ANIMAL PATHOLOGY, MORPHOLOGY, PHYSIOLOGY, PHARMACOLOGY AND TOXICOLOGY

ПАТОЛОГИЯ ЖИВОТНЫХ, МОРФОЛОГИЯ, ФИЗИОЛОГИЯ, ФАРМАКОЛОГИЯ И ТОКСИКОЛОГИЯ

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Optimization of General Anesthesia Monitoring Methods Used in Laboratory Animals during Development and Experimental Study of Veterinary Drugs

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Abstract

Introduction. Giving general anesthesia to animals is an important element of experimental research during the development and study of new veterinary drugs. Due to specific nature of general anesthesia and the need to use the potent sedatives and hypnotics, it has always been posing a risk to laboratory animals. In the frame of present-day veterinary research, the problem of proficient monitoring of anesthesia is extremely relevant, especially with regard to research involving laboratory rodents. The aim of the study is to develop and optimize laboratory monitoring methods of general anesthesia in animals during experimental studies of new veterinary drugs.

Materials and Methods. The study was conducted at the research laboratory of the Institute of Living Systems, DSTU, from January to May 2024. The experiment involved 20 Wistar rats of both sexes weighing 300–400 g that were subjected to inhalation anesthesia with isoflurane, using an RWD R520 anesthesia machine. The laboratory monitoring protocols were developed based on the electroencephalography (EEG), electrocardiography (ECG), respiratory rate, blood pressure, and body temperature parameters. The condition of animal organs and systems was assessed by means of biochemical blood and urine tests, as well as excretory function tests.

Results. An inhalation chamber connected to an anesthesia machine was designed for the study in rats. It allowed laboratory monitoring of animals' condition during inhalation anesthesia by electrophysiological and functional methods. A trial mode of the chamber using 2.0% isoflurane gas supply, at a flow rate of 2.0 l/min, and a withdrawal rate of 8.0 l/min had proved to be the optimal protocol for administering general anesthesia to laboratory rats. Electrophysiological, biochemical, and functional parameters demonstrated high tolerability of the anesthesia protocol used.

Discussion and Conclusion. The development proposed by the authors optimizes laboratory monitoring in animals during general anesthesia, contributes to improvement of quality of experimental research results, facilitates the work of researchers, and can be used during the development of new veterinary drugs.

Key words: general anesthesia, laboratory monitoring, experimental studies, laboratory rats, inhalation anesthesia, inhalation chamber, electrophysiological parameters

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Оригинальное эмпирическое исследование

Оптимизация методов контроля общей анестезии лабораторных животных при разработке и экспериментальном изучении ветеринарных препаратов

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Аннотация

Введение. Общая анестезия животных является важным элементом экспериментальных исследований при разработке и изучении новых ветеринарных препаратов. В силу своей специфики, необходимости применения сильнодействующих седативных и гипнотических средств, анестезия всегда представляет собой риск для лабораторных животных. В настоящее время в ветеринарных исследованиях остро стоит проблема грамотного анестезиологического сопровождения, особенно при работе с лабораторными грызунами. Цель данного исследования — разработка и оптимизация методов лабораторного контроля общей анестезии животных при проведении экспериментальных исследований новых ветеринарных препаратов.

Материалы и методы. Исследование проведено на базе научно-исследовательской лаборатории Института живых систем ДГТУ в период с января по май 2024 г. В эксперименте участвовало 20 крыс линии Wistar обоих полов массой 300–400 г, которых подвергали общей ингаляционной анестезии с использованием изофлурана и наркозного аппарата RWD R520. Протоколы лабораторного контроля разрабатывали на основе показателей электроэнцефалографии, электрокардиографии, частоты дыхания, артериального давления и температуры тела. Оценку состояния органов и систем животных проводили с помощью биохимических исследований крови, мочи и показателей выделительной функции животных.

Результаты исследования. В ходе исследований была сконструирована ингаляционная камера для крыс, подключаемая к наркозному аппарату, позволяющая проводить лабораторный контроль электрофизиологическими и функциональными методами в условиях ингаляционной анестезии. Апробация камеры при режиме подачи газа с концентрацией изофлурана 2,0 %, скоростью подачи 2,0 л/мин и отбора 8,0 л/мин, показала, что это оптимальный протокол осуществления общей анестезии у лабораторных крыс. Данные электрофизиологических, биохимических и функциональных параметров свидетельствовали о хорошей переносимости использованного анестезиологического протокола.

