Middle-East Journal of Scientific Research 18 (3): 352-358, 2013
ISSN 1990-9233

DOI: 10.5829/idosi.mejsr.2013.18.3.12429

The Study of Behavioural Modification of Stress Responses at the Mice Exposed by Jwh-250.

Autoecological Aspects. Part 2.

[1]*Alexander Igorevich Andreev,* [1]*Ilya Petrovich Bulatov,* [2]*Viktor Petrovich Kotegov,*
[1]*Tamara Leonidovna Malkova,* [1]*Petr Sergeevich Maschenko,* [3]*Svetlana Sergeevna Zykova*

[1]Perm State Pharmaceutical Academy, Lenin St. 48, 614990, Perm, Russia
[2]Perm State National Research University, Bukireva street, 15, 614990, Perm, Russia
[3]Perm Federal Penitentiary Service Institute of Russia, Karpinsky st. 125, 614990 Perm, Russia

Abstracts: *Explanation:* Found that a single of a white outbred laboratory mice JWH-250 at doses equivalent used by people to achieve altered states of consciousness, causes significant changes in the structure of a model of behavioral stress response, reducing the effectiveness of adaptation of animals to the changed environment. Dose - dependent effects in the probability distribution of instantaneous velocities in animals exposed to the “Open field” test with videotracking and in the analysis of spontaneous locomotor activity of single animals were also found. Objectives: in Objectives : This study provides detailed characteristic changes of normal behavioral stress response in laboratory mice under the influence of the synthetic cannabinoid JWH-250 - in the aspect of the structure of spontaneous locomotor activity of animals in the conditions of a model stresses. Results: it is found out that it is rational to analyze changes in the structure of the behavioral response using multivariate nonparametric analysis of variance methods. Spontaneous locomotor activity of animals in the conditions of model stresses - short-term change of environment in "Open field" and long - in the actografical test can be a subject of such analysis. Length of a temporary window of observation, i.e. range of scales of an estimate appears an essential factor of completeness of the description of effect. Conclusions: JWH-250 causes typical and long behavioral effect, effectively identifying and characterizing by the proposed group of complementary parameters of spontaneous motor activity. Dynamics of development and specific features of effect JWH-250 reflected in distortions of normal stressful behavioral answers at mice, are described.

Key words: JWH-250 · Spontaneous locomotor activity · Movement · Stereotypy · Ataxia · Stressful behavioural response · Energy investments into a stress.

INTRODUCTION

In this part of article, we continue the presentation of the results obtained in male white outbred mice with comprehensive study of the influence of non-classical synthetic cannabinoid JWH- 250 on behavioral factors of competitiveness of individual animals.

MATERIALS AND METHODS

Materials and methods are described in detail in the first part of the article, here we denote only the main points :

Chemicals: JWH- 250 was obtained as an alcohol flush with leaf smokable mixture, analyzed by chromatography and recrystallized. The first dose administered was determined on the basis of commonly consumed amount of the smoking mixture, a second dose was five times for her. Ratings dose - 3.71 mg / kg and 18.55 mg / kg respectively. Animals. Males are white outbred mice aged 6-7 weeks, weighing 19-22 grams, were kept in a vivarium RRC " Farmatest " SEI HPE PGFA, Perm, Russia. Type of cell - T3, dunnage - softwood sawdust. Access to food and water the animals were free, except SLA - test. Lighting-natural.

Corresponding Author: Zykova, Perm Federal Penitentiary Service Institute of Russia, Karpinsky st. 125, 614990 Perm, Russia.

Research Design: Effect of JWH- 250 on the stress response in animals has been studied in a sequence of the following steps: 1. aktographical test of spontaneous locomotor activity, 2. The test of the "Open Fields" with video tracking, 3. The analysis of the probability distribution of instantaneous velocities of animals in the "open field". For each dose, received as " longitudinal " and "cross" data. In both groups of experiments, it was presence of synchronous control - a group injected with placebo (distillate) at the same time injecting laboratory animals. In the study as a whole has been used 34 animals. This number includes two experimental groups of 5 animals and a control group of 6 animals - in the study of long-term (sucircadian) effect exerted by the normal stress response of the animals and the experimental group 2 (for each dose) and the control group - 6 animals - during the test "Open field".

