

UNIVERSITY OF PÉCS
Biological Doctoral School

**Ultrastructural, functional and molecular
aspects of the organization of the olfactory
center of gastropod species (*Helix pomatia*,
Limax valentianus)**

PhD Thesis

Izabella Battonyai

Supervisor:

Károly Elekes PhD

Doctor of Biological Sciences

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1. INTRODUCTION

In terrestrial pulmonata snails olfaction is the main sensory modality, it determinates and influences many specific behavior (Stocker 1993). We have immersed knowledge about their olfactory system, including its structural and functional organization (Gelperin, 1999, Chase, 2002). Both electrophysiological and autoradiographical methods supported that the receptor cells, located on the tip of the tentacles take part not only in mechanosensory but in olfactory information processing (Chase 1982). The olfactory epithelium is situated in the ventral part on the tip of the tentacles, from where through the receptor cells the odor information is sent to and integrated in the tentacular ganglion followed by the information processing processes in the CNS. The center of odor processing is the procerebrum (olfactory lobe, PC), which is located in the cerebral ganglion and is a characteristic unit in Stylommatophora (Pulmonata) snails (Van Mol 1974). There are also a number of morphological and physiological data proving that the PC takes part in olfaction processes, it has important role in olfactory information processing and in odor learning and memory formation as well (Gelperin, 1999; Chase, 2002). Although in early electron microscopic studies Zs.-Nagy and Sakharov (1969) identified synapses in the cell layer of the PC, our knowledge is incomplete regarding the exact ultrastructure and synaptology of the cell body layer.

Apart from some neuropeptides and the octopamin, neurotransmitters in the Gastropoda nervous system are similar to that can be found in the vertebrates (e.g. acetylcholine, serotonin, dopamine, glutamate) (Chase 2002). Among them, serotonin (5-HT) has been known for a very long time as an important signal molecule, which is widely distributed in invertebrates (Gerschenfeld, 1973; Gardner and Walker, 1982; Walker et al., 1996).

In the Gastropod nervous CNS it has been revealed that many 5-HTergic processes are present and arborizing in the medullary neuropil of the PC (Osborne and Cottrell, 1971; Inoue et al., 2004; Matsuo et al., 2009). However, the high-resolution analysis of the organization and intercellular interactions of the 5-HT containing elements within the PC, including the identification of their postsynaptic targets, is still missing.

Potassium (K^+) channels can be found virtually in all living organism, responsible for maintaining the electronic activity of the cells (Kurachi et al. 1999). Voltage-dependent potassium channels are the largest ion channel

family. They play a pivotal role in bursting of nerve cells, neuronal excitability and re-polarizing cells following action potentials (Hille, 2001; Conley and Brammar, 1999). The first K^+ -current was recorded from neurons of the slug *Onchidium* by Hagiwara et al. (1961). Although, by immunohistochemistry (IHC), Azanza et al. (2008) demonstrated the presence of different ion channels in the neurons of the subesophageal ganglion complex of *Helix aspersa*, the detailed investigation of K^+ -channels in the entire CNS, regarding their precise distribution, projection patterns as well as the visualization of possible intercellular contacts established by K^+ -channel containing neurons have completely been neglected. The precise localization and functional characterization of K^+ -channels would be indispensable to have a better insight in their possible implication in signal transduction processes in the nervous system of gastropods.

2. AIMS

It is clear that still many information and details are missing concerning the olfactory lobe (procerebrum, PC) of the terrestrial pulmonate snails, including its anatomical, ultrastructural organization and its synaptic connections. Moreover, the exact organization of the 5-HTergic innervation pattern and its synaptology is not known.. Data and the possible functional interpretation have also been missing on the presence and cellular localization of K^+ -channels in the gastropod CNS. In the globuli cells of the olfactory center, PC, the precise localization, functional characterization and synaptic connections of K^+ -channels seem to be indispensable to have a better insight in voltage-gated K^+ -channels as to their involvement in signal transduction processes in the nervous system of gastropods, including odor information processing.

The aims of our present work were the following.

1. To revise and define the ultrastructural organization of the PC cell body; layer, including the local neuropil areas and the intercellular connections
2. To describe the 5-HT-IR innervation system of the PC in details, with special attention to the cell body layer and the different forms of intercellular connections;

3. To identify voltage-gated K⁺-channels in the CNS, including the PC, and possibly reveal their functional characteristics.

