SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Local connections of neurons in the superficial laminae of the dorsal horn of rats revealed through analysis of their axonal branching patterns

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The Examination takes place at room 2.305, Dept. Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, on 18 September, 2024, 11AM

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1. Introduction

Pain transmission in the central nervous system

In normal (and also in pathological) conditions noxious and non-noxious stimuli are processed by a complex network. Transmission in the pain-associated pathway is initiated by a painful stimulus, which can be thermal, chemical, mechanical, inflammation or associated with tissue damage. This signal is transmitted by free nerve endings or nociceptors located in the connective tissue, peripheral arteriola or in the skin. Nociceptors are pseudounipolar neurons that have a peripheral axon at the site of injury, a soma in the dorsal root ganglia (DRG) and a central axon (termed as primary afferent) in the dorsal horn of the spinal cord.

The main output elements of the pathway from the dorsal horn are projection neurons (PN). Axons of these neurons form several ascending pathways, including the spinothalamic, and the spinoreticulothalamic, that forward the pain information into the thalamus and brainstem. From the thalamus, the 3rd-order neurons transmit the information to the cortex, where the pain sensation happens.

Pain sensation can be modulated at several levels of the above pathway, including the DRGs, the dorsal horn of the spinal cord, the reticular system of the brainstem and some regions of the cortex. These areas allow alteration of the transmission process before the stimulus reaches the cortex.

Structure of the spinal cord and its superficial laminae

The painful stimuli from outside the body first will reach the central nervous system in the spinal cord, where sensory information is integrated. The spinal cord has a flattened cylindrical shape, and can be divided into cervical, thoracic, lumbar, sacral and coccygeal segments.

Macroscopically the cross section of the spinal cord shows 2 distinct regions. The greyish H-shaped inner part, that surrounds the central canal is the grey matter, which contains cell bodies and processes of neurons and glial cells. Furthermore, we can distinguish ventral and dorsal horns inside the grey matter. The ventral horn contains mostly motoneurons, and the

dorsal horn plays a pivotal role in processing and forwarding sensory information. The outer part of the spinal cord is the white matter, where ascending and descending pathways are formed from myelinated and unmyelinated axons. The proportion of grey and white matter changes along the length of the spinal cord; it is widest at the cervical (C5-6) and lumbal (L3-4) intumescences due to the upper and lower limb plexuses being connected to these areas.

Historically, the grey matter of the spinal dorsal horn has been divided into 6 parallel layers first in cats, and later this division system was adapted to other species including rats, humans, and monkeys. This region plays a crucial role in pain transmission with a complex neuronal network that contains projection and interneurons (IN). The nociceptive information reaches these neurons in the dorsal horn area via primary afferents.

Primary afferents

Stimuli from the periphery can trigger sensations with different delay times, corresponding to different conduction velocities of the primary afferent fibres. These fibres can be myelinated (Aβ: 16-100m/s, Aδ: 5-30m/s), allowing faster transmission or non-myelinated (C: 0,2-2 m/s) that result in a slower conduction speed. Based on their electrophysiological properties we can distinguish low-threshold primary afferents, which transmit non-noxious, low-intensity stimuli that do not cause painful sensations, and high-threshold primary afferents, whose function is to transmit high-intensity, potentially tissue-damaging stimuli.

A-fibres are mainly present as low-threshold mechanoreceptors (A-LTMR), however, in addition, Aδ-fibers are assumed to be responsible for the perception of pricking pain and some Aβ is also able to respond to painful stimuli.

Based on their sensory modality, C fibres can be divided into low-threshold fibres that process tactile stimuli (C-LTMR) and high-threshold fibres that respond to mechanical and painful thermal stimuli. These C fibres involved in nociception can be further divided into peptidergic and non-peptidergic groups. Peptidergic C fibres (and some Aδ fibres involved in nociception) express neuropeptides and contain calcitonin gene-related

peptide, substance P and galanin. These afferents express transient receptor potential vanilloid 1 (TRPV1), and their role is in the perception of heat-induced pain. Another non-peptidergic group of C nociceptors is involved in the normal perception of mechanical pain. They typically express Mas-related G-protein coupled receptor member D, but do not express TRPV1 and do not have any neuropeptides. 2 other types of C-type fibres have been described: co-expressing somatostatin and B-type natriuretic polypeptide, and expressing MrgA3/MrgC11 (2 members of the Mas protein-linked receptor family). Both types are involved in pruriception.

The different modalities will be processed by different primary afferents which are located strictly along the dorsal horn, therefore the primary afferent inputs show a termination pattern. The thinly myelinated Aδ and non-myelinated C fibres terminate in the superficial laminae (I-II) as nociceptors. The lamina III, the deeper part of the dorsal horn, takes part in several functions. The Aδ afferents innervate the hairs, and the Aβ afferents functioning as low-threshold mechanoreceptors terminate here.

There are more and more shreds of evidence to prove the fact that could be some dorsal horn neurons, which are capable to connect low-threshold mechanoreceptors to pain-processing neurons either directly or in an indirect manner via different types of excitatory or inhibitory interneurons.

Morphological and electrophysiological characterisation of neurons in superficial laminae

Lamina I

Lamina I (LI) is situated as a thin rim along the edge of the dorsal horn of the spinal cord, bending slightly on the lateral side. Due to the entering myelinated fibers the medial end of lamina I is better defined, but there is no sharp separation from the white matter.

Lamina I contains projection and interneurons. Although projection cells are mainly located in LI, they can also appear scattered in LIII-VI. This cell type has a main axon which will cross the midline of the spinal cord and travel rostrally in the contralateral white matter to ascend to higher brain regions. The majority of neurons also in lamina I is also interneurons. These

cells do not have a main axon, their axonal arborization remains locally, mostly in the superficial laminae (LI-II). Based on their phenotypes, they can be excitatory or inhibitory.

Lamina I neurons are distributed to four (or sometimes three) classes based on their somatodendritic features: fusiform, multipolar, pyramidal-shape and flattened. This classification ignores axonal features, mainly because most labelling techniques (both the Golgi methods earlier and more recent retrograde labelling techniques) fail to label the axon in detail.

Based on the action potential (AP) series in response to current injection in electrophysiological recordings four different firing patterns of lamina I neurons can be distinguished. Tonic firing neurons fire slowly, but in a maintained manner; phasic (or adapting) neurons fire with high frequency over a variable time with decaying amplitude; delayed pattern neurons show a longer pause preceding the first AP, while single spike neurons fire only a single AP regardless of the strength of the depolarization.

According to some studies, fusiform cells show a typical tonic firing pattern, pyramidal neurons more frequently show phasic pattern while multipolar cells tend to be delayed or fire a single spike only. Firing pattern was also thought to be associated with certain functions in the dorsal horn. Cells with tonic and delayed firing patterns mostly integrate information, while the phasic and single spike firing patterns are related to supporting roles.

