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European Journal of Pharmacology 463 (2003) 163–175
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A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze

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Accepted 10 December 2002

Abstract

Fear (i.e., decreased percentage time spent on open-arm exploration) in the elevated plus-maze can be potentiated by prior inescapable stressor exposure, but not by escapable stress. The use of fear-potentiated plus-maze behaviour has several advantages as compared to more traditional animal models of anxiety. (a) In contrast to the traditional (spontaneous) elevated plus-maze, which measures innate fear of open spaces, fear-potentiated plus-maze behaviour reflects an enhanced anxiety state (allostatic state). This ''state anxiety'' can be defined as an unpleasant emotional arousal in face of threatening demands or dangers. A cognitive appraisal of threat is a prerequisite for the experience of this type of emotion. (b) Depending on the stressor used (e.g., fear of shock, predator odour, swim stress, restraint, social defeat, predator stress (cat)), this enhanced anxiety state can last from 90 min to 3 weeks. Stress effects are more severe when rats are isolated in comparison to group housing. (c) Drugs can be administered in the absence of the original stressor and after stressor exposure. As a consequence, retrieval mechanisms are not affected by drug treatment. (d) Fear-potentiated plus-maze behaviour is sensitive to proven/putative anxiolytics and anxiogenics which act via mechanisms related to the benzodiazepine–gamma-aminobutyric acid receptor, but it is also sensitive to corticotropin-releasing receptor antagonists and glucocorticoid receptor antagonists and serotonin receptor agonists/antagonists complex (high predictive validity). (e) Fear-potentiated plus-maze behaviour is very robust, and experiments can easily be replicated in other labs. (f) Fear-potentiated plus-maze behaviour can be measured both in males and females. (g) Neural mechanisms involved in contextual fear conditioning, fear potentiation and state anxiety can be studied .Thus, fear-potentiated plus-maze behaviour may be a valuable measure in the understanding of neural mechanisms involved in the development of anxiety disorders and in the search for novel anxiolytics. Finally, the involvement of corticotropin-releasing factor and corticosteroid–corticotropin-releasing factor interactions in the production of fear-potentiated plus-maze behaviour are discussed.

Keywords: Stress; Elevated plus-maze; Enhanced anxiety state; State anxiety; Fear-potentiation; Allostasis; Allostatic state; Allostatic load; Corticotropin-releasing factor; Corticosteroid; Glucocorticoid receptor antagonist; Controllability; Contextual conditioning; Diazepam

1. Different stressors produce fear potentiation in the elevated plus-maze

In the field of anxiety, the elevated plus-maze has become one of the most popular animal models (Pellow et al., 1985). The test involves placing a naive rat (or mouse or pig) in the center of an elevated plus-maze with two open and two enclosed arms, and allowing it to freely explore (Rodgers and Cole, 1993; Rodgers and Dalvi, 1997; Andersen et al., 2000). It has been suggested that the reluctance of rats to explore the open arms of the maze is caused by fear of open spaces, rather than the novelty of the maze or its height (often 50 cm) (Pellow et al., 1985; Falter et al., 1992; Treit et al., 1993; Fernandes and File, 1996). Anxiolytic compounds increase, whereas anxiogenic compounds decrease the percentage of time spent on open arms relative to time on open + closed arms (Pellow et al., 1985; Cole et al., 1995). The total number of entries and/or the number of entries into the closed arms reflects a measure of locomotor activity (Korte et al., 1999).

Although plus-maze test results are often robust within the same laboratory, the results from different laboratories can easily be inconsistent and contradictory (Hogg, 1996). This was confirmed by the finding that differences in anxiety in genetically identical mice, as tested in the elevated plus-maze, depended highly upon the specific testing lab (Crabbe et al., 1999). In contrast to traditional plus-maze testing, it

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doi:10.1016/S0014-2999(03)01279-2

has been shown that test results are very robust when fear is potentiated by prior stressor exposure, as measured in the elevated plus-maze (see Table 1).

One has to realize, of course, that the neural mechanisms involved in fear-potentiated plus-maze behaviour (state anxiety) as compared to spontaneous plus-maze behaviour (trait anxiety) are quite different because in state anxiety, a cognitive appraisal of threat is a prerequisite for the experience of this type of emotion, whereas in trait anxiety, the existence of stable individual differences are characteristic.

However, fear-potentiated plus-maze behaviour may be an (even more) interesting measure because an enhanced anxiety state is involved in many psychopathologies Korte, 2001).

Some reports, however, describe no effects of prior stressor exposure on plus-maze behaviour (Steenbergen et al., 1991; Grahn et al., 1995; Falter et al., 1992). Therefore, in Table 1, the different stressor properties which are indispensable for fear potentiation in the elevated plus-maze are given. In the present review, several critical factors that affect fear potentiation in the elevated plus-maze (including duration of the enhanced anxiety state) will be presented. Neural mechanisms involved in fear potentiation will be explained as well (see discussion).

Table 1 clearly shows that fear potentiation in the elevated plus-maze (as measured by the specific suppression of open-arm exploration) can be observed after certain types of stressor exposure, but not all. There is a growing body of evidence that prior exposure to more severe unconditioned stressors may enhance the anxiety state in rats in the elevated plus-maze. Predator stress (cat exposure) seems to be the most potent stressor (Adamec and Shallow, 1993). Cat odour alone is also effective, but the enhanced anxiety state exists for less than 1 day (Zangrossi and File, 1992), whereas the effects of predator stress last for 3 weeks. Interestingly, it is observed that fear potentiation after stress exposure (e.g., social defeat) is more pronounced if rats are isolated, since stressor exposure in group-housed animals had no effect on the percentage time spent on open arms 14 days after defeat (Ruis et al., 1999). The differences in stressor type, intensity and duration of stressor exposure might explain why unconditioned stressors, such as social defeat in isolated rats, forced swim and restraint, produce fear potentiation in the plus-maze, whereas a unconditioned stressor, such as mild footshock, does not. It is quite well possible that differences in hypothalamic–pituitary–adrenal axis (re)activity plays a crucial role. For example, social defeat (followed by approximately 30 min of psychological stress in the intruder rat caused by a resident male aggressor rat that was separated by a wire mesh), forced swim (2 min) and restraint (60 min) produce much higher plasma corticosterone stress responses (300–600 ng/ml) (Heinrichs et al., 1992, 1994; Koob et al., 1993; Koolhaas et al., 1997) than mild footshock (0.6 mA, a.c. for 3 s) (100–200 ng/ml) (Roozendaal et al., 1991; Korte et al., 1992a). Previously, it has been shown that reactive (passive) rats have a higher hypothalamic–pituitary–adrenal axis activation after stressor exposure than proactive rats (Koolhaas et al., 1999). Furthermore, social defeat is also characterized by a strong sympathetic dominance and can be considered as one of the most severe stressors in terms of neuroendocrine activation (Koolhaas et al., 1997).

Previously, it has been shown that when tailshocks were given without fear conditioning, no fear potentiation was observed (Grahn et al., 1995). Fear potentiation after mild footshock, however, was only observed if rats were reex-

Table 1
Fear potentiation in rats, as measured in the elevated plus-maze, can be produced by different stressors

Housing	Stressor	Time interval (stressor-test)	Percent time in open arms	Refs.
Group	restraint	<1 min	↓	Heinrichs et al. (1994)
Group	restraint	24 h	↓	Martijena et al. (1997), Mendonca and Guimaraes (1998)
Group	forced swim	<1 min	↓	Heinrichs et al. (1994)
Group	partial water immersion	15 min	○	Falter et al. (1992)
Group	social defeat	ca. 5 min	↓	Heinrichs et al. (1992, 1994)
Isolation	social defeat	14 days	↓	Ruis et al. (1999)
Group	social defeat	14 days	○	Ruis et al. (1999)
Group	isolation	2–24 h	↓	Maisonnette et al. (1993)
Isolation	regrouping	2 h	↔	Maisonnette et al. (1993)
Group	cat (predator stress)	30–60 min and lasting 3 weeks	↓	Adamec and McKay (1993), Adamec and Shallow (1993)
Isolation	cat odour	1 h	↓	Zangrossi and File (1992)
Isolation	cat odour	24 h	○	Zangrossi and File (1992)
Isolation	inescapable shock	<1 min, 24–72 h	○	Steenbergen et al. (1991)
Group	inescapable shock	1 min, 24 h, 7 days	○	Korte et al. (1999)
Group	prior shock box	<1 min	↓	Korte et al. (1999)

Fear potentiation is expressed as decreased (↓) percent time in open arms of the plus-maze.↔ means normalisation of percent time in open arms exploration, while ○ means no effect.

posed to the compartment where they had experienced the inescapable footshock 24 h earlier (i.e., contextual conditioning) (Korte et al., 1999).

2. Stressor controllability and fear-potentiated plus-maze behaviour

To study the effects of stressor controllability on fear-potentiated plus-maze behaviour, an experiment was set up in accordance with the methods of Weiss (1968) on the influence of psychological variables on stress-induced pathology. Three groups were used (Korte et al., 1999). The first group (no stress) of rats received no shocks (see Fig. 1). The second group of rats were trained in a two-way (shuttle box) active avoidance paradigm and were exposed to 10 controllable mild electric footshock trials; rats quickly learned that the presentation of light was followed by a mild footshock; after receiving several trials, rats jumped across the hurdle to the save compartment (active control). The third group of rats was yoked and could not escape the footshocks (loss of control). Yoked controls (loss of control) received the same amount of shocks as their escape pairs because the shock was terminated in both the escaping rat (after jumping) and yoked control. On the second day, rats of all groups were exposed to their own training compartment, but this time the rats received no shocks (contextual conditioning). Directly thereafter, the animals of the three groups were exposed to a 5-min plus-maze test.

