Pharmacology Biochemistry and Behavior, Vol. 58, No. 3, pp. 737–746, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00037-3

Anxiogenic Behavior in the Light–Dark Paradigm Following Intraventricular Administration of Cholecystokinin-8S, Restraint Stress, or Uncontrollable Footshock in the CD-1 Mouse

GLENDA MacNEIL, YAEL SELA, JUDITH McINTOSH AND ROBERT M. ZACHARKO[1]

Institute of Neuroscience, Carleton University, Life Sciences Research Building, Ottawa, Ontario, Canada K1S 5B6

Received 13 June 1996; Revised 28 January 1997; Accepted 7 February 1997

MacNEIL, G., Y. SELA, J. McINTOSH AND R. M. ZACHARKO. *Anxiogenic behavior in the light–dark paradigm following intraventricular administration of cholecystokinin-8S, restraint stress, or uncontrollable footshock in the CD-1 mouse.* PHARMACOL BIOCHEM BEHAV **58**(3) 737–746, 1997.—The influence of restraint stress (0, 15, 30, or 60 min), uncontrollable footshock (0, 15, 30, or 60 shocks), or intraventricular CCK-8S administration (0, 5, 25, or 50 ng delivered in a 1 μl volume) were evaluated on transition frequency and cumulative time in light among CD-1 mice in the light–dark paradigm. Mice exposed to restraint stress of either 15 or 60 min were indistinguishable from nonrestrained animals, while the 30-min session of restraint decreased time in light and transition scores. The presentation of 15, 30, or 60 uncontrollable footshocks were equally effective in decreasing cumulative time in light but had no effect on transition scores. Intraventricular infusion of 25 and 50 ng doses of cholecystokinin-8S reduced cumulative time in light and transition frequency in CD-1 mice relative to vehicle or 5 ng CCK-8S–treated animals in the light–dark paradigm. The time in light and transition data secured among mice with repeated light–dark exposure and 30 min of restraint were comparable to the corresponding scores secured when performance was only evaluated on trial 1. Transition scores were reduced on trial 1 of mice exposed to 30 min of footshock, but time in light was reminiscent of the performance detected among mice with prior light–dark experience. Potential neurochemical correlates associated with the anxiogenic effects associated with stressor exposure and CCK-8S administration in the light–dark task are discussed. © 1997 Elsevier Science Inc.

Light–dark paradigm CCK-8S Intraventricular administration Footshock Restraint Mouse

THERE is considerable evidence suggesting that experience with aversive life events contributes to the provocation of a variety of behavioral disturbances in infrahuman subjects (1). In recent years some investigators have argued that uncontrollable stressors provoke behavioral pathology, at least in part, by promoting anxiety (30,40). In this respect, stressors engender neurochemical alterations in central sites associated with the induction of anxiety (16), anxiogenic agents increase catecholamine (CA) turnover in brain areas responsive to stressor imposition (4), reductions in anxiety accompany pharmacological manipulations that reduce amine turnover in brain regions sensitive to stressor imposition (2), and endogenous anxiogenic agents are colocalized with CA and serotonin (5-HT) in central sites responsive to stressor exposure (25). Interestingly, release of the anxiogenic peptide, cholecystokinin (CCK), has been detected in the dopamine (DA)-containing anteromedial frontal cortex (3) and the nucleus accumbens (38) following stressor exposure in rats. It has also been demonstrated that stressors facilitate CCK release in central norepinephrine NE (44,45) and 5-HT (38) containing sites im-

[1]To whom requests for reprints should be addressed.

plicated in the promotion of anxiety. In view of the considerable inter- and intraregional variability in the sensitivity of central CA and 5-HT sites to stressor imposition, the release and/or corelease of endogenous anxiogenic agents in these identical central sites may likewise vary (47).

Behavioral paradigms employed to assess anxiety in infrahuman subjects have typically taken advantage of the innate tendency of rats and mice to avoid entry into the open arms of an elevated plus maze (28,37) or the decreased propensity of animals to explore illuminated arenas (24,31). In the latter instance, the light–dark paradigm has been shown to be sensitive to the anxiogenic and the anxiolytic influence of pharmacological agents (6,27). To a considerable extent, however, data pertaining to an evaluation of stressor effects in the light–dark paradigm and comparison with the anxiogenic influence of the CCK are lacking. Interestingly, central sites with demonstrable concentrations of CCK, including the mesocorticolimbic system, have been associated with neurochemically distinct DA release profiles that vary with the duration and the nature of stressor imposition. For example, immobilization stress and uncontrollable footshock produce alterations of mesolimbic DA activity in rats and mice that are dependent on the duration of stressor exposure (5). In particular, in vivo microdialysis revealed that immobilization stress and uncontrollable footshock in mice promoted significant but differential alterations of DA release, turnover, and metabolite accumulation over the course of a 60-min interval following initial exposure to the stressor. Interestingly, DA-associated alterations were conspicuous in the nucleus accumbens, a site of prominent DA-CCK interface (10). It should be underscored that the impact of varying durations of immobilization stress and footshock on central neurochemical change do not appear to be restricted to central DA-CCK sites (32,41,43). Indeed, stressors also promote release of the anxiogenic agents diazepam binding inhibitor (19) and corticotropin releasing factor in neurochemically diverse forebrain, midbrain, and hindbrain sites (7) and presumably augment central β-carboline availability (39). Such data suggest that behavioral alterations following encounter with aversive stimulation likely follow from the influence of multiple neurochemical variations and the putative influence of diverse anxiogenic agents. It should be considered, nevertheless, that stressor-induced neurochemical variations of central amine turnover and release of some anxiogenic agents may be paralleled by behavioral disturbances in paradigms evaluating anxiety in infrahuman subjects.

Evaluation of the anxiogenic effects of CCK administration has ordinarily involved systemic administration of the agent, although intracerebral drug administration has been accomplished in some paradigms (14). Available evidence to date suggests that (a) peripheral and central CCK receptors participate in the expression of the anxiogenic effects of systemically administered CCK (17,18), (b) CCK-8S is the predominant CCK sequence available in central sites (8,22,29), (c) the promotion or reduction of DA turnover in the nucleus accumbens induced by CCK varies in a rostral–caudal plane according to intraregional CCK_A and CCK_B receptor density (10), (d) central CCK_A and the CCK_B receptors may be associated with the induction of anxiety (36), and (e) behavioral expression of anxiety may follow from the relative activation of CCK_A and CCK_B receptors within and between relevant central sites (23).

The present experiment was designed to (a) evaluate the propensity of varying durations of immobilization and uncontrollable footshock to promote anxiogenic behavior in the light–dark paradigm in CD-1 mice, (b) compare the effects of restraint stress and uncontrollable footshock with the anxiogenic influence of central CCK-8S administration, (c) determine the threshold anxiogenic dose of intraventricularly administered CCK-8S in the light–dark paradigm, and (d) determine behavioral indices of anxiety associated with the specific anxiogenic stimulus imposed.

METHODS

Subjects

Naive, male, CD-1 mice obtained from Charles River Canada (St. Constant, Quebec) at 5 weeks of age were employed as subjects. All mice were acclimatized to the animal facility with food and water available ad lib.

Surgery

Surgical anesthesia was induced by an intraperitoneal injection of Somnotol (sodium pentobarbital, 65 mg/kg). Lateral ventricular cannulation was accomplished with the aid of a David Kopf Micromanipulator. Stereotaxic coordinates for surgical implantation of a 23-gauge cannula were: A.P +0.8 mm from Bregma, L. +0.7 mm from the midline and V. −2.8 mm from a flat skull surface. Individual cannulas were fitted with a 30-gauge stylette. Following surgery, animals were housed individually, placed on a warm heating pad, and provided a dietary supplement (Meritene) for at least 3 days. Following this postoperative recovery period, mice were returned to the main animal area for at least 7 days prior to behavioral testing.

