Review paper

Recording of the neuronal activity in the gastrointestinal tract using the Multisite Optical Recording Technique (MSORT)

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Abstract

The Enteric Nervous System (ENS) plays a key role in regulating the function of the gastrointestinal tract. The knowledge about the function of neurons is based on experiments using the intracellular recording method. Limitations of the method have become a stimulus for developing an alternative technique that would record cell membrane potential changes without mechanical interruption and with no cells bias due to their size. The recently developed neuroimaging method meets these demands. The principle of the technique is based on fluorescence of voltage sensitive dyes (VSD) specific molecules that incorporated in cell membranes react to their potential. Photodiode arrays or charge-coupled device (CCD) cameras can be used for acquiring the fluorescent light that permits to monitor fast changes in membrane potential. The Multisite Optical Recording Technique (MSORT) is capable of recording changes in membrane potential in all neurons in the ENS with sufficient spatial and temporal resolution. Studies published up to date are very promising and give an interesting view on the function of neurons in the ENS.

Key words: enteric nervous system, neuroimaging, myenteric plexus, voltage sensitive dyes, fast excitatory postsynaptic potential.

The enteric nervous system (ENS) forms a brain unto itself [1]. Millions of neurons compose an integrative network, covering the entire gut wall. These complex microcircuits aredriven by more neurotransmitters and neuromodulators that can be found anywhere in the peripheral nervous system. Discovery of the ENS dates back to Bayliss and Starling [2] who demonstrated a phenomenon that they called the "law of intestine" which we know now as the peristaltic reflex. At their time it was impossible to explain the mechanism, but the general idea that the gut itself could regulate its function by the neuronal activity was confirmed by many later studies. In spite of the considerable progress in the knowledge about the function and anatomy of the gastrointestinal tract since the first discoveries in the 19th century, there are still many challenges for neurogastroenterologists. The most urgent task is explanation of mechanisms and pathogenesis of functional disorders of the gastrointestinal tract. For instance, the irritable bowel syndrome (IBS) is the most common

disorder diagnosed by gastroenterologists and one of the more common ones encountered in general practice. The overall prevalence rate is similar (approximately 10%) in most industrialized countries; the illness has a large economic impact on health care use and indirect costs, chiefly through absenteeism [3]. One of the mechanisms that may be responsible for pain sensation in the IBS is an increase in activation of the peristaltic reflex that produce changes in motility which in turn induce diarrhea or constipation [4].

The ENS consists of two major plexuses: the myenteric plexus located between the longitudinal and circular muscle and the submucosal plexus located between submucosa and mucosa. The ENS plays a crucial role in regulating peristaltic reflexes. There is a body of evidence that both functional and anatomical features of the ENS are structured to fully regulate responses of the gut to various stimuli from the lumen [5]. It has been shown that reflex responses recorded after denervation of the gut are no different to those recorded from the control tissue. It leads to a conclusion that, in the small intestine, cell bodies of primary sensory neurons for mucosal and probably for distension reflexes are intrinsic to the organ [6].

Therefore, it is concluded that the ENS can work in separation from the central nervous system, and major activities of the gastrointestinal tract can be regulated internally in the gut wall. Based on combinations of multiple-labeling immunohistochemistry and retrograde tracing, it has been possible to account quantitatively for all of the neurons in the guinea-pig small intestine and total of 18 classes of neurons have been currently distinguishable, including primary afferent neurons, motor neurons, interneurons, secretomotor and vasomotor neurons [7]. Immunohistochemical studies show inhibitory and excitatory "hardwiring" of neurochemical coding of neurons in the ENS [8]. In short, 90% interneurons in the myenteric plexus that project orally are positive for choline acetyltransferase (enzyme involved in acetylcholine synthesis) while 46% of interneurons projecting anally are nitric oxide synthase positive. Acetylcholine is the main excitatory, while nitric oxide is an inhibitory neurotransmitter in the ENS. Therefore, the functional observations that distension of the gut wall produces a nerve-mediated transient contraction of the circular muscle on the oral side and sustained relaxation on the anal side [9] have an anatomical support.

The basic knowledge about functions of the ENS is based on the intracellular recording technique. This approach allows recording of nerve interactions, synaptic, and neuropharmacological properties on the cellular level. However, there is only one study that shows paired impalements and direct synaptic interactions between enteric neurons [10]. Most studies show reactions of single neurons and conclusions are drawn based on these findings. The reason for this "single impalement" approach is that the intracellular recording is technically demanding and time consuming. Moreover, there is sampling bias in intracellular studies because impalements depend on the cell type and morphology. In addition, impalement is traumatic for the cell and may lead to changes of physiological properties that are unknown at the moment.

