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FEEDBACK HYPOTHESIS AND THE EFFECTS OF ALTERED GRAVITY ON FORMATION AND FUNCTION OF GRAVIRECEPTORS OF MOLLUSKS AND FISH

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ABSTRACT

Popular hypothesis based on the idea of simple feedback mechanism that correlates gravity level and weight of test mass cannot explain the variety of the effects of altered gravity on development and function of gravireceptors. The reaction of organisms to the change of gravity depends on the gravisensitivity of the physical and chemical processes corresponding to specific phases of development and may have no relation to any feedback mechanisms of compensation of altered weight of the test mass. The present work analyzes the hypothesis of feedback and shows the ambiguity of possible effects of the altered gravity on formation and function of gravireceptors basing on the data from mollusks and fish.

INTRODUCTION

Altered gravity is known to influence the state of gravireceptors. Although these effects are more pronounced during the earlier stages of organism development, structural changes also take place in mature gravireceptors. Despite the number of experiments, the results have not led to a clear interpretation. Popular hypothesis says that regulation of growth of the single otolith in fish or the statoconia mass in mollusks, i.e., the test mass upon which gravity and linear acceleration act, is based on the *weight* of the mass. Thus, a larger-than-normal mass should be produced in a reduced-*g* environment and a smaller-than-normal mass would be produced in hyper-*g* (HG). It seems reasonable to assume that such reaction of gravisensing system (GS) to the change of gravity may be the result of feedback.

It also may be assumed that the possible "goal" of such self-regulation is to compensate the change of the parameters of the system caused by the external influence and to return the analyzing system in the normal physiological range. The level of complexity here suggests the existence of central (neural) feedback mechanisms.

However the supporting evidence for this hypothesis is not so coherent, because: *i)* the altered gravity is not a natural change of the environmental conditions and there are no "built-in" self-regulated programs of the development of the GS in altered gravity; *ii)* central feedback will not work until neural connections are developed; *iii)* in the absence of

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neural feedback the influence of altered gravity on local mechanisms of gravireceptor development may be determined by sensitivity of the physical and chemical processes of receptor formation to gravitational force rather than by assumed requirement of compensating the altered weight of the test mass (12).

We have to distinguish at least two kinds of feedbacks. One is connected with local mechanisms of self-regulation and specific for initial period of the GS development when the neural connections are still absent. And the other feedback is related to neural self-regulation and specific for later stages of the GS development. Complexity of the problem is enhanced by incompleteness of experiments, and consequently the experimental results have not led to a clear interpretation despite the numerous studies.

The goals of the present work are the structuring and analysis of the hypothesis of feedback and the demonstration of the ambiguity of possible effects of the altered gravity on formation and function of gravireceptors derived from the data from mollusks and fish.

METHODS

To predict the effects of altered gravity on gravireceptors we need to query the processes that participate in the earlier stages of gravireceptor development, their evolution and sequelae.

1. Gravireceptor Formation

1.1. Mollusks

The peculiarity of gravisensing system of *Aplysia californica* is found in the initial period of development when a single statolith is present in the cyst lumen. The interaction of the statolith with the receptor cells provides the necessary information for the animal's spatial orientation. This initial period corresponds to the swimming behavior of mollusks. After a critical size (~ 45 μm) of the statocyst is reached, a fast growth occurs in the number of statoconia; the increase of statoconia number proceeds at a non-monotonic rate, but the size distribution of statoconia is stable during the life of animal (24). It is assumed that the ratio of statolith to statocyst size controls the triggering of multiple statoconia generation in *Aplysia* (24).

The snail *Biomphalaria* lacks the single statolith stage and the growth in number of statoconia begins in the initial phase (9) when shell diameter D£4mm. After that phase the generation of statoconia ceases, and further evolution of size distribution of statoconia occurs due to their growth in the cyst lumen (11). The statoconia size distribution is altered slightly over the interval D ≈ 4-6 mm When D > 6mm the growth of statoconia sizes in *Biomphalaria* occurs without the change of the shape of statoconia size distribution.

