Patch-clamp study of neurons and glial cells in isolated myenteric ganglia

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Hanani, M., M. Francke, W. Härtig, J. Grosche, A. Reichenbach, and T. Pannicke. Patch-clamp study of neurons and glial cells in isolated myenteric ganglia. Am J Physiol Gastrointest Liver Physiol 278: G644–G651, 2000.—Most of the physiological information on the enteric nervous system has been obtained from studies on preparations of the myenteric ganglia attached to the longitudinal muscle layer. This preparation has a number of disadvantages, e.g., the inability to make patch-clamp recordings and the occurrence of muscle movements. To overcome these limitations we used isolated myenteric ganglia from the guinea pig small intestine. In this preparation movement was eliminated because muscle was completely absent, gigaseals were obtained, and whole cell recordings were made from neurons and glial cells. The morphological identity of cells was verified by injecting a fluorescent dye by micropipette. Neurons displayed voltage-gated inactivating inward Na+ and Ca2+ currents as well as delayed-rectifier K+ currents. Immunohistochemical staining confirmed that most neurons have Na+ channels. Neurons responded to GABA, indicating that membrane receptors were retained. Glial cells displayed hyperpolarization-induced K+ inward currents and depolarization-induced K+ outward currents. Glia showed large “passive” currents that were suppressed by octanol, consistent with coupling by gap junctions among these cells. These results demonstrate the advantages of isolated ganglia for studying myenteric neurons and glial cells.

Enteric nervous system; electrophysiology; sodium channels; calcium channels

Knowledge of the enteric nervous system has benefited greatly from studies using isolated tissues. Particularly important were studies utilizing the longitudinal muscle-myenteric plexus (LMMP) preparation, in which the physiology and pharmacology of myenteric neurons were characterized (for reviews see Refs. 9, 12, 30, 34). Still, a few disadvantages limit the usefulness of LMMP preparation: 1) substances released from the muscle tissue may interfere with the interpretation of biochemical and pharmacological experiments; 2) muscle movements can dislodge microelectrodes during electrophysiological recordings and disturb optical measurements; and 3) the muscle may reduce the visibility of the neurons. To overcome these difficulties, Yau et al. (35) developed a new preparation consisting of isolated myenteric ganglia from the guinea pig, which they used for studying acetylcholine release. This method yielded a large number of ganglia and was employed to measure cAMP synthesis in response to various agonists (36) and to study the regulation of substance P release by adenosine (28) and of vasoactive intestinal peptide release by nitric oxide (17). Fiorica-Howells et al. (7) used isolated ganglia to investigate the pharmacological mechanism by which serotonin induces cAMP synthesis in myenteric ganglia. The availability of isolated myenteric ganglia has opened up interesting possibilities for culturing myenteric ganglia from adult animals. These cultures proved to be helpful in biochemical (6, 16) and electrophysiological (1, 18, 23, 38) experiments.

A serious drawback of the LMMP preparation is the presence of a connective tissue layer (basal lamina) over the ganglia, which prevents the use of patch electrodes. Therefore, patch-clamp studies have been done only on cultured myenteric neurons (1, 3, 8, 24) and cultured myenteric glia (4). A major disadvantage of culture preparations is the disruption of the organization of the tissue, which underlies neuronal connectivity, relations between glia and neurons, and interactions within the glial network. In central nervous system (CNS) research this problem has been partly overcome by the use of slices, which enable recordings with patch electrodes. Patch-clamp recordings have also been made in intact sympathetic ganglia (14). In this study, we show that isolated myenteric ganglia are suitable for patch recordings from neurons and also from glia, whose small size has so far precluded their systematic electrophysiological investigation in LMMP preparations. In this paper we describe the isolation and recording techniques and present an account of the basic electrophysiological properties of myenteric neurons and glial cells.

