

HYPERGRAVITY SUSCEPTIBILITY OF VENTRAL ROOT ACTIVITY DURING FICTIVE SWIMMING IN TADPOLES (*XENOPUS LAEVIS*)

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INTRODUCTION

In vertebrates, the vestibular system controls body, head and eye posture (4) and swimming (7, 8, 9, 22). The vestibular system is also known to control the muscle tone that is necessary to maintain body posture. After hemilabyrinthectomy, animals show asymmetrical postures of head, body or eyes, or perform rotatory swimming (30). In adult amphibians bilateral labyrinthectomy decreased the support of head posture and forelimb support of body posture, and hind limb movements during swimming get uncoordinated (24).

Contrary to lesions, exposure of animals to altered gravity is a method to modify the activity flow within the vestibular system in a controlled manner and without interrupting neuronal pathways by lesions. Studies in adult toadfish (1) and bullfrog (2) as well as in young fish *Oreochromis mossambicus* (33) and in *Xenopus laevis* tadpoles (10, 15, 31, 32, 37) revealed significant physiological and behavioral changes of the vestibular system by modifications of the gravitational environment. The modifications usually persisted for some days after termination of altered gravity. In some instances, the capability of adaptation to altered gravity conditions is related to development. In *Xenopus*, the sensitivity of the roll-induced vestibuloocular reflex (rVOR) to hypergravity depends on the age of the embryos at onset of hypergravity (14, 15); in *Oreochromis*, the sensitivity of the rVOR to microgravity is age-related (33).

The influence of vestibular activity on swimming (7, 8, 9, 22) raises the question to which extent hypergravity affects swimming in an age-related manner like the rVOR. Instead of investigating freely swimming animals during the respective period of life shortly after hatching by means of video recordings (10), modificatory effects of altered gravity on swimming can also be studied by means of *fictive swimming* which is a rhythmic activity recorded from ventral roots in paralyzed tadpoles. The pattern of fictive swimming is characterized by the duration of swimming episodes, duration and frequency of activity bursts, and the propagation of this activity along the rostrocaudal axis (rostrocaudal delay). After hatching, ventral root activity of the tadpoles during fictive swimming correlate to actual movements of the tail during freely swimming concerning cycle period and alternating activity on either side of one segment (17, 18). The parameters of fictive swimming change during development (5, 17, 35).

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The occurrence of activity bursts is linked to the modulating effect of serotonin (5HT) released from the raphespinal projections. Membrane properties of the spinal motoneurons change, too (34). Immigration of descending pathways into the spinal cord during this developmental period (21) leads to the assumption that there is axonal growth in the raphe-spinal projections.

The study focused on the question whether rhythmic activity of spinal ventral roots (VR) during fictive swimming revealed similar sensitivity modifications after hypergravity exposure as the rVOR. The hypothesis was that if the age-related hypergravity effects on the rVOR have their origin in developmental processes within the vestibular nuclei then fictive swimming should also reveal an age-related susceptibility to hypergravity.

METHODS

Animals.

Experiments were performed with tadpoles of the clawed toad *Xenopus laevis*. Adult animals were reared under laboratory conditions at the Animal Research Center at the University of Ulm, Germany. Eggs were collected after HCG-induced mating and carefully controlled for their developmental progress. Staging of *Xenopus* tadpoles was performed according to its standard atlas of development (20). Embryos and tadpoles were reared at temperatures of 20 to 22 °C in tightly closed petriPERM dishes, which had a volume of 25 ml. One side of petriPERM dishes was covered by an air transparent Teflon folie (bioFOLIE25; in vitro/Göttingen, Germany) for air supply. At this period of life feeding is not necessary.

Embryos were controlled for their developmental progress until they had reached the appropriate developmental stage. Ventral root recordings were performed between developmental stage 37/38, which is directly after hatching and stage 47 when the hind limb buds appear. In older paralyzed stages, fictive swimming cannot be induced. The investigated tail area extended from the 4th to the 17th myotome (Fig. 1A, B). The most rostral recording sites were only used in embryos up to stage 43. In tadpoles older than stage 43, large blood vessels limited the access to these roots so that the most rostral recordings were taken from ventral roots of myotome 10 (Fig. 1B). In the majority of experiments two electrodes were used; their distance was always 4 myotomes.

Ventral root recordings.

Extracellular recordings of neuronal activity in the ventral roots started 4 hours after the end of the 3 g-treatment and were performed for 8 days. The animals were paralyzed with α -bungarotoxin, the skin of the tail was partly removed and the ventral root activity was mostly recorded with two suction electrodes placed between the 4th and 17th myotome behind the otic vesicle (Fig. 1). Fictive swimming was induced by stimulating the animals at the tail tip with a mouse whisker. The recordings were amplified, filtered (10-10,000 Hz), digitized (12.5 kHz) and stored before data analysis. Stimulation of the tail by a mouse whisker elicits several episodes of fictive swimming. Each episode which can last a few seconds is composed of short bursts that are repeated at rather regular intervals (Fig. 1). Thus, beside the duration of these episodes, the burst duration, the cycle length and the rostrocaudal delay were determined (cf. also Figure legend). All data were determined from the burst cycles 5 to 16 of the first 3 swimming episodes per animal.

Hypergravity exposure and selected embryonic stages.

