Title
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Journal

ISSN

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Publication Date

DOI

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Peer reviewed

Behavioral Analysis of Transgenic Mice Overexpressing Corticotropin-Releasing Hormone in Paradigms Emulating Aspects of Stress, Anxiety, and Depression

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Chronically elevated levels of corticotropin-releasing hormone (CRH) are implicated in human stress-related and affective disorders, including generalized anxiety disorder and major depression. To gain more insight into the relationship between hyperactivity of the CRH system and associated neuroendocrine, autonomic, physiological, and behavioral changes, we have developed a transgenic mouse model of CRH overproduction (CRH-OE). In this study, we explored the behavioral consequences of chronic CRH overproduction in mice of the two available transgenic lines (CRH-OE2122 and CRH-OE2123) in paradigms measuring behavioral aspects of stress, anxiety, and depression. These paradigms include tasks based on free exploration of novel environments (unfamiliar home-cage and unfamiliar open field), stress-induced hyperthermia, tests associated with anxiety-related behavior (elevated plus maze and light-dark box), and paradigms in which (anti-)depressant-like behaviors can be detected (tail suspension test and forced swim test). The only relatively consistent finding, although not always significant, was reduced locomotor activity in CRH-OE$_{2122}$ mice, corresponding well with the known effects of CRH on locomotion. Contrary to our predictions, CRH-OE mice did not show an altered response to stress, and a phenotype indicative of increased anxiety and/or depression was not evident in CRH-OE mice.

Corticotropin-releasing hormone (CRH) plays a pivotal role in the response of an organism to various stressors, coordinating neuroendocrine, autonomic, behavioral and immunological responses to stress (Dunn & Berridge, 1990; Holsboer, 1999; Koob et al., 1993; Koob & Heinrichs, 1999; Owens & Nemeroff, 1991; Vale et al., 1981). Chronically elevated levels of CRH are implicated in human stress-related and affective disorders, including major depression (see Arborelius et al., 1999; Mitchell 1998).

To gain more insight into the relationship between hyperactivity of the CRH system and associated neuroendocrine, autonomic, physiological, and behavioral changes, we have developed a transgenic mouse model of life-long CRH overproduction (CRH-OE), under control of a Thy-1 promotor which drives constitutive transgene expression in neurons in postnatal and adult brain (e.g.,

Morris & Grosveld, 1989; Vidal et al. 1990; Moechars et al. 1996; Lüthi et al. 1997; Wiessner et al. 1999). The procedures used to generate transgenic mice yielded two fertile, transgenic founder animals, which gave rise to two independent lines of transgenic animals. The differences between the two lines consist of the site in the genome where the transgenic construct is inserted, the number of copies inserted, and the level and location in the brain of transgene expression. In earlier experiments we have observed that despite the presence of the same transgene in their genome, in only one of the two established transgenic lines (CRH-OE2122, but not CRH-OE2123) life-long central CRH overproduction is associated with chronic stress-like alterations, including increased CRH content in the hypothalamus, changes in HPA-axis (hypothalamic-pituitary-adrenal axis) regulation, and increased heart rate and body temperature (Dirks et al., in press; Groenink et al., 2002).

In the present study, we explored the behavioral consequences of chronic CRH overproduction in CRH-OE2122 and CRH-OE2123 mice in behavioral paradigms reflecting different aspects of stress, anxiety, and depression. These paradigms include tasks based on free exploration of novel environments (unfamiliar homecage or unfamiliar open field), stress-induced hyperthermia, the elevated plus maze, the light-dark box, the tail suspension test, and the forced swim test.

