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STATE OF OXIDANT HOMEOSTASIS IN THE BODY OF DOGS WITH DIFFERENT TYPES OF HIGHER NERVOUS ACTIVITY

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The relevance of the study is due to the lack of data on the cortical mechanisms of regulation of the activity of the antioxidant defense system in the body of dogs. In this regard, this article is aimed at revealing the specifics of the activity of key enzymes of the system of antioxidant protection of the blood of dogs with various types of higher nervous activity under the influence of short-term food deprivation. It was shown that short-term food deprivation in dogs caused a stressful state, which was accompanied not only by a change in the behavior of the animals, but also reflected the state of the enzymatic link of the antioxidant defense system in their body. In particular, a significant effect of short-term food deprivation on the activity of catalase in dog blood erythrocytes was established (F = 13.7 > FU = 4.15; p < 0.001). We note the increase in the influence of the force of nervous processes on the activity of catalase in the blood of dogs during the experiment from - $gh^2_{\chi} = 0.20$ *(p < 0.05) in the intact state, to the indicator -* $\text{гх}^2_{\chi} = 0.68$ *(p < 0.001) by the third day of research. Also, under the influence of food deprivation, direct connections of the strength and balance of nervous processes with the activity of this enzyme appear (r = 0.52–0.78 (p < 0.05–0.001). Under such influence, after one day and three days after the action of the stress factor, the activity of catalase in the blood of dogs with a weak type of higher nervous activity is lower by 3.6–5.8% (P < 0.05) than that of dogs with a strong balanced mobile type of higher nervous activity blood erythrocytes also found a decrease in the activity of the glutathione link of the antioxidant protection system in the blood plasma of dogs, in particular, under the influence of a stress factor, the activity of glutathione reductase during the day in dogs with various types of higher nervous activity decreases by 11.6–16.9%, respectively (p < 0, 05–0.01), the activity of glutathione reductase also decreases. Prospects for further research consist in the development of new methods for the correction of substance metabolism based on the use of nanoaquachelates of biogenic metals, taking into account the individual characteristics of the dog's body.*

Key words: *dogs, higher nervous activity, deprivation, catalase, glutathione peroxidase, glutathione reductase.*

СТАН ОКИСНОГО ГОМЕОСТАЗУ В ОРГАНІЗМІ СОБАК З РІЗНИМ ТИПОМ ВИЩОЇ НЕРВОВОЇ ДІЯЛЬНОСТІ

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Актуальність дослідження зумовлена відсутністю даних щодо кортикальних механізмів регуляції активності системи антиоксидантного захисту в організмі собак. У зв'язку з цим дана стаття спрямована на розкриття питання щодо особливостей активності ключових ензимів системи антиоксидантного захисту в крові собак з різними типами вищої нервової діяльності за впливу короткотривалої харчової депривації. Показано, що короткотривала харчова депривація у собак викликала стресовий стан, який супроводжувався не тільки зміною поведінки тварин, але і мало своє відображення на стані ферментативної ланки системи антиоксидантного захисту в їх організмі. Зокрема, встановлено достовірний вплив короткотермінової харчової депривації на активність каталази в еритроцитах крові собак (F = 13,7 > FU = 4,15; P < 0,001). Відмітимо збільшення впливу сили нервових процесів на активність каталази в крові собак протягом експерименту з – η^2_χ = 0,20 (P < 0,05) в інтактному стані, до третьої доби досліджень до показника – η^2_χ = 0,68 (P < 0,001). Також за впливу харчової депривації з'являються прямі зв'язки сили та врівноваженості нервових процесів з активністю даного ензиму (r = 0,52–0,78 (P < 0,05–0,001). За такого впливу, через добу та три доби після дії стресового фактору активність каталази в крові собак слабкого типу вищої нервової діяльності менша на 3,6–5,8% (P < 0,05) від такої у собак з сильним врівноваженим рухливим типом вищої нервової діяльності. Поряд із зменшенням антиоксидантного захисту в еритроцитах крові встановлено і зменшення активності глутатіонової ланки системи антиоксидантного захисту в плазмі крові собак, зокрема, за дії стресового фактору активність глутатіонредуктази протягом доби у собак з різними типами вищої нервової діяльності зменшується відповідно на 11,6–16,9% (P < 0,05–0,01), аналогічно відбувається і зменшення активності глутатіонредуктази. Перспективи подальших досліджень полягають у розробці нових методів корекції обміну речовин на основі застосування наноаквахелатів біогенних металів з урахуванням індивідуальних особливостей організму собак.

