Specific Features of Learning with Nociceptive Electrical Reinforcement in Rats of Different Genetic Strains: Role of Brain Neurotransmitter Systems

A. L. Kalyuzhnyi, S. V. Litvinova, V. V. Shul'govskii, and L. F. Panchenko*

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The interaction between neurotransmitter systems and opioid system in rats of different strains was studied during learning and emotional stress. The interaction between noradrenergic, serotoninergic, and opioid systems in the brain is the main neurochemical mechanism underlying learning reinforced by nociceptive electrical stimulation, which determine individual differences in the rate and type of learning.

Key Words: *learning; nociception; monoaminergic system; endogenous opioids*

The study of general mechanisms of learning, memory, and stress-produced disturbances in these processes is difficult due to the existence of individual differences in adaptation to stress factors. They are determined by a variety of neurochemical mechanisms of the interaction between neurotransmitters and endogenous opioid system of the brain. The animals of different strains have specific antistress protective mechanisms and differ in the dynamics of learning and stress reaction [1]. Acquisition of a conditioned active avoidance response (CAAR) is a common approach to studying memory during learning and emotional stress [2]. However, little is known about the influence of genetically determined individual differences on the mechanisms of memory and stress under conditions of CAAR paradigm.

Studies of neurochemical mechanisms of learning and stress reaction showed that changes in the interaction between neurotransmitter systems depend on the type of stress factor. Monoaminergic systems, neuropeptides, ACTH, GABAergic system, and other factors interact with each other under stress conditions [3]. Individual differences in the process of learning and development of emotional stress are primarily determined by the neurotransmitter mechanism [8,9, 12,13].

Here we compared CAAR acquisition in rats of different genetic strains differing in the resistance to stress and content of opioid peptides [4,6,14,15]. Opioid peptide concentration in brain structures is lowest in WAG rats [3]. We measured the content of monoamines and their metabolites in different brain structures.

MATERIALS AND METHODS

Experiments were performed on 75 male WAG, Fischer-344, and Wistar rats weighing 220-250 g. The animals were housed in roomy cages at room temperature and natural light/dark cycle. They had free access to water and food. Ten rats of each strain were intact. Fifteen animals of each strain were learned CAAR and then decapitated.

CAAR performance and number of intersignal reactions were recorded over 1 session. The latency of reactions was estimated.

The contents of serotonin (5-hydroxytryptamine, 5-HT), its metabolite 5-hydroxyindoleacetic acid

Department of Higher Nervous Activity, Biological Faculty, M. V. Lomonosov Moscow State University; *Laboratory of Biochemistry, National Research Center of Narcology, Russian Ministry of Health, Moscow. ***Address for correspondence:*** shulg@protein.bio.msu.ru. V. V. Shul'govskii

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(5-HIAA), norepinephrine, and dopamine in the cortex, hippocampus, and striatum, as well as the concentrations of dopamine metabolites 3,4-dihydroxyphenylacetic acid (DPAA) and 3-methoxy-4-phenylacetic acid (homovanillic acid) in the striatum, were measured by high-performance liquid chromatography (HPLC) with electrochemical detection. Reversed-phase HPLC employed sodium octyl sulfate as the ion-pair agent. We used a Biophasa RP-18 column (ODS, 5 μ, 4.6×250.0 mm) and a RP-18 precolumn (ODS, 10 μ, 4.6×30.0 mm). This series was carried out at the Laboratory of Neurochemical Pharmacology (Institute of Pharmacology, Russian Academy of Medical Sciences).

The results were analyzed by Student's *t* test, Mann—Whitney test, and Wilcoxon test.

RESULTS

On days 1, 2, 3, and 4, CAAR performance in WAG rats exceeded that in Wistar rats by 40, 25, 26, and 30%, respectively. In this period CAAR acquisition did not differ between Wistar and Fischer-344 rats, as well as between WAG and Fischer-344 rats. Interstrain differences were not revealed in the follow-up period (Fig. 1, *a*). The latency was similar in animals of different strains. On day 1 the number of intersignal reaction in WAG rats 14.5-fold surpassed that in Wistar rats (Fig. 1, *b*). On day 8 the number of intersignal reaction in Fischer-344 rats was higher than in Wistar and WAG rats by 6.75 and 2 times, respectively. The number of reactions in WAG rats was 3.25 times higher than in Wistar rats.

In intact rats, 5-HT content was maximum in the cortex of intact Wistar rats (2.8-fold surpassed that in WAG rats, Table 1). 5-HT content in the cortex of Fischer-344 rats was 2.34 times higher than in WAG rats. No differences were found between Wistar and Fischer-344 rats. Hippocampal 5-HT content in Wistar rats 1.46-fold surpassed that in Fischer-344 rats.

