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Physiology 25:311-318, 2010. doi:10.1152/physiol.00019.2010

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Identifiable Cells in the Crustacean Stomatogastric Ganglion

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Neural circuits rely on slight physiological differences between the component cells for proper function. When any circuit is analyzed, it is important to characterize the features that distinguish one cell type from another. This review describes the methods used to identify the neurons of the crustacean stomatogastric ganglion.

In the study of neurophysiology, neurons are often regarded as information processors. Their role in the function of an organism is to process sensory information (the input) and respond with the command impulses for some action (the output). Of course, individual neurons cannot achieve this goal in isolation; it is the combined processing power of complex neural circuits that controls behavior. Therefore, the key to understanding any behavior lies in defining and dissecting the neural circuits involved. Although this goal can be achieved in a variety of ways, arguably the most successful techniques begin by observing the component neurons and classifying each according to a type.

That said, cell type is something of a tricky concept. Historically, cell type was defined first and foremost by morphology, or what the cell looks like; however, in neuroscience this has proven to be only partially useful. More recently, neurons have been reliably identified by a combination of several different features. For example, mammalian neocortical fast-spiking cells are identified by a combination of their morphology, inhibitory neurotransmitters, electrophysiological properties, unique protein expression, and characteristic synaptic locations targeted on postsynaptic cells (63). Increasingly, molecular markers and genetic profiles are also being used for identification of mammalian neurons (25, 32, 42). Despite this progress in identifying increasingly specific cell types, the large scale and high interconnectivity of mammalian systems makes cell-level circuit mapping extremely difficult.

The average invertebrate neural circuit, on the other hand, is substantially simpler than any mammalian system. In some cases, this means that there exists a single neuron serving a unique and necessary function in every animal of that species (hereafter referred to as identified neurons), whereas, in a typical mammalian system, this necessary function will be served by a population of neurons with similar properties (a cell type). The invertebrate advantage of readily identifiable neurons has allowed the full connectivity of several functional neural circuits to be reproducibly mapped (19, 30, 64). A well known example of this is the stomatogastric ganglion (STG) of

decapod crustaceans, such as lobsters and crabs. The STG is comprised of ~30 neurons that control the rhythmic movement of the animal's foregut musculature, many of which are readily identifiable (54). In this review, the concepts of cell type and identity will be explored by introducing how cells have been identified in the STG in the past, how the features that define cell identity in the STG have evolved over time, and how the knowledge gained from identifiable cells in the STG can be useful for the study of neurophysiology.

The History of Identified Cells in the Stomatogastric Ganglion

For over 100 years, it has been known that there is tremendous potential for nervous system study in the rhythmic muscular contractions of the crustacean foregut (12). Methylene blue staining was used in the late 19th century and early 20th century by Allen and Orlov to reveal nerve pathways and locations of cell bodies within the ganglia of the crustacean stomatogastric nervous system, and it was found that the main stomatogastric nerves were similar in all decapod crustaceans (8). However, it was not until the mid 20th century that interest in this system grew (40). During this period, a combination of anatomical and electrophysiological techniques was used to establish the identifiable neurons present in the STG, to determine the intrinsic network connectivity, and to understand how each neuron contributed to the motor pattern production.

There are two general motor patterns that are controlled by the neurons in the STG: the grinding of the gastric teeth to break down large food particles in the stomach, known as the gastric mill, and the dilation and constriction of the pylorus to filter and push food through to the digestive gland or midgut (19). The first neurons in the STG to be consistently identified were the motor neurons that drive these patterns. Motoneuron function in the STG was conclusively determined by using electrophysiology to confirm the anatomical muscle innervation patterns that had previously been

