Behavioural Brain Research 198 (2009) 69–73

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Role of the bed nucleus of the stria terminalis in the acquisition and expression of conditioned defeat in Syrian hamsters

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ARTICLE INFO

Article history:
Received 5 September 2008
Received in revised form 9 October 2008
Accepted 13 October 2008
Available online 19 October 2008

Keywords:
Social defeat
Conditioned fear
Anxiety
Stress
Defensive behavior

ABSTRACT

When Syrian hamsters (*Mesocricetus auratus*) are defeated by a larger, more aggressive opponent, they subsequently produce more defensive and submissive behaviors and less chemosensory investigation and aggression, even when they are paired with a smaller, non-aggressive intruder. This persistent change in the behavior of defeated animals has been termed conditioned defeat. In the present study, we tested the hypothesis that the bed nucleus of the stria terminalis (BNST) is important for the acquisition and expression of conditioned defeat. We found that the $GABA_A$ receptor agonist muscimol infused into the BNST immediately prior to initial defeat training failed to disrupt the acquisition of conditioned defeat, while muscimol infused prior to testing caused a significant reduction in submissive/defensive behaviors and an increase in investigatory behaviors of the non-aggressive intruder. These results indicate that (1) the BNST, unlike the amygdala, does not appear to be critically involved in the consolidation process related to the memory of social defeat and (2) the BNST may be an important site for the execution of fear behaviors associated with social defeat. Considering the high degree of connectivity between the BNST and the amygdala, these findings provide further insight into the neural circuitry governing conditioned defeat and support the view of a functional dissociation between the amygdala and the BNST in the modulation of conditioned fear in an ethologically relevant model.

1. Introduction

Agonistic interactions among members of the same species are a common, yet very potent, form of stress. These interactions typically results in a 'winner' and a 'loser', and losing has been shown to have behavioral and physiological consequences. For example, defeated animals consistently show a general increase in defensive behaviors, including freezing and risk assessment, while exhibiting reduced aggressive and pro-social behaviors [2,3]. Social stress can also cause changes in brain morphology, including reduced branching and total dendritic length of the apical dendrites in the CA3 pyramidal neurons of the hippocampus [17] as well as more peripheral changes such as increased cortisol secretion [2,3,10] and a concomitant reduction in circulating levels of testosterone [3,10,20]. The long-term consequences of social defeat can therefore have severe debilitating effects on an organism [4,5] and may result in irreversible and widespread systemic changes.

Because Syrian hamsters are territorial, they readily and reliably exhibit agonistic behaviors, even in a laboratory environment, when an intruder is placed into the home cage of another hamster. Despite the high incidence of agonistic behavior, hamsters are very rarely injured during brief interactions. These characteristics make hamsters an ideal species with which to study the consequences of social defeat. Over the past several years, our laboratory has employed an ethological model of conditioned fear called *conditioned defeat* [19]. Briefly, a hamster is placed into the home cage of a larger, more aggressive animal ("resident aggressor", or RA) wherein it is quickly defeated (usually within 1 min). When the defeated animal is subsequently exposed in its own home cage to a non-aggressive intruder (NAI), it exhibits high levels of defensive and submissive behaviors and a lack of chemosensory investigation and aggression. In contrast, a weight and age-matched hamster that has not been recently defeated reliably attacks and defeats a NAI.

Using the conditioned defeat model, we are beginning to identify the components of a closely linked circuit of structures, including various sub-nuclei of the amygdala, that are responsible for the acquisition and expression of conditioned defeat [11,12,16]. Our findings are consistent with the large number of studies [1,6,7,14] that have previously established the importance of the amygdala in conditioned fear using more traditional approaches, such as those based on Pavlovian fear conditioning. More recent

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doi:10.1016/j.bbr.2008.10.022

evidence has suggested that the bed nucleus of the stria terminalis (BNST), a structure closely connected to the amygdala, may also play an important role in fear or anxiety processes [18,21,22,25]. Our own findings [12] have demonstrated that the infusion of a corticotropin releasing factor (CRF) antagonist, D-Phe CRF, into the BNST disrupts the expression of conditioned defeat. Although these results indicate the involvement of the BNST in the neural circuitry mediating conditioned defeat, a careful examination of the role of this structure as it relates to both the acquisition and expression of conditioned defeat has yet to be conducted. The purpose of the present experiment was to therefore examine the effects of temporary inactivation of the BNST on the acquisition and expression of conditioned defeat.

