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Short Communication

Chronic predator scent stress alters serotonin and dopamine levels in the rat thalamus and hypothalamus, respectively

Eliyahu Dremencov[1,2,3], Maxim Lapshin[3], Maria Komelkova[3], Anatoli Alliluev[3], Olga Tseilikman[3], Marina Karpenko[4], Nina Pestereva[4], Eugenia Manukhina[5,6], H. Fred Downey[6] and Vadim Tseilikman[3]

[1] *Institute of Molecular Physiology and Genetics, Centre for Biosciences, Slovak Academy of Sciences, Bratislava, Slovakia*
[2] *Institute of Experimental Endocrinology, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia*
[3] *School of Medical Biology, South Ural State University, Chelyabinsk, Russia*
[4] *Institute of Experimental Medicine, Saint Petersburg, Russia*
[5] *Institute of General Pathology and Pathophysiology, Moscow, Russia*
[6] *University of North Texas Health Science Center, Fort Worth, TX, USA*

Abstract. The aim of this study was to investigate the effect of chronic predator scent stress (PSS) on monoamine levels in rat thalamus and hypothalamus. Rats were exposed to the PSS (sand containing cat urine) for ten minutes daily for ten days. Control animals were exposed to the sand containing clean water. Fifteen days later, rats' behavior and thalamic and hypothalamic levels of monoamines were analyzed. PSS rats had elevated anxiety, increased thalamic serotonin and decreased hypothalamic dopamine concentrations. This decrease in hypothalamic dopamine may explain, at least in part, lowered corticosterone levels observed in PSS animals in our previous studies.

Key words: Post-traumatic stress disorder — Predator scent stress — Serotonin — Norepinephrine — Dopamine — HPLC

Repeated exposure of the rats to the predator scent stress (PSS) has been used in our previous studies as an animal model of post-traumatic stress disorder (PTSD). We had previously reported that repeated PSS induce long-lasting behavioral changes, such as increased anxiety index (AI), observed as late as fourteen days after the last PSS exposure (Lazuko et al. 2018; Manukhina et al. 2018). Repeated PSS might be a more accurate model for PTSD than the acute one, since it minimizes the effect of uncontrolled factors, such as concentration of pheromones in each individual dose of urine. Indeed, rats repeatedly exposed to the PSS have showed some abnormalities which were not observed after the acute exposure, such as decreased plasma corticosterone levels and adrenal hypotrophy (Manukhina et al. 2018).

Correspondence to: Vadim E. Tseilikman, School of Medical Biology, South Ural State University, 76 Lenin Ave, Chelyabinsk, Russia 454080
E-mail: vadimed@yandex.ru

Monoamine (serotonin or 5-HT, norepinephrine, and dopamine) systems of the brain are fundamental in the memory, cognition, and emotions (Stahl et al. 2014), the functions which are impaired in PTSD (American Psychiatric Association. DSM-5 Task Force. 2013). Indeed, monoamine abnormalities in PTSD were reported (Feduccia and Mithoefer 2018).

Majority of studies on neurophysiology and neurochemistry of PTSD are focusing in prefrontal cortex, hippocampus, amygdala, nucleus accumbens, and related limbic brain areas. The research on these brain areas has provided highly relevant information on the etiology of PTSD. However, the role of other brain areas, such as thalamus and hypothalamus, remained relatively under-investigated. Thalamus is a hub for all sensory information entering the brain. Certain thalamic nuclei, such as paraventricular nucleus, regulates emotional response to the sensory stimuli (Hsu et al. 2014). Since the inadequate emotional response to the certain sensory stimuli is a key feature of PTSD, thalamus might play a role in pathophysiology of this disorder. Indeed,

recent studies showed that thalamic neural circuits are involved in the response to the threatening sensory stimuli (Dunkley et al. 2018) and in the fear extinction (Silva et al. 2018) in PTSD.

Hypothalamus is fundamental in the central regulation of endocrine functions, including the stress hormone signaling. Altered functioning of the hypothalamic-pituitary-adrenal axis was detected in patients with PTSD (Cooper et al. 2017) and in an animal model of this disorder (Boero et al. 2018). Finally, thalamic-hypothalamic interconnections are important in the choosing of the coping strategy to the predatory threats in rodents (Cezario et al. 2008). In this study, we aimed to investigate the role of thalamic and hypothalamic monoamine transmission in PTSD, using our animal model of this illness.

Adult male Wistar rats were used in all experiments. Rats were housed in standard cages (4–5 animals/cage) and received food and water *ad libitum*. The animals were maintained at controlled temperature (22–25°C) and humidity (55%). A 12:12 h light-dark cycle was maintained with lights on between 7:00 and 19:00. All animal procedures were performed in accordance with the U.S. National Research Council Guide for the Care and Use of Laboratory Animals (publication 85-23, revised 2011), and experimental protocols were approved by the Animal Care and Use Committee of the Institute of General Pathology and Pathophysiology, Moscow, Russia.