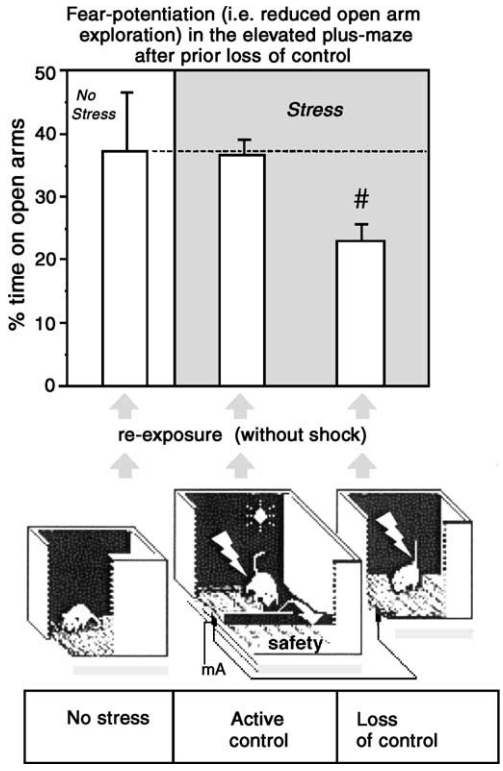

Fig. 1. Effects of prior stress history in rats with and without stressor controllability in elevated plus-maze behaviour. The no-stress group of rats received no shocks and spent almost 38% of the time on the open arms (open/open + closed) (means + S.E.M.). The second group of rats (active control) were trained in a two-way (shuttle box) active avoidance paradigm and quickly learned that the presentation of light was followed by a mild footshock. After receiving several trials, rats jumped across the hurdle to the save compartment. The third group of rats was yoked and could not escape the footshocks (loss of control). Yoked controls (loss of control) received the same amount of shocks as their escape pairs because the shock was terminated in both the escaping rat (after jumping) and yoked control. On the second day, rats of all groups were reexposed to their own training compartment, but this time they received no shock (contextual conditioning). Directly thereafter, the animals of the three groups were exposed to a 5-min plus-maze test. Only the rats that experienced loss of control (fear of inescapable footshock) showed a significant (#$P<0.05$) decrease in the time spent on the open arms of the plus-maze (i.e., fear potentiation) as compared to the no-stress group. The group that received the same amount of footshocks but learned to actively control (avoid) footshocks did not show fear potentiation. Thus, whether fear potentiation will be produced or not depends highly on the amount of active controllability.

Fig. 1 shows that fear of inescapable footshock (loss of control) produced a significant decrease in the percentage of time rats explored the open arms of the plus-maze (i.e., fear potentiation). The rats of the group that received the same amount of footshocks but learned to actively control (avoid) footshocks spent the same time on open-arm exploration as the nonshock controls group (no stress) did. Thus, whether fear potentiation will be produced or not depends highly on the amount of active controllability. It is quite well possible that this is caused by differences in stress-induced corticosterone release because we have shown previously that corticosterone release is higher in passive coping rats (no escape possible, freezing) as compared to active coping (active control) rats (Korte et al., 1992c).

3. Duration of fear potentiation

To study the duration of fear-potentiated plus-maze behaviour in group-housed rats, they received one inescapable mild footshock (0.6 mA, a.c. for 3 s.) (loss of control) (Korte et al., 1999). The next day, the rats were exposed to the prior shock compartment, but no further footshock was given (contextual conditioning). The rats were placed at the following times in the plus-maze: directly, 30 min, 60 min, 90 min, 120 min and 180 min after reexposure to former shock compartment. The control rats received no shocks (no stress).

Fig. 2 shows that reexposing rats to a prior shock compartment (stress) reduced the percentage time spent on open-arm exploration as compared to the nonstress group (no stress) if the time between reexposure to prior shock compartment and placement in the plus-maze did not exceed 90 min. Stressed rats spent significantly less time spent on open-arm exploration at 30 min ($P<0.01$), 60 min ($P<0.05$) and 90 min ($P<0.01$) as compared to nonshocked controls. This

relatively long-lasting enhanced anxiety state has many advantages. The fact that the enhanced anxiety state persists for about 90 min after the conditioned inescapable stressor has the advantage that drugs can be administered after (conditioned) stressor exposure. Thereby, drug effects can be studied that specifically affect the enhanced anxiety state instead of effects that disturb memory retrieval processes. Furthermore, in the present procedure, animals are exposed to a conditioned inescapable mild stressor, whereafter they are transported to another room where they are placed in the plus-maze test, making fear-potentiated plus-maze behaviour a sensitive measure of "generalized anxiety".

The advantage of the present paradigm with fear conditioning is that stress can be made context-dependent, and such a test may make different predictions than unconditioned stressor exposure and consequently other brain structures may be involved. Because fear conditioning processes may contribute to such disorders as phobia, excessive fear, anxiety, posttraumatic stress and panic (LeDoux, 1995), it is of interest to study fear-potentiated plus-maze behaviour. Both hippocampus and amygdala play important roles in fear conditioning. The hippocampus may relay environmental inputs pertaining to the conditioning context to the lateral amygdala, where emotional meaning is added to context (LeDoux, 1995).

Fig. 3 shows that acute social defeat (without reexposure) in rats that are group-housed produces a significant enhanced anxiety state (i.e., reduced percentage open-arm exploration) in the elevated plus-maze directly after defeat (day 0), but also 24 h later (day 1). However, 2 days later, no significant effects are observed. As can be seen in Table 1, however, if rats are isolated than the effects of social defeat, reduced open-arm exploration can still be observed 14 days later (Ruis et al., 1999). Furthermore, Table 1 shows that the

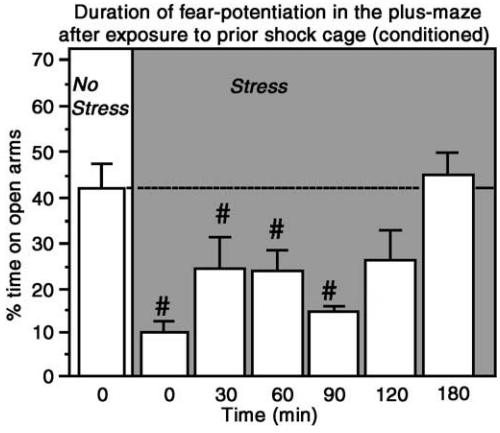

Fig. 2. Duration of fear-potentiated plus-maze behaviour (i.e., reduction of open-arm exploration, means + S.E.M.) in group-housed rats that received loss of control (see also Fig. 1). Fear potentiation in stressed rats tested in the elevated plus-maze was observed 30, 60 and 90 min after reexposure to former shock compartment (contextual conditioning). Significant differences: $\#P<0.05$ compared to nonstressed controls.

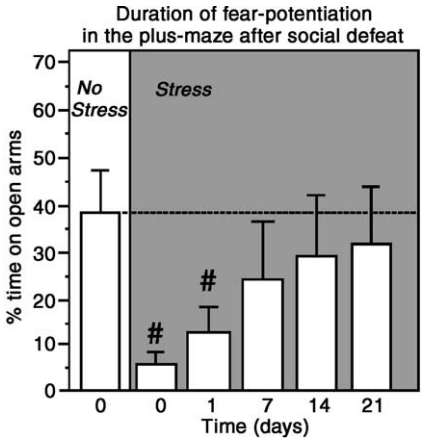

Fig. 3. Duration of fear-potentiated plus-maze behaviour (i.e., reduction of open-arm exploration, means + S.E.M.) in group-housed rats that experienced acute social defeat. Fear potentiation in stressed rats tested in the elevated plus-maze was observed directly after defeat (day 0) and 1 day after defeat as compared to nonstressed controls. No effects of social defeat were seen 7, 14 and 21 days later. Significant differences: $\#P<0.05$ compared to nonstressed controls.

effects of predator stress (cat) last for 3 weeks (Adamec and Shallow, 1993).

4. Classical anxiolytic and anxiogenic drug effects on fear-potentiated plus-maze behaviour in female rats

Previously, we have shown that the classical benzodiazepine anxiolytic diazepam (valium) and the anxiogenic inverse benzodiazepine agonist methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), respectively, decreased and increased the enhanced anxiety state as measured in the elevated plus-maze in male rats (Korte et al., 1999). To study whether these drug effects were sex-dependent, we used the same drugs in female rats. A similar stress procedure as described in "duration of fear potentiation" was used. Female rats were first placed in the prior shock compartment, then injected subcutaneously with vehicle or drug and 30 min thereafter placed in the elevated plus-maze.

