Altered emotional and locomotor responses in mice deficient in the transcription factor CREM

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Edited by Solomon H. Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved September 27, 1999 (received for review July 13, 1999)

Various transcription factors act as nuclear effectors of the cAMP-dependent signaling pathway. These are the products of three genes in the mouse, CREB, CRE modulator (CREM), and ATF-1. CREM proteins are thought to play important roles within the hypothalamic–pituitary axis and in the control of rhythmic functions in the pineal gland. We have generated CREM-mutant mice and investigated their response in a variety of behavioral tests. CREM-null mice show a drastic increase in locomotion. In contrast to normal mice, the CREM-deficient mice show equal locomotor activity during the circadian cycle. The anatomy of the hypothalamic suprachiasmatic nuclei, the center of the endogenous pacemaker, is normal in mutant mice. Remarkably, CREM mutant mice also elicit a different emotional state, revealed by a lower anxiety in two different behavioral models, but they preserve the conditioned reactiveness to stress. These results demonstrate the high degree of functional specificity of each cAMP-responsive transcription factor in behavioral control.

Transcriptional regulation by the cAMP-signaling pathway is mediated by a family of transcription factors binding to cAMP-responsive elements (CREs). These factors are the products of three genes in the mouse, CREB, CRE modulator (CREM), and ATF-1 (1, 2). The CREM gene has a complex structure and generates several products in a tissue-specific manner that may activate or inhibit cAMP-responsive genes (1). One of them, inducible cAMP early repressor (ICER) acts as a powerful transcriptional repressor (3) and was shown to be expressed in a day–night rhythmic fashion in the pineal gland (4), a finding that suggested a possible role in the control of cyclic physiological responses. CREM appears to act as a regulator of output functions of the biological clock in response to adaptive environmental changes (5). CREM directly modulates an important day–night endocrine signal, the rhythmic production of melatonin. Indeed, the activity of the enzyme serotonin *N*-acetyltransferase, which catalyzes the rate-limiting step of melatonin synthesis (6), is negatively regulated by ICER (7). The day–night switch in CREM expression is under the control of adrenergic signals originated from the suprachiasmatic nuclei (SCN) of the hypothalamus, the central circadian pacemaker structure (8–10).

CREM is also involved in the control of various neuroendocrine responses (1, 11). In particular, CREM plays a key role within the hypothalamic-pituitary-gonadal axis (1, 12–14) and in the physiological response to pituitary hormones (15, 16). Several hypothalamic–pituitary axis hormones are involved in behavioral responses, including emotional state and reactiveness to stress (17, 18).

We have generated mutant mice for the CREM gene by homologous recombination (13). These mice show drastic phenotypic changes that include spermiogenesis deficiency (13, 19) and altered oscillation in *N*-acetyltransferase gene expression (7). In this study, we have evaluated the effects of CREM gene disruption on behavioral responses. Locomotor activity was analyzed under different experimental conditions at several times of the circadian cycle. Strikingly, CREM-deficient mice are hyperactive and do not show the characteristic day–night change in locomotion. Interestingly, the emotional state of these mice indicates a decrease in anxiety-like behavior.

Materials and Methods

Animals. Wild-type and CREM-mutant mice (13) of similar age and sex were housed in groups of four with free access to food and water. A light–dark cycle (light: 8:00 to 20:00) and temperature-controlled environment (21 ± 1°C) were maintained. Mice were acclimated to a soundproof experimental room and handled during 1 wk before the experimental sequence in accordance with ethical guidelines (20). Similar results were obtained from male and female groups. The following experimental sequence was performed in a group of 21 wild-type and 21 mutant mice: first, locomotor activity box; second, elevated plus maze; third, open field. Similar order has been previously used (21) to avoid interferences between behavioral tests. A second group of 18 wild-type and 18 mutant mice was exposed at the following sequence: first, elevated zero maze; second, conditioned suppression of motility test. Different groups of wild-type and mutant mice ($n = 8$–10 per group) were used for each study with β-carboline and amphetamine and for SCN histology.

