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Corticotropin-releasing factor receptors in the dorsal raphe nucleus modulate social behavior in Syrian hamsters

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Abstract

Rationale—In Syrian hamsters (*Mesocricetus auratus*), social defeat produces a prolonged change in subsequent agonistic behavior termed conditioned defeat. This stress-induced change in behavior is marked by increased submissive and defensive behavior toward a novel, nonaggressive opponent and a complete loss of normal territorial aggression. Corticotropin-releasing factor (CRF) has been shown to affect serotonergic neurons in the dorsal raphe nucleus (DRN) and to modulate learned helplessness via a CRF type-2 receptor (CRF-R2) mechanism.

Objectives—In this study, we tested the hypothesis that a nonselective CRF receptor antagonist (experiment 1: 250 or 500 ng D-Phe CRF in 200 nl saline), or a selective CRF-R2 antagonist (experiment 2: 500 ng anti-Svg-30 in 200 nl saline), injected into the DRN would reduce the acquisition of conditioned defeat in male hamsters. We also tested similar hypotheses for the expression of conditioned defeat (experiments 3 and 4).

Results—Infusion of D-Phe CRF into the DRN significantly reduced both the acquisition and expression of conditioned defeat compared to vehicle controls, whereas infusion of anti-Svg-30 into the DRN reduced expression but not acquisition. In particular, CRF antagonism in the DRN decreased fleeing from novel opponents but did not reinstate normal territorial aggression after social defeat.

Conclusions—Our results suggest that the increased flight associated with conditioned defeat is modulated by CRF-R2 activation within the DRN. Overall, social defeat is an ethologically relevant stressor that appears to activate at least some of the same neural substrates that have been implicated in the control of learned helplessness.

Keywords

Social defeat; Stress; Aggression; Anxiety; Fear; CRH; Defensive behavior

Introduction

Social conflict represents a potent stressor for humans and other animals. Social defeat, in particular, activates the hypothalamic–pituitary–adrenal (HPA) axis and produces stress-induced behavioral changes. A striking example of the consequences of social defeat occurs in Syrian hamsters. In the laboratory, hamsters are aggressive animals that readily defend their

home cage against intruders (Albers et al. 2002). However, after experiencing a single defeat by a larger, more aggressive opponent, hamsters exhibit a loss of normal territorial aggression and instead submit without provocation even when tested with a smaller, nonaggressive opponent. We call this change in agonistic behavior conditioned defeat, and we use this model system to investigate the neurobiology of stress-induced changes in behavior (Huhman et al. 2003).

Corticotropin-releasing factor (CRF) is a neuropeptide that has been implicated in the regulation of neural, endocrine, and behavioral responses to stressful stimuli (Dunn and Berridge 1990; Owens and Nemeroff 1991). Anatomical studies indicate that CRF-immunoreactive cell bodies and receptors are distributed outside the HPA axis, suggesting that CRF does more than control the neuroendocrine response to stress (Olschowka et al. 1982; Swanson et al. 1983; Chalmers et al. 1995); furthermore, hamsters appear to have a similar distribution of CRF-immunoreactive neurons and fibers as do rats (Delville et al. 1992). Several studies indicate that CRF modulates fear-related, anxiety-related, and defensive behavior. For instance, administration of CRF, or related peptides such as urocortins, has been shown to increase acoustic startle (Liang et al. 1992), anxiety-related behavior in open-field and elevated plus maze tests (Moreau et al. 1997; Jones et al. 1998), and defensive behavior shown in response to predators (Yang et al. 2006; Carvalho-Netto et al. 2007). Likewise, several studies have shown that CRF receptor antagonists reduce fear- and anxiety-related behavior (e.g., Schulz et al. 1996; Griebel et al. 1998; Deak et al. 1999). The actions of CRF and urocortins are mediated by CRF type-1 receptors (CRF-R1) and CRF type-2 receptors (CRF-R2). Each CRF receptor subtype has a unique distribution in the brain, suggesting potential functional differences between them (Chalmers et al. 1995; Bittencourt and Sawchenko 2000; van Pett et al. 2000). Data from pharmacological and receptor knockout studies indicate that activation of CRF-R1 increases a variety of stress-related behaviors (Heinrichs et al. 1997; Timpl et al. 1998; Contarino et al. 1999; Griebel et al. 2002). On the other hand, evidence from CRF-R2 has been mixed. Some studies have reported reduced anxiety-related behavior with CRF-R2 activation (Valdez et al. 2002) and increased depression-like and anxiety-like profiles for mice lacking the CRF-R2 gene (Bale et al. 2000; Coste et al. 2000; Kishimoto et al. 2000; Bale and Vale 2003). Other studies have reported reduced anxiety-related behavior with CRF-R2 antagonism (Radulovic et al. 1999; Ho et al. 2001; Bakshi et al. 2002; Risbrough et al. 2003). More recently, Risbrough et al. (2004) have proposed a combined role for CRF-R1 and CRF-R2 in modulating emotional behavior.