Fig. 4 shows, as expected, that vehicle (0)-treated stressed female rats spent a decreased percentage time on the open arms in comparison to nonshocked vehicle-treated rats ($P<0.05$). In the stressed female rats, diazepam (1 mg/kg) increased the percentage of open-arm exploration as compared to the vehicle-treated stressed female rats ($P<0.05$). No effects of diazepam on arm entries were observed. In contrast, DMCM (1.0 mg/kg) further decreased the percentage of time spent on the open arms ($P<0.05$). DMCM in both doses (0.5 and 1.0 mg/kg) further decreased the number of open-arm entries ($P<0.05$), whereas the higher dose further increased the number of closed arm entries. Thus, fear-potentiated plus-maze behaviour is very sensitive to classical benzodiazepine anxiolytic and anxio-

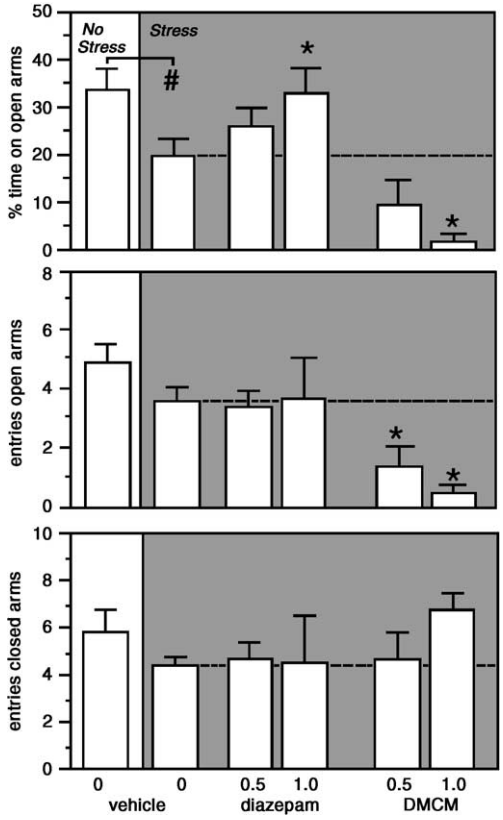

Fig. 4. Anxiolytic and anxiogenic drug effects on fear-potentiated plus-maze behavior in female rats. As expected, a prior history of inescapable stress (see also Figs. 1 and 2) produced fear potentiation in the elevated plus-maze (#$P<0.05$). The anxiolytic diazepam dose-dependently (1.0 mg/kg) increased the percentage time spent on open arms (means + S.E.M.), whereas the anxiogenic DMCM dose-dependently (1.0 mg/kg) decreased the percentage time spent on open arms and decreased the number of open-arm entries (both 0.5 and 1.0 mg/kg). Significant differences: *$P<0.05$ compared to vehicle-treated (0 mg/kg) stressed controls.

genic drugs both in male and female rats. As can be read below, fear potentiation in the elevated plus-maze is not solely influenced by gamma-aminobutyric acid (GABAergic) mechanisms.

5. Corticosteroid involvement in fear-potentiated plus-maze behaviour

To study whether glucocorticosteroids are involved in fear potentiation in the elevated plus-maze test, a similar stress procedure was used as described in Section 3. Male rats were first placed in the prior shock compartment, then injected subcutaneously with vehicle or glucocorticoid receptor antagonist RU38486 or the even more specific glucocorticoid receptor antagonist ORG34580 and 30 min thereafter placed in the elevated plus-maze.

Fig. 5 shows, as expected, that vehicle (0)-treated stressed rats spent a decreased percentage time on the open arms in comparison to nonshocked (no stress) vehicle-treated rats ($P<0.05$). In the stressed rats, both glucocorticoid receptor antagonists RU38486 and ORG34580 in the highest dose (respectively, 12.5 and 20 mg/kg) increased the percentage of open-arm exploration as compared to the vehicle-treated stressed rats ($P<0.05$). No effects of both glucocorticoid receptor antagonists on arm entries were observed. Thus, the present results suggest that corticosteroids via glucocorticoid receptors are involved in fear-potentiated plus-maze behaviour. This did not come by surprise because the corticosteroid synthesis inhibitor metyrapone (25 mg/kg. s.c.) injected 90 min before exposure to the prior shock compartment led to increased open-arm exploration, indicative of reduced anxiety state (Roozendaal et al., 1996). In support, it was found that administration of the glucocorticoid receptor antagonist RU38486 (50 ng, i.c.v.) 30 min before exposure to the prior shock compartment attenuated fear in the plus-maze, thus, suggesting that brain glucocorticoid receptors play a stimulatory role in fear

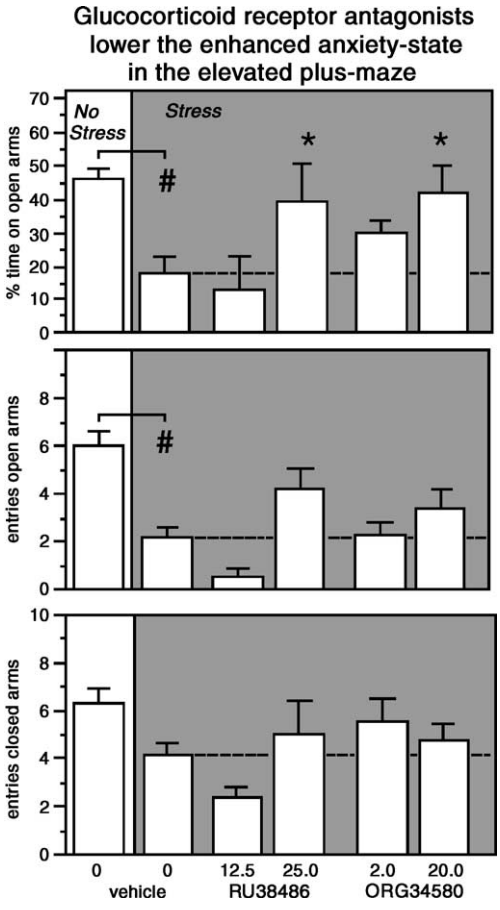

Fig. 5. Effects of glucocorticoid receptor antagonists on fear-potentiated plus-maze behaviour. As expected, a prior history of inescapable stress (see also Figs. 1 and 2) produced fear potentiation in the elevated plus-maze (#$P<0.05$). The glucocorticoid receptor antagonists RU38486 and ORG34580 dose-dependently (respectively, 25 and 20 mg/kg) increased the percentage time spent on open arms (means + S.E.M.) without affecting number of entries. Significant differences: *$P<0.05$ compared to vehicle-treated (0 mg/kg) stressed controls.

potentiation (Korte et al., 1995). It is suggested that when plasma corticosterone levels are high (Korte et al., 1992a) and glucocorticoid receptors are occupied after a stressful experience, an enhanced anxiety state is produced in which brain glucocorticoid receptors play a crucial role.

6. CRF involvement in fear-potentiated plus-maze behaviour

In order to show that fear-potentiated plus-maze behaviour is a very robust measure and corticotropin-releasing factor (CRF) receptors are involved in this enhanced anxiety state, we replicated and extended (24 h after defeat) an experiment firstly performed in the Koob laboratory of the Scripps Research Institute at La Jolla (USA) (Heinrichs et al., 1992; Koob et al., 1993; Menzaghi et al., 1994). We investigated whether the potent mixed $CRF_{1,2}$ receptor antagonist D-Phe CRF-(12-41), which was intracerebroventricularly injected 10 min prior to plus-maze testing, also blunted the social defeat (without reexposure) enhanced anxiety state in the elevated plus-maze 24 later. As can be seen in Fig. 6, social defeat produces fear potentiation (i.e., reduced open-arm exploration) in the elevated plus-maze and also in our hands, the potent mixed CRF receptor antagonist D-Phe CRF-(12-41) normalized the social stress-induced enhanced anxiety state as reflected by the increased open-arm exploration in CRF receptor antagonist-treated rats.

These data indirectly suggest that CRF is involved in stress-induced fear potentiation in the plus-maze. In agreement, it was reported that both acute and chronic CRF (intracerebroventricular) treatment reduced open-arm exploration on the elevated plus-maze (Baldwin et al., 1991; Adamec and McKay, 1993; Buwalda et al., 1997). In addition, the overproduction of CRF in transgenic mice produced decreased open-arm exploration, which could be blocked by the mixed $CRF_{1,2}$ receptor antagonist α-helical CRF-(9-41) (intracerebroventricular) (Stenzel-Poore et al., 1994). There is a growing body of evidence that the CRF_1 receptor mediates the stimulatory CRF effect on fear potentiation. Recently, it was shown that the very selective nonpeptide CRF_1 receptor antagonist R121919, administered orally, blunted the swim stress induced enhanced anxiety state in the elevated plus-maze. (Heinrichs et al., 2002). In line with these results, it has been shown that CRF_1 receptor-deficient mice spent a higher percentage of time on the open arms (Smith et al., 1998; Contarino et al., 1999). In agreement it was shown that CRF enhanced anxiety state in the plus-maze was attenuated by intracerebroventricular CRF_1 receptor antisense (Skutella et al., 1998).

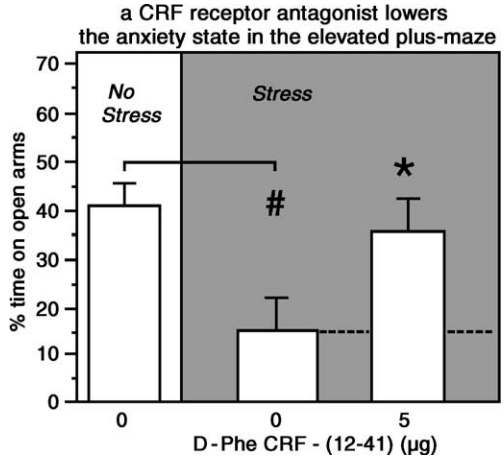

Fig. 6. Effects of a corticotropin-releasing factor (CRF) receptor antagonist on fear-potentiated plus-maze behaviour. As expected, a prior history of acute social defeat (see also Fig. 3) produced fear potentiation in the elevated plus-maze 24 h after social defeat (#$P<0.05$). The intracerebroventricular injected CRF receptor antagonist D-Phe CRF-(12-41) increased the percentage time spent on open arms (means + S.E.M.). Significant difference: *$P<0.05$ compared to vehicle-treated (0) stressed controls.