2. Methods and procedures

2.1. Animals and housing conditions

Subjects were adult male Syrian hamsters (*Mesocricetus auratus*, Charles River Laboratories, Wilmington, MA) that weighed 110–135 g and were approximately 9–10 weeks old at the time of testing. All subjects were individually housed for 2–3 weeks prior to the start of testing in a temperature ($20 \pm 2^{\circ}$ C) and humidity-controlled room with free access to food and water. Animals were kept on a 14:10 h light:dark cycle (lights out at 11:00 h). All training and testing sessions were performed under dim red illumination during the first 3 h of the dark phase of the light–dark cycle to minimize circadian effects. Resident aggressors used for defeat training were older (>6 months), singly housed males weighing between 160 and 180 g. Younger males (2 months) that weighed between 100 and 110 g were group housed (5–6 per cage) and served as non-aggressive intruders. The cages of the experimental animals and the resident aggressors were not changed for 2 weeks prior to testing. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Surgical procedures

Subjects were anesthetized with sodium pentobarbital (90 mg/kg, i.p.) and placed into a stereotaxic frame. Two stainless steel guide cannula (26-gauge) were implanted bilaterally into the brain and aimed at the bed nucleus of the stria terminalis (1.7 mm rostral and ±3.6 mm lateral relative to bregma). The guide cannula was lowered to 2.1 mm below dura to prevent tissue damage to the BNST. On the day of injection, a 33-gauge injection needle that projected 4.2 mm below the guide cannula, reaching a final depth of 6.3 mm below dura, was used for the drug injections. Following surgery, dummy stylets were placed in the guide cannula to help maintain patency. Hamsters were allowed 7–10 days to recover from surgery prior to the start of behavioral testing, and they were handled each day after surgery by gently restraining them and removing and replacing the dummy stylet in order to maintain patency and to habituate the subjects to the infusion procedure.

2.3. Social defeat and behavioral testing

On the day of social defeat training, animals were transported to the testing room and allowed to acclimate to the testing room for 1 h. Training sessions consisted of one 15-min exposure to the RA in the aggressor's home cage, upon which the RA reliably attacked the experimental hamsters within 60 s. This training duration is based on previous studies from our lab [11,12,16] showing that 15 min of defeat resulted in reliable levels of conditioned defeat when subjects were tested with a NAI 24 h later. Additionally, it should be noted that during a training session, attacks by the RA against a subject occur sporadically, or in bouts, and do not last for the entire duration of the training. All training and testing sessions were recorded on a DVD disc via a CCD camera positioned overhead. Analysis of the initial defeat sessions (see description below) revealed no significant behavioral differences between muscimol and vehicle infused animals, indicating that muscimol infusions did not alter the behavior of the RA's or experimental animals during training (data not shown). Twenty-four hours later, subjects were again transported to the testing room and a NAI was introduced into the subjects' home cage for 5 min. All testing sessions were also recorded on DVD. The trials were scored by an observer that was experimentally blind to the conditions using the behavioral scoring analysis program Hindsight (developed by Scott Weiss, PhD). The total duration of four classes of behaviors were scored during the test session: (a) social behavior (stretch, approach, sniff, nose touching and flank marking), (b) non-social behavior (locomotion, exploration, grooming, nesting, feeding and sleeping), (c) submissive/defensive behaviors (flight, avoidance, tail up, upright, side defense, full submissive posture, stretch attend, head flag, attempted escape from cage, and (d) aggressive behaviors (upright and side offense, chase and attack, including bite).