As reviewed by Holmes (2001), the behavioral profile in an unfamiliar and well-lit open field reflects the internal conflict between the innate tendency to explore a novel place and the avoidance of an aversive novel environment. High levels of exploration in an aversive environment are interpreted as reflecting low levels of anxiety-like behavior. In this test, increased anxiety-like behavior is primarily associated with avoidance of the central, exposed part of the open field, as anxious rodents tend to show more defensive thigmotactic behavior along the walls of the open field (Treit & Fundytus, 1988). The stress-induced hyperthermia paradigm in singly-housed mice (ISIH) is based on the phenomenon that in mice stress results in an acute increase in body temperature (Olivier et al., 1998; van der Heyden et al., 1997). The elevated plus maze is based on the ethologically-relevant conflict between the tendency of mice to explore a novel environment and the aversive properties of an open, elevated visual cliff (Crawley et al., 1997; Lister, 1987). It is considered a standard paradigm for testing anxiety-like responses in mice. The light-dark exploration model of anxiety-related behaviors is based on a similar conflict between the tendency of mice to explore a novel environment and the aversive properties of a brightly lit area (Crawley et al., 1997). In the light-dark box, an aversion to the brightly lit compartment produces a clear preference for the dark compartment (e.g., Griebel et al., 2000; Hascoet et al., 2001; van Gaalen & Steckler, 2000). In the tail suspension test, mice are suspended by the tail, using adhesive tape, to either a horizontal (Stéru et al., 1985) or vertical bar (present study). Typically, mice immediately engage in several "agitation- or escape-like" behaviors followed temporarily by increasing bouts of immobility that is reversed by antidepressant treatment (Dalvi & Lucki, 1999; Stéru et al., 1985). In the forced swimming test, rodents become immobile after an initial intense swimming period. Acute treatment with antidepressants reduces immobility time in mice (Dalvi & Lucki, 1999). The immobility behavior is thought to reflect either a failure to persist in escape-directed behavior or the development of passive behavior that disen-

gages the animal from active forms of coping with stressful stimuli (see Dalvi & Lucki, 1999; Lucki, 1997, 2001; Lucki et al., 2001; Porsolt et al., 1977).

Considering the behavioral effects mediated by CRH in reaction to stress and aversive events, we hypothesized that mice overexpressing CRH should show an altered response to stress and a phenotype indicative of increased anxiety and/or depression.

Method

Subjects

The CRH transgene used for the generation of the CRH-OE mice was composed of the complete coding sequence of rat CRH cDNA (0.6 kb fragment; Thompson et al., 1987), which was inserted into a 8.2 kb genomic DNA fragment encompassing the murine Thy-1.2 gene, including regulatory regions and polyadenylation signal sequence (Aigner et al., 1995). The Thy-1 regulatory sequences drive constitutive transgene expression in postnatal and adult neurons (Lüthi et al. 1997; Moechars et al. 1996; Morris and Grosveld 1989; Vidal et al. 1990; Wiessner et al. 1999). To identify transgenic founder animals, tail DNA from offspring was screened by standard Southern dot-blot analysis using the 0.6 kb CRH cDNA fragment as probe. These procedures yielded two fertile, transgenic founder animals (one female and one male) which gave rise to two independent lines of transgenic animals (C57BL/6J background). Subsequent breeding at the local breeding facilities (Central Laboratory Animal Institute, Utrecht, The Netherlands) consisted of matings between transgenic males and wildtype C57BL/6J females. Tail DNA from all offspring, extracted with the High Pure PCR Template Preparation Kit (Boehringer, Mannheim, Germany), was screened using PCR with transgene-specific primers. The forward-primers were specific for rat CRH and the reversed-primers originated from the Thy-1 promotor, thus excluding the possibility that the endogenous CRH and Thy-1 gene were amplified.

Male transgenic mice of the two established lines (n=9 CRH-OE$_{2122}$ and n=12 CRH-OE$_{2123}$) were used in these experiments. Littermate wildtype (WT, n=13) mice served as controls. The mice were 11-16 weeks old at the time of testing. Animals were individually housed at constant room temperature (21 ± 2 °C) and relative humidity (50-60 %) in standard Macrolon-I (21 x 10 x 13 cm) cages, with a tissue as cage-enrichment. Standard rodent food pellets (Hope Farms, Woerden, The Netherlands) and water were freely available. Mice were maintained on a 12-h light-dark cycle (light on at 07:00 h). All experimental procedures were conducted during the light phase of the cycle.

Apparatus and Procedure

Spontaneous Behavior in an Unfamiliar Cage. An animal was transferred to a clean unfamiliar homecage, placed in a sound-attenuated box to allow recording of undisturbed behavior. During 10 min the behavior of the mouse was recorded on video. Videotapes were analyzed using The Observer (Noldus Information Technology, Wageningen, The Netherlands). The extent of locomotion and immobility were scored as well as the time spent sniffing, burying and grooming, and the number of rearings and stretch attend postures (SAPs).