7. Central mechanisms of an enhanced anxiety state

7.1. Allostasis, allostatic state and allostatic load in relation to enhanced anxiety

Allostasis refers to the integrative adaptive processes maintaining stability through change (Sterling and Eyer, 1988; McEwen and Stellar, 1993; McEwen, 1998, 2000; Goldstein and McEwen, 2002). By controlling all the mechanisms simultaneously, the brain can enforce its command and introduce experience, memories, anticipation and reevaluation of needs in anticipation of physiological requirements (Koob and Le Moal, 2001). *Allostatic state*, as defined by Koob and Le Moal (2001), refers to a state of chronic deviation of the regulatory system from its normal homeostatic operating level. Finally, *allostatic load* refers to the cost or price the body may have to pay for being forced to adapt to an adverse psychological or physiological stressor accumulating over time (McEwen and Stellar, 1993). There is growing support for the idea that CRF and corticosteroids play crucial roles in the production of allostatic state and allostatic load. According to Schulkin et al. (1994, 1998) and Schulkin (1999), the amygdala is involved in anticipation of negative events. Chronic anticipation of negative events may lead to allostatic load. They were the first to suggest that increases in CRF, by stress or glucocorticoids, in the amygdala may have functional consequences for allostatic load, playing an important role in the development of anxiety disorders.

7.2. CRF and the production of an enhanced anxiety state

There are several studies supporting the hypothesis that hypersecretion of CRF is a crucial factor in anxiety disorders and depression (Nemeroff et al., 1984). A number of

researchers showed that when CRF was injected into the brains of rats, it produced many of the signs and symptoms seen in patients with depression and/or anxiety orders (e.g., see review, Koob and Heinrichs, 1999), but not all depressives display hypercortisolism; high endogenous glucocorticoid levels have been observed in depressive humans with anxiety (Gold et al., 1988). Furthermore, a positive correlation between hypercortisolism and increased noradrenaline levels in cerebrospinal fluid has been observed in patients suffering from major depression with melancholic features (hyperarousal, fear and anhedonia) (Wong et al., 2000).

Recently, Koob (1999) presented the feed-forward CRF–noradrenaline–CRF stress system, which might be involved in the production of an allostatic state (here, i.e., anxiety state). He described a feed-forward system whereby CRF, originating from the central amygdala, could activate brainstem noradrenergic activity in the locus coeruleus, which in turn activates forebrain CRF activity in the central amygdala and the bed nucleus stria terminalis, where noradrenaline stimulates CRF release (Koob, 1999). This view is confirmed by the findings that stress induces noradrenaline release in the central amygdala (Quirarte et al., 1998) and noradrenaline in the central amygdala stimulates release of CRF (Raber et al., 1995). Evidence that CRF originating from these noradrenaline terminal areas innervates the locus coeruleus region (Van Bockstaele et al., 1998, 1999, 2001) suggests that this last projection is effectively closing the loop, making it a powerful feed-forward system. According to Koob, this feed-forward CRF–noradrenaline–CRF stress system may be especially important for making behavioural adjustments in anticipation of changing demands (see Fig. 7). However, because such a feed-forward mechanism may be particularly vulnerable to dysfunction, it may lead to stress-sensitization and this may be the key to a variety of pathophysiologic conditions involving abnormal responses to stressors (Koob, 1999). Previously, it has been postulated that a regular production of such an enhanced anxiety state may be a key predispositional factor to anxiety disorders and depression (Korte, 2001).

There is general agreement that CRF, via CRF_1 receptors in the amygdala (Potter et al., 1994), regulates fear and anxiety (e.g., Koob et al., 1993; Heinrichs et al., 1995; Holsboer and Montkowski, 1995; Heinrichs et al., 1997a,b; Marchuk et al., 1998). This action may take place in the central amygdala and the extended amygdala, i.e., the bed nucleus of the stria terminalis (LeDoux, 1993, 1995; Lee and Davis, 1997). It has been suggested that activation of the central amygdala may mediate stimulus-specific (e.g., lights, tones, touch) fear, whereas somewhat less explicit information, such as that produced by exposure to a threatening environment for several minutes, may activate the bed nucleus of the stria terminalis (Davis, 1998). Because the nature of this information may be less specific than that produced by an explicit cue, as well as of much longer duration, activation of the bed nucleus of the stria terminalis

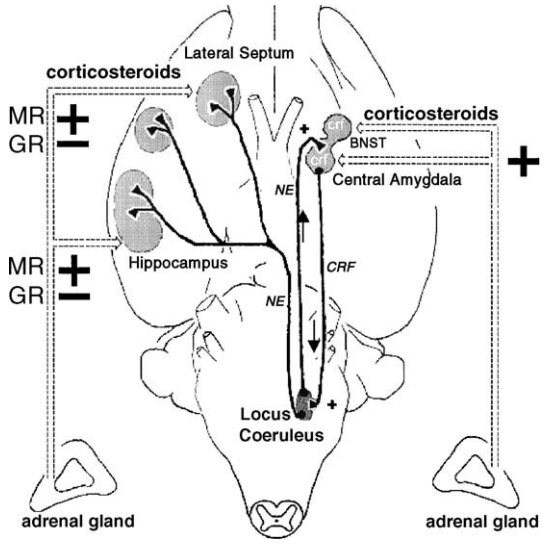

Fig. 7. Diagram illustrating the central role of corticosteroids and corticotropin-releasing factor (CRF) in the production of the enhanced anxiety state. There is a large body of evidence that stress and high levels of corticosteroids can facilitate the expression of CRF mRNA in the central amygdala and in the lateral part of the bed nucleus of the stria terminalis BNST. CRF via its binding to CRF_1 receptors in the central amygdala and bed nucleus of the stria terminalis may enhance anxiety. CRF containing projections, originating from the central amygdala, could activate brainstem noradrenergic (NE) activity in the locus coeruleus, which in turn activates forebrain CRF activity in central amygdala and the bed nucleus of the stria terminalis, where noradrenaline stimulates CRF release. CRF projections originating from these noradrenergic terminal areas innervate the locus coeruleus region again and close the loop. This feed-forward CRF–noradrenaline–CRF system might be involved in the production of an allostatic state (Koob, 1999) (here, i.e., anxiety state). Such a feed-forward mechanism may be particularly vulnerable to dysfunction and it may lead to stress sensitization and this may be the key to a variety of pathophysiologic conditions involving anxiety disorders. Previously, such a mechanism has been described for the serotonergic raphe nuclei (not shown; Korte, 2001). Corticosteroids not only affect emotional behaviour via the central amygdala and the bed nucleus of the stria terminalis but also via hippocampus and probably septum. Both brain nuclei possess corticosteroid receptors and CRF receptors. Previously, it has been suggested (De Kloet, 1991) that corticosteroids via mineralocorticoid receptors (MR) prime (+, i.e., higher neuronal excitability) the different elements of the stress response, and bring it to peaks of readiness for action (e.g., increased freezing and orientation, lower sensory detection thresholds, higher alertness) (Korte, 2001). Glucocorticoid receptor's (GR) role is especially important after stressor exposure because corticosteroids (suppress neuronal excitability) prevent the stress reactions from overshooting through their suppressive actions via glucocorticoid receptors, but glucocorticoid receptors also effect emotional behaviour. Previously, it was shown corticosteroids via hippocampal glucocorticoid receptors stimulate fear potentiation, contextual conditioning and consolidation of information (Korte, 2001) and hippocampal CRF_1 receptors mediate stress-induced enhancement of fear conditioning (Radulovic et al., 1999). It is quite well possible that corticosteroids via their mineralocorticoid receptor/glucocorticoid receptor balance influence the CRF/urocortin system. Urocortin II/III might act via CRF_2 receptors in the lateral septum to produce stress-induced fear potentiation, whereas urocortin II in other brain centres has a delayed anxiolytic-like action (Valdez et al., 2002). Thus, CRF, the urocortins and their receptors form a network in the brain involved in the acute phase (anxiogenic) as well as the recovery phase of the stress response (anxiolysis), which may be under the influence of corticosteroids.

may be more akin to anxiety than to fear (Davis, 1998). Infusion of CRF into the bed nucleus of the stria terminalis facilitates fear responses (Lee and Davis, 1997). In addition, CRF receptor antagonists administered into the central amygdala reduce several fear-related behavioural responses (Koob et al., 1993). Interestingly, it has been shown that "risk" seeking rats, which are less anxious in the Black–White box and elevated-plus maze, have lower basal levels of CRF mRNA in the central amygdala in contrast to their counterparts (Kabbaj et al., 2000). Oppositely, fawn-hooded rats exhibit more stress-induced fear (freezing) than Wistar rats and they have higher levels of CRF mRNA in the central amygdala (Altemus et al., 1994–1995).

Thus, it is quite well possible that CRF exerts its effects via the central amygdala because it has been shown that both $CRF_{1,2}$ receptor antagonists infused into the central amygdala (Heinrichs et al., 1992) and chronic infusion of CRF_1 receptor antisense into the central amygdala reduced the social defeat induced enhanced anxiety state in the plus-maze (Liebsch et al., 1995, 1999). There is some evidence that CRF also receptors in other brain areas are involved. For instance, CRF_1 receptors located in the dorsal hippocampus are involved in stress-induced associative (contextual) learning (Radulovic et al., 1999). However, CRF receptors in the paraventricular nucleus of the hypothalamus are unlikely to be involved in the present results because $CRF_{1,2}$ receptor antagonists administered into the paraventricular nucleus of the hypothalamus did not affect the enhanced anxiety state in socially defeated rats (Wotjak et al., 1996).

Recently, it has become clear that stress-induced behaviours require CRF receptors, but not necessarily CRF (Weninger et al., 1999). Recently, two new members of the neuropeptide CRF family have been discovered in the brain: urocortin II (also known as stresscopin-related peptide) and urocortin III (also known as stresscopin) (Hsu and Hsueh, 2001; Reul and Holsboer, 2002). Urocortin which binds with equally high affinity to both CRF_1 and CRF_2 receptors (Valdez et al., 2002). Intracerebroventricularly administered urocortin, like CRF, is able to enhance the anxiety state in the elevated plus-maze (Moreau et al., 1997; Spina et al., 2002; Rivier et al., 2002). Urocortin II binds highly specifically to the CRF_2 receptor (Valdez et al., 2002). It has been hypothesized that urocortin II via CRF_2 receptors exerts a compensatory coping mechanism to oppose the relatively short and fast CRF_1 receptor-mediated anxiogenic action because urocortin II increased open-arm exploration 4 h after intracerebroventricular injection, suggesting a delayed anxiolytic-like action (Valdez et al., 2002).

