The Journal of Experimental Biology 209, 2847-2858
Published by The Company of Biologists 2006
doi:10.1242/jeb.02298

# Microgravity-induced modifications of the vestibuloocular reflex in *Xenopus laevis* tadpoles are related to development and the occurrence of tail lordosis

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*Accepted 25 April 2006*

## Summary

**During space flights, tadpoles of the clawed toad *Xenopus laevis* occasionally develop upward bended tails (tail lordosis). The tail lordosis disappears after re-entry to 1*g* within a couple of days. The mechanisms responsible for the induction of the tail lordosis are unknown; physical conditions such as weight de-loading or physiological factors such as decreased vestibular activity in microgravity might contribute. Microgravity (μ*g*) also exerts significant effects on the roll-induced vestibuloocular reflex (rVOR). The rVOR was used to clarify whether tail lordosis is caused by physiological factors, by correlating the occurrence of μ*g*-induced tail lordosis with the extent of μ*g*-induced rVOR modifications.**

**Post-flight recordings from three space flights (D-2 Spacelab mission, STS-55 in 1993; Shuttle-to-Mir mission SMM-06, STS-84 in 1997; French Soyuz taxi flight Andromède to ISS in 2001) were analyzed in these experiments. At onset of microgravity, tadpoles were at stages 25–28, 33–36 or 45. Parameters tested were rVOR gain (ratio between the angular eye movement and the lateral 30° roll) and rVOR amplitude (maximal angular postural change of the eyes during a 360° lateral roll).**

**A ratio of 22–84% of tadpoles developed lordotic tails, depending on the space flight. The overall observation was that the rVOR of tadpoles with normal tails was either not affected by microgravity, or it was enhanced. In contrast, the rVOR of lordotic animals always revealed a depression. In particular, during post-flight days 1–11, tadpoles with lordotic tails from all three groups (25–28, 33–36 and 45) showed a lower rVOR gain and amplitude than the 1*g*-controls. The rVOR gain and amplitude of tadpoles from the groups 25–28 and 33–36 that developed normal tails was not affected by microgravity while the rVOR of μ*g*-tadpoles from the stage-45 group with normal tails revealed a significant rVOR augmentation. In conclusion: (1) the vestibular system of tadpoles with lordotic tails is developmentally retarded by microgravity; (2) after a critical status of vestibular maturation obtained during the appearance of first swimming, microgravity activates an adaptation mechanism that causes a sensitization of the vestibular system.**

Key words: development, space flight, adaptation, critical period, clawed toad, *Xenopus laevis*.

## Introduction

Tadpoles of the clawed toad *Xenopus laevis* subjected to gravity deprivation during space flights suffered behavioral and morphological modifications, which persisted for some days after termination of the space flight. Behavioral modifications included swimming abnormalities (Neubert et al., 1994; Snetkova et al., 1995; Fejtek et al., 1998) and a depression of both gain and amplitude of the roll-induced static vestibuloocular reflex (rVOR) (Sebastian et al., 1996) but not of the dynamic VOR induced by horizontal lateral displacements (Eßeling et al., 1994a; Eßeling et al., 1994b). Morphological modifications include a hyperextension of the tail (tail lordosis) (Fig. 1), and were observed in *Xenopus laevis* tadpoles launched before hatching (Snetkova et al., 1995; Sebastian and Horn, 1998) but not in tadpoles that developed from eggs fertilized in orbit under microgravity (Souza et al., 1995) (cf. Table 1). After re-exposure to natural 1***g*** conditions on the ground or to simulated 1***g*** conditions in space by centrifugation, the tail lordosis disappeared during further development (Sebastian and Horn, 1998).

The reduction of the rVOR is probably caused by developmental retardation of the vestibular system (Sebastian et al., 1996). Tail lordosis might be caused by weight de-loading during μ*g*-exposure because affected tadpoles showed muscle degeneration (Snetkova et al., 1995). However, other factors such as: (1) trophic effects of vestibular activity on muscle development, and (2) the rostrocaudal maturation gradient, might cause tail lordosis if they revealed a sensitivity to altered gravity. In fact, vestibular activity of bullfrogs was transiently reduced during microgravity exposure (Bracchi

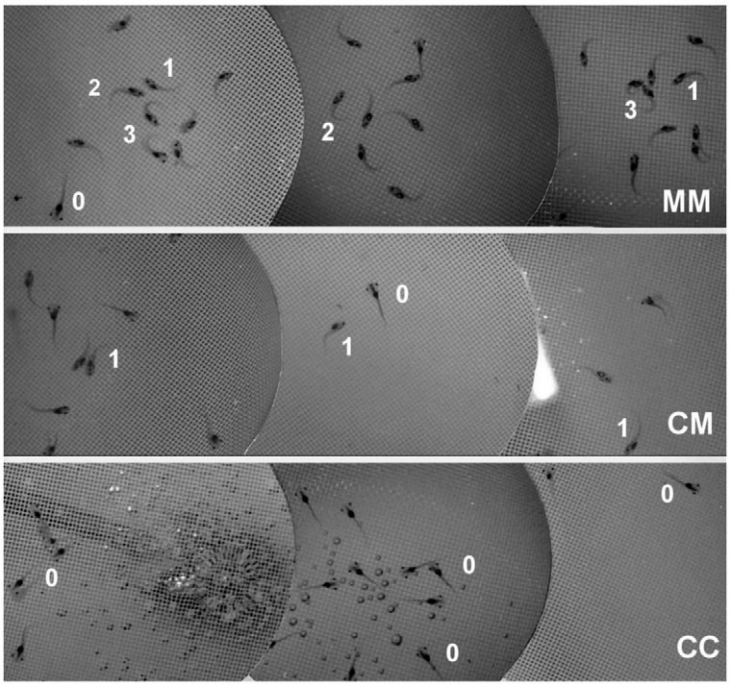


Fig. 1. Tail lordosis induced by microgravity. Lordotic body shapes of tadpoles exposed to microgravity during a spaceflight (MM, CM) or to simulated 1***g***-condition by centrifugation (CC) during a spaceflight. CM differs from MM because these tadpoles were centrifuged at 1***g*** for 4 days, and thereafter exposed to microgravity for 5 days, while MM tadpoles were exposed to microgravity for 9.2 days (observations from the STS-84 mission in 1997). The numbers indicate the lordosis score with 1 weak, 2 medium, and 3 strong. 0 indicates normal body shape.

et al., 1975). In addition, vestibular activity affects the development of extraocular eye muscles of juvenile rats (Brueckner et al., 1999). Activity-related trophic effects on developing neurons, their interneuronal connectivity patterns and the development of muscles were also described for the maturing visual system of fish, chick and rats (Schmidt, 1988; Fawcett, 1988). A rostrocaudal maturation gradient of the tail was described (Roberts and Tunstall, 1994). In addition, recordings from ventral roots of different tail segments during fictive swimming revealed a sensitivity of vestibulospinal projections to microgravity (Böser et al., 2003; Horn et al., 2003) and hypergravity (Böser and Horn, 2006) during early periods of tail development.

Thus, a rostrocaudal maturation gradient of the tail during early periods of life and a reduced or absent tonic vestibular activity may influence the development of vestibulospinal projections and body muscles, leading to transient modifications of body shape and locomotion, especially if the animals are exposed to an atypical environment such as microgravity. In deafferented bullfrog larvae, coupling between compensatory eye movements and locomotion was demonstrated (Stehouwer, 1987). This close link between vestibuloocular and vestibulospinal mechanisms in tadpoles makes it reasonable to ask the question whether rVOR depression and tail lordosis are correlated due a basic mechanism.

