Behavioural Brain Research 270 (2014) 277–286

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Maintenance of dominance status is necessary for resistance to social defeat stress in Syrian hamsters

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HIGHLIGHTS

- Hamsters show reduced conditioned defeat after 14 days of dominance experience.
- 14 days of dominance experience increases c-Fos expression in the IL, PL, and vMeA.
- Development of reduced conditioned defeat parallels increased neural activation.
- Resistance to social defeat stress requires experience-dependent neural plasticity.

ARTICLE INFO

Article history:
Received 6 March 2014
Received in revised form 16 May 2014
Accepted 19 May 2014
Available online 27 May 2014

Keywords:
Dominance
Stress
Resilience
Conditioned defeat
Ventral medial prefrontal cortex
Medial amygdala

ABSTRACT

Resilience is an active process that involves a discrete set of neural substrates and cellular mechanisms and enables individuals to avoid some of the negative consequences of extreme stress. We have previously shown that dominant individuals show less stress-induced changes in behavior compared to subordinates using a conditioned defeat model in male Syrian hamsters (*Mesocricetus auratus*). To rule out pre-existing differences between dominants and subordinates, we examined whether 14 days of dominance experience is required to reduce the conditioned defeat response and whether the development of conditioned defeat resistance correlates with defeat-induced neural activation in select brain regions. We paired hamsters in daily 5-min aggressive encounters for 1, 7, or 14 days and then exposed animals to 3, 5-min social defeat episodes. The next day animals received conditioned defeat testing which involved a 5-min social interaction test with a non-aggressive intruder. In separate animals brains were collected after social defeat for c-Fos immunohistochemistry. We found that 14-day dominants showed a decreased conditioned defeat response compared to 14-day subordinates and controls, while 1-day and 7-day dominants did not differ from their subordinate counterparts. Also, the duration of dominance relationship was associated with distinct patterns of defeat-induced neural activation such that only 14-day dominants showed elevated c-Fos immunoreactivity in the ventral medial prefrontal cortex, medial amygdala, and lateral portions of the ventral medial hypothalamus. Our data suggest that resistance to social stress develops during the maintenance of dominance relationships and is associated with experience-dependent neural plasticity in select brain regions.

1. Introduction

Social defeat is a robust stressor used to investigate behavioral and physiological responses to social stress and model the biological basis of stress-related mental illness [1,2]. Conditioned defeat is a type of social stress model in male Syrian hamsters (*Mesocricetus auratus*) in which a brief social defeat results in a loss of species-typical territorial aggression and an increase in submissive and defensive behavior when animals are later tested with a small, nonaggressive intruder. Social defeat models, including conditioned defeat, allow for investigation of neurobiological mechanisms controlling vulnerability to the effects of social stress. For instance, we have previously shown that pairs of male Syrian hamsters with established dominance relationships respond

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http://dx.doi.org/10.1016/j.bbr.2014.05.041
0166-4328/

differently to social defeat stress such that dominant animals show reduced conditioned defeat compared to subordinates [3,4].

Exposure to chronic stress is a risk factor for the development of stress-related mental illness including major depression [5,6], and exposure to an acute, traumatic stressor is an essential prerequisite for post-traumatic stress disorder [7–9]. However, in both cases not all individuals exposed to stressful events develop stress-related psychopathologies [10,11]. Understanding the neurobiological mechanisms regulating vulnerability and resistance to the effects of stress is an important step toward advancing treatment options for stress-related psychopathology. Animal models have identified several brain regions critical for stress resistance including the ventral medial prefrontal cortex (vmPFC). The vmPFC, which includes the infralimbic cortex (IL) and prelimbic cortex (PL), controls affective processing and executive function [12,13]. The vmPFC is also part of a neural circuit that inhibits neurons within the paraventricular nucleus of the hypothalamus that control the neuroendocrine stress response [14,15]. Neural activation in the vmPFC is required for the stress resistance which develops following exposure to environmental enrichment [16], social dominance [4], and controllable stress [17]. For example, exposure to controllable stress has an immunizing effect on the development of learned helplessness such that uncontrollable stress fails to produce learned helplessness when rats are pre-exposed to controllable tail-shocks. Pharmacological inhibition of the vmPFC blocks the immunizing effect of controllable stress [18], and pharmacological activation of the vmPFC has an immunizing effect in the absence of controllable stress [19]. Also, stress-induced neural activation in the vmPFC is associated with individual differences in coping strategies and baseline trait anxiety in rodents [20,21]. Overall, the vmPFC is a critical neural substrate that inhibits neuroendocrine and behavioral responses to stress.