Ключові слова: *собаки, вища нервова діяльність, депривація, каталаза, глутатіонпероксидаза, глутатіонредуктаза.*

Introduction. Like many other mammals, dogs have a complex nervous system that allows them to perceive, process and respond to a variety of stimuli. Research on higher neural activity in dogs has mainly focused on understanding their cognitive abilities, perception, learning and social behavior (Bray et al., 2021). Stress is a major problem in veterinary behavioral medicine (Martínez, Pernas, Casalta, Rey, & De la Cruz

Palomino, 2011). In the survey, 49% of owners said that their dogs are afraid of loud noises. Stresses are accompanied by manifestations of fear in dogs, which are considered to be an emotional state of anxiety and excitement caused by an imminent danger (Adolphs, 2013; Stellato, Flint, Widowski, Serpell, & Niel, 2017). Stress triggers adaptive behavioral responses that will allow the animal to mitigate or avoid the threat (Sherman & Mills, 2008). A person faced with a threat may demonstrate the behavioral reactions of the "four F" (fight, flight, freezeorflirt), that is, fight, flee, freeze or flirt (Marks, 1987). Stress and fear are potentially one of the most common causes of aggressive behavior in dogs, even if the owner does not necessarily associate this aggression with fear per se (Tiira & Lohi, 2014). The development of a stressful state is accompanied by the intensification of lipid peroxidation and a decrease in the activity of the antioxidant defense system (Danchuk et al., 2020). Oxidative stress can be the result of excess formation of radicals and a decrease in the body's antioxidant defenses (Sies, Berndt, & Jones, 2017). A constantly functioning system of antioxidant protection of the body regulates the process of free radical reactions in all its stages. Its enzymatic link includes a number of enzymes, the main of which are: superoxide dismutase, catalase, glutathione peroxidase (HP), glutathione transferase (HT), glutathione reductase (GR) and others (Pisoschi et al., 2021).

Despite a significant number of publications on food deprivation (Khoo, Taylor, & Owens, 2019; Pointer, Reisman, Windham, & Murray, 2013; Schupp & Renner, 2011), the question of the effect of short-term food deprivation on the activity of the antioxidant defense system in dogs remains out of the attention of researchers. Therefore, the actual direction of scientific research is the study of indicators of the state of the antioxidant defense system in dogs depending on the type of nervous activity, both in an intact state and under the influence of a stress factor.

Food deprivation can be short-term or long-term and is a stress factor for the body (Schupp & Renner, 2011). However, the number of studies devoted to the effects of deprivation is small. Accordingly, more systematic research efforts are needed to identify reliable and consistent findings related to deprivation (Pointer et al., 2013). Short-term (up to 2-3 days, depending on the size of the animal) food deprivation in dogs is not accompanied by the development of a pathological state of the body, but it significantly affects the behavior of animals and their metabolism (Khoo et al., 2019). This line of research is promising, on the one hand, we will get information on the effect of limiting food consumption on the metabolism in the body of dogs, and on the other hand, we will learn the role of higher nervous activity in the reactivity and adaptability of the animal body to short-term food deprivation. Food deprivation has a powerful effect on almost every aspect of behavior. Prolonged periods of starvation are among the most tragic experiences of mankind, and during these periods thoughts of food fill the mental life. Even mild periods of food deprivation are effective in eliciting eating behaviors, including increased food intake and increased food value (Schupp & Renner, 2011).