Locomotor Activity Boxes. Mice were individually placed in activity boxes (255 cm × 205 cm) with two crossed photocells, isolated and in almost complete darkness (less than 5 lx). Activity was evaluated at 14 h, during 10 min, for 3 consecutive days. On day 4, circadian locomotor activity was recorded during 10 min at 9:00, 14:00, 21:00, and 02:00. In a second experiment, the reactiveness of mice to an arousing stimulus was evaluated. Mice were habituated to the locomotor activity boxes during 3 consecutive days and on day 4 received an injection of saline (0.9%) or amphetamine SO_4 (6 mg/kg i.p.) (Calaire Chimie, Calais, France) at 2:00 or 21:00. Locomotor activity was evaluated 15 min after injection during 15 min. In a third experiment, locomotor responses were evaluated after changing the emotional state of mice. After 3 days of habituation, on day 4 different groups received an injection of vehicle (carboxymethylcellulose 0.5%) or β-carboline-3-carboxylate acid methyl ester (1 mg/kg i.p.) (Sigma) at either 14:00 or 02:00. Activity was evaluated 20 min after injection during 15 min.

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: CRE, cAMP-responsive element; CREM, CRE modulator; SCN, suprachiasmatic nuclei.

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Open-Field Test. A rectangular area (70 × 90 × 60 cm) brightly illuminated (500 lx) divided in 63 squares (10 × 10 cm) was used. Six parameters were recorded during 5 min: number of squares crossed, rears, grooming bouts, defecation boli, urination events, and the latency time to move out and to cross two squares. Mice were exposed to the test at 14:00 during 3 days. Circadian behavior was evaluated during the next 3 days, only once in each dark period to minimally affect the circadian rhythm. Thus, animals were exposed to the open field at 9:00 and 21:00 on day 4, at 14:00 on day 5, and at 02:00 on day 6.

Elevated Plus Maze. The elevated plus maze (22) was elevated by 30 cm and illuminated from the top (100 lx). Mice were placed in the middle of the maze facing one of the open arms. The number of visits and time spent in each arm were measured for 5 min with the help of a video camera. The same experimental timing as for the open-field test was applied.

Elevated Zero Maze. The zero maze (diameter, 46 cm; runway width, 5.5 cm) (23) was 50 cm above ground and illuminated from the top (100 lx). Video camera recording of the position and time spent by the mouse in each arm was measured for 5 min. Time in a new sector was measured as soon as the animal entered with four paws. General activity was assessed by counting all entries with at least two paws into a new sector. Mice were exposed to the test at 14:00 during 3 consecutive days.

Conditioned Suppression of Motility. The assay was performed as described (24). On the first day, the mouse was left in the test cage for 6 min and received electric footshocks (0, 1 Hz, 200 ms, 100 V) through an isolated stimulator (GL 260T, Société Ravia, France). On the second day, the animal was placed in the same cage without receiving footshocks, and motility changes were tested by counting the number of squares crossed plus the number of rears in 6 min. Control mice had no foot shock on the first day.

Spontaneous Alternation. In a Y maze (25) (arms 25 × 8 × 15 cm) illuminated from the top (100 lx), each arm serves as either a start or goal arm. Video camera recording was over 10-min periods divided in two 5-min intervals. Entering an arm was defined as all four legs crossing over the area dividing an arm from the triangular choice area. Nonalternation is defined as the mouse returning to the same compartment (25). Proportion of alternation is given by the ratio of alternation to nonalternation.

***In Situ* Hybridization and Immunohistochemistry.** Classical histological (NISSL stain) and *in situ* hibridization techniques were used (26). The c-*fos* probe has already been described (27).