Hypergravity by centrifugation of 3 g was used because in *Xenopus laevis* and the fish *Oreochromis mossambicus*, a gravitational force three times higher than earth attraction had a significant impact on the rVOR (15, 16, 32), while 1.4 g, 2.0 and 2.5 g were ineffective in young fish (33). The centrifuge (diameter 20 cm) was identical with that of the BIORACK, a facility for biological experiments in space developed by the European Space Agency (ESA) (19). Between 1985 and 1997, it flew six times in orbit.

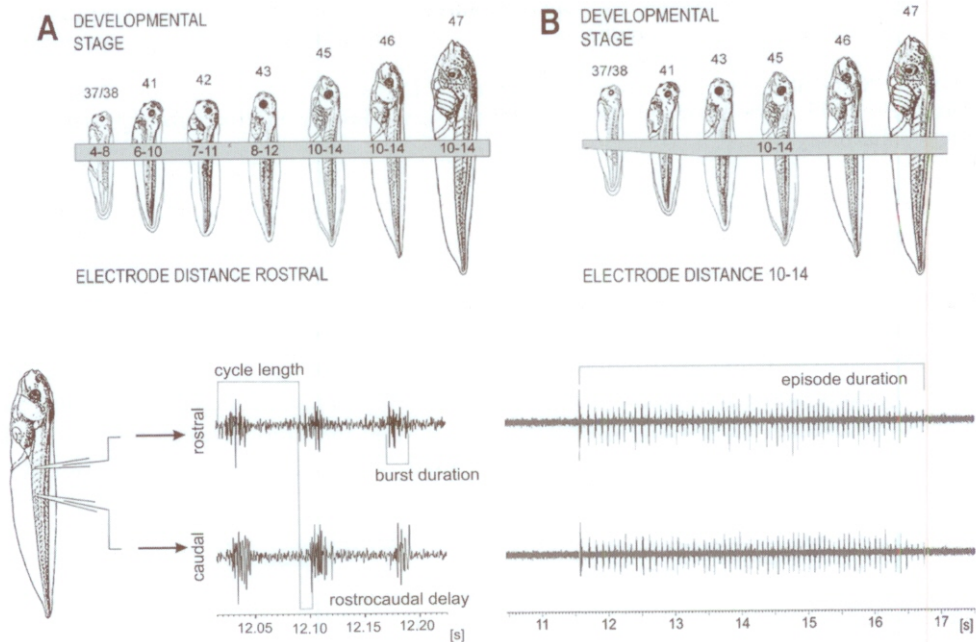


Fig. 1. - Principles to record fictive swimming in embryos and young tadpoles.

Upper: Location of electrodes in two groups of experiments. The distance of the first (= rostral) electrode was defined by the number of myotomes behind the otic vesicle; the second (= caudal) electrode was always placed 4 myotomes more caudally. A: Location as rostral as possible (limitations due to access to ventral roots). B: The rostral electrode is always at myotome 10 and the caudal one at myotome 14. - Lower: Definition of parameters characteristic for the description of tadpole's fictive swimming. *Burst duration* (= time between the first and last significant potential beyond the base noise of the VR recording); *cycle length* (= time between onset of two subsequent bursts), *rostrocaudal delay* (= time between onset of a burst recorded from the rostral and caudal electrode), *episode duration* (= time between the first and last burst of the episode).

The 3 g-treatment started when *Xenopus* embryos had reached critical ages of neuronal and motor development. The youngest group had reached the developmental stages 11 to 19, which is shortly after gastrulation. The second group was at stages 24 to 27, when rhythmical activity in the ventral roots appears for the first time. The third group was at stages 37 to 41, i.e., these tadpoles had hatched and were able to swim at onset of the 3 g-exposure. Higher stages than the 37/41-group could not be included in the hypergravity study because during the 9 to 11 days lasting 3 g-treatment, tadpoles usually develop to stages between 46 and 47. Older paralyzed tadpole stages, however, do not exhibit fictive swimming. During the 9 to 11 days lasting 3 g-exposure the animals were kept in complete darkness at a temperature of 20 °C. The 1 g-controls came from the same parent toads and the same fertilization procedure and were kept in darkness in the same incubator as the centrifuged animals.

During an exposure to hypergravity for periods up to 11 days embryos develop to tadpole stages 46 to 48 independent of the onset of the 3 g period. These stages, however, are not covered by so far known developmental characteristics for fictive swimming (5, 17, 35). Therefore, the study also examines fictive swimming changes in older tadpoles up to stage 47.

Statistics.

For statistics the u-test from Wilcoxon, Mann and Whitney (29) was used because it could not be unequivocally shown that data were distributed as a Gaussian distribution which is a requirement for applying variance analysis and Student's t-test. Differences with an error probability $p < 0.05$ or

smaller were defined as significant. An error probability of $p < 0.1$ was considered as a tendency (*trend*) for a difference. In the figures the mean values and their standard errors (SEM) are used.

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. 657 (Ulm).

