20 Gravitational Zoology: How Animals Use and Cope with Gravity

Ralf H. Anken and Hinrich Rahmann

Since the dawn of life on Earth some four billions of years ago, gravity has been a more or less stable environmental factor thus influencing the phylogenetic development of all living organisms. On the one side, gravity represents a factor of physical restriction, which compelled the ancestors of all extant living beings to develop basic achievements to counter the gravitational force (e.g., elements of statics like any kind of skeleton - from actin to bone - to overcome gravity enforced size limits or to keep form). On the other side, already early forms of life possibly used gravity as an appropriate cue for orientation and postural control, since it is continuously present and has a fixed direction.

Due to such a thorough adaptation to the Earthly gravity vector, both orientation behavior as well as the ontogenetic development of animals is impaired, when they have to experience altered gravity (Δg; i.e. hyper- or microgravity). Nevertheless, animals still can cope with Δg in a certain range based on their physiological plasticity, which varies among the different animal phyla.

20.1 Gravity as a Factor of Physical Restriction: A Brief History of Evolutionary Challenges To Surmount It

As a matter of fact, the non-linear self-organizing dynamics of biological systems are inherent in any physical theory that satisfies the requirements of both quantum mechanics and general relativity [1]. Gravity therefore has always been a challenge for biological systems to adapt or/and to cope with it. Concerning single cells, the earliest life forms, it has been stated that average cell size results, in part, from the physical equilibrium between the destructive influence of the force of gravity and the protective role of diffusion and the cytoskeleton [2]. At increased forces of gravity the cell size would thus be decreased, whereas at lower gravitational forces and weightlessness cell size would be expected to increase. Mechanisms of protection of giant cells against internal sedimentation are based on protoplasmic motion, thin and elongated shape of the cell body, increased cytoplasmic viscosity, and a reduced range of specific gravity of cell components, relative to the ground-plasma. The nucleolus, due to its higher density, is considered as a possible trigger of mitosis. Although gravity limits the size even of single cells, its impact became especially apparent with the evolution of multicellular animals. There is not much known about the first multicellular animals inhabiting our planet in the late Precambrium (earlier than 570 millions of years ago) prior to the so-called “Cambrian explosion”, which showed an extremely rapid evolutionary radiation with the development of almost all nowadays phyla of invertebrate animals. With only a few exceptions, the Precambrian animals (e.g., the forms of the Ediacara-fauna, named after the Ediacara Hills north of Adelaide, Australia) did not have any sort of inner or outer skeleton. All of them were small and many species had a worm- or jellyfish-like appearance (Fig. 20.1). In most cases, their relationships to the nowadays present invertebrate groups is unclear.

Obviously, elaborate anti-gravity systems had not yet been fully evolved, which would have allowed these animals to grow larger, to develop a directed locomotion and even to cope with the terrestrial impact of gravity at the stage of their exit from water to land (Fig. 20.2). Development of a directed locomotion might have been one of the most important evolutionary inventions. When heterotrophic animal life decreased the nutrients (e.g., autotrophic plants) in the oceans, animals were forced to cope with this evolutionary pressure; directed locomotion was therefore developed to predate other animals or, *vice versa*, to escape from predators. For exercising directed locomotion especially gravity was - besides other environmental factors such as radiation (especially light), atmospheric conditions/composition, sound and electromagnetic as well as mechanical impacts - one of the most important morphogenetic factors of animal evolution which pushed the gene to elaborate adequate mechanisms for surmounting it [3]. Directed locomotion generally requires any sort of skeleton to allow the insertion of muscles; such a skeleton then could (pre-adaptively) act as a prerequisite for animals to turn from their aquatic habitat to a terrestrial life some 440 millions of years ago, then following the green plants as a further source for their heterotrophic lifestyle (the first terrestrial animals were early ancestors of our nowadays spiders, belonging to the arthropods).

The first vertebrate animals which were able to cope with the terrestrial impact of Earth’s gravity were early ancestors of fish some 350-400 millions of years ago. Concerning modern bluefish, it has been found that these animals can accelerate at 3 ×*g* during swimming and that the vertebral column is strong enough to withstand this

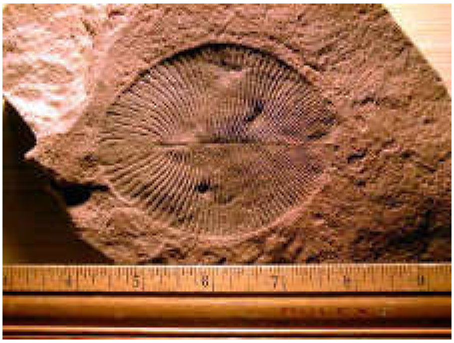

Fig. 20.1 This Ediacara-fossil may possibly represent an early jellyfish.

Fig. 20.2 Jellyfish certainly cannot cope with the terrestrial impact of gravity.

force [4]. This finding strongly indicates that the muscles and body structure of a bluefish would be able to withstand the force of gravity if the fish were otherwise equipped for terrestrial life (hypergravity experiments revealed in this context that development of larval fish is not impaired by 3 *g*; see 20.3.2.2). One can therefore speculate that early fish may also have evolved some degree of strength to overcome gravity-based inertia and drag during aquatic locomotion, and this evolution may have been a prelude to vertebrate terrestrial locomotion.

