Forebrain-Specific trkB-Receptor Knockout Mice: Behaviorally More Hyperactive Than "Depressive"

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Background: *According to the neurotrophin hypothesis of depression, decreased activity of brain-derived neurotrophic factor (BDNF) contributes to behavioral and plasticity-related alterations in depressed patients. We investigated the hypothesis that mice with a forebrain-specific knockout of the trkB receptor, the main mediator of BDNF signaling, represent a genetic animal model for depression.*

Methods: *Using the CRE-loxP system, we bred trkB$^{CaMKII\text{-}CRE}$ mice with a trkB-receptor disruption in the forebrain. We subjected trkB-mutant mice to a battery of behavioral tests, comprising open field, elevated zero maze, emergence test, novel object test, and forced swim. Additionally, we investigated the hypothalamic-pituitary-adrenal (HPA) axis immunohistochemically and by plasma analyses.*

Results: *trkB$^{CaMKII\text{-}CRE}$ mice showed a stereotyped hyperlocomotion with reduced explorative activity, and impulsive reactions to novel stimuli. The trkB-mutant mice did not exhibit depressionlike behaviors such as increased "despair" in the forced swim test, increased anxiety in the elevated zero maze, or neophobia in the novel object test. Furthermore, no HPA dysregulation was observed under normal and stressful conditions.*

Conclusions: *trkB$^{CaMKII\text{-}CRE}$ mice cannot be regarded as a genetic mouse model of depression. Instead, the behavioral symptoms of trkB$^{CaMKII\text{-}CRE}$ mice, comprising hyperlocomotion, stereotyped behaviors, and cognitive impairments, are similar to those postulated for mouse models of attention-deficit disorder.* Biol Psychiatry 2003;54: 972–982 © *2003 Society of Biological Psychiatry*

Key Words: Conditional knockout mice, trkB, behavior, depression model, HPA axis

Introduction

Recent studies have provided evidence for a neurotrophin hypothesis of depression and antidepressive treatment (Altar 1999; Duman 2002; Duman et al 1997; Licinio and Wong 2002; Manji et al 2001; Nestler et al 2002; Vaidya and Duman 2001; Wong and Licinio 2001). According to this hypothesis, decreased expression of brain-derived neurotrophic factor (BDNF) contributes to plasticity-related changes, such as hippocampal atrophy, in response to stress in depressed patients. In contrast, upregulation of BDNF is thought to mediate the action of antidepressant treatment (Saarelainen et al 2003). A critical role of BDNF in the pathogenesis of depression has been indicated by several lines of evidence: BDNF is found in high concentrations in the hippocampus and cerebral cortex (Lewin and Barde 1996), brain regions thought to be critically involved in the pathogenesis of depression. In these areas, BDNF expression is decreased by exposure to stress, which is currently the only measure to induce depressionlike states in rodents (Nestler et al 2002; Smith et al 1995). In contrast, chronic, but not acute, treatment with antidepressants as well as electroconvulsive therapy induce increased levels of BDNF, primarily in the hippocampus (Nibuya et al 1995; Russo-Neustadt et al 1999). Furthermore, local cerebral administration of BDNF itself has antidepressantlike effects in animal models of depression (Shirayama et al 2002; Siuciak et al 1997).

Despite this cumulative evidence for a critical role of BDNF in the pathophysiology of depression, mice with a heterozygous deletion of the BDNF gene and a 50% reduction of BDNF expression did not exhibit depressionlike behavior when investigated in an extensive behavioral test battery (MacQueen et al 2001). Thus, these mice did not confirm the predicted hypothesis, nor did they provide a murine model of genetic susceptibility to stress-related mood disorders such as anxiety and depression (MacQueen et al 2001). There are two major caveats in the interpretation of these data, however. The first is the notion that large reductions in BDNF, exceeding 50%,

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may be necessary to elicit depressionlike symptoms in mice. Second, in this mouse strain, developmental mechanisms may have compensated for the lifelong reduction of BDNF, which is already present during embryonic development and thus may have resulted in an essentially normal behavioral profile in adult mice. This interpretation may be supported by the recent finding that mice with a brain-specific conditional knockout of the BDNF gene revealed increased anxiety (Rios et al 2001).

To overcome the aforementioned restrictions and to study possible behavioral effects of BDNF in adult mice, we used a different approach in this study. Because the biological activity of BDNF is mediated by trkB receptors, a receptor tyrosine kinase (Barbacid 1995), an alternative strategy to study BDNF function in vivo, is the use of mice with a disruption of the trkB gene; however, conventional trkB knockout mice die between birth and weaning age and demonstrate, similar to BDNF knockout mice, severe structural central nervous system (CNS) defects (Ernfors et al 1994; Jones et al 1994; Klein et al 1993). In contrast, recently generated mice with a conditional deletion of the trkB gene restricted to the forebrain are viable and have a normal brain morphology (Minichiello et al 1999). These so-called trkB$^{CaMKII\text{-}CRE}$ mice lack trkB receptors predominantly in the hippocampus and forebrain neocortex (Minichiello et al 1999), brain regions postulated to be critically involved in the pathogenesis of depression. Postmitotic excision of the trkB gene starts at about postnatal day 20, thus minimizing the risk of causing developmental CNS abnormalities (Minichiello et al 1999). According to the predictions of the neurotrophin hypothesis, this mouse strain represents a candidate model for "murine depression." To evaluate this hypothesis, we investigated trkB$^{CaMKII\text{-}CRE}$ mice in a series of behavioral tests for the presence of characteristic depressionlike symptoms, such as behavioral despair, anxiety, and neophobia. Because dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is considered a hallmark biological marker for human depression (Barden et al 1995; Nemeroff 1996), we also analyzed the HPA-system under basal conditions and after stress.

