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BIOLOGICAL SCIENCES

THE MODIFYING EFFECT OF HYPOKINETIC STRESS ON THE PAINFUL SENSITIVITY IN THE RATS DURING THE EXPERIMENTALLY SUMMONED TONIC SOMATIC PAIN

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Abstract

The modifying effect of hypokinetic stress on the changes of the painful sensitivity in the rats during the experimentally summoned tonic somatic pain has been studied. It has been shown that the modifying effect of hypokinetic stress on the painful sensitivity in the rats in the formalin test depends on the duration of the limitation of the mobility. At the same time, the changes of the painful sensitivity (decrease and increase) in the animals during hypokinetic stress can be served as the criterion of the transition of eustress to distress.

Keywords: hypokinetic stress, painful sensitivity, tonic pain.

Introduction

The problem of the research of the pain has been devoted with the large number of the works, offering the different methodological approaches. However, the study of the painful phenomenon presents the certain difficulties. Recently, enough evidence has been obtained that at the heart of the pain is individual, genetically determined predisposition.

In the modern physiological research, the pain is an extremely complex phenomenon formed with the interweaving of the anatomical, the psychical, the physiological, the biochemical and the social components, each of which includes the number of the component elements [1, 8, 9, 10, 18].

The experimental research of the pain runs against the numerous difficulties in the people. Firstly, practically all stimuli that damaging tissue cause pain, so no one stimulus can be identified which is adequate for the pain. Secondly, due to the subjective assessment of the intensity of the pain by a person, many aspects of the quantitative determination of the painful sensitivity have not been researched. Moreover, the intensity of the painful reactions depends not only on the value of the stimulus, but also on the functional state of the organism. For example, in the extreme situations of the emotional stress (accident), a person cannot feel the pain at all. The solution of these problems can be contributed, on the one hand, the experiments on the animals, which allow estimating quantitatively intensity of the pain during the absence of the psychogenic factor accompanying the research of the painful reactions in human, and, on the other hand, to study the modifying effect of the different factors, including the stress factors, on the painful sensitivity.

One of the widespread stress-factors is hypokinesia (HK, the limitation of the mobility), which causes the development of HK stress and it is accompanied with the specific and the nonspecific changes in the functioning of practically all organs and the systems of the organism: structural and functional musculoskeletal disorders, the changes of the functions of the nervous, sympathoadrenal and hypothalamic-pituitary-adrenal, cardiovascular, digestive, respiratory systems, metabolic shifts, the decrease of nonspecific resistance and immunoreactivity, the change of the antioxidant balance, the behavioral reactions, the development of the desynchronization [2, 6, 7, 12].

HK is an important problem of medicine, because the treatment of many diseases requires the maintenance to the strict bed rest, the periods of which can be calculated for the weeks or the months. It becomes the object of the attention of space medicine and biology, studying the consequences of the space flights on the crews of the spacecraft which is in the conditions of the limited motor activity [13, 17]. Due to the stall keeping of the livestock, the problem of the limitation of the mobility is also actual for agriculture and veterinary medicine.

The ability of HK stress has been revealed in the different researches to modify the reactions of the organism of the animal to the action of the factors of the different nature and the intensity, for example, the development of the infectious process, the impact of the weak electromagnetic radiation of ultra-low and extremely high frequency [4, 5]. At the same time, the modifying effect of HK stress on the level of the painful sensitivity remains unexplored.

In this regard, the purpose of this research has been the research of the modifying effect of HK stress

on the changes of the painful sensitivity in the rats during experimentally summoned tonic somatic pain.

Material and research methods

The researches have been carried out on 135 white Wistar male rats of the weight of 180-250 g.

The animals of the same age have been chosen in the experimental groups, characterized with the same motor activity in the test of "open field" (OF) [3]. Such selection allows forming homogeneous groups of the animals with the same constitutional features, reacting in the same way to the action of the different factors.

In the experimental research, the modifying effect of HR stress on the changes of the painful sensitivity in the rats during experimentally summoned tonic somatic pain in the "formalin test" (FT) has been studied.

The adequate model of the continuous tonic pain is FT, which is a classic method of the determination of the effectiveness of the analgesic action of the pharmacological drugs, the physiotherapeutic and other factors (acupuncture, electricity, laser, Peiler-light, etc.) [14].

