The impact of trauma of the mucous membrane of the nasal septum in rats on behavioral responses and changes in the balance of the autonomic nervous system (pilot study)

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Abstract. The nasal cavity surgery are usually traumatic surgery. Septoplasty leads to reactive inflammation, edema and hypoxemia. In the present study, we study the response of the autonomic nervous system (ANS), its role in changing behavioral reactions, as well as possible mechanisms of impairment of cognitive and adaptive reactions in rats after trauma to the nasal septum mucosa. The nasal septum mucosa was scarified in 10 adult mongrel male rats. The day before surgery and 2 days after surgery animals were tested in the square-shaped "open field" (OF) and electrocardiogram (ECG) in 1 hour before OF. ANS condition was analyzed by the high-frequency component of the heart rate (HF) and the low-frequency component of the heart rate (LF). The correlation of HF & LF with rat behavior in the open field before and after surgery was performed. Simulation of septoplasty in rats provokes a powerful stress response in the form of a sharp imbalance of ANS towards its PNS on the 2 postoperative day. Changes in behavioral and research reactions of rats in OF are manifested in a decrease in research activity, a display of uneasiness, depression-like state, as well as anxiety.

1. Introduction
The autonomic nervous system (ANS) provides the appearance of rapidly reacting mechanisms in response to external and internal factors to ensure the control of a wide range of physiological functions through the innervation of the end organs [1].

It has been shown that ANS dysfunction goes beyond a set of systemic conditions, including cardiovascular and lower respiratory diseases [2, 3]. Although the single airway hypothesis points to common pathophysiological processes in both the upper and lower airways [4, 5] the role of the ANS in the symptoms of the nose and sinuses remains poorly understood. Our understanding of the pathophysiology of dysfunction is limited mainly to the data of five small studies with no new data [6]. Important potential clinical complications in the upper respiratory tract require further investigation. Description of molecular mechanisms of ANS in the nasal cavity suggests potentially new targets for interventions in neurogenic inflammation in chronic rhinosinusitis [7]. In addition, chronic airway obstruction often leads to hypoxia, hypercapnia, as well as to significant changes in intra-chest pressure. These factors can influence the activation of sympathetic and parasympathetic parts of the autonomic nervous system, as well as various heart reactions provoked by the regulatory action of ANS [8].

However, there is still insufficient data on the relationship of behavioral reactions and autonomic regulation of the body during surgical interventions on the nasal septum.
In this study, the following tasks were set: (1) to determine the effect of surgical trauma in the nasal cavity (septoplasty models) on behavioral reactions, (2) to assess the state of the autonomic nervous system and (3) to identify the relationship of anxiety in animals with changes in the activity of the sympathetic and parasympathetic departments of the ANS.

2. Materials and methods

2.1 Surgery
We performed a simulation of septoplasty in 10 adult mongrel male rats weighing 185-250 g. The day before surgery animals were tested in the square-shaped "open field" with Central and peripheral branches and burrows. The next day, 10 minutes before the operation, intraperitoneally solution was injected at a dosage of 15 mg/kg. Local anesthesia and analgesia with non-steroidal anti-inflammatory drugs were not carried out to ensure that these methods of anesthesia did not affect the stress response in the simulation of septoplasty.

Simulation of septoplasty was performed by scarification throughout the nasal septum mucosa with the probe with a sharp end. In order to prevent drowning from its own blood rats carried out its constant aspiration to complete hemostasis.

2.2 Open field
Further, on the 2nd, 4th and 6th days after surgery, the repeated observation of the behavior of animals in an open field for 15 minutes was carried out. While calculating the results, the data for every 3 minutes were averaged and 5 temporary observation points were received.

Figure 1. Scheme of an open field. 1 - central sector, 2 - central minks, 3 - peripheral sectors, 4 - peripheral minks.

