Review article

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Optogenetic analyses of neuronal networks that generate behavior in *Caenorhabditis elegans*

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Abstract: In compact brains, circuits consisting of few neurons fulfill functions of entire brain systems in mammals. Thus, studying these small circuits can provide insights and guidelines also for the study of the human brain. We developed methods and approaches to use optogenetics in the nervous and neuromuscular system of the nematode *Caenorhabditis elegans*. These include single-cell expression and/or photoactivation of optogenetic tools, to control the function of individual neurons, and behavioral, electrophysiological or electron microscopic analyses of circuit function and synaptic transmission. We studied a number of circuits involved in locomotion, navigation and food searching; we addressed new genes in synaptic vesicle recycling, and we identified a novel pathway of neuromodulatory presynaptic plasticity. In our laboratory, support by the Schram foundation allowed me to explore new avenues of research especially during the early years of my career.

Keywords: behavior; connectome; electron microscopy; electrophysiology; optogenetics; synaptic transmission.

Zusammenfassung: In kompakten Nervensystemen übernehmen Schaltkreise aus einigen wenigen Neuronen die Funktionen ganzer Hirnsysteme in Säugetieren. Daher kann die Untersuchung solcher kompakter Gehirne Leitlinien auch für die Untersuchung des menschlichen Gehirns liefern. Wir haben optogenetische Methoden für die Untersuchung des neuromuskulären Systems des Nematoden *Caenorhabditis elegans* entwickelt, u.A. für die Expression und Aktivierung von optogenetischen Werkzeugen in einzelnen Nervenzellen, sowie für Verhaltens-, elektrophysiologische und elektronenmikroskopische Analysen. Mit diesen Methoden untersuchten wir eine Reihe von Schaltkreisen für die Bewegung, Navigation und Futtersuche. Wir analysierten außerdem verschiedene Gene mit Funktionen im Recycling von synaptischen Vesikeln, und identifizierten einen neuen Mechanismus der neuromodulatorischen Plastizität. Die Schram Stiftung half bei der Implementierung dieser Projekte und der Entwicklung neuer Forschungsgebiete in meinem Labor.

Schlüsselwörter: Elektronenmikroskopie; Elektrophysiologie; Konnektom; Optogenetik; synaptische transmission; Verhalten.

Introduction

The nematode *Caenorhabditis elegans* has a compact nervous system of 302 neurons, whose connectivity has been determined by serial electron microscopy (EM) in the 70s and 80s of the twentieth century, and the data were revisited last year (Cook et al., 2019; White et al., 1986). Also, new connectomes, acquired with modern EM techniques, across development of the animal, have been determined, providing comprehensive 'wiring diagrams' of the *C. elegans* brain (Witvliet et al., 2020). This information forms a basis to the understanding of the function of neuronal circuits in the generation of behavior of 'the worm.' Due to the compactness of the *C. elegans* nervous system, single neurons often need to fulfill the function of entire circuits in higher animals, though at a lower level of complexity. Until recently, the function of individual *C. elegans* neurons was inferred from animals in which these neurons were eliminated by laser ablation and by observing the altered behaviors that resulted from the loss of the neuron. More recent methods to address the loss of (single, or entire classes of) neurons involve expression of caspases (Chelur and Chalfie, 2007) or other proteins that affect cell viability or neuronal function, like constitutively active mutated potassium channels (Kunkel et al., 2000) or photosensitizers, that generate toxic reactive oxygen species upon illumination (Qi et al., 2012). Also, optogenetic methods allow the interference with neuronal function, for example, *via* light-activated anion channels or

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ion pumps, that cause hyperpolarization during illumination (Bergs et al., 2018; Govorunova et al., 2015; Zhang et al., 2007), or inactivators of the synaptic machinery for transmitter release, like photoactivated botulinum neurotoxin (PA-BoNT) (Liu et al., 2019). Of course, also light-triggered activators like channelrhodopsin (ChR2) are used to probe neuronal function in *C. elegans* (Fang-Yen et al., 2015; Nagel et al., 2005).

Supported in part by the Schram foundation, my lab developed integrated approaches of using optogenetics, electrophysiology, imaging and behavioral analysis, as well as electron microscopy, to analyze functional neuronal networks that generate behavior, the molecules they use and also, how synaptic transmission is achieved at the physiological, ultrastructural and molecular level (Figure 1). Paradigmatically, the potential of the *C. elegans* system will be discussed in this overview article.

Single-neuron photoactivation in free-moving animals

Distinct neuronal connections that are visible in the wiring diagram can be explored by the methods described above, provided one can express or activate the tools in a cell-specific manner. Optogenetics enables this to some extent, e.g. by a special microscope and tracking system that can illuminate specific regions of the *C. elegans* body, thus restricting illumination in time and space, but also in different light colors, to cells of interest (Stirman et al., 2011) (Figure 2).

Nociceptive neurons and molecules acting within them

This methodology was used to probe a network of neurons downstream of, and the function of ion channels acting within, a nociceptor neuron termed PVD (Husson et al., 2012) (Figure 3). PVD is a harsh-touch sensor that evokes escape behavior, allowing the animal to avoid harmful stimuli. For the size of *C. elegans*, PVD is a huge neuron, covering almost the entire body with a complex, branched dendritic arbor. *C. elegans* was until recently believed to transmit electrical signals passively (Bargmann and Kaplan, 1998; Liu et al., 2018). Yet, since a nociceptor neuron must act rapidly, the question arose whether PVD has specific mechanisms to ensure fast

