Locomotor Pattern in the Adult Zebrafish Spinal Cord In Vitro

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1Department of Neuroscience, Nobel Institute for Neurophysiology, Karolinska Institutet, Stockholm; 2School of Life Sciences, Södertörns Högskola; 3Department of Biosciences and Nutrition, Novum, Karolinska Institutet, Huddinge, Sweden; and 4Department of Biology, Emory University, Atlanta, Georgia

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Gabriel JP, Mahmood R, Walter AM, Kyriakatos A, Hauptmann G, Calabrese RL, El Manira A. Locomotor pattern in the adult zebrafish spinal cord in vitro. J Neurophysiol 99: 37–48, 2008. First published October 31, 2007; doi:10.1152/jn.00785.2007. The zebrafish is an attractive model system for studying the function of the spinal locomotor network by combining electrophysiological, imaging, and genetic approaches. Thus far, most studies have been focusing on embryonic and larval stages. In this study we have developed an in vitro preparation of the isolated spinal cord from adult zebrafish in which locomotor activity can be induced while the activity of single neurons can be monitored using whole cell recording techniques. Application of NMDA elicited rhythmic locomotor activity that was monitored by recording from muscles or ventral roots in semi-intact or isolated spinal cord preparations, respectively. This rhythmic activity displayed a left–right alternation and a rostrocaudal delay. Blockade of glycinergic synaptic transmission by strychnine switched the alternating activity into synchronous bursting in the left and right sides as well as along the rostrocaudal axis. Whole cell recordings from motoneurons showed that they receive phasic synaptic inputs that were correlated with the locomotor activity recorded in ventral roots. This newly developed in vitro preparation of the adult zebrafish spinal cord will allow examination of the organization of the spinal locomotor network in an adult system to complement studies in zebrafish larvae and new born rodents.

INTRODUCTION

The neural network located in the spinal cord can produce locomotor movements in the absence of inputs from the brain or sensory afferents (Grillner 1981; Pearson 1993; Rossignol et al. 2006). The architecture of this network has been characterized electrophysiologically and anatomically in lamprey and Xenopus tadpole (Buchanan 2001; Dale and Kuenzi 1997; Grillner 2003; Roberts et al. 1998). Based on the molecular signaling responsible for their specification during development, different groups of interneurons have been identified (Goulding and Pfaff 2005; Jessell 2000). In both mice and zebrafish, the functional role of some of these interneurons in the locomotor network is examined by combining electrophysiological and molecular analysis (Bhatt et al. 2007; Gordon and Whelan 2006; Kiehn 2006; Kiehn and Butt 2003; Kimura et al. 2006).

The zebrafish embryo and larva are attractive model systems to characterize the spinal locomotor circuitry because of their accessibility for electrophysiology and genetic manipulations as well as the availability of mutant animals with locomotor deficits (see Granato et al. 1996). Rhythmic activity in embryos is thought to be generated by a network of electrically coupled motoneurons and a subset of interneurons that does not involve chemical transmission (Saint-Amant and Drapeau 2001). In larvae there is a topographic pattern not only of recruitment of motoneurons but also of excitatory and inhibitory interneurons that underlies swimming of varying speeds (McLean et al. 2007). During escape in larvae, stronger movement is produced by a graded increase in the activity of the same population of interneurons rather than by recruitment of quiescent neurons (Bhatt et al. 2007). However, it is not known whether the features of the spinal locomotor networks at early development stages and the mechanisms of recruitment of neurons are conserved or whether they are subject to refinement as zebrafish grow into adulthood. To address these issues, it will be necessary to access the adult spinal cord at the cellular and network levels using electrophysiological tools.

In this study, we developed an in vitro preparation of the isolated spinal cord from adult zebrafish. The spinal locomotor networks can be activated by perfusion with excitatory amino acid agonists to generate a rhythmic motor pattern with characteristics similar to swimming in the intact animal. This preparation is also accessible for patch-clamp recordings from motoneurons and interneurons that display rhythmic membrane potential oscillations during locomotion. We describe the motor pattern and examine the role of glycinergic inhibition to provide a foundation for future cellular studies on the architecture of the spinal locomotor network in adult zebrafish.