3. MATERIALS AND METHODS

3.1. Animals

Adult specimens of the garden (land) snail *Helix pomatia* and the slug *Limax valentianus* were used. Specimens of *Helix pomatia* were collected in the surrounding areas, kept thereafter under laboratory conditions and fed on lettuce. Slugs 11–14 weeks after hatching were maintained under laboratory conditions and fed on a mixed diet of rat chow (Oriental Yeast, Tokyo, Japan), wheat starch (Wako, Osaka, Japan) and vitamins (Oriental Yeast).

3.2. Electron microscopy

For fixation, the CNS of both species were isolated and pinned out in a Petri-dish and covered with a mixture of either 2.5% PFA and 1 % GA. Preparations were post-fixed in 1% OsO₄, dehydrated in graded ethanol and propylene oxide, and finally embedded in Araldite. Block staining was performed in 70% ethanol saturated with uranyl acetate. For orientation 1 μm semi-thin sections were stained with toluidine blue. 50-60 nanometer ultrathin serial sections from the PC region were taken with an LKB Novacut ultramicrotome, stained with lead citrate, and viewed in a JEOL 1200 EX, a JEOL 1200 EXII, and a Hitachi H-7650 electron microscope.

3.3. Immunohistochemistry

In our experiments we used two-step indirect (fluorescent dye or peroxidase-IgG) or three-step avidin-biotin complex (ABC) for visualization. In case of 5-HT IHC only the cerebral ganglia (PC), while in case of the ion channels the whole CNS were fixed in 4% PFA and cut into 16 μm slices on a cryostat. The following primary antibodies were used: monoclonal anti-5-HT (1:500-1000); polyclonal anti-K_v3.4 and anti-K_v4.3 (1:500 vagy 1:1000); and monoclonal mouse anti-K_v2.1 (1:500). After this the next secondary antibodies were used depending the primary antibody: rabbit anti-donkey IgG conjugated with FITC or TRITC (1:200), or biotin-conjugated goat anti-mouse igG or anti-rabbit IgG (1:200), following avidin-HRP (1:200) incubation. The HRP-reaction was visualized by using 0.05 %

3.3'-DAB and 0.01% H₂O₂. The sections were mounted in 1:1 mixture of glicerol:PBS and viewed in a Zeiss Axioplan compound microscope attached to a Canon PS G5 digital camera or CCD (Alpha DCM510, Hangzhou Scopetek Opto-Electric) camera. The specificity of each antibody was tested by applying both negative (omitting the primary antibody) and pre-absorption control experiments. No immunostaining could be observed following these control experiments.

3.4. Correlative light- and electron microscopic immunocytochemistry

Helix and *Limax* CNS were dissected and fixed with 2.5 % PFA and 0.1 % GA, than the PC -s (5-HT, K_v4.3) and the caudo-medial lobe of the pedal ganglia (K_v4.3) were postfixated in 10% PFA and cut into 50 μm slices with a Vibratome. Slices were incubated in mouse monoclonal anti-5-HT antiserum or rabbit polyclonal anti-K_v4.3 antiserum. In case of anti-5-HT the slices were processed for a two-step peroxidase immunocytochemistry (HRP-DAB), the secondary antibody concentration was 1:50. In case of K_v4.3 the immunoreaction was visualized with a one-step polymer-HRP IHC detection system. The slices were post-fixed in 1% OsO₄, dehydrated, and then mounted on slides in Araldite. After polymerization, the slices were analyzed in a light microscope. Following light microscopy, slices displaying high quality immunolabeling were selected and re-embedded for electron microscopy and Camera lucida reconstruction also have been made. Ultrathin sections of 50–60 nm were cut, stained with lead citrate, and viewed in the electron microscopes.

3.5. Western blot experiments

For K_v2.1 and K_v3.4 immunoblotting 10 pieces of the CNS including the surrounding neural sheath or 10 pieces of PC, meanwhile for K_v4.3 immunoblotting 10 pieces of desheathed CNS, separated connective tissue sheath and PC, respectively, were homogenized in an SDS containing lysating buffer. Electrophoresed proteins were blotted onto nitrocellulose membranes and membrane strips were incubated with the primer antiserum (anti-K_v2.1, anti-K_v3.4 and anti-K_v4.3, 24h) than HRP-conjugated to secondary antibodies. The visualization of the reaction product was carried out with an enhanced chemiluminescent (ECL) reagent, or in Tris-HCl buffer (pH 7.6) containing 0.05% DAB (Sigma) and 0.01% H₂O₂. Method control

experiments were performed by omitting the primary antibody and preabsorption specificity control was made with the recognizing peptides.