Statistical Analysis. Individual group comparisons were made by using a two-way ANOVA with repeated measures. The factors of variation were mutation (between subjects) and time (within subjects). In the case of conditioned suppression of motility, data were analyzed by two-way ANOVA between subjects (factors of variation/mutation and shocks). After significant main effect by one-way ANOVA, individual dose effects were analyzed by using Dunnett's *t* test comparisons when more than two groups were compared. The level of significance was $P < 0.05$.

Results

Spontaneous Locomotor Activity. The locomotor activity of CREM mutants in a nonstressful environment was studied. Spontaneous locomotion was analyzed in activity boxes in almost complete darkness to avoid stress (21). In the three first determinations during the light period, CREM mutants elicited a higher motility as compared with wild-type littermates. This hyperactivity was significant ($P < 0.05$) on day 2 (Fig. 1*A*). However, wild-type

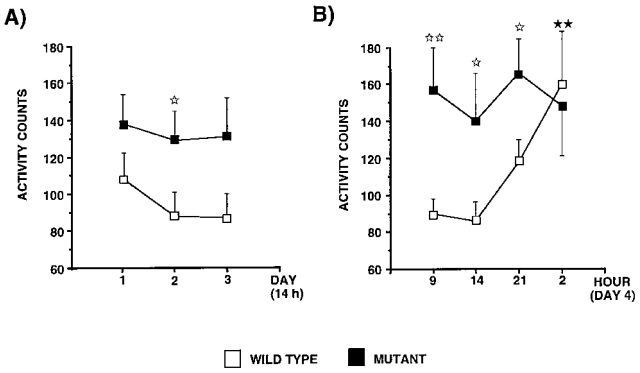

Fig. 1. Spontaneous locomotor activity of CREM-mutant mice ($n = 11$) and wild-type littermates ($n = 11$). Locomotor activity was measured at 14:00 on 3 consecutive days. On day 4, locomotor activity was recorded at 9:00, 14:00, 21:00, and 2:00. Values represent mean ± SEM. ★, $P < 0.05$, ★★, $P < 0.01$ vs. same group on day 1 (*A*) or day 4 at 9:00 (*B*) (Dunnett's *t* test); ☆, $P < 0.05$, ☆☆, $P < 0.01$, vs. wild-type group at the same time point (one-way ANOVA).

mice showed a more important habituation to the activity boxes, as revealed by the locomotor decrease on day 3 ($P < 0.05$ in the male group).

To test whether the hyperactivity has effects on circadian rhythmicity, spontaneous locomotion was evaluated at different times during the light (9:00 and 14:00) and dark (21:00 and 2:00) periods. Wild-type animals showed the expected increase in activity during the dark period. In contrast, CREM mutants elicited a homogenous activity during both light and dark periods (Fig. 1*B*). The measurements of the circadian activity during light and dark periods were repeated on 3 consecutive days, and highly similar results were obtained in all cases. Thus, the activity of the CREM-mutant group was also significantly higher than wild-type littermates on the second day of circadian activity determination at 09:00 (122.9 ± 11.5 vs. 81.2 ± 9.3, $P < 0.05$) and 14:00 (121.8 ± 19.1 vs. 77.5 ± 10.2, $P < 0.05$), but not at 21:00 [125.2 ± 19.8 vs. 103.2 ± 15.5, not significant (N.S.)] and 02:00 (121.7 ± 24.0 vs. 116.8 ± 11.5, N.S.), and on the third day at 09:00 (122.7 ± 16.3 vs. 79.5 ± 10.3, $P < 0.05$) and 14:00 (116.8 ± 15.3 vs. 71.3 ± 10.3, $P < 0.05$), but not at 21:00 (115.2 ± 23.6 vs. 88.0 ± 12.9, N.S.) and 02:00 (126.8 ± 22.1 vs. 128.8 ± 12.4, N.S.). Thus, mutant mice show a homogenous and increased locomotor activity along the whole circadian period.

