DOI 10.1007/s10517-020-04761-5

The Levels of Monoamines and Their Metabolites in the Brain Structures of Rats Subjected to Two- and Three-Month-Long Social Isolation

N. A. Krupina[1], N. N. Khlebnikova[1], V. B. Narkevich[2], P. L. Naplekova[2], and V. S. Kudrin[2]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 168, No. 11, pp. 540-545, November, 2019
Original article submitted July 5, 2019

The levels of monoamines and their metabolites in the brain structures of adult Wistar rats subjected to post-weaning social isolation for 2 and 3 months were analyzed by HPLC with electrochemical detection. We have previously shown that these rats consistently demonstrate increased aggressiveness and, as a rule, impairment of short-term habituation. Two-month-long social isolation was accompanied by a reduction in serotonin content and its increased turnover judging from the 5-HIAA/5-HT ratio in the hippocampus; three-month-long isolation was associated with increased levels of serotonin and reduction in its turnover in the amygdala. At this term, the level of dopamine metabolite 3-methoxytyramine tended to increase in the amygdala. In the frontal cortex, a tendency to a decrease in 5-HT level was found. These findings suggest that more prolonged post-weaning social isolation is accompanied by reorganization of neural networks in the brain cortex, which can serve as the pathophysiological basis for psychoemotional disorders.

Key Words: *post-weaning social isolation; model; emotional and motivational disorders; monoamines; rat brain structures*

In the early postnatal period, the brain is highly sensitive to adverse events [15]. Numerous studies demonstrated that stressing during the early period of development leads to disruption of the central nervous system and various neuropsychiatric disorders, such as depressive and psychotic symptoms, anxiety, and increased aggressiveness [12]. Early social isolation is among aversive stress-events [4]. It is often modeled by single-housed rearing of rats immediately after weaning [6,7,15]. Social isolation if started during critical pre-adolescence period (postnatal days 21-28, PND21-28) usually leads to numerous behavioral disorders [11]. The pre- and mid-adolescent periods (PND21-46) when rats are most sensitive to stress, are characterized by significant changes in the monoamine content in the brain [11,14]. Typically, social isolation in the experiment lasts for 4-9 weeks [6,7,13]. The most common consequence of early stress exposure is the development of behavioral abnormalities, such as hyperactivity, increased social interaction and aggression, cognitive impairment, deficit of prepulse inhibition (judging from acoustic startle-response, index of the psychotic state), sometimes increased anxiety and less often depression-like disorders appear [6,11,15]. The data on the effect of social isolation on monoaminergic brain systems are contradictory, which can be related to high diversity of observed behavioral disturbances and different duration of early social isolation [6,7,13,15].

In our previous studies, 2- and 3-month-long social isolation led to increased aggression in the test of social contacts and impairment of short-term habituation (non-

[1]Laboratory of General Pathology of the Nervous System, Research Institute of General Pathology and Pathophysiology; [2]Laboratory of Neurochemical Pharmacology, V. V. Zakusov Research Institute of Pharmacology, Moscow, Russia. ***Address for correspondence:*** krupina-na@yandex.ru. N. A. Krupina

associative learning) in tests assessing sensorimotor reactivity and motor activity (Table 1) [1,2].

Here we analyzed the levels of monoamines and their metabolites in the brain structures of rats subjected to 2- and 3-month-long social isolation starting from weaning.

MATERIALS AND METHODS

The study was performed on male Wistar rats (born and bred in the vivarium of the Research Institute of General Pathology and Pathophysiology). Two series of experiments were carried out by the same scheme. The day of birth was considered as postnatal day 0 (PND0). On PND1, the females were allowed to feed 3-5 pups of the same age from different litters to minimize the influence of the genetic factor and to level the developmental conditions. Animals of the experimental groups were weaned during the fourth week of postnatal development (first series: PND24, second series: PND26) and housed in individual cages over two (series I, n=15) and three (series II, n=13) months. The control animals were not separated after weaning and were housed in groups of 2-5 animals per cage (series I, n=15; series II, n=13). The rats were kept under standard vivarium conditions at natural light/dark cycle with free access to water and food. The previously described changes in the behavior of the rats in these series are presented in Table 1.