It was found (16-18) that two kinds of enzymes, urease and carbonic anhydrase (CA), play important roles in the processes of statoconia formation in *Aplysia*. However, their functions appear to be different: urease mainly controls the rate of statoconia production by the supporting cells, while CA appears to be more important in the maintenance of statoconia and statolith volume. Thus the experimental data suggest that both enzymes influence the statoconia evolution in *Aplysia* within the supporting cells of statocyst.

1.2. Fish

The critical steps in the initial formation of the zebrafish otolith have been described in (19). First, otolith growth begins at 18-18.5 h after fertilization by localized accretion of free-moving precursor (seeding) particles. Second, otolith seeding is regulated by: *a)* kinocilia of precociously forming hair cells (tether cells) that bind seeding particles, thereby localizing otolith formation, and by *b)* beating cilia that agitate seeding particles, thereby inhibiting premature conglutination. Third, seeding particles and beating cilia disappear soon after 24 h, decreasing the rate of otolith growth by nearly 90%. Fourth, precursor hair cells (HCs) at 20 h are still immature. Fifth, the developing sensory epithelium at 24 h probably cannot support transduction and is not capable of

transmitting neural signals. Sixth, most of the apparatus of functional HCs and afferent synapses appear to be present at 36 h but the hair bundle does not appear as compact as in the mature hair cell. Seven, between 24 and 52 h the primary afferents from the maculae and the semicircular canal cristae project to the brainstem vestibular nuclei (7). And eighth, after 24 h the central efferent vestibular fibers that project to the vestibular end organs pioneer the pathway from the brainstem to periphery (10), thus the timing of outgrowth of efferent fibers and the timing of ingrowth of primary afferents appear to coincide.

2. Effects of Altered Gravity

2.1. Mollusks

Pedrozo *et al.* (18) reared *Aplysia* eggs and larvae on a short-radius centrifuge. In pre-metamorphic animals the statolith was significantly smaller than that of size-matched 1-*g* control specimens and the degree of reduction was graded with increasing rotation rates, and thus the applied centrifugal forces, from 1- to 5-*g*. Post-metamorphic animals reared on a centrifuge produced smaller and fewer statoconia than those of control animals and demonstrated a reduced urease activity in the statocyst 18); and a similar result was observed even for isolated statocysts from early post-metamorphic animals placed on a centrifuge (18). The similarity between the evolution of statolith and statoconia volumes as well as urease levels *in vivo* and *in vitro* hyper-G experiments suggests that feedback(s) that which regulates these parameters have mainly the a local or peripheral origin.

According to (22), microgravity (μG) mainly increased the rate of the statoconia production in *Biomphalaria*, while the growth of the mean size and the change of statoconia size distribution were not observed. Average increase was ~ 39% for snails fixed on landing day (R + 0) and 60% for those fixed on R + 5 day.

2.2. Fish

The results of the study (21) demonstrated that zebrafish exposed to 3-*g* from 36 h to 7 d after fertilization had significantly smaller otoliths, while those exposed to 3-*g* only from 12-36 h after fertilization had slightly smaller otoliths than 1-*g* controls. The results of (19) indicate that the period after 36 h corresponds to the second (slower) phase of otolith growth. This phase resembles the growth of statoconia in the cyst lumen and depends on secretory function of supporting cells. A series of experiments on cichlid fish reared on a centrifuge to produce hypergravity have been reported in (3-5). These studies demonstrated that the radius of both the utricular and saccular otoliths of cichlid fish were significantly reduced in animals reared at 3-*g*. The amount of calcium deposited in the otoliths, measured by fluorescence of Alizarin bound to newly deposited calcium, was also reduced. Thus, as with mollusks, rearing fish in hypergravity produces smaller-than-normal otoliths. According to (1, 2), the growth of cichlid fish otolith was controlled by CNS. Experiments showed that nerve transection or altered gravity affects calcium carbonate incorporation via the efferent vestibular system (determined via acetylcholine esterase histochemistry) by a stimulation/deprivation of inner ear CA activity leading to an increased/decreased provision of endolymphatic carbonate (1). Meanwhile according to (23), the μG experiments with rearing of zebrafish show a profound increase in otolith growth in later stage embryos but either no effect or an opposite effect (smaller otoliths developed in space) in early-stage embryos.