Обсуждение и заключение. Предложенная авторская разработка оптимизирует лабораторный контроль животных при проведении общей анестезии, способствует повышению качества результатов экспериментальных исследований, облегчает работу ученых-исследователей и может быть использована при разработке новых ветеринарных препаратов.

Ключевые слова: общая анестезия, лабораторный контроль, экспериментальные исследования, лабораторные крысы, ингаляционный наркоз, ингаляционная камера, электрофизиологические параметры

Для цитирования. Шебеко С.К. Оптимизация методов контроля общей анестезии лабораторных животных при разработке и экспериментальном изучении ветеринарных препаратов. *Ветеринарная патология*. 2026;25(2):25–33. https://doi.org/10.23947/2949-4826-2026-25-2-25-33

Introduction. General anesthesia in animals is an integral part not only of veterinary practice but also of experimental studies of veterinary drugs. Laboratory monitoring of anesthesia is an important factor for its successful implementation [1]. Due to its specific nature and the need for potent sedatives and hypnotics, anesthesia evidently has always been posing a risk for laboratory animals, especially for young and elderly animal specimens and animals with induced pathologies [2, 3, 4]. Complications resulting from general anesthesia manifest not only in deterioration of animal's physiological functions and the state of all life support systems, but also in exacerbation of the pathological process, which can even lead to death [5].

According to statistics, adverse reaction to anesthesia is a quite widespread phenomenon in veterinary practice [6]. Poor recovery from general anesthesia in dogs is observed in 29.1% of cases, with 55.5% of them requiring sedation to control behavior [7]. In sick animals, anesthesia-related mortality is 3.6 times higher than this figure in the overall animal population (4.80% versus 1.35%) [2], moreover, in sick dogs the probability of cardiac arrest caused by anesthesia is 23 times higher, and the probability of death is 24.5 times higher than in healthy animals [8]. Post-operative mortality is of particular concern in sick animals. According to some data, it reaches 47% in dogs, 61% in cats and 64% in rabbits. In the overall animal population, rab-

bits are the most susceptible to anesthesia-and sedation-related mortality, with the risk of death being 8 times higher than that in dogs (1.39% versus 0.17%) [9].

Currently, the problem of proficient monitoring anesthesia when working with animals, along with proper examination, and adherence to recommendations is acute, both in experimental research and in veterinary medicine [10, 11]. This problem is especially relevant when working with small rodents, which are the main test systems in biomedical research, due to their small size and metabolic characteristics [12]. The opportunities of Russian scientists in the field of experimental veterinary research are considerably limited due to limited access to modern technologies, medicinal products, and equipment, especially those used for monitoring general anesthesia in laboratory rodents. Therefore, improving approaches to administering general anesthesia and laboratory monitoring of the process in the frame of experimental studies is of scientific importance for improving animal safety.

The aim of the study is to develop and optimize the methods for monitoring general anesthesia in laboratory animals to improve the efficiency and safety of experimental studies of new veterinary drugs.

Materials and Methods. The study was conducted at the Pharmacology and Experimental Pathology Research Laboratory of the Institute of Living Systems, DSTU (Rostov-on-Don) from January to May 2024. Twenty Wistar rats of both sexes weighing 300–400 g were involved in the experiment. All animals were kept on a standard diet with free access to water in accordance with acting sanitary standards [13].

The studies were conducted in compliance with the general principles of bioethics [14] and Directive 2010/63/EU of the European Parliament and of the Council "On the Protection of Animals Used for Scientific Purposes" (Brussels, 2010) [15]. All manipulations causing pain and suffering, and the withdrawal of animals from the experiment was conducted using adjusted dosage of anesthesia and analgesia [16]. The protocol of the study was verified by the Local Independent Ethics Committee of DSTU (Resolution No. 4 of 09.10.2023).

General anesthesia of animals was performed using an RWD R520 anesthesia machine for animals (RWD Life Science, USA) and isoflurane (Laboratories Karizoo, S.A., Spain).