elucidated with methylene blue stainings (41). Extracellular wire pin electrodes were placed on motor nerves near the point of muscle innervation, whereas sharp glass microelectrodes were used to impale cell bodies in the STG (54). In this way, intracellular neuronal activity could be correlated with the extracellular nerve activity driving particular muscles, and motoneuron connectivity to stomach musculature could be determined. Simultaneous intracellular recordings in multiple cell bodies were used to identify the connectivity and character of the few interneurons in the STG, as well as the electrical and chemical synaptic connections between motoneurons (43, 53). Furthermore, a combination of pharmacology to inspect neuronal response to applied synaptic channel agonists, electron microscopy to distinguish vesicle features at synaptic terminals, and biochemistry to identify the presence of enzymes in neurons that help produce certain neurotransmitters allowed for the identification of the different neurotransmitters released by STG neurons (28, 29, 35, 36, 38, 39). During this early period of crustacean STG study, identifiable STG neurons were defined as the cells that were functionally identical in different animals of the same species (53). Thus a functional description became the basis for the established naming system for the neurons in the STG, and by this definition it was often the case that multiple neurons were given the same name if they were electrically connected and carried axons to the same muscle, making them functionally indistinguishable.

The identified neurons in the STG are typically functionally segregated based on what motor pattern each particular neuron is found to participate in. The gastric mill network contains seven neuron types: the anterior median neuron (AM), the lateral gastric neuron (LG), the median gastric neuron (MG), the two lateral posterior gastric neurons (LPG), the four to nine gastric mill neurons (GM), the dorsal gastric neuron (DG), and interneuron 1 (Int1) (19). The membrane potentials of the electrically connected LG and MG neurons rise and fall in synchrony, alternating with the two LPG neurons, which are inhibited by both LG and MG (FIGURE 1, B AND C). Int1 fires bursts of action potentials slightly phase advanced from AM and DG, which are also electrically connected and alternate with the GM neurons. This oscillatory rhythm has a 5- to 10-s period. The pyloric network contains six neuron types: the anterior burster neuron (AB), the two pyloric dilator neurons (PD), the ventricular dilator neuron (VD), the inferior cardiac neuron (IC), the lateral pyloric neuron (LP), and the three to eight pyloric neurons (PY) (FIGURE 1, B AND C; Table 1). The variability in GM and PY neuron number depends on the species of

decapod crustacean and can even co-vary between animals of the same species, suggesting that these two cell types may arise from the same precursor (7). The pyloric circuit can be separated into three functionally distinct nodes whose inhibitory neurons fire in successive bursts to give rise to a characteristic triphasic pyloric firing pattern with a cycle period of 1–2 s. The AB/PD node, which consists of the AB neuron that is electrically coupled to the two PD neurons, is considered the circuit pacemaker; when neuromodulatory inputs from the adjacent commissural ganglia (CoG) and esophageal ganglion (OG) are intact (FIGURE 1A), AB oscillates strongly and drives the two PD neurons to fire bursts of action potentials in synchrony through gap junctions (19). The LP, IC, and VD neurons, which begin bursting immediately after the AB/PD node falls silent, comprise the next node in the circuit. The final node includes the PY neurons, which burst after the LP neuron falls silent. The AB/PD pacemaker node sends fast glutamatergic and slow cholinergic inhibitory inputs to each of these cells to drive the staggered periodic bursting in those two circuit nodes. The neurons of the pyloric circuit are referred to as conditional bursters because, if the nerve that carries the various neuromodulatory inputs (see FIGURE 1A) to the STG is blocked or cut, a technique known as decentralization, the triphasic rhythm is quickly abolished, with each cell in the circuit falling silent or spiking tonically (60) (Table 1). The LP and PY neurons of the pyloric network are also known as rebound bursters because they are not pacemaking neurons with the intrinsic oscillatory behavior of the AB/PD neurons; they instead fire bursts of action potentials when they are released from inhibitory drive. Despite the typical segregation of STG neurons into their respective networks, for some species of crustacean (e.g., the crab *Cancer borealis*), there are stronger interconnections and thus less segregation between gastric and pyloric networks than there are for lobsters (19), and some neurons can share participation in each of the networks (FIGURE 1B, labeled as gastro-pyloric neurons).