Lamina II

Lamina II (substantia gelatinosa) is located ventrally to LI. Using background stains (e.g. toluidine blue) it can be further divided into an outer part, which shows intensive staining with densely located cells and an inner part, where the cells are slightly looser. This lamina contains small-size neurons and is free from myelinated fibres. Large caliber primary afferents, entering in the medial dorsal root entry zone traverse the medial part of LII.

Based on intracellular labelling during recordings of LII cells in spinal cord slices of different species (including rats), the following main morphological groups were defined: islet, central, radial, medial-radial and

vertical. The dendritic trees of LII neurons extend mainly in the rostrocaudal direction and they are not too extensive mediolaterally and dorsoventrally. Their firing patterns are similar to those described for lamina I neurons previously (tonic, phasis, delayed and single spike) with a few existing only here: gap (irregular)- when between 2 spikes the delay is 1.5 times longer than the 2 two preceding or following two spikes; and reluctant- when the depolarisation cannot evoke any spikes. There is some evidence, that most of the excitatory LII neurons (but not all) have delayed, gap or reluctant firing patterns.

Laminae III-IV

Lamina III also contains densely located small cells, which makes it harder to define the boundary between lamina II and III. The presence of myelinated fibres here, however helps to recognise the lamina. The lateral edge of LIII follows the curvature of lamina I-II and it can blend medially as well. In the midline, the dorsoventral extent of this lamina is larger than at its sides. LIV contains medium to large morphologically heterogeneous cells. Its medial border follows the shape of the dorsal horn, but the lateral curvature previously described in laminae I-III is not characteristic of this lamina.

Neurons from this area are less characterised. One of the most examined cell types is the large antenna cell, which has a pyramidal-shaped soma located in LIII-IV. This cell type can express Neurokinin-1 receptors (NK1R) and project to the nociceptive areas of the brainstem and thalamus. However, there are also antenna cells that do not project supraspinally and function as intrinsic or propriospinal neurons.

The antenna-type neurons typically have a dorsal dendrite that crosses LII and then gives branches intensely in lamina I, while their ventral dendrites extend towards the deeper laminae. This morphological feature allows the integration of multiple primary afferent inputs. Immunohistochemical reactions have shown that their dorsal dendrites contact numerous peptidergic and non-peptidergic thin afferents, but less frequently with thick myelinated primary afferents. It is known that these cells are targets of local interneuron axons, which can suggest that the spinal cord network regulates their excitability. Little information is currently

available on the electrophysiological properties of these cells; our current knowledge is that these neurons respond to a wide dynamic range or weak mechanical stimulation, and possibly also to direct electrical stimulation.

Laminae V-VI

The lamina V is located at the neck of the dorsal horn. Its cells are heterogeneous and larger than the LIV cells. Their dendrites can dorsally reach LII-III and ventrally LVII. Lamina VI is the most ventral part of the dorsal horn but is missing in some segments (T6-L2, S2-4). The cells here are smaller and better aligned than those in lamina V. The LVI interneurons are involved in the development of reflex pathways.

A brief overview of the neurochemical characterization of dorsal horn neurons

Based on their main neurotransmitters, dorsal horn neurons can be divided into 2 groups: excitatory cells that express glutamatergic and vesicular glutamate transporter 2 (VGLUT2), and inhibitory neurons that release γ-aminobutyric acid (GABA) and/or glycine. Based on Larsson's estimate, 36-53% of the neurons in the lamina I-III of the spinal cord in rats are inhibitory cells.

In the case of lamina I-II excitatory cells, Gutierrez-Mecinas and her colleagues were able to determine 6 distinct neuron populations based on their neurochemical markers in the mouse spinal cord. Based on their results, the excitatory cells in lamina I-II contain neurotensin (9%), substance P (also known as Tachykinin 1; 24%), neurokinin B (14%), gastrin-releasing peptide (15%), cholecystokinin (7%) or neuropeptide FF (6%). An additional 25% of the excitatory cells cannot be classified into these groups.

The same working group further revealed 5 groups of inhibitory neurons. A minimal overlap was detected between galanin-dynorphin (24%) and neuronal nitric oxide synthase (nNOS, 17%) positive cells, and cells expressing galanin-dynorphin and neuropeptide Y (33%). Furthermore, inhibitory cells containing parvalbumin (11%) and calretinin (27%) were isolated in lamina I-II. It should be noted that dynorphin, nNOS, parvalbumin

or calretinin-positive cells can also be excitatory cells, the Pax2 transcription factor can be used to distinguish them as a reliable inhibitory cell marker.

Distribution of cells with NK1 receptor in the dorsal horn

All substance P-containing primary afferents are involved in nociception, and their main target is the NK1 receptor, which shows a restricted distribution in the dorsal horn. About 45% of lamina I cells express NK1R, it is largely absent in lamina II and re-appears in a smaller percentage in deeper laminae. 80% of projection cells of lamina I in rats and 90% of cells in mice show NK1R positivity. Although the receptor is also found in excitatory interneurons, its expression level is lower than in projection neurons. Antenna cells of lamina III may also contain NK1R and these cells can also be identified as projection neurons.

Axonal branching patterns revealed in the superficial laminae of the lumbar spinal cord

Ramón y Cajal described several neuron types using Golgi methods. Those cells are categorised, mainly based on the somatodendritic features and their locations in the dorsal horn of the spinal cord and their unique axons were less acknowledged. Using transgenic mice, electrophysiological recordings, and different anatomical and imaging techniques could reveal several excitatory and inhibitory neuron populations in the dorsal horn. Furthermore, combining these techniques could help identify anatomical features and postsynaptic targets, but the axonal distribution of the individual cells is still out of focus in most classification schemes.

Earlier studies tried to reveal axonal patterns using horseradish peroxidase (HRP) labelling methods in monkeys and cats. Later on, Yasaka and his coworkers tried to correlate axonal morphology and electrophysiological properties of LII excitatory and inhibitory cells of rats. Another study in LIII described axonal trees of cholinergic neurons and recently the axonal patterns of projection neurons were analysed. These experiments are pivotal to a better understanding better of the network behind the pain processing. However, these data came from spinal cord slice preparations, where unavoidably most parts of the axon of individual neurons is lost.

Using the whole spinal cord preparation allows preserving the local axonal arborization intact. Illuminate the surface of the spinal cord block with LED (Light-emitting diodes) allows visualization of live cells that are close to the surface in the unstained brain or spinal cord preparation in the case of young rats or mice. Szücs and coworkers worked out this approach and by using it they performed full cell reconstructions from individual labelled cells from lamina I and gave detailed anatomical descriptions of projection and interneurons of young rats.

Furthermore, the group suggested a novel classification system for grouping ALT (ascending anterolateral tract)-projection neurons based on the distribution of their collaterals on the ipsilateral side. The proposed categories were: dorsal, lateral, ventral or mixed collateral types. Furthermore, it is known that the axon can originate from the soma or a primary dendrite. Szücs and his collegues quantified that in a majority of the cases (64%) the axon arises from a dendrite.