The rats were randomly divided to two groups: control ($n = 7$) and PSS ($n = 9$). The sand containing fresh cat urine was collected daily from the litterbox of a domestic cat. This sand was stored at the room temperature in the closed plastic container for 3–5 h before the experiment. Twenty gram of this sand was laid in a Petri dish covered with nylon tissue and placed in the home cage of PSS rats for 15 min daily, between 13:00 and 14:00, during 10 consecutive days. Control rats were exposed to the sand containing clean water. Control and PSS rats were kept in different rooms with separate air conditioning systems. After the last exposure, the rats were left intake for 15 days. On the 15th day after the last exposure, the rats' level of anxiety was measured using the elevated plus maze (EPM) test, as previously described Lazuko et al. (2018), Manukhina et al. (2018). The AI was calculated using the formula: AI = 1 – [(time in open arms/total time on maze) + (number of entries into open arms/number of all entries)]/2.

Twenty-four hours after the EPM test the rats were decapitated and their brains were removed. The whole left and right thalami (7–10 mm posterior from the frontal pole, 3–6 mm dorsal from the ventral brain surface, and 0–3 mm lateral from the midline) and hypothalami (7–10 mm posterior from the frontal pole, 0–3 mm dorsal from the ventral brain surface, and 0–2 mm lateral from the midline; Paxinos and Watson 2014) were isolated and frozen in liquid nitrogen for the subsequent neurochemical assessments, which were performed within seven days after the tissue collection. For the quantification of monoamines, brain tissue was homogenized in 0.1 M perchloric acid. After homogenization the samples were centrifuged (7000 × *g* for 15 min at 4°C) and the supernatants were filtered through a syringe filter (0.2-micron pore size; Whatman, USA) before the high performance liquid chromatography (HPLC) analysis. The HPLC analysis was performed on a Hypersil BDS C18 reversed-phase column (250 × 4.6 mm, 5 μm) under isocratic conditions, with electrochemical detection. The mobile phase consisted of a 75 mM phosphate buffer containing 2 mM citrate acid, 0.1 mM octanesulfonic acid, and 15% (v/v) acetonitrile (pH 4.6). Electrochemical detection was achieved by setting a glassy carbon working electrode at +780 mV. The final amount of monoamines in tissue sample was expressed as pg/mg of tissue, using an external calibration curve.

PSS rats showed significant ($p < 0.05$, two-tailed Student's *t*-test) increase in time spent in closed arms and decrease in time spent in open arms during the EPM, which results in a higher AI (Table 1). The relatively small size of the PSS group did not allow statistical distinguishing between PSS-resistant and vulnerable animals as in our previous study (Tseilikman et al. 2017); however, all but one PSS animals showed the AI > 8. An exposure to the PSS increased thalamic 5-HT (259.33 ± 17.11 pg/mg of tissue *versus* 199.29 ± 12.62 pg/mg of tissue in controls, $p < 0.05$, two-tailed Student's *t*-test, Fig. 1A) and decreased hypothalamic dopamine concentrations (321.67 ± 49.22 pg/mg of tissue *versus* 472.67 ± 25.3333 pg/mg of tissue in controls, $p < 0.05$, two-tailed Student's *t*-test, Fig. 2C); norepinephrine levels were not different between the groups (Fig. 1B and 2B).

The decrease in animals AI after chronic PSS, observed two weeks after the last exposure to the stressor, is similar to this reported in our previous studies (Lazuko et al. 2018; Manukhina et al. 2018).

Table 1. Effect of chronic predator scent stress (PSS) on the rats' behavior using the elevated plus maze (EPM) test

Group	*n*	N (Open)	N (Closed)	T (Open)	T (Closed)	AI
Control	7	7.43 ± 1.19	3.71 ± 0.81	70.00 ± 11.13	530.00 ± 11.13	0.77 ± 0.02
PSS	9	7.44 ± 0.73	2.33 ± 0.29	37.78 ± 7.03*	562.22 ± 7.03*	0.85 ± 0.02*

n, number of animals; N (Open), number of entrances open arms; N (Closed), number of entrances to closed arms; T (Open), time spent in open arms; T (Closed), time spent in closed arms; AI, anxiety index; * $p < 0.05$ *vs.* control, two-tailed Student's *t*-test.