3.6. Electrophysiology

3.6.1. Patch-clamp experiments

Perforated patch-clamp recordings on *Limax* PC globuli cells were made using previously published experimental protocols (Watanabe et al. 2003). Focal application of 5-HT was performed by a glass micropipette placed beside the soma (≤ 50 μm). 5-HT was dissolved in *Limax* saline solution containing 0.05 %, Fast Green (Sigma) to allow visual confirmation. In *Helix* PC, electrophysiological experiments were made on neurons of the *Helix* PC selected from those expressing immunolabeling against Kv4.3 and Kv2.1. Current recording was performed under an inverted microscope (Leitz Labovert FS, Germany) using tight seal whole cell patch-clamp recording. For data acquisition and clamp protocols, the amplifiers were connected via a TL DMA interface to a computer supplied with pClamp 5.5 software. Currents were separated using special voltage protocols.

3.6.2. Voltage-clamp experiments

Further electrophysiological experiments were made on neurons of the *Helix* CNS expressing immunolabeling against K_v4.3 (pedal ganglion, caudomedial lobe neurons), K_v2.1 (PC), and K_v3.4 (buccal ganglion, B2 cell) channels, respectively. Current recordings were performed using a GeneClamp amplifier in two microelectrode voltage-clamp mode. Data acquisition and analysis were performed using Digidata interface and pCLAMP software (Axon Instruments). In part of the experiments for recording transient outward K-current the physiological solution was modified, containing 5 mM tetraethylammonium chloride (TEA, Sigma, Budapest) and NaCl was replaced for sucrose. In sucrose and 5 mM TEA saline, the Na-dependent inward current and part of the K-currents, except A-current, were eliminated. For blocking the following drugs were used: blood-depressing substance (BDS-II, Alomone Labs), 4-aminopyridine (4-AP) and 20 mM TEA (both from Sigma).

4. RESULTS

4.1. Ultrastructure and synaptology of the cell body layer of the procerebrum

In semi-thin section taken from both the *Helix* and *Limax* PC local neuropils were present in the entire depth the globuli cell body layer globulus. At ultrastructural level, these neuropil regions were of different size and they display a gradual branching, in the course of which the smaller neuropil units contained only a few axon profiles, meanwhile forming a more intimate contact with the surrounding globuli cell bodies. Finally, individual axons established axo-somatic contacts.

At the ultrastructural level, the form of the axo-somatic contacts in the PC cell body layer widely varied. Both specialized and unspecialized axo-somatic contacts were established by varicosities deeply embedded into the globuli cells perikarya. The axo-somatic and axo-axonic contacts observed in the local neuropils of both the *Helix* and *Limax* PC revealed different forms of synaptic configurations, such as synaptic divergence and presynaptic modulation. In addition, along the unspecialized close contacts exocytotic membrane configurations could also be observed, referring to the active release of signal molecules.

4.2. The chemical-neuroanatomy of the 5-HTergic innervation in the procerebrum

In 50 µm Vibratome slices taken from the *Helix* PC the characteristics of the 5-HT-immunoreactive (IR) innervation pattern has been visualized, establishing the determining role in the innervation of a thick labeled axon bundle of extrinsic (metacerebral) origin. Entering the PC, the 5-HT-IR fibers showed a rich arborization innervating the entire olfactory lobe. This pattern from broad modulatory system might be involved both in processing the olfactory information and execution of proper behavioral responses given to odor stimuli. The individual axo-somatic innervation of the globuli cells refers to the local role of 5-HT.

By camera lucida tracing the until unknown 5-HT-IR innervation of the cell body layer of the *Limax* PC-ben could also be visualized, and in addition the functional presence of 5-HT has also been proved in this system

by demonstrating the 5-HT sensitivity of the spiking (B) cells, using patch-clamp mode, following the local application of 1 mM 5-HT.

The ultrastructural characteristics of the 5-HT-IR elements innervating the *Helix* olfactory have been revealed by correlative light- and electronmicroscopic immunohistochemical investigations. The 5-HT-IR varicosities were shown to form frequently close but unspecialized membrane contacts with perikarya and agranular axon profiles. They formed with them synaptic configurations as well.