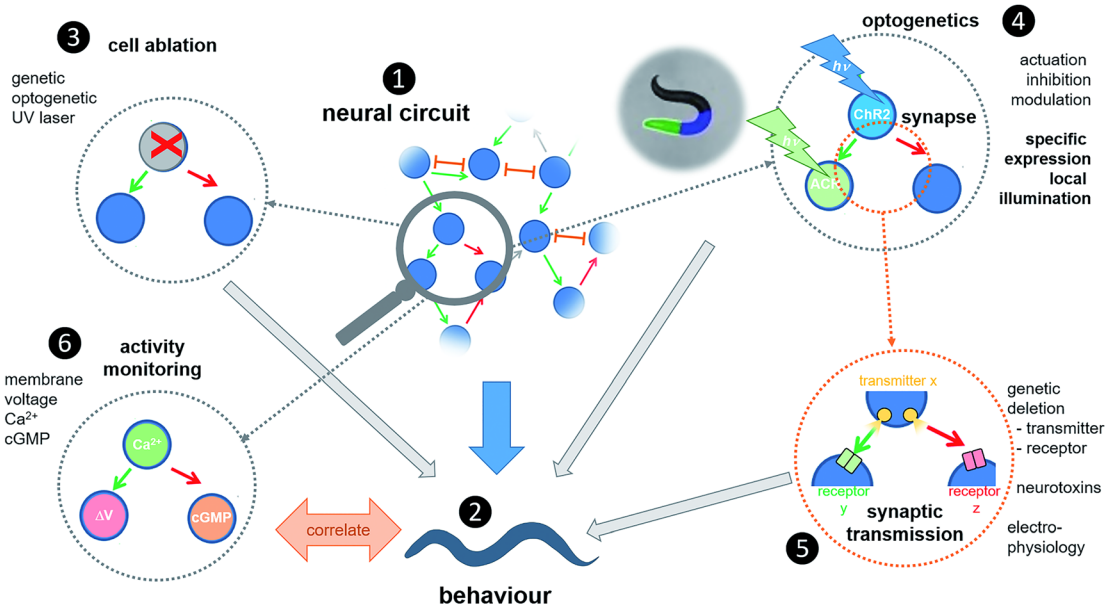

Figure 1: Functional connectomics approaches to study neural circuits and their role in generating behavior in the nematode *Caenorhabditis elegans*.
Circuits (1) drive behavior (2), and to understand the function of individual neurons in this, several approaches need to be combined: Cell ablation (3), to eliminate the neuron of interest; optogenetics (4), to stimulate, inhibit or modulate the neuron acutely (local illumination, inset from Stirman et al., 2012), and to understand its synaptic connections to its partners; synaptic details (5, e.g. transmitter and receptors used) need to be determined, by blocking transmission or by electrophysiology. Experiments to monitor evoked or intrinsic activity, correlated with behavior, involve imaging of second messengers and/or membrane voltage (6). Manipulations in (3–5) affect behavior, and information gathered in all approaches can be combined in models to enable a comprehensive understanding of the circuit.

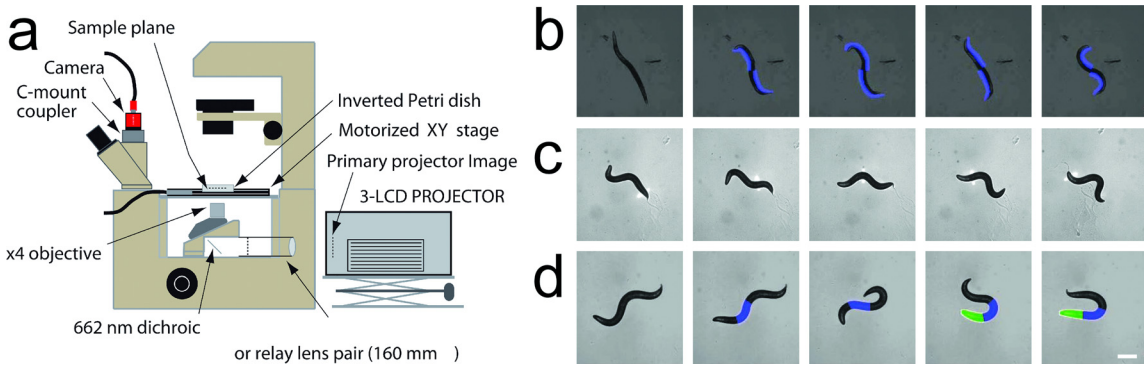

Figure 2: A tracking and illumination system for specific optogenetic manipulation of neurons in freely moving *C. elegans*. (a) Microscope setup, including a projector used to shine light patterns into the microscope, based on the body posture of the animal, obtained from a live video, while the animal is automatically tracked. (b–d) Example geometries, light colors and stimulus sequences to free-moving animals. In (b), this animal, expressing Channelrhodopsin (ChR2) in its body wall muscles, has been paralyzed by addition of ivermectin, which hyperpolarizes motor neurons. The animal can be forced to move by alternating illumination of anterior and dorsal halves, on opposing sides of the body, thus photodepolarizing muscle cells in a localized fashion. In (c), a bar of light is moved from tail to head, which hits ChR2 expressed in mechanosensory neurons, evoking forward locomotion. Once it reaches the anterior half, reverse locomotion results. In (d), the animal expresses ChR2 for photodepolarization in the mechanosensory neurons, and the green light activated hyperpolarizing proton pump Mac in interneurons that normally mediate reverse locomotion. First, blue light evokes a reversal, which is inhibited by concomitant green illumination of the head, in this optogenetic 'circuit-breaker' experiment. From Stirman et al. (2011, 2012).

electrical transmission from the most distal dendritic branches to the axon and synaptic terminals. Optogenetics and RNAi were used to identify ion channels expressed in PVD that may act in the propagation of electrical signals. This led to the identification of two channels, which either amplify signals within PVD (i.e., GTL-1, a TRPM channel) or enhance its output at the synaptic terminal (i.e., ASIC-1, an acid-sensing ion channel) (Figure 3a). Do such channels contribute to mechanisms of habituation in sensory systems? This may be assessed by voltage imaging or electrophysiology. The PVD neuron can be repeatedly photostimulated and always generates a strong behavioral response, as expected for a nociceptor. Similar experiments using gentle-touch neurons demonstrated a profound habituation, as these (repeated) signals represent irrelevant stimuli (Figure 3b and c). Interestingly, PVD optogenetic stimulation would evoke forward escape behavior, while harsh mechanical stimuli mostly evoked reversal escape. The wiring diagram shows synapses from PVD to both forward and reverse pre-motor interneurons, PVC and AVA, respectively (Figure 3d). By ablation of PVC, the response to optogenetic activation of PVD was switched from forward to reverse escape behavior, thus showing that the PVD-PVC synapses are functional (Figure 3e). Thus, during mechanical stimulation, PVD signals and signals from other touch sensors are integrated in the interneuron circuit to determine whether forward or reverse escape behavior is most appropriate.

