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Study of the dopaminergic component of stress induced behavioral adaptations

DISSERTATION

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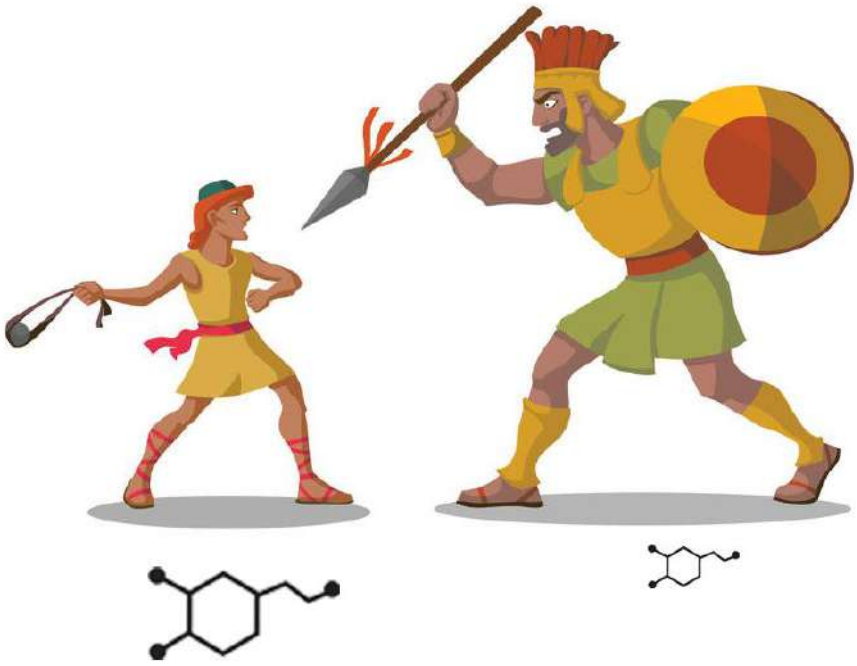
INTRODUCTION

Relevance

The topic of adaptation to various stressors is relevant due to the prevalence of depressive and anxiety disorders in the modern world, in which a person has to meet daily with unfavorable stressors of various types and duration. For effective adaptation to various adverse environmental conditions, so-called coping strategies (behavioral strategies aimed at direct coping with the stressor) are used. Actualization of a certain behavioral strategy (active or passive) in certain (stable or unstable) conditions provides effective adaptation of an individual to stress and, ultimately, to survival. Thus, in order to effectively select psychological or pharmacological methods to combat stress, it is necessary to know the neurochemical and behavioral indicators of stress in individuals with different types of stressor response. In this dissertation, different types of stressors are modeled in animals reflecting real stressors in humans (social stress, uncontrolled physical stress, traumatic psychological exposures, mild daily stress of everyday life, stress-induced alcoholism) and behavioral and neurochemical correlates of stressor response are shown. The main emphasis is placed on changes in the activity of the dopaminergic neurotransmitter system.



Theoretical and practical significance of this work

The theoretical significance of this dissertation lies in the complex substantiation of neurochemical and behavioral correlates of stressor reactions of animals with different (active and passive) behavioral strategies to stressors of different type and duration. The thesis also demonstrates behavioral and neurochemical consequences of social defeat stress and shows not only individual but also sex sensitivity to the action of such stressors. This work addresses not only the known hormonal mechanisms of stressor response regulation, but also earlier and more subtle neurotransmitter mechanisms. The combined contribution of central serotoninergic and mesolimbic dopaminergic systems to aggressive behavior and stress-induced alcohol consumption is shown. It has also been shown that not only physical but also emotional exposure can lead to long-term posttraumatic changes. Upon exposure to stressors there is a change in motivational behavior, thus this thesis focuses on the contribution of the mesolimbic dopaminergic system to the overall stressor response. It is shown for the first time that this system may serve as a possible neurochemical indicator of adaptation processes occurring both in the short and long term after the end of the stressor's action.

The behavioral and neurochemical mechanisms of adaptation to stressors of different nature and duration shown in this dissertation may in the future become the foundation for the development and selection of effective doses of new selective antidepressants designed to take into account sex and individual characteristics of the stressor response. Studies of the neurochemical basis of post-traumatic stressor disorder and pathological alcohol abuse will help in the future to select effective therapy for the treatment of these diseases.

Degree of development of the research topic

The topic of effective adaptation to stressors has not been sufficiently developed to date. There are works showing the influence of stressors of different nature on dopaminergic neurotransmission in animals with different behavioral strategies [1-3], but these works are either generalized (reviews) or focused on individual components of the stressor response. Thus, this dissertation reflects the results of a series of experiments including complex selection of animals using various known methods, behavioral activity studies, biochemical and neurochemical screening of animals before and after stressor exposure using state-of-the-art methods, including the fast-scan cyclic voltammetry method (FSCV in vivo).

Currently, the topic of the effect of prolonged stressors on behavioral and neurochemical parameters in humans and animals is quite developed. Time intervals of the appearance of a depressive-like state after chronic [4-9] uncontrolled stress [10,11], the influence of social context on the manifestation of a depressive-like state [12], sex-specific stressor response [13-15], and the presence of stressors in childhood [16] have been shown. Previously, it was demonstrated that during stress the sympatho-adrenal (SAS) and hypothalamic-pituitary-adrenal (HPA) hormonal systems are activated [17], but recent work has shown that not only hormonal but also neurotransmitter changes occur during and after a stressor event, especially changes in dopaminergic (DA) neurotransmission, as shown in the work of Prof. Miczek [5,18-21]. The effects of long-term stressors on HPA, DA systems and behavior of animals with different behavioral strategies are also reflected in this dissertation.

The thesis emphasizes on post-stressor changes in the mesolimbic DA system. It is known from the literature that this system is associated with emotional and motivational processes in the brain during the actualization of various behavioral programs [22]. Alteration of DA neurotransmission in humans and animals has been found in drug use and alcoholism [23-25], as well as in depression [26,27] and post-traumatic stress disorder (PTSD) [28-31]. During stressor aggressive interaction in rats various fast (activation of SAS, - increase in HR, pressure, body temperature, etc.) and slow (activation of HPA - changes in anxiety, motor activity, etc.) stressor behavioral and neuroendocrine reactions are observed [4,32-34]. In the works of Anstrom and Prof. Budygin E.A. using the in vivo voltammetry method it was shown that during aggressive interaction in rats there is a synchronous change in mesolimbic DA neurotransmission [19]. In this dissertation, we extend these seminal experiments and examine the remote effects of social defeat stress on mesolimbic dopaminergic system functioning and behavior in animals.

This thesis also explores the links of the dopaminergic (DA) system to processes of stress, alcohol exposure and aggression. Prof. Budygin's work shows that stress can provoke alcohol consumption and the development of alcohol dependence [35]. In this dissertation we have continued these studies on Wistar rats and on TPH2 gene knockout animals, in which central SER neurotransmission is genetically switched off (80-100%). It has been shown in the literature that such animals, not only survive, but also exhibit an altered wide range of behavioral activity [36,37]. In addition to stress-dependent alcohol consumption and aggression in TPH2 knockouts, this thesis studied their DA neurotransmission using a novel voltammetry method (FSCV in vivo). Since the dopaminergic and serotoninergic neurotransmitter systems are involved in motivational behavior in animals [26], it seems relevant to study these characteristics.

Various techniques are known to exist for modeling post-traumatic stress disorder (PTSD) in animals [38,39]. The limbic emotional system of the brain plays a major role in the neurophysiology of PTSD: amygdala, prefrontal cortex, hippocampus, etc. [40]. The dopaminergic neurotransmitter system (DA) is involved in the processes of stress and the development of depression-like states [41]. However, its contribution to the development of PTSD remains poorly understood. This dissertation shows that not only physical but also emotional stimuli can provoke the development of PTSD with characteristic long-term (up to 2 months) emotional and cognitive behavioral abnormalities.

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Scientific novelty

This dissertation provides a comprehensive analysis of the effects of stressors of different types and duration on rats with opposite sex, social dominance and behavioral strategies, and proposes neurochemical mechanisms of the observed behavioral changes. In this work we studied the effect of different types of stressors (single social, subchronic immobilization, chronic moderate) on somatic biochemical parameters (plasma corticosterone levels, etc.), on neurochemical parameters in the brain (assessment of mesolimbic DA neurotransmission functioning) and behavioral activity (assessment of depressive-like component) in animals of different sex and type of social and stressor behavior (coping strategies). A comprehensive behavioral and biochemical analysis of passive and active in relation to stress rats, both before and after stressor exposure was carried out. Along with a comprehensive approach to animal selection, a detailed behavioral, biochemical, and neurochemical analysis of the observed stress-induced changes was given, correlation analysis was performed, and hypotheses were proposed.

The thesis demonstrates the high efficiency of the lifetime voltammetry (FSCV in vivo) method for the study of mesolimbic dopaminergic (DA) neurotransmission, analyzing the effects of various stressors on the behavior and functioning of the dopaminergic (DA) system and its receptors. The contribution of not only DA but also SER system on alcohol consumption of rats, on the motivational component of both alcoholic and aggressive behavior was investigated. In a study of TPH2 knockout rats (in which SER neurotransmission in the brain is reduced by more than 80%), it is shown that stress can provoke alcohol consumption in all rats, especially in TPH2 knockout animals.

In conclusion, this paper proposes a new model of PTSD based on non-contact psychoemotional exposure of animals kept in the same cage with animals subjected to single organophosphate compound (OP) poisoning. As a result, the resulting psychotraumatic changes persist in the neighboring rats up to 2 months after a single exposure to the “poisoned” rats. This model can be applied for modeling various types of strong emotional impact.

AIM

To investigate adaptive changes in response to various stressors in rats with active and passive behavioral strategies.

OBJECTIVES OF THE STUDY

1. Perform randomization of animals according to behavioral coping strategies (active and passive) and to identify possible differences between groups in behavioral, neurochemical and biochemical parameters.

2. Investigate the effects of single social defeat (SD) on behavior and DA neurotransmission in male rats.

3. Find the behavioral and neurochemical sex differences 24 h after single social defeat stress.

4. Show different effects of SD on behavioral and DA neurotransmission indices in female rats with active and passive behavioral strategies.

5. To investigate the effects of short-term (forced swim test) stressor exposure on animals with active and passive coping behavioral strategies.

6. Influence of subchronic immobilization on behavioral, physiological, biochemical and neurochemical parameters in rats.

7. To investigate the effect of subchronic immobilization stress on the development of depression-like state in animals with active and passive behavioral strategies.

8. To investigate the effect of chronic mild uncontrollable stress on the development of depression-like behavior in animals with active and passive behavioral strategies.

9. To reveal the effect of antidepressant bupropion on the correction of behavioral consequences of chronic mild stress.

10. Demonstrate the alterations in DA neurotransmission and behavior in rats in processes related to stress, alcohol consumption and aggression using the TPH2 KO model.

11. Validation of a new model of PTSD using a vicarious psychoemotional stress.

Main scientific results

1. Social defeat causes depressive-like behavior and activation of mesolimbic DA neurotransmission in both male [41], [387] and female rats [15], [388-389] as well as behavioral changes in female rats [15] 24 h after the end of stressor exposure. These changes are especially pronounced in animals with passive type of stressor response [15].

2. Short-term and long-term uncontrolled stress has different effects on animals with different social rank and behavioral strategy during stress [42-44].

3. The “active avoidance” technique can be used both to study learning dynamics in laboratory rats and to identify active/passive behavioral strategies detectable under electroshock stress [45].

4. It was found that animals with active behavioral coping strategy are the most sensitive to the action of chronic uncontrollable stress [46].

5. Different group (active/passive behavioral coping strategies) sensitivity of rats to the antidepressant bupropion (DA and NA reuptake blocker) has been shown [47].

6. Mesolimbic DA system play an important role in the processes of alcohol dependence and stress-dependent alcohol consumption [35].

Provisions put forward for defense

1. Single social defeat stress (SD) induces significant changes in mesolimbic DA neurotransmission as well as different behavioral responses in male and female rats 24 h after stressor exposure.
2. Subchronic immobilization stress leads to depression-like changes in all stressed animals, which was manifested in characteristic changes in behavioral activity and physiological parameters, as well as to an increase and then to a maladaptive decrease in corticosterone concentration and dopamine receptor density in the cerebral cortex.
3. The ability to adapt effectively to the action of stressors of different nature and duration depends on the behavioral coping strategies of rats:
    - Animals with active coping strategy show low level of adaptation in response to the action of chronic but not single stressors.
    - Animals with passive behavioral strategy show high level of adaptation in response to chronic but not single stressors.
4. The antidepressant bupropion, which regulates mesolimbic DA levels effectively eliminates the depression-like effects of chronic stress, but only in susceptible rats.
5. Dysregulation of the DA system reflects abnormalities in the processes of aggressive and alcoholic motivational behavior in TPH2 KO animals.
6. Chronic vicarious psychoemotional stress leads to the development of pronounced post-traumatic stress disorder (PTSD) in rats, which persists for at least 60 days.

Author's personal contribution

The author was directly involved in all described methodological experimental aspects of the work. All described behavioral techniques were performed by him personally in all experiments. The author independently carried out genotyping of rats, brain sampling, determination of estrous cycle stage in female rats, measurement of physiological parameters, selection of rats by behavioral strategies using various methods, which he mastered in his bachelor's and then in master's studies at St. Petersburg State University under the guidance of Associate Professor E.P. Vinogradova. Together with V.E. Sobolev, V.I. Shmurak and V.V. Garnuk, the author performed immunohistochemical analysis of dopamine receptor density in the brain, histological verification of the registering microelectrode, as well as general biochemical analysis and analysis of corticosterone concentration in the blood plasma of animals, and wrote a paper. Selection of FOS doses, as well as the introduction of FOS, was carried out by Prof. Goncharov N.V., but all behavioral monitoring, as well as analysis of experimental data was carried out by the author. Under the guidance of Prof. Budygin E.A. and with the direct participation of Prof. Gainetdinov R.R., the author mastered and then independently planned and carried out the whole range of voltammetric studies from electrode calibration to the study of dopamine response parameters in mice and rats, the author conducted his own in-laboratory methodological studies in this area, as described in this dissertation. The author performed independent statistical processing of all obtained data, (developed protocols, analyzed data, constructed all graphs, spoke at conferences, organized experimental studies, developed concepts of new experiments). Together with professors Budygin E.A., Grinevich V.P., Gainetdinov R.R. and Vinogradova E.P. the author wrote and published articles. This dissertation was written independently, but invaluable advice for its improvement was given by: Prof. Budygin E.A., Prof. Gainetdinov R.R. and Associate Professor E.P. Vinogradova. Vinogradova E.P. On the materials of the thesis 12 works were published: 8 scientific articles in journals indexed in WoS and/or Scopus, 4 articles in RSCI, 1 methodological article - in VAK, including 3 mini articles (publications at ECNP international conferences, 2 publications of which were awarded ECNP Excellence Award 2020 and 2021, and Travel Grant). The thesis research data were approved at 8 different international scientific conferences in the form of oral and poster presentations.

LITERATURE REVIEW

Stress

- History of the origin of the concept of "stress"

The concept of "stress" was introduced by Canadian scientist Hans Selye [48] in the 1940s. In his experiments, the scientist exposed animals to various physical influences such as cold, heat, radiation, toxins and others. Selye showed that all animals under different external exposures showed the same physiological changes in the body, such as: increase in adrenal glands (responsible for the production of stressor hormones), degradation of thymus (immune response of the body), gastric ulcer. The scientist concluded that there is a certain non-specific reaction of the body to external stimuli. These changes, later, the scientist called "adaptation syndrome". Selye distinguished three stages of this syndrome:

1) the stage of anxiety, in which the animal encounters the stimulus for the first time.

2) The stage of adaptation or resistance of the organism to the new conditions of existence. In this phase, the animal or human tries to resist the environmental conditions by producing appropriate stress hormones, increasing immunity, changes in the gastric mucosa. If the body fails to cope with the stressor, the next phase occurs.

3) The stage of exhaustion occurs if the stimuli act for a long time. The body has exhausted all its potential and is no longer able to withstand environmental conditions. Changes in the adrenal glands, thymus and mucosa are fatal. Most often this stage ends with the death of the animal [48].

Selye characterized the adaptation syndrome as a nonspecific, systemic response of the whole organism, to prolonged exposure to a stimulus. These stimuli were called "stressors" by Selye. And the reaction itself - "stress". The modern definition of stress is as follows. Stress is a nonspecific systemic, adaptive reaction of the organism to deviation of conditions of existence from the usual ones (Zhukov 2004).

A) Nonspecificity is that there is no connection between the nature of the impact and the nature of the organism's response [48]. Any environmental factor affecting the organism causes a specific reaction, however, at the same time, it causes activation of several functions aimed at restoring the normal state. That is, the nonspecificity of stress is that any stimuli cause the same reaction of the organism. Stressor response does not depend on the modality of the stimulus. Muscular work, heat, loud sound, sudden news triggers a certain complex of reactions in the body, leading to an increase in heart rate, respiration rate, etc. (Zhukov 2004). (Zhukov 2004). Also an important feature of the

stressor response is the release of stressor hormones such as adrenaline [49] and glucocorticoids [48]. This dissertation shows that in addition to hormonal hormones, changes in neurotransmitter activity also occur in the body [19,41].

To confirm the concept of nonspecificity, Mason in the 1970s conducted an experiment in which animals were exposed to heat and cold stress, and different specific reactions were recorded, but the nonspecific component, such as the rise of the main stressor hormones, was the same [50]. We can observe these reactions in humans as well (Zhukov 2004).

B) Systemicity. Stressor causes a reaction in the whole organism, and this ability of stressor causes another feature of stress - systemicity. That is, under the action of a stressor, all systems of the organism are involved in the fight against the irritating agent.

Distinguish such components of the stressor response as:

- behavioral,

- endocrine,

- physiological

- immune.

- *neurotransmitter (the object of our study)*

All changes under stress are aimed at emergency adaptation of the organism to the changed environmental conditions. The stressor response was formed over many millions of years, when the main stressor stimuli were situations posing a direct threat to the animal's existence (predators, competitors for females, food, territory, etc.). Piloerection (raising body hair), for example, creates the illusion of increased body size, which serves to scare away the enemy [51]. Also, physiological changes can include: bronchial dilation, increased frequency and depth of breathing, increased heart rate, etc. All these reactions are due to the action of specific stressor hormones (see: major changes in the endocrine system under stress). Selye in his experiments found [48] the formation of ulcers on the gastric mucosa, i.e. digestive dysfunction as a consequence of impaired functioning of the immune system. The effect of stress in suppressing digestive activity is evident. In an emergency situation, for example, while running from a predator, it is wasteful to spend the organism's energy on functions not aimed at directly saving life, i.e. preserving the organism as a whole. Therefore, under stress, the digestive function is suppressed: the secretory activity of the gastrointestinal tract is inhibited, as well as the inhibition of intestinal motility. However, due to frequent stress, digestive function disorders develop, which may develop into disease [52]. There are experimental data on the development of

gastric ulcer in rats under the influence of strong stressors such as immobilization. [53]. In these experiments it is shown that already after 3 days of immobilization more than 20% of rats develop gastric ulcers [54]. People also develop gastric ulcers after severe stress, this has been shown in clinics on different groups of patients [55]. Patients who experienced post-operative, post-traumatic stress, etc. developed gastric ulcers, some within 24 hours of stress, others within 3-6 days [56]. It is known that after prolonged fasting a person is obliged to take food in small portions. This is due to the fact that due to the stress caused by starvation, the secretory and motor functions of the gastrointestinal tract are inhibited and a large food load in these conditions can cause death [57].

In experiments on animals it was shown that short-term stress causes activation of memory and immune function of the organism, but long-term stress leads to suppression of immunity, synthesis of T-cells, and cognitive decline, in case of post-traumatic stress to death of hippocampal neurons [44], as well as suppression of reproductive function (reproduction function) and growth processes [58].

It has been shown for a long time that during stress the hormonal systems SAS (sympatho-adrenal) and HPA (hypothalamic-pituitary-adrenal) are activated [17], but recent studies have shown that not only hormonal but also neurotransmitter changes occur during and after a stressor event [5,18-21]. An increase in DA neurotransmission has been shown to occur during social [19] or immobilization stress [18], in addition, our studies have shown that 24 h after social defeat stress (SD) there is also an increase in DA neurotransmission in rats [41]. Various studies suggest that these changes may be indicators of an increase in “avoidance motivation” in these rats, or they may be an indicator of adaptive processes in the body [18].

Depending on the behavioral strategy, all animals respond to acute stressor exposure by activation with a certain (active or passive) behavioral strategy. In English-language literature, the term “coping” (coping style) is used. Thus, the animal either runs/runs (active strategy) or hunkers down (passive strategy) [44]. It is noteworthy that in different situations either one or the other strategy is effective, i.e. sometimes it is more effective for an animal to chase away a predator, and sometimes it is more effective to lie low if the danger is too high. During the activation of these behavioral strategies, animals produce stress hormones and release neurotransmitters in certain brain structures, but with a different neurochemical pattern [44,59,60].

C) “Adaptability” - means that the biological significance of stress is to preserve the organism as a whole. Stressor reaction has arisen in evolution, has been fixed and is constantly improving due to the fact that with its help the organism of animals and humans adapted to changes in the environment, i.e. adapted. Damage to health is not stress, but unfavorable changes in the conditions of existence, which the animal or man could not avoid by the time when the protective resources of the organism

were exhausted. Moderate in strength stresses are necessary for the development and existence of the organism [61]. If stress leads to disease, it means that the impact was too long or too strong [62].

Behavioral changes that occur under stress always begin with an increase in anxiety (see “depression and anxiety” for more information) or vigilance. An animal becomes alert when it smells an unfamiliar odor or hears a crunching branch. A person tenses up inwardly when in an unfamiliar environment. Increases not only the sensitivity of vision, hearing, etc., but also increases attention. Information about the stressor stimulus is collected and those details that the person did not pay attention to before are now involved. The collected information is compared with the information stored in memory about similar situations. If the animal or human has a lack of information about the stimulus, or the object represents a potential danger to it, then a highly anxious state is maintained and a decision is made, and then a behavioral response is triggered, avoiding this stimulus [63].

D) Novelty. The fundamental difference between the modern definition of stress and Selye's definition is the indication that the novelty of changes in the environment is necessary for stress to develop. Deviation of the conditions of existence from the usual ones is a prerequisite for considering the organism's reaction as stress. Even strong influences, if they occur regularly, do not lead to the development of a systemic reaction. Only novelty is a factor necessary for stress to occur. Also, it should be noted that stress levels are never zero because there is an element of novelty in any situation. Thus, stimuli that cause activation of the organism must contain an element of novelty and unpredictability [64]. However, prolonged exposure to uncontrolled stress leads to the formation of depression-like state and other diseases [42].

Stress and behavioral types

Attempts to classify the human population have been made many times in history. There were different reasons for this, and to understand the peculiarities of the human psyche and to create a system of more effective management of the masses of people, as different people have different needs and stereotypes of behavior [65].

In the ancient period, the philosopher Theophrastus in his book “Characters” [66] described 30 human characters, more detailed to the issue of classification of human character traits were approached by Jung, Eysenck, Leonhard, [67] Freud [68] and others.

Carl Jung in 1921 published the book “Psychological Types” in which he distinguished two fundamentally different types of human personality: extroverts (the orientation of human motivations in the external world) and introverts (the orientation of human motivations in the inner world of imagination and reflection). Hans Eysenck borrowed the concept of extraversion and introversion from Jung and correlated the corresponding personality parameters to these psychological types [67].

The classification of human psychosomatic types was compiled by Ernst Kretschmer and later supplemented by William Sheldon. In this classification three types of physique are described: athletic, pycnitic, asthenic. Each of the types of physique, according to this classification, corresponds to a certain mental fold [69]. However, the validity of this theory is currently under discussion.

- M. Friedman and the introduction of the concept of "behavioral types"

A significant scientific classification of behavioral types was developed by cardiologists M. Friedman and R. Rosenman in 1959. In the clinic, they found that people with an active type of stressor response had a higher risk of coronary heart disease than people with a passive type. People with active and passive types of stressor response were labeled as "A" and "B" type, respectively. It should be noted that the described behavioral types are polar or "nuclear" types and most people occupy an intermediate position between the respective types [70].

To identify the predominant belonging to one or another behavioral type of people, a questionnaire of 19-25 questions is used after multifactor analysis of which it is possible to draw a conclusion about possible belonging of a person to A or B type of stressor reaction. It has been shown that neither gender nor country of residence influences the results of responses [71,72].

For people with the "A" behavior strategy (in the English abbreviation TABP - type "A" behavior pattern) [73,74], the characteristic reaction to stressor influence will be a struggle with unfavorable conditions or an attempt to escape from them, to get rid of them. However, in an uncontrolled situation, people with this reaction show confusion and depression. This reaction is the cause of an imbalance of mediator systems in the body (see humoral regulation). Many people of this type are characterized by stereotyped behavior, such people do not act according to changing environmental conditions, but according to a plan. In a stressful situation, this stereotypicality or behavioral rigidity will manifest itself to the fullest extent. If in an uncontrollable situation there is an opportunity to perceive this situation as controllable, such people will, without looking back, show all their will and persistence to cope with this situation [75-79].

People with behavioral strategy "B" (TBBP - type "B" behavior pattern) will not take active actions to get rid of the stressor agent, but will try to wait for the end of the impact. In a situation of uncontrolled exposure, such people will not experience a strong negative change in mental processes, i.e. a qualitative change in mediator balance. In ordinary life such people have no definite unified plan of actions, their plan of actions is made according to the existing conditions at the present moment. However, in a controlled stressor situation such people are lost because of the specific reaction of the humoral system (see the next chapter) and because of their inability to choose the right behavioral program suitable for the situation [74,77,80-87].

In 1996, experiments were conducted that showed the relative nature of the influence of behavioral type on the mother's behavior with her newborn infant. It was shown that regardless of behavioral type, more than 81% of mothers exhibited type “A” behavior when interacting with their newborn infant [88]. Also, some researchers emphasize the great contribution of parental upbringing factor in the formation of behavioral strategy [89].

- Animal lines selected for the activity of the behavioral strategy.

There is much data in the literature on the different rate of active avoidance production in rodents in the “active avoidance” test, and this rate varies both between animals of different species (rats and mice) and between animals of the same species and even the same line [90-92]. It is currently accepted that behavior in most animal species is the result of the manifestation of genetically laid down traits of an organism, but the environment in which the organism evolved also plays a major role [93].

In behavior geneticists, studies of the stress response of genetically selected animals selected for behavioral traits are widespread. Currently, several pairs of lines selected for opposite (high and low) rates of active avoidance production in the “active avoidance” test have been obtained. These are: Koltushi lines (Koltushi Low avoidance - KLA and Koltushi High avoidance - KHA), Roman lines (RHA, RLA), Syracusan lines (SHA/Bru, SLA/Bru), Australian lines (AHA, ALA), Japanese lines (THA) [93]. Let us dwell on some of them.

For the first time, successful selection for multidirectional rate of active avoidance production was performed in Italy [94]. The resulting lines are now known as Roman High avoidance - KHA and Roman Low avoidance - RLA. The initial material was Wistar rats. According to the results of the “active avoidance” test after 2 days of production already in the fifth generation the percentage of avoidance reactions amounted to 73% and 14% for RHA and RLA rats, respectively. The latency period of avoidance and escape was significantly lower in RHA rats than in RLA rats [94]. In RLA rats, unlike RHA rats, a lulling response is present in the setting. Motor activity is higher in RHA than RLA both in the home cage and during inter-signal intervals [95]. High levels of stereotypic behavior are exhibited by RHA rats, while RLA rats exhibit less stereotypic forward movement and show more turns in the open-field test. These findings are interpreted as a greater level of exploratory activity in these rats. Also, RHA rats frequently return to already explored chambers, which is interpreted by the authors not as stereotyped behavior but as worse short-term memory compared to RLA rats. Alcohol consumption is higher in RHA rats than in RLA rats [95]. The basal level of corticosterone, one of the main stressor hormones, is higher in RHA than in RLA, this explains the different response to stressor exposure in animals of these lines [93]. Taking into account behavioral (exploratory activity/freezing) and humoral (different activity of the HPA system in animals of these lines) differences, with a certain

degree of approximation some authors have used the behavior of RHA lines as a model of “proactive” type of behavior, and RLA as a model of “passive” type of behavior [96].

Rats of the Koltushi Low avoidance - KLA and Koltushi High avoidance - KHA lines were selected at the Institute of Physiology named after I.P. Pavlov, Russian Academy of Sciences. I.P. Pavlov Institute of Physiology of the Russian Academy of Sciences by high (KHA) and low (KLA) speed of development of the conditioned active avoidance reflex (“active avoidance”). KHA rats showed high motor activity in response to the unavoidable action of electric current, while KLA rats showed a hushing response. Sucrose consumption during the action of avoided stress (electric shocks) after 4 weeks of stressing fell in KLA rats in contrast to KHA, i.e. we can say that KLA rats developed anhedonia, the main sign of depression-like state [97]. Anxiety in KHA rats in contrast to KLA rats in response to the action of uncontrolled stress is maladaptively decreased, as the authors believe. (Zhukov 1993). And it is in these rats (KHA) that, unlike KLA, the development of learned helplessness occurs. The basal level of corticosterone in KLA and KHA rats is the same [99]. Thus, if rats are not stressed, corticosterone levels will be the same in rats with different abilities to produce active avoidance. However, under stress, the mechanism of action of HPA and SAS system is different in KLA and KHA rats, which, in turn, leads to the implementation of different behavioral programs (Zhukov 1977).

- Current approaches for modeling behavioral coping styles

An attempt to classify people according to the “activity of their neurotransmitters” such as dopamine, serotonin, oxytocin and testosterone was made by anthropologist Helen Fisher, dividing people into 4 psychosomatotypes with distinct character traits (dopamine - explorer, testosterone - director, serotonin - builder, oxytocin and estrogens - negotiator) corresponding to the functions of neurotransmitters, but this classification is not scientific and is purely speculative, but the ideas expressed by Helen Fisher are applicable to scientific research [100].

Despite the long history of attempts to classify behavioral types, there is no definitive classification in the literature that would once and for all systematize all behavioral and neurochemical knowledge about behavioral types, but a major work was done in this article [101], where the authors distinguished animals by aggressiveness in the social defeat paradigm, then gave a comprehensive behavioral assessment of active and passive animals and on the basis of data from the literature - conducted and neurochemical assessment and hypothesized about the different pro-behavioral types of behavioral types. According to their hypothesis, since the prefrontal cortex (PFC) is responsible for the control of aggression and impulsivity, through SER and DA innervation. This is proved by experiments on deletion of the PFC and increased aggression in rats [102]. Deletion or inactivation of OFCs also cause escalation of aggression; however, selective activation of m OFC neurons suppresses

aggressive behavior in mice [103]. Thus, the authors believe that cognitive control of aggression and SER levels are reduced in aggressive animals as opposed to non-aggressive animals. The authors say that PFC plays a role in behavioral flexibility and cognitive control. Regarding mesolimbic DA, the authors argue that aggressive animals are likely to have increased DA in the NAC, as evidenced by work on RHA and RLA, which showed increased DA in RHA [104] and increased aggression with ontogenetic VTA stimulation [105].

Another important work also shows the role of DA in the formation of behavioral active and passive profiling [106]. In this paper, the author hypothesizes different levels of DA in PFC and NAC in passive and active animals during stress. The main idea of the paper is the cognitive conceptualization of a stressful situation as controlled (active coping) or not controlled (passive coping), according to the hypothesis put forward by Simona Cabib, not only behavior, but also the level of DA in the corresponding limbic structures depends on it. A review of the neurochemistry and behavior of animals with different behavioral profiles was also made by the author [44].

Our work provides experimental confirmation of the hypotheses put forward, a comprehensive behavioral and neurochemical analysis of passive and active rats, along with a comprehensive approach to animal selection, which has not been used in any study before.

Neurochemistry of stress

- Major changes in the endocrine system under stress

It is now established that when an animal is exposed to a stressor, many pathways in the CNS are activated and stressor hormones are released: corticoliberin (CRH), ACTH, glucocorticoids - cortisol (in humans), corticosterone (in rats), and adrenaline. Glucocorticoids and adrenaline are the major stressor hormones.

Corticoliberin (CRH) secreted in the hypothalamus is the primary stressor hormone because the neurons in which it is synthesized receive nerve signals from extrahypothalamic brain structures rather than humoral signals. Being the primary link in the HPA, CRH stimulates synthesis and secretion of ACTH and provides the initial stage of the stressor response - the Alarm-response. Accordingly, CRH, inducing the state of anxiety, enhances the motor manifestations of this state. In addition, it increases the sensitivity of sensory systems and suppresses the alpha activity of the EEG, typical for the resting state. It is believed that CRH plays a major role in the formation of motivation, since it is the increase in anxiety (CRH affects the activation of noradrenaline neurons, especially in female rats [107]), increased anxiety that makes an animal or a person form a program of action to satisfy the actual need [108]. This fact is supported by studies on rodent brain slices, which showed that CRH increases DA

response in the NAc [109-111], since it is known that it is the mesolimbic DA, via CRH, that provides the motivational component of behavior during stress [112].

Under the influence of corticoliberin, synthesis and secretion of ACTH (adeno-corticotropic hormone) in the anterior pituitary increases [113]. ACTH activates the synthesis and secretion of glucocorticoids in the adrenal cortex [114,115]. In addition, ACTH has pronounced psychotropic effects such as: enhancing attention, memory, and learning [116].

The main function of glucocorticoids synthesized in the cortical layer of the adrenal glands is to increase the body's resistance to prolonged action of a stressor (this hormone increases the level of glucose in the body and has anti-inflammatory and anti-allergic effects), as well as to redistribute the adaptive potential of the body. Thus, glucocorticoids are responsible for the body's “adaptation” to stress. However, an excessive concentration of glucocorticoids in the blood can be harmful to the body. Elevated glucocorticoid levels can cause cardiovascular, kidney rheumatic, and psychiatric diseases. This group of diseases has been called diseases of adaptation [117].

In recent years, there has been experimental evidence that the main function of glucocorticoids is not only to increase resistance to damaging effects, but also to inhibit other components of the stressor response, in particular, to inhibit the activity of CNS stress mediator systems. Consequently, glucocorticoids participate in the inhibition of the stressor response according to the negative feedback principle [118].

The hormone adrenaline, synthesized in the brain layer of the adrenal glands, is part of the sympatho-adrenal (activating) system and has various effects in the body such as: increases heart rate, systolic output, increases excitability and conductivity of the heart muscle, dilates bronchioles, inhibits digestive functions - both secretory and motor, dilates the pupil, relaxes bladder muscles. That is, it secondary increases the feeling of anxiety by the mechanism of conditioned reflex, i.e., when the heart rate increases, a stressor reaction occurs, even if the initial stimulus causing it was not stressful [17].

When adrenaline enters the bloodstream, it expands the respiratory tract, increases the heartbeat due to increased oxygen supply to the cells, and stimulates the formation of glucose from stored substances.

An important function of cortisol and adrenaline in the body is to increase the content of glucose in the blood, also these hormones are responsible for its breakdown. Cortisol is responsible for transporting glucose to the CNS (as well as to skeletal muscles) inhibiting its entry into peripheral tissues [119]. An increase in glucose levels may signal the presence of a stress response, the components of which are the above hormones. This was shown as early as Canon, recording increases

in urinary glucose during stressor exposure [120], and later by Hall, Gold in 1990. [121], showing that after stress there is an increase in blood glucose. Accordingly, glucose levels, rather than the content of the corresponding hormones, can be measured during stress.

All major stressor hormones, to which hormones of the hypothalamus-pituitary-adrenal axis (pituitary-adrenal system, HPA) are commonly referred, directly influence behavior, i.e. have psychotropic effects. The direct influence of hormones on mental processes has been proven in experiments in which the possibility of indirect influence, i.e. by activation of other endocrine systems, is minimized - for example, when hormones are injected directly into the brain. However, at the level of the whole organism, it is extremely difficult to isolate the isolated effect of a single hormone, since the HPA (as well as the systems of other glands, as well as the whole endocrine system) is integrated by numerous direct and feedback connections [122].

The main psychotropic function of glucocorticoids is to provide the “hush” reaction. That is, under a stressful situation, the animal does not run or fight, but sits still - hush [44]. In the laboratory it was shown that the removal of the adrenal cortex in an animal, which is constantly in motion, eliminates the hushing reaction - one of the two main forms of stressor reaction. When glucocorticoids are administered at a compensatory dose, the hibernation response is restored. Since increasing the dose of hormone administered does not increase the time the animal spends in immobility, it can be concluded that glucocorticoids do not regulate but provide the hibernation response [17].

It has also been observed that when treated with high doses of glucocorticoids, most patients experience euphoria (unreasonably elevated mood), sometimes reaching psychosis. These are so-called “cortisol psychoses” [17].

Unlike glucocorticoids, which provide a “hush” reaction, adrenaline stimulates active forms of stressor behavior. As the dose of the administered hormone increases, the time and intensity of the response increases.

Through activation of all physiological mechanisms, adrenaline stimulates a stress response such as the “fight or flight” response, i.e., the animal does not hunker down, as when glucocorticoids are administered, but runs or fights if the stressor situation is consciously controlled. If the situation is not consciously controlled by the animal, then learned helplessness develops, i.e. a state of unwillingness to get rid of an uncontrollable unpleasant stressor [123].

The secretion of adrenaline increases the heart rate, which leads to a subjective feeling of anxiety, although adrenaline itself does not affect the feeling of anxiety. The feeling of subjective

anxiety and anxiety appears because of the conditioned reflex formed in humans that the increase in heart rate should be caused by stressful stimuli [17].

When adrenaline enters the bloodstream, the respiratory tract expands, heartbeat increases due to increased oxygen supply to cells, and adrenaline stimulates glucose formation from stored substances.

In addition to the hormonal stressor response, the processes associated with changes in the activity of various neurotransmitter systems, such as dopaminergic (DA), serotoninergic (SER), etc., are also triggered in the body. The functions of these neurotransmitters include providing both motor and cognitive, and motivational components of the stress response [30,124], as well as activation of coping-dependent programs of behavior in a stressful situation depending on the subjective perception of the situation as controlled or uncontrolled [1]. In experiments on animals [1] and humans [30] it was shown that the level of DA in mesolimbic brain sections correlates with this subjective assessment of stress. It is the subjective assessment of the stressor stimulus that plays a major role in the occurrence of depression or other traumatic changes.

Stress and depression

- Depression

Depression is the most common of the so-called major psychoses (the other two are schizophrenia and epilepsy) - MBC-10 and is included in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [125]. The notion of depression as an independent disease was introduced by psychiatrist Emil Kraepelin. In 1899, he put forward the concept that recurrent depression and mania are manifestations of the same disease. [126]. Kraepelin described a triad of symptoms of a depressive state:

1. Decreased mood,

2. Cognitive disturbances,

3. Inhibition of motor activity

In other words, depression is characterized by depression of emotional, cognitive, and motor functions of the personality for 2 weeks or more, as well as the manifestation of other behaviors such as suicidal tendencies, changes in sleep or activity (e.g., psychomotor disturbances), agitation or lethargy [125]. For mania, a state opposite to depression, the above triad is inverted: cheerful mood, mental, verbal and motor agitation.

The tendency to manifest these states (mania/depression) may be inherited [127]. However, like any trait, depression is influenced by a combination of genetic and environmental factors [127]. The main environmental factor that influences the formation of depression is stressors [128].

However, not every stressor can cause a depressive state. The greatest harm to the animal or human organism is caused by the so-called uncontrolled stress, i.e. stress in which the animal or human cannot actively or passively influence the environment. That is, when it is impossible to predict the unfavorable impact, avoid or get rid of it [46].

The study of uncontrolled stress was initiated in the works of Seligman and Overmire [129], who introduced the concept of “learned helplessness”. They irritated the skin of dogs with an electric current in such a way that the animal could neither avoid this effect nor predict at what point the irritation would be applied. Also, at the origin of the theory of uncontrollable stress stood an employee of I.P. Pavlov's laboratory, N.R. Shenger-Krestovnikova, who taught the dog to distinguish a circle from an ellipse, gradually changing the shape of the ellipse so that it most resembled a circle. A correct solution was reinforced with food - an incorrect solution was not. When the ratio of ellipse axes reached 8:9, the dog began to make mistakes and could not learn to distinguish the shapes even in three weeks, and then he lost the ability to distinguish even an elongated ellipse from a circle. Moreover, all other conditioned reflexes that had been developed before disappeared. In addition, the dog, previously standing calmly in the machine, was now constantly in motion and squealing [130].

It should be noted that as a result of the uncontrolled situation, changes occurred in all three personality areas of the experimental subject. The dog developed:

- Cognitive deficit - the dog lost previously developed skills

- Motor deficits - the dog was constantly on the move

- Emotional disturbances - the dog was constantly squealing.

This experiment is valuable in that it did not use aversive stimuli, i.e. those that the animal tries to avoid. The dog was not in pain, it was not frightened, it was not presented with multiple stimuli at the same time, etc. The only factor traumatizing the animal's psyche was the inability to establish control over the situation [131].

Later, when studying the effects of uncontrolled exposure, they began to use electric shocks [132]. Two animals participated in the experiment. Stimulation was performed simultaneously. Both subjects received electric shocks of the same frequency, duration, and regularity. The only difference between the situations was that one subject could control the situation and the other could not. It turned

out that only those animals exposed to the uncontrolled shock developed multiple disorders, unlike those who experienced the same stress but in a controlled situation. [133].

Animals exposed to uncontrolled stress lose the ability to acquire new skills, they have poorer performance of previously learned actions, their sleep patterns change [134], their immunity is weakened, and ulcers form on gastric and intestinal mucosa. The most dramatic effect of uncontrolled stress, however, is the formation of learned helplessness [135-137].

Learned helplessness can appear for many reasons. It can be caused by prolonged deviations of environmental conditions from the usual ones. We see this in the experiments on the dog of N.R. Shenger-Krestovnikova. For three weeks the dog tried to catch the pattern of food appearance before it developed learned helplessness. Immobilization-that is, the loss of the ability to move independently-is highly stressful in animals. Rats are placed in plastic cups, monkeys are strapped to a chair and, despite a comfortable posture and even a small possibility to change it, in a few hours the animals develop all maladaptive syndromes in a pronounced form [138-142]. Constant social pressure can also lead to the development of learned helplessness. Two unfamiliar rats or mice meet and fight. The losing individual is stressed, but all physiological and biochemical parameters return to normal in a few hours. If an animal is constantly defeated in social contacts, then, despite the minimal level of novelty with each new contact, it develops a persistent disorder of functions that persists for weeks and is characteristic of learned helplessness [135,143].

- ***Types of depression***

Depression, as a disease, in a person can take the following forms. According to the cause of occurrence, a distinction is made:

- *Reactive depression* (if the cause can be established). This disease is a reaction to a strong external impact (shocks, disasters, etc.) [144]. There is evidence that some patients with reactive depression may develop slowly progressive schizophrenia with remission and decline phases after traumatic influences [145]. A study of the dreams of rehabilitated patients after a shock showed that in this type of depression irreparable damage was done to the human psyche. In dreams, patients increasingly returned to the day of the trauma, they had increased levels of violence and masochism and hostility to the world around them. In addition, the disturbances affected the structure of dreams. [146].
- *Endogenous depression* (no external cause can be identified) [147]. Has some kind of internal cause. The cause is most often the effect of chronic stress [148].

Depression may be accompanied by somatic disorders. Patients come with complaints of pain in various parts of the body, but the doctor cannot identify serious physiological disorders that could be the cause of pain. Thus, in medical practice, it was possible to distinguish: psychogenic shortness of breath, psychogenic headache, etc. According to various researchers, from one third to one half of patients who first consult a doctor need correction of their emotional state [149].

Severe depressive disorders have two sides, as shown by Kraepelin in his works [126]. The manic phase, when the patient has increased psychoemotional excitement, elevated mood, speech excitement, etc. is replaced by the depressive phase, in which the patient has all the characteristic features of depression.

According to the ratio of these phases, a distinction is made:

- *Monopolar depression*, in which light intervals are replaced by depressive episodes [150].
- *Bipolar depression* - in which light intervals are replaced by depressive and manic episodes (as a rule, this form of the disease is severe) [151]. Different degrees of severity of the disease are distinguished. Neurosis is a condition in which the patient is a full member of society, but is in an emotionally depressed state. In psychosis, the patient switches off from social life and needs hospitalization [152].

There are several methods of treatment of depression. In clinical and everyday practice, the method of sleep deprivation is used. In which a person is deprived of night sleep several times a week for 14 days. In the clinic, reliable results have been shown in contrast to patients who have not undergone this procedure [153-155]. The sleep deprivation method is based on the alteration of somatotropin synthesis and secretion [154].

Phototherapy is also one of the methods of treatment of endogenous depression. It is known from everyday practice that in sunny weather, a person's mood improves and depressive disorders are not observed, unlike in the fall-winter period. It is during this period that doctors recommend phototherapy - exposure to light spectrally similar to the sun. In medical practice, reliable results of treatment with this method have been shown [155,156]. Also, an effective method of treatment of mild depression is physical activity, which is always a stressor for the body and leads to the production of endorphins - pleasure hormones. Phototherapy reduces the concentration of melatonin and, as a consequence, increases the content of gonadoliberin, which has an antidepressant effect [157].

- Hormonal regulation of depression.

Since the early 20th century, work has been underway to find a hormone for depression. Tissue fluid was analyzed from snails or rats with learned helplessness and injected into healthy animals. The healthy animals developed learned helplessness. Consequently, scientists concluded that there is some humoral component of depression [17]. There were versions that this hormone is cortisol, but the analysis of similar diseases in which there is an increased secretion of this hormone, namely Icenko-Cushing's, showed the implausibility of this version.

Modern data have shown that in patients with depression there is a feedback disorder in the pituitary-adrenal system (HPA). In addition, there is a decrease in receptors for glucocorticoids in the pituitary gland and in the brain [17].

Also, depressed patients have decreased production of ACTH, growth hormone, gonadoliberin, disturbances in thyroid regulation, the circadian rhythm of thyrotropin is disturbed (that is why depressed patients have increased temperature at night) and increased melatonin is observed [17].

- Contribution of neurotransmitter systems in the regulation of depression.

Not only hormones, but also neurotransmitters are involved in the processes of depressive state formation. Thus it has been shown that in depression patients there is a reduced activity of dopaminergic (DA) and serotoninergic (SER) and noradrenergic (NA) neurotransmitter systems [125,158]. Patients with depression and anhedonia have been shown to have significantly lower levels of DAT binding compared to healthy individuals, high levels of D2 autoreceptors in the striatum [125]. Experiments using animal models of depression (chronic moderate stress, learned helplessness) have also shown a decrease in mesolimbic DA in depression [125]. The mesolimbic DA system has been shown in animal studies to be a target for depression, with deletion of VTA leading to a depression-like state in rats [159]. A special line of rats (Flinders), a genetic animal model of depression (Overstreet, 1993), has also been shown to have reduced activity of the VTA DA system [160]. A modern (2023) comparison of more than 12 meta-analyses did not show that depression is formed NOT because of reduced SER content in the brain [161], thus modern studies have debunked the serotonin hypothesis of depression, which for more than 50 years was the main hypothesis [162], but the presence of SER abnormalities in depressed patients should be considered and this mechanism should be taken as an additional one. But the authors [161,162] do not recommend uncontrolled use of SER drugs, as they can, in chronic use, compensatory decrease the level of endogenous SER and thus aggravate the course of the disease.

Researchers have put forward the concept that increasing the concentration of the main neurotransmitters of depression (DA/SER/NA) reduces symptoms of anhedonia [163], in addition,

correction of the psychological state of the patient is possible with the help of combined pharmacological correction of DA/SER/NA systems depending on the type of depression [163]. During the phase of emotional and motivational depression (loss of positive mood) - drugs increasing the content of mesolimbic DA and NA should be taken. During the negative effects in the patient, such as anxiety, fear, irritability, guilt - anti-anxiety drugs, as well as drugs that increase the content of SER, in medical practice, this method is called symptom-specific antidepressant therapy [163].