Open field. Spontaneous locomotor behavior was quantified in an open field. The open field was a round, opaque plastic box (diameter 45 cm, height 29.5 cm), divided in a center, middle, and outer ring, placed in a sound-attenuated box to allow recording of undisturbed behavior (illumination ca 2400 lux). During 10 min the behavior of the mouse was recorded using the EthoVision automated tracking program (Noldus Information Technology). Total distance moved, total time moving, time spent in outer, middle, and center ring, time spent grooming, and number of rearings were scored.

Stress-Induced Hyperthermia. The stress-induced hyperthermia procedure in singly housed mice (ISIH procedure) is described extensively elsewhere (Olivier et al., 1998). Briefly, basal body temperature (T_0) was recorded using a rectal probe (Digital Thermometer 871A, Tegan Inc., Ohio, USA). Ten min later rectal temperature was measured again ($T10$). Typically, the mild stress of

the temperature measurement at t=0 causes an increase in body temperature of ca 1-1.5 °C 10 min later. The stress-induced hyperthermia is expressed as the difference $T_{10} - T_0$ (delta BT).

Elevated Plus Maze. The elevated plus maze was made of black Plexiglas with 29 cm long and 5 cm wide arms, extending from a central platform (5 cm square) elevated 75 cm above the floor. Two opposite arms were enclosed by black Plexiglas walls (15 cm high; illumination level 30 lux) and two arms were open (illumination level 60 lux) with a small ledge (0.25 cm high). The mouse was placed on the central platform facing an enclosed arm. During 5 min behavior of the mouse was recorded using EthoVision. The total distance moved, the number of open arm, closed arm and central platform entries, the time spent in the different compartments of the maze, time spent grooming, and number of rearings and stretch attend postures were scored.

Light-Dark Box. A rectangular, Plexiglas box divided into two compartments, one consisting of black Plexiglas (13.5 x 27 x 30 cm) and illuminated by red light (40 lux), and one of white Plexiglas (26.5 x 27 x 30 cm) illuminated by bright light (950 lux) was used. The compartments were connected by a 7.5 x 7.5 cm wide opening. The light-dark box was placed in a sound-attenuated box to allow recording of undisturbed behavior. A session started by placing the animal in the center of the light compartment facing the opening, and lasted 5 min. Behavior of the mouse was recorded using EthoVision. The total distance moved, the number of entries and the time spent in the light and dark compartments, time spent grooming, and number of rearings were scored.

Tail Suspension Test. In this test, six identical Mouse Tail Suspension systems (PHM-300, MED Associates, Vermont, USA) were used, consisting of white PVC cubicles (inside dimensions of 33 x 33 x 31.75 cm) with a removable tail hanger. Mice were individually suspended by the tail to the vertical tail hanger using adhesive tape (1.5 cm from the base of the tail). During 6 min the duration of immobility was measured using automatic data collection software (MED Associates).

Forced Swim Test. Clear plastic cylinders (diameter 12 cm, height 25 cm) were filled to a depth of 10 cm with water (25 °C). Mice were placed in the water for 6 min and behavior was recorded on video. The same procedure was repeated the following day. Videotapes were analyzed using The Observer and duration of immobility, swimming and climbing only during the last 3 min were scored for both days (see Dalvi & Lucki, 1999).

Experimental Design. All mice were tested in the aforementioned paradigms. Tests were separated from each other by at least one week and were conducted in the following order: unfamiliar cage, elevated plus maze, open field, light-dark box, ISIH, tail suspension test, and forced swim test.

Data Analysis. Data were analyzed by one-way analysis of variance (ANOVAs) on genotype unless otherwise stated. Posthoc tests consisted of independent t-tests, with Bonferroni correction of α for repeated between-subject comparisons. The level of significance was set at $p < 0.05$.

Results

The results of the analysis of spontaneous behavior in an unfamiliar home-cage in mice of both transgenic lines and control WT mice are listed in Table 1. The duration scores of sniffing and grooming were not different between groups (sniffing: $F(2, 31) = 2.64$; grooming: $F < 1$). Rearing activity of CRH-OE$_{2123}$ mice was significantly increased only when compared to CRH-OE$_{2122}$ mice, $F(2, 31) = 3.43$. Both CRH-OE$_{2122}$ and CRH-OE$_{2123}$ mice showed decreased time spent digging in the sawdust $F(2, 31) = 5.85$. CRH-OE$_{2123}$ made significantly fewer SAPs than WT mice, $F(2, 31) = 4.03$, but the total number of stretch-attend postures (SAP) was extremely low. The duration of locomotion and immobility were not significantly different between the genotypes $F(2, 31) = 2.32$ and $F < 1$, respectively.

