© 2015. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2015) 218, 3353-3359 doi:10.1242/jeb.113860

COMMENTARY

A classic model animal in the 21st century: recent lessons from the leech nervous system

Daniel A. Wagenaar*

ABSTRACT

The medicinal leech (genus *Hirudo*) is a classic model animal in systems neuroscience. The leech has been central to many integrative studies that establish how properties of neurons and their interconnections give rise to the functioning of the animal at the behavioral level. Leeches exhibit several discrete behaviors (such as crawling, swimming and feeding) that are each relatively simple. Importantly, these behaviors can all be studied – at least at a basal level – in the isolated nervous system. The leech nervous system is particularly amenable to such studies because of its distributed nature; sensory processing and generation of behavior occur to a large degree in iterated segmental ganglia that each contain only ~400 neurons. Furthermore, the neurons are relatively large and are arranged with stereotyped topography on the surface of the ganglion, which greatly facilitates their identification and accessibility. This Commentary provides an overview of recent work on the leech nervous system, with particular focus on circuits that underlie leech behavior. Studies that combine the unique features of the leech with modern optical and genetic techniques are also discussed. Thus, this Commentary aims to explain the continued appeal of the leech as an experimental animal in the 21st century.

KEY WORDS: Leech, Nervous system, Systems neuroscience

Introduction

The medicinal leech (*Hirudo* spp.; Fig. 1A) is a semiaquatic annelid worm that has been an important model organism in many studies in the field of systems neuroscience. As in other annelids, the leech nervous system consists of a ventral nerve cord comprising the cephalic ganglia (or ‘head brain’, which includes supra- and subesophageal ganglia) and the segmental ganglia (Fig. 1B). Additionally, leeches have a prominent ganglion at the caudal end of the nerve cord (the ‘tail brain’), the main function of which is thought to be control of the rear sucker. The majority of the neurons of a leech are located in the segmental ganglia. Each of these 21 nearly identical ganglia contains ~400 neurons, mostly as bilateral pairs. The cell bodies are organized at the ganglion surface in a shell surrounding a central neuropil. Conveniently, the geometric arrangement of those neurons is highly stereotyped (Fig. 1C,D), allowing the identification of homologous neurons both across ganglia within an individual leech and across animals. Helpfully for both electrophysiology and imaging, the somata of most leech neurons are relatively large (15–70 µm in diameter). These facts, combined with the large repertoire of simple behaviors that leeches possess, have facilitated a remarkable range of studies on the neurons and circuits that underlie specific leech behaviors.

Accordingly, this Commentary focuses on the use of the leech as an experimental organism in systems neuroscience and, in particular, on progress in the decade since the appearance of the last major review of leech systems neuroscience (Kristan et al., 2005). I discuss recent lessons in circuit function, behavior and the development of the nervous system. Of particular interest are studies that explain behaviors in terms of neuronal mechanisms. As a comprehensive review of leech cellular neuroscience and molecular biology is beyond the scope of this article, I focus primarily on works that study behavior in terms of neuronal activity.

Neuronal control of behavior

The leech is a particularly good model for level-spanning studies of behavior. Because the leech has a relatively rich behavioral repertoire and a simple, robust and readily accessible nervous system, it is frequently possible to identify specific roles for individual neurons in particular leech behaviors, such as feeding, locomotion and reproduction. Remarkably, even though the leech nervous system is obviously much simpler than vertebrate nervous systems, principles of its function have in several cases been found to have counterparts in more complex animals. To cite but one example, population coding of sensory information, a common principle in the mammalian cortex, can be studied in an attractively simple setting in the leech ‘local bend’ response system (Lewis and Kristan, 1998).

Behavioral hierarchies: don’t bother me while I’m eating

Many animals exhibit behavioral hierarchies – the execution of one behavior stops or prevents the execution of others, and behaviors are differently privileged in their ability to suppress other behaviors. For a leech, for instance, interrupting a meal can be a costly choice because the animal might only eat once every several months (Sawyer, 1986). Accordingly, it is not surprising that feeding is the top priority in the leech behavioral hierarchy (Misell et al., 1998). Stimuli that normally elicit locomotory responses such as swimming and crawling – or even escape behaviors such as local bending and full-body shortening – evoke little or no response while a leech is feeding.