TMS therapy can also be used to treat depression. Magnetic stimulation modulates the activity of different brain regions by activating or inhibiting their activity. Many randomized clinical trials have shown that daily transcranial magnetic stimulation (TMS) of the left prefrontal cortex (activation of the left prefrontal cortex is associated with positive emotional stimuli [164]) is effective in treating depressive mood symptoms with remission rates ranging from 30% to 40% of cases. Similar to antidepressants, TMS resulted in sustained improvement in anhedonia symptoms but with fewer side effects [165]. The neurobiological phenomena underlying the effectiveness of TMS as an antidepressant are poorly understood, but this method is promising for further study.

- Animal modeling of depressive disorder

When modeling depressive-like states in animals, the basic criteria for depression, such as motor, emotional, and cognitive deficits, should be considered. Otherwise, these tests are partly similar to those that model PTSD (see the chapter below), but there are some traditional models. Let's examine one of them: chronic mild uncontrollable stress.

Persistent, i.e. chronic, even mild, exposure can cause depressive disorder if the body has not been able to adapt to or escape the exposure. For example, if an animal is constantly electrocuted, it has an increased work of the HPA system, constant production of stress hormones, and as a result, the corresponding depressive disorders [166-168].

Not only severe or chronic exposures can lead to depressive disorders. It should be noted that even seemingly insignificant influences, each of which may not seriously damage a person's psychological state, in sum can cause depression in a person or animal if the person or animal cannot actively or passively influence the situation. Such stress is commonly referred to as chronic moderate uncontrollable stress.

Chronic moderate uncontrollable stress is considered to be the most adequate model of everyday stress, which a person experiences in conditions of modern metropolis, when he or she is exposed daily to unfavorable influences, but the type of these influences and the probability of their occurrence are unpredictable. As a result, a person forms depression [10,169].

To model the stress of everyday life according to Willner [170], rats or mice are exposed to different, daily changing exposures for 4 weeks. As a result, these rats develop depression which, however, is corrected with the use of various antidepressants [171]. This model will be used in this thesis as well. The drawbacks of the model include poor reproducibility possibly due to high variability in experimental protocols and individual resistance to stress in rats.

Other types of stressor stimuli such as chronic/acute social defeat stress and immobilization stress are described in detail in the next chapter (PTSD) and can be used to model both depression-like behavior and PTSD, as the boundaries between PTSD and depression, according to the literature [172] are blurred and it is acceptable to use the same tests to model both of these disease models, but for PTSD the time of symptom development (1 month and beyond) will be a crucial factor.

Post-traumatic stress disorder (PTSD)

- History and etiology of the disease

The term PTSD was defined in the 90s of the 20th century, but clinical manifestations were noticed much earlier. The first observations of characteristic symptoms were made during the Civil War in the United States, and in Russia - after the First and Second World Wars. All authors emphasized organic disorders in the central nervous system, which were caused by both physical and psychological factors [173]. After World War I, the cause of this “traumatic nervousness” was thought to be “concussion” or poor discipline. Later, it was possible to separate the physical and psychological manifestations of trauma. Researchers of that time, emphasized the similarity of war neurosis and hysteria, giving the predominant role in the manifestation of clinical symptoms of the emotional factor, having conducted a number of studies authors gave such a definition of the syndrome as “anxious heart”. [174]. It was observed that veterans with this syndrome were incapable of performing their job duties because they demonstrated an elevated HR when presented with a loud stimulus. The term “physioneurosis,” “flashbacks,” and “epileptic symptom complex” were later given [173,174]. Grinker and Spiegel in 1945 noted problems in military personnel who had experienced so-called “war neurosis”. Later in 1952 (DSM-1) and 1968 (DSM-2) recommendations were issued “Diagnostic and Static Manual of Mental Disorders” in which these symptoms are indicated as an adaptive reaction to stressor influence, but this disease was considered as a stressor reaction and not as a separate syndrome. In 1978, following the Vietnam War, in which the same previously described symptoms were observed in combat participants [174]. Kormos in (1978) in his book “The nature of combat srerss” grouped the symptoms described and gave them the name acute war reactions. Studies at that time stated that due to individual sensitivity to stress, between 10-40% of veterans had PTSD symptoms that persisted up to 10 years after the end of the war, and in some veterans (10%) for 40

years, the same findings were obtained in survivors of the Afghan war [40]. In the 1980s, PTSD symptoms were also described in survivors of not only war but also other traumatic events (violence, train wrecks, terrorism, etc.). In 1994, the updated Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) gave the name posttraumatic stress disorder (PTSD) to all the symptoms described. Only in this later revision a clear distinction is made between an acute reaction to traumatic stimuli (now called “acute anxiety disorder” OAD), expressed in an increase in anxiety, panic, etc., this reaction is controlled by antidepressants and disappears in a few days/weeks after the end of the stimulus [175] and long-term post-traumatic stress disorder (PTSD) expressed in a whole complex of mental manifestations, which can make themselves felt for several months/years after the end of the traumatic stimulus [40,173,174].

- The main clinical manifestations of PTSD:

As early as the 3rd edition of the DSM-III, manifestations such as: persistent re-experiencing of traumatic events, social isolation, and the presence of several clinical symptoms (sleep disorders, guilt, impairment in cognitive processes and memory, and a decrease in any external and internal (thoughts) activities that may recall the traumatic event) were noted. In the 4th edition, an addition was made that PTSD can develop not only in people who directly experienced the traumatic event, but also in those who witnessed it. Additions were also made about the duration of all the listed symptoms - from 3 months and onwards. A traumatic event is exposure to exceptional short- or long-term mental or physical stressors. PTSD events, include military combat, sexual or other violent abuse, human or natural disasters, and serious accidents. [173]. However, it should be reemphasized that PTSD develops in only a fraction of people; it is more likely to develop acute anxiety disorder AAD or other isolated traumatic manifestations that disappear a few days to weeks after the traumatic event [175]. The subjective “strength” (perception) of the stressor, regardless of the actual duration or physical traumatization, gender cultural and individual characteristics of the organism contribute to the likelihood of PTSD development [40,175-177]. It is the individual sensitivity to traumatic events that will be shown in this dissertation, as the degree of recovery is greatly influenced by the comprehension of the stressor event, as negative comprehension leads to “secondary traumatization” of the patient [175].

- Possible neurochemical mechanisms of PTSD development

Thus, in the studies of that time on rats and according to modern data, long-term activation of adrenergic neurons in the midbrain, cortex and hypothalamus in response to psychotraumatic effects was shown [40]. These results are explained by researchers as emotional-vegetative reactions caused by excitation of limbic and stem structures from the cerebral cortex, probably in the process of

realization of psychotraumatic impact. With the disappearance of the traumatic factor, after some time, under the influence of compensatory reactions of the central nervous system, these disorders are gradually leveled. However, according to researchers, depending on the strength of the mental trauma, the duration of its action, individual features of neurochemical and neurophysiological mechanisms and the previous functional state of the brain, other outcomes of the reaction to mental trauma are possible [174].

The limbic emotional system of the brain plays a major role in the neurophysiology of PTSD: the amygdala, prefrontal cortex, and hippocampus, among others. [40]. Many animal experiments [178] and separate studies on epileptic patients [179] show the occurrence of subjective conditioned-reflex feeling of fear (by increasing HR and HR) when the amygdala is activated. The prefrontal cortex (PFC) during stress is responsible for comprehending stressful information, and comprehending stress as “controlled” and “uncontrolled” leads to different PFC/NAc activity [1]. It has been shown in studies that activation of these particular brain regions with a certain pattern leads to the onset of PTSD [40]. However, it is noteworthy that in people with different types of stressor reaction it can occur in different directions [40]. PTSD also affects the hippocampus [40]. Experiments on animals show the death of hippocampal neurons in chronic traumatic exposure, such death of neurons is caused by the toxic effect of glucocorticoids on hippocampal neurons [40,180,181]. Studies of PTSD survivors have shown a decrease in hippocampal volume in humans compared to controls [182]. As a result, PTSD patients often experience a loss of memory of traumatic events, which may also be an adaptive response of the body [40].

The dopaminergic neurotransmitter system (DA) is involved in the processes of stress and the development of depression-like states [41]. However, its contribution to the development of PTSD remains poorly understood. Studies on humans who have suffered from PTSD [28] and on animals using various models of PTSD [183] have shown the role of the DA system in the process of diagnosing PTSD. Thus, in depressive state there is a decrease in DA neurotransmission due to VTA inhibition [184], electrical stimulation of VTA or perirhinal nucleus reduces depressive state [185]. Clinical studies have shown an increase in the DA transporter DAT in patients with elevated DA [186,187], but patients with low DA levels have a reduced amount of DAT, while anhedonia is observed in both groups (depressed and PTSD patients) [188]. PTSD patients also have decreased SER transporter in the amygdala, as well as receptors for glucocorticoids and anandamide [40], which has a negative effect on the emotional state of patients, who may experience hyper/hypo arousal depending on the activation of PFC/amygdala [40].

In the treatment of PTSD, social support, non-trauma-related psychotherapy and the prescription of possible SER antidepressants, whose efficacy, however, is debated [40,177].

PTSD patients, unlike depressed patients, have lower cortisol levels than controls [189], but other studies have shown an increase in cortisol levels 3 days after PTSD [190]. Wichmann and colleagues [191] argue that high cortisol is characteristic exclusively of depressed patients.

This ambiguity in the available neurochemical data may be explained by the ambiguity in the diagnosis of PTSD, as it is currently difficult to distinguish the diagnosis of PTSD from depressive disorder [192]. Certain criteria are necessary to accurately make a diagnosis of PTSD [193].

- PTSD criteria

- Presence of a real threat to life
- Repeated experience of traumatic events
- Avoidance of trauma
- Changes in mood indicators, anhedonia
- Cognitive impairment, memory impairment
- Disturbances of CNS excitation/inhibition processes
- Duration of 1 month or more

Some of these criteria can be used for modeling PTSD using animal models. The main such tests are presented below [193].

Basic animal models of PTSD and depression-like states

- Electrical irritation of the paws (foot shock stress)

This test aims at modeling depressive state and PTSD. The test is based on electrical stimulation of different duration (2-20 s) and current strength (1-3 mA). Varying the current strength and duration can create different stressful situations for rodents, in which they can either exhibit active or passive stress responses or show learned helplessness.

- Underwater trauma

Underwater trauma, or dive stress, involves 1 minute of forced swimming followed by 30 seconds of forced immersion in a tank of water. Rats exposed to such an exposure show immediate and persistent (7-30 days post-stress) increased arousal in the acoustic startle response and anxious behavior in the elevated cross maze (ECM) test compared to control rats that swam without immersion [193], as well as cognitive deficits three weeks after injury. Thus, such short-term exposure results in persistent posttraumatic changes in rats.

- Odor/predator presence

This test is based on the predator/prey ecological relationship. In numerous rodent experiments it has been shown that 23% of rodents exposed for 5-60 min to fox odor/predator interactions (cat, ferret) show pronounced PTSD symptoms (trauma avoidance, cognitive and emotional disturbances), as well as all neurochemical changes characteristic of PTSD. These behavioral and neurochemical changes in PTSD-exposed rats persist from 1-4 months after a single exposure to a traumatic agent. Depending on the type of PTSD planned, the experimenter can select the strength and type of stressor exposure. In the literature there is a variant with a combination of immobilization stress and the presence of a predator, to reduce the adaptive potential of the "victim", in the adaptation period after stress, additional exposure to chronic moderate unpredictable stress according to Willner [169] is possible to simulate the lack of social support after traumatic stress [193].

- Immobilization stress

This stressor test is based on the effect on the motivational sphere of the animal, i.e. it is the uncontrollability of the situation that causes a depression-like state and possibly PTSD. During the test (1-4 h) the animal is placed in a plastic pen in which the animal is not allowed to move, different protocols demonstrate different levels of immobilization of the animal. In experiments, rats have been shown to exhibit depressive-like and posttraumatic (anhedonia, social avoidance) behavioral and neurochemical (changes in spike density and amygdala, changes in the number of D2 dopamine receptors and intensity of DA response in the VTA area) after single stress [194]. Some neurochemical changes are observed even 1-10 days after stress [18,193,195]. The disadvantages of this test include the impossibility to control the magnitude of stress due to the individual stressor response of the animal. The use of this model may lead to death of rodents from excessively strong emotional impact, while other animals may be less susceptible to such a stressor. The individual differences in the perception of this uncontrolled exposure by rats are outlined in this thesis.

- Social stress and depression model - "social defeat stress"

Social defeat stress (SD) is used in modeling conditions of social stress and depression in humans [196], as well as in modeling depression-like and post-traumatic states when applying both acute and chronic stress in animals [38,39]. The animal models of SD are based on territorial aggression of male rats [197]. In the implementation of this test in rats and mice, a "resident"-"intruder" model is used in which a less aggressive intruder (an experienced animal) is planted with an aggressive "resident". During confrontation, these animals often show aggressive (attacks, aggressive stances, biting, stalking) and defensive postures (defense stances, running away,), stressor activity - freezing, displacement activity - grooming, digging, exploratory activity - stances and sniffing the

space and “partner” [197]. Also, during aggressive confrontation, both “resident” and “intruder” animals show an increase in DA responses in the ventral tegmental area (VTA) [198] and NAc [19]. Exposure to a single SD affects neurochemical processes in the synapse, causing neuroadaptation processes in DA cells in the VTA area [5]. After the action of such a stressor, animals show ambiguous neurochemical and less frequently expressed behavioral changes. More often researchers distinguish susceptible and resistant animals to the action of such a stressor. In “exposed” animals, changes in corticosterone levels are observed 39 days after exposure, but whether these changes are posttraumatic or depression-like is difficult to say because of the disparate scientific data and different subjective strength of the stressor [172]. The work reported in this dissertation shows that these adaptive changes in DA neurotransmission can be observed in intruder rats even 24 h after a single exposure to SD [41].

However, it should be kept in mind that this experimental work has its limitations. Both in nature and in laboratory practice, only 10-40% of resident males demonstrate territorial aggression [197], SD is practically not performed on females due to their very low aggressiveness. In this dissertation, special TPH 2 knockouts are used as “residents” in some experiments. Both males and females who show increased levels of aggression in the SD test.

There are also other models of PTSD (social instability, early life stress, etc.), but it should be remembered that all new tests used in the laboratory must meet at least some of the criteria described above, in addition to being reproducible, i.e. validated.

Motivational component of behavior

Behavioral act according to P.V. Simonov [199] is a movement that arises in the organism in order to satisfy various needs in case of external or internal changes in the environment, which is aimed at the return of environmental parameters to the initial values (active behavioral strategy) or adaptation to new environmental conditions (passive behavioral strategy). This movement is preceded by motivation, which forces the organism to make this movement by increasing anxiety, gathering information about the external environment, and developing an adequate program of behavior (activation of cognitive processes) [17]. Only when the organism is ready for active action - the movement itself is carried out and depending on the efficiency of satisfaction of the current need (positive or negative emotion) there is either recording of all this effective chain into memory or comprehension and change of the program of behavior.

Thus, motivation is a necessary lever for the implementation of the current need as A.A. Ukhtomsky wrote in his works on “dominance” - the main lever modulating the activity of various brain systems for the actualization of the leading need [200]. The relevance of the theory of

Ukhtomsky, who spoke about the dominant, dominant focus of excitation in the brain is proved by modern ideas about the functioning of the limbic system, about the action of narcotic substances (NS) and about the behavior of addicted people, which is aimed exclusively at finding and satisfying the current need (taking NS) to the detriment of other basic needs (food, sex, social behavior). Motivational behavior has its neurochemical correlates, the main one being dopamine (DA) as the main neurotransmitter of the "reward system" in the brain.

- Neurochemical correlates of motivation

The mesolimbic DA system participates in the motivational components of a behavioral act as a "rewarding agent".

Electrophysiological experiments on monkeys back in the 1990s showed that neurons of the mesolimbic DA system act according to the principle of "reward prediction", i.e. there are groups of neurons that are activated before the "reward" is received - these are the so-called "expectation neurons" [201,202]. This mechanism underlies both motivational behavior in the process of instrumental conditioned reflex development in animals and humans [203], but the same mechanism can be involved in the process of formation of drug and other types of addictions.

To illustrate the dopamine-dependent mechanism of instrumental learning, we can give the following example. If an animal trained to receive a "reward" (sweet solution) for pressing the pedal is then given an unlimited amount of "reward", the value of such "reinforcement" disappears, and the animal with a CNS not altered by narcotic substances naturally stops pressing the pedal altogether (the "devaluation of choice" model). The same result can be achieved by applying the "instrumental conditioned reflex (ICR) extinction" test, in which the instrumental action (pedal pressing) would not lead to positive reinforcement at all [204]. Thus the DA link is necessary for CR formation. It is noteworthy that animals chronically consuming D1,2 receptor agonist continued to press the pedal, even without receiving a "reward" for it [204].

It has been shown that DA neurons encode the entire spectrum of motivational information in the brain, but not without the help of other neurotransmitter systems. One type of DA neuron is responsible for the motivational value of a stimulus, being activated for "desirable" stimuli, and inhibited for aversive stimuli; another is activated for both of these stimuli, and a third is activated for danger signals [203]. However, not only DAs, but also the related GABA system is involved in the processes of processing motivational information in the limbic parts of the brain; it is these neurons that are activated in the ventral tegmental area (VTA) during the process of "waiting" for reinforcement. As a result, the resulting neuronal response is expressed to a greater extent during

reward anticipation and to a lesser extent during reward acquisition itself, unless it is an “unexpected positive emotionally colored reward” to which DA neurons are similarly active [202].

In animal experiments and human studies, it has been shown that not only the DA but also the SER system is involved in motivational processes when receiving both positive and negative reinforcement, and it has been shown that it is the joint action of both systems that provides the motivational value of “reinforcement” [124]. [124]. However, the role of SER neurons in the mechanisms of motivational learning is still under research. Nevertheless, some scattered data may enable some hypotheses. According to one data SERs play a role in behavioral inhibition of DA response through the action of SERs on PFC, this hypothesis is proved by experiments with the inhibitory effect of selective serotonin reuptake inhibitors [158], as multiple animal experiments and human studies showing that low SERs cause high impulsivity in decision making. In animal studies, it has been shown that activation of the DA response, (through the action of a D2 antagonist) and inhibition of SER neurotransmission (5-HT1A agonist) leads to increased impulsivity in rats and a large number of risky responses [205]. More recent work has identified a role for compartments such as the amygdala, orbitofrontal and ventromedial prefrontal cortex [206]. According to other hypotheses, the SER plays a role in the formation of tolerance for “delayed reinforcement” [124]. Subjective human evaluations have shown that an immediate increase in DA or SER levels is not felt as pleasant, but a joint increase in DA+SER is perceived as “very pleasant” [124], and studies in knockout animals have also shown that DA+SER systems are necessary for the formation of addiction [207]. Thus, only the joint action of SER and DA neurotransmitter systems ensures the formation of CR during motivational instrumental learning.

Dependent behavior

- Neurochemical correlates of addiction

The main target of narcotic drug substances (NS) action in addiction formation is the DA system, in which activation of DA neurons occurs, not only during NS intake or other need actualization, but also between events, which was proved in animal and human studies [208]. Moreover, if NS`s were taken at intervals, the emotional response to a subsequent NS intake becomes much greater than the first, even at a lower NS dosage. The possibility of NS overdose may occur due to this effect, along with the tolerance effect. This drug sensitization effect [209] has been proven by animal studies using in vivo voltammetry. In experiments it was shown that repeated (after 2 h) administration of cocaine caused a much greater rise in the DA level in the NAc than the first [210].

As it was indicated earlier, depending on the duration of NS administration, quite different neuronal responses develop in humans (Figure 1) [211]. While a single NS intake in a human shows

artificial activation of the mesolimbic DA system responsible for motivational behavior and an artificially induced state of “satisfaction and joy” is observed, chronic NS intake causes persistent suppression of the mesolimbic DA system as a result of physiological processes of neuronal adaptation to long-term NS action [211]. This neuronal adaptation is the main reason for the development of withdrawal and subsequent relapses [212]. Despite the fact that the target of various NS`s is their natural receptors, such as opioid, nicotine, and cannabinoid receptors, all of them, in one way or another, activate the mesolimbic DA system [211]. Thanks to this system, addiction is formed. Any psychological addiction goes through the following stages: the stage of interest, consumption, habit and withdrawal and then dependence. It has been shown in animal models that cortical regions and hippocampus are responsible for the emergence of the feeling of “interest”, VTA DA system is responsible for the feeling of satisfaction in the process of habit formation, during the consumption of NS and for the continuation of NS intake despite its aversive effect in the process of “hangover” and intoxication [211].

The serotoninergic neurotransmitter system (SER), like the DA system, is involved in various aspects of addiction formation. During a single administration of almost all types of NS, there is an increase in extracellular SER in the region of limbic brain structures, but, as in the case of DA, during chronic administration, there is an adaptation of SER receptors, leading to a decrease in SER neurotransmission and the occurrence of “withdrawal” syndrome of varying severity and duration [213], depression, pathological aggressiveness, and suicide [214].

It has been shown that at low activity of the SER system, both humans and animals demonstrate high impulsivity [215]. High impulsivity can be an unfavorable factor in the emergence of various forms of addiction, as it can provoke a person to the first intake of NS. However, not only the SER but also the DA system is involved in the control of impulsivity [216]. Removal of the NAc in rats has been shown to increase impulsivity [217]. One of the reasons for this high impulsivity could be a decrease in the activity of 5-HT2B receptors, which control both SER and DA release in the region of the NAc [215]. The SER neurotransmitter system, along with the DA system, is also involved in the cognitive control of impulsivity in the process of addiction formation. It has been shown in studies that prefrontal cortical areas, along with other limbic regions of the brain, acting in concert, provide an optimal level of decision making [216,218] and the SER system plays an important role in behavioral inhibition and cognitive control of motivation [216] (Figure 1).

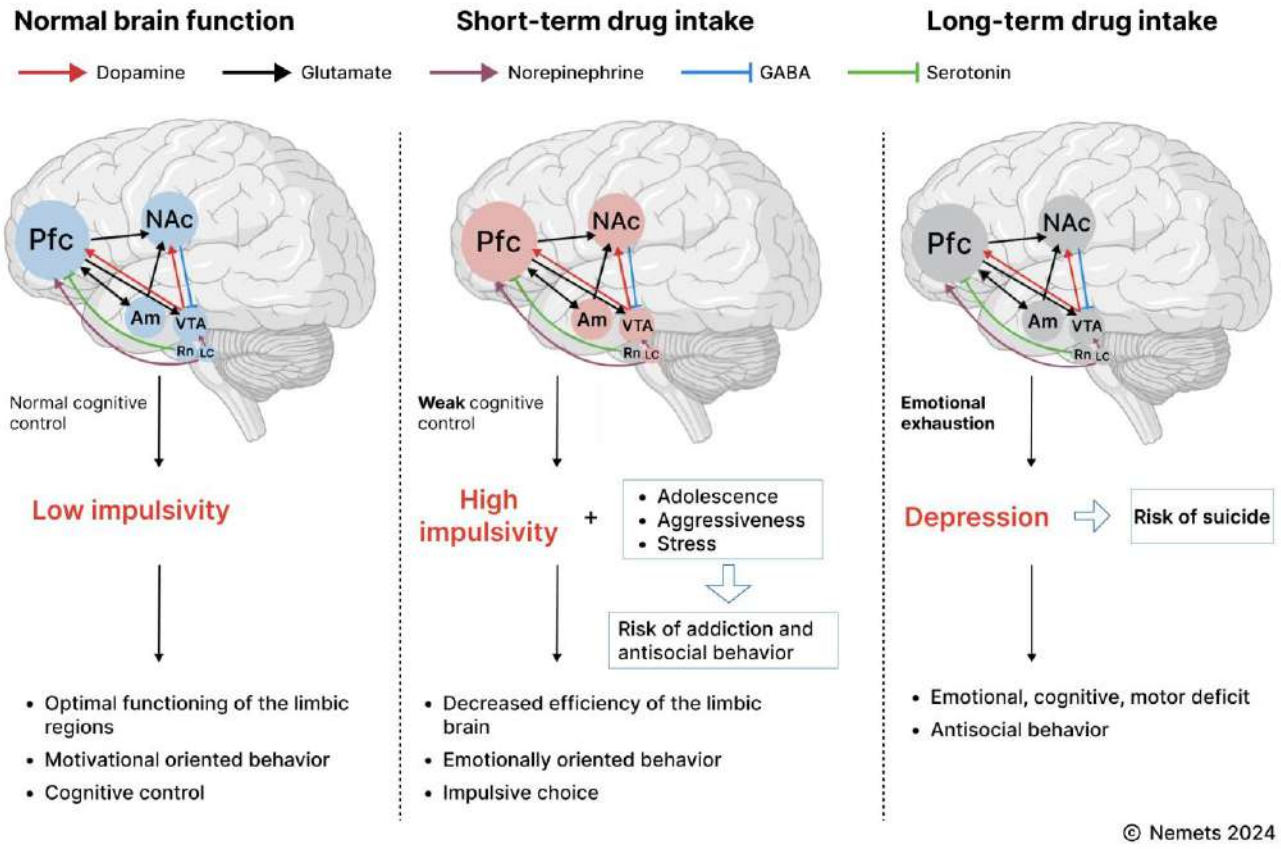


Figure 1: Schematic representation of the effect of narcotic drug substances (NS) on the functioning of different parts of the human brain. The figure shows the scheme of interaction of different parts of the human brain in the norm, during short-term intake of narcotic substances and during long-term intake, as well as the main behavioral manifestations and possible consequences of narcotic substance intake. The basis of interaction of different brain areas is neurotransmission, which has activating (DA, GLU, NA) and inhibitory (GABA, SER) effect on different brain areas. The limbic regions of the brain are indicated in the figure (Pfc - prefrontal cortex, NAc – nucleus accumbens, Am - amygdala, VTA - ventral tegmental area), as well as Rn - raphe nuclei (SER source), LC - locus coeruleus (NA source).

Figure 1 shows the human brain in normal, short-term and long-term use of NS. The literature shows that cognitive control, which is exercised by balancing the work of the PFC and basal brain nuclei, is necessary for normal motivationally oriented behavior [219]. Short-term NS intake leads to increased activity of GLU, DA systems in limbic parts of the brain, providing emotional effects of NS intake, SER system on the one hand is responsible for cognitive control of emotions [219], on the other hand - for NS occurrence together with mesolimbic DA system [207]. It has been shown that people with reduced levels of SER show greater impulsivity, emotional effects of NS, and likelihood of addiction than people with normal levels of SER [220]. Stress may be an additional trigger for NS intake [35,221]. Prolonged NS intake, as well as other prolonged depleting effects on the “reward

system" leads to depression of the limbic parts of the brain and, as a consequence, depression, which, along with other unfavorable symptoms of "withdrawal" syndrome, can lead to suicide, especially among young people [222].

We would like to dwell separately on the peculiarities of addiction formation in adolescents. In adolescence, due to underdevelopment of frontal brain regions, as well as natural dysregulation of SER and DA systems [223], there is a decrease in cognitive control and an increase in impulsivity during decision-making [223,224]. Adolescents' behavior is often emotionally oriented [219]; moreover, adolescent NS consumption itself causes strong changes in brain neurochemistry, further shifting the balance between the major limbic brain regions toward hyperactivity (Figure 1). Thus, due to the underdevelopment of the central nervous system as a whole, adolescents are more likely than adults to become targets of drug use, various fraudulent schemes that require immediate reward, and psychological addictions; moreover, they are more difficult than adults to succumb to further pharmacological and cognitive-psychological therapy [225].

- Stress in the mechanisms of addiction formation

Physiologically, the DA system is divided into the nigrostriatal (motor control), mesolimbic (emotional control), and mesocortical systems (cognitive control). However, the functions of the mesolimbic DA system are not limited only to "positive reinforcement"; recent data obtained in animals using voltammetry [19,41] and microdialysis [5] show that DA neurons are also activated under the influence of aversive events and stress. It is stress that can trigger various forms of both motivational and pathological behavior associated with substance use. Thus, stress along with other factors (interest, social identification within a group, etc.) can provoke initial NS intake and then contribute to the development and aggravation of addiction [211,226]. Stressor hormones [226], neurotransmitters, especially DA [35,41] may be involved in stress-related NS intake. The reason for NS use during stress may be biased activity, i.e., subjective opportunity to "get rid" of an unfavorable stressor event by taking NS. In addition to stress, NS`s can be used to alter feelings of other behaviors such as social and sexual behaviors [213]. These pathological behaviors become habitual and then develop into addiction [17,225].

The mechanism of this condition, otherwise known as "bad trip", may be the effect of stress hormones, namely corticoliberin norepinephrine (NA) on striatum and DA neurotransmission [23,227,228]. In animal studies, corticoliberin has been shown to directly affect the mesolimbic DA system by modulating motivational processes during the stress response [112].

Thus, stress may also influence the direct effects of NS intake, such as alcohol, and, as has been shown in recent animal studies, may be a trigger for both alcohol consumption itself [35] and the

aggressive behaviors often observed in humans after alcohol intake. Statistically, between 16-50% of alcohol-dependent men exhibit aggressive behavior. Among them, criminals, alcoholics who committed a violent crime are 5 times higher than people who did not drink alcohol [229]. Also because of the state of unmotivated alcoholic aggression, alcohol has a high risk rating for antisocial behavior after its ingestion [230]. However, not only stress, but also individual genetic characteristics such as increased impulsivity due to mutation in the SER transporter gene or age (increased aggression in adolescents) may be the cause of unmotivated aggression after acute or chronic alcohol ingestion [229]. Thus, low levels of SER and high levels of stress hormones may provoke people to antisocial behaviors. Modeling stress-dependent alcohol consumption on TPH2 knockouts and the involvement of the SER and DA systems in this process is shown in this dissertation.

Stress can accompany not only acute drugs intake but can also be observed in chronic NS intake. During prolonged intake, all neurotransmitter systems, especially the reward system, are depleted, and then the person develops apathy and depression, accompanied, however, by increased levels of stress hormones such as NA [231]. At low levels of SER, along with reduced activity of all limbic structures of the brain (Figure 1), suicidal attempts are also possible [214]. Thus, in the treatment of patients with mental disorders caused by chronic drug (NS) use, special attention should be paid to the management of depression symptoms and then at least partial restoration of the balanced work of all limbic parts of the brain and neurotransmitter systems that carry out cognitive control of emotions [216].

Fast-scan cyclic voltammetry (FSCV) method for recording DA response

- History of the voltammetry method

When A. Karlsson discovered dopamine (DA) as a neurotransmitter in the brain in 1950, there was a need for its quantitative measurement. The main areas of application of such methods became the study of neurodegenerative diseases associated with degradation of the dopaminergic system (Parkinson's disease, Alzheimer's syndrome, etc.). The techniques of the time allowed histological, electrophysiological, but not neurochemical studies of the amount and dynamics of DA neurotransmission; such techniques were highly invasive (damaging surrounding tissues) and thus poorly informative.

In the 1960s, Clark and Adams began to study the processes of catecholamine oxidation, and came to the need to use special microelectrodes made of carbon fiber as recording electrodes and in the 1970s were able to successfully record the catecholamine response in the anesthetized rat. For this experiment, the researchers used “solid electrodes” made of graphite mixed with oil, packed in Teflon in the form of a tube with a diameter of 0.5 mm. After reaching the required level, the electrodes were

cemented, further recording of DA response was done for several days. Thus, these studies gave rise to the modern method of in vivo voltammetry [232].

Later, Mark Whiteman and team improved the recording and hardware of the method and named the resulting method fast-scan cyclic voltammetry (FSCV) [233]. The recording electrode used in the FSCV technique is made of carbon fiber, which has chemical neutrality, high electrical conductivity (practically zero electrical resistance), and strength. Microscopic dimensions of modern carbon fiber (d = 6μ, L = 70-100μ) allow to register DA signal practically without traumatizing the surrounding tissues [234,235]. Fast scanning voltammetry equipment is equipped with modern sensors and software (HDCV - High Definition Cyclic Voltammetry). Thus, the modern method of fast scanning voltammetry allows to make measurements of DA with millisecond temporal resolution, recording various parameters of DA response (ejection, recapture) during the whole time of the experiment (8 hours and more).

- Principle of the voltammetry method

Fast scanning voltammetry (FSCV) is a modern electrochemical method of neurobiology. The method is based on the oxidation/reduction processes of substances at the electrode when a potential of certain electrical parameters is applied. This approach allows us to study the electrochemical properties of the studied substances occurring on a carbon microelectrode. The graph of current dependence on the applied potential is called a voltammogram (CV - cyclic voltammogram). With the help of CV it is possible to identify and quantify the substances under study (catecholamines) in a sample or nerve tissue. The shape of the voltammogram determines the ratio of oxidation/reduction processes on the surface of the working electrode (WE). The position of peaks on the voltammogram serves as a qualitative identifier of catecholamines and their metabolites, and the amplitude of the signal indicates the change in the concentration of the substance on the electrode surface (Figure 2).

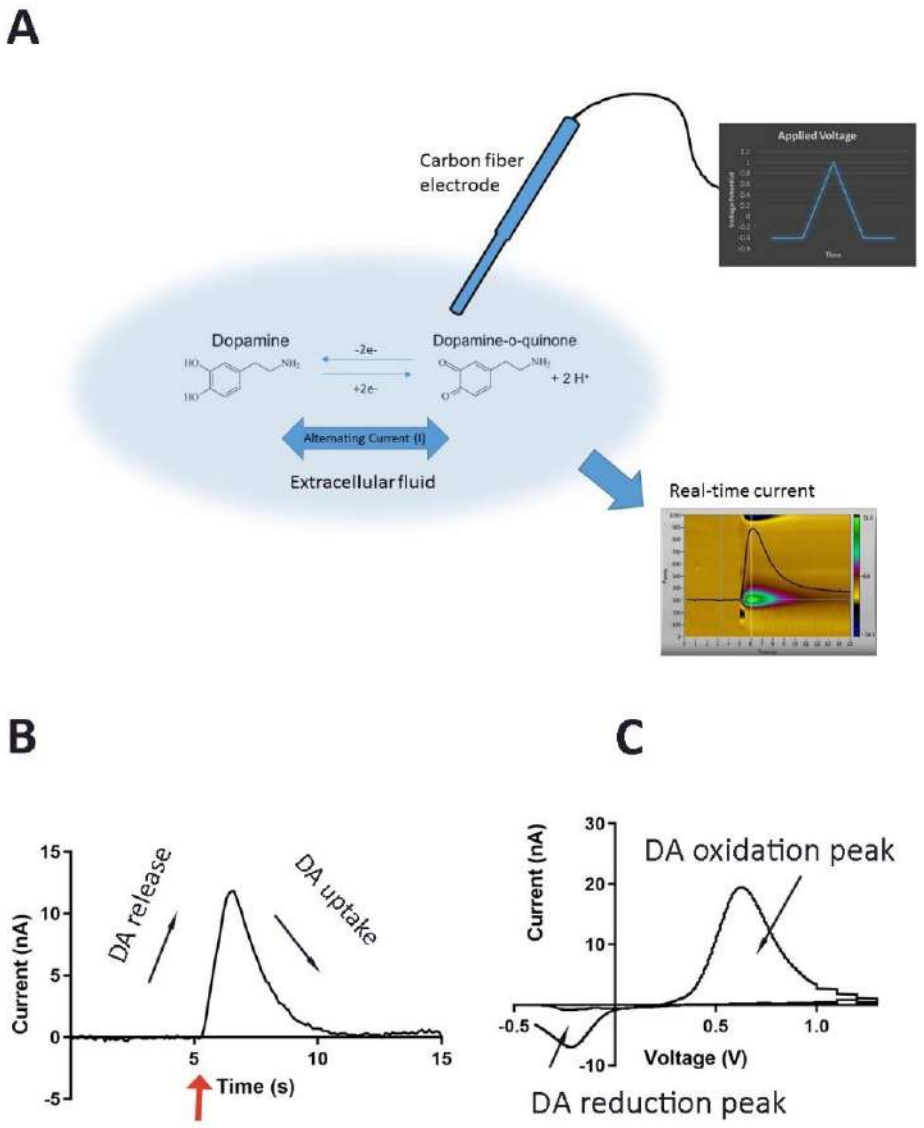


Figure 2: Schematic illustration of the principles of DA detection using the of fast-scan cyclic voltammetry method. A - electrochemical processes of DA oxidation and reduction from dopamine o-quinone on the surface of the registering carbon microelectrode (WE), the applied triangular potential (compared to Ag/Ag Cl) with parameters -0.4 +1.3 -0.4 V is also shown, below is the DA response in NAc, nA. B - DA response in NAc after stimulation of the VTA area, nA. C - cyclic voltammogram (CV) of DA with characteristic peaks of DA oxidation 0.6 V and recovery from -0.2 V. Orange arrow - electrical stimulation of VTA (330 μA, 60 Hz, 60 Pulses).

Different characteristics of the applied potential are used for different catecholamines and their metabolites (Figure 3). To determine DA, a triangular-shaped potential with characteristics of -0.2 +1.2 V or 0 to 1 V with a scanning speed of 150 mV\s was previously used. In such a modification of the device it took up to 13 s to record a voltammogram [236]. Now, as the sensitivity of the procedure increases, the same triangular-shaped potential is used, but with characteristics of -0.4 +1.3 -0.4 V and

a scan rate of 400 V/s. However, as the scanning speed increased, the surface currents increased, so it became much more difficult to register the Faraday currents (obtained in the case of DA from oxidation of DA to DA OQ and back), but this problem was solved by hardware removal of these surface currents, which can reach up to 1000 nA (Figure 12). This removal of background currents significantly reduced the sensitivity of this technique, so it became impossible to record basal values of neurotransmitter concentrations in the brain measured in nM, but an approach was invented by which electrical stimulation of brain regions far from the area of electrode recording was performed. Such stimulation significantly increased neurotransmitter release into the synaptic space and enabled the researcher to record the corresponding neuron response in real time with great temporal and spatial accuracy [232]. Also used in modern voltammetry, glass isolated microelectrodes made of carbon fiber with a diameter of 6-10μ allows (in contrast to microdialysis) the least invasive registration of DA response in the brain, in addition, as it was said earlier, hydrocarbon microelectrodes have a low chemistry of the brain (as opposed to metallic) and are more suitable for long-term electrochemical experiments.

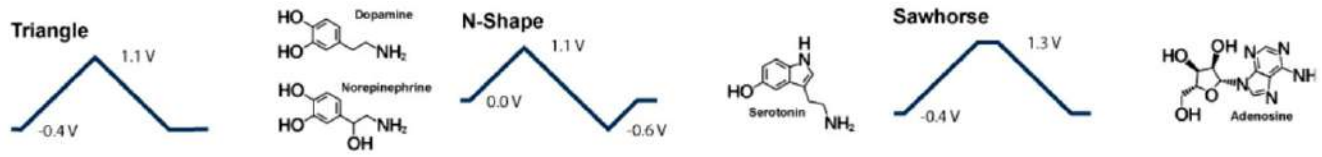


Figure 3. Potential waveforms applied to the recording microelectrode required for voltammetric recording of various compounds.

- Different variants of the voltammetry techniques (in slices, in vivo, freely-moving rats)

There are different variations of voltammetry technique in rodents. Many researchers use the identification of DA response on brain slices [237-241]. This approach is the simplest from the technical point of view (electrode placement, data stability) and is effective for revealing the regularities of work of certain tissues (brain and spinal cord, adrenal glands, etc.), as well as proteins influencing the dynamics of neurotransmitters [232]. The method using anesthetized animals (in vivo) is more complicated, as it is necessary to select an anesthetic agent that will be suitable for long-term operations and will minimally affect the functioning of the nervous system, in addition, it is necessary to ensure the stability of the signal and accuracy of the data obtained from different animals, but the obvious advantage is the study of the neurotransmitter system on the whole brain with preserved nerve pathways [41,234]. Most challenging is the study of the neurotransmitter response in freely-moving animals, in which the source of neurotransmitter release is the animal's natural behavior [19,242].

In our laboratory and in the works presented here we use in vivo voltammetry on anesthetized rats/mice, because this approach combines the advantages of in vivo methods (integral functioning

neural pathways) and work on slices (it is possible to study the dynamics of drugs affecting DA responses, the work of neurons and receptors), this method is not easy to use, but the data obtained by in vivo voltammetry are accurate, and comparable with the data obtained in the laboratory and other research groups working on the rodent.

- Features of voltammetry in comparison with the microdialysis method

Just like the microdialysis method, voltammetry registers extracellular DA. The traditional microdialysis method, performed in conjunction with high-performance liquid chromatography (HPLC), records the direct DA concentration in a given area. The voltammetry method has a lower resolution than the conventional microdialysis method. A DA peak is recorded in the NAc core region: up to about 5 nM by the FSCV method [243], versus 0.4 nM recorded using the microdialysis + HPLC method [5,244].

In contrast to microdialysis, voltammetry can be used to analyze the DA response with high temporal (400 V\s) and spatial resolution. Thus, the voltammetry method allows instantaneous analysis of DA response, while the microdialysis sample collection requires tens of minutes. This fact is a clear advantage of the voltammetry method when it is necessary to study instantaneous neurochemical changes in the brain, for example, during (or after) aggressive behavior [19]. When using in vivo voltammetry in the process of investigating the dynamics of DA neurotransmission after pharmacological injection, the researcher is able to record long-term dynamics of DA response at intervals of 10 min (this interval provides a complete recovery of DA neurotransmission in neurons [41,240,245,246] after electrical stimulation) or less (depending on the task) for many hours (up to 8 hours or more). Special software (HDCV) allows to set all time intervals of stimulation, which makes it possible to obtain an accurate pharmacodynamic picture of the drug action for a long time including in semi-automatic mode. In addition, when using voltammetry in vivo, in addition to the value of DA concentration, researchers receive information about: the type of the registered compound, the value of its release and the value of reuptake. This information is necessary to identify the specificity of pharmacological action of drugs. This information is particularly useful for the study of substances that affect dopamine reuptake parameters (e.g. GBR) [246] + (see in this dissertation), similar abnormalities can also be observed in DAT knockout animals (see in this dissertation).

Thus, microdialysis and voltammetry methods are effectively used in laboratories to record DA neurotransmission, as it was shown by Prof. Budygin and Prof. Miczek that the data obtained by these methods are comparable and complementary [19,247].

- Applications of the voltammetry method

The voltammetry method has been successfully applied in studies of neuropsychiatric disorders such as drug and alcohol addiction, Parkinson's disease and schizophrenia. This method is used to record the extracellular DA response in model animals, which is necessary to identify the mechanisms of development of these diseases [26].

Studies of cocaine addiction performed on model animals in the paradigm of cocaine self-consumption and voltammetry method revealed the main mechanism of action of this drugs, i.e. inhibition of DA reuptake in the process of cocaine self-consumption [248]. In an experiment using the voltammetry method, an instantaneous release of DA in the NAc area was shown for the first time even before the cocaine self-administration procedure (lever pressing), and it is noteworthy that this increase in DA level coincided with the onset of cocaine-seeking behavior [249], as well as alcohol [26]. Thus, studies using the voltammetry method, proved the association of increased mesolimbic DA levels (the drug expectation process) with the appetitive phase of drug-seeking behavior [249]. To reveal the mechanisms and approaches to the treatment of alcohol dependence, a combination of voltammetry and optogenetic stimulation methods was used in Prof. E. A. Budygin's experiments. It was first shown that phasic electrical stimulation of VTA-NAc triggers alcohol seeking behavior, while tonic stimulation suppresses this behavior [221]. Work using DAT knockout animals has demonstrated the role of DAT in the mechanisms of drug addiction and tolerance development [26,250]. DAT knockout rats and mice have been used in various experiments as an animal model of hyperdopaminergic states and schizophrenia. In studies using the voltammetry method by other researchers [26] and in this thesis, it has been shown that DAT KO animals, in contrast to WT, show a significant reduction in DA reuptake.

In studies of TAAR1 agonists, such agents have been shown to effectively inhibit the hyperactivity of DAT KO animals [251,252]. In clinical trials, the first tested TAAR1 agonist with 5-HT1A agonist activity “ulotaront” showed significant efficacy in the treatment of schizophrenia patients for both positive and negative symptoms, without causing the side effects observed with existing antipsychotics [253]. Using voltammetry and the use of TAAR-1 knockouts, an increase in DA response in the NAc in TAAR-1 knockouts compared to wild type was shown, as well as no change in DAT function was shown, thus it was shown that ulotaront does not directly affect D2 receptor function [254], but there is a functional relationship. This mediated (via TAAR-1) action on D2 receptors provides the most effective treatment for schizophrenia with fewer adverse symptoms than direct action on D2 via agonists [26].

Thus, the voltammetry method is widely used in the study of neurochemical mechanisms of various neuropsychiatric diseases. The main advantage of this method is the ability to perform studies of DA release and reuptake in response to the administration of pharmacological drugs in real time, and in combination with other (e.g., behavioral) techniques voltammetry effectively allows us to study in detail the subtle neurochemical mechanisms of these neurological disorders in one way or another affecting DA neurotransmission.

- The use of voltammetry in the study of neurochemical components of the stress response.

In this thesis, the voltammetry (FSCV in vivo) method is used to investigate the neurochemical consequences of social defeat stress in male [41] and female rats with different behavioral strategies [15].

Stressor events provoke a chain of biochemical and neurochemical reactions in the body. Stress-induced disorders of dopaminergic (DA) neurotransmission have received special attention due to the involvement of DA both in motivational behavior, as well as in the implementation of various pathological conditions such as drug and alcohol addiction, anxiety, depression, and posttraumatic stress disorder (PTSD) [255]. The role of mesolimbic DA neurotransmission not only in “reward” behavior, but also in aversive and stressful behavior was first shown in the works of Prof. Miczek [5]. In the works using electrophysiological and microdialysis methods, it was shown that not only “positive” but also aversive stimuli (tail prick, immobilization and social stressors) caused activation of mesolimbic DA neurons. The authors associated these processes with “neuroadaptation” often observed during the action of narcotic substances on the brain [5]. Other works link this DA activation to the motivational component of the stressor response [1,112,256]. It has also been shown in animal studies that corticotropin-releasing factor (CRF) can directly influence the observed changes in extracellular DA in the NAc[109].

A direct elevation of mesolimbic DA during immobilization stress [18] and then during social defeat stress [19] has been shown in free-living rats in Prof. Budygin's laboratory using in vivo voltammetry [19]. The fact that a single social defeat stress can lead to long-term (up to 1 month) physiological and humoral changes in the organism was shown in the works of Koolhaas [4]. Studies of the effects of social defeat stress on DA dynamics were also shown in the laboratory of Prof. Budygin [240] and reflected in this dissertation and in articles [15,41].

Use of transgenic animals to study mechanisms of stress behavior

- Use of TPH2 knockouts to study the mechanisms of alcohol and stress behavior

Studies done in Prof. Budygin's lab have shown similar changes in the DA system in the effects of stress and alcohol consumption [240]. This thesis reflects the involvement of both the DA and SER systems in the processes of alcohol stress-related ethanol consumption using tryptophan hydroxylase type 2 (TPH2) gene knockout rats.

The central serotoninergic neurotransmitter system is involved in many critical body processes such as mood, emotion and cognitive function, and social behavior. Deficiency of SER signaling, fundamentally should affect the development of all major CNS functions; however, animals knocked out by the TPH2 gene, in which central SER neurotransmission is genetically switched off (by 80-100%), not only survive but also exhibit an altered but wide range of behaviors [36,37]. Synthesis of SER in the body depends on the activity of tryptophan hydroxylase TPH enzymes. These enzymes are present in the body in two isoforms and are encoded by two genes. The TPH1 gene is responsible for the synthesis of peripheral SER, while TPH2 is responsible for the synthesis of brain SER. TPH1 and TPH2 enzymes also differ in their spatial distribution. TPH1 is predominantly expressed in intestinal cells, which is the main source of circulating SER, and in the pineal gland, where serotonin is a precursor to melatonin. TPH2, on the other hand, is expressed in the intestinal myenteric plexus and in SER neurons of suture nuclei localized in the brainstem, where it is responsible for central serotonin synthesis [37].