Materials and Methods

The isolation of myenteric ganglia was done according to the method of Yau et al. (35), with some modifications (23). Guinea pigs of either sex weighing 250–300 g were stunned and bled. The small intestine was removed and placed in cold Krebs solution containing (in mM): 120.9 NaCl, 5.9 KCl, 14.4...
NaHCO₃, 2.5 MgSO₄, 1.2 Na₂HPO₄, 2.5 CaCl₂, and 11.5 glucose. The longitudinal muscle with the attached myenteric plexus preparation was stripped and cut into 5-mm segments, which were incubated in an enzyme mixture containing (in mg/100 ml Krebs solution) 125 collagenase type IA, 100 protease type IX, and 2.5 DNase I (all from Sigma). The incubation was at 37°C for 35 min with continuous shaking. After incubation, the ganglia were allowed to settle and the supernatant was discarded. After two washes in Krebs solution, the ganglia were collected with a micropipette under a dissecting microscope and were washed for 20 min in medium 199 (Sigma) containing 500 µg/ml streptomycin sulfate, 500 U/ml penicillin G sodium, and 0.25 µg/ml amphotericin B (all from Sigma). The ganglia were mounted on a polycarbonate filter (7) or on a glass coverslip. The filters or coverslips were placed on the bottom of a chamber that was attached on the stage of a Zeiss Axioskop microscope equipped with fluorescence illumination. The chamber was superfused with Krebs solution at room temperature; the solution was bubbled with 95% O₂-5% CO₂. Drugs were applied by bath perfusion. The preparation was viewed with a water immersion ×40 objective using Nomarski optics. Patch pipettes were made from borosilicate glass capillaries using a Narishige puller. The pipettes were filled with a solution containing (in mM) 130 NaCl, 130 KCl, 1.0 CaCl₂, 2.0 MgCl₂, 10 HEPES, and 10 EGTA, pH 7.1. The electrode resistance was 5–10 MΩ.

For the electrophysiological experiments the tissues were placed on the bottom of a chamber that was attached on the stage of a Zeiss Axioskop microscope equipped with fluorescence illumination. The chamber was superfused with Krebs solution at room temperature; the solution was bubbled with 95% O₂-5% CO₂. Drugs were applied by bath perfusion. The preparation was prepared with a water immersion ×40 objective using Nomarski optics. Patch pipettes were made from borosilicate glass capillaries using a Narishige puller. The pipettes were filled with a solution containing (in mM) 10 NaCl, 130 KCl, 1.0 CaCl₂, 2.0 MgCl₂, 10 HEPES, and 10 EGTA, pH 7.1. The electrode resistance was 5–10 MΩ. Lucifer yellow (0.1%) was added to the pipette solution to enable morphological identification of recorded cells.

Whole-cell voltage clamp recordings were made using an Axopatch 200A amplifier. Currents were low-pass filtered at 1 kHz and digitized using a 12-bit analog-to-digital converter. Data acquisition and analysis and generation of voltage command protocols were performed with ISO2 software (MFK, Niedernhausen, Germany). Input resistances were measured with a 10-mV hyperpolarizing step from a holding potential of −80 mV. For all measurements series resistance compensation was used up to 30% to minimize voltage errors. At the end of the recording from each cell, its identity was established by observing its morphology with fluorescence illumination.

Lucifer yellow-injected cells were first observed and photographed with a Zeiss fluorescence microscope. For further study, they were inspected without fixation with a confocal microscope (Zeiss LSM 510). In immunohistochemical experiments, isolated ganglia were fixed in 4% paraformaldehyde for 2 h and subsequently rinsed with 0.1 M Tris-buffered saline (TBS), pH 7.4. Nonspecific binding sites were blocked with 5% normal goat serum in TBS containing 0.3% Triton X-100 (NGS-TBS-T) for 1 h. The tissue was then incubated in 5 µg/ml affinity-purified rabbit antibodies directed against Na⁺ channels (type II or Pan, Alomone Labs, Jerusalem, Israel) for 16 h at room temperature. After several rinses in TBS, immunoreactivity was visualized by incubation in Cy3-tagged goat anti-rabbit IgG (Jackson Laboratories), 20 µg/ml in TBS containing 2% bovine serum albumin for 1 h. For double immunofluorescence labeling of sodium channels and the Ca²⁺-binding protein calbindin, ganglia were fixed and blocked as described for single staining. Afterwards, mouse anti-calbindin (done CI-300, Sigma), 1:200 in NGS-TBS-T, was applied simultaneously with rabbit antibodies to Na⁺ channels (5–10 µg/ml). Rinsed tissue was then treated with a cocktail consisting of Cy2-goat anti-mouse IgG (Jackson Laboratories), 20 µg/ml in TBS containing 2% bovine serum albumin for 1 h. Finally, all tissues were extensively rinsed with TBS, briefly washed in distilled water, air dried, and coverslipped with Entellan (Merck). The omission of primary antibodies resulted in the absence of any cellular staining.