Single-cell expression of optogenetic tools

Often, neurons in *C. elegans* are located close to each other in the dense nerve ring or ventral nerve cord, thus precluding achieving single-neuron activation or inhibition by spatiotemporally restricting light and optogenetic tools. We thus implemented conditional expression of optogenetic tools (Schmitt et al., 2012), based on methods for cell-specific, conditional expression from two promoters, overlapping in the cell of interest (Figure 4), as established by several labs (Davis et al., 2008; Macosko et al., 2009).

Analysis of neuronal circuits in the generation of behavior

Neuronal circuits as defined by the anatomical connections of chemical and electrical synapses appear hard-wired and thus may be very limited in their activities and potential computations. However, even the genetically determined *C. elegans* circuits are plastic and are not restricted to a single, hard-wired connectivity. Instead, an additional 'wireless' network of neuromodulators is in effect that can alter the function of neurons or even individual synapses such that several functional networks can be superimposed on a single anatomical network (Bargmann and Marder, 2013).

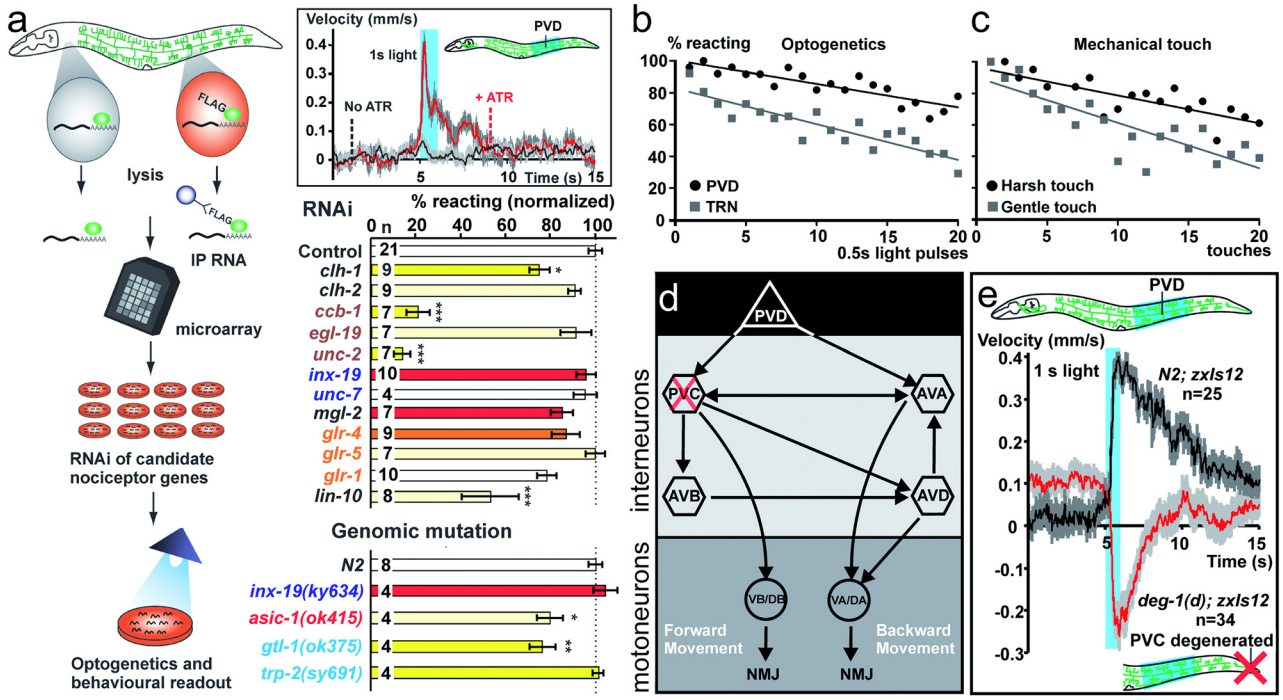

Figure 3: A reverse genetic screen for modifiers of nociceptive signaling. (a) *C. elegans* possesses nociceptive neurons, normally responding to harsh touch, termed PVD. To identify ion channels acting in these neurons, downstream of the nociceptive mechanoreceptors, mRNAs were isolated from PVD by our collaborator, David Miller (Vanderbilt University, Nashville, USA), and identified by microarray analysis. We used double-stranded RNA-mediated interference (RNAi) to knock down candidate genes and analyzed the optogenetically evoked activation of PVD and subsequent escape behavior in these animals (inset, showing increased velocity when PVD is photostimulated via Channelrhodopsin [ChR2; red graph]; no response occurs when all-trans retinal [ATR] is absent, which renders ChR2 non-functional). This highlighted several ion channels (and other genes) required for PVD function. A transient receptor potential (TRP) channel, GTL-1, and an acid-sensing ion channel (ASIC-1) amplified signals or mediated their transmission following PVD photodepolarization. (b, c) Habituation of repeated, optogenetically (b) or mechanically (c) evoked escape behavior following PVD (harsh-touch) or gentle touch receptor neuron (TRN) activation. While gentle touch responses habituate, harsh touch responses (as threat signals) do not. (d) Network downstream of PVD neurons. PVD innervates both forward (PVC) and reverse (AVA) pre-motor interneurons, causing escape behavior. Interestingly, under normal conditions, the photostimulated PVD-evoked behavior is forward escape (as quantified in e). To show whether synapses to AVA are also functional, we genetically ablated PVC (red cross in d). These animals now showed backward escape responses (red graph). From Husson et al. (2012).