They also started to explore the axon diversity of local circuit neurons and estimated the rostrocaudal extent of the axons of these neurons, revealing that they can cover 2-3 spinal segments (total length of the axon in the range of 2312 to 6113 μm). Along with other morphological evidence this finding suggested that lamina I neurons can also play an important part in the processing of propriospinal sensory information.

2. Aims

Investigation of neurons in the superficial laminae is widely performed thanks to the recently developed techniques that are helpful tools to examine cells at a population level, mostly in transgenic mice. However, the fine morphological details of individual neurons, e.g. axonal branching patterns, are usually overlooked or poorly described. The lack of such information poses a serious obstacle in understanding spinal dorsal horn network operations.

Our objective was, therefore, to carry out a detailed, quantitative study of the axons of individually labelled dorsal horn neurons from serial sections in the transversal plane. Although many similar properties have been described about rat and mouse spinal cord networks, quite a few differences have also been revealed. To be able to compare our results with the previous, detailed publications about the axonal distribution of our workgroup more reliably, we continued to use neurons from rats in our experiments, which were provided to us by our Portuguese collaboration partners.

In the first step, we performed a structure-function analysis of lamina III antenna cells, and then we carried out a series of analyses targeting lamina I cells (projection and interneurons), where our goal was a more extended comparative analysis of the morphometric parameters of the axonal and dendritic network, both in terms of their spinal cord interlaminar coverage and their symmetry. During my PhD work, I performed a morphological analysis of the labelled cells, and thus, the electrophysiological experiments that the labelled neurons originated from will only be described marginally throughout the thesis. The analyses performed on lamina I neurons were subsequently extended and adapted to the cells of lamina III. These later results were not included in the original publication of lamina III neurons, thus they are only present in this thesis.

3. Material and methods

Spinal cord preparations of young (postnatal (P)6-15 and P9-20) Wistar rats were used in this study. All animals were sacrificed under the ethical approval of the given institute. During the electrophysiological experiments in the intact spinal cord preparations (*Instituto de Biologia Molecular e Celular, Porto, Portugal*), the recorded cells were labelled with biocytin, which allowed their post-hoc detailed morphological analysis (*Debreceni Egyetem, Debrecen, Hungary*).

Short overview of the in vitro electrophysiological experiments in spinal cord blocks

The following electrophysiological expereiments that provided the labelled neurons were not performed by me, therefore they are described briefly . It is important to note that according to the best of our knowledge the recording and filling of the neurons did not have any effect on the morphology of the cells and our observations. Parts of the electrophysiological data that were used to draw conclusions about morpho-functional features of the investigated cell types will be described shortly, later in the discussion.

For the spinal cord block preparation, the Wistar rats (P9-20) were anaesthetized with Na+-pentobarbital intraperitoneally, and after confirming the lack of the pedal withdrawal reflex the animal was decapitated. The spinal cord was quickly removed and transferred into the oxygenised artificial cerebrospinal fluid (ACSF). For recording LI cells the lumbar segment was divided into 3 different separate parts (L1-L2, L3-L4 and L5-L6), meanwhile for LIII cells, the L4-5 was kept. To specify the type of primary afferent (only for LIII neurons) inputs in some of the cases, the L4 and/or L5 roots were kept for electric stimulation.

The electrophysiological measurements of the block preparation were performed in the superficial layers using the IR-LED technique. The intracellular solution contained 0,5-1% biocytin (Sigma, St. Louis, MO, USA) to diffuse into the cell during the whole-cell patch-clamp recordings. In a few cases, biocytin was suplemented with 0.5% rhodamine-red to allow verification of the neurotransmitter content of the recorded cell (University

of Glasgow, Glasgow, Scotland). Although this modification slightly affected the quality of the labelling, we could still perform detailed 3D reconstructions. Results concerning the immunocytochemical character of these few neurons, however, won't be detailed in this thesis due to their small numbers. Also, from the numerous functional parameters recorded during the electrophysiological experiments only firing patterns (AP series as a response of the neuron to a current step) were tried to be correlated with our morphological findings.

We have checked the location of the cell bodies in every single case after the histological processing.

Histological processing

After the electrophysiological recordings, the spinal cord blocks were placed in 4% paraformaldehyde. Following the immersion fixation, the blocks were washed multiple times and then embedded in 2.5% agar. The goal of this study was to determine the mediolateral and laminar distribution of the cells. For that, we decided to use transverse serial sections with 100 µm thickness cut by a vibratome (Leica VT1000). Our choice was based on the assumption, that using different orientations (like we did in our previous studies) could cover certain anatomical details.

To reveal biocytin the sections were permeabilized with 50% ethanol and treated according to the avidin-biotinylated horseradish peroxidase method followed by diaminobenzidine (DAB) chromogen reaction. The sections were also counterstained with (0.1%) toluidine blue to help determine the borders of the grey matter and laminae and to give higher contrast between the background and the processes.

Cell reconstruction

To reconstruct the neurons in 3D the Neurolucida software was used (versions 9 and 10; *MBF Bioscience, Williston, USA)*. We selected 15 inter- and 10 projection neurons from lamina I, and also (based on their somatodendritic features) 9 antenna-type cells from lamina III.

The characteristic appearance of axons and dendrites helped us identify the different processes of the labelled neurons. The dendrites' thickness is gradually becoming thinner, and their surface can contain spines. Meanwhile, the axon thickness is almost the same all along and on its thinner axonal processes can have varicosities in different sizes.

In several cases (n=25 from 34) the processes reached the rostral and/or caudal end of the specimens, thus, in this thesis I did not aim to draw any conclusions from information about the rostrocaudal extent. The proportion and shape of the grey and white matter of the first and last sections were used to identify the rostral and caudal ends.

Quantification for the morphological analyses

The distribution of the axonal and dendritic processes was quantified by the Neurolucida Explorer (versions 9 and 10, MBF Bioscience, Williston, USA). To get the values from different regions closed contours must be drawn around the regions of interest. It has to be noted, that in the case of projection neurons, the main axon was left out from the analyses, because it is myelinated and does not form any local connections.

Analysis of the interlaminar distribution

There is no available standardized atlas for the lumbar spinal cord of young rats to differentiate the laminae so we used toluidine blue background staining to distinguish different regions based on cell size and density. Seven different regions were defined in the grey matter, that corresponded to lamina I, II, laminae III-IV, V-VI and VII-X. The white matter was separated into dorsal, lateral and ventral funiculus on both (ipsi- and contralateral) side, based on the entering and leaving dorsal and ventral roots.

We also distinguished the lateral and medial halves of the dorsal horn (LI-IV) in our analysis, with an imaginary line perpendicular to the surface of the specimen at the midpoint of the mediolateral extent of the dorsal grey matter. Since all of our cell bodies were located in the lateral side of the dorsal horn, this analysis aimed to examine the mediolateral preference of the neuronal processes.