Terrestrial locomotion, of course, requires further special adaptations due to an animal's particular lifestyle. Gravitational force influences musculoskeletal systems, fluid distribution, and hydrodynamics of the circulation, especially in larger terrestrial vertebrates. The disturbance of hydrodynamics and distribution of body fluids relates largely to the effects of hydrostatic pressure gradients acting in vertical blood columns. These, in turn, are linked to the evolution of adaptive countermeasures involving modifications of structure and function. Comparative studies of, e.g., snakes [5] suggest that there are four generalizations concerning adaptive countermeasures to gravity stress that seem relevant not only to lower vertebrates: first, increasing levels of regulated arterial blood pressure are expected to evolve with some relation to gravitational stresses incurred by the effects of height and posture on vertical blood columns above the heart; second, aspects of gross anatomical organization are expected to evolve in relation to gravitational influence incurred by habitat and behavior; third, natural selection coupled to gravitational stresses has favored morphological features that reduce the compliance of perivascular tissues and provide an anatomical "antigravity suit"; fourth, natural selection has produced gradients or regional differences of vascular characteristics in tall or elongated vertebrates that are active in high gravity stress environments.

These generalizations can explain, why the position of the heart in relation to the head and the tail in different types of snakes vary: an aquatic snake will not be that affected by gravity. Consequently, the heart can be positioned relatively far away from the head and thus the brain. The heart of a terrestrial snake is positioned closer to the head. This is especially obvious in snakes living on trees, where the heart is situated almost directly behind the head in order to allow blood supply to the brain even during climbing upwards, i.e. in a direction opposite to the gravity vector [6]. Similar problems arise concerning extremely large or high-growing animals [7, 8]. The distance from the heart to the brain in the giraffe is ca. 2.8 m, whereas it reaches some 7.9 m in some herbivorous dinosaurs (human: 0.3 m), requiring an enormous blood-pressure to make the blood reach the brain, especially when the head eventually is being raised.

Bipedal walking was another challenge to cope with the force of gravity. There is not yet complete agreement, what the evolutionary pressure for bipedal walking actually had been, since bipedal walking is rather costly in terms of energetics: the short, flexed hindlimbs of chimpanzees younger than 5 years are not able to lift the body center of gravity high enough, so that these infants have a considerable energy output during bipedal walking [9]. Extension of the hindlimb is one of the bases for energy economy in human bipedalism (although lifting the legs in humans still consumes some 90% of the energy needed for locomotion [6]) and thus an important component of the evolution of human bipedalism.

An even higher ability to surmount gravity was necessary to come into being when animals made their way into the air. For certain, the potential diversity for evolution in large species (irrespective of them being invertebrates or vertebrates) is less than for medium-sized or small ones, and dwindles to zero above a body mass of about 14 kg [10]. The "World's largest flying bird", the Miocene (8-15 Ma ago) fossil *Argentavis* would require improbably high values for stress and strain in level flight, unless the air density were much higher in the Miocene times than at present, and/or the strength of gravity were much less. *Argentavis* therefore will not have been able to actively fly by flapping its wings, but will have mostly soared as do present-day condors.

Summarized, it was most probably the evolutionary pressure to develop directed locomotion which then, as a sort of prelude, allowed animals to cope with terrestrial gravity. Active, directed locomotion, and especially active maintenance of equilibrium during bodily movement (e.g., in locomotion) requires, however, appropriate sensory functions. Although many animals usually maintain their bodies with the long axis horizontal (backside up), humans being a notable exception, there are frequent departures from the usual position. A fish may dive steeply downward and a man may alter his normal orientation by lying down at full length. In no case, however, need there be any loss of equilibrium. Every deviation means an equilibrium disturbance and evokes compensatory reflex movements.

Gravity, therefore, did not only act as a factor of physical restriction, but was an environmental factor readily being available as an appropriate cue for orientation and postural control.

20.2 Gravity as a Cue for Orientation and Postural Control

Maintenance of equilibrium is based upon contact of the animal with the external world; several sensory systems may play a role in this context. When an animal moves over a solid surface, tactile stimuli usually predominate as cues. It has to be noted that also proprioceptors (i.e. sense organs allowing the perception of stimuli relating to the animal's own position, posture, equilibrium, or internal condition) in vertebrates and arthropods can also contribute to spatial orientation; bodily tissues like the club-shaped sensilla of arthropods (Figs. 20.3, 20.7) under gravity weigh vertically down and stimulate internal mechano-receptors in a way that depends on, and varies with, the animal's spatial position. When they are out of contact with the ground, many animals orient themselves in space by keeping their back (dorsal) side turned up toward the light, e.g., in the course of the dorsal light response (DLR) of fish (see 20.3.2.2). Visual cues also can serve equilibration; for example, through compensatory body movements (optomotor reflexes like the vestibulo-ocular reflex in fish and amphibians [11]) brought about by the shifts of the image of the environment over the retina of the eye. For the receptors mentioned thus far, however, equilibration is not the unique function. There are other sensory structures that are genuine organs of equilibrium in that they primarily and exclusively serve orientation of posture and movement in space: gravity receptors.

In general, gravity - or any sort of acceleration - can be transformed to a biological signal in several different ways (Fig. 20.3): already synthetic membrane bilayers containing incorporated ion-channels respond to gravity [12, 13]. The exact signal transduction chain remains, however, hitherto unresolved. Unicellular protozoans (e.g., *Paramecium*) perceive gravity obviously by their membrane or have already developed minute, intracellular crystals that function as “heavy bodies” (*Loxodes*) (compare 20.2.1 as well as Chap. 18, Bräucker et al.).

20.2.1 Graviperception in Unicellular Animals

Already archaic extantly living eukaryotic organisms (particularly phytoflagellates and ciliates) were shown to be gravitactic (cell orientation) or even gravikinetic (adjustment of swimming speed) (for review [14]; see also Chap. 18, Bräucker et al.). In the ciliate *Paramecium*, gravikinesis is obviously regulated by a pressure-gradient between the membrane and the surrounding aqueous medium. This gradient is only at equlibribium when the animal swims horizontally. Vertical tilts result in the opening/closing of mechano-sensitive ion-channels, which effect a local de- or hyperpolarisation, which in turn selectively activates cilia for propulsion. In contrast to *Paramecium*, another ciliate - *Loxodes* – seems to perceive gravity via particular intracellular organelles, called "Müller-bodies", which consist of a membraneous pouch, containing a ”heavy body” or ”statolith” of $BaSO_4$ (Fig. 20.4) [15] (see also Chap. 18, Bräucker et al.).

