Imaging neuron-glia interactions in the enteric nervous system

Werend Boesmans1,2, Michiel A. Martens1,2, Nathalie Weltens2, Marlene M. Hao1,2, Jan Tack2, Carla Cirillo1,2 and Pieter Vanden Berghe1,2*

1 Laboratory for Enteric Neuroscience (LENS), Translational Research Center for GastroIntestinal Disorders, University of Leuven, Leuven, Belgium
2 Translational Research Center for GastroIntestinal Disorders (TARGID), Department of Clinical and Experimental Medicine, University of Leuven, Leuven, Belgium

INTRODUCTION

Many aspects of gastrointestinal function are controlled without major inputs from the brain. Instead, the enteric nervous system (ENS), a ganglionic neuronal network that resides within the gut wall, autonomously controls gastrointestinal motility, secretion, and blood flow (Furness, 2000). The ENS develops from neural crest cells that migrate and proliferate extensively to eventually form a network of interconnected ganglia throughout the entire length of the gut (Sasselli et al., 2012; Obermayr et al., 2013). Similar to other parts of the nervous system, the ENS comprises both a neuronal and a glial component. Enteric glial cells are located in close contact with enteric neurons within the ganglia, along interganglionic connectives of the myenteric and submucosal plexus, and can also be found in the extraganglionic ganglia, along interganglionic connectives of the myenteric and submucosal plexus. Enteric glial cells share several features with astrocytes and are closely associated with enteric neurons and their processes both within enteric ganglia, and along interconnecting fiber bundles. Similar to other parts of the nervous system, there is communication between enteric neurons and glia; enteric glial cells can detect neuronal activity and have the machinery to intermediate neurotransmission. However, due to the close contact between these two cell types and the particular characteristics of the gut wall, the recording of enteric glial cell activity in live imaging experiments, especially in the context of their interaction with neurons, is not straightforward. Most studies have used calcium imaging approaches to examine enteric glial cell activity but in many cases, it is difficult to distinguish whether observed transients arise from glial cells, or neuronal processes or varicosities in their vicinity. In this technical report, we describe a number of approaches to unravel the complex neuron-glia crosstalk in the ENS, focusing on the challenges and possibilities of live microscopic imaging in both animal models and human tissue samples.

Keywords: enteric neuron, enteric glia, calcium, synaptic, GCaMP
NEURONS AND GLIA: CLOSE NEIGHBORS IN THE GUT

Enteric glial cells resemble astrocytes in several ways, including the expression of the intermediate filament glial fibrillary acidic protein (GFAP) (Jessen and Mirsky, 1980) and the Ca\(^{2+}\) binding protein, S100\(\beta\) (Ferri et al., 1982). The intimate association between glia and neurons within enteric ganglia has been revealed by co-immunolabeling of gut tissue with GFAP or S100\(\beta\) with neuronal markers, such as HuC/D (Figures 1A,B). In addition, the transcription factor SRY box-containing gene 10 (Sox10), which is expressed by multipotent ENS precursors (Paratore et al., 2002; Bondurand et al., 2003), is also expressed by mature enteric glia (Young et al., 2003), and is ideally suited for quantification purposes because of its selective nuclear localization (Figure 1C) (Hoff et al., 2008). Enteric glial cells closely embrace nerve fibers and varicose release sites both within enteric ganglia and along the interconnective fiber tracts (Figures 1D–F) (Hanani and Reichenbach, 1994; Vanden Berghe and Klingauf, 2007). As a consequence, the optical segregation of signals arising from either neurons or glia in live imaging experiments is not straightforward.

Microscopic imaging techniques have provided invaluable information about several aspects of neuronal signaling in both the developing and adult ENS (Schemann et al., 2002; Vanden Berghe et al., 2008; Hao et al., 2012). In particular, chemosensitivity and mechanosensitivity of various classes of enteric neurons has been uncovered using both voltage-sensitive and Ca\(^{2+}\) indicator dyes (Smith et al., 2007; Schemann and Mazzuoli, 2010). Strong evidence for communication between enteric neurons and glia comes from a series of studies using live imaging in both ex vivo and cell cultures of the ENS (Gomes et al., 2009; Gulbransen and Sharkey, 2009; Gulbransen et al., 2010). In addition, in a study by Broadhead et al., interaction between neuronal and glial cells was shown following spontaneous or induced physiological activity (Broadhead et al., 2012). In all studies, purinergic signaling pathways have been identified as the primary mechanism of transmission.

