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Cognitive Deficit in the Animal Model of Depression

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ABSTRACT. **Investigation of the factors underlying depression is essential for contemporary clinical medicine and neuroscience. A vast number of different methods and models are widely adopted for solving this problem. Among the models of depression, a long-lasting (2 and 8 months) social isolation, along with partial light deprivation should be considered as an adequate animal model of depression. In this model the learning and memory processes were studied using active and passive avoidance tests in rats. Porsolt's forced swimming test was used for evaluation of the level of depression. Analysis of the results obtained showed that long-lasting social isolation and partial deprivation of light caused significant cognitive deficit as compared to those depressive animals which remained in normal social environment. The longer was the social isolation the stronger was deficiency in learning process and consolidation deterioration. Neurochemical investigation of the brain of the depressive animals by means of ^{3}H-cetanserin (NEN, USA), has shown an increased number of the serotonergic receptors in neocortex and caudal region of the brain. This fact indicates deficit of serotonin in the above regions of the brain.**

Presumably, serotonin deficit in this animal model of depression is the cause of disrupted learning and memory processes.

Therefore, the proposed model of depression - social isolation plus partial deprivation of light - could be considered as an adequate and valid model of depression suitable for the studies of other manifestations of the brain integrative activity. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words: *cognitive defect, serotonin, animal model, depression.*

Investigation of neurophysiologic mechanisms of depression is a major challenge facing psychiatry and neurobiology. Although there is a wide variety of models of depression, they reflect the lack of a single satisfactory solution [1]. Investigation of the factors which generate depression and create respective animal models, must promote understanding of the causes of this disease. However, inappropriate neurotransport and neurotropins may lead to structural and functional disorganization [1-4] which, in its turn, evoke some cognitive disorders and deficit in the learning and memory processes [5-8]. Among the models of depression, social isolation of different duration, with partial deprivation of illumination might be a useful tool for investigation of learning and memory processes in experimental conditions [9-11]. Considering, the above notion, the aim

of this work was to study, feasibility of acquisition of active avoidance reaction, its retention, and processes of memory trace consolidation in the passive avoidance test, with further neurochemical assessment of the serotonin receptors' density in the cortical and caudal areas of the rat brain.

Materials and methods. Experiments were conducted in the outbred male adult albino rats, weighing 200-350 grams. Following natural weaning the animals were housed in separate, dimly illuminated cages with free access to food and water, and at ambient temperature of 18-20^{o}C. Duration of social isolation and partial deprivation of light was 2 months (n=12) and 8 months (n=10). Additional fifteen animals served as control subjects.

Selection of animals for depression properties. The level of depression was determined with forced swimming test (FST) [12]. The animals were immersed into 40 cm deep water (22-26^{o}C) for 15 min on the first day and for 5 min - on the second. Time of forced swimming and immobilization in the water were recorded. The animals were considered depressive if they maintained predominance of passive vertical floating.

Experimental procedures. The *active avoidance response* (AAR) was tested in the shuttle-box apparatus consisting of a rectangular chamber with two compartments (overall size 50x20x20 cm). There was a 4 cm high partition between the compartments. The animals should jump over the partition in response to electrical stimulation (0.5-1.2 mA) delivered through the metal floor grid. About 10-20 s prior to electrical stimulus a 500 Hz tone was presented. The auditory stimulus acquired thus a property of conditional stimulus. The AAR was regarded as acquired if an animal, in response to conditional stimulus, jumped over the partition in the 9 sequential trials out of 10, avoiding hence the painful stimulus.

Passive avoidance test (PAT) was conducted in a two-compartment chamber. One compartment consisted of Perspex cube (25x25x25 cm); on the one side it had a hole which allowed rats to enter from the lighted compartment to the dark one. The lighted compartment was illuminated with four incandescent lamps (60 W each). The dark compartment was 20x25x25 cm. The floor grid in the dark compartment was electrified and an animal entering the compartment received noxious stimulus – an AC electrical stimulation of 2 mA.

At the beginning of the PAT experiments an animal was placed in the light chamber with its back to the hole. Latency of entrance into the dark compartment was recorded. After entrance into the dark compartment, the hole was closed and an animal received 10 s electric shock. Then the animal was withdrawn from the dark compartment and returned to its living cage. Retention of the passive avoidance reaction was assessed after 24 h. The test response retention was evaluated by the latent period of entrance into the dark compartment. Behavior was observed for 10 min. If the latency of entrance was over 4-5 min the reaction was considered as retained.