The readaptation of structural and functional changes in mature gravireceptors of the oyster toadfish (*Opsanus tau*) caused by the μG exposure were studied in 2 shuttle orbital missions (STS-90 and STS-95) by (6). The sensitivity and directionality of response of individual utricular nerve afferents to natural translational stimulation were measured at intervals up to 5 d following return to 1-*g*. It was found that the response sensitivity (the modulation of firing rate with respect to input acceleration) was significantly increased shortly after landing. The physiological results agree with the morphological observation of Ross (20) who found an increase in the number of synapse bodies in utricular hair cells in adult rats flown in space. Both the changes in synapses in adult rats and the change of afferent response in adult fish returned to normal within a day or two of return to the 1-*g* environment.

RESULTS AND FUNCTIONAL CONSIDERATIONS

1. Common and Specific Features of Mollusks and Fish Gravireceptor Development

The available experimental data show that the development of gravireceptors may be divided into specific periods separated in time and connected functionally in such a way that the subsequent period can be realized only after the previous one is accomplished. These periods include very different physical and chemical processes, have different goals and play different, but necessary roles in gravireceptors formation. It is also evident that the absence or disturbance of one of these elements may disrupt or even destroy the whole program of gravireceptor formation. The rules of development of these elements may be called sub-programs.

Thus in *Aplysia* we can distinguish a few successive steps of gravisensing system development: a statolith period, a transition period of multiple statoconia production, followed by a period when statoconia size distribution becomes steady, but statoconia number continues to increase (13, 14). In *Biomphalria* we find three specific life periods: a transition period of an increase of statoconia number, a period of "tuning" the statoconia size distribution, and a steady state period of the growth of statoconia sizes in cyst lumen while their production by supporting cells is slowed down or stopped (11, 13).

The example of such periods in the development of otolith in fish is the existence of two different phases of otolith formation. The mechanism of the first phase (up to 24 h) is connected with appearance and further disappearance of two types of cells with different functions: the beating cilia cells and the tether cells. The mechanism of the next (slow) phase of otolith formation is determined by interaction between otolith substance and surrounding statolymph. (There is likely a third, or initial, period of otolith formation corresponding to the development of precursors (seeding particles) (19).

It should be noted that the same physical elements and processes participate in development of the statoconia in mollusks and the otoliths in fish. Hair cell cilia play a few key roles: support and move the dense particles, and affect the membrane potential of the receptor cell body during the transduction process. Further, in some yet undefined way the receptor cell transfers the information of the animal's orientation with respect to gravity to the supporting cells. In the initial phase of fish otolith formation there are also hair cells that 'trap' seeding particles and beating cilia that agitate them. There are structures (likely, supporting cells) that produce statoconia or otolith precursors. Also we may hypothesize that there are at least two different mechanisms of stone growth common to both mollusks and fish. An example of the first one is the formation of statoconia within the supporting cells and the formation of precursors in fish otolith. The second one is the growth of particles in fluid environment in *Biomphalaria* that is likely similar to the slow phase growth of fish otolith. (It is also possible that a mechanism exists that transforms the change of the state of receptor cells caused by interaction with the particles into the signal for formation of statoconia or otolith precursors in supporting cells.)

At present we may suppose that the first mechanism of growth has a local origin and is mainly determined by the process of statoconia-cilia interaction while the second mechanism may have local as well as neural (efferent) origins.

2. Sensitivity of Processes and Parameters of Development to Altered Gravity

Every subprogram consists of a number of physical processes and parameters. Some of them may depend on the alteration of gravity that can change or even destroy the fulfillment of these subprograms. Let us assume that subprograms may switch from one to another when some control condition (parameter) that corresponds to fulfillment of the previous subprogram is reached. A possible example of such critical parameter that controls the triggering of multiple statoconia in *Aplysia* is the change of statolith-cilia interaction due to the change of the ratio of statolith/statocyst sizes (24). This "control condition" may also depend on the change of gravity level. Thus there are two possible sites (sources) of the altered gravity sensitivity: the processes within subprograms and the parameters of critical conditions related to transitions between them. The types and numbers of subprograms of gravireceptor development are changing in the course of time. Thus the set and number of the processes sensitive to alteration of gravity will change too.