Using the KOKS-2 cardiorespiratory complex for small laboratory animals (Medical Computer Systems Ltd., Russian Federation), electrocardiography (ECG) was performed, and heart rate (HR) and respiratory rate (RR) were assessed [17, 18]. Blood pressure (BP) was assessed by non-invasive plethysmometric method [19] using the "Sistola" non-invasive blood pressure measurement system for rodents with the "Phlogiston" heating platform (Neirobiotiks LLC, Russian Federation). Brain activity intensity was assessed by electroencephalography (EEG) [20] using the "Neiron-Spektr-1V" computerized electroencephalograph for veterinary medicine (Neirosoft LLC, Russian Federation). Animal body temperature (T) was measured using a Zamer-1 veterinary digital rectal thermometer (version 2 – rectal, Zamer LLC, Russian Federation). During laboratory observations, the animals' pedal, swallowing, and pupillary reflexes were also assessed.

To assess the functional condition of the organs and systems of animals before and after anesthesia, a series of biochemical studies of blood and urine were performed. Blood was obtained from the caudal vein in an amount of up to 0.5 ml per draw. The glucose concentration in native blood was determined using a OneTouch Verio Reflect glucometer (LifeScan Europe GmbH, Switzerland). Creatinine content, aspartate aminotransferase (AST) and alanine aminotransferase (ALAT) activity were determined in blood serum using "Diacon-DS" kits and a SPECTROstar Nano spectrophotometer (BMG Labtech, Germany) [21]. Urine was obtained using metabolic cages (OpenScience, Russian Federation) with a duration of collection for 2 hours. The creatinine content was also determined in urine, as well as the glomerular filtration rate (GFR) was calculated based on the endogenous creatinine clearance [22].

The obtained results were processed using descriptive statistics methods, tested for normality using the Shapiro-Wilk test, and presented as the arithmetic mean ± standard error of the mean (M ± m). Statistical analysis of intergroup differences was performed using one-way analysis of variance (ANOVA) with Tukey's HSD post hoc test [23]. Calculations were performed using IBM SPSS Statistics v. 22 (IBM Corp., USA) and MS Excel 2016 (Microsoft Corp., USA) software. Differences in figures were considered statistically significant at a probability level of $p < 0.05$.

Results. Inhalation anesthesia is the preferred method of general anesthesia used in experimental studies in terms of efficacy and safety, as it enables complete control over animal's condition through a combination of two key parameters: the concentration of the inhalation anesthetic and the rate of gas mixture flow. This method not only allows quick putting an animal in a state of pharmacological hypnosis but also its immediate recovery after cessation of gas delivery, practically without adverse consequences. Therefore, the study proposes improvement of laboratory monitoring techniques used specifically for this type of anesthesia.

Inhalational anesthetics, especially isoflurane, are known to pose a hazard to professionals who are constantly in contact with them during work. The standard masks supplied with the RWD R520 animal anesthesia machine are not hermetically sealed and are prone to gas leakage. To solve the problem, a sealed inhalation chamber for rats that enabled laboratory monitoring based on measuring the electrophysiological parameters was designed. The design of the chamber had been developed to ensure enough space to place EEG electrodes on the animal's scalp as well as the

respiratory sensor of the cardiorespiratory system with a minimal working volume of a gas mixture required. At the same time, there remained the possibility to place ECG sensors on the animal's torso, a blood pressure cuff on the tail, and a rectal thermometer in the anus. Implementation of all the above-mentioned monitoring elements was possible only using a chamber, where the animal's head was placed, leaving the rest of the body free. The standard inhalation anesthesia induction chambers are not suitable for this purpose.

The chamber was made of transparent polycarbonate with external dimensions of 100 x 100 x 60 mm and an internal volume of 500 cm^3 (Fig. 1). A nozzle adhering to DIN standard with an internal diameter of 6 mm was used to deliver the gas mixture to the chamber, and a nozzle adhering to 22M standard with an internal diameter of 18 mm was used to remove the waste gas. These nozzles were positioned opposite each other to ensure a unidirectional gas flow, so that it could reach the inlets of the animal's respiratory tract. The 1:3 ratio of inlet to outlet nozzle cross-sections ensured the flow of the gas mixture from the entry to evacuation point. Six animals were involved at the stage of inhalation chamber design and assembly.