Methods and Materials

Animals

All animal procedures were approved by German and Swiss animal welfare authorities, respectively. trkB$^{CaMKII\text{-}CRE}$ mice were generated, bred, and genotyped as previously described (Minichiello et al 1999). Mice were kept on a C57BL/6N genetic background (>90%) with contributions of 129/sv and CBA/J from the transgenic crosses. Homozygous trkB$^{CaMKII\text{-}CRE}$ mice, as well as littermate control animals carrying one or two copies of the floxed trkB allele but no CRE-recombinase transgene, were used. The testing sets contained male and female animals in balanced numbers. Sexual analysis, however, will not be shown because all mutation effects were found to be independent of sex. Experimental animals were 3–6 months old. During the experimental period, animals were single housed in standard mouse cages on a 12-hour inverted light–dark cycle with lights on between 8 PM and 8 AM. Standard mouse food, water, and nesting material were available ad libitum. Behavioral analyses were carried out between 8:00 AM and 8:00 PM. The home cage rack was brought to the test room at least 30 min before each experiment, and each apparatus was thoroughly cleaned with 70% ethanol before releasing the animal. For data acquisition, animals were video tracked at 4.2 Hz and 256 × 256 pixel spatial resolution using a Noldus EthoVision 1.96 system (Noldus Information Technology, Wageningen, The Netherlands). For each sample, the system recorded x-y position, object area, and the status of defined event recorder keys on the keyboard. Raw data were then transferred to the public domain software Wintrack 2.4 (Wolfer et al 2001; www.dpwolfer.ch/wintrack) for further analysis.

Open Field Test

We investigated 10 trkB$^{CaMKII\text{-}CRE}$ mice and 16 control littermates first in the open field test and then in the elevated zero maze, followed by the emergence and novel object test, with 2 weeks interval between the tests, respectively. The round open field arena had a diameter of 150 cm, a smooth white plastic floor, and 35-cm-high sidewalls made of white polypropylene. Illumination was by indirect diffuse room light (four 40 W bulbs, 12 lux). Each subject was released near the sidewall and observed for 10 min. The same procedure was repeated the following day, resulting in a total observation time of 20 min, partitioned into four bins of 5 min for time course analysis. Recorded tracks were segmented into three motion states according to criteria modified from Drai et al (2001): 1) Progression episodes were defined by a velocity above the locomotion threshold of 8.5 cm/sec and a total distance moved >5 cm. Rapid decelerations deeper than 15 cm/sec were subtracted and classified as scanning. 2) Resting episodes were periods lasting 2 sec or longer with smoothed speed values (averaging frame .5 sec) below the system noise level of 2.5 cm/sec. 3) The remaining time was classified as scanning episodes. Whereas progression episodes typically corresponded to bouts of long-distance locomotion, scanning episodes correlated with exploratory behaviors such as brief stopping, sniffing, establishing snout contact with the substrate or an object, looking around, stretch attend postures, rearing, or leaning against the wall. Grooming episodes were mostly recorded as resting. To further characterize exploratory behavior, the arena was divided into three concentric zones: an exploration zone located in the center and comprising 50% of the arena surface, a 7-cm-wide wall zone and a transition zone in between. To obtain a measure of stereotypic movement, the arena was overlaid with a set of 25 quadratic tiles. The sequence of tile visits was scanned for repeating patterns, whereby each repetition incremented the stereotypy count of all involved tiles. Finally, the stereotypy counts of all tiles were summed. An exploration index was calculated as a measure of exploratory

efficiency by dividing the arena into quadratic tiles of approximately 7 × 7 cm^2 and by determining the percentage of tiles in which at least one scanning episode had occurred during a given period of time. To compute path linearity, an estimate of total distance moved based on only every eight-tile visit was compared with an estimate based on all visits.

Elevated Zero Maze

The zero maze consisted of a gray plastic annular runway (width 5.5 cm, outer diameter 46 cm, 40 cm above ground level). Two opposing 90° sectors were protected by inner and outer walls of gray polyvinyl-chloride (10 cm high). Animals were placed in one of the protected sectors and observed for 10 min. For time course analysis, this time was partitioned into two bins of 5 min. To complement video tracking, head dipping movements were recorded using the keyboard event-recorder function of EthoVision. Motion states were defined as in the other tests with progression thresholds adapted to the more strongly confined movements: speed threshold 4 cm/sec, distance threshold 3 cm, deceleration threshold 8 cm/sec. Three zones were defined as follows: a transition zone comprising four 30° segments at the ends of the protection walls separated the two 50°-wide protected and the two 70°-wide unprotected exploration zones. With these boundaries, the system detected entries to the unprotected sector only when the animal moved into it with all four paws. Head dips that occurred while the animal was registered to the transition zone with all or part of its body between the protection walls were classified as protected dips, all others as unprotected dips. The number of stretch attend postures was estimated based on forward–back movement sequences that occurred during scanning episodes in the transition zone and were not associated with head dips.

Emergence Test

Frames of nonreflective aluminum (37 cm high) were used to partition the earlier described open field into four square 50 × 50–cm arenas, allowing concurrent observation of four animals. Each arena had a 12 × 8 × 4–cm plastic home box with an opening of 8 × 4 cm^2, positioned in a corner at 5 cm from the nearest walls, with the opening facing away from the wall. This home box was thoroughly cleaned and placed in the home cage of each test subject 24 hours before testing. The next day, test subjects and home boxes were introduced into the arenas and observed for 30 min. For time course analysis, this period was partitioned into three 10-min bins. Motion state analysis was as in the open field. The definitions of the three zones were adapted to the test situation: a home zone of 18 × 22 cm^2 surrounded the home box and extended to the nearest portions of the sidewall. A 5-cm-wide wall zone extended along the rest of the sidewalls. The center of the arena was again defined as exploration zone. Exploration index, stereotypy, and path linearity were calculated as in the open field test. Vertical activity was estimated by counting reductions of object area by more than 250 mm^2 occurring while the animal was not progressing.

