

## “CRITICAL PERIODS” IN VESTIBULAR DEVELOPMENT OR ADAPTATION OF GRAVITY SENSORY SYSTEMS TO ALTERED GRAVITATIONAL CONDITIONS?

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### INTRODUCTION

A feature of sensory and motor systems is their susceptibility to modifications of their adequate physical and/or chemical stimuli during development. Studies in kittens deprived of the visual input have revealed that this susceptibility is restricted to a certain period of life (cf. [59]), which was called the critical (sensitive) period. Critical periods were also described for other sensory and motor systems including hearing, feeling, olfaction or walking (12, 25, 27, 33, 52, 57). They are characterized (1) by a sensitivity of the developing system to modifications of the adequate environment, preferably to stimulus deprivation, (2) by a clearly defined time window of this sensitivity and (3) by the irreversibility of anatomical, behavioral or physiological modifications induced by these altered environmental conditions. These criteria imply that developing animals have to be exposed to short- and long-lasting modifications of their environment at different periods of their life.

Modifications of the stimulus input for gravity sensory systems (GSS) include absence or augmentation of the stimulus *per se*, i.e., the gravitational environment, or any change of physiological input to the central nervous GSS structures. The most effective approach for the analysis of critical periods is sensory or motor deprivation. In contrast to deprivation, gravity augmentation provides the developing and adult animal with a gravitational reference direction throughout their life because the development of the sensory epithelium is not blocked by hypergravity (53).

Several approaches exist to deprive an animal from gravity information. (1) A gravity sense organ can be destructed so that the gravitational field cannot be detected any longer. This approach is a very selective one because the site of a lesion can be clearly defined. The disadvantage is that lesions cause an irreversible damage in vertebrates while in some invertebrates, regeneration can occur. This means that the lesion technique does not allow to define precisely a deprivation period. (2) Parts of the body or the whole body can be deloaded by counterweights or by diving to compensate for the gravitational pull. Deloading by counterweights is a selective deprivation technique. Both approaches can successfully be used in adult animals but not in developing animals because the counterweight must be steadily adapted to the weight of the growing animal, or they have to be submersed for very long periods. (3) The animal can be exposed to simulated microgravity by means of 2D- or 3D-clinostates or rotating bioreactors. By this non-selective technique, sedimentation of particles within the organism's body and, in particular, the gravity sense organs is reduced, however, gravity and therefore shearing forces still exist. (4) The animal

can be exposed to microgravity during space flights (*syn.* near-weightlessness). Actually, this approach is the most effective deprivation technique; however, its use is limited by the rather low number of space flight opportunities. Therefore, all these techniques are used to solve the problem of a sensitive period in the development of gravity sensory systems.

For several reasons, quantitative studies on critical periods during the development of gravity related vestibular systems were only performed in lower vertebrates such as fish and amphibians. Some of these studies used simulated deprivation by clinorotation (30, 31), others space flights (43, 44, 45). Animals were tested after termination of altered gravity using mainly behavioral observations. Behavioral observations looked for equilibrium control during swimming and on compensatory eye movements. *Equilibrium control during swimming* causes that the back of a swimming animal is directed upwards; the response is supported by the dorsal light reaction (55).

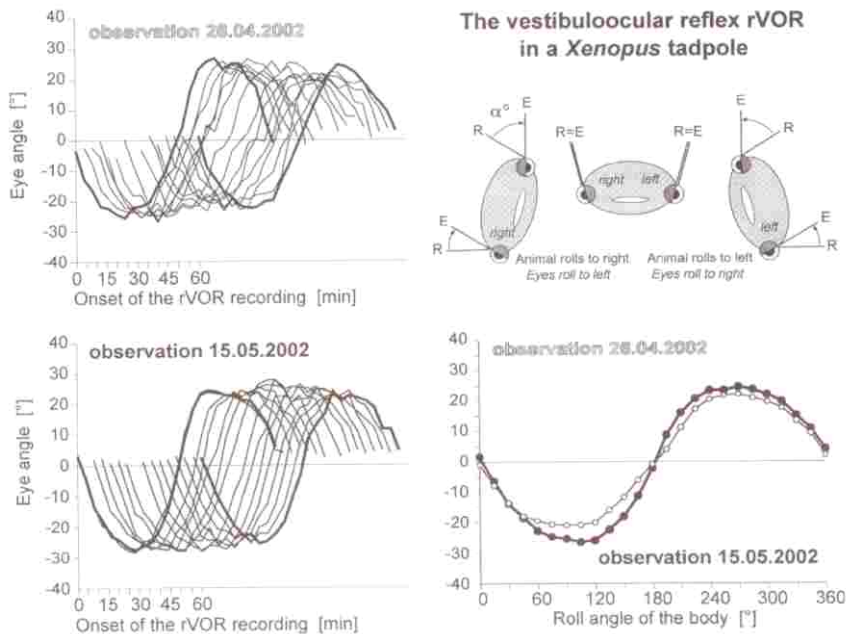


Fig. 1. - Features of the roll-induced vestibuloocular reflex (rVOR) in the clawed toad *Xenopus laevis*.

Right: The rVOR is a movement of both eyes which is directed opposite to a passive lateral roll of the animal's body. R, orientation of the margin of the eye cup before the lateral roll; E, orientation of the margin of the eye cup after the lateral roll. The angle  $\alpha^\circ$  between both lines is the compensatory eye response (rVOR). The mean response characteristic recorded from a group of animals or a sequences of recordings from an individual animal is typically sine-like for a complete 360° roll.

Left: Two sequences of each 15 rVOR measurements taken for a complete 360° lateral roll from a tadpole at a time distance of 3 weeks (days of recording are shown in each plot). Each sequence of the 15 recordings was taken within one hour. The fat curves indicate the first and the last recording of each sequence: the onset between each rVOR characteristic is shifted by 4 min along the abscissa (cf. Onset of Recording). Note that there is rather no adaptation between the first and the last recording. The means of these recordings are shown on the lower right plot.

