C106

Beat-and-glide swimming in larval zebrafish is an emergent property of their spinal network

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Larval zebrafish (*Danio rerio*) primarily use a beat-and-glide swimming strategy. Here we explore which portion of the central nervous system produces this unique motor pattern. By chemically immobilizing intact zebrafish larvae (α-bungarotoxin, 12.5 μM) and recording from axial motor nerves at 1-3 different points with suction electrodes (Masino & Fetcho, 2005), we first confirmed that electrical stimulation (1 ms, 5-10 V) was eliciting swimming and not struggling, a more powerful rhythmic behavior. 'Fictive' swimming was characterized by cyclical bursts of motor activity (bursts 10.4 ± 0.7 ms long at frequencies of 27.2 ± 1.4 Hz, n = 11, mean ± standard error) that propagated from head to tail (0.7 ± 0.1 ms/segment, n = 5) and alternated from side to side (phase, 49.2 ± 0.1%, n = 9). As in unrestrained zebrafish larvae, we observed periodic bouts of swimming in immobilized larvae (bouts 210.7 ± 24.9 ms long every 4.1 ± 1.2 s, n = 10), which were more frequent after flashes of light (bouts 278.1 ± 21.7 ms long every 1.7 ± 0.2 s, n = 11). A brief electrical stimulus reliably evoked swimming, but persistent stimuli could elicit fictive struggling, which also alternated (phase, 45.7 ± 3.4%, n = 3), but was distinctly slower (13.5 ± 1.2 Hz, n = 3), with longer motor bursts (46.3 ± 3.3 ms, n = 3) in the opposite propagation direction (-6.3 ± 0.8 ms/segment, n = 3). Strychnine (10-15 μM) transformed swimming into prolonged bursts of periodic (140.2 ± 20.5 ms in duration every 1.6 ± 0.4 s, n = 3), bilaterally synchronous (phase, 97.9 ± 0.8%, n = 3) motor activity. This suggested that glycinergic signaling regulated the 'beat', but not the 'glide' mechanism. We next used paired patch-clamp recordings from spinal motor neurons (Drapeau et al., 1999) to assess the effects of spinalization on swimming. The spinal cord was transected between the 2nd-3rd muscle segments under anesthesia (0.2% MS-222). After 20-30 minutes recovery, we observed no periodic bouts of swimming, nor did light elicit any. Electrical stimuli generated spikes in motor neurons and, at higher stimulation levels (10-15 V), evoked extended episodes of swimming (bouts 5.4 ± 1.6 s long at frequencies of 21.3 ± 0.6 Hz, n = 6). Remarkably, once swimming was started, a periodic motor pattern appropriate to generate beat-and-glide swimming continued in the absence of further stimuli (bouts 319.4 ± 57.5 ms long every 3.2 ± 1.9 s, n = 5). While the rhythmic synaptic drive (20.9 ± 3.3 Hz, n = 5) was below threshold in primary motor neurons, it was often sufficient to cause secondary motor neurons to fire. Our data suggest that beat-and-glide swimming emerges from the rhythmic firing properties and connectivity of the spinal network. This locomotor network is likely activated and modulated by descending inputs.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C107

Dorsal root potentials and intersegmental inhibition in the rat spinal cord

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Primary afferent depolarization (PAD) is a phenomenon associated with pre-synaptic inhibition of the effects of primary afferents on their synaptic targets in the spinal cord. PAD is readily evoked by stimulation of other afferents in the same, or closely neighbouring spinal segments. The mechanisms underlying this have been extensively studied (e.g. Rudomin & Schmidt, 1999; Willis, 1999). With electrical stimulation, dorsal root potentials (DRPs which are often taken as a sign of PAD) are also evoked in the roots of more distant spinal segments (e.g. Lidierth & Wall, 2001). These DRPs were the subject of the present study.

Experiments were performed in adult male Sprague-Dawley rats under urethane anaesthesia (1.25 g/kg, i.p., supplemented as required). The trachea, jugular vein and carotid artery were cannulated. The spinal cord was transected at mid-thoracic level and exposed to the cauda equina. Animals were then immobilized with gallamine triethiodide (20mg i.v.). End-tidal pCO2 and heart rate were monitored throughout.

