

## **IMMUNOLOGY CELL BIOLOGY**

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### **STUDY OF THE LARVAE GALLERIA MELLONELLA EXTRACT EFFECT ON THE BEHAVIORAL ACTIVITY OF ANIMALS BY THE METHOD OF «OPEN FIELD» AND «SUOK TEST»**

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#### **Abstract**

It was made a comparative analysis of behavioral reactions in drinking water and 40% extracts of larvae of greater wax moth (*Galleria mellonella* L.) laboratory mice. It was taken the basis such indicators as the number of crossed squares (KGC), and vertical motor activity – the number of racks, the reaction time of fading, the total number of acts of defecation, and vegetative parameters (the number of boluses). The experiment showed that the discover of the

relationship between behavioral response of the animals in "Open field" and the work of the limbic system of the brain studied in laboratory mice. This explanation is based on the known principles of functioning of the amygdala and hippocampus. It is shown the influence of multicomponent compositions of extracts obtained by different technologies on the parameters of the functioning of these parts of the brain by the behavioral reaction of laboratory mice in the "Open field".

**Keywords:** extract larvae greater wax moth, *Galleria mellonella*, behavior, open field, motor activity, open field, suok test, linear mice

### **Introduction**

The study of the laws of the adaptation process, the search for means and ways to improve the functional reserves of the body is at the heart of the priorities of modern medical and biological Sciences. More and more urgent is to increase nonspecific body resistance to infections with the help of pharmacological agents - adaptogens. The most perspective direction is development of adaptogenic preparations on the basis of raw materials of a vegetable origin possessing a number of advantages in comparison with synthetic preparations the content of biologically active substances, possess several pharmacological properties, and are characterized by smooth increase of pharmacological effect [1]. The extract, made on the basis of larvae *Galleria mellonella* L. belongs to this group of biological preparation. In 1906 S. I. Metalnikov proved antitubercular effect, which is a consequence of the non-specific immune response of the body. In addition, a group of scientists Of the Institute of theoretical and experimental Biophysics led by M. N. Kondrashova proved that larval extract *G. mellonella* has immunoprotective and antioxidant effect, protects the cardiovascular system, and increases the adaptogenicity of the body [2]. In this deep investigation of the mechanism of the extract on adaptogenic biological systems is not revealed. The aim of the research is to study the behavioral reactions of laboratory mice when drinking extract from *G. mellonella* larvae. The experiments with the larvae product have demonstrated its harmlessness in laboratory animals when using extremely high doses exceeding the therapeutic dose [3].

### **Experimental**

All procedures with animals were performed in accordance with international rules and regulations (European Communities Council Directives of 24 November 1986, 86/609/EEC) and the order of the Ministry of health of

Russia № 267 of 19.06.03 "on approval of the rules of laboratory practice in the Russian Federation".

The object of study is the extract of larvae of greater wax moth (*Galleria mellonella* L.). The rearing of larvae was carried out in the laboratory of «Udmurt Research Institute of Agricultural Federal» - Branch of the Federal State Budgetary Institution of Science «Udmurt Federal Research Center of the Ural Branch of the Russian Academy of Sciences». The larvae of *G. mellonella* were contained in the patented device "Molari" [4] at a constant temperature of  $30 \pm 2^{\circ}\text{C}$  and a relative humidity of 65-70% in total darkness in the glass jars with a volume of 7 L. The studies were conducted according to the "Methodical recommendations for laboratory maintenance and rearing of greater wax moth *Galleria mellonella* L." [5].

Since several forms of extract is known according to the literature, an aqueous solution of the extract containing preservatives (sorbic acid and sodium benzoate) and 40% water-alcohol extract of larvae *G. mellonella* has been prepared. The use of water-based extractant with the addition of preservative is due to the fact that there are legal restrictions on the use of alcohol in the pharmacological market; in addition, the presence of ethanol can prevent the extraction of a kind of active components. In parallel, a comparison was made with the traditional 40% aqueous-alcoholic solution of larvae *G. mellonella*. Preparation of extracts was carried out with the use of living (native) larvae *Galleria mellonella* in the ratio 1:4. An experiment to study the effect of the extract on the behavioral responses of laboratory mice was conducted at the Department of immunology and cell biology Department at the Udmurt state University. The experiment was performed on 48 non-hydrogen non-linear mice: six-month males weighing 27-30 g. The twelve mice's were kept on a diet of viscous oatmeal and were a control group for the study of an aqueous extract (table 1).