A common mechanism of suppressing competing behaviors is inhibition at an interneuronal level (Jing and Gillette, 2000). However, the dominance of feeding behavior in leeches is uncommonly strong, which led to the hypothesis that it might be implemented earlier, at the sensory level. Presynaptic inhibition of sensory synapses is common in other systems (McGann, 2013), but typically serves to make sensory responses more specific rather than to suppress competing behaviors. To determine how feeding suppresses other behaviors, Gaudry and Kristan (2009) recorded from mechanosensory ‘P’ neurons and their postsynaptic partners in a semi-intact leech preparation in which the anterior half of the animal could engage in normal feeding while the posterior half was accessible to intracellular electrophysiology. Feeding caused the

University of Cincinnati, Department of Biological Sciences, Cincinnati, OH 45221-0006, USA.

*Author for correspondence (daniel.wagenaar@uc.edu)

Received 12 May 2015; Accepted 10 September 2015

Glossary

Central pattern generator (CPG)
A circuit located in the central nervous system that generates the rhythm needed to produce a behavior. Although the output of a CPG is often modulated by sensory feedback, by definition a CPG can produce its output in the absence of external inputs.

Command neuron
A neuron that, when activated, autonomously triggers the execution of a particular behavior.

Connective
A bundle of nerve fibers that connects neighboring segmental ganglia.

Fictive behavior
A motor activity pattern observed in an isolated nervous system preparation that matches the patterns associated with a particular behavior in the intact animal.

Local bend
An escape response in which the leech bends away from a pressure point anywhere on its skin. Mediated by well-characterized circuitry present in each of the segmental ganglia.

Nerve cord
The collective of head brain, segmental ganglia and tail brain that form the nervous system of invertebrates. In leeches, it is located in a blood sinus on the ventral side of the body cavity.

Presynaptic inhibition
Inhibition of a synapse mediated by neurotransmitter release onto the presynaptic terminal.

Segmental ganglion
Each of the 21 nearly identical ganglia that are organized in a linear chain along the ventral nerve cord between the head and tail brains.

Sensilla
Simple sensory organs located in the skin that contain both photoreceptors and mechanoreceptors.

serotonin-dependent inhibition of the output of sensory neurons at their presynaptic terminals. Inhibited synapses included those with target neurons that would normally mediate the local bend escape response. Remarkably, normal fictive swimming could still be induced by the stimulation of a previously described swim-initiating interneuron – a technique that bypasses the inhibited sensory synapses. Evidently, feeding does not induce sufficient inhibition at the interneuronal level to suppress the initiation of other behaviors. It is therefore possible that inhibition at the sensory level is the dominant mechanism by which feeding suppresses other behaviors.

Sensing distension: how does a leech know when it's full?

Although leeches eat infrequently, their meals are large; a single meal can be up to ten times the original biomass of the leech. Consequently, the animal becomes severely distended, and for several days after feeding it will move sluggishly, becoming particularly reluctant to swim. Gaudry and Kristan (2010) used another semi-intact preparation in which the posterior of a leech and most of its digestive system was intact while several anterior ganglia were exposed for electrophysiology to demonstrate a direct relationship between the degree of distention and the duration of evoked swim bouts in response to electrical stimuli. This relationship held both when the leech was feeding naturally and when its intestines were artificially distended using a saline-filled syringe. So how do leeches sense that they are distended? Mechanically stretching an isolated nerve cord did not substantially affect swimming, excluding a role for stretch receptors embedded in the nervous system. To investigate whether stretch receptors in the gut lining or in the body wall play a role in sensing distention (Blackshaw and Thompson, 1988), Gaudry and Kristan dissected away the entire digestive tract, using a procedure in which a leech is turned inside-out (von Uexkuell, 1905). Remarkably, when turned outside-out again, gutted leeches swam with a very nearly normal rhythm, and their response to saline-mediated distension was qualitatively preserved. This experiment, which would have been impossible to perform in most other species, provided strong support for stretch receptors in the body wall as the key players for sensing satiation in the leech.

