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Research report

Sex-specific impairment and recovery of spatial learning following the end of chronic unpredictable restraint stress: Potential relevance of limbic GAD

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HIGHLIGHTS

- In males, chronic unpredictable restraint impaired spatial learning.
- Stress-induced spatial learning deficits in males improved after a recovery period.
- Spatial learning negatively correlated with medial amygdala GAD_{65} in males.
- In females, chronic unpredictable restraint had no effect on RAWM performance.
- Female RAWM spatial learning positively correlated with hippocampal CA1 GAD_{65}.

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ABSTRACT

Chronic restraint stress alters hippocampal-dependent spatial learning and memory in a sex-dependent manner, impairing spatial performance in male rats and leaving intact or facilitating performance in female rats. Moreover, these stress-induced spatial memory deficits improve following post-stress recovery in males. The current study examined whether restraint administered in an unpredictable manner would eliminate these sex differences and impact a post-stress period on spatial ability and limbic glutamic acid decarboxylase (GAD_{65}) expression. Male ($n = 30$) and female ($n = 30$) adult Sprague–Dawley rats were assigned to non-stressed control (Con), chronic stress (Str-Imm), or chronic stress given a post-stress recovery period (Str-Rec). Stressed rats were unpredictably restrained for 21 days using daily non-repeated combinations of physical context, duration, and time of day. Then, all rats were tested on the radial arm water maze (RAWM) for 2 days and given one retention trial on the third day, with brains removed 30 min later to assess GAD_{65} mRNA. In Str-Imm males, deficits occurred on day 1 of RAWM acquisition, an impairment that was not evident in the Str-Rec group. In contrast, females did not show significant outcomes following chronic stress or post-stress recovery. In males, amygdalar GAD_{65} expression negatively correlated with RAWM performance on day 1. In females, hippocampal CA1 GAD_{65} positively correlated with RAWM performance on day 1. These results demonstrate that GABAergic function may contribute to the sex differences observed following chronic stress. Furthermore, unpredictable restraint and a recovery period failed to eliminate the sex differences on spatial learning and memory.

Abbreviations: ANOVA, analysis of variance; CA1, cornu ammonis 1; CA3, cornu ammonis 3; Con, control; CUR, chronic unpredictable restraint; GABA, γ-aminobutyric acid; GAD_{65}, glutamic acid decarboxylase; RAWM, radial arm water maze; Str-Imm, Stress-Immediate; Str-Rec, Stress-Recovery.

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1. Introduction

It is increasingly apparent that chronic stress impairs hippocampal-dependent spatial learning and memory in a sex-dependent manner. In males, chronic stress impairs performance on the radial arm maze [38,76], Y-maze [54], Morris water maze [25,36,37,50,67,74,75], and the radial arm water maze task (RAWM) [31,32] (for review, see [11,48,64]). Conversely, several studies report that chronic stress enhances spatial memory ability in females on the radial arm maze [7], Y-maze [54], object placement task [2], and Morris water maze [36,37,50] (reviewed in [39,73]). These studies suggest that the sex of an individual influences the underlying neural mediators of spatial ability.

A putative target for chronic stress actions is the hippocampus, as it is essential for spatial memory [11,20,57,58] and contains an abundance of receptors for glucocorticoid stress hormones [49]. In male rats, chronic stress leads to a simplification of apical dendritic arbors within the CA3 region of the hippocampus [10,22,80,81] and reduced glucocorticoid receptor number [70,71], both of which correspond to deficits in spatial tasks [31,86]. When chronically stressed males are given time to recover following the end of chronic stress, spatial ability improves in parallel with the restoration of the CA3 dendritic architecture [31,60]. In contrast, chronically stressed, gonadally intact female rats show mild or negligible basal dendritic retraction in the CA3 region [22,55], which does not necessarily correspond with spatial memory [52]. Mitigating variables that might contribute to chronic stress effects on the hippocampus and spatial memory in females include the type of stressor [47,62], task [46,53,84], estrogen status [15,61,72], and even experimenter handling of the rats [6,18,30]. Whether females would return to their previous status in spatial ability following a post-stress recovery period has not been investigated. One goal of the present study is to investigate the potential sex differences arising from the effects of chronic stress and a post-stress recovery period on spatial learning and memory. Successful spatial memory recovery from chronic stress has been documented in male rats using the radial arm water maze [31,60] and so a similar task will be used in the current study.

