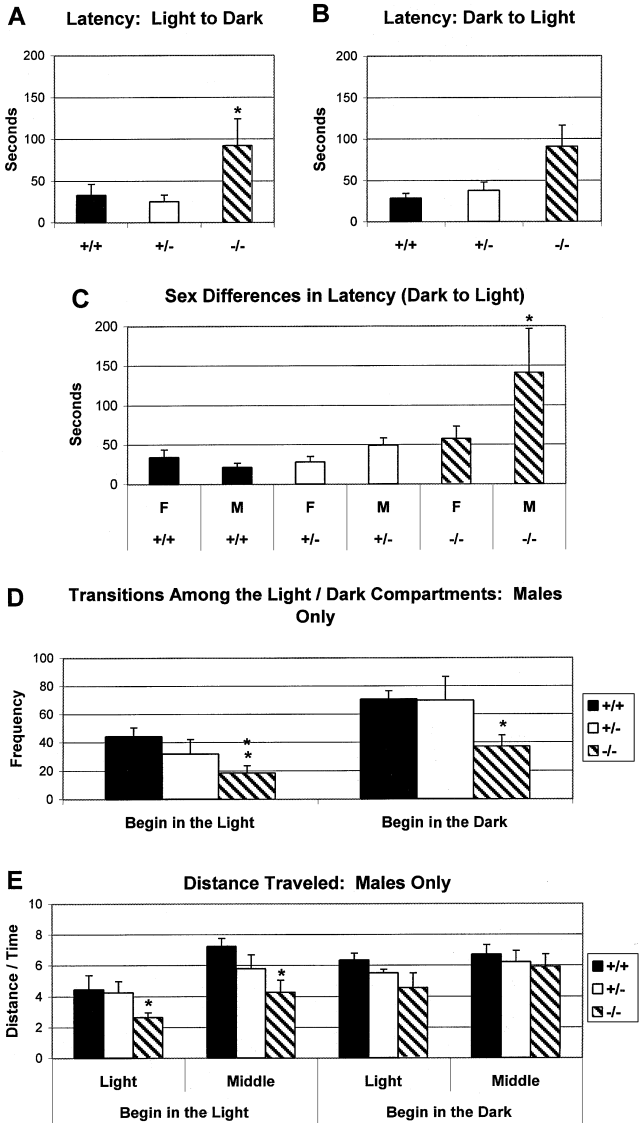

FIG. 4. Performance in the LD box. Data show the mean ± SEM. WT, CREB$^{\alpha\delta-}$ heterozygous and homozygous deficient mice are designated as +/+, +/–, and –/–, respectively. Female and male mice are designated as F and M, respectively. The middle compartment was a 10 cm wide × 4 cm long area by the door connecting the light and dark compartments. This area was only recognized by the Ethovision videotracking system and could not be seen with the naked eye. Separate groups of mice were initially placed in the light or dark compartments of the LD box. CREB$^{\alpha\delta-}$ homozygous deficient mice remained in the initial starting compartment longer than mice of the other two genotypes when initially placed in the light (A) or the dark (B). The increased latency to enter the light (B) is due to the male CREB$^{\alpha\delta-}$ homozygous deficient mice that took significantly longer to enter the light compartment than all other mice (C). Male CREB$^{\alpha\delta-}$ homozygous deficient mice also exhibited significantly fewer transitions among the LD compartments under both test conditions (D) and travelled significantly less distances when initially placed in the light compartment (E) than their WT counterparts. *$P < 0.05$, **$P < 0.01$.