Novel Object Test

Arenas were the same as for the emergence test, but without the home box. The novel object was a 12 × 4–cm semitransparent 50 mL Falcon tube positioned vertically in the center of the arena. Each subject was observed for 30 min in the empty arena. The novel object was then introduced, and observation continued for another 30 min. For time course analysis, total observation time was partitioned into six 10-min bins. The same measures were calculated as in the emergence test, with three concentric zones defined as follows: a 5-cm-wide wall zone, a circular exploration zone of 16 cm diameter around the arena center where the object was introduced, and a transition zone in between. For relevant measures, we attempted to extract object-related components by extrapolating object unrelated components from the surrounding transition zone and subtracting them from the raw values measured in the exploration zone. This method brought corrected measures close to zero in absence of the object.

Porsolt Forced Swim Test

We investigated eight $trkB^{CaMKII\text{-}CRE}$ mice and eight control littermates, all without previous treatment. Mice were placed into a glass cylinder (23 cm high, 12 cm in diameter) that was filled with water (25°C) up to a height of 8 cm, as previously described (Porsolt et al 1977; Tronche et al 1999). A testing time of 6 min was used to determine the onset and the percentage of time spent in immobility. For time course analysis, this period was partitioned into three 2-min bins. Immobility was defined as motionless floating in the water, only allowing the movements that were necessary for the animal to keep its head above water. In contrast, swimming was defined as time spent engaged in active escape or struggling movement.

Statistical Analysis

To test for main effects of genotype, parameters were averaged over the entire observation period or, in the case of the novel object test, the period when the object was present, and subjected to a one-way factorial analysis of variance (ANOVA). Two-way factorial and repeated ANOVA designs, respectively, served to test for sex–genotype and time–genotype interaction. Whenever possible, ANOVA results were verified using nonparametric tests, which produced similar results. Statview version 5.0 (SAS Institute, Cary, North Carolina) was used for all statistical calculations.

Analysis of HPA Axis

Serum levels of corticotropin (ACTH) and corticosterone were investigated in eight $trkB^{CaMKII\text{-}CRE}$ mice and eight control littermates under basal conditions. Furthermore, ACTH and corticosterone levels were analyzed after 30 min of immobilization stress in six $trkB^{CaMKII\text{-}CRE}$ and eight mice, in a manner similar to that previously described (Tronche et al 1999). All animals were sacrificed by decapitation at circadian nadir (between 8 and 9 PM), when the difference in corticosterone levels between depressed and control individuals is expected to be

Table 1. Behavioral Measures Shared among Different Tests

	Open Field Test		Elevated Zero Maze		Emergence Test		Novel Object Test	
	ANOVA Genotype[a] *p* Value (%)	Time × Genotype[b,c] (*p*)	ANOVA Genotype[a] *p* Value (%)	Time × Genotype[b] (*p*)	ANOVA Genotype[a] *p* Value (%)	Time × Genotype[c,d] (*p*)	ANOVA Genotype[e] *p* Value (%)	Time × Genotype[d,f] (*p*)
% Time Spent								
Resting	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	<.0257 (−57)	<.0213
Scanning	<.0279 (−15)	*ns*	*ns*	*ns*	*ns*	*ns*	<.0132 (54)	<.0065
Progressing	<.0393 (18)	*ns*	*ns*	*ns*	<.0320 (34)	*ns*	*ns*	*ns*
Total Distance Moved	<.0077 (33)	*ns*	*ns*	*ns*	<.0452 (23)	*ns*	*ns*	*ns*
Scanning	<.0287 (−9)	*ns*	*ns*	*ns*	*ns*	*ns*	<.0175 (54)	<.0085
Progressing	<.0073 (43)	*ns*	*ns*	*ns*	<.0159 (48)	*ns*	*ns*	*ns*
Velocity								
Scanning	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
Progressing	<.0009 (24)	*ns*	*ns*	*ns*	<.0006 (13)	<.0071	*ns*	*ns*
Total Time Spent								
Wall zone	<.0001 (23)	<.0158	—		*ns*	*ns*	<.0032 (−48)	<.0004
Protected zone[g]	—		*ns*	*ns*	*ns*	*ns*	—	
Transition zone	<.0001 (−46)	<.0253	*ns*	*ns*	—	—	<.0191 (76)	<.0027
Exploration zone[h]	<.0007 (−59)	<.0604	*ns*	*ns*	<.0552 (−35)	*ns*	<.0038 (3.5×)	<.0029
Total Path								
Wall zone	<.0001 (81)	<.0014	—	—	<.0001 (64)	<.0010	*ns*[i] (12)	<.0200
Protected zone[g]	—	—	*ns*	*ns*	*ns*	*ns*	—	—
Transition zone	*ns*	*ns*	*ns*	*ns*	—	—	*ns* (34)	<.0011
Exploration zone[h]	<.0363 (−41)	<.0805	*ns*	*ns*	*ns*	*ns*	<.0247 (85)	<.0001
Stereotypy Count	<.0001 (3.1×)	<.0015	—	—	<.0266 (35)	*ns*	<.0417 (2.1×)	<.0003
Exploration Index	<.0008 (−20)	*ns*	—	—	<.0200 (−17)	*ns*	*ns*	*ns*
Path Linearity	<.0001 (10)	*ns*	—	—	*ns* (11)	*ns*	<.0722 (−11)	<.0135
Vertical Activity	—	—	*ns*	*ns*	<.0307 (−21)	*ns*	*ns*	*ns*

All behavioral parameters listed in the left column could be analyzed across the four tests analyzed here. Statistically significant changes between trkB$^{CaMKII\text{-}CRE}$ and control mice are given in % and are listed behind the respective *p* value. Time × genotype indicates whether behavioral differences observed change over time during the experiment.

ANOVA, analysis of variance.

[a]Repeated two-way ANOVA, main effect of genotype and change relative to control group.

[b]Repeated two-way ANOVA, entire experiment partitioned into 5-min bins.

[c]Significant interactions indicate that effect decreases over time.

[d]Repeated two-way ANOVA, entire experiment partitioned into 10-min bins.