TPH2 gene knockout animals are characterized by high impulsivity and aggression [257], in addition, genetic inactivation of TPH2 function and subsequent serotonin deficiency in adulthood in mice leads to behavioral changes such as depressive behavior, reduced anxiety, but high learning scores in the “active avoidance” test were observed [257]. TPH2 knockout females exhibit impaired maternal behavior associated with serotonin deficiency. Such females are less likely to have calves surviving because they have a lower weight compared to the wild type, and canibalism has also been observed. TPH2 knockout females themselves show high aggression even during the lactation period, as well as worse than wild-type animals in terms of maternal behavior (searching for cubs, caring for cubs, building a nest) [258], thus, in this dissertation, males and females perform the role of an aggressive subject (resident) in the procedure of social stressing.

These knockouts are often used as a model for disorders such as autism, attention deficit and hyperactivity disorder [258], but in this dissertation they also act as a model subject during stress-related alcohol consumption.

It has been shown in the literature that alcohol intake increases SER and decreases cortisol (some people have increased cortisol levels) [259], [260], thus alcohol intake in some cases reduces stress levels, which may be one of the causes of stress-related alcohol consumption. However, in some individuals, a single high-dose ingestion causes an increase in cortisol levels 105 minutes after ingestion [261], in addition, aggressive behavior may be observed in a certain type of alcoholism (aggressive alcoholism) [262]. However, in chronic alcoholics the situation is the opposite, with chronic alcohol consumption they have low serotonin levels and high tryptophan hydroxylase (TPH2) [263] along with low cortisol levels [261]. A possible association in humans of the TPH2-703T polymorphism and "stressful alcoholization" has been shown, but the data are not reliable, [264], in addition, despite the fact that chronic alcoholism sometimes (15-27% of alcoholics) leads to suicide [265] the role of the TPH2 gene in the likelihood of suicide is still unclear, studies of polymorphisms have not provided reliable data [266].

In a study of rat stressor behavior, some differences in corticosterone reactivity after 1 h of immobilization stress were shown in female TPH2 knockout rats in contrast to wild-type animals, as well as slightly different patterns of PFC activation in response to stress; however, no significant neurochemical differences in stressor response between TPH2 knockout rats and wild-type animals could be identified [267].

Furthermore, the absence of central SER neurotransmission from birth in TPH2 knockouts should have induced an adaptive compensatory increase in neurotransmission of other neurotransmitter systems to ensure acceptable CNS functioning. There is scant evidence in the literature to this effect that TPH2 knockout rats exhibit a significant increase in both gene and protein expression of brain-derived neurotrophic factor (BDNF) in the prefrontal cortex compared to wild-type animals [267], similarly, it has been shown by HPLC that TPH2 heterozygote mice exhibit abnormal brain metabolism of DA and NA during stress [268]. All these scarce data allow further studies of compensatory DA mechanisms that may occur in TPH2 knockouts, in addition, the role of the SER neurotransmitter system in stress and alcoholization processes should be elucidated. All of these issues are in the active research phase, but light has been shed on all of these issues in this thesis.

- DAT knockouts as a model of increased hyperactivity and stereotypy

The dopamine transporter (DAT) is an integral membrane protein whose main function is to uptake released DA from the extracellular space into the presynaptic neuron. DAT protein is selectively expressed in DA neurons of the substantia nigra, VTA, but its density is highest in the striatum and NAc. In these compartments, DAT is a major regulator of DA signaling. DAT is an important substrate for psychostimulants such as cocaine, amphetamines, and other drugs [269,270].

Experimental alterations of DAT protein functions (creation of knockout animals/knockdown animals) are used to study the parameters of DA neurotransmission and the effect of these changes on motivational, emotional, cognitive, and motor components of rodent behavior [270]. After the creation of DAT knockout, it was found that mice without DAT protein were viable, but only about 70% of animals survived 10 weeks after birth. The mice proved to be fertile, but female KO mice were found to be incapable of lactation and impaired maternal behavior and thus were not capable of caring for a litter, but in general the animals develop normally but gain weight more slowly compared to wild type animals and adult DAT knockout mice show some body weight deficit (30% lower than wild type) [271].

The main characteristic of the DAT knockout phenotype is pronounced hyperlocomotion and impulsivity, as well as disorders in sleep regulation and learning [271]. These behavioral changes in DAT knockout animals are caused by impaired DA reuptake and, as a consequence, compensatory increase of its extracellular level. A voltammetry study showed that DA reuptake in DAT knockout mice was 300 times slower than in DAT wild-type animals [271]. The same results are replicated in this thesis, but using DAT knockout rats and behavioral and voltammetric approaches.

In the literature, in animal studies using microdialysis, it has been shown that the basal extracellular DA level is five times higher in DAT knockout mice compared to wild-type animals [272]. This fact can be explained by the fact that in DAT knockout animals, unlike wild-type animals, DA is retained for a long time in the synaptic cleft, since DA reuptake is slower (40-300 times slower), and is carried out mainly by diffusion. However, when using the DA voltammetry method, the response in the area of the NAc was also reduced (amounted to 75%) in DAT knockout animals compared to wild-type animals [273]. These results can be attributed, among other things, to the difference in the methods used. Microdialysis measures the basal level of extracellular DA, which was elevated in mutant animals, while voltammetry measures the electrically evoked extracellular phasic DA response, which was reduced due to decreased DA synthesis in DAT knockouts compared to wild-type animals [274] and neuronal depletion due to high-frequency stimulation causing phasic rather than tonic DA elevation.

The content of intracellular vesicular DA in DAT knockout animals, as shown by HPLC, was found to be reduced (up to 20-fold) compared to wild-type animals [269,275]. These changes can be explained by the fact that in DAT knockout animals' adaptive changes in synthetic processes in the DA system occur, thus in DAT knockout animals, due to neuroplasticity there are adaptations to low DAT content in tissues and practically no DA reuptake [274].

DAT knockout mice have now established themselves as the best animal model for dopamine transporter deficiency syndrome [269]. DAT knockout animals are mainly used to model hyperactivity disorder (ADHD) because they possess the hyperactivity and cognitive deficits seen in ADHD, in addition, administration of amphetamine and methylphenidate has been found to have an antihyperkinetic effect in these animals [276]. Currently, methylphenidate is widely used in the treatment of ADHD patients, reducing spontaneous hyperactivity and increasing the extracellular DA level in the prefrontal cortex. In addition, DAT knockouts are also used as a model subject in modeling hyperdopaminergic states in bipolar disorder and schizophrenia and motor manifestations of parkinsonism [269]. In addition, DAT knockouts have been investigated in drug addiction processes [271]. As might be expected, DAT knockout animals (rather than SERT knockout animals) showed a significant reduction in cocaine self-administration. Thus, studies have shown that cocaine acts on DA reuptake via DAT [277].

DAT knockout rats exhibit increased motor activity and restless exploration of the environment, which is associated with a transient anxiety profile. In addition, these rats exhibit marked stereotypic and compulsive behavior [270]. Stereotypic behavior is an important component of animal activity (sniffing, licking, strutting), pathological activity (nodding) [278], in addition, one of the important manifestations of stress response (grooming, burying, biting cage bars) [44], in any case, the DA system is directly involved in the activation processes of stereotypic behavior. It has been shown that amphetamine dose-dependently triggers different types of stereotypic behavior in rats and mice, while striatum removal inhibits this behavior [278]. In psychiatry, stereotypic is usually referred to as movements that are repeated without a purpose. Such movements can be observed in various psychiatric or neurological disorders such as schizophrenia, mental retardation, autism, obsessive-compulsive disorder, dementia, Tourette's Syndrome, and temporal lobe epilepsy in animal behavior, the term “stereotypy” often refers to components of instinctive behavior, or the “fixed patterns of behavior” described above [278]. DAT knockout animals demonstrate hyperlocomotion in a novel environment, as well as exhibit increased stereotypy behaviors [271]. This thesis demonstrates the features of the DA response in DAT knockout rats using the FSCV in vivo method, and provides evidence of their hyperlocomotion and increased stereotypy.

MATHERIALS AND METHODS

Animals

White mongrel male rats weighing 280-320 g, nursery “Pushchino”, age at the beginning of the experiment - 3 months were used in the experiments on the effect of immobilization stress. Animals were kept in the vivarium, in standard conditions (5 rats per cage) under light regime (12/12 h), with free access to water and food. All animal procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All experiments were in accordance with the international rules for the ethical use of animals according to the 1985 Geneva Convention on “International Principles for Biomedical Research Using Animals”, the 2000 Declaration of Helsinki on the Humane Treatment of Animals, and the Guide for the Care and Use of Laboratory Animals: eighth edition [279]. Work with laboratory animals was carried out according to the minutes of the closed meeting of the ethical committee of FGUP NIIGPECH No. 001010/18 05.11.2017. In other experiments, animals from the Resource Center of the Vivarium of the SPbU Science Park were used. The animals were also kept under standard conditions described above. Male and female Wistar rats weighing 280-320 g, as well as male Spread Dowley rats with an average weight of 350 g and Long Evans (450-500 g), male and female TPH2 KO and WT (Dark Agouti line), as well as DAT KO and WT (Wistar line) were used for the experiments. Work with laboratory animals was carried out according to the protocol of the Ethical Committee of the Biology Department of St. Petersburg State University (number 131-03-8 /25.09. 2023).

Behavioral techniques

- The open field test

This test has been used in neuropharmacology to study the assessment of locomotor, stressor (freezing, grooming) and exploratory activity (stance) in animals. This test is based on the natural fear of open spaces. There are different modifications of this test. The fields differ in size and shape, but the principle is common to all of them: the animal is placed in a brightly illuminated arena. In our studies, an arena (40 x 40 x 40 cm) is used. Rat behavior is recorded for 5 min using a video recording system (EthoVision XT 11.5, Noldus. Wageinen, The Netherlands). The duration of behavioral patterns (locomotor activity, freezing, stance, grooming, etc.) was calculated manually by analyzing the recorded videos [280].

- Elevated cross maze (ECM) test

This test is currently considered the most adequate test for assessing anxiety in laboratory animals [281,282]. This test is based on rodents' natural fear of open spaces (open arms), while

favoring closed ones (closed arms). Unlike the “open field”, the ECM registers not only locomotor activity but also anxiety level (time spent in the closed arms of the maze). The ECM consists of two open arms, 50 cm long and 10 cm wide, and two closed arms of the same length and width, at least 40 cm above the surface of the maze. The dimensions of the entrances to the closed sleeves are 10×10 cm. They are located opposite each other at a distance of 10 cm. The floor of each arm is drawn into 10×10 cm squares, 5 squares on each arm. The ECM is placed at least 75 cm above the floor. All animals were seated with their nose toward the center of the maze. After testing each animal, the maze was wiped with 11% hydrogen peroxide solution to destroy the odor of the previous rat.

The distance traveled and time spent in the open/closed arm of the maze were recorded using a program (EthoVision XT 11.5, Noldus. Wageinen, The Netherlands) (see Figure 4). The duration of stalls, grooming, freezing, et al. - were calculated manually using the Realtimer program of the “open science” company. Figure 4 shows the process of recording rat behavior in the ECM test using the Noldus program. In separate series of experiments, the animal's behavior was recorded using the program Rat Behavior © Nicolai Kamyshev 2007. The program recorded the duration of each behavioral act in % per 100 s of measurement. The number of squares traveled was recorded quantitatively, M ± m.

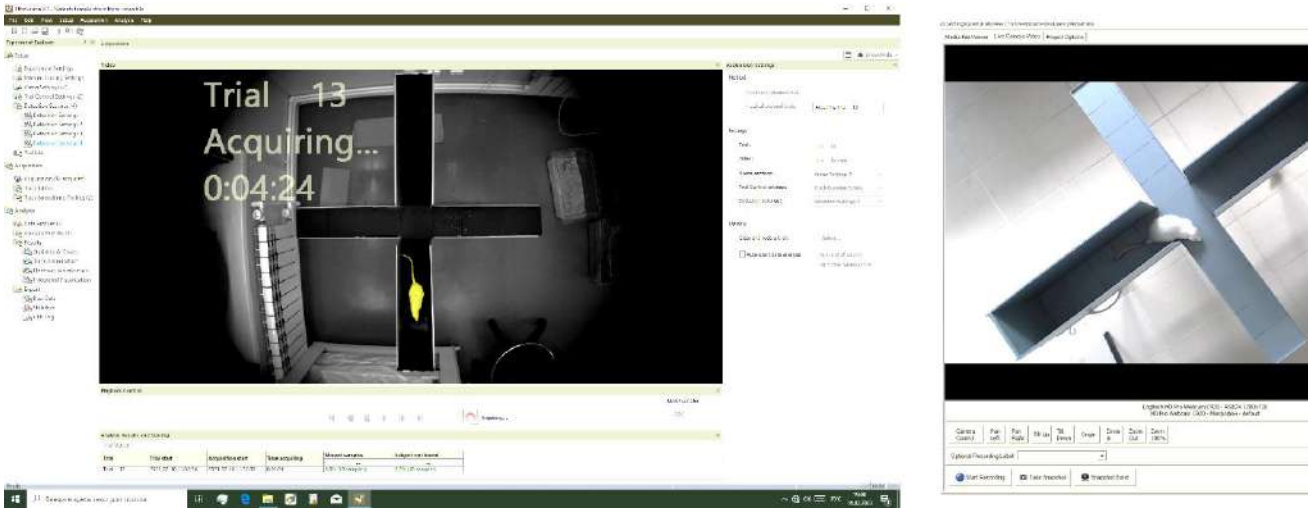


Figure 4. Registration of rat behavior in elevated cross maze using Noldus software (left) and Rat Behavior © Nicolai Kamyshev 2007 (right).

- Long-term recording of behavioral activity of rats using “Laboras” equipment

To identify differences in motor, exploratory, etc. activity of rats in separate series of experiments of this dissertation we used the LABORAS, Metris, Netherlands, automatic behavioral registration system, which allows in a “home cage” without the experimenter's participation, for a long

time to register behavior, water and food consumption in each rat. The system is based on measuring platforms that convert the animal's movements into electrical signals. Cages with the animal are placed and maintained in a fixed position on the measuring platforms. Vibrations caused by the animal's movement in the cage are converted into electrical signals collected from all platforms, which are then amplified and converted into behavioral patterns using special software. The following behavioral parameters were measured in our studies: locomotor, exploratory activity, immobility/sleep time, duration of grooming, food and water consumption. Measurements were performed in automatic mode for 18 hours (from 17 h one day to 11 h the next day). It has been shown in studies using the LABORAS system that this time period (18 hours) is the most adequate and informative for assessing the natural behavior of rodents [283].

- Sucrose preference test

In the implementation of this test, a standard two-bottle scheme was used in which rats had access to water (in one bottle) and 10% sucrose solution (in the other bottle) for 18 hours per day for 1.5 weeks. Consumption of water and 10% sucrose solution was measured during the dark phase of the diurnal cycle [284]. There are various modifications of this test for rats and mice with a range of concentrations from 2-32%. Based on the experience of our and other laboratories and the article [284], we used a concentration of 10% as the most preferred. Figure 5 shows that rats prefer 10% sucrose to water on the fifth day of consumption (see Figure 5).

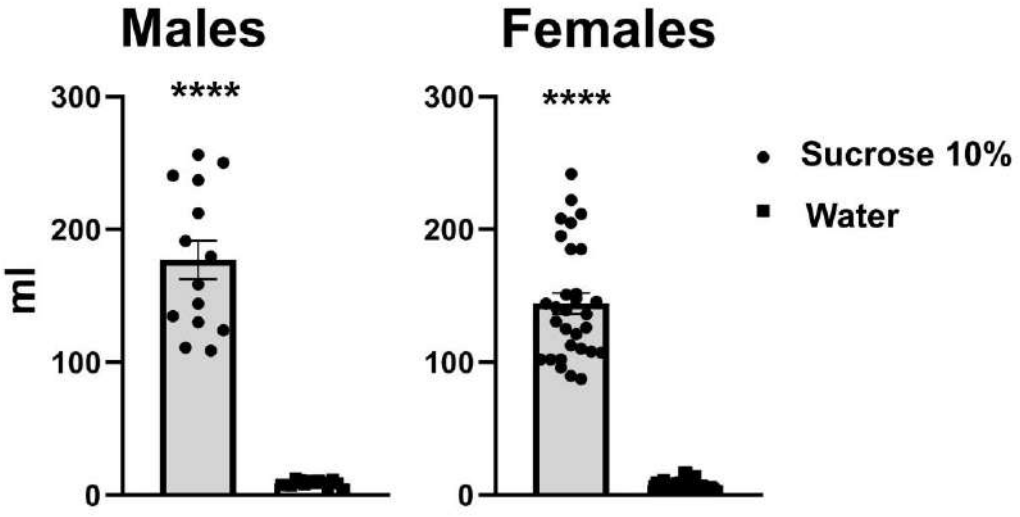


Figure 5. Consumption of sucrose/water solutions by female and male Wistar rats on day 5 after daily consumption (18h/day). **** - P<0.0001, Stewart's t-test. Horizontal - groups of animals, vertical - consumption, ml.

Sucrose preference for water in our experiments was calculated as the ratio of 10% sucrose solution (g) to the sum of total fluid intake (sucrose + water, g) × 100% [285].

- Alcohol consumption test

To investigate the neurochemistry of alcohol consumption, rats (male and female) TPH2 knockouts (KO)/wild type (WT) as well as Wistar rats aged about 1 year, weighing 350-400 g were subjected to alcoholic drinking procedure for 85 days using a two-bottle test (experimental solution/water). During the alcohol-mating procedure, rats were given 2 drinkers, one with sucrose solution, which was gradually replaced with ethanol solution, and the other with water. At the beginning of the experiment rats were given drinkers with 10% sucrose/water solution, which after 20 days was replaced by ethanol solution (6% sucrose+4% ethanol)/water, then after another 20 days (2% sucrose and 8% alcohol), and after another 20 days they were exposed to different stressors: in some experiments (Wistar rats) - a single 20-min social defeat stress (SSS), in other experiments a subchronic immobilization stress procedure (4 h) was applied.

Consumption data of solution (2% sucrose and 8% ethanol)/water were recorded for 2-3 days after stressing. To achieve optimal consumption values and to establish a stable alcohol dependence, the interval between consumption of sweet-alcohol solutions was 3 days (water drinker only). Drinkers were weighed on a scale to calculate the consumption of solutions, g. The preference of alcohol solutions to water in our experiments was calculated as the ratio of alcohol solutions consumption (g/kg) to the sum of total fluid consumption (solutions + water, g/kg) × 100% [285].

- Porsolt test

This test is used to detect depressive-like behavior in rats [286-288]. In this test, a rat is placed in a cylindrical glass container filled with water (diameter 45×28 cm, water temperature 22±2°C). This water temperature is effective in creating motivation for action in rats, but is not low enough to lead to an increased state of immobilization due to cold stress [289,290]. Evaluation of behavioral parameters began after the rat was immersed in water and the tail was lowered down. During the test, the experimenter assesses immobility time in rats during the 6 min test [287,291]. Immobility is assessed as a brief state where the rat freezes and does not move [286,287]. In this dissertation and in some literature [291-293] this test is also used to select animals with “active” and “passive” behavioral strategies. The selection criterion is the time of active/passive swimming during 6 minutes of the test. Animals showing pronounced immobilization behavior in the Porsolt test are classified as “passive”, if the rat shows pronounced active swimming - “active” behavior strategy. The results of correlations

with other behavioral tests we used to select animals with these behavioral strategies are presented in this dissertation.

- The "novel object recognition" test

This test is used to detect disorders of visual memory and cognitive processes in laboratory animals [294-296]. The visual cortex of area V1 (part of the occipital cortex responsible for visual perception) is involved in the recognition of a new object [296], and some data show the involvement of the hippocampus [297,298] and prefrontal cortex [299,300]. It has been shown in experiments that removal of these brain regions leads to an inability to identify a new object compared to a previously presented object [301-303]. The test is aimed at the natural exploratory activity of rodents, which may decrease when the functioning of the central nervous system is impaired.

This test is based on the natural curiosity of rodents to new objects. The main criterion for choosing a "new" object over an old one is its difference in shape, color, texture, and size [304]. According to my data, Wistar rats prefer a new object to an old one (see Figure 6).

Figure 6: The "new object preference" test. The figure on the left shows the proportion of rats that prefer a new object to an old one. On X axis – discrimination rating of novel object preference (calculation formula: (N – O)/(O + N)), on Y axis - individual values of each rat. The figure on the right is an illustration of object exploration by a rat, the process of sniffing a new object is noticeable.

This test consists of two parts. In the first part, two identical objects are presented. The rat examines the objects for 8-10 minutes. After an hour (in case of short-term memory study) or after 24 hours (if long-term memory is studied), the same object (old object - "O") is presented again for 3 minutes together with a new object (new object - "N") differing in color, shape and texture. A stopwatch is used to record the time of exploration of each object, s. while the animal sniffs or otherwise explores the new object at a distance closer than 1 cm [294]. The preference rating of a new object is calculated from the difference in the time of studying the objects according to the formula (N

– O)/(O + N) [294,305]. In the modification of the test that I use, the experiment is conducted in home single cages in order to eliminate the stress of a new environment in animals (Figure 6), [306].

- Test of conditioned reflex of active avoidance

To test the activity of the behavioral strategy in the situation of electric pain stress we used the test “active avoidance”. In our experiments we used a device of our own production, which was built by us specially for these studies [43] and was characterized by reliably higher “avoidance” indices than the basic version [307]. In any modification, the unit for developing the conditioned reflex of active avoidance is a chamber with transparent side walls made of organic glass (in our modification) and a floor replaced by an electrically conductive grid [308,309]. Inside the chamber is divided by a partition with a passage in the center. Outside, a panel with a switch is located on a special control unit, through which an electric current and a conditioned (tone signal 2-4 kHz 70 dB - these parameters were selected based on the auditory sensitivity of rodents [310] and/or an electric pain stimulus (current 0-3 mA) are supplied. The parameters of the electric current were adjusted by an ammeter with a scale of 0.1 μA. This sensitive scale provided smooth adjustment of pain intensity based on personal differences in skin conductivity and pain threshold of the animals. In our modification, an alternating pulsating current (2 imp/s) was used. Under such influence the animals showed pain reactions (piloerection of hair, aggressive posture, narrowing of eye slit, tail erection, teeth grinding) even under the action of current below 1 mA, thus at low currents it was possible to achieve more effective “avoidance” behavior than in the basic version of the device, besides it was possible to avoid undesirable aversive reactions (pronounced freezing, vocalization, defecation) observed in animals at presentation of higher currents. During testing in the active avoidance setting, the animal was placed in the “active avoidance” chamber for 5 minutes to reduce stress and to measure background motor, exploratory and stressor activity. At the end of the adaptation period, a conditioned stimulus (sound) was turned on for 4 seconds, followed by an unconditioned stimulus in the form of an electric current for 6 seconds. For each rat 25 attempts (measurements) were made, the inter-signal interval between measurements was from 15 to 30 seconds. The current strength (up to 1mA) and the duration of the inter-signal intervals varied depending on the degree of learning and stress level of the animal [43]. During testing, we measured the number of escapes to the unconditional stimulus (current) - “escape” and to the conditioned stimulus (sound) - “avoidance”. We also measured the number of “non-avoidances”. The testing time of each animal averaged 20 minutes. Testing in the “active avoidance” unit was carried out for 2 consecutive days. According to the data obtained by us earlier [46], the efficiency of skill development of “avoidance” reactions to the conditional signal - sound on the second day of testing determines the activity of the animal's behavioral strategy.

- "Competition for water" – social status test

It was used to reveal the social status of animals in the cage [311]. Rats in home cages (n = 5) with an established hierarchy were subjected to water deprivation for 24 h. This was followed by placing a water drinker on a special tripod. According to the literature [312] and our laboratory experience, the hierarchy in rats is usually established within 5-7 days after placement in a new cage. The stability of the hierarchy was determined by an insignificant number of social conflicts in the cage during laboratory manipulations (cage cleaning, etc.), as well as by the absence of competition for any resources (food and water in free access) and other stressors. The behavior of rats was recorded using a video camera. The time of each test was 10 min. When analyzing the video recording, the latent period of approach to the drinker, the number and efficiency of aggressive attacks were measured for each animal preliminarily marked with a bright tag. Social status was calculated by the efficiency of competition for a vital resource (water) according to a set of behavioral indicators. Testing of social status was performed three times with an interval of 2 weeks; animals with 2/3 and 3/3 efficiency were assigned to the corresponding behavioral groups. At the end of randomization procedures, the following subgroups were identified from the total group of male rats (n = 60): "active dominants" AD (n = 15) and 'passive subordinants' PS (n = 15). The animals selected for further experiment were kept in their home cages to maintain their hierarchical status [43].

- Tail flick test

The tail flick test is used to measure pain threshold in rats. The latency period of the tail flick reaction in response to thermal stimulus is recorded in seconds. During the test, the animal is placed on a heated plate for 20 min. The surface is calibrated so that a normal animal responds approximately 10 s after exposure (typically 52-53 °C). The researcher records the latent period and duration for which the animal responds to thermal stimuli. Individual responses vary: licking the hind paw is a reliable indicator of discomfort, although some animals may jump or show vocalization [313,314]. In our work, the PANLAB 7160 apparatus, Spain, was used. No adaptations to the apparatus were made, one measurement was counted, the device was heated for 20 min, and the final temperature was 50°C.

Biochemical, histologic and genetic tests

- Serum biochemical analysis

Triglycerides, creatinine a, high density lipoproteins (HDL), low density lipoproteins (LDL), cholesterol were analyzed according to the method [313] on an automated biochemical analyzer SAPPHIRE-400 (Tokyo Boeki Ltd., Japan) using reagent kits manufactured by Randox (Randox Laboratories Ltd., UK). Before analysis, blood plasma was isolated from rats 5 days (4 hours/day)

after immobilization stress. Blood serum obtained from blood collected after decapitation, then centrifuged at 2000 rpm and separated from the clot-free upper fraction, was used for analysis.

- Analysis of corticosterone in plasma

Corticosterone is a reliable indicator of the stressor state in animals. Under the influence of stress, this hormone is released into the blood from the adrenal cortex and its concentration remains elevated up to 6 hours after stress [4,316]. In our studies, we performed baseline measurement of corticosterone levels, also 4 hours after a single 4-hour immobilization stress and 5 days after immobilization stress (4 h \day). Blood plasma obtained from blood collected from the tail vein into a special tube with EDTA was used for analysis. A Termophisher Multiscan FC microplate photometer and Abnova Elisa Kit were used to measure corticosterone.

- Immunohistochemical analysis (IHC) of DA receptors

Procedure, reagents. The procedure was performed in rats 5 days after immobilization stress (4 h \day). After decapitation, the animals' brains were extracted and tissue samples of the segmental plane of the left hemisphere were excised in stereotaxic coordinates: -1.13 to -2.07 mm ant. Bregma (https://scalablebrainatlas.incf.org/). Tissue samples immediately after excision were placed on a specimen holder in the chamber of the Slee MEV microtome cryostat at -130C. A 9% sucrose solution prepared ex tempore was used as a cryoprotectant, with which the tissue sample was completely covered. Transverse brain slices 7 μm thick were obtained. Slices were fixed in acetone for 5 min, followed by a 5 min wash with 0.01 M phosphate-salt buffer TBS IHC Wash Buffer+Tween, 20X (Cell Marcue). For IHC, the following were used:

1. For D1 identification: multimeric biotin-free REVEAL-Biotin-Free Polyvalent DAB (SpringBio) chromogen diaminobenzidine detection system (SpringBio)

2. For D2 identification: multimeric biotin-free detection system REVEAL-Biotin-Free Polyvalent AP (SpringBio) chromogen fast red.

All IHC steps were performed using a Slide Master plate (SkyTek Laboratories) at an ambient temperature of 21-230C. Protein Block (phosphate buffer pH 7.6 containing 0.5% BSA and 0.5% casein) was applied to the slices for 10 min. Rinsed in TBS for 5 min.

A working solution of primary antibodies (Mouse Anti-dopamine D1a receptor Millipore© (MAB5290) and Anti-Dopamine D2 receptor (AB1558) Millipore©) was applied to the slices at titers ranging from 1:50-1:200.

Primary Antibody Diluent (Diagnostic Biosystems) was used to dilute the antibodies. Incubated from 60 minutes at room temperature to 12 hours at +4C. Washed in TBS twice for 5 minutes each. HRP Conjugate (secondary antibodies, goat anti-rabbit, horseradish peroxidase-labeled anti-rabbit) was applied to the slices. Incubated for 15 minutes. Washed in TBS five times for 5 minutes each. A pre-prepared mixture of 20 µl DAB Plus Chromogen (3,3'-diaminobenzidine) and 1 ml DAB Plus Substrate was added to the slices. Incubated for 5 minutes. Washed in TBS for 3 changes of 5 minutes each. Slices were then processed in ascending strength alcohols, clarified with ortho-xylene and encapsulated in Biomount medium (Biovitrum).

IHC evaluation protocol. Stained sections were viewed under a microscope at magnification modes of 103x; 247x and 413x. Regions of the neocortex and striatum were photographed at 103x magnification and then the number of positively stained neurons in an area of 104 µm was counted in the «Video test» program (Figure 7)

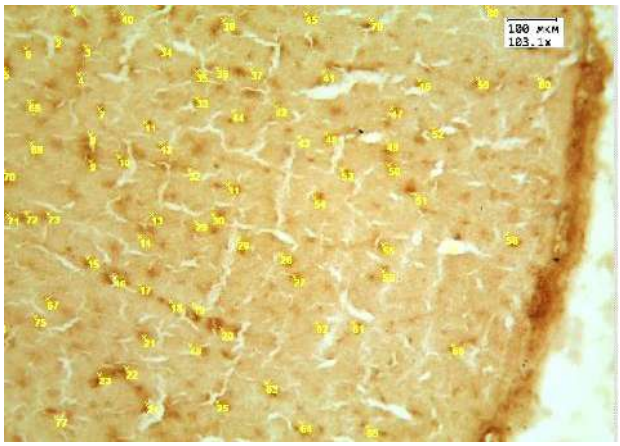


Figure 7: Illustration of dopamine cell counting in the «Video Test» program. Yellow arrows indicate the numbers of dopamine cells. The figure shows 80 cells.

- Methods of measuring the stages of the estrous cycle in rats

Vaginal swabs were collected and analyzed from female rats by the vaginal flush method. For this purpose, a plastic pipette with 60 µl of distilled water was inserted atraumatically into the vagina of a rat that was sitting in a closed tissue sleeve. After inserting the pipette into the vagina of the rat - 3 presses were made and a drop of liquid with biomaterial was placed on the slide. After each rat, the pipette spout was discarded. Then we performed the procedure of staining vaginal smears according to May Grunwald. For this purpose, 20 µl of eosin methylene blue dye was dropped onto the slide with a vaginal smear and covered with a coverslip [317]. Further, such a wet stained preparation was immediately placed under a Leica light microscope with a magnification of 300-400 times and the stage of the estrous cycle was analyzed.

The estrous cycle in rats (see figure 8) consists of 4 phases: proestrus, estrus, metestrus, and diestrus [317-320]. Diestrus (the longest stage) is characterized by the presence of leukocytes and a large amount of mucus in the smear. In the prostrus stage, the smear contained large nuclear epithelial cells, with bluish large nucleus. The estrus stage was identified by the presence of a large number of large keratinized nucleusless cells in the smear, forming significant clusters at the end of the stage (late estrus). In the metestrus phase, cells of all 3 types (leukocytes, epithelial cells, scales) are present. However, some researchers do not distinguish this stage of the cycle in rats, but refer it to early diestrus because of the difficulty in identifying it [321].

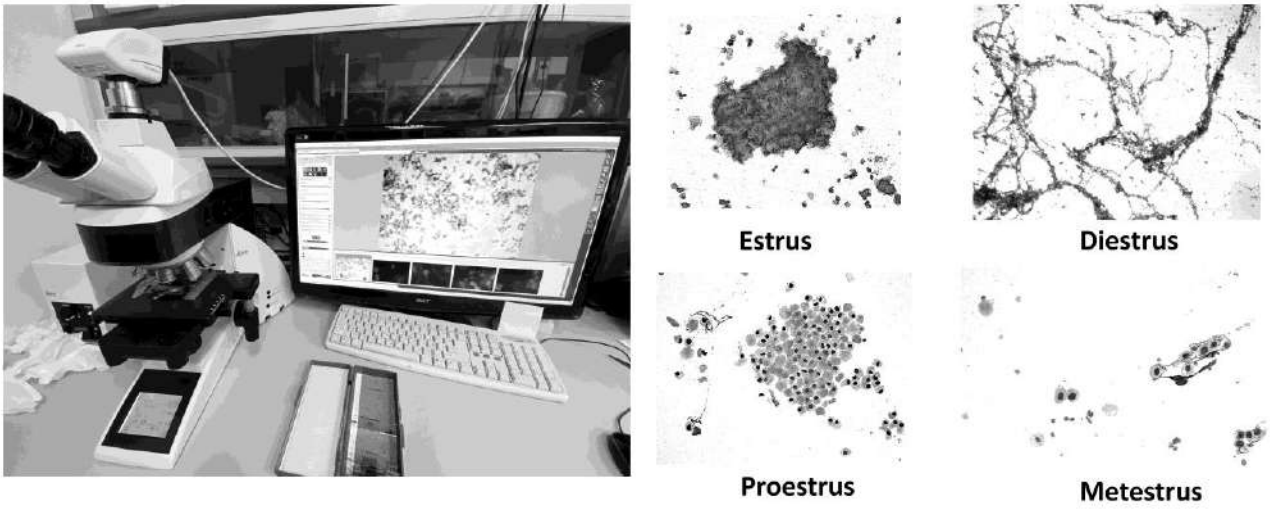


Figure 8. Illustrative scheme of identification of estrous cycle stages in rats. The picture on the left shows the process of identifying the stages of the estrous cycle in rats by photograph. The picture on the right depicts the stages of the astral cycle.

It is noteworthy that the vaginal swabbing procedure was performed at the same time (13.00) to increase the accuracy of the data obtained, since morning and night rats have a progesterone peak [322,323] and maximally atraumatic for the rat. During and after the procedure, the rats showed normal behavior (no vocalization during the procedure, no stressful behavior of freezing after the procedure). Vaginal swabs were taken at 2 weeks to eliminate the stress of swabbing [317].

In studies of social stress on females, the stages of the cycle were determined in rats before the stressing procedure and behavioral tests, in the case of division into groups (according to the type of stressor response), rats were selected so that each group had the same number of rats in the stages of diestrus (non-receptive phase of the cycle) and estrus/proestrus (receptive phase of the cycle), since the literature shows a change in anxiety in rats in such stages of the estrous cycle [319].

- TPH2 knockout genotyping methodology.

TPH2 is a key gene involved in the neuronal serotonin (5-HT) synthesis cascade playing a key role in the regulation of 5-HT neurotransmission (Gutknecht et al., 2012). Identification of rats knocked out by this gene is essential for experiments. This process is called genotyping.

The genotyping process itself consists of several steps. At the first stage, DNA is extracted from a fragment of animal tissue using lysing and neutralizing enzymes, then DNA amplification is performed using PCR (polymerase chain reaction) procedure. For PCR, a special mixture of primers and a special solution (Biolabmix) is prepared and added to the sample tubes. It is necessary to check the efficiency of the PCR run. For this purpose, an intermediate electrophoresis procedure with 2% agarose gel (3g agarose to 150 ml TAE 1x + 100 µl EtBr 1000x solution) is carried out in 80V mode for 40 min - 1 hr. ImageLab software is used to visualize the results obtained after intermediate electrophoresis (Figure 9).

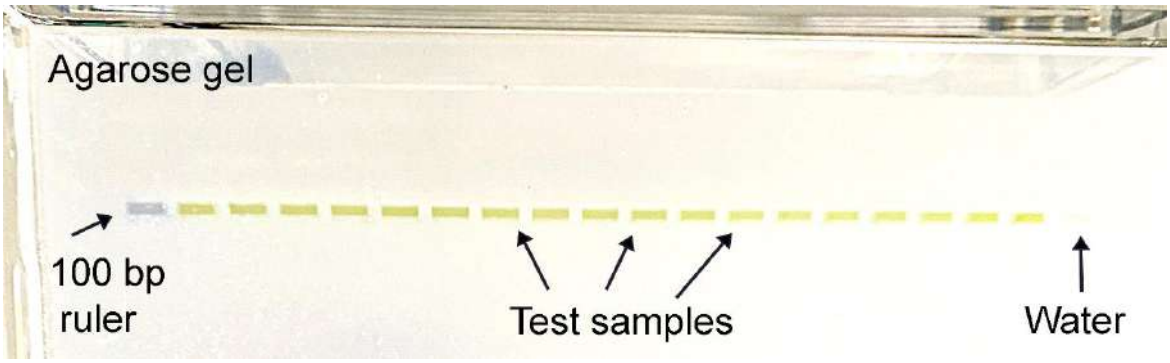


Figure 9. Initial stage of electrophoresis (before sample separation).

To identify DNA fragments corresponding to the genotype of knockout (KO), heterozygote (Hz) and wild type (WT) in this study, it is necessary to perform restriction procedure (cutting of DNA fragments). To do this, prepare a special restriction buffer (composition - Mnl1 restrictionase (NEB R0163S), CutSmart buffer and water), add this buffer to the samples, heat the samples to 37 C (2.5 h), then to 60 C (20 min). After the samples cooled down, perform gel electrophoresis procedure (3% agarose gel). ImageLab program is also used for visualization of the obtained genotyping data (Figure 10).

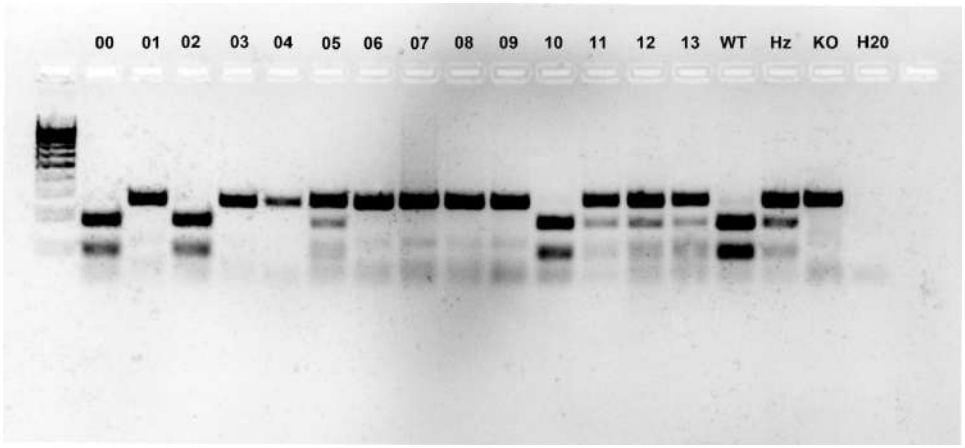


Figure 10. Final results of genotyping of knockout rats by TPH2 gene. The genotype WT (wild type) corresponds to samples (00,02,10); KO (knockouts) (01,03,04,06-09); Hz (heterozygotes) (05,11-13).

Fast-scan cyclic voltammetry (FSCV in vivo) technique

- Registration of DA response in anesthetized rats using the FSCV in vivo technique

In our laboratory, we use in vivo voltammetry in anesthetized rats and mice to study the processes of DA release and reuptake, as well as the processes of DA depletion/synthesis and record the DA response after the action of various pharmacological agents [41].

Before the beginning of experimental procedures animals were weighed, then anesthesia with urethane 1.5 g/kg was performed. Before the start of the surgical procedures, anesthesia efficacy was tested by irritation of the foot surface (plantar reflex) and tail, and during the surgical procedures, this monitoring was also performed every 10 min. If before the operation, within 30 min or in the process there are signs of withdrawal from anesthesia - the dose of anesthesia is increased by 0.01 ml (readings are recorded). Next, the skull was marked further, the appropriate voltammetric equipment was checked and connected (Figure 11), then the electrodes were lowered for adaptation in the tissues. During the adaptation process, a “cycling” procedure was performed. This procedure is necessary to increase the stability of the data obtained from the WE and reduce “noise”. During this procedure, the WE is in the brain of the animal for 10 minutes at an applied potential with a frequency of 60 Hz. After the adaptation processes of the electrodes, they are positioned in the appropriate area according to stereotactic coordinates [324].

A recording carbon microelectrode (WE) (up to 6 μm in diameter and 70-100 μm in length) was lowered into the NAc region (AP: 1.3 mm, ML: 1.3 mm, DV: -7.1 mm), a metallic stimulating electrode (SE) (Plastics One, VA) was lowered into the ventral tegmental area (VTA) (AP: -5.2 mm, ML: 1.0 mm DV: -8.4 mm). A chlorosilver reference electrode (Ref) (Ag\AgCl) (which is pre-chlorinated in 1N HCL solution) was lowered to the minimum effective depth in the tissue of the

contralateral hemisphere. The electrodes (WE and Ref) were connected to a voltammetric amplifier interfaced to a computer running specialized software for recording voltammetric data (HDCV). A triangular waveform potential (-0.4 V to +1.3 V and back to -0.4 V) was used for DA detection compared to Ag/AgCl, 400 V/s).

In the process of electrical stimulation (necessary for DA registration) in the animal only separate whisker twitch reflexes are registered, this reflex lasts only 1 s during stimulation, by the expression and symmetry of this reflex we can judge about the zone of stimulating electrode hit. It is noteworthy that all other reflexes (plantar reflex, etc.) are tested every 10 min throughout the experiment and should be suppressed, i.e. absent.

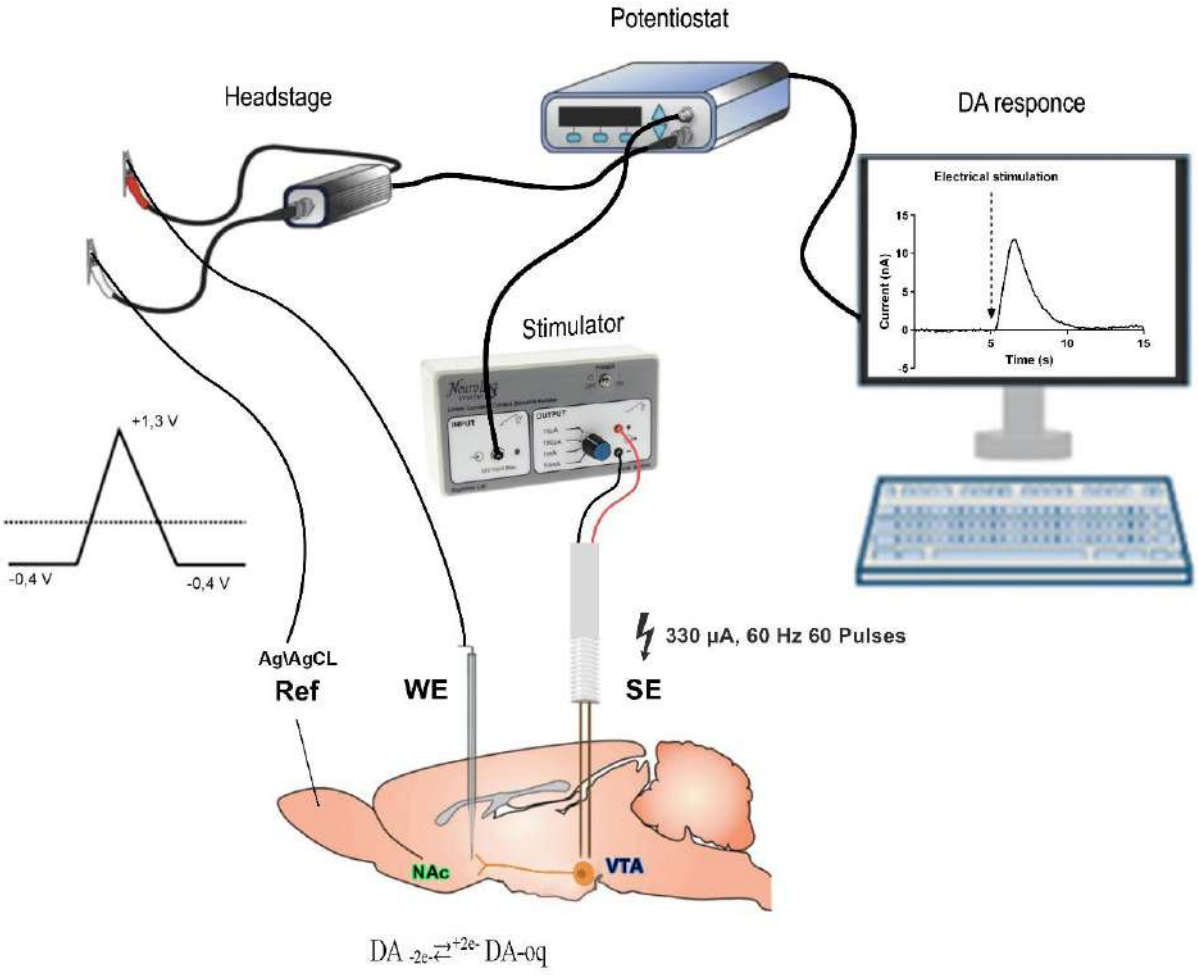
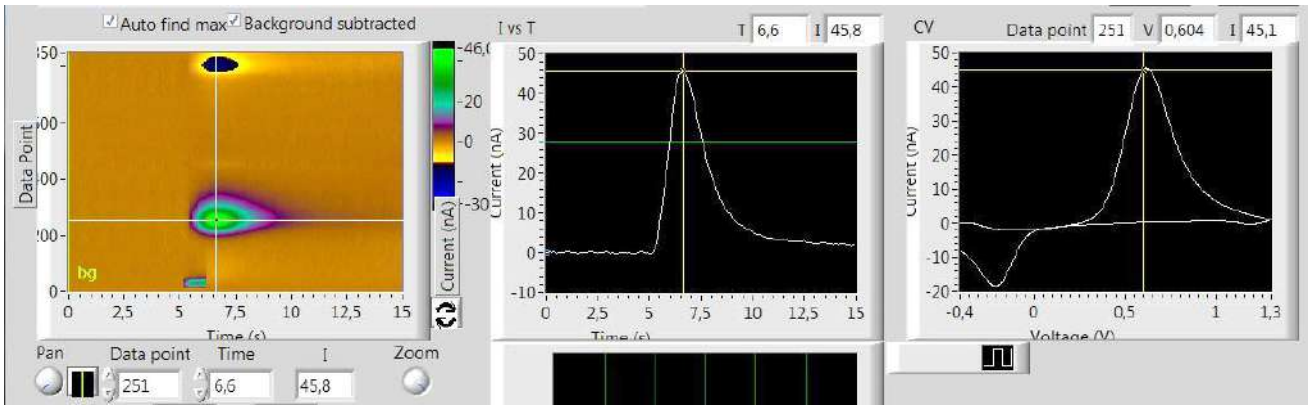


Figure 11. General scheme of voltammetric setup and electrode positions during DA registration using the FSCV in vivo method. Ref - reference electrode, WE - recording microelectrode, SE - stimulating electrode. NAc – nucleus accumbens, VTA - ventral tegmental area. In the process of registration of DA response, the corresponding potentials are applied and maintained (using a potentiostat) to the

electrodes WE and Ref. A triangular-shaped potential, -0.4 V to +1.3 V and inversely to Ref (Ag\AgCl) is used for WE. Electrical stimulation is performed by SE and delivered with different current parameters from a computer via a potentiostat and stimulator; in response to electrical stimulation of the VTA area using WE, a DA response in the NAc is recorded. The DA response data obtained after electrical stimulation, minus background, are fed to a personal computer with the HDCV program installed. The figure shows the characteristic DA response, nA after 1 s of 330 μA stimulation.