Materials and methods. A total of 20 dogs (Canisfamiliaris, or Canislupusfamiliaris) of the beagle breed (English beagle) were used for the experiment. Age-matched dogs (1-1.5 years) for the experiment were selected from kennels and from private owners. The kennels in which the research was conducted were free of infectious diseases during the dissertation work. The health status of the dogs was assessed by a general clinical examination and laboratory tests. Experiments were conducted exclusively on clinically healthy animals. Laboratory studies were carried out in the Multidisciplinary Laboratory of Veterinary Medicine of the Ukrainian

State Academy of Sciences, Odesa, and the Veterinary Clinic "VITAVET" in Kamianets-Podilskyi.

The strength, balance and mobility of nervous processes in dogs were determined by the author's modified method. On the basis of the experiment, 4 groups of animals were formed, 5 heads in each: I group - strong balanced mobile type (SVR); Group II – strong balanced inert type (SVI); Group III – strong unbalanced type of GNI (SN); Group IV – a weak type of higher nervous activity (C). Food deprivation was carried out for 36 hours, animals had free access to water. Blood samples obtained before food deprivation and one and three days after the beginning of deprivation were selected as material for research. To assess the state of the antioxidant protection system, the activity of: catalase in dog erythrocyte hemolysates was determined by the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts (Vlizlo, Fedoruk, & Ratych, 2012); glutathione reductase (GR; K.F.1.6.4.2.) and glutathione peroxidase (GP; K.F.1.11.1.9.) in the blood plasma of dogs were determined by the method of V.V. Lemeshko et al. The obtained results were analyzed statistically.

All experimental studies were carried out in compliance with the requirements of the Law of Ukraine No. 3447-IV dated 21.02.06 "On the Protection of Animals from Cruelty" and are consistent with the basic principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" (Strasbourg , 1986), declaration "On humane treatment of animals" (Helsinki, 2000).

Research results and discussion. Catalase (КФ1.11.1.6.) is a heme-containing enzyme that catalyzes the splitting of H_2O_2 with the formation of water and endogenous oxygen (Aebi, 1974). The activity of catalase in the blood erythrocytes of dogs with different types of BND in the intact state does not differ significantly (Table 1). Short-term food deprivation did not affect the activity of this enzyme in the blood erythrocytes of SVR, SVI and SN type VND animals. Along with this, one day after exposure to the stress factor, catalase activity in the blood of dogs with a weak type of VND decreases by 4.1% ($P < 0.05$) and becomes lower by 3.3–3.6% ($P < 0.05$) from such in dogs with SVR, SVI and SN of the VND type. Even three days after the beginning of the research, the activity of the enzyme is 5.8% less ($P < 0.01$) than the indicators of dogs with SVR of the VND type at this stage of the research.

Table 1

Activity of antioxidant enzymes in red blood cells of dogs with different types of VND (M±m, n=5)

Type of VND	**Research period**		
	Before the stimulus	**A day later**	**In 3 days**
Catalase activity, mkM $H_2O_2/dm^3 \times min \times 10^3$			
Strong balanced mobile	54,5±0,4	53,5±0,4	55,08±0,4
Strong balanced inert	54,8±0,3	53,7±0,4	54,9±0,2
Strong unbalanced	55,1±0,3	53,8±0,4	55,5±0,1
Weak	53,9±0,6	51,68±0,6*	51,88±1,0**
Glutathione peroxidase activity, mkmol $NADPH_2$ h/mg protein			
Strong balanced mobile	16,56±0,54	14,64±0,62	15,04±0,64
Strong balanced inert	16,84±0,57	13,84±0,20	15,24±0,30
Strong unbalanced	16,76±0,59	14,06±0,42	16,94±0,36*
Weak	16,36±0,29	13,60±0,50	15,36±0,38

Glutathione reductase activity, mkmol $NADPH_2$ h/mg protein			
Strong balanced mobile	5,15±0,23	4,74±0,23	5,11±0,26
Strong balanced inert	5,00±0,167	4,66±0,17	4,88±0,25
Strong unbalanced	5,32±0,34	4,43±0,17*	5,28±0,19**
Weak	4,95±0,24	4,52±0,25	4,84±0,17

*Note. Significant differences with SVR type VND: *P < 0.05; **P < 0.01; ***P < 0.001.*

