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RELATIONSHIP BETWEEN THE PECULIARITIES OF ELABORATION AND RETENTION OF BRIGHTNESS DISCRIMINATION AND THE SEROTONIN CONTENT IN THE RAT BRAIN

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Abstract. The role of serotonin in memory consolidation was investigated by comparing the serotonin content in the brain of young and old animals which differed in the duration of consolidation process and by comparing the serotonin content in the brain with the efficiency of retrieval of memory traces in animals of the same age. Old rats had the serotonin content higher in the hippocampus and lower in the hemispheres, diencephalon, midbrain and medulla as compared with young ones. Elaboration of brightness discrimination increased the brain serotonin content in old animals and decreased it in young ones. Inverse correlation occurred between the serotonin content in the hippocampus, midbrain and the medulla and retention of conditioned reflexes in rats of the same age. No correlation occurred between the serotonin content in the cerebral structures and the rate of conditioning.

INTRODUCTION

One of the possible ways of ascertaining the role of neuromediators in conditioned activity consists of comparing their content in the brain of experimental animals with the rate of formation, fixation and reproduction of memory traces. So far such an approach has been chiefly applied in genetic investigations in which the neuromediator content and the activity of the enzymes involved in their metabolism were considered as neurochemical correlates of genetically determined characteristics of behavior. For instance, Wimer et al. (20) discovered substantial differences in the serotonin content in the brain of C57BL/6J and DBA/2J

strains of mice, but no difference in the noradrenaline content. According to other data, the strains of mice differing in emotionality, motor activity and maze performance differ in the catecholamine and serotonin content in the brain and in its various parts (14, 16, 17). Definite relationships between the behavior of different strains of mice and activity of many enzymes participating in the metabolism of neuromediators have been found by Tunnicliff et al. (17).

The above studies indicate the existence of definite relationships between the neuromediator content in the brain and behavior of animals of different genetic lines, but also stimulate the search for such relationships in individual animals. Such an investigation was carried out by Krause et al. (10), who found that the noradrenaline content in the cerebral hemispheres of fast learning mice is lower, and that of dopamine is higher, than in slow learning mice.

The purpose of the present paper is to establish the role of serotonin in conditioning. According to some reports (5, 11) serotonin seems to play an important role in the consolidation of memory traces. In accordance with this concept, it may be surmised that the serotonin content in the brain of animals differing in the rate of the consolidation process is different, and that the differences are especially pronounced in structures directly participating in the consolidation process. As more perfect consolidation provides for more stable fixation (and hence for better retention and subsequent retrieval) of memory traces, one might likewise assume certain differences in the serotonin content in the brain of animals differing in their capacity for the retention of elaborated conditioned reflexes. The assumptions were experimentally verified by comparing the serotonin content in the brains of young and old animals which differ as is well known (3) in the duration of the consolidation process, and by comparing the serotonin content in brains with similar efficiencies of retrieval in animals of the same age.

METHODS

The experiments were carried out on albino rats of different ages. Simultaneous brightness discrimination was elaborated in a Y-shaped maze with electrified floor. Each arm of the maze was 25 cm long, 12 cm high and 12 cm wide. Sixty-watt bulbe were used to illuminate the arms. The rat was placed in one of the maze arms, and the arm to the right or to the left of the animal was illuminated. Simultaneously, an electric current ($\approx$ 20 V, 50 Hz) was applied to the grid floor of non-illuminated arms of the maze, and the rat could escape its action by running over to the illuminated arm. The light was then switched off and within 30

to 90 s was switched on again in another arm simultaneously with electrocutaneous stimulation (ES) in the non-illuminated parts of the apparatus. The arms were used in a random sequence which corresponded to Gellerman's tables (6) or to the scheme of Ott and Matthies (13), according to which the right arm was first lighted up three times in succession, then the left one three times, and so on. Only such a reaction was considered correct when in response to ES the rat ran straight into the illuminated arm without entering the non-illuminated alley first.

The effect of conditioning on the serotonin content in the brain, was studied after 50 trials of training. Retention of the discrimination was studied as follows. In the initial training, brightness discrimination was elaborated in the rats up to a criterion of 10 successive correct trials. Ten days later, the discrimination was retrained to the same criterion, and savings (A) was calculated after the formula:

$$A = \frac{n_1 - n_2}{n_3} \cdot 100,$$

where n_1 is the number of trials required to attain the criterion in the acquisition session, and n_2 is the number of trials required to attain the criterion in the relearning session. The serotonin content in the different brain structures was determined spectrophotofluorometrically according to the method of Kuntzman et al. (12).

