The Study of Behavioural Modification of Stress Responses at the Mice Exposed by JWH-250. Autoecological Aspects

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Abstract: 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

Growing rates of the directed synthesis of new biologically active compounds naturally lead as to distribution of new substances in society and to increase in volumes of their receipt in environment. In our opinion, special danger also will be represented now in the near future by distribution of the new synthetic psychoactive chemical agents possessing high specificity of action and, respectively, of active even in small quantities. Thus characteristics of their action generally carry clinically-focused character, that not always sufficiently characterizes concrete substances as modifiers of a condition and behavior of people and animals out of clinic. In this work some opportunities of complex studying of behavioral violations at animals under the influence of such specific agent, as JWH-250 operating on endocannabinoid receptors are considered.

Structure: JWH-250-synthetic substance from family of phenylacetylindols, relating to the group of nonclassical synthetic cannabinoids. Unlike many earlier synthesized connections of a number of JWH, this substance has no naphthalenic ring; holding this position 2 '-metoxyphenylacetyl the group does JWH-250 by the representative of a new class of ligands-cannabinoids [1].

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Other connections with groups in 2'-situation (such as methyl-chlorine or brome-substituted analogs) are also present and are a little more powerful [2].

Action on the human. The psychoactive effect arises much quicker, than at the marijuana use. Typical side effects are dryness in a mouth, reddening of whites of the eyes, increase a arterial pressure, the speeded-up palpitation, nausea at the overdose, anesthetizing action.

Action mechanism. JWH-250 influences both types of cannabinoid receptors-CB1 and CB2 belonging to rather recently opened endocannabinoid system [3]. It was finding that physiological properties of endocannabinoids are very similar to those synthetic cannabimimetics [4]. Now it is conventional that the endogenous cannabinoid system is universal lipidic signal system which arose at early stages of evolution and carries out important regulatory functions in an organism of all vertebrata, owing to "through" existence of receptors of CB1 [4,5]. Thus, evolutionary age of this signal system-not less than 500 million years. Receptors of CB1 belong to G-protein-related family of receptors and are widely presented in areas of the brain which functions are connected with control of locomotion activity, cognitive functions, emotional reactions, motivated behavior and a homeostasis. Considerable concentration of CB1 receptors are found in cerebral cortex, hippocampus, caudatum, reticular part of the substantia nigra, globus pallidus, cerebellum [6, 7] and also in the structures participating in perception and regulation of nociceptive signals. Distribution of CB1 receptors in CNS in a certain degree forms a basis for formation of psychotropic effects of cannabinoids [8].

CB1 receptors are studied better and with them connect psychotropic effects of THC. Settling down on axons (in CNS and on the periphery), modulate the release of excitatory and inhibitory neurotransmitters, enhancing or inhibiting the transmission of the corresponding signals [8,9]. Stimulation of the CB1-receptor in the postsynaptic membrane of pyramidal neurons in the hippocampus, leads to increased neuronal excitability by closing the potassium channels. Most likely, activation of CB1 receptor reduces the intensity of signals transmitted from cell to cell by other neurotransmitters (serotonin and dopamine). Currently, animal experiments showed that activation of CB1 receptors causes inhibition of neurotransmitter release, antinociception / analgesia, memory disorders, disorders of motor function- hypokinesia and catalepsy [10]. Nicoll and Alger [3] indicate that endocannabinoids, without affecting the appearance of fear, the animal needed to overcome it.

One of the objectives of this work was to determine the characteristics of the stress behavior of animals in which the agent through a high-affinity-JWH-250-artificially activated cannabinoid receptors.

The choice of stressful conditions is caused by several moments: first, functional relationship of endocannabinoid system with functional system of the stress response [11]. Second, the prevalence of stress, in fact, laboratory tests, such as the "open field". Third, significant energy investments in overcoming stress, making it easier to measure the behavioral parameters.

Fourth, it is often the effectiveness of behavioral stress response is crucial for the survival of the animal, which fell under the influence of a powerful factor in the natural environment, or when human social interactions with an altered state of consciousness.

It is known that a single administration of certain substances a number of synthetic cannabinoids cause changes in motor activity in rodents and, in high doses, stereotypic behavior disorders, including incoordination, head weaving, gait disturbance. These effects have a certain similarity to the effects of funds specifically modifying the level of dopamine. There is reason to believe that the basis of differences in the behavioral effects are similar changes in the functioning of the dopaminergic system of the brain [12] and nerve conduction pathways, including the permeability of synapses [13].

