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Research Article

Intranasal administration of ghrelin receptor antagonist [D-Lys-3]-GHRP-6 reduces the manifestations of impulsivity and compulsivity induced by maternal deprivation in rats

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Abstract

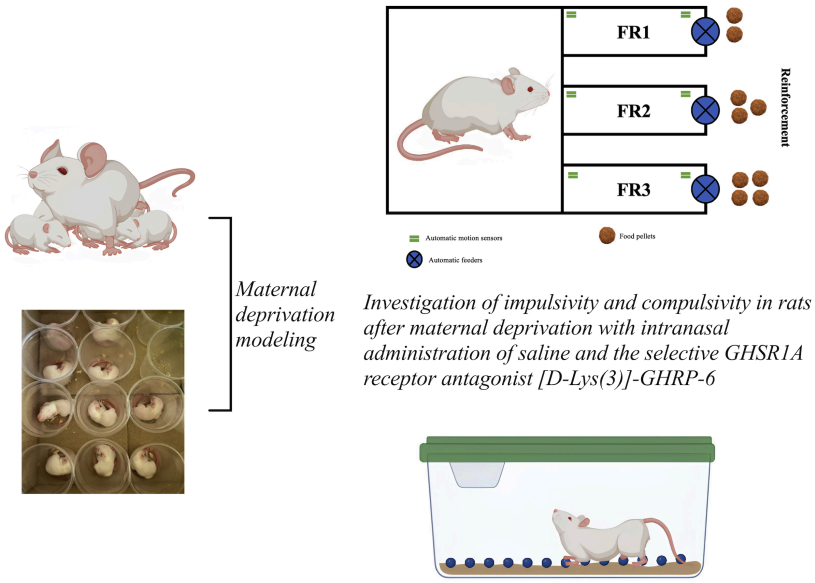
Introduction: The aim of our study was to investigate the effects of ghrelin receptor antagonist [D-Lys-3]-GHRP-6 on impulsivity and compulsivity in a model of gambling addiction in adult rats after maternal deprivation MD in early ontogenesis.

Material and Methods: The cubs were deprived of their mother daily for 3 h from days 2 to 12 postnatal. Adult Wistar rats were trained in a three-arm maze for 21 days. Two seeds were fed in arm 1 with reinforcement mode FR1. In arm 2, three seeds were presented with reinforcement mode FR2; in arms 3, 4 seeds were presented with reinforcement mode FR3 (i.e., only every 3rdrun to the feeder was reinforced with food). The animals were also examined to quantify their status of compulsive behavior in a marble test. The rat was placed in the cage with sawdust and glass beads for 30 min, after which, the number of balls covered with sawdust by more than 2/3 was counted.

Results: In the MD group, the number of entrances to arm 3 increased 1.5-fold, indicating the selection of a larger reinforcement value at its low probability. The number of entrances to arm 1 (reward with high probability (100%) but with the lowest food reinforcement) decreased compared to the control group. The intranasal administration of the selective GHSR1A receptor antagonist [D-Lys (3)]-GHRP-6 (1 mg/mL) to animals MD led to significant ($p<0.01$) increase in the number of visits to arm 1. Additionally, there was a tendency to decrease the number of buried balls in rats treated with [D-Lys (3)]-GHRP-6.

Conclusion: Thus, the work shows that chronic stress of MD causes a behavioral strategy in animals aimed at obtaining more significant food reinforcement, but with a low probability of achieving it, which is associated with an increase in the impulsive component of gambling addiction. MD also causes an increase in the number of buried balls in the marble test, which is associated with an increase in the compulsive component of gambling addiction. Intranasal administration of GHS-R1a antagonist [D-Lys (3)]-GHRP-6 to rats MD significantly reduces increased impulsive activity in Iowa test. The data obtained in rats MD opens up the possibility of creating drugs targeted at ghrelin receptors. The intranasal method of administering peptide substances used in the work substantiates the possibility of using this method of administration.