Apparatus

The light–dark apparatus consisted of a rectangular Plexiglas box, 20 × 47 × 20 cm high with the white section occupying two-thirds of the chamber and a black section comprising the remaining third. The two chambers of the apparatus were separated by a Plexiglas partition with a 12.5 × 5 cm opening allowing the animal passage from one section of the apparatus to the other. A 60 watt light bulb, situated 10 cm above the center of the white compartment provided illumination while the dark black section of the box was covered with red, translucent Plexiglas.

The shock apparatus (9.5 × 28 cm × 16 cm) consisted of black Plexiglas walls and a floor of stainless steel rods, connected in series, spaced 1 cm apart. Footshock (150 μA, 6 s duration with a 59-s intertrial interval) was delivered by a microcomputer controlled 3000 V source (Science Technology Centre, Carleton University). Restraint stress was applied in a clear semicircular Plexiglas tube, measuring 2.5 cm wide. The tube was equipped with slots at equidistant intervals to permit insertion of a rectangular plate that confined the animal in the restraining device. Once the mouse was positioned in the restraint tube, the tail was taped down to further restrict movement.

Procedure

Mice exposed to footshock or restraint were tested in the light–dark box for a 10-min interval on 3 consecutive days between 0900 and 1200 h. Each mouse was removed from its home cage and placed in the center of the light compartment facing the entrance to the dark section of the box. The latency for the mouse to enter the dark compartment, the number of transitions between the two chambers, and the time spent in the dark and light chambers were recorded for each session.

An animal was considered to have entered a chamber, and timing commenced only when all four paws were positioned in the chamber.

Following a 3-day baseline procedure, mice were assigned to either uncontrollable footshock or restraint stress. Mice of the uncontrollable footshock condition were assigned to a no-footshock treatment condition, 15 uncontrollable footshocks, 30 uncontrollable footshocks or 60 uncontrollable footshocks (n = 6/cell). Mice in the restraint condition were assigned to no restraint, 15 min of restraint, 30 min of restraint or 60 min of restraint (n = 6/group). Independent groups of mice were also assigned to an intraventricular CCK-8S condition. Following recovery from surgery, mice were challenged intraventricularly with 0, 5, 25, or 50 ng CCK-8S in a 1 μl volume employing a Hamilton microliter syringe connected to a 30-gauge injector (n = 18/cell). Sulfated cholecystokinin (CCK-8S) (Sigma) was dissolved in a 1-M sodium bicarbonate and 0.9% sterile saline vehicle solution. Intraventricular injections were accomplished over a 1-min interval and the injector was left in place for an additional minute to ensure adequate drug diffusion. The stylette was then replaced. Immediately following stressor imposition and 15 min following central drug administration, mice were tested in the light–dark paradigm employing the transition and cumulative scores previously outlined. In view of established protocols pertaining to the interaction of repeated testing in anxiogenic paradigms and subsequent drug administration (37), mice in the CCK-8S condition were not subjected to repeated baseline assessment in the light–dark box. Nanogram doses of CCK were selected in view of previous determination that such drug challenges do not enter the peripheral circulation and accordingly do not induce sedation (9,11). Behavioral testing commenced 15 min following CCK administration, a postadministration interval consistent with such anxiogenic challenge (46).

Following the completion of behavioral testing, mice exposed to the stressor conditions were sacrificed with CO_2 and cannulated mice were overdosed with sodium pentobarbital and perfused intracardially with 0.9% physiological saline followed by a 10% formalin solution. The brains of cannulated mice were removed from the skull, blocked, frozen, and sectioned at 40 μm. Coronal brain sections were stained with cresyl violet and examined under a microscope to verify cannula placement in the lateral vehicle.

Data Analysis

Behavioral scores pertaining to time spent in the light, and the frequency of transitions between the light and dark compartments were analyzed by a one-way analysis of variance with repeated measures during baseline assessment and by a one-way analysis of variance for independent groups following stressor and CCK-8S administration. Newman–Keuls multiple comparisons were employed where appropriate and the 0.05 level of significance was adopted for all statistical comparisons.

RESULTS

Histological analyses revealed that, in all cases, cannulae were appropriately positioned in the lateral ventricle. Analyses of variance of the data describing time in light and transition scores across the 3-day baseline evaluation interval failed to reveal significant differences between groups exposed to the various restraint conditions, $F(6, 40) = 1.72$, $p > 0.05$ and $F(6, 40) = 1.38$, $p > 0.05$, respectively. Accordingly, the scores describing cumulative time in light and transition frequency for all animals in the four conditions were collapsed and pooled (see Fig. 1A and B). Analysis of variance of the combined time in light data revealed a significant main effect of day attributable to repeated testing, $F(2, 40) = 13.07$, $p < 0.05$. Newman–Keuls multiple comparisons revealed that cumulative time in light was elevated on day 1 relative to the remaining test days. Likewise, the analysis of variance revealed

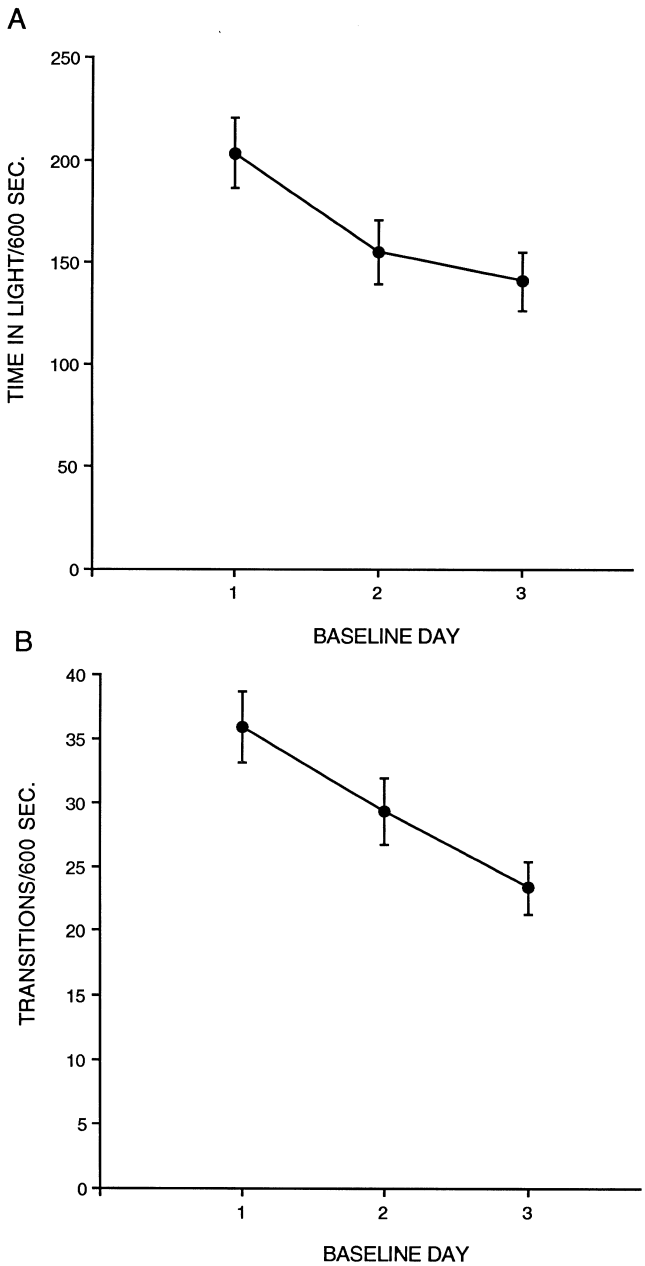

FIG. 1. Mean (±SEM) cumulative time in light (A) and transition frequency (B) of CD-1 mice during each of three baseline assessment days in the light–dark paradigm. Note: the performance of mice assigned to no restraint, 15 min of restraint, 30 min of restraint, or 60 min of restraint was not observed to differ on each of the three baseline test days and the scores of these animals were collapsed. Behavioral test were 10 min in duration.

a significant main effect of day for transitions, $F(2, 40) = 15.34$, $p < 0.05$. Newman–Keuls multiple comparisons revealed that transitions scores of mice were elevated on day 1 relative to the performance of mice on days 2 and 3.