During spaceflights, the ***g***-level is $10^{-3}$ ***g*** to $10^{-5}$ ***g***, but the term microgravity (microgravity; abbreviations $\mu$***g*** or 0***g***) is used by the scientific community, and for convenience is so designated in this paper.

## Materials and methods

Tadpoles of the southern clawed toad *Xenopus laevis* Daudin were used from the stock of the Central Animal Holding Facility at the University of Ulm and from the Institute of Aerospace Medicine of the Deutsches Zentrum für Luft- und Raumfahrt (DLR). The developmental stages were determined using published tables (Nieuwkoop and Faber, 1967). During the period of rVOR recordings, animals were kept in groups of five in small nets of 8 cm diameter at a temperature of 20°C.

### *Microgravity exposure*

Tadpoles were exposed to microgravity during three space missions, the 10-day flight STS-55 (German D-2; 1993; experiment STATEX-VOR), the 9.2-day flight STS-84 (Shuttle-to-Mir SMM-06; 1997; experiment TADPOLES), and the 10-day flight Andromède (French Soyuz Taxi Flight to the International Space Station ISS; 2001; experiment AQUARIUS-XENOPUS). During the Spacelab mission D-2, animals were kept in groups of five in petriPERM dishes, which have a volume of 25 ml. At launch, they had reached stage 33–36, which is shortly before the hatching stage. For the flight on STS-84, animals were transported in groups of 12 in miniaquaria, which have a volume of 42 ml. They were launched when they had reached stage 25–28, which is the tail bud stage. Miniaquaria were developed for the flight on STS-84, and were re-used during the Andromède flight in 2001 when two different stages were exposed to microgravity. The young group had reached stage 26–27 at launch; they were kept in groups of 12 animals in each miniaquarium. Tadpoles from the old group had reached stage 45 at launch; at this time they were able to perform the rVOR. They were kept in groups of five in each miniaquarium.

During the D-2 and SMM-06 missions, a centrifuge for in-flight 1***g***-simulation was available (CC-groups). For all missions, a parallel ground control experiment (GG-groups) was performed. Tadpoles from the D-2 and the Andromède experiment were exposed to microgravity throughout the mission (MM-groups). During the SMM-06 experiment tadpoles were divided in four groups: (1) animals exposed to microgravity for 9.2 days (MM-group), (2) animals exposed to in-flight 1***g***-simulation for 93 h, and thereafter to microgravity for 5 days until re-entry to Earth-1***g*** conditions (CM-group), (3) tadpoles treated in the opposite way (MC-group), and (4) animals exposed to in-flight 1***g***-simulation for the whole flight (CC-group) (Sebastian and Horn, 1998).

For logistic reasons, animals from the D-2 mission were

cooled for 4 days to 14°C to guarantee stage 33–36 at launch. Animals from the SMM-06 mission were always kept under laboratory temperature at a range of 22–24°C until handover to the launch team. Thereafter, they were stored at a temperature of 20°C for 17 h until launch. Animals from the Andromède mission were cooled for at least 2 days to 14°C to maintain the defined developmental stages of 26–27 and 45.

### *Recordings of the rVOR*

A detailed description is given elsewhere (Horn et al., 1986; Sebastian et al., 1996). Briefly, the tadpoles were mechanically fixed in an observation chamber and illuminated homogeneously from the frontal view. They were rolled around their longitudinal axis by 360° in 15° steps. During this stimulation procedure, the animals were continuously videotaped. From these recordings, the posture of the eyes was determined 7 s after initiation of each 15°-step. The sine-like response characteristics were used to calculate the rVOR gain and rVOR amplitude (Fig. 2). The rVOR gain is defined by the ratio between the angular counter roll of the eye and the roll angle. In this study, data for a 30° roll from the horizontal to the inclined posture are presented. The rVOR amplitude is defined by the maximal eye movement during a complete 360° roll. Previous studies in *Xenopus* and the fish *Oreochromis mossambicus* have shown that the rVOR amplitude is a sensitive indicator to describe: (1) modifications of the rVOR during its development (Horn et al., 1986; Sebastian and Horn, 1999), (2) the extent of vestibular compensation after hemilabyrinthectomy (Rayer and Horn, 1986), and (3) susceptibility changes within the vestibuloocular system to altered gravity (Horn and Sebastian, 1996; Sebastian et al., 1996; Sebastian and Horn, 1998; Sebastian et al., 2001). Recordings of the rVOR were taken during the post-flight days 1–11 and also, in the case of the STS-84 study, 5 weeks after landing. Before starting the rVOR measurements, tadpoles were evaluated for their developmental stage and body shape.

### *Data evaluation and statistics*

Animals were included in the statistical analysis only if they remained active for at least 3 h after the rVOR recording. During each recording period, each animal was investigated only once. Thus, each distribution of either rVOR gain or amplitude consists of statistically independent values. Some animals from the D-2 mission were tested twice with a delay of 7 days. Most animals from the SMM-06 mission could be tested twice during the first 2 post-flight weeks, and a third time 5 weeks later (cf. Table 2). Animals from the Andromède mission were always tested only once because they were later used for anatomical studies (Horn et al., 2006). The non-parametric *U*-test from Wilcoxon, Mann and Whitney (Sachs, 1997) was used because a transformation of the experimental distributions into a Gaussian one was not possible. Median values of samples are presented in tables. Lordotic and non-lordotic animals were compared with respect to: (1) their age

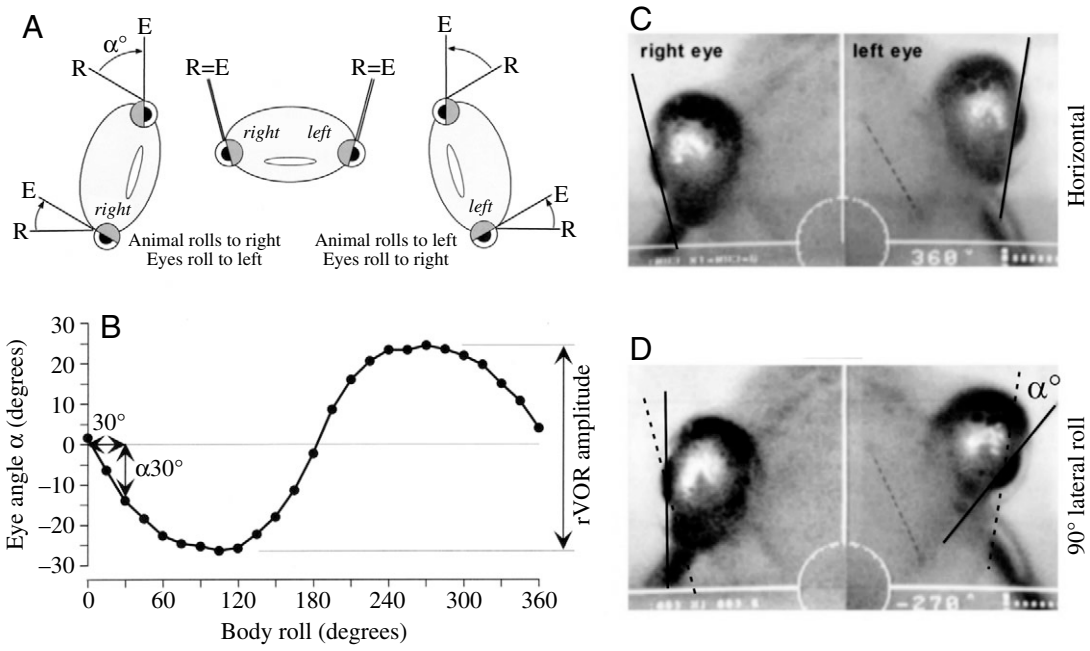


Fig. 2. The roll-induced vestibuloocular reflex in *Xenopus* tadpoles. (A) Schematic presentation of the frontal view of an animal that indicates the direction (arrow) and extent (α°) of eye movement during a right and left roll, respectively. R, reference line that is constant with respect to the animal; E, eye cup margin that is used as recording line. (B) Typical rVOR characteristic obtained during a 360° lateral roll. Definitions of the rVOR amplitude and those parameters used for the calculation of the rVOR gain (see arrows at the left side of the characteristic) are included. (C,D) Original video picture of the eyes of a tadpole in a frontal view in the horizontal (C) and 90° lateral (D) position. Because the camera is moved with the stimulation machine, only the eyes change their position. With respect to the frontal view, the animal is rotated to the left; with respect to the animal's body, to the right. The dotted line corresponds to line R.