*Compensatory eye movements* are common in vertebrates (roll-induced static vestibulo-ocular reflex, *rVOR*; cf. [9]). Compared to swimming control, recordings of the *rVOR* give a better quantitative measure to study sensitivity modifications of a GSS after exposure to altered gravity because (1) the relation between *rVOR* and stimulus is a sine-like function, and (2) the *rVOR* can be induced several times without any significant decrement (Fig. 1). Occasionally, neuroanatomical, biochemical or physiological methods were also used to analyse principles of modifications within the peripheral sense organs and central neuronal networks (cf. [2, 3, 4, 19, 34, 38]).

A fine analysis of the developmental characteristic of the behavior has to precede any study on critical periods. It allows to define more precisely the onset of preferred exposure periods of gravity deprivation. In addition, earth-bound studies has to concentrate (1) on the description of the general g-sensitivity of the developing GSS using also hypergravity exposures, and (2) on the application of microgravity simulation techniques such as clinorotation.

## RESULTS

### 1. Standard development of gravity related behavior.

Determination of developmental characteristics is useful to understand mechanisms of information processing within the adult central nervous system. These studies are mandatory for the analysis of critical periods and have to be performed at a high resolution of larval stages to detect and define the precise time window of sensitivity to altered gravity conditions. In addition, developmental characteristics of structural components and physiological mechanisms including behavior are not always in synchrony (for *Xenopus laevis*, cf. [18]). The developmental characteristics of *rVOR* were determined with a high resolution only for two lower vertebrate species (*Xenopus laevis*, *Oreochromis mossambicus*). Staging of developing fish and amphibia is based on external markers such as the size of the yolk sac, pigmentation, fin structures, shape of the gut, or appearance and shape of extremities (for *Xenopus*: cf. [32]; for *Oreochromis*: cf. [1]).

Compensatory eye movements occur within 3 days after fertilization. In *Oreochromis*, the *rVOR* can be observed for the first time at developmental stage 13; thereafter, it increases until stage 17. During stages 18 and 21, the *rVOR* is depressed but increases later again until the animals become premature at stage 25 (46). In *Xenopus*, the *rVOR* appears 3 days after fertilization (stage 42). Both *rVOR* amplitude and gain increase up to stage 52, and then decrease to an almost constant value at stage 60 and older tadpoles. In both species, *rVOR* development is retarded during early periods of life if animals are reared for some days or permanently under complete darkness (22, 23, 43) (Fig. 2).

### 2. Modifications of *rVOR* development by hypergravity exposure.

Long-lasting gravity augmentation by centrifugation revealed effects on many systems of the body including sensory, central nervous and motor systems.

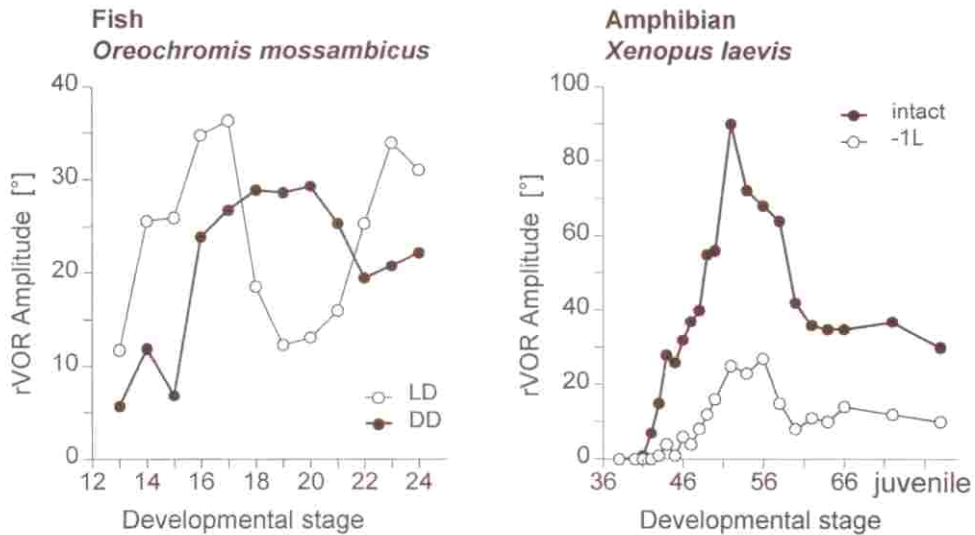


Fig. 2. - Development of the roll-induced vestibuloocular reflex in lower vertebrates.

Left: The cichlid fish *Oreochromis mossambicus*. Developmental characteristics are shown for animals reared under light-dark conditions (LD = 12:12 hours) and for animals reared either during early periods of life or during a 9 day period under permanent darkness (DD). Note the strong influence of light conditions on the rVOR development in particular a retardation. The number for each developmental stage varied between 10 and 25; the total number of animals for each characteristic was approx. 250. Definition of developmental stages, cf. (1); modified from (46).

Right: The amphibian *Xenopus laevis*. Developmental characteristics are shown for intact animals (intact) and for animals with only one labyrinth (-1L). Note the depression of the rVOR after destruction of one labyrinth. As in the fish *Oreochromis*, rearing in darkness retards the development of the rVOR (not shown in the picture; cf. [44]). The number for each developmental stage was 6; the total number of animals for each characteristic was approx. 120. Definition of developmental stages, cf. (32); modified from (22, 23, 43).

Neurophysiological recordings during exposure to centrifugation induced hypergravity will give a clear picture of adaptation processes to this new environment; however, those studies are rarely done (cf. for example, [14]). Most behavioral studies were performed after hg-termination, in particular on the rVOR in young *Oreochromis mossambicus* and *Xenopus laevis* tadpoles. In most cases, the rVOR was depressed after hypergravity exposure; rarely, it remained unchanged. Studies in *Oreochromis* revealed that a 9-day 3g-exposure was affective but not a 2.0g or 2.5g exposure of the same duration (24, 43, 44).

