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THE EFFECT OF KISSPEPTIN ON THE BEHAVIOR OF MALE RATS IN THE "OPEN FIELD" TEST

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Abstract. The aim of the work is to study the nature of the behavioral reactions of rats in test tasks with intranasal administration of kisspeptin. To analyze the effect of kisspeptin on the behavioral response of rats in the "Open Field" test. In the process of work, research methods were used: testing rats in the "Open Field" test, methods of mathematical statistics. As a result of the work, it was determined that kisspeptin affects the behavioral responses of rats in new (stressful) conditions. In the experiment conducted with the use of the "Open Field" test setup, new data were obtained on the behavioral characteristics of rats under conditions of pharmacological activation of kisspeptin-energy receptors. It is noted that kisspeptin activates motor, exploratory activity, causes an anti-anxiety effect and the likelihood of cases of memory infection. The results presented in this article indicate a high frequency of hormonal effects of kisspeptin receptors on exploratory responses, anxiety levels, and memory.

Key words: kisspeptin; behavior; open field; rats; vertical motor activity; anxious grooming; kisspeptinergic receptors (GPR54).

ВЛИЯНИЕ КИССПЕПТИНА НА ПОВЕДЕНИЕ КРЫС-САМЦОВ В ТЕСТЕ «ОТКРЫТОЕ ПОЛЕ»

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Резюме. Цель работы — изучить характер поведенческих реакций крыс в тестовых заданиях при интраназальном введении кисспептина. Проанализировать влияние кисспептина на структуру поведения крыс в тесте «Открытое поле». В процессе работы использованы методы исследования: тестирование крыс в тесте «Открытое поле», методы математической статистики. В результате работы определено, что кисспептин оказывает модулирующее влияние на поведенческие реакции крыс в новых (стрессогенных) условиях. В проведенном исследовании с использованием тестовой установки «Открытое поле» получены новые данные об особенностях поведения крыс в условиях фармакологической активации кисспептинергических рецепторов. Отмечено, что кисспептин активизирует двигательную, исследовательскую активность, обладает

противотревожным эффектом и положительно влияет на процессы пространственной памяти. Результаты, представленные в данной статье, позволяют уточнить механизмы гормонального действия рецепторов кисспептина на исследовательские реакции, уровень тревожности и память.

Ключевые слова: кисспептин; поведение; открытое поле; крысы; вертикальная двигательная активность; тревожный груминг; кисспептинергические рецепторы (GPR54).

INTRODUCTION

Kisspeptin (KP) is a neurohormone responsible for the onset of puberty and subsequent regulation of reproductive function [8]. In 2001, it became known that the peptide products of this gene exhibit biological activity by binding to the G-protein-coupled receptor — GPR54 [5, 8, 10]. In 1999, this receptor was cloned. Kiss-1 peptides were called kisspeptins [5, 8, 10].

Kisspeptin belongs to a family of neuropeptides that are encoded by the *Kiss1* gene. In humans, the *Kiss1* gene is located on chromosome 1q32, encodes 144 amino acid residues, and has four exons [11]. This peptide is cleaved into a 54-amino acid peptide known as kisspeptin-54. There are also shorter peptides that share RF-amidated bases with kisspeptin-54, such as kisspeptin-10, -13, and -14 [3, 9].

Kisspeptin has a G protein-coupled receptor GPR54, which has a high homology percentage with the galanin receptor (44–45%). GPR54 mRNA is expressed in the brain, including the hypothalamus [6], and in other organs, such as the pituitary gland, placenta, pancreas [10], and ovaries [2]. Binding of Kiss-1r to the Kiss-1 peptide leads to activation of G protein-activated phospholipase C (PLCβ), which indicates a G q/11-mediated signalling pathway [7]. These signalling molecules, in turn, act as a mediator of intracellular Ca^{2+} release and activation of protein kinase C. KP stimulates gonadotropin-releasing hormone (GnRH) secretion by activating cation channels and inhibiting potassium efflux from the channels by DAG and/or Ca^{2+}.

The study of the mechanisms of reproductive system functioning, influence of sex hormones on the functions of the central nervous system (CNS) and organisation of behaviour is one of the urgent tasks of physiology. In 1999, a new hypothalamic factor was discovered — kisspeptin, which plays a key role in the launch and subsequent control of the functioning of the hypothalamic-pituitary-gonadal axis (HPGA). In particular, the inclusion of this neuropeptide in control of GnRH production was established. The development of the hypothalamic form of hypogonadism with insufficient functioning of the kisspeptin system was revealed. These circumstances indicate the possible use of KP in the correction of sexual dysfunction [4].

Nowadays, the issue of kisspeptin participation in the regulation of adaptive behaviour (motor activity, spatial memory, anxious grooming) is poorly understood.