Cephalic and noncephalic control of behavior

The leech is well known for the degree to which its behaviors are controlled by repeated circuit motifs in the segmental ganglia rather than by the head brain; not only are the central pattern generators

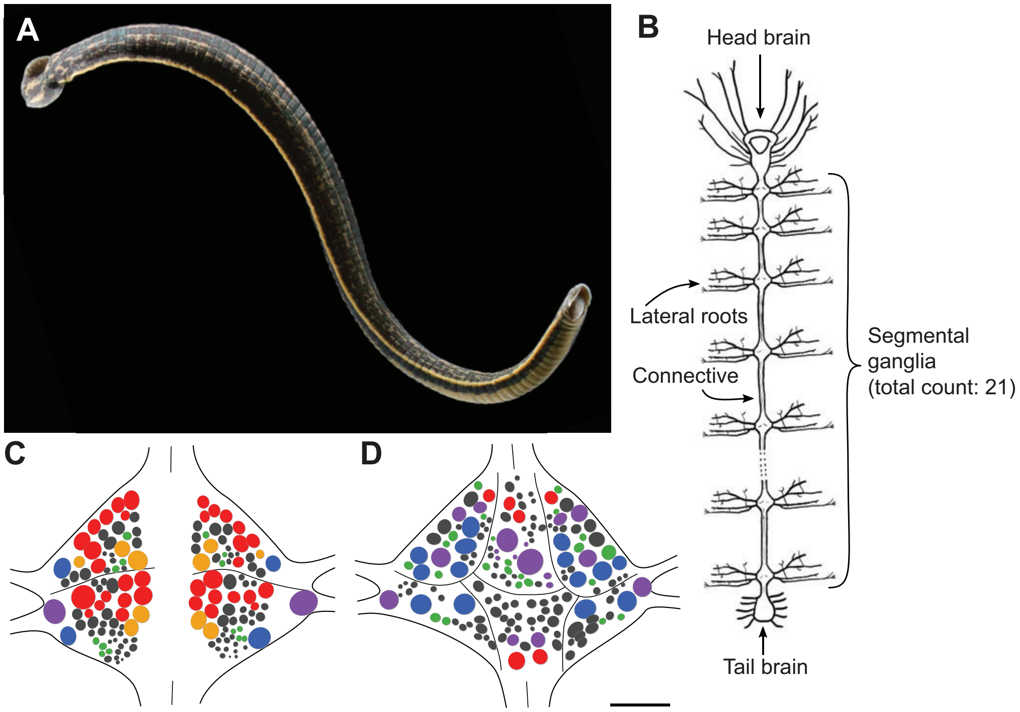

Fig. 1. The leech and its nervous system. (A) A swimming leech, *Hirudo verbana*. (B) Overview of the leech nervous system. (C,D) Canonical maps of the dorsal (C) and ventral (D) aspects of a segmental ganglion. Neurons of known function are colored: blue, sensory neurons; red, excitatory motor neurons; orange, inhibitory motor neurons; green, interneurons; purple, neurosecretory and partially characterized neurons. Gray neurons remain to be characterized. Each of these neurons has been assigned a number (not shown here) that allows researchers to conveniently identify them across experimental preparations. Scale bar: ∼200 μm.

(CPGs) for behaviors such as swimming distributed across the segmental ganglia (Stent et al., 1978), but these ganglia even contain cells that are involved in deciding whether to swim or crawl (Briggman et al., 2005). Because the segmental ganglia are also exceptionally accessible to electrophysiological recording techniques, a lot of attention has been directed at the role of the segmental ganglia in controlling leech behavior. Nevertheless, it would be a mistake to conclude that the head brain plays no role in coordinating behavior. Indeed, specific neurons in the subesophageal ganglion are known to trigger swimming (Brodfuehrer and Friesen, 1986) and crawling (Esch et al., 2002). Recently, two neurons that control whole-body posture have also been found to belong to this group of 'command neurons' (Mesce et al., 2008). Stimulation of one of these neurons causes the leech to elongate and to become rigid, whereas stimulation of the other causes the leech to stop swimming and to bend laterally. Both of these neurons project to the segmental ganglia, following a similar pattern to the neuron described above that triggers crawling.

All of these command neurons are also rhythmically active during locomotion, although their roles in shaping motor patterns remain unknown. Intriguingly, several of the neurons respond reliably and strongly to flashes of light projected onto the leech body, but their roles in visual processing also remain to be determined. One possibility is that they help to mediate the recently described aversion of leeches to UV light (see below; Jellies, 2014a).