Graphical abstract



Keywords

ghrelin, [D-Lys-3]-GHRP-6, impulsivity, compulsivity, gambling addiction, maternal deprivation

Introduction

The function of the brain is influenced by various environmental factors, signals, stimuli, and learning experiences throughout the entire lifespan. Events that take place during critical developmental stages, like the neonatal period, can have long-lasting effects that persist into adulthood. Stress is a common experience during the early years following birth. Research indicates that stressful events encountered in infancy can negatively impact behavioral and physiological functions, including growth, metabolism, and inflammatory and immune responses in later stages of life. Processes such as cell proliferation, differentiation, and neuronal migration are particularly active in the first week post-birth. In rodents, hippocampal granule cell neurogenesis peaks around the second week of life, while in humans, this peak occurs around the second month after birth (Delavari et al. 2016).

Maternal deprivation (MD) is a stressful occurrence during early childhood, typically happening at various intervals after birth, particularly in infancy. This early-life stressor, a challenging experience for young individuals, leads to cognitive, emotional, and neurochemical disruptions in adolescent rats. By impacting the hypothalamus-pituitary-adrenal axis, the disruption of the typical mother-child bond leads to enduring alterations in emotional responses, as well as physiological and neurobiological functions, throughout adolescence (Pyurveev et al. 2023a). Early traumatic experiences have been identified as factors that can increase the susceptibility to developing brain disorders in adulthood (Palma-Gudiel et al. 2020). To explore the wide range of enduring changes following exposure to various stress conditions in early postnatal life, numerous animal models have been created and confirmed over time (Murthy and Gould 2018). Various effects of early-life stress have been documented, including disruptions in memory formation and cognitive abilities (Talani et al. 2023), heightened levels of anxiety, symptoms resembling depression, and an increased susceptibility to developing substance use disorders (de Almeida Magalhães et al. 2017; Lebedev et al. 2022a; Lebedev et al. 2023b).

The neonatal maternal deprivation model is commonly used in rodents to study the effects of early-life stress on various levels such as endocrine, neurophysiological, and behavioral (Mejía-Chávez et al. 2021). In this model, pups are separated from their mother's nest for a few hours each day during the first two weeks after birth, although the specific protocols may vary in terms of separation duration and timing (Bailoo et al. 2014). Interestingly, this manipulation tends to have a stronger impact on male rodents compared to females, suggesting a sex-dependent effect (Talani et al. 2023). However, conflicting findings have been reported in other studies (Balakina et al. 2021), indicating the need for further investigation into the role of sex as a vulnerability factor in maternal separation effects. Additionally, age was another crucial factor that can influence the impact of early-life social experiences. Both social isolation and enrichment during the first three weeks after birth can have different effects on behavior in adolescent and adult rats (Pyurveev et al. 2023b).

Recent reports showing an overlap in the neural pathways responsible for obesity and those involved in pathogenesis gambling suggest that orexigenic peptides are promising targets for pharmacotherapy of gambling. The orexigenic peptide ghrelin, produced in the gastric fundic mucosa, causes the secretion of growth hormone, and acts to stimulate appetite and food intake (Lebedev et al. 2023a). Ghrelin is also expressed in the hypothalamus, namely in the lateral hypothalamus, the arcuate nucleus (Arc), the ventromedial nucleus, dorsomedial nucleus and the paraventricular nucleus (Kojima et al. 1999; Chen et al. 2009).

Researchers have extensively studied the role of ghrelin in food intake and obesity. Ghrelin levels increase before a meal and decrease after eating. Bath central and systemic ghrelin stimulate food intake, and the Arc of the brain is involved in the effects of ghrelin on feeding and weight gain. Conversely, when a peripheral of a GHSR antagonist, D-Lys3-GHRP-6, is administered, it reduces body food intake and weight gain (Lebedev et al. 2022b).

Decision-making is a process involving a choice between two or more different alternatives, which requires that individuals perceive the probabilities and risks associated with each option and estimate the consequences of each option in the short, medium, and long term. Decision-making impairments can be defined as a tendency toward risky or unwise choices and play an important role in substance use disorders and behavioral addictions (Koffarnus and Kaplan 2018), as well as other psychiatric conditions or neuropsychiatric disabilities, such as schizophrenia, obsessive compulsive disorder, attention-deficit hyperactivity disorder, and affective disorders (de Siqueira et al. 2018). One way of conceptualizing decision-making is through experimental studies using laboratory behavioral tests on both humans and animals. Studies have shown that decision-making, animals share similar preferences and biases that are seen in human choice behavior, e.g., escalating commitment and loss chasing (Winstanley and Clark 2015). These similarities enable the use of laboratory animals to understand the neurobiological underpinnings of decision-making. Several rodent tasks have been developed to enable studies of different aspects of decision-making (Izquierdo et al. 2019). Such tasks aspire to simulate human decision-making processes. However, studies examining interspecies data on human and animal behavior in these tasks are scarce, but important from a translational perspective. The Iowa gambling task (IGT) is a widely used clinical and experimental instrument for the assessment of decision-making under uncertainty and risk.

