Current Biology
Dispatches

Neural Circuits: From Structure to Function and Back

Nicholas F. Trojanowski and David M. Raizen*
Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: raizen@mail.med.upenn.edu
http://dx.doi.org/10.1016/j.cub.2015.06.048

The new field of connectomics aims to obtain fine-grained anatomical connectivity data for vertebrate brains. A recent study highlights the types of experiments that will be necessary in order to draw conclusions about function from anatomical connectivity.

There has been a recent push to determine the anatomical connectivity of vertebrate brains as a necessary step for understanding the neural basis of behavior [1]. However, four decades of work on a simple 20-neuron circuit in the pharynx of the roundworm *Caenorhabditis elegans* suggests that extrapolating from anatomical to functional connectivity will not be simple. A study by Bhatla and colleagues [2], reported in this issue of *Current Biology*, demonstrates some of the types of functional experiments necessary to bridge the gap between anatomical connectivity and behavior.

The nematode *C. elegans* ingests its food — bacteria — using a neuromuscular tube called the pharynx [3]. Feeding consists of two stereotyped behaviors, pumping and peristalsis. During a pump, bacterial food is ingested by the front of the pharynx, the corpus, and crushed and transported to the intestine by the back of the pharynx, the terminal bulb. During a peristalsis, bacteria are transported from the corpus to the terminal bulb.

Pharyngeal behavior is governed by the pharyngeal nervous system, which consists of 20 neurons of 14 types and which makes just one anatomical connection with non-pharyngeal neurons. The map of neural connectivity of the pharyngeal nervous system, or its connectome, was defined by painstaking analysis of thin section transmission electron micrographs and published nearly 40 years ago: Albertson and Thomson [3] categorized pharyngeal neurons as either motor (M) if they formed synapses on the muscle, or interneurons (I) if they did not. They proposed that two circuits govern pharyngeal behavior, a 'control circuit' and a 'pumping circuit'. In this model, the I2 interneurons initiate pumps in the 'pumping circuit', where the M1 and M2 motor neurons are the primary excitatory motor neurons and the M4 motor neuron plays a role in inhibition, while in the 'control circuit' the I1 interneurons transmit inhibitory signals from the somatic nervous system and shut down the pharynx during emergencies [3].

Observations of behavior after ablation of specific pharyngeal neurons have called this model into question [4–6]. In contrast to its proposed inhibitory role, the M4 neuron is actually required for peristalsis [4]. Ablation of the M1, M2, I1, and I2 neurons did not produce gross defects in pumping behavior, suggesting they were not essential for pharyngeal function [5,6]. However, ablation of the pair of MC neurons (and only the MC neurons), which form fewer synaptic contacts onto pharyngeal muscles than any of the motor neurons, caused a dramatic decrease in pumping frequency [5,6].

The results described by Bhatla *et al.* [2] are part of a third wave of studies on pharyngeal nervous system function, and highlight the importance of detailed functional studies to understand the neural basis of behavior [7–11]. The authors combined laser ablation of specific neurons with newer tools, such as optogenetic stimulation and calcium imaging, to demonstrate that there are at least three independent circuits for inhibiting feeding, serving the function of the "control circuit" originally proposed by Albertson and Thomson [2].

Bhatla *et al.* [2] began their studies when they noted that *C. elegans* feeding is inhibited in response to high intensity violet light [7]. By ablating individual pharyngeal neurons, they found that the pair of bilaterally symmetric I2 neurons is both necessary and sufficient for this behavioral response to light. They further showed that the I2 neurons are activated by the noxious light stimulus, and this activation does not depend on other neurons. These findings indicate that the I2 neurons have both sensory and motor neuron functions, an insight impossible to glean from original connectivity data, where the I2 motor synapses were not reported. The functional studies demonstrating a motor function for the I2 neurons motivated Bhatla *et al.* [2] to return to the electron micrographs in an attempt to discover synapses between the I2 neurons and pharyngeal muscle. Indeed, in newly generated electron micrographs, as well as in the original electron micrographs from the 1970s, they found evidence for I2-to-muscle synapses. Hence, their approach illustrates a recursive approach to understanding behavior: begin with anatomy to define connectivity, perform functional studies guided by the anatomical blueprint, then return to the anatomy if the functional studies suggest unappreciated connections.