Low intensity (<0.01N) mechanical stimulation of the central pad of the hindpaw (L4 dermatome) was found to evoke DRPs not only in the L5 segment, but also on thoracic roots The thoracic DRPs were delayed relative to those at L5. This delay was approximately equal to that seen when DRPs were evoked by an electrical stimulus to the L6 dorsal root in the same animals (averaging 8.3±5.3ms and 9.5±3.4ms, respectively; mean±S.D., n=10).

PAD evoked in cutaneous afferents of the hindpaw was examined using the technique of terminal excitability testing (Wall, 1958). Monophasic compound volleys, evoked by microstimulation in the deep dorsal horn at L4/5 level, were recorded from the cut and crushed end of the sural nerve exposed in the periphery. Typical results are shown in Fig. 1. Stimulation of both nearby (L3 and 6) and distant (T13 and S1) dorsal roots increased the areas of the sural nerve volleys. The timecourses of the DRPs and of the changes in terminal excitability were closely matched.

Finally, the synaptic potentials evoked in the deep dorsal horn by electrical stimulation of the dorsal root of the same segment were examined. The initial (monosynaptic) component of these potentials was clearly reduced by conditioning stimulation of both nearby and distant dorsal roots.

In conclusion, DRPs evoked at distance occur with low-intensity natural stimulation. They accompany PAD and are associated with inhibition at the first central synapse. While further quantification of these effects is required, it is clear that pathways mediating these DRPs may play an important, and to-date largely ignored role, in setting the functional connectivity of the spinal cord.

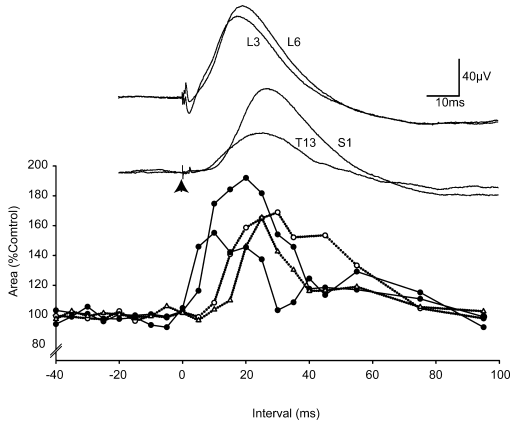

Fig 1. DRPs (above) and changes in sural nerve terminal excitability in response to stimulation of nearby and distant dorsal roots.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C108

Comparison of static fusimotor patterns in the fixed and unfixed hindlimb of the locomoting decerebrate cat

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Studies of locomotor patterns of fusimotor activity in decerebrate cats have been hampered by the need to denervate one hindlimb extensively and to restrict movement to rotation of the ankle joint (Taylor et al. 2000 a,b). It is important to know whether the consequent loss of natural afferent input affects the normal activity patterns. Here we compare this earlier data with new recordings from single spindle afferents of ankle flexor and extensor muscles with no significant denervation and with all limbs walking freely on the treadmill.

Cats (n = 13) were anaesthetised with 2% halothane in 50% nitrous oxide in oxygen (as previously described (Taylor et al. 2000 a) until after premammillary decerebration and killed at the end of the experiment with pentobarbitone overdose. Strain gauges recorded the lengths of tibialis anterior (TA) and gastrocnemius muscles and EMG electrodes were inplanted. Cats were supported by a head holder, a clamp on a low thoracic vertebra and by pins in the iliac bones. The thoracic and pelvic supports were free to move vertically and hung from springs. A small dorsal root filament of L7 or S1, exposed through a left-sided laminectomy was dissected on a floating electrode platform sutured to the vertebrae. Single spindle afferents were isolated from gastrocnemius, soleus or TA and stable recordings from up to 6 units could be achieved. They were characterised by the effects of succinylcholine and by conduction velocity measured under full sodium pentobarbitone anaesthesia after the locomotor recordings. Muscle length changes recorded on tape during walking were reproduced later with a servo, with fusimotor activity suppressed by pentobarbitone. Natural fusimotor activity was deduced from the difference in spindle firing in active and passive spindle movements.