Analysis of the cell symmetry

For every neuron analysed, when measuring medio-lateral asymmetry, a line has been defined that crossed the middle of the cell body and was perpendicular to the surface contour of the dorsal horn. This line was used as a symmetry axis through all the consecutive serial sections. Defining the rostrocaudal symmetry axis, we used the z-filter in Neurolucida 9 (depth filter in Neurolucida 10) to find the middle level of the cell body (where the contour of the cell body was the sharpest) in the section containing the soma. With the help of the axis, we could separate the processes and then quantify the length in the different regions (medial or lateral, rostral or caudal).

Finally, we combined the previously described analyses, so that the space around the cell body was divided into four quadrants: rostromedial (RM), rostrolateral (RL), caudomedial (CM) and caudolateral (CL). Neuronal processes were quantified in each of these regions.

Statistics

In every case where it was possible, the data were normalized for the length of the whole process and given in mean ± SEM. The statistical analyses were performed in Origin 9 software (Microcal Software, Northampton, MA, USA). For the different analyses, the following tests were used: one-way ANOVA (neuron symmetry comparisons) and Mann-Whitney test (interlaminar distribution).

4. Results

In this study, our main goal was to investigate the distribution pattern of axonal and dendritic branches in the spinal cord. To achieve this, we performed full cell reconstructions of 10 PNs, 15 INs from LI, and also 9 ANTs from LIII, which were labelled in lumbar segments of young rat spinal cords.

During the histochemical processing, the sections likely suffer various distortions and can even be damaged (especially on the surfaces). In case of transverse sections used in this study these consecutive mismatches in the facing surfaces makes it very difficult to connect neuronal processes, that was performed in earlier studies from this workgroup in case of sagittal sections. For this reason we changed the main concept of our analysis and focused mostly on location rather than the tree structure. Furthermore, it is important to notice that the rostrocaudal extent is usually underestimated because the axonal processes left one or both ends of the blocks in 73.5% of cases. Considering these problems, we designed new approaches to discover details previously unknown for the neurons in the superficial laminae of the spinal cord.

Lamina I neurons

Projection neurons

The main feature of PNs is that they have a main axon, which branches off either directly from the cell body or from a major dendrite and than crosses to the contralateral side in the anterior commissure. This feature was used to distinguish them from interneurons. Nine out of the 10 reconstructed neurons had the main axon crossing to the contralateral side. In one case, the main axon got faint at the level LV but since the initial part of the axon was identical to the rest of the examined PNs, we also identified this neuron as an ALT-PNs.

Since the myelinated main axon does not form synaptic contacts with cells in the spinal cord, the single PN that lacked collaterals was excluded from the analysis of axonal parameters and was only considered for the

description of dendritic distribution.

The reconstructions revealed novel types of collaterals, which have not been described previously. Of 10 cases in 3 PNs we saw collaterals reaching the contralateral side after crossing in the posterior or anterior commissure. In 2 further cases the axon collateral faded before the crossing but judging from their course these collaterals most likely also reached the contralateral side. In previous studies using sagittal sections this collateral type could easily be missed.

Interlaminar distribution

Two characteristic dendritic tree conformations were identified for the resconstructed cells. In one case, a significant part of the dendrite tree was distributed in the white matter along the posterior horn, while in the other case it extended medially and laterally but within the grey matter. Analysing selected examples from each of these 2 groups, we found that their preferred areas of dendrites are similar, even if the distribution ratio differs slightly. The distribution of their axon does not reflect this similar area distribution, but LV and LF are general targets. When comparing all PNs examined in this study, we observed that the appearance of axon collaterals in superficial laminae is less typical, they appear more in the V-VI lamina and LF, and may also extend to the contralateral side. In contrast, dendrites were present only ipsilaterally, the dendrite tree was significantly lateralized, but dendrite pieces were also present medially (in 70% of the cases studied).

Cell symmetry

The slight polarization of PN dendrite distribution raised the question whether there is a correlation between the distribution patterns of dendrites, which are the input to the cells, and the axon, which define local targets as the output. This correlation was analysed within cell groups for each cell and then compared between cell groups. For this analysis, the space around the cells was divided into medial and lateral, and rostral and caudal regions centered on the soma.

In terms of their mediolateral symmetry, the axons and dendrites of the PNs studied generally showed a same-sided dominance and were rarely close to the axis of symmetry, i.e. relatively polarized in one direction. However, examination of the rostrocaudal symmetry revealed axon dominance on the side opposite to the side of dendrite dominance and a higher degree of polarization in the PN population studied. Rostrocaudally, axon collaterals appeared in some cases (n= 3) only on one side, which could be explained by the fact that the collateral originating from the main axon branched off further from the soma and neither that collateral nor any further possible branches returned to or reached the soma region.

In the final analysis, the space around the neuron was divided into four quadrants in the horizontal plane, with the soma forming the centre, and the percentage of axon and dendrite in each quadrant was determined. In the present analysis, however, we could only detect trends, and no clear correlation between dendrite and axon distribution could be detected. One such trend was that the axons arising from dendrites originated almost exclusively from medially initiated dendrites. The axon mostly showed a caudo-lateral dominance, while in dendrites showed a mixture of more and less polarized cases. For axon collaterals a markedly laterally polarized axon collateral distribution was observed, independent of whether they originated from the soma or dendrites.

In conclusion, the axon collateral distribution of the PNs studied reflects the collateral types described previously and no clear correlation was demonstrated with the spatial distribution of the dendrites located mainly in the superficial laminae and DLF.

Interneurons

The vast majority of neurons located in the superficial laminae of the spinal cord are interneurons (90-95% of lamina I cells), whose axons cannot be clearly separated into a principal axon and local collaterals. The lamina I interneuron axons form a cloud-like dense network with multiple levels of branching, often returning to the same areas from different primary branches with a large number of axon varicosities. From the labelled neurons available to us, we were able to use 15 interneurons in lamina I.

Interlaminar distribution

The cells examined were characterised by a dense axonal cloud, mainly confined to the superficial laminae. Although it is only possible to label cells on the lateral surface of the GV when draining the entire spinal preparation, nevertheless several IN axons showed axons also appearing medially, in one case we could even trace a small section of axon in the DF.

When we compared the axon and dendrite distribution by region for 2 interneurons, one with a significant medial axon extension and the other with axons exclusively in the lateral half of the field, we observed that their spans covered almost the same area: LI, LII, LIII-IV and the lateral funiculus (with minimal axon appearing in laminae V-VI). Of these, we found the largest number of axons and dendrites in lamina II, as was also evident for all other interneurons. In the case of axon, we also found stretches in lamina I, III-IV and LF. When we examined the same in the mediolateral extent of the superficial laminae, we found that dendrites were exclusively located laterally, while 13 IN (out of 15) had all axons medially (ranging from 0.5% to 43.4%).