In this study, we present a detailed characterization of changes in the normal behavioral stress response in albino laboratory mice exposed to JWH-250 in the aspect of the structure of spontaneous locomotor activity (further-SLA). The use of special techniques for analyzing multidimensional data useful behavioral including from multiple internal relationships between behavioral parameters [14].

**MATERIALS AND METHODS**

**Chemicals:** JWH-250 was obtained as an alcohol flush with leaf smokable mixture, recrystallized and analyzed by Agilent 7890A gas chromatograph with mass spectrometer Agilent 5975C, whereby it was identified chemical structure. Water extraction for injection was prepared immediately before the experiments according to
the rules of the interspecies transfer of doses given in the [15]. The volume of injection solution linearly match the required dose. The first dose administered was determined on the basis of commonly consumed amount of the smoking mixture, a second dose was fivefold for her. Ratings dose-3.71 mg / kg and 18.55 mg/kg respectively.

**Animals:** Males of white outbred mice age of 6-7 weeks, weighing 19-22 grams, contained in a vivarium of RTC "Farmatest" of PSPA, Perm, Russia. Animals lived under standard conditions of the contents, in plastic cages of the T3 type with an saw dust laying. Before experience animals contained in cages up to 10 heads, in repartitions of circadian experiment-on one mouse in a cage, constantly being exposed to "a loneliness stress". Animals had a free access to water and food, except for days of exhibiting in the camera of registration of the spontaneous locomotor activity (SLA) when animals had an access only to water. Animals are grown up in the conditions of daily conditions of lighting and of animals in installation didn't differ from standard, however for the period of SLA-test animals contained in cages (registering cameras) within a day, singly, had a free access to water, but were deprived of a food (were fed in advance).

**Research Design:** Effect of JWH-250 on the stress program modules of a complex and also packages of response at animals was studied in sequence of the mathematical data processing, in particular, StatSoft Statistica 8.0 and R 3.0.1 [21]. Received SLA-data allows to identify and describe the deviations from the normal curve locomotor activity (called baseline, [22]) synchronously received by data from control group of animals. These results allowed to trace the daily dynamics (relative to the time axis). 2. The test of "Open field" with the video tracking, which kernel of data processing-the duration and magnitude (absolute peak value) effect. During registration of spontaneous locomotor activity of animals contained in cages (registering cameras) within a day, singly, had a free access to water, but were deprived of a food (were fed in advance).

The data created by the hardware, were processed by program modules of a complex and also packages of mathematical data processing, in particular, StatSoft Statistica 8.0 and R 3.0.1 [21]. Received SLA-data allows to identify and describe the deviations from the normal curve locomotor activity (called baseline, [22]) synchronously received by data from control group of animals. These results allowed to trace the daily dynamics of influence of studied substances on SLA in aspects of duration and magnitude (absolute peak value) effect. During registration of spontaneous locomotor activity of a condition differed nothing from those for the main herd (the same cages and the racks, identical temperature and light conditions). All data-acquisition equipment was from outer side of cages thus providing a non-invasive registration, in comparison, for example, with classic techniques of registration of mobility according to the scheme "beam crossing"[23]. Nevertheless, all animals in experience (including group with placebo) were exposed first of all to "a stress of change of environment" as were moved to unfamiliar cameras and , in the second turn, to "a loneliness stress" as individual locomotor activity was registered and animals were in individual cameras on one.
The order of carrying out experiment included 3 phases: pre-exposure, exposition, post-exposure (fig. 1). Duration of a pre-exposition made 64 minutes. Animals were located on this phase in individual cameras of registration of the physical activity, specified time record of individual parameters with the purpose to record background variability of behavior (to an exposition) in experienced groups rather control. The next 11 minutes—performance of administration of substances as placebo, a smaller dose, a big dose and putting back into the individual chambers. After the injection and the end of the experimental period lasted post-exposure, throughout which there is a deployment of a time pattern of effect lasts. This SLA test groups curves are interpreted relative to the curve of the control group (baseline), because the data is synchronous, which ensures uniformity conditions external to the experiment (primarily external pacemakers—time of day length of daylight, etc.)

**RESULTS**

Decoding of Data of Monitoring of SLA: Effective and sufficiently robust tool in this case may be the use of analysis of variance with repeated measures [26], coupled with samples bootstrap-to estimate confidence intervals for each time discrete. Results of homogeneity of variances and proper dispersion analysis are presented in Tables 1 and 2.

Here DFn—quantity of degrees of freedom of effect, DFd—quantity of degrees of freedom of residuals, SSn—the sum of squares of effect, SSd—the sum of squares of residuals.

Thus, we assume homogeneity of variances confirmed, the fact that a shift differences between the groups-identified. Build the chart with confidence intervals: Fig. 1.

