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Optical survey of vagus nerve-related neuronal circuits in the embryonic rat brainstem

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HIGHLIGHTS

- We surveyed N.X-related neural circuits in the E16 rat brainstem by optical imaging.
- N.X stimulation elicited bilateral optical response areas in addition to the NTS.
- These responses are the neural activity in the second/higher-ordered nucleus.
- The bilateral areas are considered to correspond to the PBN and the A5 group.
- The N.X-related neural circuits are established as in adults from embryonic stages.

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ABSTRACT

The multiple-site optical recording technique with a voltage-sensitive dye, NK2761, was used to survey the functional organization of neuronal networks related to the vagus nerve (N.X) in the E16-stage rat brainstem. When we stimulated N.X, in addition to the responses in the vagal sensory nucleus (nucleus of the tractus solitarius (NTS)) on the stimulated side, other response areas were bilaterally detected. Characteristics of the optical signals in these areas suggested that they correspond to neural activity in the second/higher-ordered nucleus of the vagal pathway. The first area was located at the level of the pons. Based upon morphological information, we suggest that this area corresponds to the parabrachial nucleus (PBN), which receives inputs from the NTS. The second area was located between the NTS and the PBN. We suggest that this area is the A5 noradrenergic group. These results suggest that the N.X-related neural networks are established similarly to the adult pattern from an early developmental stage.

1. Introduction

To elucidate the autonomic control of various physiological functions in living animals, it is important to examine how the neural networks are precisely formed within the central nervous system (CNS). In the autonomic neural networks, the brainstem is a pivotal region to integrate various sensory information from peripheral organs and to produce motor reflexes [for reviews, see 3,25,30,31]. The vagus nerve (N.X) transfers autonomic input and output information to/from the brainstem, and analysis of the N.X-related nuclei in the brainstem is the first step to understand the entirety of the autonomic neural networks. Although many anatomical and molecular genetic approaches have been used, electrophysiological studies of neural network organization have been hampered because of technical difficulties such as the fragility and small size of embryonic neurons.

The voltage-sensitive dye recording technique has made it possible to monitor transmembrane voltage changes from living cells that are inaccessible to conventional electrophysiological means. Furthermore, the introduction of multi-element photodiode arrays has provided powerful tools for monitoring the spatiotemporal dynamics of neural activity from a variety of invertebrate and vertebrate CNSs [for reviews, see 1,2,5,24].

Using the optical technique, we have analyzed electrical activity in embryonic nervous systems [for reviews, see 4,17–20], especially that in the brainstem related to autonomic function. In embryonic chick [8,9,14,15,28] and rat [13,27] brainstems, we examined spatiotemporal patterns of neural activity evoked by N.X stimulation and demonstrated three-dimensional profiles of the vagal sensory nucleus (nucleus of the tractus solitarius (NTS)) and motor nucleus (dorsal motor nucleus of the vagus nerve (DMNV)). Furthermore, we also optically recorded neural responses in the

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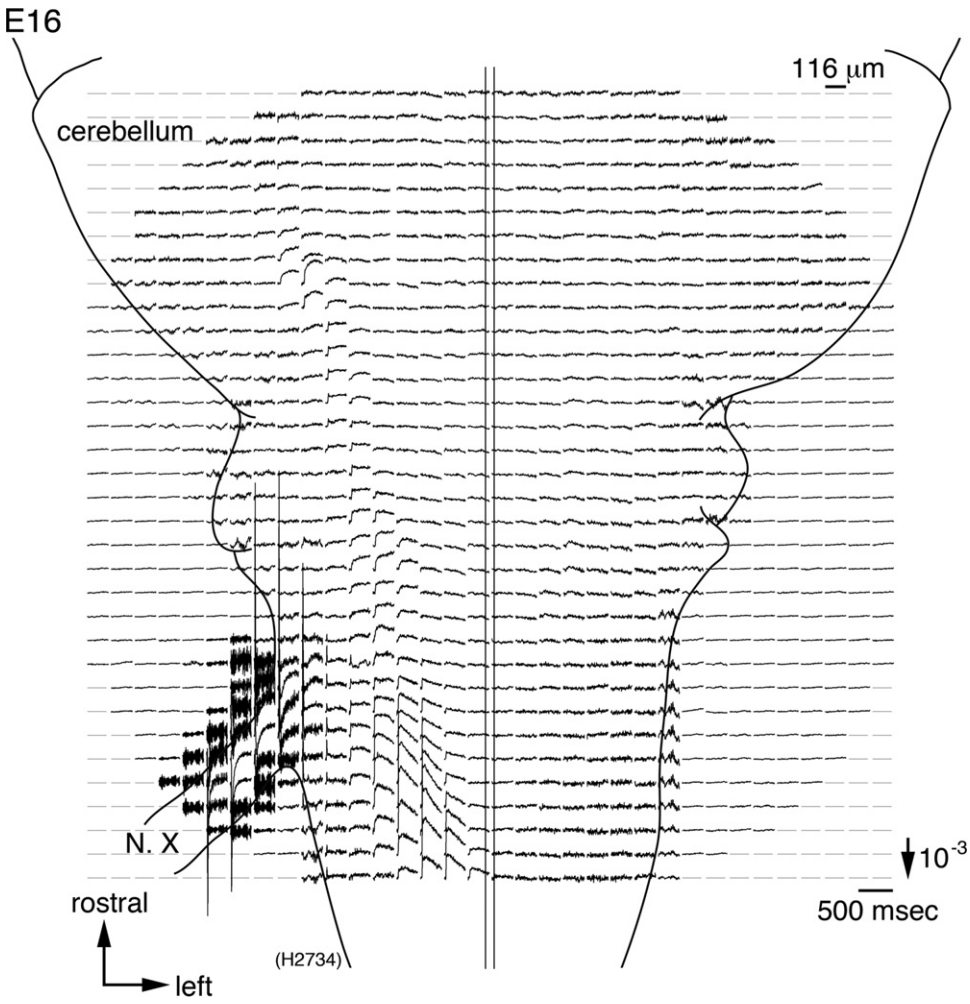