Mean values are given with standard deviations. We used the t-test for unpaired samples and the Wilcoxon test for paired samples. Analysis was done with SPSS for Windows software.

RESULTS

Morphological observations. Figure 1A shows a preparation typical of those used in this study, as seen during the experiments. The preparation consists of several ganglia interconnected by fiber tracts. With the use of Nomarski optics, single neurons were clearly visible. Glial cells could not be seen under these conditions.
although they outnumber the neurons. A Lucifer yellow-stained neuron is shown in Fig. 1B; this cell was characterized electrophysiologically as a neuron. Although the morphological identification of the neurons was unequivocal, distinction between subtypes of neuronal morphologies was not possible in most cases. Figure 1C shows a glial cell stained with Lucifer yellow; the small size of this cell and the fine, short processes are typical for glia (21). This cell had the electrophysiological properties of a glial cell.

Electrophysiology of neurons. Patch-clamp recordings in the whole cell configuration were made from 82 neurons, which were identified by their morphology after filling with Lucifer yellow (Fig. 1B). Because neurons could not be clearly classified in most cases, we pooled the data for all the cells. Input resistance was measured with a 10-mV hyperpolarizing step from a holding potential of $-80$ mV and was $392 \pm 203$ MΩ (mean ± SD, $n = 63$); the resting membrane potential was $-46 \pm 13$ mV ($n = 58$). Figure 2A shows current recordings from neurons. The neuronal current pattern was characterized by outward rectification. Hyperpolarizations evoked only small inward currents. Depolarizing steps to $-30$ mV or higher evoked fast-inactivating inward currents. In some neurons, the current response was repetitive (Fig. 2B). In most cells, the inward currents had two time constants of inactivation, a fast early time constant and a slower late one, suggesting the involvement of two types of ionic channels. The inward inactivating currents were followed by slower activating, sustained outward currents (presumably delayed-rectifier K⁺ current).

We next performed a series of experiments to learn the ionic nature of the inward currents. Figure 3A shows current responses under control conditions. Replacing the extracellular Na⁺ with choline eliminated the fast component of the inward current ($n = 28$, Fig. 3B). Tetrodotoxin (1 µM) blocked this component as well ($n = 3$, data not shown), consistent with a major contribution of Na⁺ channels. In most neurons, after
the Na\(^{+}\) current was blocked, a slower inward current was still recorded. This current was inhibited by the Ca\(^{2+}\) channel blocker Cd\(^{2+}\) (1 mM, \(n = 19\), Fig. 3C). The difference between the traces in Fig. 3, C and B, is displayed in Fig. 3D and represents the net voltage-activated inward Ca\(^{2+}\) current. Likewise, eliminating Ca\(^{2+}\) from the external solution suppressed the slow inward current (\(n = 2\)). To obtain information on the type of the voltage-dependent Ca\(^{2+}\) channels we first incubated the tissue in Na\(^{+}\)-free solution (Fig. 4A) and then added the L-type Ca\(^{2+}\) channel blocker nimodipine (5 \(\mu M\)). Nimodipine inhibited strongly (but not completely) the inward currents in all five cells tested (Fig. 4, B and C), indicating the presence of L-type Ca\(^{2+}\) channels in the myenteric neurons. In some cells, blocking Ca\(^{2+}\) currents inhibited the late outward currents, which were apparently due to K\(^{+}\) flow (Fig. 4B). Thus it appears that the neurons also have Ca\(^{2+}\)-dependent K\(^{+}\) channels.

We concluded from these experiments that all neurons displayed both Na\(^{+}\) and Ca\(^{2+}\) inward currents. In some of the experiments, we identified the morphological type of the cells and found that this conclusion holds for both Dogiel type I (\(n = 8\)) and Dogiel type II (\(n = 5\)) neurons. (In the rest of the cases the morphology of the neurons could not be determined with certainty.) However, the ratio between the Na\(^{+}\) and Ca\(^{2+}\) inward currents varied among cells. In some cells the Ca\(^{2+}\) current was barely measurable, whereas in others it was prominent.