To verify the locomotor reactiveness to an external stimulus, mice received an acute injection of saline or amphetamine (6 mg/kg, i.p.) at 21:00. Amphetamine produced a similar increase in activity in wild-type (662% ± 43 of control values) and mutant mice (509% ± 84 of control values) (not shown). Similarly, at 2:00 amphetamine (6 mg/kg, i.p.) induced an increase in locomotion in wild-type (727% ± 68 of control values) and mutant mice (772% ± 81 of control values) (not shown). Thus, in spite of their hyperactivity, mutant mice increase their locomotion when exposed to an arousal stimulus.

Because the emotional state may influence locomotor and circadian responses, locomotion was evaluated after increasing anxiety by β-carboline-3-carboxylate administration. As β-carbolines have been reported to decrease locomotion in rodents (28), the effect induced by several doses (0.5, 1 and 2 mg/kg, i.p.) was evaluated in preliminary experiments to select a dose without intrinsic motor effects. β-carboline-3-carboxylate administered at 0.5 and 1 mg/kg did not modify motility, whereas it decreased locomotion (33% ± 4, with respect to saline-injected animals, $P < 0.05$) when injected at 2 mg/kg. Therefore, the dose of 1 mg/kg (i.p.) was chosen. β-carboline-3-carboxylate did not modify motility of mutant and wild-type mice when

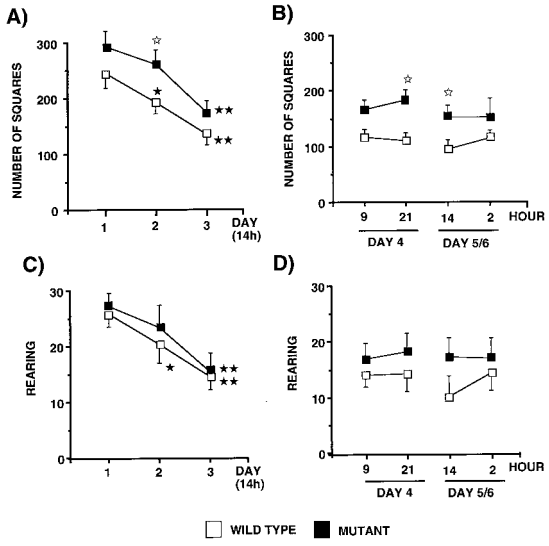

Fig. 2. Number of squares crossed (*A* and *B*) and rears (*C* and *D*) in the open field by CREM mutants ($n = 11$) and wild-type littermates ($n = 11$). The behavioral test was performed at 14:00 on 3 consecutive days. On day 4, animals were exposed to the test at 9:00 and 21:00, and at 14:00 and 2:00 on day 5 and 6, respectively. Values represent mean ± SEM. ★, $P < 0.05$; ★★, $P < 0.01$ vs. same group on day 1 (*A* and *C*) or day 4 at 9:00 (*B* and *D*) (Dunnett's *t* test); ☆, $P < 0.05$; vs. wild-type group at the same time point (one-way ANOVA).

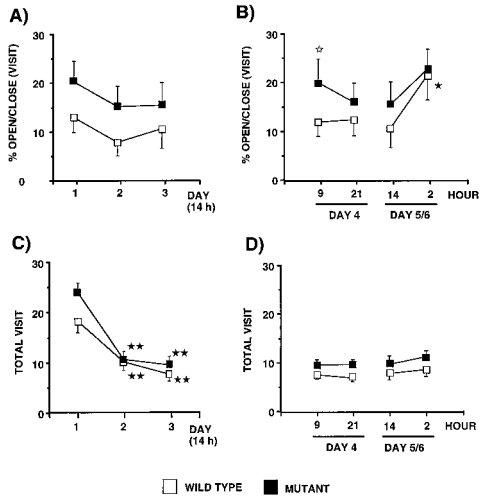

Fig. 3. Percentage of visits to the open arms of the elevated plus maze (*A* and *B*) and total number of visits (*C* and *D*) elicited by CREM mutants ($n = 11$) and wild-type littermates ($n = 11$). The behavioral test was performed at 14:00 on 3 consecutive days. On day 4, animals were exposed to the test at 9:00 and 21:00, and at 14:00 and 2:00 on days 5 and 6, respectively. Values represent mean ± SEM. ★, $P < 0.05$; ★★, $P < 0.01$ vs. same group on day 1 (*A* and *C*) or day 4 at 9:00 (*B* and *D*) (Dunnett's *t* test); ☆, $P < 0.05$; vs. wild-type group at the same time point (one-way ANOVA).