The so far most extended studies come from *Xenopus laevis* in which 6 different stages were exposed to 9- to 12-day 3g-hypergravity (Fig. 3). For technical reasons, the 3g-period started always after fertilization. The developmental stages at onset of the 3g-conditions covered the life period before and after the first appearance of the ear vesicle, the sensory epithelia and the rVOR. Recordings started 1 day after termination of the 3g-exposure and were mostly continued for 10 days. The main finding was that the median rVOR amplitudes (= angle between the extreme postures of the eyes during a complete lateral roll) from tadpoles with 3g-experience were

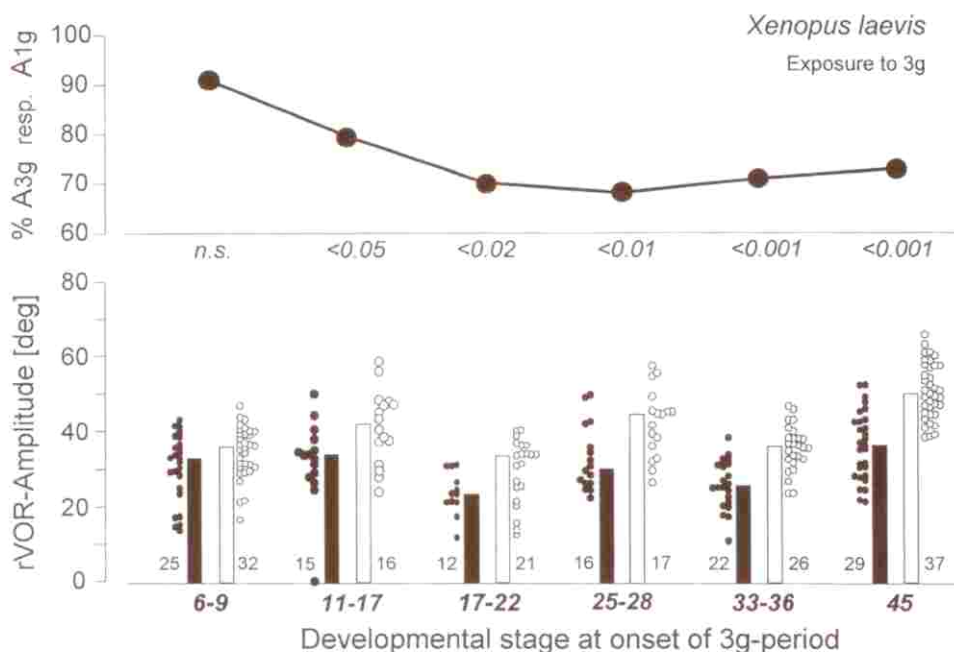


Fig. 3. - Effect of hypergravity on the development of the vestibuloocular reflex in *Xenopus* tadpoles.

Groups are defined by the developmental stages the animals had reached at onset of 3g. The 3g-exposure lasted 9 days except for the 6-9 group which were exposed for 12 days to 3g-conditions. rVOR amplitude, angle between the most extreme positions of the eye during a complete 360° lateral roll. Black columns and black dots, 3g-animals; white columns and white dots, 1g-tadpoles. Each dot represents one tadpole, each column the median value of the respective sample. Numbers at the base of each column, sample size. The upper plot presents the percentages of the median rVOR amplitude from 3g-animals with respect to that recorded from the 1g-control groups. Samples were compared by means of the U-test from Wilcoxon, Mann and Whitney. Levels of statistical significance are presented at the abscissa of the upper plot (modified from [24] with addition of so far unpublished data).

smaller with respect to those recorded from their 1g-reared siblings, and that the subsequent development depended on the stage at the onset of the 3g-period. In particular, in the stage 6/9-sample, no difference between the 3g- and 1g-samples was recorded for the first two days after 3g-exposure. During the following 4 days, the rVOR amplitude increased significantly only in the 1g-controls, so that the differences between the 3g- and 1g-reared tadpoles became significant. In all other stages, a significant 30% reduction of the median rVOR amplitudes was recorded in the 3g-exposed tadpoles with respect to their 1g-reared siblings during the first two post-3g days. These differences persisted for the next 10 days during 1g-readaptation in the stage 33/36 and stage 45 samples, but the median rVOR amplitudes increased significantly only in the stage 45-sample and not in the 33/36-group (24).

### 3. Modifications of rVOR development by microgravity.

The lesion technique is a powerful tool to determine consequences of sensory deprivation on the development of sensory systems. In rodents, it was used for the

analysis of critical periods in the somatosensory system (52). In many animals, lesions activate regeneration mechanisms, in particular during development, by which structure and/or function of a sensory system recovers to normal. Both complete and partial replacement of the nervous system has been documented in invertebrate phyla including coelenterates, flatworms, annelids, gastropods and tunicates. Some vertebrates exhibit cyclical replacement or facultative repair in sensory systems, other vertebrates are able to replace discrete portions of the central nervous system (fish, amphibia), retina (amphibia), hair cells of the auditory (birds) or vestibular organ (mammals) or olfactory neurons (mammals) (cf. [29]). Regeneration capacities within the GSSs of lower vertebrates are low; however, a physiological normalisation is activated by labyrinthine lesions (*vestibular compensation*, cf. [40]). For these reasons, this presentation will not further consider the lesion approach but instead, it will give examples of experiments under simulated microgravity and microgravity during space flights.

The limited number of flight opportunities prompted on-ground deprivation experiments using the clinostate technique. Studies in the zebrafish *Danio rerio* by means of a NASA developed rotating bioreactor gave evidence for the existence of a critical period in vestibular development (30, 31). In a first study, eggs and hatchlings stayed in the rotating bioreactor for either 72 or 96 h after fertilization of the eggs. These animals displayed a swimming behavior that was indistinguishable from control animals when illuminated from above. When incubated in the bioreactor for 72 h, some animals were missing one or more otoliths. In contrast, all control animals had two otoliths on each side. When incubated in the bioreactor for 96 h, experimental animals showed a weaker rVOR during passive head-down and head-up tilt compared to the age-matched control animals for 1 day (30). In a more detailed study, eggs were placed in the bioreactor 3, 24, 30, 36, 48, or 72 hours after fertilization and maintained there until they became 96 hours old; other eggs were placed in the bioreactor shortly after fertilization and stayed there until they reached the age of 24, 36, 48, 60, 66, 72, or 96 hours. Based on recordings of the rVOR induced by head-down or head-up tilt, the studies revealed the existence of a critical period which included the period of life from 24 to 72 hours after fertilization. This postulation was confirmed by an experiment with zebrafish eggs which were rotated in the bioreactor from 3 to 96 hours after the fertilization except for the period between 24 to 72 hours. These larvae developed a normal VOR (31) (Fig. 4).