To learn the distribution of Na\(^{+}\) channels in myenteric neurons we stained the ganglia with an antibody against Na\(^{+}\) channels. The ganglia were also stained for the Ca\(^{2+}\)-binding protein calbindin, which is a marker for most AH-type neurons (10). Action potentials in these neurons have a large component of voltage-induced inward Ca\(^{2+}\) current ("Ca\(^{2+}\) spikes"; Refs. 29, 34). Figure 5 shows a confocal image of the staining for the two proteins. It was evident from this

![Image](http://ajpgi.physiology.org/)
and other such experiments that there is a subpopulation of calbindin-positive neurons. It also appeared that most of the neurons were immunopositive to the Na$^+$-channel antibody, but the intensity of the staining varied among cells. Some of the calbindin-positive neurons showed weak staining for the Na$^+$ channels, whereas others were brightly stained for both proteins. Thus it seems that there is a continuum of intensity of staining for Na$^+$ channels. In most studies on the distribution of calbindin in myenteric neurons, the guinea pig ileum has been used. In the present work the ganglia were isolated from both the ileum and jejunum. To compare between these two parts we performed double immunostaining for calbindin and for Na$^+$ channels in the myenteric plexus of the jejunum. The results showed that a subpopulation of neurons were positive for calbindin and had the morphology of Dogiel type II neurons. Many more cells were positive for Na$^+$ channels, but there was a partial overlap between these two populations, as described above. Thus it appears that the use of calbindin as a marker for Dogiel type II cells holds for the entire guinea pig small intestine.

To find out whether neurotransmitter receptors were retained in the isolated ganglia we recorded responses to GABA. As demonstrated in Fig. 6, GABA (50 µM) evoked an inward current, which partly inactivated in the presence of octanol strongly reduced the passive currents and caused the appearance of voltage-dependent outward currents (typical for delayed-rectifier K$^+$ current). In the presence of octanol, strong hyperpolarizing voltages evoked time-dependent inactivation of inward currents (typical for inward-rectifier K$^+$ current; see Fig. 7A). Input resistance was only slightly increased by octanol (from 59 ± 23 to 71 ± 21 M$\Omega$, $n = 5$). This increase was also not significant (Wilcoxon test, $P > 0.06$). Addition of 1 mM Ba$^{2+}$ in the presence of octanol strongly reduced the inward currents (Fig. 7C) and increased the input resistance from 72 ± 18 to 490 ± 497 M$\Omega$ ($n = 9$). This difference was significant ($P < 0.02$, Wilcoxon test). This result indicated the presence of Ba$^{2+}$-sensitive, inwardly rectifying K$^+$ channels in myenteric glia. Incubating the tissues in medium with low pH (6.55), which also blocks gap junctions (27), affected input resistance and membrane currents similarly to octanol (data not shown).

Large passive currents were observed in 41 of 61 myenteric glial cells. In the other 20 cells voltage- and/or time-dependent currents were observed in normal Krebs solution. Fast-inactivating inward currents were found in a small number of glial cells ($n = 7$, Fig. 7D). The amplitude of these currents was much smaller than that of those recorded in neurons. In one glial cell that was tested, tetrodotoxin (1 µM) blocked these currents.

Cells displaying low input resistance and electrophysiological properties as shown in Fig. 7 had the morphological features of enteric glial cells (Fig. 1C). In most cases (37 of 45), glial cells were found to be dye-coupled to 2–25 other glial cells (Fig. 8). When the tissue was

![Fig. 6. Response of a myenteric neuron to GABA (50 µM), applied as indicated by line. Note inactivation of GABA-evoked current. Holding potential was −80 mV.](http://ajpgi.physiology.org/)

![Fig. 7. Current responses of glial cells in isolated myenteric ganglia.](http://ajpgi.physiology.org/)
incubated with octanol before the recording, there was no dye coupling (n = 4). Under control conditions, only 8 of 45 cells were not dye coupled and 7 of them displayed voltage-dependent currents. These eight glial cells had an input resistance of 156 ± 64 MΩ (n = 8), which is significantly higher than the resistance of coupled glia (P < 0.001, t-test). This value was significantly lower than the resistance of neurons (392 ± 203 MΩ, P < 0.001, t-test) and supports the identification of these cells as glia rather than neurons.

