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Short Communication

Chronic stress effects on working memory: Association with prefrontal cortical tyrosine hydroxylase

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HIGHLIGHTS

- Chronic stress affects working memory function in rats.
- Altered working memory is associated with prefrontal cortical tyrosine hydroxylase.
- Chronic stress effects on working memory depend on prefrontal tyrosine hydroxylase.

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ABSTRACT

Chronic stress causes deficits in cognitive function including working memory, for which transmission of such catecholamines as dopamine and noradrenaline transmission in the prefrontal cortex (PFC) are crucial. Since catecholamine synthesis depends on the rate-limiting enzyme, tyrosine hydroxylase (TH), TH is thought to play an important role in PFC function. In this study, we found that two distinct population existed in Sprague-Dawley rats in terms of working memory capacity, one with higher working memory capacity, and the other with low capacity. This distinction of working memory capacity became apparent after rats were exposed to chronic stress. In addition, such working memory capacity and alterations of working memory function by chronic stress were associated with TH expression in the PFC.

The prefrontal cortex (PFC) is one of the central brain regions that mediate cognitive function [1]. The PFC receives catecholamine inputs from the ventral tegmental area (VTA) and locus coeruleus (LC), where dopamine (DA) and noradrenaline (NA) neurons, respectively, are located [2,3]. These catecholamines in the PFC are crucial for such cognitive function as working memory [2,3]. In this regard, tyrosine hydroxylase (TH), which is the rate-limiting enzyme for catecholamine synthesis ([4]), is thought to play a critical role in PFC function. TH is expressed not only in DA and NA cell bodies, but also localized in varicosities of catecholamine axonal fibers that project into the PFC, suggesting that DA and NA are locally synthesized in the PFC.

Chronic stress yields devastating effects including cognitive dysfunction [5]. In rodents, working memory has been reported to be disrupted by chronic stress [6]. Studies with microdialysis analyses have revealed that DA transmission is decreased in the PFC of rodents exposed to chronic stress [6,7]. In contrast, chronic stress has been also shown to increase TH expression in VTA DA [8] and LC NA neuron cell bodies [9]. It has remained unknown whether TH expression in axons of catecholamine neurons projecting into the PFC is affected by chronic stress. In particular, working memory has been shown to depend on both DA and NA transmission in the PFC [2,3]. Moreover, chronic stress also affects both DA [6,7] and NA [7] release in the PFC. Collectively, the effects of chronic stress on working memory could be produced by combined alterations of both DA and NA, but neither DA or NA alone. Thus, TH appears to be a promising candidate that is influenced by chronic stress, as this enzyme is involved in synthesis of both DA and NA.

In this study, we examined whether TH expression in the PFC of rats was associated with working memory, and whether such association was modulated by chronic stress.

All experiments were conducted in accordance with the *Canadian Council on Animal Care Guide to the Care and Use of Experimental*

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Animals, and approved by the McGill University. Male adult Sprague-Dawley rats were purchased from Charles River Laboratories (St-Constant, QC, Canada).

Chronic stress was given to rats by either placing them in a clear acrylic restrainer for 6 h per day for 21 days (i.e. chronic restraint stress), or placing them on a clear transparent acrylic platform (20 × 20 cm) elevated 1 m from the ground floor for 1 h per day for 14 days (i.e. chronic elevated platform stress). These chronic stress procedures for rodents have been utilized and well-documented in previous studies [10–16].

A spatial delayed alternation (SDA) task was conducted using the T-maze (Fig. 1A) to examine chronic stress effects on working memory [6,17]. In this task, animals had to learn a rule to turn into the left and right arm of the maze alternately to obtain rewards (cereals). Before training, rats were habituated to the maze during which they freely explored the maze for 20 min per day for 3 days. Rats were then trained to perform the task. Rats received training sessions, consisting of 24 trials per session per day, until they performed over 80% correct responses per session for three consecutive sessions. No inter-trial interval (ITI) was incurred in these training sessions. When animals made incorrect responses (i.e. entering into the same arm consecutively), additional correction trials were given until they made a correct response. Once rats completed training, three test sessions were conducted, in which ITIs were set at 0, 10, and 30 s, respectively, in each session. A percentage of correct responses (%CR) in each test session was recorded. Throughout the task, food restriction was given to animals, during which body weights of both stressed and control rats were carefully monitored, and maintained not to be lower than 85% of body weights of rats with ad libitum food access.

In the SDA task, 29 rats were tested. These animals were divided into those exposed to (1) restraint stress ($n = 13$), (2) elevated platform stress ($n = 10$), and (3) without stress ($n = 6$). Rats were first trained to perform the SDA task, and then subjected for stress exposure (Fig. 1A). The SDA task was conducted again following stress exposure (started one day after the last stress exposure). Performance before and after stress exposure were recorded. All data are expressed as means ± s.e.m. Statistical analysis was conducted using two-way repeated measures analysis of variance (ANOVA), with stress exposure (pre- vs. post-stress exposure) and delay conditions (0, 10, and 30 s delays) as within-subjects factors. Post hoc Tukey test was conducted for comparison of each group in ANOVA.