Cell symmetry

The dendrite symmetry of INs in both the mediolateral and rostrocaudal directions was very similar to that observed in PNs. Two thirds of INs had a predominantly lateral dendritic tree (n=10). Axon rostrocaudally and mediolaterally were diverse in terms of symmetry, however, a higher proportion of the values were near the axis of symmetry. Although not a clear correlation, but a visible tendency was observed for a given dendrite dominance side to be associated with an axon showing dominance on the opposite side.

Analysis in the 4 quadrants around the INs, defined in the horizontal plane, also confirmed a more symmetric, less polarized appearance of the INs, suggesting that INs cover the space around the soma more uniformly, both in terms of their dendritic and axonal trees. Opposite-sided dendrite/axondominancy is also apparent in this analysis. In INs, axons originating from dendrites did not prefer medial dendrites as in PNs, but a

higher number of these axons originated from dendrites distributed in the caudal direction.

In summary, INs were generally less polarized and more symmetric than PNs. However, the lower degree of asymmetry was often associated with an opposite-sided distribution of axons and dendrites.

Lamina III neurons

Antenna cells

Quantitative morphometric analysis of lamina I cells was performed mainly with the aim of inferring the functional properties of neuronal groups by detailed structural exploration. However, the new methods used, some of which we have developed, were developed in a previous work when we performed an anatomical confirmation of a functional finding on lamina III cells. Electrophysiological measurements suggested that there are Aβ and Aδ low-threshold primary afferent cells of lamina III receiving inputs from Aβ and Aδ that reach the lamina I-II with their axons, where they modulate local excitatory transmission by establishing monosynaptic connections. To verify this pathway, a systematic study of the primary afferent input of lamina III cells, including antenna (ANT) cells (described in the discussion), and, as my own work, a quantitative analysis of their axonal and dendritic trees were performed.

A peculiar morphological feature of these cells is that they have a large dorsoventrally extending dendritic tree, typically with a dorsal dendrite extending upwards towards the surface of the dorsal horn, giving the cell a somatodendritic appearance similar to that of cortical pyramidal cells. This morphological feature was the selection criterion for the analysis of this cell group.

Interlaminar distribution

Examining the distribution of ANT cell processes showed that the dendrites, although varying in quantity, preferred the same laminae. However, axons showed two distinct patterns. While all ANT cells have axons in laminae III-IV and V-VI, they do not always have axons in

superficial lamina I. As in the PNs and INs, axons were also found in the DLF area, but at a significantly lower proportion and frequency than in case of lamina I neurons.

It should be emphasized that, for both dendrites and axons, the lateral half of the posterior horn is where the processes are concentrated, with only 3 of the 9 ANTs showing a minimal proportion of axon segments extending in the medial direction (0.4-9.5%).

Cell symmetry

The symmetry analysis relative to the soma as a centre showed the most consistent asymmetry in ANT cells, especially with respect to axons. They showed a predominantly lateral and rostral distribution, while dendrites, with the exception of one outlier, showed a more symmetrical arrangement along both axes.

The distribution in the horizontal plane centred on the soma, although relatively homogeneous, also reflected rostral and lateral dominance. It was also an interesting observation that the dendrite-derived axons of ANT cells all originated from dendrites located in the medial direction.

In conclusion, in addition to the expected uniform dendritic parameters based on the dendrite morphological criterion used for the selection of ANT cells, the axons showed 2 distinct axon distribution patterns: in some cases the axons reached lamina I (n=4), while in the other cases (n=5) the axon was mainly located in lamina III-IV and in the DLF.

Comparison of the described cell types

Although the neurons in lamina I and III presented in the previous chapters showed marked morphological diversity within their cell groups, there were a few characteristic features that made these cell groups different. For this reason, we considered it important to compare the three populations of neurons in terms of the previously investigated parameters.

The comparison of the laminar distributions showed that PNs tend to give axon collaterals in deeper laminae (V-VI), whereas INs and ANT cells

axons remain mainly superficial (I-IV). The dorsal part of the DLF was a typical target for all three cell groups. Total axon length was highest for INs, nearly three times that of the sparse axon network of PN and ANT cells. The distribution of axon varicosities was almost identical in all laminae, with the exception of ANT cells, where there were some outliers, mainly in laminae where the axon was found in a higher percentage. A higher number of contralaterally located axon collaterals was seen in PNs (n= 3, while in 2 other cases the axon moved in that direction). In the IN group only a single crossing axon was found, while in ANT cells, the axon of one cell approached the midline but did not cross it.

Based ont the normalized distribution dendrites of the three neuron groups were concentrated in the laminae expected, based on the location of the somata. For PNs and INs, this was mainly lamina I, II and the DLF, while ANT cells tended to occupy lamina II-III. The total dendrite length was similar for INs and ANT cells (mean IN= 3192.5 ± 372.4 and mean ANT= 2991.6 ± 371.3 μm), while the dendrite length of PNs was found to be slightly longer (mean PN= 3967.1 ± 518.2 μm). However, in the rostrocaudal direction ANT cells appeared to cover a larger area.

When symmetry was examined in the mediolateral and rostrocaudal directions centred on the soma, both IN and ANT cells were found to be more symmetrical (closer to the centre of symmetry - similar amount of dendrite/axon in both directions) than cells in the PN group.

In the mediolateral direction, PNs and ANTs more often showed dominance of their dendrites and axons in the same direction (so-called homologous distribution: e.g. lateral axon dominance associated with lateral dendrite dominance). In the mediolateral direction, INs showed a heterologous distribution, i.e. the axon dominating on the opposite side of the dendrite dominance. For PNs, we found many cases where axon collaterals were exclusively located on one side of the field. For laterally located ANT and PN cells, the position of the soma was associated with a dominant lateral location of the processes, whereas for INs this tendency was not observed, with a similar proportion of processes dominating both the medial and lateral halves of the space around the neuron.

In the rostrocaudal direction, a rostral preference was evident for ANT

cells. PNs with a more polarized (marked rostral or caudal dominance) rostrocaudally heterologous distribution showed a similar degree of rostral and caudal preference. INs in this direction also showed more homogeneous, varied dominance values.

Cell symmetry values showed no correlation with either their firing pattern or their somatodendritic morphological classification.

5. Discussion

In this work, 34 fully reconstructed cells were examined from the lumbar spinal cord of young rats. We aimed to reveal anatomical differences between neurons from lamina I (inter- and projection neurons) and lamina III (antenna cells). Our goal was to reveal the subtle anatomical differences that are not clear from the somatodendritic appearance of the cell but at the same time can be decisive from the point of view of the cell's network role. We considered the position of the laminar distribution of dendrites and axons relative to the cell body as such a parameter, which largely determine the inputs and targets of neurons.