In the process of voltammetric measurements, namely, during potential application, surface non-Faraday currents increase significantly. By their magnitude these currents exceed the necessary Faraday currents (more than 10 times), obtained from redox reactions of DA, which thus drown out the DA signal. In the HDCV program it is possible to hardware remove the values of these surface currents, which can reach up to 1000 nA (Figure 12) and extract the necessary DA signal in the NAc zone obtained after electrical stimulation of the VTA zone.

✓ Background substracted signal



✗ NO Background substracted signal

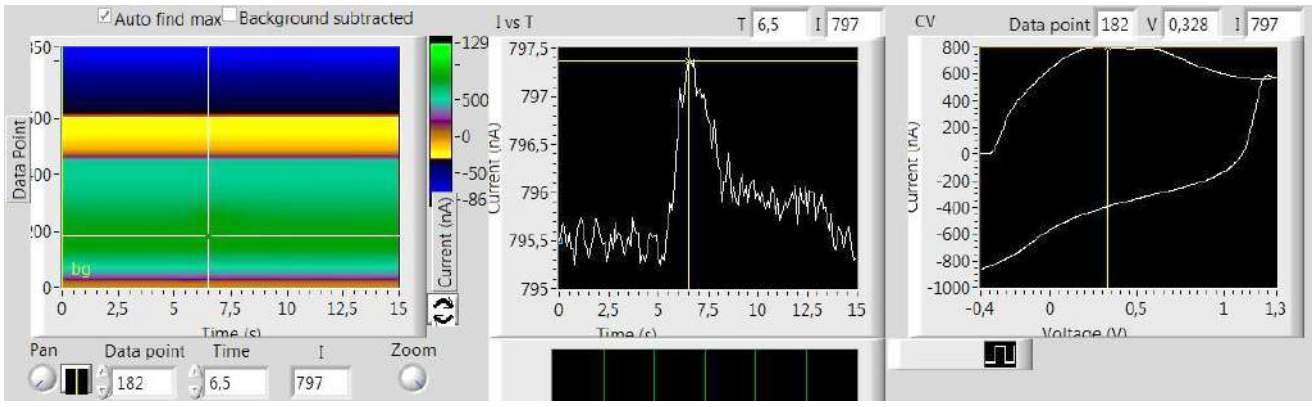


Figure 12 The figure shows voltammetric signals without and with deduction of background non-Faraday currents. The picture clearly shows that without the subtraction of such currents it is

impossible to identify the DA response, since such currents are more than 17 times greater than the DA signal.

- Characteristics of stimulations and frequency dependence of the signal

In the course of experiments using the voltammetry method, we used the following characteristics to record DA:

Length of the recording part of the working electrode: 80-165 μM
The position of WE, SE is determined by atlas, relative to stereotaxic coordinates.
Potential applied to the working electrode compared to the potential of the reference electrode Ag\AgCl: -0.4 to +1.3 V; -0.4 V, rate 400 V/s.
Stimulation frequency: 5-60 Hz
Amplitude: 198-330μ
Number of pulses: 5-60 in 1 sec
Stimulation time: 1 sec
Time between stimulations: 5-10 min.

It is noteworthy that during the search for DA signal we used lower currents (198μA) than in the experimental part (330μA). This scheme was used because the recovery process of DA after stimulation with high currents is equal to 7-10 min, and with low currents - 1 min. Thus, it is possible to find the optimal DA signal at low currents, then perform the experimental part at 330 μA.

After finding a stable DA level at the depth corresponding to the stereotactic coordinates, the procedure of frequency dependence of the DA signal on the characteristics of electrical stimulation is performed (Figure 19). Stimulation was performed at intervals of 10 minutes and with frequency characteristics of the signal from 5-60 Hz at a signal amplitude of 330 μA, stimulation time 1 s. In separate series of the experiment, the dependence of the signal amplitude on the DA of the response was produced. In this design, only the signal amplitude was varied (from 198-330 μA with a step of 33 μA) with constant stimulation time and frequency indices (60 Hz, 60 pulses).

- Use of the FSCV method to study DA depletion/synthesis processes

This procedure is performed in order to study the processes of depletion and subsequent recovery of DA levels in neurons. It has been previously shown that strong prolonged stimulation depletes DA from the terminalia [245], we used a scheme with 10 s of VTA stimulation followed by DA release recording for 180 min [41]. For this purpose, after reaching a stable basal DA level (differences in the signal level of the last two stimulations not more than 10%), rats were subjected to a DA depletion procedure (10 s, 60 Hz stimulation, 60 pulses). The depletion protocol included three consecutive stimulations (330 μA, 60 Hz, 600 pulses), which were performed 1-2 s apart. Then, conventional stimulation (330 μA, 60 Hz, 60 pulses) was used at intervals of 1 min (14 stimulations), 5

min, and 10 min to follow the process of DA signal recovery. The obtained DA signal values are converted to % of background values (the average of the first two values before stimulation is taken as 100%).

- Using the voltammetry method to study the functionality of D2 autoreceptors

The D2 antagonist raclopride, which was administered (2 mg/kg) intraperitoneally (i.p.), was used to detect the functionality of D2 autoreceptors. The DA response was recorded within 90 min after administration of this drug.

- Histologic verification of the position of the recording microelectrode (WE)

After excision, tissue samples of the brain region of interest were fixed in 10% formalin solution for 24 hours, washed in running water for 20 minutes, and then incubated at +4°C in a 30% sucrose solution in 0.1 M phosphate buffer according to the method [325] for 48-96 hours. Brain tissue sample after treatment with cryoprotectant was frozen in a cryostat chamber in 7% sucrose solution in 0.1 M phosphate buffer. Cryostat slices 15 microns thick were obtained on a SleeMev cryostat microtome (Germany) at -18 °C. Slices were stained with hematoxylin-eosin according to the standard technique. Microscopy of slices was performed using an Axiostar plus microscope (Carl Zeiss, Germany).

- Equipment and electrodes and for the voltammetry procedure

We use the following equipment in the process of recording DA by voltammetry:

A voltammetric setup (Figure 11) consisting of a potentiostat unit that generates and maintains a set point potential (PS) (UNC Chemistry Electronics Shop, Chapel Hill, NC, USA). The PS unit is connected to the Headstage unit, to which a silver reference electrode and a carbon recording microelectrode are wired. Electrical stimulation of VTA with different electrical characteristics is performed with a stimulator (NeuroLog, DIGITIMER, England) using a stimulating electrode. The stimulator unit is also connected to PS, which in turn is connected to a personal computer with special software High Definition Cyclic Voltammetry (HDCV), NC, USA. This software controls the scanning parameters (potential supplied by the potentiostat), namely the shape and electrical (we use a triangular-shaped potential to detect DA -0.4 V to +1.3 V and -0.4 V) and temporal (400 V/s) characteristics of the potential, as well as the scanning time (9.3 ms) and the time between scans (100 ms) [326]. The DA signal obtained after electrical stimulation of the VTA was calculated in hardware using this program, which removes background non-Faraday currents due to electrochemical processes on the surface of the recording electrode during scanning and extracts the Faraday DA signal. To

reduce noise, which can significantly affect voltammetric measurements - the stereotaxis and electrodes are in a Faraday chamber.

We used a bipolar stimulating electrode (Plastics One, VA, USA) as the stimulating electrode. The electrode was trimmed to a length of 8.8 mm and the distance between the electrode legs was 1 mm.

The reference electrode was made of silver wire, which was covered with insulation with a diameter of 0.5 mm. Before the experiments, the insulation layer was removed to free the silver layer, for subsequent electrolysis under a constant current of 3A 5 V with the addition of 1H hydrochloric acid solution. As a result of electrolysis, due to the electrochemical reaction on the surface of this electrode there was a chlorination process with the formation of silver chloride coating (AgCl).

The recording microelectrodes were manually fabricated (Figure 13). We use a glass capillary with a diameter of 0.8 mm (A-M Systems, K-F Technology, USA) carbon fiber, which is sucked into the capillary using a surgical suction (Armed 7E-V, RF). The ready semi-finished product is placed in a puller (PC-100, Narashige, Japan), where it is stretched and the thus obtained blank is cut manually on a microscope using a scalpel. The length of the carbon fiber thus obtained averages 100 μ. Experimenters try to use electrodes with lengths from 80-100 μ because the length of the electrode is directly related to its sensitivity. The fabrication process of the recording microelectrode is shown in Figure 13. During the process, a wire connecting to the setup is placed in a glass capillary, which was previously coated with a liquid silver solution (Silver Print, USA) for better electrical conductivity.

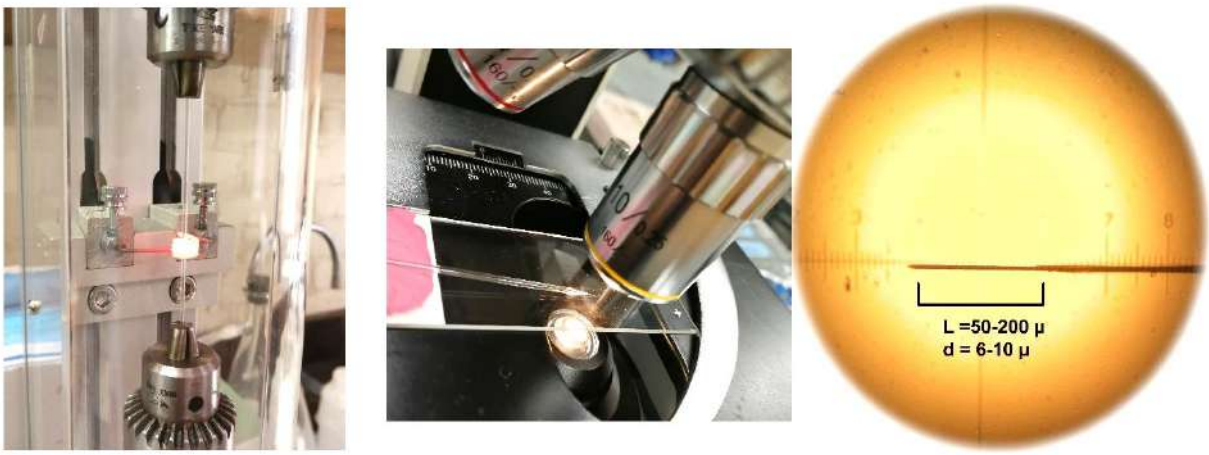


Figure 13. Fabrication process of a recording microelectrode. On the left - stretching of the electrode on the puller. In the center and on the right is the process of the electrode during and after trimming to the specified length. In the picture the electrode is 210 μ long and irregularly shaped, such an electrode is not suitable for experiments on rats as it will give off-scale due to high sensitivity.

- Calibration of recording microelectrodes (WE) in vitro

Since in the process of recording DA signal by FSCV method we use electrodes of different length, therefore, different sensitivity and as a result we get different DA signal. Thus, it is necessary to introduce an appropriate calibration factor to correct the obtained data. For this purpose, we use in vitro calibration of electrodes in DA solution with a given concentration according to the method [327]. The calibration setup was assembled manually (Figure 14) and consisted of a phosphate buffer solution supply unit (composition: KCl - 7.45g, KH2PO4 - 1.36g, d. H20 - 1L; Ph=7.4) and 10 μM DA solution. During the calibration process, the electrode was lowered into the buffer solution, then a syringe was used to uniformly deliver 10 μM DA solution for 5-10 s, and then the DA was washed off with the same buffer solution delivered from another syringe. The procedure was repeated until the maximum DA response of the electrode was reached, which was then recorded.

Figure 14 shows that the solutions (DA (10μM) and phosphate buffer) are fed into a mini compartment into which the electrode to be calibrated (WE) with a reference electrode (Ref) (Ag\AgCl) is lowered. During calibration for 5-10 s, the DA solution is first fed for 5-10 s, then washed off with buffer in the same way for 5-10 s. It is noteworthy that during calibration, the electrodes are always immersed in the buffer solution in order for appropriate electrochemical reactions to occur. The WE and Ref electrodes are supplied via the Headstage leads and maintained (using a potentiostat) at the respective potentials. The WE utilizes a triangular shaped potential required for YES detection of -0.4 V to +1.3 V and back compared to the Ref which is supplied with a constant known potential.

The DA response data obtained after feeding the DA solution, minus the background (Figure 12), is fed to a personal computer with the HDCV program installed. The figure shows such a DA response obtained after calibrating a 100 μ long electrode (Figure 14).

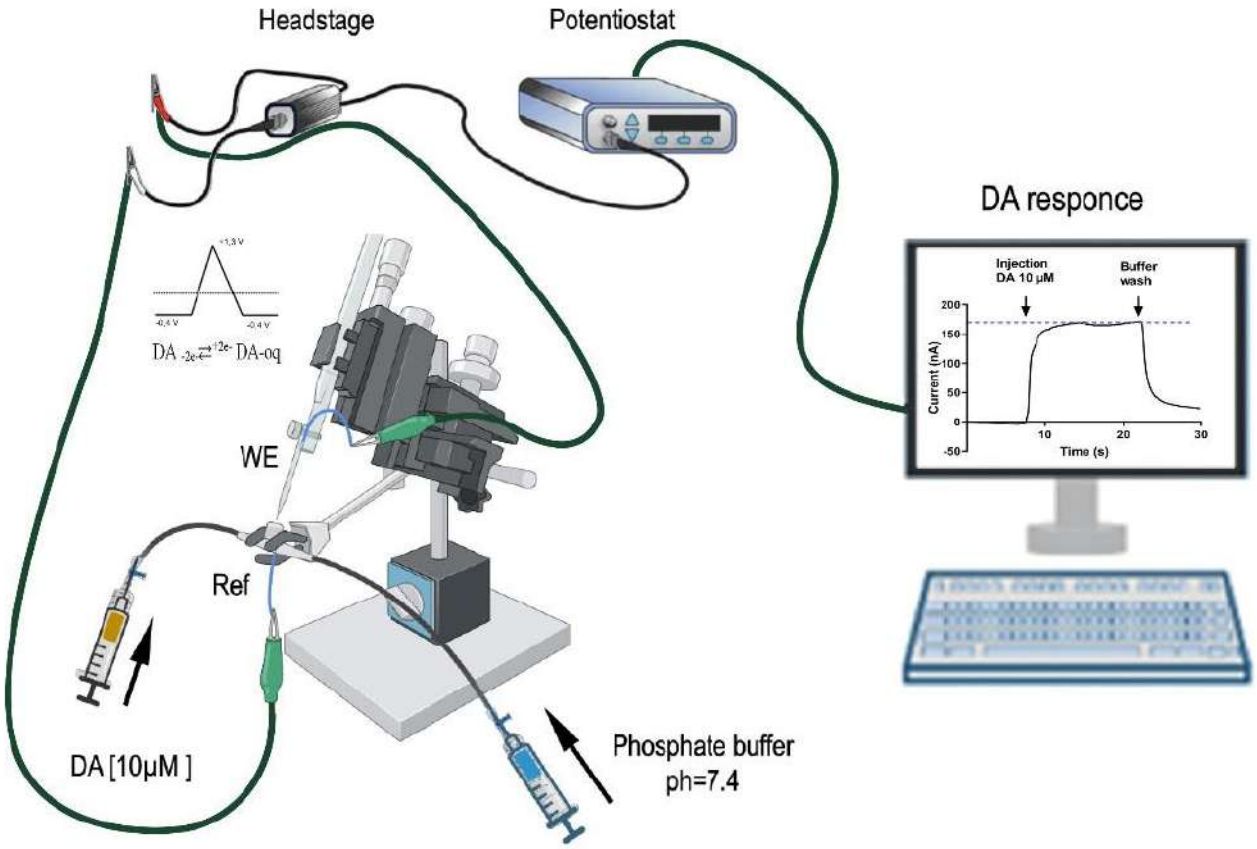


Figure 14. Schematic of the calibration setup.

Figure 15A shows the DA response of the electrode during the calibration process at the time the DA solution was applied. A significant DA response can change even real-time changes in the electrode potential, affecting the oxidation\reduction processes of DA, shifting them significantly toward oxidation (Figure 15B).

After finding the maximum DA signal at a given electrode, the values of the signal obtained in nA were converted to µM and the calibration factor was calculated per 1 µM of solution. That is, if the average DA response at the 90 µm long electrode = 233 nA, then the calibration factor when converted to 1 µM is 23.3.

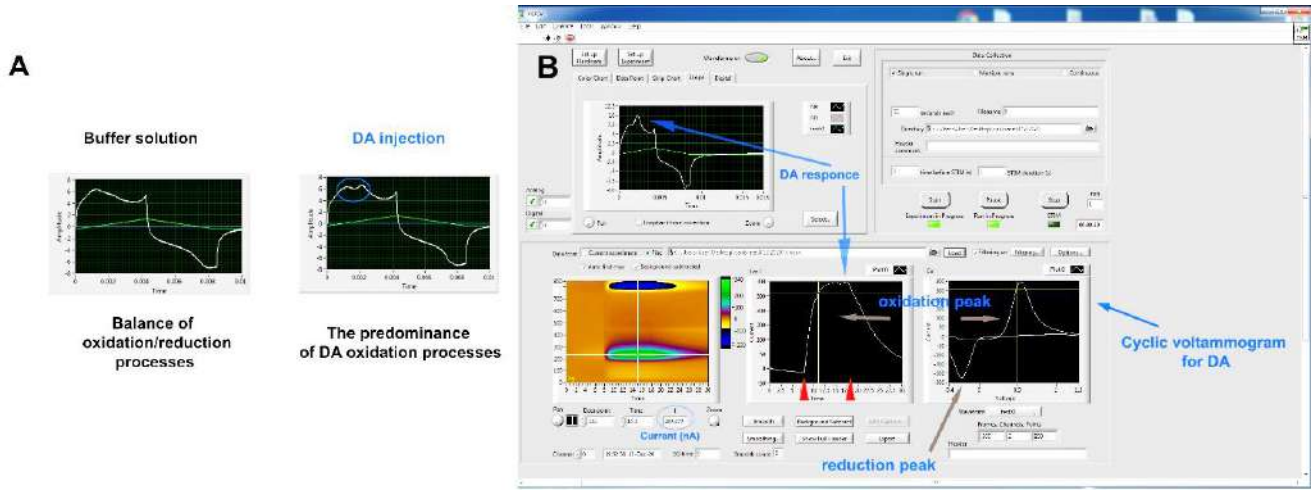


Figure 15. Display of the electrode calibration process on the computer screen. A - Change of the potential shape in the process of feeding DA solution to the carbon recording microelectrode before and after feeding DA solution. B - Cyclic voltammogram with oxidation (+0.6 V) and reduction (-0.2 V) peaks corresponding to electrochemical processes of oxidation and reduction of DA, as well as on the figure are DA responses shown on the graph and in the form of a color scheme and DA response expressed in the digital value of current.

Stress induce procedures

- Acute short-term stress

To create an acute uncontrolled stressor exposure, we used the procedure of unavoidable swimming in the Porsolt test (the testing procedure is described earlier); [43]. The literature shows the use of this procedure for modeling stress and depression-like symptoms [171].

- Acute and subchronic immobilization stress

To implement the procedure of acute and prolonged uncontrolled stressor exposure, we used the procedure of single (4h) and prolonged immobilization stress (4 h/ 5 days, daily (from 10.00 to 14.00)). Immobilization was performed in special pens. In experiments on exposure to subchronic (4 h/ 5 days) immobilization stress before and after the procedure, behavioral and neurochemical components of the stressor response were assessed [43].

These exposures have been used to model different components of posttraumatic stress such as acute stress disorder ASD [328] and depression with multiple stress exposures [192,193].

- Chronic mild stress (CMS)

To model everyday life stress (CMS) according to P. Wilner [170], male Wistar rats (n=80) were exposed to different, daily alternating exposures for 4 weeks. In our work we used the following types: food deprivation, water deprivation, cage tilt at 45 ºC, isolation, crowding, inversion of daylight hours, wet bedding. The application of these effects was varied in a pseudo-randomized order to create an uncontrollable situation. The duration of each exposure was 24 h. All animals were exposed at the same time [46]. The antidepressant bupropion (Aspen Bad Oldesloe GmbH, Germany) at a dose of 10 mg/kg animal weight for 10 days i.p. The control group was injected with saline solution of the same volume [47].

- Single Social Defeat Stress Procedure (SD)

The SD exposure experiments used mature Sprague-Dawley males (280-330g) as "intruders" who were caged to a larger and more aggressive "resident" Long Evans (>350g) to create a stable stressor environment during a 20-min SD session [20,34,35]. During the first and last 5-min segments of the experimental session (20 min), resident and intruder rats interacted via a wire cage. This cage allowed only visual, auditory, and olfactory perception of the aggressive subject. During a subsequent 10-min "free interaction" session, the defense cage was removed. During this time, the duration of all behavioral elements under study (clinch attack), defense behavior (defense), freezing (freezing - the rat does not move), running (running), exploratory behavior (exploring), submission posture (submission - the rat lies on its back), other (other - walking or standing still) were recorded. Control animals were given the opportunity to interact with an individual of their own species, from their own cage (conspecific) for 20 minutes [41].

- Social stress in acute organophosphate (OP) poisoning

The experiment included measurements of baseline behavioral parameters and three comparison groups: the "stress" group (rats in the same cage with acutely poisoned animals), the POX2x group (rats were administered paraoxon at a dose of 0.6 LD_{50} one hour after administration of paraoxon at a dose of 0.45 LD_{50}), and the CBDP group (rats were administered paraoxon at a dose of 0.6 LD_{50} one hour after administration of CBDP at a dose of 3.3 mg/kg). Observations of the animals were continued for 8 weeks after poisoning. To detect a depression-like and post-traumatic component in both the acutely intoxicated animals and the animals in the same cage with them in the "stress" group, a specific battery of behavioral tests was used, including measurements taken 8 weeks after a single poisoning, such as: cognitive test (novel object recognition), test for depression (Porsolt test); in addition testing of locomotor and other activities (see above) conducted daily and testing for 8 weeks, 18h per day (see LABORAS equipment).

Statistical methods of research

Statistical processing of the obtained materials was carried out in GraphPad Prism program version 6.05 - 8. The D'Agostino and Pearson normality criterion was used to calculate whether the values fit a normal Gaussian distribution. The unpaired two-sided Student's t-test (normal distribution) or the nonparametric Mann-Whitney U-test (non-normal distribution, small groups) was used to compare the two groups. One-factor One-Way ANOVA (normal distribution) or Kruskal-Wallis test (non-normal distribution, small groups) was used when comparing the three groups. For multivariate comparison, two-factor Two-Way ANOVA analysis of variance was used.

RESULTS AND DISCUSSION

Chapter 1: Study of the dopamine response using fast-scan voltammetry

Voltammetry is an effective method of studying various characteristics of DA response in the area of the nucleus accumbens (NAc) during electrical stimulation of the VTA area. Setting the optimal position of electrodes by stereotactic coordinates: recording (WE) and stimulating (SE) is necessary for any voltammetric studies (Figure 16).

Dependence of DA signal on the depth of electrode placement in the rat brain

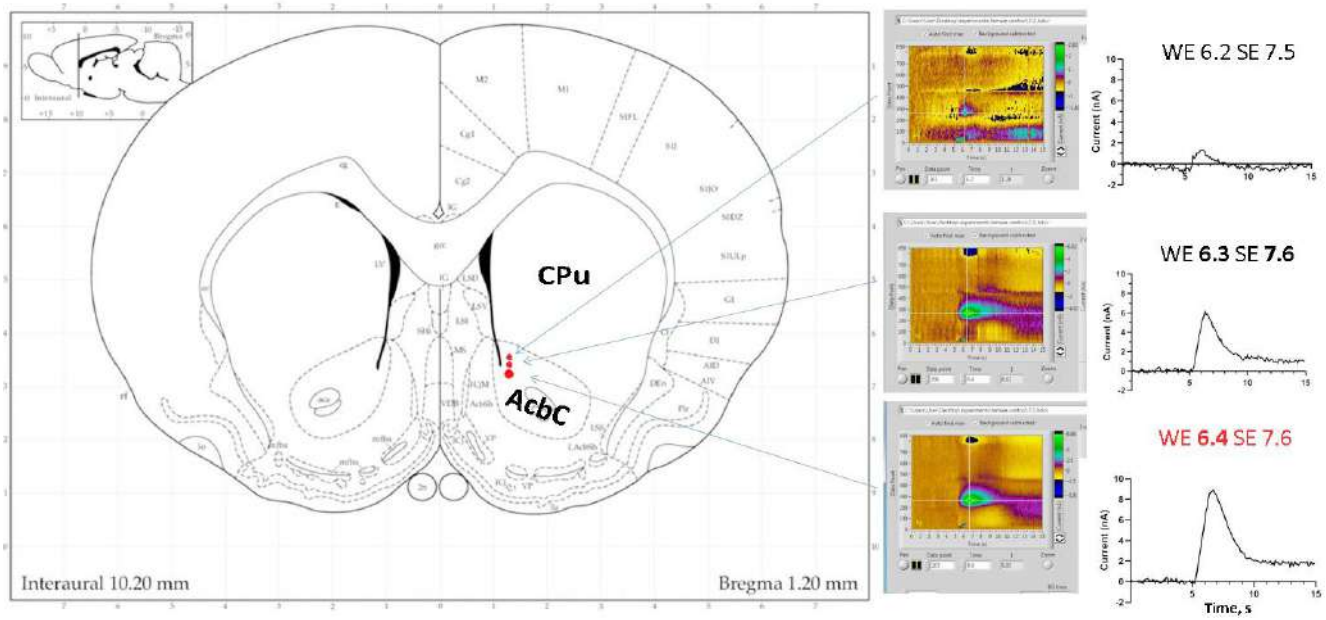


Figure 16. Dopamine (DA) response of neurons after electrode placement. In the figure, the recording electrode (WE) is positioned through the caudate nucleus (CPu) into the area of the nucleus accumbens core (AcbC), and the stimulating electrode (SE) into the ventral tegmental area (VTA). On the right are plots showing DA signals (schematics and color representation), and on the left are the position of WE motion in the stereotaxic atlas [324].

It is noteworthy that the lowering of these electrodes (WE and SE) did not occur all at once, but gradually to find the optimal maximum DA signal in a given area (Figure 16). In the process of searching for the DA signal, it is necessary to correctly position the electrodes so that the signal would be of the correct shape and magnitude characteristic of the animals of this group when the recording microelectrode is correctly positioned. The correct placement of the recording electrode is revealed by subsequent histological verification (Figure 37 D).

In Figure 16 we can see that within stereotactic coordinates of the nucleus accumbens we can observe a large scatter of signals in rats (group “control”), in addition, these signals are directly related

to the depth of placement of the recording electrode (WE), however, under the action of a stressor stimulus (group “stress”) - there is a change in DA signaling and there is an increase in DA signal at all depths within the limits of NAc, but there is a large scatter of values, which is probably associated with individual sensitivity of rats to stress.

In our laboratory, we have shown the dependence of the DA response on the depth of placement of the recording electrode (NAc area), (Figure 17).

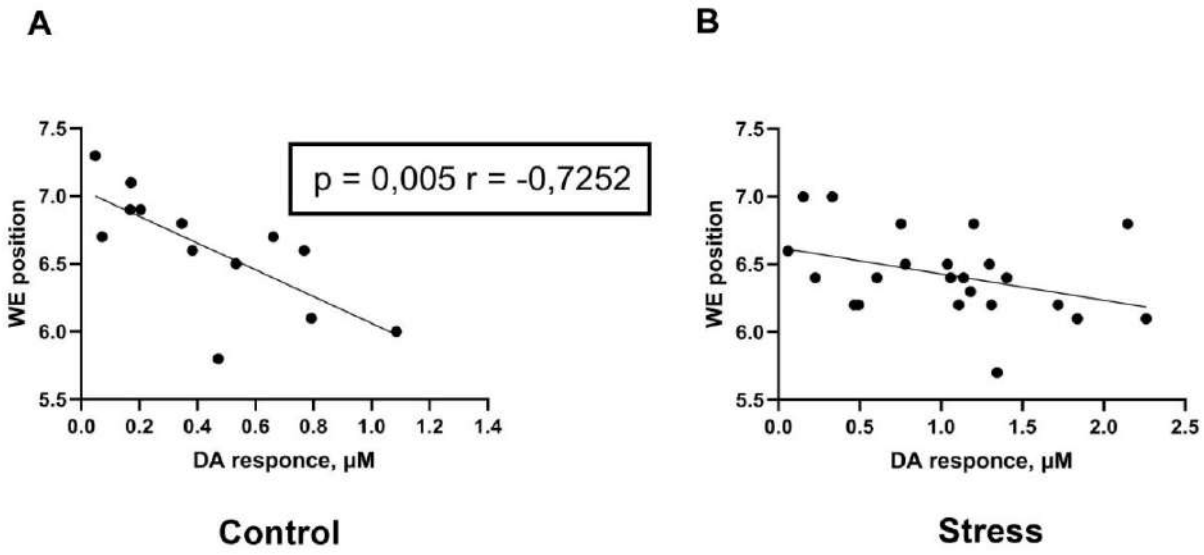


Figure 17. Correlation of DA response and electrode placement depth using the FSCV in vivo method. On the X-axis, DA response in µM; on the Y-axis, depth of lowering of the recording (WE), represented in mm from the brain. Pearson correlation analysis

In our laboratory, we have shown the correlation between rat mass and electrode placement depth (Table 1). This table is useful to find the optimal DA signal in the NAc in rats of different masses.

Rat mass, g	**Depth WE (from brain)**	**Depth SE (from brain)**
195±6.1 (n=5)	6.2±0	7.8±0.2
248.2±6.5 (n=12)	6.4±0	7.9±0.1
329.9±7.4 (n=11)	6.5±0	8.1±0

Table 1. Dependence of animal weight on the depth of electrode lowering. The table shows the masses of rats, g, as well as the depths of lowering of the main WE (recording), SE (stimulating) electrodes responsible for the appearance of DA signal (in this process also plays a role Ref (chlorosilver

reference electrode), however, the depth of lowering of this electrode does not affect the level of DA signal).

The works of our laboratory have shown the frequency and amplitude dependence of the signal in the area of the NAc during VTA stimulation [41,241]. Pearson correlation analysis for Wistar rats (n=8) showed a positive correlation between DA response and frequency of electrical stimulation (r=0.904, p=0.051) (Figures 18).

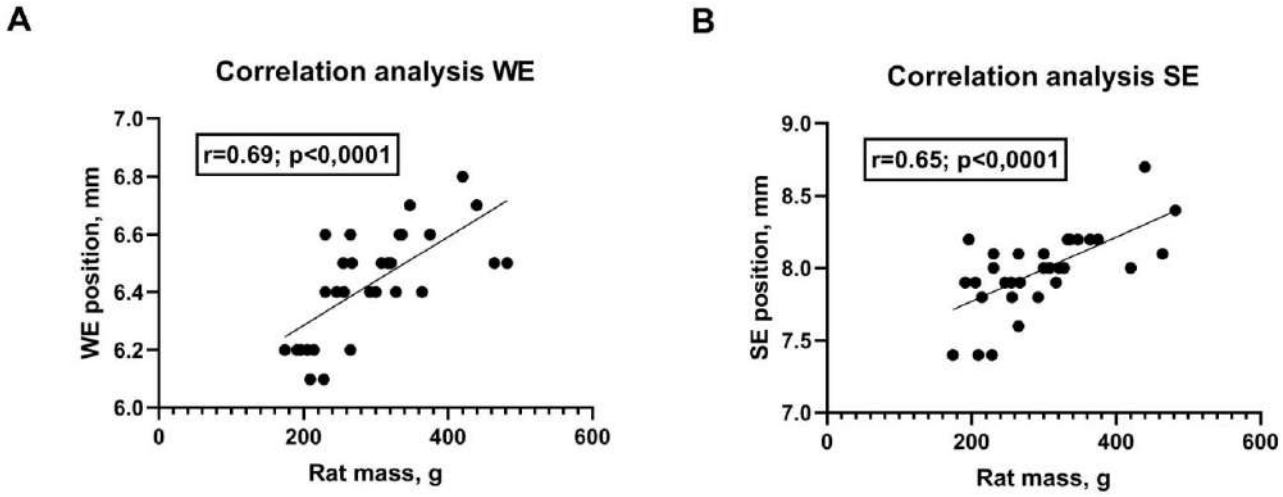


Figure 18. Pearson correlation analysis for Wistar rats (n=8). X-axis, rat weight, g; Y-axis, position of recording (WE) (A) and stimulating (SE) (B) electrodes represented in mm from the brain. FSCV in vivo method.

Previously, the linear frequency dependence of DA signal in the VTA area was shown in the works of Mark Whiteman, the ancestor of fast scanning voltammetry [245]. In this work, both frequency and amplitude dependence of the DA signal in the NAc zone during stimulation of the VTA zone with different current characteristics in anesthetized rats is presented (Figure 19).

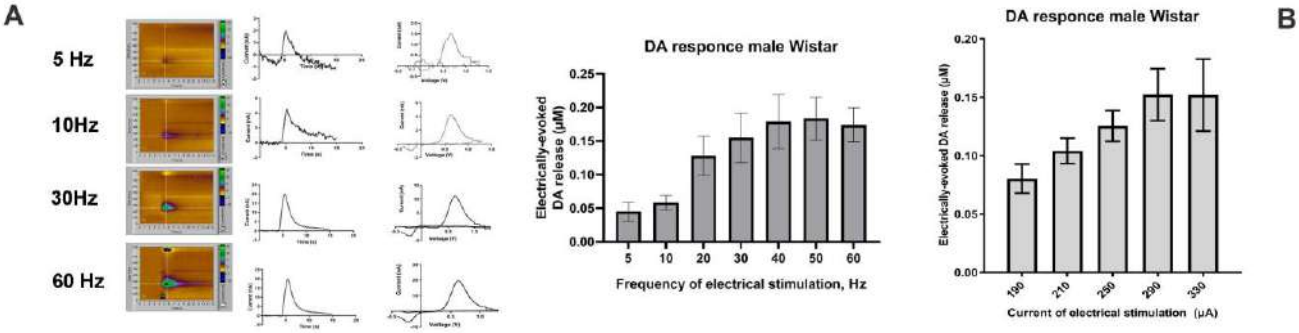


Figure 19. Dependence of DA signal on different current characteristics. A - Color representation of DA signal. B - DA response in rats depending on electrical stimulation of different frequency (5-60

Hz) at current strength of 330 μA and at different amplitude characteristics of current (190-330 μA) at electric current frequency of 60 Hz.

Figure 20 shows that the DA level does not depend on the injection duration, but the most accurate (with less noise) will be the calibration with a standard injection duration of 9-10 seconds. Figures 20C and F show the dependence of the DA response on Ph buffer, thus a buffer with Ph = 7.4 is most adequate for calibration. Figure 20C shows the dependence of the DA response on the length of the recording electrode, a significant positive correlation (P<0.0001, r=0.9) was found, thus the DA signal is directly related to the length of the electrodes used, but having the average values of the DA signal when using electrodes of known length it is possible to approximately convert nA to μM using a known formula without calibrating the electrode. It is noteworthy that the use of different DA concentration does not affect the positions of DA oxidation/reduction peaks on the voltammogram, only their magnitudes (Figure 20), however, when using buffer with different Ph, the peaks are shifted. Thus, the Ph of the medium is an important characteristic in recording the DA response. This remark was also made by other authors [329,330].

Calibration of recording electrodes (WE) in vitro

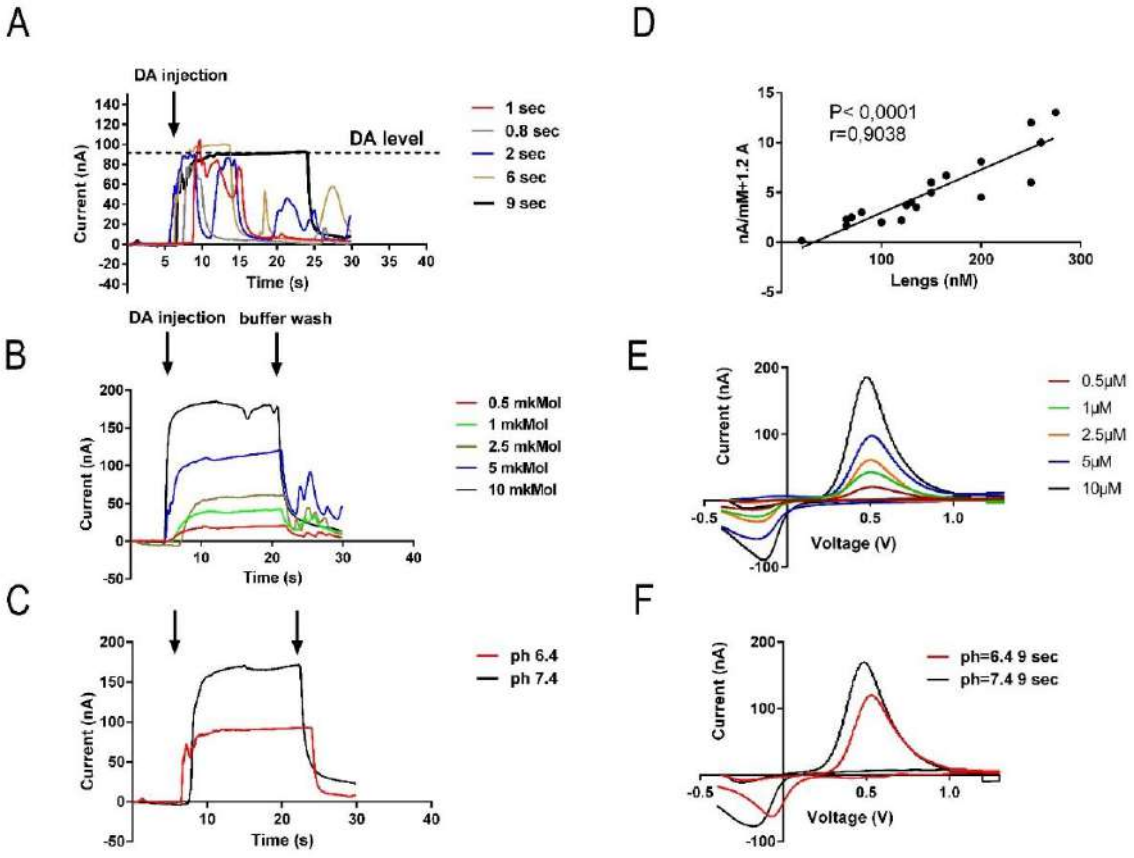


Figure 20. Dopamine (DA) signal, nA obtained during calibration of electrodes with different characteristics. A - dependence of the DA response on the duration of injection (0.8 - 9 s) of DA

solution with a concentration of 10 μM. B - DA response obtained by DA injection for 10 s. C - DA response at 10 s of DA injection using phosphate buffer of different concentrations. D - dependence of DA response on the length of the recording electrode, nM. E, F - corresponding voltammograms at 10 s of DA injection.

Dependence of DA signal shape on the parameters of DA release/reverse capture

- DAT knockouts as a model of stereotyped behavior

In rats and mice, the processes of DA release/reuptake are in a state of equilibrium; however, in DAT knockout rats (KO) with absent dopamine transporter (DAT) and, as a consequence, significantly reduced DA reuptake, the values of DA reuptake differ more than 20-fold from those observed in wild-type rats (DAT WT). Likewise, DAT KO knockout rats in contrast to wild-type (WT) rats show marked locomotor and stereotypic activity in the locomotor box P=0.016; Unpaired t-test (Figure 21).

As we know from the literature that the main characteristic of the DAT knockout phenotype is pronounced hyperlocomotion [271] being the cause of almost complete absence of DA reuptake in DAT knockout animals. Increased stereotypic activity is often observed in rodents during stressor exposure [44]; however, in this model, the authors noted pronounced stereotypy and compulsive behavior in DAT knockout animals [270]. Since the DA system is directly involved in the activation of stereotypic behavior, as shown by experiments with amphetamine administration [278], such behavior would be expected in DAT knockouts, as we have seen in our study. A voltammetry study by other authors has shown that reuptake in DAT knockout mice is 300 times slower than in wild-type animals [271]. We investigated these processes in DAT knockout (KO) rats and wild-type (WT) animals using voltammetry and behavioral techniques (locomotor boxes) and obtained similar data.

Thus, we can observe that the voltammetry method (FSCV *in vivo*) allows us to clearly see the difference in the release and recapture processes in animals (WT and KO), and in combination with behavioral techniques allows us to trace causal relationships in animal behavior and their brain neurochemistry.

Figure 21 clearly shows that almost complete absence of DA reuptake in rats knocked out by the DAT gene, detected using the voltammetry method, leads to hyperlocomotion and increased stereotypic behavior in contrast to wild-type animals.

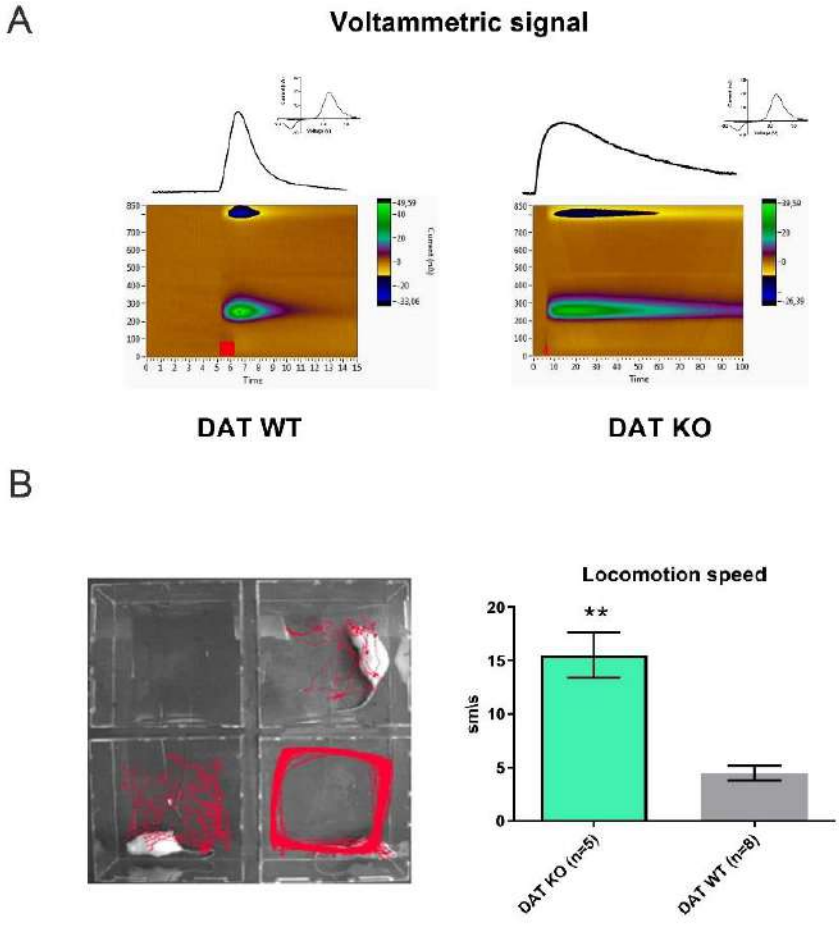


Figure 21 Features of behavior and DA neurotransmission in DAT KO rats and wild-type (WT) animals. A - voltammetric DA signal in WT DAT rats (left) and DAT KO rats (right) after 1 s of electrical stimulation of 330 μA 60 Hz 60 Pulses (representative signal display). B, Locomotor activity of rats during 10 min in locomotor boxes.

- Comparison of indices of DA dynamics in mice and rats in the background and under the influence of different pharmacological substances using the voltammetry method

Under the action of raclopride (2 mg/kg), DA release parameters increased 1.5-2-fold in mice without changes in the parameters of recapture (Figure 22). Since raclopride, being a D2 antagonist, temporarily blocks D2 autoreceptors regulating tyrosine hydroxylase synthesis, the DA content in the synapse - increases, which we can observe in our results. Exposure to GBR (10 mg/kg) administered 30 min after raclopride administration increases 4-fold DA release and 5-6-fold decreases DA reuptake in mice. Since GBR 12909 temporarily turns off DAT functions, the underlying mechanism regulating DA reuptake at the synapse becomes temporarily suspended (figure 22).

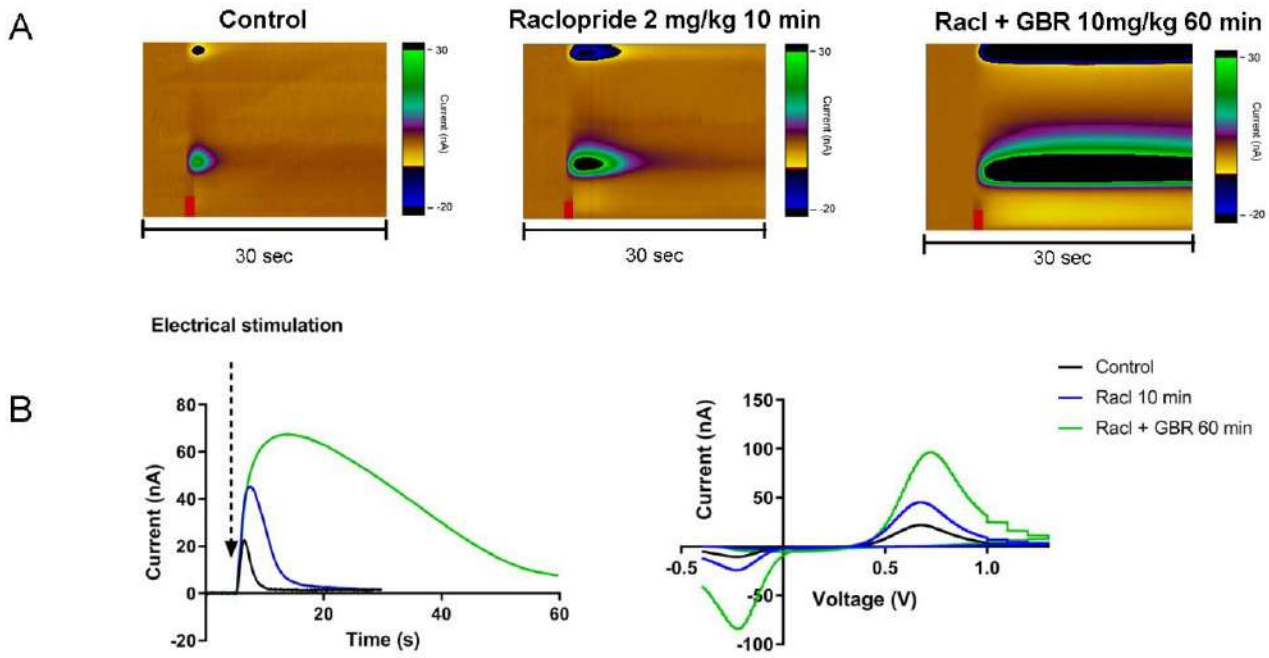


Figure 22 Representative mapping of DA signal in mice after VTA stimulation (330 μA, 60 Hz, 60 Pulses, 1 s) in the NAc area before and 10 min after administration of 2 mg/kg raclopride and 60 min after administration of 10mg/kg GBR. Part (A) depicts color plots (Color Plots) of voltammetric signals, with the red square representing the onset of electrical stimulation (1 s). Part (B) shows DA responses (nA) and cyclic voltammograms in mice showing the main electrochemical parameters of DA signal (oxidation peak 0.6 V; reduction, 0.2 V) in control, 10 min after raclopride administration and 60 min after GBR administration.

From Figure 23A we can see that, however, in rats, the dynamics of raclopride are very different from those in mice (Figure 23), even at the same doses we can observe a significantly ($P<0.0001$, two-way ANOVA with repeated measures) less pronounced effect in mice, in contrast to rats.