Analysis of variance of the effects of restraint stress on time in light just failed to reveal an acceptable level of statistical significance, $F(3, 20) = 2.88$, $p < 0.06$. However, Newman–Keuls multiple comparisons were conducted owing to the a prior prediction that restraint stress of 30 min was expected to produce a significant anxiogenic effect relative to the remaining restraint conditions. The post hoc comparisons revealed that time in light among mice in the 30-min restraint session was significantly different from the comparable measure among animals in the no-restraint condition, but this measure did not differ from the scores achieved among mice exposed to either the 15- or 60-min immobilization sessions. Analysis of variance of the effects of restraint stress on transition frequency, like the time in light data, revealed an effect that just failed to achieve statistical significance, $F(3, 20) = 2.78$, $p < 0.06$. Newman–Keuls multiple comparisons revealed that transition scores among mice in the 30-min restraint condition were significantly different from the transition scores exhibited by mice in the no-restraint session, but these data were not significantly different from those displayed my mice exposed to either the 15- or 60-min sessions of immobilization (see Fig. 2A and B).

Analyses of variance of time in light and the transition scores during baseline assessment failed to reveal a significant between-groups difference among animals subsequently assigned to the respective uncontrollable footshock conditions, $Fs(6, 40) < 1$. Accordingly, the preexperimental data were pooled for all animals for each of the three baseline test days. Analysis of variance of time in light revealed a significant main effect attributable to day of test, $F(2, 40) = 14.69$, $p < 0.05$. Newman–Keuls multiple comparisons revealed that time in light was elevated significantly on day 1 relative to the remaining test days. Analysis of variance also revealed a significant main effect attributable to day of test for transition scores during baseline, $F(2, 40) = 17.61$, $p < 0.05$, Newman–Keuls multiple comparisons revealed that, as observed with time in light, transition scores of mice were elevated on the first baseline day 1 relative to the remaining baseline days (see Fig. 3A and B).

Analysis of variance of the data pertaining to cumulative time in light following exposure to uncontrollable footshock revealed a significant main effect attributable to footshock, $F(3, 20) = 10.90$, $p < 0.05$. Newman–Keuls multiple comparisons revealed that exposure to 15, 30, or 60 uncontrollable footshocks provoked a significant reduction in the time spent in light relative to the no-shock condition. In contrast, the analysis of variance failed to reveal a significant main effect of stress when transition scores were analyzed, $F(3, 20) = 0.70$, $p > 0.05$ (see Fig. 4A and B).

Analysis of variance of the data describing time in light following intraventricular administration of CCK-8S revealed a significant main effect attributable to drug administration, $F(3, 68) = 12.42$, $p < 0.05$. Newman–Keuls multiple comparisons revealed that the 25 and 50 ng doses of CCK-8S promoted a significant reduction of time in light relative to either the vehicle or 5 ng conditions (see Fig. 5A). Analysis of variance of the transition scores of mice following intraventricular drug administration also revealed a significant main effect attributable to drug administration, $F(3, 68) = 12.23$, $p < 0.05$ (see Fig. 5B). Newman–Keuls multiple comparisons revealed that the 25 and the 50 ng doses of CCK-8S promoted a significant reduction of transition scores post-CCK administration relative to the vehicle and 5 ng CCK-8S–treated mice.

Although it is not certain whether prior experience in the light–dark task alters responsivity to pharmacological challenge comparable to that noted in the elevated plus maze [e.g., (20)], repeated apparatus exposure may have influenced reactivity to footshock and restraint. Accordingly, an additional experiment assessed the effects of restraint and footshock on performance in the light–dark paradigm among CD-1 mice with no prior experience in the light–dark paradigm. Naive mice were exposed to no stress, 30 min of restraint, or 30

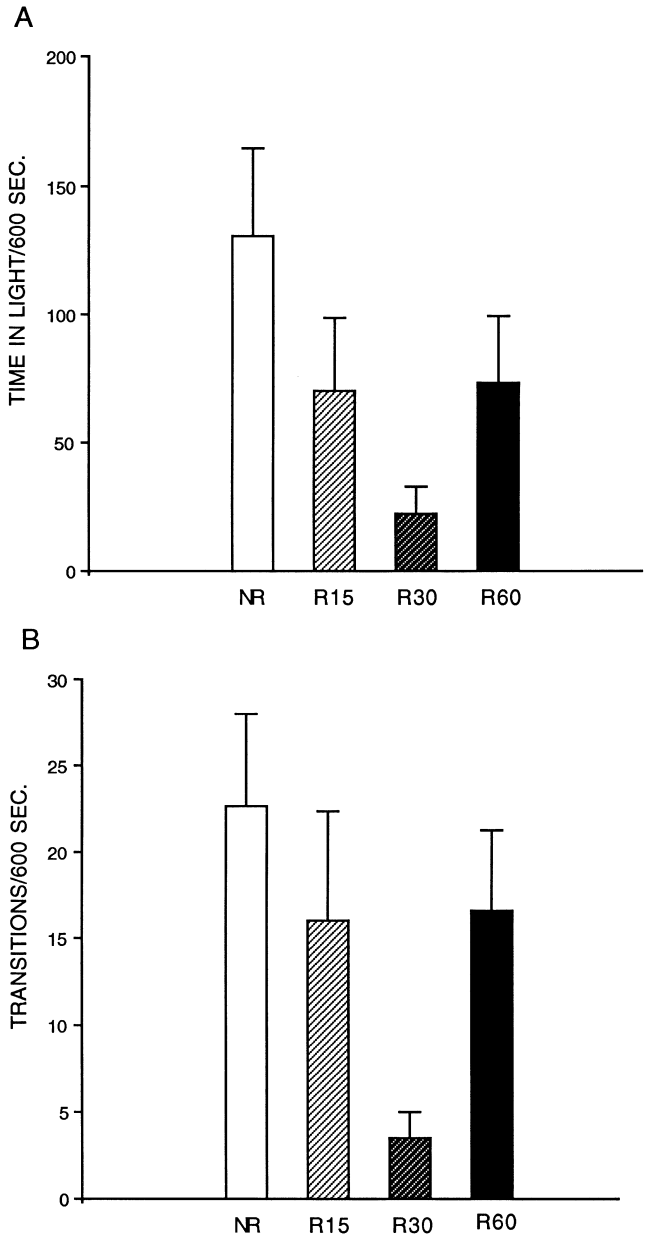

FIG. 2. Mean (±SEM) cumulative time in light (A) and transition frequency (B) of CD-1 in the light–dark paradigm immediately following exposure to no restraint (NR), 15 min of restraint (R15), 30 min of restraint (R30) or 60 min of restraint (R60). Behavioral assessment was conducted over a 10-min test period.

min of footshock. Immediately following stressor exposure mice were tested in the light–dark paradigm. Analysis of variance of the time in light and transition data revealed a significant main effect of stress, Fs(2, 40) = 8.00, 5.35, $p < 0.05$, respectively. Newman–Keuls multiple comparisons of the former effect revealed that both restraint and footshock reduced the time in light scores of animals relative to nonstressed mice. Likewise, restraint and footshock reduced transition scores of mice relative to nonstressed control subjects (see Fig. 6A and B).