For our analysis, we used neurons that were labelled with biocytin during electrophysiological measurements, which were made available to us by our Portuguese collaboration partner. The morphological analysis of the cells used here was not previously carried out. In the present work, the projection (n= 10) and interneurons (n= 15) of lamina I and the antenna cells of lamina III (n= 9) were examined. The different number of analyzed cases is partly due to the different number of cases in the original works, and partly to the fact that not all the developed cells met the conditions necessary for our 3D reconstruction and analyses. It should be noted that I did not participate in the electrophysiological measurements resulting in the cells used for the morphological examination (nor during the series of experiments performed by my supervisor), however, the description of these results is essential for the interpretation of our morphological results, so they are briefly presented in this chapter.

Our analyses revealed the following: (1) the axon collateral distribution of PNs suggest a dorsoventral information flow, while (2) the axon of INs runs more in the mediolateral direction. (3) The antenna cells are more compact compared to the 2 other cell types. ANT neurons were selected based on the somatodendritic morphology and the examination of their axon revealed that these cells are (4) capable to reach the superficial laminae with their axonal arborizations. Finally, (5) all of the cell types showed axons in the lateral funiculus (all of the lamina I cells had some), which suggests that cells could be capable of forming propriospinal connections.

Technical consideration

Although the electrophysiological recordings were carried out in spinal cord block preparations, in 73.5% of the cases, the axon left one or both ends of the blocks. Because of this, we tried to avoid interpretation of numerical values concerning rostrocaudal extent. Furthermore, although our criteria for selecting neurons was very strict on the quality of labelling, not all the labelling was to the same extent, thus the ability of following different processes could be affected. We also cannot exclude, that our observations would change in older animals, since this technique is allowing us to use only younger (P9-20) animals, where the myelinisation is not too progressed. Moreover, the superficial laminae in the grey matter are more readily approachable on the lateral side, so our recordings were performed there.

Projection neurons

Presence of axon collaterals on the contralateral side

In my supervisor's earlier works, sagittal sections were used, which did not give the opportunitiy to recognise the PN collaterals crossing to the contralateral side. In this study, we could describe collaterals which crossed the midline at the dorsal commisure, furthermore, in one case a main axon gave a branch on the contralateral side. In 1/3 of PNs we could follow crossing axons to the other side, and in one case of the INs. By the ANTs, there was one case where the axonal process got close to the midline but did not cross.

Previously, after BDA (Biotinylated dextran amines) injections, a similar crossing was hypothesised. Varicosities of this type of collaterals from LIII-IV cells could make synapsis with LX cells, which can integrate inputs from different thin primary afferent fibres.

In this study, axonal processes were found in LV (ipsilaterally) in several cases, which can suggest the PNs can modulate other supraspinal neurons and link to other ascending pathways.

Ventral collaterals of PNs might atcivate dorsal horn neurons in deeper laminae (*Complementary electrophysiological experiments)*

Our morphological results from PN axon collaterals suggesting dorsoventral information flow are well supported by the previous electrophysiological findings of my supervisor. In this series of experiments, whole-cell patch-clamp measurements were performed on 6-15 day old Wistar rat spinal cord slices on neurons of laminae V-VII, while superficial dorsal horn neurons were pharmacologically activated with SP.

Neurokinin-1 receptor is expressed by nearly 45% of lamina I neurons, with a more significant proportion being putative excitatory cells. About 80% of PNs express NK1R, but INs may also show weak NK1R positivity, and one third of them also respond to SP. However, this effect was found to be smaller than that observed in PNs. The time course of depolarization following SP use may suggest that there is a functionally significant difference between PN and IN NK1R expression. SP activation via NK1R induced a large increase in the number of EPSPs in cells recorded in laminae VII-X. This effect was blocked by both a selective NK1R antagonist (SR140333) and TTX. The latter excluded the possibility of presynaptic NK1R activation.

Our observation that only the higher concentration of NK1R antagonist was able to block SP-induced stimulation may indicate that the contribution of lamina I interneurons (with weaker NK1R expression) is likely to be smaller than that of PNs with higher NK1R expression. Nonetheless, we hypothesize that both PNs and INs may contribute to the coupling of the anterolateral ascending system with other ascending pathways. This is supported by the observation that mechanical separation of the posterior horn prevents SP-induced EPSP number enhancement in deep posterior horn and ventral laminas. Our morphological results suggest, in light of the electrophysiological results presented above, that INs that concentrate their axons mainly in superficial laminae tend to influence the network of deeper laminae indirectly through other interposed interneurons. In contrast, PNs display a significant amount of collateral networks in deeper laminae, through which they can directly influence either interneurons of motor networks or dendrites of motoneurons, where they can even directly form monosynaptic connections.

Excitatory effect of the appearing axon collaterals in LV-VI of PNs might take part in flexor reflex

Information flow from the deeper laminae towards the superficial laminae was described in normal and pathological conditions as well, using transgenic animals, where the different neuron populations from the dorsal horn were analysed. Based on the classic pain theory and results from recent studies interneurons in LII-III might be the „gate" that can transmit information between primary afferents of different modalities. Similar function of lamina I neuron axons have not been proposed yet.

The hypotheses described in previous publications from our workgroup were first supported by Browne et al. in their review, where they cite the collaterals of PNs as a hitherto overlooked player involved in the processing of spinal pain information. The authors point out that other possible functions of collaterals may include "feedforward" stimulation, as well as inhibition indirectly via certain inhibitory interneurons. Direct evidence for feedforward stimulation between adjacent PNs is provided by a publication in which electrophysiological evidence for synaptically coupled lamina I PNs were found. Measurements in spinal cord slices described above provide further evidence for the involvement of lamina I cells in feedforward stimulation. In the deep dorsal horn and intermediate gray matter areas, last-order premotor interneurons also emerge as targets of collaterals, through which sensory activation also reaches motoneurons, potentially contributing to the development of the nociceptive flexor reflex. WDR neurons in deep laminae may also form the target of ventrally directed collaterals of lamina I PNs, thus providing nociceptive input to their integrated outputs to the supraspinal centre.

Finally, both the spinocerebellar tract neurons and autonomic sympathetic preganglionic neurons in the lateral horn of the thoracolumbar segment may receive synaptic inputs from PN collaterals.

Interneurons

Dendritic and axonal distribution pattern and functional asymmetry in the superficial laminae of the spinal dorsal horn

Neurons of LI are corresponding to late-born population of spinal cord neurons. While early-born neurons migrate mostly ventrolaterally and find their final locations in deeper laminae, the late-born neurons migrate dorsolaterally to settle down in a superficial lamina. Their dendrites and axons have a spatial limitation related to the closeness of the surface and the high density of the primary afferent in the entry zone of the dorsal roots. Based on this, both projection and interneurons have an apparent dorsoventral asymmetry.