Despite of unfavourable conditions such as the limited number of space flight opportunities, a first attempt was done in developing fish (*Fundulus heteroclitus*) aboard Skylab 3. Behavioral studies were performed with fish which hatched during the 59-day space flight. Most larvae displayed normal swimming behavior (54). The postflight tests showed that young fish launched before their labyrinth was formed exhibited a decreased positive geotaxis when compared with their 1g-ground controls. In contrast, fish launched when their labyrinth had developed behaved normally (16). Sixteen to 17 months after flight, the youngest group revealed persisting physiological changes. They were less disoriented during short-term  $\mu$ g-periods (parabolic flights) but hypersensitive to hypergravity (17).

Periods of Bioreactor Rotation	Experimental Zebrafish Groups																							
	01	02	03	04	05	06	11	12	13	14	15	16	21	22	23	24								
F																								
3																								
24																								
30																								
36																								
48																								
60																								
66																								
72																								
96																								
rVOR	D	D	D	N	N	N	N	N	N	N	D	D	D	pD	pD	N								

Fig. 4. - Critical period in the development of the vestibuloocular reflex of zebrafish.

Zebrafish (*Danio rerio*) embryos were exposed to simulated microgravity by means of the rotating NASA bioreactor. Periods of bioreactor rotation are given on the left in hours after fertilization (F). Black columns indicate normal (N) rVOR development; light grey columns indicate depressed (D) rVOR development during a period of 5 days after the 96th post-fertilization hour, and dark grey columns indicate a weak rVOR modification (pD) of short persistence (modified from [29]).

Systematic observations on compensatory eye movements in developing animals using space flights are rare. They include post-flight measurements in the cichlid fish *Oreochromis mossambicus* and the amphibian *Xenopus laevis* after the missions STS-55 (German D-2 mission; 1993; Experiment STATEX-VOR), STS-84 (Shuttle-to-Mir mission SMM-06; 1997; Experiment TADPOLES) and a Soyuz taxi flight to the International Space Station ISS (Andromède mission; 2001; Experiment AQUARIUS-XENOPUS). The flights lasted 9 to 10 days. In both species, stages were selected according to the development of their roll-induced rVOR; at least one stage had not yet developed the rVOR at  $\mu$ g-onset while one stage had already developed it.

Young fish (*Oreochromis mossambicus*) flew on STS-55 or on STS-84. During the space flights, all fish embryos continued to develop at an approximately normal pace. Recordings of the rVOR taken between the postflight days 1 to 4 revealed no modification of the sine-like shape of the rVOR characteristics irrespective of the animals' age at onset of microgravity. In contrast, both rVOR gain and rVOR amplitude were significantly larger in 11/12-fish while the rVOR was not affected in the 14/16-fish (Fig. 5). Post-flight readaptation was completed in the affected animals

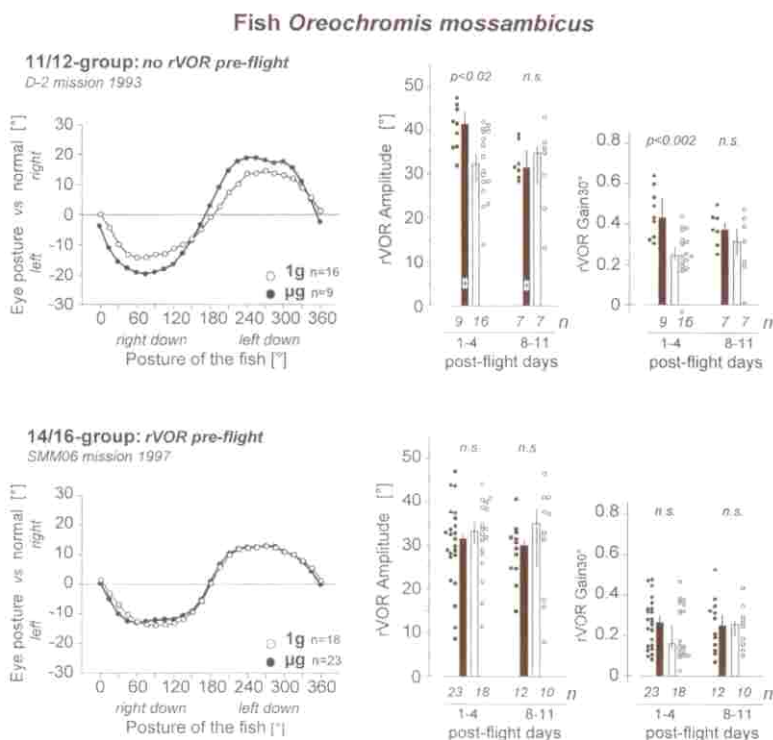


Fig. 5. - Effect of microgravity on the development of the rVOR in a cichlid fish.

Studies were performed with two developmental stages of the cichlid fish *Oreochromis mossambicus*. The animals were exposed to microgravity during the space flights STS-55 (German spacelab mission D-2; 1993) and STS-84 (Shuttle-to-Mir mission SMM06; 1997). The young stages (11/12) had not yet developed the rVOR at launch of the spacecraft; the old stages (14/16) had developed it at this time. Definition of stages, cf. (1).

Left: The mean rVOR characteristics for the  $\mu\text{g}$ -exposed young fish (black dots and heavy line) and the 1g-control animals (circles and fine line). Samples sizes are given for each experiment.

Right: The modification of the rVOR amplitude and the rVOR gain. Black columns and black dots, 3g-animals; white columns and white dots, 1g-tadpoles. Each dot represents one tadpole, each column the median value of the respective sample. Numbers at the base of each column, sample size. Data are given for the first observation period between post-flight days 1 to 4, and a second recording period between post-flight days 8-11. Each animal from the second recording period was also tested during the first period. rVOR amplitude, angle between the most extreme positions of the eye during a complete 360° lateral roll; rVOR gain, ratio between the response angle  $\alpha^\circ$  (definition, cf. Fig. 1) and the roll angle which was 30° in this study (modified from [44]).

within 11 days; during the same period, 14/16-fish with  $\mu\text{g}$ -experience continued to develop as their 1g-ground control did. Thus, microgravity exposure increased the vestibular sensitivity only in those fish embryos, which had not yet developed their rVOR at onset of the space flight (44).