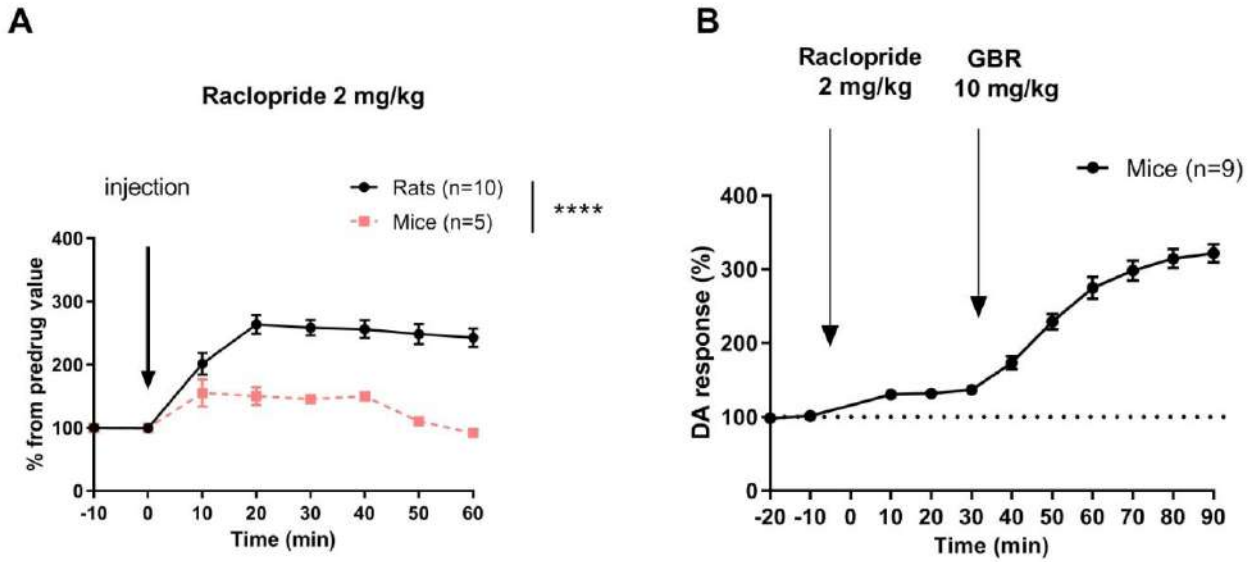


Figure 23. Dopamine response in mice and rats after i.p. administration of raclopride at a dose of 2 mg/kg and GBR at a dose of 10 mg/kg. On the x-axis is time, in min. Y axis - DA response, % of baseline values. A - DA response in mice and rats after administration of raclopride at a dose of 2 mg/kg. B - DA response in mice (n=9) after sequential administration of 2 mg/kg raclopride and 10 mg/kg GBR. Data are presented as mean ± SEM, two-way ANOVA with repeated measures; **** - P < 0.0001.

According to the literature, raclopride and GBR 12909 have different effects when administered i.p. to rodents. GBR 12909 by mechanism of action is a selective DA reuptake inhibitor acts like cocaine but more selectively, temporarily blocking DAT function. When administered to animals, a dose-dependent prolonged behavioral activation characterized by increased locomotion, rearing, sniffing and various other stereotypies with increasing dose was found; “reinforcing” various behaviors effects of GBR 12909 administration have been shown [331]. The neurochemical profile from administration of GBR 12909 using medium to high doses is similar to that of DAT knockouts as shown by voltammetry and microdialysis studies [246,332], but when administered intravenously, even lower doses result in prolonged inactivation of DAT in rats [333]. Raclopride acts as a selective antagonist of D2 atoreceptors, also increasing DA concentrations in the synaptic cleft, but not affecting DAT, which was shown by microdialysis and voltammetry methods [41,332,334]. By mechanism of action, raclopride is more selective than haloperidol and also has an antipsychotic effect [334].

In our study on mice, GBR 12909 was administered 30 min after administration of raclopride, while raclopride had already stopped acting on the DA response in mice, and there was also a group of mice and rats without GBR 12909 but with raclopride. Thus, we were able to investigate the effect of GBR 12909 and raclopride separately by voltammetry and as in the articles of Prof. E.A. Budygin,

given on rats at a dose of 20 mg/kg [246], we showed a significant and persistent (up to 2 hours) increase in DA response under the action of GBR 12909. However, in our study we used a dose 2 times lower (10 mg/kg) than in the studies of Prof. E.A. Budygin and another kind of animals (mice) and, therefore, we saw a slightly less pronounced increase in DA response (300% rather than 500%), but we also obtained a significant increase in DA response 60 min after administration. We also found significant ($P < 0.0001$) interspecies differences in DA response when raclopride was administered at the working dose (2 g/kg) between mice and rats. Additional studies by other methods are required to reveal the cause-and-effect relationship of such changes; different sensitivity of rats and mice to raclopride of a given dosage, as well as different distribution of D2 autoreceptors in the region of the NAc of the brain of rats and mice could contribute to such changes in DA response.

Thus, based on these two studies, we can conclude that the voltammetry method (FSCV in vivo) is a reliable method that is effectively used to record the instantaneous extracellular DA response in rodents. With the help of this method it is also possible to study DA neurotransmission in the NAc in DAT knockouts and other animals with altered DA neurotransmission, as well as to carry out pharmacological manipulations affecting the level of cerebral DA. However, for such registration it is necessary to take into account different electrochemical peculiarities of used materials, as well as complexities of surgical and stereotactic manipulations. It is most effective to use the voltammetry method in combination with behavioral techniques to identify cause-and-effect relationships between brain neurochemistry and behavioral activity, which will also be reflected in the following chapters of the dissertation.

Chapter 2. Neurochemical bases of alcoholic and aggressive behavior

Alcohol consumption in Wistar rats

To create alcohol dependence, a protocol was used to habituate Wistar rats (n=10) to a sweet solution and then to an alcohol solution (see the alcohol-mating test in the methods section). A standard two-bottle test was used. One bottle contained water and the other contained a sucrose solution (10%), which was gradually replaced with a 10% ethanol solution over a period of 2.5 months. Presentation of the solutions and measurement of consumption parameters occurred 2 times per week. After reaching stable consumption parameters of the 10% ethanol solution, the single social defeat procedure (SD) described previously was applied (figure 24).

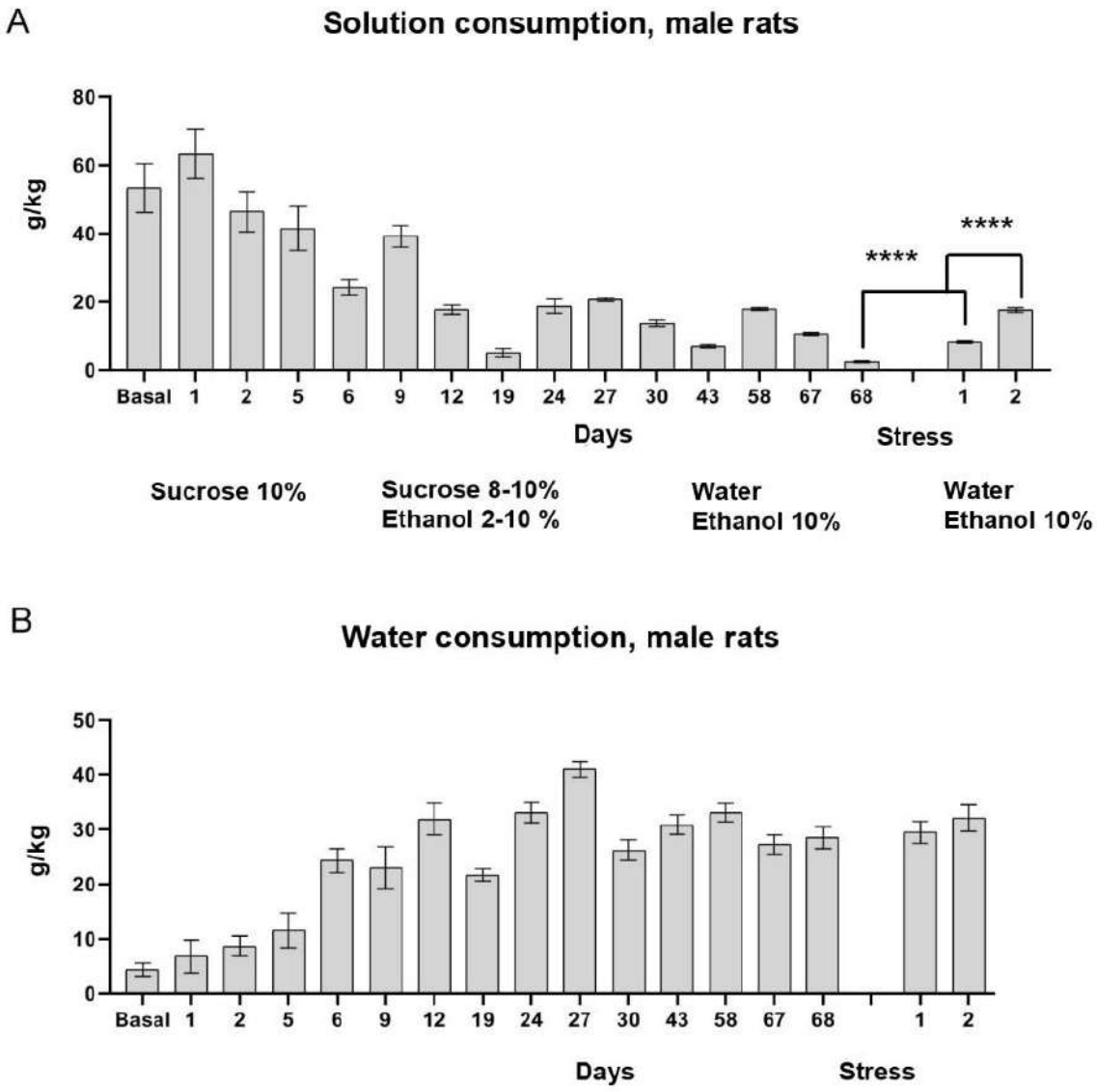


Figure 24. Solution consumption of Wistar rats (n=10) for 70 days using a two-bottle regimen. The 10% sugar solution was gradually replaced with 10% ethanol/water solution during alcoholization of

rats. Figure A shows the consumption of solutions (sugar/alcohol) in animals for 68 days, followed by 2 days after a single social defeat stress. Figure B shows water consumption. On the X axis is the time of the experiment, days, and on the Y axis is the consumption of solutes, g. The solute consumption data were converted to kg of animal weight. Data are presented as mean ± standard error of the mean; Unpaired t-test; **** - $P < 0.0001$.

After reaching stable parameters of 10% ethanol solution consumption (Figure 24), a single social stressor procedure was applied. A significant reliable increase (Unpaired t-test; $P<0.0001$) in 10% alcohol consumption was found in animals at 2 days post-SD compared with values one day before stress. No significant differences were found in water consumption of rats before and after stressor exposure. It was also shown that ethanol solution preference decreased in rats with decreasing sugar concentration in solutions.

As a result of this study, we can conclude that Wistar rats are effectively exposed to alcoholic coping, which has also been shown in the literature [335]. As shown in various animal studies, stress can serve as a trigger for increased alcohol consumption [35], which was also shown in our data.

Thus, according to the results of this experiment, a single social stress provokes alcohol consumption in male Wistar rats.

Sweet solution consumption by TPH2 KO rats

Consumption of a 10% sugar solution compared with water in a two-bottle test paradigm was measured in TPH2 KO and WT animals (n = 6 per group) over an 8-day period. A significant (Two-way ANOVA, $F (1, 40) = 65.77$; $P<0.0001$, repeated measures) increased consumption of sweet solutions and a less pronounced increased consumption of water (Two-way ANOVA, $F (1, 40) = 8.671$; $P=0.0054$, repeated measures) was shown in TPH2 KO animals compared with wild type (WT). On the first day, TPH2 KO animals compared with WT animals significantly drink more water (Two-way ANOVA, $P=0.04$; Sidak multiple comparisons), but on the following day, probably due to decreased neophobia and increased habituation to sweet solutions, there is an increase in sugar intake already consumed by TPH2 KO animals compared with WT. TPH2 KO rats compared with the WT significantly more drink 10% sugar solution, on the 4th, 5th, and 7th day (Two-way ANOVA, $P\leq0.02$; multiple Sidak comparisons); on the 8th day these differences between TPH2 KO and WT animals in consumption of sweet solutions reach maximum values (Two-way ANOVA, $P=0.0009$; multiple Sidak comparisons) (figure 25).

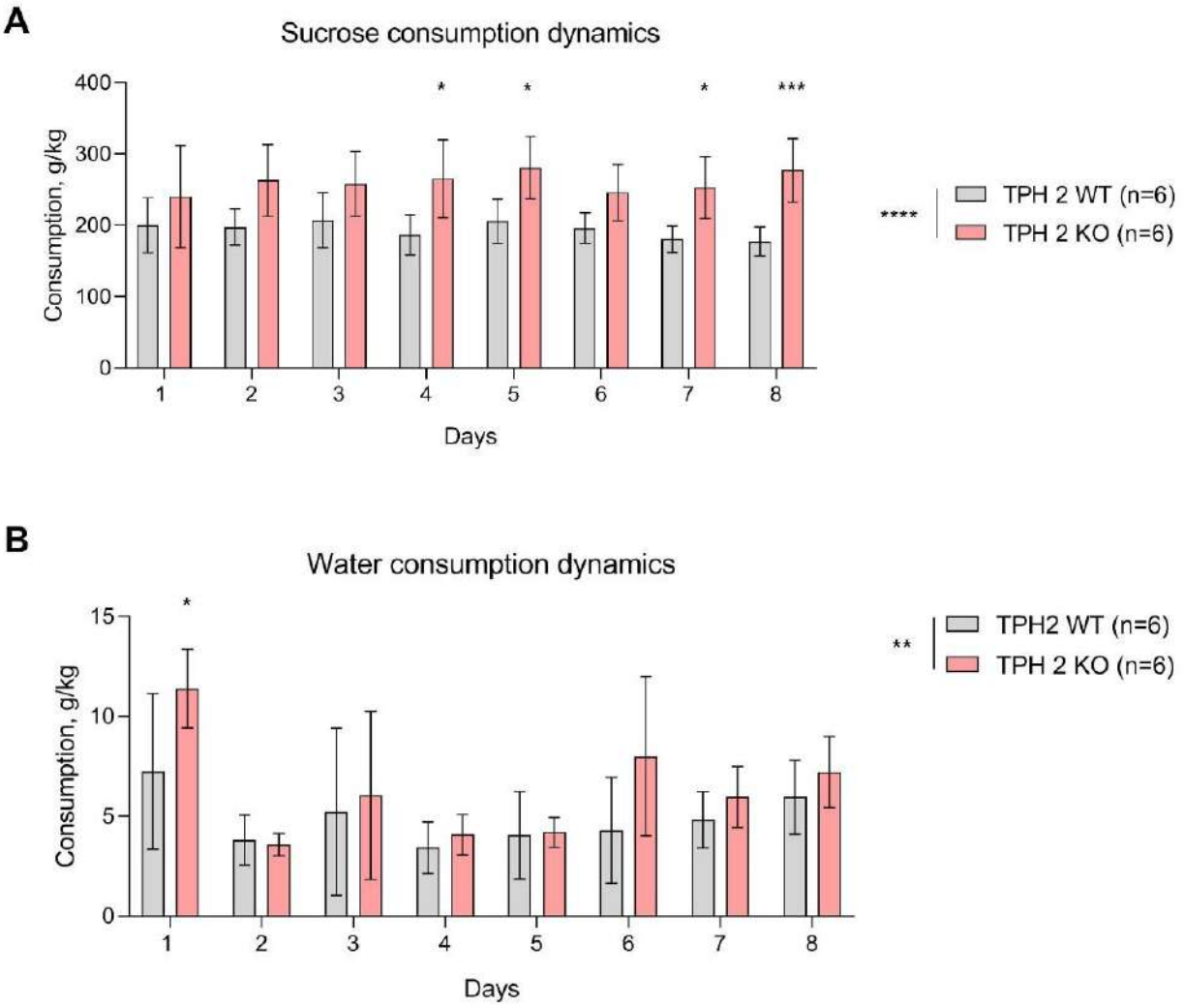


Figure 25. Results of the two-bottle test (sucrose 10%/water) in TPH2 KO and WT rats. Graphs of consumption (A) of sucrose solution (10%) and water (B) by TPH2 KO and WT animals during 8 days. X-axis - consumption time, days, Y-axis - consumption of solutions, g of solution/kg of animal weight. Data are presented as mean ± standard error of the mean; * - P≤0.04; ** - P=0.005; *** - P=0.0009; **** - P<0.0001; Two-way ANOVA.

Investigation of alcohol consumption in TPH2 KO rats before and after stress

To induce alcohol dependence in TPH2 KO and WT rats, a standard protocol was used with rats being habituated to a sweet, then to an alcoholic solution (see the "alcohol drinking test in the methods section). After habituating rats to alcohol consumption (8% ethanol + sugar 2%) / water, animals were subjected to an acute uncontrolled stress (4 h immobilization stress) procedure (IS).

During 100 days of alcohol consumption, TPH2 KO rats were more susceptible to alcoholization, in contrast to wild-type animals, as reflected in the characteristics of sugar-alcohol

preference (P=0.03; Two-way ANOVA) and total sugar-alcohol consumption (P<0.0001; Two-way ANOVA) (Figure 26) and feed consumption (P=0.008; Two-way ANOVA) (Figure 28). There was a significant increase (P=0.03; Two-way ANOVA; Sidak multiple comparisons) in the consumption of 10% sugar solution on day 6 by KO rats compared to WT (Figure 27).

As a result of the effect of IS, TPH2 gene knockout (KO) animals, in contrast to wild type (WT), showed an increase in preference for alcohol solution (8% alcohol 2% sugar) on the 2nd and 3rd day (P=0.008; nonparametric Mann-Whitney U-test) after stress compared to pre-stress values (Figure 26).

Likewise, in TPH2 KO rats at the 1-st (P=0.031; nonparametric Mann-Whitney U-criterion), 2nd (P=0.0159; nonparametric Mann-Whitney U-criterion) and 3rd (P=0.0079; nonparametric Mann-Whitney U-criterion) day after stress, as well as on the 3rd day in WT rats (P=0.0152; nonparametric Mann-Whitney U-criterion) consumption of alcohol solution (8% alcohol 2% sugar) was more pronounced compared to the values before stress (figure 26).

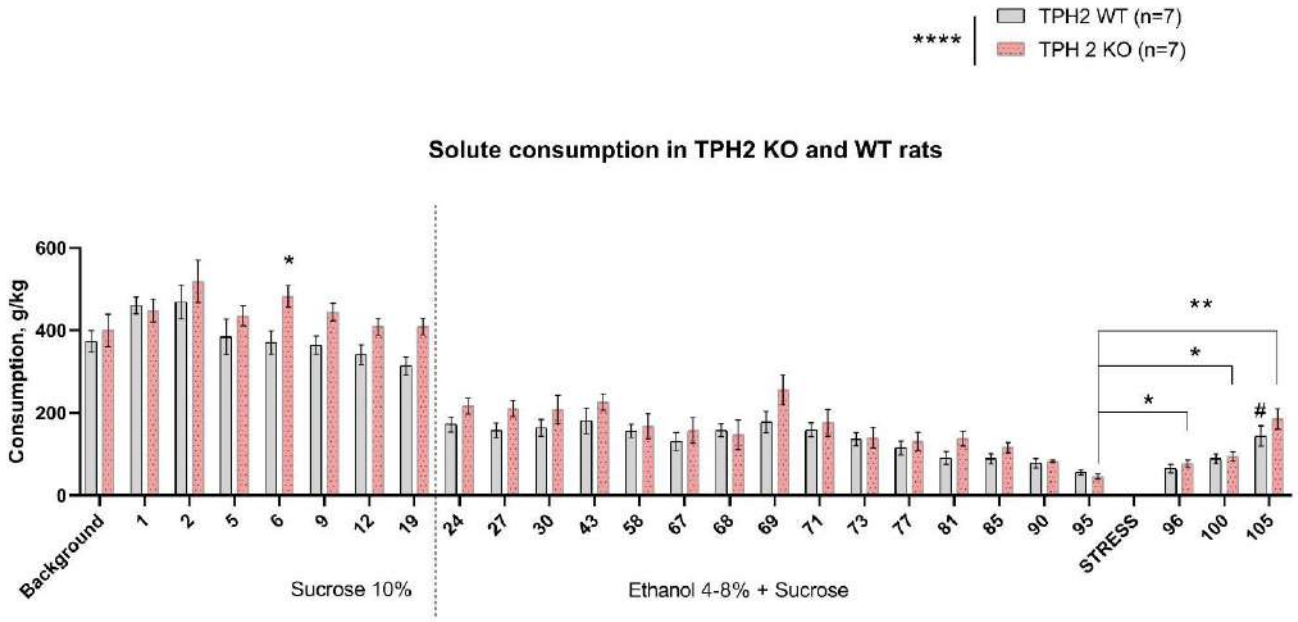


Figure 26. Results of solution consumption by rats TPH2 KO and WT. X axis - consumption of solutions by animals TPH2 KO and WT, days; Y axis - consumption of sugar-alcohol solution to water, g/kg. Solute consumption was calculated using the formula (solute consumption, g/rat weight, kg × 1000). Data are presented as mean, % ± standard error of the mean. Data KO (* - P≤0.03; ** - P=0.0079) and WT (# - P=0.0152) compared with values before stress (95 days); nonparametric Mann-Whitney U-test.

No statistically significant changes in water consumption (Figure 27) were observed between TPH2 KO and WT rats; however, stress caused a significant decrease in feed intake (Figure 28) in

animals of both groups: WT (P=0.0022; non-parametric Mann-Whitney U-test) and KO (P=0.0079; non-parametric Mann-Whitney U-test) on the 1st, 2nd and 3rd day after stress.

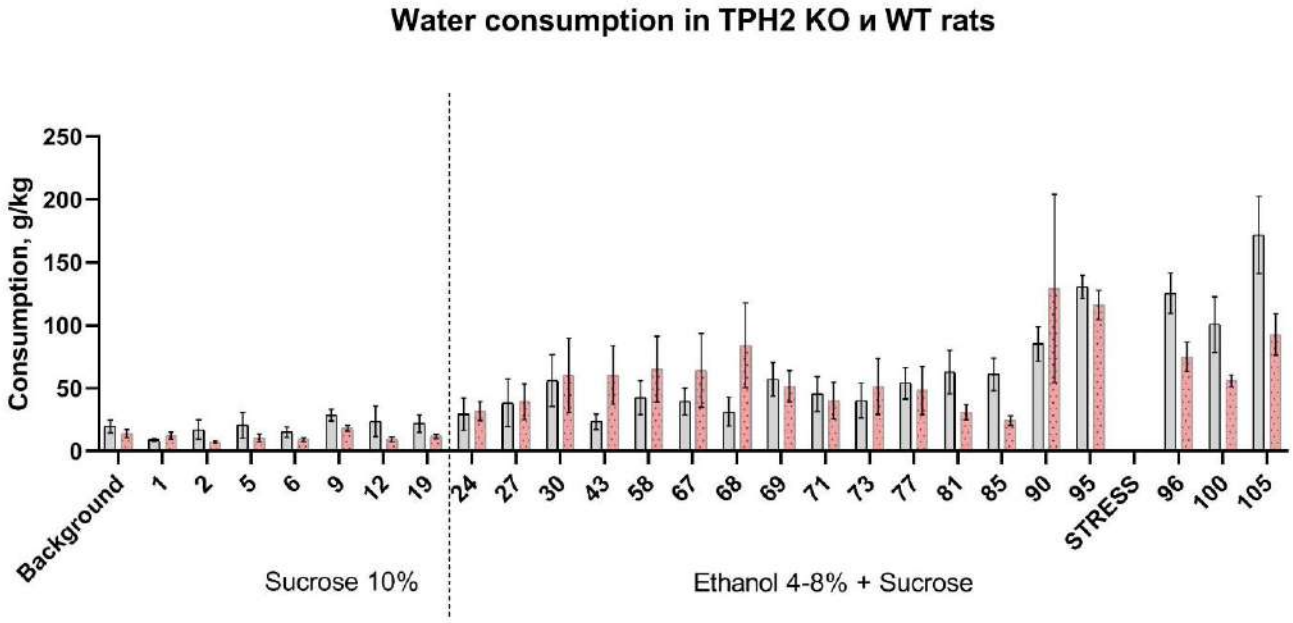


Figure 27. Results of water consumption by TPH2 KO and WT rats. X axis - water consumption of TPH2 KO and WT animals, days; Y axis - water consumption, g/kg. Water consumption was calculated using the formula (water consumption, g/rat weight, kg × 1000). Data are presented as mean, % ± standard error of the mean.

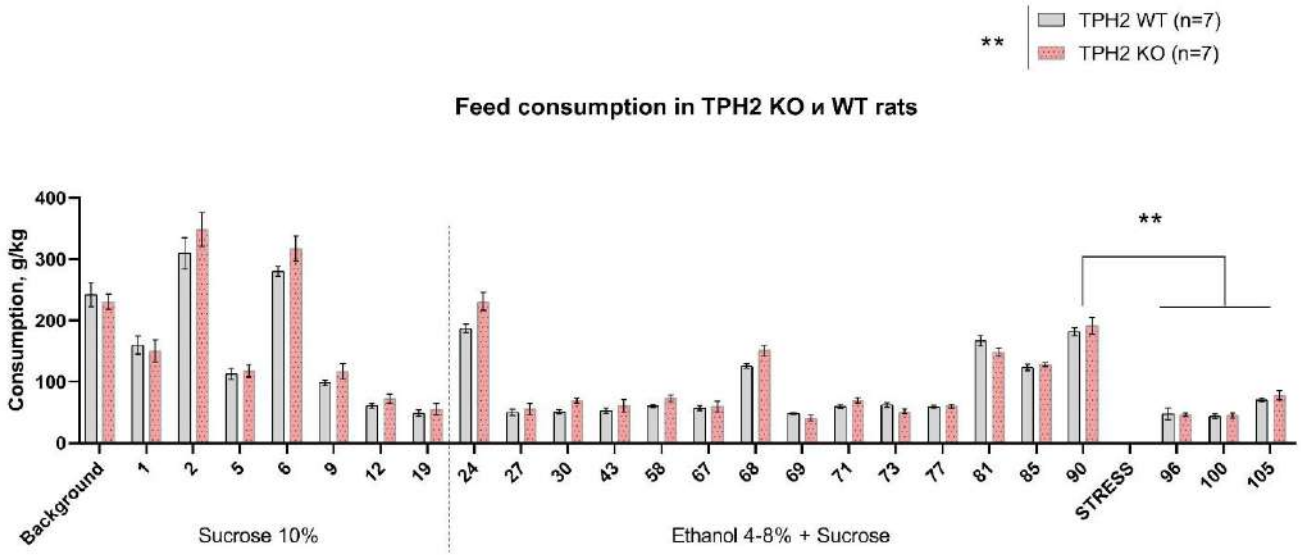


Figure 28. Results of feed consumption by TPH2 KO and WT rats. X axis - feed intake of TPH2 KO and WT animals, days; Y axis - feed intake, g/kg. Feed intake was calculated using the formula (feed intake, g/rat weight, kg × 1000). Data are presented as mean, % ± standard error of the mean. KO and

WT data on 1,2,3 days after stress compared with pre-stress values (95 days) ** - P ≤ 0.008; Mann-Whitney nonparametric U-test.

There were no differences in body weight changes throughout alcohol consumption between KO and WT groups and before and after stress (Figure 29); however, during the consumption of 10% sugar solution and then sugar-alcohol solution (6% sugar/ 4% alcohol), there was a statistically significant decrease in body weight during the first 30 days of the study (Two-way ANOVA; F (15, 159) = 1.899; P=0.0268) in KO animals compared to WT, probably due to the process of active consumption of sweet alcohol solution.

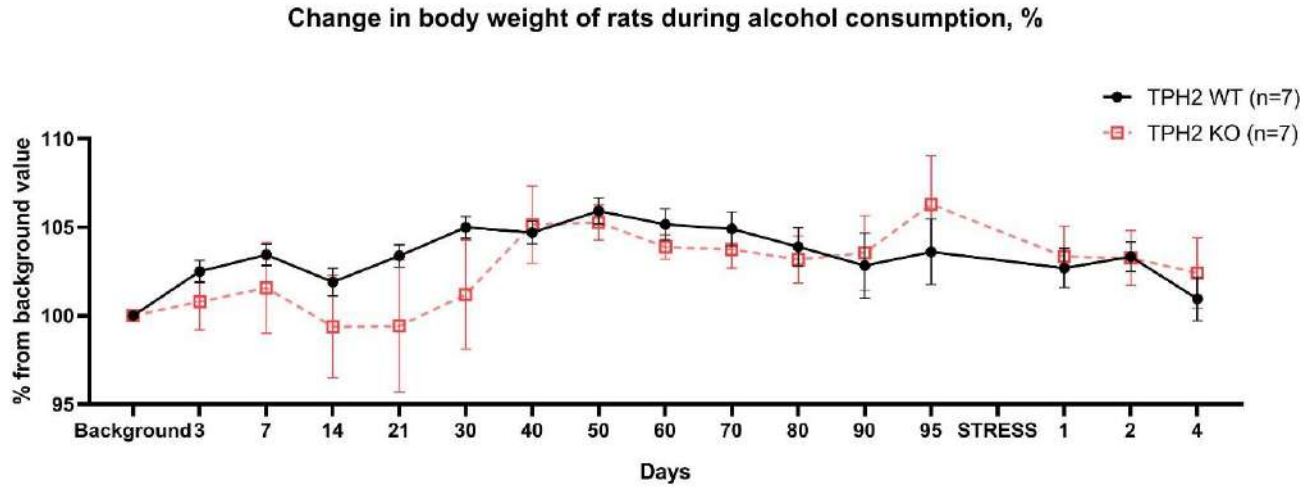


Figure 29. Dynamics of body weight changes in TPH2 KO and WT rats during alcohol consumption. X axis - change in body weight of animals, in contrast to background values taken as 100%. Y axis - time, days. Data are presented as mean, % ± standard error of the mean. * - P = 0.0268, Two-way ANOVA.

Thus, it can be concluded that TPH2 gene knockout rats are significantly more susceptible to alcohol dependence compared to the wild type. A single immobilization stress affected all animals, especially on the 3rd day after stress, but stress-induced alcoholism developed to a greater extent in TPH2 KO animals compared to the wild type. These changes are possibly related to the neurochemical features of TPH2 KO, which will be described in the next chapter.

Examination of DA neurotransmission in TPH2 KO rats

Based on the data obtained (Figure 30), we can conclude that in TPH2 KO rats, unlike wild-type rats, there are no differences in the functioning of D2 autoreceptors in the zone of the NAc. There are no differences in the parameters of DA response/reuptake in the area of the NAc during tonic and phasic VTA electrical stimulation. However, a significant decrease in mesolimbic DA recovery time after prolonged electrical stimulation was found (P = 0.0349, Two-Way ANOVA with repeated measures). These neurochemical differences, along with significant differences in SER neurotransmission (more than 80% absence of SER neurotransmission in the brain of TPH2 KO rats), may reflect behavioral abnormalities, which are shown in subsequent chapters.

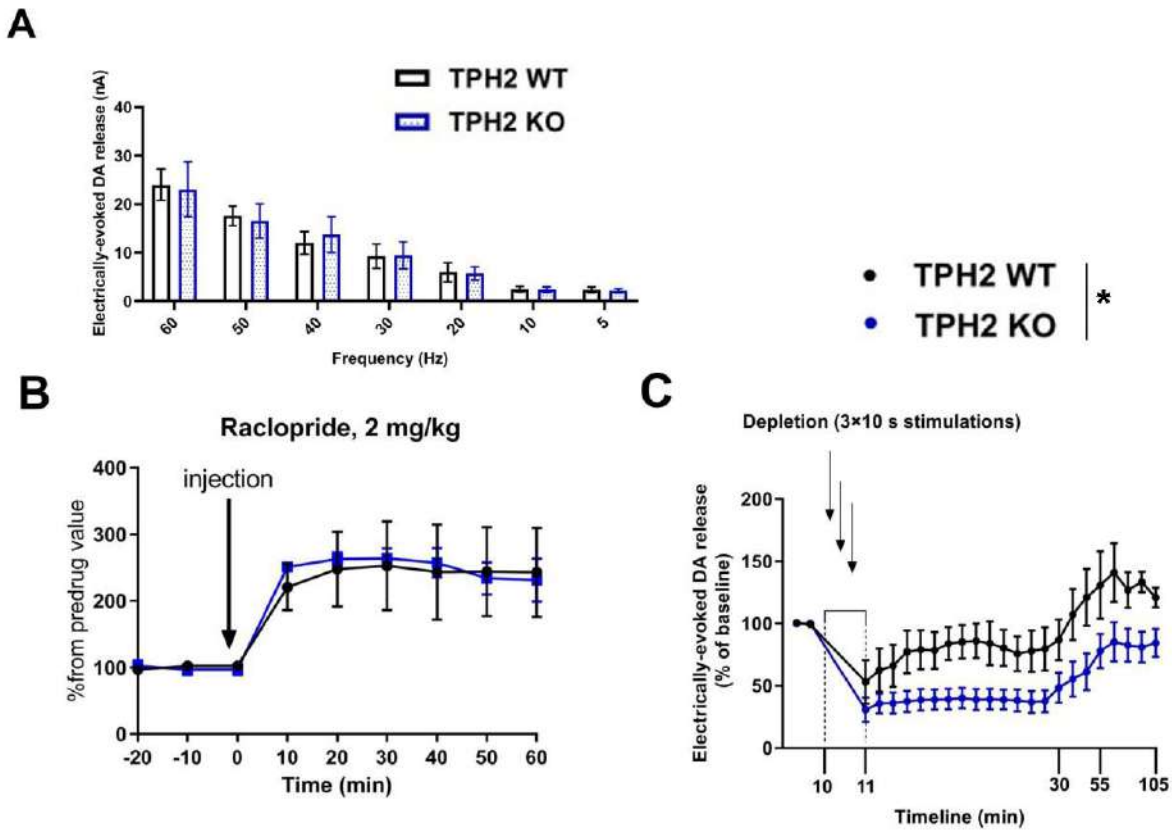


Figure 30. Features of mesolimbic dopamine (DA) neurotransmission in TPH2 knockout (KO) rats (n=7) and wild-type (WT) rats (n=7). (A) - DA signal in NAc as a function of VTA stimulation with different current frequency in KO and WT rats. VTA electrical stimulation parameters (1 s, 5-60 Hz, 5-60 pulses, 330 μA current, 10 min inter-simulation interval). (B) - DA signal in NAc before and after administration of raclopride 2 mg\kg i.p. (C) - DA depletion/recovery procedure of DA after 3 prolonged stimulations (60 Hz 600 pulses, 10 sec). Data are presented as mean ± standard error of the mean, two-way repeated-measures analysis of variance; * - P = 0.0349.

Peculiarities of aggressive behavior in TPH2 KO rats

In the process of aggressive interaction between TPH2 KO rats and Wistar rats, the following patterns of behavior were observed, presented below (Figure 31). It is noteworthy that attacks with the highest aggressiveness (violent attacks) with bites - were observed only in male resident TPH2 KO rats.

Figure 31. Behavioral elements of TPH2 gene knockout rats (KO) and wild-type (WT) rats acting as resident interactors in the interaction process with Wistar rats acting as intruders in the social defeat stress test paradigm.

Based on the data in Table 2 and Figure 32, wild-type resident females, compared with wild-type resident males, exhibit significantly ($P \leq 0.0001$; Mann-Whitney nonparametric test) greater social exploratory activity (opponent sniffing), but no differences were observed between the knockout and wild-type groups in this index; in contrast, a difference between the knockout and wild-type groups in resident males was found in space exploration (rearing) ($P \leq 0.0001$; nonparametric Mann-Whitney test) and resident females ($P \leq 0.003$; parametric unpaired t-test); grooming time was significantly reduced ($P = 0.0008$; nonparametric Mann-Whitney test) in knockout males compared to wild-type males. No changes in grooming time were observed in the female groups (KO/WT). It is likely that the observed differences in grooming parameters are explained by the high level of stress during aggressive interactions between male knockout rats - resident and wild-type males - intruders (Figure 32). Indeed, TPH2 KO males are significantly more aggressive than TPH2 KO females ($P = 0.054$; nonparametric Mann-Whitney test) in terms of total attack scores (Figure 32); however, all TPH2 KO rats are much more aggressive than male rats ($P = 0.0017$; nonparametric Mann-Whitney test) and especially than wild-type females ($P \leq 0.0001$; nonparametric Mann-Whitney test).

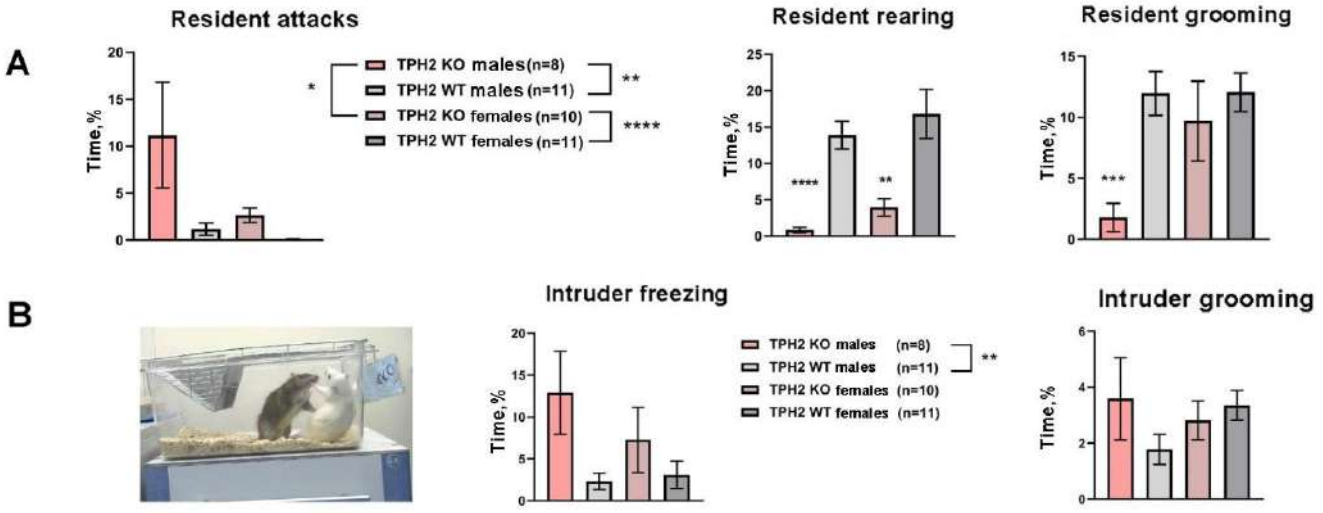


Figure 32. Behavior of TPH2 KO and WT rats acting as residents (A) and Wistar rats acting as intruders (B) during interaction with resident TPH2 KO and WT rats for 10 min in the social defeat stress test. This figure is a supplement to Table 2 and duplicates some of its data. The figure represents such behavioral elements as: attacks (weak attack + powerful attack + pseudo-attacks), grooming, rearing, and freezing. X-axis - groups of animals. Y axis - time, %. Data are presented as mean, % ± SEM. * - $P = 0.054$; ** - $P \leq 0.003$; **** - $P \leq 0.0001$; nonparametric Mann-Whitney test or parametric unpaired t-test.

If we examine in detail the aggressive interactions between groups of KO/WT animals acting as residents in the SD test, we can observe that TPH2 gene knockouts showed an increase (P = 0.0032; nonparametric Mann-Whitney test) in the rates of weak provocative attacks in male and female TPH2 KO rats (P ≤ 0. 0001; nonparametric Mann-Whitney test), as well as increases in the timing of powerful attacks in males (P = 0.001; nonparametric Mann-Whitney test) and females (P = 0.028; nonparametric Mann-Whitney test) and the number of powerful attacks in males (P = 0. 001; nonparametric Mann-Whitney test) and females (P = 0.035; nonparametric Mann-Whitney test) of TPH2 knockout rats compared with the corresponding wild-type animals.

Groups	Behavioral elements in **intruder rats**, during 10 min of aggressive interaction in the SD test with resident rats											
	Sniffing %	Rearing %	Grooming %	Attack weak %	Attack strong %	Attack strong n	Run %	Freeze %	Defence %	Pseudomatting %	Submissive %	Other %
Males TPH2 KO (n=8)	9.0± 3.0	0.9± 0.4	1.8 ±1.2	**2.9 ±1.3 ****	**8.2 ±5.8 ****	**3.5 ±1.1 ****	0.8 ±0.6	0.4 ±0.2	1.0 ±0.5	0.1 ±0.1	0.1 ±0.1	74.8 ±4.6
Males TPH2 WT (n=11)	6.0± 1.0	**13.9± 1.9 ******	**12.0±1.8 *****	0.5 ±0.5	0.0 ±0.0	0±0	0.6 ±0.3	0.5 ±0.2	1.6 ±0.9	0.7 ±0.3	0.4 ±0.2	64.1 ±3.1
Females TPH2 KO (n=10)	17.0±2.4	4.0± 1.2	9.7 ± 3.26	**2.5 ±0.8 ******	**0.1 ±0.1 ***	**0.4 ±0.2 ***	0.5 ±0.3	2.1 ±1.2	3.9 ±2.1	0.2 ±0.1	0.8 ±0.4	59.2 ±3.1
Females TPH2 WT (n=11)	**16.6±1.3 ******	**16.8± 3.4 ****	12.1±1.6	0.1 ±0.1	0±0	0±0	0.4 ±0.1	0.9 ±0.5	0.3 ±0.3	0±0	1.6 ±0.6	52.1 ±2.5

Table 2. Behavioral elements: sniffing the opponent, rearing, grooming, weak provoking attacks (weak bites, thrusts), powerful attacks (clinch attack, bite attack), running, freezing, defensive behavior, pseudo-sitting, submission postures, other behaviors (walking, standing still, etc.) in TPH2 KO and WT rats acting as residents during 10 min of interaction with Wistar rats acting as intruders in the paradigm of social stress (pseudo-sitting, submission postures, other behaviors (walking, standing in place, etc.) in TPH2 KO and WT rats acting as residents during a 10 min interaction with Wistar rats

acting as intruders in the social defeat stress test (SDS) paradigm. Data are presented as mean, % ± SEM. * - P ≤ 0.03; ** - P ≤ 0.03; *** - P = 0.0008; **** - P ≤ 0.0001; nonparametric Mann-Whitney test or parametric unpaired t-test.

TPH2 KO-resident aggression provokes a significant increase in freezing parameters in male intruder rats (P = 0.0063; nonparametric Mann-Whitney test) compared to wild-type residents (Figure 33), indicating high levels of stress in intruder rats during encounters with aggressive TPH2 KO-resident males as opposed to encounters with less aggressive wild-type males.

As experiments using animal models exhibiting aggressive behavior have shown, dysfunction or low levels of brain SER (often along with hyperfunction of the DA system) leads to the display of impulsive aggression [215,336]. The SER plays an inhibitory role in the manifestation of aggressive or addictive behavior [336], as well as the literature shows that the use of SSRIs or D2 agonists, reduces aggressive and alcoholic behavior [260,336]. In TPH2 KO mice, we showed a significant (up to 10-fold) increase in impulsive aggression in the resident-intruder test, as well as reduced anxiety and high depression [337].

In our study, we also demonstrated a 10-fold increase in the number of attacks in male TPH2 knockout (KO) rats compared to wild type (WT) animals with signs of aggressive behavior (violent attacks) - bites, these TPH2 knockout (KO) attacks resulted in pronounced freezing in the attacked residents, indicating a strong stressor response in the attacked rats. TPH2 females similarly exhibited attack behavior compared to wild-type females, which showed no aggression toward the intruder. Thus, we have shown that TPH2 KO rats (both females and males) as well as mice [337] have increased aggressiveness and can act as an aggressive agent in the resident-intruder test, especially in experiments using aggressor females, which usually do not show aggressive behavior towards the intruder, but TPH2 KO females, as our studies have shown, do.

Stress is a provocative factor for alcohol ingestion [35,221]. In the present work this has been proven in Wistar rats and in TPH2 KO and WT animals, but we have also shown that TPH2 KO rats are more susceptible to stress-induced alcohol ingestion than WT animals. The reason for this susceptibility is an imbalance of neurotransmitter systems in KO rats, especially the reward system. Evidence for this theory is provided by the fact that our data in TPH2 KO rats show an increase in the consumption of sweet solutions and sweet alcoholic solutions, i.e., all those solutions that have hedonic value.

Along with increased aggression, it can be assumed that dysregulation of the mesolimbic DA system, responsible for the motivational component of behavior together with suppressed activity of the central SER system, responsible among others for cognitive control of emotional and impulsive behavior [336], leads to altered motivational behavior (increased impulsive aggression, increased

hedonic behavior, increased susceptibility to stressful alcohol consumption) in TPH2 KO animals. The literature shows various findings in people suffering from alcoholism, patients with neuropsychiatric diseases receiving DA agonists as medication [216], people dependent on substance use - all of these individuals have impaired cognitive control of emotion and have reduced central SER levels [336]. Thus, normal functioning of the DA and SER system is necessary to ensure optimal functioning of motivational behavior through cognitive control of impulsivity.

Thus, based on the increased aggressiveness of TPH2 gene knockout rats, we can recommend these rats as an excellent model for creating an aggressive environment and social defeat stress. The obtained data testify to the importance of the functioning of the central link of the SER system, the disorder of which is also accompanied by a violation of synthetic processes in the DA system and, as a consequence, an increase in general hedonic motivation, motivation to alcohol consumption and aggressive actions.

Chapter 3: Effects of a single social defeat stress on behavior and mesolimbic DA neurotransmission in rats

Consequences of social defeat stress in male rats

This section focuses on the study of the effects of social defeat (SD). Fast-scan voltammetry and behavioral tests were used.

During these experiments, the animals of the experimental group were subjected to the procedure of single SD (the procedure of single SD is detailed in the Materials and Methods section) or voltammetric studies (Figure 33).

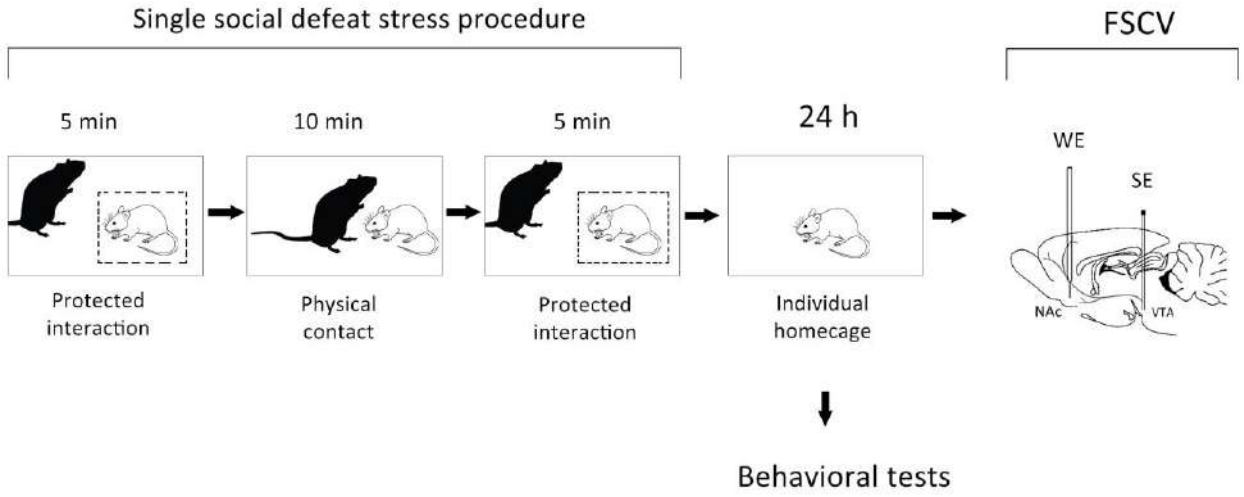


Figure 33. Schematic illustration of experimental procedures. NAc – nucleus accumbens, VTA - ventral tegmental area, FSCV - fast-scan cyclic voltammetry, WE - working recording electrode, SE - stimulating electrode.