Since the experience beginning on the 64th minute the pre-exposition period (in fig. 1 to the left of the red line designating the moment of an exposition of JWH-250) lasted. From the image it is clear that before injections of substance of group were statistically uniform. During the period from 75 to 550 minute, in control group 2 large-scale peaks of the activity, each lasting about 200 minutes and , further a long dormant period-more than 500 minutes

**Open Field Test:** For receiving "cross" concerning an arrow of time of data the Open field installation [24] (NPK Open Science, Moscow, Russia) was used. Installation is a round platform diameter 63\(\text{cm}\) with available 13 holes in a floor and divided into 19 equal parts with allocation of a peripheral, intermediate and central zone of a field. Illumination was 120 Lx. Before experience animals passed half-hour exhibiting in cages on three and in "open field" the animal was withdrawn from this group. At record of ethogram within 3 minutes of observation over animals registered the following indicators: number of the crossed squares on the periphery, in the zone “2/3” and the platform center (horizontal motor activity, HA), number of a rising on hinder legs (vertical activity, VA), number of peeping in holes (research activity), quantity fecal boluses (an emotional factor), a grooming [25].

**Video Tracking:** For details and an objectification of results of measurements "Open field" video tracking was carried out, i.e. animals during the test were recorded on video, then the video stream processed by the program part of the complex. Thus, traditional ethogram was registered in parallel and irrespective of a technical superstructure. Initial material for the detailed characteristic of HA was the flow of 2D information on position of an animal with the resolution of 0,3 Mpix and 15 fps. Calculated and studied behavioral parameters the are as follows: the distance traveled, the individual variability of speed, upper decile of acceleration, the proportion of immobility time, an asymmetry of a choice of the directions and integrated asymmetry the proportion of time spent outside the periphery of the fields, as well as the percentage of time the activity in the periphery of the field. Besides, 3 structural characteristics of time series are applied: Shannon's entropy, Hurst exponent and fractality index. In the analysis of data of videotracking we proceeded from a problem of obtaining the complex characteristic of behavior of animals in "open field", which means the device signal analysis calculated the number of behavioral parameters, both basic and supplementary, including the characteristics of chaos, structure and symmetry.
were observed, at the beginning and which end two peaks of activity with a smaller magnitude of energy consumption, with the centers on the 680th and 1000th minute were observed. After a dormant period the following large-scale peak of activity is visible—beginning from 1100th minute, terminates outside experiment. Apparently from fig. 1, changes of SLA of the single mice, being observed at single (1X) and fivefold (5X) doses of JWH-250 entered intraperitoneally, are qualitatively similar and have the main contents suppression of normal peaks of the activity observed at intact mice in control group for the considered subcircadian period. Such representation (fig.1) allows to execute a periodization of the behavioral effects of two doses: in the first 10 minutes after exposure there is no difference between the groups, then the same for 4x 10-minute cycles, we observe three types of dynamics: in control of the activity of stable high at 1X dose-consistently low, with 5X-is close to zero, with a tendency to increase with time. With 150 of 300 minutes—the first peak of activity is suppressed completely and equally. From 300 to 550 minutes—the second peak of activity was also completely suppressed in the group with a 5X dose and to a lesser degree—in 1X dose group, statistically significant differences between the three groups.

During a dormant period significant distinctions aren't found, except for activity flash in control with the center on the 670th minute—about experienced groups it absent. It is interesting that at the end of a dormant period (1000-1100 minutes) there is no significant difference between control and exposed groups with a single dose—but the group with a dose of 5X shows the phase shift—3-hour delay entering the peak of activity and the peak violation—a low level of activity during said peak. On 1600 minutes and then ill defined group dynamics.

The results of the experiments considered suggest that JWH-250 has a long and sedating effect on the structure of the central nervous system, accompanied by a decrease in the SLA as a whole in individual animals (!) during the first days after the establishment of the stress change of the environment.

As changes of a dose of JWH-250 change both amplitude characteristics and a pattern dynamic of SLA, the conclusions of the dose dependence may depend on what kind of time window is selected for the test. This confirms the view expressed in the work [23], but in making proposed in this work a 4-hour period of observation can be made wrong conclusions about the asymptotic approximation curve effect to the reference level.

**DISCUSSION**

The main part of the discussion will be presented in the second part of the article in the magazine «Middle East Journal of Scientific Research», here dwell only on some important point.
Despite a number of studies of behavioral phenomena caused by synthetic cannabinoids [8,9], 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 [23] 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.

JWH-250, unlike such agents, as MK-801 [26] 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. 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.

REFERENCES


