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Research Article

Monoaminergic effects of the unilateral blockade of orexin receptors (OX1R) in the extended amygdala under psychostimulant action

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BACKGROUND: The search for new drugs potentially effective in stopping the development of pathological addictions is an urgent task of experimental psychopharmacology.

AIM: To examine the participation of brain monoaminergic systems in the mechanisms of the blocking effect of SB-408124 on the self-stimulation of the lateral hypothalamus activated with the psychostimulant β-phenylisopropylamine (PIPA) treatment.

MATERIALS AND METHODS: Male Wistar rats pre-administered with psychostimulant (PIPA, 1 mg/kg intraperitoneally) were unilaterally injected with an orexin antagonist SB-408124 (1 μg in 1 μL) into the central nucleus of the amygdala or the bed nucleus of the stria terminalis (BNST). High-performance liquid chromatography with electrochemical detection was used to determine the levels of monoamines and their metabolites: norepinephrine (NA), dopamine (DA), serotonin (5-HT), dioxyphenylacetic (DOPAC), homovaniline (HVA), and 5-hydroxyindolacetic (5-HIAA) acids on the left and right sides of the prefrontal cortex, hippocampus, striatum, and olfactory tubercle.

RESULTS: Under the action of PIPA, microinjections of SB-408124 into the right central nucleus of the amygdala induced the following effects: prefrontal cortex, an increase in the levels of HVA and 5-HT on the right side and 5-HIAA on the left; hippocampus, bilateral increase in the levels of NA and HVA and 5-HT on the right; striatum, bilateral increase in the level of DA and the left-sided increase in HVA, 5-HT, and 5-HIAA. Microinjections into the left central nucleus of the amygdala caused a right-sided decrease in NA levels, an increase in 5-HT levels, and a left-sided decrease in DA and DOPAC levels in the striatum and a left-sided increase in HVA level in the olfactory tubercle. Microinjections into the right BNST caused a left-sided decrease in the levels of NA and DA in the prefrontal cortex, bilateral decrease in DOPAC levels, a right-sided increase in 5-HT levels, and a left-sided increase in 5-HIAA levels in the striatum; and a left-sided increase in HVA levels in the olfactory tubercle. Microinjections into the left BNST caused increased 5-HT levels in the left striatum and decreased DOPAC and 5-HIAA levels in the left olfactory tubercle.

CONCLUSIONS: Right-sided microinjections cause a greater number of changes in monoamine metabolism than left-sided ones. The introduction of SB-408124 into the right structures of the extended amygdala increases 5-HIAA levels in the left striatum, whereas microinjections in BNST lead to increased 5-HT levels in the ipsilateral striatum and in the contralateral in the central nucleus of the amygdala

Keywords: BNST; brain asymmetry; central amygdala nucleus; monoamines; orexin antagonist.

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Научная статья

Моноаминергические эффекты унилатеральной блокады орексиновых рецепторов (OX1R) в структурах расширенной миндалины на фоне системного действия психостимулятора

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Актуальность. Поиск новых препаратов, потенциально способных купировать развитие патологических зависимостей, — актуальная задача экспериментальной психофармакологии.

Цель — изучить участие центральных моноаминергических систем в механизмах блокирующего действия препарата SB-408124 на самостимуляцию латерального гипоталамуса, активированную психостимулятором β-фенилизопропиламином (ФИПА).

Материалы и методы. Крысам-самцам линии Вистар на фоне предварительно введенного психостимулятора (ФИПА, 1 мг/кг в/бр) в центральное ядро миндалины или в ядро ложа конечной полоски (bed nucleus of the stria terminalis — BNST) унилатерально вводили антагонист орексина SB-408124 (1 мкг/мкл). Методом высокоэффективной жидкостной хроматографии с электрохимической детекцией в префронтальной коре, гиппокампе, обонятельном бугорке и стриатуме левой и правой сторон мозга отдельно определяли содержание моноаминов и их метаболитов: норадреналина (НА), дофамина (ДА), серотонина (5-ГТ), диоксифенилуксусной (ДОФУК), гомованилиновой (ГВК) и 5-гидроксииндолуксусной (5-ГИУК) кислот.

Результаты. На фоне ФИПА микроинъекции SB-408124 в правое центральное ядро миндалины вызывали следующие эффекты: в префронтальной коре — повышение уровня ГВК и 5-ГТ справа, а 5-ГИУК — слева; в гиппокампе — билатеральное возрастание содержания НА и ГВК, а 5-ГТ — справа; в стриатуме — билатеральное повышение уровня ДА и левостороннее повышение ГВК, 5-ГТ и 5-ГИУК. Микроинъекции в левое центральное ядро миндалины вызывали: в стриатуме — правостороннее снижение уровня НА и возрастание 5-ГТ, а также левостороннее снижение уровня ДА и ДОФУК; в обонятельном бугорке — левостороннее повышение ГВК. Микроинъекции в правое BNST вызывали: в префронтальной коре — левостороннее снижение уровня НА и ДА, в стриатуме — билатеральное снижение уровня ДОФУК, правостороннее повышение 5-ГТ и левостороннее — 5-ГИУК; в обонятельном бугорке — левостороннее повышение уровня ГВК. Микроинъекции в левое BNST вызывали в левом стриатуме повышение уровня 5-ГТ; в левом обонятельном бугорке — снижение уровня ДОФУК и 5-ГИУК.

Заключение. Правосторонние микроинъекции вызывают большее число изменений показателей обмена моноаминов, чем левосторонние. Введение SB-408124 в правые структуры расширенной миндалины увеличивает содержание 5-ГИУК в левом стриатуме, при этом микроинъекции в BNST приводят к возрастанию уровня 5-ГТ в ипсилатеральном стриатуме, а в центральное ядро миндалины — в контралатеральном.

Ключевые слова: антагонист орексина; BNST; центральное ядро миндалины; моноамины; асимметрия головного мозга.

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BACKGROUND

The search for new drugs that can stop the development of pathological addictions is one of the urgent tasks of experimental psychopharmacology. One model of addictive behaviors in animals is the self-stimulation of the lateral hypothalamus using pre-injected electrodes, which is significantly enhanced by intraperitoneal administration of a psychostimulant [1, 2]. This effect is blocked by the local injection of the SB-408124 antagonist of the orexin type 1 receptor (OX1R) into the extended amygdala, i.e., its central nucleus [3] and the bed nucleus of stria terminalis (BNST) [4].

The *study* aimed to investigate the involvement of central monoaminergic (MA-ergic) systems in blocking SB-408124 mechanisms on the self-stimulation of the lateral hypothalamus activated by beta-phenylisopropylamine (PIPA) psychostimulant.

MATERIALS AND METHODS

Experiments were conducted using 25 sexually mature male Wistar rats weighing 350–400 g. Before the experiments, the animals were divided into 11 rats that were not subjected to surgical treatment and 25 animals modeled under conditions in which the effect of orexin antagonist on enhanced self-stimulation was previously examined [3, 4]. Under nembutal anesthesia, monopolar nichrome electrodes in glass insulation were implanted bilaterally in the lateral hypothalamus of rats, and 0.2-mm diameter stainless steel guide cannulas were implanted unilaterally in the extended amygdala. Coordinates for implantation were determined according to the atlas [5]. Thus, electrodes were implanted 2.5 mm back from the bregma (AP), 2.0 mm lateral to the sagittal suture (SD), and 8.4 mm from the cranial surface (H) [6, 7]. The guide coordinates in the central nucleus of the amygdala were 2.8 mm posterior from the bregma (AR), 3.9 mm lateral from the sagittal suture (SD), and 8.2 mm from the cranial surface (H) [3]. In BNST, implantation was performed 0.5 mm posterior from the bregma (AR), 1.5 mm lateral from the sagittal suture (SD), and 6.7 mm from the cranial surface (H) [4]. After the surgery, the animals were kept in individual cages until the end of the experiment.

Experiments were conducted at least 10 days after the surgery. On the day of the experiment, 5 unoperated and all 20 operated animals were injected intraperitoneally (ip) with the PIPA psychostimulant (1 mg/kg/ip). At 10 min later, 8 rats were injected through the implanted cannulas (4 to the left and 4 to the right) into the central nucleus of the amygdala, and 11 rats were locally injected in the BNST (5 to the left and 6 to the right) with SB-408124 (Sigma-Aldrich, MO, USA) at a dose of 1 μg/1 μL per rat for 30 s [3, 4]. The lateral hypothalamic area was not stimulated in this experiment. Six operated rats that did not receive microinjections were used as the control group of sham-operated animals exposed to the psychostimulant. At 15 min after microinjection, rats were decapitated. Sham-operated and intact rats receiving only PIPA were decapitated 25 min after drug administration. Five intact rats that did not receive PIPA injections were also decapitated.

