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Research Article

Ghrelin and orexin 1 type receptors effects on impulsive component of decision making in the Iowa Gambling Task Animal Model under stress conditions

Maria A. Netesa[1], Andrei A. Lebedev[1], Edgar A. Sekste[1], Galina P. Kosyakova[1], Victor A. Lebedev[1], Eugenii R. Bychkov[1], Petr D. Shabanov[1]

1 Institute of Experimental Medicine, 12 Academician Pavlov St., St.-Petersburg 197376 Russia

Corresponding author: Maria A. Netesa (SaintUla@gmail.com)

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Abstract

Introduction: Electronic resources and computer games are important tools for social interaction, relaxation, and learning, but according to literature, they can also cause addiction in 1.2–1.4% of users. Exposure to stress, whether a repeated mild or single severe one, contributes to the development and relapse of addictions. Therefore, it is especially important to study the effects of promising pharmacological agents aimed at combating addictive behavior under stressful conditions. The ghrelin and orexin systems modulate both reward system activity and stress, making them particularly interesting for studying addictive behaviors.

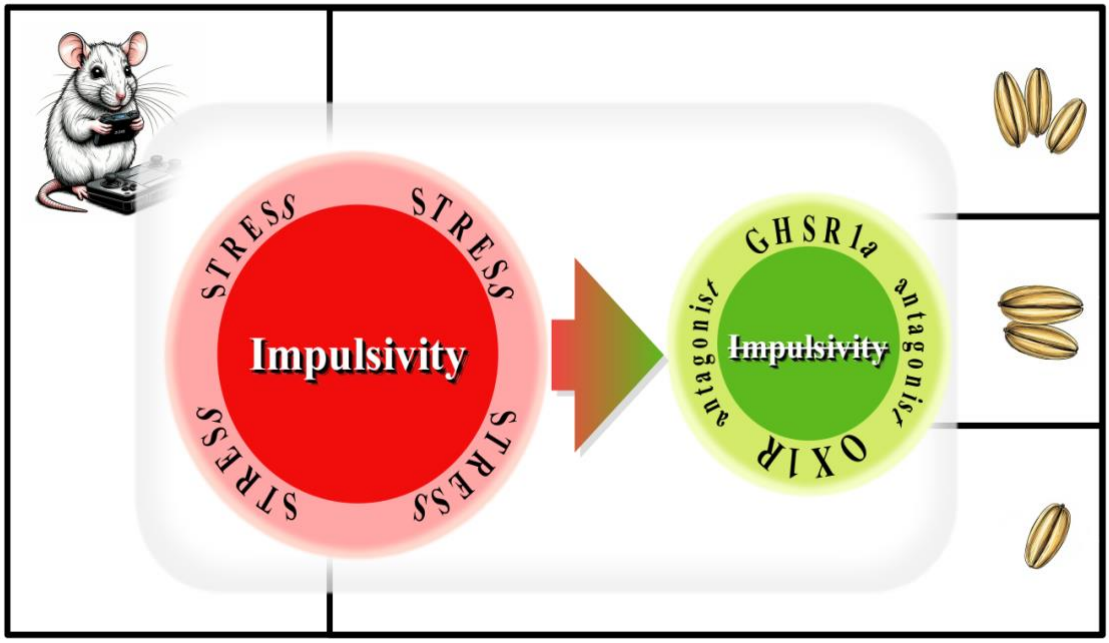
Materials and Methods: Impulsive component of decision making in rats under stress was assessed using a modification of the Iowa Gambling Task (IGT) in a setup consisting of 3 arms (C1-3), each entry into which dispensed sunflower seeds with a specific magnitude and probability (C1 – one seed per entry, C2 – 2 seeds every second entry, C3 – 3 seeds every third entry). Foot shock stress (0.6 mA, 1 min, once a week) and the formation of post-traumatic stress disorder after interaction with a predator were used as an aversive impact. A novel peptide ghrelin antagonist agrelax and the selective orexin receptor type 1 antagonist SB-408124 were administered intranasally. Gene expression in the prefrontal cortex, hypothalamus, and amygdala was assessed employing the polymerase chain reaction method.

Results: The experiments showed that foot shock and interaction with a predator influence different components of impulsivity in the rat IGT test. Foot shock (FS) affected impulse control, which was manifested by an increase in the number of transitions between arms from 29.4 ± 2.4 to 40.0 ± 2.6. At the biochemical level, FS significantly decreased the expression of ghrelin receptor gene in the prefrontal cortex (0.79 ± 0.18) and amygdala (0.15 ± 0.03), and increased the expression of orexin receptor type 1 gene in the prefrontal cortex (1330.38 ± 105.54) and hypothalamus (44.71 ± 16.24). Post-traumatic stress disorder (PTSD), formed as a result of interaction with a predator, affected the decision-making of rats by significantly ascending the proportion of choosing the most risky option C3 from 45.8 ± 1.2 to 55.3 ± 3.5%. The quantity of transitions augmented after FS was affected only by agrelax, decreasing them to 26.8 ± 3.0, and the proportion of C3 was lessened by both agrelax (43.2 ± 3.1%) and SB-408124 (44.3 ± 1.6%) in rats with PTSD.

Conclusion: This study confirms that repeated mild and single severe stress expands impulsivity in rats in the IGT test, but impacts different aspects of behavior – impulse control and decision-making, respectively. Ghrelin and orexin receptor type 1 antagonists reduce impulsivity induced by established post-traumatic stress disorder, but the effects of foot shock are counteracted only by agrelax. According to the results, foot shock reduced the ghrelin receptor gene expression in the prefrontal cortex and amygdala, and magnified orexin receptor type 1 gene expression in the prefrontal cortex and hypothalamus.

Graphical Abstract

Ghrelin and orexin 1 type receptor antagonists decrease rats' impulsivity in a modification of Iowa Gambling Task under stress conditions.



