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Влияние феназепама на паттерны поведения крыс

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Аннотация. Благодаря развитию сферы информационных технологий для многих исследователей становятся доступными новейшие методы в наблюдении, фиксации, а также анализе разного рода массивов данных. Так, компьютеризированный анализ на основе новых алгоритмов способствует сопоставлению разных схем поведения, включая изучение динамики активности животных под воздействием нейротропных препаратов, примером которых может служить феназепам. Цель исследования – сопоставление паттернов поведения двух групп лабораторных крыс: контрольной группы и группы под воздействием феназепама, с применением систем компьютерного анализа, а также изучение влияния феназепама на поведение крыс как таковое. В результате исследования были выявлены особенности в уровне выраженности общей траектории движения крыс в ходе тестирования. Также применение разработанных нами приемов цветового временного кодирования позволило обнаружить существование корреляции в поведении между двумя временными позициями. Сфера применения разработанных нами методов не ограничена научно-лабораторной практикой. Многие из этих методов применимы к людям, особенно, к пациентам, страдающим неврологическими и моторными расстройствами широкого спектра.

Ключевые слова: поведение, крысы, феназепам, анксиолитик, тревожность, «открытое поле»

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NEUROBIOLOGY

Original article

Effect of Phenazepam on Behavioral Patterns in Rats

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Abstract. Due to the development of information technology, many researchers have received access to the latest methods in the observation, recording, and analysis of various kinds of data arrays. Thus, machine analysis based on new algorithms facilitates the comparison of different patterns of behavior, including the study of the dynamics of animal activity under the influence of neurotropic drugs, an example of which is phenazepam. The aim of our study was to use computer analysis systems to compare the behavior patterns of two groups of laboratory rats: the control group and the group following phenazepam, as well as to study the overall effect of phenazepam on the behavior of rats. As the study showed, features were revealed in the level of expression of the general trajectories of rat movement during the testing. In addition, the use of home-developed color time coding techniques, made it possible to detect the existence of a correlation in behavior between two time positions of the same animal. The scope of the methods developed by us is not limited to scientific and laboratory practice. Some of these methods are applicable to humans, especially patients suffering from a wide range of neurological and motor disorders.

Keywords: behavior, rats, phenazepam, anxiolytic, anxiety, «open field»

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Introduction

The study and analysis of the behavioral aspects of animals is one of the most complex tasks in modern neuroscience. It requires the formation of qualified experience on the part of the observer, painstaking observation and a lot of time. Fortunately, the use of automated analysis systems creates the conditions for significant facilitation and timesaving in research. Thanks to the introduction of new analysis, it seems possible to more deeply study the various behavioral patterns of groups of laboratory animals, including rats [Andreev et al., 2021]. Moreover, prospects for the use of the developed methods in the diagnosis and treatment of various neuropathology in humans are opening up.

For many years, animal behavior has been studied through visual observation, which imposes certain restrictions on the research process, thereby affecting the data and results obtained during the analysis. However, in recent decades, a trend in the use of artificial intelligence systems has become noticeable, making it possible to optimize the processes of observation, recording and analysis of arrays of received data [Godec P., et al, 2019]. In particular, the comparison of behavioral patterns and dynamics of animal activity under the influence of neurotropic drugs is greatly simplified and objectified. As an example, in our study, a widely known drug in the clinic, phenazepam, has been used.

Phenazepam is a tranquilizer developed at the end of the last century and has a pronounced anticonvulsant, muscle relaxant and soporific effect. It is a white crystalline powder, insoluble in water and slightly soluble in alcohol. The mechanism of action of phenazepam is to facilitate the inhibitory action of gamma-aminobutyric acid (GABA) on the signal transmission of brain [Hepsomali at al., 2020, Oyemade, 2012]. In medical practice, phenazepam is prescribed for the treatment of nervous, neuropathic, psychopathic conditions accompanied by anxiety, fear, and increased irritability [Maskell, 2012]. In this study, the goal was to study the effects of phenazepam on the behavior of rats using the methods of home-developed computer color-coding.

Materials & Methods

Animals. Outbred laboratory rats were kept in standard cages, strictly observing the rules of animal maintanance in accordance with the international convention "International Convention for the Protection of Animals" [URL: https://www.animallaw.info/treaty/international-convention-protection-animals], following the Order by the Ministry of Health of the Russian Federation from April 1, 2016 No. 199n “On Approval of the Rules for Good Laboratory Practice”. The animals were provided with suitable nutrition, stable temperature conditions and veterinary care. The day/night cycle was set at 12/12 hours.