In the open field test, CRH-OE$_{2122}$ mice showed a significant reduction in the duration of locomotion (Figure 1; $F(2, 31) = 6.83$), due to a significant de-

crease in the time spent moving in only the outer ring of the open field (center ring: $F_{(2, 31)}=1.18$; middle ring: $F_{(2, 31)} = 1.08$; outer ring: $F_{(2, 31)} = 4.59$). These findings in CRH-OE2122 mice were paralleled by a nonsignificant decrease in the total distance moved when compared to WT mice, due to a reduction in the dis-

Table 1

Spontaneous Behavior of CRH-OE and WT Mice in Unfamiliar Homecage.

	WT (n = 13)	CRH-OE$_{2122}$ (n = 9)	CRH-OE$_{2123}$ (n = 12)
Locomotion (s)	153.6 ± 9.9	167.9 ± 13.8	187.4 ± 12.0
Immobility (s)	6.7 ± 2.1	3.9 ± 1.4	5.8 ± 2.3
Sniffing (s)	132.8 ± 21.0	184.8 ± 25.3	109.2 ± 21.7
Grooming (s)	28.3 ± 5.1	33.1 ± 4.4	35.5 ± 5.2
Rearing (#)	62.3 ± 7.7	61.3 ± 5.7	81.4 ± 3.8 ‡
Burying (s)	88.0 ± 18.0	31.1 ± 9.4 *	36.9 ± 6.0 *
SAPs (#)	2.1 ± 0.7	0.9 ± 0.6	0.1 ± 0.1 *

Note. s: duration in seconds, #: frequency; *: $p < 0.05$ vs. WT; ‡: $p < 0.05$ vs. CRH-OE$_{2122}$
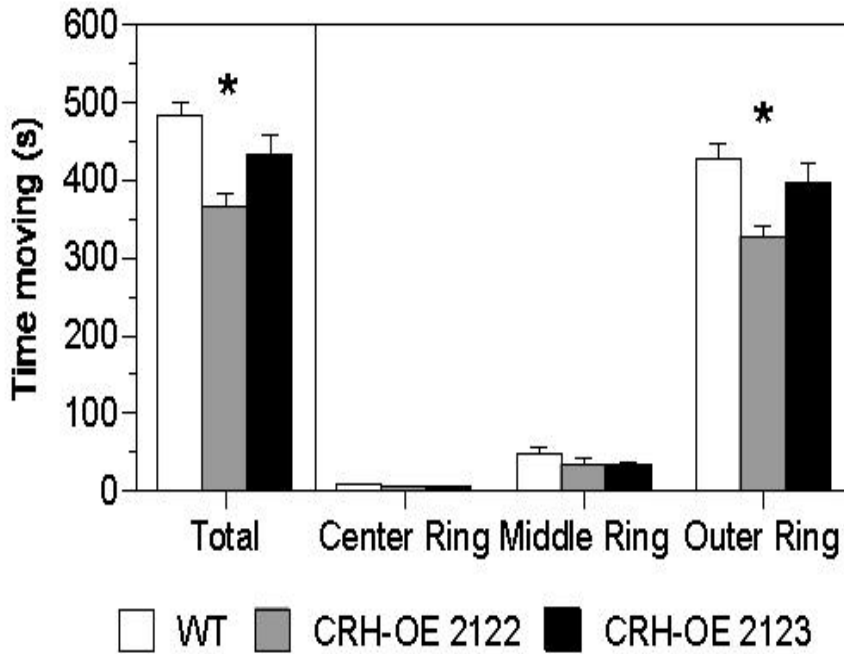
Figure 1. Locomotor activity in unfamiliar open field (diameter 45 cm). Time moving in total open field and in outer, middle and center ring in WT (white bars; n = 13), CRH-OE2122 (gray bars; n = 9), and CRH-OE2123 (black bars, n = 12) mice. Data are expressed as means + SEM. *: $p < 0.05$ versus WT.

tance moved in the outer ring only (data not shown). CRH-OE2123 and WT mice were not different.

In the ISIH procedure, basal body temperatures did not differ between genotypes (Table 2; $F < 1$). Recording rectal temperatures 10 min after the first rectal temperature measurements yielded the expected 1.0-1.5 °C increase in body temperature. However, there was no difference between CRH-OE2122, CRH-OE2123, and WT mice with regard to the magnitude of this ISIH effect, $F < 1$.