Keywords

addiction; foot shock; impulsivity; Iowa Gambling Task; orexigenic peptides; post-traumatic stress disorder

Introduction

Electronic resources are an integral part of our lives. They allow us to develop a wide range of skills, communicate with a large audience, travel, shop, and much more, all without leaving home. However, not everyone can quickly adapt to new technologies. According to research, approximately 1.2-1.4% of active computer and video game (not related to gambling) users develop symptoms of addiction (Wittek et al. 2016; André et al. 2020). Nevertheless, there are currently no pharmacological approaches that are sufficiently effective in promoting recovery from gaming addiction (Chen et al. 2023). It is known that chemical and behavioral forms of addiction have a resemblant pathogenesis; therefore, studies of the components of behavioral ones can in many aspects be relevant for chemical forms with the absence of direct drug exposure (Punia et al. 2019).

Addiction development begins from a chronic activation of the mesocorticolimbic system, followed by a withdrawal (or negative affect). During the second stage, the cut down in the reward system activity and compensatory changes in other related brain systems occurs (Koob and Volkow 2016; Sussman et al. 2018). For instance, during withdrawal, elevated excitability of the hypothalamic-pituitary-adrenal axis (HPAA) is observed; in the amygdala, levels of adrenocorticotropic hormone, corticosterone, corticotropin-releasing factor (CRF), and

norepinephrine increase while the concentration of neuropeptide Y decrease. As a result, negative reinforcement develops, and the susceptibility to stress is enhanced (Weiss et al. 1992; Delfs et al. 2000; Rasmussen et al. 2000; Olive et al. 2002; Roy and Pandey 2002). The next stage, preoccupation/anticipation, makes the process of addiction recovery extremely difficult. Notably that relapse can be promoted by both cues directly associated with the addictive chemicals/behavior, as well as exposure to stress (likely through activation of amygdala CRF and norepinephrine) (Weiss et al. 2001; Shalev et al. 2002; Shaham et al. 2003; Koob and Volkow 2016).

Impulsivity is a maladaptive, risky behavior characterized by lessened impulse control, impaired decision-making, and motor disinhibition (Bakhshani 2014; Kozak et al. 2019). The neurobiological mechanisms of addiction and impulsivity intersect in the areas of prefrontal cortex, dopaminergic system, and amygdala (Giedd 2008). Impulsivity is an important pattern for substance and behavioral addictions, correlating with relapse rates and treatment adherence (Kozak et al. 2019; Hultman et al. 2022). Therefore, reducing impulsivity is crucial for pharmacological agents aimed at treating addictive disorders. In this study, an impulsive component of rats' behavior was assessed using our modification of the Iowa Reinforcement Probability and Magnitude Task which does not include "penalties", allowing modulating specifically gaming but not gambling behavior (Sekste et al. 2021).

The orexigenic systems, orexin and ghrelin, are currently considered promising targets for the pharmacotherapy of addictions. The orexin system is thought to be involved in regulating of the reward system activity and impulse control, inasmuch as the orexin neurons located in the lateral hypothalamus are projected to various brain regions, including the ventral tegmental area (VTA), nucleus accumbens (NAc), and prefrontal cortex (PFC) (Koob and Bloom 1988; Fadel and Deutch 2002; Baimel et al. 2015). The orexin system is known to have two mediators (orexin A and B) and two receptors (types 1 and 2); however, in the issue of addictions, particularly orexin type 1 receptors (Ox1r) are currently of greatest interest (Trivedi et al. 1998; Baimel et al. 2015). Experimental data supports involvement of the orexin system both in addiction and stress pathways since not only stimuli associated with food or drugs, but also the application of foot shock stress cause excitation of orexin neurons (Harris et al. 2005). Thus, experiments on rats have demonstrated the orexin system participation in the formation of the rewarding and reinforcing properties of drugs (Shabanov et al. 2015; Tissen et al. 2020). With the benefit of morphine as an example, it was shown that orexin neurons are involved in the formation of negative affect and drug-seeking in the absence of the morphine, whereas the administration of a selective Ox1r antagonist SB-334867 significantly improved the somatic state of the animals (Georgescu et al. 2003; Azizi et al. 2010; Laorden et al. 2012). It can also be noted that in an experiment with the formation of post-traumatic stress disorder (PTSD), the Ox1r antagonist SB-408124 significantly reduced anxiety and compulsivity in animals (Tissen et al. 2018).

The hunger hormone ghrelin was shown to regulate the functioning of various structures in the central nervous system including stress and reward (Shabanov et al. 2020). Ghrelin receptor (Ghsr1a or growth hormone secretagogue receptor type 1a) is expressed in structures such as the hypothalamus, hippocampus, mesolimbic system (VTA and NAc), and amygdala (Shevchouk et al. 2021). Furthermore, Ghsr1a is known to form complexes with dopamine receptors, which may play a key role in the fine-tuning of the reward system (Kern et al. 2015; Mustafá et al. 2021). Growing body of evidence confirms the importance of ghrelin in the development of drug and alcohol addictions, as well as in the formation of negative affect, in particular through ghrelin's direct activation of HPAA (Gajewska et al. 2023). Thus, higher plasma ghrelin levels were associated with a subjectively more intense and prolonged response to alcohol in humans (Ralevski et al. 2017), whereas Ghsr1a knockout mice did not exhibit the behavioral and neurobiological changes observed in wild-type animals when those were exposed to alcohol (Bahi et al. 2013). Into the bargain, the literature describes a reduction in alcohol craving following the administration of ghrelin receptor antagonists in rodents (Gomez et al. 2015; Abtahi et al. 2018). In humans, a correlation between plasma ghrelin levels and craving as well as an accrue in ghrelin levels upon presentation of alcohol-related cues have been found (Koopmann et al. 2019; Sha et al. 2021). Regarding the interaction between stress and the ghrelin system, a growing body of studies has recently confirmed the exceptional importance of ghrelin in shaping the stress response (Shabanov et al. 2020; Shabanov et al. 2021; Reikhardt and Shabanov 2022). Specifically, studies in rats have shown that administration of ghrelin antagonists reduces anxiety, compulsivity, and impulsivity induced by both acute and chronic stress (Yakushina et al. 2017; Lebedev et al. 2023; Nadbitova et al. 2024).