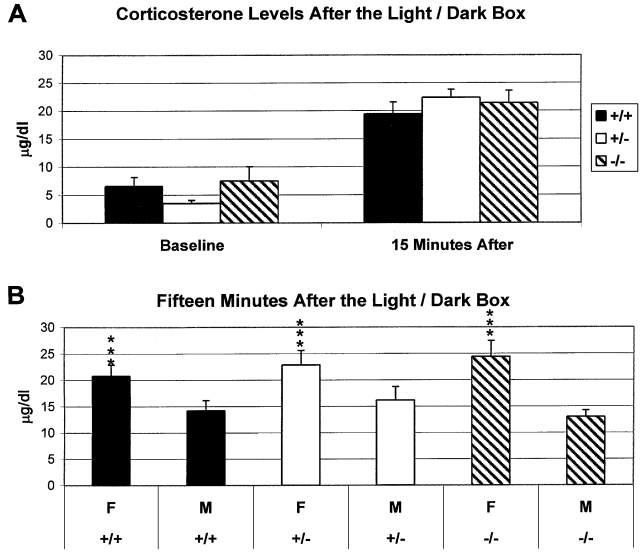

FIG. 5. CORT levels before and 15 min after the LD box test. Baseline CORT levels did not differ among the genotypes (A). A sex, but no genotype, difference in CORT level was found 15 min after removal from the LD box (B). As expected, all females exhibited higher stress levels of CORT than males. ***$P < 0.001$.

of these two factors across tests further emphasize the multidimensionality of the emotional response elicited in novel environments.

In the EPM, mice of all three genotypes spent very little time and had few entries into the open arms. These two measures have often loaded on the anxiety-related factor in other factor analyses (Grailhe *et al*., 1999; Lister, 1987; Trullas & Skolnick, 1993; Ramos & Mormede, 1998; Yilmazer-Hanke *et al*., 2003). The degree of aversiveness of the open, elevated arms appeared similarly high between the WT and CREB$^{\alpha\delta-}$ deficient mice. Interestingly, Graves *et al*. (2002) reported increased anxiety in CREB$^{\alpha\delta-}$ deficient mice, as demonstrated by a lower percentage of open arm entries and a trend towards less time spent in the open arms compared to WT mice. The EPM appears not to have been as aversive to the mice in the study of Graves *et al*. (2002) as the percentage of open arm entries and time spent for WT mice was 33% and 53%, respectively, compared to 12% and less than 10%, respectively, for WT mice in the current study. The experimental apparatus used in the study of Graves *et al*. (2002) is quite similar to that used in the current study, but illumination was not reported. Illumination differences may be responsible for this discrepancy between studies.

In contrast to time spent and number of entries into the open arms, examining locomotor activity during the 5-min test reveals that CREB$^{\alpha\delta-}$ homozygous deficient mice are less active and more inhibited. Such locomotion in the maze loads on the second factor, termed general motor activity, in the study of Trullas and Skolnick (1993). Graves *et al*. (2002) did not report locomotor activity of the CREB$^{\alpha\delta-}$ deficient mice in their study. Although not measuring locomotor activity (as measured by distance travelled in centimetres), several investigators have found that the total number of entries (into open and closed arms or usually closed arms only) loads on the activity factor (Lister, 1987; Cruz *et al*., 1994; Rodgers & Johnson, 1995; Fernandes & File, 1996; Espejo, 1997; Grailhe *et al*., 1999; Yilmazer-Hanke *et al*., 2003). Total number of entries into open and closed arms, however, was not decreased in the CREB$^{\alpha\delta-}$ homozygous deficient mice even though they travelled the least distance overall. Some investigators consider the total number of arm entries to be ambiguous when used as a main index of locomotion because it loads on both anxiety-related and activity factors in their analyses (Fernandes & File, 1996; Rodgers & Johnson, 1995; Ramos & Mormede, 1998). Thus, general ambulation in the EPM appears to be a better index of activity.

The validity of a locomotor activity factor in describing EPM behaviour has been questioned (Dawson *et al*., 1995a; Dawson & Tricklebank, 1995b; Weiss *et al*., 1998; Wall & Messier, 2001). Given