Several other brain regions, outside of the vmPFC, have been implicated in resistance to the effects of stressful events. Six weeks of voluntary wheel running has been shown to prevent the shuttle box escape deficits that occur following uncontrollable stress [22]. A series of studies has shown that voluntary wheel running alters ΔFosB immunoreactivity in the nucleus accumbens [23], brain-derived neurotrophic factor mRNA expression in the hippocampus and amygdala [24], and 5-HT1A autoreceptor mRNA expression in the dorsal raphe nucleus [25]. Chronic social defeat stress in mice leads to heightened reactivity of the hypothalamic-pituitary–adrenal axis as well as increased anxiety and depression-like behavior [26]. However, about one-third of mice exhibit a resilient phenotype insofar as they do not exhibit social avoidance and anhedonia-like symptoms. Resilient mice show changes in the expression of K+ channels and AMPA receptor subunits which normalizes firing within a ventral tegmental-nucleus accumbens circuit [26,27]. Dominant social status is also associated with coping style and neuroendocrine responses to stress. In Anolis lizards dominants have been described as adopting a proactive behavioral strategy during agonistic social encounters [28]. Dominant lizards also show elevated serotonin levels in the amygdala following restraint stress whereas subordinates show elevated dopamine levels [29]. In several species dominants show reduced basal or stress-induced glucocorticoid activity compared to subordinates [29–32]. Overall, resilience appears to be an active process that involves multiple brain regions and cellular mechanisms which facilitate coping with stress.

One limitation of studying the natural formation of dominance relationships is that subjects cannot be randomly assigned to dominant or subordinate status. Subjects may have pre-existing differences that correlate with both the probability of winning and responses to stress. The aim of this study was to examine the time course of the status-dependent changes in conditioned defeat and defeat-induced neural activation. We used the protein product of the immediate early gene *c-fos* as a marker of defeat-induced neural activation [33]. We hypothesized that dominants would show reduced conditioned defeat and elevated defeat-induced neural activation within brain regions such as the vmPFC compared to subordinates after 14 days, but not 1 or 7 days, of dominance experience. This approach allowed us to determine whether dominant and subordinate animals systematically differed in conditioned defeat and neural activation prior to dyadic dominance interactions or whether behavioral and physiological changes were experience-dependent.

2. Experimental

2.1. Subjects

Subjects were male Syrian hamsters (*Mesocricetus auratus*) obtained from our breeding colony that was originally derived from hamsters purchased from Charles River Laboratories (Wilmington, MA). Subjects were 3–4 months old (120–180 g) at the start of the study and were individually housed one week prior to the start of the study. Older hamsters (>6 months, >190 g) were individually housed and used as resident aggressors for social defeat training. Younger hamsters (approx. 2 months, <120 g) were housed in groups of four and used as non-aggressive intruders for conditioned defeat testing. All animals were housed in polycarbonate cages (12 cm × 27 cm × 16 cm) with corncob bedding, cotton nesting materials, and wire mesh tops. Food and water were available *ad libitum*. Cages were not changed for one week prior to dominant–subordinate encounters to allow individuals to scent mark their territory. Subjects were handled daily for one week prior to dominant–subordinate encounters to habituate them to the stress of human handling. Animals were housed in a temperature controlled colony room (21 ± 2 °C) and kept on a 14:10 h light:dark cycle to facilitate aggressive behavior. All behavioral protocols were performed during the first 3 h of the dark phase of their cycle. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Behavioral protocols

2.2.1. Dominant–subordinate encounters

To allow animals to establish social status, subjects within each cohort were weight-matched in resident-intruder dyads and paired in daily social encounters for 1 day, 7 days, or 14 days. Subjects were randomly assigned as a resident or intruder, and all social encounters occurred in the resident's home cage. The encounter on day 1 was 10 min in duration, while all subsequent encounters were 5 min. We have previously determined that a 10 min encounter on day 1 facilitates the formation of a dominance relationship, and that 5 min encounters on subsequent days maintain the dominance relationship and reduce the chance of wounding [3]. Dominant and subordinate animals were identified by the direction of agonistic behavior within each dyad. Subjects were pseudo-randomly assigned to the number of dominant–subordinate encounters. Dyads must have formed a dominant–subordinate relationship during their first encounter to be assigned to 1 day of social encounters. The remaining dyads, including some that formed a dominance relationship on the first day, were randomly assigned to 7 days or 14 days of social encounters. If a dyad did not form a dominance relationship after 5 daily encounters, that dyad was dropped from the study. Control subjects were individually housed one week prior to social defeat training and were handled daily during that time.