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Effect of Substance P on Behavior in the Open Field Forced Swimming Tests in Rats with Various Types of Behavior

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It was found that substance P administered to rats with active and passive types of behavior reversed their behavioral type but did not affect the behavioral parameters of rats comprising an intermediate group. The "pure" effect of substance P (discounting the influence of repeat testing) was significant only for the number of rearings and the time of passive floating.

Key Words: *individual features of behavior; open field test; forced swimming test; substance P*

Our previous studies showed that the brain's resistance to circulatory hypoxia (ischemia), evaluated by the "48-h survival" index, is in good correlation with the type of behavior. The level of depression (time of passive floating, TPF in the Porsolt test [9]) and the number of rearings (NR) in the open field test turned out to be the most informative behavioral parameters for predicting the outcome of brain ischemia [3]. Substance P (SP) administered 30 min after bilateral ligation of the carotid arteries lowered the resistance to ischemia in rats with the active type of behavior (highly resistant), raised it in rats with the passive type of behavior (low-resistant), and did not reliably affect the resistance of rats of an intermediate group (medium-resistant) [5]. The fact that SP differently affects the mortality in the "extreme" groups characterized by opposite types of behavior indicates, first, that the type of emotional and behavioral response to a stress factor plays an important role in the resistance to the pathogenic action of circulatory hypoxia, a conclusion which agrees with the data obtained on the model of experimental emotional stress [1]. On the other hand, it may be assumed that the effect of SP on the resistance to brain ischemia is mediated by its modulation of behavior type. The present study was aimed at testing this hypothesis, the specific goals being to find out how the type of behavior in the open field and forced swimming tests is modified by SP treatment and if SP similarly affects all the behavioral parameters under analysis.

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MATERIALS AND METHODS

The study was carried out on 36 male albino rats weighing 250-300 g kept under standard vivarium conditions (5-6 animals in a cage) with natural lighting. The type of behavior was determined by means of the open field and forced swimming tests [9] in accordance with a technique developed earlier [3,4] which enables animals with significantly different behavioral indices (number of squares crossed - NSC, NR, and TPF) to be distinguished within a general population. To better reveal the effects of SP on behavior during a later second testing, optimal pairs of animals ("control-experiment") were chosen on the basis of their behav-

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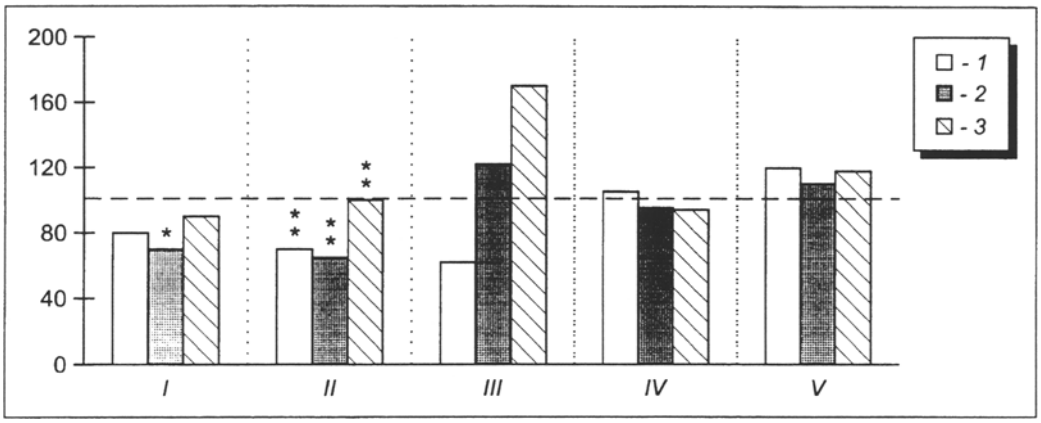

Fig. 1. Effect of repeat testing on behavioral indices in rats with different types of behavior. Here and in Fig. 2: data are presented in percent of the initial values taken as 100% (dotted line). *1*) rats with the active type of behavior; *2*) an intermediate group; *3*) rats with the passive type of behavior. *I*) number of squares crossed; *II*) number of rearings; *III*) number of forays to the center of the field; *IV*) time of passive floating; *V*) duration of first episode of active swimming. *$p<0.05$; **$p<0.01$ as compared to initial values.

