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New Morphofunctional Criteria for Resistance Profile in Post-Traumatic Stress Disorder Models as Adrenal Dysfunction Trigger

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Abstract—For the first time in modeling posttraumatic stress disorder (PTSD), we have described the morphofunctional state of adrenal glands in Wistar rats resistant and sensitive to predator stress (rodent fear of the predator). Despite the evident signs of adrenal dysfunction in both phenotypes, we have discovered the thickening of undifferentiated cell zone and high indices of functional activity of stem cells in resistant animals, suggesting ample adaptation. The most important data demonstrate the direct relationship between the reduction of corticosterone and testosterone levels and adrenal dysfunction in PTSD models. The study results allow considering the adrenal stem cells as potential therapeutic targets.

Keywords: Wistar rats, post-traumatic stress disorder, morphofunctional characteristics of the adrenal glands, adrenal stem cells, stress resistance

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INTRODUCTION

A dangerous disease provoked by the viruses of coronavirus family primarily broke out in China in the end of 2019 and quickly spread across continents. In January 2020, the World Health Organization (WHO) has officially named this coronavirus COVID-19, and in March a pandemic has been declared. Pandemics and epidemics are always characterized by numerous negative sociopolitical, economic, sociopsychological, and situational consequences, hence the negative contribution to the mental health of population. In the middle of 2020 already multiple evidences have emerged, suggesting the high incidence of posttraumatic stress disorder (PTSD) symptoms in the world population. The typical features of PTSD firstly include delayed symptom manifestation and secondly, the development of disease only in certain individuals exposed to stress [1]. Thus, for instance, according to the epidemiological research, the incidence of PTSD and depression was 2.7 and 9.0% among 2450 Chinese home-quarantined university students, respectively [2]. Therefore, currently there is an urgent need to actively study the mechanisms of adaptive response in PTSD. Presently, the studies are prioritized that consider in details the reaction of human and animal organisms sensitive and resistant to PTSD. The definition of molecular, cellular, and morphofunctional background for the individual variations in stress responses is pivotal for the understanding of PTSD pathophysiology and treatment [3, 4]. Until now the morphofunctional state remains unclear for the adrenal glands, which are the crucial source of hormone regulators of many key processes in the organisms of mammals and important elements of hypothalamic–pituitary–adrenal system. There has been no researches comparing the adrenal glands of specimens sensitive and resistant to stress.

The purpose of this study was to model PTSD, determine the integrated parameters of morphofunctional state of adrenal glands in Wistar rats, and compare these parameters in animals resistant and sensitive to stress.

MATERIALS AND METHODS

The study was conducted in autumn and winter period on mature male Wistar rats (n = 60) initially

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weighing 180–200 g. The animals were kept in groups of 10. PTSD was simulated using the generally accepted predator stress model, an evolutionary selective rodent fear of predators and their scent, established by Cohen and Zohar and improved by Tseilikman et al. [5, 6]. The researches of Voznessenskaya et al. and Apfelbach et al. discovered that the influence of scent on the predator was so significant for the rodents that it could activate both behavioral flight response and abortion in pregnant females [7–9]. The animals were divided into two groups, group 1 containing intact rats (n = 20) and group 2 of animals exposed to predator stress (cat urine) for 10 min during 10 days, further kept in the normal conditions for 14 days (PTSD) (n = 40).

To identify the behavioral symptoms of PTSD, animals were tested for 10 min (600 s) in the Elevated Plus Maze (EPM) in the end of the experiment. Anxiety index (AI) elaborated by Cohen et al. was calculated using the equation $AI = 1 - [(TOA/TTM + NEOA/TEM)/2]$, where TOA is the time spent in the open arms of EPM; TTM is the total time in the maze, s; NEOA stands for the number of entries to the open arms; TEM is the total exploration on the maze [10].