A further experiment was conducted to assess putative nonspecific effects of intraventricular CCK-8S administration in CD-1 mice. Accordingly, independent groups of mice were implanted with an intraventricular cannula as previously described, and locomotor activity was assessed in an independent behavioral task, the elevated plus-maze. The elevated plus-maze was constructed of gray Plexiglas and consisted of

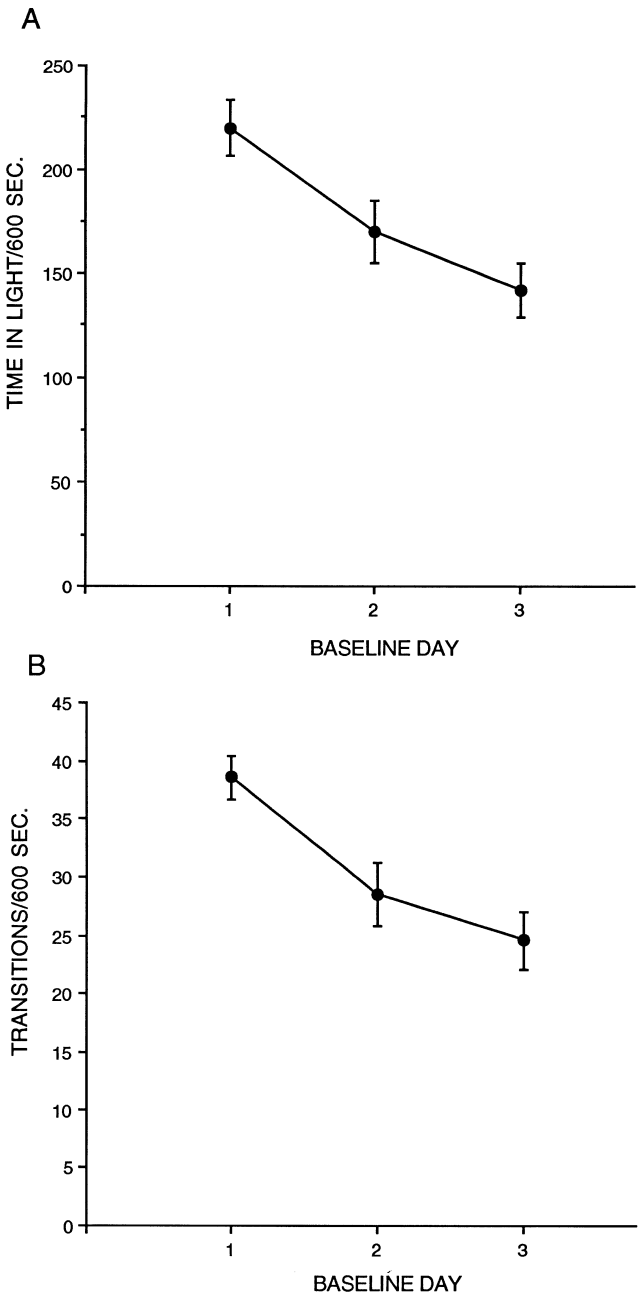

FIG. 3. Mean (±SEM) cumulative time in light (A) and transition frequency (B) of CD-1 mice during each of three baseline assessment days in the light–dark paradigm. Note: the performance of mice assigned to no footshock, 15 footshocks, 30 footshocks, or 60 footshocks was not observed to differ on each of the three baseline test days and the scores of these animals were collapsed. Behavioral test were 10 min in duration.

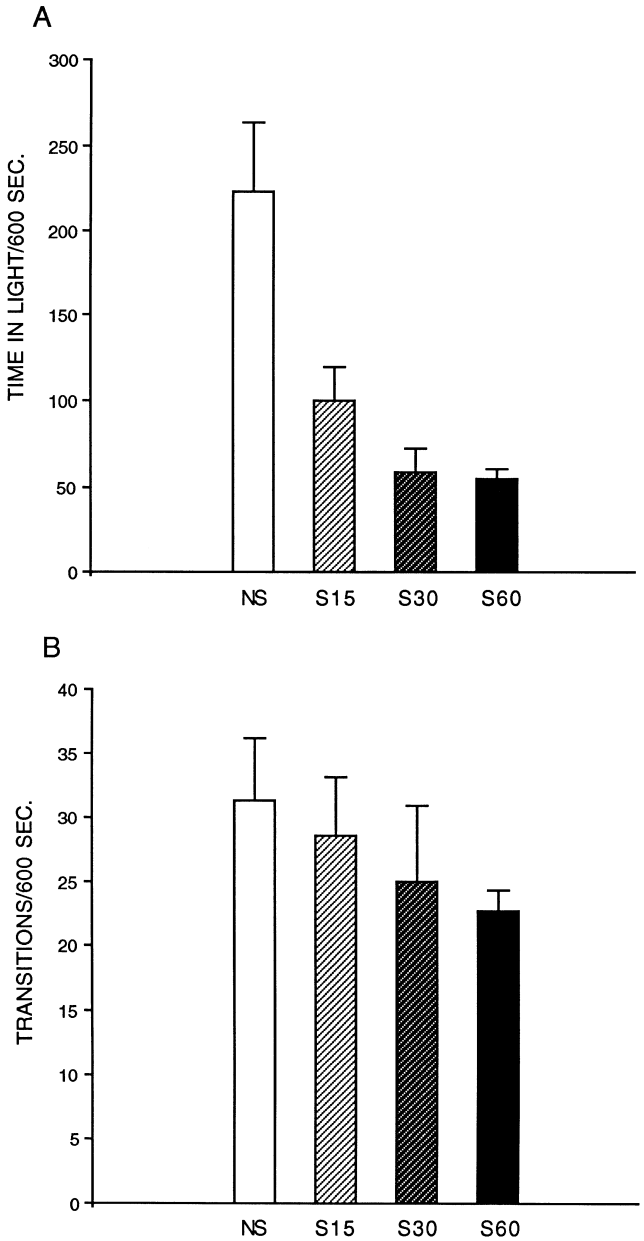

FIG. 4. Mean (±SEM) cumulative time in light (A) and transition frequency (B) of CD-1 in the light–dark paradigm immediately following exposure to no shock (NS), 15 uncontrollable footshocks (S15), 30 uncontrollable footshocks (S30), or 60 uncontrollable footshocks (S60). The behavioral test was 10 min in duration.

two opposing open arms, 5 × 25 cm, and two closed opposing arms of the same configuration with side and ends walls of 25 cm and 20 cm, respectively. Following postoperative recovery mice were administered saline (0 ng), 5, 25, or 50 ng of CCK-8S in a 1 μl volume as previously described and exposed to the elevated plus-maze. Both cumulative and closed arm entries were assessed over a 5-min test period. Analysis of variance of cumulative entries revealed a significant main effect of drug treatment, $F(3, 21) = 2.98$, $p < 0.06$. Newman–Keuls multiple comparisons revealed that only the performance of mice administered to 50 ng dose of CCK-8S was reduced relative to that of saline-treated animals. The analysis of variance of closed-arm entries failed to reveal a significant main effect of drug treatment, $Fs(3, 21) = 2.40$, $p > 0.05$ (see Fig. 7A and B, respectively).