administered during the light period (14:00). As expected (Fig. 1), untreated mutants displayed a higher spontaneous locomotion than wild-type mice at this time. Importantly, the administration of β-carboline-3-carboxylate also during the dark period (02:00) did not change the locomotor response in both groups. At this time, both wild-type and mutant mice showed a similar spontaneous motility (not shown). Thus, the altered locomotor responses of mutant mice do not seem to be linked to their emotional state.

Open Field. Behavioral responses were also evaluated in a stressful environment consisting of a large and brightly illuminated open-field test (29). CREM mutants crossed a higher number of squares than their wild-type littermates in the three first measurements performed during the light period. This effect was significant ($P < 0.05$) on day 2 (Fig. 2*A*). The number of rears was similar in both genotypes (Fig. 2*C*). Wild-type mice showed a significant habituation (decreased number of squares crossed, $P < 0.05$, and rears, $P < 0.05$) from the second day. Habituation in mutant mice was observed only on the third day (Fig. 2 *A* and *C*). No significant differences were observed in the other behavioral events in this test. Thus, the increased locomotor activity of mutant mice was more difficult to reveal in a stressful environment.

Behavioral responses were also evaluated at different time points during the light (9:00 and 14:00) and dark (21:00 and 2:00) periods. Mice were exposed to a strong illumination, which presumably disrupted the circadian rhythm, as revealed by the small differences observed during light and dark periods in both genotypes. Mutant mice crossed a higher number of squares ($P < 0.05$) than wild-type mice at 14:00 and 21:00. The number of rears was similar in mutant and wild-type mice in all measurements (Fig. 2 *B* and *D*).

Elevated Plus Maze. A well-known animal model of anxiety is the elevated plus maze (22). Rodents are exposed to an approach–avoidance conflict between exploratory behavior (total number of visits) and a natural aversion to heights and open spaces (visit to open arms). Animals tend to avoid the open parts, which is considered to reflect anxiety (22). No significant differences between wild-type and mutant mice were found in the visits to the open arms in three first determinations during the light period. However, mutants always showed a higher tendency than wild types to visit open arms (Fig. 3*A*). The total number of visits was similar in both groups during the 3 days, with a rapid habituation to the test (Fig. 3*C*).

Exposition to this illuminated test presumably disrupted the circadian rhythm, because small differences were observed during light and dark periods. Thus, the total number of visits was similar in both groups during the whole circadian period (Fig. 3*D*). The percentage of visits to the open arms also elicited small circadian modifications. When compared with the behavioral response observed at 09:00, only a significant increase in the exploration of open arms ($P < 0.05$) was observed at 02:00 in wild-type mice. Mutants have a higher tendency to visit open

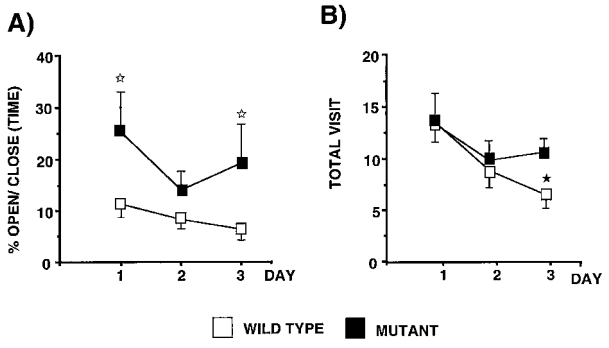

Fig. 4. Behavioral responses elicited in the elevated zero maze by CREM mutants ($n = 10$) and wild-type littermates ($n = 10$): percentage of time spent in the open arms (*A*) and total number of visits (*B*). The behavioral test was performed at 14:00 on 3 consecutive days. Values represent mean ± SEM. ★, $P < 0.05$ vs. same group on day 1 (Dunnett's *t* test); ☆, $P < 0.05$ vs. wild-type group at the same time point (one-way ANOVA).