**DISCUSSION**

Investigations of cells in isolated preparations such as brain slices and sympathetic ganglia have been extremely useful in the study of these systems. A great advantage of these preparations is the ability to make patch-clamp recordings from neurons and glial cells while these cells retain many of their original connections. This is in contrast to tissue culture preparations, in which cell interactions, when they occur, probably do not accurately reflect the original connectivity. The experiments described here clearly demonstrate the utility of isolated, intact myenteric ganglia for investigation of the physiology and pharmacology of myenteric neurons and glial cells in their original environment. A major advantage of this method is that it enables patch recording from these cells. Optical recordings of Ca²⁺ signals in neurons are also facilitated in this preparation (19).

Myenteric neurons have been studied extensively using intracellular recording, and much information on their pharmacology has been obtained (9, 34). These studies have provided important knowledge of this system and have contributed to neuropharmacology in general. However, only limited progress has been made in understanding the membrane properties of myenteric neurons and glial cells. Studies using patch recordings were done on cultured myenteric neurons (1, 3, 8, 24, 33, 38, 39). Patch recordings have not been done on myenteric neurons in the intact ganglia because the basal lamina covering the ganglia prevents the formation of a gigaseal with a patch pipette. In the present work we show that patch recordings can be made after isolating the ganglia, apparently because the enzymatic digestion breaks the basal lamina and allows access of the pipette to the cells. A possible criticism of this method is that cells may be damaged by the proteolytic enzymes. However, electron microscopic observations (7, 35) showed no damage to the ganglia after the isolation procedure. To minimize cell damage we used a lower concentration of the enzymes than those used by previous workers for the same incubation time and kept the ganglia for 3 h or more in an incubator before the recordings, to allow tissue recovery. Despite these precautions we cannot rule out that the isolation of the ganglia caused a certain degree of damage or abnormalities in the cells. This may account for the difficulty in classifying neurons according to morphology and for the variations in the ratio in Na⁺ and Ca²⁺ currents among cells.

In comparison to myenteric neurons, very little is known about the physiology and pharmacology of myenteric glia, which may function like CNS astrocytes (13, 22). The small size of these cells (cell body diameter 8 µm; Ref. 22) has so far precluded a systematic investigation of these cells with intracellular microelectrodes. In two studies, some physiological properties of cultured myenteric glia were investigated. Broussard et al. (4) made patch-clamp recordings from the glial cells, and Zhang et al. (37) used Ca²⁺ imaging to investigate their responses to endothelin. In the present study we demonstrated that patch recordings from glial cells in isolated myenteric ganglia were feasible. We found that myenteric glia have low input resistance, consistent with their being connected by gap junctions (11, 22, 27). Indeed, by injecting Lucifer yellow into these cells, we found that they were dye coupled and that this coupling was blocked by the gap junction blocker octanol. In accordance with these observations, most myenteric glia displayed large passive currents in response to voltage steps. In the presence of octanol these passive currents were reduced, allowing us to observe inward-rectifying currents, which were blocked by Ba²⁺, and also outwardly directed delayed currents. Thus it appears that these cells have at least two types of voltage-dependent K⁺ channels. Broussard et al. (4) recorded delayed-rectifying K⁺ currents in cultured myenteric glia but not inward-rectifying K⁺ channels. They also recorded inward Na⁺ currents in 4 of 20 glial cells, as found by us in a subpopulation of the glial cells. Most of the glial cells that were not dye coupled under control conditions displayed time- and voltage-dependent currents, similar to those obtained when octanol was used to uncouple the cells. It thus appears that the coupling among glia masks the voltage-dependent ionic currents. The physiological significance of these ionic channels is not yet clear, and they may not contribute to the behavior of the cells when they are strongly coupled. However, under conditions favoring the uncoupling of the cells (e.g., when intracellular pH is low), these
channels may be functionally important. Glial cells are believed to regulate many neuronal functions (26), and thus alterations in glial properties may in turn affect myenteric neurons, which are in close contact with the glial cells (11).