Table 1. *Number of lordotic tadpoles in relation to the age at onset of microgravity and the period of μg-exposure during the mission*

| | SMM-06 STS-84 | | | Andromède Soyuz-taxi/ISS | | | D-2[1] STS-55 | | | Andromède Soyuz-taxi/ISS | | | SMM-06[2] STS-84 | | | SMM-06[3] STS-84 | | | SL-J[4] STS-47 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Year | 1997 | | | 2001 | | | 1993 | | | 2001 | | | 1997 | | | 1997 | | | 1994 | | |
| Stage at launch | 25–28 | | | 26–27 | | | 33–36 | | | 45 | | | 42–43 | | | 25–28 | | | Fertil. in μg | | |
| Duration (days) | 9 | | | 9 | | | 10 | | | 9 | | | 9 | | | 9 | | | 7 | | |
| Days of μg | Whole mission | | | Whole mission | | | Whole mission | | | Whole mission | | | FD5–FD9 | | | FD1–FD5 | | | FD2–FD7 | | |
| | N | L | N% | N | L | N% | N | L | N% | N | L | N% | N | L | N% | N | L | N% | N | L | N% |
| First observation | 9 | 25 | 26.5 | 14 | 4 | 77.8 | 3 | 16 | 15.8 | 9 | 3 | 75.0 | 15 | 13 | 53.6 | 32 | 0 | 100 | 100 | | |
| Mean stage | 47.1 | 47.0 | | 46.3 | 46.0 | | 45.0 | 44.9 | | 47.0 | 46.7 | | 47.2 | 47.0 | | 47.1 | – | | 46 | | |
| Days after 0*g* | 1–4 | | | 2–4 | | | 2–6 | | | 2–4 | | | 1–4 | | | 1–4 | | | Post-flight H/O | | |
| Second observation | 22 | 7 | 75.9 | | | | 3 | 3 | 50.0 | | | | 20 | 3 | 87.0 | 29 | 0 | 100 | | | |
| Mean stage | 48.3 | 47.7 | | | | | 46.5 | 46.0 | | | | | 48.6 | 47.3 | | 48.5 | – | | | | |
| Days after 0*g* | 8–11 | | | | | | 9–12 | | | | | | 8–11 | | | 8–11 | | | | | |
| Third observation[5] | 27 | 1 | 96.4 | | | | | | | | | | 22 | 1 | 95.7 | 29 | 0 | 100 | | | |
| Days after 0*g* | 14 | | | | | | | | | | | | 14 | | | 14 | | | | | |

FD, flight day; N, normal shape; L, lordotic shape; N%, percentage of normal tadpoles.

[1]Data from the rVOR component of the STATEX project; [2]after exposure to 1*g* in the BIORACK centrifuge for 4 days; [3]after FD5, exposure to 1*g* in the BIORACK centrifuge; [4]fertilization in orbit not before 34 h after launch, stages immediately after handover (H/O) of the samples at the landing day [data from the Japanese Spacelab mission SL-J (Souza et al., 1995)]; [5]stages were not determined at 14 days after landing.

Lordotic animals were also observed in two other experiments with pre-flight fertilized eggs of *Xenopus* (stage 25 at launch). One of them was performed on the Russian Cosmos 2229 satellite (11.5-days μg), the other on the Japanese Spacelab mission SL-J (STS-47). These experiments are not included in the table because the authors (Snetkova et al., 1995) gave no numbers of lordotic tadpoles. Stages are defined according to published tables (Nieuwkoop and Faber, 1967).



(days after fertilization) and (2) their developmental stage [definition of stages (cf. Nieuwkoop and Faber, 1967)], to exclude the impact of different developmental velocities for the occurrence of rVOR differences between lordotic and non-lordotic tadpoles. Frequency distributions determined to describe the developmental progress during μg-exposure were tested by means of the $\chi^2$-test (Sachs, 1997).

## Results

During all missions, *Xenopus* embryos continued to develop. However, lordotic animals revealed slightly smaller developmental progress (Table 1) than normally developed ones. For example, during the first and fourth post-flight day both the lordotic animals from the MM- and CM-groups from the SMM-06 mission developed to stage 47.0, and the corresponding non-lordotic tadpoles to stages 47.1 and 47.2, respectively. The MC-, CC- and 1*g*-ground tadpoles had reached the stages 47.1, 47.0 and 47.1, respectively. The stage differences between flight and ground tadpoles were not significant ($\chi^2$-test: $P>0.05$). The stage difference became larger during the second week of recordings and reached values of 0.6 for the MM-group and 1.3 for the CM-group. For tadpoles from the missions D-2 and Andromède, the absolute stage differences never exceeded 0.3 (Table 1).

### *Frequency of lordosis*

In most tadpole groups from the D-2, Shuttle-to-Mir (cf. Fig. 1) and Andromède missions that were exposed to microgravity, lordotic animals could be observed after landing. The only exception was the MC-group from SMM-06, which was first exposed to microgravity and, thereafter, to 1*g*-conditions by centrifugation during the space flight (Table 1). In-flight observations of the tadpoles during flight day FD4 (mission elapse time MET 3/3:27 to 3/3:46) and FD9 (MET 7/21:40 to 7/22:8) during SMM-06 were recorded for each 12 animals from the 1*g*-inflight control and μ*g*-groups (MM, CM and MC). These recordings revealed abnormal tail development even at FD4 in the μ*g*-reared groups MM and MC, but not in CM. On FD9, some tadpoles from the CM group had lordotic tails as well as those from the MM tadpoles while no lordotic animal was observed in the MC-group. As demonstrated by the in-flight video recordings, lordotic tadpoles were also observed during the Andromède mission on FD4 and FD9.

Due to the large number of available animals after landing, recovery from tail lordosis could be studied for tadpoles from SMM-06. In the MM-group, 25 out of 34 tadpoles revealed lordosis 4 days after landing of the spacecraft, while 14 days after landing, only one out of 28 tadpoles was lordotic. In the CM-group of this mission, the respective ratios were 13 out of 28 at post-flight day 4 and one out of 23 at post-flight day 14 (Table 1). These data fit with the observations from in-flight video recordings, which revealed that lordosis developed during the first 4 days in microgravity disappeared if these tadpoles were exposed to 1***g***-centrifugation during the remaining 5 days of the flight (MC-group).

### *Tail lordosis and vestibuloocular reflex*

Logistic and developmental conditions make it impossible to follow the rVOR of individual animals during the complete experiment starting before onset and ending after termination of microgravity conditions. Therefore, all observations about modifications of the rVOR are related to a comparison of means of respective samples. Based on this limitation, the overall observation of the experiments was that the rVOR values of tadpoles with normal tails were either not affected by microgravity, or enhanced. In contrast, lordotic animals always revealed a depression of their rVOR (Table 2).