The activity of catalase in erythrocytes of the blood of dogs in an intact state is not reliably related to the main characteristics of nervous processes in dogs (Fig. 1). Within a day after the start of research, correlations of enzyme activity with the strength, balance and mobility of nervous processes in dogs increase significantly, but only with the strength of nervous processes they are reliable - r = 0.65 (P < 0.01). By the third day after the start of the experiment, the relationship between the strength of nerve processes and the activity of catalase in the erythrocytes of dog blood increases - r = 0.78 (P < 0.001) and a reliable relationship between the mobility of nerve processes and the activity of the enzyme appears - r = 0. 52 (P <0.001). It should also be noted that there is no reliable correlation between the balance of nervous processes and the activity of catalase in erythrocytes of dog blood.

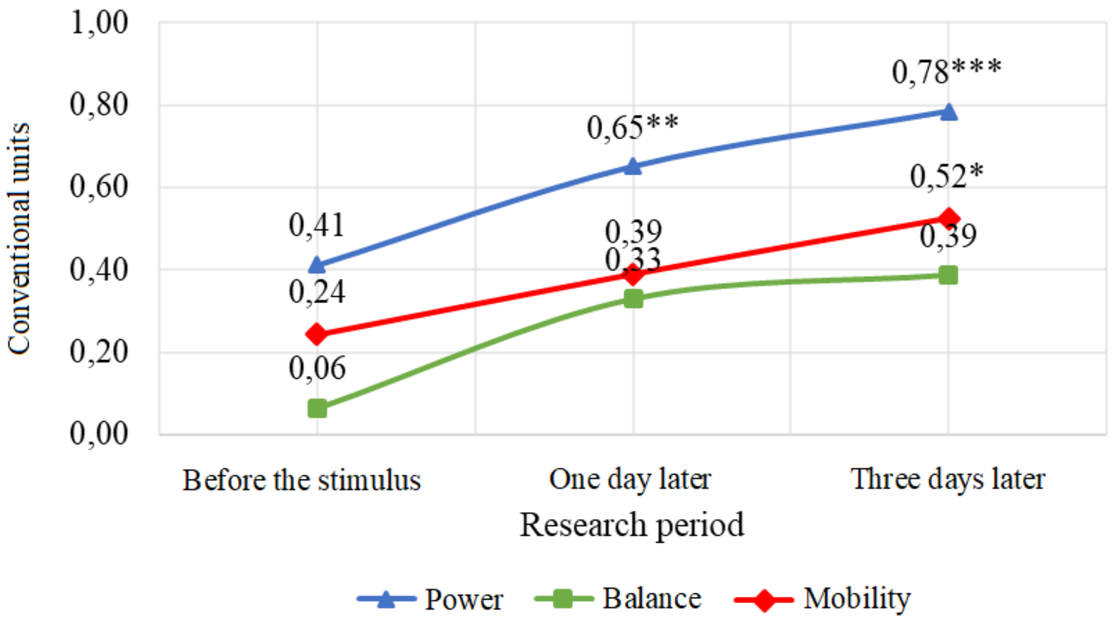

Fig. 1 **Relationships (r) of the main characteristics of nervous processes with catalase activity in erythrocytes of dog blood (n = 20; mental unit).**
*Note. Indicators are reliable for: *P < 0.05; **P < 0.01; ***P < 0.001.*

In the intact state, only the strength ($\hbar^2_\chi$ = 0.20; P < 0.05) of nerve processes reliably affects the activity of catalase in erythrocytes of dog blood (Fig. 2), while the influence of the mobility and balance of nerve processes is absent ($\hbar^2_\chi$ = 0, 00). In the future, during the experiment, the influence of the strength of nervous processes only increases, in particular, on the first day of research to the indicator - gh^2_χ = 0.51 (P < 0.01) and by the third day of research to the indicator - rgh^2_χ = 0.68 (P < 0.001). The balance and mobility of nervous processes did not reliably affect the activity of catalase in the hemolysates of erythrocytes of the blood of dogs during the entire experiment ($\hbar^2_\chi$ = 0.00–0.14).