The objective of this study was to compare the effects of the orexin and ghrelin systems on rats' impulsive behavior in a modification of the Iowa Gambling Task under conditions of foot shock and post-traumatic stress disorder.

Materials and Methods

Animals

Experiments were conducted with the employment of 36 male Wistar rats obtained from Rappolovo Laboratory Animal Nursery, Leningrad region (Russia). Fourteen rats were involved in the experiment with electric foot shock stress (R-FS), 10 – in the experiment with post-traumatic stress disorder modeling (R-PTSR), and 12 rats were an intact control (R-IC) for gene expression determination. The rats were kept in a vivarium with inverted light at a temperature of 22±2°C in standard plastic cages in groups of 5-7 individuals, after a preliminary quarantine of 2 weeks. Rats of the R-IC group were provided with water and food without restrictions. Rats of the R-FS and R-PTSD groups were fasted 20 hours before the experiment. The experiments were carried out for 5 days in a row; then there was a break of 2 days, during which food and water were available *ad libitum*. The work with the animals was carried out in accordance with The Rules of Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267, of 19 June, 2003).

Rat IGT test

The modified rat IGT test (or IOWA Reinforcement Probability and Magnitude test) was performed in a specialized setup (85 x 50 x 35 cm) consisting of a start compartment and 3 arms at the end of which there was placed a feeder that automatically dispensed cleaned sunflower seeds according to a set schedule. The schedules included 3 variants according to the magnitude and the probability of obtaining the reinforcement: C1 – a rat gets 1 seed in every entry to the first arm (least risky), C2 – a rat gets 2 seeds in every second entry into the second arm (medium risks), C3 – a rat gets 3 seeds every third entry into the third arm of the setup (most risky). Based on the proportion of C3 entries, rats were divided into 2 groups: impulsive (50%; R-FS I and R-PTSD I) and non-impulsive (< 50%; R-FS N and R-PTSD N). During the first 7 approaches rats got used to the setup – all arms operated in C1 mode. The next 14 times rats were trained applying C1-C3 schedules. Taking into account the necessary breaks in the experiments, training of animals took 4 weeks. To assess impulsive behavior, rats were placed in the setup for 10 minutes no more than once a day (Fig. 1) (Sekste et al. 2021).

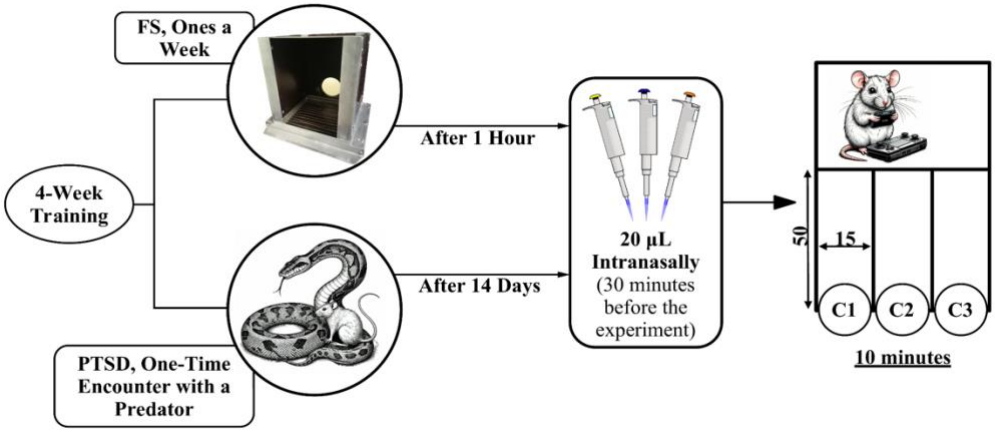


Figure 1. Experimental design. ***Note:*** FS – foot shock stress, PTSD – post-traumatic stress disorder. The dimensions of the installation are given in centimeters. C1-C3 denote variants of the magnitude and the probability of obtaining the reinforcement, where C1 – rat gets 1 cleaned sunflower seed in every entry to the first arm (least risky), C2 – rat gets 2 seeds in every second entry into the second arm (medium risky), C3 – rat gets 3 seeds every third entry into the third arm of the setup (most risky).

Electric foot shock

The electric foot shock (FS) stress was performed in a specialized box with the electrified floor with the current of 0.6 mA for 1 minute once a week 1 hour before the behavioral experiment.

Post-traumatic stress disorder modeling

To form post-traumatic stress disorder (PTSD), rats and a tiger python were placed at the opposite ends of a terrarium (1.2 m x 0.7 m x 1 m) divided by a transparent partition. After 5 minutes, the partition was removed and the snake grabbed one of the rats, after which the partition was returned to its place, and the remaining rats could observe the death of their fellow

rat. The effect of PTSD on rats' behavior was assessed 2 weeks after the traumatic event (Avaliani et al. 2022).

Drugs

For inhibition of the ghrelin receptor, agrelax was used, a novel promising peptide consisting of 13 amino acids (Experimental Medicine Institute, St. Petersburg, Russia) (Lebedev et al. 2023a, 2023b, 2024) and for inhibition of orexin 1 type receptor – SB-408124 (Tocris, England). Agrelax (1 mg / mL), SB-40812 (1 mg / mL), and the physiological saline (control) were administered intranasally – 10 μL in each nostril 30 min before exploring impulsive behavior. The substances were administered with a 7-day interval, which was enough to stabilize behaviour after previous influencing.