Our electrophysiological results show that myenteric neurons in the isolated ganglia display several types of voltage-sensitive ion channels. We recorded fast inward currents in all the cells identified morphologically as neurons. In all the neurons examined, these inward currents consisted of both Na$^+$ and Ca$^{2+}$ currents. Ca$^{2+}$ currents were identified in Na$^+$-free solution, using the Ca$^{2+}$ channel blocker Cd$^{2+}$. Ca$^{2+}$ currents were smaller than Na$^+$ currents and had slower decay times. The L-type Ca$^{2+}$ channel blocker nimodipine strongly inhibited the Ca$^{2+}$ currents, indicating the existence of L-type Ca$^{2+}$ channels in myenteric neurons. This blockade was not complete, suggesting the presence of other types of Ca$^{2+}$ channels. The presence of L-type Ca$^{2+}$ channels has been reported in rat myenteric neurons (8, 24). In contrast, Grafe et al. (15) reported that the L-type Ca$^{2+}$ channel blocker D-600 had no effect on the electrophysiological properties of AH-type myenteric neurons of guinea pigs. A possible explanation for this discrepancy is that D-600 is a verapamil derivative and not a dihydropyridine like nimodipine. Baidan et al. (2) recorded Ca$^{2+}$ currents in cultured myenteric neurons and found that nifedipine was not very effective in blocking these currents. Nevertheless, they concluded that both L- and N-type currents were present. The quantitative difference from our results may be caused by the fact that we used intact, isolated ganglia rather than cultures. Using a Ca$^{2+}$ imaging technique, Simone et al. (31) found that acetylcholine caused large increases in intracellular Ca$^{2+}$ concentration ([Ca$^{2+}]_i$) in cultured myenteric neurons from guinea pigs, which were completely blocked by 10 µM nifedipine. They concluded that the [Ca$^{2+}]_i$ increases were caused by Ca$^{2+}$ influx through voltage-gated L-type Ca$^{2+}$ channels, which is in accord with our observations. It is interesting to note that in many studies of myenteric neurons in the LMMP preparation Ca$^{2+}$ blockers of the dihydropyridine family were used to prevent muscle contraction (see, e.g., Ref. 32). Obviously, it was assumed that these drugs have no direct effects on myenteric neurons, although the discussion above suggests that these drugs may, at least partly, block L-type Ca$^{2+}$ channels in neurons.

The observation of voltage-gated Ca$^{2+}$ currents in virtually all myenteric neurons is consistent with the findings of Hanani and Lasser-Ross (20), who measured Ca$^{2+}$ transients in single myenteric neurons simultaneously with intracellular recordings. They showed that both S- and AH-type myenteric neurons had voltage-activated Ca$^{2+}$ currents. Previous studies on myenteric neurons showed that S-type neurons have pure Na$^+$ spikes, whereas spikes in AH neurons have a prominent Ca$^{2+}$ component (25, 29, 34). Our results do not contradict, but modify, these observations. We showed that all myenteric neurons studied have voltage-gated Na$^+$ and Ca$^{2+}$ channels. The contributions of these channels to inward currents vary among cells. These conclusions are supported by immunohistochemical staining for Na$^+$ channels, which showed that most neurons possess these channels, but to varying degrees. Many calbindin-positive neurons, which were shown to be mostly AH-type neurons (10), were immunopositive for Na$^+$ channels. These conclusions are supported by Ca$^{2+}$-imaging studies showing that both neuron types have voltage-activated Ca$^{2+}$ channels (5, 20). Thus the distinction between AH- and S-type neurons on the basis of the nature of the action potentials is not as sharp as thought previously and is more a matter of degree.

The fast inward currents were followed by currents that appeared to be delayed-rectifying K$^+$ currents, as found in cultured myenteric neurons (38). We also obtained evidence for the presence of Ca$^{2+}$-induced K$^+$ currents, as found in studies using intracellular recordings (34). We expect that the ability to make patch recordings will lead to studies in which the ionic and molecular mechanisms of neurotransmitter and drug effects on myenteric neurons will be investigated in detail.

The recordings from myenteric glia can shed new light on the role of these cells in the enteric nervous system. Glial cells in the CNS have been shown to possess voltage-sensitive ion channels and neurotransmitter and hormone receptors, and they are able to synthesize and release a variety of neuroactive compounds (26). It is now widely accepted that glia have more than a mechanical role in the nervous system and may have key physiological functions in normal and pathological conditions. Our observations indicate that myenteric glia share some physiological properties with central glia. We found that these cells possess a number of voltage-gated ion channels—inward-rectifier and delayed-rectifier K$^+$ channels as well as voltage-activated Na$^+$ channels. In view of the complex pharmacology of myenteric neurons, it will be interesting to investigate the effect of putative neurotransmitters on myenteric glia. We expect that future studies using the isolated ganglia will reveal how myenteric neurons and glia interact to produce the overall integrative ability of this system.

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