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«OPEN-FIELD» TEST BEHAVIORAL PROFILE IN RATS SUBJECTED TO NEONATAL HANDLING OR UNSTRESSED STIMULATION OF MATERNAL CARE

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Introduction. Studying environmental events with postnatal ontogenesis is extremely significant for understanding the biological determination of postnatal development [1]. Nowadays, it is known that the nervous system of the neonate mammal is in the state of being ready for adaptation. It is most typical of the altricial species. During the time between the birth and the first socialization stages, the development of the mammalian higher nervous system undergoes a rapid completion. The environment such as the maternal surroundings represents the adaptation zone for the forming nervous system [2].

The effects of early environmental factors on the future profile of the adult animal’s behavior tend to correlate in a way that is complicated and difficult to explain. However, it has been shown that the alteration of either direction or intensity of some environmental parameters can be persistent and affect trivial behavioral acts that can be observed for a long-term animal’s life [3]. In particular it concerns such aspect as animal’s behavior in stressful condition.

The study of an early environmental event impact upon mammalian behavioral status will be incomplete unless some factors like the level of expression of maternal care are taken into account [4]. Mammal pups generally depend on maternal care for a long time, but it is of a special concern in species whose newly born pups are not mature. Maternal care factors include such dams-litters manipulation as licking and grooming, temperature regulation as well as the quantity and composition of the dam’s milk [1]. The grooming is of critical importance as a component of the maternal behavioral complex which deals with the pups nursing. It has been suggested that grooming, being a comfort behavior, has also a signaling function for the pups indicating they are in safety. Thus, maternal grooming is able to influence greatly the formation of animal stress-reactivity in future life.

Nowadays, handling-effect formation by maternal grooming and the physiological mechanisms of this phenomenon remains least studied aspects of this problem. In our opinion, future investigations of handling-effect in this particular direction are of greater interest for both the search of physical ways of information transmission into the care-emotional stability system and the study of proper signal mechanisms used by mammals in mother-offspring relationships.

Objects and methods. The handling was realized as a pup deprivation. On a daily basis from birth on, the animals were taken away from their mothers for several minutes and then placed back into the living cages [5]. In order to stimulate grooming, a method involving the application of an excrements drop of on the animal’s back in the tail region was used.

In the experiments we used white rats from 10 litters. The amount of the random was 116 animals of both sexes. The experimental group «Handling» (H) was subjected to 10-minutes handling one time a day since its birth until the thirtieth day of their life. The animals were placed into an isolated wooden box sized 20×30 cm. The group «Grooming» (G) was exposed to the treatment described above once a day while reducing physical contact with the hands of the experiment conductor.
The tests were carried out after 35 life days in the course of three successive days. The open-field had the dimensions of 100×100 cm and was parted into 25 squares and illuminated with 200 Watt at 1,5 m above [6]. During the two minutes’ test we registered such gauges as moving activity (MA), searching behavioral acts (SB’ – central square visiting, hind legs standing) and stress behavior (SB – peripheral square visiting, side lean standing) [7].

We used the nonparametric statistics method Mann-Whitney U test to analyze the obtained data.

**Results and their discussion.** The behavior in the open-field test conditions (OF) was used as a simple emotional test. We supposed that rats subjected to the handling would be less emotional and the SB amount would be considerably lower than in intact animals [6]. The mean values of the registered data from the different groups are given in table.

We found that animals that were subjected to the handling as well as exposed to the grooming showed the significant decreased emotionality in the open-field test. The increased MA of experimental animals ($P = 0.004093$) (fig. 1) and their higher activity in the center of the open-field were the results of lowered fright motivation and enhanced search behavior motivation compared to the control.

**Behavioral activity in rats that were exposed to either Handling (H), Grooming (G) or left abandoned (C)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Number of animals</th>
<th>U</th>
<th>D</th>
<th>MA</th>
<th>SB</th>
<th>SB’</th>
<th>K_ma</th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>m</td>
<td>24</td>
<td>2.11</td>
<td>2.45</td>
<td>39.4</td>
<td>34.6</td>
<td>6.56</td>
<td>0.08699</td>
</tr>
<tr>
<td>H</td>
<td>f</td>
<td>16</td>
<td>1.8</td>
<td>3.13</td>
<td>40.8</td>
<td>35.5</td>
<td>7.03</td>
<td>0.10504</td>
</tr>
<tr>
<td>G</td>
<td>m</td>
<td>17</td>
<td>1.43</td>
<td>2.88</td>
<td>38.3</td>
<td>32.6</td>
<td>7.52</td>
<td>0.11370</td>
</tr>
<tr>
<td>G</td>
<td>f</td>
<td>20</td>
<td>1.73</td>
<td>3.86</td>
<td>33</td>
<td>29.1</td>
<td>6.93</td>
<td>0.07625</td>
</tr>
<tr>
<td>C</td>
<td>m</td>
<td>18</td>
<td>1.43</td>
<td>2.18</td>
<td>34.7</td>
<td>31.1</td>
<td>6</td>
<td>0.06445</td>
</tr>
<tr>
<td>C</td>
<td>f</td>
<td>21</td>
<td>1.13</td>
<td>3.42</td>
<td>33.1</td>
<td>30.4</td>
<td>4.44</td>
<td>0.05452</td>
</tr>
</tbody>
</table>


It is significant to note that the groomed rats’ MA was a statistical magnitude less ($P = 0.008345$) than the one registered in the handled rats.