Determination of gene expression of ghrelin and orexin type 1 receptors

Rats were decapitated 7 days after the last drug administration. Brains were isolated in the cold, immediately frozen in liquid nitrogen and stored at -80°C until analysis. The content of ghrelin and orexin type 1 receptors genes were determined in the prefrontal cortex, amygdala, and hypothalamus. For R-PTSD, the hippocampal region was also examined. Gene expression was analyzed by reverse transcription followed by real-time polymerase chain reaction. Total RNA was isolated from 20 mg of brain sample using TRIzol reagent (Ambion, USA) in full accordance with the manufacturer's instructions. cDNA synthesis was performed by reverse transcription in 25 μL of the reaction mixture using RNA-dependent DNA polymerase of Moloney murine leukemia virus (M-MuLV reverse transcriptase, Promega, USA). PCR with real-time detection (Mx3005P, Stratagene, USA) was performed in 20 μL of the reaction mixture containing SYBR Green (Synthol, Russia) and a mixture of specific forward and reverse primers selected and synthesized by Beagle (Russia) (Table 1). The data obtained were normalized to the expression level of the glyceraldehyde-3-phosphate dehydrogenase (Gapdh) gene and calculated in relative units (Livak and Schmittgen 2001). Gapdh was chosen based on the fact that previously conducted studies indicate an insignificant change in the expression of this gene under various experimental conditions (Wang et al. 2018).

Table 1. Polymerase chain reaction primer sequences

Gene	Forward Primer	Reverse Primer
Gapdh	5′-AGACAGCCGCATCTTCTTGT-3′	5′-CTTGCCGTGGGTAGAGTCAT-3′
Ghsr1a	5'-CCTGGTGTCCTTTGTCCTTCTTAC-3'	5'-GTAAGAAGGACAAAGGACACCAGG-3'
Ox1r	5′-GTGGCAAATTTCGGGAGCAG-3′	5′-GCTCTGCAAGGACAAGGACT-3′

Note: Gapdh – glyceraldehyde-3-phosphate dehydrogenase, Ghsr1a – ghrelin receptor, Ox1r – orexin 1 type receptor.

Statistical analysis and graphical visualization

Statistical analysis and graphical visualization of the results were performed on Python 3.13.5 language (https://www.anaconda.com/) at the Jupyter Notebook platform (https://jupyter.org/). The effects of drugs in rat gambling test were evaluated employing ANOVA test with Dunnet post hoc criterion, and to compare gene expressions, the Kruskal-Wallis H-test tests with subsequent Dunn test were performed. The values are presented as a mean ± standard error of the mean. The difference between means was considered significant at $p<0.05$. The figure illustrating experimental procedures was made using LibreOffice 7.3 Draw and the Shedevrum.ai v.2.5.

Results

FS significantly increased the total number of transitions between the arms of the setup to 40 ± 2.6 relative to 29.4 ± 2.4 (p=0.019) (Table 2, Fig. 2). Administration of agrelax mitigated the effect of stress, reducing it to 26.8 ± 3.0% (p=0.022), while SB-408124 did not significantly change the number of transitions. In our experiment, FS had no effect on the preference for the maze arms. Agrelax augmented the rats' preference for C2 to 32.2 ± 3.5% (p=0.01) whilst SB-408124 reduced the proportion of C1 (22.6 ± 3.5%, p=0.016) and increased the proportion of C2 33.8 ± 3.1%, p=0.002) (Table 2, Fig. 3).

Table 2. Effects of the ghrelin receptor antagonist agrelax and the orexin 1 type receptor antagonist SB-408124 on behaviour in rats gaming test after foot shock stress

Impact	SUM	C1	C2	C3
No stress	29.4 ± 2.4	29.9 ± 2.1	24.3 ± 2.4	45.8 ± 1.2
Foot Shock	40.0 ± 2.6 *	33.6 ± 1.3	22.2 ± 1.4	44.2 ± 0.8
Saline	37.5 ± 3.1	29.5 + 2.8	25.6 ± 1.9	45.0 ± 2.7
Agrelax	26.8 ± 3.0 *	25.0 ± 2.8	32.2 ± 3.5 *	42.8 ± 1.2
SB-408124	31.5 ± 3.4	22.6 ± 3.5 *	33.8 ± 3.1 *	43.6 ± 1.5

Note: SUM – total quantity of entries in all arms of the maze, C1 – the first arm, C2 – the second arm, and C3 – the third arm of the maze. Values are mean ± standard error of the mean. * – p<0.05.

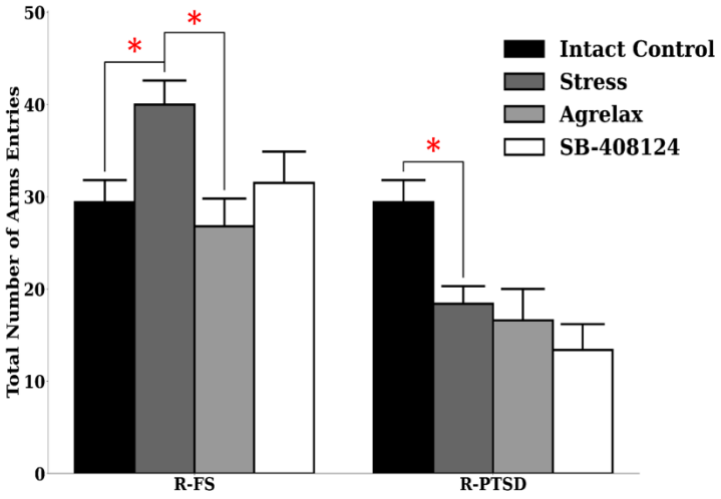


Figure 2. Changes in the number of arms entries. upon exposure to stress and ghrelin antagonists. ***Note:*** R-FS – rats underwent foot shock stress, R-PTSD – srats formed post-traumatic stress disorder. * – p<0.05. Values are mean ± standard error of the mean.