An antagonistic network of peptidergic neurons in the regulation of food-motivated behavior

One such network regulates *C. elegans* locomotion as well as navigation behavior, for example, when the animal encounters or searches food (Oranth et al., 2018) (Figure 5). Two peptidergic neurons, called 'AVK' and 'DVA', which have opposing influence on the motor nervous system, either enhance or reduce the extent of body bending and the rate of directional changes. This way, the animal can stay more local (i.e. when food is present), or can initiate long-range search behavior. Further, these two interneurons integrate information from sensory neurons, which detect the presence of food, and signal via dopamine and two opposing dopamine receptors in AVK and DVA, respectively. The neuropeptides released by these two neurons are either excitatory (DVA) or inhibitory (AVK). AVK function gets inhibited in the presence of food (or by optogenetic inhibition using halorhodopsin – NpHR), and the resulting disinhibition of motor neurons leads to increased body bending and dwelling behavior. Similar neuropeptides and dopamine regulate motivated behavior in mammals.

A locomotion stop neuron

Another circuit we studied involves the peptidergic neuron RIS (Steuer Costa et al., 2019) (Figure 6a). The RIS neuron was previously implicated in sleep regulation. Upon photoinhibition of RIS, the animals stopped all muscular behaviors, including locomotion (Figure 6b). This was a rapid and brief response, only as long as the photostimulus,

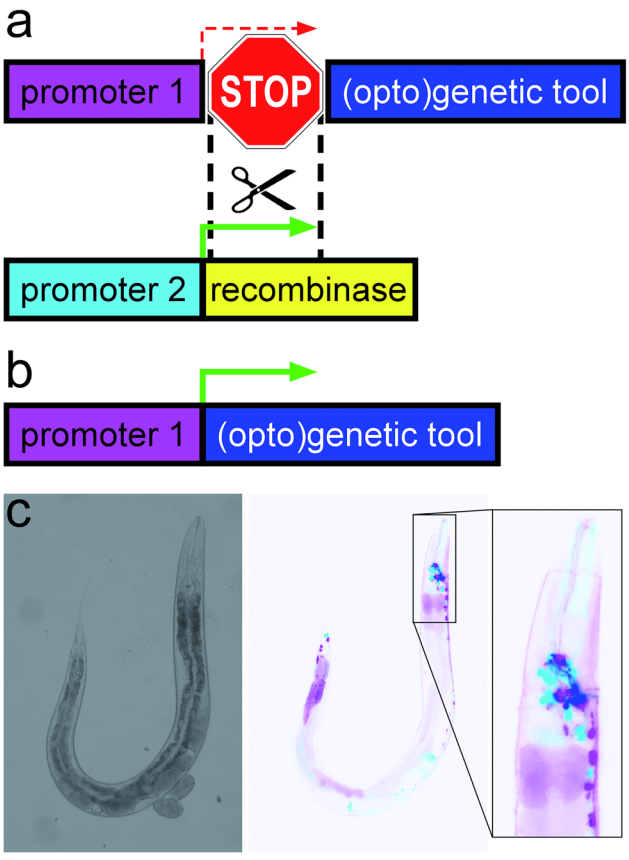

Figure 4: Conditional approach to achieve cell-specific expression of (opto)genetic transgenes. (a) Two promoters with overlapping expression in the cell of interest are used to express (promoter 1) the cDNA for an optogenetic tool, behind a stop cassette, flanked by recombination sites, or (promoter 2) a recombinase like FLP or *Cre*. (b) The stop cassette prevents expression unless it is removed by the recombinase. (c) Example expression patterns of two promoters (pink and cyan), overlapping in the cells of interest (SMB neurons, blue), in the animal's head. Differential interference contrast (DIC) micrograph, (false-colored) overlaid fluorescence micrograph, as well as enlarged section showing the head. Modified from the studies by Oranth et al. (2018) and Schmitt et al. (2012).

unlike a typical sleep episode. We thus wondered if the RIS neuron, which is involved in sleep mainly during development (Turek et al., 2016), functions also in the fine-control of locomotion in adult animals. Animals frequently change their direction, and this involves slowing and stopping, before a reverse locomotion can be initiated, and finally forward locomotion resumes in a different direction. This directional change of locomotion is orchestrated by a motor program (Donnelly et al., 2013; Roberts et al., 2016), and we speculated that a brief episode of RIS function may induce this locomotion stop during the reversal behavior. RIS activity in free-moving animals (Figure 6d), i.e. the onset of the rise of cytosolic Ca^{2+}, preceded the slowing event; thus RIS plays an active role in this behavior or is part of a sequence of neuronal events involving additional cells. The connectome of RIS (Figure 6c) indicates which neurons these could be. How does RIS actually affect locomotion stop? We found that activity of cholinergic motor neurons became desynchronized during RIS activation, which releases neuropeptides and gamma-amino butyric acid (GABA). This appeared to stop locomotion without losing the muscular tone (unlike in sleep), such that movement can quickly resume. RIS shows locally different Ca^{2+} signals in the axon and a branch of the axon, depending on whether just slowing or slowing and reversal occur (Figure 6e). Since the branch receives innervation by three neurons (Figure 6c), we speculate that upstream, as well as RIS-intrinsic events, are integrated at branch and axon, to determine different output of the neuron.