RESULTS

1. Developmental characteristics of fictive swimming.

The tadpole's motor pattern changes during the period from hatching to the beginning of metamorphosis. The recording from the most rostral part of the tail as well as from the 10th and 14th myotome show similarities in burst duration, cycle length and episode duration but also show differences concerning rostrocaudal delay and the ratio between the two burst duration values that were recorded 4 myotomes apart. The individual recordings revealed

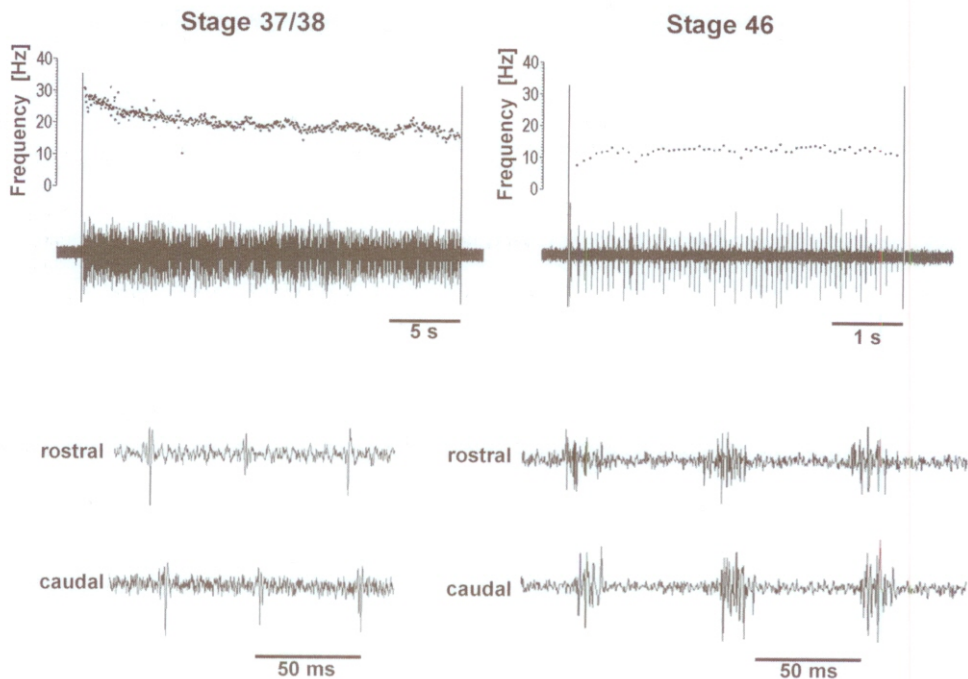


Fig. 2. - Typical patterns of fictive swimming in *Xenopus* embryos and tadpoles.

Representative original recordings from the ventral roots showing fictive swimming in the rostral part of the tail of *Xenopus* tadpoles directly after hatching (stage 37/38) and before the start of metamorphosis (stage 46). Electrode position was 4 and 8 myotomes behind the otic vesicle in stage 37/38, and 10 and 14 myotomes in stage 46. In stage 37/38 the motor pattern shows long episodes with a higher burst frequency (i.e., the reciprocal value of cycle length) at the beginning. In stage 46 the episodes are shorter and the burst frequency remains almost stable. Note different time calibrations for the young and the old tadpole in the upper plots. The representative original recordings presented in the lower part of the figure reveal that burst duration is more extended in the old animals.

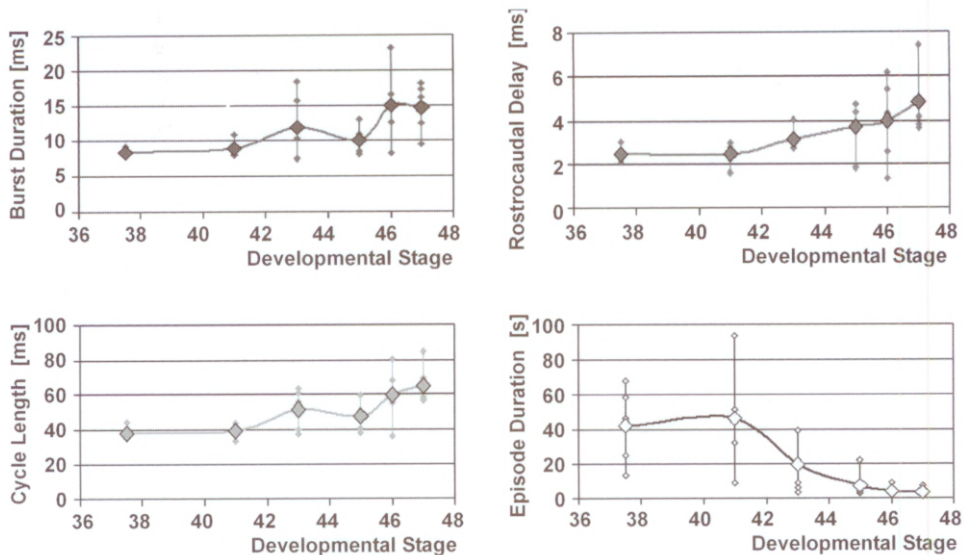


Fig. 3. - Developmental characteristics of fictive swimming in *Xenopus laevis* tadpoles.