Animal studies were performed in accordance with the Rules of Good Laboratory Practice approved by the Order No. 199n of the Ministry of Health of the Russian Federation (January 4, 2016) and the GOST Nos. 33215-2014 and 33216-2014 (Guidelines for Accommodation and Care of Animals) that fit European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986, appl. of June 15, 2006). The studies were carried out under the control of the Ethics Committee of Research Institute of General Pathology and Pathophysiology.

After completion of behavioral testing, the animals were decapitated at the age of 3 months (series I; 2 months of social isolation; PND97-98) and 4 months (series II; 3 months of social isolation; PND133). The brain was extracted, and frontal cortex, striatum, amygdala, and hippocampus were isolated on ice under visual control. In series II, the level of monoamines and their metabolites was determined in half of the animals in each group: 7 rats in the social isolation-group and 6 rats in the control group. Tissue samples were immediately frozen in liquid nitrogen, weighed, and then stored at -83°C until analysis by HPLC with electrochemical detection on an LC-304T chromatograph (BAS) with ReproSil-Pur ODS analytical column ReproSil_Pur ODS (C18, 100×4 mm, 3 μ; Dr. Maisch). An electrochemical detector LC_4B (BAS) was used. Sample preparation and methods for determining the level of monoamines in rat brain structures are described in detail previously [3]. The contents of norepinephrine (NE), DA and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine (3-MT), serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured. Monoamine concentrations in samples were calculated by the "internal standard" method, based on the ratio of peak areas in the standard mixture and in the sample, and expressed in nmol/g tissue.

Statistical data processing was performed using the algorithms of the Statistica 7.0 software. According to the results of the preliminary verification using Kolmogorov—Smirnov and Lilliefors tests, the hypothesis on normality of data distribution was rejected, and nonparametric unpaired Mann—Whitney U test for independent variables (two-sided) was used for comparative analysis. The accepted significance level was 5%. If the achieved level of statistical significance exceeded the critical value, the null hypothesis was accepted. The data are presented as $M \pm SEM$.

RESULTS

Three-month-old rats subjected to 2-month-long social isolation (series I) showed a statistically significant decrease in 5-HT levels and an increase in its turnover judging from the 5-HIAA/5-HT ratio in the hippocampus. No changes in the level of monoamines and their metabolites were detected in the frontal cortex, striatum, and amygdala (Tables 2, 3).

In 4-month-old rats subjected to 3-month-long social isolation (series II), a statistically significant increase in 5-HT levels and a decrease in its turnover judging from the 5-HIAA/5-HT ratio was revealed in the amygdala (Table 3). Also, a trend to an increase in the level of the DA metabolite 3-MT was observed in the amygdala. Moreover, a pronounced tendency to a decrease in the level of 5-HT in comparison with the control (rats housed in group) was found in the frontal cortex. No changes in the level of monoamines and their metabolites were detected in the striatum and hippocampus.

By the time of brain isolation for monoamine assay (*i.e.* after 2-month isolation in series I *vs.* 3-month isolation in series II), the pattern of behavioral disturbances in rats subjected to social isolation was similar in both series: increased locomotor activity, increased non-aggressive social interaction, increased aggressiveness, and decreased short-term habituation; no signs of depressive-like behavior and increased

TABLE 1. Behavioral Disorders in Rats Exposed to Early Social Isolation in Comparison with Animals Housed in Groups [1,2]

Parameter	Age of rats		
	2 months	3 months	4 months
	Duration of social isolation		
	1 month (series I)	2 months (series I и II)	3 months (series II)
Locomotor activity	=	Series I: ↑ Series II: =	↑
Depressive-like behavior	=	Series I: = Series II: =	=
Sensitivity to positive reinforcement	=	Series I: ↑ Series II: —	—
Non-aggressive social contacts	=	Series I: ↑ Series II: ↑	↑
Anxiety	=	Series I: = Series II: ↑	=
Aggression	↑	Series I: ↑ Series II: ↑	↑
Sensorimotor reactivity	—	Series I: amplitude, PPI = Series II: amplitude ↑ PPI ↓	—
Short-term habituation	—	Series I: ↓ Series II: ↓	↓