The State of the Art: What We Know Now About Identified Neurons

With network connectivity presently established in the STG, neurons are often identified in practice by comparing their intracellular electrical activity profile to extracellular nerve recordings in locations where specific neurons are known to send axons. As with the network activity of the gastric and pyloric networks, the intracellular activity of each neuron that comprises these networks is known

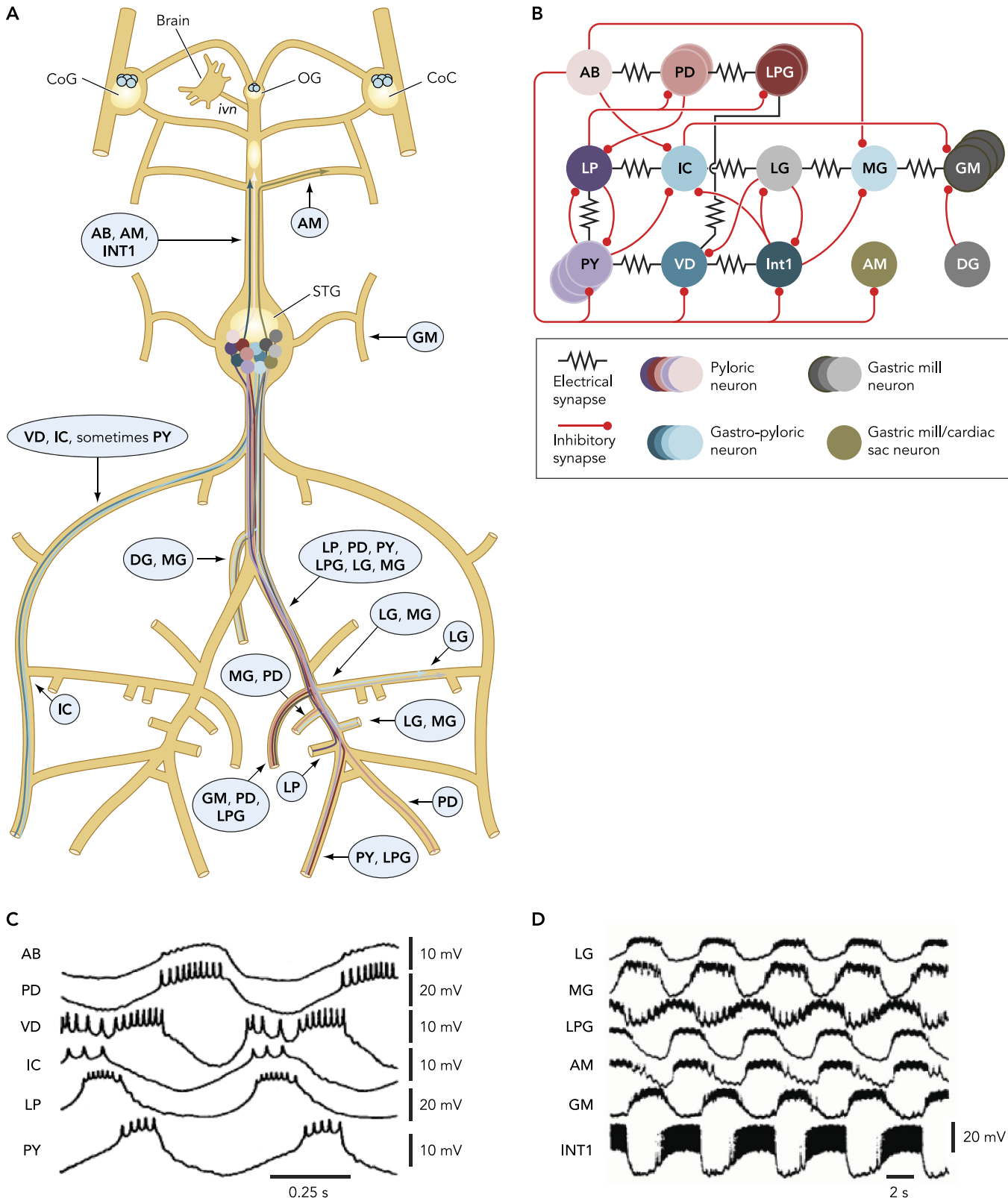


FIGURE 1. The cells of the stomatogastric ganglion

A: the STG is part of a larger stomatogastric nervous system including upstream ganglia such as the commissural ganglia (CoG) and the esophageal ganglion (OG). The neurons of the STG are color coded according to type identified in B. Although the exact location of each neuronal cell body within the STG varies from animal to animal (7), the illustrated axonal projections are known and often used for neuronal identification. Note that relative ganglia and nerve sizing is not drawn to scale. B: the circuit configuration of the STG that is typical for *C. borealis* is shown. Neurons are color coded based on their participation in the pyloric rhythm (warm colors), the gastric rhythm (gray), or both (cool colors). The electrical connections between cells are also illustrated. Figure is adapted from Ref. 56 and used with permission from Springer. C: the neurons of the STG have robust periodic electrical activity when within the circuit. Shown are two cycles of the pyloric rhythm when the gastric rhythm is absent [used with permission from *Scholarpedia* (52)]. D: electrical activity of the gastric mill circuit [used with permission from *Scholarpedia* (52)].

Table 1. The intrinsic activity of identified stomatogastric neurons is variable and may depend on the method of isolation, among other factors

Neuron Name	Abbreviation	Intact STNS, Intact Circuit	Decentralized, Intact Circuit	Decentralized, Synaptic Isolation	Intact STNS, Synaptic Isolation
Anterior burster	AB	Bursting (47)	Silent (44) Bursting (11)	Spiking (1,2) Bursting (21)	Bursting (2)
Pyloric dilator	PD	Bursting (47)	Silent (1) Bursting (11,21)	Silent (47) Spiking (2,21,47) Bursting (47) Irregular (2)	Bursting (2)
Pyloric neuron	PY	Bursting (47)	Silent (21) Spiking (1)	Silent (21,47) Spiking (19)	Bursting (2)
Lateral pyloric	LP	Bursting (47)	Silent (21) Spiking (2,21) Bursting (1,11)	Silent (21,47) Spiking (2) Irregular (21)	Spiking (2) Bursting (2)