This study devoted to finding the features of rats' behaviour under conditions of activation of kisspeptin receptors of the brain, on various components of the holistic behaviour of laboratory rats.

MATERIAL AND METHODS

The experiment was conducted on 20 mature male Wistar rats. The animals were kept in a separate room on a standard balanced diet.

At the first stage, the animals were tamed to hands for 14 days.

At the second stage, we studied the behavioural status of rats depending on the functional state of kisspeptin receptors. For this purpose, we formed an experimental group of rats, which were administered an activator of kisspeptin receptors (kiss-1 68-80, 10^{-4} M; Sigma) intranasal in a volume of 10 μl in each nostril. The control group was administered a physiological solution according to the similar scheme.

During testing in the "Open Field" setup, horizontal motor activity, vertical activity, anxiety level, latent transition time to the first square, and latent transition time to the central square were recorded for three minutes.

The data obtained during the experiment were processed using the SigmaStat program, One Way ANOVA test and Student's t-test. The graphs were constructed using the SigmaPlot program.

RESULTS

The results of the study revealed some features of the influence of activation of kisspeptin brain receptors on behaviour of rats when performing various experimental tasks.

Horizontal motor activity was less pronounced when rats received kisspeptin. In 3 minutes, rats that were administered kisspeptin mastered an average of 35 sectors, rats from the control group — 47 sectors.

The most experimentally significant differences were revealed in vertical motor activity. On the 4th and 5th days of observation, this indicator was almost 2 times more pronounced in rats of the experimental group (Fig. 1).

Exploratory activity was also higher in the kisspeptin-treated group. On the 3rd, 6th, and 11th days of observation, the differences between the groups were 25, 37, and 78 %, respectively (Fig. 2).

Under the conditions of "Open Field" test, it was not possible to identify a statistically significant difference in the level of anxiety in the incomplete grooming reaction. In the group of rats under the influence of kisspeptin, the number of such grooming acts on the 11th day of the experiment was 0, and in rats from the control group — 2. Thus, the anxious grooming reactions in the control individuals did not undergo significant changes, while the experimental rats demonstrated relatively low anxiety a day after receiving kisspeptin.

The latent time of first transition to the adjacent sector indicates the activation of exploratory behaviour in animals from the experimental group (Fig. 3).

Thus, on the 11th day, the first indicator in controls averaged 6.9 s, while in the first group it was less than 1 s. On 11th day, the latent time of the first visit to the central sector in controls averaged 73 s, while in group 1 it was 20 s. In addition, the "Open Field" test established the effect of activation of kisspeptin receptors on behaviour.

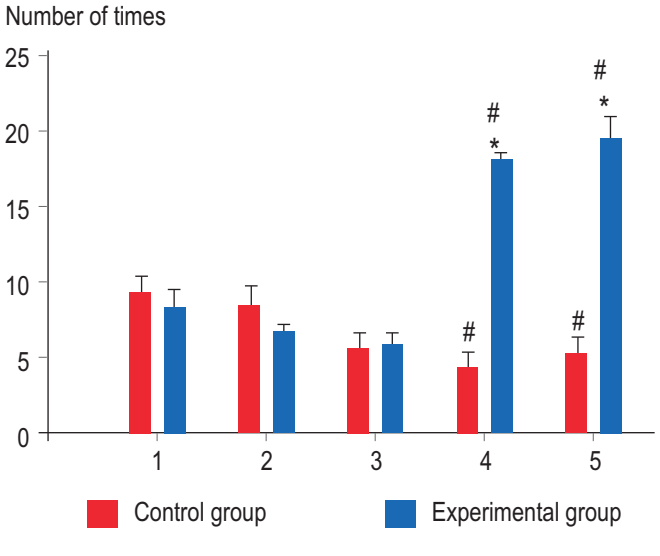

Fig. 1. **Peculiarities of behavioural reactions of rats in the "Open Field" test. Vertical activity with support: 1 — baseline testing; 2 — testing 40 minutes after kisspeptin administration; 3 — testing on day 3; 4 — testing on day 11. # p < 0.05 — statistically significant differences from baseline at p <0.01; * — statistically significant differences between control and experience at p < 0.05**