FT has been carried out with the subcutaneous injection of 5% formalin solution (0.08 ml per 100 grams of the weight) into the dorsal surface of the rat's foot of the hind extremity. After the formalin injection, each rat has been returned to its cage and, with the help of the special computer program, the duration of the behavioral manifestations has been recorded within the set sequential intervals (the minimum interval is 1 minute) and within all observation period of 90 minutes. The duration and the frequency (number of cycles) of the licking of the injured extremity have been the indicators of the intensity of the painful reaction in the rats during experimentally tonic pain.

The non-painful behavioral manifestations have been considered according to the duration of the motor activity and the passive behavior. In this case, the motor activity has been assessed with the sum of the time of the movement of the animals in the cage and the time spent by the animals for eating and grooming. The duration of the passive behavior has calculated with the sum of the time spent by the animals for the sleep and the rest.

The experimental animals have been divided into three equal groups of 45 animals in each. The animals of the first control group (Cf) have been subjected to subcutaneous injection into the back surface of hind extremity of saline (0.9% NaCl solution is in 0.08 ml per 100 grams of weight). In the animals of the second (FT) and the third (HK + FT) groups, the tonic pain has been summoned in FT. The animals of the third group (HK + FT) have been subjected preliminarily to the action of HK stress, which is created with the placement of the rats in the special pencil-case of plexiglass, consisting of five cells, in which they locate for nine days for 22 hours daily.

Considering the fact that the painful threshold varies during the day in the rodents, the experiments have been carried out at the same time during the light half of the day (from 9.00 to 11.00). The rats have been kept in the condition of the vivarium at temperature of 18-22 °C on the standard food ration and on the standard condition of the lighting (12 hours is dark: 12 hours is light). The light phase began at 7.00 am.

During the experiments, the "Rules of the conducting of the works with the use of the experimental animals" have been followed. The experiments have been carried out in maintenance of the principles of the "European Convention for the Protection of Vertebrate Animals used for Research and Scientific Purposes".

The working and the analysis of the experimental data have been carried out and analyzed with the help of the parametric methods. The Student's t-criterion has been used as the criterion of the assessment of the reliability of the observed changes. The working of the results has been processed on a PC using with the standard statistical programs.

We have used a multidimensional statistical method such as the cluster analysis, which is an adequate tool for the assessment of the multicomponent reactions of the organism and it allows finding the hidden connections both within the functional systems and between them. As the result of the application of the procedures of the cluster analysis, the initial complex of the objects (indicators) is divided into the clusters, or the groups of the similar objects (indicators), then the closest objects are combined sequentially into one cluster. In this research, one of the methods of the cluster analysis such as the Ward method has been used. The use of the agglomerative strategy of the analysis allows building the tree of the classification (dendrogram) of all objects by means of hierarchical combination them into the clusters based on the criterion of the minimum distance in the space of the variables describing the objects. The names of the objects (indicators) and the distance have been shown on the dendrogram, at which the combination of the objects has been taken place in each cluster.

The calculations and the graphic design of the obtained data in the work have been carried out with the use of the Microsoft Excel program and the software package of "STATISTICA – 8.0".

Results

As the results of the research has showed, the introduction of saline (0.9% of NaCl solution) into the dorsal surface of the hind extremity in the rats of the first group (Cf) has caused the slight painful reaction for the first 15 minutes of the observation of the total duration of 29.45 ± 8.10 s, that, apparently, is a typical reaction to an injection (Fig. 1). The duration of the motor activity in the animals of this group amounts 725.00 ± 94.16 s, and the passive behavior amounts 4626.33 ± 114.70 s.

In the animals of the second group (FT), which have been subjected to the isolated injection of formalin, the total duration of the painful reaction for 90 minutes of the observation has increased by 34.59 times ($p < 0.001$) concerning to the corresponding meanings of this indicator in the first control group of the animals (Cf) (Fig. 1). Moreover, in the animals of this group, the injection of 5% formalin solution has caused a typical two-phase reaction of the licking of the injured extremity, which is conformed with the literature data [14]. The first phase of the painful reaction has been recorded for the first ten minutes of the observation, and its duration amounts 177.82 ± 19.41 s. In 10 minutes after the introduction of formalin, the development of

the second phase has been observed, the duration of which amounts 840.84 ± 25.19 s.