Note. Motor activity was assessed in the automated open-field test (under non-stress conditions) and the elevated plus-maze test; sensitivity to positive reinforcement was determined in an alternative sucrose-water selection test by sucrose consumption; anxiety was assessed in the elevated plus-maze test by a decrease in activity in the open arms of the maze; depressive-like behavior was assessed in the forced swimming test by the development of behavioral despair and in the alternative sucrose-water test by reducing sucrose preference, as well as by reducing the level of vital drinking motivation; non-aggressive social interaction and aggression were evaluated in the social contact test; sensorimotor reactivity — in the acoustic startle response by the amplitude and pre-pulse inhibition (PPI); short-term habituation — in the acoustic startle response according to the dynamics of reducing the relative amplitude of the acoustic startle-response or in the automated open-field test according to the dynamics of reducing motor activity. ↑ — increase, ↓ — decrease in at least one of the tests used; = no differences from the control. "—" — not assessed.

anxiety were found (Table 1). By these indicators, we revealed no aggravation of behavioral disturbances with increasing the duration of social isolation [2]. However, the pattern of changes in monoamine levels in rat brain structures differed in these series.

The decrease in serotonin content and increase in its turnover in the hippocampus after 2-month isolation observed by us were partially consistent with the results of previous studies [4] demonstrating a decrease in the serotonin content in the hippocampus of male Sprague-Dawley rats subjected to social isolation on PND28-100, which is comparable to 2-month social isolation in our study. In this work, the authors also observed an increase in serotonin and dopamine turnover in the hippocampus, but the rats showed signs of depressive-like behavior in the forced swimming test, while aggression was not evaluated. Impairment of presynaptic 5-HT-ergic function in the hippocampus of rat subjected to 2-month social isolation has been shown by many authors [10], but in our study, no signs of 5-HT system impairment in the hippocampus were observed after prolongation of social isolation to 3 months (judging from the levels of monoamines and their metabolites). More studies are required to explain this phenomenon.

When summarizing the results of many studies on the state of monoaminergic brain systems in social isolation of rodents [15], no data were found on the state of the 5-HT-ergic system in the amygdala of animals subjected to social isolation. In light of this,

TABLE 2. Contents of Norepinephrine, Dopamine, and Its Metabolites and Dopamine Turnover in Brain Structures of Rats Subjected to 2- and 3-Month-Long Social Isolation (Experiment) and Control Group-Housed Animals (Control) (*M±SEM*; nmol/g tissue)

Experimental conditions	Norepinephrine	Dopamine	DOPAC	3-MT	HVA	DOPAC/DA	HVA/DA
Frontal cortex (social isolation for 2 months)							
Experiment	1.87±0.08	0.77±0.05	0.32±0.05	0.27±0.06	0.28±0.05	0.42±0.06	0.38±0.07
Control	1.69±0.11	0.75±0.08	0.27±0.03	0.20±0.03	0.22±0.03	0.40±0.05	0.34±0.06
Frontal cortex (social isolation for 3 months)							
Experiment	2.06±0.16	0.50±0.07	0.22±0,03	0.47±0.20	0.18±0.04	0,55±0.15	0.38±0.08
Control	2.17±0.27	0.34±0.12	0.17±0.03	0.31±0.08	0.10±0.03	0.65±0.22	0.45±0.20
Striatum (social isolation for 2 months)							
Experiment	0.53±0.13	61.15±3.39	7.19±0.48	1.79±0.19	4.74±0.34	0.12±0.00	0.08±0.00
Control	0.94±0.21	60.68±6.72	7.12±0.80	1.79±0.21	3.87±0.42	0.16±0.04	0.09±0.02
Striatum (social isolation for 3 months)							
Experiment	2.67±0.52	53.38±4.56	8.06±1.20	1.20±0.08	4.56±0.66	0.16±0.02	0.09±0.01
Control	2.13±0.72	42.27±15.57	8.13±3.03	1.35±0.44	3.22±1.19	0.19±0.01	0.10±0.02
Hippocampus (social isolation for 2 months)							
Experiment	2.20±0.13	0.27±0.03	0.08±0,01	0.08±0.01	0.07±0.01	0.32±0.04	0,28±0.05
Control	2.27±0.10	0.24±0.02	0.10±0.02	0.08±0.01	0.07±0.01	1.38±1.04	0.43±0.13
Hippocampus (social isolation for 3 months)							
Experiment	2.57±0.20	0.31±0.08	0.20±0.02	0.25±0.09	0.24±0.04	1.38±0.58	1.60±0.67
Control	2.62±0.08	0.35±0.11	0.31±0.06	0.22±0.05	0.23±0.05	1.89±0.83	1.35±0.54
Amygdala (social isolation for 2 months)							
Experiment	2.81±0.18	4.00±0.40	0.38±0.04	—	—	0.10±0.01	
Control	3.18±0.27	4.87±0.53	0.51±0.08	—	—	0.11±0.01	
Amygdala (social isolation for 3 months)							
Experiment	1.18±0.13	2.87±0.26	0.65±0.23	0.56±0.14^{+}	0.55±0.16	0.26±0.11	0.19±0.05
Control	1.37±0.07	3.21±0.51	0.80±0.11	0.22±0.08	0.46±0.07	0.29±0.08	0.14±0.01