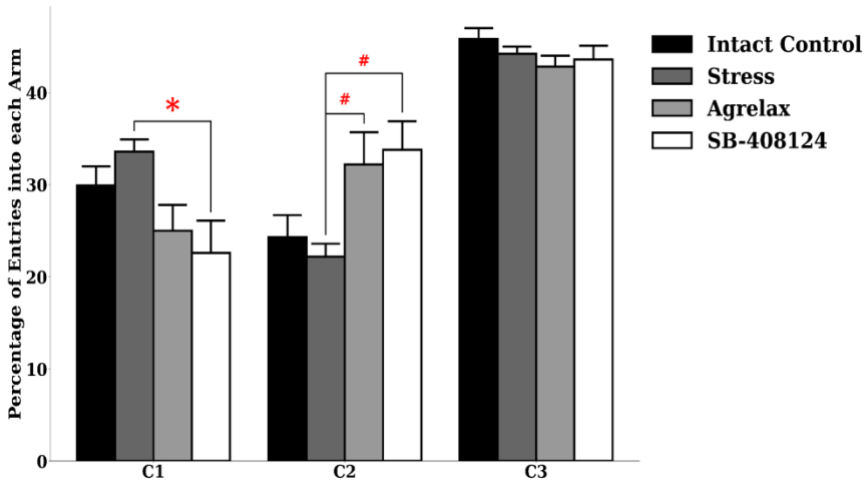


Figure 3. Changes in the arm preference upon exposure to foot shock stress and ghrelin antagonists. ***Note:*** C1-C3 denote variants of the magnitude and the probability of obtaining the reinforcement, where C1 – rat gets 1 cleaned sunflower seed in every entry to the first arm (least risky), C2 – rat gets 2 seeds in every second entry into the second arm (medium risky), C3 – rat gets 3 seeds every third entry into the third arm of the setup (most risky). * – p<0.05, # – p<0.01. Values are mean ± standard error of the mean.

PTSD, on the contrary, reduced the number of transitions between the arms from 35.0 ± 2.3 to 18.4 ± 1.9 (p=0.012), and neither agrelax nor SB-408124 significantly affected this parameter (Table 3, Fig. 2). Further, PTSD altered the rats' preference for the arms in favor of the riskier option C3 – 55.3 ± 3.5 compared to 45.8 ± 1.2% before stress (p=0.02). Administration of agrelax declined the percentage of C3 option choices (43.2 ± 3.1%, p=0.027) and additionally enlarged the probability of choosing the least risky C1 (39.1 ± 3.9%, p=0.023). SB-408124 also

diminished the percentage of the most risky C3 choices to 44.3 ± 1.6 (p=0.036), increased the percentage of C2 option (41.7 ± 2.1%, p<0.001) and decreased the percentage for C1 (14.0 ± 2.6%, p=0.011) (Table 3, Fig. 4).

Table 3. Effects of the ghrelin receptor antagonist agrelax and the orexin 1 type receptor antagonist SB-408124 on behavior in rats gaming test after developing post-traumatic stress disorder (PTSD).

Impact	SUM	C1	C2	C3
No stress	29.4 ± 2.4	29.9 ± 2.1	24.3 ± 2.4	45.8 ± 1.2
PTSD	18.4 ± 1.9 *	22.7 ± 2.7	22.1 ± 2.2	55.3 ± 3.5 *
Saline	14.9 ± 3.0	24.5 ± 4.0	25.6 ± 2.6	50.0 ± 3.8
Agrelax	16.6 ± 3.4	39.1 ± 3.9 *	17.7 ± 2.6	43.2 ± 3.1 *
SB-408124	13.4 ± 2.8	14.0 ± 2.6 *	41.7 ± 2.1 *	44.3 ± 1.6 *

Note: SUM – total quantity of entries in all arms of the maze, C1 – the first arm, C2 – the second arm, and C3 – the third arm of the maze. Values are mean ± standard error of the mean. * – p<0.05.

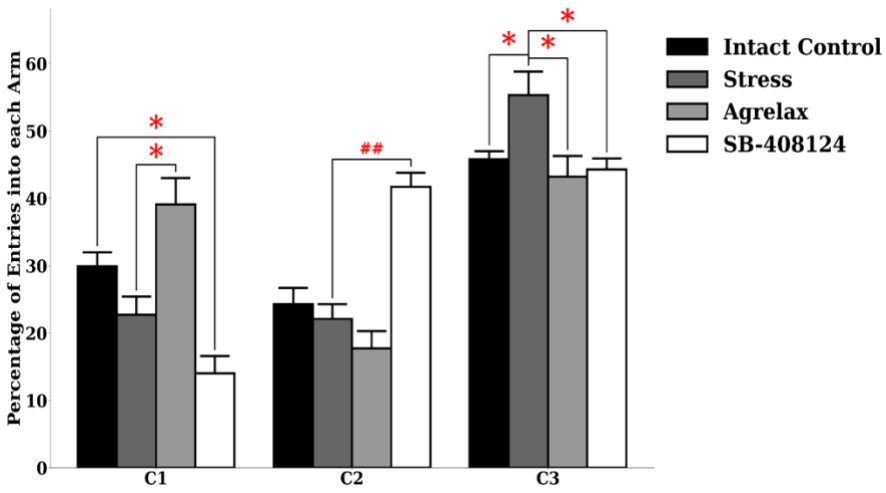


Figure 4. Changes in the arm preference upon exposure to post-traumatic stress disorder and ghrelin antagonists. ***Note:*** C1-C3 denote variants of the magnitude and the probability of obtaining the reinforcement, where C1 – rat gets 1 cleaned sunflower seed in every entry to the first arm (least risky), C2 – rat gets 2 seeds in every second entry into the second arm (medium risky), C3 – rat gets 3 seeds every third entry into the third arm of the setup (most risky). * – p<0.05, # – p<0.001. Values are mean ± standard error of the mean.