Table 1 – Scheme of experience

Groups	The number of animals	The substance supplied	Dose
Control 1	12	water	rate of 0.5 ml per day for each animal as an additive to oatmeal
Control 2	12	40% ethanol alcohol	
1 experimental group	12	Water solution of larvae <i>Galleria mellonella</i>	
2 experimental group	12	40% water-alcohol extract <i>Galleria mellonella</i>	

The next 12 control animals received ethyl alcohol and served as control for exposure to alcohol extract of larvae. Animals were kept in standard vivarium conditions with free access to water and food. Determination of the effect of biologically active substance (BAS) on behavioral activity, emotionality and anxiety of rats was carried out by the method of "Open field" in standard light conditions for 5 minutes with an interval of 4 days and souk – test [6].

To study the effect of larvae *G. mellonella* extract on the behavioral activity of animals by the open field method, the following behavioral parameters were registered: horizontal motor activity - the number of crossed squares (NCS), and vertical motor activity – the number of racks, the fading reaction time, the total number of defecations and vegetative parameters (the number of boluses) [7].

The mice in a new space experience usually contradictory feelings. On the one hand, they are drawn to the study of a new unfamiliar space, and try to learn to navigate it. On the other hand, they feel fear and discomfort in this new space. The struggle of these opposites and defines the behavior of the mouse. The impact of multicomponent BAS obtained by modification of the extractant from the same biological object – greater wax moth larvae will change the ratio of reactions.

It is known that to a large extent the behavior of the mouse is determined by the limbic system of the mouse brain. Feeling discomfort and fear, as well as vegetative reaction in the form of boluses formed almond-shaped body, which is part of the limbic system, and orientation in space and memorization formed hippocampus, which is also part of the limbic system and anatomically adjacent to the almond-shaped body. These two components of the limbic system, competing with each other, can largely determine the behavior of the mouse in the "Open field".

Almond-shaped body and hippocampus have differences in structure and biochemistry. In nature between the amygdala and hippocampus there is a certain antagonism, when the fear inhibits cognitive activity and Vice versa. But the effect of BAS water and traditional extracts will have on the almond-shaped body and hippocampus due to their different structure and biochemistry different influence, forming different behavior of mice in the "Open field" as a result of the impact of different components composition of extracts. That is, theoretically, a variety of combinations are possible. For example, with the simultaneous increase due to the extract and the activity of the hippocampus, and the amygdala, theoretically, the behavior of animals can change so that it will lead to increased defecation and research activity at the same time. And

maybe the opposite reaction, when the mouse will not experience much discomfort, but their research activity will be lowered or changed somehow.

Statistical processing of the results was carried out using the software package MS Office and Statistica 6.0. We calculated the average value of the parameter and the average error.

#### **Results and consideration**

The main results of the research are given in table 2. The activity values of animals in the center of the open field differ slightly in all groups. At the same time, the activity on the periphery of the labyrinth of the "Open field" is higher in the control –  $76.58 \pm 9.64$  s. this activity is noticeably reduced for animals on the periphery of the "Open field", which indicates a certain decrease in the research motivation even in the field of relative animal safety for both extracts. At the same time, the standard deviation for the water-alcohol extractant is less in comparison with the pure water extractant. The significant differences in the control groups were not detected, so further research is a comparison of the results obtained for one control group.

Table 2 – Results of the study of "Open field" and "Suok test" on laboratory mice after evaporation of solutions extract of larvae *G. mellonella*

An indicator of the behavioral act	Control group	1 experimental group	2 experimental group
Test «Open field»			
Center	22,16±4,01	20,08±4,04*	22,83±3,75*
Periphery	76,58±9,64	64,83±9,05*	65,66±4,75*
Racks	16,66±2,92	20,08±4,04*	22,83±3,75*
Minks	15,91±2,92	24,41±5,95*	19,83±2,77*
Fading	0,41±0,14	0,91±0,33*	0,33±0,12*
The total number of acts of defecation	3,83±0,42	2,16±0,48*	1,33±0,15*
«Suok-test»			
Number of sectors	118,83±14,21	166,91±27,90*	182,41±43,30*
Slippages	19,00±3,06	10,00±2,53*	14,00±1,82*
Looking down	35,33±3,34	84,25±8,64*	46,83±6,13*
Way out of the centre	15,41±4,21	8,58±2,72*	46,83±2,48*