The maximum manifestation of the painful reactions in the rats of the third group (HK+FT) has been

observed at 20-40 minutes after the formalin injection, after which the linear decrease of the pain with the attenuation to the 65th minute of the experiment has been noted (Fig. 1).

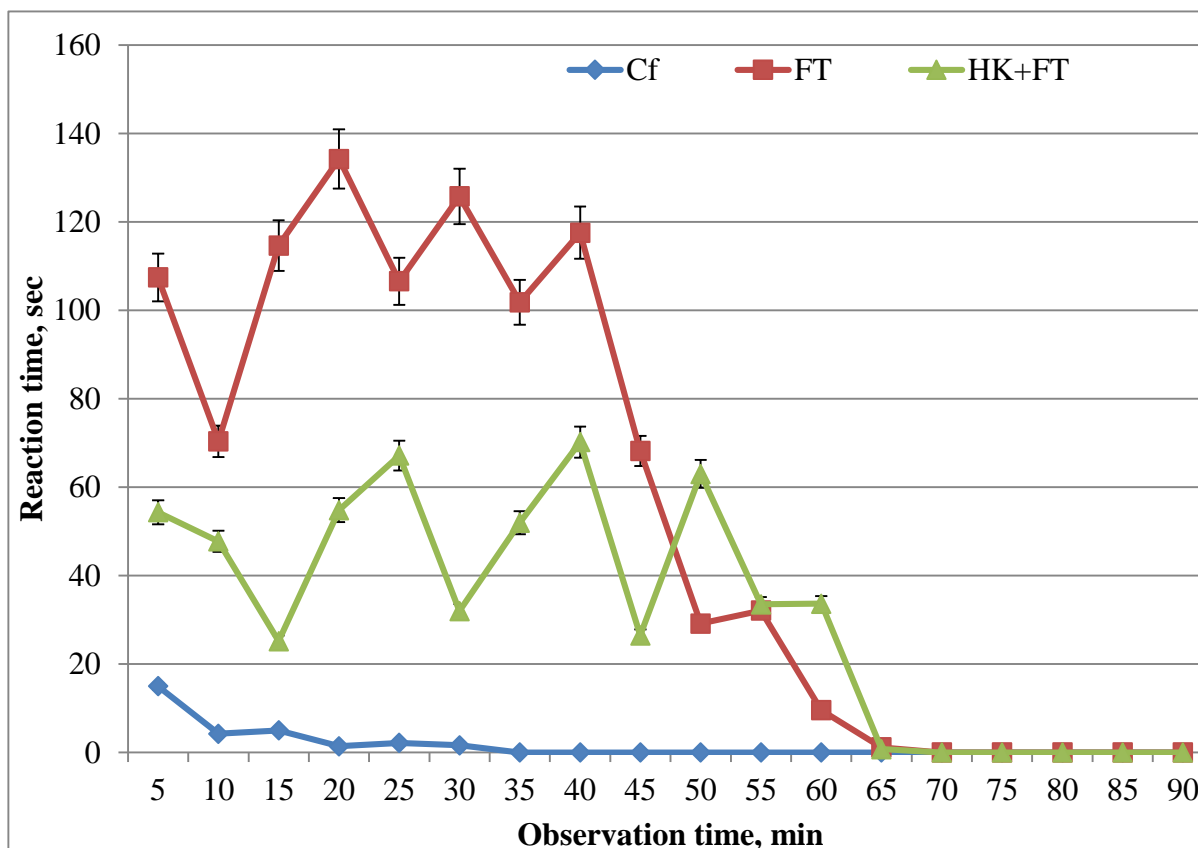


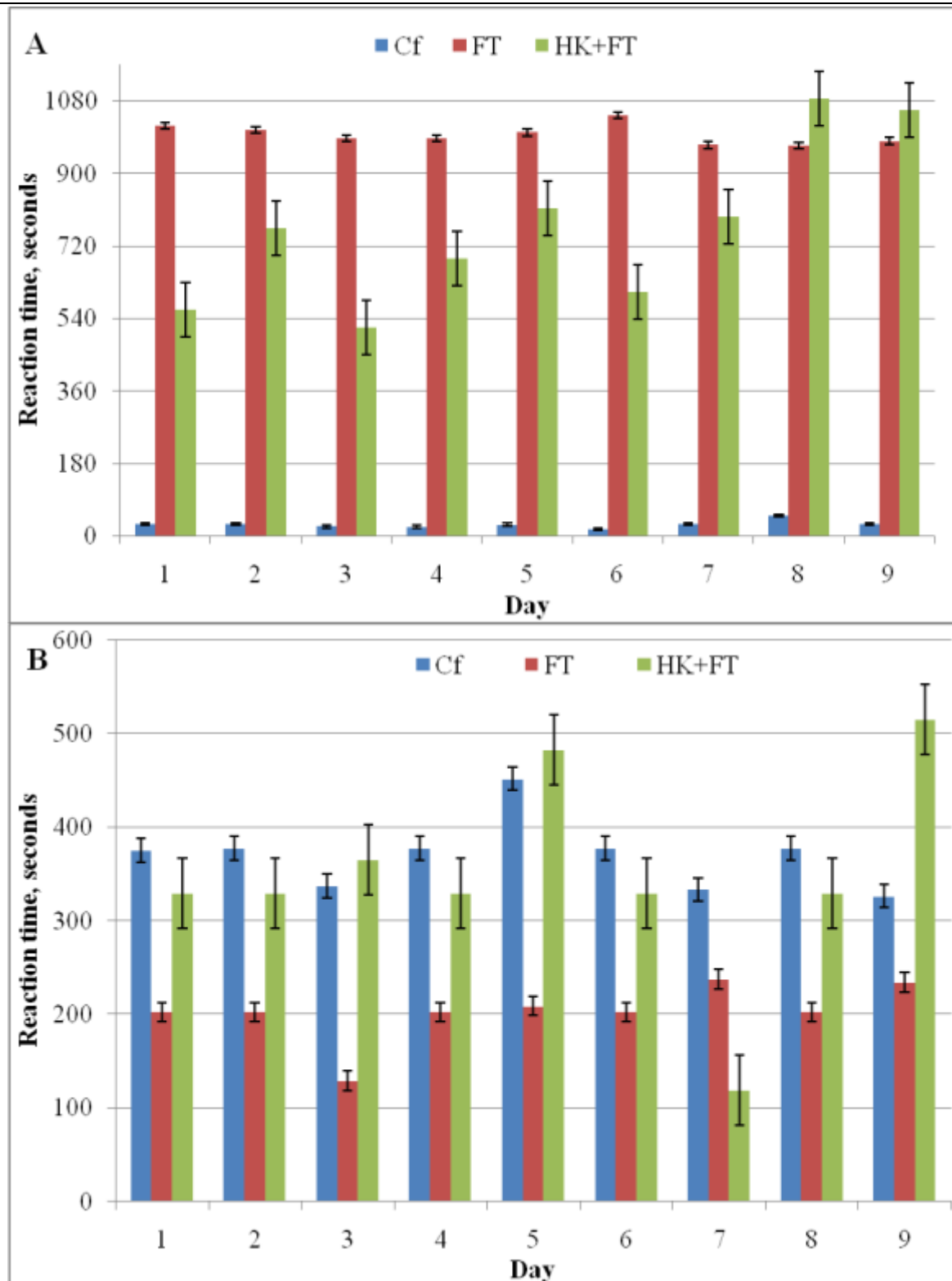
Fig. 1. Changes of duration of painful reaction of licking of injured extremity in rats subjected to introduction of saline solution (Cf), isolated (FT) and combined with daily hypokinesia (HK+FT) of introduction of 5% formalin solution with experimentally summoned tonic pain

The duration of the non-painful, both the motor and the passive behavioral manifestations in the animals of the second group (FT) after the injection of formalin has been also differed from the corresponding indicators in the animals which were injected with saline (Cf). So, there is the decrease of the duration of the reactions of the run by 46.67% ($p < 0.01$), of eating by 85.05% ($p < 0.001$), of grooming by 56.89% ($p < 0.01$), of sleep by 69.19% ($p < 0.05$), and the duration of the rest reaction has the tendency to the increase by 3.78% ($p > 0.05$) concerning to the meanings of the corresponding indicators in the rats of the first group (Cf) (Fig. 2-B, C).

The duration of the non-painful behavioral phenomena, on the contrary, has increased: the duration of the reaction of eating is by 15.67 times ($p < 0.001$), run is by 1.63 times ($p < 0.01$), grooming is by 2.11 times ($p < 0.05$), on the background of the decrease of the duration of the sleep is by 4.88 times ($p < 0.01$) concerning to the meanings of these indicators in the animals of the second group (FT) (Fig. 2-B, C).