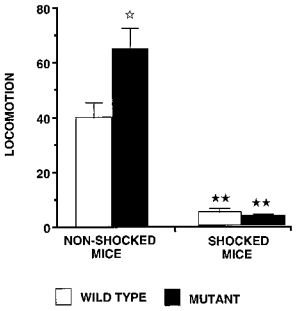

Fig. 5. Conditioned suppression of motility elicited by CREM mutants and wild-type littermates (n = eight per group). The behavioral test was performed at 14:00 on 2 consecutive days. Values represent mean ± SEM of the number of squares crossed plus the number of rears. ★★, $P < 0.01$ vs. same group (Dunnett's t test); ☆, $P < 0.05$ vs. wild-type group at the same time point (one-way ANOVA).

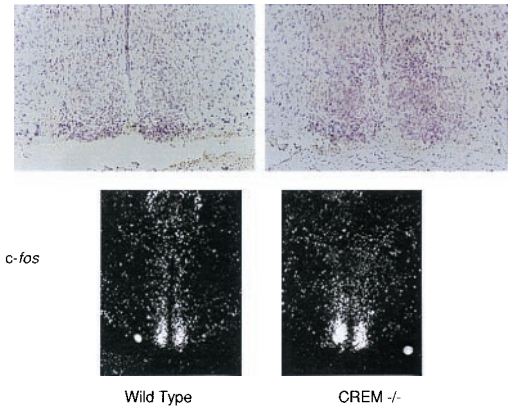

Fig. 6. Anatomy of the suprachiasmatic nuclei in wild-type and CREM mutants. (*Upper*) Comparative histological sections showing no apparent anatomical alteration in the SCN. Several serial sections were analyzed. Only representative results are shown. (*Lower*) Inducibility of c-*fos* 30 min after a light-pulse (400 lx) is comparable in wild-type and CREM mutants. The kinetics of c-*fos*-induced expression are equivalent in both animals (not shown).

arms than wild-type mice, this effect being significant at 09:00 ($P < 0.05$) (Fig. 3*C*). These observations indicate that mutant mice have a lower anxiety state.

Elevated Zero Maze. The elevated zero maze, a methodological modification of the elevated plus maze, helps evaluate the anxiety state and allows uninterrupted exploration (23). Mutant mice showed a higher percentage of visits to the open arms than wild-type controls. This effect was significant in the first ($P < 0.05$) and third ($P < 0.05$) determinations (Fig. 4*A*). No differences were observed in the total number of visits in any of the behavioral determinations. However, wild-type mice showed a stronger habituation to this test because the total number of visits on day 3 was significantly lower than on day 1 ($P < 0.05$) (Fig. 4*B*). This analysis confirmed that mutants present a decrease in their anxiety state.

Conditioned Suppression of Motility. The conditioned suppression of motility paradigm is used to evaluate the reactiveness to stress. Mice exhibit a marked suppression of motility when they are placed in the same environment where they previously had received an electric shock. This corresponds to an emotional response to environment stressful stimuli (30). Mice were tested in the same test cage where they previously received, or did not receive, electrical footshocks. When nonshocked, mutants presented a higher locomotion than wild-type controls ($P < 0.05$), as in previous nonstressful environments (Fig. 1). Mutant and wild-type mice exhibited an equivalent and significant suppression of motility ($P < 0.01$) when previously exposed to footshocks (Fig. 5).