The DRN is innervated by CRF-immunoreactive fibers (Swanson et al. 1983; Sakanaka et al. 1987) and contains mRNA for CRF-R1 and CRF-R2 (Potter et al. 1994; Chalmers et al. 1995). The effect of CRF on the activity of DRN serotonin neurons is complex and is likely related to the heterogeneity of the DRN. Recognition of subregions within the DRN is important because these subregions have unique, topographically organized afferent inputs and efferent projections (van Bockstaele et al. 1993; Peyron et al. 1998). Data indicate that CRF primarily inhibits the firing of DRN serotonin neurons (Price et al. 1998; Kirby et al. 2000) and decreases extracellular levels of serotonin in the lateral septum and striatum (Price and Lucki 2001). In contrast, CRF has been shown to excite a subpopulation of serotonin neurons in the caudal DRN (Lowry et al. 2000). One possible explanation for these contrasting effects, as suggested by Lowry et al. (2000), is that Price and colleagues recorded primarily from neurons in rostral portions of the DRN while Lowry's study focused on a small cluster of neurons in the caudal DRN. Also, it is possible that CRF-R1 might mediate inhibitory responses in the rostral DRN, whereas CRF-R2 might mediate excitation more caudally (Hammack et al. 2003a). More recently, however, it has been shown that CRF-R2 activation produces inhibitory, as well as excitatory, effects in DRN serotonin neurons (Pernar et al. 2004). CRF modulation of serotonin neurons in the DRN appears to contribute to the impaired escape behavior and increased fear conditioning associated with learned helplessness. Researchers have shown that injection of a

CRF-R2 antagonist into the DRN reduces learned helplessness when given before inescapable shock (i.e., acquisition) but not when given before behavioral testing (i.e., expression) (Hammack et al. 2003b).

Conditioned defeat shares some behavioral characteristics with learned helplessness in the sense that both models involve stressors that cause animals to surrender to stimuli they would otherwise control. Researchers using the learned helplessness model have identified a neural circuit in the DRN where CRF modulates the acquisition but not expression of stress-induced changes in behavior. In the present study, we test the hypothesis that injection of a nonselective CRF receptor antagonist, and a selective CRF-R2 antagonist, into the DRN will reduce the acquisition but not expression of conditioned defeat.

Materials and methods

Subjects

We used male Syrian hamsters (*Mesocricetus auratus*) that weighed 120–140 g at the start of the study (3–4 months), and we individually housed them for 10–14 days before testing. Older hamsters (>6 months) that weighed 160–180 g were housed individually and used as resident aggressors. Younger hamsters (~2 months) that weighed 90–110 g were group-housed (five per cage) and used as nonaggressive opponents. All animals were housed in polycarbonate cages (20×40×20 cm) with corn cob bedding, cotton nesting materials, and wire mesh tops. Animal cages were not changed for at least 1 week before testing to allow individuals to scent mark their territory. Animals were housed in a temperature-controlled colony room (20±2°C) and were maintained on a 14:10-h light–dark cycle with food and water available ad libitum.

Stereotaxic surgery

Hamsters were anesthetized with sodium pentobarbital (90 mg/kg) and were stereotaxically implanted with a 4-mm, 26-gauge guide cannula aimed at the DRN. Lambda and bregma were leveled before guide cannula implantation. The angle of approach was 20° from vertical to avoid penetrating the fourth ventricle, and the stereotaxic coordinates were 4.5 mm posterior to bregma, 1.8 mm lateral to bregma, and 2.3 mm below dura. These coordinates aimed the guide cannula toward the midpoint of the rostral–caudal extent of the DRN. The guide cannula was aimed short of the DRN to avoid damaging the nucleus and a 33-gauge injection needle projected 3 mm below the guide cannula for a final depth of 5.3 mm below dura. After surgery, dummy stylets were placed in the guide cannula to help prevent clogging. All animals were given 10–14 days to recover from surgery before behavioral experiments. Hamsters were repeatedly handled after surgery by gently restraining them and removing and replacing the dummy stylet, which helped habituate them to the experimental procedure.