The results of the elevated plus maze test are depicted in Figure 2. CRH-OE$_{2122}$ mice tended to show a reduction in locomotor activity, evidenced in non-significant reductions in the total distance moved when compared to WT mice (Figure 2A; $F(2, 31) = 1.23$) and the total number of entries (Figure 2C; $F(2, 31) = 1.18$). CRH-OE2123 and WT mice were not different. Mice from the three genotypes spent equal percentages of total test time on the open arms (Figure 2B; $F < 1$). Although the percentage of open arm entries did not differ between genotypes (Figure 2D; $F < 1$), the absolute number of entries into the open arms was significantly reduced in CRH-OE2122 when compared to WT mice (data not shown).

Table 2
Behavior of CRH-OE and WT Mice in ISIH Procedure.

	WT (n = 13)	CRH-OE$_{2122}$ (n = 9)	CRH-OE$_{2123}$ (n = 12)
Body temperature T_0 (°C)	36.1 ± 0.2	36.3 ± 0.3	36.4 ± 0.2
Delta BT (°C)	1.4 ± 0.2	1.6 ± 0.3	1.3 ± 0.2
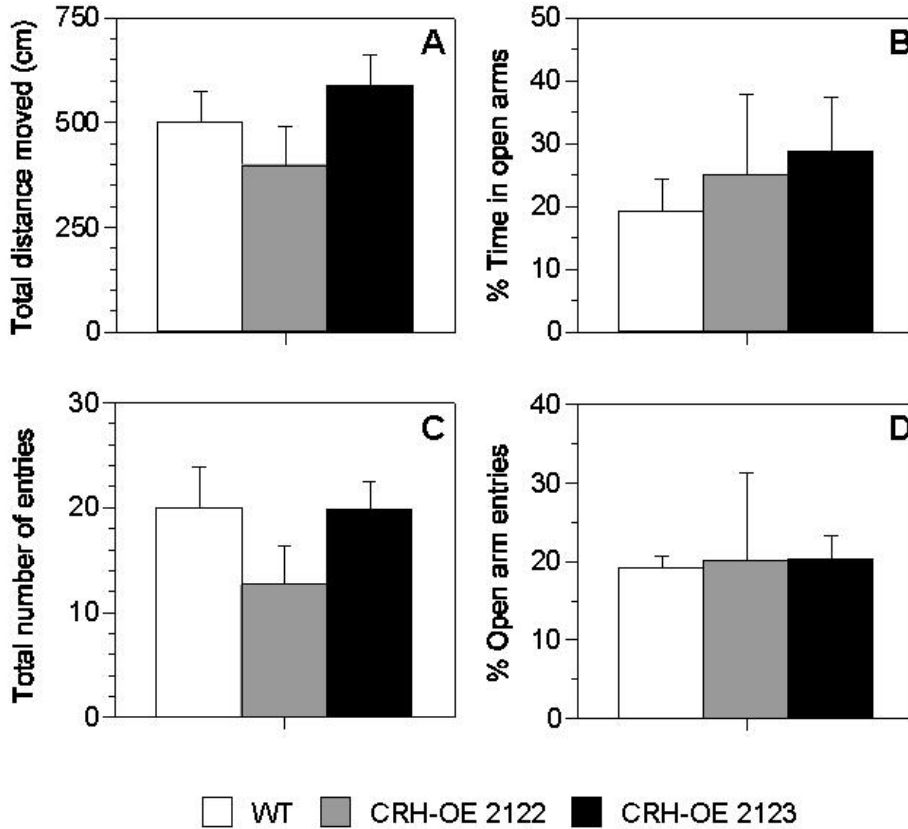
Figure 2. Behavior on elevated plus maze. Total distance moved (A), relative time spent in open arms (B), total number of entries (C), and relative number of open arm entries (D) are shown for WT (white bars; $n = 13$), CRH-OE2122 (gray bars; $n = 9$), and CRH-OE2123 (black bars, $n = 12$) mice. Data are expressed as means + SEM.