#### *Tadpoles from the Shuttle-to-Mir mission SMM-06*

The tadpoles of this flight had reached stage 25–28 at launch. After landing of the spacecraft, tail lordosis was observed only in the MM- and CM-groups. Tadpoles from the other three experimental groups MC, CC and GG, had developed normal tails. For all groups, rVOR recordings were taken 3 times in most animals, the first between post-flight days 1–4, and the last between post-flight days 38–41.

During the first recording period, the rVOR was depressed only in tadpoles with lordotic tails. Normally developed MM-tadpoles had nearly the same median rVOR amplitude as the other groups without or short microgravity experience (MM: 51.9°; GG: 52.0°; CC: 47.8°; note that CC-animals were exposed to microgravity for about 20% of the mission because the 1***g***-centrifuge was started not before FD2, and switched off 12 h before landing). However, within samples with microgravity experience that contained lordotic and normal tadpoles, the rVOR amplitude was always smaller in lordotic than in normal animals (MM-group: 35.9° *vs* 51.9°, $P<0.01$; CM-group: 45.9° *vs* 54.1°, $P<0.02$) (Fig. 3). For the rVOR gain, the observations were similar; however, the gain differences between lordotic and normal animals were never significant (Fig. 4).

During the second post-flight recording period, lordotic tails were still present in both MM- and CM-tadpoles but in a lower number of tadpoles. However, the number of tadpoles with normal tails was higher then during the first recording, i.e. normalization of tail development took place. The tadpoles with lordotic tails revealed a reduced rVOR performance compared to normally developed tadpoles with μ***g***-experience.

Table 2. *Survey of the median values for rVOR amplitude and rVOR gain obtained from the different space flight experiments with special reference to differences between the rVOR of animals with normal tail and lordotic tail*

| | Lordotic | Normal | | |
|---|---|---|---|---|
| | 0***g*** | 0***g*** | G1***g*** | F1***g*** |
| **D-2 Mission STS-55** | | | | |
| Stage 33–36 at launch | | | | |
| Post LD 2–6 | | | | |
| rVOR amplitude | 27.9 | 31.5 | 49.0 | 38.6 |
| rVOR gain | 0.191 | 0.242 | 0.439 | 0.347 |
| *N* | 16 | 3 | 23 | 15 |
| Post LD 9–12 | | | | |
| rVOR amplitude | 18.7 | 48.4 | 53.0 | 44.3 |
| rVOR Gain | 0.064 | 0.310 | 0.515 | 0.321 |
| *N* | 3 | 3 | 15 | 11 |
| **Shuttle-to-Mir Mission STS-84** | | | | |
| Stage 25–28 at launch | | | | |
| Post LD 1-4 | | | | |
| rVOR amplitude | 35.9 | 51.9 | 52.0 | 47.8 |
| rVOR gain | 0.378 | 0.412 | 0.508 | 0.473 |
| *N* | 25 | 8 | 32 | 35 |
| Post LD 8–11 | | | | |
| rVOR amplitude | 36.0 | 53.8 | 63.1 | 60.6 |
| rVOR gain | 0.228 | 0.392 | 0.565 | 0.589 |
| *N* | 7 | 22 | 30 | 32 |
| Post LD 38–41 | | | | |
| rVOR amplitude | – | 78.0 | 74.9 | 73.7 |
| rVOR gain | – | 0.593 | 0.635 | 0.633 |
| *N* | – | 13 | 17 | 21 |
| **Andromède Mission to ISS** | | | | |
| Stage 26–27 at launch | | | | |
| Post LD 2–4 | | | | |
| rVOR amplitude | 18.1 | 33.2 | 35.7 | – |
| rVOR gain | 0.123 | 0.378 | 0.303 | – |
| *N* | 4 | 14 | 18 | – |
| **Andromède Mission to ISS** | | | | |
| Stage 45 at launch | | | | |
| Post LD 2–4 | | | | |
| rVOR amplitude | 29.2 | 43.0 | 33.0 | – |
| rVOR gain | 0.223 | 0.433 | 0.257 | – |
| *N* | 3 | 9 | 11 | – |

0***g***, microgravity animals; F1***g***, in-flight control in a 1***g***-centrifuge; G1***g***, 1***g***-ground control. post LD, days after landing of the spacecraft.

*N*, number of animals; –, no test group.

These differences were significant for both rVOR amplitude (MM-group: 36.0° *vs* 53.8°, $P<0.002$; CM-group: 36.3° *vs* 61.2°, $P<0.01$) and rVOR gain (MM-group: 0.228 *vs* 0.392, $P<0.01$; CM-group: 0.325 *vs* 0.529, $P<0.02$) (Fig. 4). Five weeks after landing, both rVOR gain and rVOR amplitude

were on the same level for all five groups. At that time, only tadpoles with normal tails were alive.

Comparison of the median rVOR amplitudes and gains in the time frame revealed developmental progress of the rVOR in all groups, but only for tadpoles with normal tails. During the period between the first and third recording period, the