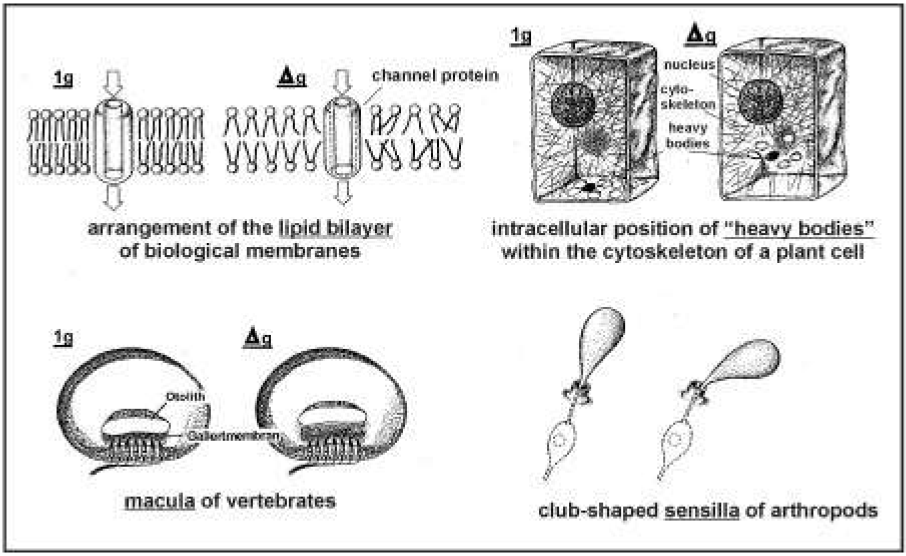

Fig. 20.3 Possibilities of graviperception in biological systems. Already (simulated) biological membranes can respond to the gravity vector. Plants and most animals use “heavy bodies” (called statoliths in plants, invertebrates and most vertebrates; the compact statoliths of fish are called otoliths), whereas arthropods use body extensions to perceive gravity.

20.2.2 Graviperception in Multicellular Animals

All multicellular animals hitherto analyzed, who exhibit active (not necessarily also directed) locomotion, use so-called stato- or otoliths, being localized in differently specialized organs (e.g., the vestibular organs within the inner ear of vertebrates) or body extensions (e.g., club-shaped halters and sensilla of insects; i.e. proprioceptors, see above) for the transformation of an acceleration into a body-own signal.

Specific sensory abilities do not show a clear evolutionary progression, most likely because the development of any type of sense depends on many other factors in the total ecology of a given organism. Vision, for instance, is sometimes poor or absent in a species of a class in which other members have a highly developed visual system: examples include cave-dwelling species, who are relatives of sighted emergent species. Since mechanical stimuli rather than optical ones are effective in all forms of life, specialized organs already appear very early in animal evolution. In accomplishment of the "heavy body"-strategy of the unicellular protozoans, some fungi, some lower but most of the higher plants, almost all invertebrate animals and virtually all vertebrates use different types of heavy bodies to orient themselves towards the direction of earth gravity.

Depending on the function-morphologic characteristics of the body, the respective sense organs (sensilla, inner ear maculae etc.) have been positioned in different ways during the phylogenetic development (Figs. 20.5-20.7). Gravity receptors already appear in jellyfish. Ctenophoran jellyfish (the so-called Comb Jellies), e.g., are biradial-symmetric (two axes of symmetry) and possess one statocyst at the top of the body (Fig. 20.5). The calcareous statolith is supported by four, long, spring-like tufts of cilia called balancers. The whole structure is enclosed in a transparent dome that is apparently derived from fused cilia. From each balancer arises a pair of ciliated furrows, each of which connects with one of the so-called comb rows. Thus, each balancer innervates the two comb rows of its particular quadrant. Tilting the animal causes the statolith to press more heavily on one of the balancers, and the resulting stimulus elicits a vigorous beating of the appropriate comb rows to right the body.

Radial-symmetric cnidarian jellyfish with multiple axes of symmetry have many statocysts located around the mantle (Fig. 20.6). In contrast to the circumstances as observed in Comb Jellies, the statoliths in cnidarians have connections to epidermal neurons which transmit the sensory information to a nerve ring, which connects to muscles stimulating rhythmic pulsations of the bell and thus locomotion and postural control.

A bilateral-symmetric organization (one axis of symmetry) is found in most of the other animals. In the more advanced members of the phyla Mollusca and Arthropoda, greatly developed sense organs occur; whereas arthropods use mostly proprioceptors (Figs. 20.3, 20.7), the gravity-sensing organ of, e.g., many snails such as *Aplysia* consists of bilaterally paired statocysts. They are composed of supporting cells and receptor cells, forming a sac which contains calcium carbonate inclusions (called statoconia). The receptor cells are hair cell-like neurons (an analogue to the hair cells in the inner ear of vertebrates) whose cilia are motile and mechanosensory [16]. In the statocyst, the continuos beating of the mechanosensory cilia keeps the statoconia in constant motion. Gravity pulls the statoconia down, obstructing the beating of the cilia on

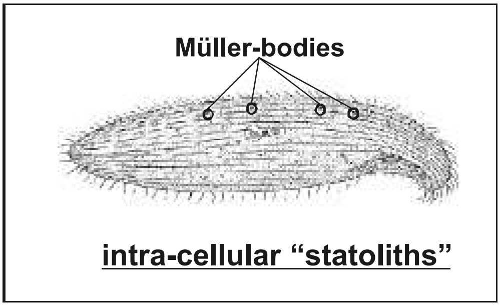

Fig. 20.4 The ciliate *Loxodes*, who possibly perceives gravity via intracellular statoliths.