After the second day of the limitation of the mobility, the duration of the painful reaction has the tendency of the decrease (by 24.21%; $p > 0.05$) concerning to the meanings of this indicator in the animals which have only been injected with formalin (Fig. 2-A). The duration of the motor activity has increased by 321.68% ($p < 0.001$), and the passive behavior, on the contrary, has decreased by 13.93% ($p < 0.05$) concerning to the meanings of these indicators in the animals of the second group (FT) (Fig. 2-B, C).

The maximum decrease of the duration of the painful reaction has been noted on the third (by 47.49%; $p < 0.05$) and the sixth (by 41.99%; $p < 0.05$) days of the limitation of the mobility concerning to the meanings of this indicator in the animals of the second group (FT). In the dynamics of the changes of the duration of the non-painful behavioral manifestations, the maximum increase of the duration of the motor activity has been observed on the sixth day of the observation (by 446.59%; $p < 0.001$) concerning to the meanings of these in the animals which have been subjected to formalin injections only (Fig. 2-B).



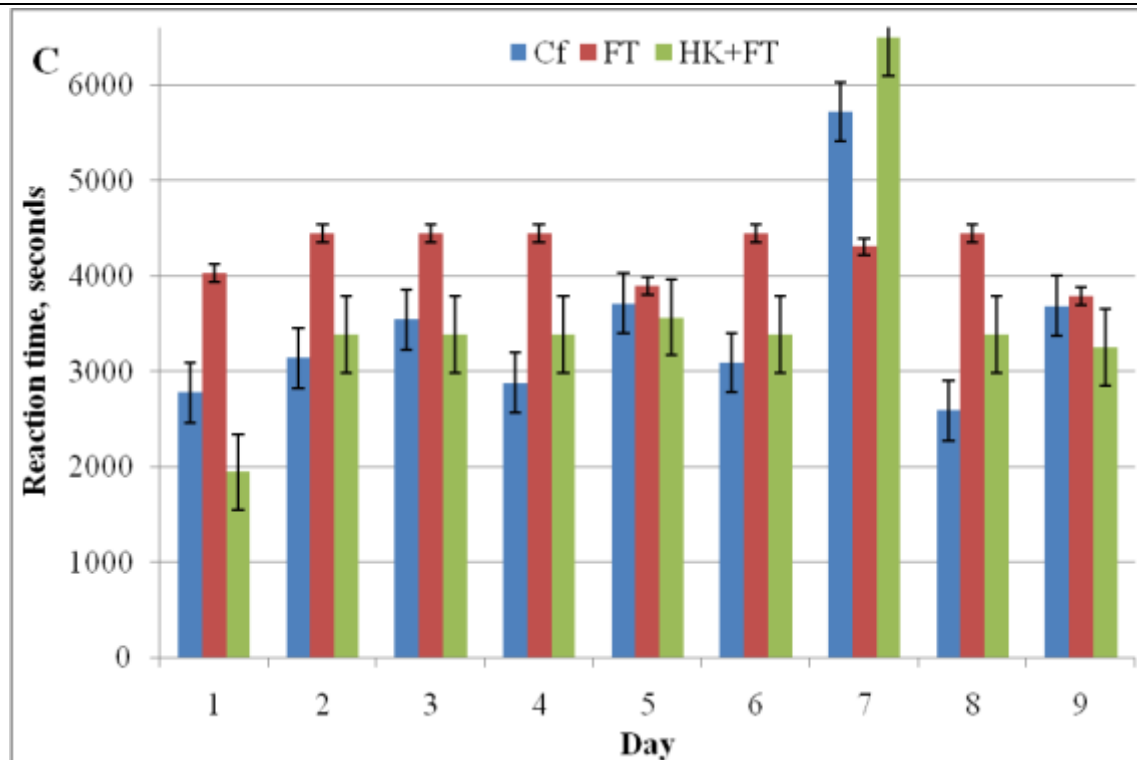


Fig. 2. Dynamics of durations of painful reaction (A), motor (B) and passive (C) non-painful behavioral phenomena in rats subjected to introduction of saline solution (Cf), isolated (FT) and combined with daily hypokinesia (HK+FT) of introduction of 5% formalin solution for nine days of hypokinesia of licking of injured extremity

The maximum decrease of the duration of the passive behavior has been recorded on the seventh day of the observation (by 36.27%; $p < 0.05$) concerning to the meanings of the corresponding indicator in the rats which have not been subjected to the additional HK stress (FT) (Fig. 2-C).