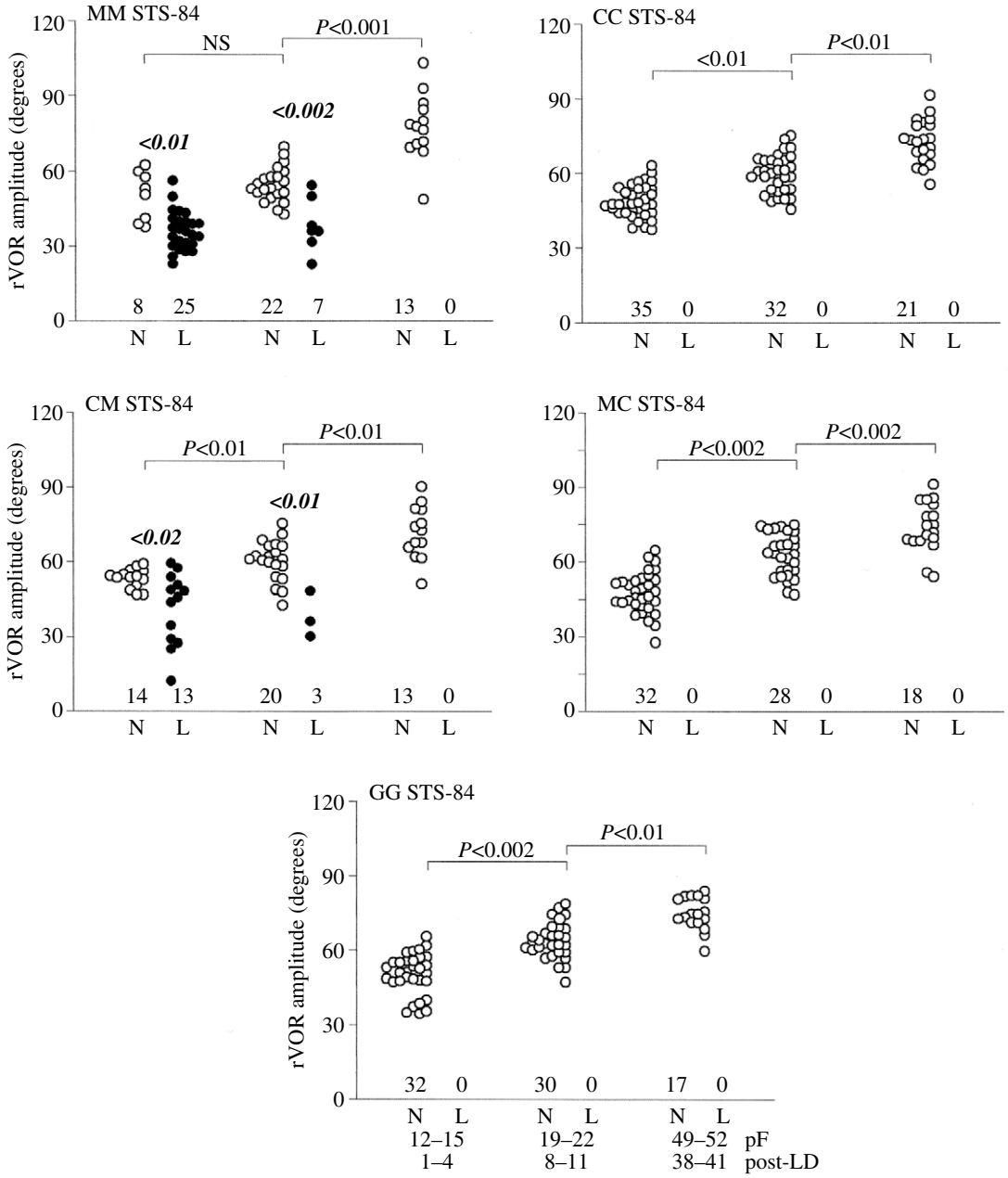


Fig. 3. The amplitude of the roll-induced static vestibuloocular reflex (rVOR) in *Xenopus laevis* tadpoles with lordotic tail. Observations after the 9.2-day SMM-06 Shuttle-to-Mir mission 06 (STS-84, 1997). At the onset of the mission, embryos had reached the developmental stages 25–28. For the recordings after landing, tadpoles were grouped according to the development of tail lordosis (L, filled circles) or normally developed tails (N, open circles). rVOR amplitude = maximal angular roll of the eyes during a 360° lateral body roll. MM, tadpoles that were exposed to microgravity throughout the 9.2-day mission; CM, tadpoles exposed to in-flight 1***g***-simulation for the first 4 days (93 h) and thereafter to microgravity until the end of the mission; MC, tadpoles exposed to microgravity for the first 4 days (93 h) and thereafter to in-flight 1***g***-simulation until deactivation of the centrifuge, 12 h before the end of the mission; CC, tadpoles exposed to microgravity throughout the 9-day mission except the time between launch and activation of the centrifuge and after deactivation of the 1***g***-centrifuge; GG, tadpoles from the 1***g***-ground control. Observation periods are defined below the lowest plot; pF, days after fertilization; post-LD, days after landing of the spacecraft. Each filled and open circle represents an individual animal. Numbers at the bottom of each plot give the numbers of tadpoles; levels of statistical significances in bold italic letters indicate differences between normal and lordotic animals of the respective observation period; NS, not significant. Numbers above brackets indicate differences between samples of the two observation periods.

median values for the rVOR amplitude recorded for all five experimental groups MM, MC, CM, CC and GG, increased from a range between 45.8° and 54.1° to a range between 71.5° and 78.0° (Fig. 3). In most groups, the developmental progress of the rVOR amplitude from one to the next recording period was significant except for the lordotic and normal MM-groups between the first and second recording period. Similar to the rVOR amplitude, the rVOR gain also increased from the first to the third recording period. But due to larger variations of data, these increases are often not significant, especially for tadpoles with microgravity experience during the second 4.5-day period of the mission (Table 2; Fig. 4). Lordotic tadpoles could be observed only for 2 weeks after landing. In contrast to the normal animals, they never revealed a significant change of the rVOR between the first and second recording period; instead, both rVOR and rVOR gain declined (Figs 3 and 4).

The rVOR depression was not related to a general retardation in development. Comparison between the rVOR amplitude in stage-matched samples revealed a weaker response of lordotic compared to normal tadpoles. This result was clearly

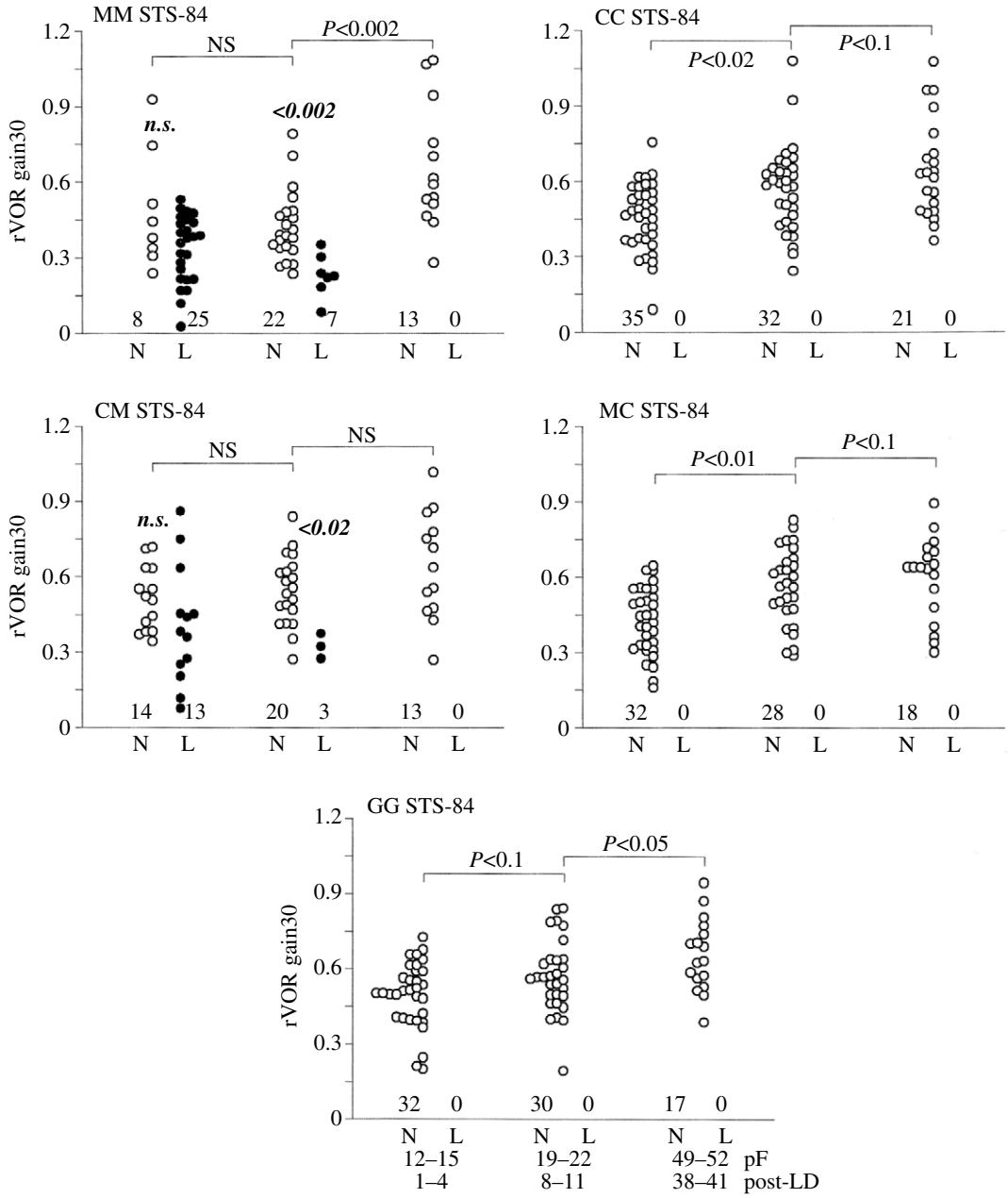


Fig. 4. The gain of the roll-induced static vestibuloocular reflex (rVOR) in *Xenopus laevis* tadpoles with lordotic tail. Post-flight observations about the rVOR gain from tadpoles that flew on the 9.2-day SMM-06 mission compared to their ground-reared siblings. Same animals as in Fig. 3. rVOR gain30 = ratio between the angular eye movement and the lateral roll of 30° from the horizontal to the inclined position. For further explanations, see Fig. 3.

