

Series: Seminal Neuroscience Papers 1978–2017

Science & SocietyTaking a Big Step
towards Understanding
LocomotionClaire Wyart^{1,*}

Locomotion is generated by intrinsically oscillating circuits in the spinal cord that are modulated by information from the brain and periphery. In their seminal 1987 publication, Buchanan and Grillner provided for the first time evidence for excitatory spinal neurons receiving inputs from descending commands and sensory afferents, and synapsing onto motoneurons and commissural inhibitory interneurons. These critical findings established the circuit model for central pattern generators incorporating excitatory interneurons' role in the rhythm-production mechanism.

In the 1980s, after multiple decades of a heated debate that inflamed the field of circuits underlying locomotion, a consensus seems to finally emerge [1]. At its core, the debate focused on the locus of the minimal neural circuit required for generating the rhythmic pattern controlling locomotion. Some researchers had argued that the oscillatory activity of motoneurons driving muscle contractions during locomotion relied on the recruitment of mechanosensory feedback at each cycle. Others defended the idea that locomotion was primarily generated centrally, that is, in the spinal cord, as shown by evidence from multiple species of rhythmic activity of motor neurons induced in the absence of sensory feedback. Eventually, the field came to an agreement that locomotion is generated centrally in the spinal cord, by circuits referred to as central pattern generators

(CPGs) [1,2]. Previous experiments of electrical stimulations had indicated that spinal CPGs could be triggered by descending commands from the brain to initiate or stop locomotion, and were modulated by sensory feedback associated with stimulation of skin afferents in particular [3,4]. One of the open key questions that remained, however, pertained to the precise roles of excitatory and inhibitory spinal interneurons. Despite indirect evidence for their importance in locomotion the overall neuronal organization of spinal CPGs, and the specific contribution of different interneuron types to their operation, was mostly unknown in the early 1980s.

Investigations in simple aquatic animals with undulating locomotion have been instrumental to tackle this question. Along with the *in vivo* *Xenopus* tadpole spinal cord preparation [5], the investigation of cellular mechanisms underlying fictive locomotion in the lamprey spinal cord *in vitro* [6] was a game changer. When the lamprey spinal cord is isolated *in vitro*, application of agonists of glutamatergic receptors induces 'fictive' locomotion in which the properties of the oscillatory activity of motoneurons strikingly resembled those of innate locomotion [7]. The group of Sten Grillner implemented single and double intracellular recordings of neurons combined with multiple extracellular recordings of the ventral nerve root taking advantage of the fact that the *in vitro* lamprey spinal cord preparation remained functional over long time periods. They also developed innovative 'split bath' methodology to perform local manipulation using pharmacology and challenging mechanical manipulation of the spinal cord to mimic locomotor entrainment during active movement. The lamprey spinal cord preparation offered seemingly endless possibilities for novel experiments. While out-of-phase inhibition and in-phase *excitation* onto motor neurons relative

to the ipsilateral ventral nerve root had been observed [8], the source of excitation was unknown. The nature of the excitatory drive onto motoneurons, and its function in the overall circuit organization remained unclear.

By performing audacious double intracellular recordings of interneurons and motor neurons combined with ventral nerve root recordings, James Buchanan and Sten Grillner discovered excitatory interneurons in the spinal cord projecting monosynaptically onto motoneurons and receiving indirect inputs from skin afferents and descending pathways from the brainstem [9]. By simultaneously recording these excitatory premotor interneurons with lateral inhibitory interneurons, the authors discovered that these excitatory premotor interneurons project onto inhibitory commissural interneurons involved in left/right alternation.

Altogether, based on these heroic double intracellular recordings, Buchanan and Grillner proposed an elegant schematic of how a relatively simple circuit map—constituted of reciprocal excitation and inhibition between interneurons, which together with commissural inhibitory interneurons project onto motor neurons—could lead to rhythmic activity patterns in motoneurons with left/right alternation. Consequently to this CPG schematic drawn from the lamprey spinal cord, a similar overall circuit organization has been identified in the *Xenopus* spinal cord by Alan Roberts, Wang-Chang Li, and collaborators (reviewed in [10]).

Subsequently, molecular studies led by the groups of Tom Jessell and Martyn Goulding, and many other teams who implemented fictive locomotion in the mouse, chick, and zebrafish, harnessed the power of genetic approaches and revealed that the vertebrate spinal cord is organized in about a dozen progenitor

domains, each expressing a specific cascade of transcription factors [11,12]. Electrophysiological recordings from genetically identified neurons have identified multiple links between Buchanan and Grillner's schematic model of spinal cord organization and interneurons originating from each progenitor domain [13,14]. These joint discoveries—of a functional map for central pattern generators, and a topographic organization of spinal interneurons based on a cascade of transcription factors—were some of the breakthroughs that led to the nomination of Sten Grillner and Thomas Jessell as co-recipients, along with Pasko Rakic, of the inaugural Kavli Prize in Neuroscience in 2008.