The free limb showed movements very similar to those found in intact cats walking on a treadmill (e.g. Bélanger et al. 1996). EMG bursts in gastrocnemius were more prolonged than in the fixed limb and this corresponded to the stance phase and weight bearing. Despite this difference the active minus passive spindle firing indicated very similar static fusimotor patterns, with firing frequency matching muscle shortening and with phase advance of 0.1 cycle length. In TA, static fusimotor patterns also appeared to match muscle shortening and with similar phase advance, in contrast to the fixed limb, which showed no phase advance in TA.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA44

The functional organization of spinal interneurons revealed by optical, genetic, and physiological studies of zebrafish

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Spinal interneurons are numerous and diverse. Some of their roles in the generation of swimming motor behaviors of Xenopus tadpoles and lampreys are well understood. Two major classes of problems regarding spinal interneurons have, however, proven more elusive. The first is the issue of how patterns of activity in populations of interneurons vary during different behaviors. Much is known about recruitment of motoneurons and their involvement in a range of behaviors, but much less is understood about recruitment of interneurons. The second, more difficult problem has been establishing links between the neuronal classes in relatively simple vertebrates and those in more complex ones, such as mammals. Both of these issues are now tractable through the application of optical and genetic methods in zebrafish.

By labeling different classes of neurons with calcium indicators in transparent larval zebrafish we have explored the patterns of activity in groups of interneurons during different motor behaviors such as swimming and escape. Our work indicates that gradations in the rapid movements of an escape bend are accomplished largely through changes in the activity level in an active pool of interneurons rather than by recruitment of inactive cells. Changes in the classes of active interneurons are associated with the production of very different motor behaviors such as swimming and escape.

One strategy for labeling spinal interneurons for these functional studies is to use the promoters of genes involved in the differentiation of spinal cord to drive expression of genetically encoded fluorescent markers or calcium indicators in subsets of cells. This approach has allowed us to show that Engrailed-1 expression marks a subset of spinal interneurons that are active in swimming and that serve, in part, to gate the flow of sensory information during swimming. These neurons probably play multiple roles both in rhythm generation and in sensory-motor gating. Interneurons with a similar morphology are labeled in transgenic mice in which the Engrailed-1 expressing neurons are targeted. These neurons comprise multiple functional classes in mammals, suggesting that a primitive cell type, similar to the Engrailed positive neurons in fish, may have differentiated into multiple types with more specialized functions in mammals. Optical and genetic tools along with the shared developmental history of vertebrate spinal cord thus allow us to begin to relate neuronal classes in fish to those in mammals. This should help to direct functional studies of the even more diverse array of interneurons in mammalian spinal cord.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA45

Excitatory interneurons controlling swimming in frog tadpoles

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Understanding the source of excitatory drive is critical in deciphering how a vertebrate locomotion central pattern generator (CPG) works. However, the interneurons providing this drive are poorly understood in most vertebrates. *Xenopus* tadpoles at hatching stage 37/38 are capable of swimming away when touched and the locomotion rhythm is generated and maintained by spinal cord and hindbrain neurons (Roberts, 2000). Recently, we have devised a way to make whole-cell recordings from tadpole spinal neurons and to label them with neurobiotin. We have obtained anatomical and physiological evidence that a group of spinal interneurons with ipsilateral descending axons produce excitatory drive during tadpole swimming.

Paired recordings showed that these spinal excitatory interneurons (dINs), with anatomical features defined by neurobiotin filling, directly excited more caudal CPG neurons of all types including: (1) motorneurons, (2) commissural reciprocal inhibitory interneurons, (3) ascending interneurons providing inhibitory feedback to the skin sensory pathway and other CPG neurons, and (4) other dINs. Remarkably, we discovered that dINs corelease both acetylcholine and glutamate as transmitters (Li *et al.*, 2004). This corelease occurred during both miniature EPSCs and unitary EPSCs produced by dINs. Apart from chemical synapses, dINs are also electrically coupled exclusively to other dINs. This coupling is weak and dye-coupling was not seen. Gap junction blockers Carbenoxolone, Heptanol and Flufenamic acid (FFA) could block the coupling but all had clear side effects.

The electrical properties of dINs were examined. Importantly, when the current injection exceeded firing threshold, most dINs only fired one spike at the onset of current injection even when the current was increased up to 6 times above the threshold. dINs didn't fire rebound spikes after negative current injection when the membrane potential was at rest. However, when the membrane potential was depolarised above firing threshold, reliable rebound firing was observed.