An important variable that could impact sex differences in spatial memory performance is the type of stressor used. Restraint stress is commonly used in rodents due to its relative ease of use, however chronic restraint elicits detriments in spatial learning and memory in male [23,31,60,75], but not female rats [36,55]. Stressors can be categorized as being physical, psychological, or both, and chronic restraint is thought to include components of both physical and psychological stressors [8]. In humans, psychological stressors produce a stress response when the stressor consists of any one of three characteristics: loss of control, novelty, and unpredictability [9,43,56]. In rodents, administration of chronic restraint stress might consist of a loss of a sense of control, but lacks components in the other categories (i.e. novelty and unpredictability). Furthermore, repeatedly administering restraint leads to predictability, as male rats demonstrate habituation of the stress hormone response [22]. Therefore, one purpose of the present study was to determine whether enhancing the unpredictable nature of chronic restraint would lead to impaired spatial ability in both male and female rats.

Another purpose of this study was to examine the role of inhibitory tone as it pertains to chronic stress-induced changes in spatial learning and memory. Glutamate and γ-aminobutyric acid (GABA) neurotransmitters are primarily responsible for regulating inhibitory tone. In male rats, chronic stress leads to significant increases in hippocampal extracellular glutamate [35] and decreases in hippocampal GABA [27,59]. Hippocampal GABA levels in males might be low following chronic stress, in part, due to decreases in glutamic acid decarboxylase (GAD) levels, the synthesizing enzyme for GABA [21]. Furthermore, other limbic areas may display a dysregulated inhibitory tone following chronic stress, which may also impact spatial learning and memory. MeA input into the HPA axis is critical for HPA axis activation following restraint stress [16]. Cells in the MeA exhibit the greatest level of *c-fos* activation following a psychological stressor compared to other amygdala nuclei (e.g. central amygdala). These findings suggest that the MeA is implicated in the response to stressors. Taken together, these studies provide support for the notion that the spatial memory impairments induced by chronic stress are mediated by changes in inhibitory tone the limbic system. Whether these changes contribute to the sex differences observed in spatial learning and memory tasks following chronic stress are unknown and will be investigated.

The current study tested the hypothesis that the unpredictability of a chronic stress paradigm impacts the sensitivity of females to the immediate and long-term (i.e., recovery from) effects of chronic stress. The behavioral endpoint of spatial learning and memory was chosen due to the well-established immediate and long-term effects of chronic restraint on male rats. In addition, this study explored the effect of chronic unpredictable restraint stress on GAD expression in limbic regions, as a likely mediator of chronic stress effects and a potential mediator of sex differences.

2. Material and methods

2.1. Animals

Sixty young adult Sprague–Dawley male and female rats (males = 30, females = 30) approximately 2 months of age were purchased from Charles River Laboratories (Wilmington, MA) and were pair housed with a same-sex cage mate at Arizona State University housing facilities. Male and female rats were housed in separate rooms, and control and experimental groups were additionally separated into different chambers on a reverse light cycle (12:12; lights off at 06:00 am). Rats were given 1 week to acclimate before any behavioral procedures were performed. Food and water were available *ad libitum* except during the restraint procedure when both control and stressed animals did not have access. Rats were weighed once a week throughout the experiment. Behavioral testing was conducted during the dark phase of the light cycle. All procedures were conducted according to federal guidelines outlined in the Guide for the Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources on Life Science, National Research Council, 1996) and institutional guidelines set forth by the Institutional Animal Care and Use Committee at Arizona State University.

2.2. Group assignments

Rats were randomly divided into six groups (n = 10/group): male and female non-stressed control (Con), male and female stressed rats that were tested immediately (Str-Imm), and male and female stressed rats that were given a 21-day post-stress recovery period (Str-Rec). The experimental groups and timeline are depicted in Fig. 1A.

2.3. Chronic unpredictable restraint stress procedure

Rats were transported to different rooms each day and were restrained using restrainers made from wire mesh (purchased from Flynn and Enslow, San Francisco, CA, USA) with the ends sealed with grip guard sealer (ACE Hardware). The ends were secured with black binder clips. The chronic unpredictable restraint (CUR) schedule was comprised of different start time periods (ranges: 05:00–07:00, 08:00–10:00, 11:00–13:00, 14:00–16:00, 17:00–19:00), physical contexts (four different rooms), extract odors (almond, banana, orange), and restraint durations (no