METHODS

Zebrafish preparations

Zebrafish (ABC and AB/Tuebingen strains) were raised and kept according to established procedures (Westerfield 2000). All experimental protocols were approved by the animal research ethical committee, Stockholm. Juvenile (early juvenile stage: age 30–44 days; late juvenile stage: age 45–89 days) and adult zebrafish (age ≥90 days) were cold-anesthetized in a slush of frozen fish saline [containing (in mM): 134 NaCl, 2.9 KCl, 2.1 CaCl2, 1.2 MgCl2, 10 HEPES, and 10 glucose (pH 7.8 with NaOH, 285–290 Osm)]; Drapeau et al. 1999; Masino and Fetcho 2005]. All steps of the dissection were performed in the slush of frozen saline. The fish were pinned down ventral side up and quickly eviscerated. For the semi-intact preparations, the animals were glued dorsal side up to the recording chamber. The skull was opened and the brain was cut caudal to the tectum. The muscles were cut dorsally and laterally with fine scissors over 50–
The spinal cord was fixed for 1 h [4% formalin/14% picric acid in 0.01 M phosphate-buffered saline (PBS) and washed in 0.3% Triton-X in 0.01 M PBS (X-PBS)]. The cord was then incubated overnight in streptavidin-Cy3 in X-PBS (1:2,000), washed in PBS, dehydrated in ethanol, and cleared in methylsalicylate. When the tissue was completely cleared, the spinal cord was mounted lateral side up on a glass petri dish. Cells were visualized on a confocal microscope (Zeiss LSM 510 Meta).

**Data acquisition and analysis**

Data were digitized at 10 kHz (extracellular recordings) or 40 kHz (patch recordings) with a Digidata 1200/1322A AD converter (Axon Instruments) and acquired on a personal computer using pClamp software (version 9, Axon Instruments). Data analysis was performed in Spike2 (version 5, Cambridge Electronic Design). For the correlation analysis, extracellular traces were rectified, smoothed (time constant 0.01 s), and down-sampled to 1 kHz. From the peak times of these correlation traces, period and phase values of the motor pattern were extracted (Fig. 1Cii). The locomotor period (T) was determined from the time-of-peak of the autocorrelation. The contralateral phase (ϕcontra) was determined from the time-of-peak of the cross-correlation of right recording versus left recording divided by the locomotor period (ϕcontra = Δtcontra/T). The ipsilateral phase (ϕipsi) was determined from the time-of-peak of the cross-correlation between caudal recording and rostral recording divided by the locomotor period (ϕipsi = Δtipsi/T). The value for ϕipsi was then divided by the number of segments that separated the recording sites to obtain the ipsilateral phase lag for one body segment.

The maximum and minimum burst frequencies during wash-in of strychnine were analyzed by marking the bursts using a threshold criterion and measuring the instantaneous frequency of the burst onsets. To analyze the motor pattern in strychnine, the frequency of the large-amplitude bursts in the semi-intact and in vitro preparations was determined by measuring the interburst intervals. The amplitude of the membrane potential oscillation in spinal neurons was measured using a waveform average of 25 cycles triggered on the peak of the depolarization.

All values are given as means ± SE. The significance of differences of means between experimental groups and conditions was analyzed using Student’s two-tailed t-test. P values of <0.05 were regarded as statistically significant.

**RESULTS**

**Motor pattern elicited by NMDA in the semi-intact preparation**

To analyze the basic features of the motor pattern underlying swimming in adult zebrafish (age 127–136 days), locomotor activity was elicited in a semi-intact preparation with exposed spinal cord by superfusion with the excitatory amino acid agonist NMDA. Both left–right and rostrocaudal coordinations were examined using three extracellular suction electrodes. Two electrodes were placed on the surface of the myotomal muscles on opposite sides of the animal, whereas the third was placed caudally (Fig. 1A). Persistent rhythmic locomotor activity was reliably induced by application of NMDA (50–100 μM) and was accompanied by alternating left–right movement of the freely moving tail (Fig. 1A). The recorded motor pattern showed left–right alternation between muscle activity in the same segment and a rostrocaudal delay between different segments (Fig. 1, B and C).