During each swimming cycle, dINs fired one spike reliably and their spikes appeared earlier than other CPG neurons at similar longitudinal positions. Since dINs can directly excite all types of CPG neurons, this spike timing difference suggests that dINs are the source of the excitation that normally drives swimming. The column of dINs extends into the hindbrain area where some of them also have ascending axon branches. These ascending axons can form the base for excitatory feedback connections among these hindbrain interneurons. Such connections could underlie mechanisms to sustain swimming activity.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA46

Mechanisms of long-term plasticity in the lamprey locomotor network

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To understand the neural basis of behaviour, identified cellular, synaptic, and molecular mechanisms must be examined in the neuronal networks that generate specific behaviours. The lamprey, a lower vertebrate, allows activity in the locomotor network that generates swimming to be examined in the intact spinal cord in vitro.

A single 10min application of the neuropeptide substance P evokes a long-term (>24 h) increase in the frequency of segmental network activity in adult, but not larval lampreys (Parker 2000). The burst frequency modulation has three phases: an induction phase (~2 h) that is associated with the protein kinase C-mediated potentiation of NMDA receptors; an intermediate phase (~2-15 h) that requires protein synthesis, but not de novo RNA synthesis; and a final phase (>15-20 h) that requires de novo RNA synthesis. At the synaptic level, substance P converts the activity-dependent depression of excitatory network interneuron (EIN) inputs to motor neurons during physiologically relevant spike trains into facilitation, an example of metaplasticity. This effect is associated with a reduction of the transmitter release probability (to allow facilitation), but an increase in the number of docked synaptic vesicles (which ensures that the facilitation does not develop from a reduced initial EPSP amplitude; Bevan And Parker 2004. The synaptic metaplasticity shares the same induction and maintenance mechanisms as the burst frequency modulation, suggesting that the two effects are linked.

While interneuron inputs to motor neurons provide some insight into the plasticity of the network output, it is the connections between network interneurons that probably pattern network activity. The effects of substance P on these connections between network interneurons are now being examined. The analysis is complicated by the uncertainty over the network organisation (i.e. what types of neurons and synapses are involved in generating the network output; Parker 2000). It seems likely that interneurons involved in generating segmental network activity are relatively small, the larger interneurons (lateral and crossed caudal interneurons) that were originally examined having a role in intersegmental coordination. This analysis shows that substance P has synapse specific effects on connections between putative network interneurons: it reduces the strength of connections between the EINs, but increases the strength of feedback inhibition onto the EINs.

The connections between identified network interneurons must now be examined in detail, and the changes at these synapses related to effects on the network output. The molecular mechanisms underlying the protein synthesis-dependent synaptic and network effects are being examined, and should facilitate these analyses.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA47

Rhythmic activity of Renshaw cells during locomotor-like rhythm in the mouse spinal cord

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Inhibitory synaptic transmission is an essential element for the central pattern generator (CPG) for locomotion in the spinal cord. However, the actual role of inhibitory neurons in the mammalian CPG and how they are modulated remains unclear. We examined the synaptic inputs to Renshaw cells (RCs) which provide recurrent inhibition to motoneurons. We performed whole cell recordings from visually identified GABAergic neurons in the lumbar (L2 segment) ventral horn using isolated spinal cord preparations taken from glutamic acid decarboxylase-green fluorescence protein (GAD67-GFP) knock-in mouse neonates (Tamamaki et al. 2003). Animals were humanely killed before isolating the spinal cord. Among the recorded neurons, RCs were identified by 1) electrically stimulating the adjacent ventral root to evoke a short latency EPSP which was blocked by bath-application of a nicotinic receptor blocker mecamylamine or d-tubocurarine with glutamatergic antagonist CNQX (Nishimaru et al. in press), and 2) filling the cell with Alexa-dyes and confirming their expression of calbindin after recording (Carr et al. 1998). Locomotor-like rhythmic activity evoked by bath-application of 5-HT and NMDA was monitored in the L2 ventral root that represents the flexor activity. About 50% of the recorded RCs fired in phase with the ipsilateral L2 rhythm while the other remaining RCs fired out of phase with the ventral root rhtyhm. RCs received both excitatory synaptic inputs and inhibitory synaptic inputs during the locomotor-like rhythmic activity. Blocking the nicotinic receptors by mecamylamine markedly reduced the amplitude of the excitatory synaptic inputs indicating that these inputs are mainly from motoneurons. The inhibitory input, on the other hand, persisted in the presence of the nicotinic blocker suggesting that they are from other sources. Such rhythmic synaptic inputs in RCs could also be observed in a split hemicord indicating that these inputs are from neurons located on the same side. These results suggest that during locomotor activity, RCs are modulated not only by motoneurons but also by the CPG itself.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA48

