Effect of Static and Dynamic Influences on Receptors of Equilibrium Organ of *Helix Lucorum* after 163-Day Orbital Flight in "Mir" Station

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A 163-day orbital flight increased the baseline impulse activity of statocyst receptor cells in terrestrial pulmonata snail *Helix lucorum*. The maximum of reaction to step static stimuli (changes in body position in the range from 0 to 180°) was significantly shifted by 30° from gravitational vertical, while the reaction to dynamic stimuli (dumped sinusoidal oscillations) faded more rapidly than in control animals.

Key Words: *weightlessness; gravireception; organ of equilibrium; statoreceptor; snail*

Evaluation of the role of gravitational factor in the functioning of equilibrium organ is an important problem of space biology. According to current views, dysfunction of this organ is responsible for various disorders during flight and after landing [2,4].

Our aim was to study impulse activity of specialized gravitation-sensitive receptor structures after long-term exposure to weightlessness. The examined object was statocyst, the equilibrium organ of terrestrial pulmonata snail *Helix lucorum*, which has no more than 13 primary sensory hair cells, statoreceptors [1,3]. The cells are large and can be isolated. In addition, the snails require no particular maintenance conditions, and they can live in closed containers in spaceship for a long time.

MATERIALS AND METHODS

Experiments were performed on mature pubertal snails *Helix lucorum* exposed to weightlessness, and also on control vivarium and control synchronous snails (n=75 snails in each group, body weight 11-15 g). The space group snails were placed in a special container and launched to the «Mir» complex by a "Progress" freight carrier spaceship. The snails were exposed to weightlessness for 163 days (March 15 to August 25, 1998). During the flight, the telemetric data on microclimate near the container were monitored daily. The snails of synchronous control group were placed in a similar container and were kept at temperatures close to on-board temperatures (17-22°C). They were also subjected to dynamical loads simulating spaceship start and landing. Vivarium control snails were given food and water *ad libitum*.

The shell of the snails were removed, the anterior part of the foot except cephalic end was dissected along the midline, and the statocysts were found on the dorsoventral surface of the pedal ganglia under a binocular microscope. They looked like the dark spots with a diameter of about 180 μ (Fig. 1). The specimen was placed on a special platform of a mechanical stand. Extracellular impulse activity of the receptor cells located in the equatorial zone of the statocyst was recorded with tungsten microelectrodes. The platform with a specimen was oriented horizontally, inclined in a stepwise mode, or subjected to relaxation oscillations. The tilts were made about the longitudinal axis of

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snail body to ipsi- or contralateral sides relatively to the recorded cell. The platform was fixed for 20 sec every 30° within the range from 0 to 180°. The sinusoidal damped oscillations (0.15 Hz, 52 sec, damping factor 0.04) were performed in the same plane with initial amplitude of platform tilt of 60°. Activity of receptor cells in space snails was recorded 10, 11, 12, 13, 15, 16, 20, 24, 31, 34, 36, 48, 50, and 55 h (one cell per test), 3, 5, 6 days (3 cells per test), and 7 and 8 days (one cell per test) after landing.

RESULTS

Body weight of vivarium snails before the experiments was 13.0±0.3 g. After 163 days it increased by 1.2 g and became 14.2±0.5 g (p>0.05). Baseline activity of individual cells varied from 0.1 to 1.5 pulses per sec with the amplitude of some spikes of 300-400 μV. When the platform was tilted in the ipsilateral direction, the discharge rate increased gradually, and after reaching the maximum value, it gradually decreased (in both cases, extinction-augmentation coefficients were 0.01-0.02). In a half of the examined cells the maximum activity was observed at a tilt angle of 90°, when impulse activity 11-fold surpassed the baseline value. In other cells the maximum activation was observed at tilt angles of 120° (35%) and 150° (15%). When the platform was tilted through 180°, the discharge rate was still significantly surpassed the baseline value.

The tilt of the platform to contralateral side increased the discharge rate from 0.3 to 1.3 pulses per sec (Figs. 2 and 3).

When the platform oscillated, the continuity of receptor cell discharges disappeared: period of activation alternated the periods of inhibition. Activation was observed when the platform moved in such a way that the cell transited to the "bottom" position; when the platform moved in the opposite direction and the cell transited to "top" position, impulse activity moderated and disappeared completely. While oscillation of the platform damped, the rate and duration of evoked discharges decreased with a coefficient of 0.05. As a rule, the baseline impulse activity characteristic of a particular cell