Two series of experiments were carried out. In the first series the serotonin content was examined in the cerebral structures of naive and trained rats aged 8 to 10 wk and 15 to 18 mo. The animals were decapitated either immediately after conditioning or 7 to 10 days after conditioning. In the second series of experiments, performed on females aged 8 to 10 wk, a comparison was made between the serotonin content in the cerebral structures and the proficiency in learning and relearning brightness discrimination. Decapitation of the animals was carried out immediately after the relearning test.

The experimental data were evaluated with Student's t-test, Spearman's coefficient of range correlation was used to establish the relationships between the serotonin content in the various parts of the rat brain and the rate of learning and savings.

RESULTS

A comparison between the serotonin content in the cerebral structures of untrained rats of different ages (Table I, col. I, IV) shows that the serotonin content in the hippocampus of old rats is higher; whereas

TABLE I

Serotonin content (μg/g of wet tissue) in cerebral structures and its changes under the influence of defensive conditioning in rats of different ages (n — number of animals used)

Brain structure	Young animals			Old animals			P		
	Naive animals ($n = 7$)	Immediately after conditioning ($n = 9$)	7 days after conditioning ($n = 8$)	Naive animals ($n = 10$)	Immediately after conditioning ($n = 9$)	7 to 10 days after conditioning ($n = 8$)	I–IV	II-V	III–VI
	I	II	III	IV	V	VI			
Hippocampus	0.56±0.03	0.37±0.048*	0.41±0.04	0.77±0.07	0.79±0.03	0.79±0.03	<0.05	<0.01	<0.01
Hemispheres	0.51±0.046	0.45±0.016	0.55±0.01	0.40±0.022	0.60±0.036*	0.67±0.04	<0.05	<0.01	<0.05
Diencephalon	0.67±0.042	0.49±0.042*	0.56±0.07	0.57±0.044	0.82±0.05*	0.79±0.08	<0.05	<0.01	<0.05
Mesencephalon	0.69±0.019	0.55±0.045*	0.59±0.05	0.54±0.04	0.83±0.03*	0.75±0.082	<0.05	<0.01	>0.05
Medulla	0.67±0.011	0.55±0.045*	0 74±0.036	0.49±0.036	0.72±0.036*	0.67±0.04	<0.05	<0.05	<0.05

* $P_{II\text{-}I;\ V\text{-}IV} < 0.05$

in the hemispheres, diencephalon, mesencephalon and medulla it is lower than in young animals.

To investigate the influence of brightness discrimination on the serotonin content in the brain, 50 trials were applied to each group. Under such conditions the young animals showed 35.7 ± 1.0 and the old rats 13.4 ± 1.1 correct reactions ($P < 0.01$). This means that the brightness discrimination learning in young animals proceeded two and a half times faster than in the old animals. At the same time, no correlation was found in the animals of either age group between the serotonin content in different parts of the brain and the acquisition rate estimated by the number of correct reactions in the 50 trials.

Conditioning induces opposite changes in the serotonin content of the central structures examined in young and old animals. It follows from Table I (col. II, V) that immediately after conditioning, the serotonin content in the brain of young animals decreases, while in the old animals conditioning produces a substantial increase in the serotonin content in all the examined parts of the brain. The shifts in the serotonin content induced by conditioning persist for a long time and are still present 7 to 10 days after conditioning (Table I, col. III, VI). By that time the young animals exhibit a tendency towards normalization of the serotonin content, which is clearly expressed in the hemispheres, diencephalon and medulla; while in the old animals such a tendency is less pronounced, if at all (the hemispheres and the hippocampus).

The aim of the second series of experiments was to study the relationships between the learning and relearning rates and the serotonin content in different parts of the brain in females of the same age. Preliminary investigations did not reveal any difference in the serotonin content in the cerebral structures of the untrained males and females (Table II). The brightness discrimination was elaborated in female rats up to a criterion of 10 successive correct reactions in 24.00 ± 2.0 trials,

TABLE II

Serotonin content in the brains of male and female rats (n — number of animals used)

