Behavioural Brain Research 256 (2013) 188–196

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Olfactory cues increase avoidance behavior and induce Fos expression in the amygdala, hippocampus and prefrontal cortex of socially defeated mice☆

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HIGHLIGHTS

- Social defeat increased Fos expression in mouse brain regions implicated in stress response.
- FosB/ΔFosB expression was increased in amygdala of socially defeated mice.
- An olfactory cue increased Fos expression in brain regions implicated in fear memory.
- An olfactory cue increased FosB/ΔFosB in prefrontal cortex of socially defeated mice.
- An olfactory cue increased avoidance behavior in socially defeated mice.

ARTICLE INFO

Article history:
Received 30 May 2013
Received in revised form 10 August 2013
Accepted 13 August 2013
Available online 19 August 2013

Keywords:
Social stress
Basolateral amygdala
Hippocampus
Prefrontal cortex
Avoidance
Fos

ABSTRACT

Genes and proteins of the Fos family are used as markers of neuronal activity and can be modulated by stress. This study investigated whether social defeat (SD) or exposure to an olfactory cue associated with the SD experience activated Fos and FosB/DeltaFosB (ΔFosB) expression in brain regions implicated in the development of post-traumatic stress disorder. Mice exposed to acute SD showed more Fos positive cells in the basolateral amygdala (BLA), CA1 of the hippocampus and the medial prefrontal cortex (mPFC) 1 h after SD, and had greater expression of the more persistent FosB/ΔFosB protein in the BLA 24 h after SD compared to controls. Mice exposed to an olfactory cue 24 h or 7 days after SD had higher levels of Fos expression in all three regions 1 h after exposure to the cue, and displayed increased avoidance behavior compared to controls. While the avoidance response dissipated with time (less at 7 day *vs* 24 h after social defeat), Fos expression in the mPFC and CA1 in response to an olfactory cue was greater at 7 days relative to 24 h after social defeat. The results suggest additional processing of the cue-stress association and may provide further support for a role of the mPFC in fear inhibition. These findings may have implications for brain regions and circuitry involved in the avoidance of cues associated with a stressful event that may lead to context-dependent adaptive or maladaptive behavior.

Published by Elsevier B.V.

1. Introduction

Stress is defined as a reaction to any environmental or bodily demand [reviewed in [1,2]], and is characterized by activation of the sympathetic nervous system and the hypothalamic-pituitary-adrenal-axis (HPA-axis) [reviewed in [3,4]]. Social defeat stress is a naturalistic model of stress that involves an agonistic encounter between conspecifics. It induces prolonged behavioral changes in mice and has been used as an animal model of depression [5]. Social defeat induces robust physiological, behavioral and endocrine responses including circadian rhythm disturbances, avoidance behavior and elevated levels of corticosterone [6–8]. Some behavioral changes induced by social defeat, such as a tendency to avoid stimuli and situations reminiscent of a traumatic event, may be analogous to symptoms of post-traumatic stress disorder (PTSD) [9]. Human patients with PTSD experience a variety of symptoms including anxiety, avoidance, despondency, emotional numbing, enhanced physiological reactivity, intrusive thoughts involving trauma, increased startle response, memory deficits, and sleep disturbances (*e.g.*, nightmares) [reviewed in [10]].

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0166-4328/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbr.2013.08.020

Changes in neuronal activity have been reported for many brain regions in response to social and restraint stress, in multiple rodent species. To measure these changes, genes and proteins of the Fos family are commonly used as markers of neuronal activation. Exposure to a stressor can influence the expression of the immediate early gene c-*fos* and its protein product Fos, indicating stress-induced alterations in neuronal activity [reviewed in [11,12]]. Restraint stress increases Fos-positive cells in the paraventricular nucleus, central and medial amygdala, and the paraventricular thalamic nucleus [13]. In addition, after a single social defeat in rats, Fos expression increases in multiple brain areas including the locus coeruleus, regions of the amygdala and extended amygdala, the paraventricular nucleus, and the lateral hypothalamic area [14]. Similar results have been observed in mice [15]. Using a social defeat paradigm, Matsuda et al. [15] found that acute and chronic defeat resulted in elevated Fos expression across many brain regions. The effect of acute defeat impacted Fos levels for up to 2 h in four brain regions: the anterior hypothalamic area, the dorsal premammillary nucleus, the paraventricular hypothalamic nucleus, and the superior colliculus; however, chronic defeat (20 attacks/day for 5 consecutive days) elevated Fos expression 24 h after defeat in nearly 50 brain regions including the CA1 and CA3 of the hippocampus, the medial prefrontal cortex (mPFC), the central and basolateral amygdala (BLA), and the nucleus accumbens. In rats, 5 days of repeated social stress increases FosB/DeltaFosB (ΔFosB) protein expression in the frontal cortex, nucleus accumbens, and in the medial, central and basolateral amygdala, with elevated levels observed on day 3 and continuing up to 14 or 21 days after exposure, depending on the brain region [16]. The FosB/ΔFosB protein is more stable and activation may persist for weeks, compared to Fos expression, which typically degrades within hours [17–19].