2.3. HRV.
The electrocardiogram (ECG) was recorded in the free field. To assess the state of the autonomic nervous system, a spectral analysis of rat cardiointervalograms was carried out before surgery and in 1 hour before testing in the open field on the second day after the surgery intervention. Rats were stitched with piercing in three places - in the area of withers and back. The state of the parasympathetic division of the ANS was analyzed by analyzing the high-frequency component of the heart rate (HF, parasympathetic nervous system); the predominant state of the sympathetic division of the ANS by analyzing the low-frequency component of the heart rate (LF, mainly the sympathetic nervous system). The state of parasympathetic and sympathetic parts of the autonomic nervous system was calculated as a percentage of each frequency index of their sum. The correlation of sympathetic and parasympathetic components with rat behavior in the open field before and after surgery was performed. To assess the validity of the differences in the results, the criterion for connected Wilcoxon samples was used.
Animal studies were conducted in accordance with the requirements of the Order of the Ministry of higher and secondary special education of the USSR No 742 "Rules of work with experimental animals".

3. Study results

The behavior of rats in the open field. Central sectors. Rats' visits to the central sectors on the second postoperative day were significantly less frequent in the first three minutes, and from 4 to 6 minutes they were not at all in the center of the open field, compared with preoperative indicators (CI 95% 1.00-3.00, p = 0.032). However, from 7 to 9 minutes there was an inversion in activity in the central sectors: in the control there was a complete extinction of activity, while after the operation the rats in this period of testing began to actively examine this area (CI 95% -2.00 (-2.00), p = 1.000). There was no significant difference in the fourth three – minute interval of testing (CI 95% -1.00-2.00, p = 0.854), and in the fifth increased activity before surgery and a significant decrease after were observed.

Peripheral sectors. Reliable differences in the vicinity of the peripheral sectors between the results dehydrogenase test and on the second day after modeling septoplasty we were found (CI 95% -3.50 - 8.00, p = 0.462).

Central minks. At 4-6, 7-9, and 12-15 minutes of testing operation has resulted in rarer research of the central minks rats, compared with before surgery data, while the animals were exploring the central burrows only for 7-9 minutes more, compared with preoperative data (CI 95% 1.00 - 2.00, p = 0.004). It should be noted that in the first three minutes of testing there were no differences between the two terms of examination of rats (CI 95% -1.00 - 0.50, p = 0.783).

Peripheral minks. After surgery, the rats studied peripheral Minks significantly less frequently than before (CI 95% 6.50 - 18.50, p = 0.002) at the period from 1 to 6 minutes of testing. However, there was no difference in the following minutes of testing (CI 95% -18.0 - 14.0, p = 0.688). Research activity was fading in rats before the operation, whereas after it, on the contrary, this activity increased and decreased in General, compared with 1 observation period, only by 12-15 minutes of testing (CI 95% 1.50 - 5.00, p = 0.022).

Fading. In testing conditions, after the operation, the rats were fading longer than before. This was reliably observed from 1 to 6 and from 12 to 15 minutes (p = 0.043).

Grooming. Throughout the test, the rats were washing themselves for longer time after surgery, compared with preoperative parameters (CI 95% -27.0 - (-2.0), p = 0.031).

Stands. The number of stands was observed significantly more frequently in rats before surgery from 1 to 11 minutes of testing, compared with the data after surgery (p = 0.027).

Boluses (defecation). The number of acts of defecation was observed significantly more often from 4 to 9 and from 12 to 15 minutes of testing in rats before surgery than after it (p = 0.047).

Analysis of HRV. On the second day after the operation activity of the sympathetic part of autonomic nervous system in rats significantly decreased compared to the control parameters (0.34±0.02% and 0.44±0.03%, respectively) (p<0.01). The influence of parasympathetic nervous system in this period, on the contrary, increased from 0.55±0.03% to 0.65±0.02%, respectively (p<0.001).

Correlation before surgical intervention between indices of HRV and data from the open field have not been identified. On the second day after the simulation of septoplasty and postoperative inflammation, comparing the proportion of the sympathetic division of the ANS (LF%) and OF data, we revealed a number of dependencies. Thus, the value of reliability approximation of the number of crossings of the central sector of tonus of the sympathetic division of the ANS has made 0.80, number of crossings of the peripheral sector with LF% 0.70, research central mink with LF% 0.71, studies of peripheral mink with LF% 0.77, grooming with LF% 0.71. Negative relationship between these parameters was recorded in this case.

The tone of the parasympathetic division of the ANS strongly correlated with the indicators of the study of the central sectors (0.78) and with grooming (0.71). Good correlation was observed in HF% with data on the peripheral sector, central minks and peripheral minks – 0.57, 0.60 and 0.68, respectively.
4. Discussion

Influence of research methods on the behavior of rats in the open field. It is known that the half-life of Zoletil components (tiletamine hydrochloride and zolazepam hydrochloride) is up to 4.5 hours (in cats) [9]. In this study, on the second day, the effects of tiletamine hydrochloride and zolazepam hydrochloride were minimized and, in our opinion, these drugs could not have any effect on the test results in the open field.