Although the mediolateral and rostrocaudal growth of lamina I neurons is less restricted, there may be as yet unexplored anatomical obstacles in these directions. For example, dense bundles of thick primary afferents entering the dorsal root entry zone medially may form a physical barrier to the growth of lamina I neurons. This asymmetry in the anatomical and functional mediolateral direction has been reported previously for lamina I cells. Using laser scanning photostimulation, the stimulating input zones of some neurons showed marked medial asymmetries that were consistent with the structural asymmetries of the dendritic fields of neurons. A later finding of the same group is that the medial and lateral parts of the dorsal horn differ in their responses to local electrical stimulation, which may result from their internal structure, such as the orientation of local connections. Their hypothesis was that the more medial neurons of the superficial dorsal horn tend to establish stimulatory synaptic connections with neurons located laterally from them and that this direction may reverse as they move laterally. Our morphological result that laterally located INs show a heterologous distribution of dendrite and axon dominance provides excellent support for this previous hypothesis.

Some neurons in lamina I have axon segments containing varicosities that overlap with the area occupied by the dendrites of the neurons there. In the present work, we found that in the majority of INs, the axon occupied space around the dendrite tree. However, in a relatively distinct group of INs, the axon, which also extended medially, was associated with dendrites

that remained lateral. This axon configuration has been described previously by our group for reconstruction from sagittal sections, but reconstructions from transverse sections are a very useful contribution to a thorough understanding. The presence of similar mediolateral and lateromedial projections in deeper laminae has also been previously demonstrated, but the resulting cells of the projections have not been identified. In a recent study investigating the radiating pain syndrome and certain types of headache, the investigators concluded that specific dorsal horn neurons are able to connect the input zones of lateral and medial primary afferent inputs and serve as an anatomical basis for providing the radiating pain and lateromedial information flow. Evidence for this hypothesis may be provided by the medially spanning axon network INs described in the present work, which may provide lateromedially directed information flow. This hypothesis is supported by the fact that most of the IN showed a heterologous axon/dendrite distribution. Of course, it cannot be ruled out that these polarized neurons, which have a medial axon and lateral dendrites, would be extreme examples of lateromedial information flow. However, this raises the possibility that axons from medially located INs could similarly reach the lateral edge of the dorsal horn, or even the lateral white matter. However, the in vitro spinal cord block preparation used by our collaborator and our team is less suitable for testing medially located neurons, therefore a different experimental approach will be needed to verify this hypothesis.

For INs, several classifications based on neurochemical markers or genetic background are already available in the literature. It would be desirable to compare the morphological traits we have identified with these classifications, but the vast majority of these classifications are derived from experiments in mice, which may be an obstacle due to potential interspecies differences. Another obstacle to comparison is that for our morphometric studies, we needed the most complete, yet stable labelling possible to perform detailed reconstructions.

In our reconstructions, however, we did not find any correlation between the parameters we tested and the somatodendritic type and firing pattern, but we believe that these parameters (possibly when examining a larger number of cases) may help to classify lamina I cells into more precise functional groups.

Antenna cells

Morphologic characteristics

In our experiments with ANT neurons, about 8% of the candidate lamina III cells were found to be antenna cells, which were invariably of smaller size. Although it is widely accepted that antenna cells belong to the group of projection neurons, none of the neurons presented here had a principal axon crossing to the opposite side, despite the fact that the somatodendritic nature of the cells examined matched the description of antenna cells. ANT cells have a very extensive dorsoventrally oriented dendrite tree, suggesting a broad integrative function. Their dorsal dendrites receive numerous synapses from primary afferents as well as spinal interneurons, whereas the ventral dendrites prefer to connect with axons of the intrinsic spinal network and primary afferents terminating in deeper laminae. Their uniform appearance therefore makes it likely that they process information from the same sensory inputs.

The axons of neurons located in lamina III-V are known to either branch near their cell body or have a ventrally originating axon that branches and then runs rostrally and caudally. In the present work, the antenna cells studied were divided into 2 groups based on their axon distribution. In one case, the axon was mainly located near the cell body and in deeper laminae and in the dorsolateral funiculus, while in the other group the axon network reached the laminae I-II. Although the amount of axon that reached the superficial laminae was generally small, it still carries the potential to form synapses on the ventral dendrites of lamina I cells. Since the so-called multipolar somatodendritic morphology with ventral dendrites is primarily a feature of lamina I INs, PNs with this morphology less frequently are likely to be targets of dorsal axons from ANT cells.

Axon distribution pattern of ANT neurons does not correlate with their primary afferent input (*Complementary electrophysiological experiments)*

Dorsal root stimulation revealed that the axon distribution pattern of the cells does not show a clear correlation with their primary afferent input type.

Neurons with a certain percentage of their axon (2-13.4%) appearing in lamina I often receive Aδ and C inputs, but the same is true for ANT cells with axon concentrated around the cell body. In this measurement, primary afferent inputs were further subdivided into low and high thresholds by our collaborators, taking into account the conduction velocity and activation threshold.

The Aβ afferents terminate in laminas III-VI and function as low threshold mechanoreceptors. Therefore, it could be assumed that they target proximal or ventral dendrites of antenna cells. Nevertheless, the ANT cells studied rarely received monosynaptic Aβ input, which is also in agreement with immunocytochemical data showing a relatively low number of synaptic connections between thick myelinated fibers and dendrites of NK1 positive antenna cells. However, the observed Aδ fiber inputs were likely derived from two primary afferent populations, Aδ nociceptors and low-threshold Aδ mechanoreceptors, based on their activation thresholds.

Superficial members of Aδ nociceptors terminating in lamina I and V may form synapses on the dorsal dendrites of antenna cells. Consistent with this data is the fact that the threshold of ANT cell inputs identified as Aδ nociceptors coincided with those measured for lamina I PNs and INs. Functionally, high threshold Aδ afferents include a special class of nociceptors, the high threshold mechanoreceptors.

Low-threshold Aδ afferents, including hair follicle afferents, terminate in lamina III, so that these Aδ afferent fibers can also reach ANT dendrites in deeper laminae. High-threshold Aδ inputs were also observed, suggesting that ANT cells receive high-threshold Aδ afferents on their dorsal dendrites extending into lamina I, whereas low-threshold Aδ inputs are primarily received on their dendrites occurring in deeper laminas around the cell body.

It is known from the literature that dorsal dendrites of lamina III cells form synapses with thin non-myelinated C fibres in their passage through lamina II. Electrophysiological measurements have also demonstrated that ANT cells receive strong input from primary afferents with activation thresholds and conduction velocities characteristic of C fibers. These inputs effectively elicited firing that was similar to the response of deep dorsal horn neurons and some small lamina I neurons to nociceptive stimuli. The

prolonged firing observed here was apparently facilitated by tonic firing patterns, which are important for the conversion of long-lasting synaptic connections into a series of action potential discharges.

Primary afferent inputs to the dorsal and deeper dendrites of ANT cells in a potentially segregated manner are particularly interesting given the fact that not all ANT cell axons reach the superficial laminae. Further detailed research will be required to clarify whether superficial axons in any way select-or modulate-inputs to the dorsal dendrites of ANT cells, and in this way may interfere with the integration with inputs received on deep dendrites that will ultimately determine the output of ANT cells.