Neurochemical analysis of the brain serotonergic system. In this series of experiments the animals were beheaded in order to determine the number of serotonin receptors. The brains were removed and frozen. Frontal and caudal brain slices were made. The number of serotonin receptors was assessed with the ^{3}H-cetanserine (NEN, USA). Synthetic membranes were incubated with radioactive ligand (1-10nM), during 30min at 20ºC, in the Tric-HCl buffer (pH-7.4). Incubation medium was decanted through Whatman CF/C and quantities of radioactivity on the filters were determined by means of the liquid-spring scintillation counter. Nonspecific binding of radioactivity was calculated using 1 mM serotonin.

The obtained data were statistically processed with the Student *t*-test.

Experiments were approved by the Ethical Committee of Animal Experiments at the I.Beritashvili Institute of Physiology

The Results and Discussion. In the first series of experiments depression index was determined in the forced swimming test in the control group, and in the experimental animals, after 2 (n=8) and 8-months (n=10) of social isolation and light deprivation. In the control group (n=12) the animals with a high index of depression-like condition (above 75% of passive floating in the water) were selected. After 2- and 8-months of isolation, all the animals of experimental groups showed passive behavior in the water (immobilization index about 80% and over). Testing in the open field showed that isolated animals developed less motor and exploration activity.

In the second series of experiments the possibility of learning in the AAR 120 trials in the two-way test was studied. The AAR was regarded as achieved if in response to auditory stimulus an animal jumped over the partition in 9 sequential trials out of 10.

In addition the animals were tested in the open field as well. The correspondence between the indices of FST and the open field was discovered only in those animals of the third group which were kept in conditions of social isolation and partial light deprivation for 8 months.

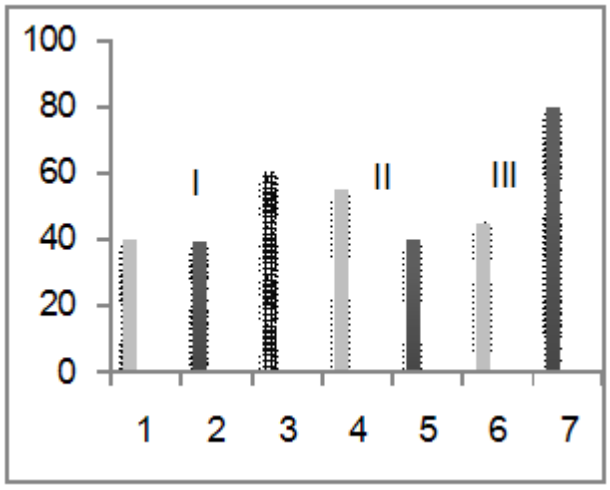

Fig. 1. Acquisition of active avoidance reaction in 120 trials two-sided test in the three groups of animals: intact animals (I); animals after two months of isolation (II); animals after eight months of isolation (III).
I . The intact group of the animals - 1. Control (immobility in the FST <60%), (n=12); 2. Non-depressive (immobility in the FST <50%), (n=8); 3. Depressive, (immobility in the FST >75%), (n=8). **II**. The second group - 4.Control (random) (n=10); 5. After two months of isolation (immobility in the FST >75%) (n=12). **III.** The third group - 6. Control (random) (n=8); 7. After eight months of isolation (immobility in the FST >75%) (n=10). **p<0.001.
The average number of trials is shown on the ordinate.

The experiments indicated that learning and memory processes were disturbed in the animals which were housed in social isolation and partial light deprivation. These disturbances were more significant in the group of animals which spent more time in isolation.

Figure 1 shows the results of the AAR acquisition within 120 trials in the two-way test in the following groups of animals: (I) Intact animals – 1. Control (immobility in the FST <60%) (n=12); 2. Non-depressive (immobility in the FST <50%) (n=8); 3. Depressive, (n=8). (II) Second group - 4. Control (random, home caged) (n=10); 5. Two months following isolation (n=12). (III) Third group - 6. Control (random, home caged) (n=8); 7. Eight months following isolation (n=10). The mean number of trials is shown on the ordinate. *p<0.001.