Putting animals in an open field is itself a stress factor for animals for a number of reasons: for rats, the field is a new and unexplored environment. Furthermore, the installation we used was white in our experiment, the study was conducted in daylight, while rats are nocturnal animals. In this regard, the question which arose was about the time of ECG monitoring—before or after the OF? We decided that ECG removal was less stressful than OF, and 1 hour between studies was likely sufficient for the effect of the previous study to be leveled.

Changes in the behavior of rats in the open field. Behavioral studies in humans and animals have shown that stress tends to disrupt various memory tasks depending on the hippocampus. In animal studies it was found that stress changes the subsequent synaptic plasticity and excitatory properties of hippocampal neurons [10], which in this study can characterize a significant decrease in research activity in rats after modeling septoplasty, in particular central and peripheral sectors, peripheral minks, compared with the data before surgery. It is known that a visit to the central sectors itself is a stress for the tested animals [11], additional effects of complications after surgery (mucosal edema, inflammation, hypoxemia) cause including a decrease in locomotor activity in the Central sectors of OF.

Morphological studies in humans and animals have shown that stress changes the structure of neurons, inhibits the proliferation of neurons and reduces the volume of the hippocampus. Since the beginning of stress studies almost 80 years ago, much attention has been paid to different levels of neuroendocrine hormones of the hypothalamus-pituitary-adrenal axis, glucocorticoids in particular, as mediators of numerous stress effects on the hippocampus and factors contributing to stress-related psychopathology, such as post-traumatic stress disorder. It has also been shown that prolonged exposure of glucocorticoids by injection, implantable granules or drinking causes morphological and molecular changes, reduces neurogenesis and disrupts synaptic plasticity in the hippocampus, the physiological results of which are thought to accelerate hippocampal-dependent memory disorders such as anxiety and depressive behavior [11]. In our opinion, the absence of postoperative analgesia and subsequent inflammation in rats undergoing surgery may indicate indirectly that the pain syndrome may have provoked anxiety, which was manifested by the suppression of motor activity, a decrease in the number of stands in operated animals, which may indicate the presence of depressive-like disorder in animals. So, any surgical intervention except pain syndrome provokes emotional reaction, in particular irritation, anxiety, anxiety, etc. [12].

Heart rate variability and surgical stress. This study does not use techniques that determine the specific mechanisms of cause-effect relationships between posttraumatic inflammation and changes in the balance of ANS. However, in a study by A. Woody et al. it was confirmed that the change in HF in HRV was associated with a change in levels of proinflammatory cytokines in an hour after the stress factor [13] in accordance with the neuroimmune reflex [14]. If the subsequent effects of the stressor, which reduce HF HRV, are a reflex increase in the number of inflammatory cytokines, it may provide a better understanding of the pathway between stress and disease [15]. These data may also serve as evidence that interventions in homeostasis that increase parasympathetic activity may be of particular interest for the treatment or prevention of many diseases caused by inflammation [14]. In our study, the shift in the balance of the ANS toward the parasympathetic component in rats after surgery can also be explained by post-surgical inflammation, since rats were not analgesic and anti-inflammatory therapy. In addition, it is known that rats cannot breathe through the mouth. In the nasal cavity after septoplasty, as a rule, inflammation is accompanied by swelling of the mucous membrane, which leads to narrowing of the nasal passages. The reduction in the flow of air, disturbance of the mucociliary system of the epithelium of the nasal cavity always leads to hypoxemia [16], which in turn may increase the influence of the parasympathetic component of vegetative balance [17]. However, according to modern concepts,
prolonged exposure of hypoxia on the body leads to the opposite effect – activation of the sympathetic nervous system and an increase in angiogenesis [18].

The sympathetic-adrenomedullary axis can rapidly increase heart rate and blood pressure (in seconds) by stimulating the cardiovascular system [19]. It is important to note that in the acute phase of stress, the excitement of ANS rapidly decreases due to reflex parasympathetic activation, which leads to short-term reactions [1]. The pronounced predominance of the high-frequency component of the heart rate shows that on the second day after the simulation of septoplasty, the processes of reparation and anabolism, for which the parasympathetic system is responsible, prevailed [21].