To solve the problem, it was necessary not just design a chamber itself, develop the method of putting the upper part of the animal's body into it, but also to invent the way of forced gas removal from the chamber using a gas evacuation device and replaceable gas filters supplied with the anesthesia machine. Such devices are designed to absorb and bind excess inhalational anesthetic, thereby ensuring operational safety. In the current experiment, two RWD Life Science gas evacuation apparatuses with the gas filter weighing function were used: R546-Pro with forced gas removal and R548 without it, with corresponding gas filters R510-31-6 and R510-31S-6.

During general anesthesia, the animal was exposed to isoflurane in the induction chamber. The animal was then placed on the experimental heating platform, fixed, and its head was placed in the inhalation chamber. EEG electrodes were attached, a respiration sensor was installed, and the ECG electrodes were applied. The inhalation chamber was then closed, and the gas mixture started to be delivered (Fig. 2). The animal's tail was then placed on a "Phlogiston" heating platform, a blood pressure cuff was applied, and a rectal thermometer was inserted. The animal remained in this position throughout the general anesthesia procedure to enable laboratory monitoring, piloting this technique, and recording the respective physiological parameters. The resulting data were analysed for the efficacy of the proposed monitoring methods and safety of the tested general anesthesia protocol.

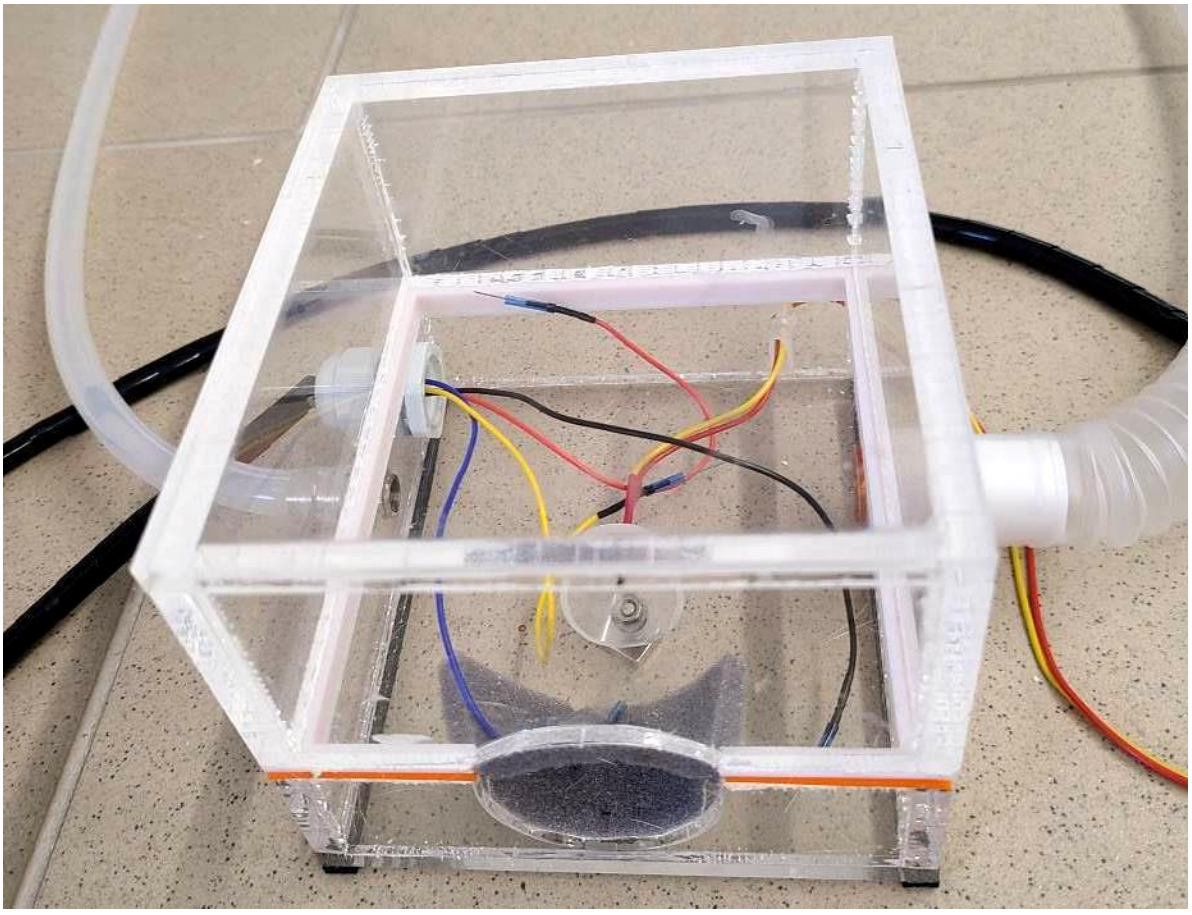

Fig. 1. A prototype of an inhalation chamber for general anesthesia in rats

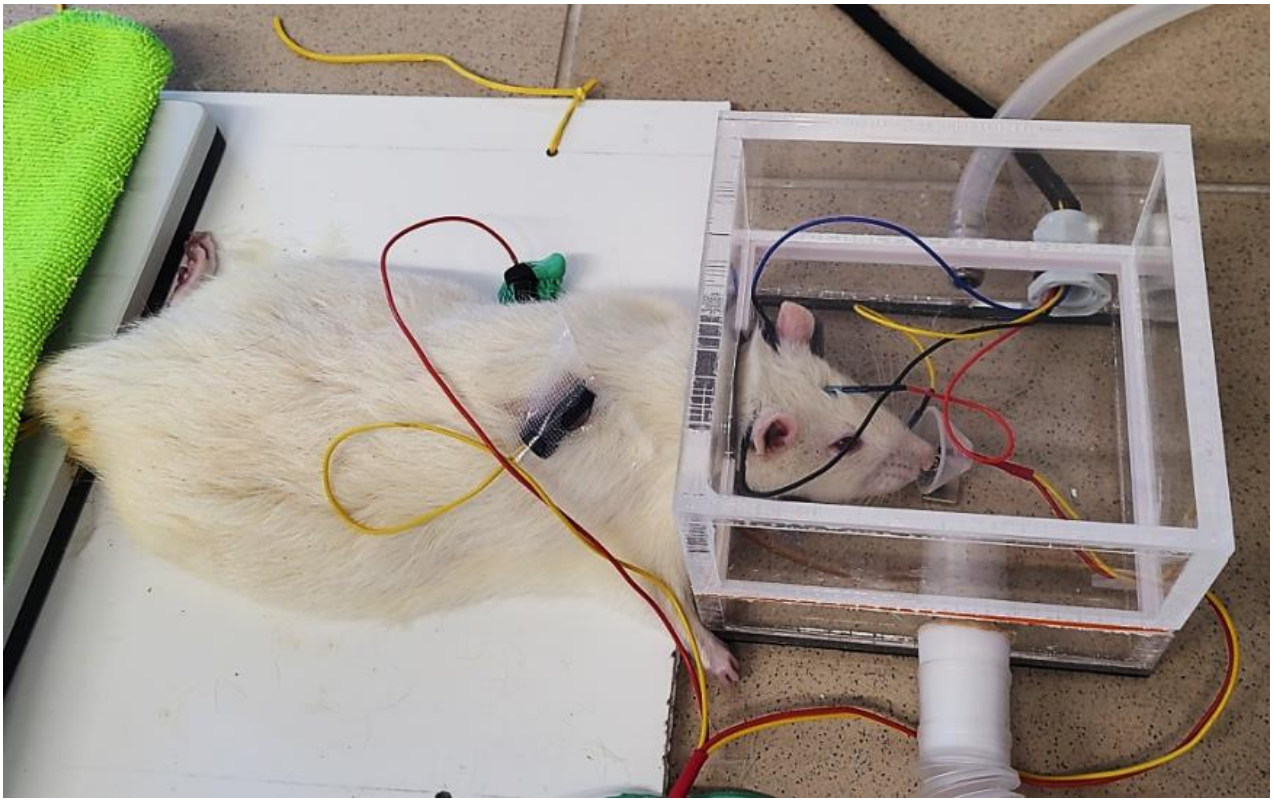

Fig. 2. A Wistar rat monitored during inhalation anesthesia using the developed inhalation chamber

During the study, two modes of anesthesia administration were used — using active and passive gas removal, whereas the concentration ratio and flow rate were adjusted to minimize the effect on the animal. During the adjustment process, we judged about the depth of anesthesia in rats by determining the stages based on the animal condition, the level of reflexes and physiological parameters.