Gambling addiction, also known as a behavioral addiction, can manifest in impulsive and compulsive behaviors in rats, mirroring aspects observed in human gambling addiction. Impulsivity refers to a tendency to act without forethought, often leading to risky or detrimental behaviors. Compulsivity involves repetitive behaviors driven by an inner urge or compulsion, often despite adverse consequences (Sekste et al. 2021).

In studies with rats modeling gambling addiction, impulsive behaviors can be observed through actions such as rapid decision-making in gambling tasks, prioritizing immediate rewards over long-term gains, and increased risk-taking behavior. Rats exhibiting impulsive tendencies may show heightened activity in brain regions associated with reward processing, such as the nucleus accumbens (Pyurveev et al. 2022a).

On the other hand, compulsive behaviors in gambling addiction among rats may involve repetitive responses towards gambling-related cues or activities, even in the absence of rewards. This compulsive behavior can be linked to alterations in cortico-striatal circuits, affecting decision-making and response inhibition mechanisms (Grant and Kim 2014). The interplay between impulsive and compulsive elements in gambling addiction in rats can shed light on the complex neurobiological underpinnings of this behavioral disorder. Understanding these mechanisms through animal models helps researchers explore potential interventions and treatments targeting impulsivity and compulsivity in gambling addiction, offering insights into the development of more effective therapeutic strategies for treating gambling addiction in humans (Grant and Potenza 2006).

The aim of our study was to investigate the effects of ghrelin receptor antagonist [D-Lys-3]-GHRP-6 on impulsivity and compulsivity in a model of gambling addiction in adult rats after maternal deprivation.

Materials and Methods

Ethical rules and regulations

This work was conducted in accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (adopted in Strasburg, 18.03.1986 and confirmed in Strasburg, 15.06.2006) and approved by the local ethical committee. The study plan, standardized operating procedures, and the accompanying documentation were subjected to ethical review by the local ethical committee at St. Petersburg State Pediatric Medical University, Minutes #17/05 dated 14.10.2022, Ministry of Health Care of the Russian Federation.

Obtaining off spring

At the first stage of the study, 10 sexually mature female Wistar rats of bodyweight 270±20 g were obtained from the nursery of laboratory animals at Pushchino Branch of the Institute of Bioorganic Chemisnry of the Russian Academy of Sciences (Moscow Region). After a two-week quarantine period, a male was added to the groups of females during the estrus phase for one day. This procedure was repeated until spermatozoa were detected in the vaginal smears; this day was considered as day zero (0) of pregnancy. Next, the females were seated in individual plastic containers with free access to water and food under inverted light conditions 8:00–20:00 at 22±2°C.

Maternal deprivation (MD) modeling

In the second stage of this study, only P1 male offspring were included. After their delivery, the rats were counted, weighed, and sexually differentiated, and the general condition of the newborns, their mobility, and skin coloration were evaluated. Thirty male rats were directly included in the work. The pups were deprived of their mother (MD) daily for 3 h from days 2 to 12 postnatal. At this time, the rats were placed in individual plastic containers to avoid contact with each other, assigned an individual number, applied to the skin of the rats (Fig. 1). Upon completion of the deprivation session, the brood was returned to its home cage. At the end of the deprivation period, two experimental groups of animals were created: a control group without MD (Control) and

an experimental group with MD, each with 15 rats. The experimental and control groups were created through randomization using the closed envelope method in the ratio of 1:1 (Balakina et al. 2021).