During the single SD procedure, intruder animals were subjected to multiple aggressive clinch attacks (sometimes involving biting) during the course of the resident. The mean time of direct physical contact was 436 ± 81.5 s (this time varied depending on the degree of aggressiveness of the resident rat). The mean latent period of the first attack was 1.3 ± 0.2 s. Animals of the experimental group subjected to SD exhibited active defensive (12.1 ± 3.0 %), running (0.7 ± 0.2 %), exploratory behavior (26.6 ± 5.8 %), as well as freezing (25.6 ± 4.6 %), subordinate (1.7 ± 0.6 %) behavior. During interactions with conspecifics, animals of the control group showed high rates of exploratory activity (27.0 ± 4.1%) and practically no freezing behavior (0.1 ± 0.1%), i.e. stress was absent in this relationship. In contrast, resident animals in the “stress” group (Figure 34) lacked “grooming” during the stressful interaction, showed high values of halting behavior (Mann-Whitney U-criterion, $p = 0.0006$) in stressed animals (unpaired t-test, $p = 0.0001$) compared to the control group, however, no

differences in exploratory behavior were observed between experimental and control group animals (27.0 ± 3.6 vs. 26.6 ± 5.1; p = 0.09546).

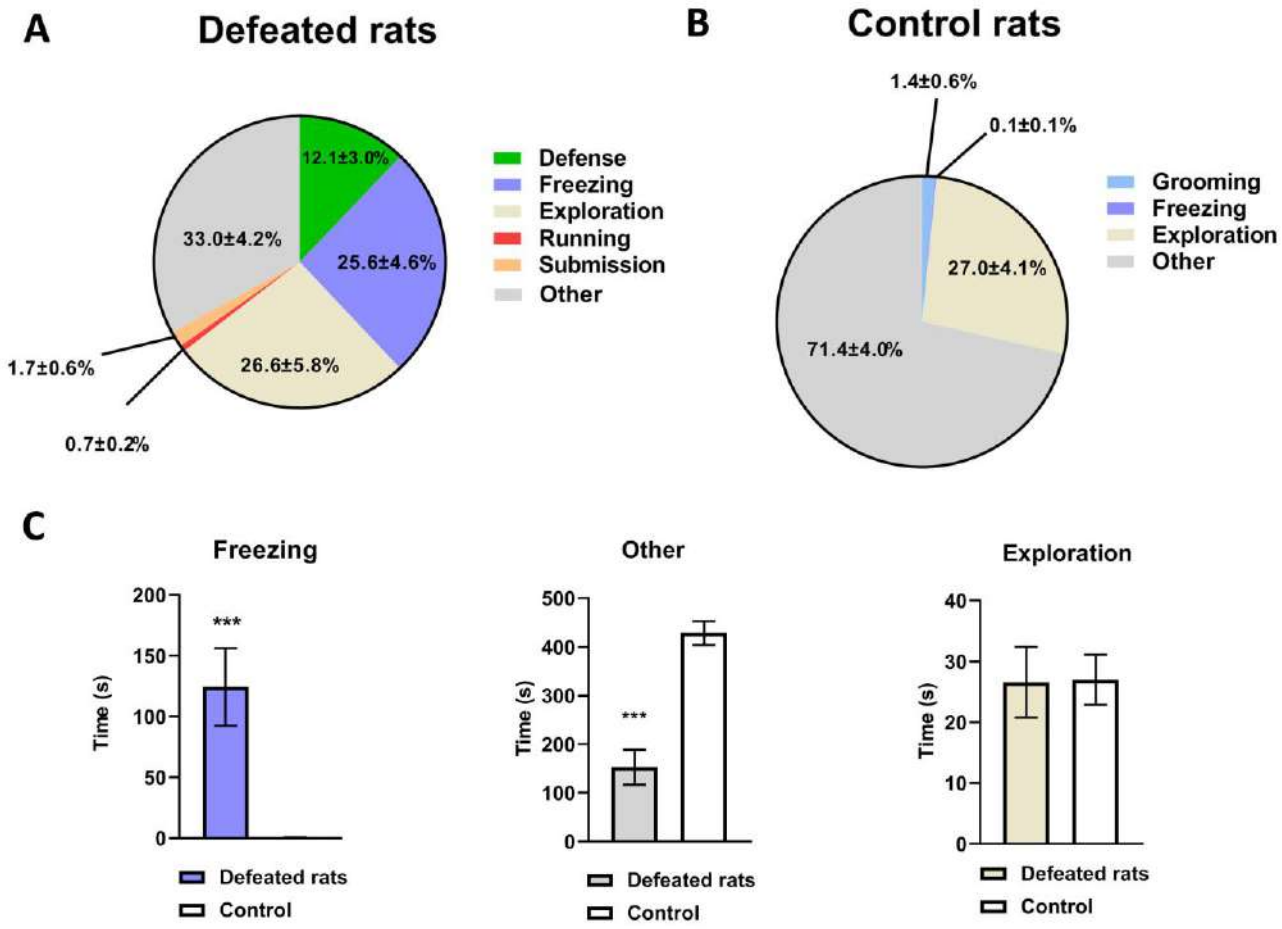


Figure 34. Rat behavior displayed during social defeat stress. Behaviors were characterized as defensive, freezing (rat does not move), explorative (rearing and sniffing), running, submissive (rat lying on the back), or other (walking, standing). A – Distribution of behaviors exhibited by intruder rats during social defeat stress presented as a percent of total session time exposure (n = 8), (mean ± SEM). B – Distribution of behaviors exhibited by intruder "control" rats during a 20-min exposure to a non-aggressive counterpart presented as a percent of total session time (n = 8), (mean ± SEM). C – Effect of social defeat stress on the display of select behaviors compared to non-stressed controls. Data presented as mean ± SEM, Unpaired two-tailed t-tests or Mann-Whitney U test was used, (n = 8 per group), *** - $p \leq 0.0006$.

Several tests did not find significant alterations in behaviors of intruder rats following the SD experience (Figure 35). There were no differences between defeated rats and control in sucrose preference (84.2 ± 3.9 % vs 80.4 ± 4.9 %, p = 0,8048, Mann-Whitney U test) in two-bottle choice test. Similarly, behavioral measures in the open field test were not significantly different between groups,

including distance moved (27.6 ± 2.0 m vs 26.1 ± 1.6 m, p=0.5819, unpaired two-tailed t-test), time spent in the center of arena (53.7 ± 10.6 s vs 43.4 ± 5.8 s, p=0.2176, Mann-Whitney U test), rearing (63.9 ± 6.1 s vs 50.7 ± 3.6, p = 0.1051, Mann-Whitney U test), grooming (9.8 ± 3.2 s vs 14.2 ± 4.6 s, p=0.2176, Mann-Whitney U test) and locomotor activity (123.1 ± 9.3 m vs 129.0 ± 13.7 s, p = 0.8559, unpaired two-tailed t-test). However, socially-defeated rats showed a significant increase in the immobility time during the forced swim test (0.8 ± 0.2 vs 0.4 ± 0.1; p = 0,0427, unpaired two-tailed t-test) when compared to controls (Figure 35 F).

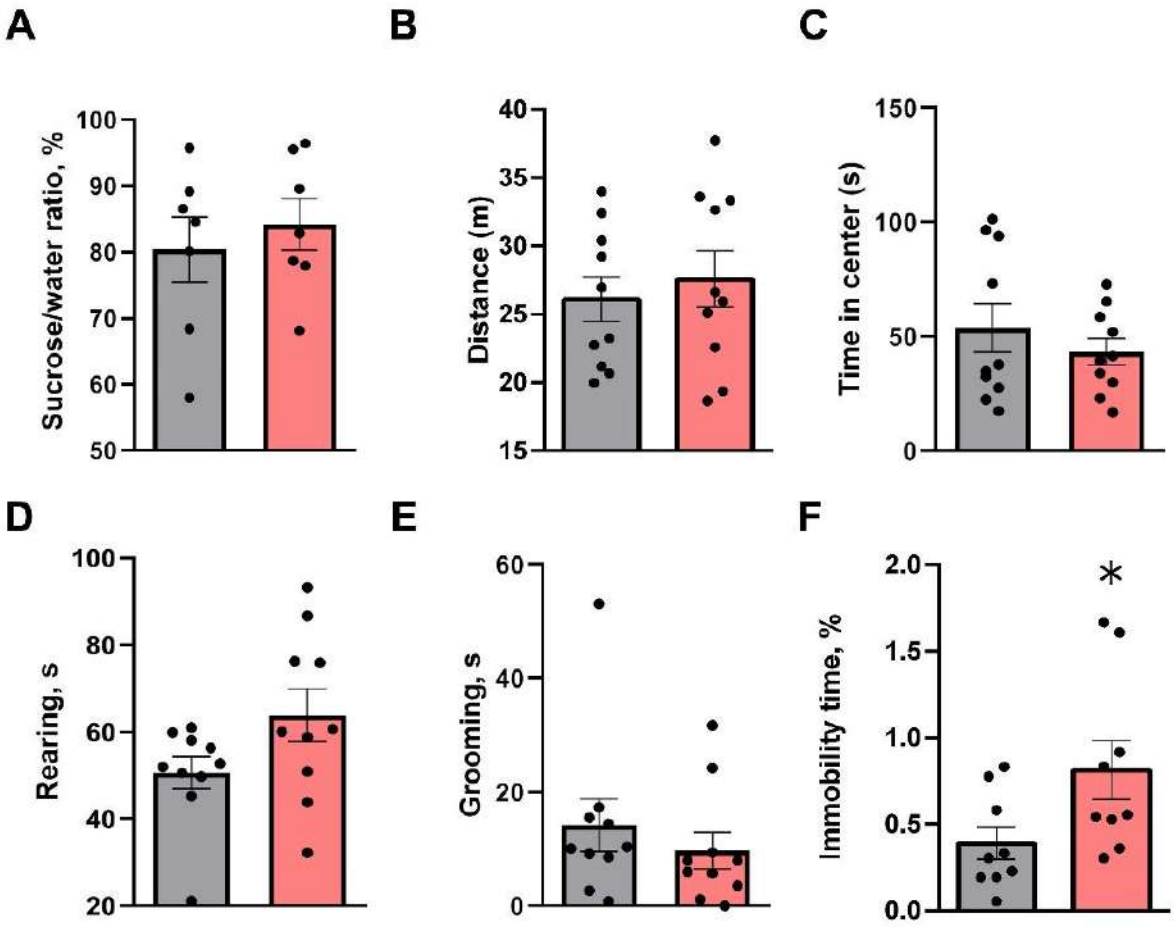


Figure 35. Behavior of animals 24 hours after a single SD. (A) Preference for 10% sucrose solution measured 24 h later (n = 7); (B-E) Locomotor and anxiety behaviors recorded during the open-field test (n = 10), (F) Immobility time during the Porsolt forced swim test (n = 9 per group). Gray bars, control group animals; Red bars, intruder defeated rats. All data are presented as mean ± SEM. Unpaired two-sided t-test or Mann-Whitney U-test was used, * - p = 0.0427.

The voltammetry method was used to evaluate the changes in DA response in animals 24 hours after the SD procedure compared to a group of control animals (conspecific interaction). Figure 36 shows the DA response (60 Hz, 60 pulses, 1 s) in animals of the stress and control groups as color

schemes and graphs. Figure 36 D also shows histologic verification of the position of the recording microelectrode.

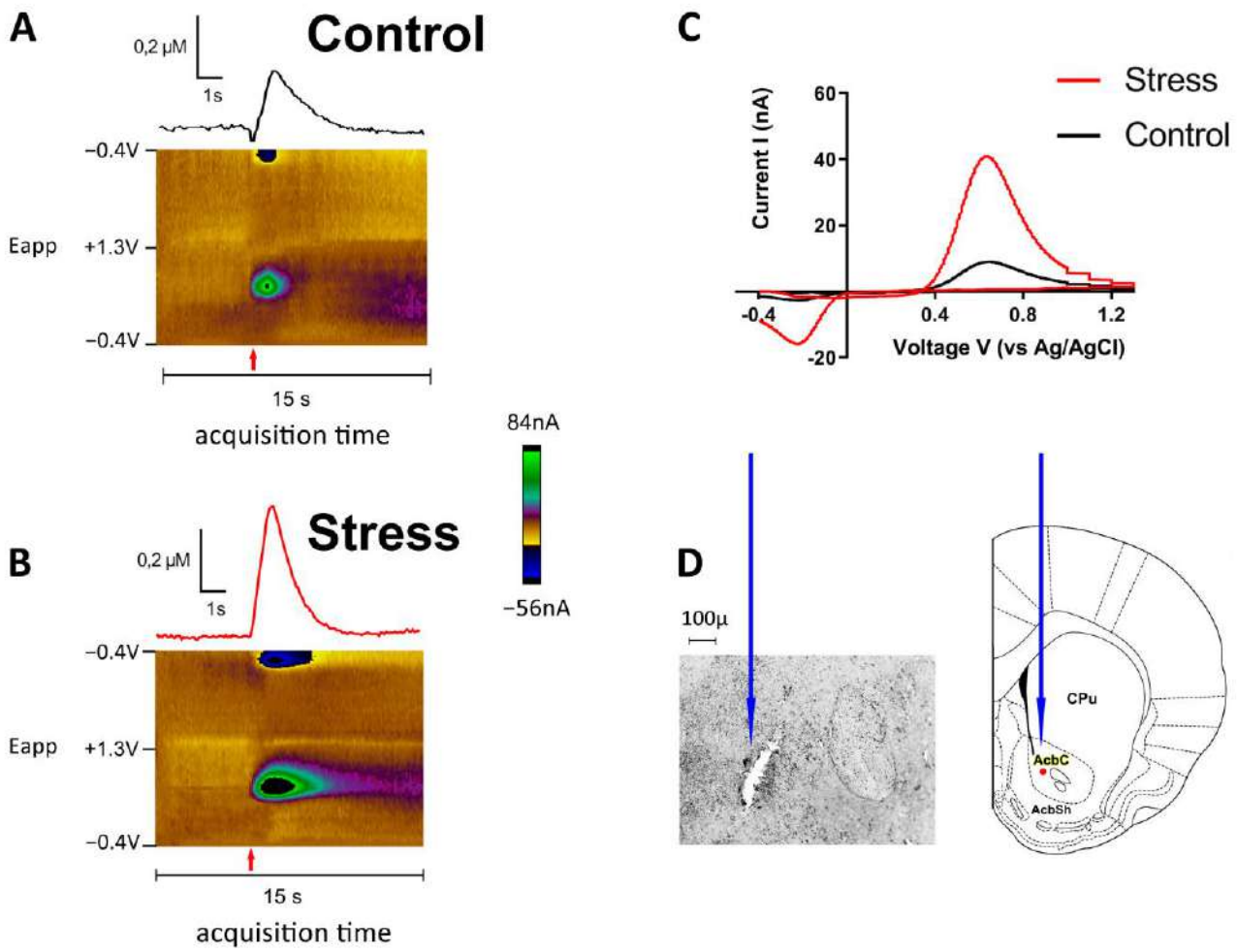


Figure 36. Dopamine release as measured by FSCV in the nucleus accumbens during electrical VTA stimulation. Representative color plots and traces of electrically-evoked dopamine release in the brain of defeated (A) and control (B) animals are shown with time of acquisition along the X-axis, applied potential (Eapp) along the Y-axis, and background-subtracted faradaic current in pseudo color shown in the z-axis. Red arrows indicate the onset of electrical stimulation (1s, 60Hz, 60 pulses, current 330µA). (C) Representative cyclic voltammogram showing oxidation and reduction peaks at ~ 0.6 and ~ −0.2 V (respectively), confirming the signal as dopaminergic. (D) Histological verification of working electrode placement in the NAc as shown by a blue arrow. (Right) Electrode path observed on a coronal section 1.60 mm from Bregma; (Left) tissue damage indicating placement of electrode was observed on a coronal tissue section. CPu – Caudate Putamen; AcbC - nucleus accumbens core; AcbSh - nucleus accumbens shell.

Figure 37 shows that the amplitude of the DA response was increased in both (stress and control) groups as a function of the frequency of electrical VTA stimulation (repeated measures Two-

Way ANOVA; F (6, 78) = 15.59, p<0.0001); however, significantly more pronounced DA was observed in animals of the stress group compared to the control group (repeated measures two-factor ANOVA; F (1, 13) = 7.005, p=0.0201). A higher DA response was shown in animals of the "stress" group compared to controls at higher (60, 50 and 40 Hz; p<0.05) but not at lower frequencies (30, 20, 10 and 5 Hz; p>0.05).

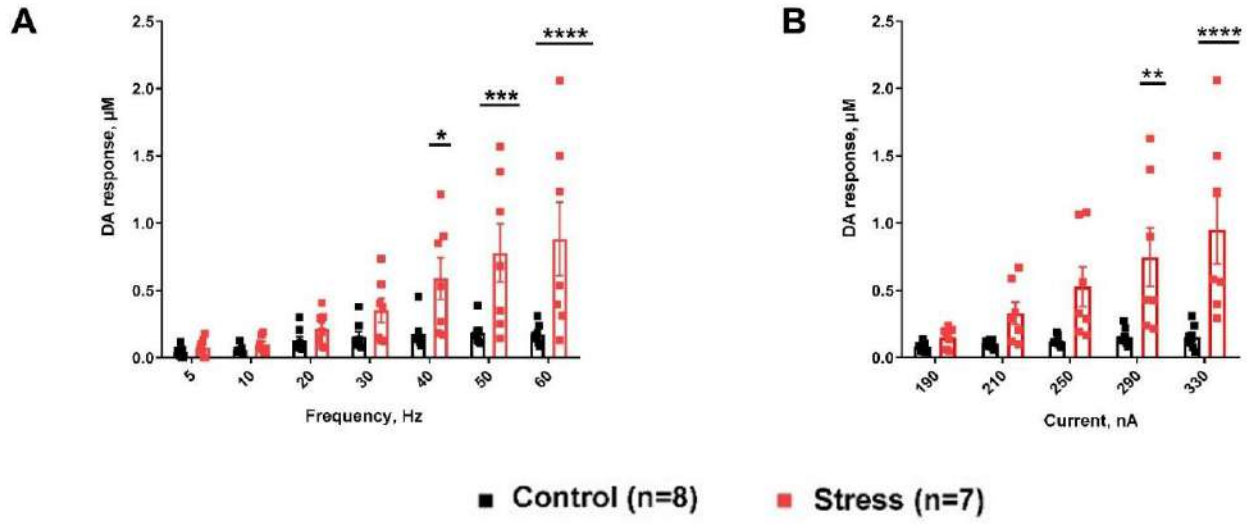


Figure 37. DA response in NAc with different frequency (A) and amplitude (B) characteristics of VTA electro-stimulation (1 s, 5-60 Hz, 5-60 pulses, 330 µA current) 24 hours after SD. DA response in animals subjected to the SD procedure (n = 7) and control group animals (n = 8). Data are presented as mean ± SEM, two-way ANOVA with repeated measures; * - p < 0.05; *** - p 0.001; **** - p<0.0001.

Administration of raclopride, a selective DA D2 receptor antagonist (2 mg/kg, i.p.), resulted in a significant increase in DA response in both groups (repeated measures two-factor ANOVA; F (8, 88) = 30.26; p<0.0001); however, this response was more pronounced in animals of the "control" group than in the defeated group (F (1, 11) = 5.321; p<0.05) (Figure 38).

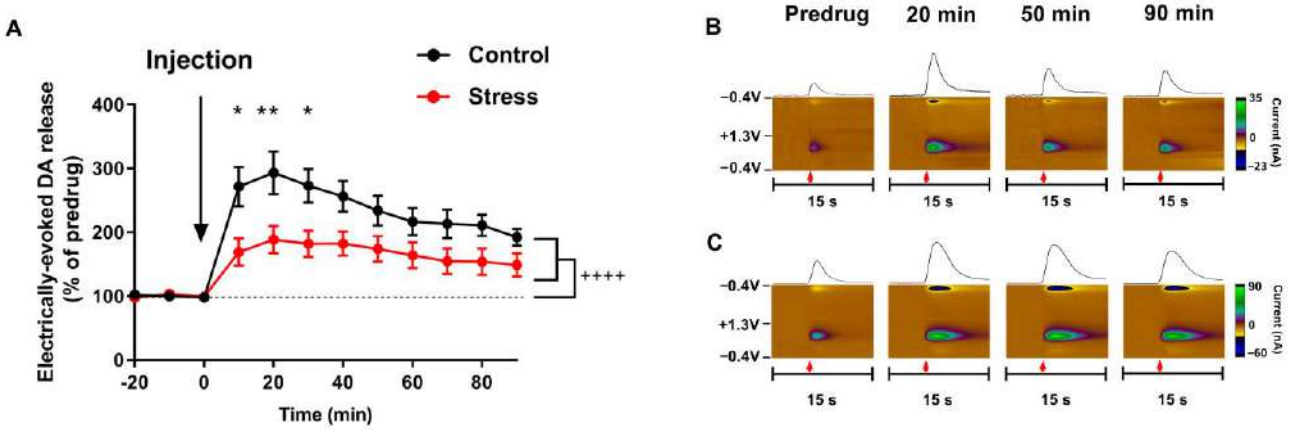


Figure 38. Raclopride (2 mg/kg) had a significantly smaller effect on DA response in NAc in rats exposed to social defeat stress in contrast to control group animals. (A) Mesolimbic DA response after

administration of raclopride. Data are presented as mean ± SEM. Control group (n = 8), animals of the stress group (n = 5). Repeated measures Two-Way ANOVA, * - p<0.05; ** - p<0.01; ++++ - p<0.0001. (B, C) Representative color plots showing DA response in NAc in animals of control (B) experimental (C) groups. Red arrow indicates the onset of electrical stimulation (1 s, 60 Hz, 60 pulses, current 330 μA).

Following the DA depletion procedure (Figure 39), both groups showed a similarly dramatic decrease in DA response (repeated measures Two-Way ANOVA; F (21, 210) = 14.76; p < 0.0001), no difference was observed between groups (F (1, 10) = 1.141; p > 0.05).

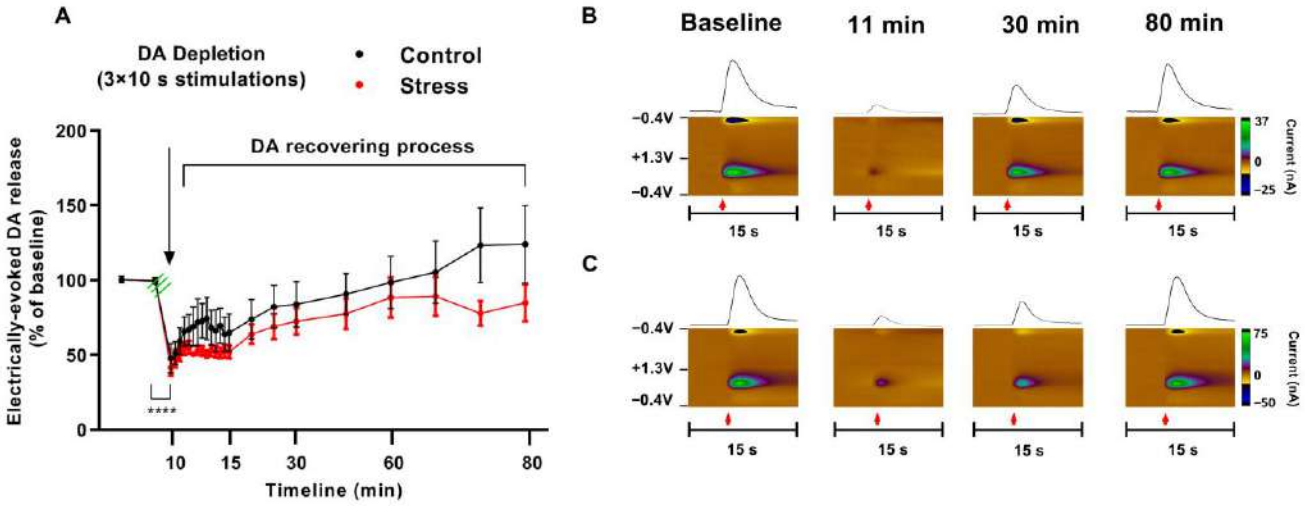


Figure 39. Social defeat stress did not affect DA depletion processes. (A) Electrically evoked DA response obtained at intervals of 1 min (14 stimulations), 5 min (3 stimulations), and 10 min (5 stimulations) after application of a series of pulses designed to deplete DA in the NAc region. Data are presented as mean ± SEM; (n = 6 per group); **** - P<0.0001 (repeated-measures two-factor analysis of variance). (B, C) Representative color plots and DA responses in the brains of control (B) and stressed (C) rats obtained 1, 20, and 70 min after DA depletion. Red arrow is the onset of electrical stimulation.

In spite of the fact that most of the behavioral tests performed did not reveal significant changes in the animals' behavior 24 h after aggressive interaction, nevertheless, in the forced swimming test a significant increase in immobility time was observed in the animals of the “stress” group compared to the control 24 h after SD.

In this experiment, it was also shown that the effect of the DA D2-receptor antagonist, raclopride (2 mg/kg, i.p.) on the DA response was significantly reduced in animals exposed to ERP compared to animals in the control group (Figure 38). The DA depletion treatment (Figure 39) did not reduce or restore the amplitude of the DA response after social defeat stress.

SD in rodents produces acute physiological adaptations that include activation of the cardiovascular and endocrine systems [33,338] along with neurochemical changes such as increased DA and noradrenalinergic neurotransmission [19,339,340] as well as dramatic changes in patterns of behavioral activity [19,340]. Most of these changes diminish or disappear within 24 hours of stressor exposure. However, some behavioral and neurobiological effects can be observed even after a single episode of social defeat [340]. Also, the literature shows that prolonged aggressive interaction between resident and intruder leads to significant behavioral changes such as: decreased amplitude of the diurnal temperature rhythm, expressed as an increase in body temperature in the diurnal resting phase, decreased spontaneous activity in the home cage and decreased locomotion in the new environment. [32,341]. However, the current study showed a significant increase in immobility time in the Porsolt forced swim test in the "stress" group already after a single SD treatment. The Porsolt test is known to be one of the indicators of a depression-like state [286,287]. In the current study, this behavior was accompanied by an increase in DA response in the NAc.

The literature shows that a single stress can promote neuronal adaptive processes occurring in the mesolimbic DA system. In animal models, stress has been shown to induce LTP blockade in VTA DA neurons [342,343]. It has also been shown that for at least 24 hours after acute stress there is an increase in AMPA/NMDA receptors in the VTA area [344,345]. Thus, the literature shows that the enhancement of neuroplasticity in the VTA area that occurs under various stressors can persist 24 hours after the end of the stressor. Corticotropin-releasing factor (CRF) may be responsible for the observed changes in extracellular DA. This neuropeptide is released in response to acute stress, contributing to both adaptive and maladaptive behavior [346,347] through its action on central CRF receptors [111]. The changes shown in VTA neurons are directly related to the increase in DA concentration in NAC in response to the stressor [5].

Changes in extracellular DA concentration could potentially lead to presynaptic neuroadaptation to compensate for such changes by affecting the processes of synthesis, autoreceptor regulation of neurotransmitter release and reuptake.

To investigate whether the consequence of a single SD could influence DA dynamics at the presynaptic level, we applied a DA depletion protocol. Such a protocol allowed us to identify possible changes in DA synthesis, reuptake, and autoreceptor regulation [348-350]. This approach is based on the fact that a certain time is required to restore the electrically-evoked DA response after prolonged high-frequency electrical stimulation. The dynamics of DA reduction and recovery should reflect the processes of general presynaptic neuronal regulation. Despite the significant difference found in DA responses between non-SD-exposed and SD-exposed rats, the DA depletion protocol resulted in

complete depletion and then recovery of DA signaling. Thus, these data may suggest that the balance of intrasynaptic processes in the rat NAc is not disrupted by a single social stress.

Nevertheless, a single SD led to a decrease in the efficacy of the D2 antagonist raclopride in rats of the experimental group subjected to SD compared with controls. Thus, stress caused an alteration in the regulation of DA response carried out by D2 receptors in the NAc area. Interestingly, there is evidence that acute aggressive exposure increases the density of D2 receptors in the NAc zone in intruder rats [351]. According to another hypothesis raclopride and DA compete for D2 receptors, due to the increase in the amount of intrasynaptic DA after SD there was a decrease in the pharmacological effect of the drug due to the processes of competition for the substrate (receptor). Thus, in the "stress" group, an attenuation of presynaptic autoreceptor blockade and consequent decrease in DA concentration was observed after administration of raclopride, in contrast to the control group, which showed a significant increase in DA response in NAc under the action of raclopride during the entire 90 min of observation.

Thus, our data demonstrated marked changes in DA neurotransmission in the NAc area, as well as isolated signs of depression-like state without changes in other behaviors 24 h after exposure to SD. It is noteworthy that these changes were observed after a single exposure to social defeat stress.

Consequences of social defeat stress in female rats

A similar study was conducted on the effects of SD in female rats. In this study, the same temporal parameters of stressor exposure and electric current values were used as were used on males in a previous study. All experimental groups consisted of equal numbers of animals in different stages of the estrous cycle. This was done to avoid the influence of the stage of the cycle on the behavior or dynamics of the DA response [319].

Figure 40 shows a statistically significant decrease in total exploratory activity (on average by 30.23±3%) in female rats exposed to SD compared to the control group of rats. Also in the "stress" group has a such behavioral element as: running, defensive, attacking behavior and grooming. During SD action, the mean time of direct physical contact in female rats was 644.2 ± 25.1 s (Figure 40). This time varied depending on the degree of aggressiveness (presence of biting, attacking behavior) of the resident rat. On average, females were exposed to 0.8 ± 0.2 aggressive attacks without biting during the SD procedure.

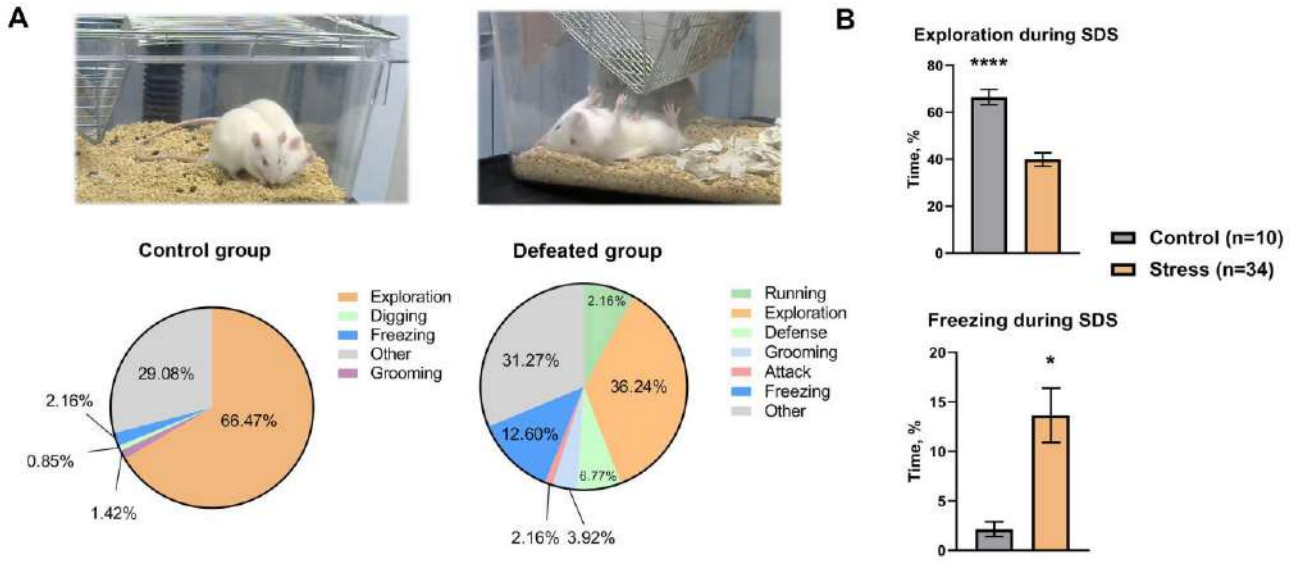


Figure 40. Behavior of female rats during social defeat. A - behavioral patterns (exploratory activity, digging, freezing, grooming, running, defending, attacking, and other) demonstrated by animals during SD (right) and during interaction with a non-aggressive conspecific (left - control group). B - statistically significant differences in exploratory activity of both groups. **** - $p<0.0001$, unpaired t-test.

In contrast to males, female rats exhibited different, sometimes polar behavioral patterns during a single SD. This fact allowed us to divide the animals into two groups: those with high and low exploratory activity during the SD procedure, i.e. rats with active and passive behavioral strategies during SD. The animals of the active and passive groups demonstrated different behavioral profiles during the SD (Figure 41). Thus, the exploratory activity of passive animals during SD was reduced by 40% on average compared to the control, while in the group with an active behavioral strategy these values did not exceed 20%. These groups of animals differed significantly not only in the parameters of general exploratory activity (stance, space and stranger sniffing) between the groups (unpaired t-test, $P \leq 0.0003$, Figure 42) and control rats (Unpaired t-test, P.) <0.0001), but also on the freezing parameters between groups (Mann-Whitney U-test, $p \leq 0.05$) and controls (Mann-Whitney U-test, $p = 0.0026$).

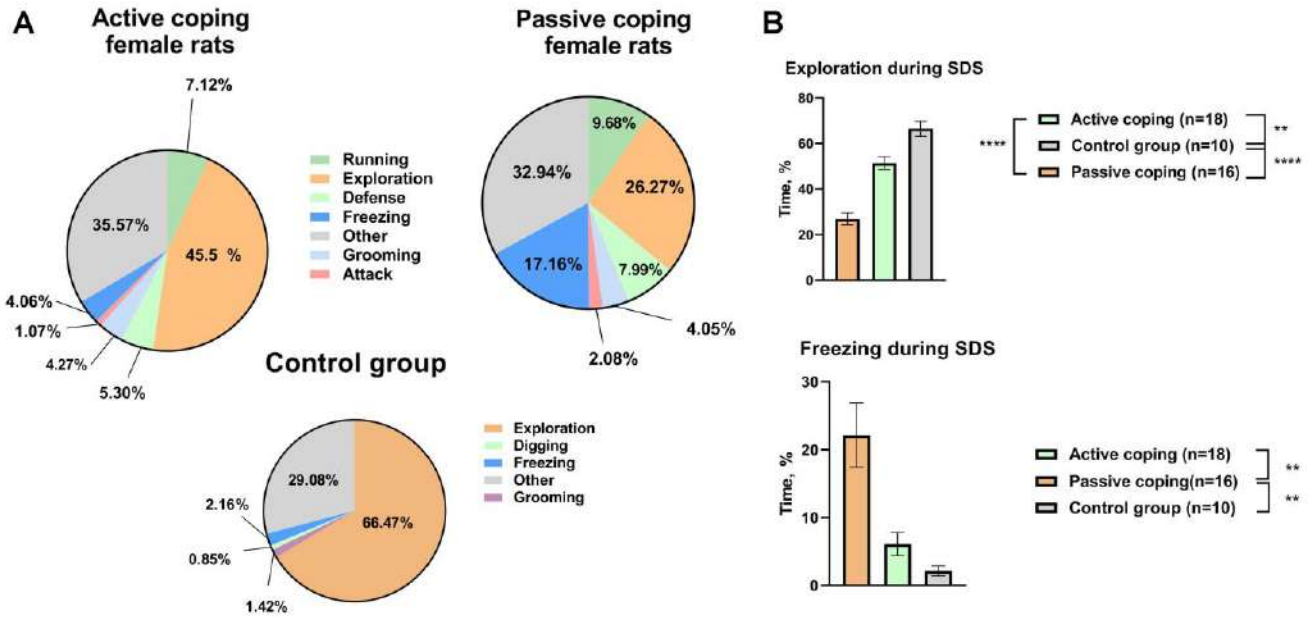


Figure 41. Behavior of rats with active and passive behavioral strategies during a single social defeat stressor. Social defeat behaviors manifested as: defensive, attacking (aggressive postures and attacks), exploratory (sniffing and rearing), running, freezing, digging, grooming and other (walking, standing). (A) - Behavioral patterns in rats with passive (n = 16), (mean ± SEM) and active (n = 18), (mean ± SEM) behavioral strategies during a 20 min SD procedure and in control animals (n = 10), (mean ± SEM) during a 20 min exposure with a non-aggressive conspecific. (B) - Exploratory and freezing behavior during the SD procedure in female rats with different behavioral strategies. Data are presented as mean ± SEM, unpaired two-sided t-test or Mann-Whitney U-test was used, **** - $P < 0.0001$; *** - $P \leq 0.0003$; ** - $P < 0.0012$.

24 hours after stress exposure, a statistically significant increase in total exploratory activity (rearing) (Mann-Whitney U-criterion, p = 0.04, Figure 42) was found in cross maze test in female rats, along with decreased consumption (Mann-Whitney U-criterion, p = 0, 01) and a trend toward a decreased preference (Mann-Whitney U-criterion, p = 00.7) for sweet solutions (10% sucrose solution), indicating the development of a state of anhedonia along with increased exploratory activity (rearing) in rats 24 h after SD.

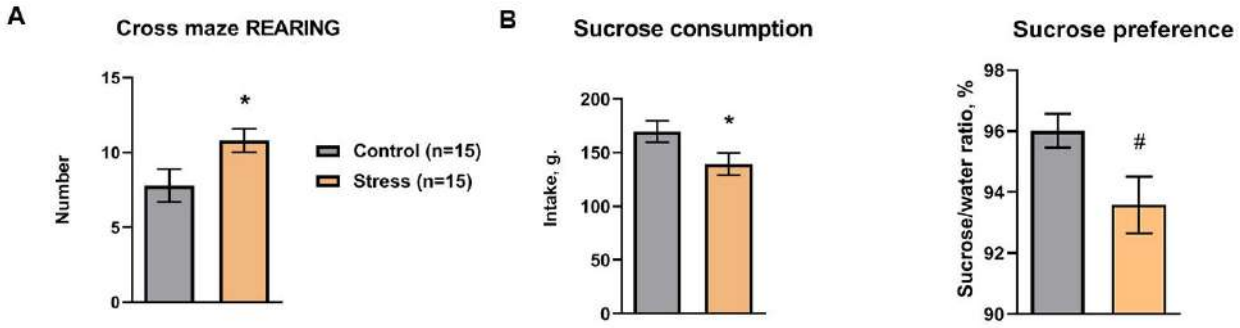


Figure 42. Behavior of female rats 24 h after a single social defeat stress. A - increase in stereotypic behavior (rearing) in stressed rats compared to controls (cross maze test); B - decrease in sweet solution consumption in rats in contrast to the control group. * - $p \leq 0.03$, # - $p \leq 0.1$, unpaired t-test.

When dividing animals by behavioral strategies, it was found that the state of anhedonia was more pronounced in animals with a passive behavioral strategy 24 h after SD compared to the control (Kruskal-Wallis test, $p = 0.02$, Figure 43). Without group separation, we cannot detect differences in depression-like state scores in the Porsolt forced swim test 24 h after SD; however, after group randomization, we can observe a strong tendency to increase the latency period of the first immobilization in females with a passive behavioral strategy compared to animals of the active group (Kruskal-Wallis criterion, $p = 0.06$, Figure 43) and controls (Kruskal-Wallis criterion, $p = 0.04$, Figure 43). Twenty-four hours after SD, no changes were found in anxiety behavior in the elevated cross maze in female rats of both passive and active groups compared to controls; however, there was a significant increase in exploratory behavior (rearing) in all female rats compared to controls (Mann-Whitney U-test, $p = 0.04$, Figure 43). The observed changes were more pronounced in animals of the passive group (Kruskal-Wallis criterion, $p = 0.07$, Figure 43). A cognitive test of novel object recognition, performed 24 hours after SD, showed a significant increase in the novel object recognition rate in the passive group of female rats compared to the unstressed control (Kruskal-Wallis criterion, $p = 0.03$, Figure 43) and the active group (Kruskal- Wallis criterion, $p = 0.02$). There were no statistically significant differences between the groups in terms of the total time of object exploration.

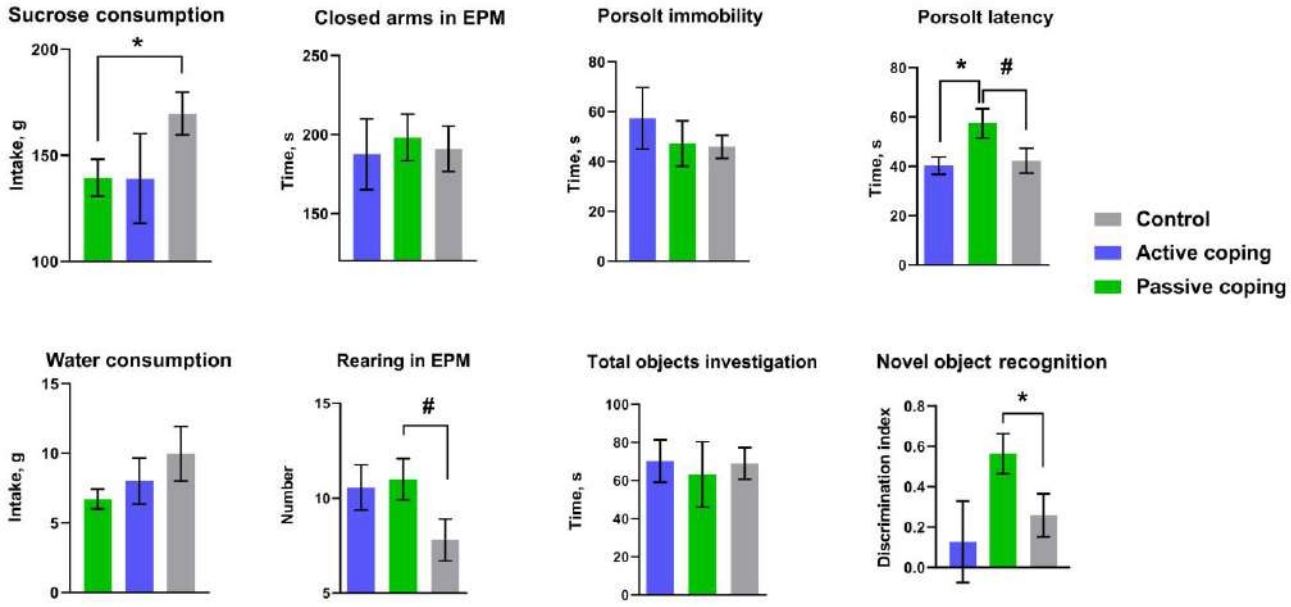


Figure 43. Behavior of female rats 24 h after a single social defeat (SD). The figure shows: consumption of water and 10% sucrose, behavior of rats in the elevated cross maze, forced swimming test and novel object recognition test. (Active (n = 7) per group; Passive (n = 8) per group; Control (n = 15) per group) (n = 9 per group). All data are presented as mean ± SEM. The Kruskal-Wallis test with multiple comparison test was used to compare groups, * - $P \leq 0.05$; # - $P \leq 0.08$.

However, it should be noted that there was a tendency to increase the preference for sweet solutions (10% sucrose solution) to water in animals with a passive coping behavioral strategy even before SD (Kruskal-Wallis test, p = 0.07, Figure 44), which indicates individual background differences in solution consumption and may serve as a behavioral predictor of post-stressor disorders detected later on.

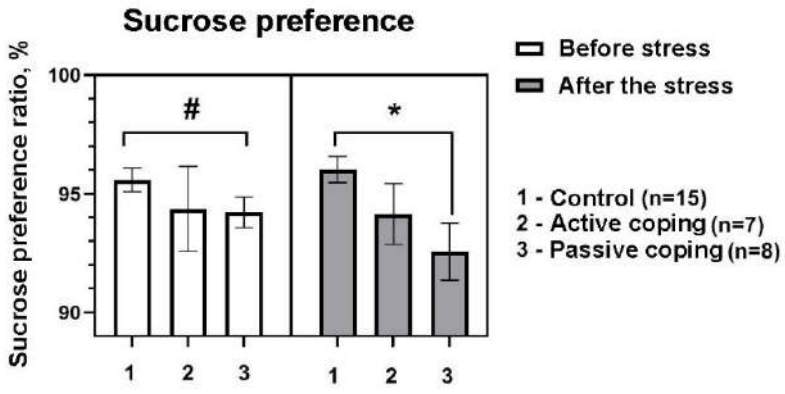


Figure 44. Preference for 10% sucrose solution to water by animals before and 24 h after single social defeat stress. Kruskal- Wallis test with multiple comparisons test was used to compare groups, * - $P \leq 0.05$; # - $P \leq 0.08$.

The relatively low aggressiveness of TPH2 KO females (compared to TPH2 KO males) during SD enabled the researcher to divide study animals into appropriate groups (active and passive). During SD, rats with an active coping strategy were subjected to an average of 1 ± 0.4 attacks; passive ones - 0.6 ± 0.3 attacks; it is noteworthy that 62.5% of females with passive behavioral strategy compared to 18% of active females demonstrated aggressive behavior (aggressive postures and attacks) towards the resident rat; however, the high level of freezing and low level of general exploratory activity in "passive" rats may indicate a high level of stress in such animals, which directly correlates with the level of DA in them 24 h after SD (Figure 47).

Examination of DA neurotransmission in NAc showed that this signal depends on the frequency response of the current (repeated measures, two-way analysis; F (6, 126) = 56.41; p<0.0001 Figure 45) during electrical stimulation of the VTA, as well as showing a statistically significant increase in the DA response in stressed rats in contrast to controls 24 h after stress (repeated measurements, two-way analysis; F (1, 22) = 4473, p=0.0460), especially significant were the differences in the phasic DA response obtained after high-frequency stimulation of the VTA. No differences in DA synthesis processes were observed after DA depletion; however, both groups showed depletion of DA by more than 50% of the baseline value.