As a result, two gas delivery modes were used:

1st mode: isoflurane concentration 2.0%, flow rate 2.0 l/min, removal rate 8.0 l/min;

2nd mode: isoflurane concentration 1.5%, flow rate 2.0 l/min, without forced gas removal.

With respect to these modes, two groups of seven animals were formed and exposed to general anesthesia with assessment of laboratory monitoring methods based on physiological parameters recorded.

During the study, electroencephalograms (EEG) were continuously recorded in the animals in the state of inhalation anesthesia (Fig. 3), and EEG indices were computed using the software (Table 1).

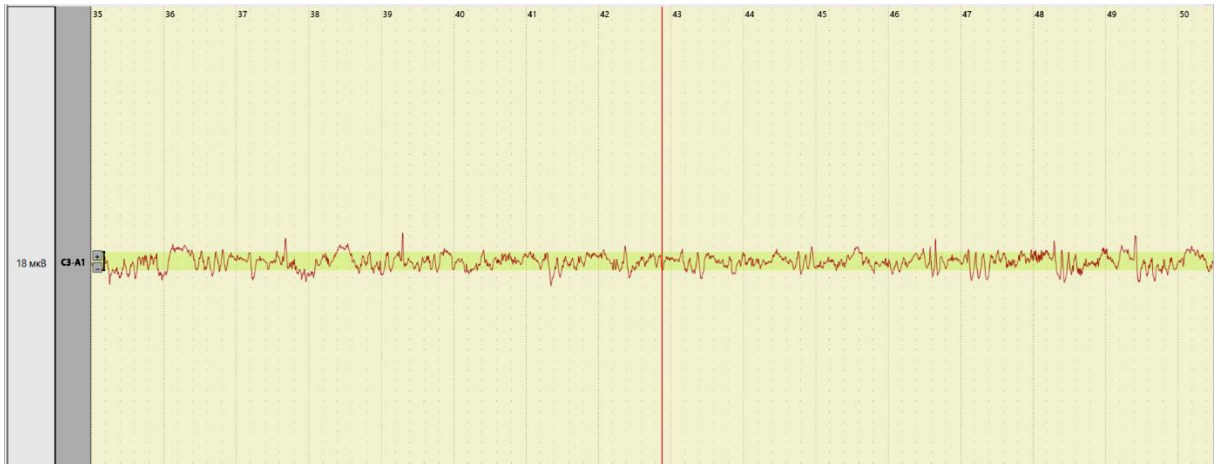

Fig. 3 Example of an electroencephalogram of a rat in the state of general anesthesia

Table 1

Electroencephalogram indices in rats in condition of general anesthesia 30 minutes after induction

EEG indices	1st group (n=7)	2nd group (n=7)
Maximum amplitude, μV	130.2±13.7	112.7±11.0
Mean amplitude, μV	19.3±2.2	15.8±1.6
Delta rhythm, μV	45.6±4.5	58.7±5.5
Theta rhythm, μV	33.5±3.5	37.2±4.0
Alpha rhythm, μV	31.4±3.2	20.3±2.7*
Beta-LF rhythm, μV	11.7±1.5	10.2±1.3
Beta-HF rhythm, μV	10.7±1.2	5.9±1.0*

Note: * – differences are significant relative to animals of the 1st group ($p<0.05$).

The EEG was analysed by the general indices of maximum and mean amplitudes, as well as the indices of mean amplitudes of five main rhythms: delta rhythm with a frequency of 0.5–3.0 Hz, theta rhythm (4.0–6.0 Hz), alpha rhythm (8.0–13.0 Hz), low frequencies of the beta band (13.0–18.0 Hz) and high frequencies of the beta band (18.0-32.0 Hz) [20]. Analysis of the EEG spectral amplitudes makes it possible to determine the predominant type of EEG rhythm and, based on this, draw a conclusion about the degree of animal immersion in anesthesia, weakening the hypnotic effect of anesthetics, or, conversely, excessive depth of anesthesia with the risk of a lethal outcome, as well as to record the moment of agonal phase or clinical death development.