Animals were sacrificed by Zoletil overdose. Left adrenal glands were fixated in Bouin's solution and embedded in paraffin. Histological equatorial sections of adrenal glands, 4–5 μm in thickness were stained with hematoxylin and eosin. The AxioVision morphometric software was applied to microphotographs, obtained with an Axioplan 2 imaging microscope, to perform 10 thickness measurements of functional zones of adrenal cortex and the karyon diameter of endocrionocytes. The index of functional activity (FAI) of adrenocorticocytes of each zone was calculated using the equation $FAI = (FZT \times MCD)/100$, FZT standing for functional zone thickness; MCD, mean karyon diameter calculated as $\sqrt{Max \times Min}$ [1].

With the help of immune enzyme-linked immunosorbent assay (ELISA), the levels of IBL corticosterone (Germany) and DBC testosterone (Canada) were measured in the blood serum of rats using an ANTHOS immunoassay analyzer (Austria).

The experimental groups were compared by means of ANOVA using the Kruskall–Wallis test for multiple comparisons and Mann–Whitney U test for pairwise comparison. The results were presented as Me (LQ; UQ), i.e., the median, lower and upper quartiles. The differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

It is well known that PTSD does not develop in all subjects, humans or animals, exposed to the equal amount of stress [1, 2]. Tests of rats in EPM showed that subjects sensitive to stress were characterized by more prominent anxiogenic effect compared to the control and stress-resistant animals, manifesting in the decrease of time spent in the open arms of EPM, number of entries to the open arms, and locomotor activity (Table 1). The parameters of resistant subjects differed from the control ones only by the time spent in the open arms that was 5.6 times higher than in sensitive subjects, though also tended to decrease. The population phenotyping in addition to the aforementioned parameters was justified by the integrated parameter, particularly the anxiety index that was statistically higher in stress-sensitive animals, whereas in resistant subjects it was not different from the control (Table 1).

The comparison of corticosterone and testosterone levels in the blood serum demonstrated that the decrease in measured hormone levels of all rats exposed to stress (Table 1). The reduction of corticosterone level is a generally accepted index of PTSD-like state development [6, 10, 12, 13]. Nevertheless, the values of corticosterone levels in resistant rats were statistically higher than in sensitive ones (Table 1). Our previous experiment showed that rats later becoming sensitive to stress prior to stress modeling had a higher corticosterone level. We managed to determine that cytochrome P4503A-mediated metabolism of glucocorticoids was prevailing in stress-resistant animals, whereas the metabolism of stress-sensitive animals depended from 11β-hydroxysteroid dehydrogenase type 2 [13].

The lowest testosterone level was registered in sensitive subjects (Table 1). Similar decrease in testosterone level was detected in combatants diagnosed with severe PTSD [14, 15]. Moreover, the reduction of testosterone level, the major anabolic hormone, is normally related to the risk of coronary artery disease and other cardiovascular disorders [16, 17].

It should be emphasized that the integrated parameter of testosterone to corticosterone ratio decreased in the animals sensitive to stress (Table 1). This suggested the preponderance of catabolic processes over anabolic ones in stress-sensitive rats, predisposing to the failure of functional systems in the presence of additional risk factors. Besides, this parameter did not differ from the control in resistant animals (Table 1).

The analysis of morphological state of adrenal cortex representing the condition of hypothalamic–pituitary–adrenal system discovered that the development of PTSD-like state was associated with the thinning of cortical layer of adrenal glands by 46.1–44.3%, caused by the reduction of thickness of zona fasciculata by 48.1–50.2% and zona reticularis by 43.9–50.1% (Table 2). In addition, the integrated parameter of cell functional activity index (CFAI) of these zones declined statistically in PTSD models (Table 2). Furthermore, CFAI of the zona fasciculata in resistant rats was statistically higher than in sensitive ones, whereas CFAI values in the zona reticularis were similar (Table 2). Nevertheless, more extensive morphofunctional transformations were seen in the animals

Table 1. Behavioral parameters of Wistar rats in Elevated Plus Maze, testosterone and corticosterone levels in blood serum in PTSD models Me (LQ; UQ)