Conditioned defeat protocol

Our conditioned defeat protocol has been extensively described elsewhere (Potegal et al. 1993; Huhman et al. 2003) and is briefly described here. Social defeat training consisted of one 15-min encounter with a resident aggressor in the aggressor's home cage. Resident aggressors reliably attacked and defeated the experimental hamsters. To equalize the duration of defeat, 15-min encounters began at the first attack by the resident aggressor, which usually occurred within the first 30 s. Any hamster bitten such that it bled was removed from the study and was examined by a veterinarian, and 1.9% of animals were excluded due to wounding. During social defeat, we recorded the total duration of aggression displayed by the resident aggressor, the number of attacks, and the total duration of submissive and defensive behavior displayed by the experimental subjects. No-defeat controls were given a 15-min exposure to a resident aggressor's empty cage. We performed all training and subsequent testing under red light during the first 3 h of the dark phase of the light–dark cycle.

Behavioral testing occurred 24 h after training and consisted of one 5-min encounter with a novel, nonaggressive opponent in the experimental animal's home cage. Testing sessions were later scored by a researcher blind to the experimental conditions using behavioral definitions adapted from the study of Albers et al. (2002). We recorded the total duration of four classes of behavior during the 5-min tests: (a) social (attend, approach, investigate, sniff, nose touch, and flank mark), (b) nonsocial (locomotion, exploration, self-groom, nest build, feed, and sleep), (c) submissive and defensive (flee, avoid, tail up, upright and side defense, full submissive posture, stretch-attend, head flag, and attempt to escape from cage), and (d) aggressive (upright and side offense, chase, and attack including bite). For a more detailed analysis of submissive and defensive behavior, we recorded the frequency of fleeing and stretch-attend. A second researcher separately scored a subset of testing sessions, and interrater reliability was 92% with $r = 0.98$.

Experiments 1 and 2: acquisition of conditioned defeat

We designed experiment 1 to test the hypothesis that injection of a nonselective CRF receptor antagonist ($\text{D-Phe CRF}_{(12-41)}$, Bachem) into the DRN would reduce the acquisition of conditioned defeat. We infused D-Phe CRF (250 or 500 ng in 200 nl saline) or vehicle (200 nl saline) into the DRN 10 min before social defeat. Likewise, for no-defeat controls, we infused D-Phe CRF (500 ng in 200 nl saline) or vehicle (200 nl saline) into the DRN 10 min before novel cage exposure. We performed infusions with a 1- μl Hamilton syringe connected to a 33-gauge needle via polyethylene tubing. The syringe was mounted onto a syringe pump (Harvard Apparatus PHD 2000, Natick, MA, USA), programmed to infuse 200 nl/min. The needle remained in place for an additional 1 min to allow diffusion of the solution. An air bubble separated the water in the tubing from the solution, and movement of the air bubble down the tubing indicated a successful injection. Hamsters that did not receive a successful injection were excluded from data analysis. Animals were tested for conditioned defeat 24 h later as described above.

We designed experiment 2 to test the hypothesis that injection of a selective CRF-R2 antagonist (anti-Svg-30 Amide, Polypeptide Laboratories) into the DRN would reduce the acquisition of conditioned defeat. We infused anti-Svg-30 (500 ng in 200 nl saline) or vehicle (200 nl saline) into the DRN 10 min before social defeat training.

Experiments 3 and 4: expression of conditioned defeat

We designed experiment 3 to test the hypothesis that injection of D-Phe CRF into the DRN would reduce the expression of conditioned defeat. At training, animals either experienced a 15-min social defeat or a novel cage exposure. The next day, D-Phe CRF (250 and 500 ng in 200 nl saline) or vehicle (200 nl saline) was infused into the DRN of previously defeated hamsters 10 min before behavioral testing. Similarly, we infused D-Phe CRF (500 ng in 200 nl saline) or vehicle (200 nl saline) into the DRN of no-defeat controls 10 min before testing.

We designed experiment 4 to test the hypothesis that injection of anti-Svg-30 into the DRN would reduce the expression of conditioned defeat. At training, animals experienced a 15-min social defeat. The next day, anti-Svg-30 (500 ng in 200 nl saline) or vehicle (200 nl saline) was infused into the DRN 10 min before behavioral testing.

