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Optical survey of initial expression of synaptic function in the embryonic chick trigeminal sensory nucleus

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HIGHLIGHTS

- We examined the initial expression of synaptic function in the embryonic chick CNS.
- We applied voltage-sensitive dye imaging to the N.V-brainstem preparation.
- We optically identified N.V-related sensory and motor nuclei in the brainstem.
- Synaptic function in the sensory nuclei emerged earlier than previously considered.
- Development of synaptic function differs among nerves and between sensory nuclei.

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ABSTRACT

We examined the initial expression of synaptic function in the embryonic chick trigeminal nucleus using voltage-sensitive dye recording. Brainstem preparations with three trigeminal nerve afferents, the ophthalmic nerve ($N.V_1$), maxillary nerve ($N.V_2$) and mandibular nerve ($N.V_3$), were dissected from 5.5- to 6.5-day-old chick embryos. In our previous study [Sato et al., 1999], we detected slow signals corresponding to glutamatergic excitatory postsynaptic potentials and identified the principal sensory nucleus of the trigeminal nerve (Pr5), spinal sensory nucleus of the trigeminal nerve (Sp5) and trigeminal motor nucleus. In this study, we examined the effects of removing Mg^{2+} from the physiological solution, which enhanced *N*-methyl-D-aspartate receptor function in the sensory nuclei. In 6.5-day-old (St 29) embryos, the slow signal was observed in Pr5 and Sp5 only when $N.V_1$ was stimulated, whereas it appeared in Mg^{2+}-free solution with every nerve stimulation. In 6-day-old (St 28) embryos, the slow signal was observed in Sp5 with $N.V_1$ stimulation, and the appearance of synaptic function in Mg^{2+}-free solution varied, depending on the nerves and preparations used. In 5.5-day-old (St 27) embryos, synaptic function was not detected even when external Mg^{2+} was removed. These results indicate that the initial expression of synaptic function in the trigeminal system occurs earlier than previously considered, and that the developmental organization of synaptic function differs among the three trigeminal nerves and between the two sensory nuclei.

1. Introduction

The trigeminal nerve (N.V) is the largest cranial nerve and contains sensory and motor fibers. The sensory fibers of the N.V are the general somatic afferents, which transfer sensory information mainly from the face and forehead regions. The trigeminal sensory system has a precise somatotopic organization in the brainstem, thalamus and somatosensory cortex [20,25]. In the field of developmental neuroscience, this system is one of the most interesting models in which to examine how the neuronal network in the central nervous system (CNS) is established during embryogenesis. Therefore, it is important to elucidate the developmental sequence of synaptic function in the brainstem trigeminal nuclei.

We have investigated functional development of the CNS by using an ontogenetic approach and applying optical imaging with voltage-sensitive dyes (VSDs) (for reviews see Refs. [4,13–16]). The VSD imaging technique has several advantages over conventional electrophysiological techniques. First, it is possible to monitor

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changes in intracellular membrane potential in living systems that are inaccessible to microelectrodes. Second, electrical activity can be recorded simultaneously from many sites in the preparation, which enables us to analyze spatiotemporal patterns of a response (for reviews see Refs. [2,3,5,18]).

In our previous study, we applied the VSD imaging technique to the N.V system of chick and rat embryos [11,24]. We examined optical responses in the chick N.V system during 5–8 days of incubation, and identified three N.V-related nuclei, namely, the principal sensory nucleus of the trigeminal nerve (Pr5), spinal sensory nucleus of the trigeminal nerve (Sp5) and trigeminal motor nucleus (Mo5). We showed that glutamatergic synaptic function was detectable in Sp5 from day 6 with ophthalmic (V_1) nerve stimulation and from day 7 with maxillary (V_2) and mandibular (V_3) nerve stimulation. It has been reported in other chick cranial nuclei that the glutamatergic synaptic function is latently generated but is suppressed by external Mg^{2+} at the stage just before functional synapses are first expressed [9,10,12,21,22]. However, it remains unknown whether a similar mechanism operates in the chick N.V system. In the present study, we examined optical responses in the chick N.V nuclei during 5.5–6.5 days of incubation, and pursued the initial expression of synaptic function in more detail, focusing on the regulatory function of Mg^{2+} on the *N*-methyl-D-aspartate (NMDA) receptor. The results showed that synaptic responses were detected in the Mg^{2+}-free solution with every nerve stimulated at 6–6.5 days, demonstrating earlier expression of synaptic function in latent form than had previously been considered.

Preliminary results have appeared in abstract form [23].

