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Research report

Altered function in medial prefrontal cortex and nucleus accumbens links to stress-induced behavioral inflexibility

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HIGHLIGHTS

- Single prolonged stress (SPS) impaired set-shifting.
- SPS-induced set-shifting impairments coincided with activity reduction in NAc core.
- Neurons in mPFC exhibited higher activity in SPS-induced set-shifting dysfunction.
- Enhanced phospho-GluA1-Ser845 in mPFC was related to the impaired set-shifting.

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ABSTRACT

The medial prefrontal cortex (mPFC) and its output area, the nucleus accumbens (NAc), are implicated in mediating attentional set-shifting. Patients with posttraumatic stress disorder (PTSD) exhibit difficulties in the disengagement of attention from traumatic cues, which is associated with impairments in set-shifting ability. However, unknown is whether alterations in corticostriatal function underlie deficits in this behavioral flexibility in individuals with PTSD. An animal model of single prolonged stress (SPS) has been partially validated as a model for PTSD, in which SPS rats recapitulate the pathophysiological abnormalities and behavioral characteristics of PTSD. In the present study, we firstly found that exposure to SPS impaired the ability in the shift from visual-cue learning to place response discrimination in rats. Conversely, SPS induced no effect on a place-to-cue set-shifting performance. Based on SPS-impaired set-shifting model, we used Western blot and immunofluorescent approaches to clarify SPS-induced alternations in synaptic plasticity and neuronal activation in the mPFC and NAc. Rats that were subjected to SPS exhibited a large increase in pSer845-GluA1 and total GluA1 levels in the mPFC, while no significant change in the NAc. We further found that exposure to SPS significantly decreased c-Fos expression in the NAc core but not the shell after set-shifting behavior. Whereas, enhanced c-Fos expression was observed in prelimbic and infralimbic cortices. Collectively, these findings suggest that abnormal hyperactivity in the mPFC and dysfunction in the NAc core underlie long-term deficits in executive function after traumatic experience, which might play an important role in the development of PTSD symptoms.

1. Introduction

Deficits in behavioral flexibility are found in posttraumatic stress disorder (PTSD) [1]. The capability of behavioral flexibility can be examined in an attentional set-shifting task, which requires a subject to disengage from a once relevant set of stimulus dimensions and begin responding to a previously irrelevant set of stimulus dimensions to achieve optimal performance [2]. Impairments in set-shifting are observed in patients with severe symptoms of PTSD [3], including difficulties in disengaging attention to trauma-related stimuli, leading to a reliance on avoidant coping strategies that contribute to the development of PTSD symptoms [4,5].

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Many imaging studies have highlighted alterations in neural activity in the prefrontal cortex (PFC) in PTSD patients [6–8]. Stress-induced alterations in the PFC function may underlie the deficit in behavioral flexibility. Several studies have reported the involvement of the PFC in attentional switching tasks [9,10]. In humans and primates, lesions of the dorsolateral PFC (dlPFC), which performs a similar cognitive function as the medial PFC (mPFC) in rodents, slow the acquisition of set-shifting [11]. Rodent studies revealed that the prelimbic cortex (PL), rather than the anterior cingulate cortex, plays an important role in set-shifting, suggesting differential contributions of different subregions of the mPFC [12,13]. The core and shell segment of NAc receive neuronal projections from the mPFC [14]. The involvement of the NAc core but not the shell in attentional set-shifting has been reported in our and other previous studies [15,16]. Moreover, asymmetric disconnections have been found using pharmacological methods, suggesting that the PL-nucleus accumbens (NAc) core connection may play a role in strategy shifting [17]. Nevertheless, still unclear is whether functional changes in mPFC and NAc neurons is associated with deficits in set-shifting ability in the individuals who are exposed to traumatic stress.