The rVOR was also studied in *Xenopus laevis* tadpoles which flew either on the space shuttle flights STS-55 or STS-84 or on the International Space Station ISS. The flights lasted 9 or 10 days. Studies included young animals (stages 25-36) which had not yet developed the rVOR at  $\mu\text{g}$ -onset and older ones (stages 45) which had



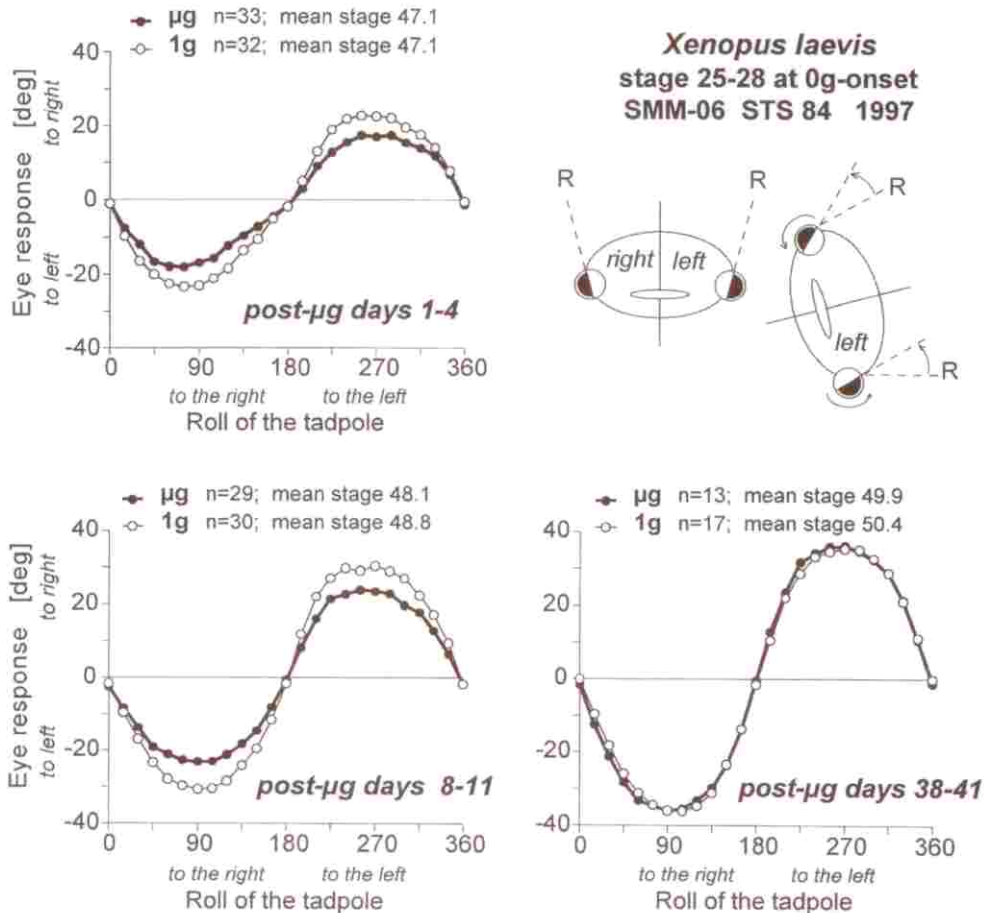


Fig. 6. - Effect of microgravity on the development of the rVOR in *Xenopus* and its readaptation to normality after re-exposure to 1g-conditions.

Tadpoles flew on the Spacehab mission STS-84 to the then Russian Space station Mir. At onset of microgravity, animals had reached stages 25 to 28. The mission lasted 9.5 days. Recordings were taken between day 1 and 4, 8 and 11 and 38 and 41 after landing of the space craft. Note the depressed rVOR characteristic of  $\mu$ g-tadpoles with respect to their 1g-controls during the first and second observation period and the adjustment between both groups during the third recording period. Note also the increase of the amplitudes for the rVOR characteristics in all samples between the first and last observation period. All animals from the third observation period were also tested during the first and second ones.

already developed the rVOR. The over-all analysis revealed an approximately 20 to 30% depression of the rVOR by microgravity. The depression disappeared after re-exposure to 1g-conditions; the period of 1g-readaptation could last up to 5 weeks (Fig. 6). The detailed analysis of the data from  $\mu$ g-exposed animals revealed that the rVOR modification was clearly linked to changes of the body shape which developed during the space flight. In particular, exposure to microgravity can induce malformations of the tadpole's body (45, 50). The most obvious modification is a hyper-

extension of the tail. It was observed in tadpoles which developed from eggs fertilized on ground before the space flight (45, 50) but not in tadpoles which developed from eggs fertilised in microgravity during a spaceflight (51). After re-exposure to 1g-conditions the hyperextension of the tail disappeared (45) (Fig. 7).

The reason for these malformations is unknown although; a vestibular origin via a depressed trophic action on muscle development might be included. However, this observation prompted the separate analysis of  $\mu g$ -effects on the rVOR in both normally and abnormally developed tadpoles. All studies revealed a significant depression of the rVOR in tadpoles which developed an upward bended tail during microgravity exposure (47). In contrast, tadpoles with normal tails which had not devel-