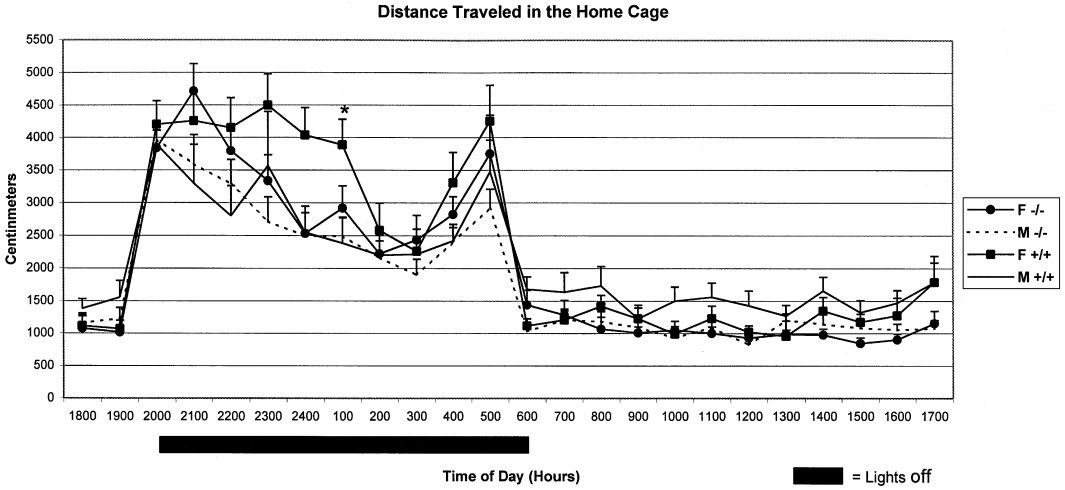

FIG. 6. Locomotor activity in a familiar environment – the home cage. Data show the mean ± SEM. WT and CREB$^{\alpha\delta-}$ homozygous deficient mice are designated as +/+ and –/–, respectively. Female and male mice are designated as F and M, respectively. In the home cage, male and female CREB$^{\alpha\delta-}$ homozygous deficient mice exhibited similar locomotor activity to that of male WT mice. As expected, all mice were most active during the lights off period (from 20:00 h to 06:00 h). Female WT mice exhibited higher locomotor activity during a portion of the lights off period, which only became significant during one hour. Area under the curve values for each of the four groups did not reveal any significant differences. *$P < 0.05$.

the disagreement among investigators about what measure(s) is (are) pure locomotor activity indicator(s) in the EPM and the results of their confirmatory factor analysis, Wall & Messier (2000) purport that 'locomotor activity' is not a valid EPM construct. Their second factor of a two-factor model, consisting of the number of closed arm entries and closed arm time ratio, is called 'closed exploration.' Dawson *et al.* (1995a, b) have shown that two anxiolytic drugs simultaneously increase locomotor activity (as measured by distance travelled) and open arm exploration, while one anxiogenic drug decreases locomotor activity and open arm exploration. These findings led Dawson *et al.* (1995a, b) to conclude that changes in the level of 'anxiety' in the EPM cannot be adequately dissociated from changes in locomotor activity. The distance travelled by CREB$^{\alpha\delta-}$ deficient mice in the current study, however, was significantly lower than that of WT mice without changes in anxiety-like indices. Locomotor activity (as measured by the distanced travelled) may be a valid EPM construct when examining dimensions of emotionality under drug-free conditions.

A number of 'ethological' parameters of the EPM have been examined in addition to traditional measures to increase the sensitivity of the EPM as a test for anxiety-like behaviour (Cruz *et al.*, 1994; Rodgers & Johnson, 1995; Fernandes & File, 1996; Espejo, 1997; Wall & Messier, 2000; Ohl *et al.*, 2001; Carola *et al.*, 2002). Common ethological measures, such as stretch attend postures, head dipping, sniffing, and rearing, are often considered risk assessment behaviours as they are a means of gathering information from the environment about potential sources of danger (Rodgers & Dalvi, 1997). As a result, several investigators have reported multiple factors describing EPM behaviour (Cruz *et al.*, 1994; Rodgers & Johnson, 1995; Fernandes & File, 1996; Holmes & Rodgers, 1998; Ohl *et al.*, 2001). Although the inclusion of some risk assessment behaviours enhances the sensitivity of the EPM to detect subtle changes in anxiety-like behaviour, Wall & Messier (2001) report that little evidence exists to support the inclusion of numerous variables in an analysis and a two-factor analysis best explains EPM behaviour. Risk assessment behaviours were not measured in the current study, except for time spent in the middle area of the EPM.