The prefrontal cortex, olfactory tubercle, striatum, and hippocampus were isolated from the left and right halves of the rat brain. The levels of noradrenaline (NA), dopamine (DA), serotonin (5-HT), and their metabolites, i.e., dioxyphenylacetic (DOPAC), homovanilinic (HVA), and 5-hydroxyindoleacetic (5-HIAA) acids were determined by reversed-phase high-performance liquid chromatography with electrochemical detection on a Beckman Coulter chromatograph (Beckman Coulter Inc., CA, USA) [8]. The chromatographic system included a Rheodyne 7125 injector (Rheodyne LLC, CA, USA) with a 20-μL loop for sample application, a Phenomenex column (4.6 × 250.0 mm) with a Sphere Clone 5 sorbent and ODS(2) column (Phenomenex Inc., CA, USA), and an LC-4C BAS amperometric detector (Bioanalytical Systems Inc., IN, USA). The concentrations of the investigated substances were determined at a potential of +0.70 V. The mobile phase contained 5.5 mM citrate-phosphate buffer, with 0.7 mM octanesulfonic acid, 0.5 mM ethylenediamine tetraacetic acid, and 7.5% acetonitrile (pH 3.0). In the mobile phase, the elution rate was 1 mL/min, and the time required for analyzing one sample was approximately 20 min.

The indices measured in the left and right structures were analyzed separately. Overall, at least 72 variables were subjected to statistical analysis. Of these variables, 48 were completely independent (concentrations of the substances analyzed), and 24 represented metabolite/mediator ratios.

The results were processed using GraphPad Prism version 6.0 (GraphPad Software Inc., La Jolla, CA, USA). The Kolmogorov–Smirnov test was applied to determine the normality of the distribution, combining all data relating to a particular index measured in each brain structure on a particular side. Between-group differences were assessed using one-factor analysis of variance (ANOVA) by applying Fisher's least significant difference test as a posteriori. Similar data corresponding to the left and right sides of the brain were compared using Student's paired *t*-test. Differences were considered statistically significant at $p < 0.05$.

The efficiency of right- and left-sided microinjections was assessed based on the proportion of detectable differences between groups receiving the psychostimulant out of the total number of independent variables ($n = 48$). Differences in the efficiency of left- and right-sided microinjections were estimated by comparing the probabilities of two binomial distributions.

RESULTS AND DISCUSSION

The study showed no differences in monoamine metabolic parameters in the non-operated and sham-operated group that received PIPA. Therefore, all PIPA-injected groups that

did not receive SB-408124 microinjections were combined into one group. Amphetamine-type psychostimulants predominantly affect the catecholaminergic systems [10]. However, PIPA did not affect the NA levels in any of the brain regions in our experiments, and its effect on DA concentration was manifested only in subcortical structures (Tables 1–8). Moreover, DA levels were bilaterally increased only in the olfactory tubercle ($p < 0.05$; Fig. 1 and Tables 3 and 7), whereas the striatum showed this effect only on the left side ($p < 0.05$; Fig. 2 and Tables 4 and 8). Since the HVA/DA ratio was decreased in the left striatum ($p < 0.01$; Fig. 2 and Tables 4 and 8), the increased DA levels may be associated with a left-sided reduction in the release of the mediator.

In the prefrontal cortex, PIPA significantly decreased the levels of 5-HIAA ($p < 0.05$; Fig. 3 and Tables 1 and 5). In the hippocampus, PIPA did not induce statistically significant changes in monoamine metabolism indices but led to the right-sided asymmetry with a predominance of DA, 5-HT, and 5-HIAA levels ($p < 0.05$; Fig. 4 and Tables 2 and 6).

The effects of SB-408124 injection into the central nucleus of the amygdala were dependent on the side of injection and are most frequently manifested on only one side of the brain. The only "mirror-symmetrical" response to this exposure was an increase in 5-HT levels in the contralateral striatum (left, $p < 0.05$; right, $p = 0.0524$; Fig. 5 and Table 4). Moreover, two opposite effects were reported in the striatum and hippocampus when SB-408124 was injected into the left and right central nuclei of the amygdala. In the striatum, DA levels decreased ($p < 0.05$) on the microinjected side during left-sided injection and increased bilaterally during right-sided injection ($p < 0.05$; Table 4). Injection into the left central nucleus of the amygdala tended to decrease 5-HIAA levels in the contralateral (right) hippocampus ($p = 0.0728$), whereas right-sided injection increased the corresponding index in the contralateral (left) hippocampus ($p < 0.05$; Table 2).

Other effects of right- and left-sided microinjections into the central nucleus of the amygdala, although not opposite, were only observed on one side. Left-sided SB-408124 injections induced changes in the monoamine levels in forebrain structures. NA levels decreased in the left cortex (compared with intact rats, $p < 0.05$; Table 1), whereas DOPAC ($p < 0.01$) and DA ($p < 0.05$; Table 4) levels decreased

Table 1. Levels of monoamines and their metabolites (ng/mg of tissue) in the prefrontal cortex in male Wistar rats upon unilateral injection of SB-408124 into the central amygdala nucleus

Таблица 1. Содержание моноаминов и их метаболитов (нг/мг ткани) в префронтальной коре у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в центральное ядро миндалины

Psychostimulant effects	–		1 mg/kg β-phenylisopropylamine, intraperitoneally					
Microinjected side[1]	–				Ipsilateral	Contralateral	Contralateral	Ipsilateral
Animal groups	Intact		β-phenylisopropylamine		1 μg/μL SB-408124 into the left central nucleus of the amygdala		1 μg/μL SB-408124 into the right central nucleus of the amygdala	
Side of the brain	Left	Right	Left	Right	Left	Right	Left	Right
NA	0.516 ± 0.050	0.335 ± 0.077 $^{(\#)AC}p = 0.0578$	0.417 ± 0.061	0.357 ± 0.060	0.173 ± 0.134$^{\#*}$	0.368 ± 0.251	0.495 ± 0.117$^{\#}$	0.523 ± 0.103
DA	0.438 ± 0.114	0.396 ± 0.112	0.424 ± 0.076	0.352 ± 0.076	0.244 ± 0.145	0.294 ± 0.210	0.472 ± 0.202	0.409 ± 0.176
DOPAC	0.443 ± 0.142	0.461 ± 0.126	0.392 ± 0.097	0.404 ± 0.085	0.246 ± 0.197	0.339 ± 0.279	0.674 ± 0.232	0.569 ± 0.187
DOPAC/DA	1.045 ± 0.114	1.294 ± 0.235	0.885 ± 0.108	1.097 ± 0.149	0.675 ± 0.249	0.812 ± 0.169	1.194 ± 0.123	1.205 ± 0.244
HVA	0.042 ± 0.028	0.003 ± 0.003	0.018 ± 0.012	0.012 ± 0.011	0.099 ± 0.099	0.011 ± 0.011$^{\#}$	0.060 ± 0.060	0.083 ± 0.053$^{\#*\&}$
HVA/DA	0.425 ± 0.380	0.087 ± 0.086	0.081 ± 0.052	0.034 ± 0.031	0.717 ± 0.717	0.097 ± 0.097	0.988 ± 0.988	0.429 ± 0.254
5-HT	0.110 ± 0.026	0.085 ± 0.012	0.097 ± 0.017	0.084 ± 0.005	0.119 ± 0.030	0.125 ± 0.044	0.175 ± 0.061	0.149 ± 0.039$^{*\&}$
5-HIAA	0.293 ± 0.088	0.206 ± 0.039	0.125 ± 0.017*	0.125 ± 0.015*	0.192 ± 0.113	0.256 ± 0.161	0.407 ± 0.140$^{\&}$	0.320 ± 0.149
5-HIAA/5-HT	2.779 ± 0.572	2.585 ± 0.604	1.745 ± 0.390	1.555 ± 0.217	1.309 ± 0.489	1.760 ± 0.526	2.014 ± 0.324	2.730 ± 1.078

*$p < 0.05$, differences from the corresponding index measured in intact animals; $^{\&}p < 0.05$differences from the corresponding index measured in beta-phenylisopropylamine-treated animals; $^{\#}p < 0.05$, reliable differences of ipsi- and contralateral SB-408124 effects (difference between the index measured on a given side of the brain after microinjections into the central nucleus of the amygdala on the same side of the brain and the same index after a similar exposure on the opposite side) by ANOVA; $^{\#AC}p = 0.0578$, manifestations of asymmetry (differences between the corresponding indices of the left and right sides of the brain) according to paired Student's *t*-test. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine

[1] In relation to the studied side of the brain.