Remarkable progress in our understanding of the function of the ENS has been achieved for last two decades, however there are many concepts that are still speculative because there is no appropriate method allowing for direct investigation of signal spread in neuronal circuits in the ENS. Therefore, it is very important to develop an alternative method to intracellular recording that will be able to show transmission of signals in populations of neurons and nerves in neuronal network in the gut as well as in the central nervous system. In the mid-seventies the first publications showing a new approach based on optical recording of cell membrane activity were published [11-14]. The concept that electrical activity of various organs can be recorded using optical methods was further investigated and developed [14, 15]. The new method has been based on the principle of fluorescence of voltage sensitive dyes (VSD) specific molecules that incorporated in cell membranes react to their potential. Photodiode arrays or charge-coupled device (CCD) cameras can be used for acquiring the fluorescent light that permits to monitor fast changes in membrane potential with sufficient spatiotemporal resolution at the subcellular level [16]. Recording in the brain has been proven to be also possible [17, 18], however the cellular resolution has not been reached yet. On the other hand, in the intestine it is possible to investigate single neurons using the optical method [19-22].

In comparison to intracellular recording the optical method is relatively easier to manage. In the ENS, the membrane-bound styryl voltage sensitive dye 1-(3-sulfonatopropyl)-4-[β[2(di-n-octylamino)-6--hapthyl]vinyl]pyridinium betaine (Di-8-ANEPPS) has been used to monitor fast changes in membrane potentials [19-21]. The method has been developed for last few years and reproducible recordings with low toxicity were achieved. Finally, it is possible to record reproducible responses to electrical stimulations [23, 24]. Myenteric ganglia from guinea pig small intestine, colon, or stomach stained locally with di-8-ANEPPS show clearly an internal structure and it is possible to distinguish cell bodies and interganglionic nerves that are visualised by fluorescent light (Figure 1). The dye outlines cell bodies in the ganglion and visualises interganglionic nerve strands. The ability to see cell bodies stained

by VSD is crucial for further analyzing signal transmission and its pharmacological properties. The principle of the method is based on the recording of changes in the fluorescent light emitted by VSD that is excited by a stable light source (xenon lamp) attached to a fluorescent microscope (Figure 2). Excited VSD changes the emitted fluorescent light spectrum dependently on the membrane potential that is observed as changes in light intensity in certain wavelength. Electrical stimulation of interganglionic nerve strand induces changes in membrane potential that can be divided into compound action potential and fast excitatory postsynaptic potential (EPSP) (Figure 3) [23, 24]. Compound action potential is a signal recorded from nerves lying around the analyzed cell body while fast excitatory postsynaptic potential (EPSP) is a depolarization of the cell membrane that is induced by a release of neurotransmitters [19]. The inhibition of fast EPSP by ω-conotoxin GVIA supports the above conclusion. Similar effects are observed in the presence of the buffer with depleted calcium and

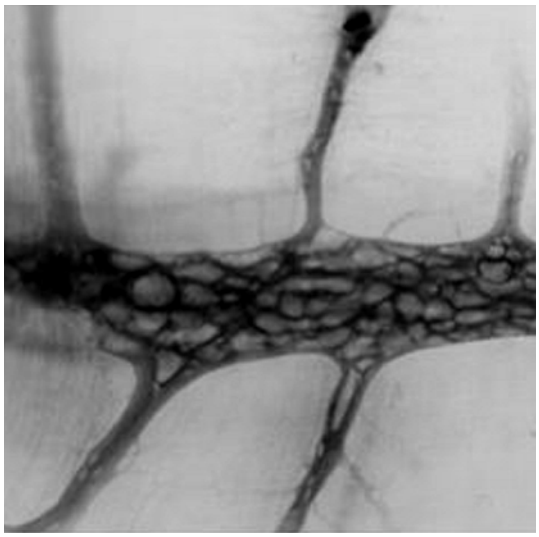

Figure 1. Myenteric ganglion from guinea pig ileum stained with Di-8-ANEPPS. The dye was introduced by microinjection directly into the ganglion. Cell bodies and interganglionic nerves are visible in the preparation