The hind legs standings availability in the center of the open-field both in the handled rats and in the groomed ones was the evidence of a lower fright level and a marked search motivation. Those standings were extremely rarely detected in the intact rats. The hind legs’ standings near the wall of the open-field area is known to be the manifestation of the active avoid reaction. Such behavior in the OF was noticeably less frequently observed in the handled rats than in the control.

The moving activity coefficient ($K_{ma}$) was computed as a ratio of the amount of search behavior acts to the amount of stress behavior. This ratio is statistically higher ($P = 0.000240$) in experimental groups (fig. 2).

![Fig. 1. Moving activity index in handled (H), groomed (G) and control (C) rats](image-url)
Fig. 2. Moving activity coefficient in handled (H), groomed (G) and control (C) rats

We did not find any deviation from this dependence when we were comparing the first test minute with the data of the second one which is often stated in other reviews [e.g. 8]. Handled rats’ $K_{ma}$ was noticeably higher throughout the test time and the search behavior constantly prevailed over the avoid behavior (fig. 3).

We compared $K_{ma}$ values of males and females. The described above regularity remained both in males and females. In the bar graph which was made on the basis of the data obtained from the experiment we saw a higher $K_{ma}$ level in the groomed animals both males and females. However, this difference was not reliable (males $P = 0.09$; dams $P = 0.096$).

When we were comparing treatment effects in males with females inside the groups we found no remarkable difference of behavioral profile between the sexes.

Handling is a multicomponent treatment. The pups are taken from the cage for several minutes, which results in maternal deprivation in their early life. This handling significantly alters the affected adult’s behavior and the most important systems and functions of organism are involved in those changes. The effects of this kind of treatment are said to be observable in at least two offspring generations of the treated rats [1]. The above effects can be directly caused by the fact that the early natal ontogenesis period plays an important role in morphologic and functional development of mammal’s brain, cortex’ layers differentiation and the establishment of interneuronic connections in the cortex [9]; and as a consequence the environmental parameters in which the first neonatal adaptation of brain structures takes place can acquire the ability to indirectly influence the animal behavior in test conditions during its all adult life. In addition, the handling causes changes in the hormonal balance of the hypothalamic-pituitary-adrenal axis [10]. The altered balance is characterized by an increased basal level and a decreased

Fig. 3. Moving activity coefficient during the first and the second test minutes in handled (H), groomed (G) and control (C) rats
reaction to an acute emotional stress. The neuron and endocrine reorganization of mammals subjected to neonatal handling includes both the increase of hippocampus, cortex cytosol glucocorticoid receptor expression and the change of serotonin synthesis level [10–12].

In this context, the handling represents a convenient model for understanding the mechanisms by which an environmental stimulus can regulate the nervous system development in mammals. In accordance with some previous reported data [4] the increased maternal grooming level is supposed to lead to the decreased fearfulness in rats.

The purpose of these experiments was to study the correlation between the shot time maternal deprivation in early life (handling) and animal behavioral profile after its separation from the nursing mother. Furthermore we intend to verify the hypothesis that the dominant factor for the forming of the handling-effect in mammals was the increase of maternal grooming.

In the context of our research we proceeded from the assumption that the dominant treatment for the forming of the handling-effect is the maternal grooming that occurs enhanced as a response to the vocalization of stressed pups [13]. In this case it is not improbable that the stimulation of the maternal grooming that is not accompanied by stress can cause similar effects. These effects should be observed in the open-field test like the analogous ones in the handling case. In our experiment we found out that the no handled rats differ from the rats which were subjected to stimulation of maternal grooming in that case at least if their testing is realized in open-field test conditions. These findings completely answer the above assumptions.

We found no remarkable difference between treated males and females inside the groups comparing their fearful pattern in the open-field. In our opinion the reason for this phenomenon was the fact that we have tested the animals before their pubescence (between the first and 37th days of their life). It does not exclude the possibility of finding the above difference in adults. We suppose this aspect of the problem requires subsequent studying.

**Conclusion.** Analysis of rats' behavior in an open-field test suggests that the early postnatal handling can positively influence the forming of stress-response regulation systems and decrease the animals' reaction to acute emotional stress. However, such conventional indexes as urination or defecation do not always yield information suitable for the interpretation because of their small magnitude.

Sex does not influence the handling-effect manifestation in rats in the case of pre-puberty animals.

The handled rats' moving activity coefficient is significantly higher in comparison with the control animals during both the first and the second minute of the test. Thus we can assert that the search behavior motivation in the experimental rats is constant but the fright motivation is lower from the very outset.

We found that the behavioral effects of the classic handling could be reproduced by the non-stress maternal grooming stimulation method. In this case, the groomed animals are intermediate between the handled and the intact rats in some activities, for example the moving activity.

The easy reproduction of the handling-effect by maternal grooming stimulation allows us to suppose that it is the maternal care depth that determines the stress-sensitivity in rats which they display when adults.

It is not improbable that the maternal influences are the critically important neonatal environmental factor for the development of such fundamental survival mechanisms as a stress response.

**Literature**

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Summary

During the investigation of the neonatal handling influence on the white rats behavioral profile in the open-field test, the significant lowering of the test animals stress reactivity has been detected. This has been found along with the augmented expression of the exploration behavior with a general rise in moving activity. It was shown that it is possible to reproduce behavioral effects of the neonatal handling by unstressed stimulation of maternal care.