Acquisition of the AAR was feasible in all depressive animals, although in the first and the second groups the difference between control and experimental groups was statistically insignificant. The worst learning was observed in third experimental group after eight months of isolation**,** compared to the random control group and the group of the intact animals**.**

Retention of the AAR within 120 trials was assessed after 24 h. Retention of the AAR in the first group was ambiguous. The animals of the second group needed fewer trials. Retention in the third experimental group, with high index of immobility, was significantly lower. Several animals (Fig. 1, column 7), who could not achieve the learning criterion at the first test, learned to avoid the stimulation at the second test, but they required more trials, compared to the animals in the same and control groups.

Figure 2 illustrates the possibility of the AAR retention in experimental and control groups of animals. **(**I) Intact animals – 1. Control; 2. Non-depressive animals; 3. Depressive animals (immobility in the FST >75%). (II) Second experimental group - 4. Control (random, home caged); 5. Two months after isolation. (III) Third experimental group – 6. Control (random, home caged), 7 – Eight months after isolation, 8. The animals of the second experimental group, which did not achieve the learning criterion in the first test. *p<0.001. The average number of trials is shown on the ordinate.

In the next series of experiments, for investigation of the possibility of consolidation of memory traces, the PAR was studied in all the mentioned groups of animals. Although the test of PAR is considered as suitably severe [13], it reflects sufficiently well the possibility of retaining the reaction to the aversive stimulus in rodents, the more so if it is used for depressive individuals.

The PAR was considered acquired if an animal did not enter the dark chamber for 5-10 min, this behavior being persistent. In Fig.3 the percentage of the errors in

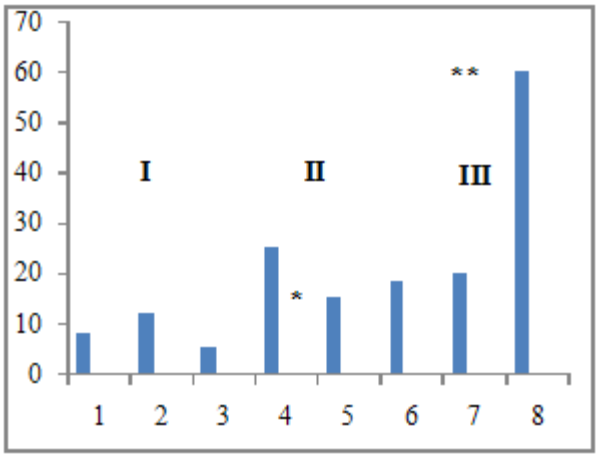

Fig. 2 . Retention of the active avoidance reaction in 120 trials two-sided test.
I. Intact animals - 1. Control (first) group (immobility in the FST <60%) 2. Non-depressive animals (immobility in the FST<50%; 3. Depressive animals (immobility in the FST >75%)**. II.** The second experimental group - 4.Control (random), 5. After two months of isolation (immobility in the FST >75%). **III.** The third experimental group - 6. Control (random), 7. After eight months of isolation (immobility in the FST>75%). 8. The animals of the second experimental group, who could not achieve the learning criterion at the first testing. ** p<0.001. The average number of trials is shown on the ordinate.

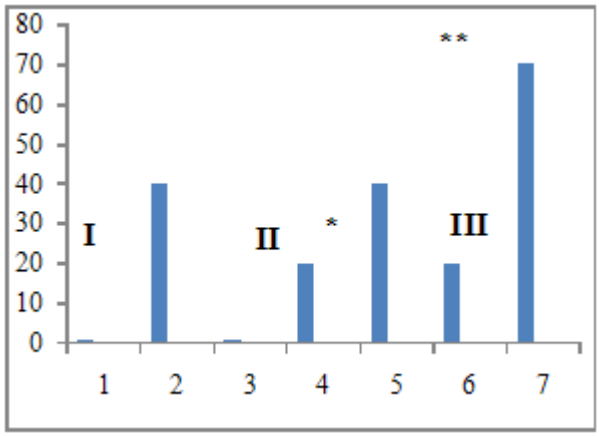

Fig. 3. Percent of the mistakes in the passive avoidance task.
I. The intact animals – 1.Control (immobility in the FST<60%); 2. Non-depressive, immobility in the FST<50%); 3. Depressive (immobility in the FST>75%); **II.** The group after two months of isolation – 4. Control (random), 5.After isolation, immobility in the FST>75%. **III.** The group after eight months of isolation - 6. Control; 7. After isolation (immobility in the FST>75%). **p<0.001.
The percentage (%) of mistakes is shown on the ordinate.

the PAR task is shown. (I) - Intact animals, 1.Control (immobility in the FST<60%), 2. Non-depressive, (immobility in the FST<50%); 3. Depressive (immobility in the FST>75%); (II) -The group after two months of isolation; 4.Control (random, home caged); 5. After isolation (immobility in the FST>75%). (III**)** - The group after eight months of isolation; 6. Control (random, home caged); 7. After isolation (immobility in the FST>75%). *p<0.001. The percentage of errors is shown on the ordinate.