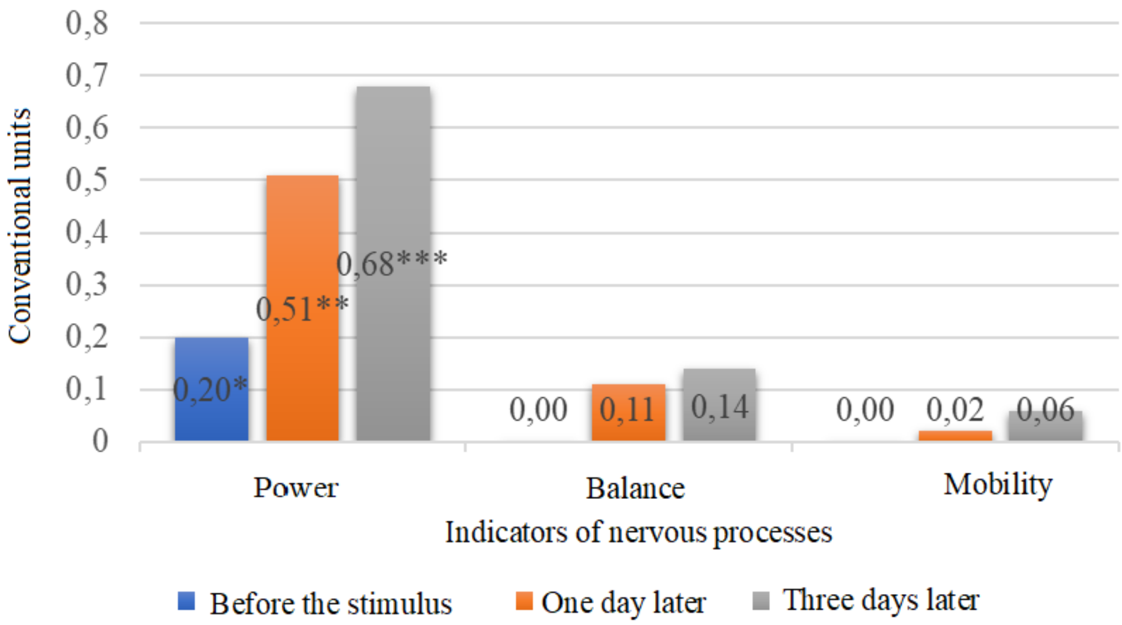

Fig. 2. **The influence (η^2_{χ}) of the main properties of cortical processes on the activity of catalase in erythrocytes of dog blood (individual units; n=20).**

*Note. Indicators are reliable for: *P < 0.05; **P < 0.01; ***P < 0.001.*

Two-factor variance analysis (Table 2) showed that the type of higher nervous activity has a significant effect on catalase activity in dog blood erythrocytes (F=17.5> FU=2.90; P<0.001). The effect of short-term food deprivation on enzyme activity was also established (F=13.7>FU=4.15; P<0.001). However, no interfactor interaction was established.

Table 2

Two-factor variance analysis of the activity of enzymes of the antioxidant defense system in the blood erythrocytes of dogs with different types of higher nervous activity under the effects of food deprivation

Source of variation	SS	df	MS	F	P- value	F critical
Catalase						
Type of VND	52,4	3	17,5	17,5	< 0,001	2,90
Action of the stimulus	13,7	1	13,7	13,7	< 0,001	4,15
Interconnection	3,46	3	1,15	1,16	0,34	2,90
Internal	31,9	32	1,00	–	–	–
In total	101,5	39	–	–	–	–
Glutathione peroxidase						
Type of VND	6,55	3	2,18	2,69	0,06	2,90
Action of the stimulus	25,9	1	25,9	31,9	< 0,001	4,15
Interconnection	7,86	3	2,62	3,22	0,35	2,90
Internal	26,0	32	0,81	–	–	–
In total	66,3	39	–	–	–	–

Glutathione reductase						
Type of VND	0,33	3	0,110	0,59	0,62	2,90
Action of the stimulus	1,91	1	1,91	10,3	0,003	4,15
Interconnection	0,60	3	0,20	1,07	0,38	2,90
Internal	5,96	32	0,19		–	–
In total	8,80	39			–	–

Notes: df – number of factor levels (-1); F is the criterion for evaluating the influencing factor on the dependent variable; F critical – critical value of the influence factor; MS – mean square; SS – sum of squares; P is reliability.