LIVE IMAGING OF NEURON–GLIA INTERACTIONS: In vitro vs. Ex vivo

Optical information generated by multiple sources is always convoluted due to the diffraction limitations of optical microscopy. To address this, we estimated the contribution of any given signal in the pixels directly neighboring a structure of interest as recorded by a widefield fluorescence microscope equipped with a CCD camera. Primary ENS cultures (including both neurons and glia) were loaded with the Ca\(^{2+}\) indicator dye, Fluo-4-AM, and stimulated by 75 mM K\(^{+}\) depolarization (Figure 2). To measure the change in fluorescence, a region of interest (ROI) was drawn over a neuronal bouton. We found that even with limited optical resolution (widefield, 20\(\times\), NA = 0.75, pixel width: 623 nm), the signal contribution drops sharply outside of any structure that can be picked out intuitively by an observer (Figure 2C). Simple rectangular ROIs are sufficient to calculate the cellular signals and polygon-shaped ROIs do improve signal to noise ratios due to inclusion of larger cell areas (Figures 2D–F). Thus, at least in in vitro experiments, careful drawing of ROIs at least 1 \(\mu\)m away from each other may be sufficient to separate signals coming from structures situated in each other’s vicinity.

The ENS, due to its planar organization in ex vivo preparations, is very attractive to investigate with imaging techniques. However, the assumption of 2D structure is only valid when entire cells are considered. Once synaptic contacts and cellular processes are of interest, the analysis faces all the technical problems that are associated with 3D organization and ROIs will easily incorporate scattered light emanating from structures close by. Hence, the intimate relationship between enteric glia, neurons and their processes (Figure 1) entails the risk that signals arising from enteric varicosities and fibers are interpreted as being of glial origin. This is an important confounding factor that may cause the false impression that glial cells respond as fast to electrical and depolarizing stimuli as do neurons. Therefore, to make use of the fundamental physiological difference between neurons and electrically passive non-excitable glia (Hanani et al., 2000; Gulbransen and Sharkey, 2012) we suggest, in combination with using lenses with sharp focal depths, to consistently apply known stimuli (e.g., electrical stimulation) to identify neuronal structures, which then serve as a guide to draw regions at least 1 \(\mu\)m away from other structures in order to minimize false interpretation.

The clear delineation of the cells and compartments of interest in ex vivo gut preparations is further complicated by the fact that these ganglia are on a contractile muscle layer, which even with pharmacological inhibition and mechanical restraining can still cause movement artifacts. This further complicates accurate analysis, especially of smaller structures and cell compartments (e.g., varicosities, parts of glial processes). To correct for residual movements, we use translation stabilization routines (Bischofs et al., 2006; Gallego et al., 2008), which recently have been expanded to also correct for more complex movements like rotation and torque.
DIFFERENTIATING BETWEEN NEURONAL AND GLIAL RESPONSES: RESPONSE TIMING AND SHAPE

Although originally not intended for studying neuron-glia interactions, data from pioneering studies showing that several neurotransmitters can elicit Ca$^{2+}$ transients in cultured enteric glial cells have already indicated the potential for glial participation in enteric neurotransmission (Kimball and Mulholland, 1996; Zhang et al., 1997, 1998; Garrido et al., 2002). In an alternative approach to directly measure the sensitivity of enteric glial cells to neuronal transmitters, we used an immortalized rat enteric glia cell line (CRL-2690) (Ruhl et al., 2001) and confirmed that neurotransmitters known to elicit fast excitatory potentials in enteric neurons can directly induce [Ca$^{2+}$]$_i$ changes in enteric glial cells (Boesmans et al., 2013). In contrast, enteric glia did not respond to high K$^+$ depolarization. The absence of neurons in these cultures obviously eliminates the problems illustrated above, but also excludes the possibility that these cells are activated secondary to neuronal activation. However, in mixed cultures of neurons and glia this is not the case (Gomes et al., 2009). By specifically stimulating enteric neurons while monitoring the secondary glial responses, an adenosine triphosphate (ATP)-dependent paracrine communication pathway between enteric neurons and glia was revealed.

This typical ATP sensitivity was also found in enteric neuron-glia co-cultures obtained from adult mouse gut where the neuronal and glial Ca$^{2+}$ fingerprint was used to identify specific cell types (Laranjeira et al., 2011). Indeed, enteric neurons display a strong and fast Ca$^{2+}$ response to high K$^+$ depolarization, electrical field stimulation (EFS) and the nicotinic agonist dimethylphenylpiperazinium (DMPP). Enteric glial cells, on the other hand, do not respond to these stimuli directly, but show delayed responses that can be modulated by intervening with several components of purinergic signaling (Gomes et al., 2009; Laranjeira et al., 2011) (Figure 3). Due to these timing differences, it is possible to construct “activity-over-time” (AoT, Figure 3D) images that identify cells which exhibit a change in fluorescence intensity above baseline noise. These images appear similar to immunostainings, but a physiological response is represented instead of the structural information (custom developed algorithm in Igor Pro, Wavematrics). To further characterize the timing of the responses, we developed a routine in which the shortest distance to an active neuronal fiber was computed and transformed in a color coded image (Figures 3F–J). The computation of distance can be performed either on an immunostaining or on one of the AoT images generated from live recordings. In this way we are able to test whether secondary responses, for instance responses in glial cells, emerge earlier if they are physically closer to active neuronal fibers. We found that the timing of glial responses does correlate with spatial aspects since cells closer to a neuronal component have a higher likelihood of responding to a neuronal stimulus (Figure 3I), thus further corroborating the fact that it is a diffusible factor that mediates the communication from enteric neurons to glia.