Inferior cardiac	IC	Bursting (47)	Silent (1)	Silent (2,21)	Bursting (2)
Ventricular dilator	VD	Bursting (47)	Silent (1) Bursting (11)	Silent (2) Spiking (19) Bursting (21)	Bursting (1)
Interneuron 1	INT1	Bursting (20,47)			
Gastric mill neuron	GM	Bursting (20,47)	Silent (20)	Silent (47)	
Lateral gastric	LG	Bursting (20,47)	Silent (20)		
Lateral posterior gastric	LPG	Bursting (20,47)	Spiking (20)	Irregular (47)	
Medial gastric	MG	Bursting (20,47)	Silent (20)		
Dorsal gastric	DG	Bursting (20,47)	Silent (20)		
Anterior median	AM	Bursting (20,47)	Silent (20)		

and stereotyped (FIGURE 1, C AND D), making this method of identification convenient, but neuron identification based on electrical activity alone has its limits. For example, in the lobster *Homarus americanus*, there are six STG neurons that are considered silent neurons and display no membrane potential fluctuations or only show a few hyperpolarizing postsynaptic potentials (7). These neurons are naturally unidentifiable using simple intracellular electrical activity recordings because of the lack of stereotyped features. Also, as mentioned earlier, the crab *Cancer borealis* has less segregation between gastric and pyloric networks, and this could also present a challenge in definitively identifying neurons through a combination of stereotyped intracellular and extracellular activity alone. In *Cancer borealis*, the gastric neuron LPG, for instance, is electrically coupled with the two PD neurons and may fire bursts of action potentials in near-synchronous rhythm with them (FIGURE 1), making consistent LPG and PD identification in *Cancer borealis* difficult. Neuron identification based on stereotyped electrical activity is also disrupted in instances where the motor pattern is no longer present, as is the case when all external neuromodulatory input to the STG is lost through decentralization, or when neurons are disconnected from their synaptic partners either with drugs or by physical cellular isolation. In fact, STG neurons exhibit different electrical activity in different states: in the normal circuit, decentralized

but retaining STG-intrinsic synaptic connections, decentralized and in synaptic isolation through chemical synaptic block, and in isolation but with normal neuromodulatory inputs (Table 1). Furthermore, activity characteristics are often indistinct within many of these conditions, preventing consistent identification.