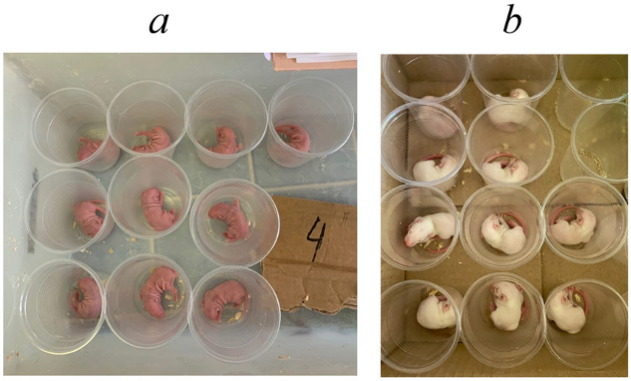


Figure 1. 3-hour deprivation session: **a** - baby rats in individual plastic cups on the 3 postnatal day; **b** - baby rats in individual plastic cups on the 10th postnatal day.

Experiment design

Rats were divided into groups of 10 animals each:

Experiment 1.

Group 1 – animals without maternal deprivation who were tested in marble test and in test of reinforcement probabilities and magnitudes;

Group 2 – animals surviving maternal deprivation who were tested in marble test and in test of reinforcement probabilities and magnitudes.

Experiment 2.

Group 3 - animal survivors of maternal deprivation tested in marble test and in test of reinforcement probabilities and magnitudes receiving intranasal ghrelin antagonist at a dose of 20 µlL (10 µlL in each nostril) [D-Lys-3]-GHRP-6;

Group 4 - animal survivors of maternal deprivation tested in marble test and in test of reinforcement probabilities and magnitudes receiving intranasal administration of a 0.9% sodium chloride solution (isotonic solution).

A variant of the Iowa Gambling Task test of "reinforcement probabilities and magnitudes"

The food reinforcement setup included a starting platform (33×50×35 cm) and 3 arms (50×15×35 cm) (Fig. 2). At the end of each arm was an automatically controlled feeder. Food reinforcement was provided when the feeder was reached in each arm of the three-arm maze. When the animal exited the arm to the starting area, the next reinforcement was given. Training was performed each day. Runs to the feeder and returns to the starting chamber were tested for 10 min and no additional cues were given. Animals were fed daily, limiting feeding time to 4 h, with free access to water (Lebedev et al. 2023a). Food deprivation was maintained before each experiment for 20 h. Animals were trained in a three-arm maze for 21 days. A sunflower seed served as reinforcement. A training regimen of food reinforcement was used during the first days of the experiment. At each choice of arm 1, animal received one seed. At each choice of arm 2, two seeds were presented, and at each choice of arm 3, three seeds were presented. The training regimen of food reinforcement feeding lasted for 5 days. No experiments were performed for the next two days (Lebedev et al. 2022b). On the 8th day of training, the mode of food reinforcement was changed. Two seeds were fed in arm 1 (reinforcement mode FR1_2). At the same time, each reach to the feeder was reinforced with food. In arm 2, three seeds were presented in FR2_3 mode and every second run to the feeder was reinforced with food; in arms 3, 4, seeds were presented in FR3_4 mode (i.e. only every 3rd run to the feeder was reinforced with food). Thus, 1/2 of the runs to arm 2 and 2/3 of the runs to arm 3 of the mazes remained without reward. Rats were trained for 2 weeks in this regimen. Rats that did not enter the maze arms were removed from the experiment (no more than 15%) (Lebedev et al. 2022b).

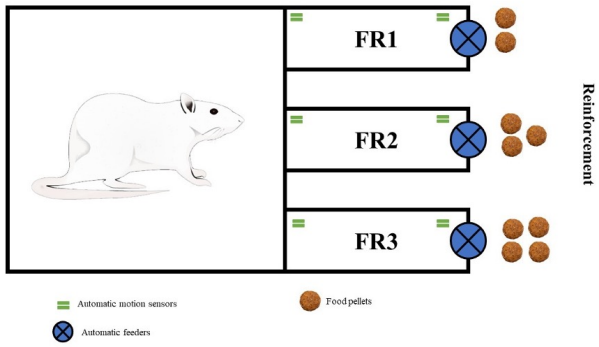


Figure 2. Installation diagram for determining the probability and magnitude of food reinforcement in a three-arm maze.