[e]Factorial one-way ANOVA, on average over period when object is present.

[f]Significant interactions indicate that effect is restricted to or much larger during 10 min after addition of novel object.

[g]Zone around home box in emergence test, closed sectors on zero maze.

[h]Center field in open field and emergence test, object zone in novel object test, open sectors on zero maze.

[i]Baseline above controls, similar to controls after object introduction.

greatest (Holsboer 2000). Trunk blood was taken within 30 sec after the animal's removal from the cage. Corticosterone and ACTH serum levels were analyzed using commercially available radioimmunoassay kits (ICN Biomedicals, Eschwege, Germany). On a subset of six mutant and six control animals, immunostaining of the paraventricular hypothalamic nucleus, the median eminance and the pituitary gland was done using 6-μm paraffin sections obtained from brains fixed in 4% paraformaldehyde. Primary antibodies against corticotropin releasing hormone (CRH; diluted 1:200, UCB, Braine l'Alleud, Belgium); Neurophysin (1:10.000, Dr. Lang, Heidelberg, Germany), and ACTH (1:500, DAKO, Hamburg, Germany) and biotinylated secondary antibodies (VECTOR, Petersborough, United Kingdom) were used. The antigen–antibody complexes were visualized by 3′3-diaminobenzidine (Sigma, Munich, Germany) as chromogen.

Results

The description of the behavioral data obtained is restricted to the results most relevant for the scientific questions addressed here. Additional data with other behavioral changes and all statistical significance levels are shown in two tables. Table 1 summarizes behavioral measures shared among open field test, elevated zero maze, emergence test, and novel object test. Table 2 gives a synopsis of behavioral measures specific for individual tests, respectively.

Open Field Test

In the open field test, the general locomotor and exploratory behavior of mice is assessed by exposing the animals

Table 2. Test-Specific Behavioral Measures

	ANOVA Genotype[a] *p* Value (%)	Time × Genotype[b] (*p*)
Elevated Zero Maze		
Entries to closed sectors	*ns*	*ns*
Entries to open sectors (%)	*ns*	*ns*
Head dipping frequency	<.0078 (43)	*ns*
Protected head dips (%)	*ns*	*ns*
Estimated stretch attend postures	*ns*	*ns*
Falls off maze	<.0318 (8.3×)	*ns*
Deceleration to open zone	<.0341 (−12)	*ns*
Emergence Test		
Visits to box	*ns*	*ns*
Time in box (%)	*ns*	*ns*
Latency to exit box	*ns*	*ns*
Novel Object Test		
Object-related scanning episodes	<.0562 (77)	<.0346
Object-related scanning distance	<.0327 (89)	<.0078
Time near object	<.0368 (94)	<.0151
Object approaches	<.0013 (2.5×)	<.0001
Vertical activity toward object	<.0542 (92)	<.0742
Porsolt Swim Test		
Latency to float	<.001 (2.9×)	—
Floating time	<.0015 (−69)	—

ANOVA, analysis of variance.

[a]Factorial one-way ANOVA on averages over entire experiment or period when object is present (novel object test), change relative to mean of control group.

[b]Repeated two-way ANOVA with experiment partitioned into 10-min bins or 5-min bins (elevated zero maze). Significant interactions indicate that effect is restricted to or much larger during 10 min after addition of novel object.

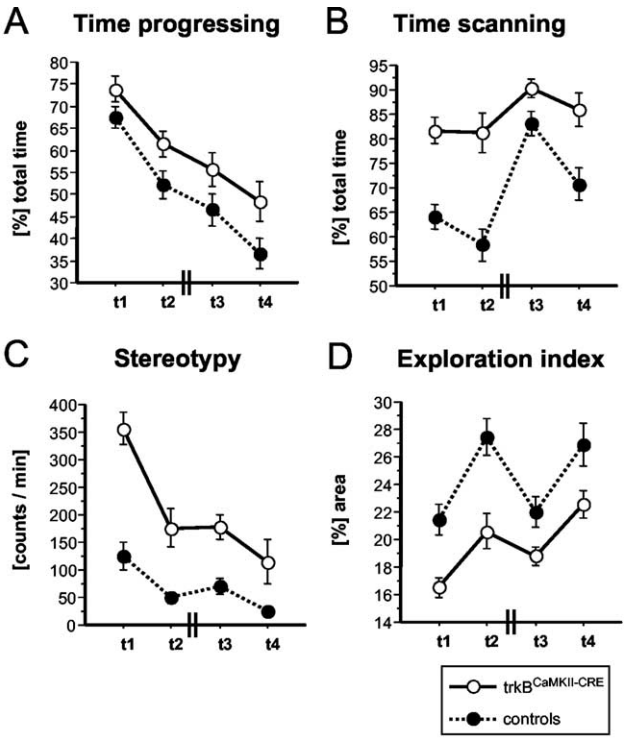

Figure 1. In the open field test, trkB$^{CaMKII\text{-}CRE}$ mice exhibit increased locomotion and decreased exploratory behavior. **(A, B)** In each phase of the test, trkB$^{CaMKII\text{-}CRE}$ mice spend significantly more time progressing ($p < .04$) and less time scanning ($p < .03$) than littermate control mice. **(C, D)** trkB$^{CaMKII\text{-}CRE}$ mice investigate a smaller fraction of the arena surface, resulting in a reduced exploration index ($p < .001$), due to more frequent stereotypic movements ($p < .001$). t1–t4 represent 5-min bins, t3–t4 was carried out 24 hours after t1–t2.

to a large open arena under dim light conditions. In this test, trkB$^{CaMKII\text{-}CRE}$ mice moved overall a longer distance than control subjects (Table 1). In addition, differential analysis of motion states revealed that progressive locomotion was drastically increased at the expense of small scanning movements in a speed range that is associated with exploratory behaviors, such as brief stopping, sniffing, looking around, stretch attend postures, rearing, or leaning against the wall (Figure 1A-D, Table 1). Resting time was not significantly altered (Table 1). trkB$^{CaMKII\text{-}CRE}$ mice spent significantly less time in the center field, and this was associated with an abnormal pattern of locomotion. The trkB-mutant mice moved in a highly linear and stereotyped way (Figure 1C), mostly following the wall, resulting in hypolocomotion in the central exploration zone compared with control mice. Thus, despite overall increased locomotor activity, trkB$^{CaMKII\text{-}CRE}$ mice actually explored a smaller fraction of the arena than did control mice, as evidenced by a reduced exploration index (Figure 1D).

