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Locomotion: Electrical Coupling of Motor and Premotor Neurons

Andrew Prendergast and Claire Wyart*
Institut du cerveau et de la moelle épinière (ICM), 83, bld de l'hopital, 75013 Paris, France
*Correspondence: claire.wyart@icm-institute.org
http://dx.doi.org/10.1016/j.cub.2016.02.021

A new study has found that zebrafish motor neurons can contribute to the generation of locomotor rhythms by feedback modulation of their premotor interneurons via gap junctions.

Motor neurons, while essential for the activation of muscle groups that participate in locomotion, are not classically thought of as active participants in the oscillatory spinal cord activity that leads to their rhythmic activation. Rather, locomotor rhythms are believed to emanate from an upstream network of premotor interneurons that is collectively referred to as spinal central pattern generators, with motor neurons acting solely as output neurons. However, a new study [1] now suggests that, in the zebrafish (*Danio rerio*), motor neurons could contribute to rhythm generation and pattern formation by directly modulating the premotor interneuron pool.

In the spinal cord, *Chx10*$^+$ V2a neurons (also referred to as dIN neurons in *Xenopus* and CiD neurons in fish) are glutamatergic premotor interneurons that exhibit an oscillatory firing pattern during locomotion in phase with and preceeding motor neuron spiking. These neurons constitute a major drive to motor neuron activation during locomotion [2–4] and contribute to the maintenance of left–right locomotor alternation [5,6]. V2a neurons show a wide range of intrinsic properties, connectivity, and recruitment frequency, indicating that they include multiple subtypes that could account for different locomotor speeds and distinct patterns of motor neuron recruitment [7–9]. V2a premotor interneurons have been assumed to lead motor neuron activity in order to generate locomotion.

Some V2as and Motor Neurons Are Electrically Coupled beyond Early Development

Other groups [4,10] had previously reported electrical coupling between V2as and motor neurons in zebrafish, but at early stages of development. Generally, widespread electrical coupling was thought to be a general feature of developing networks that is lost in adulthood. Paired recordings of motor neurons in mammals suggested that electrical coupling in the spinal cord was eliminated gradually over developmental time [11,12], but paired recordings between motor neurons and premotor V2a interneurons have not been performed in mammals.

In their new study, Song *et al.* [1] combine dye-coupling experiments with immunohistochemistry and paired whole-cell recordings in juvenile/adult zebrafish to show that the mature spinal cord remains networked to an extraordinary degree by gap junctions. In addition to observing V2a–V2a and motor neuron–motor neuron coupling, the authors observe that approximately 30% of V2a-motor neuron pairs are dye-coupled, suggesting the two populations are also electrically coupled. In paired recordings, applying a current step to either partner correspondingly alters the membrane voltage of the other cell (Figure 1).

How Might This Electrical Coupling Influence the Locomotor Central Pattern Generator?

Pharmacological blockade of gap junctions in the spinal cord indicates that electrical coupling is necessary to synchronize functional elements in order to generate rhythmic activity, even postnatally [13]. The recent study [1] in juvenile/adult zebrafish now suggests that gap junctions might additionally act as a feedback mechanism from motor neurons onto premotor pools. Within pairs of neurons connected with mixed synapses, depolarizing currents applied to motor neurons reliably increased V2a spiking whereas hyperpolarizing currents decreased V2a spiking. Crucially, this direct feedback mechanism appears to be effective during induced bouts of locomotion as well. When the authors induced fictive locomotor bouts by stimulating descending brainstem projections, the hyperpolarization of motor neurons was sufficient to inhibit, and in some cases completely abrogate, V2a spiking.

Previous studies have shown in mouse and in zebrafish that motor neurons and their cognate V2as are

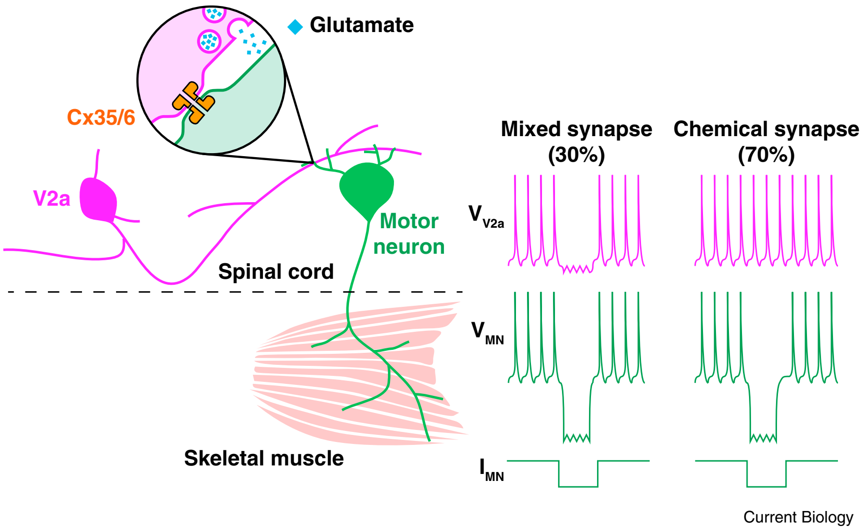

Figure 1. Electrical coupling of motor neurons and V2a premotor interneurons in adult zebrafish spinal cord.
In zebrafish juveniles/adults, excitatory premotor V2a interneurons form either chemical or mixed (chemical and gap-junction-coupled) synapses on the motor neurons that activate skeletal muscle during locomotion. Via paired recordings, Song *et al*. [1] have shown that, at mixed synapses, hyperpolarization of motor neurons is sufficient to inhibit firing of V2as that drive them. This phenomenon does not occur at purely chemical synapses.

functionally organized according to speed (as so-called 'modules') that are progressively recruited at increasing locomotor frequencies [4,9,14–17]. In their recent study, Song *et al.* [1] found that V2a–motor neuron electrical coupling could occur within neurons of a given module type (slow, intermediate and fast) as well as between neurons of adjacent modules. This is consistent with previous work from the same group showing a high degree of within-module connectivity [15]. Electrical coupling could provide an additional mechanism by which adjacent modules might recruit or modulate one another during acceleration or deceleration. More needs to be done to characterize the specificity of the fraction of V2a interneurons showing mixed synapses with motor neurons.