To achieve the goals of the study, a series of experiments was carried out on 20 outbred rats, divided into 2 groups according to the administered drug. The rats of the control group were injected with a physiological solution of 0.5 ml. Rats of the second group were injected with phenazepam in a volume of 1 mg/kg, with a similar injection volume [Sysoev et al., 2022]. The exposure time of the drug before registration was 20 minutes.

Experimental setup and registration. The "open field" setup was a round, flat arena of black color, equipped with sides of sufficient height to prevent the escape of animals, 97 cm in diameter, divided into 19 sectors of equal area, with a number of small holes in the floor, attractive for their exploration by rats. Video recording was carried out using a Canon 5D camera, at a frequency of 30 frames per second, for 3 minutes, at 320-lux illumination. The camera was controlled remotely, without a person being in the room, with the image of the field displayed on the monitor.

Methods of image acquisition. In the course of a study conducted with each of two groups of rats, a number of behavioral parameters were analyzed in the open field test [Walsh and Cummins, 1976; Crusio, 2001; Seibenhener and Wooten, 2015]: mobility, anxiety, and orienting-exploratory behavior.

Each animal test was recorded on a video camera, resulting in 20 video recordings. The resulting videos were processed in the Google Colaboratory service [URL: https://colab.research.google.com]. Each video was processed using the program code. These video files included images consisting of three time positions expressed using color coding. To obtain a color video recording, each video was divided into frames. Thus, at each moment of time, 3 frames were combined: 1st frame - the present time, 2nd frame - the near future (in 10 frames relative to the first one or after 0.33 s), 3d frame - the distant future (in 50 frames relative to the first, i. e. after 1.66 s). Further, all three frames were combined into a single frame in sequence while maintaining the frame rate. The need to introduce color coding was to determine the relationship between behavior patterns in the near and distant future. Then the goal was to predict the individual patterns of animal behavior based on the combined frames.

In addition to the above methods, when studying the behavior of rats, an additional analysis tool was used, which allowed analyzing motor activity in general, without taking into account specific patterns. For these purposes, using Fiji software from ImageJ [Schindelin et al, 2012] we created a Java code that processed 540 frames obtained from test video files at equal intervals, with a total recording duration of 3 minutes. As a result, after the execution of the program code, a single collage of six images per video file was obtained.

Results and Discussion

The temporal color coding method made it possible to trace the dynamics and trajectory of movements of individuals in the control and phenazepam groups of rats throughout the entire video recording (Fig. 1). The video file with each animal was divided into 6 parts of 90 frames each (that is, a total of 540 frames) using the original program code we created.

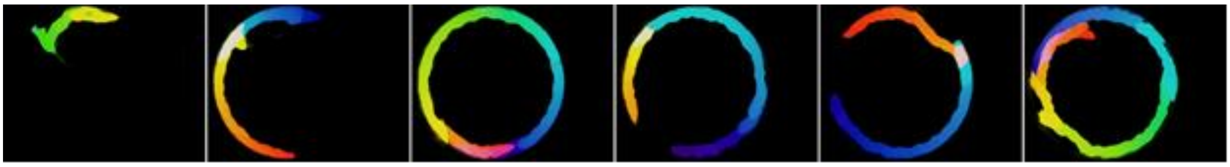

Fig. 1. A montage showing the trajectory of an individual rat during a three-minute recording ((Temporal Color Code).

To form a collage, every tenth frame was used, that is, 540 out of 5400 consecutive frames. For the formation of each of the six elements of the collage, 90 selected frames were used. Thus, the entire recording period was divided into six 30-second segments. The beginning of the movement in each element was indicated in dark blue, then in blue, green, yellow, orange and red. The absence of one or another color in the trajectory of movement meant the imposition of several shades, which occurred when the animal froze in one position

From the images generated in this way, it can be seen that the orienting-exploratory activity of each of the control animals was generally very intense: most of the rats during the behavioral test made long runs both in the center and around the periphery of the arena. Thus, the temporal color coding of the control group of laboratory rats showed quite diverse and rich dynamics of movements, which in all cases corresponded to normal orienting-exploratory activity.

The use of temporal color coding for the phenazepam injection group revealed significantly different results. First, in this group, abnormal orienting-exploratory behavior was clearly manifested, expressed in long runs, mainly along the periphery of the arena. Moreover, most of the animals moved exclusively in a circular trajectory throughout the recording. Secondly, by the end of video recording, most treated animals showed a noticeable decrease or complete absence of motor activity. In many cases, the animals would suddenly stop moving, entering a state known as "freezing". Animals became almost completely immobile, only occasionally sniffing the environment or floor holes.