Behavioral Measurements: To study the spontaneous motor activity of single animals used 16-channel installation " Activity 2" (NSI PSU - PSPA, Perm, Russia), the installation of the "Open Field" ("Open Science", Moscow, Russia). In addition to commonly detected, investigated characteristics of chaos, structure and symmetry of the behavior of animals.

RESULTS

The results concerning the evaluation of the differences in the dynamics of spontaneous locomotor activity were presented in the first part of the article in the magazine «World Applied Sciences Journal», here we continue our presentation of the results, "cross" concerning time arrow tests.

"Cross" Concerning Time Arrow a Cut of Behavioral Activity: Orientation and Research Behavior: "Cross" cut of behavioral activity we call the description (signature) of the behavioral response to model "impulse" stressful influence. "Cross" it is concerning an arrow of time and is opposed in this sense "longitudinal" concerning time arrow to a cut, namely - to the description of dynamics of behavioral effect of studied substance. If data of a "longitudinal" cut - we obtain performance of the methodical complex "exposition + long stressful influence + SLA-test", data of a "cross" cut - result of the "exposition studied substance + pulse stressful influence + test of "open field" + video tracking" complex.

Actually data acquisition of a "cross" cut on a phase of implementation of the test of "open field" consists of two parallel processes: records of ethogram expert and synchronous video tracking. Duplication here carries out a number of important tasks, in particular, provides big completeness and a detail of the description of observed behavior and also mutual control of the person and the machine. Record of ethogram in the test of "open field" - standard ethologic procedure, here we won't stop on it. Its result is record of sequence of the behavioral acts executed by an animal. In this work we will concentrate attention on video tracking data.

For a detailed analysis of behavioral changes the data processing of video tracking consisting of primary and secondary decryption: primary decoding includes procedures of preprocessing of a video stream, pattern recognition (separation of subject and background), obtaining. The results of the primary decryption are time series. Secondary decryption includes identifying the behavioral characteristics of animal by calculating them based on the results of the primary decryption and adjustments to the original observations. The result of secondary decryption is a multidimensional data array, where (each) one parameter per animal behavior corresponds to a single numerical value.

After performing the decryption for JWH- 250 data were obtained that contain behavioral profiles of the effect of the agent JWH- 250 in two doses. Further, the data processing is constructed as follows : 1) ad-hoc analyzes (if necessary) and 2) a multivariate analysis of variance, which is the kernel to identify / describe the effect and 3) post-hoc tests are determined based on specific values of the differences for each group and characteristic. Further analyzes are performed individual disclosing certain essential aspects of the observed effect.

Studying of Modifications of the Stress Response to a "Pulse" Stress at JWH-250 Exposition: At test of animals performance by animals of repeated rotation round its pivot-center with reduction of productive horizontal movement and also - at other mouse was noted at a fivefold dose - a freezing within 25 seconds We briefly describe the results of behavior parameters (the results of expert observation): significant decrease in parameters of research activity of animals - the vertical activity (VA) - p=0,02 by Mann-Whitney's test and inspections of holes (p=0,05) by the same criterion is noted. These changes are noted only at a fivefold dose.

Analysis of Data of Videotracking: Calculated and studied behavioral parameters the are as follows: the distance traveled (*Path*), the individual variability of velocity (*IndVarV*), upper decile of acceleration

(*UpDecA*), the proportion of immobility time (*akinesis*), an asymmetry of a choice of the directions (*Assim Decision*) and integrated asymmetry (*AssimIntegr*), the proportion of time spent outside the periphery of the fields (*CD_all*), as well as the percentage of time the activity in the periphery of the field (*CD_act*). Besides, 3 structural characteristics of time series are applied: Shannon's entropy (*Shannon_E*), Hurst exponent (*H*) and fractality index (*m*).