To quantify the phase relationships of the locomotor activity, the EMG traces were rectified, smoothed, and subsequently subjected to correlation analysis. Figure 1Cii shows the autocorrelation of the EMG activity from the rostral right electrode (Rr) and its cross-correlation with that on the contralateral side of the same segment (Lr) and on a caudal ipsilateral segment (Ri). The period and the phase values of the locomotor pattern were calcu-
lated from the peak time of these correlation traces. The locomotor frequency was $5.2 \pm 0.3$ Hz ($n = 8$) in preparations in which swimming activity was induced using $100 \mu M$ NMDA (Fig. 1D). The left–right phase was $0.49 \pm 0.03$ and the ipsilateral phase lag per body segment was $0.017 \pm 0.003$ ($n = 7$ of 8) (Fig. 1D). Thus in the semi-intact preparation NMDA elicits alternating contractions in left and right myotomal muscles that progress caudally, similar to the motor pattern observed in the freely swimming zebrafish (Müller et al. 2000).

Influence of strychnine on the NMDA-induced motor pattern in the semi-intact preparation

To examine the role of glycineergic inhibition in controlling frequency and coordination of the motor pattern, the effect of the glycine receptor antagonist strychnine was tested on NMDA-induced locomotor activity in the semi-intact preparation from late juvenile and adult animals (age 80–136 days). In control conditions, NMDA (100 μM) induced motor activity with a frequency of $5.0 \pm 0.3$ Hz, a left–right phase of $0.47 \pm 0.03$, and a phase lag per segment of $0.018 \pm 0.005$ ($n = 4$) (Fig. 2A). During wash-in of 0.5–1 μM strychnine, the frequency and pattern of the rhythmic activity changed. The individual bursts were initially patterned into recurring episodes of coordinated activity (Fig. 2B, i and ii), which had a mean duration of $2.0 \pm 1.3$ s. These episodes occurred simultaneously at all recording positions, whereas the individual bursts within the episodes still showed left–right alternation (phase: $0.46 \pm 0.02$) and rostrocaudal delay (phase lag per segment: $0.026 \pm 0.009$) (Fig. 2B, ii and iii). The burst frequency was maximal during the first 30% of the episode duration ($25.8 \pm 6.5$ Hz) and decreased to reach minimal values at the end of the episodes ($13.4 \pm 3.8$ Hz).

With prolonged application of strychnine, the episodes of coordinated motor pattern were then replaced with a slow and synchronous rhythmic activity with amplitude larger than that in control (Fig. 2C). The mean burst frequency was $0.07 \pm 0.04$ Hz, the left–right phase was 0.000, and the phase lag per segment was 0.000. During these bursts a stiffening, but no rhythmic movements of the tail, could be observed visually (not shown). These results show that glycineergic inhibition not only controls swimming frequency and left–right alternation but also influences the rostrocaudal phase lag.

Fictive locomotion elicited by NMDA in the in vitro preparation

To examine whether the spinal network is able to generate coordinated rhythmic activity underlying locomotion in the
absence of sensory feedback, we developed an in vitro preparation of the juvenile and adult zebrafish spinal cord. For this, the spinal cord was dissected out from early juvenile, late juvenile, and adult zebrafish (age 30–139 days) and motor activity was recorded from two opposite ventral roots in the same segment and from a caudal ventral root (Fig. 3A).

Application of NMDA (20–50 μM) reliably elicited a persistent rhythmic locomotor pattern (Fig. 3B, i–iii). In the in vitro preparation, the NMDA concentrations necessary to induce locomotor activity were lower compared with those used in the semi-intact preparation, probably due to the better access of the drug to the isolated spinal cord. Because of a low signal-to-noise ratio, left–right alternation and rostrocaudal delay of locomotor activity within the episodes were not always visible from the raw ventral root recordings. However, the correlation analysis of the rectified, smoothed traces shows that motor activity induced by NMDA was alternating between ventral roots on opposite sides and displayed a rostrocaudal delay between ventral roots on the same side (Fig. 3Ci). The frequency of the locomotor rhythm induced by 50 μM NMDA was 7.1 ± 0.4 Hz (n = 24) (Fig. 3D). The contralateral phase was 0.56 ± 0.06 (n = 9 of 24) and did not significantly differ from that obtained in the semi-intact preparation (P ≥ 0.05, unpaired t-test, two-tailed). The ipsilateral phase lag per segment was 0.024 ± 0.010 (n = 7 of 24), and was not significantly different from that of the semi-intact preparation (P ≥ 0.05). Thus the pattern of activity induced in the isolated spinal cord in vitro is similar to that obtained in the semi-intact preparation, indicating that it corresponds to locomotion.