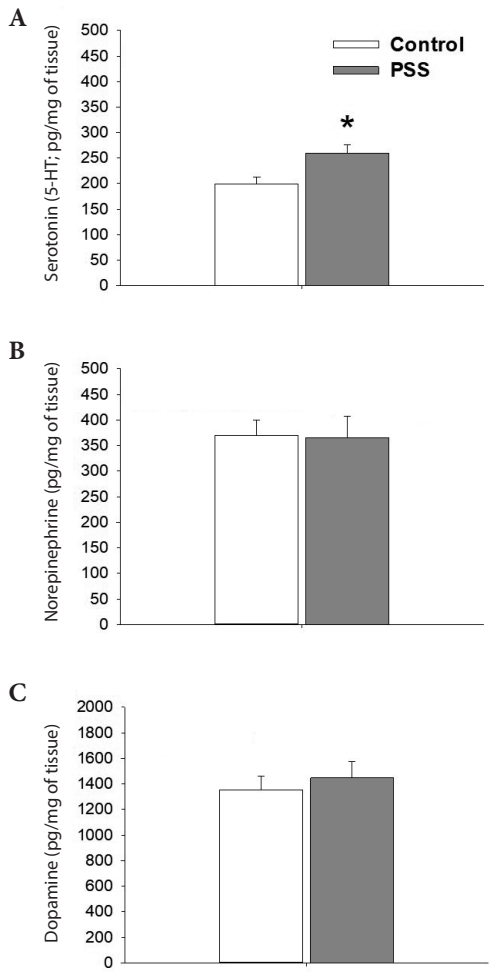

Figure 1. Effect of chronic predator scent stress (PSS) on serotonin (5-HT, **A**), norepinephrine (**B**), and dopamine (**C**) levels in rat thalamus. * $p < 0.05$ *vs.* control, two-tailed Student's *t*-test.

Our funding on the altered 5-HT transmission in the thalamus is consistent with previous studies by other groups. Thus, Nikolaus and colleagues (Nikolaus et al. 2010) reported decreased 5-HT transporter (SERT) density in the mesencephalon of patients with PTSD.

We found that PSS did not affect thalamic or hypothalamic norepinephrine and decreased hypothalamic dopamine levels. However, a previous study by Browne and colleagues (Browne et al. 2014) reported a stress-induced decrease in the hypothalamic catecholamines in mice with low fear-sensitized acoustic startle reflex. These differences might be explained by different types of stressors (PSS *versus* acute swim test) and different animals (rats *versus* mice).

Previous clinical studies reported that the dopamine agonist bromocriptine lowered adrenocorticotropic hormone (ACTH) concentrations in patients with Cushing's and Nelson's syndromes, and subsequent *in vitro* studies conformed that dopamine decreased ACTH release from cultured human corticotropic adenoma cells (Engler et al. 1999). It is therefore possible that the decreased corticosterone levels, observed in our previous studies with PSS animals, are caused, at least partially, by abnormal hypothalamic dopamine transmission.

To the authors best knowledge, this study was first to examine the effect of PSS on monoamine transmission in the thalamus and hypothalamus. Based on the results provided in this study, it can be suggested that the alterations in thalamic 5-HT and hypothalamic dopamine might play a role in pathophysiology of PTSD. As a pilot study, it did not distinguish between different thalamic and hypothalamic nuclei, as well as between intracellular and extracellular monoamines. Intracellular and extracellular levels of monoamines within the specific thalamic and hypothalamic nuclei should be evaluated in the future studies, using more

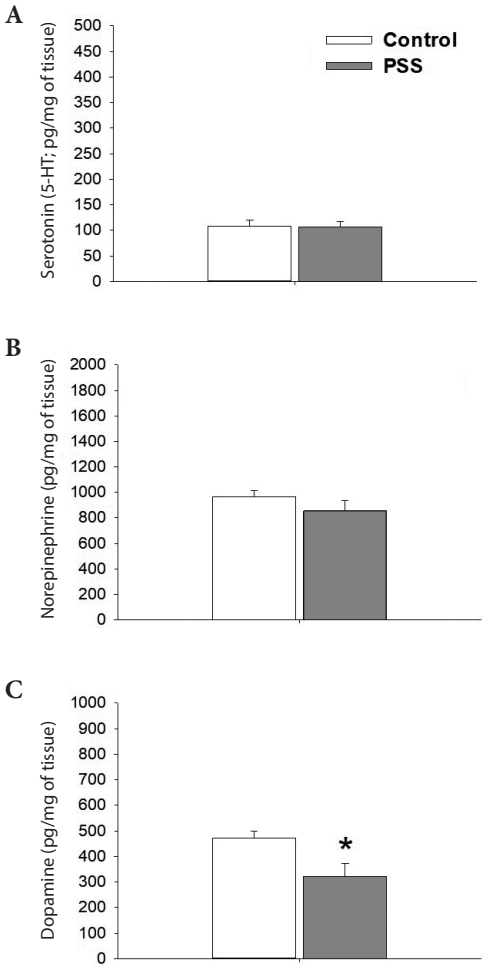

Figure 2. Effect of chronic predator scent stress (PSS) on serotonin (5-HT; **A**), norepinephrine (**B**), and dopamine (**C**) levels in rat hypothalamus; * $p < 0.05$ *vs.* control, two-tailed Student's *t*-test.

advanced research methodologies, such as combination of brain imaging and microdialysis.

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