Let's execute search of "global" differences in behavior between experimental groups of animals on the basis of our data. Conditions and limitations are as follows: multi-dimensional data, possible variations from the normal distribution of the individual (or all parameters), including possible failure to comply with the conditions of sphericity and equality of variances, small sample sizes. At such limitations it is expedient to apply a nonparametric method of comparison of multidimensional homogeneity of group variances (the "betadisper" procedure similar to calculation of beta diversity) and nonparametric version of the analysis of variance (the "Adonis" procedure), based on permutable algorithm. The number of iterations is set - 10^3. Implementations of these methods are based on the now classical work on the application of Marty Anderson commuting and bootstrap-procedures for multivariate analysis of variance [1,2]. The need for a specific multivariate analysis methods due, including the existence of different relationships between variables (behavioral) response to the studied effects. For preparation of data, we will execute normalization of the data table and we will construct from it a distance matrix. As a reference metrics we use Euclidean distance. Data presentation in the form of a distance matrix conveniently for carrying out the analyses directed on indicate of similarity/dissimilarity between multiparameter objects, since the latter can be represented as a point in feature space. In addition, the dimension of the matrix is determined by the distance (in our case) is the number of animals in the experience, not the number of input parameters and in this sense is versatile and compact.

After completing the analysis of the homogeneity of group variances, we obtain the result : p = 0,296 and accept the null hypothesis - the differences between multidimensional variances groups have been identified. Performing variance analysis, we determine the significance of the differences of the central characteristics. The calculated value p = 0,015. Let us accept the alternative hypothesis - Global differences were found. For post-hoc of testing it is applicable described in [3] robust analysis methodology

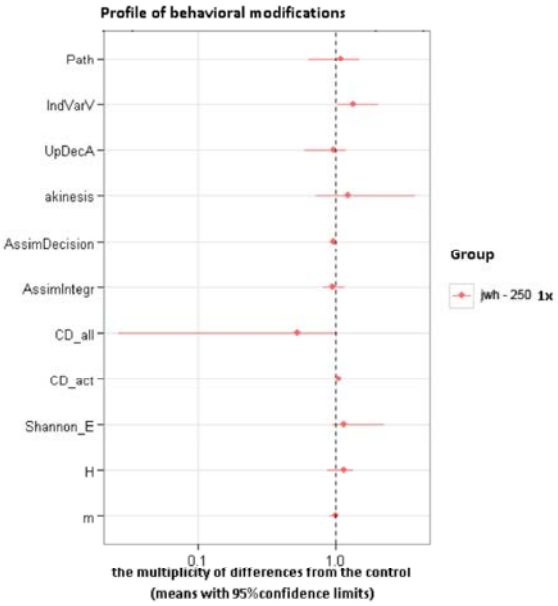


Fig. 1a: Profiles of behavioral modification at an exposition a one hold dose of JWH-250 (by results of Wilcox post-hoc testing)

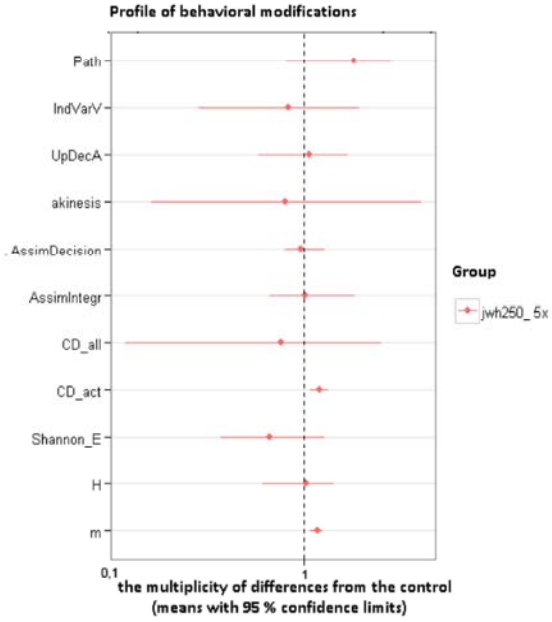


Fig. 1b: Profile of behavioral modification at an exposition a fivefold dose of JWH-250 (by results of Wilcox post-hoc testing)