### Fictive locomotor pattern in different NMDA concentrations

To determine the optimum range of NMDA concentration for eliciting locomotor activity in vitro, different concentrations were tested using late juvenile and adult animals (age 55–112 days). At 20 μM, NMDA-induced rhythmic locomotor activity had a frequency of 5.2 ± 0.3 Hz (n = 6) (Fig. 4A–C). During the wash-in the frequency increased initially and sometimes, especially with higher NMDA concentrations (50 μM), a peak was reached within the first 10 min of application (Fig. 4B). After 10–20 min
the frequency remained stable in most cases, but during longer applications in some cases a decrease of the frequency could be observed. In 50 μM NMDA the locomotor frequency was 5.3 ± 0.4 Hz (Fig. 4, A–C). A further increase of the NMDA concentration to 100 μM significantly (P < 0.05) sped up the locomotor frequency to 6.7 ± 0.7 Hz (Fig. 4, A–C). The amplitude of the rectified, smoothed ventral root bursts also increased as a function of NMDA concentration, suggesting that more motoneurons were recruited at higher locomotor frequencies (Fig. 4A). When the NMDA concentration was increased to 100 μM, the rhythm became disrupted (Fig. 4A) and no clear peak in the autocorrelation could be detected (not shown).

We also compared the influence of the age of the animal on the frequency of fictive locomotion induced by 50 μM NMDA in the in vitro preparation. As shown in Fig. 4D, in early juveniles (age 30–44 days) the locomotor frequency was 10.0 ± 1.1 Hz (n = 5), which was significantly higher (P < 0.01, unpaired t-test, two-tailed) than that in late juveniles (6.8 ± 0.5 Hz, n = 11; age 45–89 days). In adults (age ≥90 days) the frequency was 5.9 ± 0.3 Hz (n = 8).

Effect of strychnine on the fictive locomotor pattern

To examine the role of glycinegic reciprocal inhibition for left–right and rostrocaudal coordination in the isolated spinal cord, the effect of strychnine was tested on NMDA-induced locomotor activity in late juvenile and adult animals (age 57–117 days). In control conditions, 50 μM NMDA induced a rhythmic locomotor pattern (frequency: 6.3 ± 0.5 Hz, n = 7) (Fig. 5Ai) that showed left–right alternation (contralateral phase: 0.56 ± 0.07) and rostrocaudal delay of motor activity with the ipsilateral phase lag per segment of 0.011 ± 0.005 (n = 4 of 7) (Fig. 5Aii). When strychnine (0.5 μM) was added, the frequency of the locomotor pattern decreased to 0.69 ± 0.09 Hz and the coordination changed to a synchronous bursting without left–right alternation (contralateral phase: 0.008 ± 0.005) and rostrocaudal delay of motor activity (ipsilateral phase lag per segment: 0.002 ± 0.001, n = 4 of 7) (Fig. 5B). Superimposed on the slow, synchronous bursting large ventral root bursts could be observed after 10–20 min of strychnine application (frequency in 50 μM NMDA and 0.5 μM strychnine: 0.02 ± 0.01 Hz) that were followed by a silent phase before the smaller bursts reappeared and increased in amplitude until the next large burst occurred (Fig. 5C). Together, these results indicate that glycinegic inhibition is important for determining timing, left–right coordination, and rostrocaudal delay.

Activity of primary motoneurons during fictive locomotion

To examine the cellular and synaptic mechanisms underlying locomotor activity, it is necessary to record the activ-
ity of single identified neurons using whole cell recording techniques. To enable the identification of motoneurons (MNs), the fluorescent tracer Alexa-488 conjugated to dex-
tran was injected into the myotomal muscle in the intact animal, one to several days before dissecting out the spinal cord. In those segments where cells were labeled, one to three primary MNs were visible that could easily be recog-
nized by their large cell bodies and dorsal position (Fig. 6 A).