Taking all these technical issues into consideration, it is possible to isolate the net responses from neurons and glial cells in complex tissues. This reveals that the Ca$^{2+}$ transients in both cell types have typical shapes and kinetics to stimuli like EFS, high K$^+$ depolarization and fast neurotransmitters: in neurons, fast and linear upstrokes reaching their maximum in a couple of seconds are followed by a (bi)-exponential decay, while in glia a secondary close-to-Gaussian shaped response is observed. This typical fingerprint can thus be used also in tissue to identify different cell types (Figure 4). Provided that sufficient spatial resolution is achieved, fast imaging approaches (Michel et al., 2011; Martens and Vanden Berghe, 2012) can help discriminating...
between neuronal and glial signals because the extra data points allow more reliable fitting of the response upstroke.

It is of note that enteric glial cells display higher baseline fluorescence after Fluo4 loading compared to neurons (arbitrary fluorescence units, neurons: 260.7 vs. glia: 339.6, \( p < 0.05, n = 103 \), data from three animals, unpaired \( t \)-test), a difference that is more pronounced in \textit{ex-vivo} tissue preparations in comparison to primary culture. Using the ratiometric dye Fura2, we tested whether this difference was due to higher resting level of intracellular \( \text{Ca}^{2+} \). We found the differences (340/380 ratio, neurons: 0.3442 vs. glia: 0.3623; \( p < 0.05, n = 120 \), data from three animals, unpaired \( t \)-test) to be only very small and definitely not sufficient to explain the large differences in resting Fluo4 fluorescence. This was further confirmed by using Rhod2, a non-ratiometric \( \text{Ca}^{2+} \) indicator with an even higher \( K_d \), which is classically used in astrocyte research (Mulligan and Macvicar, 2004; Takano et al., 2006) and can also be used to load enteric glial cells (Gulbransen and Sharkey, 2009). Again, higher resting levels were observed in glial cells compared to neurons (arbitrary fluorescence units, neurons: 330.8 vs. glia: 419.4, \( p < 0.05, n = 113 \), data from three animals, unpaired \( t \)-test). Taken together, this suggests that glial cells take up and/or metabolize the AM ester more easily, which is probably a reflection of a higher metabolism.

**NOVEL GENETIC TOOLS FOR STUDYING ENTERIC NEURON-GLIA INTERACTIONS**

Apart from identification during live recordings and \textit{post-hoc} analysis, many new genetic tools are available to label specific cells. Given the analogy between enteric glia and astrocytes, transgenic animals in which reporter proteins, such as green fluorescent protein (GFP) derivatives, have been placed under the direct control of \textit{GFAP} or \textit{S100\beta} regulatory elements to study...
astrocytic function can also be used to visualize enteric glial cells in live imaging experiments. Furthermore, the conditional expression of fluorescent reporters by Cre-Lox recombination technology enables identification of enteric glia as illustrated by Joseph et al. (2011), who combined GFAP-Cre (Zhuo et al., 2001) and GFAP-CreERT2 (Hirrlinger et al., 2006) mice with Rosa26R6YFP reporter mice (Srinivas et al., 2001) for lineage tracing purposes. Time-dependent induction of Cre in the Sox10-iCreERT2 transgenic mouse line generated by Laranjeira et al., not only allows fate mapping of multilineage ENS precursors and labeling of enteric neurons (Sasselli et al., 2013), but also elegantly enables marking enteric glial cells only (Laranjeira et al., 2011). A big advantage of such a genetic system is the fact that individual cells can be labeled, thus allowing appreciation of the cellular morphology as opposed to immunostaining of adjoining cells. These transgenic mouse lines can aid in the examination of enteric neuron-glia interactions in several ways. A non-exhaustive overview of mouse lines that could be used to visualize enteric glial cells is listed in Table 1.

Fluorescent reporter lines are favorable over post-hoc immunohistochemistry for the identification of glia in live imaging experiments since imaging can be performed directly in the cells of interest. It is for these reasons that $S100\beta$-eGFP mice have (Vives et al., 2003) been used in Ca$^{2+}$ and nitric oxide (NO) imaging studies (Gulbransen and Sharkey, 2009; Lavoie et al., 2011; Maceachern et al., 2011). Also, ENS cultures have been generated from Sox10-iCreERT2;R26RFP mice to characterize the Ca$^{2+}$ responses of newborn neurons and genuine enteric glial cells upon a number of stimuli (Laranjeira et al., 2011). Here, the conditional expression of the red fluorescent protein FP635 (Shcherbo et al., 2009) was used to identify neurons that were derived from cultured enteric glial cells.