The results presented in Table 1 indicate a greater depth of anesthesia in rats anesthetized with the second mode of gas mixture delivery compared to the first group. This is confirmed by a non-significant decrease in the maximum and mean EEG amplitudes, growing predominance of the delta rhythm ($p>0.05$), and a significant decrease in the amplitudes of the alpha rhythm and beta-high-frequency rhythm ($p<0.05$).

Moreover, during general anesthesia, cardiorespiratory parameters were recorded in rats (Fig. 4), as well as blood pressure and body temperature 10 minutes after induction of anesthesia and after 30 minutes (before the end of the experiment). The data are presented in Table 2.

The obtained results indicate that in animals exposed to general anesthesia using the second experimental mode, the significant decrease in heart rate (HR) by 18.5%, as well as decrease in the R wave voltage by 1.6 times, and respiratory rate (RR) by 20.7% ($p < 0.05$) were observed. This pattern indicates a more pronounced suppression of cardiovascular and respiratory systems than in the first group (Table 2).

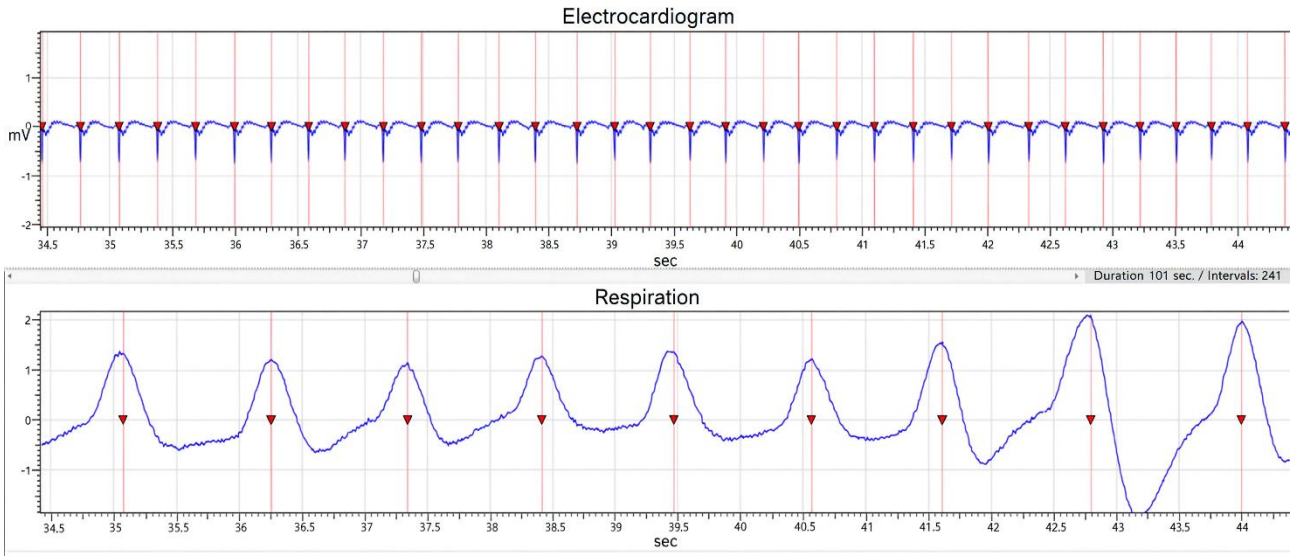

Fig. 4. Example of recording the cardiorespiratory data in rats in the state of general anesthesia

Table 2

The effect of the experimental modes of inhalation anesthesia on cardiorespiratory parameters and temperature in rats

Parameter studied		1st group (n=7)		2nd group (n=7)	
		10 min	30 min	10 min	30 min
HR, beats/min		225±12	215±10	222±13	181±11*/**
R wave voltage, mV		0.85±0.03	0.78±0.05	0.80±0.05	0.52±0.07*/**
RR, breaths/min		55±3	50±4	53±5	42±3*
BP, mmHg	systolic	121.2±4.4	115.1±3.5	119.7±3.8	99.4±2.3*/**
	diastolic	78.1±2.8	75.6±2.5	79.2±3.0	71.3±3.3
	mean	99.6±3.5	95.3±3.1	98.1±4.2	84.9±3.1*/**
T, °C		37.8±0.2	35.2±0.2*	37.5±0.3	33.1±0.5*/**

Notes: *— differences are significant relative to the baseline data (p<0.05);
**— differences are significant relative to animals of the 1st group (p<0.05).