The marble tests

On day 90 of the postnatal period, the animals were examined to quantify their status of anxiety, obsessive-compulsive behavior, or repetitive behavior. A 5-cm layer of sawdust was placed into a 20 × 25 × 17-cm cage and twenty, 1-cm-diameter glass beads were placed equidistantly. The rat was placed in the cage for 30 min, after which, the number of balls covered with sawdust by more than 2/3 was counted. The number of buried balls per experiment was determined. In this experiment, each animal was tested thrice (Lebedev et al. 2022a).

Drugs

[D-Lys-3]-GHRP-6 (Tocris, Bristol, United Kingdom) was used in this study. Solution of ghrelin antagonist was administered intranasally at a dose of 20 µL (10 µL in each nostril), course introduction of 7 days. As a control, the intranasal administration of a 0.9% sodium chloride solution (isotonic solution) was used.

During 7 days after the end of the training, the animals in the MD group were intranasally injected with 0.9% NaCl solution 20 µL (10 µL in each nostril); the animals in the MD + [D-Lys3]-GHRP-6 group were intranasally injected with the antagonist of ghrelin receptor [D-Lys3]-GHRP-6 (20 µg/20 µL/rat). Fifteen minutes after the last administration, the rats were tested in the installation for 10 minutes. The data were used for statistical analysis.

Statistical analysis

Statistical analysis was performed with Microsoft Office Excel 2021 (Microsoft, USA) and Prism 8 (GraphPad Software Inc., USA). The intergroup differences were assessed using the Kruskal-Wallis rank analysis and the Dunn post-hoc test. All data are presented as the mean and standard error of the mean (SEM). The differences were considered statistically significant at $p<0.05$.

Results

The effect of MD stress in early ontogeny on the impulsive component of gambling addiction was investigated. In the test of magnitude and probability of reinforcement (a variant of the IOWA test), the number of runs into each arm of a three-arm maze was determined. Two groups of animals were studied: intact rats (control) and MD rats (experimental group). In the MD group (from the 2nd to the 12th day), the number of entrances to the arm 3 increased 1.5-fold, indicating the selection of a larger reinforcement value at its low probability, 31.48±1.01 and 51.32±2.04 of the control and experimental groups, respectively.

The number of entrances to arm 1 (reward with high probability (100%), but with the lowest food reinforcement) decreased compared to the control group, 34.37±1.47 and 19.67±1.69 of control and experimental groups, respectively. (Fig. 3a). In accordance with the results of statistical analysis, the numbers of balls buried more than 2/3 in the experimental animals was higher than in the control animals, 10.00±0.97 and 6.10±0.48 of the control and experimental groups, respectively (Fig. 3b).

Ghrelin interacts with its specific GHSRs receptors, which are expressed in some brain structures associated with the reward response, the ventral tegmental area (VTA), the nucleus accumbens (NAcc), the prefrontal cortex and the lateral hypothalamus. This may indicate the role of ghrelin in non-homeostatic, hedonistic aspects of nutrition. In our modified version of the IOWA test, the magnitude and probability of amplification test, we estimated the number of visits to each link of the experimental setup at different probabilities of amplification.

The administration of the intranasally selective GHSR1A receptor antagonist [D-Lys(3)]-GHRP-6 (1 mg/mL) to animals that had been temporarily isolated from their mother in the early stages of development led to a statistically significant ($p<0.01$) increase in the number of visits to the radiologist with the highest probability of amplification (1 ray) was 20.60±2.81 and 38.48±3.25 in the group receiving an intranasally equivalent dose of saline solution and in the group receiving the test drug, respectively (Fig. 4). Visiting this ray guaranteed the experimental animal 100% receipt of a food object. However, there were statistically significant differences in visits to the second (50% probability of reinforcement) and third (33% probability of reinforcement) bundles between animals that experienced temporary isolation from their mother in the early stages of development, who received intranasal saline, and animals that experienced temporary isolation from their mother in the early stages of development. The stages of development with the introduction of a selective ghrelin receptor antagonist [D-Lys (3)]-GHRP-6 (1 mg/mL) were not determined, while there was a tendency to decrease the proportion of visits to these departments after administration of the studied drug (Fig. 4).