We also examined TH expression in the PFC using immunohistochemistry. On a next day of the last stress exposure or the last test session of the SDA task, rats were euthanized with a lethal dose of sodium pentoberbital (100 mg/kg, i.p.), and transcardially perfused with ice-cold 0.1 M phosphate buffer saline followed by 4% paraformaldehyde. Brains were removed from the skulls, and post-fixed with 4% paraformaldehyde. These brains were cryoprotected with 30% sucrose solution, and cut into 40 μm sections with the microtome. The sections were washed and incubated with 0.5% H_2O_2 to remove endogenous peroxides. Then, they were incubated with 2.5% horse serum to block non-specific binding of antibody. A primary antibody for TH (diluted at 1:1,000; the catalog number AB152; Millipore, Billerica, MA, USA) was incubated with the sections overnight at 4 °C. The sections were further incubated with secondary biotinylated goat anti-rabbit IgG (1:1,000; the catalog number ab6721; Abcam, Cambridge, MA, USA) for 2 h followed by avidin-biotin-peroxidase complex for another 2 h, and visualized by reaction with 3′3-diaminobenzidine (DAB; Sigma–Aldrich, Oakville, ON). DAB reaction on the sections were further intensified by the silver-gold intensification method, for which the sections were incubated in 1% silver nitrate for 1 h at 55 °C followed by 0.1% gold chloride for 10 min, and then 5% sodium thiosulfate for another 10 min at the room temperature. The sections were mounted on slide glasses, and hydrated by ethanol and xylene. Antibody bindings against TH varicosities were examined using the light microscope. Images were acquired and stored into the computer through the CCD camera connected to the microscope for later off-line analysis of images. Using the particle analysis function of the ImageJ software (National Institutes of Health, USA), dot-like TH immunostaining that corresponded varicosities on catecholamine fibers in the superficial (layer II–III) and deep (layer V–VI) layers of the prelimbic (PL) and infralimbic (IL) cortex were quantified. Such quantification of varicosities resulted in more accurate TH immunostaining measurements than optodensitometric analysis, as it could exclude background artifact (e.g. blood vessels) staining.

Twenty-two rats, which were a different bunch of rats from those 29 rats used in the first SDA task, were used for TH immunohistochemistry. Among these 22 rats, 10 rats were exposed to elevated platform stress, and the other 12 rats were stress-naive, controls. Rats were first subjected to perform the SDA task to examine a working memory capacity, which was expressed as the difference of %CR between the 0 and 30 s delay conditions. Such a working memory capacity was subsequently correlated with quantification of TH immunostatining in each rat. Statistical analysis was conducted using unpaired *t*-test or two-way ANOVA with stress (chronic stress vs. non-stress) and sub-regions within the PFC (PL vs. IL + superficial vs. deep layers) as independent variables.

Rats before chronic stress exposure exhibited delay-dependent performance, with %CR getting lower as longer the delays were ($n = 13$; Fig. 1B). After three weeks of chronic restraint stress, these rats exhibited significantly lower %CR than that before stress exposure at the 0 s delay (stress, $F_{1,36} = 5.62$, $p = 0.021$; delay, $F_{2,36} = 5.87$, $p = 0.005$; stress × delay, $F_{2,36} = 1.44$, $p = 0.245$; post hoc test, $p = 0.007$ between pre- vs. post-stress at the 0 s delay), whereas %CR was not different between before and after stress exposure at the 10 and 30 s delays (Fig. 1B). This worsened performance after chronic stress exposure at the 0 s delay was not due to sub-optimal performance, since %CR at the 0 s delay in stress-naive rats ($n = 6$), which were trained for the SDA task and subjected for the task again 3 weeks after a blank period, was comparable between before and after the blank period (paired *t*-test comparing before and after the blank period, $p = 0.61$ at the 0 s delay; $p = 0.50$ at the 10 s delay; $p = 0.47$ at the 30 s delay; Fig. 1B). Moreover, motivation for food rewards in stressed rats was not decreased compared to that of control rats, as indicated by comparable engagement and performance of the SDA task at the longer delay (10 and 30 s) conditions between stressed and control rats. A similar alteration pattern on performance of the SDA task was also observed in rats exposed to chronic elevated platform stress (i.e. significant %CR decrease at the 0 s, but not other delay conditions after stress exposure; $n = 10$; stress, $F_{1,27} = 3.48$, $p = 0.066$; delay, $F_{2,27} = 4.56$, $p = 0.018$; stress × delay, $F_{2,27} = 1.66$, $p = 0.199$; post hoc test, $p = 0.023$ between pre- vs. post-stress at the 0 s delay; Fig. 1 C). Therefore, rats with restraint and elevated platform stress were combined for further analysis. These results were unexpected, as a working memory deficit based on previous studies [6,17,18] was expressed as a greater impairment of performance in a longer delay than that in a shorter or no delay condition. Therefore, we investigated further details of the chronic stress-induced alterations of SDA task performance.

Further analysis unveiled a bi-modal distribution of SDA task performance in chronically stressed rats ($n = 23$, rats with restraint and elevated platform stress; Fig. 1D). In one group of rats ($n = 13$; denoted "Group 1" hereafter), %CR at the 30 s delay was significantly lower than that at the 0 s delay (Fig. 1D). In contrast, rats in the other group ($n = 10$; denoted "Group 2" hereafter) exhibited lower %CR at the 0 s delay than that at the 30 s delay (Fig. 1D). In the Group 1, chronic stress worsened performance at the 30 s delay (stress, $F_{1,36} = 9.35$, $p = 0.003$; delay, $F_{2,36} = 24.6$, $p < 0.001$; stress × delay, $F_{2,36} = 1.97$, $p = 0.148$; post hoc test, $p = 0.002$ between pre- vs.