Another application of transgenic methods lies in the recent development of several optogenetic tools, an opportunity that has yet to be exploited in ENS research. The core instruments of these novel techniques are genetically-encoded optical indicators (GECIs), such as GCaMPs, allowing imaging of Ca$^{2+}$ activity with cellular to subcellular resolution (Miesenbock, 2009). Among the optogenetic reporter molecules, genetically-encoded Ca$^{2+}$-indicators (GEcis), such as GCaMPs, allow imaging of Ca$^{2+}$ signaling in genetically defined cell populations, thus providing a powerful means to study neuron-glia interactions in the ENS. Recently, a reporter mouse was developed that expresses GCaMP3 (Tian et al., 2009) in a Cre-dependent manner in the Rosa26 locus (Zariwala et al., 2012). By using the Wnt1-Cre transgene (Danielian et al., 1998) to conditionally express GCaMP3 in neural crest derivatives, we found that this system can also be used to perform Ca$^{2+}$ imaging in enteric neurons and glia (Figure 5).

Table 1 | Non-exhaustive list of mouse lines that can be used to visualize enteric glial cells.

| Enteric glia promoter | Mouse line | References |
|----------------------|------------|------------|
| $S100\beta$          | $S100\beta$-GFP | Vives et al., 2003; Zuo et al., 2004 |
| GFAP                 | GFAP-Cre   | Zhuo et al., 2001 |
|                     | GFAP-CreERT2 | Ganat et al., 2006; Hirrlinger et al., 2006 |
|                     | GFAP-GFP   | Zhuo et al., 1997; Kuzmanovic et al., 2003 |
|                     | GFAP-tTA   | Wang et al., 2004 |
|                     | GFAP-DsRed | Norberg et al., 2007 |
| Sox10                | Sox10-iCreERT2 | Laranjeira et al., 2011; Simon et al., 2012 |

Tissue of each of these mouse lines (or in case of Cre and tTA lines: tissue from the correct offspring obtained from crosses with reporter lines) can be used either as a source of primary cell cultures, or to directly visualize enteric glial cells in whole mount preparations for live imaging, or finally, to combine with immunohistochemistry after fixation.
Because tissue loading steps are omitted, tissue viability can be increased and background signals (e.g., from the underlying smooth muscle layers) reduced. However, to fully employ the advantages of these genetically-encoded indicators, they should ideally be expressed in enteric neurons or glial cells specifically. This will help to overcome the earlier illustrated problems caused by the close proximity between enteric neurons and glia. In addition, depending on the specifics of the transgenic method used, they can potentially enable monitoring of events in cellular subtypes. GECIs that tether to specific membrane proteins can be used to examine activity in thin glial processes and endfeet (Shigetomi et al., 2012, 2013). These are the cellular compartments that most likely interact with varicose fibers but are difficult to study using bulk loading dyes or normal cytosolic GECIs. This would yield important information about the signaling events in potential glial release sites and microdomains near the membrane.

**IMAGING NEURON-GLIA CROSSTALK IN THE HUMAN ENTERIC NERVOUS SYSTEM**

Because most investigations have been carried out using *in vitro* and *ex vivo* animal models, and given the difficulty to obtain healthy human gut tissues for experimental purposes, our current knowledge about enteric neuron–glia interactions in the human gut is rather poor. The limited information about human enteric glia function originates from *in vitro* studies using enteric glia isolated from surgical resection specimens. These studies indicated that human enteric glia actively participate in inflammatory responses (Cirillo et al., 2011) and host-bacteria crosstalk (Turco et al., 2013). However, even though these glial cells were obtained from ‘macroscopically normal’ tissues, there is still the possibility that measurements were influenced by the fact that the resection specimens were collected from patients suffering from a variety of severe diseases.

For these reasons we have recently developed a method to culture human enteric glial cells isolated from routine intestinal biopsies (Boesmans et al., 2013). After careful removal of the mucosa, the submucosal plexus is enzymatically digested following previously described procedures (Cirillo et al., 2011), ganglia are selected and cells cultured on glass coverslips to perform live imaging studies. By implementing this technique we found that similar to rat enteric glia, also human enteric glial cells can be activated by neurotransmitters known to elicit fast excitatory responses in the ENS (Boesmans et al., 2013).