No significant differences were found in the effects of stress and the substances on either the R-FS I and R-FS N or the R-PTSD I and R-PTSD N groups, so the paper presents only data for the entire sample.

PCR study of rat brain structures showed a significant decrease in Ghsr1a expression in the prefrontal cortex of the R-FS group (0.79 ± 0.18 relative to 3.87 ± 1.2 in R-CI, p =0.027), as well as in the amygdala region in R-FS (0.15 ± 0.03, p<0.0001) and R-PTSD (0.88 ± 0.16, p=0.002) compared to the R-CI group (1.06 ± 0.11) (Table 4, Fig. 5). Ox1r expression, conversely, was significantly higher in the R-FS group in the prefrontal cortex and hypothalamus regions (1330.38 ± 105.54 and 44.74 ± 16.24) relative to the R-CI (2.9 ± 1.7 and 1.15 ± 0.27, p<0.001) and R-PTSD (0.9 ± 0.14 and 0.9 ±0.28, p<0.0001) groups (Table 4, Fig. 6). Moreover, the expression levels of the studied receptors were compared in impulsive and non-impulsive animals from the R-FS and R-PTSD groups, which showed that in R-PTSD I, the expression of Ghsr1a in the amygdala (0.56 ±0.13, p=0.018) and hippocampal regions (0.59 ± 0.11, p=0.023) and Ox1r in the hypothalamus (0.52 ± 0.06, p=0.011) was significantly lower than in R-PTSD N – 1.08 ± 0.13, 1.1 ± 0.18 and 1.28 ± 0.34, respectively (Table 5, Fig. 7). Moreover, the expression levels of the studied receptors were compared in impulsive and non-impulsive animals from the R-FS and R-PTSD groups, which showed that in R-PTSD I, the expression of Ghsr1a in the amygdala (0.56 ±0.13, p=0.018) and hippocampal regions (0.59 ± 0.11, p=0.023) and Ox1r in the hypothalamus (0.52 ± 0.06, p=0.011) was significantly lower than in R-PTSD N – 1.08 ± 0.13, 1.1 ± 0.18 and 1.28 ± 0.34, respectively (Table 5, Fig. 7).

Table 4. Expression of the ghrelin and orexin 1 type receptors genes in brains of the intact (R-IC), underwent foot shock stress (R-FS) and formed post-traumatic stress disorder (R-PTDS) rats

Brain area	Ghsr1a		
	R-CI	R-FS	R-PTSD
Prefrontal Cortex	3.87 ± 1.2	0.79 ± 0.18 *	1.96 ± 0.62
Amygdala	1.06 ± 0.11	0.15 ± 0.03 ###	0.88 ± 0.16 #
Hypothalamus	1.02 ± 0.07	1.34 ± 0.18	1.12 ± 0.14
	Ox1r		
	R-CI	R-FS	R-PTSD
Prefrontal Cortex	2.9 ± 1.71	1330.38 ± 105.54 ##	0.9 ± 0.14 ###
Amygdala	1.18 ± 0.33	0.9 ± 0.11	2.03 ± 1.36
Hypothalamus	1.15 ± 0.27	44.71 ± 16.24 ##	0.9 ± 0.28 ###

Note: Ghsr1a – ghrelin receptor, Ox1r – orexin 1 type receptor. The data is normalized by the glyceraldehyde 3-phosphate dehydrogenase expression. Values are mean ± standard error of the mean; * – p<0.05, # – p<0.01, ## – p<0.001, ### – p<0.0001.

Table 5. Expression of the ghrelin and orexin 1 type receptors genes in brains of the not impulsive (R-FS N) and impulsive (R-FS I) rats underwent foot shock stress, and not impulsive (R-PTDS N) and impulsive (R-PTDS I) rats formed post-traumatic stress disorder

Brain area	Ghsr1a			
	R-FS N	R-FS I	R-PTSD N	R-PTSD I
Prefrontal Cortex	1.12 ± 0.37	0.18 ± 0.09	3.24 ± 1.71	0.68 ± 0.1
Amygdala	0.13 ± 0.01	0.19 ± 0.06	1.08 ± 0.13 *	0.56 ± 0.13 *
Hypothalamus	1.53 ± 0.12	0.99 ± 0.26	1.09 ± 0.15	1.15 ± 0.09
Hippocampus	-	-	1.1 ± 0.18 *	0.59 ± 0.11 *
	Ox1r			
	R-FS N	R-FS I	R-PTSD N	R-PTSD I
Prefrontal Cortex	1308.29 ± 93.72	1370.13 ± 122.11	1.1 ± 0.16	0.71 ± 0.07
Amygdala	0.99 ± 0.09	0.74 ± 00.13	3.24 ± 1.81	0.82 ± 0.27
Hypothalamus	48.82 ± 16.87	37.32 ± 11.13	1.28 ± 0.34 *	0.52 ± 0.06 *
Hippocampus	-	-	1.19 ± 0.21	6.24 ± 3.69

Note: Ghsr1a – ghrelin receptor, Ox1r – orexin 1 type receptor. The data is normalized by the glyceraldehyde 3-phosphate dehydrogenase expression. Values are mean ± standard error of the mean; * – p<0.05.

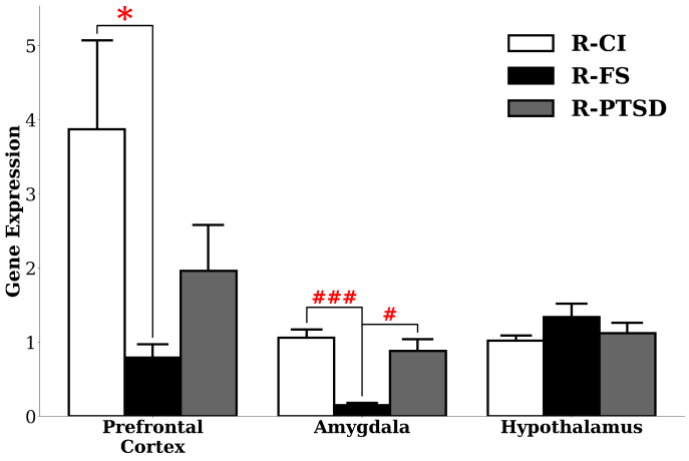


Figure 5. Expression of the ghrelin receptor gene in brain structures. ***Note:*** R-CI – intact control, R-FS – rats underwent foot shock stress, R-PTSD – rats formed post-traumatic stress disorder. * – p<0.5, # – p<0.01, ### – p<0.0001. Values are mean ± standard error of the mean.