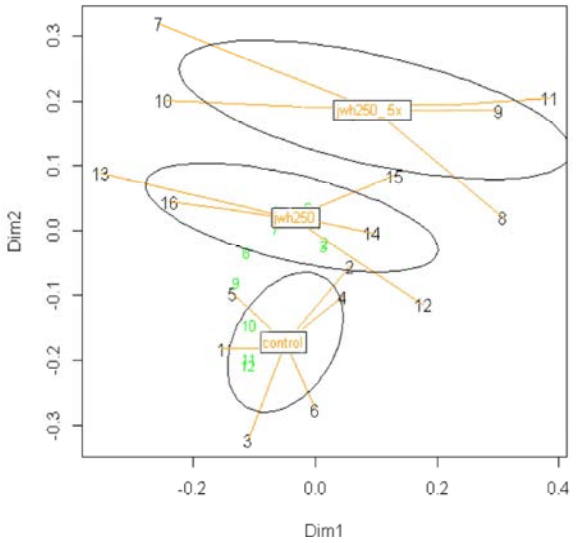
(resistant to emissions), namely - post-hoc the procedure constructed on determination of confidence intervals based on the results of a bootstrapping samples and for the characterization of central tendency are used trimmed mean (trimmed means). Since both the exposed group

compared to the same control, to the individual values for each parameter of behavior, for *p-value* is applied Bonferroni correction for multiple comparisons and the results can be understood as profiles of behavioral modification. They are represented in Fig. 1 a,b.

For both doses by Wilcox test a significant increase in the proportion of active time when outside periphery of "open field" (parameter CD_act, Fig. 1), indicating that the dose-dependent reduction in the exposed animal care, with the values of p- value (probability of false rejection of the null hypothesis) from 0.006 to < 0.001. In addition, there is a significant (p <0,001) increase in the index at fivefold the dose of the fractal, indicating a relatively monotonous behavior of these animals, reducing their interest to external objects and influences attentiveness. Animals, while at the periphery in a state of periodically relative immobility but not catalepsy operate since small movements, including stereotyped (small multiple jiggling head [4]).Symmetry violations at movement of animals aren't revealed. Decrease in attentiveness causes the overall low effectiveness of inspection of the environment surrounding an animal, in combination with a little raised power investments into a stress.

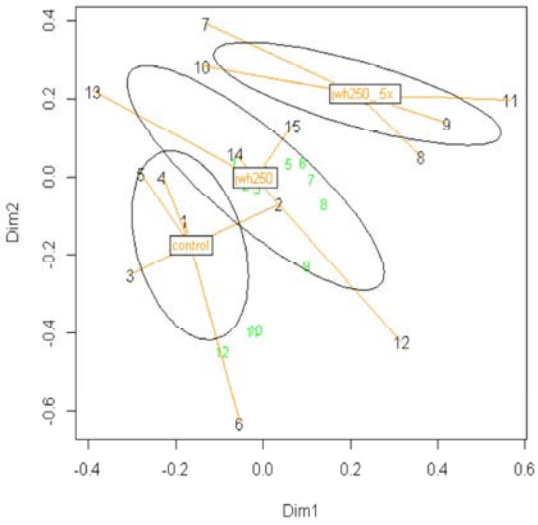
Concrete decryption of behavioral modifications will be such: the animals exhibited by a fivefold dose, while the open areas and exposed to immediate danger of being discovered, a large proportion of their time in a state of mobility, even more easier to own discovery. The diversity of mobility of animals in the group exposed by a fivefold dose, is very important - from the expressed motor excitation to interrupt behavioral "words" long breaks. This is clearly seen on the profiles of behavior modification - increasing confidence intervals compared to the group exposed with a single dose. In other words, when exposed to a low dose, we are seeing a weak motor excitement, accompanied by some loss of care, with a stronger exposure we see expressed by the human perception of the adequacy of the external risks to the individual cleavage reactions.