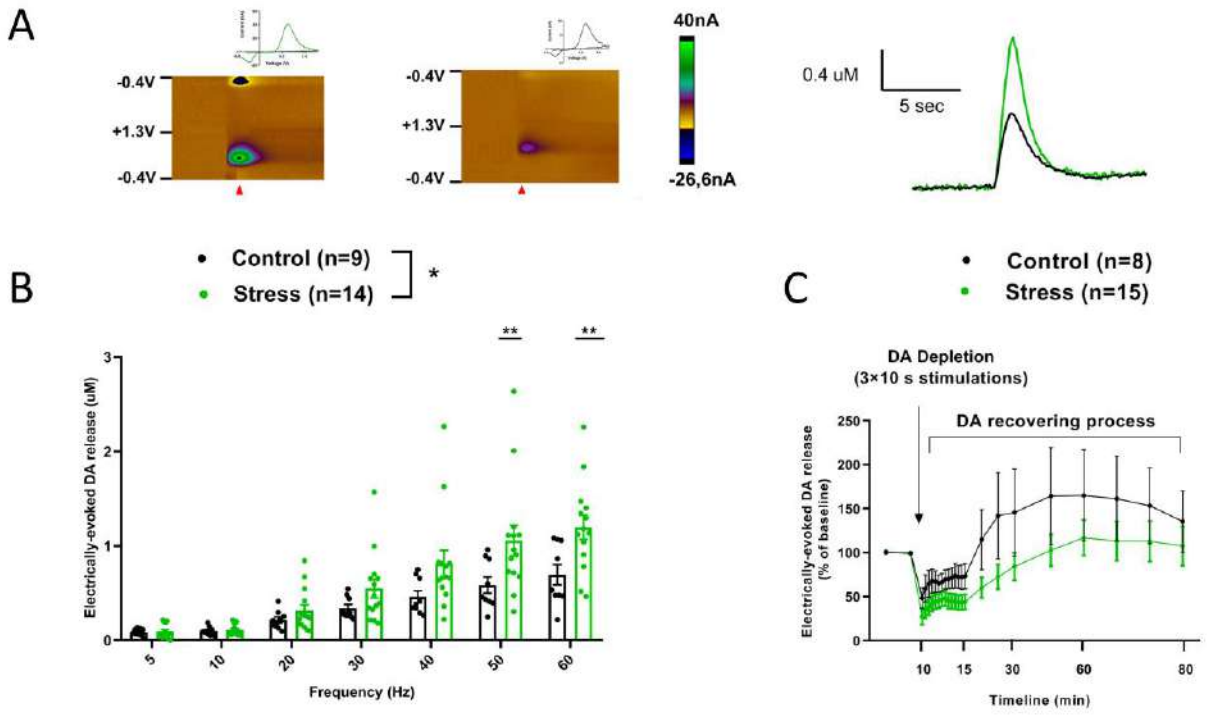


Figure 45. Changes in DA neurotransmission parameters 24 h after single social defeat stress. (A) - Representative pattern of DA response (μM) of experimental and control rats shown as a graph and color scheme. VTA electrical stimulation parameters (1 sec, 60 Hz, 60 pulses, 330 μA). (B) - Increase in DA response in animals after VTA compared with the control group. (C) - DA response obtained at

intervals of 1 min, 5 min, and 10 min after application of a series of pulses designed to deplete DA in the NAc. The onset of depletion stimulation is indicated by the arrow, followed by the recovery of DA signal, % at 80 min. Data are presented as ± SEM.

To investigate individual differences in the effect of SD, female rats of the experimental group were divided into subgroups according to behavioral strategies (active and passive strategies). In addition to the behavioral changes observed during and after SD, we can see (Figure 46) that the electrically evoked DA response in animals of the passive and active groups also differed significantly.

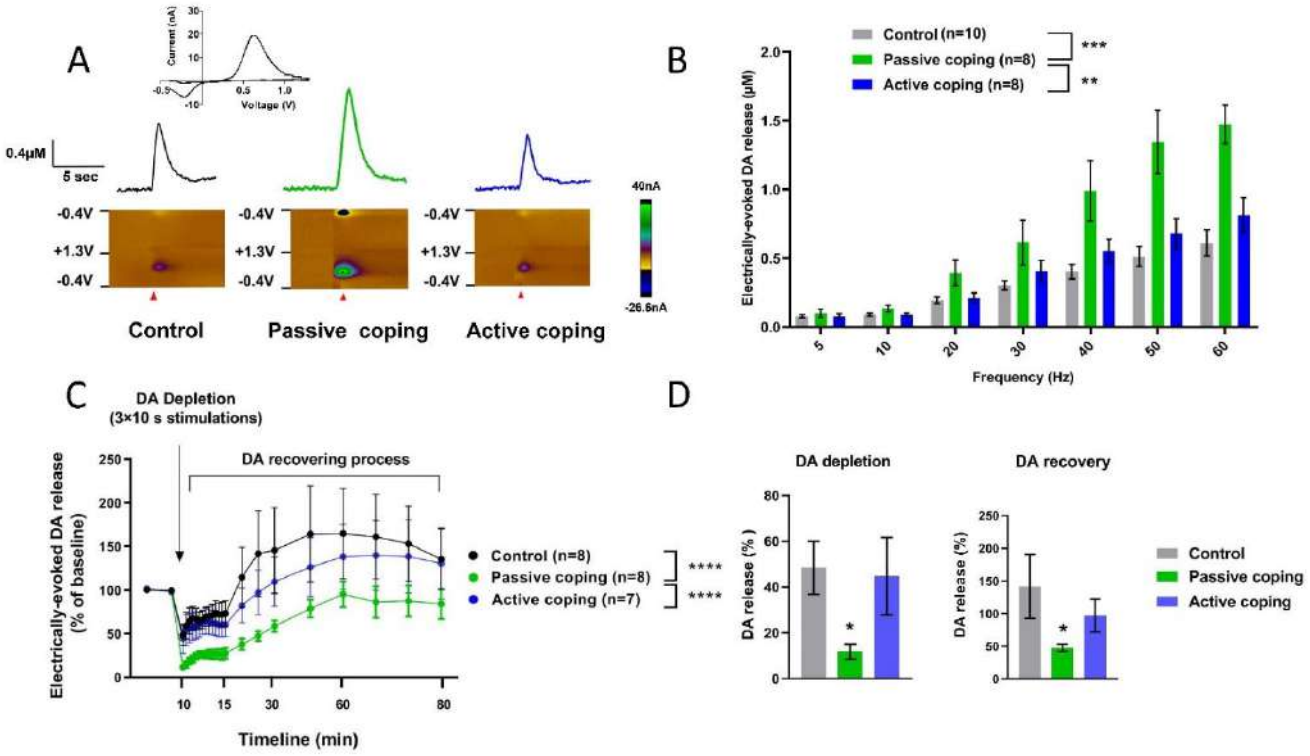


Figure 46. Changes in DA neurotransmission in female rats with different behavioral strategies observed 24 h after a single SD. (A) - representative DA signals in response to stimulation (60 Hz, 60 pulses, 330 μA current). (B) - DA signal in NAc in response to VTA stimulation with different current frequency in rats with different behavioral strategies. VTA electrical stimulation parameters (1 s, 5-60 Hz, 5-60 pulses, 330 μA current, 10 min inter-simulation interval). (C, D) - DA depletion/recovery procedure of DA in female rats with different behavioral strategy 24 h after a single SD. Data are presented as mean ± standard error of the mean, Two-Way ANOVA analysis with repeated measures and Kruskal-Wallis non-parametric test; * - $p < 0.05$; ** - $p<0.01$; *** - $p = 0.001$; **** - $p < 0.0001$.

We can observe the most pronounced changes in behavior and in DA neurotransmission in the NAc in female rats with a passive behavioral strategy 24 hours after SD (Figure 46 A). Animals with a passive behavioral strategy had significantly, compared with controls, increased levels of electrically evoked DA compared with controls (Tukey multiple comparisons test, Two-Way ANOVA; p =

0.0001) and active group rats (Tukey multiple comparisons test, Two-Way ANOVA; p = 0.0055). Likewise, female rats with passive behavioral strategies exhibit a significant decrease in DA recovery time after exhaustion, compared to rats of the active group (Sidak's multiple comparisons test, Two-Way ANOVA; p<0.0001) and control (Sidak's multiple comparisons test, Two-Way ANOVA; p<0.0001) both in total calculations and when calculating these processes (depletion and DA recovery) separately (Kruskal-Wallis nonparametric test; * - p < 0.05).

The negative correlation demonstrated between active exploring and passive freezing in rats exhibited directly during SD suggests that these behavioral indicators, along with other indicators, may serve to identify behavioral strategy (Figure 47B). as well as themes. Notably, a similarly negative correlation was found between exploratory behavior (sniffing and strutting) in female rats during a single episode of SD, %, and DA signal (nA) in NAc obtained 24 h after a single SD in female rats (Figure 47A). The changes obtained suggest that DA may be a reliable indicator of behavioral changes associated with SD in rats.

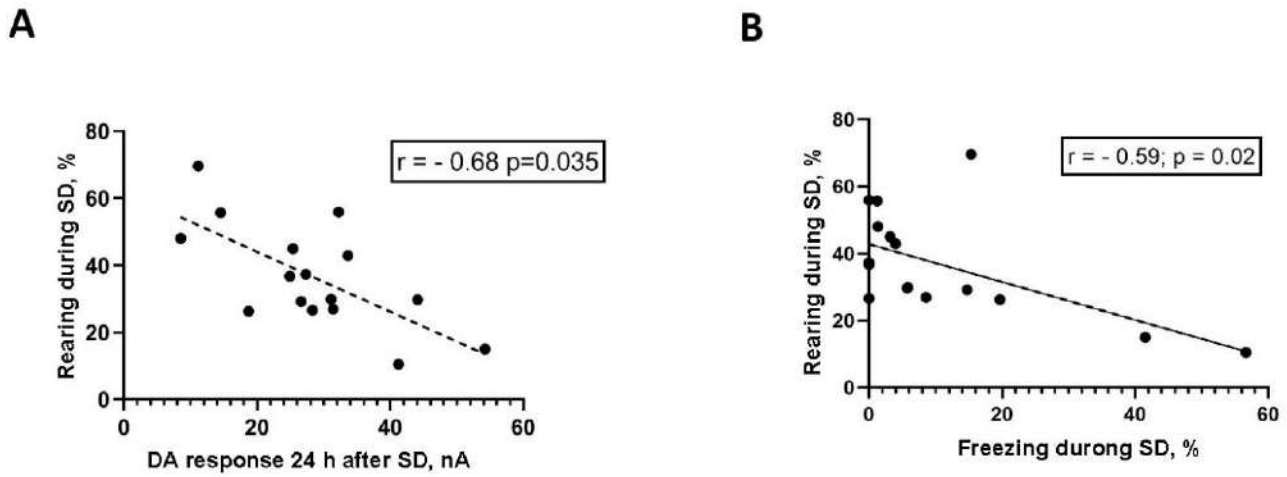


Figure 47. Correlation analysis. A - Negative correlation between exploratory behavior (sniffing and strutting) in female rats during a single episode of SD, % and DA signal (nA) in NAc obtained 24 h after a single episode of SD in female rats. B - Negative correlation between exploratory and stressor (freezing) behavior in rats during SD, %. Two-sided Pearson's analysis was used, 95% confidence interval.

The results of this study, as in males in the previous series, showed that a single social defeat stress resulted in significant neurochemical and behavioral changes in stressed animals. TPH2 KO-resident females exhibited high levels of aggression towards intruder females, thereby ensuring high levels of stress in experimental animals. Thus, rats subjected to SD exhibited increased levels of hibernation and decreased levels of exploratory activity during SD. 24 h after SD, these animals

showed various neurochemical and behavioral changes, such as anhedonia, but also increased exploratory behavior (rearing) and memory activation. Anhedonia is the most common indicator observed in depressive disorders [352], but the increase in stereotypic/exploratory behavior may be partially explained by the observed neurochemical changes. In the literature, administration of DA stimulants has been shown to cause a dose-dependent increase in stereotypy [246,278], and individual differences in stereotypy have been shown [353], but it is worth noting that the observed changes may be adaptive. It is known that acute stress affects males and females differently; unlike males, females show motor and cognitive activation in response to acute stressors [354-357], and there are several potential mechanisms by which sex hormones in males and females are activated in response to acute stressors [354-357], by which sex hormones in females may influence stress and anxiety-related behaviors by modulating serotoninergic, oxytocinergic, GABAergic systems and the entire hypothalamic-pituitary-adrenal axis (HPA) [358-361]. In fact, all these systems are interconnected to some extent with mesolimbic DA neurotransmission.

Females showed a large variation in individual responses to stressor exposure (SD), which enabled the researchers to divide the animals according to behavioral strategies (active and passive). As a result, the study showed that animals with a passive behavioral strategy demonstrated greater susceptibility to a single SD than animals in the active group, i.e., the “active” animals, compared to the passive group, did not show pronounced behavioral and neurochemical changes after a single SD procedure. In animals of the passive behavioral strategy a state of anhedonia was detected after SD, the signs of which were also shown before SD (see Figure 44), which may indicate a predisposition to anhedonia in these animals, due to their behavioral strategy. Activation of stereotypic behavior and visual memory was also found in “passive” female rats. The literature shows that individual behavioral features (different levels of exploratory activity) may indicate different levels of mesolimbic DA in animals [353], which in turn may cause different behavioral response of such animals to stress [1].

As shown in the previous series on males, females showed increased values of the mesolimbic DA response, but group differences due to different behavioral strategies were also observed. As discussed previously, the changes shown are likely adaptive in nature, that is, stress-induced changes in DA neurotransmission mechanisms may lead to subsequent presynaptic adaptations (see exposure to the D2 antagonist raclopride after SD, Figure 38) aimed at reducing the level of elevated extracellular DA. To investigate the mechanisms of release/reuptake, a DA depletion test was used to examine the dynamics of recovery of DA response values. Differences in these values between animals with different types of stressor response were shown. Thus “passive” but not “active” rats showed stronger depletion and slower recovery of DA. These results are consistent with findings in humans that the ability to synthesize dopamine correlates with the physiological response to an acute psychosocial stressor [30]. As discussed previously [30], the decrease in synthesis could be explained by activation

of an inhibitory feedback mechanism via presynaptic autoreceptors. However, we cannot exclude other possible mechanisms.

Thus, 24 h after a single SD procedure, female rats responded with different behavioral strategies (active and passive). A single SD caused a state of anhedonia and other behavioral abnormalities in female rats, most pronounced in rats with a passive behavioral strategy. Also in animals with passive behavioral strategy significant changes in DA neurotransmission in NAc were found 24 h after stress. The fact that insignificant changes in behavior ($p<0,05$) or the absence of these changes in animals 24 h after SD can be accompanied by significant neurochemical changes ($p<0,0001$), as well as the detection of correlation between these changes, proves that mesolimbic dopamine can be a reliable indicator of delayed adaptive changes in rats after the action of SD.

Chapter 4: Investigation of neurochemical correlates of coping behavior strategies

Conducting correlations of behavioral tests for optimal randomization of rats with active and passive coping behavioral strategies

Currently, there are many approaches to select rats with active and passive behavioral strategies. Some researchers prefer testing the social hierarchy of rats [311], others use the «active avoidance» test [46] to study the behavioral response of rats during the electro-pain condition ("active avoidance test"), others use the Porsolt test (single uncontrollable stressor) [292] or even locomotor activity [44]. Corticosterone is a reliable indicator of stressor response and activity of the HPA system [93]. We decided to investigate all these parameters to detect correlation using GraphPad Prism 6 program between the above mentioned tests in male Wistar rats, (n = 36).

A negative significant correlation (Table 3) was shown between immobility time in the Porsolt test of Wistar rats and such items as: the number of "freezing" behaviors (not go) on the 1st day of testing (R = - 0.3952), freezes in the «active avoidance» test on the 1-st day (R = - 0.4431), running in the ECM test (R = - 0.4847), as well as a positive significant correlation with the number of "GO" on the electric current (Avoidance) in the «active avoidance» test (R = 0.4598) on 2 day of testing. Other indices in the ECM test, such as: grooming, freezing, hovering, resting, rearing, running, time in closed and open arms, passed squares, n/100 with a reliable correlation with the time of immobility in the Porsolt test - did not reveal. Also no correlation was found between background levels of corticosterone, cholesterol and behavioral indices in all tests («active avoidance» test », ECM, Porsolt test).

"NOT GO" "active avoidance" test, n. 1 day	"Freezing" "active avoidance" test, n. 1 day	"Avoidance" "active avoidance" test, n. 2 day	"Running" ECM test, %.
P = 0,0170*	**P = 0,0098****	**P = 0,0048****	**P = 0,0049****
R = - 0,3952	R = - 0,4431	R = 0,4598	R = - 0,4847

Table 3 Correlation between **immobility time in the Porsolt test** (sec) with other parameters in the ECM (elevated cross maze) test and the «active avoidance» test on day 1 and day 2 of testing. Hereinafter «Escaping» (GO on light) - running to the other side of the set-up ("active avoidance test") in response to a light stimulus; «Freezing» and (NOT GO on current) - didn't go away or freezing

behavior on one side of the rig (“active avoidance test”) in response to an electric shock; “Avoidance” - running to the other side of the set-up (“active avoidance test”) in response to an electric current stimulus. Pearson correlation analysis. Data are presented as mean, % or n ± standard error of the mean. *,** - statistical significance of changes.

The number of escapes to the sound signal on the second day is the main indicator of the learning ability of rats in the «active avoidance» test, as well as the activity of the behavioral strategy [46]. A significant (P < 0.0001, R = - 0.7218) negative correlation was shown (Table 4) between the number of “escapings” to the sound signal on day 2 in the «active avoidance» test with “avoidance” to the electric current stimulus, as well as background indicators in the ECM test, such as “grooming” (R = - 0.4350) and “hanging” (R = - 0.3548).

“Avoidance” “active avoidance” test, n. 2 day	“Freezing” “active avoidance” test, n. 2 day	Grooming, ECM, %	Hanging, ECM, %
P = 0,0219*	**P < 0,0001****	**P = 0,0128***	**P = 0,0463***
R = - 0,3810	R = - 0,7218	R = - 0,4350	R = - 0,3548

Table 4 Correlation between **the number of escapes («active avoidance» test) to the sound signal** on the second day, with and other parameters. (n = 36). Data are presented as mean, % or n ± standard error of the mean. Pearson correlation analysis. *,**** - statistical significance of changes.

Social status is also an important indicator of the rats' behavioral strategy, the correlation of this test with such tests as ECM, «active avoidance» test, as well as with the background concentration of corticosterone in blood (R = 0.3772) and after 5 days of immobilization stress (R = 0.4606) is shown (Table 5).

“Escaping” “active avoidance” test, n. 1 day	Time in open arms ECM, %	Time in closed arms ECM, %	Grooming ECM, %	Corticosterone concentration (background), ng/ml	Corticosterone concentration (stress), ng/ml
P = 0,0358*	**P = 0,0213***	**P = 0,0209***	**P = 0,0120***	**P = 0,0333***	**P = 0,0472***

R = - 0,3511	R = - 0,4056	R = 0,4067	R = 0,4390	R = 0,3772	R = 0,4606

Table 5 Correlation between rat **social status** and other parameters. (n = 36). Data are presented as mean ± standard error of the mean. Pearson's correlation analysis. *,**** - statistical significance of changes. 1 - dominant, 2 - subdominant, 3 - subordinant status of the rat.

Thus, most of the known behavioral strategy activity tests (active avoidance test, inevitable swim test, cage social status test) showed good correlation with each other and with other parameters (anxiety, locomotor, bias activity, corticosterone levels before and after stress). However, the social status test, performed 2 times, showed good correlation with other active avoidance test, as well as behavioral parameters and corticosterone level both before and after stress, thus, along with other tests can be recommended for use in determining the type of stress response of animals. It is worth noting that for accurate determination of the type of stressor reaction a complex assessment of all parameters is required, i.e. combination of other mentioned tests (Social status test (competition for water) + active avoidance test + Porsolt's test) (Figure 49).

Behavioral and neurochemical features of animals with active and passive behavioral strategies revealed before and after the action of subchronic immobilization stress

In this section we will discuss the results of baseline behavioral and neurochemical differences between active dominant (AD) and passive subordinate (PS) animals selected using different behavioral protocols (Active avoidance test + Social status test) and after the action of subchronic uncontrolled stress (Figure 48).

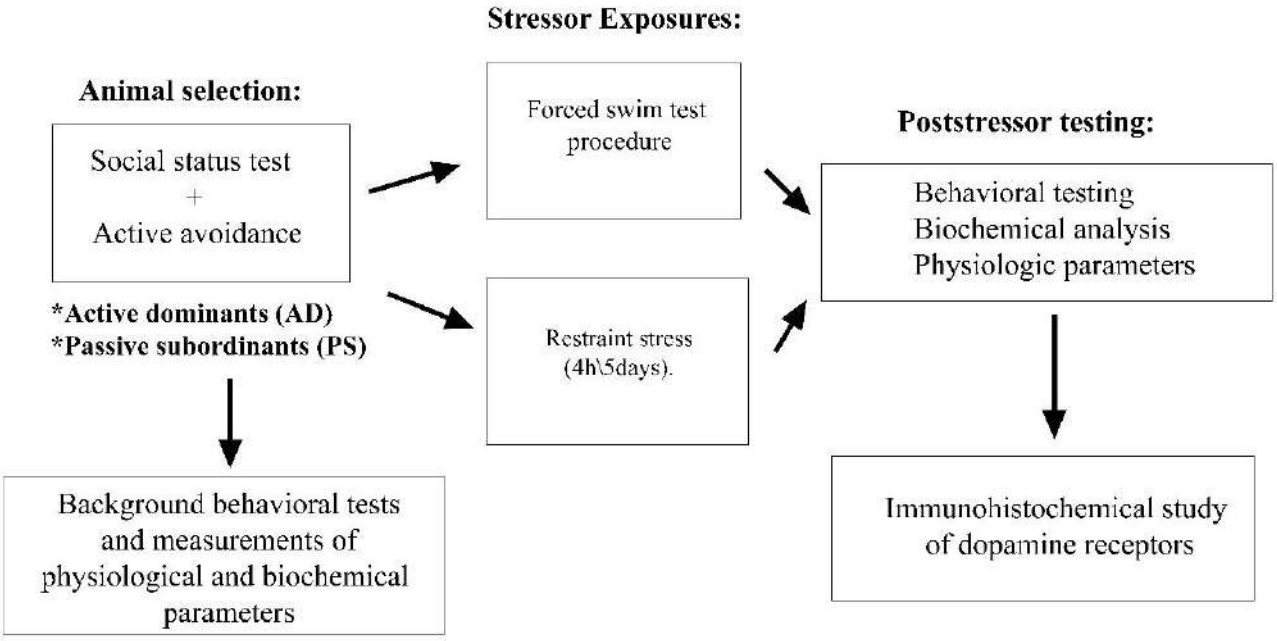


Figure 48. Schematic of the experiment. This figure shows the scheme of the experiment, including selection of animals by behavioral strategies, background and post-stressor studies.

To implement the procedure of acute and long-term uncontrolled stressor exposure - we used the procedure of single (4h.) and long-term immobilization (restraint) stress (4 h/ 5 days, daily (from 10.00 to14:00)). Immobilization was performed in special pens. In experiments on exposure to subchronic (4 h/ 5 days) immobilization stress before and after the procedure, behavioral and neurochemical components of the stressor response were assessed [43].

These exposures were used to model different components of PTSD, such as acute stress disorder ASD [328] and depression [192,193].

Figure 49 shows the process of selecting animals by behavioral strategies using the **integrated approach** outlined in Chapter 4 + See Supplemental materials.

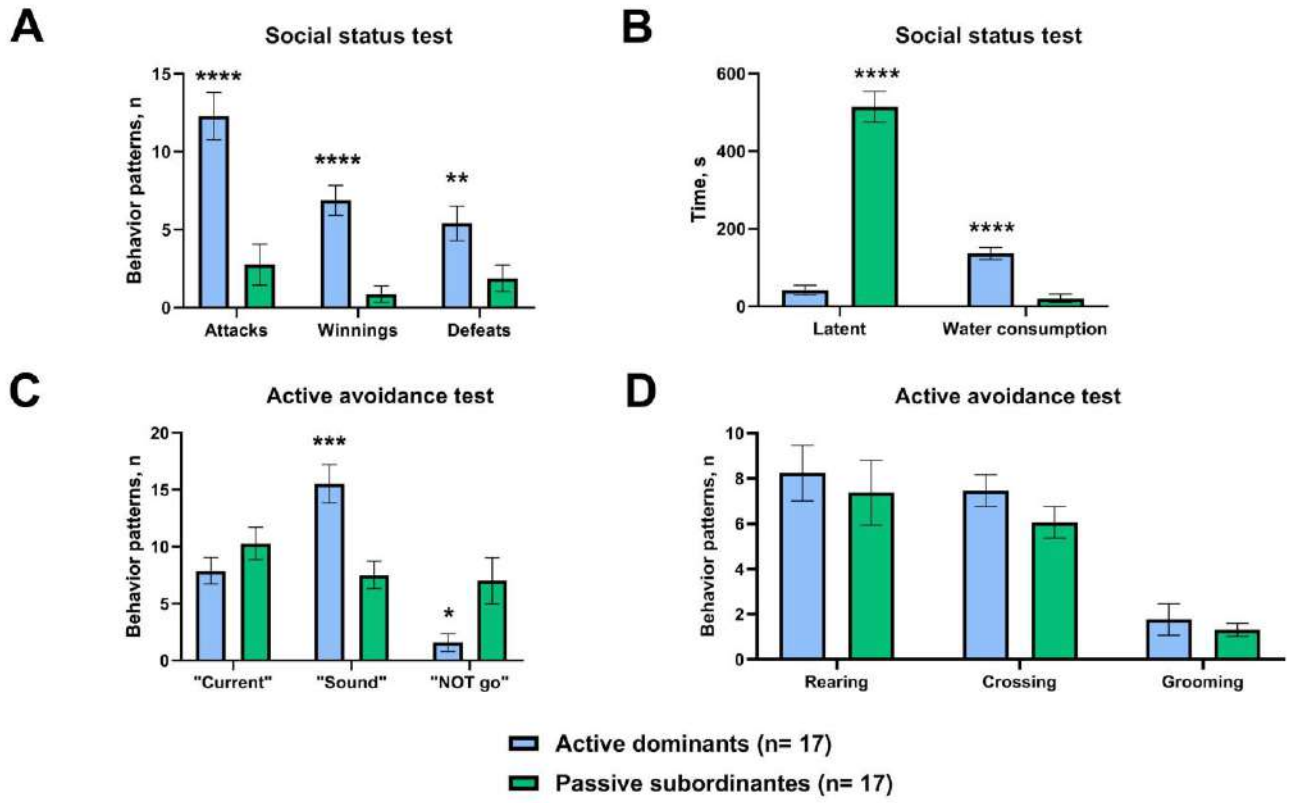


Figure 49. Behavioral characteristics of animals of the groups “active dominants” and “passive subordinants” in the “active avoidance” and “social status” (competition for vital resource – water) tests. LP - latent period of approach to the drinker. Data are presented as mean, % ± standard error of the mean. * - P = 0.0254; ** - P ≤ 0.002; **** - P ≤ 0.0001; nonparametric Mann-Whitney test or parametric unpaired t-test.

In the group of male Wistar rats (n = 60), animals with high and low activity under stressful conditions were distinguished using the “active avoidance” test, and their social status in the cage was determined using the “competition for vital resource” - water test. As a result of comparing the data of the two tests, it was shown that 42% of highly active animals identified by the “active avoidance” test were carriers of dominant social status, and 75% of animals with a passive behavioral strategy occupied a subordinant social status (Figure 50 + Supplementary).

Using the tests: “active avoidance” and “competition for vital resource” (Figure 50), experimental groups were formed (Figure 50) (17 animals per group): “active dominant” and ‘passive boarder’. Selection criterion - more than 70% of “avoidance” reactions - “active animals” (“active avoidance” test), high number of aggressive contacts, latent period of approach to the drinker - less than 10% of the total testing time. As a result of selection, the number of dominants in the formed group “active dominants” amounted to 84%, and subordinants in the group “passive subordinants” - 88% (Figure 49).

As a result of this selection, the “active dominants” AD animals in contrast to the “passive subordinants” PS animals showed a significant number ($P \leq 0.0001$; nonparametric Mann-Whitney test) of ($P = 0.0014$; nonparametric Mann-Whitney test) with the longest water consumption time ($P \leq 0.0001$; nonparametric Mann-Whitney test) and low latent period of approach to the drinker ($P \leq 0.0001$; nonparametric Mann-Whitney test) than the PS. In the “active avoidance” test, on the second day of testing, ADs demonstrated, in contrast to PS, a reduced number of “no-avoidance” responses to electric pain stimulus ($P = 0.0254$; nonparametric Mann-Whitney test) and a high number of active avoidance responses to sound stimulus ($P = 0.0005$; unpaired t-test), which are the main criterion of rat learning ability in the active avoidance test [45].

To identify individual behavioral features of AD and PS animals, other behavioral tests were performed, showing background values of: cognitive features, anxiety, motor activity, depression, as well as pain thresholds and others.

Animal groups	**Background behavioral and physiological parameters in rats of AD and PS groups**											
	ECM, locomotion, %	ECM,CA, %	ECM, OA, %	ECM, grooming, %	Sucrose 1%.	1h NOR, exploration, s	1h NOR, ratio.	24 h NOR, exploration, s	24 h NOR, ratio.	HR /60 s	Pain threshold, s	Immobility time, s
AD	26.4 ± 3.4	70.6 ± 7.2	29.4 ± 6.2	7.2± 1.6	2.9 ± 0.8	83.9 ± 12.1	0.3 ± 0.1	76.5 ± 10.7	0.3 ± 0.1	182.4 ± 4.4	12.1 ± 0.9	85.2 ± 12.0
PS	26.3 ± 3.5	83.3 ± 5.1	16.8 ± 2.4	**14.6 ±2.6 ***	2.5 ± 0.8	58.4 ± 14.6	0.1 ± 0.1	62.0 ± 15.0	0.2 ± 0.2	184.7 ± 4.3	**10.4 ± 0.7 #**	102.8± 13.7

Table 6. Background behavioral characteristics of animals of the groups “active dominants” AD and “passive subordinants” PS in the tests: ECM (elevated cross maze), OA - time in open arms in ECM, CA - time in closed arms in ECM; sugar solution preference 1%, NOR (novel object recognition) for 1 h (short-term memory) and 24 h (long-term memory), HR (respiratory rate) in 60 seconds, pain threshold (tail flick or hot plate test, measured at 20 degrees C); Porsolt's test (immobility time). Data

are presented as mean, % ± standard error of the mean. # - P = 0.080; * - P ≤ 0.05; Mann-Whitney nonparametric test.

According to the results of the performed studies (Table 6), there was a tendency to increase the pain threshold in rats - active subordinates in the background (P = 0. 080, Table 6), AD rats had increased grooming indices in ECM compared to PS (P = 0.0439; nonparametric Mann-Whitney test) other statistically significant background differences in behavior or other physiological, or biochemical indices (corticosterone, cholesterol, etc.) were not found (see Supplemental).

	Animal groups	Number of squares traveled	Rearing, %	Grooming, %	OA, %	CA, %	HR 60 sec
Before stress	**AD**	26.4±3.4	3.5±0.9	7.2±1.6	29.4±6.2	70.6±6.2	182.4 ± 4.4
	PS	26.3±3.5	4.5±0.8	14.6±2.6	16.6±2.5	83.3±5.1	184.7 ± 4.3
After stress	**AD**	**17.6±3.0#**	4.0±1.0	**20.3±4.0***	15.9±5.8	78.2±7.5	172.7 ± 6.6
	PS	**13.8±2.1****	4.7±1.1	24.2±5.6	22.9±7.7	77.1±7.71	179.7 ± 4.1

Table 7. Behavioral parameters in rats “active dominants” AD and “passive subordinants” PS after a single short-term stressor. A 6-min unavoidable swimming procedure (Porsolt's test) was used to create a short-term stressor exposure (single stressor). OA - time in open arms in ECM, CA - time in closed arms in ECM, HR (respiratory rate) in 60 seconds. Data are presented as mean, % ± standard error of the mean. # - Trend compared with baseline values in the group (P < 0.1); * - significance of differences compared with baseline values in the group; ** - P ≤ 0.05; nonparametric Mann-Whitney test or parametric unpaired t-test.

In our study, we used the Porsolt test as an unavoidable short-term stressor stimulus (Table 7). Examination of the behavioral performance of AD and PS rats after the action of such a stressor revealed a significant decrease in total locomotor activity in the ECM test in rats of the PS group (P = 0.0048; parametric unpaired t-test) and a strong tendency for such a decrease in the AD group (P = 0.0576; parametric unpaired t-test) after stress compared with the baseline group performance. A significant increase in grooming as an indicator of biased response after stressor action was found in the AD group (P = 0.023; Mann-Whitney nonparametric t-test) compared to the background group

values. No statistically significant change in anxiety after the action of unavoidable short-term stressor was found.

Immobilization (4h/day/ 5 days) was used as a subchronic 5-day unavoidable stressor. As a result of this exposure, all rats of the experimental groups showed a strong stressor response observed in the ECM test, but the nature and severity of this response differed between the groups (Table 8).

	Animal groups	Number of squares traveled	Rearings, %	Grooming, %	«Rest», %	OA, %	CA, %
Before stress	**AD**	26.4±3.4	3.5±0.9	7.2±1.6	47.3±5.5	29,4±6,2	70,6±6,2
	PS	26.3±3.5	4.5±0.8	14.6±2.6	48.0±6.0	16,6±2,5	83,3±5,1
After 1 day of Stress	**AD**	20.1±3.4	5.4±1.4	**21.2±5.1****	40.1±5.4	**11,7±3,2***	**88.3±3.2***
	PS	**15.1±3.7***	**8.4±1.2****	25.5±6.6	44.3±6.0	**7.6±2.3***	**92.2±2.3***
After 5 day of Stress	**AD**	**14.2±2.8***	3.8±0.7	**31.1±6.9******	42.1±5.6	**11,7±6,2***	**88,3±6,2***
	PS	**13.4±3.1***	5.4±1.6	**30.1±3,4****	48.6±6.9	**3,7±1,2*****	**96,3±3,7***

Table 8 Behavioral parameters in rats “active dominants” AD and “passive subordinants” PS in the ECM test after 5-day immobilization stress. Data are presented as mean, % ± SEM. # - Trend compared with baseline values in the group ($P < 0.1$); * - significance of differences compared with baseline values in the group; ** - $P \leq 0.007$; **** - $P \leq 0.0005$ nonparametric Mann-Whitney test or parametric unpaired t-test.

On the first day of stress exposure, animals in the PS group showed a stress response expressed as an increase in anxiety (significantly increased time in the closed arms of the ECM; $P = 0.0174$; parametric unpaired t-test and decreased time in the open arms of the ECM compared with background; $P = 0.0154$; parametric unpaired t-test) and exploratory activity in the ECM test ($P = 0.0067$; parametric unpaired t-test), along with decreased motor activity (significant decrease in the number of squares walked compared to background; $P = 0.045$, parametric unpaired t-test). In contrast, in active dominants (ADs), on the first day of stress, this response was expressed as an increase in

“grooming” compared to background (P = 0.0047; nonparametric Mann-Whitney test) as an indicator of biased activity during and after stress and as an increase in anxiety scores (decreased time in open arms compared to background; P = 0. 0193; nonparametric Mann-Whitney test; increased time in closed arms compared to background; P = 0.0194; nonparametric Mann-Whitney test).

On the fifth day of unavoidable stress exposure, animals in both groups showed a significant decrease in motor activity, expressed as a decrease in the number of squares traveled in the ECM compared to background (AD and PS: P = 0.016, parametric unpaired t-test). The duration of grooming, as a component of biased activity, was significantly increased in the AD group compared with background (P ≤ 0.0001; nonparametric Mann-Whitney test) on the 5th day of stress and strongly increased compared with background (P = 0.0010; parametric unpaired t-test). Time in the open arms of the ECM was significantly decreased on day 5 of stress in the AD (P = 0.0035; parametric unpaired t-test) and PS (P = 0.0004; parametric unpaired t-test) groups. Time in the closed arms of the ECM was significantly reduced in the AD (P = 0.0034; nonparametric Mann-Whitney test) and PS (P = 0.0005; parametric unpaired t-test) groups. Thus, PS animals increased anxiety values in the ECM test on day 5 of stress to a greater extent than AD animals. Stress had an unfavorable effect on body weight dynamics (Figure 50) of both groups of animals, however, it had the greatest effect on animals of the PS group (P ≤ 0.0001), but it should be noted that the scatter of values in the PS group is greater than in AD, in addition, the graph shows that the 3rd day of stress had the most unfavorable effect on animals of both groups.

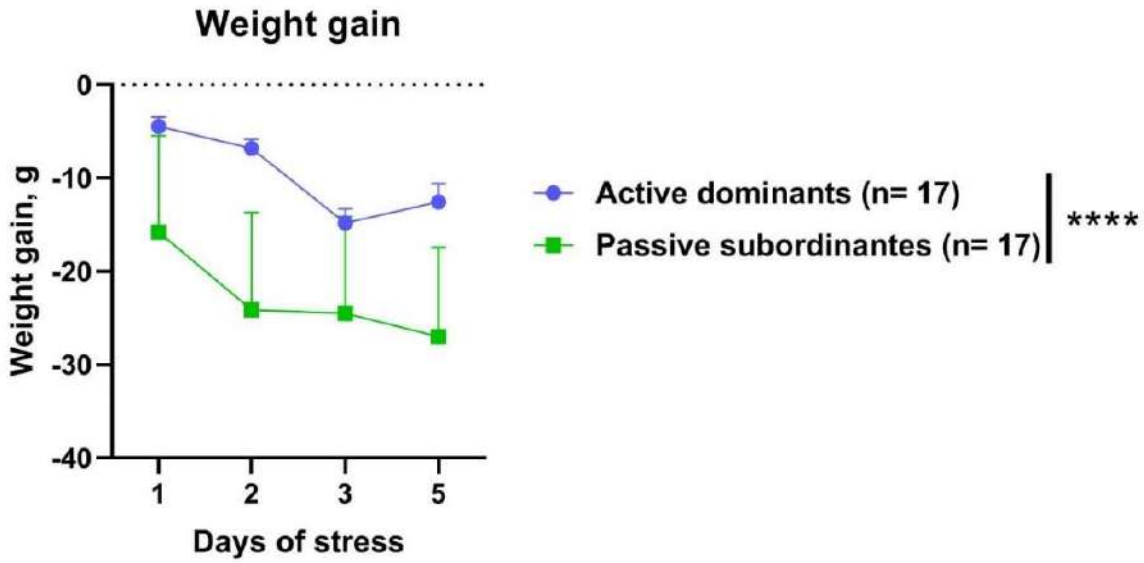


Figure 50. Change in body weight gain in animals during subchronic immobilization stress. X-axis, time scale of stress, days; Y-axis, body weight gain of rats, g. Data are presented as mean, % ± standard error of the mean. **** - P ≤ 0.0001; Two-Way ANOVA, repeated changes.

In general, the animals of the AD group were more susceptible to stress than the SP group, which we can observe from the behavioral experiments conducted before and after the 5-day immobilization stress (Figure 51). Thus, in the AD group, in contrast to the PS group, there was a significant decrease in the study time of both objects (P = 0.0010; nonparametric Mann-Whitney test) compared to the background, with no significant changes in the rating of preference for a new object one hour after the presentation of new objects (short-term memory).

In addition, in AD in contrast to PS there was a decrease in pain threshold under the influence of 5-day immobilization stress, which we can see in the test “hot plate”, set at 20 ° C. In animals of the AD group there was a significant decrease in the time of tail retraction compared to background (P = 0.0011; nonparametric Mann-Whitney test). Likewise, an increase in preference for 1% sugar solution (P = 0.0028; nonparametric Mann-Whitney test) was found in the AD group along with a decrease in water consumption (P = 0.0006; nonparametric Mann-Whitney test) compared to the background groups, in contrast to the PS (Figure 51). It is possible that the observed changes in the AD group may be a consequence of maladaptation processes.

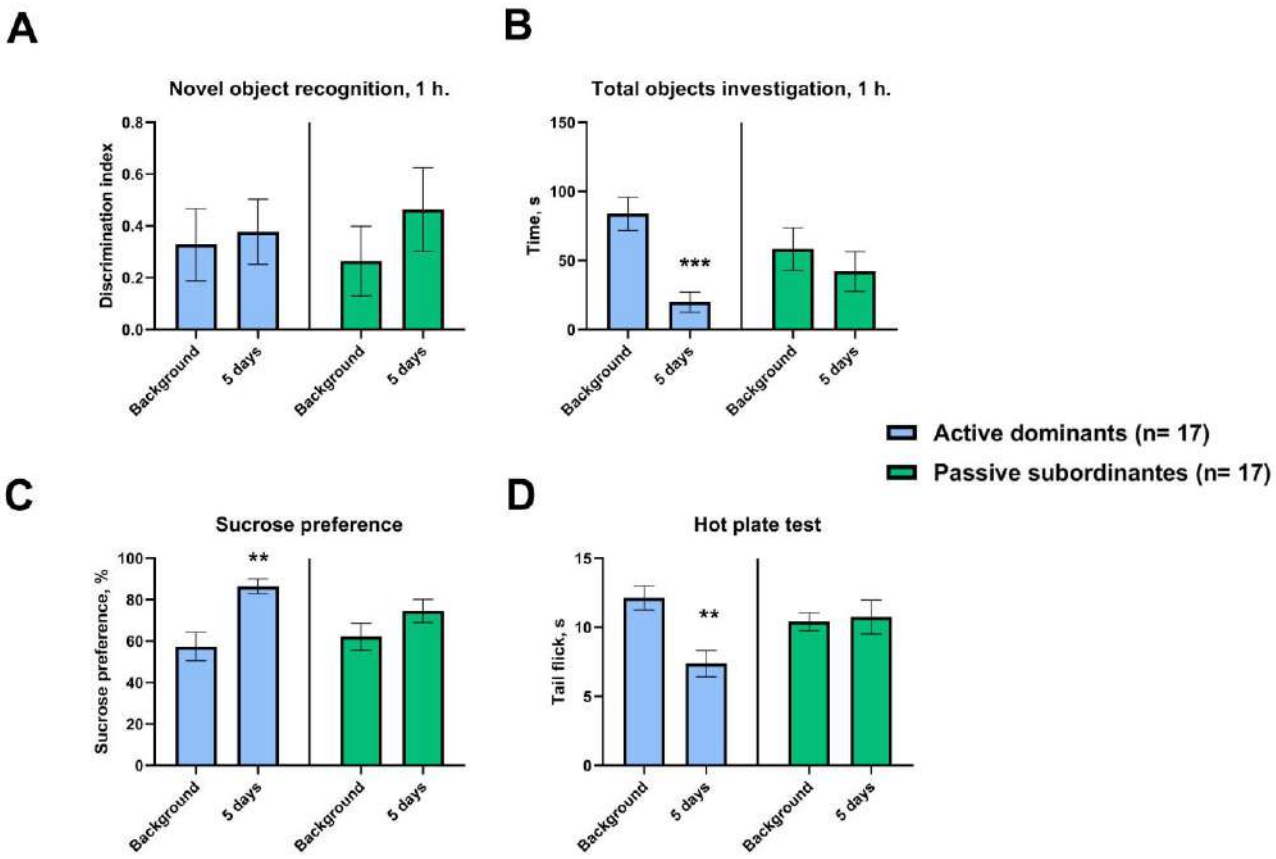


Figure 51. Behavioral indices in rats in the background and under the action of subchronic immobilization stress. X-axis - time scale of stress in days; Y-axis - corresponding indices. Data are

presented as mean, % ± standard error of the mean. ** - reliability of differences compared to background values in the group P ≤ 0.004; **** - P ≤ 0.0010 nonparametric Mann-Whitney test.

Stress did not affect the concentrations of lipoproteins, cholesterol, et al. (see Supplemental 1), however, a significant increase in blood corticosterone concentration was found in all experimental rats under 5-day immobilization stress (Figure 52). Thus, a significant increase in corticosterone concentration was shown on the 1st day of stress compared to background (P = 0.0159; nonparametric Mann-Whitney test) and a decrease on the 5th day of stress (P = 0.0497; nonparametric Mann-Whitney test), which was most pronounced in the AD group (P = 0.0485; parametric unpaired t-test) in contrast to the PS, where no significant changes compared to background were shown. This decrease in corticosterone concentration may be indicative of posttraumatic changes in the AD group under the influence of 5-day immobilization stress.

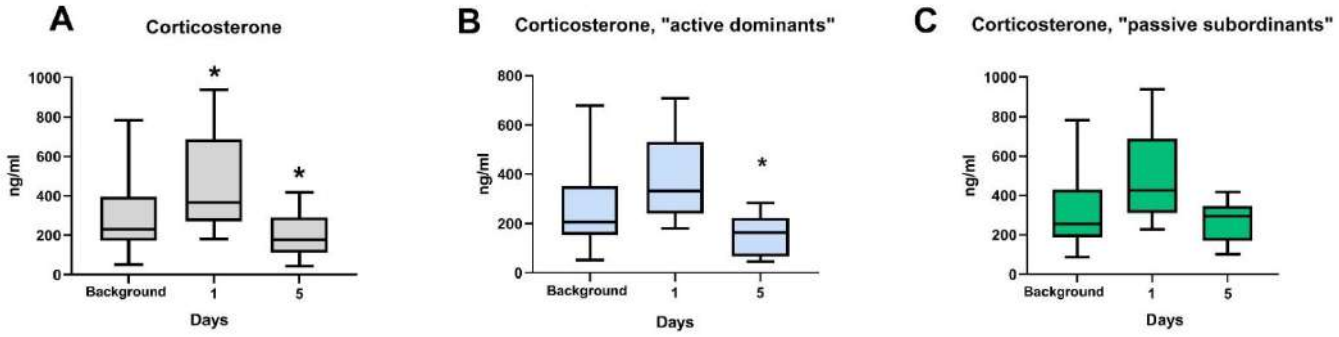


Figure 52. Changes in blood corticosterone concentration in “active dominants” (AD) and “passive subordinants” (PS) rats under subchronic immobilization stress. X axis - time scale of stress in days; Y axis - corticosterone concentration in blood plasma, ng/ml. The graph at the bottom reflects the cumulative values (AD+PS), other graphs reflect the values for the corresponding groups. Data are presented as mean, % ± standard error of the mean. *, significance of differences compared with baseline values in the group; P < 0.05; One-way ANOVA + Mann-Whitney nonparametric test or parametric unpaired t-test.

Examination of the number of D1 and D2 receptors in the cerebral cortex (Figure 53) of control and rats exposed to 5-day immobilization stress showed a significant decrease (P = 0.0031; Mann-Whitney nonparametric test) of D1 and a trend (P = 0.090; Mann-Whitney nonparametric test) toward a decrease of D2 receptors in all animals of the experimental group compared with the intact control. In a group study, it was shown that this trend was pronounced in the PS group (P = 0.0004; nonparametric Mann-Whitney test) in contrast to AD, where no significant change in the number of dopamine receptors under stress was found.

Contribution of DA receptors to the formation of a depression-like state in animals of different behavioral strategies after the action of uncontrolled subchronic immobilization stress

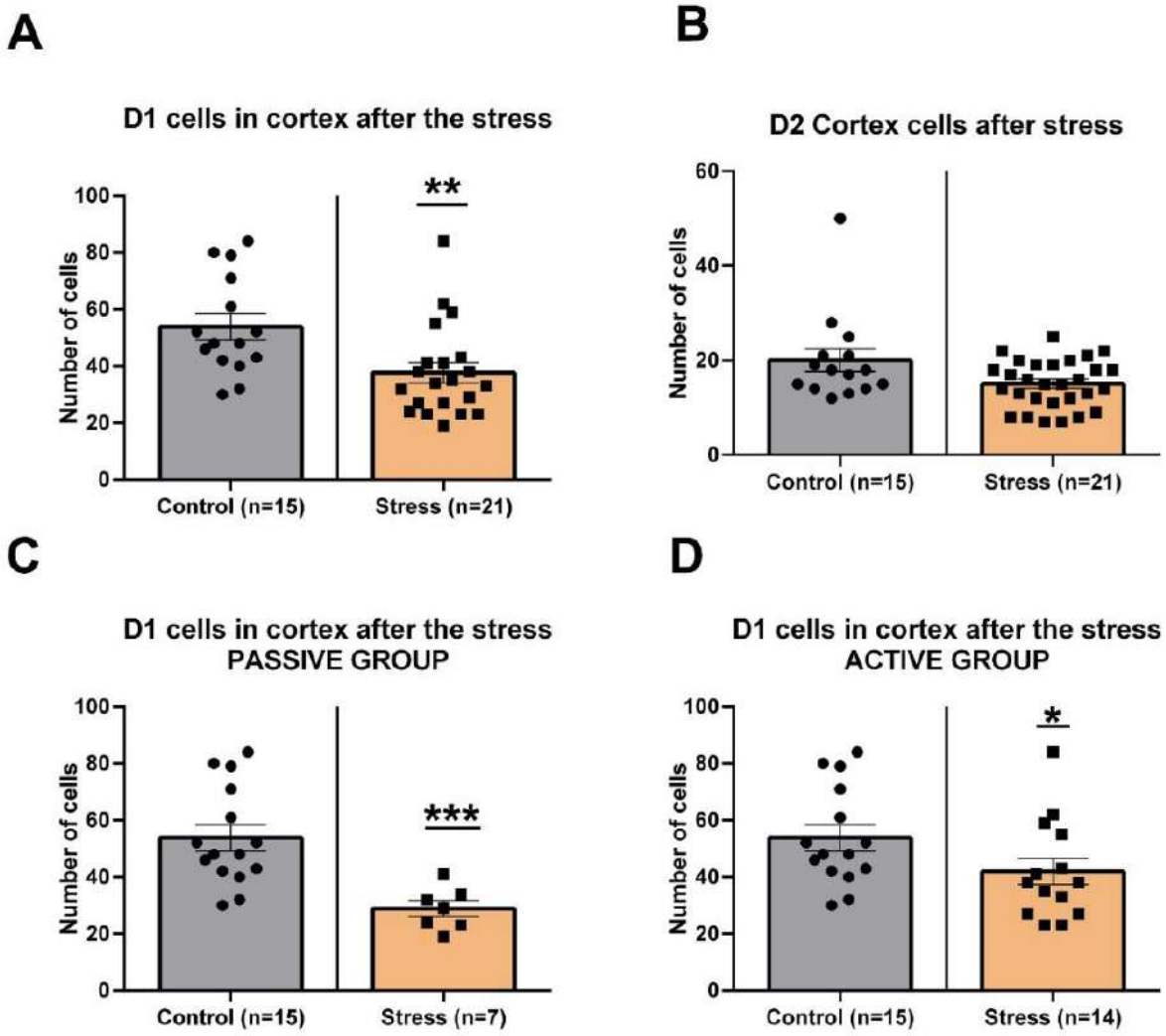


Figure 53. Decrease in the number of dopamine receptors in the rat cerebral cortex after 5-day immobilization stress. Data are presented as mean, % ± standard error of the mean. # - Trend vs. control (P = 0.090); * - significance of differences vs. control; ** - P = 0.0031; **** - P = 0.0004 nonparametric Mann-Whitney test.