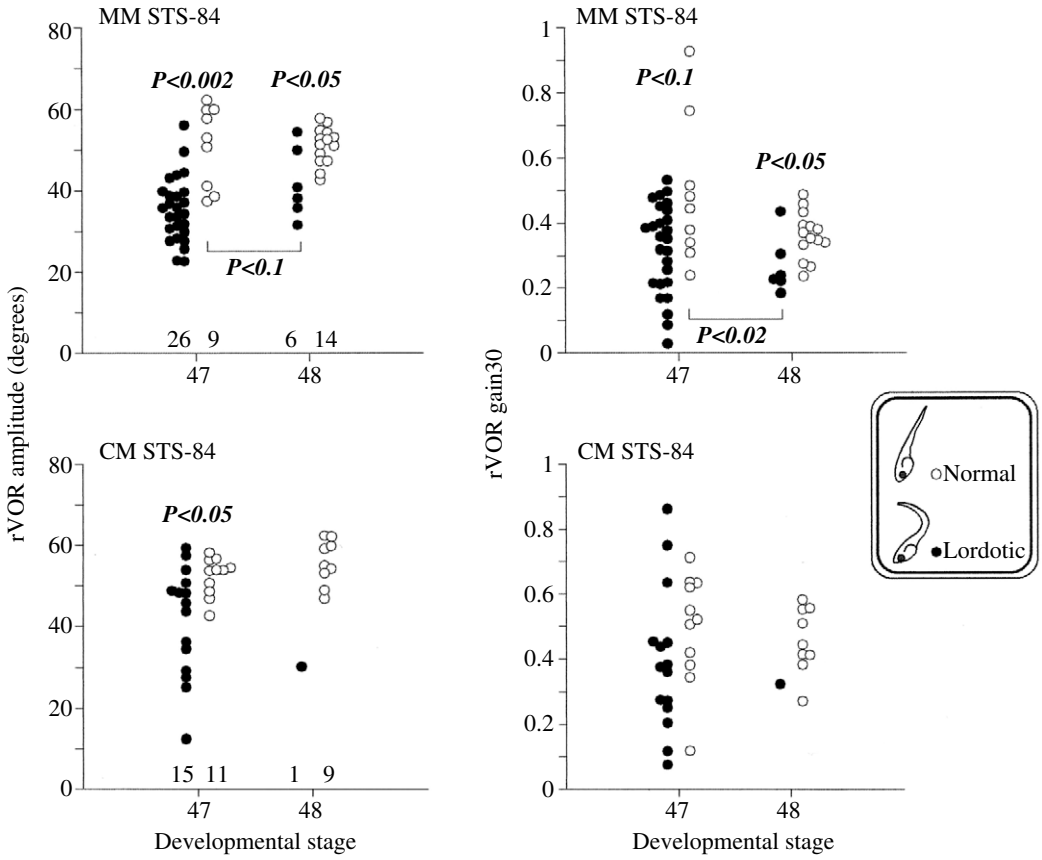


Fig. 5. The static vestibuloocular reflex (rVOR) in *Xenopus laevis* tadpoles with lordotic tail compared to 1***g***-controls in stage-matched samples. Tadpoles are grouped according to their developmental stages 47 and 48 at the time of the rVOR recording. Note the depressed rVOR in lordotic tadpoles within each stage group, in particular for the MM-group, and also that in this group lordotic stage 48 tadpoles have a weaker rVOR than the normal stage 47 tadpoles. For further explanations, see Fig. 3.

demonstrated for the rVOR amplitude in the MM-group. For both stage-47 and stage-48 tadpoles, the lordotic animals always revealed a lower rVOR amplitude than for tadpoles with normal tails (MM stage 47: 35.2° *vs* 53.1°, $P<0.002$; MM stage 48: 39.6° *vs* 52.0°, $P<0.05$). The difference was less pronounced or absent for the rVOR gain (Fig. 5).

*Tadpoles from the STS-55 (D-2) mission*

Experiments from this particular mission have recently been published (Sebastian et al., 1996). However in the context of the observations from the Shuttle-to-Mir experiment, the data were re-evaluated with respect to correlations between occurrence of tail lordosis and rVOR expression. Due to the lower number of specimens, the chances of seeing significant differences were low; however, the available data clearly show tendencies in the same direction as observed after SMM-06: depression of the rVOR in lordotic tadpoles but no microgravity effect in normally developed ones. In particular, the rVOR amplitudes from the ground controls always differed significantly from those recorded from lordotic animals for both recording periods. This also held for the in-flight 1***g***-control (Fig. 6A,B). Normal *vs* lordotic tadpoles of the microgravity group always revealed lower rVOR amplitudes in the lordotic animals, but the differences can only be considered as a trend for the second recording period (normal: 18.7° *vs* 48.8°, $P<0.1$; $N=3$ for each sample). Similar observations were obtained for the rVOR gain (Fig. 6C,D).

*Tadpoles from the Andromède mission to the International Space Station*

At launch, embryos had reached stages 26–27 and tadpoles stage 45. During this mission, specimen from both stage groups developed lordotic tails. The number was low but high enough to get reliable statistical conclusions. The main result was that the stage 26–27 group confirmed the observations from the SMM-06 mission. Lordotic tadpoles revealed the significant depression of the rVOR amplitude while normally developed animals performed the rVOR at the same median level as did 1***g*** controls (μ***g***: 18.1° *vs* 33.2°, $P<0.02$). In the stage-45 groups, lordotic tadpoles revealed a depressed rVOR (μ***g***: 29.2 *vs* 43.0°; $P=0.05$) while normally developed animals showed a significant augmentation of the rVOR (μ***g***: 43.0; 1***g***: 33.0; $P<0.02$) (Fig. 7). Significant differences were also obtained for the rVOR gain. The augmentation of the rVOR in microgravity tadpoles with respect to the 1***g***-ground controls resembled the observation in fish (*Oreochromis mossambicus*) after a 9-day space flight and points to a sensitization of the neuronal network underlying the rVOR (Sebastian et al., 2001).