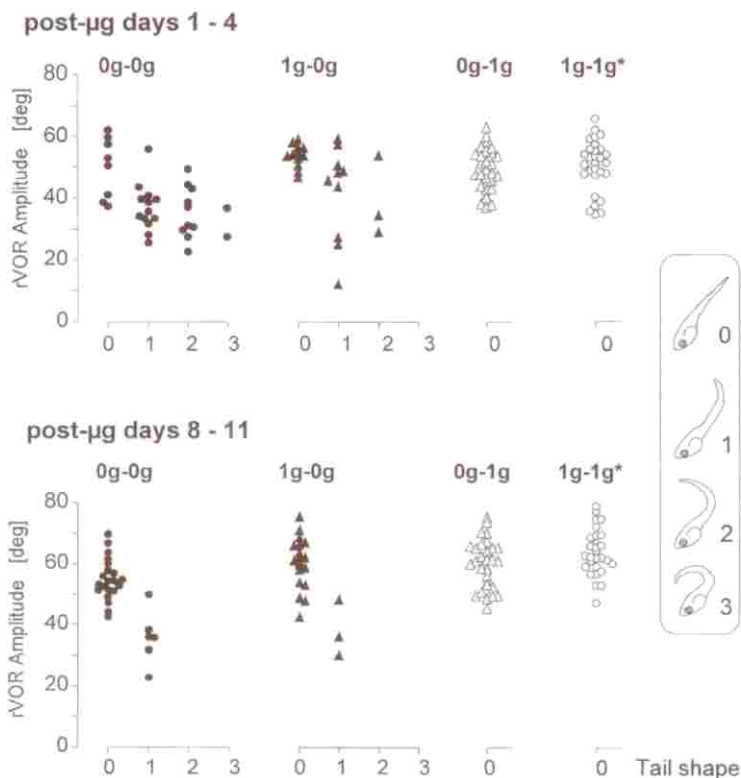
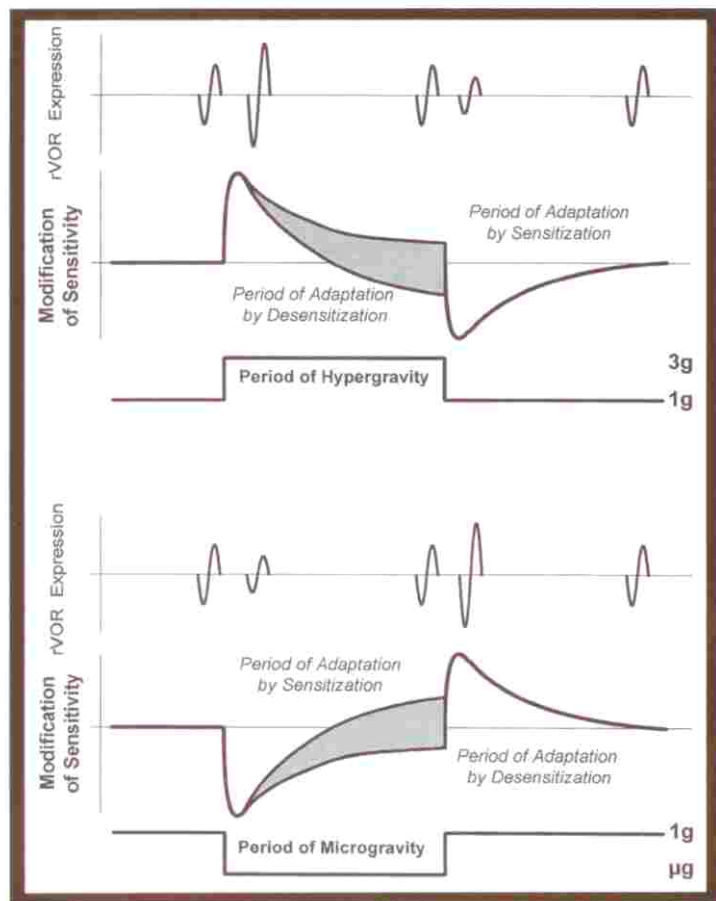


Fig. 7. - Microgravity induced mal-formation of the tadpoles' tail and its relation to rVOR depression.

Tadpoles flew on the Spacehab mission STS-84 to the then Russian Space station Mir. At onset of microgravity, animals had reached stages 25 to 28. The mission lasted 9.5 days. A first rVOR recording was taken between day 1 and 4, a second one between day 8 and 11 after landing of the spacecraft. All tadpoles from the second post-flight week were also tested during the first week. Each marker represents one animal. 0g-0g, animals were exposed to microgravity throughout the mission; 1g-0g, animals were exposed to simulated 1g by centrifugation during the first half of the mission, thereafter to microgravity; 0g-1g, animals were treated in the opposite way as 1g-0g animals; 1g-1g\*, tadpoles from the 1g-ground control. Classification of the tail's mal-formation is shown in the inset. Note that the number of animals with bended tails decreased during readaptation to 1g-condition.

Fig. 8. - Hypothesis about adaptation mechanisms of the vestibuloocular reflex during and after exposure to altered gravity.

It is assumed that the vestibular systems is sensitized or desensitized during exposure to hypergravity (upper plot) and microgravity (lower plot), respectively, to adapt to the base level of the rVOR obtained before the onset of altered gravity. After termination of altered gravity, the opposite adaptation process occurs. Normalization of the rVOR after termination of altered gravity was observed; the time courses of adaptation during exposure to gravity are hypothetically and included the possibility of over-compensation (shaded area).



oped their rVOR at onset of microgravity behaved as their ground reared controls while tadpoles with normal tails which had developed their rVOR at onset of microgravity revealed an augmented rVOR after their flight which persisted for 3 days after return to Earth 1g-conditions (19, 20, 47).

#### 4. Residual effects.

One of the 3 criteria for critical periods is the existence of residual effects; i.e., modifications in behavior or physiology persists for a long time after termination of the modified environmental conditions or they are even irreversible. So far, irreversibility was never observed in the development of vestibular functions. For swimming behavior, some, long-lasting effects became visible in the fish *Fundulus heteroclitus* (17). For the roll-induced vestibuloocular reflex, modifications mostly returned to normal independent of whether the rVOR was a depressed or augmented (24, 43, 44).

A detailed analysis, however, revealed some data which point to long-lasting deficiencies even for the rVOR. One observation was that slowly developing *Xenopus*

tadpoles obviously need longer to normalize their rVOR than fast developing ones of the same age after a 3g-exposure of 9 days. In particular, during the first recording period, the median VOR amplitude in the hg-group of stage 46 was  $31.5^\circ$  and significant ( $p < 0.01$ ) lower than that of the 1g-controls ( $36.9^\circ$ ). One week later, the 3g-group had a median VOR amplitude of  $28.4^\circ$ , while the VOR amplitude in the 1g-controls amounted to  $53.0^\circ$  ( $p < 0.001$ ). The corresponding values for tadpoles which had developed to stage 47 during the first post-3g week were  $26.5^\circ$  for the 3g-group, but  $38.5^\circ$  for the 1g-control ( $p < 0.001$ ) while the recording during the second post-3g week revealed complete normalization ( $59.1^\circ$  versus  $58.8^\circ$ ) (43).