Developmental stages ranged from stage 37/38 to stage 47 (definition of stages, cf. [20]). In older tadpoles, fictive swimming cannot be recorded because "struggling" dominates ventral root activity. The recording site was always 10 myotomes behind the otic vesicle. For the determination of the rostrocaudal delay recordings from segments 10 and 14 were used in all stages. Mean values and single data of each animal ($n = 5$; for each stage, the vertical line connects the minimum and maximum values) are shown. The differences between stage 37/38 and stage 47 are significant for all 4 parameters ($n = 5$; $p < 0.02$, at least).

that immediately after hatching motoneurons of *Xenopus* embryos produce only 1 impulse per cycle (cf. also [34]) while they produce several impulses (= burst) per cycle in older stages. In young tadpoles, the burst frequency decreases during an episode (Fig. 2 upper left) but not in older tadpoles (Fig. 2 upper right).

The developmental characteristics of the ventral root activity recorded at the myotomes 10 and 14 revealed that between stages 37/38 and 47 burst duration, cycle length and rostrocaudal delay increased while the episode duration became shorter (Fig. 3). Mean values for the burst duration increased from 8.3 ms to 14.7 ms, the cycle length from 38 ms to 65 ms, and the rostrocaudal delay from 2.5 ms to 4.8 ms. Episode duration decreased by more than 90% from 42 s in stage 37/38 to 3.6 s in stage 47. All differences between stage 37/38 and stage 47 were significant ($n = 5$, $p < 0.02$).

If the electrodes were positioned more rostrally, similar results were obtained (Table 1), except for the rostrocaudal delay, which remained at about 4 ms for all stages (Fig. 4, upper right). The ratio between the rostral and caudal burst duration values showed an age-dependent alteration. At the most rostral recording sites the ratio increased from 1.07 at stage 37/38 to a maximum of 1.33 at stage 43 (cf. Fig. 4, lower right); in older stages it decreased to 1.09 at stage 47. This phenomenon was not observed when the electrodes were placed 10 and 14 myotomes behind the otic vesicle in all stages. At these sites the burst duration ratio was 1.1 to 1.2 (Fig. 4, upper left). A ratio higher than 1.0 indicates that the bursts in the caudal roots were always shorter than those recorded from the rostral ones.

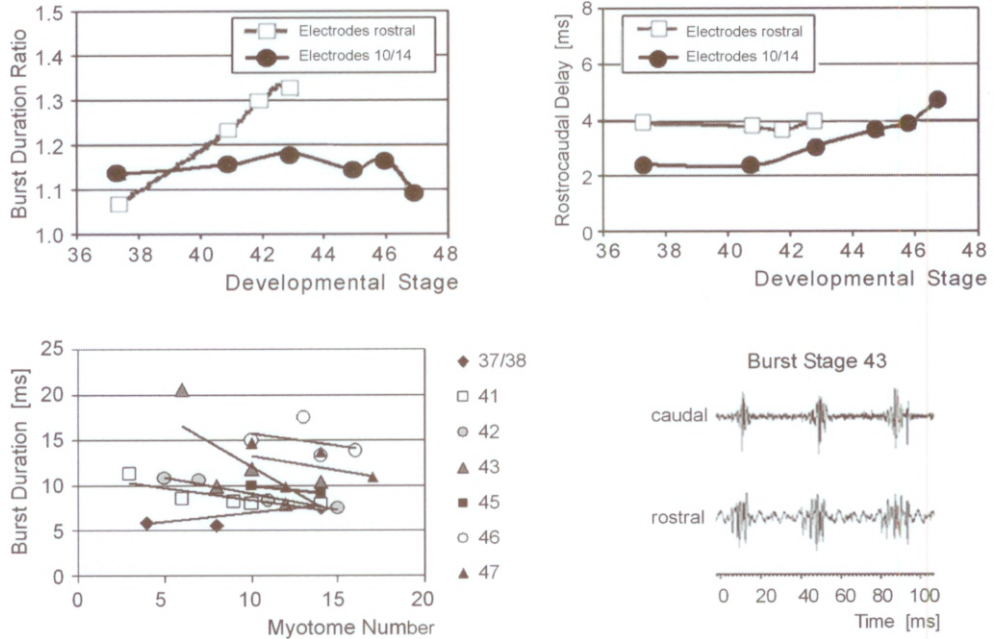


Fig. 4. - Development of fictive swimming patterns in relation to the recording site.

Upper plots: The ratio between rostrally and caudally recorded bursts, and for the rostrocaudal delay if the rostral and caudal recording electrodes were located at myotomes 10 and 14, respectively (cf. Fig. 1B) (black dots) or if they were shifted to more rostrally located myotomes (cf. Fig. 1A) (squares). In all tests, the electrode distance was 4 myotomes (mean values of $n = 5$ tadpoles for each stage). The ratio between the rostral and caudal burst duration was independent of the stage if they were located at myotome 10 and 14, but increased in younger stages up to 1.3 if they were moved more rostrally (left). In contrast, the rostrocaudal delay maintained at an almost constant level during early development if the more rostrally both electrodes are located while if electrodes were positioned at the intermyotomal clefts of myotome 10 and 14, the rostrocaudal delay was significantly lower for stage 37/38 ($p = 0.01$) but increased during further development in this part of the tail. A representative recording from a stage 43 tadpole reveals the longer burst duration at the rostral electrode compared to the caudal one (lower right). - Lower left: Relation between the recording site (myotome number) and burst duration. For the burst duration, two aspects can be demonstrated. For stages 37/38 to 43, the regression lines calculated for the burst durations at each recording site and for each tested stage (cf. right handed definitions of markers) converge to one level at myotome 15, indicating a progressing burst extension in the rostral part of the tail, while the caudally recorded values remain almost stable. The values of the stages 46 and 47 show an increase of burst duration also in the caudal ventral roots; therefore the regression lines have a weaker slope. Each dot represents the value of burst duration at a defined myotome (number and stage of tadpoles, cf. also Table 1).