Two effects of HG on *Aplysia* statocyst were observed in *in vitro* experiments (18): a decrease in calcium carbonate granule rate of generation (a decrease in statoconia number), and a decrease in calcium carbonate granule size (statolith and statoconia sizes). These results suggest that *i)* some mechanism(s) might control the rate of statoconia formation by supporting cells and another one that controls the stone growth within these cells, *ii)* feedbacks of principally local origin regulate both mechanisms, and *iii)* both mechanisms are sensitive to the change of gravity.

It was also found that μG mainly increases the rate of the statoconia production in *Biomphalaria* while the growth of the mean size and the statoconia size distribution were unaffected (22). In μG conditions the statoconia will not sink to the cyst bottom due to the absence of gravity and the motion of statolymph fluid produced by beating cilia. As a result the interaction of the statoconia with the hair cell cilia will likely be less frequent and the effect of entrainment will be less pronounced because the statoconia mass will be near the center of cyst. Thus μG may result in the decrease of loading the cilia or in the change of loading the cilia in different parts of statocyst compared with that in 1-*g* conditions. This may lead to an "instruction" *sent*, perhaps through gap junctions, from receptor to supporting cells to increase the production of statoconia. (Note that the alteration of statolith-cilia interaction due to the growth of statocyst is assumed to be responsible for triggering statoconia production in *Aplysia* (24). A possible explanation of this observed increase in statoconia production in *Biomphalaria* (22) may be due to the time in development in which the statocysts were harvested: the statocysts were taken from animals having 1, 1.5 and 2 mm shell diameters. These sizes of shells of the flight animals correspond to the phase of continuous statoconia production by supporting cells under normal conditions (9). At the critical shell diameter of ~ 4 mm the production of statoconia stops (9). Let us suggest that in order to stop statoconia production in *Biomphalaria* it is necessary to have a specific number of statoconia at the critical diameter of statocyst corresponding to a shell diameter ~ 4 mm (11). It is possible that the observed increase in the number of statoconia produced in μG (22) was nevertheless insufficient to arrest the period of statoconia production and initiate the period of statoconia size growth in cyst lumen.

If the effect of an applied external force is critical for further development of the receptor, the time-interval of development corresponding to such subprogram may be called "cri-

tical periods" relative to this force (15, 21). The possible example of such critical period in fish gravireceptor development relative to μG conditions may be the first phase of otolith formation (~ 24 h) (21).

3. Peculiarities of Feedbacks

1. It may be assumed that some subprograms of gravireceptor development are auto-regulated based on feedback mechanism(s). These feedbacks might have local or more distal (neural) origins or both. The local feedbacks are confined by the small volume of precocious receptors, while more distal feedback depends on the existence of neural connections. Until the point in time is reached when the neural connections are functionally developed, the "neural" feedback cannot work. Thus, any effect of the change of gravity on the gravireceptor during this initial period has a local origin.

Therefore at least two kinds of feedbacks may be distinguished: local and neural. They work on different time intervals, are based on different physical processes, and subserves different purposes. Auto-regulation, especially at the earlier stages of development when the neural control is absent or weak, provides the stability of subprogram fulfillment relative to the natural variations of those parameters of environment that participate in formation of gravireceptor. In this case feedback is an adaptive response of the organism to such natural instabilities. With respect to the neural feedback we may suppose that its "goal" may be not only to compensate for a change of the parameters of the system caused by an external influence, but also to return the analyzing system into its normal physiological range. We cannot exclude that in some conditions the observed effects of altered gravity on gravireceptor are the results of both neural and local feedbacks. (This possibility has to be taken into account in comparison of the results of hyper-G experiments with *Aplysia californica* in *in vivo* and *in vitro* experiments (16, 18).