Control experiments

The effect of CRF receptor antagonists on the acquisition of conditioned defeat could be due to their effect on conditioned defeat expression if they are biologically active 24 h later at behavioral testing. In a carryover control experiment, we investigated the hypothesis that injection of D-Phe CRF into the DRN 4 h after social defeat would reduce the expression of conditioned defeat. We performed injections 4 h after social defeat, a time that we supposed

would be outside the consolidation time window for conditioned defeat. At training, animals experienced a 15-min social defeat. Four hours later, we infused *D*-Phe CRF (500 ng in 200 nl saline) or vehicle (200 nl saline) into the DRN, and the next day, animals were tested for conditioned defeat. Moreover, reduction in the acquisition and expression of conditioned defeat could be due to animals being trained and tested in different physiological states. In a state-dependency control experiment, we investigated the hypothesis that injection of *D*-Phe CRF into the DRN both before training and before testing would reinstate conditioned defeat. In this case, we infused *D*-Phe CRF (500 ng in 200 nl saline) or vehicle (200 nl saline) into the DRN 10 min before social defeat and again the next day 10 min before behavioral testing.

Histology

Hamsters were given a lethal dose of sodium pentobarbital and were infused with 200 nl of India ink to verify the placement of the injections. Brains were removed, frozen on dry ice, and stored at -80°C . Later, brains were sliced at $30\text{ }\mu\text{m}$ on a cryostat, and sections were stained with neutral red and were coverslipped with DPX mountant. Brain sections were examined under a light microscope for evidence of ink in the DRN. Only hamsters with ink injections $200\text{ }\mu\text{m}$ or less from the DRN were included in data analysis (see Fig. 1). Hamsters with ink injections further than $200\text{ }\mu\text{m}$ from the DRN were used as anatomical controls.

Statistical analysis

Total durations (in seconds) of submissive and defensive, social, nonsocial, and aggressive behaviors were analyzed separately using independent sample *t* tests or one-way between-subjects ANOVAs. Likewise, frequencies of attack, flee, and stretch-attend were analyzed separately. Tukey tests were used for pairwise comparisons when necessary. All comparisons were two-tailed, and alpha was set at $p < 0.05$.

Results

Experiments 1 and 2: acquisition of conditioned defeat

Hamsters injected with 500 ng of *D*-Phe CRF into the DRN before social defeat showed reduced conditioned defeat (Fig. 2). They showed less submissive and defensive behavior ($F_{(4,41)} = 9.36, p < 0.05$, Tukey, $p < 0.05$) and more social behavior ($F_{(4,41)} = 6.41, p < 0.05$, Tukey, $p < 0.05$) than did vehicle controls. They did not show a change in aggressive or nonsocial behavior compared to vehicle controls ($p > 0.05$). Differences in the frequency of fleeing for individuals injected with vehicle (15.0, SE = 3.1), 250 ng (8.7, SE = 2.6), or 500 ng (6.4, SE = 1.8) of *D*-Phe CRF approached, but did not reach, statistical significance ($F_{(2,27)} = 3.15, p = 0.059$). The frequency of stretch-attend posture in individuals injected with vehicle (2.8, SE = 0.38), 250 ng (1.9, SE = 0.51), or 500 ng (1.8, SE = 0.39) of *D*-Phe CRF was not significantly different ($p > 0.05$). No-defeat animals differed from defeated animals in that they showed less submissive and defensive behavior ($F_{(4,41)} = 9.36, p < 0.05$), more aggressive behavior ($F_{(4,41)} = 6.74, p < 0.05$), and more social behavior ($F_{(4,41)} = 6.41, p < 0.05$) than did socially defeated animals. However, there was no effect of *D*-Phe CRF on the behavior of no-defeat controls ($p > 0.05$).

Reduced conditioned defeat acquisition was not due to variation in the intensity of social defeat or the response of subjects during defeat. Resident aggressors directed 330.2 s (SE = 34.6), 299.5 s (SE = 35.8), and 339.9 s (SE = 29.7) of aggression toward subjects that received vehicle, 250 ng, or 500 ng of *D*-Phe CRF, respectively ($p > 0.05$). Also, subjects did not significantly differ in the number of attacks they received during social defeat (vehicle: 14.8, SE = 2.0; 250 ng: 15.0, SE = 1.8; 500 ng: 15.7, SE = 1.3) ($p > 0.05$). Furthermore, animals injected with vehicle, 250 ng, or 500 ng of *D*-Phe CRF displayed 535.6 s (SE = 48.8), 514.6 s (SE = 61.8), or 540.7 s (SE = 35.1) of submissive and defensive behavior during social defeat, respectively ($p > 0.05$).