To get an overview of the spinal topography of ventral root activity, data from all experiments were plotted in relation to the myotome number of the recording site (Fig. 4, lower left). It is observable that there was a clearly pronounced increase of burst duration in the rostral part of the tail, while burst values remained almost stable at the caudal recording sites during the period of life ranging from stage 37/38 to stage 43. For stages 46 and 47, the caudal values of burst duration increased as the rostral ones (Fig. 4, lower left). This type of topographic relation did not occur for cycle length. Here there was a general increase during the observed developmental period with no distinct differences between rostral and caudal recording sites.

2. Effects of hypergravity on fictive swimming.

During the 3 g-exposure embryos developed at nearly the same pace as the 1g-controls. Body lengths as well as developmental stages were in the same range. The developmental stages determined from the 3 g- and 1 g-tadpoles during the first two days were 46 for the 11/19-group, 46.5 and 46.4, respectively, for the 24/27-group, and 47.0 and 46.3, respectively, for the 37/41-group. During further development, existing differences between 3 g- and 1 g-groups of the respective developmental group disappeared.

For all ventral root recordings with 3 g-tadpoles stage-matched 1 g-controls were used to exclude that developmental progress might cause differences between both groups. The main results was that 3 g-exposure during early development affects the burst duration of ventral root activity in an age-related manner and that in the affected stage adjustment to the 1 g-group occurs after re-exposure to 1 g condition. Embryos that have reached the gastrulation stage at onset of 3 g-centrifugation showed an increased burst duration, while the older embryos were not affected. In particular, in the 11/19-group, the mean burst duration recorded from the nerve at myotome 10 during the first 5 days after termination of the 3 g-period was 21.1 ms (minimum-maximum range: 10.8 to 33.9 ms, $n = 16$), but only 15.9 ms in the stage-matched 1 g-control group (range: 6.1 to 27.6 ms, $n = 18$). The difference was significant ($p < 0.01$). The values obtained from the recordings at the caudal nerve located close to myotome 14 were shorter (mean duration 17.2 ms for 3 g and 12.9 ms for 1 g) but also differed significantly ($p = 0.01$). Cycle length, rostrocaudal delay and episode duration were not significantly affected by 3 g-hypergravity (Table 2). The mean values for the cycle length recorded at the rostral electrode were 82.1 ms (3 g) and 75.0 ms (1 g), the rostrocaudal delay 4.7 ms (3 g) and 4.4 ms (1 g) determined during the first 5 days of recordings. The mean episode durations for this period were 2.7 s for the 3 g- and 2.1 s for the 1 g-group (Fig. 5, left).

If all burst duration data determined during the 8 day lasting observation period were plotted with respect to the day of post-3 g recording, a clear adjustment between both groups

Table 2. - Effects of 3g-hypergravity on the activity recorded from the spinal ventral root during fictive swimming in *Xenopus laevis* tadpoles.

Parameter	Stage 11-19		Stage 24-27		Stage 37-41	
	3 g $n = 16$	1 g $n = 18$	3 g $n = 11$	1 g $n = 12$	3 g $n = 6$	1 g $n = 6$
Episode [s]	2.73 ± 1.50	2.06 ± 0.73	1.95 ± 0.62	2.21 ± 0.77	2.25 ± 1.00	2.85 ± 1.41
Burst, rostral [ms]	21.05 ± 5.66	15.91 ± 4.68	22.48 ± 2.03	18.98 ± 4.73	29.90 ± 8.91	34.55 ± 16.35
Burst, caudal [ms]	17.42 ± 5.05	12.88 ± 3.86	20.43 ± 2.03	17.45 ± 5.00	26.05 ± 6.89	32.82 ± 17.38
Cycle, rostral [ms]	82.09 ± 14.45	75.03 ± 13.28	81.53 ± 10.99	74.28 ± 10.44	117.23 ± 27.34	122.15 ± 23.64
Cycle, caudal [ms]	81.18 ± 13.40	75.12 ± 13.33	81.88 ± 11.05	74.28 ± 10.37	116.63 ± 27.12	122.12 ± 23.69
Rostrocaudal Delay [ms]	4.66 ± 2.15	4.41 ± 1.66	2.91 ± 1.01	3.13 ± 0.81	5.27 ± 3.11	4.48 ± 2.78

Means and standard deviations (SD) are given. For explanation of parameters, cf. Fig. 1.