Spontaneous Alternation in Y Maze. To further evaluate the habituation to the environment, the behavioral responses were tested in the spontaneous alternation task. In the absence of response reinforcement, mice tend to enter the least recently visited arm of the Y maze (25). This alternation behavior is thought to be a consequence of habituation to the most recently visited arm (31). Spontaneous alternation was similar in wild-type and mutant mice during the whole observation period. No differences were observed in the total number of visits, which reflects locomotor activity, during the first 5 min of observation, whereas a higher locomotion ($P < 0.05$) was observed in mutants during the second observation period (5–10 min) (Table 1) in agreement with the hyperactivity shown in nonstressful environments (Fig. 1).

Anatomy of the Suprachiasmatic Nuclei in CREM-Null Mice. The phenotypic changes in the circadian behavior of the CREM mutants (Fig. 1*B*) and the implication of inducible cAmp early repressor in regulating output clock functions (7, 10) prompted us to analyze the anatomical integrity of the SCN. These hypothalamic structures contain the circadian pacemaker, central for overt circadian rhythmicity (8, 32). The anatomical structure of the SCN is unaltered in the CREM mutants (Fig. 6*A*). c-*fos* expression is known to be pulsatile and light inducible in the SCN and to follow a strict day/night oscillation program (27, 33). c-*fos*-induced expression is evident in both wild-type and mutant mice (Fig. 6*B*) and follows the same temporal profile (not shown).

Discussion

Experiments in *Aplysia*, *Drosophila,* and mouse indicate that cAMP-responsive transcription factors play a central role in the molecular processes controlling key neuronal functions (34–41). Here we show that genetic disruption of the CREM gene in the mouse results in significant alterations of locomotor activity and emotional response. Mutant mice show a higher level of spon-

Table 1. Behavioral responses elicited in the spontaneous alternation task by mice lacking CREM and their wild-type littermates

	0–5 min		5–10 min	
	Wild type	Mutant	Wild type	Mutant
Alternance, %	60.45 ± 3.06	59.91 ± 2.17	51.55 ± 4.31	53.25 ± 2.15
Locomotion (visits)	24.05 ± 2.15	29.06 ± 1.99	12.78 ± 1.68*	17.25 ± 1.26*†

Values represent mean ± SEM. *, $p < 0.01$ vs. same group (Dunnett's t test); †, $p < 0.05$ vs. wild-type group at the same time point (one-way ANOVA). Number of animals: 18 in wild-type group and 16 in mutant group.

taneous locomotion when exposed to a nonstressful environment during the light period. In this environment, mutant mice display a homogenous locomotor activity along the whole circadian period, a feature distinctly uncharacteristic of rodents. The activity levels of the CREM mutants during the day were similar to those displayed by wild-type mice during the dark interval (Fig. 1). Importantly, whereas the anatomical integrity of the SCN was confirmed in the mutant mice (Fig. 6), the absence of circadian locomotion could be related to an alteration in its function. In addition, the recent finding of peripheral pacemakers (42–44) could suggest that their function could also be altered in the CREM-deficient mice.

The small differences observed in the open field and the elevated plus-maze tests are presumably caused by the disruption of the circadian cycle by the exposition to these illuminated environments and to the high habituation. Interestingly, the behavioral responses of mutant and wild-type mice in the two tests were similar at 02:00 (Fig. 2), in agreement with the response in the activity boxes (Fig. 1).

The hyperactivity of CREM mutants was also revealed in other behavioral paradigms. One possible explanation for the absence of circadian locomotor oscillation could be that, because of their hyperlocomotion during the light interval, the mutants are unable to increase their activity to higher levels during the dark period. Interestingly, amphetamine was able to induce similar hyperactivity in mutant and wild-type mice, indicating equivalent ability to respond to an arousal stimulus.