In the light-dark box, it appeared that CRH-OE2122 moved around less, as reflected in the nonsignificant reductions in total distance moved (Figure 3A; $F(2,$

31) = 1.38) and number of entries into the light compartment (Figure 3B; $F(2, 31)$ = 1.41). CRH-OE2123 and WT mice were not different. Genotypes were similar with regard to relative distance traveled in light compartment (Figure 3C; $F(2, 31)$ = 1.07) and the percentage of total test time spent in light (Figure 3D; $F < 1$).
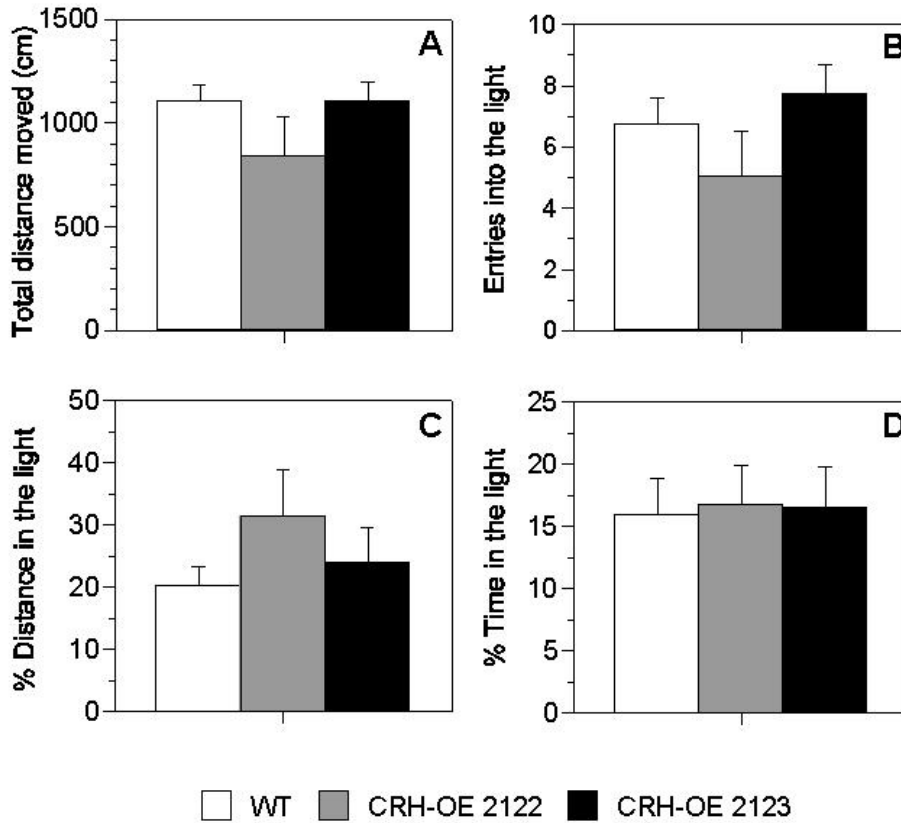
Figure 3. Behavior in light-dark box. Total distance moved (A), number of entries into light compartment (B), relative distance moved in light compartment (C), and relative time spent in light compartment (D) are shown for WT (white bars; $n = 13$), CRH-OE2122 (gray bars; $n = 9$), and CRH-OE2123 (black bars, $n = 12$) mice. Data are expressed as means + SEM.

Table 3 lists the results regarding behavior in the tail suspension test and the forced swim test. In the tail suspension test, all genotypes exhibited similar amounts of immobility, $F(2, 29) = 1.42$. This was also the case on both days in the forced swim test (day 1: $F(2, 31) = 1.70$; day 2: $F(2, 31) = 1.15$). Furthermore, on the first day of the forced swim test there were no differences between the genotypes in the duration of climbing behavior, $F < 1$, while differences between genotypes in swimming time nearly reached significance , $F(2, 31) = 3.07$, $p = 0.061$. On day 2, both genotypes did not differ from WT mice with regard to swimming time, $F(2, 31) = 2.00$, or climbing time, $F(2, 31) = 2.01$). When comparing the two test days, mice were significantly more immobile on Day 2 (repeated-measure ANOVA; day $F(1, 31) = 15.48$, no main effect genotype or interaction). Furthermore, both CRH-OE2122 and CRH-OE2123 mice displayed less swimming behav-

ior on Day 2 when compared to Day 1 (repeated-measure ANOVA; day $F(1, 31) = 20.78$). There was a nonsignificant genotype effect, $F(2, 31) = 2.31$, but a significant Day x Genotype interaction $F(2, 31) = 3.73$. Time spent climbing did not differ across days per genotype.

Table 3
Behavior of CRH-OE and WT Mice in Animal Models for Depression.