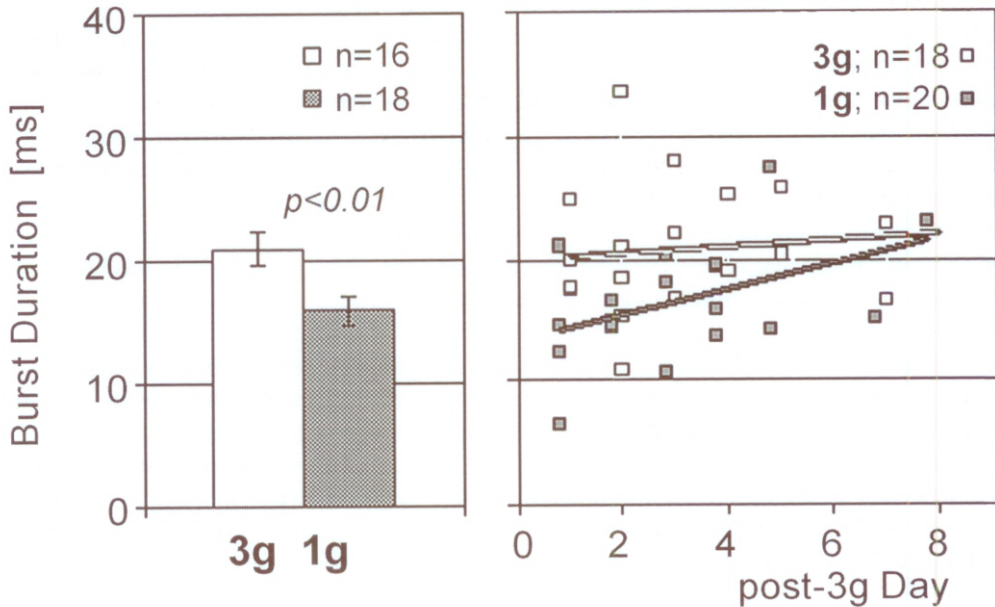


Fig. 5. - Effect of hypergravity on fictive swimming in *Xenopus laevis* tadpoles.

Exposure to 3 g-hypergravity by centrifugation started when the embryos had reached stages 11 to 19; after termination of the 10 days lasting 3 g-period and during the first 5 days of the post-3 g recordings, most animals were at stage 46; the mean stage was 46.2 for both groups. – Left: Mean values of burst duration and their standard errors of mean (SEM) obtained for the first 5 recording days. On the 7th and 8th post-3 g-day, animals had developed to stage 47 in each group; recordings of fictive swimming, however, was possible only in two tadpoles. Note the shorter burst durations for the control group during the first 5 days (left). – Right: The regression lines determined for both tadpole groups converge at post-3 g day 8, indicating a normalization of burst duration in the tadpoles with 3 g-experience. The recording electrode was placed 10 myotomes behind the otic vesicle. Recordings from the VR at myotome 14 revealed similar significant results for burst duration. n, number of tadpoles.

became visible. In particular, the regression line for the 3 g-group was located above the regression line determined for the 1 g-controls and had a lower inclination (Fig. 5, right) than that from the 1 g-tadpoles. At the time of adjustment, all tadpoles had reached stage 47.

When 3 g-exposure started after neurulation there was a lack of statistical significances between 3 g- and 1 g-tadpoles for all parameters determined from the VR-recordings. In the 24/27-group, there was only a tendency ($p < 0.1$) for an increase of burst duration. If the tadpoles were exposed to 3 g after hatching (37/41-group), there was a slight but not significant depression of burst duration compared to the stage-matched 1 g-controls (Fig. 6). Like in the youngest experimental group, cycle length, rostrocaudal delay and episode duration were not influenced in the older groups. Recordings from the VR at the myotome 14 revealed similar results (Table 2).

DISCUSSION

The investigations revealed significant changes of fictive swimming during early periods of life as well as significant effects of augmented gravity on the early postembryo-

nic development of fictive swimming. Developmental changes include an increase of burst duration, cycle length and rostrocaudal delay of fictive swimming up to stage 47, while simultaneously episodes of fictive swimming became shorter (Fig. 3). In addition, rostrocaudal delay and burst revealed topographic differences between rostral and caudal recording sites that were related to the age of the tadpoles (Fig. 4). Hypergravity effects included predominantly a significant increase of burst duration but normalization during further development (Fig. 5). In addition, the stimulating effect of 3 g-centrifugation depended on the age at onset of this period of augmented gravity (Fig. 6). These observations are discussed (1) concerning maturation of myotome connectivity, and (2) concerning modifications of neuronal plasticity within the vestibular system.

1. Maturation of the physiological properties of myotome connectivity topography.

The tail of *Xenopus* tadpoles is characterized by a rostrocaudal maturation gradient (27). Myotome formation starts in the rostral part of the tail; a production rate of 0.9 myotomes per hour was recorded between stage 17 and 40. Neuronal activity could be recorded only in the first 3 intermyotomal clefts in stage 25, while at stage 32/33 this is possible for the first 16 myotomes (38). In addition, the number of brainstem projections to the spinal cord increases during early development of *Xenopus* (21). Our recent stu-