Three brain regions that are involved in or affected by the stress response are the amygdala, hippocampus, and the prefrontal cortex [reviewed in [3]]. In patients with PTSD, increased amygdala activation is associated with symptoms such as fear, social avoidance, anxiety, and depression, that are thought to be due to alterations in the amygdala and hippocampus [reviewed in [20]]. In addition, acute corticosterone treatment, repeated restraint stress and social stress increase fear and anxiety-like behaviors, and these changes are correlated with dendritic hypertrophy in the BLA [21–24]. With a high concentration of glucocorticoid receptors and its ability to stimulate the HPA axis [25], it is likely that the amygdala is involved in the social defeat experience and some of the behavioral effects of social defeat stress. Our lab previously showed that administration of antalarmin, a corticotropin-releasing hormone antagonist, into the BLA immediately after social defeat, prevented fear responses in a resident intruder test 24 h after defeat, with antalarmin in the BLA likely blocking memory consolidation [26].

In contrast, the hippocampus and prefrontal cortex, which also have glucocorticoid receptors, exhibit dendritic atrophy in response to stress [22,27]. In the hippocampus, reduced dendritic arborization and spine density have been observed in the CA1 and CA3 [22,28]. The hippocampus is particularly sensitive to stress, as it is susceptible to stress-induced alterations in the expression of neurotrophins [29], atrophy of dendrites, suppression of neurogenesis [30], and even neuronal loss [31]. The effects of stress on the hippocampus and the PFC are important because both regions are involved in regulating the response of the HPA-axis to stress and consequently the hormones secreted as a result of HPA-axis activation [25,32,33]. Therefore, stress-induced atrophy in the hippocampus and PFC may lead to prolonged corticosterone release because of their reduced ability to provide negative feedback and regulate the HPA-axis response to stress.

The effect of olfactory cues associated with a stress experience on Fos and FosB/ΔFosB expression has yet to be elucidated. In patients with PTSD, reminders of traumatic events can influence physiological measures such as heart rate and can elicit intrusive and unwanted memories of the trauma [reviewed in [20]]. Additionally, in mice that received social defeat, avoidance and exaggerated fear responses were observed after exposure to a stress-related olfactory cue [34,35]. In the present experiment, we evaluated Fos and FosB/ΔFosB expression induced by stress and olfactory cues in brain regions of mice involved in emotion and memory, including the BLA, which plays a role in conditioned fear and anxiety [36], as well as the mPFC and the hippocampal CA1, which provide negative feedback to the HPA-axis [27] and are thought to be involved in various aspects of memory such as consolidation and retrieval [37–39].

Table 1
Group and sample size.

Group	Euthanize (1 h)	Euthanize (24 h)	Euthanize (7 days)
No SD/no cue	$N = 11$	$N = 11$	$N = 11$
SD/no cue	$N = 11$	$N = 10$	$N = 10$
No SD/cue at 24 h	$N = 8$	$N = 8$	*
No SD/cue at 7 days	$N = 8$	*	*
SD/cue at 24 h	$N = 13$	$N = 14$	$N = 10$
SD/cue at 7 days	$N = 14$	$N = 14$	$N = 9$

* Groups omitted from study based on data from initial study runs. Elimination of these groups allowed for fewer control animals to be used in the experiment, as an attempt to reduce the numbers of animals used in research.
SD = social defeat; No SD = no social defeat.

2. Material and methods

2.1. Subjects

Six-week-old male C57BL/6 mice (20–25 g; Jackson Laboratory, Bar Harbor, ME) were group housed in polycarbonate cages measuring 46 cm × 25 cm × 15 cm. Mice were kept on a 12-h reverse light/dark cycle (08:00 h–20:00 h), and food and water were available *ad libitum*, except during the 10-min stress exposure and 12-min challenge test. Ten days after the mice were received, and 7 days prior to the stress exposure, mice were individually housed to reduce the effects of a social hierarchy, which typically develops in group-housed mice [40]. This brief period of isolation was insufficient to induce increased aggressive behavior associated with long-term individual housing. The cages used for individual housing were larger than those used for initial group housing, measuring 46 cm × 25 cm × 20 cm. Mice received social defeat or no social defeat and were euthanized 1 h, 24 h, or 7 days later; in addition, subgroups were exposed to an olfactory cue 24 h or 7 days after social defeat (see Table 1). The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense and all procedures were conducted in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

2.1.1. Stimulus mice

Male SJL aggressor stimulus mice (20–25 g; Jackson Laboratory) were individually housed for two months prior to use in the study because isolation promotes aggressive behavior in mice [40]. One month prior to testing, the SJL mice were trained to be highly aggressive by repeated pairings (three times/week) with olfactory bulbectomized (OBX) C57BL/6 mice; OBX mice were removed from the aggressor's cage after an attack or after 2 min passed without an attack. The OBX mice were used because OBX mice rarely fight back when attacked and almost never initiate an attack [41]. Attack latencies were recorded during training; SJL mice with attack latencies of less than 30 s were used as aggressors in the experiment and paired with subject mice.

2.2. Acute stress paradigm

In the acute social stress paradigm, subject mice were defeated by trained aggressor mice (SJL). Subject mice were paired with aggressors for three 2-min encounters with a 2-min break in between each defeat for a total of 10 min and 100–105 attacks. Each encounter occurred with a different aggressor. Control subjects received no stress manipulation, and remained in their home cage in the colony room with continued access to food and water.

2.3. Olfactory cue procedure

Prior to placement of the olfactory cue, subjects were allowed to acclimate for 5-min to a perforated barrier placed within the home cage of the resident subject.