The general architecture of CPGs as drawn by Buchanan and Grillner in their seminal 1987 study largely stood the test of time. Today, innovative approaches, for instance, single-cell RNAseq combined with electrophysiological characterization of single classes of interneurons, reveal an astonishing and unexpected level of molecular and physiological diversity among interneurons, even those originating from a single progenitor domain [15]. Clarifying the relevance of such molecular and physiological diversity to the intrinsic organization of CPGs discovered by the group of Sten Grillner is a major quest for future research.

Acknowledgments

C.W. is a New York Stem Cell Foundation (NYSCF) Robertson Neuroscience investigator (<https://wyartlab.org>). Her work is supported by NYSCF and the HFSP Program Research grant RGP0063/2018.

¹Sorbonne Universités, UPMC Univ Paris 06, Inserm, CNRS, AP-HP, Institut du Cerveau et de la Moelle épinière (ICM) – Hôpital Pitié-Salpêtrière, Boulevard de l'hôpital, F-75013, Paris, France

*Correspondence:

claire.wyart@icm-institute.org (C. Wyart).

<https://doi.org/10.1016/j.tins.2018.09.010>

References

1. Delcomyn, F. (1980) Neural basis of rhythmic behaviour in animals. *Science* 210, 492–498
2. Grillner, S. and Wallén, P. (1985) Central pattern generators for locomotion, with special reference to vertebrates. *Annu. Rev. Neurosci.* 8, 233–261
3. Rospignol, S. *et al.* (2006) Dynamic sensorimotor interactions in locomotion. *Physiol. Rev.* 86, 89–154
4. Hultborn, H. (2006) Spinal reflexes, mechanisms and concepts: from Eccles to Lundberg and beyond. *Prog. Neurobiol.* 78, 215–232
5. Roberts, A. *et al.* (1981) Neural control of swimming in a vertebrate. *Science* 213, 1032–1034
6. Rovainen, C.M. (1979) Neurobiology of lampreys. *Physiol. Rev.* 59, 1007–1077
7. Wallén, P. and Williams, T.L. (1984) Fictive locomotion in the lamprey spinal cord *in vitro* compared with swimming in the intact and spinal animal. *J. Physiol.* 347, 225–239
8. Dale, N. (1986) Excitatory synaptic drive for swimming mediated by amino acid receptors in the lamprey. *J. Neurosci.* 6, 2662–2675
9. Buchanan, J.T. and Grillner, S. (1987) Newly identified 'glutamate interneurons' and their role in locomotion in the lamprey spinal cord. *Science* 236, 312–314
10. Roberts, A. *et al.* (2012) A functional scaffold of CNS neurons for the vertebrates: the developing *Xenopus laevis* spinal cord. *Dev. Neurobiol.* 72, 575–584
11. Jessell, T.M. (2000) Neuronal specification in the spinal cord: inductive signals and transcriptional codes. *Nat. Rev. Genet.* 1, 20–29
12. Goulding, M. (2009) Circuits controlling vertebrate locomotion: moving in a new direction. *Nat. Rev. Neurosci.* 10, 507–518
13. McLean, D.L. and Dougherty, K.J. (2015) Peeling back the layers of locomotor control in the spinal cord. *Curr. Opin. Neurobiol.* 33, 63–70
14. Grillner, S. and El Manira, A. (2015) The intrinsic operation of the networks that make us locomote. *Curr. Opin. Neurobiol.* 31, 244–249
15. Bikoff, J.B. *et al.* (2016) Spinal inhibitory interneuron diversity delineates variant motor microcircuits. *Cell* 165, 207–219

Series: Seminal Neuroscience Papers
1978–2017

Science & Society

Sonic Hedgehog Signaling is Blue: Insights from the Patched Mutant Mice

Daniel J. Merk^{1,2} and
Rosalind A. Segal^{1,2,*}

The Hedgehog (Hh) pathway is a highly conserved signaling system

regulating a range of developmental processes. A 1997 paper by Goodrich and colleagues provided major contributions to understanding the Hh pathway by mutating the gene encoding the Hh receptor, Patched, and thereby developing a mouse model for a human cancer predisposition syndrome, known as Gorlin syndrome. These studies provided one of the first genetically engineered mouse models for brain tumors.

Classic studies in *Drosophila* provided a framework for understanding complex developmental processes and identified critical and highly conserved signaling pathways responsible for intercellular communication. In particular, the discovery of segment polarity genes in *Drosophila* by Nüsslein-Volhard and Wieschaus in 1980 revealed many signaling molecules and pathways and provided mechanistic understanding of pattern formation [1]. These studies enabled the identification of the secreted factor Hh as an inducer of patterning. Subsequent molecular analyses suggested that *hh* and another segment polarity gene, *patched* (*ptc*), antagonize each other during *Drosophila* development and indicated that this opposition orchestrates pattern formation by regulating the expression of target genes such as *cubitus interruptus* (*Ci*) and *ptc* itself [2].

During vertebrate development, inductive tissues including the notochord and the zone of polarizing activity play important roles in patterning the adjacent tissue – the neural tube and limb bud, respectively. The discovery that Sonic hedgehog (Shh), one of three homologs of Hh, is produced in these inductive tissues suggested that Shh may be responsible for pattern formation in vertebrates [3]. A series of experiments by Lisa Goodrich and Ronald Johnson in Matthew Scott's