2. If there are distinctive periods (subprograms) of gravireceptor development, several feedbacks corresponding to these periods may exist. For instance, the period of statolith formation in *Aplysia* is being replaced by the period of formation of statoconia size distribution. At present it is not clear how the whole structure of feedbacks in developing gravireceptors might be organized. It is natural to suppose that plasticity of the system to the change of environmental parameters at the earlier stages of development is greater than that for fully developed and mature system. Also we assume that in the course of maturation the center of control moves toward central neural feedbacks.

3. It should be taken into account that although each sensory organ has a specific sensory modality their functions are strongly correlated. Examples of this correlation are vestibulo-ocular and vestibulo-neck interactions. Therefore, the vestibular sensorimotor systems do not develop as isolated structures, but in concert with each other and in balance with the development of other sensory organs related to a given modality. As a result, the effect of the altered gravity on the development of gravisensing system may be controlled (supported or damped) by the requirement of the correlated development of this system, i.e. by the requirements of other sensory systems involved in gravisensing, whose sensitivity and response to the altered gravity might be quite different. Because of this we may suggest that the process of the change of the gravisensing system at the stage of mature neural links has to be rather limited.

4. Also we must bear in mind that the altered gravity is not a natural change of the environmental conditions and there are no "built-in" self-regulated programs of the statocyst response to the altered gravity. The response may be based only on the existing reactions of the statocyst that have been developed (designed) for other "purposes". For instance, the size and composition of statoconia may vary due to the variation of the necessary components in environment or misbalance in gravireceptor biochemistry. It is possible that there is a local feedback system that controls statoconia production to compensate for environmental deviations and to maintain the proper statoconia-receptors interaction by the regulation of statoconia number and size. It means that the reaction of developing gravireceptor to the gravity alteration is realized, for instance, in terms of the deficit of mineral or organic supplies for otolith formation. From this point of view the altered gravity cannot be considered as a change of a particular environmental parameter because such change cannot occur under natural conditions. Because of this the altered gravity may cause such effects in the structures of developing gravireceptors that may be not related to any feedback mechanisms designed for compensating the possible changes in parameters of environment.

5. In the case of the neural feedback, the reaction of mature gravireceptor to altered gravity will be determined by two factors: by the change of gravireceptor stimulation under new conditions and the perturbation of correlation between the altered signal of gravireceptor and signals derived from other sensor modalities that provide the spatial orientation and dynamics cues used by the animal. For instance, in the case of μG the stimulation of receptor cells of gravireceptors in mollusks and fish drops and the correlation between the signal and stimuli specific for normal conditions is broken. To amplify the signal from gravireceptor it is necessary to increase the sensitivity of afferents innervating the gravireceptor. This may be accomplished, in particular, by increasing the number of synaptic bodies in the gravireceptors per synaptic contact sites or the number of synaptic contact sites in peripheral neural endings or both.

The structural and functional changes in mature gravireceptors of toadfish caused by μG were found by Boyle *et al.* (6). The readaptation of vestibular function following space flight was measured by evaluating the sensitivity of the response function of vestibular utricular afferents to inertial acceleration stimulation. Initially after landing the response sensitivity showed an upregulation compared to earth-based controls: the afferent's response sensitivity was significantly elevated. This finding agrees with the morphological observations made by Ross (20) regarding a significant increase in the number of synaptic bodies in utricular type II hair cells to vestibular nerve fibers in adult rats exposed to the μG environment. Both the changes in synapses in adult rats and the change of sensitivity in adult fish returned to normal within a day or two of return to the 1-*g* environment of Earth. It was supposed that neural feedbacks might be based on efferent regulation (2, 6).

It is necessary to consider that a regulation by the neural feedback suggests that some "comparison circuit" exists that provides an error estimation of the gravireceptor signal. Some Authors assume that such alternative information regarding gravity force might come from the weight distribution along the animal body (8) or from asymmetry of the left and right statocysts and statoliths found by (18).