Up until now, we have been describing how neurons in the STG are identified functionally, but as mentioned earlier this functional method is not easily scalable when the neural networks are as complex as mammalian nervous systems. This has led to a push to identify neurons based on molecular-level cellular properties, because they may be used to distinguish different neuron types even in the absence of stereotyped oscillatory electrical activity or clear innervation patterns. For instance, the ion channels that ultimately control the unique electrical properties of any cell can be created, modulated, replaced, or destroyed as a result of many converging molecular pathways or events (reviewed in Ref. 51). Consequently, slight differences in the intracellular milieu from one cell to another can mean the difference between a silent or a bursting cell. As molecular techniques become more advanced, it is increasingly clear that there are a variety of notable biochemical differences underlying the cell type-specific electrophysiological properties of cells in the STG. These differences can be loosely divided into two categories: those that are intrinsic to the cell and those that depend on extrinsic input.

There is considerable variability in intrinsic properties between the cell types of the STG. For example, it has been observed that the kinetics of some ionic membrane conductances are subtly different from one cell type to another, which suggests slight differences in the probability of the ion channels mediating the conductance to open or close as a function of the membrane potential (4, 15). Recent research points to a combination of alternative splicing of the ion channel genes and various posttranslational modifications as underlying these differences. Although there is still much work to be done to link specific splice variants to expressed activity, evidence for alternative splicing in the STG is widespread (3–5, 26, 27, 46). The most intensely studied case of alternative splicing in the STG is the *shal* gene, which has at least 14 possible isoforms (4, 5). The *shal* gene

codes for the ion channel mediating a transient K^+ conductance (g_A), and this conductance has graded kinetics between cell types (FIGURE 2B). However, it has been shown that many of the identified isoforms of this gene have few distinguishable electrical properties, and, therefore, they cannot be solely responsible for the large diversity of g_A . Other mechanisms, such as posttranslational modifications or cell-specific auxiliary proteins (65), must be involved, and this is an interesting area for further research.

There is also considerable variability in intrinsic properties within each cell type. When identified cells between different animals of the same species are compared, the maximal expression of each conductance (G_{max}) is highly variable (14, 49, 50) (FIGURE 2A). As mentioned, the G_{max} of each class of ion channel directly influences the electrical