## Discussion

The present study shows a clear correlation between the occurrence of microgravity-induced tail lordosis and a depression of the roll-induced vestibuloocular reflex (rVOR). Tail lordosis was accompanied by a depression of the rVOR,

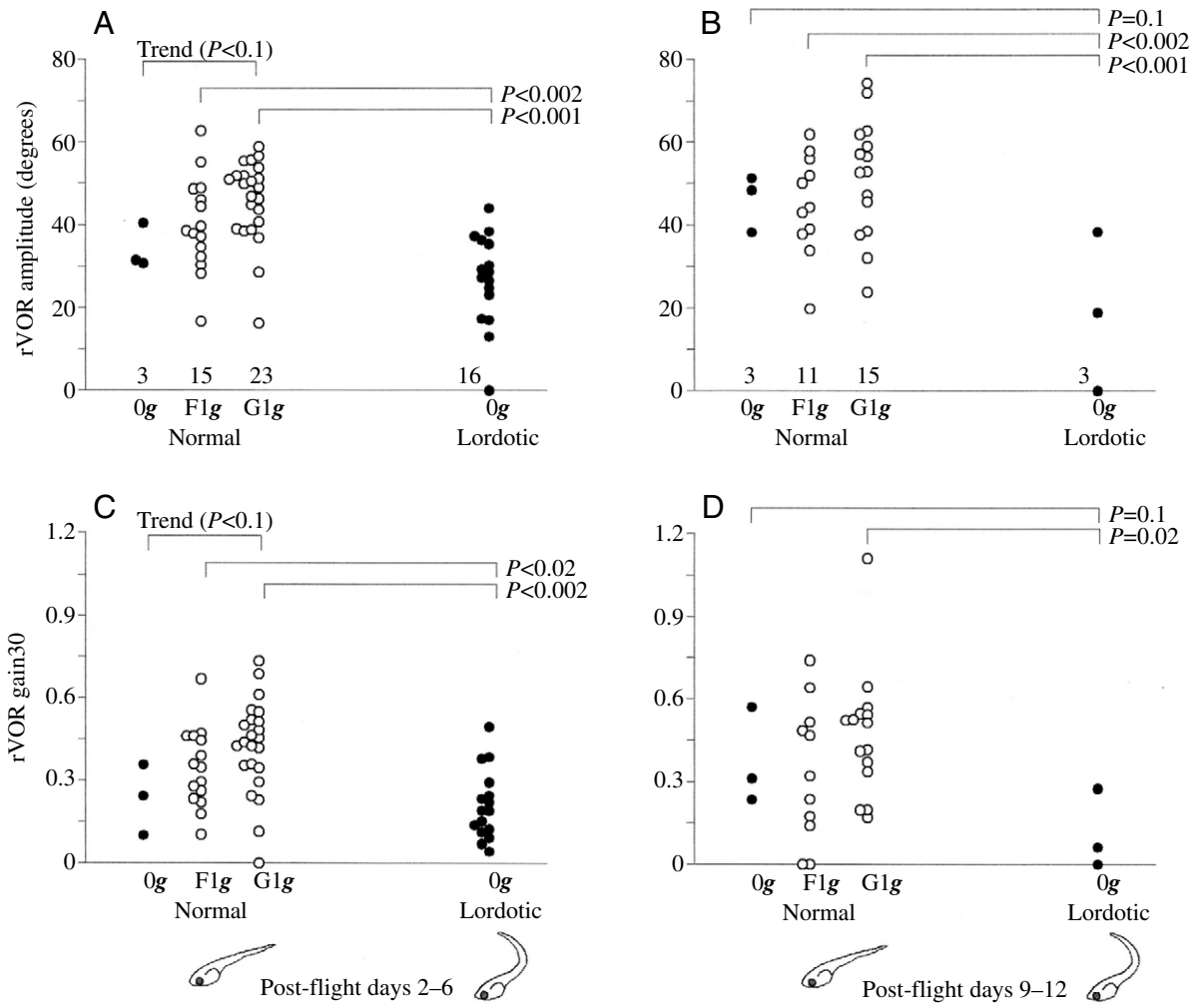


Fig. 6. Post-flight observations of the rVOR amplitude (A,B) and rVOR gain (C,D) from tadpoles that flew on the 10-day German D-2 mission (STS-55, 1993) compared to their ground-reared siblings. At the onset of the mission, tadpoles had reached the developmental stages 33–36. Observations were from post-flight days 2–6 (A,C) and 9–12 (B,D). During each period, animals were tested once. rVOR amplitude and rVOR gain30, cf. Fig. 2. Filled circles, microgravity tadpoles (0***g***); 0***g***-animals were grouped according to the development of lordosis or normal development. Open circles, 1***g***-animals from the in-flight control (F1***g***) and from the ground control (G1***g***). Numbers at the abscissa indicate sample size. Groups that differ significantly are connected by brackets; levels of significance (*P*) are shown.

while normal development of the body in microgravity was accompanied by no rVOR modification in those tadpoles launched at embryonic stages before hatching (Figs 3, 4, 6 and 7, upper plot) or with an augmentation of the rVOR in tadpoles launched when they have already developed their rVOR (Fig. 7, lower plot). Tail lordosis was first described as some kind of body malformation (Sebastian and Horn, 1998) based on the de-loading effects on muscles in microgravity (Snetkova et al., 1995). However, in the context of the observation that tail lordosis is coupled with a depression of the rVOR, neurophysiological components need to be taken into consideration. These might include (1) a general developmental retardation and loss of synchrony in the rostrocaudal developmental gradient of the motor tail system [this gradient was described elsewhere (Roberts and Tunstall, 1994)] as well as (2) a lack or failure of trophic effects of vestibular activity in microgravity. A completely different approach for the understanding of the microgravity effects on body shape and rVOR comes from a consideration of a microgravity susceptibility of secreted growth factors.

### *Neurophysiological aspects of tail lordosis and rVOR development*

A striking observation was that in contrast to normally developing tadpoles, lordotic animals revealed no developmental progress in their rVOR during the observation period of 11 days after termination of microgravity exposure (Figs 3, 4 and 6) in spite of morphological progression as determined by the external morphological markers (Nieuwkoop and Faber, 1967). In some instances, normally developed stage 47 tadpoles performed a stronger rVOR than lordotic stage 48 animals (Fig. 5, upper plots). This

observation points to a significant retardation of vestibular development in the lordotic animals.

In *Xenopus laevis*, swimming and the underlying neuronal network develop when animals have reached stages 27 to 33. This early developmental period is characterized by a rostrocaudal maturation gradient (van Mier, 1988; van Mier et al., 1989), and the separation of the ear vesicle. During this period, the proper development of the vestibular system is initiated and the vestibuloocular system becomes functionally sensitive to macular stimulation by stage 42 (Horn et al., 1986) [definition of developmental stages in *Xenopus* (see Nieuwkoop and Faber, 1967)]. Thus, there is a clear overlap in the development of the spinomotor, vestibular and vestibuloocular system that requires and/or causes a tuning between these developing systems at this period of life to produce a physiologically stable organism. In fact, observations in deafferented bullfrog larvae revealed coordination between ocular counter roll and motor activity in the spinal ventral roots (Stehouwer, 1987). Environmental disturbances such as microgravity might disrupt – in some embryos – this coupling, leading to different morphological and physiological expressions of these systems, as demonstrated by the depressed rVOR and tail lordosis that contrasts the coupling of normal tail development with normal rVOR development (Figs 3–7). Thus, a coordinated development of spinal and oculomotor systems under standard earth conditions might require normal vestibular activity as a neurotrophic factor, which is known from the development of extraocular eye muscles in rats (Brueckner et al., 1999).