The glutathione link of the antioxidant defense system includes glutathione peroxidase (HP, KF 1.11.1.9), glutathione transferase (HT, KF 2.5.18), glutathione reductase (GR KF 1.6.4.2), glutathione, glucose-6-phosphate dehydrogenase (KF 1.1.1.43) and lactate dehydrogenase (KF 1.1.1.27). This system ensures the utilization of hydrogen peroxide and hydroperoxides of organic compounds (Robaczewska et al., 2016). The conducted studies established that the activity of glutathione peroxidase (GP) in the blood serum of dogs with different types of BND in an intact state does not reliably differ (Table 1). Under the influence of a stress factor, the activity of the enzyme during the day in dogs with SVR, SVI, SN and weak type of VND decreases by 11.6% ($P < 0.05$), 17.8% ($P < 0.001$), 16.1% ($P < 0.01$) and 16.9% ($P < 0.05$). A tendency towards lower activity of the enzyme in blood serum of dogs with SVI, HF and weak type of VND (by 4.0–7.1%) compared to the indicators of dogs with SVR type was established. Later, until the third day of the experiment, the activity of HP in the blood serum of dogs with SVI, HF and weak type of VND increases by 10.1% ($P < 0.01$), 20.5% ($P < 0.001$) and 12.9% ($P < 0.05$). We note a significantly higher level of enzyme activity in the blood of dogs with SN of the BND type by 12.6% ($P < 0.05$), in accordance with that of dogs with SVR of the BND type three days after the start of the experiment.

No reliable correlations between the activity of glutathione peroxidase in the blood plasma of dogs both in the intact state and under the effects of short-term food deprivation were established. The strength, balance and mobility of the processes of excitation and inhibition in the cerebral cortex in an intact state did not reliably affect the activity of glutathione peroxidase in the blood plasma of dogs (Fig. 3). Within a day after the beginning of food deprivation, the influence of the main characteristics of nervous processes increases (from the index $r^2_\chi = 0.00–0.03$ to $r^2_\chi = 0.05–0.15$), but remains unreliable. On the third day of the experiment, only the balancing of nervous processes has a significant effect on the activity of HP in the blood plasma of dogs - $gh^2_\chi = 0.21$ ($P < 0.05$).

The conducted two-factor analysis of variance (Table 2) established that the type of higher nervous activity does not have a significant effect on the activity of glutathione peroxidase in the blood of dogs ($F = 2.69 < FU = 2.90$; $P < 0.06$), while food deprivation reliably affects the activity of this enzyme in the blood plasma of dogs ($F = 31.9 > FU = 4.15$; $P < 0.00$).