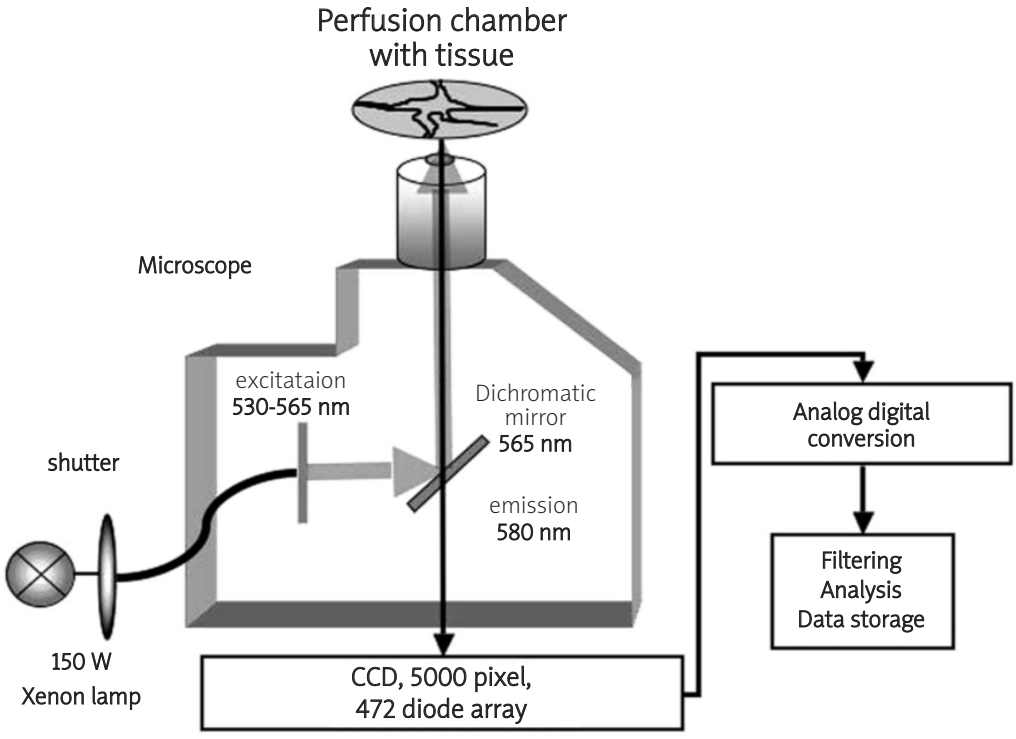

Figure 2. Principles of multisite optical recording technique (MSORT). 1-(3-sulfonatopropyl)-4-[β[2-(di-n-octylamino)-6-hapthyl]vinyl]pyridinium betaine (Di-8-ANEPPS)-stained tissue is excited with a xenon arc lamp. The fluorescence changes are detected either with a 468-photodiodes array or a cooled charge-coupled device (CCD) camera made of 70x70 pixels Optical signals are processed with a computer; frame rate is from 500 Hz to 2.7 kHz enabling the detection of action potentials. With a x40, we detected fractional changes in fluorescence (divided by the resting light level) in the range of 0.05-4%. With the x40 objective, the photodiode system has a spatial resolution of 280 μm^2 per diode, whereas the one of the CCD system is 24 μm^2 per pixel. Both systems allow resolution at a cellular range, the CCD system, even at a subcellular range

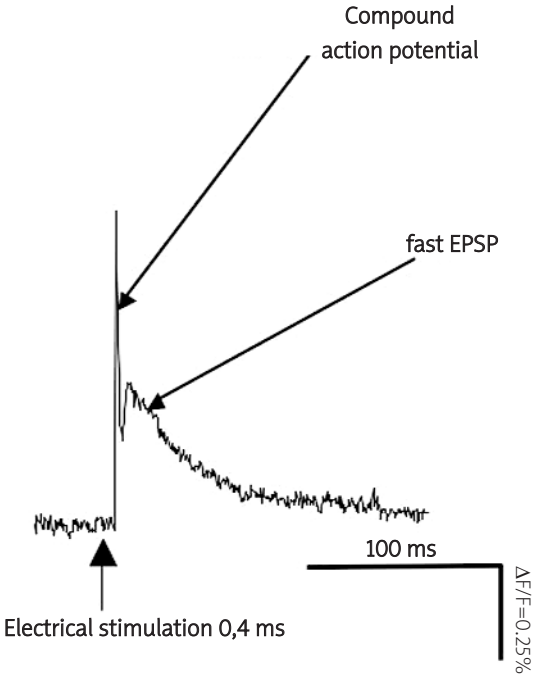

Figure 3. Response of the cell membrane of the myenteric neuron to electrical stimulation of interganglionic nerve strand recorded by the optical method. Compound action potential and fast excitatory postsynaptic potential (EPSP) are marked by arrows

increased magnesium ions content (unpublished author's data).

In summary, the optical method has certain advantages such as simultaneous recordings in a relatively large neuronal population (approximately 30 cells). The method is non-invasive in contrast to microelectrode recordings, which may damage the cell membrane and thereby alter ion concentration and cell homeostasis (the dye does not affect resting membrane potential recorded by the intracellular electrode). The optical method enables recordings from cells that are inaccessible either because they are too small or hidden between tissue layers. The additional advantage of the optical method is the ability of recording from interganglionic nerves that was unavailable for the conventional intracellular method. Nevertheless, there are certain limitations of the optical method that have to be taken in account such as non-specific labelling of cell membranes (neurons and glial cells) with the VSD, phototoxicity and bleaching of the VSD. Additionally, the necessity to reach a decent signal-to-noise ratio is crucial for the interpretation of optically recorded signals. It means that sufficient concentration of the VSD has to be reached on the neuronal membrane to record a signal while the surrounding tissues have to be almost free from the dye.

The new method gives a powerful tool for investigating neuronal transmission in the ENS and, probably in the future, in the central nervous system. It has been already possible to record from tissues that were inaccessible for conventional intracellular recording – human submucosal plexus [21]. Thus, the next step toward understanding the function of the gastrointestinal tract in health and disease has been already done.

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