4.3. Immunohistochemical identification and distribution of voltage gated K⁺-channels in the *Helix* CNS

4.3.1. Distribution of K_v2.1 immunoreactive elements

K_v2.1-IR neurons could be observed in all ganglia of the CNS. In the PC numerous K_v2.1-IR globuli cells were present. At higher magnification small immunopositive varicosities were seen, surrounding unlabeled globuli cells. In the labeled cells the immunoreactivity was frequently concentrated only in a smaller part of the cytoplasm. The majority of the K_v2.1-IR neurons projected to the medullary neuropil.

4.3.2. Distribution of K_v3.4 immunoreactive elements

K_v3.4-IR neurons have been found in the entire *Helix* CNS. The cytoplasm of the labelled cells contained dot-like precipitates that were frequently localized at the periphery, nearby the cell membrane. The axon processes of the labeled neurons projected to the neuropils, where they arborized. K_v3.4 immunoreactivity was displayed by a single giant neuron, the B2 cell in the buccal ganglion. In the PC, a few K_v3.4-IR globuli cells could be identified, located along the borderline between the cell body layer and the terminal medulla.

4.3.3. Distribution K_v4.3 immunoreactive elements

K_v4.3 immunoreactive neurons occurred in the entire CNS but the visceral ganglion, sending several labeled fibers to the neuropils. K_v4.3-IR elements have also been identified in non-neuronal tissue as well, concentrated in the connective tissue sheath, surrounding the ganglia, and in the aorta wall. In the buccal ganglion, the B2 motoneuron was labeled again, in addition to a significant group of neurons of larger size. In the PC, the

immunopositive globuli cells were partly distributed throughout the cell body layer, and partly lined up along the borderline between the cell body layer and the medullary neuropil. In the cell body layer, labeled axon processes were collected in septum-like bundles running among the cell bodies. The subesophageal ganglion complex contained numerous $K_v4.3$ -IR cell groups, mainly located in the pedal and pleural ganglia. At ultrastructural level, the labeled elements were the same type both in the PC and the pedal ganglion, containing numerous 80-100 nm granular vesicles. The character of the labeling was also similar, showing dot-like precipitates on the surface of the granular vesicles and/or distributed in the axoplasm.

4.4. Identification of the ion channels by Western blot

In the case of the $K_v2.1$, $K_v3.4$ channels the PC and the whole CNS, whereas in the case of the $K_v4.3$ channel the homogenates of the ganglionic connective tissue were also analyzed, regarding the presence of the ion channels. After blotting the specific bands appeared at the molecular weights expected on the basis of the literature data. In case of $K_v2.1$ antibody it was 110 kDa, for $K_v3.4$ antibody it was found at 240 kDa, and the $K_v4.3$ antibody showed labeling at 73 kDa.

4.5. Electrophysiological characterization of K^+ -channels in the central neurons of *Helix*

In the B2 neuron of the buccal ganglion displaying $K_v3.4$ immunoreactivity a fast, A-type K^+ -current was registered. The current could be inactivated by adding 5 mM TEA, that blocks the inward Na^+ - and the delayed rectifier Ca^{2+} -activated K^+ -currents. The A-type current could also be blocked effectively with 4-AP and BDS-II, whereas delayed rectifier currents were blocked by 20 mM TEA. However, low concentration (4 mM) 4-AP did not result in significant changes. From the neurons in the caudo-medial lobe of the pedal ganglion characterized by $K_v4.3$ immunoreactivity another type of transient A-type K^+ -current could be recorded, having a smaller amplitude and a slower kinetic. On the other hand, its voltage-current character resembled very much that of the fast transient A-current. This current type could also be blocked by 4-AP, whereas it was less sensitive against TEA. K^+ -currents were successfully recorded from the PC globuli cells both in voltage-clamp, and patch-clamp mode. Using voltage-

clamp technique, the K^+ -currents of globuli cells were analyzed that displayed $K_v2.1$ and $K_v4.3$ immunoreactivity, respectively. In a part of the slow, A-type K^+ -current was recorded, although a delayed rectifier current component could also be observed.