Parameter	Control (1)	Stress-resistant rats (2)	Stress-sensitive rats (3)
Time spent in the open arms, s	178.6 (136.3; 228.8)	106.7*# (55.1; 165.1) $p1-2 = 0.0029$ $p2-3 = 0.0001$	19.2 (67.1; 38.3)*# $p1-3 = 0.0001$
Number of entries to the open arms, c.u.	4.9 (2.8; 9.8)	5.2 (3.9; 6.3)# $p1-2 = 0.7103$ $p2-3 = 0.0001$	1.7 (1.0; 2.7)*# $p1-3 = 0.0011$
Total exploration on the maze, c.u.	16.2 (11.1; 22.8)	17.5 (15.7; 25.1)# $p1-2 = 0.5416$ $p2-3 = 0.0001$	8.6 (4.2; 12.1)*# $p1-3 = 0.0004$
Anxiety index, c.u.	0.72 (0.64; 0.81)	0.72 (0.64; 0.79)# $p1-2 = 0.9250$ $p2-3 = 0.0007$	0.91 (0.67; 0.75)*# $p1-3 = 0.0001$
Testosterone (T), nmol/L	14.3 (8.5; 22.9)	8.7 (5.6; 13.7)*# $p1-2 = 0.0401$ $p2-3 = 0.0014$	4.1 (2.8; 4.8)*# $p1-3 = 0.0004$
Corticosterone, (Cor) nmol/L	436.5 (309.9; 524.9)	275.5*# (194.3; 343.2) $p1-2 = 0.0004$ $p2-3 = 0.0014$	166.8*# (116.2; 233.2) $p1-3 = 0.0004$
T/Cor ratio	0.039 (0.021; 0.065)	0.035# (0.024; 0.051) $p1-2 = 0.7192$ $p2-3 = 0.0235$	0.025*# (0.018; 0.041) $p1-3 = 0.0412$

* Significant differences from the control; # significant differences between the experimental groups.

sensitive to stress, showing more prominent reduction of thickness of the zona glomerulosa compared to the control and resistant rats by 18.2 and 14.4%, respectively, in addition to the mentioned changes, accompanied by the lowest CFAI values of this zone (Table 2). On the contrary, CFAI values of the zona glomerulosa in resistant rats were the highest, exceeding the control values by 24 and 29.2% for stress-sensitive subjects (Table 2).

In 1994 a group of Japanese scientists have identified and characterized a new zone of adrenal glands in rats located between the zona glomerulosa and zona fasciculata called undifferentiated cell zone [18]. Further researches have found that cells of this zone are stem cells maintaining the cellular composition of the zona glomerulosa and zona fasciculata [19]. In our experiment the thickness of undifferentiated cell zone approximated the control values in sensitive subjects, whereas the resistant animals showed a substantial enlargement of undifferentiated cell zone exceeding the control values by 89.5% and values in sensitive animals by 77.1% (Table 2). In addition, the integrated parameter CFAI was the highest in undifferentiated cell zone of stress-resistant rats among all groups, exceeding the control values 2.5 fold and values in sensitive animals 2.7 fold. The thickening of undifferentiated cell zone and increase in CFAI of resistant subjects most likely represented expressed activation of adaptive processes in hypothalamic-pituitary-adrenal system.

It is known that the adrenal glands are enveloped by a fibrous capsule consisting of bundles of collagen and elastic fibers, several layers of fibrocytes and smooth muscle cells parallel to the outer gland surface. It is thought that the adrenal capsule contains stem/progenitor cells capable of producing gonadal-like cells [16]. PTSD modeling in our research detected the thinning adrenal capsule in rats resistant and sensitive to stress compared to the control ones. Nevertheless, it should be emphasized that the capsule thickness in stress-sensitive rats was 43.4% less than in resistant animals. Probably, the higher level of testosterone in more resistant subjects than in sensitive ones is associated with the ability of capsular stem/progenitor cells to produce sex steroids.