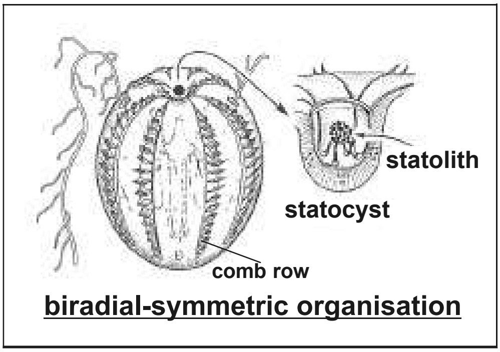

Fig. 20.5 Comb Jellies (ctenophorans) reveal two axes of symmetry, exhibiting one statocyst on the top of their body which directly regulates the beating of the comb row cilia.

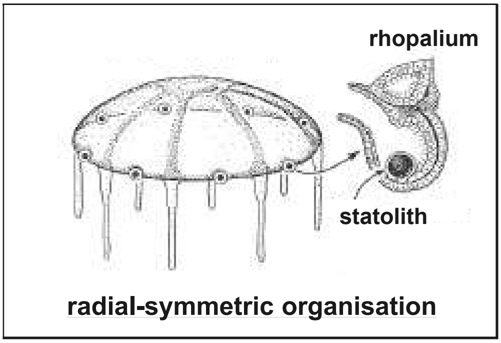

Fig. 20.6 Jellyfish (medusae of cnidarians) are organized radial-symmetrically (many axes of symmetry), exhibiting numerous statocysts (i.e. rhopalia). The sensory cells are hair cell-like neurons transmitting the gravity information to a nerve ring, which in its turn contacts to muscles.

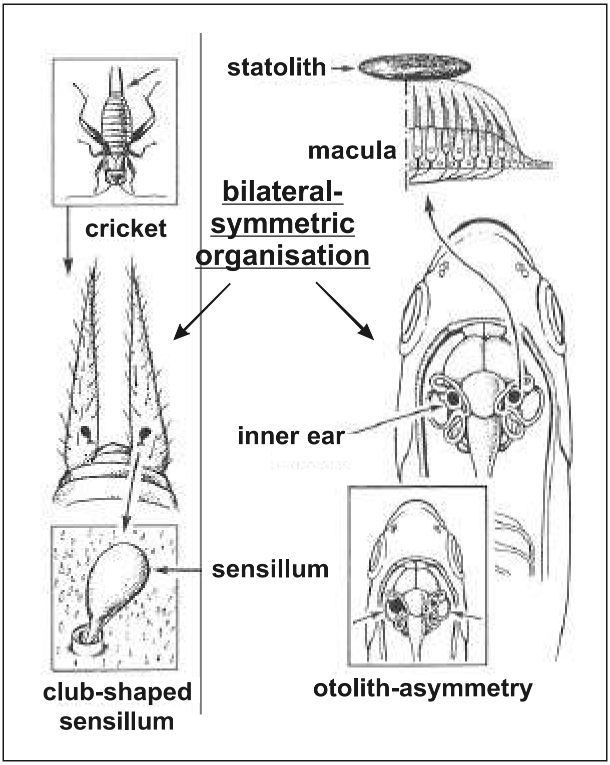

Fig. 20.7 Bilaterally organized animals (one axis of symmetry) usually exhibit gravireceptors on both sides of the body such as proprioreceptors (e.g., club-shaped sensilla in arthropods) or macula organs (vertebrates). An otolith asymmetry in the inner ear of vertebrates is believed to be the basic cause of motion sickness (e.g., sea-sickness, space sickness; see 20.3.2.2).

the bottom of the statocyst, which causes an increase in membrane conductance to Na^+ and the formation of an action potential [17]. The gravity receptors of some other mollusks show an amazingly close resemblance to vertebrate organs, e.g., the semicircular canals for equilibrium in *Octopus.*

In both inner ears of vertebrates (Figs. 20.7, 20.8), three semicircular canals (or cupular organs), located perpendicularly to each other for the detection of angular acceleration, are completed by three pouches containing either three oto-/statolith organs in lower vertebrates (utricle, saccule and lagena for the perception of linear acceleration/gravity as well as for sound) or two statolith organs and a lagena / cochlea without a stato-/otolith for sound perception in higher vertebrate animals. Sound or the movement of a statolith results in the bending of hair cell-cilia, which mechanically opens or closes ion channels [18] altering the electrical current of the respective sensory cell in the inner ear. Here, the transformation to computable action potentials takes place. Finally, a signal transduction on the level of the brain causes a motor response (Fig. 20.9). Generally, the brain integrates informations from the inner ear vestibular organs together with tactile, proprioceptive and visual cues for spatial orientation and postural control in the environment [19] (comp. Fig. 20.10).

Not only in vertebrates but in all animals exhibiting a central nervous system, there is always a close relationship between the presence of highly developed sense organs and a specialized region of the brain, since the latter is needed to process the incoming information in order to abstract the cues of importance to a given animal, which lastly results in behavior (comp. Fig. 20.9). An example in vertebrates is their cerebellum, which is responsible for the regulation of postural control; it is especially large and efficient in such animals who need to orient themselves in all three directions of space, like birds. (The fact that such elaborate systems exist does not exclude the possibility of much shorter and simpler pathways, which provide for more localized and quicker reactions; for instance, gravireceptor-mediation of postural control in Comb Jellies seems not even to require neurons.)