5. SUMMARY

The procerebrum (PC) of terrestrial pulmonates is the center of odor processing. We have little information about the ultrastructure organization of the cell body layer and the 5-HTerg innervation system of the PC at high resolution. Therefore, we have performed both routine electron microscopy and correlative light- and electron microscopic immunohistochemistry, in order to resolve the fine structural organization, and 5-HT immunoreactive innervation of the PC. Data have also been sporadic on the presence, distribution and cellular localization of ion channels, especially the K^+ -channels in the snail CNS. To have a better insight in the distribution of K^+ -channels, we have applied immunohistochemical and electrophysiological methods. The precise localization and functional characterization of K^+ -channels would be indispensable for our understanding of cell-to-cell signaling processes in the nervous system of gastropods, important models of comparative neurobiology.

Our results can be summarized as follows:

1. We have described the detailed ultrastructural organization in the globuli cell layer of the PC in two prominent terrestrial pulmonate gastropod species, *Helix pomatia* and *Limax maximus*. We identified local neuropil regions of different size which are characterized by axo-axonic and axo-somatic contacts, displaying different forms of synaptic configurations, (divergence and presynaptic modulation). Along unspecialized axo-somatic contacts exocytotic membrane configurations were also observed, indicating the process of extrasynaptic release. These observations widen the interpretation possibilities concerning the modulation of integrative olfactory processing networks in the PC.
2. Immunohistochemistry revealed a rich innervation established by a 5-HT-IR network in the *Helix* PC. The cell body layer received a massive innervation by varicose axon processes, forming a perisomatic basket-like

arrangement. The neuropil regions also contained many 5-HTergic processes. At ultrastructural level, 5-HT-IR fibers often formed axo-somatic and axo-axonic connections in the PC, establishing different synaptic configurations. Our results suggest that 5-HT is a pivotal signal molecule in the PC, having a general modulatory role.

3. Immunohistochemical experiments revealed neurons displaying labeling against three voltage-gated potassium channels ($K_v2.1$, $K_v3.4$ and $K_v4.3$) throughout the *Helix* CNS. The distribution pattern of the different channel expressing cells was similar. The antibodies used labeled distinct sets of neurons, which innervated the neuropil of the ganglia. The overall discrete number of labeled cell groups suggests a well-defined specific role for each K^+ -channels studied. At ultrastructural level we have identified $K_v4.3$ -IR varicosities in some part of the *Helix* CNS (PC, pedal ganglion). The labeled varicosities were characterized by a rather uniform fine structure, which also suggests a different role of K^+ -channels in modulating processes.

4. Patch-clamp and voltage-clamp experiments have proven the functional existence of the different K^+ -channels in the *Helix* CNS. We successfully identified a typical fast transient K^+ -current (A-current) in the $K_v3.4$ -IR B2 neuron of the buccal ganglion. Using the same voltage-protocol and the same saline, different transient (slower, A-type) K^+ -current was recorded from neurons located in the caudo-medial lobe of the pedal ganglion. In the PC, delayed rectifier K^+ -current in neurons expressing $K_v2.1$ channel, and transient A-type K^+ -current in neurons expressing $K_v4.3$ channel have been demonstrated. It is assumed that both ion channels are involved in odor information processing.

6. List of publications and presentations

6.1. Publications

Battonyai I, Krajcs N, Serfözö Z, Kiss T, Elekes K. (2014) Potassium channels in the central nervous system of the snail, *Helix pomatia*: localization and functional characterization *Neuroscience* 268:87-101 **IF: 3,389**

Battonyai I, Serfőző Z, Elekes K. (2012) Potassium channels in the *Helix* central nervous system: preliminary immunohistochemical studies. *Acta Biol. Hung. 63 (Suppl. 2)*, 146-150 **IF: 0,504**

Battonyai I, Elekes K. (2012) The 5-HT immunoreactive innervation of the *Helix* procerebrum. *Acta Biol. Hung. 63 (Suppl. 2)*, 96-103. **IF: 0,504**

Elekes K, **Battonyai I**, Kobayashi S, Ito E. (2013) Organization of the procerebrum in terrestrial pulmonates (*Helix, Limax*) reconsidered: cell mass layer synaptology and its serotonergic input system, *Brain Struct. Funct.* 218:477-490 **IF: 7.837**

Pirger Z, **Battonyai I**, Krajcs N, Elekes K, Kiss T (2013) Voltage-gated membrane currents in neurons involved in odor information processing in snail procerebrum. *Brain Struct. Funct.* 219:673-682 **IF: 7.837**