Of course, in order to fully understand neuron-glial interactions in the human gut, also these should ideally be studied in intact ex vivo preparations. Although the interplay between enteric neurons and glia was not specifically envisaged, Mueller and colleagues were able to record enteric glial cell activity in resection specimens obtained from patients undergoing surgery (Mueller et al., 2011). With the recently developed optical imaging approach (Cirillo et al., 2013), we have shown that it is also feasible to record neuronal activity by means of Ca²⁺ imaging in submucosal ganglia dissected from duodenal biopsies obtained from healthy volunteers. This technique also allows exploring human enteric glia function (Figure 6). Again, analysis is not straightforward, even in comparison to the ENS of animal models, and given the difficulty to obtain animal models, as the cells in the human enteric ganglia are organized in a far more three dimensional manner than in small animals. Nerve bundles and glial projections together form a complex structure (the ganglionic capsule) that surrounds neurons and glial cells (Figures 6A,B). Moreover, the presence of fasciculated bundles interconnecting adjacent ganglia adds to the intricacy of optical recordings from such ganglia. This makes correct interpretation of glial activation and discrimination between neuronal and glial signaling difficult, but not impossible. By analogy with the animal tissue experiments, it is still feasible, with careful attention
to focus, movement, and analysis issues, to distinguish between signals originating from glia and neurons (Figures 6C–E). Here again, glial signals are delayed with respect to the responses observed in neuronal compartments.

CONCLUSIONS AND PERSPECTIVES

Despite significant progress in understanding enteric glia function, the exact signaling mechanisms and possible “glio-transmitters” that act in a pharmacological context such as during the different gastrointestinal motility patterns remain elusive. Nonetheless, several studies indicate that enteric glial cells are active partners in enteric neurotransmission. In particular, the aforementioned live imaging studies have provided invaluable information about how enteric glial cells can detect neuronal activity (Gomes et al., 2009; Gulbransen and Sharkey, 2009; Gulbransen et al., 2010, 2012; Broadhead et al., 2012). As illustrated, the intimate association between enteric glia and neurons warrants careful experimental and analytic considerations. Enhancing optical resolution by using confocal recordings would obviously help toward avoiding false interpretation of the signal’s origin especially when two structures truly overlap. However, this should be combined with deconvolution methods as resolution along the optical axis is never as high as in the XY plane. Confocal recordings can definitely improve the spatial resolution, but as we have shown, also timing is important to tell different responses apart, an advantage that is lost in classical point scanning confocals due to limitations in scan speed. Therefore, spinning disk confocal recordings may offer an intermediate solution. The toolbox of optical, off-line analysis and genetic approaches that we present here, can aid in disentangling the complex interplay between neurons and glia, thereby producing experimental access to bridge differing conclusions.

AUTHOR CONTRIBUTIONS

Concept and design, interpretation of data, statistical analysis, drafting and editing of the manuscript were done by Werend Boesmans and Pieter Vanden Berghe; Werend Boesmans, Michiel A. Martens, Nathalie Welteins, and Marlene M. Hao performed experiments, image analysis and contributed to paper writing; Carla Cirillo performed and analyzed experiments on human enteric glia and was involved in paper writing; Jan Tack was responsible for mucosal biopsy collection; Pieter Vanden Berghe and Michiel A. Martens wrote analysis software; and Jan Tack and Pieter Vanden Berghe obtained funding.

ACKNOWLEDGMENTS

We would like to thank the members of LENS for their critical comments and skilled technical assistance. Werend Boesmans, Carla Cirillo, and Marlene M. Hao are postdoctoral fellows of the Fonds voor Wetenschappelijk Onderzoek (FWO, Belgium). This work was funded by BOF, University of Leuven (Methusalem Jan Tack; OT ZKC1808, Pieter Vanden Berghe) and FWO (KN 1.5.135.06; G.0501.10, Pieter Vanden Berghe). We thank Vassilis Pachnis (National Institute for Medical Research, MRC, London, UK) for providing the Wnt1-Cre mice. Confocal recordings were made on the equipment of the Cell Imaging Core supported by Hercules foundation grants (to Pieter Vanden Berghe).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fncel.2013.00183/abstract

REFERENCES

Bisschops, R., Vanden Berghe, P., Sarnelli, G., Janssens, J., and Tack, J. (2006). CRF-induced calcium signaling in guinea pig small intestine myenteric neurons involves CRF-1 receptors and activation of voltage-sensitive calcium channels. Am J Physiol. Gastrointest. Liver Physiol. 290, G1252–G1260. doi: 10.1152/ajpgi.00349.2004

Boesmans, W., Cirillo, C., Van Den Abbeel, V., Van Den Haute, C., Depoortere, I., Tack, J., et al. (2013). Neurotransmitters involved in fast excitatory neurotransmission directly activate enteric glial cells. Neurogastroenterol. Motil. 25, e151–e160. doi: 10.1111/nmo.12065

Bonduard, N., Natarajan, D., Thapar, N., Atkins, C., and Pachnis, V. (2003). Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. Development 130, 6387–6400. doi: 10.1242/dev.00857