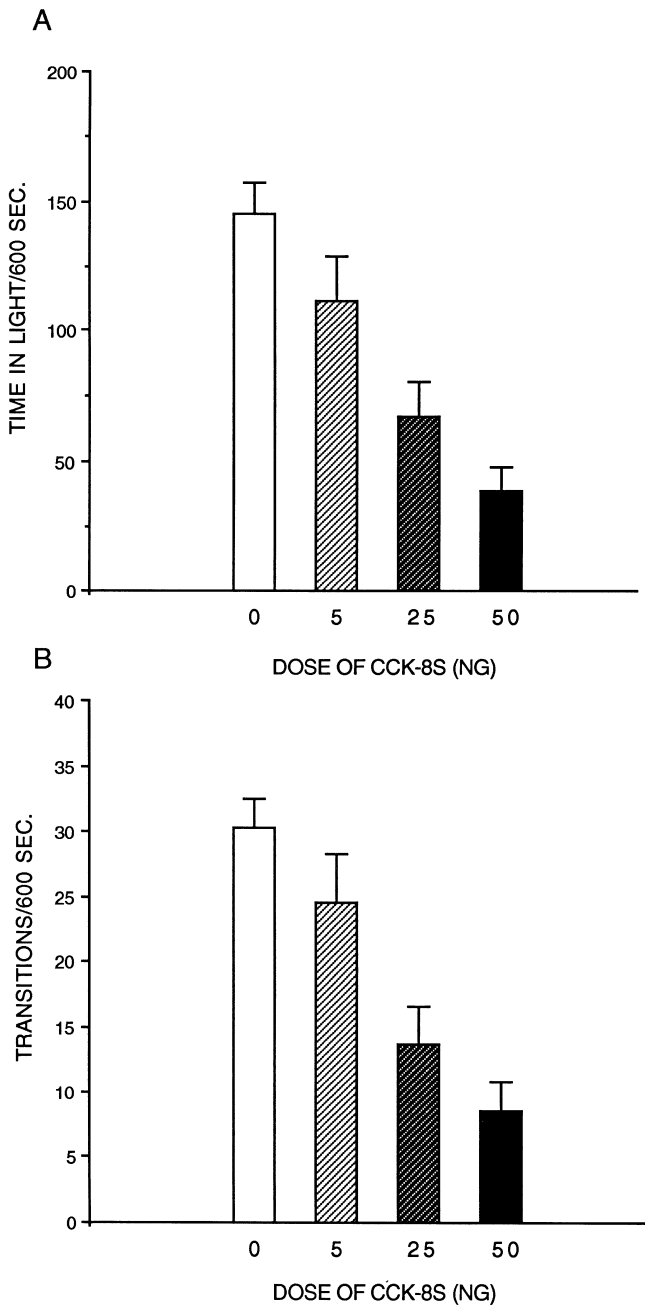

FIG. 5. Mean (±SEM) cumulative time in light (A) and transition frequency (B) of CD-1 in the light–dark paradigm following intraventricular administration of 0, 5, 25, or 50 ng CCK-8S administered in a 1 μl volume, 15 min prior to behavioral testing.

DISCUSSION

The data of the present investigation revealed that the nature of the stressor employed produced differential behavioral effects in the light–dark paradigm in CD-1 mice. In particular, exposure of mice to acute uncontrollable footshock did not influence transition frequency between the bright and dark compartments of the arena, but decreased cumulative time in the illuminated portion of the apparatus. Such an effect did

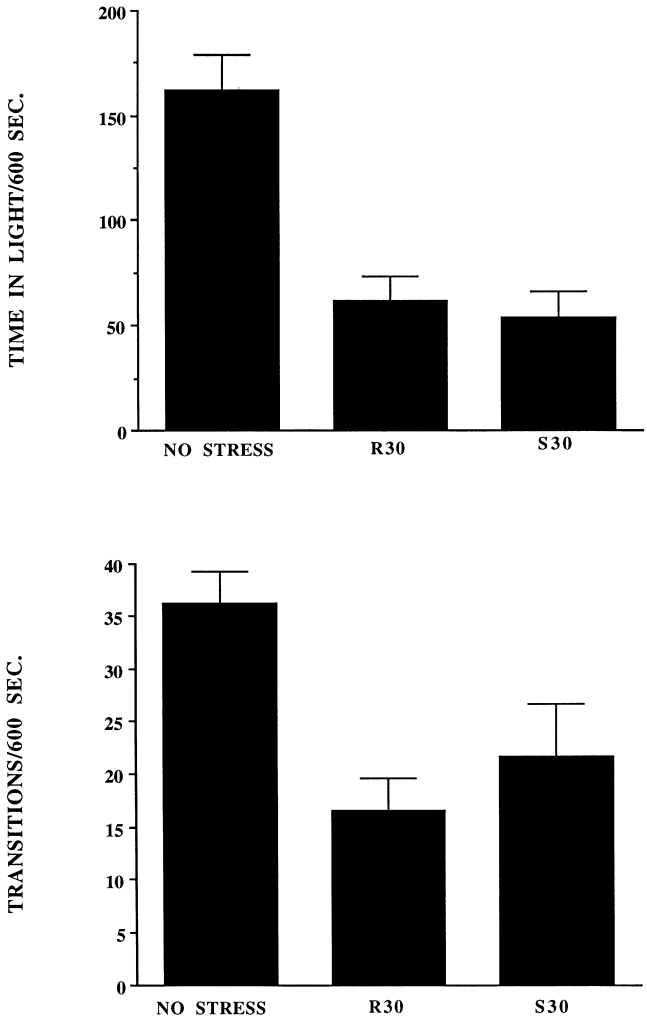

FIG. 6. Mean (±SEM) cumulative time in light (A) and transition frequency (B) of CD-1 in the light–dark paradigm immediately following exposure to no stress, 30 min of restraint (R30) or 30 uncontrollable footshocks (S30). Behavioral assessment was conducted over a 10-min test period in light–dark paradigm naive CD-1 mice.

not vary with the number of footshocks imposed prior to behavioral testing. These data depicting the effects of footshock in the light–dark paradigm are consistent with previous observations that time in light, or conversely, that time in dark is more sensitive to the anxiogenic influence of pharmacological manipulation relative to transition scores (27). In particular, these data suggest that with the stressor severity employed, footshock produced anxiogenic effects in mice paralleling those of FG-7142 administration (27). In contrast to the behavioral effects of footshock, immobilization stress provoked time-dependent behavioral alterations in the light–dark paradigm. In particular, 30 min of restraint effected a reduction of cumulative time in the light portion of the apparatus and a reduction in transition scores relative to nonrestrained mice. It should be emphasized that both the 15- and 60-min immobilization sessions produced considerable variability in the scores describing compartmental transitions and cumulative time in the light. Interestingly, variability in these measures was reduced among mice exposed to 30 min of restraint stress. In effect, the influence of the 15-min session appears to mirror emergence of anxiogenic influence of restraint, which peaks at 30 min. It should be underscored, however, that 30 min of restraint reduced time in light and transition scores in the light–dark task on trial 1, paralleling the performance of mice exposed to three prior sessions in this paradigm. In contrast, while 30 min of footshock effected a comparable reduction of time in light on trial 1 reminiscent of the effects detected with multiple light–dark task experience, the impact of the stressor on transition scores was attenuated. It would appear that closer examination of the nature of the stressor is required to verify the variables that contribute to such an effect. Nevertheless, these data are consistent with the proposition that time in light rather than transition scores may be more sensitive to the putative anxiogenic effects of stressor imposition.