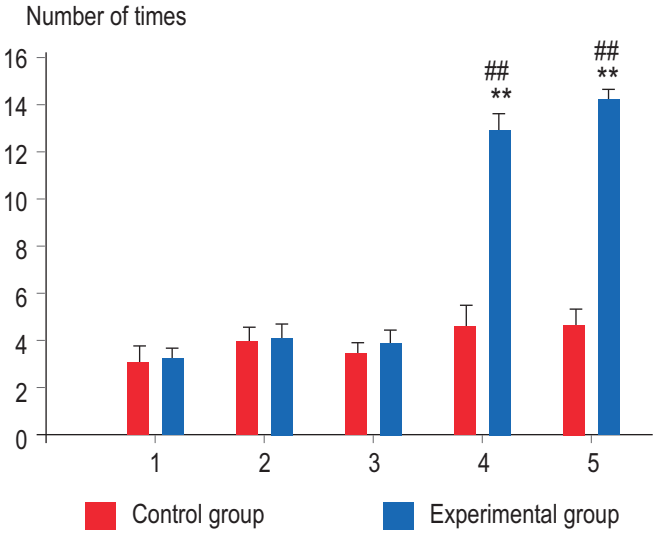

Fig. 2. **Peculiarities of behavioural reactions of rats in the "Open Field" test. Vertical activity with support: 1 — baseline testing; 2 — testing 40 min after kisspeptin administration; 3 — testing on day 3; 4 — testing on day 11. ## — statistically significant differences from baseline at p < 0.01; ** — statistically significant differences between control and experience at p < 0.01**

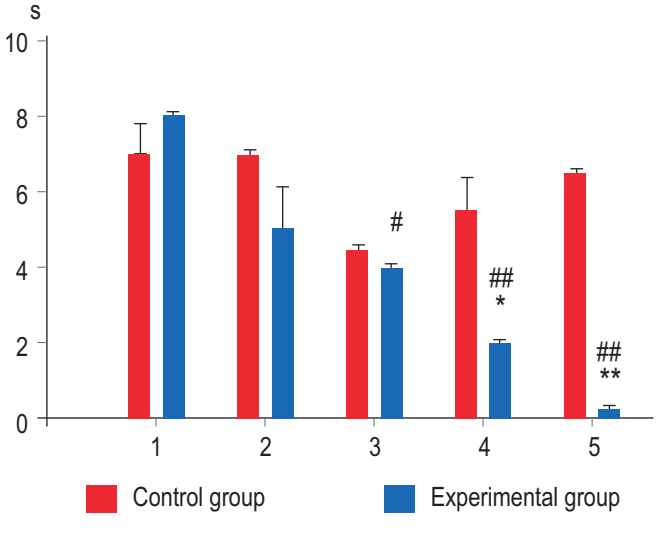

Fig. 3. **Peculiarities of behavioural reactions of rats in the "Open Field" test. Latent time (s) of the first transition: 1 — baseline testing; 2 — testing 40 min after kisspeptin administration; 3 — testing on day 3; 4 — testing on day 6; 5 — testing on day 11. # p < 0.05 — statistically significant differences from baseline; ## — statistically significant differences from baseline at p < 0.01; * — statistically significant differences between control and experience at p < 0.05; ** — statistically significant differences between control and experience at p < 0.01**

DISCUSSION

In conducted experiments, the features of the behavioural reactions of rats upon activation of kisspeptin receptors were analysed. In connection with the activation of kisspeptin receptors (GPR54), there is an active stimulation of the hypothalamic-pituitary-gonadal axis and increase in the production of the corresponding gonadotropins (luteinising and follicle-stimulating hormones). Taking into account the results of the studies, we can talk about an increased production of these sex hormones, because of which there is an increase in testosterone synthesis in male rats.

High levels of testosterone have a specific effect on the state of the central nervous system, including the structures of the cerebral cortex and limbic system. The central effects of testosterone provide a special state of "hunting", during which the animals become more active.

The results indicate a relatively low level of anxiety in rats upon activation of GPR 54 receptors. With the accumulation of kisspeptin in the body, the level of anxiety decreases. It is possible that activation of GPR 54 increases the activity level of hypothalamic neurons secreting GnRH.

CONCLUSION

Activation of the kisspeptin system had a pronounced effect on the behaviour of rats in various test setups. In the Open Field test, under the influence of kisspeptin, representation in behaviour structure of vertical motor and exploratory activity increased, and more active mastering of its sectors was also noted. During the experiment, it was found that intranasal administration of kisspeptin has a positive effect on exploratory behaviour and memory, and reduces the level of anxiety. The obtained results can probably be explained by the activation of specific receptors (GPR54) to this neuropeptide presented in the structures of cerebral cortex and limbic system.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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All procedures complied with ethical standards approved by legal acts of the Russian Federation, the principles of the Basel Declaration, and the recommendations of the bioethics committee of the biological faculty of Samara National Research University (protocol N 11 from 3.10.2013).

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Все процедуры соответствовали этическим стандартам, утвержденным правовыми актами РФ, принципам Базельской декларации и рекомендациям комитета по биоэтике биологического факультета Самарского национального исследовательского университета им. академика С.П. Королева (протокол № 11 от 03.10.2013 г.).

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