Starting from the seventh day of HK, there is a tendency to the increase of the duration of the painful reaction. So, on the eighth and the ninth days of the observation, the duration of the painful reaction has exceeded the meanings of this indicator in animals of the second group by 12.06% ($p > 0.05$) and 7.85% ($p > 0.05$), respectively (Fig. 2-A).

The duration of the non-painful behavioral phenomena has also changed such as the motor activity has increased on the average by 219.00% ($p < 0.01$), and the passive behavior has the decreasing tendency concerning to the meanings in the animals subjected to the isolated action of the painful stress (FT) (Fig. 2-B, C). However, compared with the meanings of these indicators in the animals of the third group (HK+FT) recorded on the seventh day of the observation, the duration of the motor activity on the eighth-ninth days has decreased on the average by 150.81% ($p < 0.001$), the duration of the passive behavior, on the contrary has increased on the average by 14.01% ($p > 0.05$) (Fig. 2-B, C).

Thus, HK stress modifies the painful sensitivity of the rats during the tonic somatic pain. However, the modifying effect of HK stress on the level of the painful sensitivity in the rats during FT depends on the duration of the limitation of themobility. During the adaptation of the rats to the short-term HK stress (the first-sixth days), the decrease of the painful sensitivity has been

noted, which points out the increase of resistance to the painful stress. Thus, the maximum decrease of the duration of the painful reaction in FT has been observed after three-day limitation of the mobility (by 52.51% ($p < 0.01$)). So, as the result of the action of the modifying factor (HK), the manifestations of the painful reaction in FT has decreased, therefore, there is a positive effect of the modification.

Together with the decreasing of the painful sensitivity, in the experimental animals from the first to the sixth day of the mobility, the increase of the motor activity in FT has been taken place (on the average by 324.44%; $p < 0.001$), which conforms the research of A.D. Slonim and his schools [16], in which it has been shown that concerning to the short limitation of the mobility is accompanied with the compensatory increase of the motor activity in the remaining time of the day with the aim of the maintaining of the constancy of the daily volume of the total activity. Moreover, the changes of the behavior at the early stages of the adaptation to the action of the different extreme factors are most often associated with the increase of the general excitability, which usually characterizes the development of the first stage of the stress [11].

So far as, the study of the behavioral phenomena is closely interconnected (the increase of the duration of some leads to the decrease of the duration of others), it is the certain interest to retrace the change of the relationship of these indicators in the painful test in the animals of the different experimental groups. Such relationships can be established with the applying of the cluster analysis. Thus, the dendrogram of the cluster analysis of the durations of the studied behavioral manifestations in the intact animals subjected to the false

action of the painful factor (the injections of saline into the dorsal surface of the hind extremity) (Cf), which is built with the help of hierarchically combination them into the clusters of higher community based on the criterion of the minimum distance in the space of the variables, contains 3 clusters, which has combined the studied indicators (the first cluster is the duration of the reactions of the pain and grooming, the second is run and eating, the third is sleep).

Thus, it has shown in the intact animals that characterizing the painful and non-painful behavioral manifestations have the close connections and they have united into the common clusters.

The dendrogram describing the data of the cluster analysis of the behavioral manifestations in the animals of the second group (FT), which are subjected to the isolated action of the painful factor in FT, has differed from that in the rats of the control group: the number of the clusters has not changed, but there are the significant changes in their structure such as the painful reaction is isolated into the detached cluster from the non-painful behavioral phenomena, which form two other thick clusters. What is more, between the first two and the third clusters, the link length has increased, which testifies about the significant disunity of the non-painful and painful behavioral reactions in FT in the rats of this group.

During the sequential action of the HK stress and the painful factor in the animals on the first-sixth days of the limitation of the mobility, the greater similarity of the dendrogram of the cluster analysis of the behavioral manifestations with that in the rats of the control group has been found than with the dendrogram in the animals that also are subjected to the experimental tonic pain, but without the effect of HK. At the same time, the indicator of the duration of the painful reaction has been united into the common clusters with the non-painful behavioral manifestations, as in the dendrogram of the cluster analysis in the rats of the control group, and the distance between the indicators of the duration of the painful and the non-painful manifestations has decreased in comparison with the dendrogram in the rats in FT without preliminary impact of HK, which should be regarded as the decrease of the connection between the considered behavioral reactions.