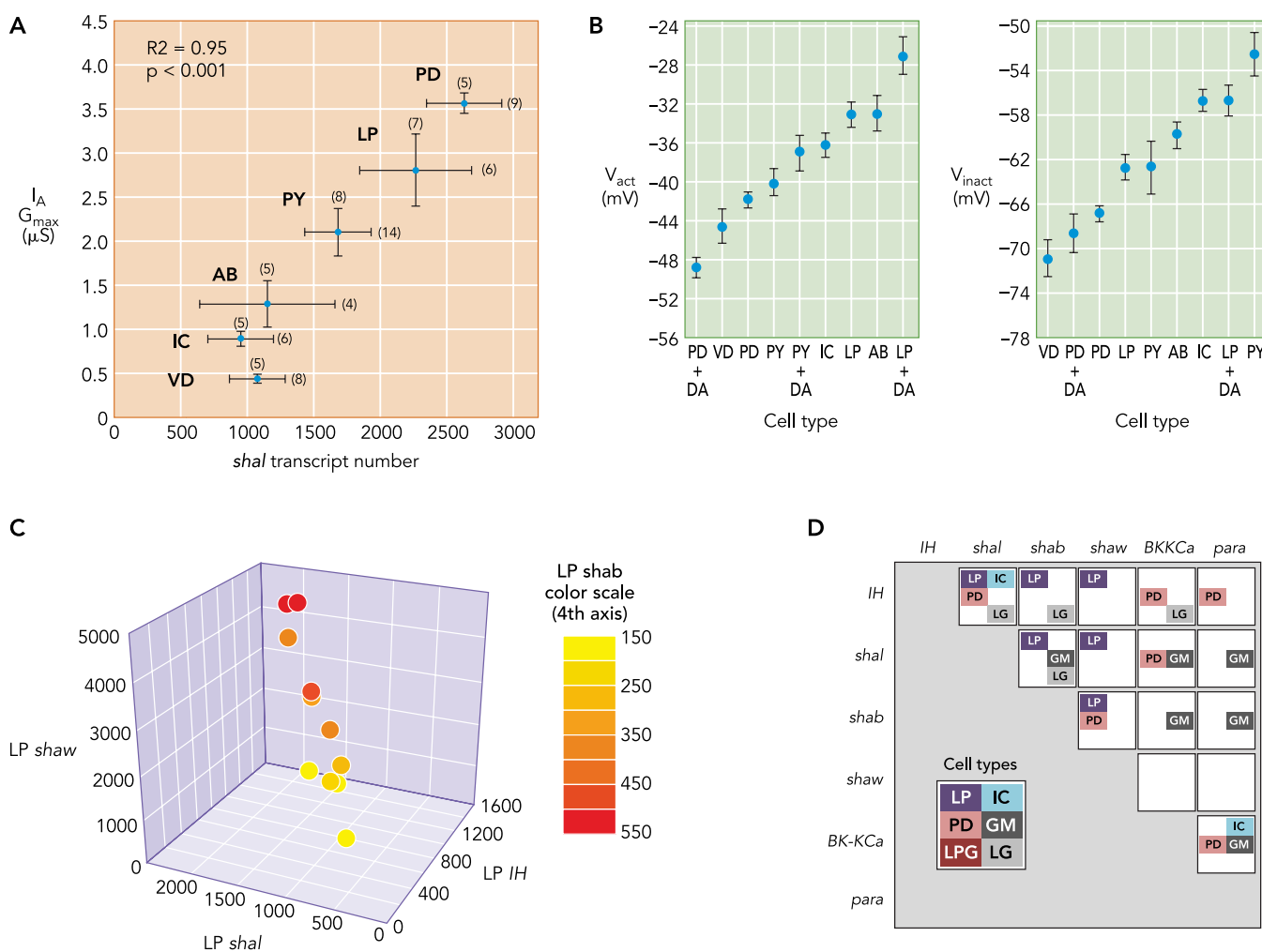


FIGURE 2. STG neurons exhibit cell-type specific constraints despite conductance variability
 A: conductance variability exists within and between cell types. This figure shows the maximal conductance of the A-current (I_A) for each cell type as a function of *shal* gene expression. The numbers in parentheses represent the number of cells used to measure either the G_{max} or the number of *shal* transcripts. Figure used with permission from *Journal of Neuroscience* (4). B: neuromodulators can affect cell-type-specific characteristics that may lead to differences in maximal conductances between different cell types. Shown are the voltages at which I_A activates and inactivates for each cell type, with and without the addition of exogenous dopamine (DA). Figure used with permission from *Neuroscience* (5). C: STG neurons show linear correlations of ion channel expression. Shown is a unique four-way correlation between ion channel mRNA transcript numbers measured in the LP neuron. D: pairwise correlations of ion channel mRNA also exist among STG neurons. Axis labels correspond to six different ion channel mRNAs present in STG neurons. Colored labels depict existence of significant pairwise linear correlation for the corresponding mRNA pair. Note that the color scheme in this figure corresponds to that used in FIGURE 1. C and D adapted from Ref. 50 and used with permission.

phenotype of a cell, so determining how a cell type with variable conductances can have dependable activity is an interesting question. Recently, several type-specific constraints on this variability have been discovered. For example, although crab populations of PD and LP cells both have large (two- and threefold, respectively) ranges of *shal* mRNA expression, these ranges do not overlap (49). In fact, each cell type studied has been found to have a unique pattern of ion channel mRNA expression, with often distinct ranges of expression (4, 50). Furthermore, the expressed conductance values for an individual cell are not as random as these large ranges might suggest. It has recently been shown that members of several STG cell types conform to fixed ratios between two or more conductances (13, 24, 33, 34, 50) (FIGURE 2, C AND D). Every cell type studied shows a unique combination of conductance relationships; both the conductances involved and the exact ratio of each conductance pair vary between cell types. These conductance ratios provide a likely mechanism for maintaining cell type-specific electrical activity in the face of conductance variability (22, 61), although the functional benefits of these ratios may depend on the characteristics of activity that are maintained by each cell type (59).