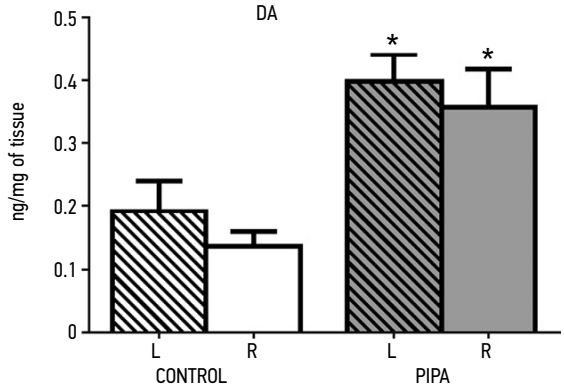

Fig. 1. Changes in the level of dopamine (DA) in the olfactory tubercle under the action of β-phenylisopropylamine. Animal groups: control, received physiological solution; PIPA, received β-phenylisopropylamine. The shaded bars (L) show the value of the corresponding parameter in the left hemisphere and the unshaded (R) in the right hemisphere. *$p < 0.05$, significant differences from the corresponding parameter measured in intact animals by Student's *t*-test

Рис. 1. Изменение уровня дофамина (ДА) в обонятельном бугорке под действием β-фенилизопропиламина. Группы животных: контроль — получавшие физиологический раствор, ФИПА — получавшие β-фенилизопропиламин. Заштрихованными столбиками (Л) показано значение соответствующего параметра в левом полушарии, незаштрихованными (П) — в правом. *$p < 0,05$ — достоверные отличия от соответствующего показателя, измеренного у интактных животных по *t*-критерию Стьюдента

Table 2. Levels of monoamines and their metabolites (ng/mg of tissue) in the hippocampus of male Wistar rats with the unilateral injection of SB-408124 into the central nucleus of the amygdala

Таблица 2. Содержание моноаминов и их метаболитов (нг/мг ткани) в гиппокампе у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в центральное ядро миндалины

Psychostimulant effects	**–**		**1 mg/kg β-phenylisopropylamine, intraperitoneally**					
Microinjected side[1]	**–**				**Ipsilateral**	**Contralateral**	**Contralateral**	**Ipsilateral**
Animal groups	**Intact**		**β-phenylisopropylamine**		**1 μg/μL SB-408124 into the left central nucleus of the amygdala**		**1 μg/μL SB-408124 into the right central nucleus of the amygdala**	
Side of the brain	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
NA	0.397 ± 0.141	0.250 ± 0.092	0.393 ± 0.108	0.452 ± 0.072$^{\#AC}$	0.383 ± 0.088	0.445 ± 0.096$^{\#}$	0.952 ± 0.314$^{*\&}$	0.894 ± 0.112$^{\#***\&\&}$
DA	0.311 ± 0.106	0.253 ± 0.086	0.229 ± 0.056	0.307 ± 0.061$^{\#AC}$	0.272 ± 0.083	0.339 ± 0.088	0.473 ± 0.369	0.495 ± 0.052
DOPAC	0.435 ± 0.183	0.354 ± 0.182	0.334 ± 0.079	0.445 ± 0.074	0.367 ± 0.059	0.458 ± 0.177	0.801 ± 0.372 $^{(\&)}p = 0.0658$	0.820 ± 0.253 $^{(*)}p = 0.059$ $^{(\&)}p = 0.0895$
DOPAC/DA	1.096 ± 0.272	1.254 ± 0.438	1.277 ± 0.191	1.963 ± 0.326	1.462 ± 0.181	1.195 ± 0.249	2.200 ± 0.000	1.792 ± 0.446
HVA	0.090 ± 0.031	0.134 ± 0.054	0.069 ± 0.020	0.074 ± 0.014	0.136 ± 0.042	0.105 ± 0.055$^{\#}$	0.201 ± 0.048$^{*\&\&}$	0.376 ± 0.107$^{\#**\&\&}$
HVA/DA	0.477 ± 0.160	0.450 ± 0.108	0.273 ± 0.097	0.320 ± 0.111	0.509 ± 0.140	0.291 ± 0.183$^{\#}$	0.546 ± 0.308	0.907 ± 0.100$^{\#*\&}$
5-HT	0.098 ± 0.028	0.082 ± 0.026	0.085 ± 0.018	0.121 ± 0.022$^{\#\#AC}$	0.122 ± 0.021	0.086 ± 0.021$^{\#\#}$	0.131 ± 0.038	0.287 ± 0.098$^{\#\#**\&\&}$
5-HIAA	0.234 ± 0.038	0.247 ± 0.034	0.177 ± 0.019	0.217 ± 0.012$^{\#AC}$	0.212 ± 0.047	0.139 ± 0.027$^{\#\#*(\&)}$ $p = 0.0728$	0.289 ± 0.021	0.282 ± 0.058$^{\#\#}$
5-HIAA/5-HT	3.063 ± 0.689	3.386 ± 0.741	2.778 ± 0.480	2.697 ± 0.602	1.754 ± 0.281	1.88 ± 0.50$^{\#AC}$	1.832 ± 0.257	1.271 ± 0.278 $^{(*)}p = 0.0780$

*$p < 0.05$, ***$p < 0,001$, $^{(*)}p > 0,05$ differences from the corresponding index of intact animals; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{(\&)}p > 0.05$, differences from the corresponding index of β-phenylisopropylamine-treated animals; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ significant differences in the ipsi- and contralateral SB-408124 effects by ANOVA; $^{\#AC}p < 0.05$, $^{\#\#AC}p < 0.01$ differences between the corresponding indices of the left and right sides of the brain according to paired Student's *t*-test; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine.

[1] In relation to the studied side of the brain.

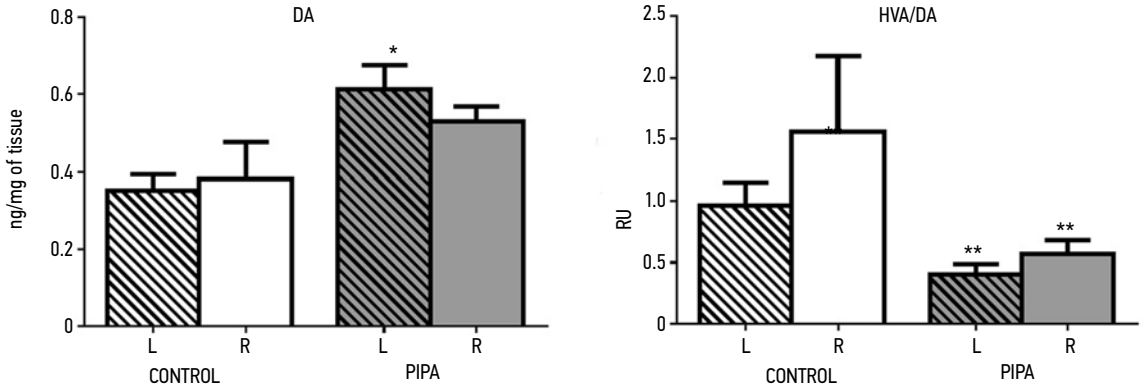

Fig. 2. Changes in dopamine (DA) level and the homovanilinic acid-to-dopamine ratio (HVA/DA) in the striatum under the action of β-phenylisopropylamine. Animal groups: control animals received saline, whereas ФИПА animals received β-phenylisopropylamine. The shaded bars (L) show the value of the corresponding parameter in the left hemisphere and the unshaded (R) in the right hemisphere. *$p < 0.05$, **$p < 0.01$, significant differences from the corresponding parameters measured in intact animals by Student's *t*-test

Рис. 2. Изменение уровня дофамина (ДА) и соотношения гомованилиновой кислоты и дофамина (ГВК/ДА) в стриатуме под действием β-фенилизопропиламина. Группы животных: контроль — получавшие физиологический раствор, ФИПА — получавшие β-фенилизопропиламин. Заштрихованными столбиками (Л) показано значение соответствующего параметра в левом полушарии, незаштрихованными (П) — в правом. *$p < 0,05$, **$p < 0,01$ — достоверные отличия от соответствующего показателя, измеренного у интактных животных по *t*-критерию Стьюдента

Table 3. Levels of monoamines and their metabolites (ng/mg of tissue) in the olfactory tubercle in male Wistar rats with the unilateral injection of SB-408124 into the central nucleus of the amygdala

Таблица 3. Содержание моноаминов и их метаболитов (нг/мг ткани) в обонятельном бугорке у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в центральное ядро миндалины