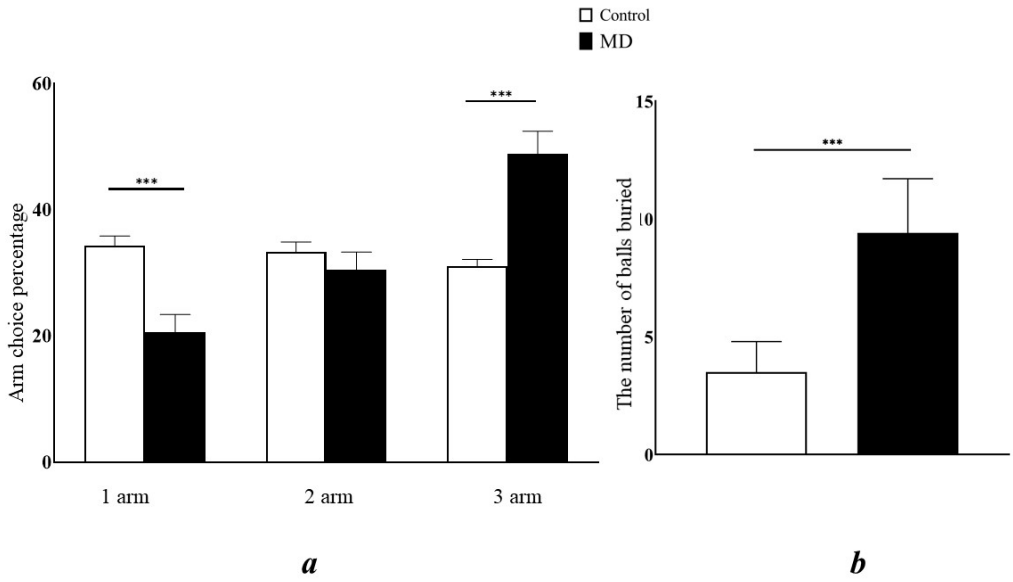


Figure 3. Behavior of experimental animals in behavioral tests. ***Note:*** a) the influence of maternal deprivation on the behavior of rats in a situation of choosing the probability and strength of food reinforcement in a three-arm maze; b) the effect of maternal deprivation on compulsive behavior in the balloon marble test; MD – group of animals subjected to maternal deprivation, receiving intranasal saline; MD+[D-Lys (3)]-GHRP-6 – group of animals subjected to maternal deprivation, receiving intranasal [D-Lys (3)]-GHRP-6; *** – $p \leq 0.001$ relative to the group after temporary isolation from the mother in the early stages of development, who received an equivalent dose of saline.

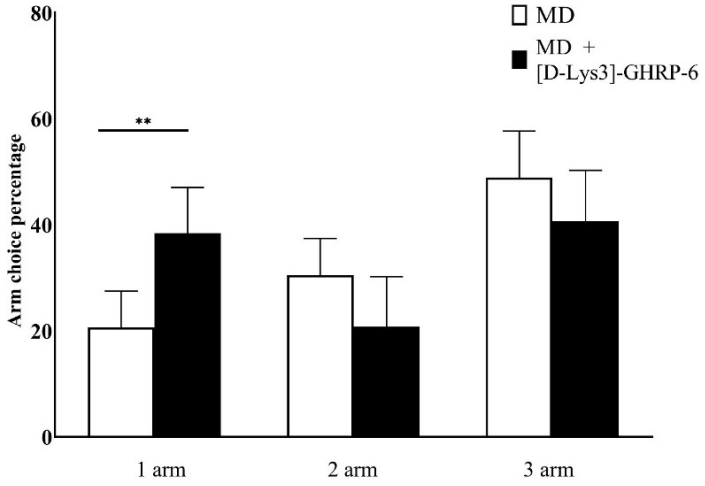


Figure 4. The effect of intranasal administration of GHS-R1a antagonist [D-Lys (3)]-GHRP-6 on the activity of rats after temporary isolation from their mother in the early stages of development in a situation of choosing the strength and probability of food reinforcement. ***Note:*** MD – group of animals subjected to maternal deprivation, receiving intranasal saline; MD+[D-Lys (3)]-GHRP-6 – group of animals subjected to maternal deprivation, receiving intranasal [D-Lys (3)]-GHRP-6; ** – $p \leq 0.01$ relative to the group after temporary isolation from the mother in the early stages of development, who received an equivalent dose of saline.