You Could Even Say That the Individual Response to a Strong Exposure Can Be Unpredictable: Thus it is necessary to recognize results of JWH-250 of adaptive tactics realized under the influence of a one-fold dose satisfactory as an animal, having partially lost ability to the jerk movement (Fig. 1, 2), which plays a crucial role in avoiding rodent variety of threats, the most part of time moves along borders of obstacles.



Dose of JWH-250 (by results of Wilcox post-hoc testing)

Fig. 3 a: "Subjective" DPiV: results of multidimensional scaling with $\chi 2$ metrics.



250 (by results of Wilcox post-hoc testing)

Fig. 3b: "Objective" DPiV: results of multidimensional scaling with $\chi 2$-metrics.

In natural environments, this type of movement is usually secretive means (the threat from above) movement. Without ensuring strategic safety through environment development, the animal thus ensures the short-term (tactical) safety. Under the influence of a fivefold dose at animals the specified aspect of care ceases to be observed and the increase in frequency of exits at open sites isn't accompanied by reduction of time in open sites, reducing competitiveness.

Analysis of Distribution of Probabilities of Instant Velocities: Distribution of probabilities of instant velocity (DPiV) is in this case one of the particular analyses which are carried out on the basis of data of videotracking. For the analysis of this distribution the following scale is used: 12 classes, first of which the probability of a full immovability of an animal describes and is characterized as "the zero speed"; with second on the eleventh classes the scale is the instant speed evenly broken on increase, with a step of 8,5 mm/sec.; the twelfth class includes all speeds which were higher included in the 11th class, so-called "ultrahigh speeds". Let's designate names of high-speed classes: class 1 - motionless (motionless), 2 and 3 - slow (slow), with 4 on 6 - "average" (med), with 7 on 9 - moderately fast (speed), 10 and 11 - fast (fast), 12 - superfast (ultrafast).

In 3 minutes of experience the animal has for environment 3min*60sec*15fps inspection = 2700 temporary discretes, each of which is characterized by stay in a certain microcondition; and, respectively, is in each of them a certain share of time of experience. It allows to speak about observed frequencies of stay in each of microcondition. As the number of observation is great in comparison with number of conditions, observed frequencies characterize probabilities of finding of an animal in one of them. Let's note that here it is applied also "objective", calculated by the rule stated above and so-called "subjective", rated on the way passed by an animal in "open field", DPiV.

Between expected (control) and observed frequencies it is convenient to apply a metrics to the description of distinctions $\chi 2$; in our case expected frequencies is DPiV at intact animals (for group of control), observed - DPiV in experience.

Distinctions in a metrics $\chi 2$ can be used as a distance measure between individual DPiV, having applied the received matrix of distances for multidimensional scaling (Fig. 2). The schedules received in such a way describe distinctions of the frequencies of microconditions saved up during experience for the individuals, graphically generalized for groups. Unlike the analysis of a set of separate behavioral characteristics, from the schedule of such scaling obviously follows: 1. dose dependence of expressiveness of effect (corresponding to remoteness of experimental group from control), 2. linearity of dose dependence of extent of distortions of DPIV; 3. discriminating role of fast and superfast movements, minor (for discrimination of groups) role of decrease in probability of slow movements; from the methodical moments we will pay attention on: 4. great resolution of the "subjective" DPIV in this case; 5. smoothness of the curves describing reference symbols. The last observation indicates existence of normal probabilistic interrelations between the next high-speed classes in behavior of animals. Let's note that in case of application of characteristics of DPiV on schedules in extreme positions there are also components at the expense of which there is probabilistic compensation (5 and 6 high-speed classes in this case). They can be read as follows : the animal larger than normal most of the time in a state with an average speed of movement. The suppression of high-energy component - characteristics, specifically indicating a demotivation (a slackness/ inattention, lack of uneasiness up to euphoria) an animal. By sight the animal, isn't present any need quickly to leave an open site, to check suitability of a surface for jumps, to try to jump through walls limiting the arena.