Psychostimulant effects	**–**		**1 mg/kg β-phenylisopropylamine, intraperitoneally**					
Microinjected side[1]	**–**				**Ipsilateral**	**Contralateral**	**Contralateral**	**Ipsilateral**
Animal groups	**Intact**		**β-phenylisopropylamine**		**1 μg/μL SB-408124 into the left central nucleus of the amygdala**		**1 μg/μL SB-408124 into the right central nucleus of the amygdala**	
Side of the brain	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
NA	0.184 ± 0.037	0.096 ± 0.026	0.344 ± 0.054	0.283 ± 0.074	0.417 ± 0.144	0.525 ± 0.062** $^{(\&)}p = 0.0690$	0.396 ± 0.055	0.455 ± 0.172*
DA	0.192 ± 0.049	0.136 ± 0.023	0.398 ± 0.042*	0.358 ± 0.059*	0.392 ± 0.175	0.475 ± 0.133**	0.396 ± 0.114	0.387 ± 0.071*
DOPAC	0.556 ± 0.102	0.475 ± 0.110	0.800 ± 0.097	0.669 ± 0.120	0.736 ± 0.250	1.028 ± 0.072* $^{(\&)}p = 0.0789$	0.671 ± 0.149	0.854 ± 0.187 $^{(\#AC)}p = 0.0584$ $^{(*)}p = 0.0887$
DOPAC/DA	2.732 ± 0.682	2.409 ± 0.438	2.081 ± 0.204	1.779 ± 0.239	1.719 ± 0.279	3.989 ± 2.182	1.835 ± 0.388	2.156 ± 0.253
HVA	0.060 ± 0.038	0.103 ± 0.052	0.050 ± 0.027	0.124 ± 0.038	0.250 ± 0.142 $^{\#*\&}$	0.161 ± 0.087	0.034 ± 0.028$^{\#}$	0.131 ± 0.044
HVA/DA	0.759 ± 0.491	0.794 ± 0.366	0.182 ± 0.107	0.408 ± 0.175	0.794 ± 0.472	0.333 ± 0.123	0.164 ± 0.149	0.313 ± 0.115
5-HT	0.142 ± 0.031	0.138 ± 0.027	0.193 ± 0.025	0.191 ± 0.018	0.176 ± 0.046	0.158 ± 0.024	0.169 ± 0.025	0.183 ± 0.038
5-HIAA	0.304 ± 0.031	0.261 ± 0.052	0.362 ± 0.040	0.316 ± 0.019	0.339 ± 0.068	0.401 ± 0.044*	0.313 ± 0.035	0.375 ± 0.073
5- HIAA /5-HT	1.665 ± 0.398	2.126 ± 0.521	1.896 ± 0.245	1.783 ± 0.171	2.057 ± 0.243	2.685 ± 0.405	1.926 ± 0.266	2.153 ± 0.28

$^{(*)}p = 0.0887$, *$p < 0.05$, ***$p < 0.001$, differences from the corresponding index of intact animals; $^{\&}p < 0.05$, $^{(\&)}p > 0.05$ (pronounced trends and specific p-values are shown), differences from the corresponding index of beta-phenylisopropylamine-treated animals; $^{\#}p < 0.05$, differences in the ipsi- and contralateral SB-408124 effects by ANOVA; $^{(\#AC)}$ $p = 0.0584$, differences between the corresponding indices of the left and right sides of the brain according to paired Student's *t*-test. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine

[1] In relation to the studied side of the brain.

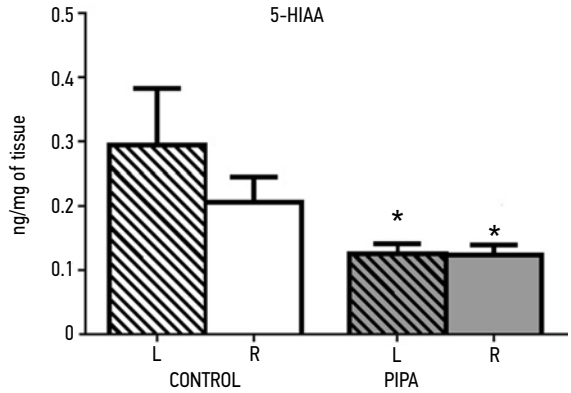

Fig. 3. Change in 5-hydroxyindoleacetic acid (5-HIAA) level in the prefrontal cortex under the action of β-phenylisopropylamine. Animal groups: control animals received saline, whereas PIPA animals received β-phenylisopropylamine. The shaded bars (L) show the value of the corresponding parameter in the left hemisphere and the unshaded (R) in the right hemisphere. *p < 0.05, significant differences from the corresponding parameter measured in intact animals by Student's t-test

Рис. 3. Изменение уровня 5-гидроксииндолуксусной кислоты (5-ГИУК) в префронтальной коре под действием β-фенилизопропиламина. Группы животных: контроль — получавшие физиологический раствор, ФИПА — получавшие β-фенилизопропиламин. Заштрихованными столбиками (Л) показано значение соответствующего параметра в левом полушарии, незаштрихованными (П) — в правом. *p < 0,05 — достоверные отличия от соответствующего показателя, измеренного у интактных животных по t-критерию Стьюдента

Table 4. Levels of monoamines and their metabolites (ng/mg of tissue) in the striatum of male Wistar rats with the unilateral injection of SB-408124 into the central nucleus of the amygdala

Таблица 4. Содержание моноаминов и их метаболитов (нг/мг ткани) в стриатуме у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в центральное ядро миндалины

Psychostimulant effects	**–**		**1 mg/kg β-phenylisopropylamine, intraperitoneally**					
Microinjected side[1]	**–**				**Ipsilateral**	**Contralateral**	**Contralateral**	**Ipsilateral**
Animal groups	**Intact**		**β-phenylisopropylamine**		**1 μg/μL SB-408124 into the left central nucleus of the amygdala**		**1 μg/μL SB-408124 into the right central nucleus of the amygdala**	
Side of the brain	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
NA	0.497 ± 0.095	0.467 ± 0.078	0.434 ± 0.050	0.473 ± 0.063	0.356 ± 0.130	0.230 ± 0.083 $^{(\#)}p$ = 0.064 $^{(*)}p$ = 0.0633$^{\&}$	0.471 ± 0.084	0.489 ± 0.051 $^{(\#)}p$ = 0.0640
DA	0.353 ± 0.040	0.381 ± 0.095	0.614 ± 0.061*	0.530 ± 0.039	0.253 ± 0.073$^{\#\#\&}$	0.609 ± 0.209	0.952 ± 0.286$^{\#\#**\&}$	1.069 ± 0.496$^{*\&}$
DOPAC	1.338 ± 0.198	1.269 ± 0.178	1.251 ± 0.109	1.295 ± 0.107	0.556 ± 0.156$^{\#\#\#**\&\&}$	0.693 ± 0.255	1.700 ± 0.182$^{\#\#\#}$	2.392 ± 1.008
DOPAC/ DA	2.670 ± 0.244	3.441 ± 0.780	2.274 ± 0.313	2.626 ± 0.337	1.625 ± 0.730	1.179 ± 0.353$^{**\&}$	2.060 ± 0.366	2.381 ± 0.175
HVA	0.342 ± 0.027	0.344 ± 0.046	0.233 ± 0.050	0.247 ± 0.047	0.262 ± 0.017$^{\#}$	0.315 ± 0.070	0.550 ± 0.113$^{\#*\&\&}$	0.376 ± 0.107
HVA/DA	0.959 ± 0.189	1.558 ± 0.620	0.400 ± 0.084*	0.569 ± 0.111	1.563 ± 0.341$^{\#*\&\&\&}$	0.698 ± 0.284	0.703 ± 0.224	0.560 ± 0.196$^{\#}$ $^{(*)}p$ = 0.0612
5-HT	0.168 ± 0.024	0.098 ± 0.033	0.125 ± 0.030	0.112 ± 0.021	0.165 ± 0.026 $^{(\#)}p$ = 0.0583	0.194 ± 0.029* $^{(\&)}p$ = 0.0524	0.259 ± 0.033$^{*\&(\#)}$ p = 0.0583	0.177 ± 0.036$^{\#AC}$
5-HIAA	0.268 ± 0.028	0.206 ± 0.027	0.231 ± 0.022	0.231 ± 0.018	0.188 ± 0.016$^{\#}$	0.238 ± 0.023	0.361 ± 0.136$^{\#}$ $^{(\&)}p$ = 0.0533	0.241 ± 0.080$^{\#AC}$
5-HIAA/5-HT	1.661 ± 0.132	3.251 ± 0.827	1.772 ± 0.419	2.218 ± 0.354	1.215 ± 0.190	1.260 ± 0.311*	1.738 ± 1.233	1.283 ± 0.154*

*p < 0.05, **p < 0.01, $^{(*)}p$ > 0.05, differences from the corresponding index of intact animals; $^{\&}p$ < 0.05, $^{\&\&}p$ < 0.01, $^{\&\&\&}p$ < 0.001, $^{(\&)}p$ > 0.05, differences from the corresponding index of beta-phenylisopropylamine-treated animals; $^{\#}p$ < 0.05, $^{\#\#}p$ < 0.01, $^{\#\#\#}p$ < 0.001, $^{(\#)}p$ > 0.05, differences in the ipsi- and contralateral SB-408124 effects by ANOVA; $^{\#AC}p$ < 0.05, differences between the corresponding indices of the left and right sides of the brain according to paired Student's t-test. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine.