4. Feedback Hypothesis: Basic Assumptions and Comments

Summarizing these data the feedback hypothesis may be formulated as a set of the following basic assumptions (Model): *1)* The gravisensing system development consists of a set of successive time-dependent parts (subprograms of development) acting during specific periods. *2)* There are some mechanisms of feedbacks of local and neural origins to control the course of subprogram fulfillment and the transition from one program to another. The local feedbacks are confined by small volume of precocious receptors. *3)* If the afferents and efferents are still immature, the "neural" feedback cannot work and any effect of the change of gravity on gravireceptor during this period has local origin. It is possible that different feedbacks are involved at different periods in the development of the gravisensing system in altered gravity: at the earlier stages they are local and in the mature state they may have neural-like efferent nature (2, 6). *4)* The altered gravity may cause direct effects in developing gravireceptors that are not related to any mechanisms designed to compensate for the possible changes of environment normally experienced by the organism. *5)* Altered gravity influences those mechanisms of the system whose parameters and processes depend on gravity level. These may be internal parameters of subprograms or control parameters that determine transition from one program to another. It is possible that several feedbacks corresponding to different stages of gravireceptor development would be sensitive to altered gravity. The examples are the changes of statolith and statoconia in *Aplysia* in HG conditions observed in *in vitro* experiments (16,18). *6)* It has to be stressed that every response of the gravisensing system to the change of altered gravity in the course of gravireceptor formation may be realized within the limits of the existing subprograms designed, possibly, for absolutely different purposes. (It was supposed that "otolith mineralization hitherto is the only biomineralization process known, which is neuronally regulated in adaptation to an environmental parameter (i.e., gravity)" (1). Our opinion is that the possible neural regulation of otolith growth cannot be consider as some kind of adaptation to the change of gravity as an environmental parameter, because the gravity was never changed during animal development, but this neural feedback may be the response to the effect of the altered gravity on some physical or chemical processes participating in otolith formation).

DISCUSSION

The experimental data related to the effects of the altered gravity on developing gravireceptors of mollusks and fish are not complete. Because of this we will assume that the processes that are likely common for mollusks and fish will experience the same effects of the altered gravity. This assumption allows us to use the altered gravity data obtained from one species to fill a gap in the data corresponding to another species.

The data listed above allow us to outline at least two different mechanisms of the growth of statoconia in mollusks: the first one works within supporting cells and the second one takes place in the cyst lumen. It is assumed that the second mechanism in mollusks is analogous to the mechanism of slow phase growth of otolith in fish. The peculiarity of the first mechanism is its local origin. We believe that the change of statoconia-cilia interaction cau-

sed by altered gravity yields a change in the state of receptor cells that transforms the state of supporting cells and thus the rate of statoconia production. Meanwhile the second mechanism of statoconia growth is determined by the interaction of statoconia (or otolith in fish) with its surrounding fluid. It is not clear whether local or neural feedbacks (or both) control this mechanism. It is possible that neural efferent modulation of the second mechanism of the growth is more effective than the local one whose role is to maintain the chemical balance within supporting cells. The determination of the contributions of local and neural feedbacks in the second mechanism of statoconia or otolith growth is one of the most intrigue problems of biophysics of gravireceptors.

It should be noted that these two mechanisms of statoconia or otolith growth have different rates but they are working in sequence. The sizes of statoconia or otolith are the result of their joint work. Both mechanisms are sensitive to altered gravity, but their dependencies on the altered gravity are different. Because of this there is a variety of the final results of the altered gravity influence on the statoconia and otolith development.

1. Statolith Period in Aplysia

It was assumed (24) that "statolith-cilia" interaction prevents the statoconia production until the statolith can stimulate all the receptors. The authors of (24) believed that as the statocyst grows beyond critical size (~ 42-44 μm), the statolith will stimulate only a few receptors cells rather than all receptor cells and this change of cell activation is what initiates the multiple production of statoconia. This assumption has to be tested because at present it is not clear: *i)* what parameters of "statolith-cilia" interaction are important for such regulation, *ii)* what parameters of the states of spatial distribution of receptor cells control the state of supporting cells and thus statoconia production.