5-HIAA concentration in the cortex of Fischer-344 rats far surpassed that in WAG rats ($p<0.01$), but did not differ from the corresponding parameter in Wistar rats. Hippocampal 5-HIAA concentration in Wistar rats was higher than in Fischer-344 and WAG rats by 2.15 ($p<0.01$) and 1.57 times ($p<0.01$), respectively.

Striatal norepinephrine concentration in WAG rats was higher than in Fischer-344 and Wistar rats by 2.06 ($p<0.001$) and 1.44 times ($p<0.01$), respectively.

The concentration of homovanillic acid in the striatum of Wistar and Fischer-344 rats surpassed that in WAG rats by 1.56 ($p<0.01$) and 1.77 times ($p<0.001$), respectively.

In trained animals the following peculiarities were revealed in the distribution of brain monoamines (Table 1): 5-HT content in the cortex of Wistar rats was higher than in WAG and Fischer-344 rats by 2.2 ($p<0.001$) and 1.35 times ($p<0.01$), respectively. 5-HT concentration in Fischer-344 rats 1.63-fold surpassed that in WAG rats ($p<0.05$). Hippocampal 5-HT concentration in Wistar rats was higher than in WAG and Fischer-344 rats by 2.31 and 2.39 times, respectively ($p<0.001$). Thus, 5-HT content in the cortex and hippocampus was maximum in Wistar rats. No interstrain differences were revealed in the concentration of 5-HT in the striatum.

5-HIAA concentration in the cortex of Fischer-344 rats 1.65-fold surpassed that in WAG rats ($p<0.05$), but did not differ from that in Wistar rats. Hippocampal 5-HIAA concentration in Wistar rats was 1.74-fold higher than in Fischer-344 rats ($p<0.01$), but did

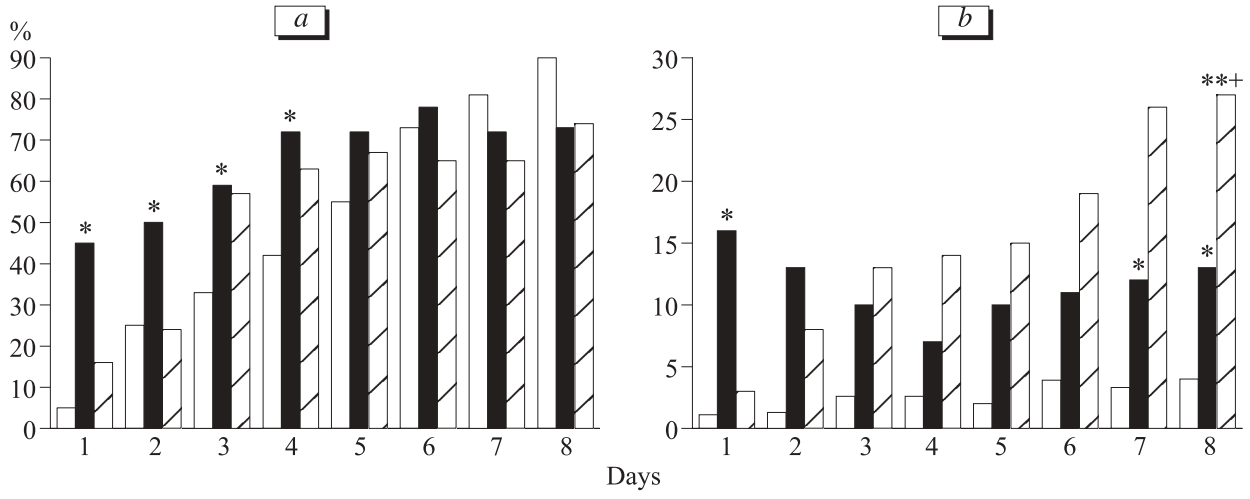

Fig. 1. Active avoidance conditioning (*a*) and number of intersignal reactions over 1 session (*b*) in Wistar (light bars), WAG (dark bars), and Fischer-344 rats (shaded bars). *$p<0.05$ and **$p<0.01$ compared to Wistar rats; $^{+}p<0.05$ compared to WAG rats.