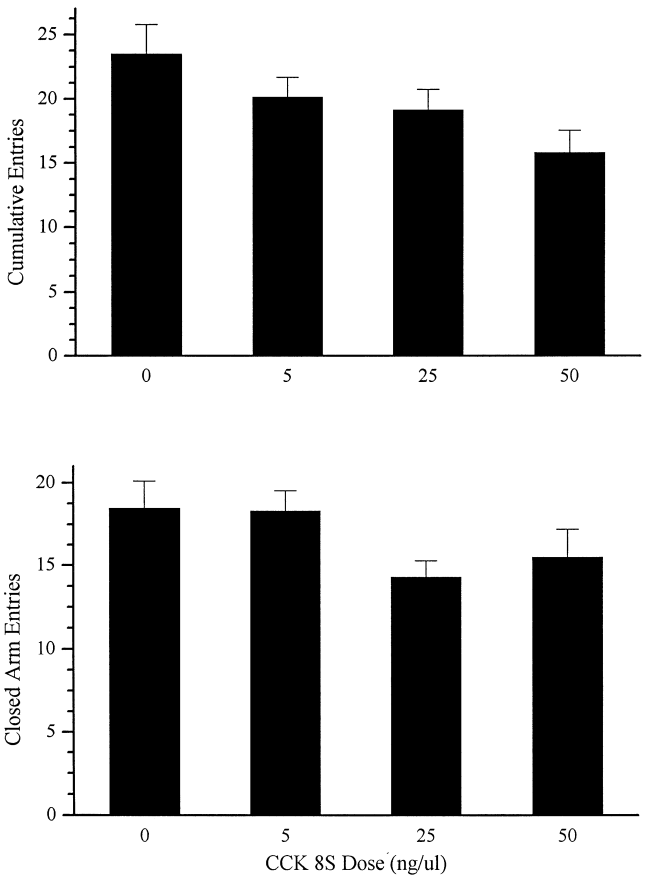

FIG. 7. Mean (±SEM) cumulative (open and closed arm) (A) and closed-arm (B) entries of CD-1 mice in the elevated plus-maze following intraventricular administration of 0, 5, 25, and 50 ng CCK-8S administered in a 1 μl volume, 15 min prior to behavioral testing.

In comparison with either restraint stress or uncontrollable footshock, intraventricular administration of CCK-8S produced a profile of anxiogenic effects that was paralleled in both transition scores as well as cumulative time in light. It should be noted parenthetically that pilot data collected in this laboratory revealed that doses of 0.5 and 2.5 ng of CCK-8S produced effects that were indistinguishable from those of the 5 ng dose, while 10 ng and 15 ng doses of CCK-8S failed to produce a significant margin of anxiety in the light–dark paradigm. The anxiogenic consequences of central CCK-8S administration were initially detected with the 25 ng dose, but were not behaviorally differentiated from the anxiogenic influence of the 50 ng dose of CCK in CD-1 mice. These data suggest a rather narrow window associated with the behavioral effects of escalating CCK-8S administration and potentially with the endogenous activity of this peptide. It should also be considered that intraventricular administration of the 25 ng dose of CCK-8S was ineffective in altering cumulative or closed-arm entries in the elevated plus-maze. While the former measure has been employed as an index of locomotor activity and anxiety, alterations in the latter variable assess variations in locomotor activity [e.g., (12,21)]. The detection of a significant effect of cumulative arm entries following intraventricular administration of the various CCK-8S doses was attributable to the anxiogenic influence of the 50 ng dose in the elevated plus-maze. Nevertheless, none of the doses of CCK-8S employed was effective in influencing locomotor activity in CD-1 mice or by extension provoking sedation. Indeed, systemic doses of CCK-8S producing such effects are ordinarily accomplished following administration of doses in excess of 500 ng in mice [e.g., (22)]. In retrospect, the behavioral effects of central CCK administration appear to be paradigm specific. The increase in anxiogenic activity with progressive increases in the doses of CCK-8S in the light–dark box contrasted with the attenuation of anxiogenic behavior induced by longer durations of immobilization stress and the lack of graded effects with footshock. Taken together, several interesting facets of these behavioral data emerge including demonstration that (a) a threshold dose of 25 ng CCK-8S favors development of anxiety in the light–dark paradigm in CD-1 mice, (b) a restraint duration of 30 min emerges as conspicuously anxiogenic in the light–dark task, while an attenuation of such behavior is evident with more protracted durations of the stressor, and (c) the anxiogenic effects of footshock are demonstrably different from those produced by either restraint or intraventricular CCK-8S administration.

Some procedural variables pertinent to this investigation should be addressed at this juncture. Multiple exposure of ICR mice to the light–dark task did not compromise behavioral reactivity (31). The data of the present investigation suggest that while the performance of CD-1 mice on day 1 of testing in the light–dark task is elevated relative to days 2 and 3, performance of mice on the latter days are comparable. It might be noted parenthetically that in an independent series of experiments conducted in this laboratory, performance of CD-1 mice in the elevated plus maze was only observed to

vary on trial 2 of testing relative to a 7-day consecutive assessment interval. Such variability in paradigm responsivity and the influence of strain variables may be pertinent to an assessment of subsequent responsivity to environmental or pharmacological challenge. For example, it has been argued that repeated exposure to the elevated plus maze alters responsivity to pharmacological challenge and benzodiazepine administration, in particular, in rats and mice, and that the critical variable appears to be maze experience on trial 1 (20). Although it is not certain that variables describing plus maze performance apply to the light–dark paradigm, altered behavioral responsivity following trial 1 exposure may be peculiar to anxiolytic drug administration and not necessarily applicable to anxiogenic drug administration or stressor imposition. For example, alterations in time in light following either 30 min of restraint or 30 min of footshock among animals tested on day 1 were comparable to the scores observed on day 4 in this experiment. Evidently, not all the variables selected for investigation in a particular paradigm may be differentially sensitive to the influence of repeated testing.

The effects of varying durations of immobilization and footshock on central DA activity in the mesolimbic system have been documented in some strains of mice (e.g., DBA/2J) (34). These neurochemical data provide a potential parallel for the behavioral effects reported in this investigation in the light–dark task. For example, in the case of footshock, levels of DOPAC and HVA were comparable following 15-, 30-, and 60-min sessions of footshock, yet parallels with the parameters of restraint stress are lacking. Clearly, strain differences notwithstanding, it would be of considerable advantage to determine whether altered CCK availability following gradients of immobilization and footshock can be detected in mesolimbic sites in the CD-1 mouse strain and whether such peptide alterations are relevant to a description of the behavioral consequences of stressor exposure in the light–dark task. It should be underscored, however, that it may well be the case that the profile of several neurochemical events define the behavioral repercussions of stressor imposition in the light–dark paradigm. For example, in addition to the influence of stressors on DA and CCK release in mesocortical and mesolimbic sites, enkephalin availability is also modified by such stimuli (26), and there is also evidence linking the δ receptor with the anxiogenic effects of CCK administration (15).