Thanks to another approach, namely frame-by-frame temporal coding with the combination of the current position, near and far future in one frame, it was possible to study the patterns of rats at different time positions.

To analyze the correlation between these time points, 4 standard patterns of movement were chosen: farther forward, turns right, left or back. As predictors of these future movements, we paid attention to the position of the tail, head, and body of rats in the context of their co-directional position and alignment with future movement.

Subsequent statistical analysis [Stanton et al, 1998] showed significant differences in percentage of positive predications between the "forward" pattern and the "right", "left" or "back" patterns in the control group of rats as well as in the group injected with phenazepam (Fig. 2).

This finding indicates that tail, head, and body positions may serve positive predictors of farther forward movement. Moreover, in the anxiolytic group, this trend was even more pronounced (see higher level of statistical significance for phenazepam group, compared to control). At the same time, considering the remaining three patterns (to the right, to the left, and back), we concluded that the positions of the tail, head, and body of rats were not reliable predictors of actual motor patterns in both the near and distant future. In our opinion, this innovative method of dynamic analysis has demonstrated significant potential in the analysis of the effect of psychoactive substances on the nervous system and behavior of the laboratory animals tested.

Thanks to the development of new research methods, we found that phenazepam, as a tranquilizer, inhibits exploratory activity in rats. We have established the features of the level of expression, related to the general trajectories of rat dynamics in “open field” test, executed in two different groups. It has been demonstrated that phenazepam in most cases promotes stereotypic circular motion along the arena wall, and in some cases completely suppresses the exploratory interest of the animals.

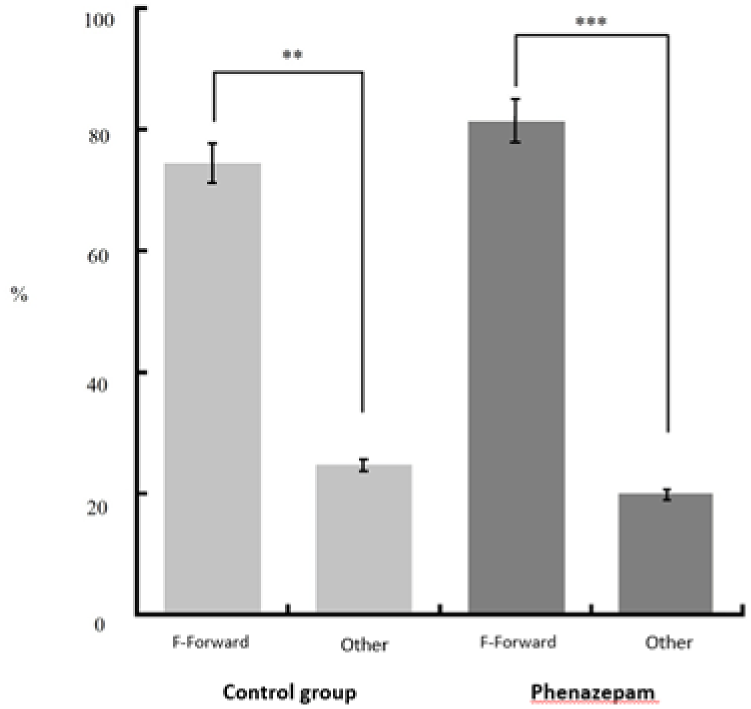

Fig. 2. Comparison of indicators predicting the patterns of animal dynamics in the “open field” test, such as movement straight forward (marked as “F-forward”) and turns right, left and back taken together (marked as “Other”) in the "Control group" (light gray) and "Phenazepam" (dark gray). When the positions of head, body or tail of the animal were co-directed with its near or farther future movements they were considered as “positive predictors”. Y-axis - overall percent of positive predictions cases

Some more unexpected results were obtained by using color temporal registration of frames, which made it possible to detect the presence of a correlation in behavior between two time positions. In particular, it turned out that the pattern of forward movement can be reliably predicted based on the position of the animal's head, body, or tail. Moreover, this trend is true for both studied groups of rats. In general, the future coding method can be used in studies whose purpose is to reveal the dynamics of the behavior of entire range of laboratory animals after the use of different psychoactive substances.

The combination of both of our innovative machine-based behavioral analysis methods opens up exciting prospects for the early diagnosis of neurological movement disorders in human patients. We are confident that the color coding of different motor phases and general motor patterns of patients will allow further development of methods for the early diagnosis of neurological disorders.

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Korkotian E. – scientific management; research concept; creation of methodology; data analysis; illustration design; writing the final text.