In contrast, the CRF_2 receptors have also been reported, as a result of site-specific brain (septum) actions, to be involved in anxiogenic-like actions on the elevated plus-maze because antagonism of the CRF_2 receptor by anti-sauvagine-30 produced an increase in open-arm exploration (Takahashi et al., 2001). Furthermore, it was shown that anti-sauvagine-30 injected in the lateral intermediate septum reduced the immobilization stress (1 h) induced enhanced anxiety state, suggesting that septal CRF_2 receptors are involved in the production of a stress-induced fear potentiation (Radulovic et al., 1999). Interestingly, several major urocortin III terminal fields have been identified, including the lateral septum, which is known to express high levels of CRF_2 receptors (Li et al., 2002). Thus, it is suggested that urocortin III is also an endogenous ligand for CRF_2 receptors in these areas. Thus, CRF, the urocortins and their receptors form a network in the brain involved in the acute phase (anxiogenic) as well as the recovery phase of the stress response (anxiolysis).

7.3. Corticosteroid–CRF interactions in relation to fear potentiation

Under healthy conditions, corticosteroids mediate behavioural adaptation via central mineralocorticoid receptor and glucocorticoid receptor mechanisms (Korte, 2001) It is important to realize that corticosteroids, in contrast to CRF(-like) neuropeptides, do not regulate emotional behaviour and physiology; rather, they induce chemical changes in particular sets of neurons, making certain behavioural and physiological outcomes more likely in a certain context in time, as a result of the strengthening or weakening of particular neural pathways (McEwen, 1993; De Kloet et al., 1994; Sapolsky et al., 2000; Korte, 2001). For instance, after a threat has disappeared and the limbic–hypothalamic–pituitary–adrenal axis is activated, brain glucocorticoid receptor mechanisms (especially hippocampal glucocorticoid receptors) promote processes underlying contextual fear conditioning and consolidation of acquired information (Sandi, 1998; Cordero and Sandi, 1998; Cordero et al., 1998; Roozendaal, 2000; Korte, 2001) (e.g., the predator's appearance, smell and sound, location) and such memories are helpful to predict the occurrence and nature of the next encounter and thereby maximize the likelihood of survival (by "fight or flight"). When visiting the prior dangerous location, the animal's first reaction is conditioned freezing behaviour, which is made possible by a permissive mineralocorticoid receptor action. When the environment is save (e.g., no predators present), extinction of passive avoidance via a mineralocorticoid receptor mechanism (Bohus and De Kloet, 1981; Bohus et al., 1982; Bohus, 1987; Korte, 2001) and extinction of active avoidance via a brain glucocorticoid receptor mechanism (Bohus, 1970) will take place. It is speculated, however, that if the stressor is strong and the extinction period is too short (in the absence of social support, i.e., isolated animals), some individuals may become more sensitive to stressors. Increased plasma corticosteroid steroid levels, probably via brain glucocorticoid receptor mechanisms, promote these processes underlying fear potentiation (Korte, 2001). Here, we suggest that the above-described mechanisms can be observed after inescapable stress, social defeat and predator stress (see also Section 5).

The underlying mechanisms of allostatic state and allostatic load are complex, and there is a fine line between adaptation and psychopathology. Previously, it has been shown that mineralocorticoid receptors rather than glucocorticoid receptors are reduced in number when corticosteroid levels are elevated (Lopez et al., 1998; Herman and Spencer, 1998). It has been shown that down-regulation of the glucocorticoid receptor requires extensive and prolonged exposure to extremely high levels of corticosteroids (Spencer et al., 1991). In contrast, the mineralocorticoid receptor may rather easily tonically inhibit the glucocorticoid receptor biosynthesis in dorsal hippocampus (Herman and Spencer, 1998) by way of binding to glucocorticoid response elements present in the glucocorticoid receptor promotor region (Arriza et al., 1988). Thus, due to down-regulation of the mineralocorticoid receptors after stress, as a consequence, glucocorticoid receptor numbers may increase. The initial mineralocorticoid receptor down-regulation and up-regulation may be functional. Corticosteroids via mineralocorticoid receptors prime the different elements of the stress response, and bring it to peaks of readiness for action (e.g., increased freezing and orientation, lower sensory detection thresholds, higher alertness), which is of no longer use after stressor exposure (Korte, 2001). Glucocorticoid receptor's role is especially important after stressor exposure because corticosteroids prevent the stress reactions from overshooting through their suppressive actions via glucocorticoid receptors (e.g., higher sensory detection thresholds). After prolonged (acute traumatic) stress, mineralocorticoid receptors are down-regulated, whereas glucocorticoid receptors are increased in number. Similar changes can be observed in many patients with posttraumatic stress disorder (Yehudam et al., 1993). However, it has been postulated that a reduction in the population of mineralocorticoid receptors presents a risk of reduced fear extinction, whereas elevated numbers of glucocorticoid receptors presents a risk of increased contextual fear conditioning, strong consolidation of traumatic memories and increased fear potentiation (Korte, 2001).

Chronic psychosocial stress and prenatal/postnatal stress down-regulate the number of central mineralocorticoid receptors and glucocorticoid receptors, which may sometimes result in, respectively, elevated baseline plasma corticosteroid levels (due to decreased mineralocorticoid receptor function) and increased stress corticosteroid levels that remain high longer after stress (due to decreased glucocorticoid receptor function: feedback resistance) (Ratka et al., 1989).

We were the first to show that glucocorticoid receptor antagonists reduced the enhanced anxiety state, produced by prior stress exposure, as measured in the elevated plus-maze (Korte et al., 1995). There is some evidence that mineralocorticoid receptors may also be involved (Korte et al., 1995, 1996b; Calvo and Volosin, 2001). In the present review, we present new data (Fig. 6) of a more specific glucocorticoid receptor antagonist (ORG34580) that confirm our earlier findings of glucocorticoid receptor involvement. The reviewed data strongly suggest that elevated levels of corticosterone, as measured earlier by Korte et al. (1992a), act via brain glucocorticoid receptors to play an important role in fear potentiation. In agreement with this assumption, it was shown that administration of corticosterone, at a dose that mimics stress-induced physiological levels, reduced open-arm exploration in the elevated plus-maze 24 h later, thus, suggesting that corticosterone produces a long-term enhanced anxiety state (Calvo et al., 1998). Additional support comes from the finding that in rats high (but not low) doses of corticosterone potentiate freezing behaviour to an explicit auditory cue that had been previously been paired with footshock (Coromidas et al., 1994). It has been suggested that an enhanced anxiety state reflects an increased anxiogenic influence of both CRF projections from the amygdala to the locus coeruleus and the ascending noradrenaline projections from the locus coeruleus to forebrain structures (Caldji et al., 1998). Interestingly, it has been suggested that the Barrington's nucleus (micturition centre), the central amygdala and the bed nucleus of the stria terminalis are glucocorticoid-sensitive sources of CRF that can influence the locus coeruleus–noradrenergic system (Lechner and Valentino, 1999).

In the beginning of the 1990s, we found that adrenalectomised rats showed a reduced behavioural stress response to intracerebroventricular CRF injection, which was normalized after corticosterone replacement (Korte et al., 1992b). These data suggested that corticosteroids are involved in the CRF-induced behavioural stress response. Schulkin et al. (1994, 1998) and Schulkin (1999) nicely reviewed that corticosteroids may stimulate CRF action to enhance fear. This hypothesis is further supported by the finding that chronic injection of corticosterone augmented CRF-enhanced startle using a dose of CRF (0.25 μg) that normally is ineffective in increasing startle amplitude (Lee et al., 1994). It has been shown that in the central amygdala, the majority of CRF-containing neurons also contain glucocorticoid receptors (Honkaniemi et al., 1992).

There is a large body of evidence that stress and high levels of corticosteroids can facilitate the expression of CRF mRNA in the hypothalamic paraventricular nucleus , central amygdala and in the lateral part of the bed nucleus of the stria terminalis (see Fig. 7) and Barrington's nucleus (Swanson and Simmons, 1989; Imaki et al., 1991; Makino et al., 1994a,b, 1995, 2002; Watts and Sanchez-Watts, 1995; Palkovits et al., 1998). This effect was positively correlated with the dose of corticosterone and negatively correlated with thymus weight suggesting the involvement of a glucocorticoid receptor mechanism (Watts and Sanchez-Watts, 1995). Rapid increases in CRF mRNA levels have also been observed in the rostral central amygdala region after acute restraint stress (Kalin et al., 1994; Hsu et al., 1998). It is known that restraint stress increases extracellular levels of CRF-like immunoreactivity in the amygdala, as measured by microdialysis in freely moving rats (Pich et al., 1995).

Recently, however, it has become clear that not only CRF in the amygdala system is involved, but also septal CRF_2 receptors mediate stress-induced anxiety, whereas hippocampal CRF_1 receptors mediate stress-induced enhancement of fear conditioning (Radulovic et al., 1999).

It is quite interesting that in the brain centres, which are involved in the modulation of emotions) including emotional memories and mood, large quantities of both mineralocorticoid receptor and glucocorticoid receptor have been observed (Reul and De Kloet, 1985; De Kloet, 1991; De Kloet et al., 1998, 1999). As a consequence, corticosteroids not only affect emotional behaviour via the central amygdala and the bed nucleus of the stria terminalis but also via hippocampus and probably septum. Both brain nuclei also possess corticosteroid receptors and CRF receptors (Radulovic et al., 1999). For instance, recently, it was shown that a low level of hippocampal glucocorticoid receptor expression in rats was responsible for decreased anxiety in exploring novelty (Kabbaj et al., 2000). In addition, in our hands, glucocorticoid receptor antisense infused into the dorsal hippocampus reduced immobility-floating behaviour in the porsolt swim test (Korte et al., 1996).