Neuroendocrine control of reproductive behavior

Leeches are hermaphrodites – each individual has both male and female gonads. At the onset of mating, two leeches twist around each other, exploring one another with their oral suckers. Mating can involve reciprocal fertilization, in which both partners receive sperm from the other. It has long been understood that supernumerary neurons in the 5th and 6th segmental ganglia control the male and female gonads located in the respective segments (Macagno, 1980). However, until recently, reproductive behavior and its neuronal control had not been described in any detail, in part because leeches are relatively shy breeders – even slight disruptions cause them to abandon their courtship and mating. Electrophysiological recordings from mating leeches therefore remained out of reach for many years.

This situation improved when a chance discovery revealed that hirudotocin (Salzet, 2006) and conopressin (Cruz et al., 1987), two members of the vasopressin/oxytocin family of peptide hormones, elicit motor behaviors in leeches that are associated with procreation (Wagenaar et al., 2010). This made it possible to determine that these behaviors are controlled by linked CPGs located in the 4th, 5th and 6th segmental ganglia. The role of the 4th segmental ganglion was especially remarkable, because that ganglion had not previously been associated with reproductive function. Subsequently, multiple neurons in these and other ganglia have been found to stain positively for vasopressin and for vasopressin receptors (Bratka, 2012).

Remarkably, the application of conopressin to isolated nerve cords elicited fictive motor output that matched the intact behavior very closely (Wagenaar et al., 2010), establishing *Hirudo* as one of very few species in which reproductive behaviors can be studied in an isolated nervous system (*Aplysia* being another example; Wayne et al., 2004).

Newly described behaviors

Despite the fact that the leech has been the subject of biological investigation for over 100 years, studies performed over the past decade have led to the identification of two newly described behaviors, which I discuss in turn: one is the first social behavior described in the leech (Bisson and Torre, 2011), the other is a newly discovered sensitivity to UV light (Jellies, 2014a).

Social behavior

It was long thought that leeches mostly ignore each other except for the purposes of procreation and, occasionally, for cannibalism (Kutschera and Roth, 2005). Informal observations suggested that captive leeches tend to aggregate in their holding tanks, but it was never clear whether they specifically prefer to be touching other leeches or simply to be touching something other than smooth glass. However, a study by Bisson and Torre (2011) showed that leeches aggregate together specifically when the density of conspecifics is above a certain threshold. Additionally, when several leeches in a tank were swimming, they found that other individuals became more likely to also start swimming. Both of these effects appear to be under neuromodulatory control – elevating the blood concentration of serotonin lowers the threshold for both aggregation and group swimming. The presence of other swimming animals (such as goldfish) did not cause leeches to swim, giving credence to the authors' argument that the effect is not due to mere mechanical stimulation, but is indeed a social phenomenon. The remarkable implication is that social behavior requires substantially less neural 'real estate' than one might imagine, and poses the intriguing question of how an animal with such a simple nervous system can tell the difference between a swimming conspecific and another animal.

Novel responses to visual stimuli

Leeches have five pairs of non-image-forming eyes on their heads and a grid of visual sensilla on each of their midbody segments. They use this visual system to locate prey, particularly as juveniles (see below). The first modern study of the photoreceptors in both head eyes and midbody sensilla (Kretz et al., 1976) measured their responses to light between 430 nm (blue/violet) and 660 nm (red), and found that their sensitivity is relatively narrowly concentrated around 540 nm (green). Accordingly, it was generally believed, although not explicitly tested, that leeches could detect neither ultraviolet (UV) nor infrared (IR) light. However, invertebrate opsins that are sensitive to green light commonly have a substantial secondary sensitivity peak at UV wavelengths (Stavenga, 2010), suggesting that this assumption might be false. Indeed, Jellies (2014a) found that leeches retract or move away from bright UV light (400 nm) projected onto their dorsal surfaces. Such responses were rarely seen to visible light.

In an elegant follow-up study with more tightly controlled light intensities, Jellies (2014b) found that leeches also respond strongly to UV light projected onto their ventral surface, primarily by locally twisting away. Surprisingly, at the level of the central nervous system, leeches respond more strongly to UV light than to green light when ventral sensilla act as the input pathway. Dorsal sensilla showed the opposite pattern of sensitivity. Whether this difference is due to the presence of multiple opsins (Doring et al., 2013) or due to differences in pigmentation between the ventral and dorsal surfaces remains an open question.