Elevated Zero Maze

The elevated zero maze is a standard test to analyze anxiety-related behavior by measuring the avoidance of the unsheltered open sectors. Percent entries to and percent time spent on open sectors (i.e., basic pharmacologically validated measures of anxiety in this paradigm) were not altered in trkB$^{CaMKII\text{-}CRE}$ mice (Figure 2A, Table 1). The proportion of protected head dips (i.e., dips made from the transition zone while the body of the animal was still between the protection walls) and the number of stretched attend postures toward the open sectors were more recently introduced as ethological anxiety measures on the zero maze (Shepherd et al 1994). They were not increased in trkB$^{CaMKII\text{-}CRE}$ mice (Figure 2B,C). Rather, the mutants performed overall more head dips than control mice and were so careless during this activity that they frequently fell off the maze (Table 2) (Figure 2D). According to our database of more than 1000 mice (different genetic backgrounds and mutations), total number of head dips is not a correlate of locomotor activity on the zero maze or in other tests. Most likely, it reflects the impulsivity of these mice. Detailed analysis of locomotion on the zero maze revealed only minimal changes, which were too small to result in

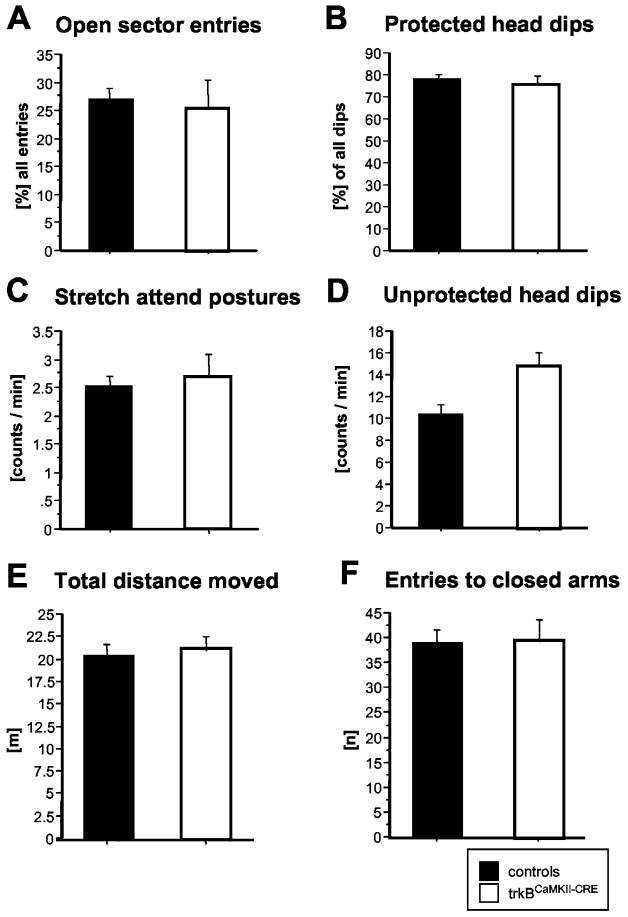

Figure 2. In the elevated zero maze, trkB$^{CaMKII\text{-}CRE}$ mice do not show increased anxiety-related behavior. **(A)** The relative frequency of open sector entries is not reduced. **(B)** The anxiety-related percentage of protected head dips performed without fully leaving the protected sector is not increased. **(C)** trkB$^{CaMKII\text{-}CRE}$ mice do not show increased risk assessment in the form of stretched attend postures. **(D)** On the contrary, they are more careless and perform more head dips while on the open sectors ($p < .01$). **(E)** trkB$^{CaMKII\text{-}CRE}$ mice do not show increased locomotor activity as judged by the total distance moved and **(F)** by entries to the closed sectors, a more "classical" measure of general activity.

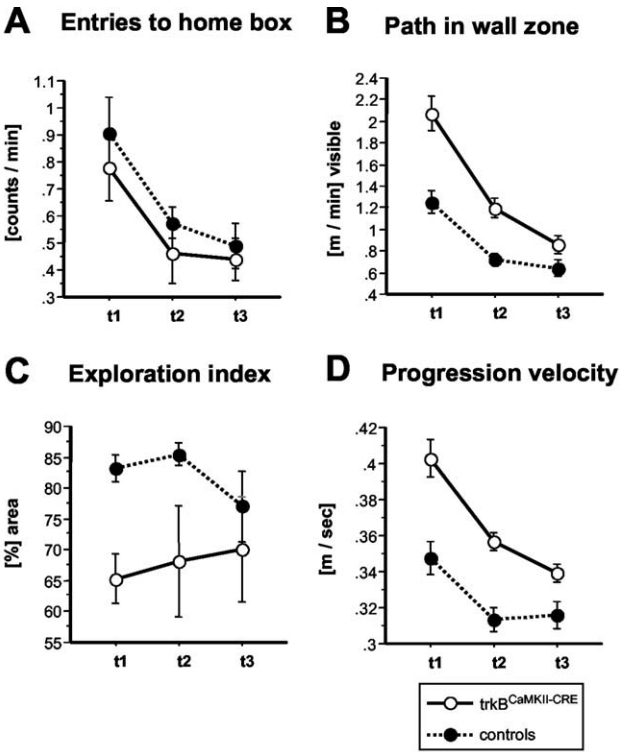

Figure 3. In the emergence test, trkB$^{CaMKII\text{-}CRE}$ mice do not demonstrate increased anxiety-related behavior. **(A)** Their number of entries to the sheltered home box was not altered. **(B, C)** As in the open field, trkB$^{CaMKII\text{-}CRE}$ mice move more in the wall zone ($p < .001$) and exhibit a reduced exploration index ($p < .01$), **(D)** while progressing with a higher velocity ($p < .001$). t1–t3 represent consecutive 10-min bins, respectively.

statistically significant differences (Figure 2E,F); however, analysis of velocity revealed that the deceleration shown by normal mice when they approached the open sectors was strongly diminished in trkB$^{CaMKII\text{-}CRE}$ mice.