Thus, the behavioral response to stress with increasing dose becomes less and less adequate to the challenges of the changing environment in several " orthogonal " aspects: a)motor stimulation and visible for the observer / predator disruption of normal locomotion, outstanding abnormal animal; b) loss of a sense of fear, which leads to activity in open areas; loss of the opportunity to adequately apply the jerk movement, expressed in reducing the likelihood of fast and ultra-fast movements. Besides, It should be noted a dose-dependent growth of variability indicating a divergence of adaptive opportunities in group under the influence of a studied factor. Taken together, these observations indicate a redistribution of energy (decrease in a share a component with high energy consumption) in process of increase in a dose and redistribution specific time resources in the central part of a probabilistic curve (more time is spent for movement with low speeds). It indicates decrease in efficiency of adaptation of an animal in the changed conditions under the influence of JWH-250. It once again confirms that a preliminary conclusion that the effect rendered by JWH-250 on probabilistic patterns of distribution of energy consumption, is dose-dependent.

DISCUSSION

Despite a number of studies of behavioral phenomena caused by synthetic cannabinoids [5, 6], integrated studies, in which on the basis of model stresses are in parallel investigated both "longitudinal"

and "cross" characteristics of effects, isn't known to us. It is difficult to disagree with opinion of J. Wu [4] that in combination with dynamics of a locomotion it is required to consider and the stereotypical phenomena and "with the increased time window of observation". It is represented to us that this period has to be at least about daily. The combination of conditions, including a stress of change of environment and the subcircadian period of measurements recommended by us, is based, on the one hand, that the circadian period as natural discrete existence of live system, including with rather stable and natural pattern of energy consumption and, on the other hand, energy consumption raised in comparison with normal activity in the conditions of a stress that facilitates a task of the description of redistribution of energy consumption. From the point of view of human ecology, is also separate observation over an exposition by psychoactive factors of single individuals and groups of animals and also presentation of stressful conditions before and after an exposition. This may be a fixed non-obvious from the curves of the dynamics and dose response - moments, such as increased aggression in groups or, for example, smoothing / transformation effect in groups.

JWH-250, unlike such agents, as MK-801 [4] or amphetamine, causes after introduction long suppression of the locomotion attached to normal peaks of activity, with partial compensation during the periods of normal rest and the effect of suppression amplifies with increase in a dose As in the case of MK-801 with increasing doses in individual animals grow manifestations ataxia. In this case, naturally - and like other members of the group of synthetic cannabinoids - there hypokinesia is observed, but a catalepsy by us wasn't recorded.

It is curious that when exposed in groups and presentation of stressful loading in the process of exposing, horizontal locomotor activity of animals wasn't suppressed through 30 minutes at the high magnitude of the effect of suppression SDA in solitary animals, despite likeness of compared indicators. This fact confirms, on the one hand, expediency of the combined carrying out the specified tests and, on the other hand, fixes one of stability conditions of the primary stressful behavioral response to the test exposure- more effective overcoming of a psychoactive factor by group of animals at the expense of tactile contacts and a motive induction (peculiar "returns to reality") is possible. It corresponds to observation, for example, [7]. On our observation, the effect is characteristic for sedative factors (synthetic cannabinoids, barbiturates) and is mirror for the amphetamine-similar stimulators causing induced motor agitation and aggression.