Several additional caveats should be introduced. The intermittent nature of footshock notwithstanding it should be emphasized that 1.5, 3, and 6 min of footshock in the present investigation delivered over the course of approximately 15-, 30-, and 60-min sessions, respectively, produced behaviorally comparable data. We have observed that two shock presentations (or the cumulative effects of 12 s of footshock) delivered over a 2-min session are sufficient to induce anxiogenic effects in the light–dark paradigm. Additional experiments are required to determine whether graded behavioral effects can be established in CD-1 mice in the light–dark task employing milder stressor intensities. We have observed, however, that Fos induction in some mesolimbic sites is not differentially influenced by the gradients of footshock employed in the present investigation. In contrast, darkly stained Fos positive staining of mesolimbic areas were conspicuous following 30 but not 15 or 60 min of restraint. Finally, it should be noted that comparison of the profile of Fos induction in mesolimbic areas induced by intraventricular CCK administration relative to that induced by footshock or restraint was compromised owing to the appearance of Fos activity following cannula placement. Clearly, additional experiments are required to determine the proactive effects of cannulation on Fos activation and that provoked by the anxiogenic agent CCK alone.

Some comment should be provided with respect to the ability of CCK-8S to activate central CCK receptors. It will be recalled that the predominant central CCK receptor type underlying promotion of anxiety appears to be the CCK_B type. Nevertheless, there are data to suggest that both central CCK_A and CCK_B receptors favor expression of anxiety in infrahuman subjects (47). In addition, there also appear to be intraregional variations in the distribution of the CCK_A and the CCK_B receptor type in mesolimbic sites that are responsive to pharmacological and environmental manipulations (11). It should be considered that intraventricularly administered CCK-8S influences both receptor subtypes, but the conspicuous concentration of the central CCK_B receptor and the absence of peripheral effects with the selected doses of CCK-8S administered favor a prominent role for the CCK_B receptor. Several caveats, nevertheless, should be introduced. Previous research has demonstrated that CCK-8S can provoke anxiogenic effects in the elevated plus maze following administration in the posterior but not the anterior nucleus accumbens of rats that lacks DA-CCK colocalization and the anxiogenic effect of such drug administration was attenuated by administration of the CCK_A receptor antagonist devazepide (14). Nevertheless it has been demonstrated that CCK_A antagonism fails to modify anxiety in the elevated plus-maze in the mouse (35). Accordingly, species variations the route, and dose of CCK administration and, consequently, the location of central CCK receptor activation and the particular paradigm should also be considered in evaluating behavioral change.

In conclusion, it would appear that immobilization stress and uncontrollable footshock provoke varying behavioral profiles of anxiety in the light–dark paradigm in the CD-1 mouse. These data provide evidence for the suggestion that behavioral expression of anxiety is intrinsically associated with the nature of the aversive stimulus imposed, the parameters of the stressor, and the paradigm employed to assess anxiety. In view of the observation that exposure of animals to specific anxiogenic stimuli (e.g., predatory scent) favors release of particular CCK fragments in a limited number of brain area (33), that endogenous anxiogenic agents displace central anxiolytic ligands (4), and that central concentration of endogenous anxiolytic agents are differentially decreased in specific brain sites following encounter with aversive stimulation (13), it is intriguing to consider that the neurochemical correlates of paradigms assessing anxiety in infrahuman subjects may provide parallels for human disorders. Nevertheless, it should be underscored that anxiety induction in the light–dark paradigm following intraventricular CCK challenge simulates the neurochemical repercussions of some yet to be defined anxiogenic stimulus. The differential distribution of central CCK receptors and variations in CCK receptor sensitivity in diverse sites coupled with cascading neurochemical variations of related anxiogenic agents require consideration. It would appear imperative in this respect to define the task and stressor specific parameters that simulate the effects of CCK administration in the light–dark paradigm.

ACKNOWLEDGEMENTS

This research was supported by grants from the Gustavus and Louis Pfeiffer Research Foundation and the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