Broadhead, M. J., Bayguinov, P. O., Okamoto, T., Heredia, D. J., and Smith, T. K. (2012). Ca2+ transients in myenteric glial cells during the colonic migrating motor complex in the isolated murine large intestine. J. Physiol. 590, 335–350. doi: 10.1113/jphysiol.2011.219519

Cirillo, C., Sarnelli, G., Turco, F., Manga, A., Grosso, M., Aprea, G., et al. (2011). Proinflammatory stimuli activates human-derived Bisschops, R., Vanden Berghe, P., Bisschops, R., Vanden Berghe, P., Boesmans, W., Cirillo, C., Van Den Abbeel, V., Van Den Haute, C., Depoortere, I., Tack, J., et al. (2013). Neurotransmitters involved in fast excitatory neurotransmission directly activate enteric glial cells. Neurogastroenterol. Motil. 25, e151–e160. doi: 10.1111/nmo.12065

Boesmans, W., Cirillo, C., Van Den Abbeel, V., Van Den Haute, C., Depoortere, I., Tack, J., et al. (2013). Neurotransmitters involved in fast excitatory neurotransmission directly activate enteric glial cells. Neurogastroenterol. Motil. 25, e151–e160. doi: 10.1111/nmo.12065

Gulbransen, N., Natarajan, D., Thapar, N., Atkins, C., and Pachnis, V. (2003). Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. Development 130, 6387–6400. doi: 10.1242/dev.00857

Broadhead, M. J., Bayguinov, P. O., Okamoto, T., Heredia, D. J., and Smith, T. K. (2012). Ca2+ transients in myenteric glial cells during the colonic migrating motor complex in the isolated murine large intestine. J. Physiol. 590, 335–350. doi: 10.1113/jphysiol.2011.219519

Cirillo, C., Sarnelli, G., Turco, F., Manga, A., Grosso, M., Aprea, G., et al. (2011). Proinflammatory stimuli activates human-derived

Boesmans et al. Neuron-glia interactions in the gut
entero- and glial cells and induces autocrine nitric oxide production. Neurogastroenterol Motil. 23, e372–e382. doi: 10.1111/j.1365-2982.2011.01748.x

Cirillo, C., Tack, J., and Vandenberg Berge, P. (2013). Nerve activity recordings in routine human intestinal biopsies. Gut 62, 227–235. doi: 10.1136/gutjnl-2011-301777

Cook, R. D., and Burnstock, G. (1976). The ultrastructure of Auerbach’s plexus in the guinea-pig. I. Neuronal elements. J. Neurocytol. 5, 171–194. doi: 10.1007/BF00118165

Daniellian, P. S., Muccino, D., and Polak, J. M. (1982). Evidence for the presence of S-100 protein in the glial component of the human enteric nervous system. Neurogastroenterol Motil. 21, 870–872. doi: 10.1111/j.1365-2982.2009.01302.x

Garrido, R., Bains, J. S., and Rothman, T. P. (2013). Nerve activity recordings in routine human intestinal biopsies. Hum. Mol. Genet. 22, 859–870. doi: 10.1093/hmg/ddd455

Sharkey, K. A. (2010). Enteric glia in health and disease. Nat. Rev. Gastroenterol. Hepatol. 7, 43–57. doi: 10.1038/nrgastro.2010.293

Mulligan, S. J., and Mavric, B. A. (2004). Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. Nature 431, 195–199. doi: 10.1038/nature02827

Furness, I. B. (2000). Types of neurons in the enteric nervous system. J. Auton. Nerv. Syst. 81, 87–96. doi: 10.1016/S0169-1533(00)00127-2

Gabella, G. (1981). Ultrastructure of the nerve plexuses of the mammalian intestine: the enteric glial cells. Neuroscience 6, 425–436. doi: 10.1016/0306-4522(81)90135-4

Gallego, D., Vandenberg Berge, P., Farre, R., Tack, J., and Jimenez, M. (2008). P2Y1 receptors mediate inhibitory neurotransmission and enteric neuronal activation in small intestine. Neurogastroenterol Motil. 20, 159–168. doi: 10.1111/j.1365-2982.2007.01040.x

Ganat, Y. M., Silbereis, J., Cave, C., Ngu, H., Anderson, G. M., Okhobo, Y., et al. (2006). Early postnatal astroglial cells produce multilineage precursors and neural stem cells in vivo. J. Neurosci. 26, 8609–8621. doi: 10.1523/JNEUROSCI.2552-06.2006

Garrido, R., Segura, B., Zhang, W., and Mulholland, M. (2002). Presence of functionally active protease-activated receptors 1 and 2 in myenteric glia. J. Neurosci. 23, 556–564. doi: 10.1046/j.1471-4141.2002.01119.x

Gershon, M. D., and Roothan, T. P. (1999). Enteric glia. Glia 4, 195–204. doi: 10.1002/(SICI)1098-1136(199907)21:2<195::AID-Glia2>3.0.CO;2-W