Therefore, our study is the first to discover personalized morphofunctional differences in the adrenal glands of Wistar rats resistant and sensitive to the predator stress. Despite the evident signs of adrenal dysfunction in both phenotypes, thickening of undiffer-

Table 2. The parameters of morphofunctional state of adrenal cortex in Wistar rats resistant and sensitive to stress in PTSD models Me (LQ; UQ)

Parameter	Control (1)	Stress-resistant rats (2)	Stress-sensitive rats (3)
Thickness of adrenal cortex, μm	1094.1 (699.4; 1451.9)	608.2* (549.1; 645.6) $p1{-}2 = 0.0001$ $p2{-}3 = 0.2822$	589.5* (550.7; 634.5) $p1{-}3 = 0.0001$
Thickness of adrenal capsule, μm	12.9 (8.9; 16.5)	10.8 (8.1; 12.6)*# $p1{-}2 = 0.037$ $p2{-}3 = 0.0001$	7.2 (6.9; 8.8)*# $p1{-}3 = 0.0001$
Thickness of undifferentiated cell zone, μm	17.1 (12.3; 21.4)	32.4 (27.9; 36.4)*# $p1{-}2 = 0.0001$ $p2{-}3 = 0.0001$	18.3 (15.2; 21.6)# $p1{-}3 = 0.3042$
Thickness of zona glomerulosa, μm	58.5 (53.9; 64.7)	55.5 (49.1; 62.6)# $p1{-}2 = 0.1093$ $p2{-}3 = 0.0029$	47.5 (38.4; 57.2)*# $p1{-}3 = 0.0001$
Thickness of zona fasciculata, μm	421.3 (433.5; 609.9)	219.1* (186.8; 266.2) $p1{-}2 = 0.0001$ $p2{-}3 = 0.3320$	209.9* (183.2; 253.3) $p1{-}3 = 0.0001$
Thickness of zona reticularis, μm	467.6 (369.7; 570.9)	280.3* (258.4; 337.5) $p1{-}2 = 0.0001$ $p2{-}3 = 0.0463$	308.7* (261.1; 333.2) $p1{-}3 = 0.0001$
Cell functional activity index of undifferentiated cell zone, c.u.	0.85 (0.56; 1.31)	2.07 (1.71; 2.78)*# $p1{-}2 = 0.0001$ $p2{-}3 = 0.0031$	0.79 (0.48; 1.2)# $p1{-}3 = 0.4712$
Cell functional activity index of zona glomerulosa, c.u.	2.5 (1.8; 2.8)	3.1 (2.8; 3.7)*# $p1{-}2 = 0.0001$ $p2{-}3 = 0.0006$	2.4 (2.1; 2.9)# $p1{-}3 = 0.6621$
Cell functional activity index of zona fasciculata, c.u.	22.7 (15.5; 27.5)	12.1 (10.6; 15.1)*# $p1{-}2 = 0.0001$ $p2{-}3 = 0.0019$	9.9 (8.3; 11.2)*# $p1{-}3 = 0.0001$
Cell functional activity index of zona reticularis, c.u.	20.5 (17.1; 24.9)	12.3 (11.1; 14.7)* $p1{-}2 = 0.0001$ $p2{-}3 = 0.4031$	12.9 (10.4; 14.4)* $p1{-}3 = 0.0001$

* Significant differences from the control, # — significant differences between the experimental groups.

entiated cell zone and high indices of functional activity of stem cells for this zone in resistant animals suggested the recovery of morphofunctional state of the adrenal glands in these animals, i.e., ample adaptation. The most important data demonstrated the direct relationship between the reduction of corticosterone and testosterone levels and adrenal dysfunction in PTSD models. The study results allow considering the adrenal stem cells as potential therapeutic targets.

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COMPLIANCE WITH ETHICAL STANDARDS

All experimental procedures were conducted in accordance with the Regulations for the Conduct of Research with Experimental Animals (1984) and the Directive

2010/63/EU on the Protection of Animals Used for Scientific Purposes (September 22, 2010).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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