[1] In relation to the studied side of the brain.

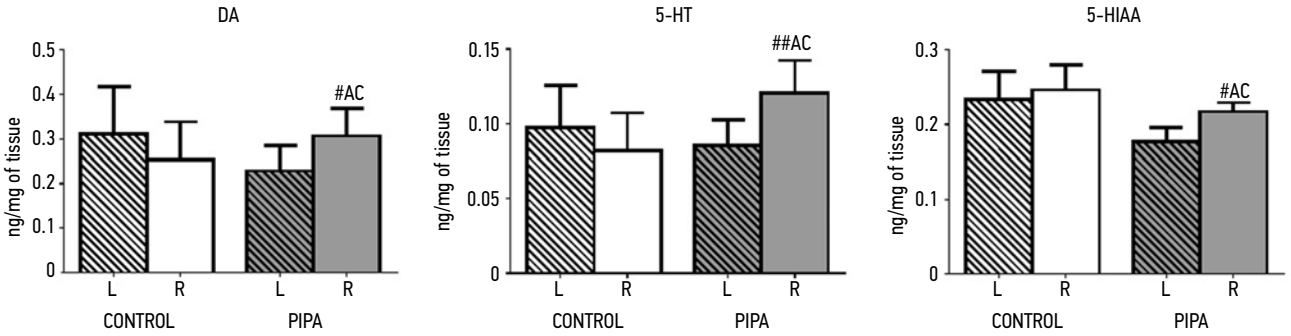

Fig. 4. Asymmetry in the levels of dopamine (DA), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus under the action of β-phenylisopropylamine. Animal groups: control animals received saline, whereas PIPA animals received β-phenylisopropylamine. The shaded bars (L) show the value of the corresponding parameter in the left hemisphere and the unshaded (R) in the right hemisphere. $^{\#AC}p < 0.05$, $^{\#\#AC}p < 0.01$, manifestations of asymmetry (differences between similar parameters on the left and right sides of the brain) by Student's *t*-criterion

Рис. 4. Появление асимметрии содержания дофамина (ДА), серотонина (5-ГТ) и 5-гидроксииндолуксусной кислоты (5-ГИУК) в гиппокампе под действием β-фенилизопропиламина. Группы животных: контроль — получавшие физиологический раствор, ФИПА — получавшие β-фенилизопропиламин. Заштрихованными столбиками (Л) показано значение соответствующего параметра в левом полушарии, незаштрихованными (П) — в правом. $^{\#AC}p < 0{,}05$, $^{\#\#AC}p < 0{,}01$ — проявления асимметрии (различия между аналогичными показателями левой и правой стороны мозга) — по *t*-критерию Стьюдента

Table 5. Levels of monoamines and their metabolites (ng/mg of tissue) in the prefrontal cortex in male Wistar rats with the unilateral injection of SB-408124 into the bed nucleus of the stria terminalis

Таблица 5. Содержание моноаминов и их метаболитов (нг/мг ткани) в префронтальной коре у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в BNST

Psychostimulant effects	**–**		**1 mg/kg β-phenylisopropylamine, intraperitoneally**					
Microinjected side[1]	**–**				**Ipsilateral**	**Contralateral**	**Contralateral**	**Ipsilateral**
Animal groups	**Intact**		**β-phenylisopropylamine**		**1 μg/μL SB-408124 into the left central nucleus of the amygdala**		**1 μg/μL SB-408124 into the right central nucleus of the amygdala**	
Side of the brain	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**	**Left**	**Right**
NA	0.516 ± 0.050	0.335 ± 0.077	0.417 ± 0.061	0.357 ± 0.060	0.331 ± 0.031$^{\#}$	0.425 ± 0.051$^{\#}$	0.063 ± 0.044$^{\#***\&\&\&}$	0.127 ± 0.062$^{\#}$ $^{(\&)}p = 0.0530$
DA	0.438 ± 0.114	0.396 ± 0.112	0.424 ± 0.076	0.352 ± 0.076	0.450 ± 0.192$^{\#}$	0.472 ± 0.104	0.078 ± 0.049$^{\#*\&}$	0.267 ± 0.142
DOPAC	0.443 ± 0.142	0.461 ± 0.126	0.392 ± 0.097	0.404 ± 0.085	0.524 ± 0.138$^{\#}$	0.492 ± 0.084	0.100 ± 0.068$^{\#}$	0.334 ± 0.162
DOPAC/ DA	1.045 ± 0.114	1.294 ± 0.235	0.885 ± 0.108	1.097 ± 0.149	1.080 ± 0.217	1.149 ± 0.232	0.926 ± 0.278	1.131 ± 0.266
HVA	0.042 ± 0.028	0.003 ± 0.003	0.018 ± 0.012	0.012 ± 0.011	0.065 ± 0.065	0.083 ± 0.080	0.026 ± 0.026	0.024 ± 0.021
HVA/DA	0.425 ± 0.380	0.087 ± 0.086	0.081 ± 0.052	0.034 ± 0.031	0.187 ± 0.187	0.102 ± 0.097	0.000 ± 0.000	0.189 ± 0.116
5-HT	0.110 ± 0.026	0.085 ± 0.012	0.097 ± 0.017	0.084 ± 0.005	0.154 ± 0.069	0.116 ± 0.041	0.085 ± 0.021	0.091 ± 0.041
5-HIAA	0.293 ± 0.088	0.206 ± 0.039	0.125 ± 0.017*	0.125 ± 0.015*	0.180 ± 0.046	0.154 ± 0.042	0.049 ± 0.015**	0.088 ± 0.023*
5-HIAA/5-HT	2.779 ± 0.572	2.585 ± 0.604	1.745 ± 0.390	1.555 ± 0.217*	1.937 ± 0.824	1.658 ± 0.506	0.930 ± 0.290*	1.303 ± 0.293*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, differences from the corresponding index of intact animals; $^{(\&)}p = 0.0530$, $^{\&}p < 0.05$, $^{\&\&\&}p < 0.001$, differences from the corresponding index of β-phenylisopropylamine-treated animals; $^{\#}p < 0.05$, differences in the ipsi- and contralateral SB-408124 effects (the difference between the index measured on a given side of the brain after microinjections into the BNST on the same side of the brain and the same index after a similar exposure on the opposite side) by ANOVA. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine.

[1] In relation to the studied side of the brain.