The missing link: AS motor neurons in coordination of locomotor circuits

The locomotion circuits contain a class of cholinergic motor neurons, termed AS (Figure 7a). These had not been analyzed previously, due to the lack of promoters specifically expressed in these neurons. Using a combination of promoters as well as transcriptional activators and repressors, optogenetic tools could be expressed in a 'subtractive' way, specifically only in the AS motor neurons (Tolstenkov et al., 2018; Wei et al., 2012). We characterized locomotion behavior in animals in which AS neurons were ablated, acutely photoinhibited, or photostimulated, and monitored AS neuron activity in moving animals. Inhibition of AS neurons blocked locomotion. They activate the musculature asymmetrically, i.e. only the dorsal muscles, and they trigger GABAergic neurons that concomitantly release GABA on the ventral side. This way, AS neurons may regulate navigation and bending, as a means of fine-tuning the general motor program (Figure 7a and b). The AS neurons are connected to premotor interneurons for reverse (AVA) and forward (AVB) locomotion, by chemical and electrical synapses (Figure 7b).

Analyses of chemical synaptic transmission at the neuromuscular junction

The second interest of my lab is the analysis of synaptic transmission at the neuromuscular junction (NMJ). The

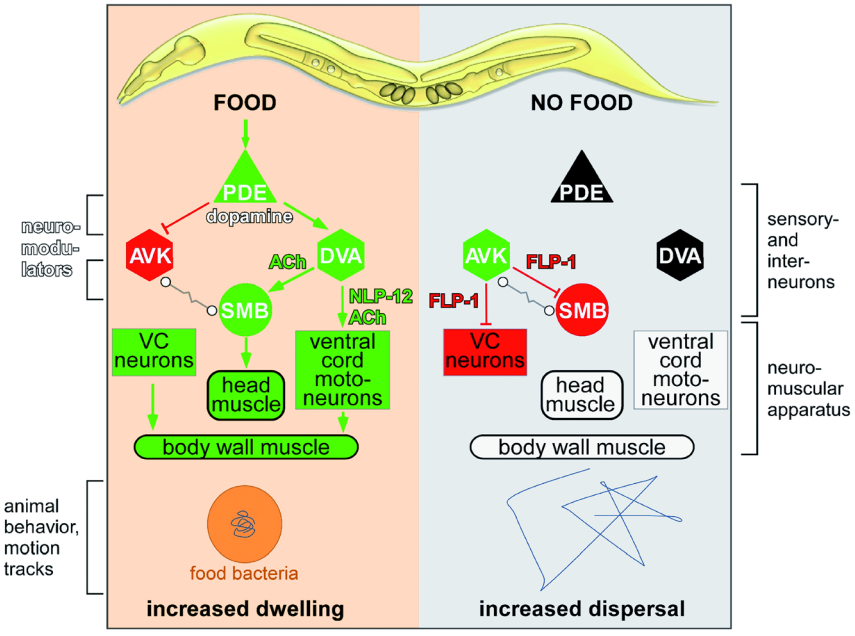

Figure 5: A neuronal circuit for navigation during food search behavior.
C. elegans navigates its environment to find, or remain on, a food source (bacterial lawn). It can also initiate search behavior, once it lost a food source. As we could show, this behavior is in part orchestrated by neuropeptidergic interneurons (AVK and DVA, symbolized by hexagons, releasing FLP-1 or NLP-12 (neuropeptide-like protein) neuropeptides, respectively) that antagonize each other in their effects on the motor system (circles, boxes), thus promoting dwelling (in the presence of food, left) or long-range search behavior (without food, right). Food is sensed by dopaminergic, sensory PDE neurons (triangles). Signals are inhibitory (red) or excitatory (green), sometimes depending on the postsynaptic receptors used. Activity states of the neurons in one or the other condition are also indicated by color. Food inhibits AVK, which stops releasing the inhibitory FLP-1 neuropeptide, thus disinhibiting motor neurons. Modified from Oranth et al. (2018).