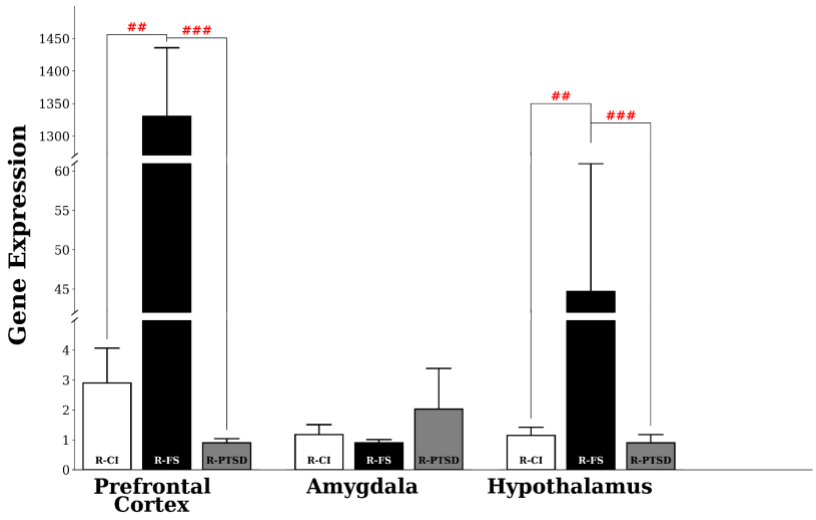


Figure 6. Expression of the type 1 orexin receptor gene in brain structures. ***Note:*** R-CI – intact control, R-FS – rats underwent foot shock stress, R-PTSD – rats formed post-traumatic stress disorder. # – p<0.01, ### – p<0.0001. Values are mean ± standard error of the mean.

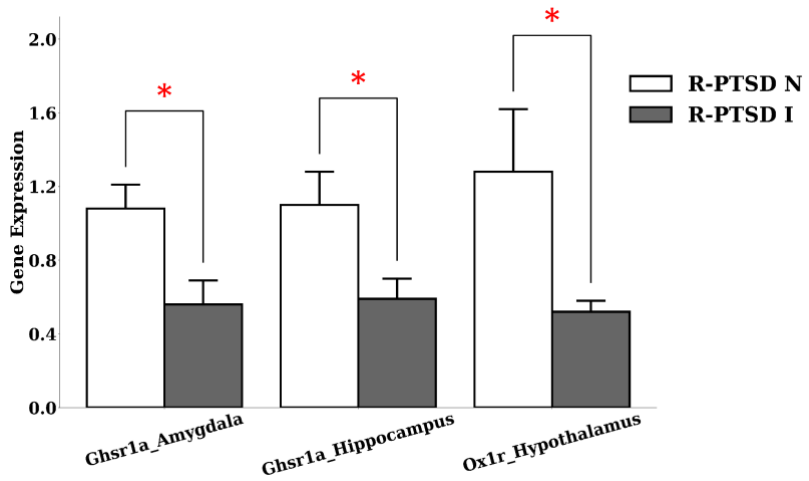


Figure 7. Differences in genes expression of ghrelin and orexin type 1 receptors depending on impulsivity. ***Note:*** R-PTSD N – non-impulsive rats with post-traumatic stress disorder, R-PTSD I – impulsive rats with post-traumatic stress disorder; * – p<0.05. Values are mean ± standard error of the mean.

Discussion

The reward brain system has always been critical for the survival of humans as a species. Although, excessive stimulation of the reward system can cause addiction to pleasurable substances or behaviors in susceptible individuals (Lewis et al. 2021). Addiction disrupts not only the dopaminergic mesocorticolimbic system but also a number of other related neural cycles. Specifically, accelerated activation of the stress hormones is observed (Koob and Volkow 2016; Sussman et al. 2018). A reverse relationship is also present: stress increases the likelihood of addiction/relapse and intensifies craving (Volkow et al. 2016). Therefore, it is crucial to evaluate the effectiveness of potential addiction medicines under various types of stress. Behavioral forms of addiction are of particular interest today – gambling disorder became the first condition to be included in the Diagnostic and Statistical Manual of Mental Disorders (5th Edition) as a ”Substance-Related and Addictive Disorder” only in 2013 (Mann et al. 2016). With the development of computer technology, the prevalence and accessibility of computer video games (which can also cause a special form of addiction in some users) is spreading like wildfire. At the same time, there are currently not many methods that allow one to evaluate gaming, rather than gambling behavior (Wittek et al. 2016; Sussman et al. 2018; André et al. 2020). Like stress, impulsivity is a concept closely related to addiction: impulsivity stimulates the formation and maintenance of addiction, and vice versa – impulsive behavior is often developed against the background of an existing addiction (Bakhshani 2014; Kozak et al. 2019).

In this study, we assessed the impulsive component of gaming behavior in rats utilising a modification of the Iowa Reinforcement Magnitude and Probability task. Since it does not contain ”penalties”, it allows one to model specifically gaming behavior in rodents (Sekste et al. 2021). Two stress approaches were employed in the current research: mild intermittent foot shock (FS) stress and the development of post-traumatic stress disorder (PTSD). The experimental results indicate that FS and PTSD have different effects on rat behavior. FS increased rats' motivation to receive a reward, having a greater effect on impulse control, as rats demonstrated a significant enhancement in the number of transitions between maze arms compared to intact controls. However, rats with PTSD showed greater changes in decision-making, manifested by a higher preference for the riskiest arm (the largest reward, but the lowest probability). In addition, rats with PTSD showed a significant reduction in the number of transitions between arms, which may be explained by the general deprivation in motor activity after interaction with a predator described in the literature (Wang et al. 2012); nevertheless, further research is needed to more accurately interpret the behavior in this concrete test.