Recently, it was shown that corticosterone implants (for 7 days) produced an enhanced anxiety state in the elevated plus-maze and produced a concomitant increase in both basal level of CRF mRNA per neuron and the number of neurons with CRF hybridization signal in the central amygdala (Shepard et al., 2000). These findings support the idea that chronically elevated glucocorticoids may increase anxiety by inducing CRF expression in the central amygdala. In agreement, it was shown that stereotaxic delivery of corticosterone to the central amygdala again produces fear potentiation in the elevated plus-maze, but also a colonic hypersensitivity, probably via descending neuronal pathways from the central amygdala (Greenwood-Van Meerveld et al., 2001).

8. Seven reasons why fear-potentiated plus-maze behaviour should be used

The present review clearly shows the many advantages of the use of fear-potentiated plus-maze behaviour as a valuable measure in the understanding of neural mechanisms involved in state anxiety and in the search for novel anxiolytics.

- In contrast to the normal elevated plus-maze, which measures innate fear of open spaces, fear-potentiated plus-maze behaviour reflects an enhanced anxiety state (allostatic state).
- Depending on the stressor used (e.g., fear of shock; predator odour, swim stress, restraint, social defeat, predator stress (cat)), this enhanced anxiety state can last from 90 min to 3 weeks. Stress effects are more severe when rats are isolated in comparison to group housing.
- Fear-potentiated plus-maze behaviour is sensitive to proven/putative anxiolytics and anxiogenics which act via mechanisms related to the benzodiazepine–gamma-aminobutyric acid receptor complex, but it is also sensitive to CRF receptor antagonists and glucocorticoid receptor antagonists and serotonin receptor agonists/antagonists (high predictive validity).
- Drugs can be administered in the absence of the original stressor and after stressor exposure. As a consequence, retrieval mechanisms are not affected by drug treatment.
- Fear-potentiated plus-maze behaviour is very robust and experiments can easily be replicated in other labs.
- Neural mechanisms involved in contextual fear conditioning, fear potentiation and state anxiety can be studied.
- Fear-potentiated plus-maze behaviour can be measured both in males and females.

References

Adamec, R.E., McKay, D., 1993. Amygdala kindling, anxiety, and corticotrophin releasing factor (CRF). Physiol. Behav. 54, 423–431.

Adamec, R.E., Shallow, T., 1993. Lasting effects on rodent anxiety of a single exposure to a cat. Physiol. Behav. 54, 101–109.

Altemus, M., Smith, M.A., Diep, V., Aulakh, C.S., Murphy, D.L., 1994–1995. Increased mRNA for corticotrophin releasing hormone in the amygdala of fawn-hooded rats, a potential animal model of anxiety. Anxiety 1, 251–257.

Andersen, I.L., Boe, K.E., Foerevik, G., Janczak, A.M., Bakken, M., 2000. Behavioural evaluation of methods for assessing fear responses in weaned pigs. Appl. Anim. Behav. Sci. 69, 227–240.

Arriza, J.L., Simerly, R.B., Swanson, L.W., Evans, R.M., 1988. The neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. Neuron 1, 887–900.

Baldwin, H.A., Rassnick, S., Rivier, J., Koob, G.F., Britton, K.T., 1991. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. Psychopharmacology (Berl.) 103, 227–232.

Bohus, B., 1970. Central nervous structures and the effect of ACTH and corticosteroids on avoidance behaviour, a study with intracerebral implantation of corticosteroids in the rat. Prog. Brain Res. 32, 171–184.

Bohus, B., 1987. Limbic–midbrain mechanisms and behavioral physiology interactions with CRF, ACTH and adrenal hormones. In: Hellhammer, D., Florin, I., Weiner, H. (Eds.), Neurobiological Approaches to Human Disease. Hans Huber Publishers, Toronto, pp. 267–285.

Bohus, B., De Kloet, E.R., 1981. Adrenal steroids and extinction behaviour, antagonism by progesterone, deoxycorticosterone and dexamethasone of a specific effect of corticosterone. Life Sci. 28, 433–440.

Bohus, B., De Kloet, E.R., Veldhuis, H.D., 1982. Adrenal steroids and behavioral adaptation, relationship to brain corticoid receptors. In: Ganten, D., Pfaff, D. (Eds.), Adrenal Actions on Brain. Springer-Verlag, Berlin, pp. 107–148.

Buwalda, B., de Boer, S.F., Van Kalkeren, A.A., Koolhaas, J.M., 1997. Physiological and behavioral effects of chronic intracerebroventricular infusion of corticotropin-releasing factor in the rat. Psychoneuroendocrinology 22, 297–309.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M., Meaney, M.J., 1998. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proc. Natl. Acad. Sci. 95, 5335–5340.

Calvo, N., Volosin, M., 2001. Glucocorticoid and mineralocorticoid recep-

tors are involved in the facilitation of anxiety-like response induced by restraint. Neuroendocrinology 73, 261–271.
Calvo, N., Martijena, I.D., Molina, V.A., Volosin, M., 1998. Metyrapone pretreatment prevents the behavioral and neurochemical sequelae induced by stress. Brain Res. 800, 227–235.
Cole, B.J., Hillmann, M., Seidelmann, D., Klewer, M., Jones, G.H., 1995. Effects of benzodiazepine receptor partial inverse agonists in the elevated plus maze test of anxiety in the rat. Psychopharmacology (Berl.) 121, 118–126.
Contarino, A., Dellu, F., Koob, G.F., Smith, G.W., Lee, K.F., Vale, W., Gold, L.H., 1999. Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. Brain Res. 835, 1–9.
Cordero, M.I., Sandi, C., 1998. A role for brain glucocorticoid receptors in contextual fear-conditioning, dependence upon training intensity. Brain Res. 786, 11–17.
Cordero, M.I., Merino, J.J., Sandi, C., 1998. Correlational relationship between shock intensity and corticosterone secretion on the establishment and subsequent expression of contextual fear-conditioning. Behav. Neurosci. 112, 885–891.
Coromidas, K.P., LeDoux, J.E., Gold, P.W., Schulkin, J., 1994. Corticosterone potentiation of conditioned fear in rats. Ann. N.Y. Acad. Sci. 746, 392–393.
Crabbe, J.C., Wahlsten, D., Dudek, B.C., 1999. Genetics of mouse behavior, interactions with laboratory environment. Science 284, 1670–1672.
Davis, M., 1998. Are different parts of the extended amygdala involved in fear versus anxiety? Biol. Psychiatry 44, 1239–1247.
De Kloet, E.R., 1991. Brain corticosteroid receptor balance and homeostatic control. Front Neuroendocrinol. 12, 95–164.
De Kloet, E.R., Rots, N.Y., van den Berg, D.T., Oitzl, M.S., 1994. Brain mineralocorticoid receptor function. Ann. N.Y. Acad. Sci. 746, 20–21.
De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S., Joels, M., 1998. Brain corticosteroid receptor balance in health and disease. Endocr. Rev. 19, 269–301.
De Kloet, E.R., Oitzl, M.S., Joels, M., 1999. Stress and cognition, are corticosteroids good or bad guys? Trends Neurosci. 22, 422–426.
Falter, U., Gower, A.J., Gobert, J., 1992. Resistance of baseline activity in the elevated plus-maze to exogenous influences. Behav. Pharmacol. 3, 123–128.
Fernandes, C., File, S.E., 1996. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol. Biochem. Behav. 54, 31–40.
Gold, P.W., Goodwin, F.K., Chrousos, G.P., 1988. Clinical and biochemical manifestations of depression. Relation to the neurobiology of stress. N. Engl. J. Med. 319, 413–420.
Goldstein, D.S., McEwen, B., 2002. Allostasis, homeostats, and the nature of stress. Stress 5, 55–58.
Grahn, R.E., Kalman, B.A., Brennan, F.X., Watkins, L.R., Maier, S.F., 1995. The elevated plus-maze is not sensitive to the effect of stressor controllability in rats. Pharmacol. Biochem. Behav. 52, 565–570.
Greenwood-Van Meerveld, B., Gibson, M., Gunter, W., Shepard, J., Foreman, R., Myers, D., 2001. Stereotaxic delivery of corticosterone to the amygdala modulates colonic sensitivity in rats. Brain Res. 893, 135–142.
Heinrichs, S.C., Pich, E.M., Miczek, K.A., Britton, K.T., Koob, G.F., 1992. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res. 581, 190–197.
Heinrichs, S.C., Menzaghi, F., Pich, E.M., Baldwin, H.A., Rassnick, S., Britton, K.T., Koob, G.F., 1994. Anti-stress action of a corticotropin-releasing factor antagonist on behavioral reactivity to stressors of varying type and intensity. Neuropsychopharmacology 11, 179–186.
Heinrichs, S.C., Menzaghi, F., Pich, E.M., Britton, K.T., Koob, G.F., 1995. The role of CRF in behavioral aspects of stress. Ann. N.Y. Acad. Sci. 771, 92–104.
Heinrichs, S.C., Lapsansky, J., Lovenberg, T.W., De Souza, E.B., Chalmers, T., 1997a. Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior. Regul. Pept. 71, 15–21.
Heinrichs, S.C., Min, H., Tamraz, S., Carmouche, M., Boehme, S.A., Vale, W.W., 1997b. Anti-sexual and anxiogenic behavioral consequences of corticotropin-releasing factor overexpression are centrally mediated. Psychoneuroendocrinology 22, 215–224.
Heinrichs, S.C., De Souza, E.B., Schulteis, G., Lapsansky, J.L., Grigoriadis, D.E., 2002. Brain penetrance, receptor occupancy and antistress in vivo efficacy of a small molecule corticotropin releasing factor type I receptor selective antagonist. Neuropsychopharmacology 27, 194–202.
Herman, J.P., Spencer, R., 1998. Regulation of hippocampal glucocorticoid receptor gene transcription and protein expression in vivo. J. Neurosci. 18, 7462–7473.
Hogg, S., 1996. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. Pharmacol. Biochem. Behav. 54, 21–30.
Holsboer, F., Montkowski, A., 1995. Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. Regul. Pept. 59, 229–239.
Honkaniemi, J., Pelto-Huikko, M., Rechardt, L., Isola, J., Lammi, A., Fuxe, K., Gustafsson, J.A., Wikstrom, A.C., Hokfelt, T., 1992. Colocalization of peptide and glucocorticoid receptor immunoreactivities in rat central amygdaloid nucleus. Neuroendocrinology 55, 451–459.
Hsu, S.Y., Hsueh, A.J., 2001. Human stresscopin and stresscopin-related peptide are selective ligands for the type 2 corticotropin-releasing hormone receptor. Nat. Med. 7, 605–611.
Hsu, D.T., Chen, F.L., Takahashi, L.K., Kalin, N.H., 1998. Rapid stress-induced elevations in corticotropin-releasing hormone mRNA in rat central amygdala nucleus and hypothalamic paraventricular nucleus, an in situ hybridization analysis. Brain Res. 788, 305–310.
Imaki, T., Nahan, J.L., Rivier, C., Sawchenko, P.E., Vale, W., 1991. Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. J. Neurosci. 11, 585–599.
Kabbaj, M., Devine, D.P., Savage, V.R., Akil, H.J., 2000. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat, differential expression of stress-related molecules. Neuroscience 20, 6983–6988.
Kalin, N.H., Takahashi, L.K., Chen, F.L., 1994. Restraint stress increases corticotropin-releasing hormone mRNA content in the amygdala and paraventricular nucleus. Brain Res. 656, 182–186.
Koob, G.F., 1999. Corticotropin-releasing factor, norepinephrine, and stress. Biol. Psychiatry 46, 1167–1180.
Koob, G.F., Heinrichs, S.C., 1999. A role for corticotropin releasing factor and urocortin in behavioral responses to stressors. Brain Res. 848, 141–145.
Koob, G.F., Le Moal, M., 2001. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24, 97–129.
Koob, G.F., Heinrichs, S.C., Pich, E.M., Menzaghi, F., Baldwin, H., Miczek, K., Britton, K.T., 1993. The role of corticotropin-releasing factor in behavioural responses to stress. Ciba Found. Symp. 172, 277–289.
Koolhaas, J.M., De Boer, S.F., De Ruiter, A.J., Meerlo, P., Sgoifo, A., 1997. Social stress in rats and mice. Acta Physiol. Scand. 640, 69–72 (Suppl.).
Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A., Blokhuis, H.J., 1999. Coping styles in animals, current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935.
Korte, S.M., 2001. Corticosteroids in relation to fear, anxiety and psychopathology. Neurosci. Biobehav. Rev. 25, 117–142.
Korte, S.M., Buwalda, B., Bouws, G.A., Koolhaas, J.M., Maes, F.W., Bohus, B., 1992a. Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioral passivity and activity in aged and in young rats. Physiol. Behav. 51, 815–822.
Korte, S.M., Eisinga, W., Timmerman, W., Nyakas, C., Bohus, B., 1992b. Behavioral and cardiac responses after intracerebroventricular cortico-