A number of secondary MNs with smaller cell bodies
located more ventrally could also be identified (asterisks in
Fig. 6 Ai). Visually guided patch-clamp recordings were
performed from primary motoneurons using a setup
equipped with IR-DIC optics (Fig. 6Aii). The intracellular
solution contained 0.1% neurobiotin for subsequent identifica-
tion and morphological analysis of the recorded neurons (Fig.
6B). With increasing age, the spinal cord tissue became less
compliant and it was more difficult to move the patch elec-
trode. Thus we performed these experiments in early and late
juvenile animals (age 30–55 days).

We analyzed the pattern of activity of primary moto-
neurons during the locomotor rhythm. In these experiments,
locomotor activity was recorded in one ventral root in the
isolated spinal cord and patch recordings were made ipsilater-
ally three or four segments caudally (Fig. 7A). During fictive
locomotion elicited by NMDA, primary motoneurons showed
oscillations of the membrane potential. The depolarizing phase
of the oscillations occurred in phase with the ventral root burst
(Fig. 7B). The correlation analysis revealed a phase lag of the
peak depolarization in motoneurons in relation to the ventral
root burst (phase lag per segment in 30 μM NMDA: 0.072 ±
0.023; n = 4 of 5; phase lag per segment in 50 μM NMDA:
0.091 ± 0.024; n = 4 of 5) (Fig. 7C).
Effect of different NMDA concentrations on the activity of primary motoneurons

We analyzed how the pattern of activity of primary motoneurons changes by increasing NMDA concentrations. In the absence of NMDA, the membrane potential of primary MNs showed no significant modulation (Fig. 8A, left). After application of 30 μM NMDA, the membrane potential of MNs depolarized and showed oscillations that were correlated with the locomotor pattern recorded extracellularly in the ventral root (Fig. 8A, middle). Application of 50 μM NMDA increased the locomotor frequency and the synaptic inputs received by the motoneurons (Fig. 8A, right). The amplitude of the membrane potential oscillations in primary MNs was 0.36 ± 0.12 mV in 30 μM NMDA and 0.61 ± 0.12 mV in 50 μM NMDA (P > 0.05; n = 4 of 5). These results indicate that the increase in the locomotor frequency in response to increasing NMDA concentration is associated with an increase in the synaptic drive to motoneurons.

Influence of strychnine on NMDA-induced activity in spinal neurons

Finally, the change of activity of primary motoneurons and other unidentified spinal neurons by blocking glycinergic inhibition during expression of the locomotor rhythm was examined. In NMDA (40–50 μM), the frequency of the locomotor pattern was 8.5 ± 0.7 Hz and membrane potential oscillations had an amplitude of 3.6 ± 1.5 mV (n = 7; range 0.1–11.4 mV) (Fig. 8B, left). When glycinergic inhibition was blocked by 0.5 μM strychnine, the locomotor rhythm slowed down to 1.2 ± 0.1 Hz and the amplitude of the membrane potential oscillations was 4.5 ± 1.1 mV (range 0.8–8.1 mV) (Fig. 8B, right). In those experiments where the cell could be held for >10–20 min large depolarizations occurred (amplitude 28.3 ± 2.9 mV; n = 5 of 7) that were accompanied by large ventral root bursts.

DISCUSSION

The aim of this work was to develop both semi-intact and in vitro preparations of the juvenile/adult zebrafish spinal cord that are able to generate a rhythmic motor pattern with characteristics similar to swimming in intact animals. In both preparations, application of NMDA induced rhythmic locomotor activity displaying left–right alternation and rostrocaudal delay. In the in vitro preparation, spinal cord neurons were accessible for patch-clamp recordings to study their pattern of activation during fictive locomotion induced by NMDA. Motoneurons displayed membrane potential oscillations that were correlated with ventral root bursts. The ability of the isolated...
zebrafish spinal cord to produce locomotor activity together with its accessibility for cellular and synaptic analyses will allow us to examine the organization of the spinal locomotor network in an adult system to complement studies undertaken in zebrafish larvae and newborn rodents.