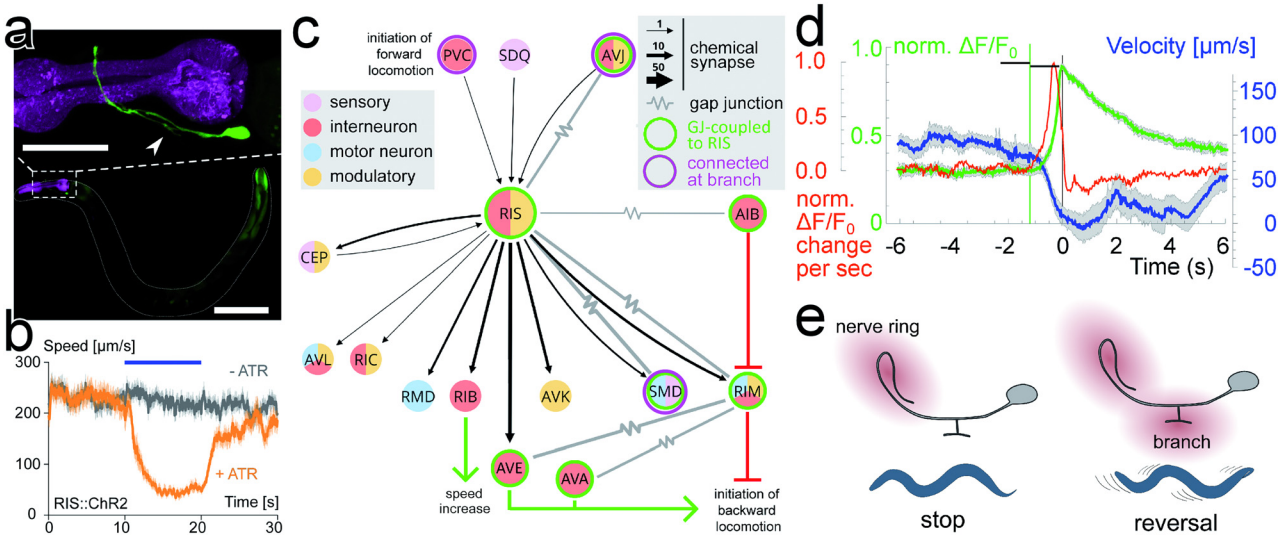

Figure 6: A single neuron, RIS, transiently inhibits the motor circuit for initiation of slowing or reversal behavior. (a) RIS (green) is a single neuron located in the animals' head, with a branched axon (arrowhead). (b) Locomotion speed is reduced when RIS is photoactivated via ChR2. (c) RIS connectome, showing input from (upper half) and output to (lower half) all its partners, with cell types and nature and the number of synaptic connections indicated. (d) Ca^{2+} activity in the axon of the RIS neuron (green) was measured in free-moving animals, and peak Ca^{2+} events were used to align these events and the concomitant locomotion velocity (and direction) of the animals (blue curve; negative indicates reverse locomotion). The Ca^{2+} events and the first derivative (rise rate of the Ca^{2+} signal, red curve) precede the slowing and onset of the slowing event, sometimes leading to a reversal (on average, a transient stop results). (e) RIS has an axon extending to the nerve ring, and a branch that dips into the ventral nerve cord. Ca^{2+} signals in the nerve ring always occurred when the animals slowed down but were accompanied by additional Ca^{2+} activity in the branch upon a reversal. Modified from the study by Steuer Costa et al. (2019).

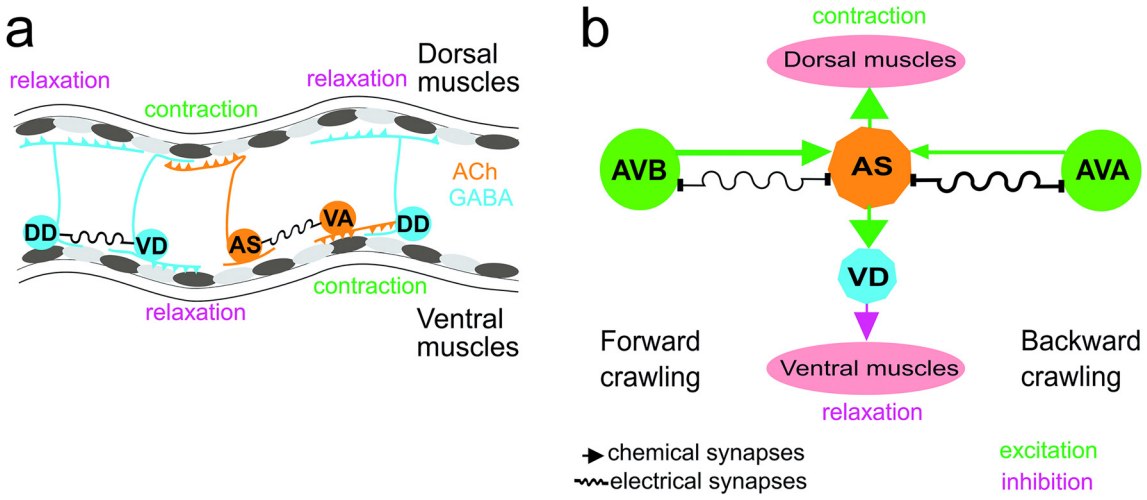

Figure 7: The previously unstudied motor neuron class AS is important for the coordination of the locomotion motor program and possibly for navigation.
(a) Integration of AS motor neurons in the motor nervous system of *C. elegans*, found in sub-circuits, repeated 6–11 times in a segmented fashion along the body. Note that only some neuron classes are shown. Anterior is to the left. (b) AS neurons are connected to premotor interneurons for reverse (AVA) and forward (AVB) locomotion via gap junctions. They are asymmetrically innervating only dorsal muscle, as well as ventral inhibitory GABAergic neurons (VD class); thus, AS neuron activation causes as strong bias to dorsal bending. From the study by Tolstenkov et al. (2018).

C. elegans NMJ is a well-accessible synapse, enabling pharmacological assays which report on postsynaptic and presynaptic functionality. Also, the NMJ arrangement of motor neurons and muscles is easily addressed by fluorescence microscopy in the live animal. The *C. elegans* NMJ is special in that the muscle cells, which extend dendritic spine-like protrusions, directly receive cholinergic and GABAergic input; thus, they integrate excitatory and inhibitory motor activity (Figure 8a). This is opposed to the mammalian system, where activity of excitatory and inhibitory interneurons is integrated by (excitatory) motor neurons. However, for *C. elegans* this means that stimulation of cholinergic neurons will also trigger GABA neurons (on the contralateral side), as this reciprocal innervation is required for coordination of body undulations. The different classes of cholinergic and GABAergic neurons and their ventral or dorsal innervation patterns have been characterized, and to some extent, specific genetic markers exist (see above). Electrophysiology of the NMJ is demanding, due to the dissection of the small animal (Figure 8b).

We developed additional – optical – assays to analyze NMJ activity (Figure 8a, c and d). To this end, we expressed (red) fluorescent sensors of cytosolic Ca^{2+} (e.g. RCaMP; Akerboom et al., 2013) or of membrane potential (rhodopsin-based voltage indicators like Arch(D95N), QuasAr or electrochromic FRET sensors; Gong et al., 2014; Azimi Hashemi et al., 2019; Kralj et al., 2012), in muscle cells (or neurons), and used optogenetic actuators like ChR2 in the motor neurons, to trigger acetylcholine (ACh) or GABA release. The resulting effects can be imaged in the postsynaptic muscle. This is not as acute as electrophysiological recordings. Yet, particularly voltage imaging enables an important alternative to electrophysiology since it can be performed in the intact animal, thus not requiring use of artificial solutions that may not reflect the endogenous ionic compositions and could thus falsify results. The all-optical electrophysiology at the NMJ also enables a more accurate analysis of the integration of excitatory and inhibitory signals in muscle cells, as no dorsoventral motor neuron commissures are severed, like in the 'filet' preparation for electrophysiology. These methods allow a straightforward comparison of wild type and mutants in presynaptic or postsynaptic proteins required for synaptic transmission, neurotransmitter detection or synaptic vesicle recycling (Kittelmann et al., 2013; Liewald et al., 2008; Wabnig et al., 2015).