A test for burying balls

In this test, we analyze the severity of stereotypical behavior of experimental animals that have been exposed to stress. According to the test results, we obtained the following results: the number of hidden balls in the group of animals that survived deprivation from a mother receiving intranasally GHS-R1a antagonist [D-Lys(3)]-GHRP-6 at a dose of 1 mg/mL, and animals that survived deprivation from their mother receiving an intranasally equivalent dose of saline solution. It does not change statistically significantly; however, there is a tendency to decrease the number of buried balls in rats treated with the studied drug. Thus, intranasal administration of GHS-R1a antagonist [D-Lys (3)]-GHRP-6 at a dose of 1 mg/mL dissolved in 20 mL of saline solution to rats which experienced deprivation from their mother in early ontogenesis significantly reduces increased impulsive activity in terms of magnitude and probability of reinforcement of the test; however, it did not statistically affect the compulsive behavior of experimental animals in the balloon burying test.

Discussion

Despite the pervasive human clinical literature information on influences of early stress on impulsivity in adolescence and young adulthood, little direct behavioral or neurobiological evidence exists to support this hypothesis. In the present study, MD directly led to increased game-addiction risk illustrated by enhanced acquisition and maintenance of increased impulsivity and compulsive behavior. Thus, this study emulates human gambling due to protracted effects of childhood stress on adult behavior.

Impulsivity is a behavioral phenotype associated with vulnerability to game-addiction initiation. The study showed that chronic stresses of maternal deprivation in early ontogenesis or isolation rearing caused a behavioral strategy of animals aimed at obtaining more significant food reinforcement, but with a low probability of its achievement, which is associated with an increase in the impulsive component of gambling. In the group of MD rats, the number of entries into arm 3 of the maze increased 1.5-fold, indicating a preference for choosing a higher reinforcement value with a low probability of obtaining a reward. The number of entrances to arm 1 (reward with high probability (100%), but with the lowest food reinforcement) decreased. The number of entrances to arm 2 (reward with higher probability (50%), but with less food reinforcement) decreased, as well. At the same time, the number of entrances to the maze arms did not differ significantly from those in the group of MD rats. The obtained data largely agree with the literature data.

Relatively few studies have examined how prenatal stressors affect impulsive action. One study did find that male and female rats exposed to prenatal stress made more premature responses on a challenging version of the 5-CSRTT (Wilson et al. 2012). Specifically, when the intertrial interval times increased, these rats were unable to withhold responding as compared to controls. In regard to postnatal stressors, work from Lovic et al. (20110 found that male and female rats that undergo a severe form of early life deprivation-artificial rearing with low simulated grooming - are unable to withhold premature responses as adults compared to control rats while performing DRL tasks. Similarly, male rats which experienced maternal deprivation make significantly more premature responses while performing the 5-CSRTT as compared to controls (Kentrop et al. 2016). Females, however, were not examined in that study. Together, these studies indicate that ELS can increase impulsive action.

Neonatal MD also impacts burying behavior, a natural behavior observed in laboratory rodents when they bury harmless items like glass marbles using bedding material. However, excessive burying behavior is considered a potential indicator of perseveration or compulsive disorders (Taylor et al. 2017). Recent research has demonstrated that mice exposed to maternal separation

exhibit a notable increase in the number of buried marbles, indicating heightened perseverative behavior, reinforcing the connection between early-life adversity and the emergence of impulsive or compulsive traits (Ou et al. 2021). Interestingly, studies have reported sex-specific differences in the marble burying test, but with conflicting findings (Emtyazi et al. 2022).

The present study investigated the role of the pharmacological inhibition ghrelin receptor in the mechanisms of vulnerability to risk-taking behavior and impulsivity in behavior in a model of gambling addiction in animals with different individual experiences. The effectiveness of ghrelin antagonists in suppressing impulsivity in behavior was tested following intranasal administration of ghrelin receptor antagonist [D-Lys (3)]-GHRP-6.

The present study shows that intranasal administration of the ghrelin receptor antagonist [D-Lys3]-GHRP-6 increased the number of runs into the first arm of the maze in MD animals relative to the MD group receiving saline solution, which suggests that the animals exhibit the less risky behavior observed in the situation of choosing differently probable reinforcements.