Basic features of the locomotor pattern

In general, a motor pattern could be elicited in the in vitro preparation at lower NMDA concentrations (20 μM) than in the semi-intact preparation (50–100 μM), probably due to the better access of the drug to neurons in the isolated spinal cord. In the semi-intact preparation the locomotor frequency in 100 μM NMDA was on average about 5 Hz, whereas in the isolated spinal cord the frequency in 50 μM NMDA was about 6 Hz. These are within the range of frequencies measured in freely swimming adult zebrafish (Mueller et al. 2000).

In our experiments we observed that the locomotor frequency was higher in preparations from early juvenile animals (age 30–44 days) compared with late juvenile and adult zebrafish (age ≥45 days). In zebrafish larvae 4–5 days postfertilization (dpf), a locomotor rhythm can occur spontaneously or could be induced by light with a frequency of about 30 Hz (Masino and Fetcho 2005). Application of NMDA to spinalized zebrafish larvae 3 dpf induced a rhythmic motor pattern with a frequency of about 18 Hz (McDearmid and Drapeau 2006). In that study, however, very high concentrations of NMDA were required to produce fictive locomotion that, when used in the adult zebrafish preparations, would disrupt the rhythmic motor pattern (see Fig. 4A). A kinematic study in freely swimming zebrafish has shown that the range of tailbeat frequencies was 30–100 Hz at 3 dpf and decreased to 40–55 Hz at 14 dpf (Mueller and van Leeuwen 2004).

In adult zebrafish, swimming frequencies of about 15–50 Hz were measured (Liu and Westerfield 1988), which suggests that during development there is a gradual change within the locomotor network that results in a decrease in the frequency of the motor output as the animal matures.

Activity of primary motoneurons during locomotion

In our recordings from primary motoneurons in the in vitro preparation we could observe small membrane potential oscillations that were in phase with ipsilateral ventral root activity and tended to increase in frequency and amplitude with higher NMDA concentrations. Unlike primary motoneurons recorded in the larvae that readily fire action potentials during fictive locomotion (McDearmid and Drapeau 2006) in the adult spinal cord these motoneurons received only subthreshold synaptic inputs. This is not surprising because primary motoneurons are recruited in the freely swimming adult mainly at very fast swimming speeds (>20 Hz; Liu and Westerfield 1988). We could not drive the spinal pattern-generating networks over the entire range of possible swimming frequencies because at high NMDA concentrations (>50 μM) the locomotor pattern became disrupted.
In general we could observe that ventrally positioned cells (which also had smaller cell bodies) showed larger membrane potential modulations than the larger more dorsally located cells such as primary motoneurons (unpublished observation). In a recent study using larval zebrafish it was shown that there is a ventrodorsal order of recruitment of spinal interneurons as a function of increased locomotor frequency (McLean et al. 2007). If the same order applies to interneurons and motoneurons in the adult spinal cord it would explain the subthreshold activity in the dorsally positioned primary motoneurons.

**Phase relationship of the locomotor pattern**

The semi-intact preparation of the zebrafish displayed a rhythmic undulation of the tail that was correlated with the EMG activity when the spinal locomotor central pattern generator was activated with NMDA (Fig. 1A). EMG recordings from the myotomal muscles in the semi-intact preparation and ventral root recordings from the isolated spinal cord showed both a left–right alternation and a rostrocaudal delay of motor bursts. The rostrocaudal delay per body segment was not...
significantly different in the semi-intact preparation and the in vitro preparation and was on average 0.020 ± 0.005 (n = 14).

The propulsion in aquatic vertebrates like the lamprey, Xenopus tadpole, and zebrafish is mediated by body undulations generated by alternating contractions of myotomal muscles that originate in rostral segments and propagate to the caudal part of the animal. Juvenile and adult zebrafish have about 30 body segments (van Eeden et al. 1998), which means that the phase delay would correspond to 60% for a full wave of activity along the body. In larval zebrafish during fictive locomotion a full wave of activity is generated along the body at any given point in time (cf. Masino and Fetcho 2005 and references therein). However, adult goldfish do not generate a complete wave of activity (63%; Fetcho and Svoboda 1993), which seems to be due to a lower flexibility of the body in the mature fish than in larval zebrafish. We hypothesize that during zebrafish maturation the rostrocaudal delay of motor activity decreases so that the spinal motor network adapts to the constraints imposed by the stiffening of the body.