Bhatla *et al.* [2] identified a second circuit for feeding inhibition in response to light that involves the RIP and I1 interneurons and the MC motor neurons, and functions in parallel to the I2 neurons [2]. The two extrapharyngeal RIP neurons each make a gap junction with the pharyngeal I1 neurons, the only connections between the somatic and pharyngeal nervous systems. When the RIP, I1, or MC neurons are killed, the acute inhibitory effect of noxious light on pumping is attenuated. Importantly, ablation of the I1 and I2 neurons together causes a stronger attenuation of the blue light effect than ablation of either neuron class individually. The I1

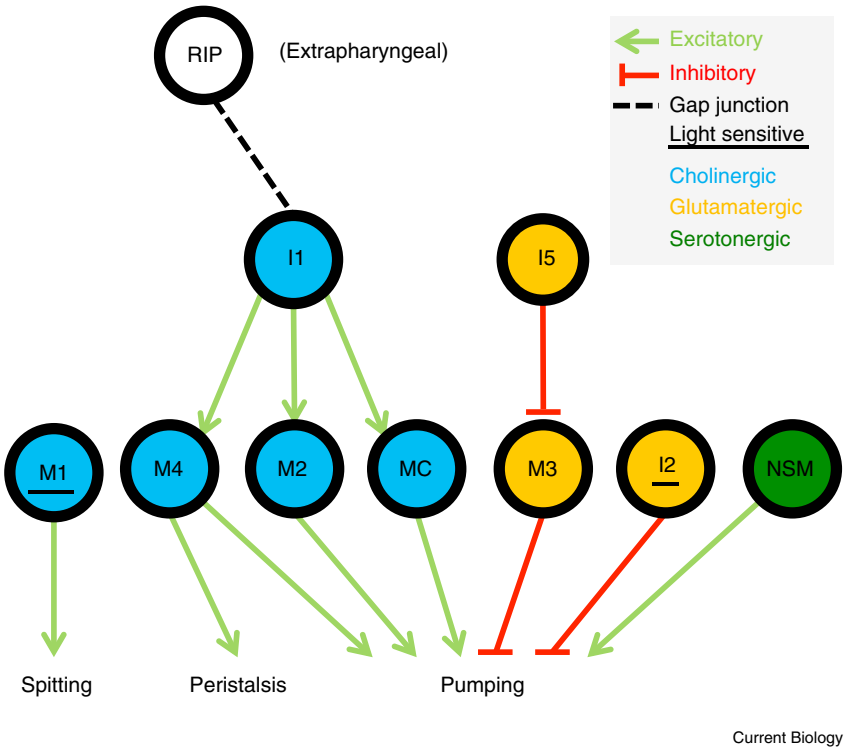

Figure 1. Functional connectivity of the *C. elegans* pharyngeal nervous system.
Green arrows represent excitatory synapses, red lines represent inhibitory synapses, and the black dashed line represents a gap junction connection. Cholinergic neurons are shaded blue, glutamatergic neurons are shaded yellow, and serotonergic neurons are shaded green. Light sensitive neurons are marked with an underline. See [2–9,14–19] for more details.

neurons stimulate feeding via the MC and M2 motor neurons [2,8].

Finally, Bhatla *et al.* [2] went beyond analysis of pumping rate and examined the fine motions of the pharynx. During a single pharyngeal pump, which lasts about 200 milliseconds, the anterior tip of the corpus relaxes a few milliseconds before the remainder of the corpus [12]. This staggered relaxation endows the corpus with its filtering capability: when it contracts it sucks in bacteria suspended in liquid, and when it relaxes it spits out the liquid and retains the bacteria. The authors noted that, after exposure to noxious light, the corpus no longer traps the bacteria: instead, it spits out the bacteria along with the liquid during muscle relaxation. In an earlier paper this year, Bhatla and Horvitz [7] showed that the violet light generates hydrogen peroxide. They therefore suggest that the spitting of bacteria might serve to minimize ingestion of hydrogen peroxide. Regardless of the reason for the spitting, Bhatla *et al.* [2] showed that this behavior is neurally controlled, as when the M1 neuron is killed the spitting in response to noxious light no longer occurs. M1 also expresses LITE-1, a violet light-sensitive gustatory receptor [7], suggesting that, like I2, M1 is also a sensory-motor neuron (Figure 1).