Both the orexin receptor type 1 (Ox1r) and the ghrelin receptor (Ghsr1a) are positioned in the scientific literature as promising targets for the pharmacotherapy of addictive disorders (Baimel et al. 2015; Shabanov et al. 2020). Indeed, for both neurotransmitters, there is evidence supporting this peptide's involvement in the formation of reward, negative affect, and relapse (Harris et al. 2005; Bahi et al. 2013; Shevchouk et al. 2021). Also, both systems may be strongly engaged in the response to stress. Case in point, experiments with the formation of PTSD demonstrated anxiolytic and compulsivity reducing properties for Ghsr1a and Ox1r antagonists (Laorden et al. 2012; Yakushina et al. 2017; Tissen et al. 2018). In view of the above, it seems interesting to conduct a comparative analysis of the Ghsr1a and Ox1r antagonists effects on behavior under various conditions, in the same manner as to evaluate how the activity of these systems changes in different areas of the brain.

In the FS experiment, agrelax significantly reduced the number of transitions between arms stimulated after stress exposure and increased the proportion of the C2 arm choices (the medium-risk option). The Ox1r antagonist SB-408124 did not significantly affect the quantity of transitions, increased the proportion of C2 choices, and decreased it for C1 (the least risky). In the PTSD experiment, neither drug accrued the number of transitions between arms, which was lessened after interaction with a predator; however, both drugs reduced the percentage of the riskiest arm (C3) choices by magnifying the proportion of C1 for agrelax and C2 for SB-408124. Polymerase chain reaction analysis revealed that changes in the expression of Ghsr1a and Ox1r genes were caused only by FS stress, with Ghsr1a gene content in the PFC and amygdala being diminished, while Ox1r gene expression in the PFC and hypothalamus was raised multiple times. Beyond that, impulsive rats with established PTSD were found to have lower levels of Ghsr1a gene (in the amygdala and hippocampus) and Ox1r gene (in the hypothalamus) expression compared to non-impulsive rats with PTSD.

Conclusion

The obtained data indicate that both repeated mild or single severe stress influence the impulsive component of gambling behavior in rats in a modified Iowa Gambling Task, but affect different components: impulse control for foot shock and decision making for post-traumatic stress disorder. Agrelax was more effective than SB-408124 in the foot shock experiment, while their effects were comparable with the post-traumatic stress disorder. Foot shock had different effects on the gene expression of the studied receptors: it was decreased for the ghrelin receptor in the prefrontal cortex and amygdala, and increased for orexin receptor type 1 in the prefrontal cortex and hypothalamus.

Additional Information

Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethics statement

The work with the animals was carried out in accordance with The Rules of Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation No. 267, of 19 June, 2003).

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Data availability

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

Abbreviations and symbols

C1 – the arm of the setup where rat gets 1 cleaned sunflower seed in every entry
C2 – the arm of the setup where rat gets 2 seeds in every second entry
C3 – the arm of the setup where rat gets 3 seeds every third entry
CRF – corticotropin-releasing factor
FS – foot shock
Gapdh – glyceraldehyde-3-phosphate dehydrogenase
Ghsr1a – growth hormone secretagogue (ghrelin) receptor
HPAA – hypothalamic-pituitary-adrenal axis
NAc – nucleus accumbens
Ox1r – orexin 1 type receptor
PFC – prefrontal cortex
PTSD – post-traumatic stress disorder
R-CI – control group of rats
R-FS – rats experienced foot shock stress
R-PTSD – rats developing post-traumatic stress disorder after encounter with the predator
VTA – ventral tegmental area

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Author Contribution

- **Mariya A. Netesa**, graduate student, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: saintula@gmail.com; **ORCID ID:** https://orcid.org/0009-0002-7353-1745. Conducting experiments, data analysis, article writing.

- **Andrei A. Lebedev**, Doctor Habil. of Biological Sciences, Professor, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: aalebedev-iem@rambler.ru; **ORCID ID:** https://orcid.org/0000-0003-0297-0425. Conducting experiments, data analysis, article writing.
- **Edgar A. Sekste**, Ph.D in Biology, senior research fellow, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: sekste_edgar@mail.ru; **ORCID ID:** https://orcid.org/0000-0002-9753-8303. Conducting a polymerase chain reaction, data analysis, article writing.
- **Galina P. Kosyakova**, Ph.D in Biology, research fellow, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: pharm@iemspb.ru; **ORCID ID:** https://orcid.org/0000-0001-7211-7839. Conducting a polymerase chain reaction, data analysis, article writing.
- **Victor A. Lebedev**, PhD in Biology, research fellow, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: pharm@iemspb.ru; **ORCID ID:** https://orcid.org/0000-0002-1525-8106. Data analysis, article writing.
- **Eugenii R. Bychkov**, Doctor Habil. of Medical Sciences, head of the laboratory, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: pharm@iemspb.ru; **ORCID ID:** https://orcid.org/0000-0002-8911-6805. Data analysis, article writing.
- **Petr D. Shabanov**, Doctor Habil. of Medical Sciences, Professor, Institute of Experimental Medicine, St.-Petersburg, Russia; e-mail: shabanov.pd@iemspb.ru; **ORCID ID:** https://orcid.org/0000-0003-1464-1127. Development of the general concept.

All authors confirm that their authorship complies with the international ICMJE criteria (all authors made a significant contribution to the development of the concept, the study and preparation of the article, read and approved the final version before publication).