To address this issue, we firstly identify the effects of traumatic stress on set-shifting performance. We subjected rats to a single prolonged stress (SPS) paradigm and then performed two types of set-shifting tasks. The SPS paradigm is widely used as a reliable animal model of PTSD. SPS rats exhibit behavioral features of PTSD, including exaggerated startle responses [18], heightened fear [19], disrupted fear memory extinction [20], and cognitive dysfunction [20]. SPS also induces many pathophysiological characteristics of PTSD, such as time-dependent dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis [21]. It also enhances the reactivity of locus coeruleus-norepinephrine (LC-NE) neurons [22] and decreases the tone of excitatory neurons in the mPFC [23]. To investigate whether alterations in synaptic plasticity and neuronal activity in the mPFC and NAc underlie the effects of SPS on executive function, the phosphorylation of the AMPA receptor GluA1 subunit and expression of the immediately early gene c-*fos* were assessed by Western blot and immunohistochemistry, respectively. GluA1 phosphorylation is required for the induction of many forms of synaptic plasticity, including long-term depression (LTD) and long-term potentiation (LTP) [24]. Specifically, GluA1 phosphorylation at Ser845 can stabilize LTP by inhibiting the internalization of newly inserted GluA1 receptors [24]. GluA1 phosphorylation at Ser845 and Ser831 is implicated in spatial memory [24,25] and appetitive incentive learning [26]. c-Fos, the protein product of c-*fos*, has been introduced as a tool for determining activity changes within neurons of the nervous system [27,28].

In the present study, exposure to SPS slowed the shift from visual-cue learning to place response discrimination but did not impact reversed shift from place response discrimination to visual-cue learning. Rats that were exposed to SPS exhibited a robust increase in pSer845-GluA1 level and total GluA1 level in the mPFC. No changes in GluA1 phosphorylation were found after SPS exposure followed by a place-to-cue set-shifting task or after SPS exposure without further behavioral testing. Furthermore, c-Fos expression increased in the PL and infralimbic cortex (IL) and decreased in the NAc core in SPS rats after the cue-to-place set-shifting task but not in the place-to-cue set-shifting task.

2. Materials and methods

2.1. Subjects

One hundred and twenty seven male Sprague-Dawley rats (Vital River Animal Center, Beijing, China), weighing 180–200 g upon arrival, were used in this study. The rats were individually housed in stainless steel sliding-drawer-type cages (50 cm length × 32 cm width × 22 cm height) with a wire mesh floor and pine wood shavings below the cages. The rats were maintained on a 12 h/12 h light/dark cycle (lights on at 7:00 A.M.), with food and water available *ad libitum*. All of the rats were gently handled daily for 1 week before the behavioral experiments began. The experimental procedures were approved by the Institutional Review Board of the Institute of Psychology, Chinese Academy of Sciences, and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Single prolonged stress treatment

Rats were exposed to a single prolonged session that consisted of three stressors (restraint, forced swim, and ether vapor), returned to their home cages, and left undisturbed for 7 or 14 days [20,21]. The rats were first restrained for 2 h, followed by 20 min of forced swimming in a glass tub (20 cm diameter × 50 cm height) with water at 24 ± 1 °C. After 15 min of recuperation, the SPS rats were exposed to ether vapor in a glass desiccator (30 cm diameter) until they lost consciousness. The control group was only handled for the duration of the SPS procedure.

2.3. Behavioral flexibility test

2.3.1. Apparatus

The behavioral flexibility test was conducted in eight operant chambers (29 × 29 × 26 cm, Anilab, Jiangsu, China) that were housed in sound-attenuating boxes with a fan to mask external noise. The operant chambers were equipped with two nosepoke operandi on each side of the food dispenser and a yellow LED light (20 mW) that was centrally located inside each nosepoke hole. A house light was mounted on the same wall as the dispenser, 20 cm above the right nosepoke hole. The house light was turned on when reinforcers (0.08 ml of 20% sucrose solution) were delivered into the dispenser through a metal spout that was attached with tubing to a 60-ml syringe pump. A 15 s timeout period followed each reward, during which subsequent responding produced no effect. A computer with Anilab software controlled the equipment and recorded all experimental data.

2.3.2. Procedure

2.3.2.1. Habituation. The test procedure was the same as in a previous study [15]. The rats were food-deprived for 18 h before the habituation session and allowed access to food for 1 h after each daily session to maintain bodyweight at 80–85% of baseline [29]. Water was removed 2 h before each daily session.

The habituation session comprised two parts. The rats were first placed in the operant chamber for 15 min in the dark and allowed to roam freely without reinforcer delivery. During the following 60 min, the rats were trained to associate stimuli with the delivery of 0.08 ml of fluid. The house light was illuminated for 4 s while 0.08 ml of 20% sucrose solution was delivered into the dispenser on a variable interval 40 s schedule.

Rats usually present place preferences between left and right nosepoke holes. To balance the effects of a possible place preference, the total number of left nosepokes and right nosepokes was recorded to determine side biases. If the ratio of one nosepoke on one side over the other side was greater than 2:1, then that was considered a side bias. When the total number of left nosepokes and right nosepokes were comparable, the side at which nosepokes occurred ≥ 4-times more over the initial seven total trials was considered a side bias.