Gomes, P., Chevalier, J., Boesmans, W., Roosen, L., Van Den Abbeel, V., Neunlist, M., et al. (2009). ATP-dependent paracrine communication between enteric neurons and glia in a primary cell culture derived from embryonic mice. Neurogastroenterol Motil. 21, 193–196. doi: 10.1111/j.1365-2982.2009.00820.x

Gulbransen, B. D., Bains, J. S., and Sharkey, K. A. (2010). Enteric glia target receptors are the sympathetic innervation of the myenteric plexus in the guinea pig distal colon. J. Neurosci. 30, 6801–6809. doi: 10.1523/JNEUROSCI.0603-10.2010

Gulbransen, B. D., Bashashati, M., Hirota, S. A., Gouri, A., Roberts, J. A., Macdonald, J. A., et al. (2012). Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during postnatal development. J. Neurosci. 32, 81655–81665. doi: 10.1523/JNEUROSCI.0612-12.2012

Hirrlinger, P. G., Scheller, A., Braun, C., Hirrlinger, J., and Kirchhoff, F. (2006). Temporal control of gene recombination in astrocytes by transgenic expression of the tamoxifen-inducible DNA recombinase variant CreERT2. Glia 54, 11–20. doi: 10.1002/glia.20342

Hoff, S., Zeller, F., Von Weyhern, C. W. H., Wegner, M., Schermann, M., Michel, K., et al. (2008). Quantitative assessment of glial cells in the human and guinea pig enteric nervous system with an anti-sox9/4D10 antibody. J. Comp. Neurol. 509, 356–371. doi: 10.1002/cne.21769

Jessen, K. R., and Mirsky, R. S. (1988). Glial cells in the enteric nervous system contain gli fibrillary acidic protein. Nature 334, 766–737. doi: 10.1038/334766a0

Joseph, N. M., He, S., Quintana, E., Kim, Y. G., Nunez, G., and Morrison, S. J. (2011). Enteric glia are multipotent in culture but primarily form glia in the adult rodent gut. J. Clin. Invest. 121, 3398–3411. doi: 10.1172/JCI58186

Kimball, B. C., and Mulholland, M. W. (1996). Enteric glia exhibit P2U receptors that increase cytosolic calcium by a phosholipase C-dependent mechanism. J. Neurochem. 66, 604–612. doi: 10.1046/j.1471-4159.1996.66020604.x

Knopfel, T. (2012). Genetically encoded optical indicators for the analysis of neuronal circuits. Nat. Rev. Neurosci. 13, 687–700. doi: 10.1038/nrn3393

Kuzmanovic, M., Dudley, V. J., and Sarthy, V. P. (2003). GFAP promoter drives Muller cell-specific expression in transgenic mice. Invest. Ophthalmol. Vis. Sci. 44, 3606–3613. doi: 10.1167/iovs.02-1265

Larajaneira, C., Sandgren, K., Kassar, N., Richardson, W., Potocnik, A., Vanden Berge, P., et al. (2011). Glial cells in the mouse enteric nervous system can undergo neurogenesis in response to injury. J. Clin. Invest. 121, 3412–3424. doi: 10.1172/JCI58200

Lavoie, E. G., Gulbransen, B. D., Martin-Satue, M., Aliagas, E., Sharkey, K. A., and Sevigny, J. (2011). Ectonucleotidases in the digestive system: focus on extracellular nucleotides. Front. Cell. Infect. Microbiol. 1, 32. doi: 10.3389/fcimb.2011.00032

Lavoie, E. G., Gulbransen, B. D., Martin-Satue, M., Aliagas, E., Sharkey, K. A., and Sevigny, J. (2011). Ectonucleotidases in the digestive system: focus on extracellular nucleotides. Front. Cell. Infect. Microbiol. 1, 32. doi: 10.3389/fcimb.2011.00032

Maceachern, S. J., Patel, B. A., McKay, R. L., and Foong, J. P. (2012). The enteric glia in health and disease. Nat. Rev. Gastroenterol. Hepatol. 10, 90–100. doi: 10.1038/nrgastro.2012.221

Narang, I., Jensen, C. V., Bonde, C., Montero, M., Nielsen, J. V., Jensen, N. A., et al. (2007). The developmental expression of fluorescent proteins in organotypic hippocampal slice cultures from transgenic mice and its use in the determination of excitotoxic neurodegeneration. Altern. Lab. Anim. 35, 61–70.

