Cross-resistance in animals with early postnatal cold imprinting

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Abstract. The article describes the cross-resistance to cold, hypoxia and physical activity of adult animals with early postnatal cold imprinting. It was shown that short-term fractional cooling in the first seven days of the life of Wistar rats increased the resistance of adult animals to low temperatures, but reduced it to hypoxia and physical static performance.

1. Introduction
The early period of development is the most important factor in individual formation, affecting the health of the adult body throughout life. In pre- and early postnatal ontogenesis, when the body’s sensitivity to various external influences is extremely high, changes in lability in the activity of various organ systems can occur, providing adaptation to future environmental conditions, increasing the survival chances and procreation [1-3]. This process is called programming or imprinting in the literature.

Numerous literature data indicate that effects of environmental factors and upbringing in early postnatal ontogenesis affect the manifestation of stress reactions, the state of neurohormonal regulation of vegetative functions, modification of behavioural acts and adaptive abilities in adult animals [4-6]. According to I.A. Arshavsky [1], precisely in this period, a newborn organism reaction to cold such as physiological stress is detected, which ends with a phase of excessive restoration of plastic and energy reserves, which is combined with an increase in the body's adaptive capabilities with respect to the action of some other factors. Physiological adaptation to environmental stressors is often studied in isolation, but these stressors often combine outside of laboratory conditions, such as cold and altitude hypoxia.

The interest in the cold factor action in early ontogenesis is due to the fact that the environment temperature is below the heat-indifferent zone is specific early age stress, which the body meets immediately after birth and can encounter throughout life. In the domestic literature, considerable data on the integrating of thermoregulatory mechanisms during the young farm animals hardening has been accumulated [7].

In the study of the formation of the body’s resistance to low ambient temperature with short-term cooling during the functional immaturity of neurohormonal systems, i.e. in the first days of life, the problem of changing its resistance to the action of other previously indifferent factors arises.

This work aimed to characterize the cross-resistance of adult animals that underwent short-term cooling in the early period of postnatal ontogenesis.
2. Methods and materials
The work was carried out on white laboratory rats of the Wistar population. Pups on the first day after birth were divided by broods into 3 groups:

- 1st - control group - rat pups were not exposed to any effects;
- 2nd - Cold Imprinting group - rat pups were subjected to cooling every day, starting from the first day of life, for seven days at a temperature of 2 .. 4oC for 15 minutes, placing them in metal bath frozen in ice, which bottom was covered with filter paper;
- 3rd - the Handling group, used to control the stressful effects of moderate strength - rat pups were picked up for seven days daily, starting from the first day of life, for 15 minutes.

After the period of feeding, the rat pups of all experimental groups were kept by litters, and at the age of 2-2.5 months, they were weighed, placed in individual cages and kept in a vivarium at a temperature of 20 .. 22°C with adjustable light mode: 12 h - light, 12 hours - darkness. Rats received a standard diet of vivarium with free access to water.

Some of the animals from all three groups were subjected to a test for survival under extreme environmental conditions, measuring the lifetime under conditions of acute cooling at -14°C without limitation of mobility.

To measure resistance to acute hypoxia, animals were individually placed in a pressure chamber, in which atmospheric pressure was reduced for 180 seconds to a level corresponding to an altitude of 11,000 m, and the time in seconds until the appearance of agonal breathing was measured, after which the pressure in the pressure chamber for 150-180 seconds restored to normal.

To measure physical static performance, we measured the duration of the animals hangs up to failure on a pole, which was placed at a height of at least 1 meter above the floor surface.

To test the resistance of the food reflex to external inhibition for 3 days, we determined the amount of food in grams that the rat ate in 5 minutes immediately after feed gets. On the 4th day, the amount of food eaten in 5 minutes under stress was determined on the same day time in an unfamiliar cage, in a new room and bright light. Data were expressed as eaten food % under stress relative to the amount of eaten food in the usual environment.

An open field test was conducted in a dark room, illuminating only the field itself. Test time is 5 minutes. Latent time (in seconds) was measured - from the moment of disembarkation of the animal in the center of the circle to the beginning of movements, motor activity - horizontal (the number of intersections of squares), vertical (number of racks), the number of visits to the central zones, the number and grooming duration. After the test, the number of defecation boluses per field was counted.