Thus, the obtained data of the cluster analysis evidence that in the modifying effect of HK stress on the painful sensitivity of the rats in the early stages of the limitation of the mobility (1-6 days), the great meaning has the restoration of the relationship between the painful and the non-painful behavioral manifestations during the action of the painful factor.

But, the long-term limitation of the mobility (the seventh-ninth days) has led to the increase of the painful sensitivity of the rats in FT concerning to the meanings in the animals subjected to the isolated action of the painful factor. So, the durations of the painful reaction have increased on the average by 9.95% ($p > 0.05$), accordingly. Thus, as the result of the action of the modifying factor (HK), the manifestations of the painful sensitivity of the rats have been increased; therefore, it can be spoken about the negative effect of the modification.

Together with the increase of the duration of the painful reaction, the significant decrease of the motor activity in FT has been taken place (on average by 2.96 times; $p < 0.01$) concerning to the corresponding meanings in the animals subjected to the action of the short-term (1-6 days) HK stress and the painful factor, which, obviously, is associated with the painful stress on the background of the continuous of HK stress which causes the increase of the general motor deficit in the animals and the development of the protective reaction of "harbouring", which is the result of the emotional reaction of the fear, the state of the general oppression of the animal's central nervous system [16] or the manifestations of the depressive-like state [15], which also testifies about the increase of the painful sensitivity of the animals during the continuous of HK stress.

The cluster analysis has confirmed the received results. Thus, on the dendrogram of the indicators of the behavioral manifestations in the animals subjected to the combined action of the painful factor in FT and HK stress on the seventh day of the limitation of the mobility, the unification of the indicators of the painful and the non-painful behavioral manifestations into the general clusters (pain – run – grooming, etc.) is still observed, however, the distance between the clusters has increased significantly. On the ninth day of the limitation of the mobility, the painful reaction has been isolated into the individual cluster from the non-painful behavioral manifestations, and the structure of the dendrogram has become more similar to that in the animals subjected to the painful factor than in the rats of the control group.

Thus, the obtained data of the cluster analysis testify that the modifying action of HK stress during the action of the painful factor in the later periods of the limitation of the mobility disunites the relationship between the behavioral manifestations. Such changes of the studied behavioral phenomena during the action of the painful factor on the background of HK can be associated with that the seven-nine-days limitation of the mobility has led to the development of the first stage of the general adaptation syndrome such as the anxiety reaction [12].

Therefore, the received results testify about the modifying effect of HK stress on the painful sensitivity of the animals. However, the modifying effect of HK stress on the level of the painful sensitivity in the rats in FT depends on the duration of the limitation of the mobility, which confirms the data about the two-component current of the first stage of anxiety of HK stress. At the same time, the changes of the painful sensitivity (decrease and increase) in the animals during hypokinetic stress can be served as a criterion of the transition of eustress to distress.

4. Conclusions

Hypokinetic stress modifies the painful sensitivity of the rats during experimentally summoned tonic somatic pain.

The modifying effect of hypokinetic stress on the painful sensitivity in the rats in the formalin test depends on the duration of the limitation of the mobility. At the same time, the changes of the painful sensitivity

(decrease and increase) in the animals during hypokinetic stress can be served as the criterion of the transition of eustress to distress.

During the adaptation of the rats to the short-term hypokinetic stress (the first - sixth days), the increase of the resistance to the painful factor is noted, which is expressed in the decrease of the duration of the painful reaction on the average by 32.30% ($p < 0.01$) on the background of the increase of the motor activity on the average by 324.44% ($p < 0.001$) in the "formalin" test.

The continuous limitation of the mobility (the seventh - ninth days) leads to the decrease of the resistance to the painful factors, which manifests itself in the increase of the duration of the painful reactions on the average by 9.95% ($p > 0.05$) on the background of the decrease of the motor activity on the average by 2.96 times ($p < 0.01$) in the "formalin" test.

Conflict of interest

All authors declare that they have no conflict of interest in the publication of this paper.

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