7. Summary

In total, we analysed the full axonal and dendritic distribution of 34 spinal dorsal horn neurons from complete 3D neuronal reconstructions. To the best of our knowledge this is the first work that performed such detailed analyses. Our findings, based on the distinct local axon collateral distributions, suggest divergent roles of lamina I and III neurons in the dorsal horn somatosensory circuitry.

Our results showed anatomical asymmetry of lamina I interneurons (IN), that is in line with functional asymmetries and the explanation of radiating pain based on a theoretical mediolateral information flow, suggested by the literature. We suggest that ventral axon collaterals of projection neurons (PN) can directly mediate a dorsoventral excitatory drive to spinal neurons of deep layers upon sensory activation. These anatomical connections are likely to link different ascending systems of the spinal cord.

The dendritic features of the antenna (ANT) cells make them capable of integrating different type of modalities from Aβ, Aδ and C afferent inputs and to function as wide-dynamic range neurons. ANT cells with axon collaterals reaching lamina II and I probably connect them to lamina I PNs and INs and may even modulate synaptic input arriving to their own dorsal dendrites. The putative pathway based on our anatomical findings would enable lamina III neurons to participate in the nociceptive processing units and to relay low-threshold mechanoreceptor input to lamina I neurons.

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List of publications related to the dissertation

1. **Kókai, É.**, Luz, L. L., Fernandes, E. C., Safronov, B. V., Poisbeau, P., Szűcs, P.: Quantitative spatial analysis reveals that the local axons of lamina I projection neurons and interneurons exhibit distributions that predict distinct roles in spinal sensory processing.
J. Comp. Neurol. 530 (18), 3270-3287, 2022.
DOI: http://dx.doi.org/10.1002/cne.25413
IF: 2.5

2. Fernandes, E. C., Santos, I. C., **Kókai, É.**, Luz, L. L., Szűcs, P., Safronov, B. V.: Low- and high-threshold primary afferent inputs to spinal lamina III antenna-type neurons.
Pain. 159 (11), 2214-2222, 2018.
DOI: http://dx.doi.org/10.1097/j.pain.0000000000001320
IF: 6.029

List of other publications

3. Gutierrez-Mecinas, M., **Kókai, É.**, Polgár, E., Quillet, R., Titterton, H. F., Weir, G. A., Watanabe, M., Todd, A. J.: Antibodies Against the Gastrin-releasing Peptide Precursor Pro-Gastrin-releasing Peptide Reveal Its Expression in the Mouse Spinal Dorsal Horn.
Neuroscience. 510, 60-71, 2023.
DOI: http://dx.doi.org/10.1016/j.neuroscience.2022.12.023
IF: 3.3 (2022)

4. Davis, O. C., Dickie, A. C., Mustapa, M. B., Boyle, K. A., Browne, T. J., Gradwell, M. A., Smith, K. M., Polgár, E., Bell, A. M., **Kókai, É.**, Watanabe, M., Wildner, H., Zeilhofer, H. U., Ginty, D. D., Callister, R. J., Graham, B. A., Todd, A. J., Hughes, D. I.: Calretinin-expressing islet cells are a source of pre- and post-synaptic inhibition of non-peptidergic nociceptor input to the mouse spinal cord.
Sci. Rep. 13 (1), 1-22, 2023.
DOI: http://dx.doi.org/10.1038/s41598-023-38605-9
IF: 4.6 (2022)

5. Luz, L. L., Lima, S., Fernandes, E. C., **Kókai, É.**, Gömöri, L., Szűcs, P., Safronov, B. V.: Contralateral Afferent Input to Lumbar Lamina I Neurons as a Neural Substrate for Mirror-Image Pain.
J. Neurosci. 43 (18), 3245-3258, 2023.
DOI: http://dx.doi.org/10.1523/JNEUROSCI.1897-22.2023
IF: 5.3 (2022)

6. **Kókai, É.**, Alsulaiman, W. A. A., Dickie, A. C., Bell, A. M., Goffin, L., Watanabe, M., Gutierrez-Mecinas, M., Todd, A. J.: Characterisation of deep dorsal horn projection neurons in the spinal cord of the Phox2a::Cre mouse line.
Mol Pain. 18, 1-16, 2022.
DOI: http://dx.doi.org/10.1177/17448069221119614

7. Mészár, Z. M., **Kókai, É.**, Varga, R., Ducza, L., Papp, T., Béres, M., Nagy, M., Szűcs, P., Varga, A.: CRISPR/Cas9-Based Mutagenesis of Histone H3.1 in Spinal Dynorphinergic Neurons Attenuates Thermal Sensitivity in Mice.
Int. J. Mol. Sci. 23 (6), 3178, 2022.
DOI: http://dx.doi.org/https://doi.org/10.3390/ijms23063178
IF: 5.6

8. Fernandes, E. C., Carlos, F. J., Luz, L. L., **Kókai, É.**, Mészár, Z. M., Szűcs, P., Safronov, B. V.: Processing of trigeminocervical nociceptive afferent input by neuronal circuity in the upper cervical lamina I.
Pain. 163 (2), 362-375, 2022.
DOI: http://dx.doi.org/10.1097/j.pain.0000000000002342
IF: 7.4

9. Papp, T., Ferenczi, Z., Szilágyi, B., Petró, M., Varga, A., **Kókai, É.**, Berényi, E., Oláh, G., Halmos, G., Szűcs, P., Mészár, Z. M.: Ultrasound Used for Diagnostic Imaging Facilitates Dendritic Branching of Developing Neurons in the Mouse Cortex.
Front. Neurosci. 16, 2022.
DOI: http://dx.doi.org/10.3389/fnins.2022.803356
IF: 4.3

10. Varga, A., Mészár, Z. M., Sivadó, M., Bácskai, T., Végh, B., **Kókai, É.**, Nagy, I., Szűcs, P.: Spinal Excitatory Dynorphinergic Interneurons Contribute to Burn Injury-Induced Nociception Mediated by Phosphorylated Histone 3 at Serine 10 in Rodents.
Int. J. Mol. Sci. 22 (5), 1-28, 2021.
DOI: http://dx.doi.org/10.3390/ijms22052297
IF: 6.208

11. Fernandes, E. C., Pechincha, C., Luz, L. L., **Kókai, É.**, Szűcs, P., Safronov, B. V.: Primary afferent-driven presynaptic inhibition of C-fiber inputs to spinal lamina I neurons.
Prog. Neurobiol. 188, 1-15, 2020.
DOI: http://dx.doi.org/10.1016/j.pneurobio.2020.101786
IF: 11.685

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12. Luz, L. L., Fernandes, E. C., Sivadó, M., **Kókai, É.**, Szűcs, P., Safronov, B. V.: Monosynaptic convergence of somatic and visceral C-fiber afferents on projection and local circuit neurons in lamina I.
Pain. 156 (10), 2042-2051, 2015.
DOI: http://dx.doi.org/10.1097/j.pain.0000000000000267
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