Statistical processing of the results was carried out in the program Statistica 6.0. The Newman-Cales test was used. The data are presented in the form M ± m, where M is the sample mean, m is the standard error. The probability of validity of the null hypothesis was taken at a 5% significance level.

3. Results and discussion
Baby rats short-term cooling, starting from the first life days, did not lead to the newborn animals’ death, they tolerated these effects without visible developmental abnormalities. It was noted that the 2nd group pups were covered with hair faster. At the age of two months, when keeping animals in conditions of thermal comfort, the mass of rats in the control group was 155+5 g, rats subjected to cold imprinting, 152+4 g.

The table 1 shows the results of functional tests conducted in adult animals.

| Experimental Group | Survival Time at -14°C, min | Survival Time at 11000 m, sec | Physical performance, sec |
|--------------------|-----------------------------|------------------------------|--------------------------|
| 1. Control         | 320±22                      | 398±33                       | 215±20                   |
A significant difference in survival time of the control group rats and the Cold Imprinting group (60% increase) indicates that the short-term cooling procedure in early ontogenesis significantly increases the resistance of adult animals to low temperatures.

In animals exposed in early ontogenesis, there was a decrease in resistance to hypoxia compared to control rats, and this effect is most pronounced in rats subjected to the handling procedure. If rats with high (life time> 5 min) and low (lifetime <2.5 min) resistance to hypoxia were distinguished in the experimental groups, their ratio in the group of control animals was 52% and 17% in the "Cold imprinting" group - 25% and 33% and in the Handling group - 13% and 67%, respectively. Thus, a decrease in the adult animals’ resistance to hypoxia occurred due to a decrease in the number of highly resistant rats and an increase in the number of low-resistant individuals in groups of rats exposed in early postnatal ontogenesis.

The Cold Imprinting group rats’ retention time on the pole decreased by almost 30%, and the Handling group rats did not statistically significant change compared to control animals.

In the test for the stability of the food reflex in animals of all three groups, no differences were found. The values of the indicator in the “Control”, “Cold imprinting”, “Handling” groups amounted to: 62.6±6.7%, 67.4±5.3% and 57.0±6.6, respectively. Similarly, no difference was found between rats of all three groups according to the results of studying the behaviour of animals in an open field test.

Cold is one of the first environmental factors that the body encounters immediately after birth and there is an evolutionarily fixed functional system for responding to it, which is why it should have been activated in the first place. The cooling procedures we used, both in terms of time (starting from the first to the seventh days of life, that is, during the formation of relations between the central and peripheral links of the endocrine regulation), and in intensity (at 2...4°C for 15 minutes) are programming signals, but do not act on the path of reactions activation within the boundaries of "physiological stress" [1]. At the same time, this stress kind, like handling, is new, for which a specific response program is not fixed.

The literature actively discusses the relationship of adaptation mechanisms to hypoxia, physical activity, and the nature of thermogenesis in humans and animals [8-11].

Based on the results of experimental animal studies, it is suggested that the general autonomous adaptation provided by a short repeated exposure to cold air can lead to a decrease in the sympathetic response to new environmental incentives, such as, for example, acute hypoxia Information available in the literature on the effects of moderately cold effects on the body's response to hypoxia are contradictory [8]. Our results indicate that in animals exposed in the early ontogenesis to both short-term cooling and handling decreased resistance to hypoxia.

Studies of the cold effect on physical performance, especially aerobic, suggest that aerobic performance deteriorates in cold conditions [9]. It is known that the adaptation of homoiothermic organisms to cold leads to a decrease in the efficiency of physical activity [10]. According to our results, a decrease in resistance to physical activity decreased in animals with early postnatal cold imprinting.

4. Conclusion
The results obtained on cross-resistance in adult rats subjected to short-term cooling in early ontogenesis indicate that such a procedure reduces the range of possible fluctuations in physiological parameters, and, consequently, adaptive capabilities of the body. The use of short-term fractional
cooling in the first seven days of life led to a decrease in the parameters of nonspecific resistance and a
decrease in the response range to other types of stress, which is probably due to stable changes in the
functioning of regulatory hormonal systems.

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