Statistically significant changes were observed in the second and third experimental groups only, i.e. in animals which were housed in conditions of social isolation and partial light deprivation. Most of them could not recall the presence of aversive, noxious stimulus in the dark compartment. These animals followed their natural instinct - avoidance of bright illumination - and entered the dark compartment of the apparatus. As the analysis of the results obtained showed, consolidation of the memory traces was impaired in the animals, whose index of immobility was > 75%, regardless of how long they carried out under the imposed conditions.

For more detailed analysis of the results obtained alterations of the latent period of entering into the dark compartment of the passive avoidance apparatus was studied. The latent period of the PAR serves as an additional parameter for understanding the nature of consolidation - whether fear can interfere with the process of the consolidation in the stressful situation (bright illumination in the first chamber). If the animal, being tested repeatedly, did not enter the dark chamber at all, or the latent period of entrance was over 5-10 min, consolidation of memory trace was considered successfully completed.

Figure 4 illustrates alterations of these data. (I) - Intact animals, 1. Control, (immobility in the FST<60%), 2. Non-depressive (immobility in the FST<50%), 3. Depressive (immobility in the FST >75%), (II) - Animals after two months of isolation, 4 – Control (random), 5 - After isolation, depressive (immobility in the FST>75%); *p<0.01, **(**III) - Following eight months of isolation, 6 – Control (random), 7 – After isolation, depressive (immobility in the FST >75%); **p<0.001. The latency (in minutes) of entering into the dark compartment is shown on the ordinate.

Thus, alterations of the latent period of entering the dark compartment in the passive avoidance apparatus show the possibility of memory trace consolidation. Statistically significant abnormalities (p<0.001) were only observed in the animals with high index of immobility, following long-lasting social isolation and partial light deprivation. These animals did not recall the threat of electric shock and some of them still entered the dark compartment, hence avoiding the light.

For the last group of animals – depressive ones, after 8 months of social isolation and partial light deprivation, neurochemical analysis of serotonin level in the frontal and caudal areas of the brain was conducted. The investigation has shown that the amount of serotonin in the above areas of the brain in depressive subjects

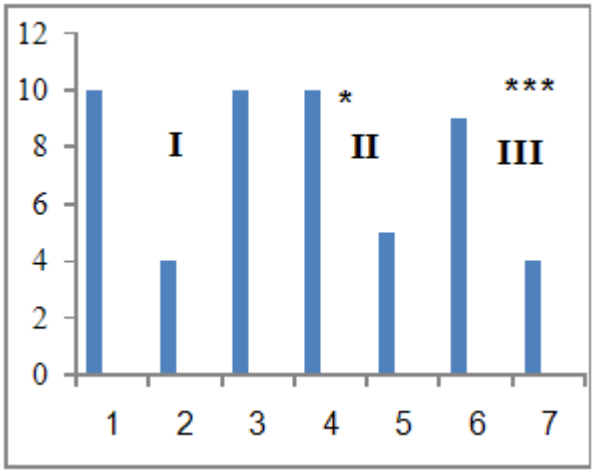

Fig. 4. Alternations of the latent period of entering the dark compartment of passive avoidance apparatus.
I. Intact animals - **I**- Control (immobility in the FST<60%); 2. Non-depressive (immobility in the FST<50%); 3.Depressive (immobility in the FST >75%)**; II. A**nimals after two months of isolation - 4. Control (random); 5. After isolation, depressive (immobility in the FST>75%).** p<0.01; **III**. After eight months of isolation – 6. Control; 7. After isolation, depressive (immobility in the FST >75%). **p<0.001. The time of entering in min is shown on the ordinate.