Because the emotional state may have some influence on behavioral responses, it was investigated by using the elevated plus maze and zero maze. Novelty is not a critical determinant of rodent behavior in these tests (22). Our mice did not show a lesser avoidance of the open arms after repeated exposure to the test, because only a decrease in the total number of visits was observed. Mutant mice showed a more significant increased exploration to the open arms than did wild-type animals in both tests since the first exposure. This higher exploration was observed in all behavioral measurements, being significant at 09:00. The high variability observed with the elevated plus maze in mice (45) explains the difficulty in obtaining significant differences. Thus, compounds classically used to modify emotional states (46) merely showed trends toward anxiolytic and anxiogenic effects in this test in mice. An even clearer response was obtained when using the zero maze (Fig. 4), a procedure that increases sensitivity by allowing an uninterrupted exploration (23). The increased locomotor activity of the CREM mutants has no relevance in the elevated plus maze and zero maze, because the total number of arm/sector visits was similar in both groups of animals. This could be caused by the short-time observation period (5 min), the lower sensitivity of these tests to measure locomotion (only number of visits are counted), and their stressful characteristics. In agreement, the increased locomotor activity of CREM mutants was stronger in a nonstressful environment (locomotor activity boxes) than in the open field. Taken together, these data indicate that the anxiety state of CREM mutants is lower than in wild-type mice. Although the molecular mechanism involved in the decreased anxiety of the CREM mutants is unclear, it is known that CREM is involved in the control of various neuroendocrine responses within the hypothalamic-pituitary axis (1). Hormones secreted by this axis are important in the control of emotional states and the adaptation to changes in the environment (17, 18). However, significant changes in corticosterone levels in CREM mutants were found (15). Because lower anxiety levels may influence circadian locomotor responses, we tested the effect of β-carboline-3-carboxylate. Our results suggest that the emotional state cannot justify the differences in locomotor responses observed in the mutant mice.

The emotional state of mutant mice was further investigated by using the conditioned suppression of motility paradigm (30). The conditioned response on this test does not seem to depend on the anxiety state, because it is sensitive to antidepressant (24) but not to benzodiazepine treatment (30). Therefore, the change in the emotional state of mutant mice did not impair the conditioned response to stress.

Habituation appeared to be diminished in CREM mutants exposed to nonstressful and stressful environments, but in the spontaneous alternation task in the Y maze (25, 31), the groups behaved similarly. Mouse performance in this test appears sensitive to drugs affecting memory function (25, 47), but the test is not sensitive enough to exclude a possible impairment in memory processes. However, it is established that at least long-term potentiation is unaltered in the CREM mutants (T. Abel, M. Barad, Y. Y. Huang, P.S.-C., and E. R. Kandel, unpublished observations).

Our results seem to match well recent experiments in *Drosophila* where dCREB2 gene mutations result in an aberrant circadian cycle (48). That transcriptional response to cAMP is linked to endogenous clock function is also confirmed by the use of transgenics mice carrying a CRE reporter (49). We provide evidence that the targeted mutation of a CRE-responsive transcription factor results in altered circadian rhythmicity in the mouse.

CREM encodes only a subset of the transcription factors responsive to cAMP (1, 2, 40). Thus, other cAMP-responsive factors may be committed to alternative or overlapping functions. A partial mutation of CREB in the mouse leads to impairment of memory consolidation (37, 41) and reduction of morphine abstinence (39), suggesting that distinct genes encoding CRE-binding proteins preside over specific physiological functions. Our results reveal the important role played by CREM in the control of specific behavioral responses, such as locomotor activity, circadian rhythmicity, and emotional/stress response.

We thank E. Borrelli, E. Lalli, and N. S. Foulkes for advice and discussions; S. H. Snyder, J. Yin, D. Storm, G. Schutz, T. Abel, M. Barad, Y. Y. Huang, and E. R. Kandel for privileged communications; and E. Heitz and G. Duval for technical assistance. This study was funded by grants from Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, Centre Hospitalier Universitaire Régional, Fondation de la Recherche Médicale, Association pour Recherche sur le Cancer, and European Commission BMH4-98-2267.

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