Emergence Test

The emergence test is a free exploration paradigm designed to assess approach or exploratory behavior of rodents (Dulawa et al 1999) in an environment that provides a safe refuge (i.e., a small home box to which the animals had been familiarized for 24 hours in their home cage). Latency to exit and total time spent inside the home box as well as number of entries to the box, which are generally considered measures of fear, were not increased in trkB$^{CaMKII\text{-}CRE}$ mice (Figure 3A); however, analysis of the behavior inside the visible arena confirmed the wall-bound stereotyped hyperlocomotion (Figure 3B) combined with a reduced exploration index (Figure 3C) that had already been observed in trkB$^{CaMKII\text{-}CRE}$ mice in the open field test. In addition, they showed again a significantly elevated progression speed (Figure 3D) at the expense of their vertical activity (Table 1). The emergence test also confirmed the tendency of trkB$^{CaMKII\text{-}CRE}$ mice to exhibit abnormal stereotyped behavioral patterns (Table 1).

Novel Object Test

The novel object test is another free exploration paradigm that exposes the animals to a novel stimulus in a familiar arena and inflicts an approach avoidance conflict (Belzung 1992; Renner et al 1992). In this test, depressionlike

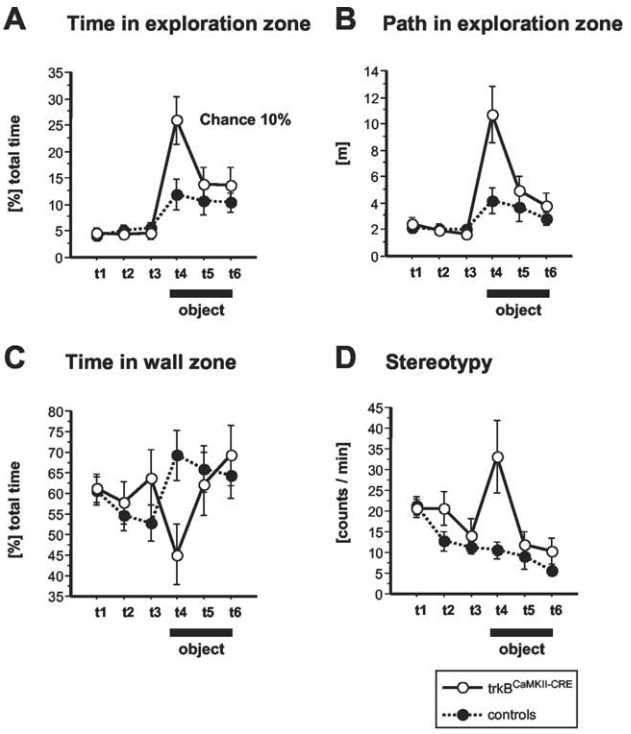

Figure 4. In the novel object test, trkB$^{\text{CaMKII-CRE}}$ mice reveal increased activity directed toward this object. **(A, B)** After introduction of the object into the arena during bins t4–t6, trkB$^{\text{CaMKII-CRE}}$ mice spend more time exploring the object ($p < .05$) and cover more distance during that time ($p < .05$). **(C)** As a result trkB-mutant mice spend less time in the wall zone ($p < .01$). **(D)** After introduction of the object, there is a strong increase of stereotypic movements ($p < .05$). The object-related activity of the mutants rapidly declines after 5–10 min. t1–t6 represent consecutive bins of 10 min, respectively.

behavior of rodents usually correlates with a reduced exploration of the novel object. Before introduction of the novel object into the arena (which was the same as in the emergence test), trkB$^{\text{CaMKII-CRE}}$ mice showed fewer abnormalities of locomotor behavior than in the emergence test, most likely due to habituation (Figure 4A–D, Table 1). Introduction of the novel object elicited a radically new pattern of anomalous behavior. The hyperlocomotion shifted away from the sidewall and toward the object (Figure 4A–C). Activity near the object was not only quantitatively increased but also qualitatively altered (Figure 4D). Whereas control mice mainly sniffed at the object and touched it with their whiskers, trkB-mutants approached it with high frequency, ran around it, and attempted to climb or jump on it more often than control mice. The typical retreat to one of the corners of the arena displayed by most control mice was much less evident in trkB$^{\text{CaMKII-CRE}}$ mice (Table 1). The object-related activity of trkB-mutant mice rapidly declined after 5–10 min.

Porsolt Forced Swim Test

The forced swim test is a well-established paradigm that assesses the tendency to give up attempting to escape from an unpleasant environment, with fewer attempts to escape interpreted as evidence of behavioral despair. This test possesses high predictive validity and good face validity as a screen for depressionlike behavior (Cryan et al 2002; Porsolt et al 1978; Willner 1991). The trkB$^{\text{CaMKII-CRE}}$ mice revealed a highly increased latency to start floating as well as a drastically reduced total floating time when compared with their control littermates (Figure 5). Thus trkB$^{\text{CaMKII-CRE}}$ mice showed distinctly less despair behavior than control mice.