A third 'decision making or risk assessment' factor comprised of the time spent in the middle area of the maze has been reported (Trullas & Skolnick, 1993; Cruz *et al.*, 1994; Rodgers & Johnson, 1995), but not necessarily considered a stand alone measure of EPM behaviour (Wall & Messier, 2000). Mice in the present study did not spend much time in the middle area or the open arms of the EPM, but spent the majority of the time in the closed arms. This was surprising as the lighting in the room was dim and decreased open arm exploration has been reported under high illumination (Bertoglio & Carobrez, 2002). In another study comparing several strains of mice, C57BL/6 mice (one of the two progenitor strains of the CREB$^{\alpha\delta-}$ deficient mice) were reported to show high emotional reactivity in the EPM as they spent very little time in the open arms (Griebel *et al.*, 2000). Although some studies have also demonstrated the importance of the experimental set-up in the extent of responsiveness of the animal to anxiolytic compounds (Fernandes & File, 1996; Hogg, 1996), other investigators have found fear responses in the EPM to be fairly resistant to experimental manipulations (Pellow *et al.*, 1985; Falter *et al.*, 1992). For example, Falter *et al.* (1992) did not find differences in behaviour after changing the light intensity, the height of the apparatus, or the physical disposition of the arms. Regardless of these discrepancies, proprioceptive cues inherent to the EPM (i.e. contact with the walls of the closed arms vs. lack of contact in the open arms) under any experimental set-up are likely most salient. Indeed, rodents show much higher CORT levels when confined to an open arm as opposed to a closed arm (Pellow *et al.*, 1985). Thus, altering the experimental set-up of the EPM in the present study may not have changed the behaviour observed in the WT and CREB$^{\alpha\delta-}$ deficient mice.

Historically, the two most commonly used and accepted emotionality measures in the OF have been locomotion and defecation (Ramos & Mormede, 1998). Originally, the fear response of an animal exposed to a novel, potentially dangerous environment was believed to be characterized by a high defecation rate and low ambulation. According to this controversial view, ambulation should increase with repeated exposure to the OF, but several investigators report a decrease in ambulation with habituation (Vadasz *et al.*, 1992; Ramos & Mormede, 1998). Our data with WT and CREB$^{\alpha\delta-}$ deficient mice also show a

decrease in locomotor activity over three consecutive days of OF exposure (data not shown). In addition, intensifying the aversiveness of the environment by increasing light and noise levels has been demonstrated either not to change or to increase locomotor activity (Ramos & Mormede, 1998). In another study, C57BL/6 mice demonstrated much more ambulation in a bright vs. a dimly lit OF (Trullas & Skolnick, 1993). Under initial exposure to bright regular room illumination, which promotes increased ambulation, CREB$^{\alpha\delta-}$ homozygous deficient mice exhibited significant inhibition relative to their WT littermates. Thus, locomotion in the OF may not be a reliable index of anxiety but may reflect more of a particular manner of coping with environmental challenges. Along this view, reduced levels of CREB alter the way in which an organism copes with novelty.

CREB$^{\alpha\delta-}$ homozygous deficient mice travelled a much shorter distance in both the centre and periphery of the OF when compared to WT mice. Several studies report distance travelled in the whole OF or just the periphery as well as number of rears and hole pokes to be part of the activity factor (Trullas & Skolnick, 1993; Ramos & Mormede, 1998; Grailhe *et al*., 1999). Time in the centre and relative distance in the centre are considered part of the anxiety-related factor in Grailhe *et al*., 1999) study. Accordingly, the central area of the OF is considered more aversive because of rodents' tendency to avoid open spaces. In the present study, time in the centre did not differ among genotypes, but CREB$^{\alpha\delta-}$ homozygous deficient mice travelled less distance in the centre compared to WT mice. As CREB$^{\alpha\delta-}$ homozygous deficient mice were less active in both the centre and the periphery, however, their phenotype in novel environments points to an alteration in general activity instead of activity with a higher emotional component.

The behavioural inhibition of male CREB$^{\alpha\delta-}$ homozygous deficient mice is limited to the initial exposure to novel environments. When they were placed in the centre of the OF two weeks after the first exposure they stayed in the centre longer than all other mice, suggesting that male CREB$^{\alpha\delta-}$ homozygous deficient mice did not remember the environment. After that initial period, however, they behaved like WT mice with similar levels of locomotor activity, mainly in the periphery. Thus, their altered initial reaction to a novel environment soon gives way to normal behaviour once they familiarize themselves with that environment.