Moreover, in the second group, a significant decrease in systolic and mean blood pressure by 16.7% and 14.3%, respectively, was also observed (p < 0.05). By the end of the experiment, body temperature in rats in this group significantly decreased to 33.1°C, which was statistically lower than in animals in the first group (p < 0.05). This also indicates an impairment in the function of cardiovascular system and general depression of body functions, which was more typical for animals in the second mode of general anesthesia, and correlates with the previous data.

Additionally, a number of biochemical parameters, including the renal function indices, were assessed in animals before and after anesthesia, and are presented in Table 3.

Table 3

Biochemical parameters and renal function indices in rats in the state of general anesthesia

Parameter	1st group (n=7)		2nd group (n=7)	
	before anesthesia	after anesthesia	before anesthesia	after anesthesia
Blood glucose, mmol/l	4.61±0.18	4.24±0.17	4.80±0.19	3.76±0.19*/**
Blood creatinine, μmol/l	45.65±3.17	39.04±2.68	42.15±2.89	55.56±3.86*/**
AST, μkat/l	0.75±0.02	0.71±0.02	0.69±0.07	0.78±0.05
ALT, μkat/L	0.36±0.02	0.38±0.01	0.40±0.03	0.66±0.02*/**
Urine creatinine, mmol/l	2.64±0.37	2.11±0.18	2.26±0.18	3.54±0.32*
GFR, ml/day	410.4±28.5	380.5±18.6	389.4±14.3	315.6±8.2*/**

Notes: * – differences are significant relative to the baseline data (p<0.05);
**– differences are significant relative to animals the 1st group (p<0.05).

The data obtained show that in the state of general anesthesia using the 1st mode of gas mixture delivery, no statistically significant changes in key blood biochemical parameters or renal function indices were observed in animals. Only single trend-based changes were observed. This indicates the good condition of organs and systems, such as the liver, heart, and urinary system, after general anesthesia. This pattern demonstrates good tolerability of the anesthetic protocol used.

At the same time, when using the 2nd mode of gas mixture delivery, a significant decrease in the blood glucose level by 21.7% ($p < 0.05$), an increase in ALT by 1.7 times ($p < 0.05$), an increase in blood creatinine by 31.8% ($p < 0.05$), urine creatinine — by 1.6 times ($p < 0.05$) and a corresponding decrease in the GFR by 18.8% ($p < 0.05$) were observed. All of the above indicates damage to some of the organs and systems of animals as a result of general anesthesia. First of all, it is worth to note the suppression of renal function, as evidenced by the GFR indicator. The activation of cytolysis, which may occur due to liver damage caused by inhalation anesthetic is also worth noting. A drop in glycemia also fits into the overall picture and indicates exhaustion of animals exposed to anesthesia.

This picture correlates with the laboratory observations. The animals were in a very deep phase of anesthesia (approximately 3–4), characterised by pronounced depression of respiratory system, with infrequent and deep breathing, and depression of cardiovascular system. EEG indices were also depressed. On the whole, this indicates poor tolerability of the second mode of anesthesia with the chosen duration time. This effect of isoflurane can be explained by stagnation of the gas mixture in the inhalation chamber due to the lack of forced gas removal, decrease of air content in the chamber, and consequent decrease of partial pressure of oxygen. This ultimately has led to deep hypoxia in the animals and, consequently, to deterioration of organ and tissue resistance to this mode of anesthesia.

Discussion and Conclusion. The obtained results demonstrate the high efficacy of the developed device—an inhalation chamber for rats connected to an anesthesia machine—for laboratory monitoring animals' condition during general anesthesia induced by inhalation. Analysis of the results reveals a significant correlation between animals' electrophysiological, functional, and biochemical parameters, which together enable a comprehensive assessment of the anesthesia protocol safety.

Thus, the proposed inhalation chamber enables monitoring general anesthesia using electrophysiological methods that are objective, sensitive, highly informative, and correlate well with the results of biochemical and functional studies in animals. This approach has good perspective for practical implementation in the experimental study of veterinary drugs, and may contribute to improving the quality of research results, as well as may facilitate the work of researchers.

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