Analysis of synaptic transmission by combined optophysiology and electron microscopy

These methods allowed analyzing proteins required for synaptic vesicle (SV) endocytosis and recycling. Particularly under conditions of prolonged stimulation, the synapse needs to efficiently recycle SV membrane and

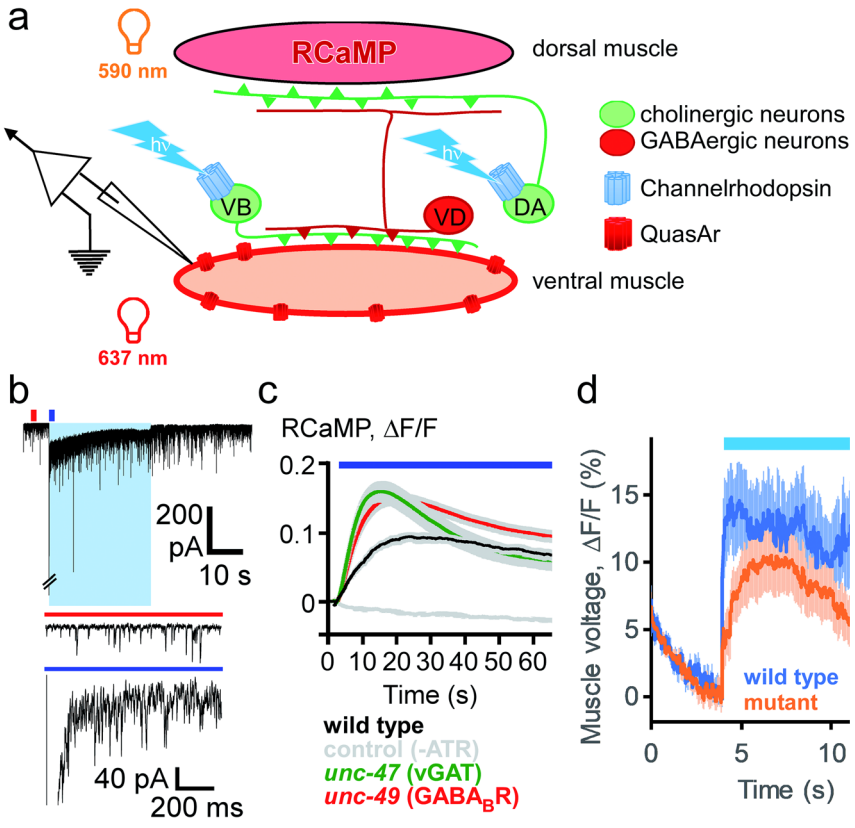

Figure 8: Optophysiology methods to study synaptic transmission at the NMJ. (a) NMJ organization of *C. elegans* (simplified). Cholinergic neurons innervate muscle, as well as GABAergic neurons that innervate the opposite side. Optogenetic tools for actuation (ChR2) expressed and activated in cholinergic neurons cause release of acetylcholine but also trigger GABAergic neurons. As an alternative to patch recordings, postsynaptic RCaMP (a red-fluorescent Ca^{2+} indicator) or QuasAr (a rhodopsin-based fluorescent voltage indicator) can be combined with presynaptic ChR2 photostimulation to monitor NMJ transmission. (b) Example electrophysiology (evoked post-synaptic currents) of light-evoked transmission (blue shaded region). Red and blue bars indicate region enlarged in the lower panels. (c) Mean ± SEM RCaMP fluorescence ($\triangle F/F$) in muscle, before and during a photostimulus to cholinergic motor neurons (blue bar). Compared are wild type and two mutants lacking GABA transmission, resulting in higher signals in muscle. (d) Experiment similar to (c), but using QuasAr voltage sensor and comparing wild type and a mutant with a presynaptic defect. Modified from the study by Kittelmann et al. (2013), Wabnig et al. (2015), and Bergs and Gottschalk, unpublished (d).

proteins, in order to keep up with ongoing release of neurotransmitter. To study these processes in more detail, optical stimulation of neurons is combined with electron microscopy of rapidly, high-pressure frozen, freeze-substituted and metal-stained animals, during or following light stimulation of the motor neurons (Kittelmann et al., 2013; Yu et al., 2018) (Figure 9a).

This methodology was also developed by other labs for use in mammalian neurons and termed 'flash-n-freeze' electron microscopy (Watanabe et al., 2013a, b). In my lab, we further analyzed the effects of cyclic adenosine monophosphate (cAMP) signaling in cholinergic motor neurons, following optogenetic stimulation of cAMP generation by photoactivated adenylyl cyclase (bPAC) (Steuer Costa et al., 2014). We found that cAMP has several effects (Figure 9b). It mobilized synaptic vesicles from the reserve pool, and it causes increased fusion due to the higher amount of fusion-competent vesicles. Furthermore, we found that cAMP causes the fusion of neuropeptides from dense core vesicles, which could not be evoked by mere depolarization using ChR2. The neuropeptides had an autocrine effect in motor neurons, which caused the filling of existing SVs with additional neurotransmitter (ACh), thereby increasing quantal size (Steuer Costa et al., 2017) (Figure 9c and d). This was due to increased activity of the vesicular ACh transporter (vAChT) and markedly enlarged the SVs. This novel neuromodulatory presynaptic plasticity may enable the motor system to integrate signals as determined by different internal states, or signals in response to external stimuli, which cause cAMP