It was shown earlier that motor development of *Xenopus* embryos continued independently of functional activity (Haverkamp, 1986). Embryos immobilized by chloretone or

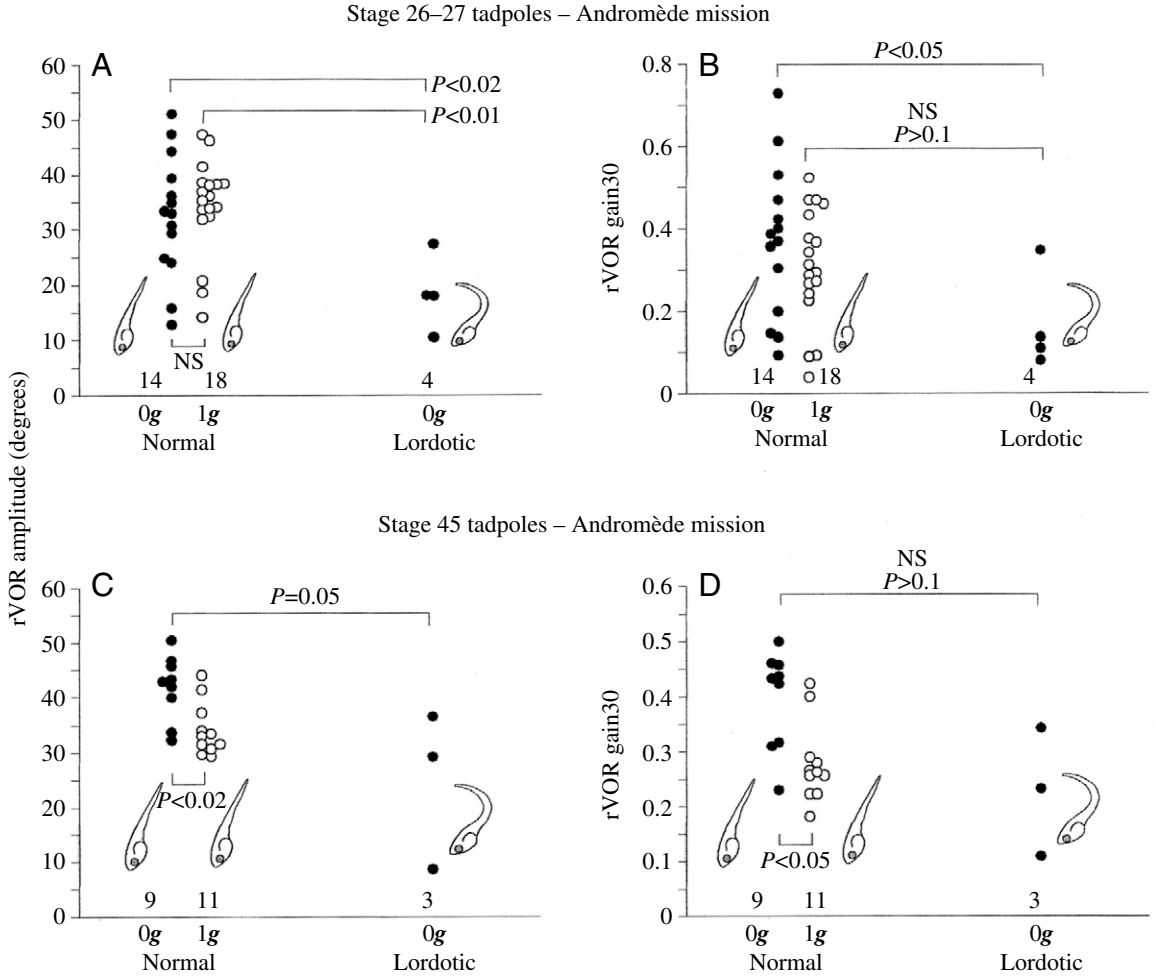


Fig. 7. Post-flight observations of the rVOR amplitude (A,C) and rVOR gain (B,D) from tadpoles that flew on the 10-day French Soyuz taxi flight Andromède to the International Space Station (2001) compared to their ground-reared siblings. At onset of the mission, tadpoles had reached developmental stages 26–27 (A,B) or stage 45 (C,D). Observations were from the post-flight days 2–4. Each animal was tested only once. rVOR amplitude and rVOR gain30, cf. Fig. 1. Filled circles, microgravity tadpoles (0***g***); 0***g***-animals were grouped according to the development of lordosis or normal development. Open circles, animals from the ground control (1***g***). Numbers at the abscissa indicate sample size. Groups that differ significantly are connected by brackets; levels of significances are shown. Note the increase of the rVOR in the old group with normal tails.

lidocain during a period between the late neurula stage and the time of hatching demonstrated normal motor output in the spinal ventral roots during fictive swimming and normal size of motor neurons and their dendritic arborization. However, space tadpoles developed in a freely moving condition. They were active during their exposure to microgravity and, in fact, they revealed an increased motor activity compared to their ground controls (Dournon et al., 2002).

The most likely mechanism for reflex augmentation in the older tadpoles (cf. Fig. 7) is sensitization of the vestibular system by gravity deprivation, as also shown in bullfrogs (Bracchi et al., 1975), fish (Boyle et al., 2000; Sebastian et al., 2001; Wiederhold et al., 2003) and men (Clément et al., 2001). Recordings from the vestibular nerve of bullfrogs during microgravity revealed an initial depression of spontaneous activity but a recovery and overcompensation during ongoing microgravity exposure (Bracchi et al., 1975). Assuming a similar process in the normally developing tadpoles, this recovery and overcompensation of vestibular baseline activity sensitizes the vestibular system and thus augments the reflex after re-entry into 1***g***-conditions.

### *Distribution of secreted growth factors and tail lordosis-rVOR relations*

Developmental processes such as axis formation are strongly affected by the distribution of growth factors. Any disturbance of this gradient can cause morphological modifications, including the body shape (cf. Gilbert, 2003). The neural tube revealed dorsoventral gradients of secreted growth factors. For example, a gradient from a Wnt and BMP (bone morphogenetic proteins) source dorsalizes the neuroectoderm while a BMP antagonist and Shh (Sonic hedgehog) source at the notochord reveals ventralizing features (Wilson and Edlung, 2001; Wessely and De Robertis, 2002). Interestingly, *Xenopus* tadpoles with tail lordosis show notochord abnormalities due to microgravity exposure during space flight (Snetkova et al., 1995). In fact, preliminary studies in *Xenopus* demonstrated a correlation between growth factor and rVOR modification. It was shown that knock-down of the transcription factor Tcf-4 of the Wnt-pathway by morpholino injection in one cell of the 2-cell stage caused tail lordosis and, additionally, depression of the rVOR (Horn et al., 2005). This morpholino-induced lordosis cannot be attributed to a Wnt pathway defect alone because in general, a balance of Wnts, BMPs, and Shh expression levels and patterns determines the establishment of the body axes. Knocking down the level of one factor would give a phenotype similar to the overexpression of one of the other factors. Interestingly, the dorsal/ventral axis of the inner ear is also patterned by a balance of Wnt and Shh (Riccomagno et al., 2005), i.e. the observed modifications of the vestibular system could also be due to Shh overexpression. In conclusion, if microgravity is a disturbing factor of secreted growth factors involved in axis formation, the development of ocular and spinal projections and thus, the development of the rVOR and tail, are probably affected by a spaceflight.

This work was supported by Deutsches Zentrum für Luft- und Raumfahrt (DLR), grants no. 01QV8925-5, 50WB9553-7 and 50WB0140. I thank the BIONETICS team for experimental support at Hangar L/Kennedy Space Center in 1993 and 1997, and the CNES team and, in particular, Dr Michel Viso and Didier Chaput for technical and logistic support during the Andromède mission, the ESA, OHB/Bremen and EADS Space Transportation (former Dornier)/Friedrichshafen teams for technical support during the D-2 and SMM-06 missions, my co-workers Sybille Böser, Konrad Eßeling and Claudia Sebastian for their help during the performance of the experiments during the missions and during the rVOR recordings and analysis, and the Crews of STS-55, STS-84 and the Andromède mission for their careful handling of animal samples and equipment during the flights. All experiments comply with the 'Principles of Animal Care', publication No. 86-23, revised 1985 of the National Institutes of Health, and with the 'Deutsches Tierschutzgesetz', BGBl from February 17, 1993. Permission for the experiments was given by the Regierungspräsidium of Tübingen (Germany), no. 399/Ulm, no. 506/Ulm and 657/Ulm, as well as by the Animal Care and Use Committee (ACUC) at Kennedy Space Center/Florida.

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