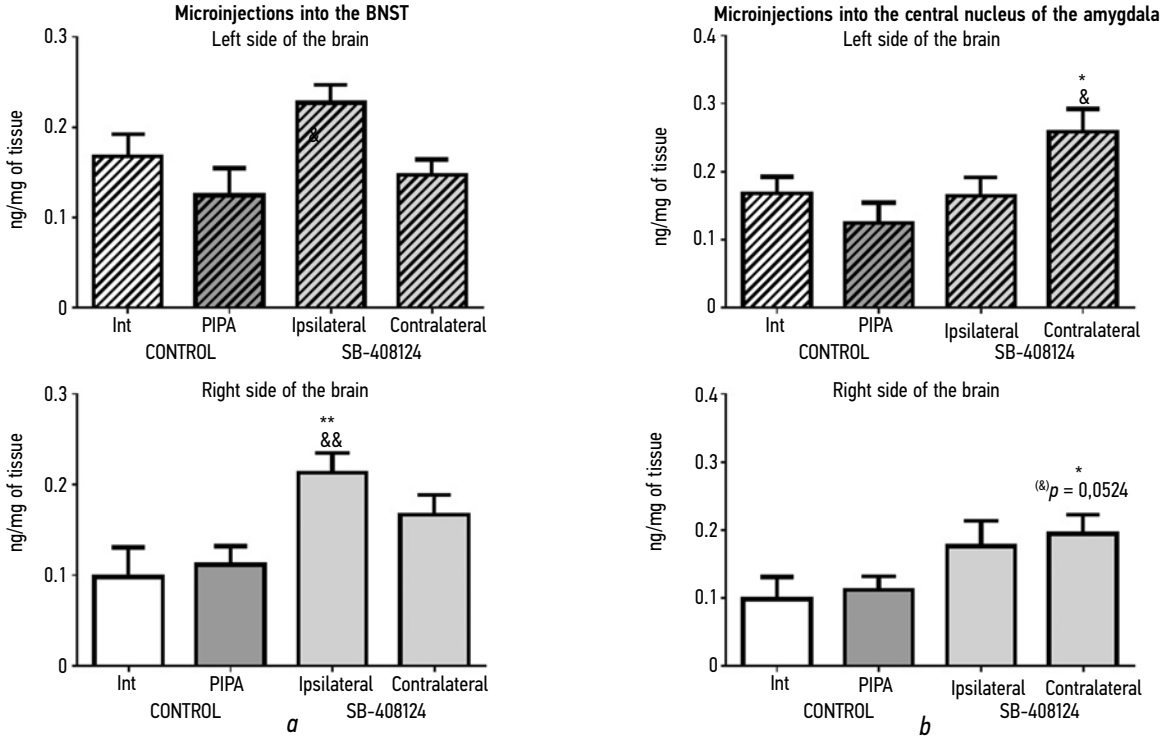

Fig. 5. Changes in serotonin (5-HT) levels in the striatum following unilateral microinjections of SB-408124: (*a*) in BNST (ipsilateral effects) and (*b*) in the central nucleus of the amygdala (contralateral effects). Animal groups: int, intact; ФИПА, after intraperitoneal injection of phenylisopropylamine (1 mg/kg weight); ipsi-, after microinjection of SB-408124 on the corresponding side of the brain; and contra-, after microinjection on the contralateral side. $^{*}p < 0.05$, $^{**}p < 0.01$, significant differences from the corresponding index measured in intact animals; $^{(\&)}p = 0.0524$, $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, differences from the corresponding index measured in animals receiving phenylisopropylamine, based on analysis of variance

Рис. 5. Изменения содержания серотонина (5-ГТ) в стриатуме под влиянием унилатеральных микроинъекций SB-408124: *а* — в BNST (ипсилатеральные эффекты); *b* — в центральное ядро миндалины (контралатеральные эффекты). Группы животных: инт — интактные, ФИПА — после внутрибрюшинного введения ФИПА (1 мг/кг веса), ипси- — после микроинъекции SB-408124 на соответствующей стороне мозга, контра- — после микроинъекции с противоположной стороны. $^{*}p < 0,05$, $^{**}p < 0,01$ — достоверные отличия от соответствующего показателя, измеренного у интактных животных; $^{(\&)}p = 0,0524$, $^{\&}p < 0,05$, $^{\&\&}p < 0,01$ — отличия от соответствующего показателя, измеренного у животных, получавших ФИПА — по результатам ANOVA

in the left striatum. In addition, left-sided injection of the orexin antagonist into the left olfactory tubercle increased the levels of HVA ($p < 0.05$) and 5-HIAA ($p < 0.05$) in the right olfactory tubercle, and similar trends were observed for HA ($p = 0.0690$) and DOPAC ($p = 0.0789$; Table 3).

SB-408124 injections into the right central nucleus of the amygdala induced other changes. The levels of HVA ($p < 0.05$) and 5-HT ($p < 0.05$) increased in the right (ipsilateral) cortex, whereas 5-HIAA levels ($p < 0.05$; Table 3) increased in the left (contralateral) cortex. In the hippocampus, right-sided injections resulted in bilateral HA increases (right, $p < 0.05$; left, $p < 0.01$) and right-sided increases in 5-HT ($p < 0.01$) and HVA ($p < 0.01$) levels (Table 2). A similar trend was evident for DOPAC levels ($p = 0.0895$).

Importantly, this exposure increased 5-HIAA levels in the left (contralateral) hippocampus, whereas symmetrical exposure obtained opposite results. In addition to the above-mentioned increase in 5-HT levels, right-sided injection into the central nucleus of the amygdala resulted in increased 5-HIAA ($p = 0.0533$) and HVA ($p < 0.01$) levels in the left (contralateral) striatum (Table 4). In the olfactory tubercle, this exposure led to increased levels of NA and a tendency for a right-sided asymmetry in DOPAC content ($p = 0.0584$; Table 3).

Overall, SB-408124 microinjections into the right central nucleus of the amygdala showed significant differences in 25.00% of the measured indices and only 4.17% when injected in the left central nucleus compared with rats receiving phenamine only (labeled "&" in Tables 1–4). In the comparison of the probabilities of the two corresponding binomial distributions, right-sided microinjections induced significantly more effects than those on the left side ($p < 0.0001$).

The results of the analysis of the parameters of MA-ergic systems in rats after unilateral SB-408124 microinjections into the BNST along with PIPA administration are presented in Tables 5–8. As described above, quantitative indices of MA-ergic systems did not change significantly in the hippocampus because of PIPA; however, an asymmetry appeared with the predominance of DA, 5-HT, and 5-HIAA on

the right side ($p < 0.05$; Tables 2 and 6). In the hippocampus, the only effect of SB-408124 injection into the BNST that was independent of the microinjected side was the disappearance of PIPA-induced asymmetry (Table 6). Levels of 5-HT levels also increased ($p < 0.05$) and the 5-HIAA/5-HT ratio decreased ($p < 0.05$) in the ipsilateral striatum under the influence of the orexin antagonist (Table 8 and Fig. 5). The effects of SB-408124 injection into the BNST were dependent on the injection side, which was most commonly manifested on only one side of the brain and were not mirror-symmetrical.

Left-sided SB-408124 injections increased the DOPAC/DA ratio in the hippocampus ($p < 0.05$; Table 6) and decreased the levels of DA (DOPAC) and 5-HT (5-HIAA) metabolites in the olfactory tubercle on the microinjected side ($p < 0.05$; Table 7). No such effects were observed when SB-408124 was injected into the right BNST. Right-sided SB-408124 injections caused a bilateral NA decrease in the prefrontal cortex ($p < 0.05$) and a DA decrease in the left cortex ($p < 0.05$; Table 5). In the left olfactory tubercle, the exposure significantly increased HVA (DA metabolite) levels, which may indirectly indicate an increase in DA release in this structure ($p < 0.05$; Table 7).

In the striatum, parameters of MA-ergic systems were mostly similarly changed after microinjections into the right BNST, with bilateral decreases in DA metabolism (DOPAC and DOPAC/DA, $p < 0.05$; Table 8) compared with intact rats and PIPA-treated rats. In addition, right-sided microinjections affected the 5-HT-ergic system of the striatum, i.e., asymmetry with predominance of 5-HT on the left side ($p < 0.05$; Table 8) occurred.

When counting the total number of differences from the groups receiving phenamine, right-sided injections in the BNST changed 14.58% of the indices, whereas left-sided injections changed only 6.25% (labeled "&" in Tables 5–8). The comparison of the probabilities of the two corresponding binomial distributions proved the greater effectiveness of right-sided microinjections ($p < 0.0001$).

Since SB-408124 injection into both the BNST and central nucleus of the amygdala induces a similar physiological effect, i.e., inhibition of activated self-stimulation of the lateral hypothalamus [3, 4], self-stimulation blockade may be related to the changes that would appear to be common to these two exposures. Such "coincident" effects included changes in 5-HT metabolism in the striatum. Right-sided SB-408124 injection into any of the examined structures of the extended amygdala led to an increase in 5-HIAA levels in the left (contralateral) striatum (Fig. 6). However, SB-408124 exposures to different regions of the extended amygdala

Table 6. Levels of monoamines and their metabolites (ng/mg of tissue) in the hippocampus of male Wistar rats with the unilateral administration of SB-408124 in bed nucleus of the stria terminalis

Таблица 6. Содержание моноаминов и их метаболитов (нг/мг ткани) в гиппокампе у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в BNST