tropin-releasing hormone (CRH) administration, role of adrenal cortical hormones. Horm. Behav. 26, 375–384.
Korte, S.M., Bouws, G.A., Bohus, B., 1992c. Adrenal hormones in rats before and after stress-experience: effects of ipsapirone. Physiol. Behav. 51, 1129–1133.
Korte, S.M., de Boer, S.F., de Kloet, E.R., Bohus, B., 1995. Anxiolytic-like effects of selective mineralocorticoid and glucocorticoid antagonists on fear-enhanced behavior in the elevated plus-maze. Psychoneuroendocrinology 20, 385–394.
Korte, S.M., De Kloet, E.R., Buwalda, B., Bouman, S.D., Bohus, B., 1996. Antisense to the glucocorticoid receptor in hippocampal dentate gyrus reduces immobility in forced swim test. Eur. J. Pharmacol. 301, 19–25.
Korte, S.M., De Boer, S.F., Bohus, B., 1999. Fear-potentiation in the elevated plus-maze test depends on stressor controllability and fear conditioning. Stress 3, 27–40.
Lechner, S.M., Valentino, R.J., 1999. Glucocorticoid receptor-immunoreactivity in corticotrophin-releasing factor afferents to the locus coeruleus. Brain Res. 816, 17–28.
LeDoux, J.E., 1993. Emotional memory systems in the brain. Behav. Brain Res. 58, 69–79.
LeDoux, J.E., 1995. Emotion, clues from the brain. Annu. Rev. Psychol. 46, 209–235.
Lee, Y., Davis, M., 1997. Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. J. Neurosci. 17, 6434–6446.
Lee, Y., Schulkin, J., Davis, M., 1994. Effect of corticosterone on the enhancement of the acoustic startle reflex by corticotropin releasing factor (CRF). Brain Res. 12, 93–98.
Li, C., Vaughan, J., Sawchenko, P.E., Vale, W.W., 2002. Urocortin III-immunoreactive projections in rat brain, partial overlap with sites of type 2 corticotrophin-releasing factor receptor expression. J. Neurosci. 1, 991–1001.
Liebsch, G., Landgraf, R., Gerstberger, R., Probst, J.C., Wotjak, C.T., Engelmann, M., Holsboer, F., Montkowski, A., 1995. Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. Regul. Pept. 59, 229–239.
Liebsch, G., Landgraf, R., Engelmann, M., Lorscher, P., Holsboer, F., 1999. Differential behavioural effects of chronic infusion of CRH1 and CRH2 receptor antisense oligonucleotides into the rat brain. J. Psychiatr. Res. 33, 153–163.
Lopez, J.F., Chalmers, D.T., Little, K.Y., Watson, S.J., 1998. A.E. Bennett Research Award. Regulation of serotonin1A, glucocorticoid, and mineralocorticoid receptor in rat and human hippocampus, implications for the neurobiology of depression. Biol. Psychiatry 43, 547–573.
Maisonnette, S., Morato, S., Brandao, M.L., 1993. Role of resocialization and of 5-HT1A receptor activation on the anxiogenic effects induced by isolation in the elevated plus-maze test. Physiol. Behav. 54, 753–758.
Makino, S., Gold, P.W., Schulkin, J., 1994a. Corticosterone effects on corticotropin-releasing hormone mRNA in the central nucleus of the amygdala and the parvocellular region of the paraventricular nucleus of the hypothalamus. Brain Res. 640, 105–112.
Makino, S., Gold, P.W., Schulkin, J., 1994b. Effects of corticosterone on CRH mRNA and content in the bed nucleus of the stria terminalis, comparison with the effects in the central nucleus of the amygdala and the paraventricular nucleus of the hypothalamus. Brain Res. 657, 141–149.
Makino, S., Schulkin, J., Smith, M.A., Pacak, K., Palkovits, M., Gold, P.W., 1995. Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. Endocrinology 136, 4517–4525.
Makino, S., Hashimoto, K., Gold, P.W., 2002. Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. Pharmacol. Biochem. Behav. 73, 147–158.
Marchuk, Y., Hauser, C., Bentley, C.A., Sawchenko, P.E., Koob, G.F., Vale, W., Lee, K.F., 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20, 1093–1102.
Martijena, I.D., Calvo, N., Volosin, M., Molina, V.A., 1997. Prior exposure to a brief restraint session facilitates the occurrence of fear in response to a conflict situation: behavioral and neurochemical correlates. Brain Res. 752, 136–142.
McEwen, B.S., 1993. Endocrine effects on the brain and their relationship to behavior. In: Siegel, S.J. (Ed.), Basic Neurochemistry, Molecular, Cellular, and Medical Aspects. Raven Press, New York, pp. 1003–1023.
McEwen, B.S., 1998. Stress, adaptation, and disease. Allostasis and allostatic load. Ann. N.Y. Acad. Sci. 840, 33–44.
McEwen, B.S., 2000. Allostasis and allostatic load, implications for neuropsychopharmacology. Neuropsychopharmacology 22, 108–124.
McEwen, B.S., Stellar, E., 1993. Stress and the individual. Mechanisms leading to disease. Arch. Intern. Med. 153, 2093–2101.
Mendonca, F.H., Guimaraes, F.S., 1998. Intra-hippocampal administration of cycloheximide attenuates the restraint-induced exploratory deficit of an elevated plus maze. Behav. Brain Res. 91, 207–211.
Menzaghi, F., Howard, R.L., Heinrichs, S.C., Vale, W., Rivier, J., Koob, G.F., 1994. Characterization of a novel and potent corticotropin-releasing factor antagonist in rats. J. Pharmacol. Exp. Ther. 269, 564–572.
Moreau, J.L., Kilpatrick, G., Jenck, F., 1997. Urocortin, a novel neuropeptide with anxiogenic-like properties. NeuroReport 8, 1697–1701.
Nemeroff, C.B., Widerlov, E., Bissette, G., Walleus, H., Karlsson, I., Eklund, K., Kilts, C.D., Loosen, P.T., Vale, W., 1984. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science 226, 1342–1344.
Palkovits, M., Young III, W.S., Kovacs, K., Toth, Z.S., Makara, G.B., 1998. Alterations in corticotropin-releasing hormone gene expression of central amygdaloid neurons following long-term paraventricular lesions and adrenalectomy. Neuroscience 85, 1351–1447.
Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open, closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.
Pich, E.M., Lorang, M., Yeganeh, M., Rodriguez de Fonseca, F., Raber, J., Koob, G.F., Weiss, F., 1995. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J. Neurosci. 15, 5439–5447.
Potter, E., Sutton, S., Donaldson, C., Chen, R., Perrin, M., Lewis, K., Sawchenko, P.E., Vale, W., 1994. Distribution of corticotropin-releasing factor receptor mRNA expression in therat brain and pituitary. Proc. Natl. Acad. Sci. 91, 8777–8781.
Quirarte, G.L., Galvez, R., Roozendaal, B., McGaugh, J.L., 1998. Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. Brain Res. 808, 134–140.
Raber, J., Koob, G.F., Bloom, F.E., 1995. Interleukin-2 (IL-2) induces corticotropin-releasing factor (CRF) release from the amygdala and involves a nitric oxide-mediated signaling, comparison with the hypothalamic response. J. Pharmacol. Exp. Ther. 272, 815–824.
Radulovic, J., Ruhmann, A., Liepold, T., Spiess, J., 1999. Modulation of learning and anxiety by corticotropin-releasing factor (CRF) and stress, differential roles of CRF receptors 1 and 2. J. Neurosci. 19, 5016–5025.
Ratka, A., Sutanto, W., Bloemers, M., De Kloet, E.R., 1989. On the role of brain mineralocorticoid (type I) and glucocorticoid (type II) receptors in neuroendocrine regulation. Neuroendocrinology 50, 117–123.
Reul, J.M.H.M., De Kloet, E.R., 1985. Two receptor systems for corticosterone in rat brain, microdistribution and differential occupation. Endocrinology 117, 2505–2511.
Reul, J.M., Holsboer, F., 2002. Corticotropin-releasing factor receptors 1 and 2 in anxiety and depression. Curr. Opin. Pharmacol. 2, 23–33.
Rivier, J., Vale, W., Koob, F., 2002. Time-dependent induction of anxiogenic-like effects after central infusion of urocortin or corticotropin-releasing factor in the rat. Psychopharmacology (Berl.) 160, 113–121.
Rodgers, R.J., Cole, J.C., 1993. Anxiety enhancement in the murine ele-