Another neuronal property that can help distinguish different neuron types is responsiveness

to external input, such as neuromodulation. Neuromodulation generally refers to a diffuse chemical signal sent from one area of the nervous system to another. This signal can be mediated by axonal projections into the region of interest or neuromodulators may be released into the blood stream and circulated throughout the animal as hormones. The STG is an ideal system for studying the effects of neuromodulation due to its physical isolation from all modulatory influences, such as the neurons of its neighboring ganglia (reviewed in Refs. 37, 56). This physical isolation has allowed the detailed comparison of cellular behavior with and without neuromodulation or with a subset of the large number of neuromodulators usually present in the STG. When all neuromodulators are removed from the ganglion, each cell type responds differently (Table 1), but the overall result is the temporary loss of network activity. Over time, the activity is restored via cell type-specific changes in conductance levels (17), which in some cases may mean the loss of the conductance ratios discussed earlier (24). This raises the question, what is causing each cell to react differently to the loss of neuromodulator? It is clear from experiments that bath-apply single neuromodulators to an otherwise isolated STG that different cell types respond to the application of

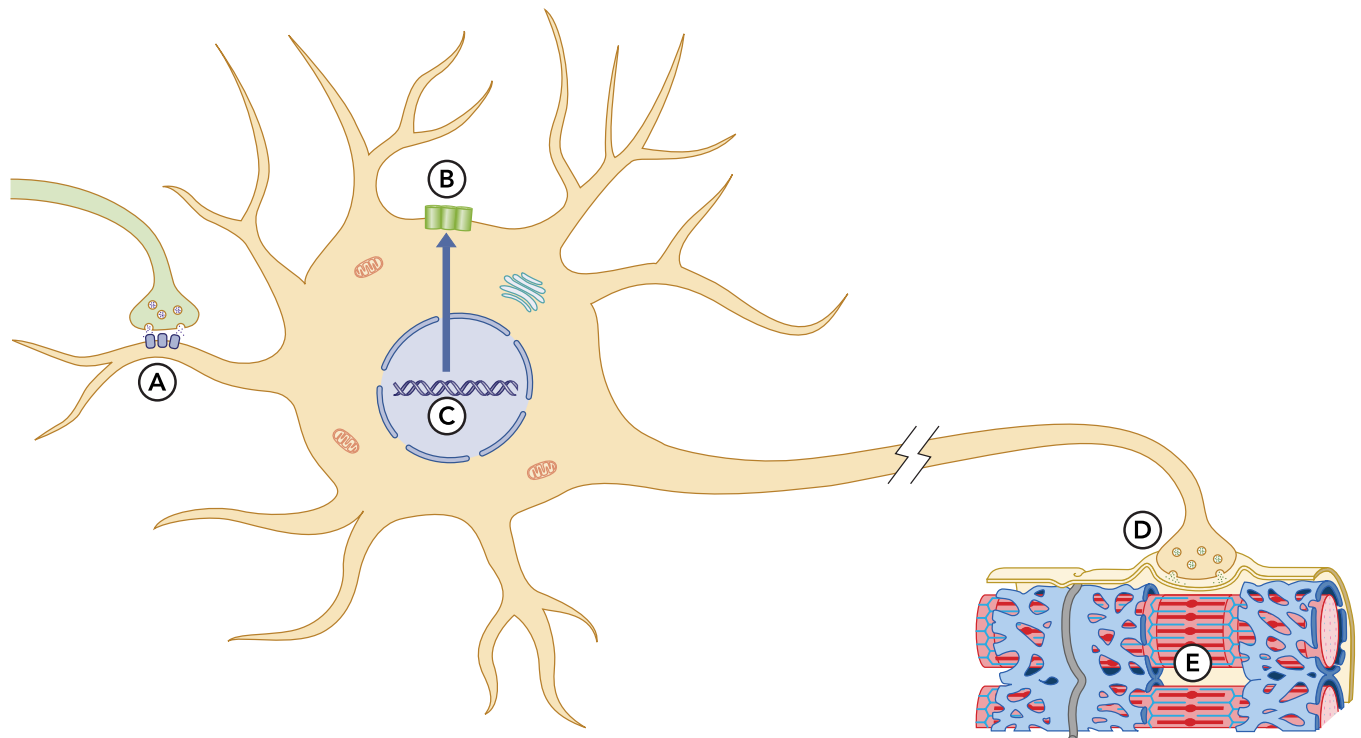


FIGURE 3. Summary of cell-level identification techniques

A: the surface proteins expressed at the synapse determine the cellular response to neurotransmitters or neuromodulators. B and C: the proportions of each kind of ion channel expressed by each cell determine the intrinsic electrical properties for that cell. D: the type of neurotransmitter released can be an identifying characteristic. E: in the STG, it is easy to identify motoneurons based on which muscle they innervate. Not shown: morphology and connectivity can also be used as identifying characteristics.

each chemical differently (18, 57, 58). For example, dopamine increases the peak amplitude of the calcium current in PY, LP, and IC cells, whereas it decreases this same property in AB, PD, and VD cells (23). Further investigations suggest that cell type-specific differences in the expression of dopamine receptors are behind these differences (10, 45). Furthermore, each dopamine receptor has been linked to a particular G protein, which hints at differences in the intracellular cascades active in each cell type. This is only one example of the widespread cell type-specific expression of neuro-modulator receptors in the stomatogastric ganglion (reviewed in Refs. 37, 56).

The Future of Cellular Identification and Circuit Analysis