The HG *in vitro* experiments resulted in the decrease of statolith diameter while the diameter of statocyst remains almost unchanged (18). Because the formation of the statolith is assumed to occur in the cyst lumen (24), the fact that the size of statolith in the hyper-G conditions is smaller than in control animals may indicate a direct influence of HG stimulation on the statolith production (18) that likely has a local origin. However, the change of the statolith diameter is very small to compensate for the change in acceleration magnitude (2G, 3G, 5G) (18). Because of this we may expect that the assumed "falling" of statolith that triggers statoconia production (24) would occur earlier than in normal conditions. In other words, we may expect that in HG the multiple production of statoconia will start at the smaller sizes of statocyst than in control animals.

We may assume also, that in the case of μG the decrease (lack) of stimulation of receptor cells may result in statolith growth. In this case the triggering of statoconia production will correspond to the larger sizes of statocyst.

2. Statoconia and Altered Gravity

2.1. Aplysia

The reaction of the post-metamorphic animals or even isolated statocysts from early post-metamorphic *Aplysia californica* to HG conditions were studied by Wiederhold and his colleagues (16, 18). However, the influence of μG on *Aplysia* gravireceptor has not been investigated. What kind of effects do we expect to observe in μG in *Aplysia*?

We know that formation of statoconia occurs in supporting cells and their generation continues throughout the animal's life. The results of both *in vivo* and *in vitro* experiments show that at least in part a mechanism of feedback responsible for the smaller statoconia size in hyper-G conditions has a local origin. We also know that the effect of μG on *Biomphalaria* results in an increase in the rate of statoconia generation. This increase occurred at the stage of *Biomphalaria* evolution (development?) corresponding to statoconia production by supporting cells (9, 11). According to (17, 18), the rate of statoconia production is controlled by the level of urease. Therefore we may expect that in μG conditions the rate of production and hence the number of statoconia, as well as the level of urease, will be greater than those in 1-*g* control.

In respect to μG experiments with *Biomphalaria* size distribution of statoconia remained almost identical between the flight and the ground-reared snails. As it was supposed (17, 18) the level of carbonic anhydrase (CA) is important for maintenance of statoconia and statolith volume in *Aplysia*. If the mechanisms of statoconia growth in *Aplysia* are similar to those in *Biomphalria* we may expect that the level of CA in statocyst of *Aplysia* will not be influenced by μG.

2.2. Biomphalaria

Microgravity. It was found that μG mainly increases the rate of the statoconia production in *Biomphalaria* while the growth of the mean size and the change of statoconia size distribution were not observed (22). The statocysts were taken from *Biomphalaria* with shell diameters ≤ 2 mm that correspond to the phase of statoconia generation by supporting cells that continues until the shell diameter reaches ~ 4 mm. It would be interesting to carry out this μG experiment with the snails whose sizes of shells would be more close to the sizes corresponding to cessation of statoconia generation. If the cessation of statoconia generation is determined by the number of statoconia per statocyst, we may expect that μG will stimulate early (relatively to 4 mm shell diameter in 1-*g* conditions) cessation of statoconia production. Assuming that urease controls the state of supporting cells whereas CA participates in the maintenance of statoconia and statolith volume in *Aplysia* and suggesting that the roles of these enzymes in *Biomphalaria* are the same, we may expect that at the stage of statoconia generation where shell diameter is < 4 mm the level of urease will be higher in snails reared in μG after landing than that in 1-*g* control counterparts and the level of CA will be unchanged between the two groups.

Another kind of μG experiments may be related to the stages of snail development that correspond to 'tuning" of statoconia distribution (i.e. for shell diameters in the range of 4-6 mm) and to the stable statoconia distribution (i.e. for shell diameters > 6 mm) when generation of statoconia ceases but the growth of statoconia continues in the cyst lumen. We may expect that μG will change statoconia-cilia interaction that will result in the growth of statoconia size and the change of size distributions of statoconia on these intervals of shell diameters. Since statoconia growth on this stage of development occurs in cyst lumen, we may anticipate an increase of CA level at this stage. Also we cannot exclude that efferent regulation may control statoconia size and size distribution on these time intervals with the help of modulation of the CA and urease levels.