METACHRONAL PROPAGATION OF BURSTING ACTIVITY IN ISOLATED SPINAL CORD OF NEWBORN RAT

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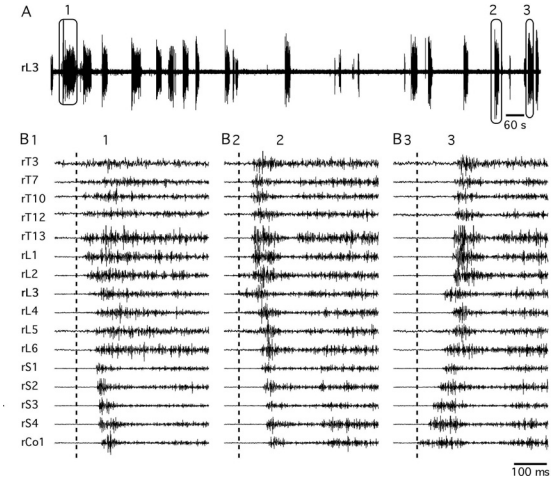
The adequate functioning of the central nervous system requires that the various areas of the body operate together. For example, quadrupedal as well as bipedal locomotion is a complex motor behaviour which requires the simultaneous activation of most body parts including the trunk and neck, forelimbs, hindlimbs and tail. On this basis, therefore, the anatomical specialization of body parts involved in movements should be reflected at the neuronal level. However, the mammalian spinal cord should not be considered as an homogeneous chain of equipotent networks, as is the case, for example, in organisms which exhibit anguilliform locomotion such as lamprey, but as an interconnected ensemble. What are the central mechanisms responsible for coordinating such distributed network activity and to what extent does coupling arise at the spinal level?

The present study was therefore undertaken to address the functioning of the entire spinal cord, studying it as a whole, rather than as separate elements, and to see how its various regions might interact to coordinate motor activity. To this end, simultaneous multisite extracellular recordings were performed at the thoracic, lumbar and sacral levels in an isolated spinal cord preparation of humanely killed newborn rat. Based on a method initially used by others (Bracci et al. 1996), a pharmacological approach involving bath-application of inhibitory synaptic blockers, strychnine and bicuculline, was employed for several reasons: (1) powerful and stereotyped bursts of action potentials are spontaneously produced; (2) the sharp onset of the bursts allows accurate determination of their timing; (3) the suppression of inhibitory connections within the spinal cord reveals underlying connectivity. It was therefore postulated that motor output recorded under these restrictive conditions might reveal coupling and other hard-wired properties of the system.

Motor activity, elicited in the disinhibited network by bath-applying strychnine (glycinergic blocker) and bicuculline (GABAergic blocker), consisted of slow spontaneous bursting. Under these conditions, the recorded bursts were coordinated in 1: 1 relationships at all segmental levels. For each cycle, a leading segment initiated the activity that then propagated in a metachronal way through adjacent segments along the length of spinal cord. There was both regionality nonlinearity and directional asymmetry in this burst propagation: motor bursts propagated most rapidly in the thoracic spinal cord and the rostro-caudal wave traveled faster than the caudo-rostral one. Propagation involved both long projecting fibres and local intersegmental connections. Our results show that motor activity propagates along the spinal cord with a specific temporal pattern and that there is an asymmetry in the propagating characteristics from rostral to caudal, versus caudal to rostral directions. Moreover, it was found that intersegmental coupling relies on a combination of local circuit connectivity as well as long projection fibres. This preparation offers as simplified model for studying network interactions in the mammalian nervous system.



Spontaneous motor bursts are initiated at different segmental levels. Multi-site ventral root recordings were performed in the isolated spinal cord; T, thoracic; L, lumbar; S, sacral; Co, coccygeal. A, single slow time-base recording from the right lumbar ventral root. B, expanded view of three cycles (1, 2 and 3) indicated in A and monitored on 16 ventral roots. Sequential propagation of activity occurredipsilateral side of the cord. In B1 the activity was initiated at the thoracic level, in B2 at the lumbar level and in B3 at the coccygeal level.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.