		WT (n = 13)	CRH-OE$_{2122}$ (n = 9)	CRH-OE$_{2123}$ (n = 12)
Tail Suspension Test				
Immobility (s)		223.6 ± 7.9	245.5 ± 6.3	223.8 ± 14.4
Forced Swim Test				
Immobility (s)	Day 1	126.1 ± 4.2	105.5 ± 11.2	107.7 ± 11.0
	Day 2	143.9 ± 6.0	132.1 ± 9.6	126.2 ± 10.6
Swimming (s)	Day 1	40.1 ± 4.5	67.6 ± 10.4	60.0 ± 9.7
	Day 2	30.5 ± 5.3	33.5 ± 5.3 §	49.8 ± 10.1§
Climbing (s)	Day 1	13.7 ± 2.6	6.9 ± 1.8	12.3 ± 5.3
	Day 2	6.0 ± 2.7	14.4 ± 6.5	4.0 ± 1.8

Note. s: duration in seconds; §: $p < 0.05$ vs. Day 1

Discussion

The present set of experiments did not reveal an apparent phenotype of the transgenic mice overexpressing CRH in paradigms measuring stress-, anxiety- or depression-related behaviors. One finding, although not always significant, was reduced locomotor activity in CRH-OE2122 mice in most of the behavioral procedures used. The large variability between groups, in part due to the small and unequal sample sizes could have masked putative behavioral differences between genotypes.

The behavioral repertoire displayed in an unfamiliar homecage consisted mainly of exploratory behavior, including locomotion, rearing, and sniffing. There were no evident differences between CRH-OE2122, CRH-OE2123, and WT mice, with the exception of burying and rearing behavior, and, although extremely low, the number of stretch attend postures, which is a measure of anxiety-related risk-assessment (Rodgers & Cole, 1993). Sawdust digging or burying is considered a form of displacement behavior and, usually, an increase is thought to reflect anxiety-like behavior (see van Gaalen & Steckler, 2000). The findings that in a unfamiliar homecage CRH-OE2123 mice and, to a lesser extent, CRH-OE2122 showed significant decreases in time spent burying as well as in the number of stretch attend postures, suggest that predominantly CRH-OE2123 mice might exhibit a phenotype associated with reduced anxiety. However, in the elevated plus maze and light-dark box, both standard paradigms to assess anxiety, CRH-OE mice did not differ from WT and did not appear less anxious (see below). Locomotion in a unfamiliar open field, more specifically in the outer perimeter of the open field, was significantly reduced in CRH-OE2122 mice. Furthermore, there was a nonsignificant trend towards decreased locomotor activity in CRH-OE2122 mice in several of the other procedures, including elevated plus maze and light-dark box. These data are consistent with findings that rodents pretreated with CRH show decreases in locomotor behavior in novel environments (Sutton et al., 1982; Takahashi et al., 1989).

In the elevated plus maze and the light-dark box, overexpression of CRH was not associated with changes in behavior indicative of altered anxiety levels. These findings were quite unexpected given the well established anxiogenic-like effects of CRH (see Dunn & Berridge, 1990; Koob & Heinrichs, 1999; Koob et al., 1993; Owens & Nemeroff, 1991; Steckler & Holsboer, 1999). For example, rats exhibit decreased exploration in the elevated plus maze after CRH administration (Baldwin et al., 1991). Furthermore, in mice, CRH pretreatment results in a decrease in light-dark transitions (Guanowsky et al., 1997). Interestingly, when tested in the footshock-induced sensitization of the startle paradigm, which is an animal model for unconditioned fear (e.g., Davis 1989; Dirks et al., 2001), both CRH-OE2122 and CRH-OE2123 mice show increased startle magnitudes after footshock presentation, indicative of increased fear or anxiety (Dirks, 2001). The data of the present study, however, indicate that CRH-OE mice do not exhibit an anxious phenotype in conflict-based procedures.

In the ISIH procedure, which assesses physiological responses to stress, mice from all groups showed the expected 1-1.5 °C increase in rectal temperature 10 min after the stress of a first rectal temperature measurement (Olivier et al., 1998; van der Heyden et al., 1997;). However, there was no effect of CRH overexpression on the magnitude of this stress-induced hyperthermia, neither were differences in basal temperature observed. These results are in contrast to the pronounced elevation of core body temperature demonstrated in rats after acute (e.g., Buwalda et al., 1998; Heinrichs et al., 2001; Rothwell, 1990), or chronic CRH administration (Buwalda et al., 1997; Linthorst et al., 1997). In a study assessing the 24-h pattern in body temperature using radiotelemetry, we have observed that body temperature was increased in CRH-OE2122 mice predominantly during the second half of the light (inactive) phase, compared to WT and CRH-OE2123 mice (Dirks et al., 2001). It should be emphasized that the ISIH procedure was performed in the first half of light period in which no differences in body temperature between CRH-OE2122 and CRH-OE2123 have been demonstrated.