Psychostimulant effects	–		1 mg/kg β-phenylisopropylamine, intraperitoneally					
Microinjected side[1]	–				Ipsilateral	Contralateral	Contralateral	Ipsilateral
Animal groups	Intact		β-phenylisopropylamine		1 μg/μL SB-408124 into the left central nucleus of the amygdala		1 μg/μL SB-408124 into the right central nucleus of the amygdala	
Side of the brain	Left	Right	Left	Right	Left	Right	Left	Right
NA	0.397 ± 0.141	0.250 ± 0.092	0.393 ± 0.108	0.452 ± 0.072$^{\#AC}$	0.367 ± 0.115	0.438 ± 0.114	0.384 ± 0.081	0.316 ± 0.076
DA	0.311 ± 0.106	0.253 ± 0.086	0.229 ± 0.056	0.307 ± 0.061$^{\#AC}$	0.250 ± 0.080	0.202 ± 0.060	0.268 ± 0.053	0.257 ± 0.065
DOPAC	0.435 ± 0.183	0.354 ± 0.182	0.334 ± 0.079	0.445 ± 0.074	0.398 ± 0.125	0.364 ± 0.108	0.424 ± 0.094	0.391 ± 0.116
DOPAC/DA	1.096 ± 0.272	1.254 ± 0.438	1.277 ± 0.191	1.963 ± 0.326	1.883 ± 0.078*	2.029 ± 0.740	1.555 ± 0.214	1.467 ± 0.171
HVA	0.090 ± 0.031	0.134 ± 0.054	0.069 ± 0.020	0.074 ± 0.014	0.012 ± 0.010	0.116 ± 0.047	0.053 ± 0.044	0.077 ± 0.039
HVA/DA	0.477 ± 0.160	0.450 ± 0.108	0.273 ± 0.097	0.320 ± 0.111	0.085 ± 0.078	0.376 ± 0.266	0.177 ± 0.135	0.266 ± 0.155
5-HT	0.098 ± 0.028	0.082 ± 0.026	0.085 ± 0.018	0.121 ± 0.022$^{\#\#AC}$	0.075 ± 0.017	0.056 ± 0.014$^{\#}$	0.094 ± 0.013	0.089 ± 0.014$^{\#*}$
5-HIAA	0.234 ± 0.038	0.247 ± 0.034	0.177 ± 0.019	0.217 ± 0.012$^{\#AC}$	0.167 ± 0.018	0.273 ± 0.038	0.207 ± 0.027	0.158 ± 0.040
5-HIAA/5-HT	3.063 ± 0.689	3.386 ± 0.741	2.778 ± 0.480	2.697 ± 0.602	2.769 ± 0.630	3.552 ± 0.802	2.508 ± 0.663	2.292 ± 0.888

*$p < 0.05$, differences from the corresponding index of intact animals; $^{\#}p < 0.05$, differences in the ipsi- and contralateral SB-408124 effects (the difference between the index measured on a given side of the brain after microinjections into the BNST on the same side of the brain and the same index after a similar exposure on the opposite side) by ANOVA; $^{\#AC}p < 0.05$, $^{\#\#AC}p < 0.01$, manifestations of asymmetry (differences between the corresponding indices of the left and right sides of the brain). 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine.

[1] In relation to the studied side of the brain.

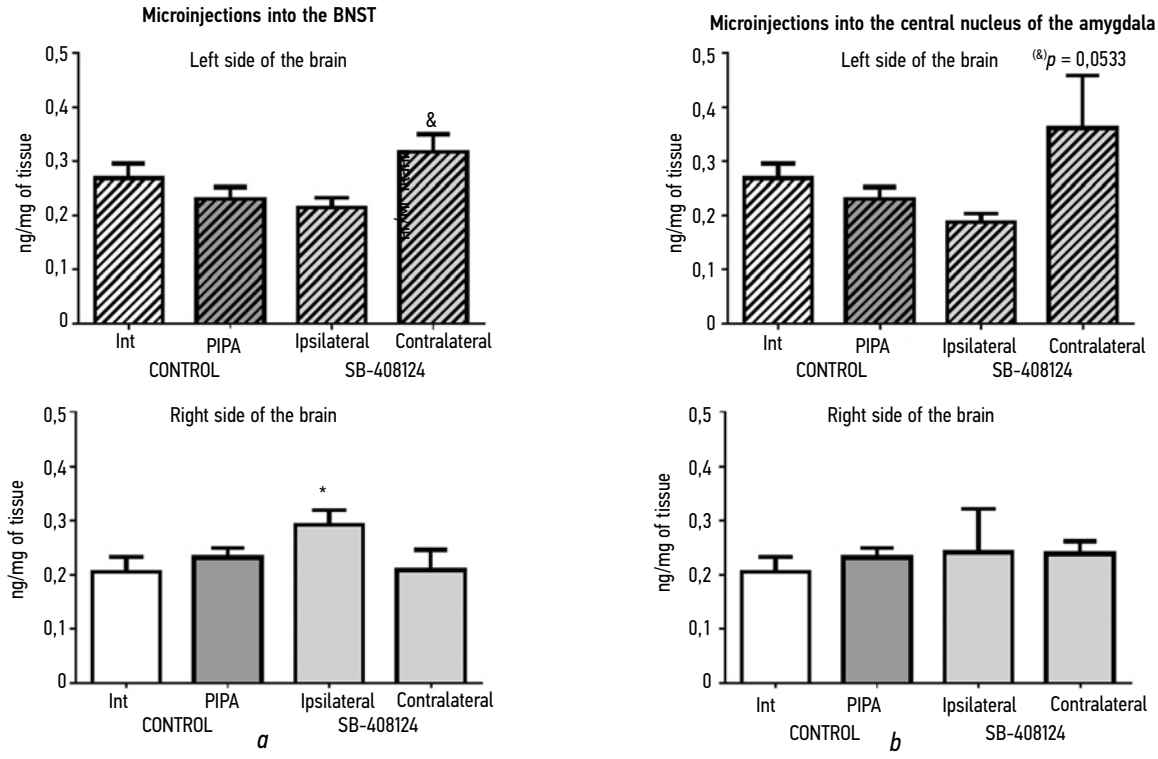

Fig. 6. Similar changes in 5-hydroxyindoleacetic acid (5-HIAA) levels in the striatum following unilateral microinjections of SB-408124 into the bed nucleus of the stria terminalis (BNST) (*a*) and central nucleus of the amygdala (*b*), right-sided injection of an orexin antagonist causes an increase in 5-HIAC levels in the left (contralateral) striatum compared with animals that received PIPA. Animal groups: int, intact; PIPA, after intraperitoneal injection of phenylisopropylamine (1 mg/kg weight); ipsi-, after microinjection of SB-408124 on the respective sides of the brain; and contra-, after microinjection on the contralateral side. $^{*}p < 0.05$, significant differences from the corresponding index measured in intact animals. $^{\&}p < 0.05$; $^{(\&)}p = 0.0533$, differences from the corresponding index in rats receiving phenylisopropylamine by analysis of variance

Рис. 6. Аналогичные изменения содержания 5-гидроксииндолуксусной кислоты (5-ГИУК) в стриатуме под влиянием унилатеральных микроинъекций SB-408124 в ядро ложа конечной полоски (bed nucleus of the stria terminalis — BNST) (*a*) и центральное ядро миндалины (*b*): правостороннее введение антагониста орексина вызывает повышение 5-ГИУК в левом (контралатеральном) стриатуме по сравнению с животными, получавшими ФИПА. Группы животных: инт — интактные, ФИПА — после внутрибрюшинного введения ФИПА (1 мг/кг веса), ипси- — после микроинъекции SB-408124 на соответствующей стороне мозга, контра- — после микроинъекции с противоположной стороны. $^{*}p < 0,05$ — достоверные отличия от соответствующего показателя, измеренного у интактных животных. $^{\&}p < 0,05$; $^{(\&)}p = 0,0533$ — отличия от соответствующего показателя у крыс, получавших ФИПА — по результатам ANOVA

caused laterally specific 5-HT changes in the striatum. Thus, microinjections into the BNST and central nucleus of the amygdala resulted in high 5-HT levels in the ipsilateral striatum and contralateral amygdala, respectively (Fig. 5).

Data on the blocking SB-408124 effect on activated self-stimulation [3, 4] were obtained when the substance was injected through a right-sided cannula. No data on the effect of left-sided injections were found. Probably, the authors of these studies believed that the effects of injecting drugs into symmetrical points of the brain would be similar. However, the present study favors that left-sided injections would be less effective. Previously, an irritation of the left hypothalamus was more likely to elicit proximity responses and produce self-stimulation responses compared with the right hypothalamus [10]. Moreover, contralateral (right-sided) SB-408124 microinjections caused an increase in 5-HT levels in the left hypothalamus [11]. The obtained data indicate that positive reinforcement includes interactions between the right and left brain halves; however, the participation of right and left brain structures is not equal. Thus, the key role in the blocking mechanism of the orexin receptor SB-408124 antagonist on PIPA-activated self-stimulation is played by its effect on the 5-GT-ergic system of the striatum.

CONCLUSIONS

1. The MA-ergic effects of unilateral microinjections of the orexin SB-408124 antagonist into the structures of the extended amygdala were dependent on both brain structures into which the drug is injected and exposure side.