6.2. Oral presentations and posters

Battonyai I, Krajcs N, Kiss T, Elekes K.: Potassium channels in the central nervous system of the snail, *Helix pomatia*, XIV. MITT Konferencia, Budapest, január 17-19., 2013

Battonyai I, Elekes K.: Synapses and neurotransmitters in the olfactory center of the snail, 7th European Conference on Comparative Neurobiology, Budapest April 25-27, 2013

Battonyai I, Krajcs N, Kiss T, Elekes K: Distribution of voltage-gated potassium channels in the snail central nervous system, FFRM, Prague, September 11 - 14, 2013

Battonyai I, Krajcs N, Serfőző Z, Kiss T, Elekes K: Potassium channels in the central nervous system of the snail, *Helix pomatia*. MITT 14th Conference of the Hungarian Neuroscience Society, January 17-19, 2013, Budapest, Hungary. *Abstract: MITT Abstract Book, ISBN 978-963-88224-2-0, p. 84-85.*

Krajcs N, **Battonyai I**, Hernádi L, Elekes K, Kiss T: Neural control of string muscles responsible for patterned tentacle movements of the snail, *Helix pomatia*. International IBRO Workshop, 2012. január 19-21, Szeged. *Abstract: Ideggyógy. Szemle/Clin. Neurosci. 2012;65(S1):39*

Battonyai I, Serfőző Z, Krajcs N, Kiss T, Elekes K: Immunohistochemical visualization of potassium channels in the central nervous system of the snail. 8th FENS Forum of Neuroscience, July 14-18, 2012, Barcelona, Spain

Battonyai I, Elekes K: Is serotonin a pivotal modulator in the snail olfactory center? MITT 13th Conference of the Hungarian Neuroscience Society, January 20-22, 2011, Budapest, Hungary. Abstract: *Front. Neurosci. Conference Abstract: 13th Conference of the Hungarian Neuroscience Society (MITT)*. doi: 10.3389/conf.fnins.2011.84.00094

Battonyai I, Serfőző Z, Elekes K: Potassium channels in the *Helix* central nervous system: preliminary immunohistochemical studies. 12th Symposium on Invertebrate Neurobiology (ISIN), August 31 – September 04 2011, Tihany, Hungary

Battonyai I, Elekes K: The 5-HT immunoreactive innervation of the *Helix* procerebrum. 12th Symposium on Invertebrate Neurobiology (ISIN), August 31 – September 04, 2011, Tihany, Hungary

Battonyai I, Krajcs N, Kiss T, Elekes K: Serotonin is involved in both the central and peripheral regulation of snail olfaction. 3rd European Synapse Meeting, October 13-15, 2011, Balatonfüred, Hungary

Elekes K, Fekete ZN, **Battonyai I**, Mikite K, Kobayashi S, Ito E: Organization of the olfactory center of terrestrial snails (*Helix*, *Limax*) revisited: massive axo-somatic innervation of the globuli cells in the procerebrum. International IBRO Workshop 2010, Pécs, 2010. január 21-23. Abstract: *Front. Neurosci. Conference Abstract: IBRO International Workshop 2010*. doi: 10.3389/conf.fnins.2010.10.00034

Pirger Z, Kiss T, **Battonyai I**, Elekes K: Sodium-channel and membrane current characteristics of the procerebral neurons of *Helix pomatia*. International IBRO Workshop 2010, Pécs, 2010. január 21-23. Abstract: *Front. Neurosci. Conference Abstract: IBRO International Workshop 2010*. doi: 10.3389/conf.fnins.2010.10.00117

6.3. Other publications

Kiss T, **Battonyai I**, Pirger Z. (2014) Down regulation of sodium channels in the central nervous system of hibernating snails. *Physiol. Behav.* 131:93-98 **IF: 3.160**

Talapka P, Bódi N, **Battonyai I**, Fekete E, Bagyánszki M. (2011) Subcellular distribution of nitric oxide synthase isoforms in the rat duodenum. *World J Gastroenterol.* 17:1026-1029. **IF: 2.471**

Bódi N, **Battonyai I**, Talapka P, Fekete E, Bagyánszki M. (2009) Spatial pattern analysis of nitrergic neurons in the myenteric plexus of the duodenum of different mammalian species. *Acta Biol. Hung.* 60:347-358. **IF: 0.593**