Summarized, animals did not only acquire systems to cope with gravity during evolution (comp. 20.1), but they also evolved receptors (and respective brain parts) in order to use gravity as the only virtually constant environmental factor for orientation, maintenance of equilibrium and postural control. On the one hand, gravitational environments which are not experienced by animals, disturb the neuronal processing of incoming information. This is especially the case, when at altered gravitational sensations (such as hypergravity or microgravity/weightlessness) the statolithic or otolithic organs transmit information to the brain, which do not necessarily match with others, e.g., the visual cues needed for a correct postural control. Thus “normal” behavior is inevitably affected. In human subjects, such an “intersensory conflict” can result in orientation problems, often accompanied by motion sickness and vomiting (kinetosis). On the other hand, altered gravity may have a strong influence on some aspects of development, since all systems (from sub-cellular organelles to complete organs) should be fully adapted to normal earth gravity.

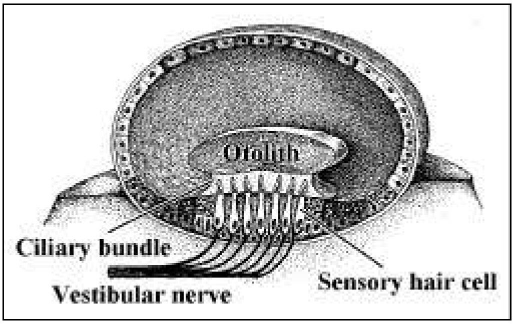

Fig. 20.8 Macula of a bony fish. Note the compact otolith. Amphibians, reptiles, birds and mammals reveal so-called statoliths, which are composed of hundreds of thousands of minute calcium carbonate cristals. A dislocation of the oto- or statolith causes shearing forces on the ciliary bundles of the sensory hair cells. These shearing forces alter the cellular ion current, which results in an increase or decrease (depending on the direction of the shearing) of the spike rate being transmitted to the brain by nerve fibers.

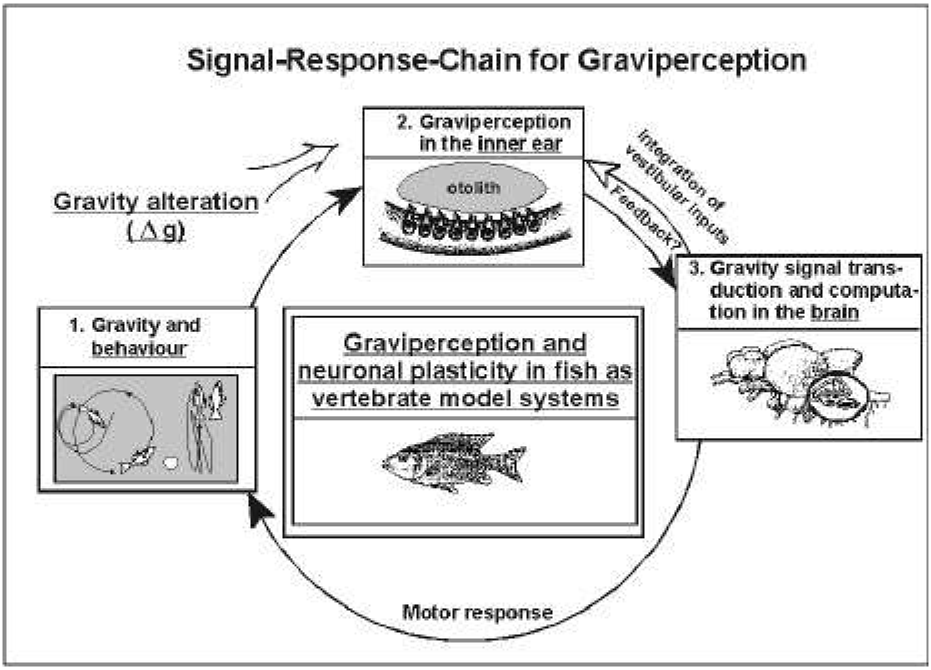

Fig. 20.9 The signal-response-chain for graviperception in vertebrate animals (fish). The basic stept of this response-chain (i.e. graviperception followed by a gravity signal transduction and computation, resulting in a motor response/behavior) are found in all animals who exhibit a central nervous system.

In order to understand the role of gravity on the (ontogenetic) development of animals as well as on their behavior, experiments at altered gravity (Δ*g*) yielded valuable clues and insights.

20.3 Behavior and Differentiation of Animals at Altered Gravity

Whilst studies investigating the influence of Δ*g* (hypergravity - h*g* - in centrifuges and microgravity - µ*g* - during drop tower, parabolic aircraft flight, sounding rocket and spaceflight experiments) on unicellular systems (protozoa, cell cultures) and on plants are focused mainly on basic gravireceptive mechanisms on subcellular and cellular level (see Chaps. 18, Bräucker et al., and 19, Schnabl), the aim of a large number of experiments using animals, particularly vertebrates, is concerned with the analysis of the physiology of cardiovascular, respiratory, intestinal, endocrine, immune and muscular systems as well as with the calcification of the skeleton (for review, see [20]) in order either to elucidate aspects of the role of gravity during evolution or/and to understand the effects of weightlessness concerning medical problems of human space exploration.

The so-called microgravity syndrome [21], a prognosticated complex arising in reduced gravity environments such as the surfaces of the Moon and Mars and principally encompassing muscle atrophy, cardiovascular deconditioning and bone demineralization ("space osteoporosis"), stands to replace physics and rocketry as the fundamental challenge of interplanetary astronautics. Mirroring our past few million years of changing climate and resources, the mobility of humans between diverse gravitational environments on the high frontier will critically depend on our ability to adapt.

One major goal of gravitational zoology therefore is to elucidate the effect of long-term Δg on the behavior and development of animals as model systems and by this to clarify particularly the basic mechanisms of perception, transformation and central computation of a gravitational stimulus within the organism, including the clarification of the animals' possibilities to adapt and compensate. In this regard, results obtained using vertebrate animals – due to the homology of the morphological and physiological systems – can widely be transferred upon the conditions given in humans (the present review will, however, not deal with mammals, since respective biomedically relevant reviews abound).