Detailed network models of behavioral circuits

For researchers interested in realistic models of small but not tiny circuits, the leech has long been an attractive middle ground between the very small circuits that control e.g. locomotion in *C. elegans* on the one hand and e.g. mammalian cortical microcircuits on the other. In particular, the networks in the leech

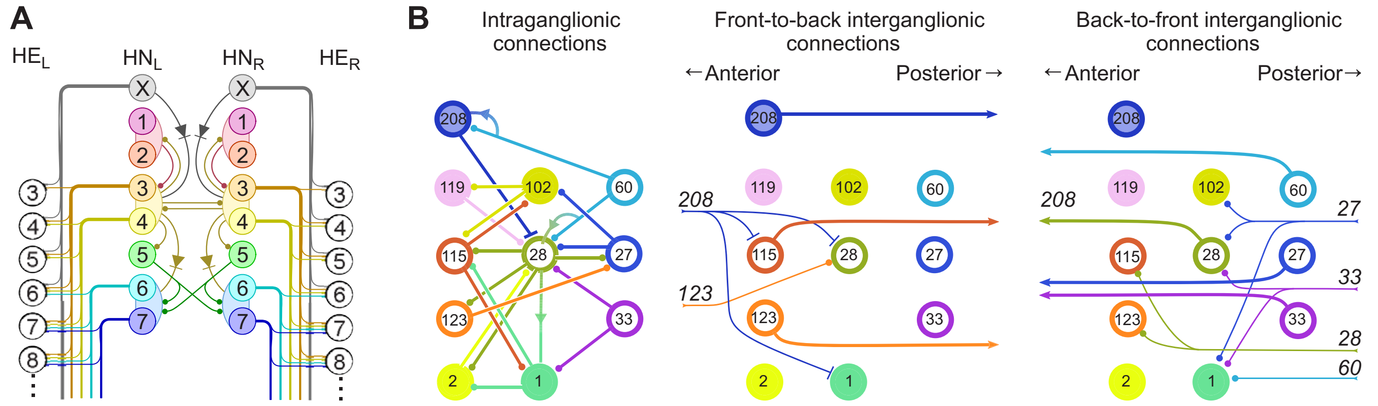

Fig. 2. The leech heartbeat and swimming neural circuits. (A) Circuit diagram of the heartbeat neural circuit, redrawn with permission from Norris et al. (2007). Circles represent neurons. Labeling indicates the bilateral [left (L) and right (R)] interneurons (HN) in segmental ganglia 1–7 plus the unidentified HN(X) and excitatory motor neurons (HE) in ganglia 3 and up. Lines represent synaptic connections; dots mark inhibitory chemical synapses; diode symbols mark rectifying gap junctions. Because HN(1) has the same connectivity pattern as HN(2), these neurons are shown as grouped. The same applies for HN(3) and HN(4), and for HN(6) and HN(7). (B) Circuit diagrams of the swimming neural circuit, redrawn with permission from a figure and data in Friesen (1989). Conventions as in A. Additionally, lines ending in bars represent excitatory chemical synapses and resistor symbols mark nonrectifying gap junctions. Cell numbers refer to the canonical maps of the ganglion (Fig. 1C,D). Cells 1, 2, 102 and 119 are motor neurons that are also part of the CPG; the others are interneurons (cell 208 is a swim initiator).

nervous system that control circulation and swimming have received much attention as relatively self-contained circuits that nearly completely control a given behavior.

Neuronal control of circulation

The circuit that controls heartbeat in the leech is an intriguing example of a system of modest complexity that has been extremely well characterized, yet continues to pose fundamental intellectual challenges. Currently, the main question is how the system reliably produces functional output despite extreme variation in synaptic parameters between animals.

Leeches circulate their blood by means of two lateral heart tubes, both of which beat 6–15 times per minute. The heart rhythm is generated by nine pairs of identified interneurons (Calabrese et al., 1995; Wenning et al., 2011). The existence of one additional (but yet to be identified) pair of interneurons, named HN(X), can be inferred from its postsynaptic effects on the rest of the circuit (Calabrese, 1977).