Long readaptation periods were also observed if the rVOR of tadpoles with 3g-experience was elicited only by small lateral roll. Compared to the rVOR amplitude and  $90^\circ$  lateral rolls, these analyses revealed that no 1g-readaptation occurred for a roll of  $15^\circ$  for more than 8 weeks after 3g-termination (47, 48), while normalization was complete for the large roll stimuli. These data show that 3g-reared animals have an impaired sensitivity to detect postural changes in the threshold range compared to 1g-reared siblings.

#### DISCUSSION

All studies give clear evidence for a general sensitivity of the developing GSS for modifications of the gravitational environment; but these observations do not imply that gravity is necessary for normal development. Most obvious is the sensitization of the GSS by gravity deprivation which was observed in man as well as in adult and young animals. Stimulation of the macular organ of adult toad fish (*Opsanus tau*) by translational linear acceleration revealed a higher nervous activity of the 8th nerve in animals with a 16-day microgravity experience than their 1g ground controls (5). Astronauts overestimated their tilt sensation during the first days after their return to 1g-condition on Earth (7). Sensitization of the GSS was also recorded by our experiments in developing cichlid fish *Oreochromis mossambicus* (44) and *Xenopus* tadpoles (19, 20) after a 9- to 10-day space flight. The up-regulation of the immediate early gene *c-fos* within the afferent vestibular nuclei of rats after exposure to microgravity during a space flight (34) and the down-regulation of *c-fos* within the efferent parts of the vestibular nuclei of rats (2) also indicates an overall sensitization of the vestibular system by gravity deprivation. The over-compensation of the activity in the VIIIth nerve of frogs during a space flight (6) points in the same direction. In astronauts and all animal species, the  $\mu$ g-induced sensitisation as well as the 3g-induced desensitization (cf. depressed rVOR after 3g-exposure in *Xenopus*, Fig. 3) mostly disappeared after some days making it more likely that vestibular g-related modifications are caused by adaptation instead of a critical period.

Does this mean that the question: "Critical periods" in vestibular development or adaptation of gravity sensory systems to altered gravitational conditions? does not exist at all and that, therefore, each microgravity and hypergravity induced modification of anatomical, physiological and behavioral features can be exclusively

explained by adaptation. In fact, from the physiological point of view, adaptation is the most simple mechanism for vestibular sensitization under microgravity. Most receptor cells and neurons decrease their physiological activity after a step-like decrease of the stimulus level, but adapt to a more or less higher activity level during maintained stimulation (*phasic-tonic response pattern*). Vestibular neurons also decrease their resting activity transiently after they were deprived from labyrinthine input, but they recover it to higher levels thereafter (36). Neurophysiological studies in alert guinea pigs (37), anesthetized cats (39) and awake monkeys (56) had also revealed that despite absence of any labyrinthine input, vestibular nuclei were able to restore the initially depressed neuronal activity. In contrast to bilateral labyrinthectomy, the macula systems remains intact in microgravity, however the otolithic mass is unweighted so that the otolith cannot be displaced by body roll and tilt. Absence of weight-stimulation of utricular sensory hair cells makes it likely that macular afferents and central vestibular neurons decrease their resting discharges after  $\mu\text{g}$ -onset, but restore normal activity during further space flight due to their neuroplastic properties (cf. [13, 36, 49]). Macula activity originated by translatory accelerations during swimming or other movements (cf. [8]) might initiate the increase of spontaneous neuronal activity, similar to the initiation of vestibular compensation following hemilabyrinthectomy (13). In fact, observations during the STS-84 mission in 1997 and the Andromède flight to ISS in 2001 revealed that tadpoles increased the frequency of swimming but not its total duration during the space flights. If neuroplastic capabilities are also used for the adaptation to hypergravity and if adaptive processes are generally guided by genetically pre-programmed set points within the nervous network to which neuronal activity adjusts during development – similar to those for the adaptation to microgravity (cf. [6]) –, animals with hypergravity experience have to show transiently lower response levels if they are tested in 1g after 3g-termination. This prediction was confirmed (Fig. 3).

Biochemical and structural studies also point to adaptive sensitization or desensitization. In fish (*Fundulus heteroclitus*) creatine kinase activity – a biochemical marker for neuronal activity – was increased at primary vestibular projection sites of the cerebellar cortex after a space flight (28). In rats, the number of synapses of Type II hair cells was increased by 57% after  $\mu\text{g}$ -exposure (38), but decreased by 30% after 2g-exposure (10). In neonate swordtail fish (*Xiphophorus helleri*), the otolith became significantly larger in microgravity during a 16-day space flight (STS-90) compared to their ground-reared siblings (58). In the same species, the number of synapses within the N. descendens, a vestibular integration center, became larger by the spaceflight while the N. magnocellularis which receives inputs from the lateral line and the visual N. corticalis was not affected (26). If synapse numbers are directly correlated with the sensitivity of the vestibular system, changes such as these might explain sensitivity modifications.

On the other hand, the existence of a "critical period" during vestibular development is not unlikely as some observations revealed long-lasting modifications (Table 1). Postflight studies in young *Fundulus* revealed that their geotactic behavior was affected by microgravity if eggs were fertilized 32 hours prior to launching of the

Table 1. - Microgravity effects on the development of vestibular function in lower vertebrates based on studies after exposure to real and simulated microgravity.