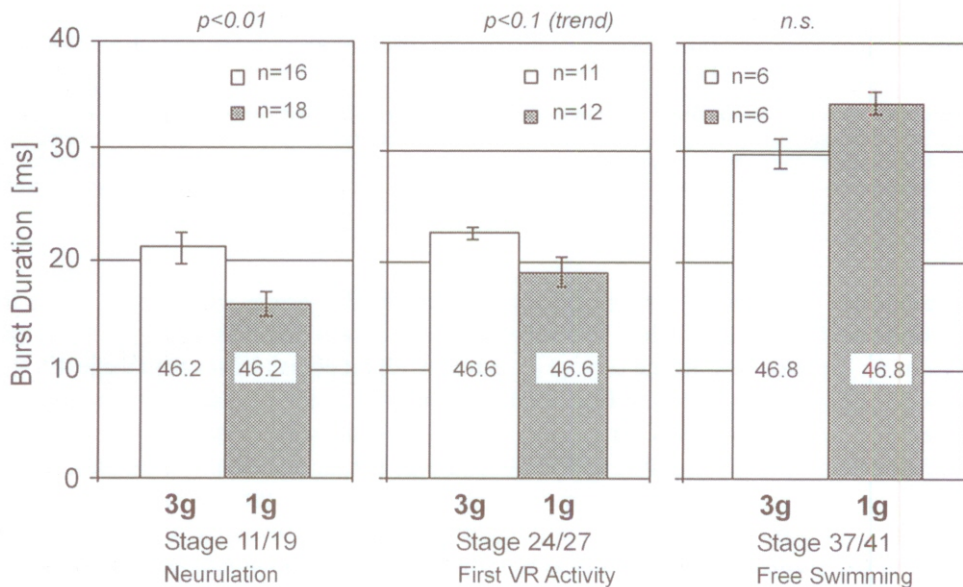


Fig. 6. - Age-dependent sensitivity of burst duration to 3 g-hypergravity.

Mean burst duration in tadpoles with 3g-experience and the stage-matched 1 g-reared controls. The developmental stages at onset of 3 g-exposure are given below the columns. The VR recordings were performed during the days 1 to 5 (stage 11-19 and 24-27) or 1 to 6 (stage 37-41) after the end of the 3 g-exposure. The numbers in the columns give the mean stage of the animals at the time of fictive swimming recording. The electrode position was 10 myotomes behind the otic vesicle. For the more caudally placed electrode, the same observation was obtained (cf. Table 1). In the youngest group the increase of burst duration after 3 g-exposure was significant ($p < 0.01$), in the second youngest group there only was a tendency of an increase ($p < 0.1$) and in the oldest group there was no effect observable (mean values with standard error of mean, SEM).

dies revealed that between stage 37/38 and 43 burst duration increased only in rostral tail parts while for caudal parts it remains unchanged (Fig. 4, upper right and lower left), and that the rostrocaudal delays were longer in rostral than in caudal parts (Fig. 4, upper right).

The gradient of the rostrocaudal delay points to a rostrocaudal gradient in neuronal complexity that is shifting in the caudal direction with increasing age of the tadpole. In particular, a well-established model for the generation of the swimming pattern of tadpoles is the half centre oscillator (5). There is anatomical evidence for this hypothesis, like the existence of glycinergic commissural interneurons in the spinal cord (6). It has been observed that the rostrocaudal delay constitutes 1-2 ms per myotome (36). Therefore it is likely that the coordination of segmental activity is performed by monosynaptic connections between segmentally arranged neuronal networks. Descending interneurons in the spinal cord already have been found (28). If they project to motoneurons and descending interneurons of the following segment a monosynaptic activity transport between the segments of the spinal cord is likely.

The results of this study point to a lack of segmental isolation of functional subunits in the caudal tail area in young animals. Although recorded over a distance of 4 myotomes, the rostrocaudal delay was only 2 ms in the very young embryos, which would correspond to a synaptic delay of 0.5 ms/myotome. Instead of accepting these short transmission times from myotome to myotome, it is more likely that immature segments share one functional subunit (Fig. 7, lowest), but develop their own network during maturation (Fig. 7, middle and top). This hypothesis is confirmed by the observation that glycinergic commissural interneurons in the spinal cord also show a decrease in number from rostral to caudal in stage 37/38 (6).

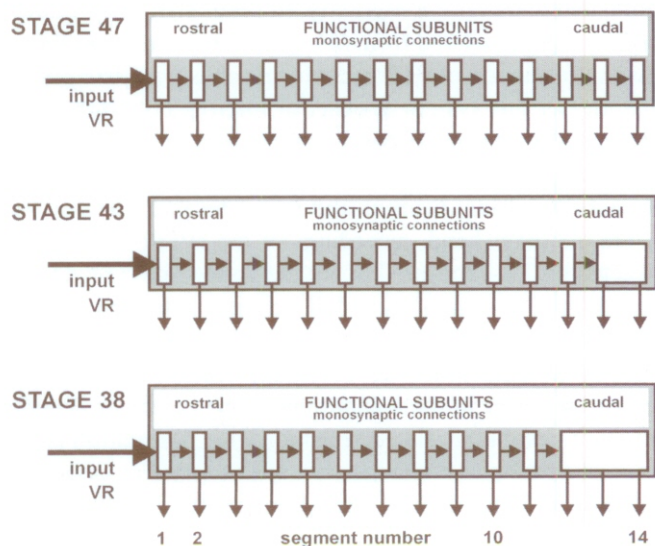


Fig. 7. - Hypothesis about developmental changes in neuronal organization during tadpole development from stage 37/38 to 47.

The picture presents an explanation for the low value of rostrocaudal delay in the caudal tail area of young tadpoles (cf. Fig. 4C). To explain the differences in rostrocaudal delay, the theory assumes that during early periods of life, the more caudally located motor subunits are not isolated in contrast to the more rostral ones.