The heart interneurons project to heart motor neurons that innervate the heart tubes. All connections between interneurons and onto motor neurons have been described in detail (Fig. 2A), along with their activity patterns. This has enabled the construction of a highly detailed model of the heartbeat control system that successfully describes the major qualitative features (Garcia et al., 2008). However, this model fails to explain some quantitative details. Why? Remarkably, the parameters that characterize connections within the heartbeat system (such as synaptic strengths) vary up to fivefold between animals (Norris et al., 2011). The model is based on average parameter values from many animals, and does not incorporate possible correlations between parameters. Could it be that correlated deviations from the average are important? For instance, one might expect to find that when one parameter deviates strongly from its population average in a particular animal, other changes develop that ensure consistent motor output. Such 'compensatory changes' have been observed in the stomatogastric ganglion of crustaceans (Marder, 2011). However, in the leech, the variability of motor output patterns was at least as great as the variability of the neuronal parameters. Additionally, correlations between neuronal parameters were relatively weak and failed to explain how the circuits produced functional output (Norris et al., 2011). All this work ultimately led to the conclusion that 'essentially, each animal arrives at a unique solution for how the network produces functional output' (Calabrese et al., 2011). A truly remarkable mechanism for a function such as the heartbeat that is critical to the everyday survival of an animal.

It would be fascinating to know how the animal finds this solution in development, particularly as this might inform how more complex animals develop control over their individual bodies. Hints to the answer might be found in recent work showing that the actual rhythm of contractions of the heart tube results not just from central control but is significantly modified by peripheral circuitry (Jellies and Kueh, 2012). Perhaps, then, information from the periphery might inform the fine-tuning of the central circuits.

Swimming: a CPG modulated by sensory feedback and the environment

Another example of a leech behavior that has been studied in great quantitative detail is swimming. Classic papers in the 1970s and 1980s (reviewed in Brodfuehrer et al., 1995) established both the dynamics of swimming and the neuronal circuitry that gives rise to it. It was found that the swim rhythm is produced by a series of interconnected CPGs located in the segmental ganglia. The neurons that make up these CPGs, their connections and their projections onto the motor neurons are all known (Fig. 2B). In the past decade, this has led to the construction of abstract models that capture the essence of the CPG dynamics (Zheng et al., 2007).

However, not all aspects of swimming can be explained only by the activity of a CPG; it has long been known that natural swimming behavior differs quantitatively from fictive swimming in isolated nerve cords. Sensory feedback from peripheral stretch receptors in the muscles has been implicated in this difference. To better understand this, a physical model of muscle activation was constructed (Chen et al., 2011a), followed by models of the interaction between the biologically generated rhythm and the fluid environment (Chen et al., 2011b). These models showed that even though the swimming motion appears to be continuous and similar along the length of the body, the actual muscle activation is strongly concentrated in the middle segments of the body, travels along the body in waves much faster than the motion itself, and causes most of the forward-propelling net force to be applied to the water near the tail.

In a further modeling effort synthesizing a career-spanning series of experimental studies, Friesen et al. (2011) constructed a network model comprising multi-compartmental neurons of the entire swimming circuit, including trigger and gating neurons not shown in Fig. 2B. This model was detailed enough to capture even subtleties such as the observation that fictive swimming in sections of isolated nerve cords is sustained longer as the number of preserved ganglia in the section is increased. It also explained quantitatively how body–fluid interaction and sensory feedback cause the intact behavior to differ from fictive swim rhythms. Leech swimming is thus a useful model system to improve our understanding of the interplay between neural activity and the physical interaction between body and environment, and its role in shaping locomotory behaviors.

Development of nervous system and behavior

Medicinal leeches lay cocoons in peat moss near the edge of a pond, but after hatchlings emerge, they spend most of the rest of their lives in the water (Sawyer, 1986). Newly hatched leeches are about 1.7 cm long and weigh around 24 mg. In the lab, they can reach reproductive maturity after about a year, at which point they weigh around 10 g – a 400-fold increase that they gain from as little as six feedings. In general, the developmental biology of *Hirudo* has not been studied nearly as extensively as that of *Helobdella robusta*, which has been favored, among other reasons, because its embryos can be produced in large quantities and maintained much more conveniently in the lab (Weisblat and Kuo, 2014). Nevertheless, two very different recent studies of the development of *Hirudo* should not be omitted here.

Role of gap junctions in the establishment of chemical synapses

Because of its well-characterized developmental biology, it was *Helobdella robusta* rather than *Hirudo* that was chosen as the first, and so far only, leech to have its genome completely sequenced (Simakov et al., 2013). However, this does not mean that the genomic revolution has passed by *Hirudo*. In particular, RNA interference (RNAi) has proved to be a powerful technique. The stereotyped topography of the leech nervous system means that RNAi can be targeted to individual cells, enabling the silencing of selected genes in individual neurons of known identity, all in a living animal whose longevity is not affected by the procedure.