Fig. 1. 1020-site optical recording of neural responses to N.X stimulation in an E16 brainstem intact preparation. The preparation was stained with a merocyanine–rhodanine dye (NK2761), and the optical signals were evoked by electrical stimulation (8 μA/5 ms) to the right N.X with a micro-suction electrode. The interval between stimulations was at least 10 min, and signal averaging of five recordings was performed offline. The relative position of the image of the preparation is drawn on the recording. The two straight lines indicate the floor plate (midline). Each trace represents a signal detected by one photodiode from a two-dimensional 116 μm × 116 μm area of the preparation. Outputs of the individual detectors were divided by the resting light intensity. The direction of the arrow in the lower right of the figure indicates an increase in transmitted light intensity (a decrease in dye absorption), and the length of the arrow represents the stated value of the fractional change.

second/higher-ordered nucleus, including the parabrachial nucleus (PBN), in the embryonic chick brainstem [12,16,26].

The main aim of the present study is to survey the N.X-related neuronal circuits in the embryonic rat brainstem. Similarly to that in the embryonic chick brainstem, we succeeded in detecting optical responses in the N.X-related second/higher-ordered nuclei in addition to the NTS.

Preliminary results have appeared in abstract form [29].

2. Materials and methods

Experiments were carried out in accordance with the guidelines of the US National Institutes of Health, Kanto Gakuin University and Komazawa Women's University for the care and use of laboratory animals. All efforts were made to minimize the number of animals used and their suffering. In the present experiments, we used embryonic day (E)16 rat intact brainstem preparations (the morning on which a vaginal plug was observed was called day 0 of gestation, E0). Wistar rats of 16 days gestation ($n = 7$) (Saitama Experimental Animals Supply Co., Ltd.) were deeply anesthetized with ether, and fetuses were removed surgically. The embryos were decapitated, and the brainstems with N.X were dissected from the embryos. The isolated brainstem preparation was attached to the silicone (KE 106LTV; Shin-etsu Chemical Co., Tokyo, Japan) bottom of a simple chamber by pinning it with tungsten wires. The meningeal tissue surrounding the brainstem was carefully removed under a dissecting microscope. The preparations were kept in a bathing solution with the following composition (in mM): NaCl, 149; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; glucose, 10; and Tris–HCl buffer (pH 7.4), 10, which was equilibrated with oxygen. Two preparations were kept in modified Kreb's solution with the following composition (in mM): NaCl 128, KCl 5, $MgSO_4$ 1, $CaCl_2$ 1.5, NaH_2PO_4 0.5, $NaHCO_3$ 24 and glucose 30, which was continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). The results obtained did not differ between these bathing solutions.

The isolated preparation was stained by incubating it for 20 min in a bathing solution containing 0.2 mg/ml of the voltage-sensitive merocyanine–rhodanine dye, NK2761 (Hayashibara Biochemical Laboratories Inc./Nippon Kankoh Shikiso Kenkyusho, Okayama, Japan [7,21]). After staining, the excess (unbound) dye was washed away with dye-free bathing solution, and the preparation was attached to the recording chamber with the dorsal side up for five preparations or with the ventral side up for two preparations. The preparations were continuously perfused with the solution at a