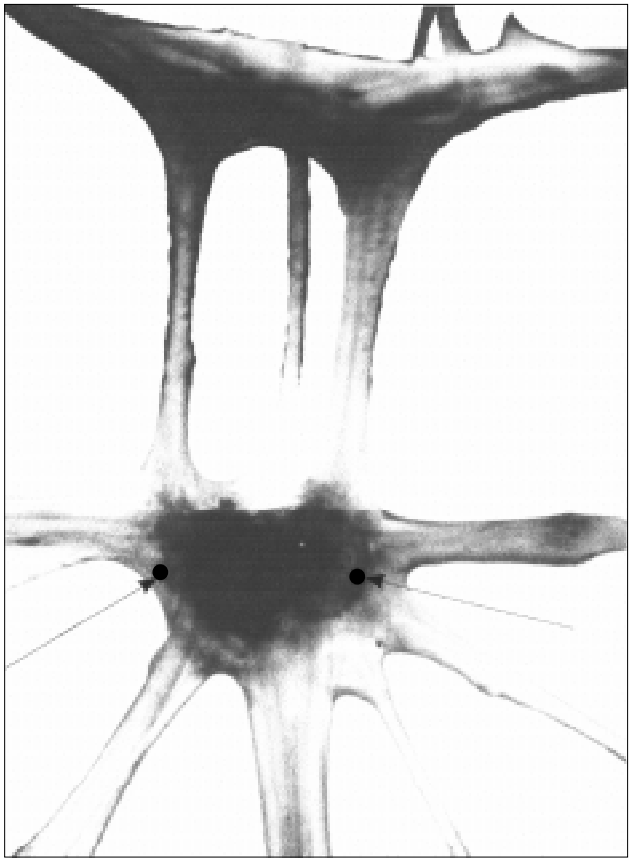

Fig. 1. Ganglionic neural complex isolated from a snail, ×20. The arrows mark the statocysts.

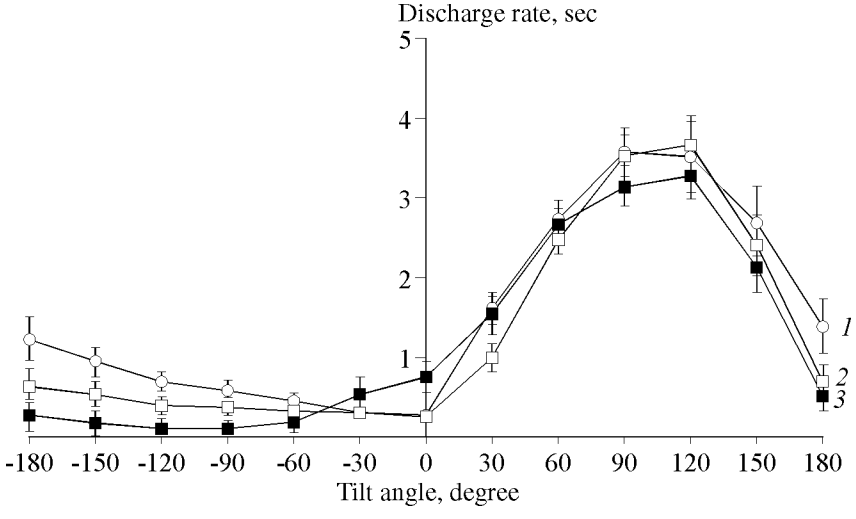

Fig. 2. Averaged discharge rate curves of statoreceptors from snails of the control vivarium group (*1*), control synchronous group (*2*) and space group (*3*) recorded in response to graded ipsi- and contralateral tilts.

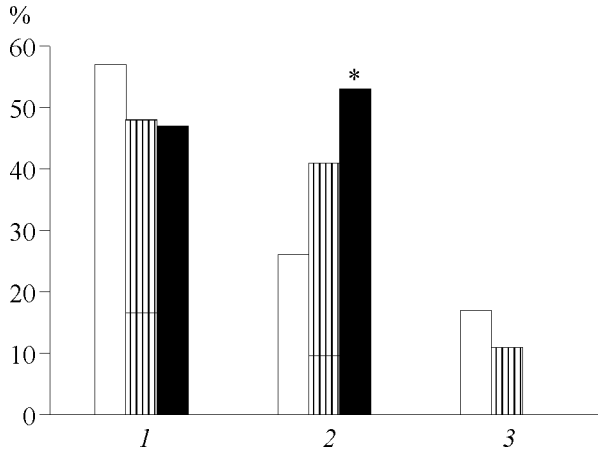

Fig. 3. Distribution of statoreceptors from snails of the control vivarium group (open bars), control synchronous group (shading) and space group (solid bars) in respect to maximum reaction produced by graded ipsilateral tilts through 90° (*1*), 120° (*2*), and 150° (*3*). *$p<0.05$ compared to the control.

recovered 15-20 sec after the platform was stopped. The intensity of reaction evaluated by the total number of spikes during one oscillation cycle was 91.8±7.6. The distribution curve was characterized by insignificant positive asymmetry and moderate direct excess (Table 1).