	Response	Sensitivity to microgravity	Insensitivity to microgravity	1g-Readaptation	Microgravity	Ref.
<i>Danio rerio</i> (Zebrafish)	rVOR	30 - 66 hpf	0 - 24 hpf	complete within 5 days	simulated $\mu$ g: NASA bioreactor	30, 31
<i>Fundulus heteroclitus</i>	swimming	if no labyrinth was formed preflight	72 - 96 hpf if labyrinth was formed preflight	incomplete re-tests during parabolic flights	space flight: Skylab 3	15, 16, 54
<i>Xenopus laevis</i>	rVOR	lordotic tadpoles; at least up to stage 45 normal tadpoles; if preflight rVOR	normal tadpoles; if no rVOR preflight	complete within 1 to 5 weeks	$\mu$ g-duration 59 days space flight: STS-55, STS-84, Soyuz taxi to ISS	43, 45, 47
<i>Oreochromis mossambicus</i>	rVOR	if no rVOR preflight	if rVOR preflight	complete within 1 week	$\mu$ g-duration 9 to 10 days space flight: STS-55, STS-84 $\mu$ g-duration 9 to 10 days	44

For zebrafish *Danio rerio*, time intervals (hours after fertilization, hpf) define periods of exposure in the rotating bioreactor which caused a rVOR depression (sensitivity) or not (insensitivity); its otic vesicle is closed for the first time at 18 hpf and first sensory cells appear at 24 hpf (cf. [15]). In the fish *Fundulus heteroclitus*, irregular swimming was tested. In the amphibian *Xenopus laevis*, periods of  $\mu$ g-exposures started between stages 24 to 45; its otic vesicle is closed for the first time at stage 27; its rVOR appears for the first time at stage 42 (definition of stages, cf. [32]). In the cichlid fish *Oreochromis mossambicus*,  $\mu$ g-periods started between stages 11 and 16; its rVOR appears for the first time at stage 13; its ear vesicle containing for the first time a few otoconia can be seen firstly at stage 8 (definition of stages, cf. [1]). 1g-Readaptation starts after  $\mu$ g-termination. rVOR, roll-induced vestibuloocular reflex;  $\mu$ g, microgravity; lordotic, tail bended dorsally.

spacecraft but not if fertilization occurred at or prior to 66 hours before launch (16, 17). These times means, that in the sensitive group, the labyrinth was not formed pre-flight, while in the insensitive group, it existed at onset of microgravity. The rVOR studies in *Xenopus* and *Oreochromis* revealed the existence of sensitive and insensitive developmental stages with respect to both hypergravity (Fig. 3) and microgravity exposure (Figs. 5 and 6). Exposure of zebrafish (*Danio rerio*) to simulated microgravity by means of the NASA bioreactor (30, 31) revealed a defined period of  $\mu\text{g}$ -sensitivity (Fig. 4). It is remarkable that this (critical) period of high sensitivity started shortly after the ear vesicle becomes closed and hair cells are formed (15). Thus, labyrinth formation and the first appearance of the rVOR are important milestones in the development of the vestibular system (Tab. 1). They can, therefore, considered as indicative of the existence of a sensitive period in vestibular development of lower vertebrates.

The results on the rVOR development in *Xenopus* and *Oreochromis* are not definitive concerning the existence of a critical period because two conditions are not yet verified: (1) the period with the high susceptibility to environmental modifications covers a limited period during postembryonic development (cf. [59]); and (2) anatomical, behavioral or physiological modifications persist for long periods or are even irreversible. In our experiments only two (fish) or three (*Xenopus*) developmental stages were studied. But although this number is insufficient to define precisely borders of a critical period, the data do indicate that in the fish the end of a putative sensitive period occurs at approximately stage 16. In addition, since it was observed that both  $\mu\text{g}$ - or 3g-induced rVOR-modifications return to normal after re-exposure to 1g, it is not yet clear that the functional changes are irreversible. There are observations which indicate the existence of long-lasting effects. The most striking result came from hypergravity studies in *Xenopus*. They showed that (1) slowly developing tadpoles did not readapt to normal development during the observation period of two weeks while fast growing tadpoles of the same age did (43), (2) that tadpoles which hatched during hypergravity revealed a delayed 1g-readaptation compared to their 1g-reared siblings (24), and (3) 1g-readaptation after 3g-exposure seem to need longer if tadpoles are tested by small lateral roll angles than by large ones (47). Similar observations as described in (1) and (2) were received from studies in crickets (*Acheta domesticus*), which were exposed to microgravity during the 16-day Neurolab mission (21).

In summary, the present studies give good arguments for both adaptive processes during exposure to altered gravity as well as for the existence of a critical period in vestibular development. The most critical aspect to find the answer to this question is the rather low number of possibilities for deprivation experiments by means of space flights. Another difficulty is the strong reorganisation capacity of living systems, in particular during early embryogenesis (cf. for *Xenopus* during space flight: [11]). Additional experiments with at least 5 to 7 stages are necessary to demonstrate in more than two stages a sensitivity to gravitational deprivation and, finally, to determine the beginning and end of a critical period precisely if it exists at all. Alternatively, long-lasting deprivation or augmentation periods are needed to describe the kinetics of adaptation to altered gravity during the exposure.

## SUMMARY

1. A feature of sensory, neuronal and motor systems is the existence of a critical period during their development. Modification of environmental conditions during this specific period of life affects development in a long-term manner, or even irreversibly. Deprivation is the preferred approach to study the existence and duration of critical periods. For gravity sensory systems, space flights offer the only opportunity for deprivation conditions.

2. Studies in a fish (*Oreochromis mossambicus*) and an amphibian (*Xenopus laevis*) revealed a significant sensitivity of their roll-induced static vestibuloocular reflex (rVOR) to a 9- to 10-day gravity deprivation (microgravity) during a space-flight. In some instances, the rVOR was augmented after the flight as demonstrated in young *Oreochromis* which were launched when their rVOR had not been developed, and in *Xenopus* tadpoles launched after their rVOR had developed. Fish which could perform the rVOR at launch were insensitive to microgravity exposure. A similar insensitivity to microgravity was observed in *Xenopus* tadpoles with normal body shape which had not yet developed their rVOR at launch. Some tadpoles, however, developed an upward bended tail during their space flight; their rVOR was significantly depressed after termination of microgravity independent of the age at onset of the flight. Hypergravity depressed the rVOR for all so far tested developmental stages in both *Oreochromis* and *Xenopus*.

3. Both adaptive processes during exposure to altered gravity as well as the existence of a critical period in vestibular development might be responsible for the modulation of the rVOR recorded after exposure to altered gravity. Deprivation studies have to be extended to older developmental stages to test the possibility of a critical period; however, this approach is limited due to the low number of space flights.

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