2. Right-sided microinjections into the central nucleus of the amygdala and BNST have greater effects on monoamine metabolism than left-sided ones.

3. Right-sided SB-408124 administration increases 5-HIAA levels (5-HT metabolite) in the left striatum.

Table 7. Levels of monoamines and their metabolites (ng/mg of tissue) in the olfactory tubercle in male Wistar rats with the unilateral administration of SB-408124 in bed nucleus of the stria terminalis

Таблица 7. Содержание моноаминов и их метаболитов (нг/мг ткани) в обонятельном бугорке у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в BNST

Psychostimulant effects	–		1 mg/kg β-phenylisopropylamine, intraperitoneally					
Microinjected side[1]	–				Ipsilateral	Contralateral	Contralateral	Ipsilateral
Animal groups	Intact		β-phenylisopropylamine		1 μg/μL SB-408124 into the left central nucleus of the amygdala		1 μg/μL SB-408124 into the right central nucleus of the amygdala	
Side of the brain	Left	Right	Left	Right	Left	Right	Left	Right
NA	0.184 ± 0.037	0.096 ± 0.026	0.344 ± 0.054	0.283 ± 0.074	0.381 ± 0.190	0.372 ± 0.136	0.348 ± 0.215	0.398 ± 0.158
DA	0.192 ± 0.049	0.136 ± 0.023	0.398 ± 0.042*	0.358 ± 0.059*	0.265 ± 0.110	0.366 ± 0.144	0.302 ± 0.084	0.456 ± 0.130
DOPAC	0.556 ± 0.102	0.475 ± 0.110	0.800 ± 0.097	0.669 ± 0.120	0.406 ± 0.117$^{\&}$	0.775 ± 0.227	0.558 ± 0.138	0.668 ± 0.173
DOPAC/DA	2.732 ± 0.682	2.409 ± 0.438	2.081 ± 0.204	1.779 ± 0.239	1.493 ± 0.370	2.022 ± 0.472	1.910 ± 0.099	1.150 ± 0.093$^{\#\#AC}$
HVA	0.060 ± 0.038	0.103 ± 0.052	0.050 ± 0.027	0.124 ± 0.038	0.052 ± 0.026$^{\#}$	0.094 ± 0.055	0.234 ± 0.136$^{\#*\&}$	0.148 ± 0.050
HVA/DA	0.759 ± 0.491	0.794 ± 0.366	0.182 ± 0.107	0.408 ± 0.175	0.271 ± 0.174	0.424 ± 0.321	1.037 ± 0.695	0.417 ± 0.204
5-HT	0.142 ± 0.031	0.138 ± 0.027	0.193 ± 0.025	0.191 ± 0.018	0.144 ± 0.042	0.134 ± 0.042	0.225 ± 0.027	0.188 ± 0.034
5-HIAA	0.304 ± 0.031	0.261 ± 0.052	0.362 ± 0.040	0.316 ± 0.019	0.206 ± 0.032$^{\&}$	0.291 ± 0.072	0.311 ± 0.043	0.255 ± 0.054
5-HIAA/5-HT	1.665 ± 0.398	2.126 ± 0.521	1.896 ± 0.245	1.783 ± 0.171	1.689 ± 0.313	1.815 ± 0.556	1.411 ± 0.157	1.606 ± 0.499

*p < 0.05, differences from the corresponding index of intact animals; $^{\&}p$ < 0,05, differences from the corresponding index of beta-phenylisopropylamine-treated animals; $^{\#}p$ < 0.05, differences in the ipsi- and contralateral SB-408124 effects (the difference between the index measured on a given side of the brain after microinjections into the BNST on the same side of the brain and the same index after a similar exposure on the opposite side) by ANOVA; $^{\#\#AC}p$ < 0.01, manifestations of asymmetry (differences between the corresponding indices of the left and right sides of the brain) according to Student's paired t-test. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine

[1] In relation to the studied side of the brain.

In this case, microinjections into the BNST and central nucleus of the amygdala lead to high 5-HT levels in the ipsilateral and contralateral striatum, respectively. Thus, the ability of the orexin antagonist to block enhanced self-stimulation is associated with its lateral-specific effects on the serotoninergic system of the striatum.

ADDITIONAL INFORMATION

Authors contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study. The contribution of each author: I.V. Karpova, E.R. Bychkov, A.A. Lebedev — manuscript drafting, writing and pilot data analyses; I.V. Karpova, P.D. Shabanov — general concept discussion.

Competing interests. The authors declare that they have no competing interests.

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ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией. Вклад каждого автора: И.В. Карпова, Е.Р. Бычков, А.А. Лебедев — написание статьи, анализ данных; И.В. Карпова, П.Д. Шабанов — разработка общей концепции.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Table 8. Levels of monoamines and their metabolites (ng/mg of tissue) in the striatum of male Wistar rats with the unilateral administration of SB-408124 in bed nucleus of the stria terminalis

Таблица 8. Содержание моноаминов и их метаболитов (нг/мг ткани) в стриатуме у самцов крыс линии Вистар при унилатеральном введении препарата SB-408124 в BNST

Psychostimulant effects	–		1 mg/kg β-phenylisopropylamine, intraperitoneally					
Microinjected side[1]	–				Ipsilateral	Contralateral	Contralateral	Ipsilateral
Animal groups	Intact		β-phenylisopropylamine		1 μg/μL SB-408124 into the left central nucleus of the amygdala		1 μg/μL SB-408124 into the right central nucleus of the amygdala	
Side of the brain	Left	Right	Left	Right	Left	Right	Left	Right
NA	0.497 ± 0.095	0.467 ± 0.078	0.434 ± 0.050	0.473 ± 0.063	0.378 ± 0.108	0.485 ± 0.095	0.312 ± 0.122	0.432 ± 0.181
DA	0.353 ± 0.040	0.381 ± 0.095	0.614 ± 0.061*	0.530 ± 0.039	0.563 ± 0.112	0.622 ± 0.229	0.511 ± 0.080	0.580 ± 0.084
DOPAC	1.338 ± 0.198	1.269 ± 0.178	1.251 ± 0.109	1.295 ± 0.107	$1.582 \pm 0.388^{\#\#}$	$1.625 \pm 0.350^{\#}$	$0.624 \pm 0.145^{\#\#*\&}$	$0.717 \pm 0.160^{\#}$ $^{(*)}p = 0.050^{\&}$
DOPAC/DA	2.670 ± 0.244	3.441 ± 0.780	2.274 ± 0.313	2.626 ± 0.337	$2.902 \pm 0.408^{\#\#}$	2.027 ± 0.074	$1.234 \pm 0.266^{\#\#*\&}$	$1.214 \pm 0.169^{*\&\&}$
HVA	0.342 ± 0.027	0.344 ± 0.046	0.233 ± 0.050	0.247 ± 0.047	0.340 ± 0.056	$0.363 \pm 0.077^{\#}$	0.291 ± 0.131	$0.124 \pm 0.069^{\#*}$
HVA/DA	0.959 ± 0.189	1.558 ± 0.620	0.400 ± 0.084**	0.569 ± 0.111**	0.726 ± 0.208	0.468 ± 0.108	0.582 ± 0.210	0.224 ± 0.132
5-HT	0.168 ± 0.024	0.098 ± 0.033	0.125 ± 0.030	0.112 ± 0.021	$0.227 \pm 0.020^{\&}$	0.167 ± 0.021	0.147 ± 0.017	$0.213 \pm 0.022^{**\&\&}$
5-HIAA	0.268 ± 0.028	0.206 ± 0.027	0.231 ± 0.022	0.231 ± 0.018	0.215 ± 0.019	0.209 ± 0.037	$0.317 \pm 0.033^{\#\&}$	0.291 ± 0.027*
5-HIAA/5-HT	1.661 ± 0.132	3.251 ± 0.827	1.772 ± 0.419	2.218 ± 0.354*	0.936 ± 0.119* $^{(\#)}p = 0.0589$	1.285 ± 0.436	1.995 ± 0.393 $^{(\#)}p = 0.0589$	1.464 ± 0.277*

$^{(*)}p = 0.050$, $^{*}p < 0,05$, $^{**}p < 0.01$, differences from the corresponding index of intact animals; $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, differences from the corresponding index of beta-phenylisopropylamine-treated animals; $^{(\#)}p = 0.0589$, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, differences in the ipsi- and contralateral SB-408124 effects by ANOVA. 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; BNST, bed nucleus of the stria terminalis; DA, dopamine; DOPAC, dioxyphenylacetic acid; HVA, homovanillic acid; NA, norepinephrine

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