Mice in the current study were exposed to different test conditions when placed in the LD box; some were initially placed in the light and others were initially placed in the dark. Initial placement in the light has been reported to be more aversive than initial placement in the dark (Chaouloff *et al*., 1997). The WT mice in the present study, however, did not show this aversion to the light compartment when first placed in the light, even though test room illumination was brighter in this condition compared to when mice were initially placed in the dark. Chaouloff *et al*. (1997) also conveyed that locomotion-related variables prove insensitive to the protocol used (i.e. begin in the light vs. begin in the dark). In contrast, WT and CREB$^{\alpha\delta-}$ homozygous deficient mice (although to a lesser extent than the WT mice) in the current study exhibited more transitions among the LD compartments and travelled more distance in the light if they were initially placed in the dark. No matter which test condition was present, however, male CREB$^{\alpha\delta-}$ homozygous deficient mice remained in the compartment they were initially placed longer than all other mice. Some investigators may have missed this immediate inhibition because they do not begin recording behaviour until the animal has entered the dark compartment for the first time (Beuzen & Belzung, 1995; Griebel *et al*., 2000). This inhibition continued throughout the 5-min test in which the male CREB$^{\alpha\delta-}$ homozygous deficient mice made fewer transitions between compartments and travelled significantly less distances in the light compartment, although the distance measure was test-condition dependent (i.e. significant when the mice began in the light).

Many investigators disagree as to which measures best indicate anxiety level in the LD box (Crawley & Goodwin, 1980; Costall *et al*., 1989; Chaouloff *et al*., 1997; Ramos & Mormede, 1998). In general, however, increased latency to enter the dark compartment, increased time spent in the light compartment, and increased number of transitions are all viewed as indices of reduced anxiety in the LD box (Clement & Chapouthier, 1998). CREB$^{\alpha\delta-}$ homozygous deficient mice showed an increase in the latency to enter the dark, but a decrease in the number of transitions. Time spent in the light compartment was increased when initially placed in the light, but decreased when initially placed in the dark compartment. Thus, CREB$^{\alpha\delta-}$ homozygous deficient mice do not show consistent anxiety-like behaviour in the LD box, but demonstrate an altered method of coping with this novel environment by being inhibited (especially in the initial phase) and exhibiting diminished locomotor activity.

In the current study, we observed a number of gender specific differences in responsiveness to novelty. The behaviour of female CREB$^{\alpha\delta-}$ homozygous-deficient mice was no different from that of WT mice under some conditions; however, male CREB$^{\alpha\delta-}$ homozygous-deficient mice were always less active and more inhibited in novel environments. Although investigators of previous studies using CREB$^{\alpha\delta-}$ deficient mice do not find sex differences (Bourtchuladze *et al*., 1994; Kogan *et al*., 1997), the sex differences observed in the current study emphasize the importance of comparing sexes when studying various transgenic and knockout strains of mice. Sex differences may be observed in some behavioural tests but not others.

The novel environments used in the current study for measuring anxiety-like behaviours are all unconditioned response tests that involve locomotion. Normal anxiety-like behaviour of CREB$^{\alpha\delta-}$ deficient mice in tests that do not involve locomotion, such as the Vogel conflict test or fear conditioning, would further substantiate the present results. Although the current authors have not performed these tests, CREB$^{\alpha\delta-}$ deficient mice have been reported to show normal levels of initial freezing during cued and contextual conditioning with a deficit only after delayed intervals (Bourtchuladze *et al*., 1994; Kogan *et al*., 1997). Thus, CREB$^{\alpha\delta-}$ deficient mice display normal fear and anxiety-like behaviour when exposed to novel environments and aversive stimuli.