20.3.1 Invertebrates

The morphogenetic development of various invertebrates in general is not heavily impaired by Δg: the shell of fresh-water snails (*Biomphalaria*), e.g., obviously develops normally at microgravity [22] indicating that the basic mineralization processes in mollusks are not affected by 16 days microgravity (this result is strongly contrasting to findings concerning human space osteoporosis and bone growth in rats!), which in its turn leads to the assumption that gravity did not play an important role in this context during evolution. Concerning squids (*Loligo* and *Octopus*), it has been argued that altering gravitational forces might not necessarily lead to morphogenetic aberrance but just to gradual deviations from the normal developmental speed of the embryos [23]. Microgravity, on the other hand, was shown to negatively affect the efficacy of fertilization processes in sea urchins [24].

In addition to this, major focus has been attributed to the physiology of the gravity sense system, to the corresponding neuronal computation and the resulting behavior: altered gravity influences statolith growth in the marine snail *Aplysia*, accompanied by changes in urease activity [25] indicating that the latter might regulate $CaCO_3$ deposition in statoliths. In contrast, Δg seems to have no effect on the development of gravireceptors in crickets, in which gravity is internalized by club-shaped sensilla; microgravity, however, mediates the sensilla-induced compensatory head-rolls [26, 27].

In conclusion, the results concerning the investigation of the effect of altered gravity – particularly on insects – clearly demonstrate the usefulness of these animals since they are by far less complex than, e.g., vertebrates. Due to their different lifestyles, the various invertebrate groups reveal a varying resistance/adaptability towards gravity. However, invertebrates, as a matter of fact, allow a comparison with vertebrate (including human) gravity related systems only in the broadest sense.

20.3.2 Vertebrates

Deeper clues and insights into the basic causes of gravity-stimulated effects in human beings can only be expected from studies performed using vertebrate animals, since the peripheral and central vestibular systems as well as the basic mechanisms of development are homologous among all animals with backbones and by this with humans.

With regard to this, most studies on the effect of altered gravity particularly on mammals had been focused on biomedical aspects comprising the physiology of cardiovascular, respiratory, intestinal, endocrine, immune and muscular systems as well as on calcification of the skeleton and related topics on which comprehensive reviews abound [7, 20, 28, 29]. Results using mammals as test subjects will therefore not be reviewed here.

Rather, in the following, we will briefly review some major results hitherto obtained using amphibians, reptiles and birds. Thereafter, focus will be laid on research results using fish as vertebrate model systems with regard to the components of their signal response chain for graviperception.

20.3.2.1 Amphibians, Reptiles, Birds

For over a century, embryologists using amphibians have debated as of whether gravity is required for normal embryonic development and, in particular, for the establishment of embryonic polarities such as pattern formation, morphogenesis and organogenesis (for review, see [30, 31]). Since their normal development at 1 ×*g* earth gravity is comparatively well understood, amphibians such as the clawed toad *Xenopus* and the salamander *Pleurodeles* are well suited animals to answer the aforementioned questions. The first fertilization of a vertebrate (*Xenopus*) in microgravity was successfully carried out in 1988 [32] showing that fertilization was monospermic as it is on Earth, and that development proceeded up to gastrulation (= end of the respective sounding rocket experiment). 1 ×*g* earth gravity thus seems not to be required for the early ontogenetic axis formation [33-35]. In elder developmental stages of amphibians, microgravity, however, normally results in non-inflated lung buds and tracheae, possibly being the result from the failure of the animals to inflate their lungs in a timely adequate fashion. Furthermore, microgravity induced some kind of malformed (typically) lordotic tails [31] with consequences for behavior (e.g., optomotoric responses [33]) and retarded larval growth. The effect on optomotor response suggests that tadpoles raised at microgravity may receive less vestibular information to control their position [11, 30].

Summarized, experiments both with salamanders and frogs indicate that amphibian egg maturation, fertilization, and embryonic development is not significantly influenced by microgravity, but larval growth might be retarded or even abnormal in microgravity. So far, however, no spaceflight mission had been long enough to investigate as of whether these animals can complete a full life cycle under space conditions. Comprehensive clues on the complete developmental life cycle are thus still lacking.

Few research at altered gravity has hitherto been conducted with reptiles and birds. There have been reviewed disorientation responses of various vertebrate animals exposed to microgravity produced by parabolic aircraft flights and in space experiments [36]. Like in mammalian species and frogs, coordinated performance can be easily compensated by visual function also in turtles. Like in fish (see below), in birds (pigeons, Japanese quail), who can move three-dimensionally in their environments, exposure to parabolic flight microgravity induces irregular tumbling with the eyes open and regular looping with the eyes closed, although the loop direction is the opposite in these two animals. A centrifuge experiment – conducted on Japanese quail – showed that hatchability is negatively affected by hyper-g [37]. Further, it has been reported based on a spaceflight experiment that the difference in specific gravity between the yolk and the albumen appeared to play a critical role in early chick embryogenesis [38]. In elder embryos, all the tissues, including cartilage and bone, however, were formed normally. Microgravity also seems to have no effect on normal eye development [39].

20.3.2.2 Fish

Fish have been proved to be the most suited vertebrate animals for basic gravity-research [40]: they can be characterized by an absence of body weight related proprioperception (in comparison with surface-bound terrestrial vertebrates), a reduced influence of gravity on supporting tissue, muscles, vascular tonus system etc., a relative higher sensitivity for gravity due to larger otoliths etc., a high reproduction rate (combined with higher genetic homogeneity of individuals), and mostly an external development, thus enabling better access to defined developmental stages. Moreover, there is rich information about genetics and developmental mutations available (Zebrafish *Danio*, Medaka *Oryzias*, Swordtail Fish *Xiphophorus*), the developmental pattern of large numbers of genes are known and there exists an extensive homology to mammals not only at the molecular level but also concerning the central and peripheral vestibular system. Last not least, fertilization and development is not significantly impaired by altered gravity [41].