1. Anisman, H.; Zalcman, S.; Shanks, N.; Zacharko, R. M.: Multisystem regulation of performance deficits induced by stressors. In: Boulton, A. A.; Baker, G. B.; Martin-Iverson, M. T., eds. Animal models of psychiatry. Clifton, NJ: Humana Press; 1991:1–59.
2. Antelman, S. M.; Knopf, S.; Kocan, D.; Edwards, D. J.: Persistent sensitization of clonidine-induced hypokinesia following one exposure to a stressor: Possible relevance to panic disorder. Psychopharmacology (Berlin) 98:97–101; 1989.
3. Benoliel, J. J.; Nevo, I.; Becker, C.; Lanfumey, L.; Hamon, M.: Stress-induced release of cholecystokinin (CCK)-like material from the frontal cortex in awake, freely moving rats. Soc. Neurosci. Abstr. 21:757; 1995.
4. Biggio, G.; Concas, A.; Corda, M. G.; Giorgi, O.; Sanna, E.; Serra, M.: Gabergic and dopaminergic transmission in the rat cerebral cortex: Effect of stress, anxiolytic and anxiogenic drugs. Pharmacol. Ther. 48:121–142; 1990.
5. Cabib, S.; Puglisi-Allegra, S.: Opposite effects of mesolimbic dopamine system to controllable and uncontrollable aversive experience. J. Neurosci. 14:3333–3340; 1994.
6. Cancela, L. M.; Bregonzio, C.; Molina, V. A.: Anxiolytic-like effect induced by chronic stress is reversed by naloxone pretreatment. Brain Res. Bull. 36:209–213; 1995.
7. Chappell, P. B.; Smith, M. A.; Kilts, C. D.; Bisette, G.; Ritchie, J.; Anderson, C.; Nemeroff, C. B.: Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat brain regions after acute and chronic stress. J. Neurosci. 6:2908–2914; 1986.
8. Crawley, N. M.: Comparative distribution of cholecystokinin and other neuropeptides. Why is this peptide different from all other peptides? Ann. NY Acad. Sci. 448:1–8; 1985.
9. Crawley, J. N.: Behavioral analyses of antagonists of the peripheral and central effects of cholecystokinin. In: Wang, R. Y.; Schoenfeld, R., eds. Cholecystokinin antagonists. New York: Alan Liss; 1988:243–262.
10. Crawley, J. N.: Cholecystokinin–dopamine interaction. Trends Pharmacol. Sci. 12:232–236; 1991.
11. Crawley, J. N.; Corwin, R. L.: Biological actions of cholecystokinin. Peptides 15:731–755; 1994.
12. Cruz, A. P. M.; Frei, F.; Graeff, F. G.: Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol. Biochem. Behav. 49:171–176; 1994.
13. De Cunha, C.; De Stein, M.; Wolfman, C.; Koya, R.; Izquierdo, I.; Medina, J. H.: Effect of various training procedures on performance in an elevated plus-maze: Possible relation with brain regional levels of benzodiazepine-like molecules. Pharmacol. Biochem. Behav. 43:677–681; 1992.
14. Dauge, V.; Steimes, P.; Derrien, M.; Beau, N.; Roques, B. P.; Feger, J.: CCK8 effects on motivational and emotional states of rats involve CCKA receptors of the postero-medial part of the nucleus accumbens. Pharmacol. Biochem. Behav. 34:157–163; 1989.
15. Derrien, M.; Durieux, C.; Roques, B. P.: Antidepressant-like effects of CCK_B antagonists in mice: Antagonism by naltrindole. Br. J. Pharmacol. 111:956–960; 1994.
16. Deutch, A. Y.; Roth, R. H.: The determinants of stress-induced activation of the prefrontal cortical dopamine system. In: Uylings, H. B. M.; van Eden, C. G.; de Bruin, J. P. C.; Corner, M. A.; Feenstra, G. P., eds. Progress in brain research. New York: Elsevier; 1990:367–403.
17. Drugan, R. C.; Basile, A. S.; Crawley, J. N.; Paul, S. M.; Skolnick, P.: Inescapable shock reduces (^{3}H)-Ro 5-4864 binding to "peripheral-type" benzodiazepine receptors in the rat. Pharmacol. Biochem. Behav. 24:1673–1677; 1986.
18. Drugan, R. C.; Basile, A. S.; Crawley, J. N.; Paul, S. M.; Skolnick, P.: Characterization of the stress-induced alterations in ^{3}H-Ro 5-4864 binding to "peripheral-type" benzodiazepine receptors in the rat. Pharmacol. Biochem. Behav. 27:1015–1020; 1988.
19. Ferrarese, C.; Menini, T.; Pecora, N.; Pierapoli, C.; Apollino, I.; Frigo, M.; Ftattola, L.: Stress induced changes of diazepam binding inhibitor (DBI) in rat brain and adrenal gland of rat. Neuropharmacology 30:1445–1452; 1991.
20. File, S. E.; Zangrossi, H.: "One trial tolerance" to the anxiolytic actions of benzodiazepines in the elevated plus-maze, or the development of a phobic state? Psychopharmacology (Berlin) 110:240–244; 1993.
21. Harro, J.: Measurement of exploratory behavior in rodents. In: Conn, P. M. ed. Paradigms for the measurement of behavior: Methods in neuroscience New York: Academic Press; 1993:359–376.
22. Harro, J.; Pold, M.; Vasar, E.: Anxiogenic-like action of caerulein, a CCK-8 receptor agonist, in the mouse: Influence of acute and subchronic diazepam treatment. Arch. Pharmacol. 341:62–67; 1990.
23. Harro, J.; Vasar, E.; Bradwejn, J.: CCK in animal and human research in anxiety. Trends Pharmacol. Sci. 141:244–249; 1993.
24. Hendrie, C. A.; Neill, J. C.; Shepherd, J. K.; Dourish, C. T.: The effects of CCK_A and CCK_B antagonists on activity in the black/white exploration model of anxiety in mice. Physiol. Behav. 54: 689–693; 1993.
25. Hokfelt, T.: Neuropeptides in perspective: The last ten years. Neuron 7:867–879; 1991.
26. Kalivas, P. W.; Abhold, R.: Enkephalin release into the ventral tegmental area in response to stress: Modulation of mesocorticolimbic dopamine. Brain Res. 414:339–348; 1987.
27. Kilfoil, T.; Michel, A.; Montgomery, D.; Whiting, R. L.: Effects of anxiolytic and anxiogenic drugs on exploratory activity in a simple model of anxiety in mice. Neuropharmacology 28:901–905; 1989.
28. Korte, S. M.; De Boer, S. F.; De Kloet, E. R.; Bohus, B.: Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus maze. Psychoneuroendocrinology 20:385–394; 1995.
29. Marshall, F. H.; Barnes, S.; Hughes, J.; Woodruff, G. N.; Hunter, T. N.: Cholecystokinin modules the release of dopamine from the anterior and posterior nucleus accumbens by two different mechanisms. J. Neurochem. 56:917–922; 1991.
30. McBlane, J. W.; Handley, S. L.: Effects of stressors on behaviour in the elevated X-maze: Preliminary investigation of their interaction with 8-OH-DPAT. Psychopharmacology (Berlin) 116:173–182; 1994.
31. Onaivi, E.; Martin, B. R.: Neuropharmacological and physiological validation of a computer controlled two-compartment black and white box for the assessment of anxiety. Prog. Neuropsychopharmacol. Biol. Psychiatry 13:963–976; 1989.
32. Pacak, K.; Armano, I.; Fukuhara, K.; Kvetnansky, R.; Palkovits, M.; Kopin, I. J. Goldstein, D. S.: Noradrenergic activation in the paraventricular nucleus during acute and chronic immobilization stress in rats: An in vivo microdialysis study. Brain Res. 589:91–96; 1992.
33. Pavlasevic, S.; Bednar, I.; Qureshi, G. A.; Sodersten, P.: Brain cholecystokinin tetrapeptide levels are increased in a rat model of anxiety. Neuroreport 5:225–228; 1993.
34. Puglisi-Allegra, S.; Imperato, A.; Angelucci, L.; Cabib, S.: Acute stress induces time-dependent responses in dopamine mesolimbic system. Brain Res. 544:217–222; 1991.
35. Rataud, J.; Darche, F.; Piot, O.; Stutzmann, J. M.; Bohme, G. A.; Blanchard, J. C.: "Anxiolytic" effect of CCK-antagonists on plus-maze behavior in mice. Brain Res. 548:315–317; 1991.
36. Ravard, S.; Dourish, C. T.: Cholecystokinin and anxiety. Trends Pharmacol. Sci. 11:271–273; 1990.
37. Rodgers, R. J.; Shepherd, J. K.: Influence of prior maze experience on behaviour in response to diazepam in the elevated plus-maze and light/dark tests of anxiety in mice. Psychopharmacology (Berlin) 113:237–241; 1993.
38. Rosen, A.; Brodin, K.; Eneroth, P.; Brodin, E.: Short-term restraint stress and s.c. saline injection alter the tissue levels of substance P and cholecystokinin in the peri-aqueductal grey and limbic regions of rat brain. Acta Physiol. Scand. 146:341–348; 1992.
39. Roth, R. H.; Tam, S.-Y.; Ida, Y.; Yang, J.-X.; Deutch, A. Y.: Stress and the mesocorticolimbic system. In: Kalivas, P. W.; Nemeroff, C. B., eds. The mesocorticolimbic system. New York: New York Academy of Sciences; 1988;138–147.

40. Roth, R. H.; Wolf, M. E.; Deutch, A. Y.: Neurochemistry of midbrain dopamine systems. In: Meltzer, H. Y., eds. Psychopharmacology: The third generation of progress. New York: Raven Press; 1988:138–147.
41. Saavedra, J. M.: Changes in dopamine, noradrenaline and adrenaline in specific sepal and preoptic nuclei after acute immobilization stress. Neuroendocrinology 35:397–401; 1982.
42. Saito, A.; Goldfine, I. D.; Williams, J. A.: Characterization of receptors for cholecystokinin and related peptides in mouse cerebral cortex. J. Neurochem. 37:483–490; 1981.
43. Shimizu, T.; Tanaka, M.; Yokoo, H.; Gondoh, Y.; Mizoguchi, K.; Matsuguchi, N.; Tsuda, A.: Differential changes in rat brain noradrenaline turnover produced by continuous and intermittent restraint stress. Pharmacol. Biochem. Behav. 49:905–909; 1994.
44. Siegel, R. A.; Duker, E. M.; Pahnke, U.; Wittke, W.: Stress-induced changes in cholecystokinin and substance P concentrations in discrete regions of the rat hypothalamus. Neuroendocrinology 46:75–81; 1987.
45. Siegel, R. A.; Duker, E. M.; Fuchs, E.; Pahnke, U.; Wuttake, W.: Responsiveness of mesolimbic, mesocortical, septal and hippocampal cholecystokinin and substance P neuronal systems to stress in the male rat. Neurochem. Int. 6:783–789; 1985.
46. Singh, L.: Evidence for an involvement of the brain cholecystokinin B receptor in anxiety. Proc. Natl. Acad. Sci. USA 88:1130–1133; 1991.
47. Zacharko, R. M.; Koszycki, D.; Mendella, P.; Bradwejn, J.: Behavioral, neurochemical, anatomical and electrophysiological correlates of panic disorder: Multiple transmitter interaction and neuropeptide colocalization. Prog. Neurobiol. 47:371–423; 1995.