The EPM, OF, and LD box are all based on a similar conflict between the tendency of mice to explore a novel environment and its aversive properties, but the main stressful stimulus of each test is somewhat different (bright light vs. open, elevated spaces vs. open, large spaces). Although the nature of the stressful stimulus varied, male CREB$^{\alpha\delta-}$ homozygous deficient mice were inhibited under all novel conditions. Thus, the similarity of behaviour of these mice under several novel, stressful conditions depended more upon how the animal assessed and reacted to novel stimuli than on the types of stressful stimuli it encountered. Interestingly, the behavioural inhibition of the CREB$^{\alpha\delta-}$ homozygous deficient mice was not associated with higher stress levels of CORT than that of WT mice. The maximum stress levels of CORT achieved for all mice in the current study (i.e. 20–30 μg/dL) are consistent with other mouse studies reporting CORT levels after the EPM (Rodgers *et al*., 1999) and forced swim test (Anisman *et al*., 2001; Droste *et al*., 2003) and lower than other mouse studies using more severe stressors (Dunn & Swiergiel, 1999; Karolyi *et al*., 1999; Anisman *et al*., 2001; Meerlo *et al*., 2001; Droste *et al*., 2003). For example, maximum CORT levels in mice are 30–45 μg/dL after 30–60 min of restraint stress, 30–60 μg/dL after footshock, and 60 μg/dL after IL-1β administration.

LHPA axis activity is associated with anxiety-like or fear-related behaviour. Higher CORT levels and more anxiety-like behaviours have been reported in animals confined to the open arms of an EPM (Pellow *et al*., 1985). Similar stress reactivity, as measured by CORT levels, in all three genotypes of the present study is consistent with the fact that CREB$^{\alpha\delta-}$ deficient mice do not show more or less anxiety-like behaviour in the EPM, OF, or LD box. Even baseline CORT levels did not differ in CREB$^{\alpha\delta-}$ deficient mice of the present study, suggesting that under both nonstress and stress conditions, a CREB deficiency does not alter this aspect of the LHPA axis. Interestingly, cAMP response element modulator (CREM)-null mice also exhibit a preserved reactivity to stress (as measured by conditioned suppression of motility; Maldonado *et al*., 1999). In mice with CREB or CREM alterations, compensation with other members of the CREB/ATF family of transcription factors likely occurs to preserve stress reactivity at the behavioural and hormonal levels.

Given the behavioural profile of the CREB$^{\alpha\delta-}$ deficient mice observed in the present study, we asked whether these mice exhibit, at a neuronal level, an altered stress system under basal conditions. The stress-related molecule glucocorticoid receptor is normally found in abundance in the paraventricular nucleus of the hypothalamus, the main integrator of responses to stress in the LHPA axis, and in the CA1 and dentate gyrus of the HPC, a major brain structure involved in the feedback inhibition of the stress response (Morimoto *et al*., 1996). Another stress-related molecule, corticotropin releasing hormone, is important for modulating endocrine and metabolic responses to stress in the paraventricular nucleus of the hypothalamus (Davis, 1998). Using *in situ* hybridization histochemistry, we found that CREB$^{\alpha\delta-}$ deficient mice do not show an alteration in glucocorticoid receptor or corticotropin releasing hormone mRNA expression in these brain areas (data not shown). This normal expression is not surprising as the mutant mice exhibit normal basal and stress levels of CORT. Thus, our studies show no evidence of changes in the standard elements of the stress circuits.

The decreased locomotor activity of the CREB$^{\alpha\delta-}$ homozygous deficient mice observed in this study is not due to a gross motor impairment. CREB$^{\alpha\delta-}$ deficient mice displayed similar activity levels to that of WT mice during 24-h monitoring in familiar home-like cages, the least stressful of the four environments tested. Normal home cage activity of CREB$^{\alpha\delta-}$ deficient mice is consistent with that reported by Graves *et al*. (2003). Locomotor response to a novel environment can predict the vulnerability of rodents to drugs of abuse (Hooks *et al*., 1991, 1994), with high responders to novelty acquiring amphetamine self-administration more readily (Piazza *et al*., 1989) and exhibiting greater behavioural activation in response to amphetamine (Hooks & Kalivas, 1994). Thus, the decreased locomotor activity of CREB$^{\alpha\delta-}$ homozygous deficient mice in novel environments suggests that they would exhibit less behavioural activation in response to drugs of abuse. Findings obtained in our laboratory (unpublished data), however, show that CREB$^{\alpha\delta-}$ deficient mice show a similar increase in locomotor activity to that of WT controls with repeated doses of morphine. This behavioural response to morphine in CREB$^{\alpha\delta-}$ homozygous deficient mice is consistent with their behaviour in the current study; locomotor inhibition in a novel environment gives way to normal locomotor activity (relative to WT controls) once the environment becomes familiar.