Since chronic hyperactivity of the CRH system is implicated in human affective disorders, including major depression (see Arborelius et al., 1999; Mitchell, 1998), and given the depression-like dysregulation of the HPA axis in our CRH-OE2122 mice (Groenink et al., 2002), we predicted increased depression-like behaviors in CRH-OE2122 mice in animal models in which antidepressant-like behaviors can be detected (Dalvi & Lucki, 1999). However, no differences were observed in immobility between CRH-OE2122, CRH-OE2123, and WT mice in the tail suspension test. To our knowledge, effects of CRH in this test have not been reported. It has been suggested that C57BL/6J, the background strain of our transgenic mice, may be unsuitable for the tail suspension test because of a propensity of these animals to climb up their tails during the testing session (Mayorga & Lucki, 2001). In our experiments only 2 out of 34 mice showed this climbing behavior. It should be noted that by using a flat vertical bar to suspend the mice, as in the present study, the opportunity to grasp the tail and climb onto the bar is greatly reduced.

We hypothesized that the CRH-OE mice should exhibit increased immobility behavior in the forced swim test, another paradigm for assessing depression-like behavior. However, both genotypes did not differ in immobility time when compared to WT mice, consistent with results in CRH-OE mice under control of a

different promotor (Heinrichs et al., 1996). In rats, acute ICV CRH administration decreased floating time, indicative of behavioral activation and opposite to the suggested increased immobility behavior in "depressed" animals (Butler et al., 1990). To our knowledge, effects of chronic administration of CRH on behavior in the forced swim test, which would suit the hypothesized hypersecretion of CRH in depression better, have not been reported. In contrast to rats, the forced swim test in mice typically consists of a single test session (Dalvi & Lucki, 1999). Results from the present study indicate that a different behavioral profile may emerge the second day, which could provide interesting additional information. The results in the tail suspension test and forced swim test together, indicate that overexpression of CRH in the transgenic mice is not associated with increases in depression-like behaviors.

In contrast to the transgenic mice used in the present study, mice overexpressing CRH under control of a different promotor show enhanced responsiveness to novelty and behavior indicative of increased anxiety. These CRH transgenic mice exhibit reduced baseline and stress-induced exploration of a unfamiliar environment, decreased activity, and open arm exploration on elevated plus maze (Stenzel-Poore et al., 1994), and decreased overall activity or reduction in number of light-dark transitions in the light-dark two-compartment test (Heinrichs et al., 1996, 1997). In contrast, mice lacking the CRH gene display activity levels and anxiety-related behavior remarkably similar to those of WT animals (Dunn & Swiergiel, 1999). Taken together, these reports and the results of the present study in CRH-OE mice do not point to a clear-cut anxiogenic or depression-like effect of CRH excess. Rather, these data indicate that the behavioral effects of chronic CRH overproduction appear to be very subtle.

The paradigms used in the present study emulate aspects of the presumed physiology or behavioral expression of stress, anxiety, and depression. These behavioral paradigms have a well established predictive validity towards anxiolytic and antidepressant properties of drugs. However, these paradigms have never been validated for mutant mice. Moreover, construct validity is less well developed, which complicates the theoretical interpretation of the behavior displayed by transgenic and knockout mice in these procedures. Therefore, statements about stress-, anxiety-, or depression-like phenotypes in mutant mice should be made with caution.

Since CRH-OE mice are overproducing CRH throughout postnatal development, numerous neurochemical and developmental changes may have occurred to compensate for the increased levels of this neuropeptide. Thus, it cannot be ruled out that the observed lack of phenotypic effects is the result of compensatory adaptations in CRH receptor number or sensitivity, or in other neurotransmitter systems involved in stress-, anxiety-, and depression-related processes.

In conclusion, the results of the present experiments in CRH-OE mice indicate that life-long CRH overproduction is not associated with an apparent behavioral phenotype indicative of enhanced stress, anxiety, and/or depression.

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