Obermayr, F., Hotta, R., Enomoto, H., and Young, H. M. (2013). Development and developmental disorders of the enteric nervous system. Nat. Rev. Gastroenterol. Hepatol. 10, 43–57. doi: 10.1038/nrgastro.2012.234

Paratore, C., Eichenberger, C., Suter, U., and Sommer, L. (2002). Sox10 haploinsufficiency affects maintenance of progenitor cells in a mouse model of Hirschsprung disease. Hum. Mol. Genet. 11, 3075–3085. doi: 10.1093/hmg/11.24.3075
Neuron-glia interactions in the gut

Vanden Berghe, P., and Klingauf, J. (2007). Spatial organization and dynamic properties of neurotransmitter release sites in the enteric nervous system. Neuroscience 145, 88–99. doi: 10.1016/j.neuroscience.2006.11.048

Shigetomi, E., Tong, X., Kwan, K. Y., Corey, D. P., and Khakh, B. S. (2012). TRPA1 channels regulate astrocyte resting calcium and inhibitory synapse efficacy through GAT-3. Nat. Neurosci. 15, 70–80. doi: 10.1038/nn.3000

Simon, C., Lickert, H., Gotta, M., and Dimou, I. (2012). Sox10-GCaERT2: a mouse line to inducibly trace the neural crest and oligodendrocyte lineage. Genesis 50, 506–515. doi: 10.1002/dvg.22003

Smith, T. K., Spencer, N. J., Hennig, G. W., and Dickson, E. J. (2007). Recent advances in enteric neurobiology: mechanosensitive interneurons. Neurogastroenterol. Motil. 19, 869–878. doi: 10.1111/j.1365-2982.2007.01019.x

Srinivas, S., Watanabe, T., Lin, C. S., William, C. M., Tanabe, Y., Jessell, T. M., et al. (2001). Cre reporter strains produced by targeted insertion of EYFP and ECFP into the ROSA26 locus. BMC Dev Biol 1:4. doi: 10.1186/1471-2121-1-4

Takano, T., Tian, G. F., Peng, W., Lou, N., Lbinoka, W., Han, X., et al. (2006). Astrocyte-mediated control of cerebral blood flow. Nat. Neurosci. 9, 260–267. doi: 10.1038/nn1623

Tian, L., Hires, S. A., Mao, T., Huber, D., Chiappe, M. E., Chalasani, S. H., et al. (2009). Imaging neural activity in worms, flies and mice with improved GCaMP calcium indicators. Nat. Methods 6, 875–881. doi: 10.1038/nmeth.1398

Turco, F., Sarnelli, G., Cirillo, C., Palumbo, I., De Giorgi, F., D’Alessandro, A., et al. (2013). Enteroglial-derived S100B protein integrates bacteria-induced Toll-like receptor signalling in human enteric glial cells. Gut. doi: 10.1136/gutjnl-2012-302090. [Epub ahead of print].

Vanden Berghe, P., and Klingauf, J. (2007). Spatial organization and dynamic properties of neurotransmitter release sites in the enteric nervous system. Neuroscience 145, 88–99. doi: 10.1016/j.neuroscience.2006.11.048

Zhao, L., Sun, B., Zhang, C. L., Fine, A., Chiu, S. Y., and Messing, A. (1997). Live astrocytes visualized by green fluorescent protein in transgenic mice. Dev. Biol. 187, 36–42. doi: 10.1006/dbio.1997.8601

Zhao, L., Theis, M., Alvarez-Maya, I., Brenner, M., Willecke, K., and Messing, A. (2001). hGFAP-cre transgenic mice for manipulation of glial and neuronal function in vivo. Genesis 31, 85–94. doi: 10.1002/gene.10008

Zuo, Y., Lubischer, J. L., Kang, H., Tian, L., Mikesch, M., Marks, A., et al. (2004). Fluorescent proteins expressed in mouse transgenic lines mark subsets of glia, neurons, macrophages, and dendritic cells for vital examination. J. Neurosci. 24, 10999–11009. doi: 10.1523/JNEUROSCI.3934-04.2004

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 August 2013; accepted: 01 October 2013; published online: 21 October 2013.

Citation: Boesmans W, Martens MA, Weltens N, Hao MM, Tack J, Cirillo C and Vanden Berghe P (2013) Imaging neuron-glia interactions in the enteric nervous system. Front. Cell. Neurosci. 7:183. doi: 10.3389/fncel.2013.00183

This article was submitted to the journal Frontiers in Cellular Neuroscience.

Copyright © 2013 Boesmans, Martens, Weltens, Hao, Tack, Cirillo and Vanden Berghe. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.
Author/s: Boesmans, W; Martens, MA; Weltens, N; Hao, MM; Tack, J; Cirillo, C; Berghe, PV

Title: Imaging neuron-glia interactions in the enteric nervous system

Date: 2013-10-21

Citation: Boesmans, W., Martens, M. A., Weltens, N., Hao, M. M., Tack, J., Cirillo, C. & Berghe, P. V. (2013). Imaging neuron-glia interactions in the enteric nervous system. FRONTIERS IN CELLULAR NEUROSCIENCE, 7 (OCT), https://doi.org/10.3389/fncel.2013.00183.

Persistent Link: http://hdl.handle.net/11343/258256

File Description: Published version

License: CC BY