
MORPHOLOGICAL BASICS
FOR EVOLUTION OF FUNCTIONS

Efferent Projections of Dorsal Root Afferents in the Spinal Cord of the Lamprey *Lampetra fluviatilis*

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Received November 21, 2016

Abstract—Arborization of dorsal root afferents was studied in the lamprey spinal cord by the method of horseradish peroxidase transport. Direct evidence was obtained for the presence of efferent fibers in dorsal roots, representing collaterals that depart from ascending and descending intraspinal branches of sensory axons and travel towards the periphery through the adjacent roots.

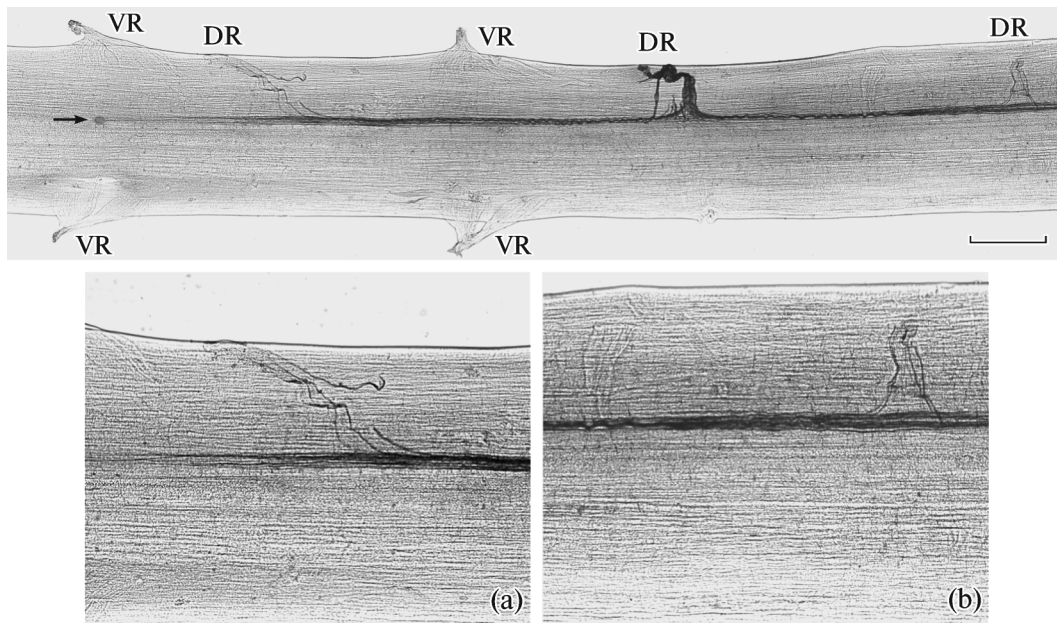
DOI: 10.1134/S0022093017050088

Key words: dorsal roots, primary afferents, collaterals, spinal cord, lamprey.

INTRODUCTION

Information about the origin and the very fact of the existence of efferent fibers within dorsal roots is discrepant, despite numerous studies. In earlier works conducted on mammals (dog, cat, rabbit) and based on transections/ligations and electrophysiological recordings, it was hypothesized that dorsal roots not only fulfil the afferent function, but also serve as a conduit to transmit efferent impulses towards skeletal muscles, blood vessels and other visceral organs [1–3], as if they were neuronal axons of the autonomous nervous system [4]. Both antidromic stimulation and injury of dorsal root fibers evoked different abnormalities at the periphery where they end: increased blood flow, vasodilation, hemorrhage caused by neurogenic inflammation due to a changed vascular permeability, hyperalgesia and allodynia [9, 10]. There is an opinion, based on various dorsal root transection experiments, that efferent fibers have a ganglionic descent with no relation to the spinal

cord [1, 4]. Dorsal root ganglia are assumed to harbor neurons that fulfil no sensory function and represent a separate class of autonomous neurons, having no preganglionic input and acting as efferent units [4]. However, this assumption conflicts with electrophysiological data showing that centrifugally conducted action potentials result from neural activity in the spinal cord [11]. Moreover, morphological studies demonstrated the anatomical substrate of such a centrifugal conduction. Specifically, the centrifugal conducting fibers are collaterals of the dorsal column fibers, heading for the periphery [12]. In outline, it was shown that afferent fibers, entering the spinal cord within a single root, send a collateral to the adjacent dorsal root. However, the experiments with dorsal root transections gave discrepant results. While some authors observed degenerating fibers following transection of the adjacent dorsal root [2], others reported neither morphological nor electrophysiological changes in neurons of the adjacent ganglion [13, 14] and attributed the changes in elec-



Fragment of the lamprey spinal cord; dorsal root (DR) labeled by horseradish peroxidase. VR—ventral roots. Scale, 500 μm . (a), (b)—(magnified details)—collaterals of primary afferent axons, traveling to adjacent spinal cord roots.

trophysiological characteristics of intact dorsal root neurons to Wallerian degeneration spreading to adjacent intact fibers [13].