Note. —, the substance cannot be detected by the specified method. ^{+}p=0.07 in comparison with the control (unpaired Mann—Whitney *U* test).

the increase in serotonin content and a decrease in its turnover in the amygdala of Wistar rats after 3-month social isolation is of particular interest.

The review [15] also provides data that the content of 5-HIAA (but not serotonin) in the prefrontal cortex of Sprague-Dawley subjected to social isolation during PND28-100 was reduced, whereas in our study, no changes in the content of serotonin and its metabolite in the frontal cortex of Wistar rats were detected at this term (PND24-(97-98), while after 3-month isolation (PND26-133), a tendency to a decrease in serotonin content was revealed. Some differences in the duration of social isolation and different rat lines can explain minor discrepancy of the results.

In Long-Evans rats, a decrease in dopamine levels in the basolateral amygdala was found after 6-week social isolation started at PND28 [8]. In our work, only a pronounced tendency to an increase in the content of dopamine metabolites 3-MT was detected after 3 months isolation. Further studies are needed to determine the nature dopaminergic system disturbances in the amygdala of rats subjected to long-term social isolation.

At the moment, we cannot directly relate the observed neurochemical changes in the CNS with particular behavioral disturbances. However, consistently observed increased aggressiveness in rats subjected to isolation deserves particular attention (Table 1). Changes in the state of the 5-HT-ergic system in cortical-limbic circuits are considered as a neurobiological mechanism of aggressive behavior [5]. It was shown that serotonin can modulate neuronal plasticity in the early ontogeny [9]. Based on our data on the dynamics of behavioral disorders and neurochemical changes in brain structures that mediate emotional activity, we can assume that early 2- and 3-month-long social isolation is accompanied by reorganization of neural networks in the cortex and limbic structures, which

TABLE 3. Contents of Serotonin and Its Metabolite and Serotonin Turnover in Brain Structures of Rats Subjected to 2- and 3-Month-Long Social Isolation (Experiment) and Control Group-Housed Animals (Control) (*M*±*SEM*; nmol/g tissue)

Experimental conditions	5-HT	5-HIAA	5-HIAA/5-HT
Frontal cortex (social isolation for 2 months)			
Experiment	4.69±0.30	3.31±0.15	0.72±0.03
Control	4.34±0.32	3.10±0.25	0.72±0.03
Frontal cortex (social isolation for 3 months)			
Experiment	1.72±0.17°	1.14±0.23	0.65±0.07
Control	2.98±0.10	2.14±0.11	0.72±0.01
Striatum (social isolation for 2 months)			
Experiment	3.84±0.18	6.50±0.35	1.69±0.06
Control	4.15±0.16	6.89±0.27	1.67±0.06
Striatum (social isolation for 3 months)			
Experiment	3.53±0.77	7.55±0.34	2.48±0.32
Control	3.49±1.85	5.35±1.53	2.07±0.50
Hippocampus (social isolation for 2 months)			
Experiment	2.18±0.15*	3.70±0.18	1.75±0.10*
Control	2.74±0.12	3.78±0.17	1.39±0.05
Hippocampus (social isolation for 3 months)			
Experiment	2.07±0.19	3.59±0.29	1.76±0.09
Control	2.09±0.29	3.55±0.37	1.76±0.17
Amygdala (social isolation for 2 months)			
Experiment	5.76±0.35	4.53±0.17	0.82±0.05
Control	5.79±0.34	5.04±0.29	0.88±0.03
Amygdala (social isolation for 3 months)			
Experiment	5.86±2.13*	4.52±0.41	0.96±0.15*
Control	2.65±0.33	4.15±0.67	1.54±0.16

Note. *p<0.05, °p=0.056 in comparison with the control (unpaired Mann—Whitney U test).

can serve as a pathophysiological basis for the development of psychoemotional disorders.