We have previously shown that multidirectional effects of ghrelin and [D-Lys3]-GHRP-6 in the three-arm maze. Ghrelin administration increased the ratio of escapes toward the third arm of the maze with low probability but higher reward magnitude (Shabanov et al. 2017).

We have previously shown intranasal administration of the ghrelin receptor antagonist [D-Lys3] -GHRP-6 (20 μL, 1 mg/mL) increased the number of entries into the 1st arm (with a high (100%) probability, but less reinforcement). No significant changes were found in the content of dopamine and serotonin after [D-Lys3]-GHRP-6 administration in the hypothalamus. The serotonin content significantly increased in the left hippocampus. The turnover of monoamines in the olfactory tubercle and striatum changed only in the right hemisphere, while the ratio of the content of 5-hydroxyindoleacetic acid to the content of serotonin increased in both structures. In the right striatum, these changes were also accompanied by an increase in the content of serotonin and dopamine metabolites. Thus, the ability of [D-Lys3]-GHRP-6 to change the strategy of choice in favor of the greatest probability, but the least amount of reinforcement, is based on an increase in the activity of the dopaminergic and serotoninergic systems in the dorsal and ventral striatum of the right hemisphere of the brain and the serotonin content in the hippocampus of the left hemisphere (Lebedev et al. 2022b).

Possible targets of ghrelin action in response to chronic stress seem to be neurons of the paraventricular nucleus of the hypothalamus (PVN), in which a high concentration of corticotropin releasing hormone (CRH) is noted (Cabral et al. 2012). Ghrelin receptors were found in the PVN. Ghrelin administration increased the concentration of c-fos protein in CRH-containing neurons of the PVN and caused activation of the hypothalamic-pituitary-adrenal axis (HPA) (Cabral et al. 2016). The target of ghrelin action under stress also appears to be the extended amygdala system, which includes the central nucleus of the amygdala (CeA), the bed nucleus of the stria terminal (BNST), the zona inserta and the accumbens nucleus (NAc) and is an extrahypothalamic CRH system (Roik et al. 2019). Structures of the extended amygdala receive inputs from dopaminergic neurons of the ventral tegmental area (VTA) and constitute the main functional system for realizing the emotional-motivational effects of various drugs (Koob 2009). CRH blockade of neurotransmission in CeA, BNST and NAc eliminates or significantly reduces the activating effects of addictive drugs (Klein et al. 2017). Structures of the extended amygdala appear to be important for the implementation of reinforcement mechanisms (Pina and Cunningham 2017). CeA and BNST have been shown to exert a regulatory effect on the hypothalamus. Psychogenic stress causes activation of pathways in CeA, BNST, NAc, pFc and then in CRH neurons of the PVN (Aguilera and Liu 2012). Ghrelin administration induces spiking activity in CRH-containing neurons, increases CRH mRNA levels in the PVN and increases serum corticosterone levels in rodents.

Thus, the work shows that chronic stress of being weaned from the mother in early ontogenesis causes a behavioral strategy in animals aimed at obtaining more significant food reinforcement, but with a low probability of achieving it, which is associated with an increase in the impulsive component of gambling addiction. Weaning from the mother in early ontogenesis also causes an increase in the number of buried balls in the marble test, which is associated with an increase in the compulsive component of gambling addiction. Intranasal administration of GHS-R1a antagonist [D-Lys (3)]-GHRP-6 at a dose of 1 mg/mL dissolved in 20 μL of saline solution to rats which experienced deprivation from their mother in early ontogenesis significantly reduces increased impulsive activity in terms of magnitude and probability of reinforcement of the test, however. There is a tendency to decrease the number of buried balls in rats treated with [D-Lys (3)]-GHRP-6. The data obtained in rats MD opens up the possibility of creating drugs targeted at ghrelin receptors. The intranasal method of administering peptide substances used in the work substantiates the possibility of using this method of administration as gentle in the sense of using small doses of substances, which will prevent the toxic effects of drugs.

Conflict of interests

The authors declare no conflict of interests.

Data availability

All of the data that support the findings of this study are available in the main text.

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