Anatomical controls did not show reduced acquisition of conditioned defeat since the duration of their submissive and defensive behavior did not significantly differ from vehicle controls ($p>0.05$). Moreover, they did not significantly differ from vehicle controls in the frequency of fleeing ($p>0.05$) or stretch-attend postures ($p>0.05$). Anatomical controls most often had injection placements inside the fourth ventricle, but some received an injection into the periaqueductal gray, tegmental nucleus, or trochlear nucleus.

Individuals injected with anti-Svg-30 into the DRN before social defeat training did not show a significant reduction in the duration of submissive and defensive behavior at testing ($p>0.05$; Fig. 3). However, they displayed more social behavior ($t_{(19)} = 2.14$, $p<0.05$) and less nonsocial behavior than did vehicle controls ($t_{(19)} = 2.25$, $p<0.05$). Similarly, individuals injected with anti-Svg-30 or vehicle did not significantly differ in the frequency of fleeing (9.8, SE = 2.9; 9.6, SE = 2.2; $p>0.05$, respectively) or stretch-attend postures (2.5, SE = .53; 2.6, SE = .50; $p>0.05$, respectively). Because conditioned defeat is defined in terms of agonistic behavior, anti-Svg-30 injected into the DRN did not reduce the acquisition of conditioned defeat.

Experiments 3 and 4: expression of conditioned defeat

Hamsters injected with 500 ng of D-Phe CRF into the DRN before testing showed reduced conditioned defeat (Fig. 4). They displayed less submissive and defensive behavior than did vehicle controls ($F_{(4,42)} = 6.79$, $p<0.05$). They did not significantly differ from vehicle controls in the duration of aggressive, social, and nonsocial behaviors ($p>0.05$). Hamsters injected with vehicle, 250 ng, or 500 ng of D-Phe CRF displayed 13.6 (SE = 2.4), 7.4 (SE = 2.2), 6.2 (SE = 1.7) flees during testing, respectively. Statistical analysis showed that 500 ng of D-Phe CRF significantly reduced fleeing compared to vehicle controls ($F_{(2,29)} = 3.64$, $p<0.05$, Tukey, $p<0.05$). In contrast, the frequency of stretch-attend postures did not significantly differ between vehicle (2.5, SE = 0.45), 250 ng (2.7, SE = 0.75), and 500 ng (2.2, SE = 0.54) groups ($p>0.05$). No-defeat controls injected with D-Phe CRF did not significantly differ from no-defeat vehicle controls in any behavioral class ($p>0.05$), although together, no-defeat controls often differed from defeated animals (Fig. 4). Specifically, no-defeat animals showed less submissive and defensive behavior ($F_{(4,42)} = 6.79$, $p<0.05$), more aggressive behavior ($F_{(4,42)} = 4.13$, $p<0.05$, Tukey, $p<0.05$), and more social behavior ($F_{(4,42)} = 3.52$, $p<0.05$, Tukey, $p<0.05$) than did socially defeated animals.

Anatomical controls did not show reduced expression of conditioned defeat, as evidenced by no change in their submissive and defensive behavior compared with vehicle controls ($p>0.05$). Also, they did not differ from vehicle controls in the fleeing ($p>0.05$) or stretch-attend ($p>0.05$). As in experiment 1, anatomical controls most often received an injection into the fourth ventricle but some received an injection into the periaqueductal gray, tegmental nucleus, or trochlear nucleus.

In the carryover control experiment, animals that received D-Phe CRF into the DRN 4 h after social defeat did not show reduced conditioned defeat compared to vehicle controls ($p>0.05$; Fig. 5a). Likewise, animals injected with D-Phe CRF or vehicle did not significantly differ in fleeing ($p>0.05$) or stretch-attend ($p>0.05$). Together, these results suggest that the effect of D-Phe CRF on the acquisition of conditioned defeat is not due to residual drug still present at testing. In the state-dependency control experiment, animals injected with D-Phe CRF into the DRN both before social defeat training and before testing showed reduced conditioned defeat compared to vehicle controls (Fig. 5b). They showed significantly less submissive and defensive behavior than did vehicle controls ($t_{(18)} = 2.24$, $p<0.05$), and no significant change in social and nonsocial behavior ($p>0.05$) was observed. Furthermore, animals that received D-Phe CRF showed less fleeing (5.8, SE = 1.9) than did vehicle controls show (12.1, SE = 2.6) ($t_{(18)} = 2.43$, $p<0.05$). Stretch-attend postures did not significantly differ ($p>0.05$). Thus,

training and testing the animals in the same physiological state did not reinstate conditioned defeat.