In a sense, studies of the pharyngeal nervous system have come full circle, as roles for M1, M2, I1, and I2, the neurons that Albertson and Thompson [3] originally suggested had important functions, have been uncovered [2,8]. However, the roles of these neurons are very different from those originally hypothesized, as the pharyngeal connectome on its own provided little useful insight into how the pharynx worked. It was only after multiple generations of functional studies that it became clear that synaptic weights and functional importance need not be correlated, as neurons with few neuromuscular synapses can have a stronger effect on behavior than those with many such synapses. Further, the pharyngeal connectome — and all other connectomes — provides no information about humoral modulation of circuits, which can play a pivotal role in circuit function [11,13]. While the anatomical connectivity will provide a framework for forming and testing hypotheses, many of these hypotheses will prove incorrect, and it may be decades before the true value of a connectome — whether from worm or human — is realized.

REFERENCES

1. Seung, H.S. (2009). Reading the book of memory: sparse sampling versus dense mapping of connectomes. Neuron *62*, 17–29.
2. Bhatla, N., Droste, R., Sando, S.R., Huang, A., and Horvitz, H.R. (2015). Distinct neural circuits control rhythm inhibition and spitting by the myogenic pharynx of C. elegans. Curr. Biol. *25*, 2075–2089.
3. Albertson, D.G., and Thomson, J.N. (1976). The pharynx of Caenorhabditis elegans. Phil. Trans. R. Soc. Lond. B. Biol. Sci. *275*, 299–325.
4. Avery, L., and Horvitz, H.R. (1987). A cell that dies during wild-type C. elegans development can function as a neuron in a ced-3 mutant. Cell *51*, 1071–1078.
5. Avery, L., and Horvitz, H.R. (1989). Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. Neuron *3*, 473–485.
6. Raizen, D.M., Lee, R.Y.N., and Avery, L. (1995). Interacting genes required for pharyngeal excitation by motor neuron MC in Caenorhabditis elegans. Genetics *141*, 1365–1382.
7. Bhatla, N., and Horvitz, H.R. (2015). Light and hydrogen peroxide inhibit C. elegans feeding through gustatory receptor orthologs and pharyngeal neurons. Neuron *85*, 804–818.
8. Trojanowski, N.F., Padovan-Merhar, O., Raizen, D.M., and Fang-Yen, C. (2014). Neural and genetic degeneracy underlies Caenorhabditis elegans feeding behavior. J. Neurophysiol. *112*, 951–961.
9. Li, Z., Li, Y., Yi, Y., Huang, W., Yang, S., Niu, W., Zhang, L., Xu, Z., Qu, A., Wu, Z.-X., *et al.* (2012). Dissecting a central flip-flop circuit that integrates contradictory sensory cues in C. elegans feeding regulation. Nat. Commun. *3*, 776–778.
10. Dillon, J., Franks, C.J., Murray, C., Edwards, R.J., Calahorro, F., Ishihara, T., Katsura, I., Holden-Dye, L.M., and O'Connor, V. (2015). Metabotropic glutamate receptors: modulators of context-dependent feeding behaviour in C. elegans. J. Biol. Chem. *290*, 15052–15065.
11. Flavell, S.W., Pokala, N., Macosko, E.Z., Albrecht, D.R., Larsch, J., and Bargmann, C.I. (2013). Serotonin and the neuropeptide PDF initiate and extend opposing

behavioral states in C. elegans. Cell *154*, 1023–1035.

12. Fang-Yen, C., Avery, L., and Samuel, A.D.T. (2009). Two size-selective mechanisms specifically trap bacteria-sized food particles in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA *106*, 20093–20096.

13. Bargmann, C.I., and Marder, E. (2013). From the connectome to brain function. Nat. Methods *10*, 483–490.

14. Avery, L. (1993). Motor neuron M3 controls pharyngeal muscle relaxation timing in Caenorhabditis elegans. J. Exp. Biol. *175*, 283–297.

15. Raizen, D.M., and Avery, L. (1994). Electrical activity and behavior in the pharynx of Caenorhabditis elegans. Neuron *12*, 483–495.