HPA Axis Analysis

The trkB$^{\text{CaMKII-CRE}}$ mice showed baseline corticosterone and ACTH levels similar to those of control littermates (Figure 6A,B). After 30 min of immobilization stress, a significant increase of both ACTH and corticosterone levels was observed in both genotypes, but again no difference was seen between trkB$^{\text{CaMKII-CRE}}$ mice and the controls (Figure 6A,B). Immunohistochemical analysis of the HPA axis revealed no differences in the detectable amounts of CRH in the paraventricular hypothalamic nucleus and in the median eminence (Figure 6C). Furthermore, similar amounts of ACTH were found in the pituitary gland of control and trkB$^{\text{CaMKII-CRE}}$ mice (data not shown).

Discussion

In this study, we investigated the behavior of mice mutant for trkB receptors, to test the prediction of the "neurotrophin hypothesis of depression" that such mice would show depressionlike behavior. To avoid the problem of early mortality and severe CNS defects associated with a general ablation of the trkB gene, we used conditional mutant mice in which trkB receptors were eliminated in a brain-region and temporal-specific manner using the CRE-loxP recombination system under the control of a CaMKII transgene. Disruption of trkB receptors in trkB$^{\text{CaMKII-CRE}}$ mice is restricted to the forebrain and does not start before postnatal day 20 (Minichiello et al 1999). Behavioral analyses of conditional mutant mice revealed a stereotyped hyperlocomotion with an increase of large distance locomotion at the expense of exploration activity. Small movements associated with exploratory behaviors such as brief stopping, sniffing, looking around, stretch attend postures, rearing, or leaning against the wall were significantly reduced (Figure 1); however, trkB$^{\text{CaMKII-CRE}}$ mice did not reveal any kind of depressionlike behavior. In our study, various established indicators of anxiety-related

behavior showed strikingly inconsistent results both across and within tests. In the open field, center exploration was largely reduced (Figure 1). In a pharmacologic model, this would be interpreted as an anxiogenic effect. On the zero maze, however, entries to open arms, protected head dips, and number of stretched attend postures were unchanged, whereas the increased frequency of unprotected head dips even suggested an anxiolytic effect (Figure 2). In the emergence test, the short latency to exit the home box and the unchanged total time spent inside the box also argued against increased anxietylike behavior, yet again the mice did not often venture to the center field (Figure 3). Finally, taken alone, the massively increased exploration of a novel object suggested reduced anxietylike behavior (Figure 4).

During past studies, we have repeatedly observed that anxiety-related behavior in mutant mice varied in different test situations and according to different indicators, especially if the same animals were tested in a battery of procedures. Generally, the trends could be interpreted either as an increase or decrease in anxiety-related behaviors; however, the pattern of changes found in $trkB^{CaMKII\text{-}CRE}$ mice includes both extremes of the behavioral spectrum. Thus, they can hardly be interpreted in terms of anxiolytic or anxiogenic effects. A reconciling interpretation is that these peculiar behavioral patterns of $trkB^{CaMKII\text{-}CRE}$ mice are caused by impulsive behavior and stimulus-bound hyperactivity. When exposed to a novel stimulus or environment, their activity tends to increase and becomes bound to the most salient stimulus. If the arena is empty or contains only familiar objects, $trkB^{CaMKII\text{-}CRE}$ mice run along the wall in a stereotyped manner. If a novel object appears in the center of the arena, their activity is diverted toward that object (Figure 4). On the zero maze, they are so strongly attracted by the cliff that they tend to fall down. In the Porsolt forced swim test, a standard test for depressionlike behavior, mice did not show despair, but on the contrary demonstrated significantly increased struggling, likely as a consequence of their tendency to respond to environmental changes by increased activity (Figure 5). Alternatively, primary hyperlocomotion could have also reduced the despair-related floating scores. Finally, no HPA axis dysregulation, which is a key biological marker for a subset of patients with severe depressive episodes, could be demonstrated in $trkB^{CaMKII\text{-}CRE}$ mice under normal and stressful conditions (Figure 6).

The behavioral findings observed here in $trkB^{CaMKII\text{-}CRE}$ mice are in accordance with observations in BDNF heterozygous mice, if one takes into account that the latter mice do not have an ablation but only a gene dose reduction of BDNF (i.e., that behavioral effects may not be as prominent as in animals with a homozygous deletion). Similar to $trkB^{CaMKII\text{-}CRE}$ mice, BDNF heterozygous mice show hyperlocomotion and a trend to "neomania" in a novel object test, but at the same time an evident lack of depressionlike behavior in the forced swim test as well as in the learned helplessness paradigm (Kernie et al 2000; MacQueen et al 2001; Saarelainen et al 2003). Thus, our findings in $trkB^{CaMKII\text{-}CRE}$ mice make it unlikely that developmental compensation or a gene dose effect are responsible for the lack of depressionlike symptoms in BDNF heterozygous mice (Wolfer et al 2002). Mice with a brain-specific conditional knockout of the BDNF gene also demonstrated hyperactivity and a dysregulated eating behavior as main abnormalities, the latter also being observed in a subset of BDNF heterozygous mice (Lyons et al 1999; Rios et al 2001). With the caveat that hyperlocomotion could have masked some depressive features, these data suggest that the lack of BDNF/trkB expression in the forebrain is not related to the development of an overt depressionlike syndrome in mice.

Current concepts suggesting that a reduced BDNF expression as a pathogenetic factor in the development of depression are mainly based on the observation that BDNF expression is downregulated in the rodent hippocampus by stress (Altar 1999; Duman et al 1997; Nestler et al 2002; Smith et al 1995; Vaidya et al 1999). On the other hand, stress, which can be applied in different forms, is currently the only behavioral measure to induce depressionlike syndromes in rodents (Nestler et al 2002; Porsolt 2000; Willner 1997). Therefore, it remains an open question whether BDNF downregulation in rodents correlates with the development of depressionlike symptoms, or whether it represents a spurious effect of the stress procedure. The present study using $trkB^{CaMKII\text{-}CRE}$ mice indicates that an ablation of BDNF signaling in the forebrain, mainly in the hippocampus and neocortex, does not lead to depression. Our results, however, cannot exclude the possibility that BDNF signaling in other brain areas is crucial for the pathogenesis of depression. Furthermore, it remains possible that $trkB^{CaMKII\text{-}CRE}$ mice show an increased sensitivity to stress and that alterations of BDNF signaling represent a risk factor for the development of depression following stress or other aversive events.