This work was partially supported by the Russian Foundation for Basic Research (grant No. 15-04-08784).

REFERENCES

1. Krupina NA, Khlebnikova NN, Orlova IN. Early social isolation increases aggression and impairs a short-term habituation in acoustic startle reflex in rats. Patol. Fiziol. Eksp. Ter. 2015;59(4):4-15. Russian.
2. Khlebnikova NN, Medvedeva YS, Krupina NA. Early Social Isolation Causing Emotional Motivational Alterations in Rats, is Accompanied by a Deficit of Short-term Habituation, but does not Affect Spatial Memory. Zh. Vyssh. Nervn. Deyat. 2018;68(5):647-662. Russian.
3. Khlebnikova NN, Orshanskaya EV, Narkevich VB, Kudrin VS, Krupina NA. Dipeptidyl Peptidase 4 Inhibitors Diprotin A and Sitagliptin Administered on Weeks 2-3 of Postnatal Development Modulate Monoamine Metabolism in the Striatum of Adult Rats. Bull. Exp. Biol. Med. 2017;163(2):190-194. doi: 10.1007/s10517-017-3763-5
4. Brenes JC, Fornaguera J. The effect of chronic fluoxetine on social isolation-induced changes on sucrose consumption, immobility behavior, and on serotonin and dopamine function in hippocampus and ventral striatum. Behav. Brain Res. 2009;198(1):199-205.
5. Coccaro EF, Fanning JR, Phan KL, Lee R. Serotonin and impulsive aggression. CNS Spectr. 2015;20(3):295-302.
6. Fone KC, Porkess MV. Behavioural and neurochemical effects of post-weaning social isolation in rodents-relevance to developmental neuropsychiatric disorders. Neurosci. Biobehav. Rev. 2008;32(6):1087-1102.
7. Han X, Wang W, Shao F, Li N. Isolation rearing alters social behaviors and monoamine neurotransmission in the medial prefrontal cortex and nucleus accumbens of adult rats. Brain Res. 2011;1385:175-181.
8. Karkhanis AN, Alexander NJ, McCool BA, Weiner JL, Jones SR. Chronic social isolation during adolescence augments catecholamine response to acute ethanol in the basolateral amygdala. Synapse. 2015;69(8):385-395.
9. Kraus C, Castrén E, Kasper S, Lanzenberger R. Serotonin and neuroplasticity — links between molecular, functional and structural pathophysiology in depression. Neurosci. Biobehav. Rev. 2017;77:317-326.
10. Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA. Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neurosci. Behav. Physiol. 2003;33(1):13-29.
11. Lukkes JL, Watt MJ, Lowry CA, Forster GL. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. Front. Behav. Neurosci. 2009;3. ID 18. doi: 10.3389/neuro.08.018.2009
12. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat. Rev. Neurosci. 2009;10(6):434-445.
13. Strauss L, Brink CB, Möller M, Stein DJ, Harvey BH. Late-life effects of chronic methamphetamine exposure during puberty on behaviour and corticostriatal mono-amines in social isolation-reared rats. Dev. Neurosci. 2014;36(1):18-28.
14. Suri D, Teixeira CM, Cagliostro MK, Mahadevia D, Ansorge MS. Monoamine-sensitive developmental periods impacting adult emotional and cognitive behaviors. Neuropsychopharmacology. 2015;40(1):88-112.
15. Walker DM, Cunningham AM, Gregory JK, Nestler EJ. Long-Term Behavioral Effects of Post-weaning Social Isolation in Males and Females. Front. Behav. Neurosci. 2019;13. ID 66. doi: 10.3389/fnbeh.2019.00066