16. Dent, J.A., Davis, M.W., and Avery, L. (1997). avr-15 encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in Caenorhabditis elegans. EMBO J. *16*, 5867–5879.

17. Chiang, J.-T.A., Steciuk, M., Shtonda, B.B., and Avery, L. (2006). Evolution of pharyngeal behaviors and neuronal functions in free-living soil nematodes. J. Exp. Biol. *209*, 1859–1873.

18. Song, B.-M., and Avery, L. (2012). Serotonin activates overall feeding by activating two separate neural pathways in Caenorhabditis elegans. J. Neurosci. *32*, 1920–1931.

19. Steciuk, M., Cheong, M.C., Waite, C., You, Y.-J., and Avery, L. (2014). Regulation of synaptic transmission at the Caenorhabditis elegans M4 neuromuscular junction by an antagonistic relationship between two calcium channels. G3 *4*, 2535–2543.

Vision Science: Can Rhodopsin Cure Blindness?

Russell N. Van Gelder[1,2,3,*] and Kuldeep Kaur[1]
[1]Department of Ophthalmology, University of Washington School of Medicine, Seattle, WA 98104, USA
[2]Department of Biological Structure, University of Washington School of Medicine, Seattle, WA 98104, USA
[3]Department of Pathology, University of Washington School of Medicine, Seattle, WA 98104, USA
*Correspondence: russvg@uw.edu
http://dx.doi.org/10.1016/j.cub.2015.07.004

Outer retinal degeneration is the leading cause of blindness in the developed world. A new study now demonstrates that ectopic expression of human rhodopsin in the inner retina, mediated by viral gene therapy, can restore light sensitivity and some vision to mice blind from outer retinal degeneration.

Blindness remains a major public health challenge. Worldwide, 285 million people are visually impaired, and about 39 million legally blind [1]. In the developed world, the leading causes of acquired and hereditary blindess — age-related macular degeneration and retinitis pigmentosa, respectively — both share a common pathophysiology. In each, the rod and cone photoreceptors of the outer retina undergo irreversible degeneration (Figure 1A,B). While the inner retina remains largely intact, in the absence of outer retinal phototransduction no visual information can be transmitted from the retinal ganglion cells to the brain. In this issue of *Current Biology*, Cehajic-Kapetanovic and colleagues [2] show that virally mediated gene therapy of human rhodopsin, expressed in the surviving cells of the inner retina, can restore vision-like physiology and behavior to mice blind from outer retinal degeneration.

A number of approaches to restore vision by conferring light sensitivity to the remaining inner retinal cells have been pursued in the past decade. Approaches that have shown promise include use of gene therapy with microbial opsins such as *Chlamydomonas* channelopsin to introduce light-regulated ion channels to inner retinal cells [3–5]; gene therapy to introduce the non-visual pigment melanopsin to the inner retina [6]; one-component and two-component optochemical photoswitches, which utilize light-isomerizable channel agonists to confer light sensitivity to remaining inner retinal photoreceptors [7–9]; and opto-electronic prostheses that stimulate retinal ganglion cells directly [10]. The latter approach is now approved in the US for clinical use. While these methods have all resulted in reconstituted light-dependent firing of optic nerve fibers and restored behavioral responses to light, each has a number of potential limitations. Use of channelopsins incurs risks inherent in expressing foreign proteins chronically in the retina, and lack of signal amplification necessitates relatively bright light for function. Relative to native rod and cone opsins, melanopsin has slow kinetics and relatively low sensitivity, which would likely limit acuity. One-component photoswitches lack cell-type specificity while two-component photoswitches require both gene therapy and a chemical adjunct, which may limit practical application. And opto-electronic approaches are limited by the physics of external stimulation of cells with resultant low spatial resolution.

Ideally, a photopigment used to restore visual functions via inner retinal expression should be very light-sensitive, have strong signal amplification, be native to the organism, and be expressible in the remaining cells of the degenerated retina. Gene therapy with rhodopsin would seem to fit these requirements. However, *a priori*, rhodopsin would seem to be a poor candidate, as its photocycle is intrinsically tied to the G-protein transducin (which is not expressed at high levels in the retina outside the photoreceptors), and requires continual chromophore replenishment from the retinal pigment epithelium (RPE) through a mechanism thought to be