Hypergravity. The results related to the HG effects on *Biomphalaria* are absent. Based on the results of HG experiments with *Aplysia* we may expect the following scenario. If the

experiment in HG would correspond to the stage of statoconia generation (D < 4 mm), then we will observe a decreased rate of statoconia production, a decreased volume of statoconia and a lower level of urease. Since the slow phase of fish otolith growth resembles the growth of statoconia in the cyst lumen in *Biomphalaria*, we may expect also that in HG conditions statoconia of snails with shells diameters > 6 mm will be smaller in size than 1-*g* controls. The investigation of the effects of HG on statoconia development in *Biomphalaria* with shells > 6 mm may be helpful for understanding the nature of feedback in the slow phase of fish otolith growth.

3. Fish Otoliths

Two different reactions of otolith development dependent on embryo age were observed in zebrafish reared in μG (23). A noticeable increase in otolith growth was found in later stage embryos while the absence of otolith growth or even smaller otoliths were observed in early stage embryos. Since there are two rates corresponding to two mechanisms of fish otolith growth specific for initial (up to 24 h) and later (after 36 h) stages after fertilization, the effects of μG on these stages may be different. The first mechanism is based on the balance of two processes: binding of otolith seeding particles by tether cells and agitation of these precursors by beating cilia. After 24 h tether cells and beating cilia disappear. Thus the absence of gravity during this time interval destroys this balance. When gravity is absent but beating of cilia continues, the precursors will move away from the tether cells and thus the formation of otolith protostructure will be slowed. The hair cells are still immature at this stage and the sensory epithelium cannot provide signal transduction, possible, until 36 h or even later. Thus the neural feedback likely is absent in this period. After disappearance of the tether cells and cilia, the continued formation of otolith occurs due to a slow phase mechanism of growth determined by the interaction of otolith substance with statolymph.

In the case of juvenile fish the μG influences the second mechanism of otolith growth while the protostructure of otolith is already formed. This influence results in much larger otoliths compared to control. However it is not clear if this effect demonstrates stimulating influence of μG on the secretory function of supporting cells or the growth of otolith in juvenile fish in μG is the result of neural self-regulation of the otolith formation.

The similar age dependent effects of altered gravity took place in HG experiments (23). Zebrafish exposed to 3G from 36 h to 7d after fertilization did have significantly smaller otoliths than those of 1-*g* control fish, while those exposed to 3G from 12-36 h after fertilization had slightly smaller otoliths, but this difference was not significant from the controls. This result may be interpreted as a demonstration of the weak influence of HG on the balance between trapping of settling seeding particles by tether cells and the agitation of these particles by beating cilia. Meanwhile the strong effect of HG on slow phase of otolith growth demonstrated the inhibition of secretory function of supporting cells. This fact correlates with the decrease of statolith growth in *Aplysia* in HG (18) that occurs in cyst lumen.

Since the slow phase of fish otolith growth resembles the process of statoconia growth in cyst lumen of *Biomphalaria*, we may expect that in HG conditions the statoconia of snails with shells diameters > 6 mm will have smaller sizes than those in 1-*g* controls.

Although the contribution of local and neural feedbacks in HG influence on the second mechanism of otolith or statoconia growth remains unclear, the results of (1, 2) indicate significant role of neural efferent regulation.

SUMMARY

The effect of exposure of altered gravity on the gravisensing system depends on the stage of development of the receptors and the particular susceptibility of the existing individual processes to the gravity force. Different feedbacks with different gravisensitivities are involved at different periods in the development of gravisensing system in altered gravity: at the earlier stages they are local and in mature state they may have a neural-like efferent component.

Some effects of the altered gravity may be not related to any feedback mechanisms participating in gravisensing system formation and result in an abnormality of gravireceptor development. The analysis shows that there are similar elements and processes in gravireceptor formation in mollusks and fish. It suggests that we may use the information obtained from one species to explain or predict the effects of the altered gravity on the similar elements and processes in other species. The use of changes in absolute gravity levels and transitions between gravity states are powerful tools to identify the mechanisms of the gravisensing system development and function in normal and abnormal (e.g. disease, space) conditions.

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