ioral indices. To this end, the animals initially divided into three groups with active (n=14), passive (n=12) and intermediate (n=10) types of behavior were pooled together. Using the method of principal components, we found the factorial loads for the first principal component accounting for over 90% of the total variability. In other words, we introduced a new NSC-, NR-, and TPF-dependent variable which reflects the relations between these characteristics and maximally changes its values along with changes in behavioral activity. Then all the animals were divided into pairs according to close values of this variable. Paired diagrams of all the parameters under study showed that in all cases both rats within each pair belonged to the same group. The two behavioral tests carried out 7 days apart. Thirty minutes before the second test one randomly chosen rat was injected with SP (i.p., 250 μg/kg in 0.5 ml saline) while the other received just saline (0.5 ml). This approach made it possible to compare behaviorally matched SP- and saline-injected animals. The analysis of behavioral characteristics of animals from control subgroups allowed us to evaluate time-related changes in the main behavioral indices upon repeat testing and to eliminate their influence when comparing the data of the repeat test in the control and experimental subgroups, thus determining the "pure" behavioral effect of the substance under study. In addition, we calculated both the absolute and relative (in relation to the initial or control values) changes in behavioral parameters with time and determined the coefficient of variation for these values, which shows the variability of the parameters relative to their mean values, $CV = s/\bar{x} \times 100\%$, where CV is the coefficient of variation, s is the standard deviation, and $\bar{x}$ is the mean value of the parameter. The significance of differences between the mean values of each parameter of interest in the 3 groups of animals was tested by standard one-factor analysis of variance (ANOVA). The data were treated statistically with "STATGRAPHICS" software. Significant differences were assessed with Student's t test. The data of ANOVA were assessed routinely with Fisher's F test.

RESULTS

Rats with different types of behavior initially demonstrated significant difference in NSC, NR, and TPF.

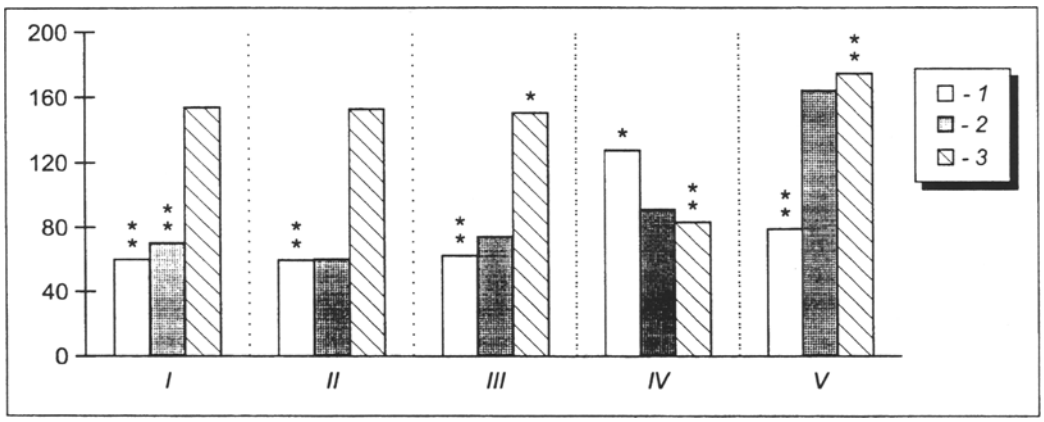

Fig. 2. Effect of SP on behavioral indices in rats with different types of behavior.

TABLE 1. Initial Values of Behavioral Indices in Rats with Different Types of Behavior

Behavioral indices (over 10 min)	Group					
	1 (active type of behavior)		2 (intermediate type)		3 (passive type of behavior)	
	control (n=7)	experiment (n=7)	control (n=5)	experiment (n=5)	control (n=6)	experiment (n=6)
Number of squares crossed	240.0±17.7	261.1±16.8	175.8±11.8*	185.4±15.9**	115.2±24.5***	89.5±23.4*****
Number of rearings	37.5±4.2	34.7±2.9	26.6±1.2*	32.6±6.1	12.2±1.7******	14.5±2.5****
Number of forays to center	11.8±1.2	9.8±1.7	4.6±0.8**	6.2±1.8	3.3±9.9***	2.5±0.5**
Time of passive floating, sec	375.5±29.8	372.5±22.4	460.0±20.8*	458.0±13.9*	500.3±11.3***	510.3±8.7*****
Duration of 1st episode of active swimming, sec	105.7±14.0	125.0±7.2	92.6±11.4	66.0±11.2***	57.2±8.8**	47.6±3.9***

Note: *p<0.05; **p<0.01; ***p<0.001 as compared to group 1; +p<0.05; ++p<0.01; +++p<0.001 as compared to group 2.