Role of glycine in generation of the motor pattern

Blocking glycinerergic inhibition during expression of the motor pattern results in synchronous motor bursts on both sides and along the rostrocaudal axis in both the semi-intact and the in vitro preparations, indicating that inhibitory synaptic transmission underlies both the alternating pattern and the delayed propagation of the motor activity. In vertebrates, alternating activity of antagonistic motoneuron pools is maintained mainly through mutual inhibition of segmental spinal half centers by glycinerergic commissural interneurons (Xenopus: Roberts et al. 1998; lamprey: Grillner 2003; mouse: Butt et al. 2002). When these connections are weakened by low doses of strychnine in the lamprey, left–right alternation is maintained and a reduction of cycle period can be observed (Grillner and Wallén 1980; McPherson et al. 1994). However, when glycine receptors are completely blocked by high doses of strychnine in the bath, the contralateral alternation of motor activity changes into a synchronous activation of motoneurons on both sides of the body (rat: Cowley and Schmidt 1995) that is accompanied by a dramatic increase in cycle period (lamprey: Cohen and Harris-Warrick 1984; McPherson et al. 1994).

In our experiments, 0.5 μM strychnine greatly reduced the frequency of the motor pattern in the semi-intact and the in vitro preparations. In the zebrafish larva, left–right alternation was lost without a concomitant change in cycle period (McDearmid and Drapeau 2006). These different actions of strychnine may reflect a difference in network archi-
tecture between larval and juvenile/adult zebrafish. In the semi-intact preparation only large bursts with a frequency of 0.07 ± 0.04 Hz were recorded. In the in vitro preparation small bursts (frequency: 0.69 ± 0.09 Hz) could be recorded in the interval between the large bursts (frequency: 0.02 ± 0.01 Hz). The small bursts were accompanied by small membrane potential oscillations in primary motoneurons and other spinal neurons, whereas the membrane potential strongly depolarized during the large bursts. Only during these depolarizations were action potentials generated in primary motoneurons. We can only speculate why the small bursts that were observed in the in vitro preparation could not be recorded in the semi-intact preparation. It is conceivable that the access of strychnine to the spinal locomotor network was better in the in vitro preparation, causing a motor pattern different from that in the semi-intact preparation, where the small bursts were absent or too weak to record with surface EMG electrodes.

In the semi-intact preparation during the wash-in of strychnine (probably representing a situation when the strychnine concentration inside the cord is still low), there was a transitional period when the continuous motor activity was patterned into individual episodes. Also, bursting frequency within these episodes was increased from 5.2 ± 0.3 Hz to a maximum frequency of 25.8 ± 6.5 Hz. In these respects the motor pattern was reminiscent of fictive locomotion in the zebrafish larva (Masino and Fetcho 2006; McDearnmid and Drapeau 2006). The bursts with significantly larger amplitude that were generated during the slow strychnine rhythm in the in vitro preparation also occurred periodically. Thus two rhythms with different cycle periods and in some cases different phase relationships (synchronous or alternating activity on contralateral sides) can be present at the same time. The presence of two discrete rhythms, previously reported in the lamprey (Cangiano and Grillner 2003; McPherson et al. 1994), shows that strychnine is acting on left–right alternation and cycle period through two separate mechanisms that will have to be investigated in detail in future studies.

Prospects

It was the aim of this study to provide a basic description of the locomotor pattern generated through activation of spinal networks with NMDA in juvenile and adult zebrafish and to demonstrate the potential of both semi-intact and in vitro preparations for future studies. Because of the good accessibility of the spinal neurons and the large spectrum of genetic tools, the zebrafish embryo and larva have emerged as successful new model systems for studying the neuronal control of locomotion. The in vitro preparation of the juvenile and adult zebrafish offers the same combination of molecular tools and accessibility of neurons as in larvae, but in a later developmental stage. It can therefore add a new dimension to the investigation of spinal networks for locomotion.

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