In an elegant example of such a study, Todd et al. (2010) used RNAi to suppress the synthesis of gap junction proteins (innexins) during a particular stage of embryogenesis. Although the suppression itself was transient (the injected cells formed gap junctions that functioned normally days later and into adulthood), it nevertheless had lasting effects; the injected cells never formed chemical synapses with their normal postsynaptic partners, thus demonstrating a causal role for gap junctions in the establishment of chemical synapses. This is a remarkable achievement, because it probably generalizes widely to other nervous systems; in many systems, including the developing mammalian cortex, transient gap junctions are found shortly before chemical synapses appear (Connors et al., 1983). Based on this and other correlational evidence, a causal role for gap junctions in synaptogenesis had long been suspected but never conclusively demonstrated, probably because of the difficulty of manipulating and following individual neurons through development in more complex nervous systems.

In this context, it is worth mentioning that the role of gap junctions is by no means limited to development. In adult leeches, gap junctions are found between many pairs of bilateral neurons. In addition, many excitatory motor neurons receive inhibitory input through rectifying gap junctions from an interneuron known as the ‘nonspiking cell’ (Wadepuhl, 1989). Recently, it was shown that the nonspiking cell, through these connections, can selectively modulate the CPG for crawling but not for swimming (Rodriguez et al., 2012).

Changing modalities of prey localization

Leeches apparently cannot undergo the huge increase in body mass mentioned above without at least a few meals of mammalian blood. However, small leeches, with their correspondingly small jaws, cannot readily pierce mammalian skin and, instead, sustain themselves by feeding on frogs (Sawyer, 1986). How, then, does a leech find its prey, and how is the necessary switch in the choice of prey made?

It has long been known that leeches can use both visual and mechanical information to localize the source of water surface waves and, hence, to locate their prey (Dickinson and Lent, 1984). However, until recently, little was known about differences in prey localization behavior at different life stages. A recent study (Harley

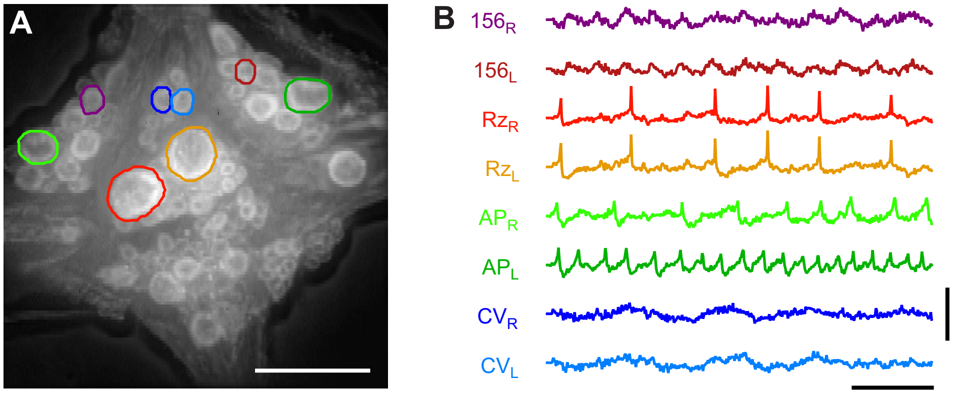

Fig. 3. Voltage-sensitive dye imaging in the leech ganglion. (A) Fluorescence micrograph of the ventral aspect of a leech ganglion stained with a voltage-sensitive dye. The image was digitally processed for contrast and sharpness from multiple frames of a *z*-stack. Scale bar: 200 μm. (B) Selected examples of optical signals simultaneously recorded during fictive swimming from: a pair of neurons that participate in the swim rhythm (cell 156 on the canonical map); the two Retzius cells (main providers of serotonin to the ganglion), which exhibit synchronized action potentials but do not participate in the rhythm (Rz); two interneurons with smaller action potentials that also do not participate (AP); and two motor neurons that have their own rhythm (CV). Colors match the regions of interest indicated in A. R, right; L, left. Scale bars: 2 s (horizontal) and 0.5% fluorescence change, which corresponds to about 20 mV of voltage change (vertical). Unpublished data, Yusuke Tomina and D.A.W.

et al., 2011) investigated the behavior of leeches at several stages of development and found that hatchlings responded more strongly to waves of lower frequencies (and hence longer wavelengths) than did adults. Surprisingly, this difference vanished when the animals were tested in the dark, demonstrating that the response of leeches to exclusively mechanically sensed waves was constant across the two life stages. This constancy is especially remarkable when one considers that leeches – and their mechanosensory arrays – grow over tenfold in length as they mature.