\* $p \leq 0.005$

The number of «mink reactions» is the highest index of research activity in the evaporation of an aqueous solution extract of *G. mellonella* larvae, amounting to  $26.41 \pm 5.95$ , which is 1.6 times higher than the control values. While the number of racks decreased slightly for the animals under the action of an aqueous extract and was significantly lower for animals exposed to ethanol-water extract. Particularly strong mink reaction reacted to an aqueous extract. What he says about the motivation of orientation in the external space. That is, there was some suppression of the response of the hippocampus aimed at identifying external reference points and the growth reaction of the hippocampus in search of shelter. Apparently can also be interpreted increasing physical activity, increasing the search of shelters. Moreover, this difference was significantly different not only in quantitative terms, but also in qualitative difference in reactions. In particular, in the almond-shaped body, the reaction of the Central group of nuclei associated with the vegetative reaction of the mouse to external irritation seems to have been more inhibited. While other groups of nuclei became more active, this led to a corresponding change in behavior associated with the search for shelters.

The same can be said about the increased number of fades. For water extract there was a significant, almost twice the increase in the number of fading. This is indicating an increased amount of discomfort, which is in conflict with the reduction in the number of boluses. The number of boluses very strongly decreased for animals under the action of water extract and even stronger for animals under the action of water-alcohol extract. This suggests a significant suppression of the vegetative reaction to fear, that is, a decrease in the activity of the corresponding sections of the amygdala. The magnitude of the dispersion for an aqueous extract in the reaction in two times above compared to the aqueous-alcoholic extract, which testifies about the presence of divergent factors (ingredients aqueous extract) compared to the aqueous-alcoholic extract.

The table 2 shows that the number of acts of defecation (indicator of anxiety, emotional reactivity) is minimal in the second experimental group, which is 2.5 with lower control values. This indicator, formally related to the repertoire of hygienic behavior, is often considered as an indicator of the level of conflict in behavior between the motives of fear and the study of the environment. We can assume that from the point of view of the response control group has the most anxiety, in comparison with the prototype. Peek down-the research activity index is higher than in the control in the first experimental group.

Motor activity related to the study of sectors has significantly increased in comparison with the control group. For water-alcohol extract it was the highest. This is also a characteristic of the research reaction. At the same time, for water extract, dramatically reduced the time of stay of the animal in the center, while for hydroalcoholic extract increased it.

In General, it can be concluded that the extracts having multi-component composition have complex and contradictory effects. They can strengthen or weaken different aspects of the amygdala and hippocampus, affecting the orientation in space and emotional motivation of this orientation. It can simultaneously be both weakened and strengthened the individual symptoms of the action of the amygdala and likewise can simultaneously be both weakened and strengthened the individual symptoms of the action of the hippocampus to orientation in space and memorizing the characteristics of this space.

#### **Summary**

Both of the extract, although to a different extent have stressprotective at the level of autonomic manifestations, although they may at the same time to strengthen a feeling of discomfort and phobias, which further have transformation in behavioral response of search for a way out of the situation.

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## **MEDICINE, STOMATOLOGY**

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### **BIOMINERALIZATION AS A NEW CONCEPT IN TREATMENT OF THE ENAMEL CARIES IN ITS WHITE SPOT STAGE**

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#### **Abstract**

The purpose of this study is to evaluate the clinical effectiveness of the application of the InnoDent remedy for the biomineralization (TOO, InnoDent, Kazakhstan) in the treatment of the enamel caries in the "white spot" stage. Clinical studies have been carried out on the basis of the dental polyclinic of the FSBEI HE KubSMU of the Ministry of Health Care of Russia. 53 volunteers aged 20-30 years diagnosed with the enamel caries in the "white spot" stage with different levels of oral hygiene have taken part. To evaluate the totally effectiveness of the InnoDent remedy for the biomineralization (TOO, InnoDent, Kazakhstan) in the treatment of the enamel caries in the "white spot" stage the volunteers have been diagnosed by means of the vital staining and the laser-fluorescent method with the "Diagnodent Pen" device (KaVo). The comparison of the received study indices has been carried out by means of the Student's test. In all patients of the first and second subgroups before treatment,