CREB$^{\alpha\delta-}$ homozygous deficient mice, who exhibit decreased locomotor activity in novel environments but normal activity in a familiar environment, have increased levels of both the activator (tau) and repressor (alpha/beta) isoforms of the CREM gene (Hummler *et al*., 1994). Interestingly, mice with a mutated CREM gene are hyperactive and do not show the characteristic day-night change in locomotion (Maldonado *et al*., 1999). These CREM null mice are also more active in the OF and have a higher tendency to visit the open arms of the EPM than WT mice, unlike the CREB$^{\alpha\delta-}$ homozygous deficient mice in the current study. Such contrasting results leads one to speculate about levels of CREM in the brain; low levels result in increased locomotion and high levels result in decreased locomotion. However, the locomotor inhibition of CREB$^{\alpha\delta-}$ homozygous deficient mice is only observed in novel environments. As these mice appear normal in the home cage, the mechanism does not appear to be entirely due to CREM levels. The mechanism is more likely due to a combination of factors in which elevated CREM levels may play a role.

The phenotype of CREB$^{\alpha\delta-}$ deficient mice observed in the present study suggests that anxiety-like behaviours and locomotor activity are dissociable. CREB$^{\alpha\delta-}$ deficient mice represent the first genetic model that may dissociate anxiety-like vs. activity-related behaviours specific to novelty. Moreover, CREB$^{\alpha\delta-}$ deficient mice show a selective decrease in locomotor activity with no changes in anxiety or stress reactivity, suggesting that the LHPA response might segregate with anxiety. Some of the neural circuits underlying exploratory activity are likely distinct from those underlying fear and anxiety and involve various neurotransmitter systems in the brain.

The HPC, as part of the learning and memory process, is involved in assessing context and detecting novel stimuli. As HPC function is most affected in CREB$^{\alpha\delta-}$ deficient mice, the data from the current study suggest that the HPC is involved in exploration of novel environments without playing a strong role in their affective appraisal. Activity in a novel situation and spatial memory may be highly tuned, but the affective tone of the situation is likely moderated by nonhippocampal structures and engages other behaviours.

In summary, we have shown here that the spontaneous initial behaviour of CREB$^{\alpha\delta-}$ deficient mice in a novel environment is inhibition. These animals are disoriented in space and have poor spatial memory (Bourtchuladze *et al*., 1994; Kogan *et al*., 1997; Hebda-Bauer *et al*., unpublished results). We know HPC place cell activity of CREB$^{\alpha\delta-}$ deficient mice is altered, lending physiological evidence to their behavioural disorientation (Cho *et al*., 1998). While CREB$^{\alpha\delta-}$ deficient mice may be forgetful and disoriented, they do not encode novelty as being any more stressful than normal animals as they have lived with minimal CREB levels from birth and do not know otherwise. As they have an impaired ability to code space, CREB$^{\alpha\delta-}$ deficient mice may compensate by being more cautious and displaying low locomotor activity when exposed to novel environments. Importantly, we are the first to report that CREB$^{\alpha\delta-}$ deficient mice (i) show altered behaviour, not related to learning and memory-associated behaviours, upon initial exposure to environments and (ii) serve as an animal model that can dissociate locomotor activity from anxiety-like behaviour in novel environments.

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Abbreviations

CORT, corticosterone; CREB, cAMP response element binding protein; CREB$^{\alpha\delta-}$, CREB alpha, delta deficient; CREM, cAMP response element modulator; EPM, elevated plus maze; HPC, hippocampus; LD, light/dark; LHPA, limbic-hypothalamic-pituitary-adrenal; OF, open field; WT, wild-type; +/+, wild type; +/–, heterozygous; –/–, homozygous.

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