At the same time, no significant differences were initially revealed within each group between the control and experimental subgroups in any of the parameters (Table 1). The repeat test of control subgroups showed a significant decrease in locomotor activity (a smaller NSC) in the rats of the intermediate group, a decrease in exploratory activity in the rats with the active and intermediate types of behavior, and a smaller number of forays to the center of the field in the rats with the active type of behavior. None of the groups demonstrated significant changes in behavioral parameters in the forced swimming test (Fig. 1). An increase in the number of forays to the center of the field, an increase in the duration of the first episode of active swimming, and a decrease in TPF were observed during the repeat test in the passive rats injected with SP. On the other hand, SP-injected rats of the active type demonstrated lower locomotor activity, a shorter first episode of active swimming, and increased TPF. The behavioral parameters of the rats of the intermediate group were nearly the same (Fig. 2). The analysis of variance showed that significant differences in NSC ($F_{(2.15)}$=17.5, p=0.0001), NR ($F_{(2.15)}$=26.8, p=0.0001), and TPF ($F_{(2.15)}$=8.1, p=0.004) between control subgroups within all the groups were also present upon the repeat testing. On the other hand, the repeat testing of SP-injected rats did not reveal any significant differences between the groups with regard to any of the parameters: $F_{(2.15)}$ for NSC, NR, and TPF was 1.2 (p=0.3), 0.4 (p=0.7), and 0.3 (p=0.7), respectively. In other words, SP smoothed the differences among the various behavioral types. This is due to the fact that in the "extreme " groups SP affects locomotor activity and depressivity in opposite directions, whereas it has no effect on the behavioral parameters in the intermediate group. The relative effect of the repeat testing on all the behavioral parameters in the control subgroups was not found to be significant. The relative effect of SP injection is significant for NSC ($F_{(2.15)}$=12.9, p=0.0005), TPF ($F_{(2.15)}$=6.0, p=0.01), the duration of the first episode of active swimming ($F_{(2.15)}$=4.3, p=0.03), and the number of forays to the center ($F_{(2.15)}$=12.7, p=0.001) and insignificant for NR ($F_{(2.15)}$=2.9, p=0.08) and the number of groomings ($F_{(2.15)}$=1.4, p=0.3). The "pure" effect of SP is significant only for NR ($F_{(2.15)}$=4.0, p=0.04) and TPF ($F_{(2.15)}$=5.4, p=0.02) and insignificant for all the other parameters.

Thus, the data obtained indicate that SP affects the behavioral parameters of the rats of the "extreme" types in an opposite direction. Its "pure" effect turned out to be significant only for exploratory activity (NR) and the level of depression (TPF), although the absolute and relative effects of SP were also significant with regard to NSC and the number of forays to the center of the field, as has also been observed by other researchers [7,10,11]. Since NR and TPF are fundamental characteristics which determine the type of behavior [3,4], we may conclude that SP exerts modulating effects on the type of behavior. The coefficients of variation for the relative effects of SP on NR were smaller than those for the absolute effects, indicating that SP may regulate exploratory activity in accordance with the principle of "the effect being proportional to the extent of parameter deviation." This ties in with the concept of the regulating mechanisms of SP action [13] and its effects on the central mechanisms of regulation of a given type of behavior. The difference in its effects on the behavioral parameters of rats with different types of behavior may be attributed to variations in SP breakdown by the body's enzymes, because the C- and N-terminal fragments of SP are known to have different behavioral effects [12]. Taking into

consideration the reciprocal relations between the activity of the central monoaminergic systems and NR or TPF [2,9], we may assume that the tranquilizing and antidepressant effects of SP on rats with the passive type of behavior are due to its activating effect on the catecholaminergic systems of the brain, while the sedative and depressant action on rats with the active type of behavior is explained by SP-induced suppression of these same systems. It is known that the level of brain catecholamines plays an important role in the brain's resistance to ischemia [8]. This explains why NR and TPF, which are indicative of the level of brain catecholamines [2,9], are the most valid indices for predicting the brain's resistance to ischemia [3] and why SP, which is capable of changing the concentration of brain catecholamines [6], exerts modulating effects on the resistance to circulatory cerebral hypoxia [5].

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