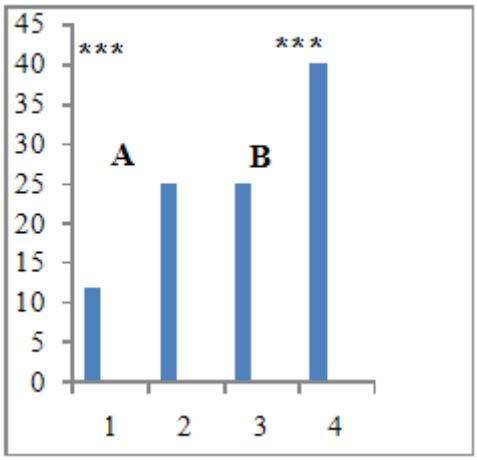

Fig. 5. Changes in the amount of serotoninergic receptors.
Amount of serotoninergic receptors in the frontal A) and caudal (B) brain areas in the control (1, 3) and experimental groups (2, 4) (after 8 months of isolation) (2).
The obtained data are statistically significant p<0.001.

was lower than in the brains of the control animals. The difference in the amount of serotonergic receptors in the frontal brain area in the control (1) and in the experimental groups after 8 months of isolation (2) (Fig.5-A) was statistically significant (p<0.001). The same is true regarding changes of the serotonergic receptors in the caudal brain area. (Fig.5-B, 1-control group, 2-experimental animals; p<0.001).

Significant increase in the amount of serotonergic receptors in the above brain regions after eight months of social isolation and partial light deprivation point to a deficit of serotonin in these regions. At the same time it is assumed that these fields of the brain are involved in regulation of the learning processes and subsequent consolidation of the memory traces [14, 15]. Moreover, they may be responsible for learning and memory processing. The data obtained give ground to assert that the lack of serotonin in the brain of experimental animals is caused by prolonged social isolation and partial deprivation of light. The latter is reflected in the cognitive possibilities and it is the reason of hampering of the learning process and disturbance of memory processing, observed in this animal model of depression.

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ადამიანისა და ცხოველთა ფიზიოლოგია

კოგნიტიური დეფიციტი დეპრესიის ცხოველურ მოდელში

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დეპრესიის გამომწვევი ფაქტორების კვლევა მნიშვნელოვანია როგორც კლინიკური მედიცინისათვის, ასევე ნეირომეცნიერებისათვის. ამ პრობლემის გადასაჭრელად მრავალრიცხოვანი და განსხვავებული მეთოდი გამოიყენება. მათ შორის, ხანგრძლივი (2 და 8 თვე) სოციალური იზოლაცია სინათლის ნაწილობრივი დეპრივაციით - დეპრესიის ადეკვატურ მოდელად შეიძლება ჩაითვალოს. ცხოველებში (ვირთაგვებში) დეპრესიის მოდელირებით შეისწავლებოდა დასწავლისა და მეხსიერების კვალის კონსოლიდაციის შესაძლებლობა აქტიური და პასიური განრიდების მეთოდების გამოყენებით. ცხოველების დეპრესიულობა განისაზღვრებოდა პორსოლტის იძულებითი ცურვის ტესტით. მიღებული შედეგების ანალიზმა აჩვენა, რომ ხანგრძლივი სოციალური იზოლაცია

და სინათლის ნაწილობრივი დეპრივაცია იწვევს მნიშვნელოვან კოგნიტიურ დეფიციტს იმ დეპრესიულ ცხოველებთან შედარებით, რომლებსაც შენარჩუნებული ჰქონდათ ნორმალური სოციალური გარემო. რაც უფრო ხანგრძლივი იყო ექსპერიმენტული იზოლაცია, მით უფრო მეტად გამოვლინდა დასწავლისა და მეხსიერების კვალის კონსოლიდაციის პროცესის გაუარესება. იზოლაციაში მყოფი ცხოველების თავის ტვინის ნეიროქიმიურმა კვლევამ $_3$**H-cetanserin-ით (NEN, USA)** აჩვენა, რომ ტვინის ახალ ქერქში და კაუდალურ უბანში გაზრდილია სეროტონინის რეცეპტორების რაოდენობა, რაც ამ ნეიროტრანსმიტერის დეფიციტზე მიუთითებს.

საბარაუდოდ, დეპრესიის ამ მოდელში ტვინში სეროტონინის რაოდენობის შემცირება დასწავლისა და მეხსიერების პროცესის გაურესებაში ვლინდება. შესაბამისად, შემოთავაზებული მოდელი – ხანგრძლივი სოციალური იზოლაცია და სინათლის ნაწილობრივი დეპრივაცია - დეპრესიის ადეკვატურ და ღირებულ მოდელად შეიძლება ჩაითვალოს და მისი გამოყენებით თავის ტვინის ინტეგრაციული მოქმედების სხვა გამოვლინებების შესწავლაცაა შესაძლებელი.

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