Nowadays, some authors persist in holding the opinion that all fibers entering the spinal cord from dorsal-root ganglionic neurons are afferent [15–17], while primary afferent neurons are considered as an independent element of sensory communication between the peripheral organs and CNS.

Most of the previous studies were carried out using the silver impregnation technique either on the normal material or degenerating axons. The aim of the present study was to scrutinize the details of arborization and distribution of primary afferent axons as identified by horseradish peroxidase (HRP).

MATERIALS AND METHODS

The study was carried out on a preparation of the isolated superfused spinal cord of the lamprey *Lampetra fluviatilis*. Sensory axons were identified morphologically using the HRP method, which is more accurate than the silver impregnation techniques. Ten lamprey individuals (30–35 cm long) were used in experiments. Animals were anesthetized by submerging to 0.01% solution

of tricainemethane sulfonate (MS-222, Sigma, USA). Following decapitation, the spinal cord fragment (2–2.5 cm) was excised and submerged to a running physiological solution with the following composition (mM): 115 NaCl, 2.0 KCl, 0.2 Na_2HPO_4 , 0.8 NaH_2PO_4 , 8.0 NaHCO_3 , 2.0 CaCl_2 , 0.9 MgCl_2 , 5.5 glucose, pH 7.3–7.4, carbogen aerated (98% O_2 + 2% CO_2). The solution temperature was maintained at 10–12°C. 10% HRP solution was introduced iontophoretically via a sucking electrode (constant current: 5–7 nA) intermittently during 1–1.5 h. The sample was left thereafter in the solution for 15–18 h at 4–5°C and then fixed in 3% glutaraldehyde. The enzyme was visualized on a total preparation with cobalt-intensified diaminobenzidine as a chromogen. To do this, the spinal cord tunic was preliminary removed, occasionally leaving some dorsal root fibers damaged. Then followed standard sample processing for light microscopy.

All observations were made on a total preparation, because the lamprey spinal cord is too small and transparent due to the lack of myelin and blood vessels.

RESULTS AND DISCUSSION

Primary afferent neurons in the lamprey, like

in all higher vertebrates, are localized in the spinal ganglia. However, some neurons reside inside the spinal cord in the ventral part of the dorsal column. The ganglion is not compact, since individual cell bodies are arranged along the length of the dorsal root and even at its entry to the spinal cord. The intramedullary location of sensory neurons appears to be primary, while their integration into a ganglion throughout the evolution of vertebrates should be considered as a result of migration of cells towards the periphery.

Like in all vertebrates, axons of dorsal root neurons split up in the spinal cord into the descending and ascending branches. Our observations showed that some of the labeled axons send off a collateral that enters the adjacent of more distant dorsal roots. Sometimes, there are several collaterals per root (Fig.1a, 1b). It is obvious that collaterals belong to axons of different dorsal sensory cells. They were found to depart both from the rostral and caudal branches of afferent axons. It is noteworthy that the observed patterns are irregular. Apparently, not all, but only a certain class of sensory neurons has the efferent function.

Thus, the data obtained support the fact of the existence of efferent fibers within dorsal roots and indicate a spinal descent of the centrifugal fibers, representing axonal collaterals of primary afferents. Besides, these data are consistent with those obtained on the lampreys *Lampetra planery* and *Lampetra fluviatilis* [3], where silver impregnation helped trace the branches of dorsal sensory cells towards blood vessels on the dorsal surface of the spinal cord. The presence of efferent axons within the dorsal root that travel through the ganglion to the periphery accounts for the existence of peripheral primary afferent depolarization (PAD) [18, 19]. Like the central, peripheral PAD is mediated by GABA_A receptors, which occur not only on the central, but also on peripheral axonal segments of dorsal-root ganglionic neurons [19, 20], and which are involved in the regulation of glutamate release at the periphery. An increase in the peripheral glutamate level activates peripheral glutamate receptors (AMPA, kainate, NMDA and non-NMDA [21]) that enhance its toxic effect. The effect on peripheral receptors is assumed to be able to reduce the release of algogenic substances and hence to relieve painful sensations [22].

Supported by the Program of the Presidium of the Russian Academy of Sciences no. 19 and RFBR grant no. 15-04-05782.

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