Thus, it can be said that this change indicates the plasticity of neurotransmitter systems in PS, in contrast to AD, which and above showed a clear maladaptation under the influence of subchronic unavoidable stress.

- Is the reaction observed in the AD group - post-traumatic stress

According to the literature, one of the important characteristics of posttraumatic stress disorder (PTSD) that distinguishes it from depression is the reduced corticosterone levels observed during or after stressor exposure [362]. In our experiment, rats were exposed to immobilization stress for 5 consecutive days. On the first day of stress, we see a significant increase in corticosterone levels in all rats, which clearly indicates the activation of the HPA system, however, on the 5th day we see a significant decrease observed only in the AD group. Thus we can speak about characteristic posttraumatic changes in the AD group compared to the PS. In addition, inadequate increase in sugar preference in the anhedonia test, increased pain threshold, and decreased exploratory activity in the object exploration test - all these behavioral changes suggest the development of PTSD in AD rats in response to uncontrolled prolonged stress.

- Contribution of DA receptors to the formation of PTSD in animals of different behavioral strategies after the action of uncontrolled subchronic immobilization stress

A decrease in D1 and a tendency to decrease D2 cells in the cortex after the action of a 5-day immobilization stress were found. However, it can be said that this change indicates the plasticity of neurotransmitter systems in PS in contrast to AD, which showed obvious maladaptation under the action of subchronic unavoidable stress. Increased adaptability of SP, in contrast to AD, was also shown in the literature [93], but such a comprehensive study involving behavioral and neurochemical techniques was conducted for the first time.

In contrast, rat-controlled social stress, where the animal has the opportunity to run away or fight, results in smaller behavioral and neural consequences in “active” Wistar females that were exposed to a single social stressor (see above) or we see similarly, small changes in anxiety and activation of exploratory activity in male PS`s after a single “Porsolt”. Thus, it is worth noting that not only the nature, but also the duration of the stressor influences the manifestations of stress in animals with different behavioral strategies, which cannot be observed before the stressor, where it is impossible to distinguish animals with different behavioral strategies neither at the behavioral nor at the neurochemical level.

- An increase in DA neurotransmission as an adaptive process observed during and after a traumatic stressor event

During [19] and after a stressor event [4,41] there is an increase in mesolimbic dopamine; this increase is due to comprehending stressor information [1] and creating motivation to escape the unfavorable stimulus [363].

Activation of VTA DA neurons in rats exposed to uncontrolled stress by P. Willner has been shown to eliminate depressive symptoms in rats, especially anhedonia; in contrast, inhibition of these neurons leads to depression [21,364]. Thus, we can conclude that the increase in DA concentrations observed in our work plays an adaptive role, the non-significant changes in the anhedonia test in females and the absence of these changes in males after a single SD, may also indicate a positive effect of dopamine on the emotional sphere of the animal 24 h after stress.

Trying to find the translational significance of the obtained data for humans, I would like to mention that there are rare references in the literature about nonspecific mood elevation in humans after psychotraumatic effects [255]. Probably, this "mood elevation" is a consequence of the activation of the DA system, along with the activation of the HPA [30] and thus plays a predictive role, ensuring the normal functioning of the nervous system in response to a psychotraumatic stimulus.

However, another explanation of this phenomenon is also possible. The literature shows that [17] there are different types of reaction to stress: "fight and flight" or "hushing reaction". The "fight and flight" reaction is provided by the SAS system, and the "hush" reaction by the HPA. Expression of the action of these systems provides a different neurochemical and behavioral profile of the observed reactions. However, PET studies in humans have shown a positive correlation between cortisol and dopamine levels [30,365].

Thus, it is currently difficult to identify a specific neural circuit reflecting individual differences in stressor response, but it is clear that DA neurotransmission plays an important role in the process of realizing stressor responses and adaptation.

- As a result, we can conclude that subchronic immobilization stress adversely affected all rats, causing them multidirectional depressive-like changes.
- The "active dominants" (AD) rats demonstrated reduced adaptive potential compared to "passive subordinates" (PS) to the action of uncontrolled immobilization stress.
- "Passive subordinate" (PS) rats demonstrate greater adaptive potential than AD in response to uncontrolled immobilization stress.

Efficacy of the antidepressant bupropion for the management of symptoms induced by chronic uncontrolled stress in animals with different behavioral strategies

Chronic moderate uncontrolled stress is considered the most adequate model of everyday life stress. To model such stress in Wistar rats, we used the classical scheme of P. Wilner [10,169]. To select rats with active and passive strategies of stressor behavior, we used the conditioned active avoidance reflex test, which, as the previous chapter showed, is effective. Bupropion is a selective DA and NA reuptake blocker and is effective as an antidepressant when administered chronically and acutely increasing mesolimbic DA levels in the NAc [366-368], in this study it is used after the stress at a dose of 10 mg/kg. The schematic of the experiment is shown in Figure 54.

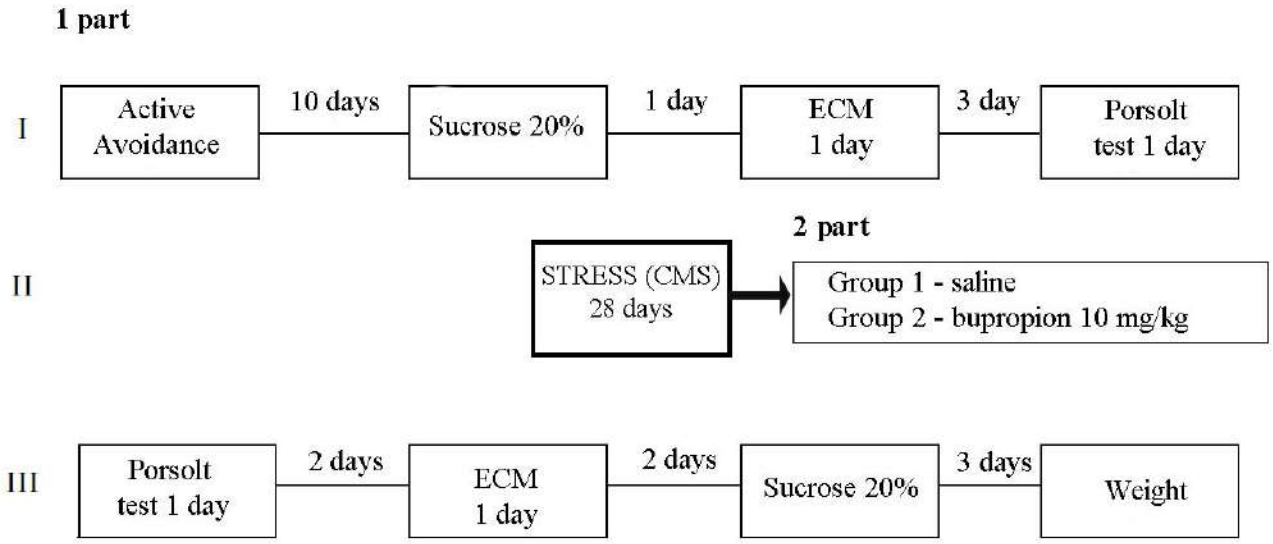


Figure 54. Scheme of experiments of the 1st and 2nd series (parts). In the first part the effect of stress chronic mild stress (CMS) was studied, in the second - after stress half of Wistar rats were injected with saline solution, the other half - with antidepressant bupropion 10 mg/kg of animal weight for 10 days, then behavioral testing was performed. The same behavioral tests as in III were conducted after the administration of substances. I, II, III are the order of behavioral tests and stressor procedures. ECM - “elevated cross maze” test.

It is a known fact that rats are characterized by a constant weight gain during life [369]. In our results, it was shown that during one month during which the stressor effect was realized, no significant increase in body weight was found in animals exposed to stress (Figure 55), both in the first and second series of experiments for animals of active and passive groups, also under the influence of bupropion (BUP). A significant ($P = 0.030$, nonparametric Mann-Whitney test) decrease in sucrose

solution consumption in rats of the active and passive groups was found, indicating a state of anhedonia in all rats after a one-month CMS procedure.

The ECM test showed a statistically significant decrease in the time rats spent in the open arms of the maze (P = 0.045, nonparametric Mann-Whitney test), which may indicate a maladaptation of the rats of the “active group” after the action of CMS and a decrease in the possible posttraumatic changes observed in the “passive” group. CMS did not affect statistically significantly the motor activity indices in the ECM in any of the groups.

According to the results of the Porsolt test, we can see that the CMS significantly negatively affected the rats with active behavioral strategy in contrast to the “passive rats”. The immobility time in this test was significantly reduced in the groups: after CMS (P = 0.0004, unpaired t-test), siline (P = 0.0007, nonparametric Mann-Whitney test), as well as, least of all, in the BUP group (P = 0.017, nonparametric Mann-Whitney test).

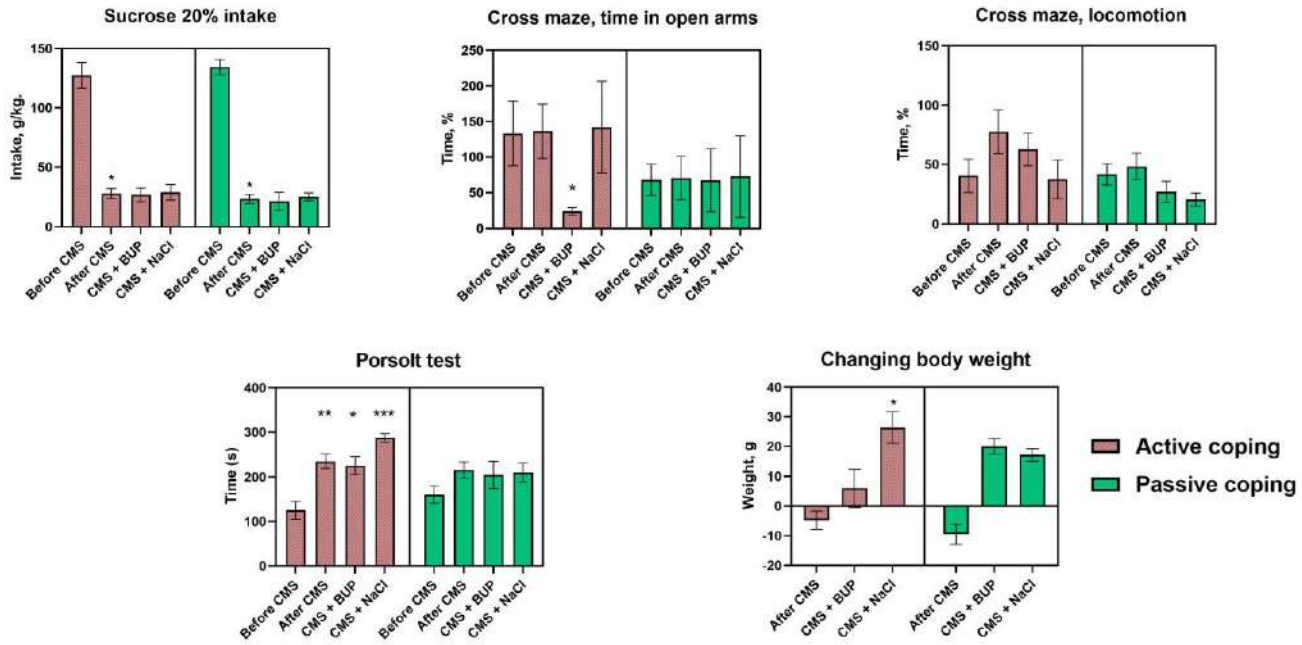


Figure 55. Behavioral changes particularly pronounced in “active” rats after 28 days of chronic uncontrolled stress (CMS) and subsequent administration of the antidepressant bupropion (BUP) for 10 days at a dose of 10 mg/kg. Data are presented as mean ± standard error of the mean. * - $P \leq 0.03$; ** - $P \leq 0.002$; *** - $P \leq 0.0005$, Mann-Whitney nonparametric test.

Based on the data obtained, it can be concluded that chronic moderate uncontrolled stress presented for 1 month caused depressive-like behavior in rats with an active behavioral strategy, whereas the behavior of rats with a passive behavioral strategy was more weakly influenced.

Thus, the effect of chronic moderate uncontrolled stress on the behavior of rats depends on their behavioral strategy [46].

We have shown that the antidepressant bupropion, administered for 10 days after one month of chronic moderate uncontrolled stress (CMS), had no effect on anxiety of rats in ECM, but had a pronounced antidepressant effect (reduction of immobility time in the Porsolt test) on animals with an active behavioral strategy, i.e. on animals that developed a depressive-like state after stress. Animals with passive behavioral strategy were not affected by the antidepressant [47].

Using the microdialysis method on free-ranging rats, it was shown that bupropion increased the concentration of extracellular DA in the NAC and caudate nucleus to a greater extent during chronic and to a lesser extent during acute administration [370]. Thus, the efficacy of this antidepressant used in clinical practice may be related to its mechanism of action. This work shows the selectivity of action of this drug, which was published by us earlier [47].

Thus, the antidepressant bupropion showed its efficacy for animals with an active behavioral strategy.

Chapter 5. Behavioral consequences of emotional stress induced by acute organophosphorus poisoning (a new model of PTSD?)

Exposure to organophosphorus compounds (OPs) results in inhibition of acetylcholinesterase (AChE) and consequent uncontrolled increases in synaptic acetylcholine levels [371]. Depending on the dose and duration of exposure to OP, paralysis, convulsions, cardiac arrhythmias, respiratory arrest and death may occur for several hours after acute poisoning [371]. Mood changes, depression, cognitive impairment and memory impairment due to the death of frontal cortex and hippocampal neurons [372], as well as impaired coordination of movements and locomotion [373] are observed in people who survive acute OP poisoning. Cognitive disorders related to decision-making, attention and motivation in patients may persist for several years after a single poisoning with OP, which may indicate, according to the authors, the development of posttraumatic stressor disorder [373,374].

In model experiments on rats during OP poisoning, a decrease in motor and stereotypic (grooming) activity was found, and motor, emotional, and cognitive deficits were observed, indicating the presence of a depression-like state [375,376]. One of the reasons for the development of depression on post-exposure to OP may be stress. Severe emotional exposure can cause serious long-term post-traumatic changes in the body (depression, increased reactivity, decreased motivation, apathy) even one month after a single exposure [38,39,177,377].

This dissertation shows that OP poisoning can cause a depression-like state not only in animals experiencing OP exposure, but also in animals in the same cage as “poisoned” animals. Such animals are constantly exposed to social stress, which has been shown in the literature to cause depressive disorders and PTSD symptoms in chronic presentation [378-380].

Using the Labs 24-hour animal activity monitoring system and other behavioral tests, we were able to show that in response to a single exposure to organophosphorus substances (OPs), animals experience significant behavioral changes that persist for up to 1 month (decreased motor, exploratory-emotional activity, increased depressivity, and decreased cognitive activity). Significant behavioral depressive-like changes are also observed in animals of the “stress” group, being in the same cage with the experimental animals during 40-60 days after intoxication.

Within several hours after a single administration of paraoxon in experimental groups POX2x (rats were administered paraoxon at a dose of 0.6LD_{50} one hour after administration of paraoxon at a dose of 0.45LD_{50}) and CBPOX (rats were administered paraoxon at a dose of 0.6LD_{50} one hour after administration of CBDP at a dose of 3.3 mg/kg), the animals showed primary clinical manifestations of poisoning (convulsions and possible respiratory arrest) characteristic of OP intoxication. In 24 h

after acute OP poisoning, the surviving rats showed secondary neurological manifestations of OP poisoning, manifested in an increased reaction to a sound stimulus expressed in an intense startle in the POX2x group in 62% of rats, and in the CBPOX group - in 25%, in contrast to background parameters. One week after poisoning, in 75% of animals of both groups, CNS arousal indices did not differ from background indices.

Compared to the baseline values, up to the 4th week after OP poisoning, all rats of the experimental groups showed a decrease in general motor (approximately 2-fold in the first day) and exploratory activity (15-20% decrease in the first day), as well as an increase in immobility (sleep) time ($P < 0.001$, Two-Way ANOVA); (Figure 56).

Cognitive deficit (new object recognition test) ($P < 0.05$; Mann-Whitney nonparametric test) and depressive-like state (Porsolt test) persisted up to 60 days after OP poisoning in all experimental groups, especially in CBPOX group ($P < 0.005$; Mann-Whitney nonparametric test); (Supplemental 1).

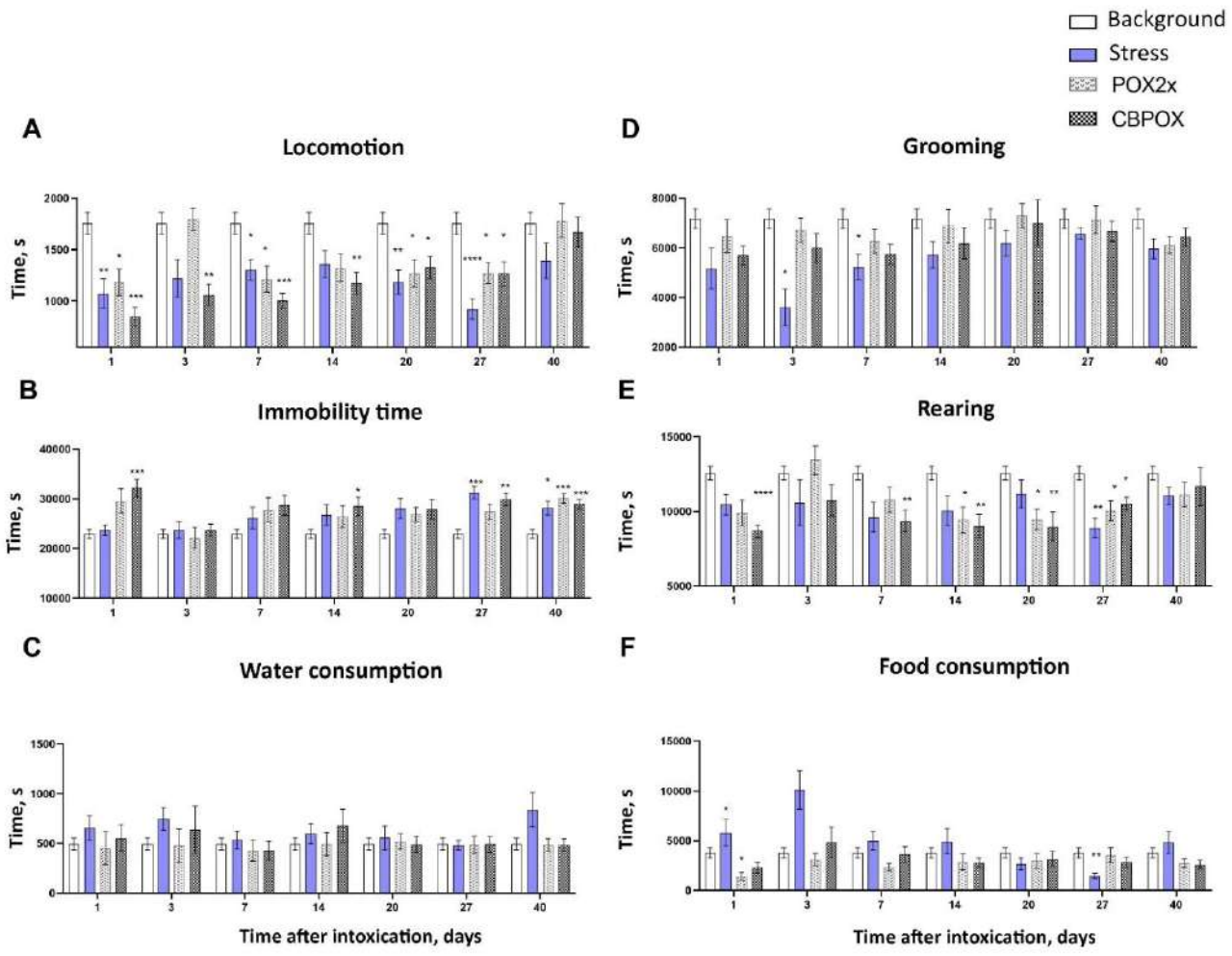


Figure 56. Suppression of behavioral activity in rats after acute poisoning with organophosphorus substances POX and CBPOX. Behavior of animals (A - E) during 40 days in groups: POX2x (n=15)

and CBPOX (n=15), Stress (n=15), Background (n=30). X-axis - time after intoxication, days; Y-axis - time, manifestation of a given behavioral element within 18 h. Differences are statistically significant (* - $P < 0.05$; ** - $P < 0.005$, *** - $P < 0.001$, **** - $P < 0.0001$, Two-Way ANOVA) compared to baseline. Data are presented as $M \pm m$.

It is noteworthy that the animals of the "stress" group, housed in the same cage as the FOS-exposed rats, showed manifestations of depression and post-traumatic stress disorder (PTSD). On the first day, a decrease in motor ($P < 0.005$, Two-Way ANOVA) and exploratory (object recognition test (* - $P < 0.05$; nonparametric Mann-Whitney test) activity was found in animals of the "stress" group compared to background. But particularly significant changes indicative of the development of PTSD in these rats were seen 27 days after stress: significant decreases in daily motor ($P < 0.0001$, Two-Way ANOVA), exploratory activity ($P < 0.005$, Two-Way ANOVA), increased immobility (sleep) time ($P < 0.001$, Two-Way ANOVA), and decreased food intake ($P < 0.005$, Two-Way ANOVA).

OP intoxication leads to a significant deterioration of the neurological status of rats on the first day after intoxication. The primary clinical symptoms (seizures) are associated, according to the literature, with stimulation of brain cholinergic muscarinic receptors (mAChRs), which causes persistent tonic-clonic seizures [381], as well as with glutamate release [382]. Possible respiratory arrest may be associated with inhibition of the corresponding parts of the medulla oblongata.

Further behavioral changes are associated with the death of neurons of various brain regions due to intoxication, oxidative stress, and inflammatory processes. Different parts of the limbic system: amygdala, hippocampus, thalamus, cortex, etc. become the target for such changes. Thus, these disorders become the cause of depressive and posttraumatic changes in rats [382]. In our work, we have shown that these changes persist up to 60 days after a single injection of OP.

Thus, during the first day after poisoning in rats of both experimental groups, general motor and exploratory activity (in the cage and in the process of recognizing a new object) decreases, immobility time increases, fine motor performance deteriorates, feed consumption time decreases, body weight decreases, and cognitive deficit develops. Decreased rearings, as well as exploration of a novel object, in poisoned animals throughout the observation period after poisoning indicates a decreased motivation to explore the surrounding space [383], which may also be one of the reasons for reduced feed intake and decreased body weight for up to 2 weeks [384]. The depressive-like state in animals is characterized by a decrease in motor, emotional, and cognitive components [385]. The development of depressive-like state in rats after OP intoxication with signs of toxicological poisoning is evident. It is important to note that after the period of recovery of behavioral and physiological indices on 3-7 days after poisoning, there is a deterioration of indices in later periods (4-11 weeks).

These data are consistent with the data [384], according to which, periodic deterioration of animal condition in the long term is characteristic of acute OP poisoning.

We found that the joint maintenance of animals of the “stress” group with the animals subjected to OP intoxication leads to significant emotional changes in these rats. These changes indicate the development of PTSD in these animals. According to the literature [38,39,386] PTSD exactly 1 month is the critical period at which characteristic depressive-like signs are observed.

- In our studies it is shown that even non-contact emotional exposure can lead to significant and long-lasting changes in the rat organism

The main criteria of PTSD in animal modeling of this disease are: participant or witness of psychotraumatic event (+), depressive-like symptoms (+), cognitive impairment (+), sleep disturbances (+), symptoms persist or manifest in a month (+), no effectiveness of pharmacological correction (don't know), reduced corticosterone level (don't know) [38,177].

- Single intoxication with OP`s leads to behavioral and cognitive disturbances. The animals show clear signs and depressive-like state. These disorders are pronounced in the first day after intoxication, with a slight recovery on the 3rd-7th day and deterioration of indicators on the terms of 4 - 11 weeks.
- The model of long-term non-contact psychoemotional exposure of rats after a single acute intoxication with OP of rats-neighbors can be considered another model of PTSD and meets most validation criteria.

Concluding remarks

1) Single social defeat stress resulted in significant changes in DA neurotransmission and behavior in male and female rats 24 h after exposure. These changes were more pronounced in rats with a passive coping strategy of stressor behavior

Single social defeat stress (as well as single stress of unavoidable swimming) influenced fewer behavioral parameters (increased anxiety, changes in cognitive abilities) than other types of stress (subchronic, prolonged, etc.).

In animals with an active coping behavioral strategy, short-term stress did not have any significant (statistically significant) effects at the behavioral or neurochemical levels.

All observed post-stressor changes were expressed predominantly in animals with a passive behavioral strategy (during stress). It should be separately emphasized that in contrast to small but statistically significant behavioral changes, animals with a passive strategy also showed significant changes in DA neurotransmission (increased DA response, decreased rate of DA recovery after its forced depletion) compared to animals with an active behavioral strategy and controls.

2) Chronic moderate and subchronic immobilization stress adversely affected all animals, but pronounced depression-like changes were found only in animals with active coping strategy of stressor behavior

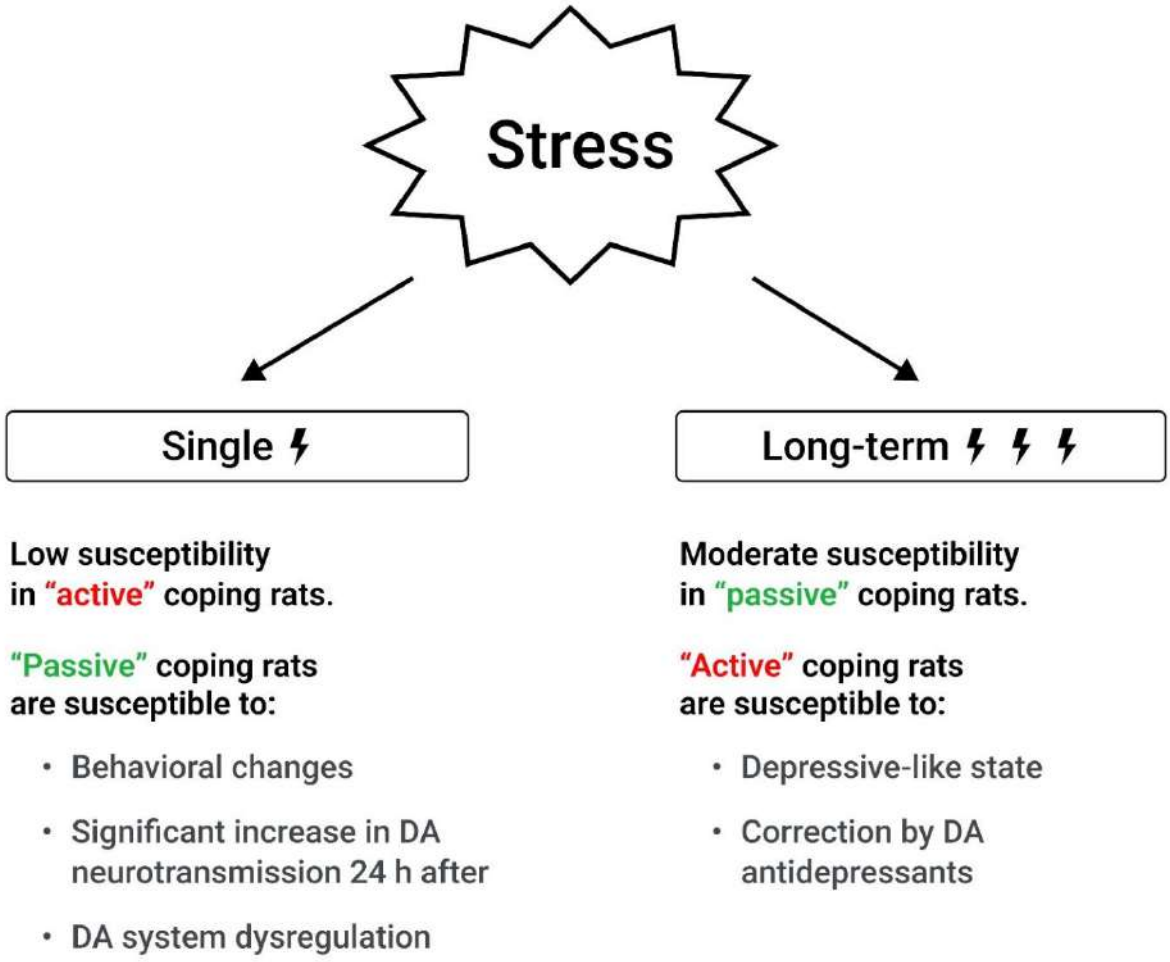
According to the results of the conducted studies on the effects of chronic stressors, it can be concluded that, depending on the duration of stressor exposure, different physiological, neurochemical and behavioral responses were observed in animals, which also differed depending on the behavioral strategy. Chronic stress resulted in increased anxiety scores, decreased motor activity, body weight and impaired cognitive functions, in addition to an initial decrease (1 day) and subsequent increase (5 day) in corticosterone concentration, changes in DA receptor density and other abnormalities in the animals. However, not all animals were susceptible to adverse effects from chronic stressor exposure.

Prolonged stressor exposure induced a depressive-like state in animals with an active behavioral strategy, along with physiological changes (body weight, pain sensitivity) and changes in blood biochemical parameters (decreased corticosterone concentration). Some of these observed changes in “active rats” were effectively corrected by the antidepressant bupropion.

Despite the fact that in animals with passive behavioral strategy no depression-like state was observed, but only anxiety and behavioral symptoms were detected along with significant changes in the number of dopamine D1 receptors, these facts allowed us to conclude about the presence of

increased neuronal adaptation in animals with passive behavioral strategy in response to the action of subchronic unavoidable stress.

It can be concluded that the effectiveness of a behavioral (coping) strategy depends on the duration and type of stressor impact in which it is implemented (scheme 1).



Scheme 1. Different susceptibility of animals with active and passive behavioral strategies to stressors of different duration.

3) TPH2 KO animals show increased aggression, stress sensitivity and increased alcohol consumption in compare to TPH2 WT animals.

TPH2 KO animals were found to exhibit increased aggression and alcohol consumption compared to TPH2 WT animals. In addition, a single immobilization stress applied after the animals had been habituated to alcohol for 3 months provoked a further increase in alcohol consumption in these animals. It is suggested that the disturbances in the motivational sphere in such rats are related to the dysregulation in dopamine neurotransmission in TPH2 KO animals observed by us. The data obtained indicate a disruption of synthetic processes in the DA system, and as a consequence, an

increase in alcohol consumption and aggressive actions. In addition, female and male TPH2 KO animals are adequate model subjects that can be used to create aggressive environments in social defeat stress modeling studies.

4) Chronic psychoemotional stress leads to the development of pronounced post-traumatic stress disorder (PTSD) in rats, which persists for at least 60 days.

A single intoxication with OP leads to behavioral and cognitive disturbances. The animals show clear signs of a depressive-like state. The model of chronic non-contact psychoemotional exposure of rats after a single acute intoxication with OP of neighboring rats can be considered an acceptable model of PTSD and meets most validation criteria.

Thus, changes in dopaminergic neurotransmission (along with behavioral, physiological, and biochemical changes) may serve as indicators of adaptation processes in the body aimed at overcoming stress and bringing the organism to homeostasis.

CONCLUSIONS

1. A set of behavioral methods resulted in baseline behavioral differences between animals with active or passive behavior coping strategies.

2. Twenty-four hours after a single social defeat stressor, all animals (males and females) showed significant activation of the mesolimbic DA response, and maladaptive changes in D2 autoreceptor regulation of DA were found in stressed males compared to control animals.

3. Males and females exhibited different behavioral abnormalities 24 h after a single social defeat stressor.

4. Single social defeat stress induced a state of anhedonia and other behavioral abnormalities, also significant changes in mesolimbic DA neurotransmission in female rats after 24 h, most pronounced in rats with a passive behavioral coping strategy in contrast to animals with the opposite active coping strategy and controls.

5. Single unavoidable stress had a greater effect on animals with a passive behavioral strategy, which showed a significant decrease in motor activity compared to baseline values in contrast to animals with an active behavioral coping strategy, showing only an increase in biased activity in response to stress.

6. Subchronic immobilization stress (5 days) resulted in significant depression-like changes in all stressed animals, as manifested by characteristic changes in behavioral activity and physiological parameters, as well as an increase and then a maladaptive decrease in corticosterone concentration and D1 receptor density in the cerebral cortex.

7. The observed significant depression-like behavioral, physiological, biochemical and neurochemical changes caused by the action of subchronic immobilization stress were pronounced only in animals with an active coping behavioral strategy. In animals with a passive strategy no pronounced depression-like state was found, but only signs of anxiety disorder were observed. The reason for this phenomenon may be the significant adaptive neuronal DA rearrangements found in “passive” rats (decreased density of D1 receptors in the cortex), compared to “active” coping rats.

8. Disadaptation and depressive-like state were demonstrated in animals with active behavioral strategy after the action of chronic unavoidable stress. These stresses did not induce a depressive-like state in animals with a passive behavioral strategy.

9. The antidepressant bupropion effectively corrected behavioral abnormalities induced by the action of chronic moderate uncontrollable stress in animals with an active coping behavioral strategy.

10. TPH2 KO animals showed increased consumption of sweet and alcohol-containing solutions, as well as increased aggression and stress-induced alcohol consumption compared to WT animals. TPH2 KO animals in contrast to WT animals showed decreased parameters of DA recovery after DA depletion procedure.

11. Chronic psycho-emotional stress led to the development of pronounced post-traumatic stress disorder (PTSD) in animals, which persisted for at least 60 days.

List of abbreviations used

AD - active dominants

AS – acute stress

ACh - acetylcholine

BUP - bupropion

HPLC - high performance liquid chromatography

GABA - gamma-aminobutyric acid

HPA - hypothalamic-pituitary-adrenal system (HPA)

IS – immobilization stress

GLU - glutamate

D1, D2, D3 - dopamine receptor types 1,2,3

DA - dopamine

DA OQ - dopamine orthoquinone

DAT - dopamine transporter

IGC - immunohistochemical analysis

CR – conditioned reflex

CRF - corticolebirin

NA - norepinephrine

NS - narcotic substances

OFC - orbitofrontal cortex

ECM - elevated cross maze

PS - passive subordinates

PTSD - post-traumatic stressor disorder

PFC - prefrontal cortex

SAS - sympatho-adrenal system (SAS)

SER - serotonin

SD - conditioned reflex

OP - organophosphorus compounds

CMS - chronic mild uncontrollable stress

HR - heart rate

5-HT1A - serotonin type 1 receptors

5-HT2B - serotonin type 2 receptors

CBDP - plasma carboxylesterase inhibitor

CBPOX - paraoxone + plasma carboxylesterase inhibitor

DSM - diagnostic and static manual of mental disorders

FSCV in vivo – fast-scanning voltammetry method

HAB - High avoidance rat line

KHA - Roman High avoidance rat line

KHA - Koltushi High avoidance rat line

KLA - line of Koltushi Low avoidance rats

LAB - line of low avoidance rats in URAI

NAc – nucleus accumbens

POX - paraoxon

Ref - reference electrode (Ag/Ag Cl)

RLA - Roman Low avoidance rat line

SE - stimulating electrode

TAAR 1 - trace amine receptor type 1

TPH1,2 - tryptophan hydroxylase genes TH types 1 and 2

VTA - ventral cap region

WE - working electrode.

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12.2020 - Best abstract at ECNP international conference.

2017 - 2018. - Member of the Russian Science Foundation grant. Grant 16-15-00199

05.2010 - Winner of the scholarship of the 15th Congress of Young Scientists of SPbU.

List of own publications

1. **Nemets, V.V.**; Deal, A.L.; Sobolev, V.E.; Grinevich, V.P.; Gainetdinov, R.R.; Budygin, E.A. Short-Term Consequences of Single Social Defeat on Accumbal Dopamine and Behaviors in Rats. Biomolecules 2023, 13, 35. https://doi.org/10.3390/biom13010035
2. **Nemets, V.V.**, Shmurak, V.I., Sobolev, V.E. et al. Effects of Transient and Prolonged Uncontrollable Stress on Animals of Dominant and Subordinate Social Status with Different Types of Stress Reactions. Neurosci Behav Physi 50, 618–624 (2020).
3. **Немец, В. В.**, Шмурак, В. И., Соболев, В. Е., Гарнюк, В. В., Рован, Е. Д., & Виноградова, Е. П. (2019). Влияние кратковременного и длительного неконтролируемого стресса на животных доминантного и субординантного социального статуса с различным типом стрессорной реакции. Физиологический журнал им. И.М. Сеченова, 105(5), 608-618. https://rusjphysiol.org/index.php/rusjphysiol/article/view/108/187
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7. Vinogradova, E.P., **Nemets, V.V.** & Zhukov, D.A. 2013, 'Active behavioral strategy as a risk factor for depression-like disorders after chronic mild stress', Journal of Higher Nervous Activity. I.P. Pavlov, Vol. 63, no. 5, pp. 589-596. https://doi.org/10.7868/S0044467713050109

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9. **Nemets, V.V.**; Vinogradova, E.P., Zavialov, V.; Grinevich, V.P.; Budygin, E.A. Gainetdinov, R.R.; Accumbal dopamine responses are distinct between female rats with active and passive coping strategies. Biomolecules 2024, 14, 1280. https://doi.org/10.3390/biom14101280

Other publications on the subject of the PhD thesis

10. **Nemets V.,** Zavyalov V., Chepik P., Gainetdinov R., Budygin E., P.0850 Different dopamine responses in female rats with aggressive and defensive stress coping, European Neuropsychopharmacology, Volume 53, Supplement 1, Page S622, ISSN 0924-977X, https://doi.org/10.1016/j.euroneuro.2021.10.709. (Award - best abstracts at ECNP conference, travel grant).
11. **Nemets V.,** Deal A., Gainetdinov R. & Budygin E. 2020, 'Consequences of a single social defeat on accumbal dopamine measures: in vivo voltammetric study', European Neuropsychopharmacology, vol. 40, no. S1, pp. S479-480. https://doi.org/10.1016/j.euroneuro.2021.10.709 (Award - best abstracts at ECNP conference).
12. **Nemets V.,** Zavyalov V., Budygin E., & Gainetdinov R. (2023). Influence of single social defeat stress on accumbal dopamine dynamics in female rats. Journal of the Neurological Sciences, 455. https://doi.org/10.1016/j.jns.2023.122134

List of presentations at international conferences

1. **Nemets V.V.**, Vinogradova E.P., Zhukov D.A. “Effect of the antidepressant bupropion on the depressive-like state caused by chronic mild stress”. pp. 20-21. Partisipate in Bion school “Neurobiotechnology 2010: Bioeconomy based on knowledge” September 28, 2010 Moscow Bekasovo (poster presentation).
2. **Nemets V.V.**, Zavyalov VA, Chepik PA, Gainetdinov RR, Budygin EA, Exploring the consequences of a single social defeat stress on accumbal dopamine in male and female rats. //28th Multidisciplinary International Neuroscience and Biological Psychiatry Conference “Stress and Behavior” – 2021. P. 21 (oral report)
3. **Nemets V.V.,** Zavyalov V.A., Chepik P.A., Gainetdinov R.R. Determination of dopamine release in rats by fast-scan voltammetry/ All-Russian Conference with international participation Almazovsky Youth Medical Forum May 2021, (poster report), 2nd place in the poster competition.

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Supplementary materials 1

A - Biochemical indices of rats after the action of subchronic immobilization stress

Groups of animals (coping)	Experimental groups of animals	TRIG	CREA	HDL	LDL	CHOL
AD	Control (n=5)	1.01±0.20	62.68±1.23	0.59±0.11	0.30±0.06	1.23±0.14
	Stress (n=11)	0.93±0.06	61.95±1.01	0.64±0.05	0.19±0.01	1.33±0.10
PS	Control (n=5)	0.93±0.08	61.88±0.77	0.60±0.01	0.23±0.04	1.18±0.33
	Stress (n=10)	1.19±0.23	60.32±0.91	0.67±0.08	0.21±0.22	1.31±0.15

Changes in various blood biochemical parameters in rats measured in mmol/L. Groups: “active dominants” (AD) and ‘passive subordinants’ (PS) under subchronic immobilization stress. Data are presented as mean, % ± standard error of the mean. TRIG - Triglycerides: CREA - Creatinine; HDL; LDL - High and low density lipoprotein cholesterol; CHOL - Total cholesterol.

B – Analysis of corticosterone in rat plasma before the action of subchronic immobilization stress

Groups of animals (coping)	Corticosterone concentration values, ng/mL
AD (Background)	261,13±39,4
PS (Background)	339,06±48,7

Background changes in blood corticosterone concentration in rats.

Groups: “active dominants” (AD) and ‘passive subordinants’ (PS). Data are presented as mean, % ± SEM

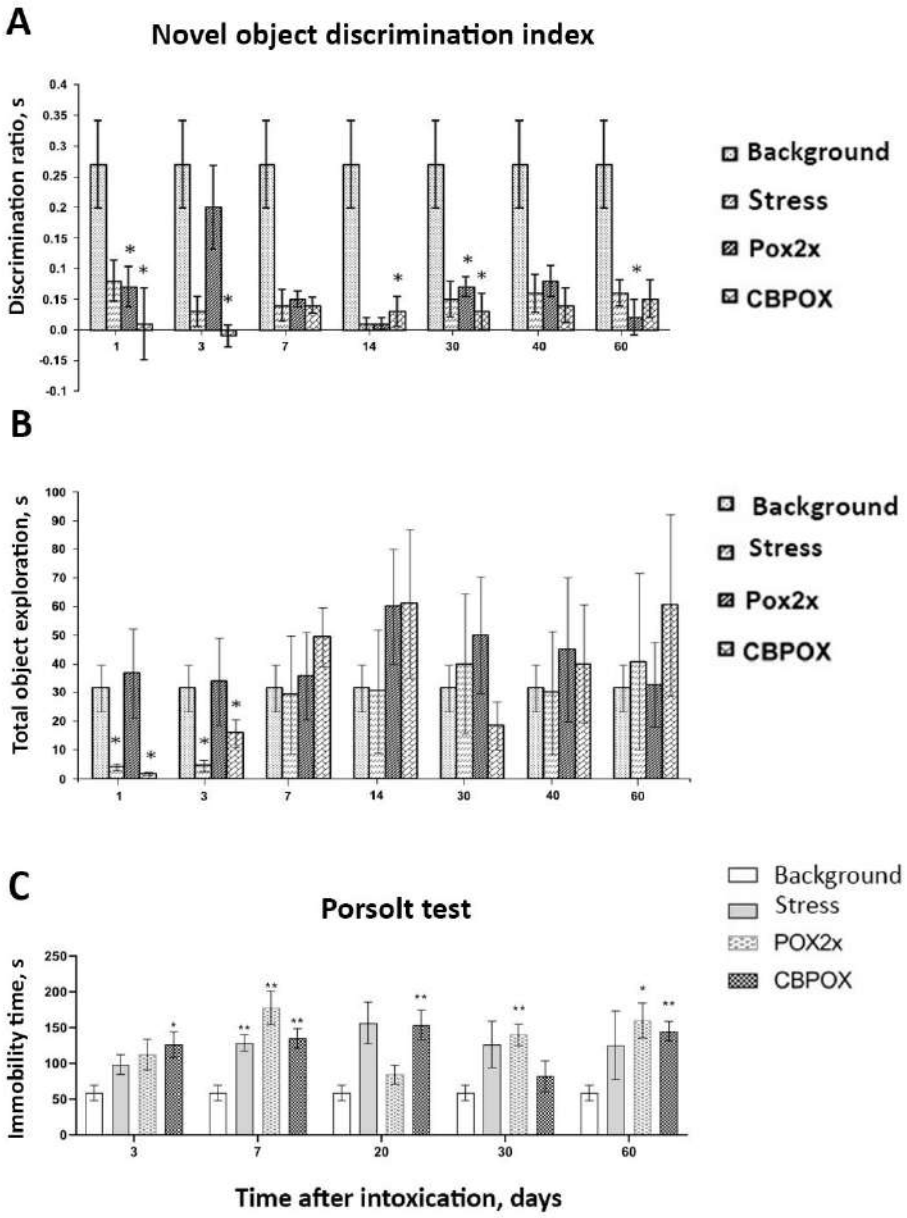
C - Ratio of animals of different social status in the groups of “active” and “passive” animals in the rat population

Behavioral strategy in a stressful situation (coping)	**Social groups**
«Active animals» (n=21)	**42%** dominants
	28 % subdominants
	28 % subordinants
«Passive animals» (n=37)	**5%** dominants
	20 % subdominants
	75 % subordinants

The “active avoidance” test was used hereafter to identify the coping strategy of behavior. Social status of the animal in the cage was determined using the “competition for vital resource” test.

D - Correlation of animals of different social status and behavioral strategies

Groups of animals	“Active avoidance” test results, %	Results of the “water deprivation” test, %
Active dominants (n = 17)	100% Highly active animals	**84%** dominants
		6 % subdominants
		10 % subordinants
Passive subordinants (n = 17)	100% Low-active animals	**0%** dominants
		12 % subdominants
		88 % subordinants



E - Decrease of cognitive activity indices and increase of depression in rats after acute poisoning with OP (POX and CBPOX), as well as rats-neighbors (group "stress")

Behavior of animals (A - C) during 60 days in groups: POX2x (n=6) and CBPOX (n=6), Stress (n=6), Background (n=11).

On the Y-axis, rating of preference of the new object to the previously presented object (A); total exploration time of all objects in 3 min, s (B); immobility time in 6 min of exploration, s (C).

On the X-axis, time after intoxication, days. Differences are statistically significant (* - $P < 0.05$; ** - $P < 0.005$, *** - $P < 0.0001$, nonparametric Mann-Whitney test) compared with baseline.

Data are presented as Mean ± SEM.