The data obtained throughout the subcircadian period, as well as results of multidimensional and a posterior tests on "Open field", are useful for establishing the similarity of the behavioral effects of JWH- 250 with the first time studied substances. We assume that in a behavioral pattern of effect of JWH-250 can be understood as suppression of interest to environment, the animal has a power inflation of stressful reaction in combination with suppression of research activity, in particular, suppression of cognitive functions and as well as result of motor impairment and reduced care.

In addition, individual animals are observed deep and lasting disturbances in circadian rhythm of energy expenditure. An analysis of published data and based on their own experiments becomes clear idea of what is commonly discussed the need [4,8, 9] a comprehensive assessment of behavioral changes under the influence of anticipated substance filling concrete content should eventually including, besides the actual detection of psychoactivity of a substance, determining the toxicity and dose dependency, some autecological principles: first, the subcircadian time windows of observation doing by possible consideration of effect of psychoactive agent as modification of a normal behavioral pattern of an animal, for which circadian period - natural discrete, including considerable (and somewhat full) part of a variety of behavior of an animal; secondly, accounting of specific features of animals and specifics of group interactions; thirdly, monitoring not only traditional behavioral parameters (ethogram), but also randomness/organization characteristics in behavior of animals. The last point relates to the fact that the tactical adaptive response must meet strict requirements whether spending time and energy, said the need for certain sets, it is enough to narrow the scope of the behavioral characteristics of chaotic time series. In our opinion, the stability of entropy parameters of functioning of healthy organisms [10] as well as distinct manifestations of fractal organization in animal behavior [11,12] are sufficient grounds for the proposal.

It is necessary to agree with authors [4] that a necessary condition of the correct description of behavioral effects of new synthetic substances is the parallel analysis of parameters of mobility, cognitive functions, stereotypical behavior of animals. For actografical studies recommended the use of at least subcircadian periods of registration.

REFERENCES

1. Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics, 62(1): 245-253.
2. Anderson, M.J., K.E. Ellingsen, B.H. McArdle, 2006. Multivariate dispersion as a measure of beta diversity. Ecology Letters, 9(6): 683-693.
3. Wilcox, R.R., 2012. Introduction to Robust Estimation and Hypothesis Testing. Oxford: Academic Press, pp: 690.
4. Wu Jinhua, Zou Hong, Strong Judith A. Yu Jun, Zhou Xuedong, Xie Qinglian, Zhao Guoping, Jin Meilei and Yu Lei, 2011. Bimodal effects of MK-801 on locomotion and stereotypy in C57BL/6 mice. Psychopharmacology, 177(3): 256-263.
5. Gebremedhin, D., A.R. Lange and W.B. Campbell, 1999. Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca2+ channel current. American Journal of Physiology, 276(45): 2085-2093.
6. Fernandez-Ruiz, J. and S. Gonzales, 2005. Cannabinoid control of motor function at the basal ganglia. Handbook of Experimental Pharmacology. 168(1): 479-507.
7. Korytin, M., 1991. Tiger under anesthesia. Animal-drugs-man. M.: Znanie, pp: 240.
8. Carlsson, M. and A. Carlsson, 1989. The NMDA antagonists MK-801 causes marked locomotor stimulation in monoamine- depleted mice. Journal of Neural Transmission. 75(3): 221-226.
9. Chichinadze, K.N., 2004. Motor and neurochemical correlates of aggressive behavior in male mice. Neurophysiology, 36(4): 262-269.
10. Saraev, I.A. and V.M. Dovgal, 2008. New possibilities of diagnostics on the basis of the analysis of nonlinear properties homeokinesis. Clinical medicine, 4(1): 64-74.
11. Kuzmenko, A.A., I.A. Saraev, S.A. Sumin, I.I. Dolgina and A.A. Perekonyuk, 1999. Analysis of nonlinear (chaotic) dynamic systems and prospect of its application. News of higher education institutions "Applied Nonlinear Dynamics", 2(1): 88.
12. Andreev, A.I., 2011. Assessment of opportunities of application of the wavelet-analysis for research of physical activity small laboratory animals. Science Prospects, 7(22): 165-182.