Individuals injected with anti-Svg-30 into the DRN before behavioral testing showed reduced conditioned defeat (Fig. 6). They displayed a lower duration of submissive and defensive behavior ($t_{(20)} = 2.81, p < 0.05$) and a greater duration of social behavior ($t_{(20)} = 2.25, p < 0.05$) than did vehicle controls. Similarly, anti-Svg-30 animals showed less fleeing (4.0, SE = 1.1) than did vehicle controls (10.1, SE = 2.2) ($t_{(20)} = 2.65, p < 0.05$). They also showed less stretch-attend (1.5, SE = 0.40) than did vehicle controls (2.8, SE = 0.39) ($t_{(20)} = 2.31, p < 0.05$). We did not have enough injections outside of the DRN for an analysis of anatomical controls.

Discussion

We found that blockade of CRF receptors in the DRN with a nonselective antagonist reduced the acquisition and expression of conditioned defeat. Data from anatomical controls suggest that D-Phe CRF did not leak into the ventricular system or diffuse into adjacent brain regions to reduce conditioned defeat. Also, a selective CRF-R2 antagonist reduced the expression but not acquisition of conditioned defeat. The effect of both D-Phe CRF and anti-Svg-30 on expression suggests that CRF-R2 activation of DRN neurons is part of the neural circuitry controlling the increased submissive and defensive behavior which marks conditioned defeat. Because injection of anti-Svg-30 into the DRN did not reduce the acquisition of conditioned defeat, it leaves open the possibility that D-Phe CRF could have acted through CRF-R1 to affect acquisition, although this possibility needs to be tested. Together, these data suggest similarities in the neurobiological mechanisms regulating conditioned defeat and learned helplessness.

CRF receptors in the DRN are part of the neural circuit modulating conditioned defeat in hamsters and learned helplessness in rats. The acquisition but not expression of the behavioral consequences of inescapable shock is blocked with an injection into the DRN of D-Phe CRF (Hammack et al. 2002) or anti-Svg-30 (Hammack et al. 2003b). In contrast, we found that blockade of CRF-R2 in the DRN reduced the expression of conditioned defeat, but that the effect of CRF antagonism was less clear-cut for the acquisition of conditioned defeat. The contrasting effects of CRF antagonism on the expression of learned helplessness and conditioned defeat may be due to differences in testing procedures. Learned helplessness testing involves escape training and fear conditioning, whereas conditioned defeat testing requires a behavioral response to a novel intruder. For learned helplessness, CRF activity in the DRN appears to sensitize DRN neurons during inescapable shock, but testing conditions apparently do not release CRF within the DRN (Maier and Watkins 2005). We may speculate that exposure to a novel intruder after social defeat leads to the release of CRF-related peptides within the DRN, which makes CRF receptors a necessary component of the neural circuit regulating the expression of conditioned defeat. The contrasting findings could also be related to species differences in the distribution and sensitivity of CRF receptors within the DRN. In any case, CRF or urocortin activity in the DRN appears to be an important component of the neural circuitry controlling conditioned defeat and learned helplessness, although the precise role of CRF-related peptides in the DRN likely differs.

Injection of anti-Svg-30 into the DRN produced a modest reduction in the expression of conditioned defeat. We interpret the modest effect of CRF antagonism to mean that CRF-related mechanisms modulate but do not mediate the neural circuitry underlying conditioned defeat. We have previously found that an intracerebroventricular injection of D-Phe CRF produces a similar 50% reduction in the submissive and defensive behavior (Jasnow et al. 1999). Likewise, we have shown that CRF antagonism in the BSNT produces a comparable reduction in submissive and defensive behavior (Jasnow et al. 2004b; Cooper and Huhman 2005). Thus, CRF-related mechanisms appear to modulate conditioned defeat at multiple brain

sites, while non-CRF mechanisms likely mediate conditioned defeat. It seems unlikely that a higher dose of α -Phe CRF or anti-Svg-30 would produce a greater reduction in conditioned defeat because the highest doses used in this study are at the top end of the range of doses that produce behavioral effects in other models of stress-induced changes in behavior (Bakshi et al. 2002; Pelleymounter et al. 2002; Hammack et al. 2003b; Funk et al. 2006).