vated plus maze by immediate prior exposure to social stressors. Physiol. Behav. 53, 383–388.
Rodgers, R.J., Dalvi, A., 1997. Anxiety, defence and the elevated plus-maze. Neurosci. Biobehav. Rev. 21, 801–810.
Roozendaal, B., 2000. 1999 Curt P. Richter Award. Glucocorticoids and the regulation of memory consolidation. Psychoneuroendocrinology 25, 213–238.
Roozendaal, B., Koolhaas, J.M., Bohus, B., 1991. Attenuated cardiovascular, neuroendocrine, and behavioral responses after a single footshock in central amygdaloid lesioned male rats. Physiol. Behav. 50, 771–775.
Roozendaal, B., Bohus, B., McGaugh, J.L., 1996. Dose-dependent suppression of adrenocortical activity with metyrapone, effects on emotion and memory. Psychoneuroendocrinology 21, 681–693.
Ruis, M.A., te Brake, J.H., Buwalda, B., De Boer, S.F., Meerlo, P., Korte, S.M., Blokhuis, H.J., Koolhaas, J.M., 1999. Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. Psychoneuroendocrinology 24, 285–300.
Sandi, C., 1998. The role and mechanisms of action of glucocorticoid involvement in memory storage. Neural Plast. 6, 41–52.
Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.
Schulkin, J., 1999. Corticotropin-releasing hormone signals adversity in both the placenta and the brain, regulation by glucocorticoids and allostatic overload. J. Endocrinol. 161, 349–356.
Schulkin, J., McEwen, B.S., Gold, P.W., 1994. Allostasis, amygdala, and anticipatory angst. Neurosci. Biobehav. Rev. 18, 385–396.
Schulkin, J., Gold, P.W., McEwen, B.S., 1998. Induction of corticotropin-releasing hormone gene expression by glucocorticoids, implication for understanding the states of fear and anxiety and allostaticload. Psychoneuroendocrinology 23, 219–243.
Shepard, J.D., Barron, K.W., Myers, D.A., 2000. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. Brain Res. 861, 288–295.
Skutella, T., Probst, J.C., Renner, U., Holsboer, F., Behl, C., 1998. Corticotropin-releasing hormone receptor (type I) antisense targeting reduces anxiety. Neuroscience 85, 795–805.
Smith, G.W., Aubry, J.M., Dellu, F., Contarino, A., Bilezikjian, L.M., Gold, L.H., Chen, R., Marchuk, Y., Hauser, C., Bentley, C.A., Sawchenko, P.E., Koob, G.F., Vale, W., Lee, K.F., 1998. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. Neuron 20, 1093–1102.
Spencer, R.L., Miller, A.H., Stein, M., McEwen, B.S., 1991. Corticosterone regulation of type I and type II adrenal steroid receptors in brain, pituitary, and immune tissue. Brain Res. 549, 236–246.
Spina, G., Merlo-Pich, E., Akwa, Y., Balducci, C., Basso, M., Zorrilla, P., Britton, T., Rivier, J., Vale, W., Koob, G.F., 2002. Time-dependent induction of anxiogenic-like effects after central infusion of urocortin or corticotropin-releasing factor in the rat. Psychopharmacology (Berl.) 160, 113–121.
Steenbergen, H.L., Farabollini, F., Heinsbroek, R.P., Van de Poll, N.E., 1991. Sex-dependent effects of aversive stimulation on holeboard and elevated plus-maze behavior. Behav. Brain Res. 43, 159–165.
Stenzel-Poore, M.P., Heinrichs, S.C., Rivest, S., Koob, G.F., Vale, W.W., 1994. Overproduction of corticotropin-releasing factor in transgenic mice, a genetic model of anxiogenic behavior. J. Neurosci. 14, 2579–2584.
Sterling, P., Eyer, J., 1988. Allostasis, a new paradigm to explain arousal pathology. In: Fisher, S., Reason, J. (Eds.), Handbook of Life Stress, Cognition and Health. Wiley, Chichester, pp. 629–649.
Swanson, L.W., Simmons, D.M., 1989. Differential steroid hormone and neural influences on peptide mRNA levels in CRH cells of the paraventricular nucleus, a hybridization histochemical study in the rat. J. Comp. Neurol. 285, 413–435.
Takahashi, L.K., Ho, S.P., Livanov, V., Graciani, N., Arneric, S.P., 2001. Antagonism of CRF(2) receptors produces anxiolytic behavior in animal models of anxiety. Brain Res. 902, 135–142.
Treit, D., Menard, J., Royan, C., 1993. Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44, 463–469.
Valdez, G.R., Inoue, K., Koob, G.F., Rivier, J., Vale, W., Zorrilla, E.P., 2002. Human urocortin II, mild locomotor suppressive and delayed anxiolytic-like effects of a novel corticotropin-releasing factor related peptide. Brain Res. 943, 142–150.
Van Bockstaele, E.J., Colago, E.E., Valentino, R.J., 1998. Amygdaloid corticotropin-releasing factor targets locus coeruleus dendrites, substrate for the co-ordination of emotional and cognitive limbs of the stress response. J. Neuroendocrinol. 10, 743–757.
Van Bockstaele, E.J., Peoples, J., Valentino, R.J., 1999. A.E. Bennett Research Award. Anatomic basis for differential regulation of the rostrolateral peri-locus coeruleus region by limbic afferents. Biol. Psychiatry 46, 1352–1363.
Van Bockstaele, E.J., Bajic, D., Proudfit, H., Valentino, R.J., 2001. Topographic architecture of stress-related pathways targeting the noradrenergic locus coeruleus. Physiol. Behav. 73, 273–283.
Watts, A.G., Sanchez-Watts, G., 1995. Region-specific regulation of neuropeptide mRNAs in rat limbic forebrain neurones by aldosterone and corticosterone. J. Physiol. 484, 721–736.
Weiss, J.M., 1968. Effects of coping responses on stress. J. Comp. Physiol. Psychol. 65, 251–260.
Weninger, S.C., Dunn, A.J., Muglia, L.J., Dikkes, P., Miczek, K.A., Swiergiel, A.H., Berridge, C.W., Majzoub, J.A., 1999. Stress-induced behaviors require the corticotropin-releasing hormone (CRH) receptor, but not CRH. Proc. Natl. Acad. Sci. 96, 8283–8288.
Wong, M.L., Kling, M.A., Munson, P.J., Listwak, S., Licinio, J., Prolo, P., Karp, B., McCutcheon, I.E. , Geracioti Jr., T.D., DeBellis, M.D., Rice, K.C., Goldstein, D.S., Veldhuis, J.D., Chrousos, G.P., Oldfield, E.H., McCann, S.M., Gold, P.W., 2000. Pronounced and sustained central hypernoradrenergic function in major depression with melancholic features, relation to hypercortisolism and corticotropin-releasing hormone. Proc. Natl. Acad. Sci. 97, 325–330.
Wotjak, C.T., Kubota, M., Liebsch, G., Montkowski, A., Holsboer, F., Neumann, I., Landgraf, R., 1996. Release of vasopressin within the rat paraventricular nucleus in response to emotional stress, a novel mechanism of regulating adrenocorticotropic hormone secretion? J. Neurosci. 16, 7725–7732.
Yehudam, R., Southwick, S.M., Krystal, J.H., Bremner, D., Charney, D.S., Mason, J.W., 1993. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am. J. Psychiatry 150, 83–86.
Zangrossi Jr., H., File, S.E., 1992. Behavioral consequences in animal tests of anxiety and exploration of exposure to cat odor. Brain. Res. Bull. 29, 381–388.