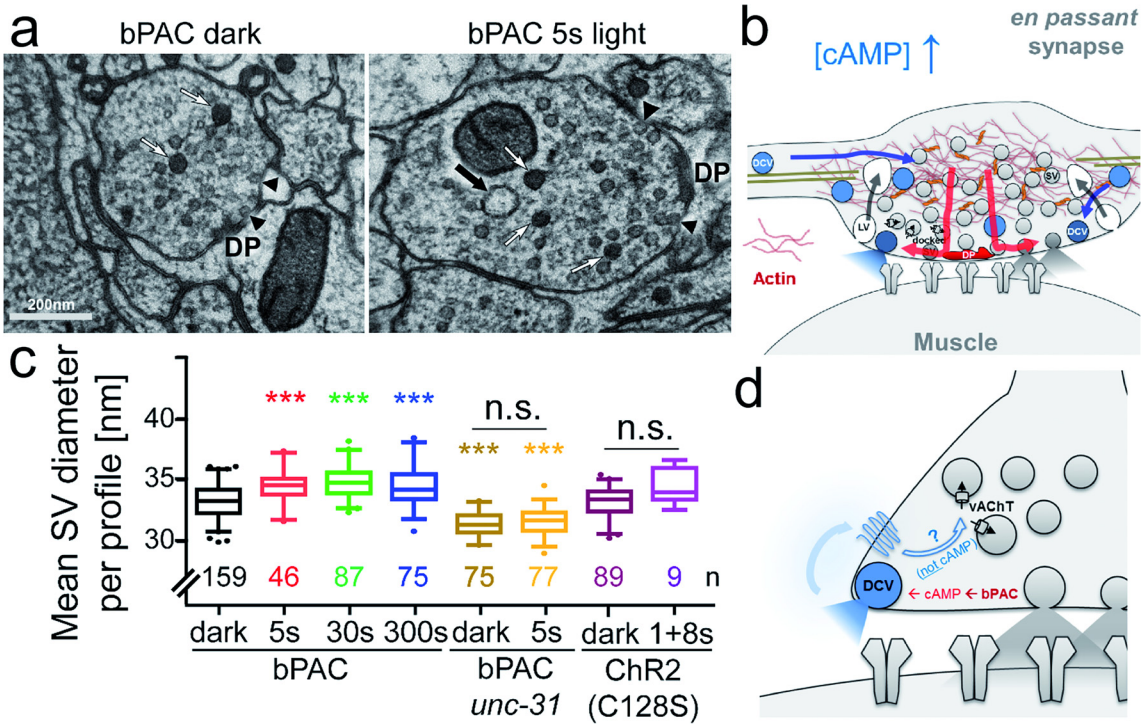

Figure 9: Cyclic adenosine monophosphate (cAMP) signaling enhances synaptic transmission at the *C. elegans* NMJ via neuropeptidergic signals and increased synaptic vesicle (SV) filling. (a) Transmission electron micrographs (40-nm sections) from cholinergic synapses expressing the blue light–activated adenylyl cyclase bPAC, without and with 5-s photostimulation. Arrowheads: Docked SVs; white arrows: dense core vesicles (DCVs), containing neuropeptides; DP: dense projection, protein machinery at the center of the active zone; black arrow: endosome, evoked by SV fusion and compensatory endocytosis. (b) Summary of cAMP evoked effects in the synapse: SVs are mobilized from the actin cytoskeleton and more readily released upon neuronal depolarization; increased DCV fusion causes neuropeptide release, and enhanced endocytosis causes formation of endosomes. (c) Diameter of SVs, averaged per profile, shows that photoevoked cAMP signaling causes a significant increase in SV diameter. This is not found in response to ChR2 photostimulation and is absent in *unc-31* mutants lacking Calcium-dependent activator protein for secretion (CAPS), a protein required for DCV fusion. Moreover, CAPS mutants have significantly smaller SVs, indicating that neuropeptide signaling may generally regulate SV size (filling state). (d) cAMP causes release of neuropeptides, which are sensed by autoreceptors and trigger a second, non-cAMP signaling pathway affecting SV filling via the vesicular acetylcholine transporter (vAChT). Modified from the study by Steuer Costa et al. (2017).

increase in the motor neurons, thus enabling a more potent output of the motor system.

Conclusion

C. elegans is an elegant system to develop integrated approaches for studies of neuronal networks and synaptic transmission by optogenetics, which allows us to make important contributions to the field in clarifying the role of some circuits and analyzing novel aspects of transmission between its neurons. We could provide methods and reagents for other labs, to address additional parts of the *C. elegans* nervous system. The Schram foundation helped us to bring forward or sparked ideas for a number of projects, and this funding was particularly important during the early days of my lab.

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Bionote

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Alexander Gottschalk studied chemistry in Frankfurt and Marburg and initially specialized in biochemistry of spliceosomes, before he turned to the neurobiology of *Caenorhabditis elegans*. After a postdoc at the University of California, San Diego, where he studied nicotinic acetylcholine receptors, he returned to Goethe University as a junior professor. In Frankfurt he learned about a novel protein, which proved to be a light-activated ion channel – channelrhodopsin-2 (ChR2). With Georg Nagel and Ernst Bamberg, he could demonstrate that ChR2 can mediate rapid depolarization of muscles and neurons in response to light, in live animals, and that it can trigger coordinated behavior. This sparked a new direction for his lab, and he began to develop and apply numerous light-sensitive proteins as optogenetic tools in *C. elegans* and to use them for analyses of neuronal network function, as well as to study mechanisms of synaptic transmission. In 2009, he became a Heisenberg Professor, and since 2016, he is a full professor at Goethe University. Furthermore, he is the speaker of the DFG priority program SPP1926 – Next Generation Optogenetics – tools and application.