Conclusions and Perspectives

The new study by Song *et al.* [1] reinforces two essential lessons. First, gap junctional networks, though often thought of as transient embryonic phenomena, are likely to persist into adulthood and retain great importance in the adult nervous system. The authors convincingly show that, in the juvenile/adult preparation, this gap junctional network gives motor neurons the potential to modulate the activity of premotor interneurons thought to actively generate the locomotor rhythm. More work will be necessary to investigate whether this V2a–motor neuron coupling persists in adult mammals as well. Second, the concept of the central pattern generator, currently fixated on premotor intermeurons, might need to include motor neurons themselves.

Although retrograde control is not a new concept in circuit neurobiology, it is one that has not frequently been applied to the study of motor neurons. Renshaw cells — glycinergic interneurons receiving collateral afferents from motor neurons — are one long-studied pathway by which motor neurons might exert autoregulatory control. Renshaw cells synapse back onto motor neurons, modulating their firing by shunting inhibition [18]. Whether these connections participate significantly in central pattern generator activity in more physiological conditions remains unclear.

Endocannabinoids, perhaps the most famous retrograde signaling molecules, have been shown by the El Manira lab to be relevant to the motor neuron/central pattern generator interface as well. Excitatory glutamatergic activation of motor neurons induces them to release cannabinoids that can modulate premotor interneurons [19,20]. The new study [1] presents another mechanism by which direct inhibitory input onto motor neurons might retrogradely modulate the premotor neuron pool via electrical coupling (Figure 1). One is left to wonder what other means of feedback between motor neurons and their upstream partners may yet exist.

Altogether this discovery suggests a possible feedback pathway by which some motor neurons could modulate premotor neurons involved in rhythm generation and pattern formation. The relevance of this mechanism on locomotion now remains to be investigated.

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Palaeontology: Scrapes of Dinosaur Courtship

David. W.E. Hone
School of Biological and Chemical Sciences, Queen Mary University of London, Mile End Road, London, E1 4NS, UK
Correspondence: d.hone@qmul.ac.uk
http://dx.doi.org/10.1016/j.cub.2016.02.015

Analysis of numerous trace fossils reveals ‘nest scrape displays’ made by dinosaurs that are analogous to those left by modern birds.

Inferring how extinct animals might have behaved is very difficult. Even when numerous lines of evidence are combined, the results can be equivocal, and because animal behaviour is plastic, firm conclusions can be difficult to reach [1]. Much of the evidence for the behaviour of long dead animals comes from trace fossils that show evidence of an animal’s actions such as footprints, bitemarks, nests, burrows, eggs and so on [2]. Such traces are known for many Mesozoic dinosaurs and can be combined with the body fossil record and our understanding of living species to infer the actions and ecology of these ancient animals. Traces can provide exact records of behaviour, although correctly interpreting even simple behaviours can be difficult [3], and ascribing traces to a given species (or even major clade) has its own problems since traces are only very rarely preserved alongside body fossils [4]. Even so, convincing cases can be made for important behaviours based on the trace fossil record such as in a recent study by Lockley *et al.* [5] reporting on a novel trace fossil for Mesozoic dinosaurs. These are ‘nest scrape displays’ that are known to be produced by a number of living birds but were previously unknown in the dinosaur fossil record.

That birds evolved from dinosaurs is now well established and numerous anatomical traits are shared by modern birds and their dinosaurian ancestors [6] (hereafter the term ‘dinosaurs’ excludes birds). This also holds for numerous behaviours, as many bird-like behaviours are recorded in the fossil record for dinosaurs. This includes communal nesting by duck-billed dinosaurs [7], adults preserved brooding on nests of eggs [8], and some adopting similar resting postures [9] to modern birds with the head tucked to the side and legs folded under the body. Living birds — as the direct descendants of the dinosaurs — therefore form an important part of our understanding of the behaviour of dinosaurs.

The new traces described by Lockely *et al.* [5] are pairs of deep scratches that are up to two metres across made in the ground with the clawed feet of large bipedal dinosaurs (Figure 1). The size of these traces indicates that these were not left by small animals, and the nature of some traces show the outline of large tridactyl feet that clearly point to theropod dinosaurs — the group of primarily carnivorous bipeds that include famous genera such as *Tyrannosaurus*, *Allosaurus* and *Velociraptor*.

Most dinosaur nests were bowl-shaped depressions in the ground,

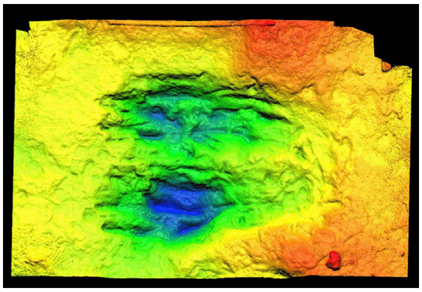

Figure 1. Nest scrape display.
Colourised image of a nest scrap display trace from a larger theropod dinosaur showing the unusual paired scratches (deeper areas are in blue; image: Martin Lockley).