The increased submissive and defensive behavior associated with conditioned defeat includes behaviors which result in approach toward threatening stimuli, such as stretch-attend posture, and those which result in avoidance, such as flight. We found that CRF antagonism in the DRN reduced fleeing during conditioned defeat testing but had less consistent effects on stretch-attend posture. These data are consistent with research showing that infusion of CRF into the dorsal periaqueductal gray increases escape attempts in mice exposed to predator-stress situations (Carvalho-Netto et al. 2007). Furthermore, our results support McNaughton and Corr's (2004) hypothesis that shows that separate neural mechanisms regulate defensive approach, which may indicate anxiety, and defensive avoidance, which may indicate fear. Moreover, although CRF antagonism reduced fleeing in our study, it did not restore normal territorial aggression. In fact, we have shown that several pharmacological treatments reduce submissive and defensive behavior after social defeat but fail to affect the suppression of aggression (Jasnow and Huhman 2001; Jasnow et al. 2004a; Cooper and Huhman 2005). Also, CRF antagonism did not alter the agonistic behavior of no-defeat controls. Peripheral administration of a CRF-R1 antagonist has been shown to reduce aggression in Syrian hamsters, suggesting a role for CRF in enhancing aggression (Farrokhi et al. 2004). However, CRF may not modulate aggression via its activity in the DRN. Although research indicates that serotonin acts within the anterior hypothalamus to inhibit hamster aggression and that DRN neurons account for at least part of the serotonergic innervation of the anterior hypothalamus (e.g., Ferris et al. 1999), our results suggest that CRF receptor blockade in the DRN is not sufficient to alter the serotonergic neural circuitry regulating aggression. Overall, it appears that submissive, defensive, and aggressive behaviors are regulated by separate, albeit interacting, neural circuits and that CRF antagonism in the DRN affects primarily a defensive avoidance circuit.

Injection of α -Phe CRF reduced the acquisition of conditioned defeat, and carryover and state-dependency control experiments indicate that the effect of α -Phe CRF is due to pharmacological blockade of CRF receptors during social defeat. However, the effect of CRF antagonism in the DRN was less consistent for acquisition than it was for expression. Although α -Phe CRF infusion into the DRN before social defeat reduced the duration of submissive and defensive behavior at testing, the effect on the frequency of fleeing approached but did not reach statistical significance. Also, the effect of α -Phe CRF was not reproduced with a selective CRF-R2 antagonist. It is possible that CRF-R1 activation in the DRN, or a combination of R1 and R2 activation, contributes to the acquisition of conditioned defeat. For instance, CRF or urocortin activation of DRN neurons might desensitize 5-HT_{1A} autoreceptors, as has been suggested for learned helplessness (Greenwood et al. 2003; Maier and Watkins 2005). The possibility that CRF-related neural plasticity in the DRN contributes to conditioned defeat, however, does not preclude a critical role for neural plasticity in other brain areas. In fact, the data showing that α -Phe CRF in the DRN reduced, but did not completely block, the acquisition of conditioned defeat suggests that other neural mechanisms are involved. For example, the basolateral amygdala (BLA) has been implicated as a site of neural plasticity controlling conditioned defeat. Phosphorylation of cyclic AMP response element-binding protein (CREB) has been shown to be critical in learning and memory for stressful events, and we have demonstrated that overexpression of CREB in the BLA enhances the acquisition but not expression of conditioned defeat (Jasnow et al. 2005).

In conclusion, our results indicate that antagonism of CRF-R2 in the DRN reduces the expression of conditioned defeat. CRF-R2 antagonism in the DRN mainly reduced defensive avoidance, which is consistent with the hypothesis that the DRN modulates conditioned defeat via serotonergic projections to fear circuits in the extended amygdala (Lowry 2002; Amat et al. 2004). In sum, social defeat is a psychosocial stressor that appears to activate at least some of the same neural substrates that have been implicated in the control of learned helplessness.

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Fig. 1.