Brain structure	Serotonin content, μg/g wet weight	
	Males ($n = 8$)	Females ($n = 9$)
Hemispheres	0.51±0.046	0.57±0.057
Hippocampus	0.56±0.031	0.55±0.017
Diencephalon	0.67±0.041	0.62±0.036
Mesencephalon	0.69±0.019	0.75±0.05
Medulla	0.67±0.011	0.69±0.037

retraining — in 18.25 ± 3.11 trials. Under these conditions the savings index was 25%, ranging from 0 to 58%. The results of studying the above relationships, as measured by the coefficient of Spearman's range correlation, are given in Table III, which shows that on the whole there

TABLE III

Correlation (ϱ) between the learning and relearning rates (savings) of a brightness discrimination and the serotonin content in different parts of the rat brain

Brain structure	Acquisition rate		Savings	
	ϱ	P	ϱ	P
Hemispheres	+0.10	> 0.05	−0.34	> 0.05
Hippocampus	−0.074	> 0.05	−0.57	< 0.05
Diencephalon	+0.20	> 0.05	−0.42	> 0.05
Mesencephalon	+0.20	> 0.05	−0.50	< 0.05
Medulla	+0.13	> 0.05	−0.47	< 0.05

is a negative correlation between savings and the serotonin content in the brain. This correlation is significant in the hippocampus ($\varrho = -0.57$), mesencephalon ($\varrho = -0.50$) and medula ($\varrho = -0.47$).

At the same time, as in the first series, no regular relationships were revealed in the comparison of the serotonin content in the examined structures with the acquisition rate.

DISCUSSION

The main facts obtained in the present investigation are:

1. The serotonin content in the cerebral structures of young and old animals is different.

2. Brain serotonin content is influenced by defensive training in young and old animals in different ways.

3. There is a negative correlation between the serotonin content in the cerebral structures and savings, though there is no mutuality between the serotonin content in the brain and the rate of conditioning.

It is common knowledge that the rate of the consolidation process decreases with age (3). It is also known that the hippocampus plays an important role in the consolidation of temporary connections (4, 8, 18). The assumption seems to be justified, therefore, that slow consolidation in old rats may be due to high serotonin content in the hippocampus, which was found in our experiments. Defensive training in old animals leads to a further rise in the serotonin content in the brain; but in young

animals, under similar conditions, there is a drop in the serotonin content. This indicates that training generates conditions which prevent the consolidation of temporary connections in the old rats, but enhance consolidation in young animals. Since an excess of serotonin has an inhibitory effect on RNA and protein synthesis (5, 19), it may be assumed that the underlying mechanism accounts for the age-related slowing of consolidation. This view is to a certain extent supported by the findings of Hyden et al. (9), according to which conditioning is accompanied by activation of the synthesis of "specific" proteins in many cerebral structures in fast learning animals, while no such activation is found in slow learning. According to Altschuler et al. (1) conditioning increased content of RNA in the brain in young rats but diminished it in old animals.

Another important finding is the lack of correlation between the serotonin content in the brain and the rate of conditioning. Deep inhibition of protein synthesis in the brain does not affect the rate of conditioning, but impairs or prevents consolidation of the corresponding engrams (2). The same effect is produced by excessive accumulation of serotonin in the brain (11), which may support the assumption that serotonin influences the consolidation process by controlling protein synthesis in the brain (5).

It is worth noting that alimentary conditioning does not cause a decrease, but rather an increase of serotonin content in different parts of the rat brain (7, 15). It is possible that the differences in the direction of the shifts of serotonin content in the brain, apart from the animal's age, depend on the conditioning paradigm and, notably, in the nature of reinforcement. This point calls, however, for additional study, and any conclusions on this score appear to be premature at present.

The fact that the differences in the distribution of brain serotonin in animals of different age correspond to different rates of conditioning provides grounds for believing that there exists a definite relationship between these parameters. A comparison between the serotonin content in the brain and the rate of conditioning did not, however, reveal any consistent relationships between these values in either individual group. In other words, serotonin distribution in the brain and the rate of conditioning in animals of different age are not mutually dependent but are probably both influenced by a third factor which calls for special investigation. Particular attention should be given to inverse correlation between the degree of retention of conditioned reflexes and the serotonin content in the hippocampus, midbrain and medulla. Complete preservation of the memory traces evidently reflects the efficiency of the consolidation processes. From this point of view, the savings may be re-

garded as a quantitative measure of consolidation. The results support the proposition that an excess of serotonin in certain cerebral structures hinders the consolidation process; while a reduction in the serotonin content, quite the contrary, facilitates consolidation at any rate in the case of aversively motivated brightness discrimination learning.

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