When leeches are stimulated with visible waves that they cannot sense mechanically, hatchlings once again show responses similar to those of adults. However, the responses to visual stimuli differ from the responses to mechanical stimuli; both hatchlings and adults prefer slower visible waves and faster mechanical waves (Harley et al., 2011). Taken together, these findings indicate that leeches adapt their behavior to match their nutritional needs not by developmental changes in individual sensory systems, but rather by a change in the way sensory information is integrated as it is processed to form the basis of behavioral choice. The neural basis of this change, however, remains unknown.

New tools and technologies: the leech as a ‘guinea pig’

Thanks to the easy accessibility of its nervous system, *Hirudo* has been a useful model for pilot experiments of novel techniques and technologies. Voltage-sensitive dyes are arguably the most successful examples of this. Leeches have been used in pioneering studies for several generations of these dyes, recently leading to a class of dyes that is fast enough to detect individual action potentials in large cells, yet sensitive enough to detect subthreshold voltage changes even in small cells (Fig. 3; Miller et al., 2012; Woodford et al., 2015). These dyes, which also work in mammalian cells, make it possible to study neural processing at the scale of an entire leech ganglion with sufficient resolution to record individual neuronal events in individually identified neurons (Moshtagh-Khorasani et al., 2013).

Another example of the use of the leech as a test subject for new techniques is in the optical control of neuronal activity. Although genetically encoded optical actuators like channelrhodopsin have yet to be used in the leech because of the lack of a genetic toolkit, alternative approaches to optically controlling neuronal activity are being considered. For instance, a photoactivated switch has been developed that regulates the conductivity of endogenous potassium channels (Fortin et al., 2008). This molecule readily incorporates into cell membranes upon bath application and can be used to individually modulate the excitability of specific neurons in a leech ganglion or in other preparations.

For the same reasons that leeches are attractive test subjects for new technologies, they are also attractive for safety testing, and have been used to investigate the effects of millimeter wave devices. Romanenko et al. (2014) exposed leech ganglia to millimeter wave radiation to investigate its effects on the nervous system. In recent years, millimeter wave scanners have appeared in airports for security screening and millimeter wave sources have been used for crowd control. The study found that even relatively modest intensities of millimeter wave radiation (no higher than the safety standard for short-term human exposure set by the Institute of Electrical and Electronics Engineers) caused substantial changes to neuronal activity that could not be attributed to thermal heating. However, it is worth noting that millimeter wave detectors used in airport security are believed to use intensities 1000 to 100,000 times weaker than those used in this study (Moulder, 2012).

Conclusion

Recent research on the leech has yielded multiple instances where function and behavior can be explained in a detailed and quantitative manner in terms of the neuronal mechanisms involved – I have discussed swimming and the heartbeat system as two striking examples. One other behavior, mating, has just recently been quantified and is now amenable to the same treatment. Furthermore, some highly general questions in biology – I have mentioned the neural implementation of behavioral hierarchies, the detection of satiation, and the role of gap junctions in the establishment of chemical synapses – have been addressed in the leech. A new specific sensitivity to UV light has been found, indicating that the visual system of the leech has more secrets awaiting discovery. The past decade has expanded our understanding of prey localization and yielded the first description of social behavior in the leech, paving the way for investigation of the neural basis of more complex behavior. In addition, the leech has been an important model organism in the development of optical tools that may be broadly useful to neuroscience, and even in safety testing.

It has been a very rewarding decade for researchers working with the leech, and the future is even brighter. Progress in voltage-sensitive dye imaging continues at an ever increasing pace, enabling activity mapping at the scale of the entire ganglion with cellular detail and useful temporal resolution (Fig. 3). From farther afield, the CRISPR revolution may soon make any animal genetically tractable, which will open a world of new possibilities for all those animals, including the leech, that previously did not have a wide array of genetic tools available. Applying these developments to exciting questions such as those posed above could make the next decade of leech research the most exciting yet.

Acknowledgements
The author wishes to thank Jim Angstadt for insightful comments.

Competing interests
The author declares no competing or financial interests.

Funding
The author is the recipient of a Career Award at the Scientific Interface from the Burroughs Wellcome Fund.

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