A dissociation between the presence of depressionlike behavioral symptoms and the levels of BDNF expression was also observed in $CREB^{\alpha\Delta}$ mice, a mouse strain with 90% reduced cAMP (adenosine monophosphate)-response element binding (CREB) transcriptional activity (Blendy et al 1996; Conti et al 2002). These mice demonstrate a significantly reduced despair reaction in the forced swim test as well as in the tail suspension test, but no alterations of BDNF expression in hippocampus and neocortex. In contrast to baseline conditions, BDNF is a target gene of the transcription factor CREB following chronic antide-

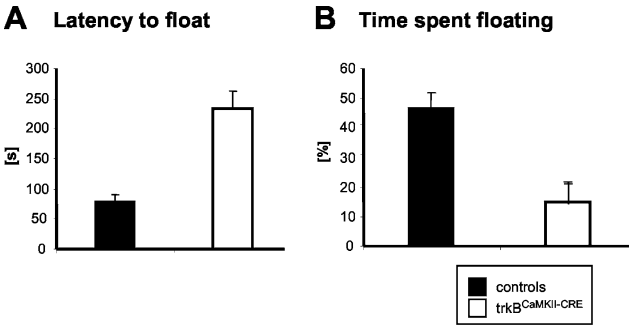

Figure 5. In the Porsolt forced swim test, trkB$^{CaMKII\text{-}CRE}$ mice demonstrate highly diminished despair behavior. trkB-mutant mice exhibit (**A**) a significantly increased latency to start floating ($p < .01$) and (**B**) a severely reduced total floating time ($p < .01$).

pressant therapy. Treatment with the tricyclic antidepressant desipramine caused a significant induction of BDNF in the hippocampus and neocortex in wildtype mice, whereas this upregulation was absent in CREB$^{\alpha\Delta}$ mice (Conti et al 2002). Together with the data from our study and studies on BDNF mutant mice, this may indicate that BDNF expression levels in the forebrain are less critical for the development of depression, but crucial for the effect of antidepressant therapy. This concept is supported by experiments that show a direct antidepressantlike effect in the forced swim test and the learned helplessness paradigm following intrahippocampal injection of BDNF (Shirayama et al 2002). It is further substantiated by recent

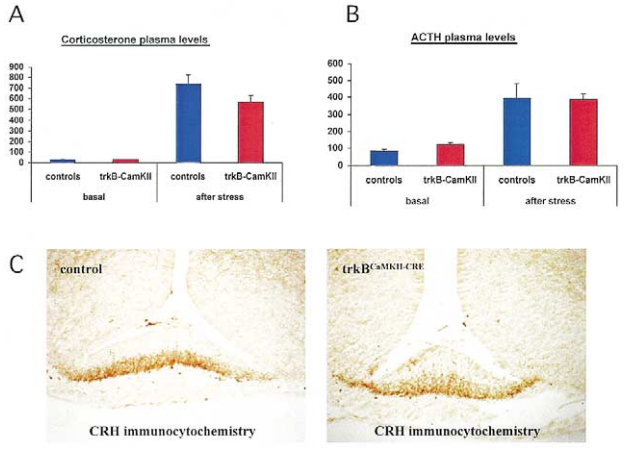

Figure 6. The hypothalamic-pituitary-adrenal system is not altered in trkB$^{CaMKII\text{-}CRE}$ mice. **(A, B)** trkB-mutant mice demonstrate the same plasma levels of corticosterone and adrenocorticotropic hormone (ACTH), both in basal conditions and 30 min after immobilization stress, as their control littermates. **(C)** Immunohistochemical stainings of corticotropin releasing hormone (CRH) in the median eminence reveal no differences between control and trkB$^{CaMKII\text{-}CRE}$ mice.

evidence that trkB activation is induced by antidepressant drugs and required for antidepressant induced behavioral effects (Saarelainen et al 2003).

Dysregulation of the HPA axis is a biological marker for depression as well as for an altered sensitivity to stress (Barden et al 1995; de Kloet 2000; Holsboer 2000). Because the HPA axis is partly controlled by higher telencephalic regions such as the hippocampus (de Kloet 2000; De Kloet et al 1998), it seemed possible that also mice with a disruption of trkB receptors restricted to the forebrain would show alterations of the HPA axis; however, trkB$^{CaMKII\text{-}CRE}$ mice exhibited normal basal and stress-induced corticosterone and ACTH plasma levels (Figure 6). Furthermore, immunohistochemical analysis of the HPA axis did not show any difference between wildtype and trkB$^{CaMKII\text{-}CRE}$ mice concerning CRH expression in the hypothalamic paraventricular nucleus and in the median eminence, as well as the ACTH protein amount in the anterior lobe of the pituitary (Figure 6). Thus, these key biological markers for depression are also negative in trkB$^{CaMKII\text{-}CRE}$ mice.

In conclusion, the forebrain-specific disruption of trkB receptors in mice does not entail depressionlike symptoms. Thus, this mouse strain should not be regarded as a genetic mouse model for depression as expected by the neurotrophin hypothesis (Altar 1999; Duman et al 1997; Manji et al 2001; Nestler et al 2002; Vaidya and Duman 2001; Wong and Licinio 2001). Instead, we observed a behavioral syndrome including hyperlocomotion, stereotyped behaviors, and increased attraction by a novel object. Together with the previously described cognitive impairments of trkB$^{CaMKII\text{-}CRE}$ mice (Minichiello et al 1999), these behavioral features are similar to those that have been postulated for mouse models of attention-deficit disorder (Gainetdinov et al 1999; Gainetdinov and Caron 2000). Further experiments will determine whether trkB$^{CaMKII\text{-}CRE}$ mice respond to pharmacotherapy with psychostimulants by a normalization of their behavioral alterations.

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