A representative photomicrograph for cannula site verification is shown. Histological maps of DRN injection placements are shown for experiments 1 and 3 (D-Phe CRF) and experiments 2 and 4 (anti-Svg-30). *Closed circles* represent acceptable injection placements, whereas *open circles* represent unacceptable sites. *Circles* indicate the center of one or more ink injections. Diagrams are adapted from the work of Morin and Wood (2001), and coordinates are reported from bregma. *DRN* Dorsal raphe nucleus, *MLF* medial longitudinal fasciculus, *4V* fourth ventricle

Fig. 2.

The mean duration ($s \pm SE$) of submissive and defensive, aggressive, social, and nonsocial behaviors is shown during a 5-min test with a novel, nonaggressive opponent. Social defeat animals received an injection of D -Phe CRF (250 ng/200 nl, $N = 9$; 500 ng/200 nl, $N = 10$) or vehicle ($N = 11$) into the dorsal raphe nucleus (DRN) 10 min before defeat training. Likewise, no-defeat controls received an injection of D -Phe CRF (500 ng/200 nl, $N = 8$) or vehicle ($N = 8$) into the DRN before novel cage exposure. These data demonstrate reduced submissive and defensive behavior and increased social behavior with increasing doses of D -Phe CRF (asterisk, $p < 0.05$). Although no-defeat controls showed less submissive and defensive behavior, more aggressive behavior, and more social behavior than did socially defeated vehicle animals (caret, $p < 0.05$), D -Phe CRF did not significantly alter the behavior of no-defeat controls ($p > 0.05$).

Fig. 3.

The mean duration ($s \pm SE$) of submissive and defensive (*sub/def*), aggressive, social, and nonsocial behaviors is shown during a 5-min test with a novel, nonaggressive opponents. Ten minutes before social defeat training, animals received an injection of anti-Svg-30 (500 ng/200 nl, $N = 11$) or vehicle ($N = 10$) into the dorsal raphe nucleus. Anti-Svg-30 did not significantly alter submissive and defensive behavior but did significantly increase social behavior and decrease nonsocial behavior compared to vehicle controls (*asterisk*, $p < 0.05$)

Fig. 4.

The mean duration ($s \pm SE$) of submissive and defensive, aggressive, social, and nonsocial behaviors is shown during a 5-min test with a novel, nonaggressive opponent. Social defeat animals received an injection of D -Phe CRF (250 ng/200 μ l, $N = 10$; 500 ng/200 μ l, $N = 11$) or vehicle ($N = 11$) into the dorsal raphe nucleus (DRN) 10 min before behavioral testing. Likewise, no-defeat controls received an injection of D -Phe CRF (500 ng/200 μ l, $N = 8$) or vehicle ($N = 7$) into the DRN. These data demonstrate a significant reduction in submissive and defensive behavior with 500 ng of D -Phe CRF compared to vehicle controls (*asterisk*, $p < 0.05$). No-defeat controls showed less submissive and defensive behavior, more aggressive behavior, and more social behavior than did socially defeated vehicle animals (*caret*, $p < 0.05$). Nodefeat controls injected with D -Phe CRF or vehicle did not significantly differ in any behavioral class ($p > 0.05$).

Fig. 5.

The mean duration ($s \pm SE$) of submissive and defensive (*sub/def*), aggressive, social, and nonsocial behaviors is shown during a 5-min test with a novel, nonaggressive opponent. In the carryover control experiment (**a**), animals received an injection of D -Phe CRF (500 ng/200 nl, $N = 6$) or vehicle ($N = 6$) into the dorsal raphe nucleus (DRN) 4 h after social defeat. D -Phe CRF did not significantly alter any behavioral class ($p > 0.05$). In the state-dependency control experiment (**b**), animals received an injection of D -Phe CRF (500 ng/200 nl, $N = 10$) or vehicle ($N = 10$) into the DRN both 10 min before social defeat and 10 min before behavioral testing. D Phe CRF significantly reduced the duration of submissive and defensive behavior compared to vehicle (asterisk, $p < 0.05$)

Fig. 6.

The mean duration ($s \pm SE$) of submissive and defensive (*sub/def*), aggressive, social, and nonsocial behaviors is shown during a 5- min test with a novel, nonaggressive opponent. Ten minutes before behavioral testing, animals received an injection of anti-Svg-30 (500 ng/200 nl, $N = 12$) or vehicle ($N = 10$) into the dorsal raphe nucleus. Anti-Svg-30 significantly reduced the duration of submissive and defensive behavior and increased the duration of social behavior compared to vehicle controls (asterisk, $p < 0.05$)