Stress also changes behavioral responses. Under the conditions of experimental study of the behavior of rats in the open field under the influence of stress, the activation reactions of space study were described [22]. Thus, during acute stress, compared with chronic stress, rats more often demonstrate stands with support on the wall of the open field [23]. According to the data obtained in this study, it can be assumed that the presence of a good correlation between the low-frequency component of the heart rate and the study of the open field and mink space also indicates the predominance of parasympathetic influence. As a rule, frequent animal stands indicate strong stress in animals, but on the 2nd day after surgery their number significantly decreased compared to the control data (p<0.05). The presence and severity of grooming also indicates expressed concern and anxiety in rodents. The quantitative reduction of this component also shows that the balance of the ANS was shifted towards the parasympathetic division. More rapid extinction of research activity in relation to peripheral mink after surgery also indicates the absence of sympathetic influence.

In this study, we have found indirect correlations between the activity of sympathetic NS by studying central and peripheral sectors, grooming. Also, these indicators of research activity showed a high connection with the parasympathetic nervous system as well. Based on this, it can be assumed that the rats developed a depressive-like condition and anxiety disorder against the background of surgical stress. It is known that grooming, in particular, is a complex behavioral act, very sensitive to various stress and pharmacological effects, genetic changes [24]. An increase in grooming also confirms an increase in stress-related anxiety in animals on the 2nd day after surgery. It is possible to draw a parallel with the data obtained by other authors. Thus, it was suggested that a complex context-specific modulation of grooming behavior may include both a path through the basolateral nuclei–the Central nucleus of the amygdala–the nucleus of the terminal plate bed (the front path), which mediates stress, anxiety and conditioned protection, as well as a path through the medial nuclei of the amygdala–the nucleus of the terminal plate bed (the back path), projected onto the hypothalamus, and this path is responsible for innate social behavior and response-protection to the predator – defensive reflex][25,26].

The brain receives data that signal serious homeostatic disorders such as blood loss, respiratory failure, visceral or somatic pain and inflammation [27]. The sympathetic response to incoming pulses involves reflex arcs, which are associated with different areas in the brain and the preganglionic sympathetic neurons in the intermediate column of the lateral cells of the spinal cord [20]. Coordination changes in the parasympathetic part of the ANS also occur after exposure to stress factors. This is manifested as a change in "wandering tone " for the heart and lungs [20] and helps to control the duration of vegetative reactions. In our study, changes in the tone of the parasympathetic division of VNS are obvious on the basis of HRV analysis. We can draw Parallels with our previous studies of HRV after septoplasty in people in the early postoperative period. Thus, it was found that in patients with inadequate anesthesia, there was increased activity of the parasympathetic nervous system [28].

In the presented experiment it was found that on the second day after the simulation of septoplasty in rats simultaneously there were coordinated processes – postoperative decrease in the research activity of rats in the open field (a rare survey of central sectors, central and peripheral minks), a decrease in locomotor activity (reducing the number of stands), increasing the fading time and duration of grooming. At the same time, the activation of the parasympathetic nervous system was shown, which was due to the following factors: post-surgical inflammation, a decrease in the volume of nasal passages due to untreated edema of the injured mucous membrane of the nasal cavity and the subsequent hypoxemia. This relationship suggests that the operated rats developed a distress syndrome. In our opinion,
subsequent studies should show how stress in surgical interventions on the nasal septum in animal models affects the formation of the distress syndrome. In addition, it is important to understand the place and role of General, local anesthesia, analgesic therapy in reducing the subsequent reactions of the body during surgical trauma in the nasal cavity.

5. Conclusion
Simulation of septoplasty in rats provokes a powerful stress response in the form of a sharp imbalance of the autonomic nervous system towards its parasympathetic component on the 2nd postoperative day. Changes in behavioral and research reactions of rats in the open field are manifested in a decrease in research activity, a display of uneasiness, depression-like state, as well as anxiety. Such reactions, compared with preoperative data, are probably associated with hyperactivation of the parasympathetic nervous system. Most likely, the emerging postoperative stress is closely related to metabolic, physiological and morphological changes in the hippocampal region.

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