Basically, fish use visual and vestibular cues for postural equilibrium maintenance and orientation as do all other vertebrates and many invertebrates. Already in 1935, the so-called DLR (Fig. 20.10) was described [42]: illuminated from the side at normal 1 ×*g* earth gravity, a fish tilts its back towards the light source. In general, the DLR expresses a balance between the tilting force induced by visual information and the so-called v̲estibular r̲ighting r̲esponse (VRR) [43] induced by gravitational information. Interestingly, the performance of the DLR depends on the visual performance (visual acuity) of an individual, suggesting that there are more "vestibular" and more "visual" individuals [44].

During microgravity, fish are often seen performing an abnormal swimming behavior, such as down- or upward pitching, inward loopings, spinning movements etc., especially following the transition from 1 ×*g* to microgravity [36, 45, 46]. This behavior has most likely the same source like motion sickness (a kinetosis) in humans. Subsequent experiments at microgravity using fish provided clues and insights into the understanding of the neurobiological basis of vertebrate gravity sensation and kinetosis.

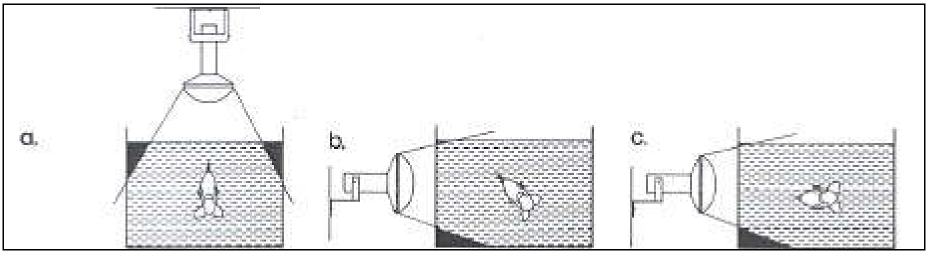

Fig. 20.10 The dorsal light response of fish. Illuminated from the side (light from above is the normal case, a) at normal 1 ×*g* earth gravity (b), a fish tilts its back towards the light source. The angle of the tilt increases when the light intensity is being raised. Increasing the force of gravity in a centrifuge at constant illumination decreases the tilt angle. After bilateral labyrinthectomy or under microgravity conditions, the tilt is consequently guided by light alone (c). It is a general feature of animals that the brain integrates informations from the inner ear vestibular organs together with further sensory cues for spatial equilibrium.

If, for instance, the sensitivity of the otolith system of humans in space is increased as compared to the ground, any brisk translation of the head would be interpreted erroneously. Such illusionary tilts in turn would conflict with visual and other information and possibly generate motion sickness [47]. Especially the spinning movements, which are induced by microgravity in some individual fish are assumed to indicate a possible source of illusionary tilts: individually asymmetric vestibular maculae (e.g., based on side to side differences in the weight of otoliths) might, at rest and under 1 ×*g* earth gravity, possibly cause asymmetric shearing forces on the sensory epithelia. A normal posture then would require neurovestibular compensation for the asymmetric discharge rates. At microgravity, however, there would be no weight differences in the otoliths from one side of the body to the other, and the (primary) discharge rates should no longer be asymmetrical. A continuing, but now unnecessary compensation, however, would then cause erroneously (secondary) asymmetric discharge rates.

It is the particular strength of this ”asymmetry-hypothesis” [48] based on [49], that it could explain the great interindividual differences in kinetotic behavior of fish or even in motion sickness susceptibility of humans.

Several findings speak in favor of the asymmetry-hypothesis (Fig. 20.11): indeed, asymmetrically weighed otoliths are a common feature in fish (for review [43, 50]). Larval cichlid fish, who were hatched at 3 ×*g* hypergravity, morphogenetically developed normally and showed a normal swimming behavior. However, as soon as the hyper-g-centrifuge was stopped, many individuals revealed looping responses and spinning movements, as had also been frequently observed in the course of spaceflight- and parabolic aircraft flight-experiments after the transfer from 1 ×*g* earth gravity to microgravity conditions [45, 46, 51, 52]. This kinetotic behavior normally disappears within several hours or days like the so-called space adaptation syndrome of humans [19, 51, 52]. As predicted by the asymmetry-hypothesis, kinetotically behaving individual fish larvae after hyper-g and at microgravity in the course of para-

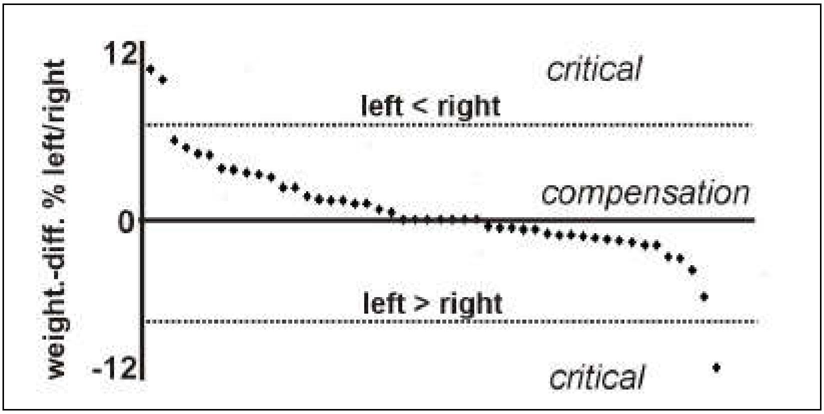

Fig. 20.11 Otolith asymmetry (weight differences between left and right otoliths) in Swordtail Fish, which were maintained in a normal aquarium. According to the "asymmetry hypothesis", a moderate range of asymmetry can be compensated for by the brain (compensation). Asymmetries on a level beyond the respective compensation capabilities (critical), however, might induce kinetotic behavior at altered gravity.

bolic aircraft flights indeed revealed a larger otolith asymmetry than normally behaving ones [48, 52].