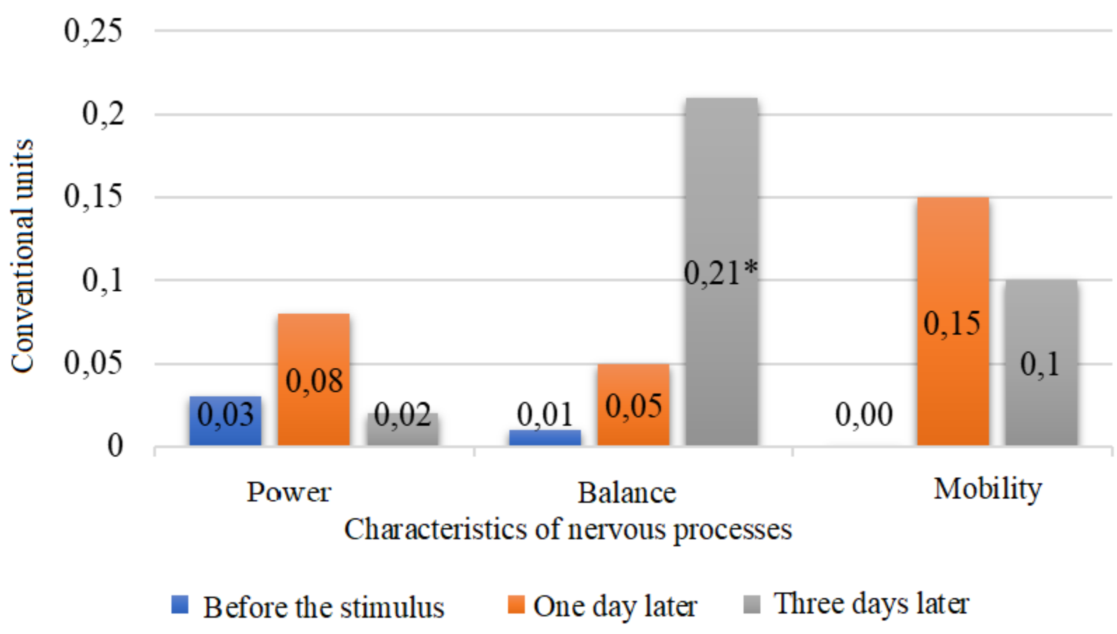

Fig. 3. **The influence (η^2_χ) of the main properties of cortical processes on the activity of glutathione peroxidase in the blood serum of dogs (individuals; n=20).**
*Note. Indicators are reliable for: *P < 0.05; **P < 0.01; ***P < 0.001.*

The activity of glutathione reductase (GR) in the blood serum of dogs with different types of VND during the experiment did not differ significantly (Table 1). A tendency to decrease the activity of the enzyme during the day under the influence of a stress factor in dogs with SVR, SVI and weak type of VND by 6.8–8.6% was established. Meanwhile, in dogs with HF of the VND type, this indicator decreases by 16.7% ($P < 0.05$). A decrease in the activity of enzymes of the glutathione chain of SAZ is accompanied by an increase in free radical processes in the body, which provoke the development of oxidative stress (Gizi et al., 2011). Later, until the third day of the experiment, the activity of GR in dogs with HF of the VND type increases by 19.2% ($P < 0.01$). We note a tendency towards lower activity of the enzyme in the blood serum of dogs with SVI and a weak type of VND, in accordance with the indicators of animals with HF and SVR type three days after the start of the research.

No reliable correlations of glutathione peroxidase activity in blood plasma of dogs were established throughout the experiment. It is only necessary to note the inverse dynamics of the relationship between the activity of this enzyme and the balance and mobility of nervous processes.

The conducted studies established that the strength, balance and mobility of nervous processes do not reliably affect the activity of glutathione reductase in the blood plasma of dogs both in an intact and stressed state. As shown in table. 2, the type of higher nervous activity does not influence the activity of HP in the blood of dogs ($F = 0.59 < FU = 2.90$; $p = 0.62$). Then, short-term food deprivation has a significant effect on the activity of the enzyme and blood plasma of dogs ($F = 10.3 > FU = 4.15$; $P < 0.01$).

Erythrocytes are vulnerable to oxidative stress (González-Domínguez et al., 2022), and antioxidant enzymes are the main defenders against free radicals and prooxidants, so a decrease in the activity of SAZ can lead to the accumulation of reactive oxygen species and lipid peroxidation products that can induce oxidative changes in the cytosol and membrane components (Melo, Coimbra, Rocha, & Santos-

Silva, 2023). Thus, the reactivity of the dog's body under food deprivation is limited by the state of the antioxidant defense system and the main characteristics of the animal's nervous system.

Conclusions. Short-term food deprivation affects the activity of catalase in erythrocytes of dogs (F = 13.7 > FU = 4.15; P < 0.001), and is accompanied by the formation of direct connections between the strength and balance of nervous processes with the activity of the enzyme (r = 0.52 –0.78 (P < 0.05–0.001). Under the influence of food deprivation, the activity of glutathione peroxidase in the blood plasma of dogs decreases by 11.6–16.9% (P < 0.05–0.01), depending on the type of higher nervous activity.

Prospects for further research consist in the development of new methods of correction of metabolism based on the use of nanoaquachelates of biogenic metals, taking into account the individual characteristics of the body of dogs.

Conflict of interest

The authors declare no conflict of interest.

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