2.4. Drug infusion

Muscimol (Sigma, 1.1 nmol or 2.2 nmol in 200 nL saline) or vehicle control (200 nL saline) was infused bilaterally into the BNST over a 1 min period using a 1 μL syringe and Hamilton mini-pump connected to a 33-gauge needle via polyethylene tubing. The needle was kept in place for an additional 1-min before being removed to ensure complete diffusion of the drug after which the dummy stylet was replaced. Training or testing began 5 min after infusion.

2.5. Site verification

At the end of each experiment, hamsters were administered an overdose of sodium pentobarbital and infused with 200 nL of India ink to verify the placement of the injection needle. The brains were then rapidly removed and fixed in 10% buffered formalin for 3 days before being sectioned on a cryostat. Thirty micrometer sections were taken and stained with neutral red and coverslipped with DPX. Sections were then examined under a light-microscope for placement verification. Only animals with injection sites within 0.3 mm of the BNST were included in the statistical analysis.

2.6. Experiment 1: role of the bed nucleus of the stria terminalis in the acquisition of conditioned defeat

The goal of Experiment 1 was to determine whether the temporary inactivation of the BNST using the $GABA_A$ receptor agonist muscimol would block the *acquisition* of conditioned defeat. Animals ($n = 40$) were matched by weight and randomly assigned to one of three conditions. Hamsters received one of two doses of muscimol (1.1 or 2.2 nmol in 200 μL saline) or vehicle control (200 μL saline) 5 min prior to being placed into the home cage of a resident aggressor for 15 min. On the following day, animals were tested in their own cage against a non-aggressive intruder for 5 min.

In Experiments 1 and 2, we considered the possibility that a difference in drug state between acquisition and expression animals may account for any behavioral differences observed in the subjects. However, previous work from our lab [11] demonstrated that animals with infusions of muscimol both before acquisition and expression still exhibit low levels of submissive/defensive behaviors at levels that were not significantly different compared to those observed in animals receiving muscimol only before defeat or testing. Thus any effect observed in the present study is not likely to be due to a state dependency effect.

2.7. Experiment 2: role of the bed nucleus of the stria terminalis in the expression of conditioned defeat

The goal of Experiment 2 was to determine whether the temporary inactivation of the BNST using muscimol would block the *expression* of conditioned defeat. Animals ($n = 50$) were matched by weight and randomly assigned to one of three conditions and placed into the home cage of the resident aggressor for 15-min. On the following day, animals received one of two doses of muscimol (1.1 nmol or 2.2 nmol in 200 μL saline) or vehicle control (200 μL saline) 5 min prior to conditioned defeat testing by placing the non-aggressive intruder into its cage for 5 min.

2.8. Statistical analysis

The total duration (s) of each behavior displayed (Submissive/Defensive, Social, Nonsocial) was determined, and the mean total duration of each behavior was then analyzed using a one-way between-subjects analysis of variance (ANOVA) with dose as the between-subjects factor. Statistically significant differences were further analyzed using a Tukey–Kramer multiple comparison post hoc test to compare all pairwise differences among group means. Significant differences for all analyses were set at $P < 0.05$.

3. Results

3.1. Experiment 1: effect of muscimol in the BNST on acquisition of conditioned defeat

Fig. 1a shows the injection sites for animals in Experiment 1. The injection needles were localized primarily within the area of the BNST surrounding the anterior commissure. Only infusion sites within 0.3 mm of the BNST were included in the analysis and 34 of the 40 animals met these criteria. The remaining animals had cannula placements that were localized outside of the BNST, including the caudate nucleus ($n = 2$) and the lateral ventricle ($n = 1$). There were no differences in submissive behaviors between the group with missed cannula placements and vehicle control animals dur-