TABLE 1. Content of Biogenic Amines in Rat Brain Structures (*M*±*m*)

Parameter		WAG		Fischer-344		Wistar	
		intact	experi-mental	intact	experi-mental	intact	experi-mental
5-HT	cortex	0.57±0.05	0.51±0.03	1.00±0.28*	0.83±0.11**	1.32±0.10***	1.12±0.08**$^{+++}$
	hippocampus	0.35±0.02	0.32±0.07	0.28±0.06	0.31±0.06	0.41±0.08^{+}	0.74±0.06***$^{+++}$
	striatum	0.38±0.03	0.36±0.04	0.37±0.02	0.44±0.08	0.28±0.12	0.39±0.03
5-HIAA	cortex	0.36±0.05	0.29±0.07	0.51±0.01**	0.48±0.01*	0.49±0.1	0.43±0.09^{+}
	hippocampus	0.44±0.02	0.41±0.04	0.32±0.09	0.31±0.06	0.69±0.08**$^{++}$	0.54±0.05^{++}
	striatum	0.59±0.80	0.52±0.05	0.46±0.08	0.54±0.04	0.43±0.05	0.31±0.08*$^{+}$
Norepinephrine	cortex	0.48±0.06	0.67±0.04	0.46±0.04	0.87±0.09*	0.56±0.03	0.46±0.04*$^{++}$
	hippocampus	0.59±0.08	0.53±0.07	0.42±0.09	0.38±0.03	0.52±0.02	0.48±0.10
	striatum	0.58±0.05^{+++}	0.51±0.08	0.33±0.06***	0.33±0.07	0.48±0.04**	0.44±0.04
Dopamine	striatum	10.5±1.3	11.70±1.16	10.1±0.4	11.7±1.4	10.6±0.4	11.50±0.78
DPAA	striatum	0.85±0.16	0.83±0.08	0.65±0.09	0.63±0.04	0.70±0.02	0.83±0.07^{+}
Homovanillic acid	striatum	0.52±0.04	0.84±0.02	0.92±0.03**	0.55±0.07	0.81±0.09**	0.71±0.05

Note. *p<0.05, **p<0.01, and ***p<0.001 compared to WAG rats; ^{+}p<0.05, ^{++}p<0.01, and ^{+++}p<0.001 compared to Fischer-344 rats.

not differ from that in WAG rats. 5-HIAA concentration in the striatum of WAG and Fischer-344 rats was higher than in Wistar rats by 1.68 and 1.74 times, respectively (p<0.05).

Norepinephrine concentration in the cortex of Fischer-344 rats was higher than in WAG and Wistar rats by 1.3 (p<0.05) and 1.89 times (p<0.001), respectively. Norepinephrine concentration in the cortex of WAG rats 1.45-fold surpassed that in Wistar rats (p<0.05).

Striatal DPAA concentration in Fischer-344 rats was 1.31 times lower than in WAG and Wistar rats (p<0.05). The concentration of homovanillic acid in Fischer-344 rats was lower than in WAG rats by 1.53 times (p<0.001).

Wistar rats more slowly learned CAAR compared to WAG and Fischer-344 rats. Our results show that 5-HT content was higher, while norepinephrine concentration was lower in the brain of Wistar rats, which can be explained by the involvement of decarboxylase in the synthesis of 5-HT and norepinephrine. Moreover, monoamine oxidase catalyzes oxidative deamination of both amines. The decrease in 5-HT content is accompanied by an increase in met-enkephalin concentration in the striatum [5]. This structure plays a key role in the endogenous antinociceptive system. The data suggest that changes in the interaction between serotoninergic and opioidergic systems contribute to slow acquisition of CAAR in Wistar rats.

The amount of dopamine metabolites in the striatum of Wistar and Fischer-344 rats decreased after CAAR acquisition, which was probably associated with variations in the synthesis of this compound. It should be emphasized that changes in dopamine metabolism can result from the increase in 5-HT content. Previous studies showed that local administration of 5-HT and agonists of 5-HT_1 receptor into the striatum of rats facilitates dopamine release [10].

During acquisition of CAAR the number of intersignal reactions in Fischer-344 rats was higher than in WAG and Wistar rats (Fig. 1, *b*). Published data suggest that activation of the brain noradrenergic system coincides with the increase in locomotor activity of rats in the open field [12]. In our experiments norepinephrine concentration was maximum in Fischer-344 rats, which probably determined greater number of intersignal reactions in these animals.

Our experiments show that the interaction between serotoninergic and noradrenergic systems plays the major role in learning reinforced by nociceptive electrical stimulation. The opioidergic system also modulates this process. Met-enkephalin and its analogue increase the content of 5-HT [7], which impairs learning reinforced by nociceptive electrical stimulation [4]. Rapid acquisition of CAAR in WAG rats is probably associated with less significant effect of endogenous opioids on the serotoninergic system in these animals (compared to Fischer-344 and Wistar rats).

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