2. Modifications of neuronal plasticity within the vestibular system.

Burst duration increases during the early development of tadpole embryos to larvae (34, 35) and during the subsequent maturation to stage 47 (Fig. 3). The increase of burst duration by 3 g-exposure (Fig. 5, left) makes it likely that hypergravity causes an acceleration of motor development, i.e., the 3 g-effect on burst duration points to a neurotrophic effect of the vestibular tonic base activity on the development of spinal motor activity which is mediated by the descending pathways from the brainstem to the spinal cord. As serotonin application increase burst duration with rostrocaudal progression from stage 37/38 to stage 42 (35), the developing 5HTergic raphespinal projections might be a target for the 3 g-stimulation.

The hypergravity effect was limited to a certain age. It was significant when 3 g-exposure started before neurulation but not significant when 3 g-exposure started thereafter (Fig. 6). This can be an evidence for a decrease of neuronal plasticity in the motor system with increasing age; similar observations were described for balance and gain compensation of the rVOR and compensation of rotatory swimming behavior after hemilabyrinthectomy in *Xenopus* tadpoles (25, 26).

The onset of synaptogenesis is probably a critical milestone in the adaptation properties of the vestibular system. In fact, at onset of hypergravity the affected embryos were younger than those in which synaptogenesis is seen for the first time. The period from initial to mature stages of neuromuscular synaptogenesis ranges from stages 23 to 48 (11). Nerve fibres were seen in the myotomes only in some embryos at stage 19, and discrete patches of high ACh receptor density began to appear at the ends of the myotomes at stage 22 (3, 23). Furthermore, presynaptic specializations such as the synaptic vesicle clusters and the presence of calcitonin gene-related peptide (CGRP) were first demonstrated in stage 32 (23).

Thus it is likely, that the developing motor system demonstrates its strongest adaptability if gravitational changes start before the first appearance of synapses at the developing tail muscles, and if further maturation occurs completely in altered gravity. This postulation is supported by results from the synapse formation in cell co-cultures exposed to both simulated and real microgravity. In particular, in co-cultures of spinal neurons and myotomal myocytes isolated from *Xenopus laevis* embryos that were exposed to simulated microgravity formation of ACh receptor patches was strongly affected depending on the level of maturity of this system at onset of microgravity. Inhibition of incidence and area of these patches was obvious if nerve contact took place during or shortly before onset of simulated microgravity but not if microgravity started after formation of stable synaptic contacts between the spinal neurons and myocytes (12, 13).

On the other hand, recordings of the rVOR revealed that older tadpoles were strongly affected by 3 g-exposure but not the young ones (14, 15). This observation is in a clear contrast to the 3 g-sensitivity of VR activity. Thus, gravity might exert its trophic influence on the development of the vestibuloocular and the vestibulospinal systems during different periods of embryonic development leading to the formation of more rigid neuronal networks earlier in the spinal than in the ocular projections, and consistently in the maturation of vestibular related behavior. This postulation is strongly supported by the observations of vestibular compensation after unilateral labyrinthectomy (25, 26) after which the swimming abnormalities are compensated for earlier than the roll-induced vestibular reflex.

SUMMARY

1. Fictive swimming is an experimental model to study early motor development. As vestibular activity also affects the development of spinal motor projections, the present study focused on the question whether in *Xenopus laevis* tadpoles, the rhythmic activity of spinal ventral roots (VR) during fictive swimming revealed age-dependent modifications after hypergravity exposure. In addition, developmental characteristics for various features of fictive swimming between stages 37/38 and 47 were determined. Parameters of interest were duration of fictive swimming episodes, burst duration, burst frequency (i.e., cycle length), and rostrocaudal delay.
2. Ventral root recordings were performed between developmental stage 37/38, which is directly after hatching and stage 47 when the hind limb buds appear. The location of recording electrodes extended from myotome 4 to 17.
3. Hypergravity exposure by 3 g-centrifugation lasted 9 to 11 days. It started when embryos had just terminated gastrulation (stage 11/19-group), when first rhythmical activity in the ventral roots appeared (stage 24/27-group), and immediately after hatching (stage 37/41-group). Ventral root recordings were taken for 8 days after termination of 3 g-exposure.
4. Between stage 37/38 (hatching) and stage 47 (hind limb bud stage) burst duration, cycle length and rostrocaudal delay recorded between the 10th and 14th postotic myotome increased while episode duration decreased significantly. In tadpoles between stage 37 and 43, the rostrocaudal delay in the proximal tail part was as long as in older tadpoles while in caudal tail parts, it was shorter. During this period of development, there was also an age-dependent progression of burst extension in the proximal tail area that could not be observed between the 10th and 14th myotome.
6. After termination of the 3 g-exposure, the mean burst duration of VR activity increased significantly ($p < 0.01$) when 3 g-exposure started shortly after gastrulation but not when it started thereafter. Other parameters for VR activity such as cycle length, rostrocaudal delay and episode duration were not affected by this level of hypergravity.
7. It is postulated that (i) functional separation of subunits responsible for intersegmental motor coordination starts shortly after hatching of young tadpoles; and that (ii) gravity exerts a trophic influence on the development of the vestibulospinal system during different periods of embryonic development leading to the formation of more rigid neuronal networks earlier in the spinal than in the ocular projections.

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