Recently, evidence has been provided for the existence of a feedback mechanism adjusting size, asymmetry and Ca-content of fish inner ear otoliths towards altered gravity [48, 53]: both in the hypergravity- (h*g*-) animals and in the 1 ×*g* controls, the morphogenetic development was identical. Evaluating the otoliths′ growth during h*g* in comparison to the 1 ×*g* controls, however, it was found that the growth both of utricular and saccular stones (lapilli and sagittae, respectively) was slowed down by h*g* and that the development of bilateral asymmetry was considerably decreased.

This can be explained from a functional point of view as follows: under increased gravity, given otoliths will be heavier and thus cause increased shearing forces during tilts. However, any tilts need to be computed by the brain and asymmetric shearing forces need to be compensated (neuro-vestibular compensation). A feedback mechanism thus might slow down otolith growth at h*g*, so that an otolith formed at h*g* will possibly cause about the same shearing forces during tilts as a respective normally sized otolith at 1 *g*. Without a feedback mechanism, a given bilateral asymmetry between two otoliths would be increased at h*g*, but for a correct interpretation of the afferent inputs to the brain for postural control and spatial orientation, any asymmetry ought not to be too pronounced in order to stay in the range of the compensation.

Therefore, the feedback mechanism mentioned above might reduce a h*g*-based increased functional asymmetry in order to fulfill the requirements for a sufficient compensation. Moreover, the feedback ought to be activated immediately after the onset of altered gravity. Indeed, recent investigations showed that otolith growth is obviously regulated by the brain: Ca-incorporation (and thus growth) stops after transection of the vestibular nerve [54].

Summarized, the basis of a disorientated (kinetotic) behavior in individual fish at a sudden reduction of the g-force is relatively well understood concerning the level of swimming behavior and concerning morphometrical studies on otoliths. The neuronal mechanisms, however, that possibly underly the adaptation to altered gravity by means of a feedback mechanism, remain so far poorly understood.

General neurochemical effects due to altered gravity abound (for review [51]). Respective investigations yielded the general finding that, e.g., enzyme activities are affected by altered gravity (from approx. 0.01 $\times g$ in the fast rotating clinostat to 3 $\times g$ in the centrifuge) in a dose dependent manner and that the effects are especially pronounced in larval fish as compared to adult ones [55]. This latter finding, according to which larval fish may react gross-biochemically to a larger extent to altered gravitational forces than adult animals may be due to their wider range of neuronal plasticity. Histochemical and electronmicroscopical investigations focused on single brain centers showed that special inner ear related nuclei in the brain react specifically towards altered gravity [56, 57], even on morphological level (form and number of synaptic contact zones [58, 59]).

Taken together, the results of these investigations suggest that the excitatory inputs from the inner ear are resembled in the vestibular system of the brain by its neuronal activity. Effects of altered gravity on the plasticity of the inner ear sensory epithelium were by far less pronounced [60].

Taken together, investigations of fish, who had developed at altered gravity, reveal a variety of short lasting, adaptive effects following Δg. The behavioral adaptation to Δg can be correlated with a compensatory otolith growth, based on a (negative) feedback mechanism between the inner ear and the brain. The neuronal control of this feedback mechanism seems to be effected by stimulation dependent enzyme activities (i.e. neuronal activity) as well as synaptic plasticity, especially in vestibular brain centers.

20.4 Conclusion

During evolution of animals, they have managed both to cope with and use the environmental gravity vector for orientation. Mechanisms concerning the latter (function of gravireceptors and the central nervous computation of respective inputs) is comparatively well investigated. Yet, however, it is not completely clear, to which extent gravity is necessary for a normal development and in which range adaptive mechanisms are efficient enough at altered gravity to guarantee a normal lifestyle.

The various data being obtained using especially vertebrates as model systems concerning gravistimulated effects reveal first of all that the normal development seems not to be significantly influenced by altered gravity. The studies undertaken so far, however, do not yet answer the question whether a complete life cycle can be completed for instance under weightlessness. Secondly, the results regarding gravity-effects, particularly on fish, speak in favor of the following concept of possible interactions: short-term altered gravity (up to around 1 day) can induce transitional aberrant behavior due to malfunctions of the inner ear, originating from asymmetric otoliths or, generally, from a mismatch between canal and otolith afferents. The vanishing aberrant behavior is due to a reweighing of sensory inputs and neurovestibular compensation, probably on bioelectrical basis. During long-term altered gravity (several days and more), step by step neuroplastic reactivities on molecular basis (i.e. molecular facilitation) in the brain and inner ears possibly activate feedback mechanisms between the CNS and the vestibular organs for the regain of normal behavior. It was shown that such a mechanism is involved in adjusting (assimilating) the otolith weights in fish.

The experimental data on the effect of altered gravity on developing and adult animals help understanding the role of gravity during evolution. The consequences for animal life at altered gravity are well in concordance with a concept about an evolution of the gravi-resistance and gravi-reactivity of animals of the known Ukrainian space physiologist N. Sirotonin (1896-1977): *There is a high tolerance to altered gravity in arthropods (especially insects), the presence of responses in fish and amphibians mainly in most early developmental stages and a wide range of the responses in birds and mammals* [61]. The reasons for this varying range of gravity-tolerance have hitherto not been disclosed.

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