Before placing into the container, body weight of control synchronous snails was 13.0±0.4 g, while 163 day later it became 9.9±0.3 g ($p<0.001$). The baseline activity of receptor cells virtually did not differ from that characteristic of the control vivarium group (Table 1). The step-like tilts of the platform to ipsilateral direction produced changes in the discharge rate and extinction-augmentation coefficients factors, which were similar to those obtained in receptor cells of control vivarium snails. In a half of examined cells the maximum activity was observed at a tilt of 90°, while in other cells the maximum of activation was observed at tilts of 120° (39%) and 150° (11%). When the platform was tilted to the contralateral side, the discharge rate still tended to increase, but to a lesser extent than in the control (Figs. 2 and 3).

When the platform oscillated, the intensity of reaction was significantly smaller in comparison with reaction of statoreceptors in control vivarium group. The distribution curve was characterized by a pronounced positive asymmetry and weak reverse excess (Table 1).

Before placing into the container, body weight of the space group snails was 13.5±0.3 g, while after landing it was 10.3±0.2 g ($p<0.001$). The baseline activity of receptor cells significantly surpassed the control values (Table 1). The step-like tilts of the platform to ipsilateral direction produced reactions whose maximums were distributed only in the range between 90° and 120°. At the same time, the number of cells with maximum activity at the tilt of 120° was significantly greater than that in control snails (Fig. 3). The rate of discharges 4.5-fold higher surpassed the baseline activity. The weakest reaction was observed on post-flight hours 10, 11, 21, and 31, while the greatest response was observed on post-flight days 2, 3, 6, and 8. By contrast to the control groups, the contralateral tilts decreased the discharge rate of the receptor cells from the space group and virtually eliminated firing at tilts 90° and 120° (Fig. 2).

When the platform performed damped oscillations, the average reaction intensity, asymmetry and excess of distribution curve did not significantly differ from the corresponding parameters in synchronous group, while reaction extinction factor was pronouncedly greater than that in both control groups (Table 1). The weakest reactions were observed on post-flight hours 11, 13, 20, and 50 and on post-flight days 3, 5, and 8, while the greatest

TABLE 1. Baseline and Evoked Impulse Activity of Statoreceptors in Snails of Space and Control Groups in Response to Platform Sinusoidal Relaxation Oscillations

Index	Control		Space group (n=21)
	vivarium group (n=28)	synchronous group (n=29)	
Baseline discharge rate, pulses per sec	0.31±0.04	0.33±0.05	0.70±0.16
Intensity of reaction to platform oscillations, the number of spikes			
$M±m$	91.8±7.6	61.5±8.2*	69.9±7.8**
asymmetry factor	0.2	1.0	0.8
excess factor	0.6	-0.2	-0.3
Reaction extinction factor	0.05±0.01	0.013±0.003	0.17±0.06

Note. *$p<0.01$, **$p<0.05$ compared to vivarium control group.

responses were recorded on post-flight hours 12, 15, and 36 and on post-flight day 6.

Therefore, a long-term weightlessness affected functional state of gravisensitive structures (snail statoreceptors) after returning to normal Earth's gravity: the baseline discharge rate increased and the responses to step-like static stimulation changed. The maximum discharge rate in most statoreceptors of the control synchronous group was at a tilt of 90°, while in the space group the corresponding angle was shifted by 30° from the gravitational vertical. Dynamical stimulation produced similar responses in statoreceptors of synchronous control and space groups, but they differ significantly from the reaction of statoreceptors of the control vivarium group. Probably, this fact results from specific conditions of the experiments. The snails "hibernated" in closed containers, which is attested by the fact that many snails had one to three epiphragms tightly closing the orifice of the clam-shells. In this state, their body weight decreased to the end of experiment by about 25% mainly due to loss of fluid. Indeed, when these snails were placed in humid medium, their initial body weight rapidly returned to baseline. Presumably, such pronounced dehydration increased viscosity of the statolymph and consequently impeded movements of statoconia which attenuated the responses of statoreceptors to oscillations of the platform. The effect of weightlessness was manifested by more rapid damping of reaction of statocysts to dynamic stimulation in snails of space group in comparison to snails of control groups, which could result from increased size of statoconia.

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REFERENCES

1. Ya. A. Vinnikov, O. G. Gazenko, L. K. Titova, *et al*., *Gravitation Receptor* [in Russian], Leningrad (1971).
2. G. I. Gorgiladze, I. I. Bryanov, and E. M. Yuganov, in: *Physiological Problems of the Weightlessness* [in Russian], Moscow (1990), pp. 198-215.
3. V. A. Kovalev, O. V. Zaitseva, and L. S. Bocharova, *Evol. Biokhim. Fiziol.*, **17**, No. 1, 18-24 (1981).
4. M. V. Reshke, L. N. Kornilova, D. L. Kharm, *et al*., in: *Space Biology and Medicine* [in Russian], Vol. 3, Pt. 1, Moscow (1997), pp. 233-328.