Identified neurons simplify the analysis of complex neural systems by adding an element of reproducibility to each experiment. When a neural circuit contains identifiable cells, targeted perturbations can be made directly to universally known circuit components. In this way, inferences can be made about the structure of the circuit and the mechanisms behind generating a particular stereotyped behavior. Identifiable cells also allow long-term observations regarding adaptation to a changing environment or response to sensory input that might not otherwise be possible. In addition, the consistent identification of individual cells allows for especially fruitful comparison of data among laboratories working on the same system. Therefore, a detailed knowledge of the component neurons in any circuit will benefit its analysis.

Yet, what level of detail is sufficient? How can those analyzing more complicated vertebrate systems benefit from what has been learned in the simpler invertebrate systems? As we have shown, there are many ways to identify a cell or cell type, and the most effective protocols use a combination of several identifying characteristics (FIGURE 3). Furthermore, the numerical value of each cellular property may not contain as much information as the relationships between properties, as is the case for the conductance correlations found in the STG (13, 22, 50). With this in mind, genetic methods of identification appear to be universally appealing as the next frontier in neuronal circuit analysis because they may provide a much larger repertoire of identifying characteristics. Work is underway to identify cell-specific transcription factors, promoters, surface receptors, or other molecular machinery that can be used to target a subset of cells in a circuit. For example, fluorescence-activated cell sorting of retrograde-labeled cells has identified several cell type-specific molecular markers in mammalian cortical and corticospinal neurons (55). Cell populations subjected to

multiplex RT-PCR may also produce type-specific genetic profiles (62). In transgenic animals, this kind of cell type-specific genetic information has proven to be an invaluable tool for reproducibly identifying neurons, tracking connections, and directing perturbations in cellular activity (6, 25, 32). In invertebrates that take years to mature, such as the lobster and crab, transgenics are impractical; however, work has recently been done utilizing electroporation, viral transduction, or direct injection of genetic material into adult neurons (9, 16, 31, 33, 48).

As this work progresses, the presence or lack of molecular differences between cells may reveal a more detailed picture of how functional cellular identity is achieved and maintained and may answer some lingering questions about the nature of cellular identity. Are two cells with identical circuit function also identical in their patterns of gene expression and molecular makeup? If not, are the differences consistent and identifiable between animals of a species? We expect the concepts of cell type and identity to continue to evolve, depending on the answers to these questions. ■

No conflicts of interest, financial or otherwise, are declared by the author(s).

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