2. Materials and methods

Experiments were carried out in accordance with the guidelines of the US National Institutes of Health for the care and use of laboratory animals, with approval from the ethical committees of Kanto Gakuin University and Komazawa Women's University. All efforts were made to minimize the number of animals used and their suffering. Fertilized eggs of White Leghorn chickens (Saitama Experimental Animals Supply Co. Ltd., Saitama, Japan) were incubated for 5.5–6.5 days in a forced-draft incubator at a temperature of 38 °C and 60% humidity. In the experiments reported here, day 5.5 corresponded to Hamburger–Hamilton (H–H) stage (St) 27, day 6 to St 28 and day 6.5 to St 29 [6]. The embryos were anesthetized using cold physiological solution. The brainstems with three trigeminal afferent fibers, the ophthalmic nerve ($N.V_1$), maxillary nerve ($N.V_2$) and mandibular nerve ($N.V_3$), together with motor nerve fibers, were dissected from the embryos. The meningeal tissue attached to the brainstem was carefully removed. The isolated intact brainstem preparation was attached to the silicone bottom of a simple chamber with the ventral side up. The preparation was kept in normal physiological solution with the following composition (in mM): NaCl, 138; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 0.5; glucose, 10; and Tris–HCl buffer (pH 7.3), 10. $MgCl_2$ was replaced with $CaCl_2$ in the Mg^{2+}-free experiments. The solution was equilibrated with oxygen.

The preparations were stained by incubation for 20 min in normal solution containing 0.2 mg/ml of a voltage-sensitive merocyanine–rhodanine dye, NK2761 (Hayashibara Biochemical Laboratories Inc./Nippon Kankoh-Shikiso Kenkyusho, Okayama, Japan [7,17,19]). The preparations were continuously perfused with the solution at a rate of 1 ml/min at 26–28 °C. The cut ends of the three branches of N.V were drawn into glass micro-suction electrodes. Depolarizing square current pulses (7–8 μA/5 ms) were applied to the nerves.

The methods used for multiple-site optical recording of electrical activity in embryonic excitatory systems are described in detail elsewhere (for reviews see Refs. [2,7,17]). In brief, bright-field illumination was provided by a 300 W tungsten–halogen lamp (Type JC-24V/300W, Kondo Philips Ltd., Tokyo, Japan) driven by a stable dc-power supply. The infrared light from the tungsten–halogen lamp was cut off by a heat filter, and incident light was collimated and rendered quasi-monochromatic with an interference filter with a transmission maximum at 703 ± 12 nm (half-width) (Asahi Spectra Co., Tokyo, Japan). The objectives (10×) and photographic eyepiece (2.5×) projected a real image (magnification: 25×) of the preparation onto a 34 × 34-element silicon photodiode matrix array mounted on a microscope. The focus was set to the upper surface of the preparation. Changes in transmitted light intensity through the preparation were detected with the photodiode array, and were recorded with a 1020-site optical recording system constructed in our laboratory. Each pixel (element) of the array detected light transmitted by a square region (54 μm × 54 μm) of the preparation. The time resolution of the system was ≈1 ms. The optical signal was recorded in a single sweep because the excitatory postsynaptic potential (EPSP) in the embryonic CNS was easily fatigued by repetitive stimulation [15].

3. Results

Fig. 1A shows examples of multiple-site optical recording of neuronal responses to N.V stimulation in a 6.5-day-old (St 29) chick brainstem intact preparation. The brainstem was translucent, and neuronal voltage responses could be detected as changes in transmitted light intensity. When a stimulating current pulse (8 μA/5 ms), which gave the maximum response, was applied individually to the $N.V_1$, $N.V_2$ and $N.V_3$, optical responses were observed on the stimulated side of the preparation. The optical signals were detected in a single sweep in two positions (positions a and b shown in an inset of Fig. 1A) and are partially illustrated in Fig. 1A. The action spectra of the optical response were similar to those of NK2761-dependent extrinsic absorption signals, and the responses were eliminated at 620–630 nm, at which the NK2761-dependent extrinsic absorption signal is absent [7,17] (data not shown). This result indicates that the optical responses were indeed dye-absorption changes related to the membrane potential and did not correspond to changes in light scattering related to mechanical or other factors.

Optical responses induced by $N.V_1$ or $N.V_2$ stimulation were observed in two areas as shown in Fig. 1A: one was located cephalic to the level of the trigeminal ganglion (G.V) (area I shaded red) and the other caudal to the level of G.V (area II shaded blue). $N.V_3$ stimulation elicited another response area near G.V (area III shaded green) in addition to these two areas. Color-coded spatiotemporal maps of optical responses evoked by $N.V_1$ stimulation (Fig. 1B) revealed that the optical responses first appeared in area I and then in area II, which seems to have been due to delays reflecting signal propagation. Similar results were also obtained with $N.V_2$ and $N.V_3$ stimulation (data not shown). Compared with our previous results and anatomical information [1,8,24], areas I, II and III were considered to correspond to Pr5, Sp5 and Mo5, respectively. In the following study, we examined the initial expression of synaptic function in the sensory nuclei of the N.V, namely, Pr5 and Sp5.

In Fig. 2A, optical signals detected in Pr5 and Sp5 shown in Fig. 1A are enlarged. The signals evoked by $N.V_1$ stimulation consisted of two components, fast spike-like signals and slow long-lasting signals, in both Pr5 and Sp5 in normal physiological solution (Fig. 2A top, left). We have shown previously that (1) the fast signals correspond to sodium-dependent action potentials, and that (2) the slow signals reflect glutamatergic EPSPs that consist of NMDA and non-NMDA receptor-mediated components [24]. When external Mg^{2+} was removed, the $N.V_1$-induced slow signals became larger than