Order in the developing rat trigeminal nerve

REHA S. ERZURUMLU and HERBERT P. KILLACKEY*

Department of Psychobiology, University of California, Irvine, CA 92717 (U.S.A.)

(Accepted October 12th, 1981)

Key words: trigeminal system — vibrissae — development — pattern formation

We provide evidence that the developing trigeminal nerve is characterized by a high degree of order. Trigeminal nerve fibers which innervate a single row of vibrissae follicles are fasciculated into distinct and identifiable bundles. Further, within the brain stem, afferent clusters related to given vibrissae within a row develop in a sequential fashion from lateral to medial. On the basis of this evidence, we hypothesize that the 180° rotated topography and vibrissae-related segmentation in the brain stem trigeminal complex is brought about by spatial and temporal factors intrinsic to the developing trigeminal nerve.

In general, the connections between peripheral receptor organs and the central nervous system are characterized by a high degree of topographic order. An excellent example of this principle is the organization within the rodent trigeminal system. Since the initial observation of an homeomorphic relationship between punctate receptor distribution on the muzzle of the mouse and a subpopulation of nerve cells within the somatosensory cortex, a similar relationship has been described at each level of the rat trigeminal system. The mystacial vibrissae of the rat are arranged in a pattern of 5 separate rows, each of which consists of 5–8 whiskers. Vibrissae-related afferent inputs and their target cells replicate this pattern in the brain stem trigeminal complex, the ventrobasal complex of the thalamus, and the somatosensory cortex. Within the brain stem trigeminal complex, the vibrissae representation is rotated 180° with respect to the face. We have studied the formation of this rotated map and conclude that spatio-temporal factors play a major role in its formation.

We utilized a reduced silver stain for nerve fibers and their terminals and an histochemical assay, succinic dehydrogenase histochemistry (SDH), which has previously been used to demonstrate the mode of afferent termination in the trigeminal system. We studied the trigeminal nerve in fetal and postnatal Sprague-Dawley rats which varied in age from embryonic day 17 (E17) to postnatal day 7 (PND 7). In addition, we selectively cut branches of the trigeminal nerve on the day of birth, sacrificed these animals on PND 5 and assayed the effect of this manipulation on the nerve. The heads of the animals were sectioned coronally or parasagittally with the
nerve in situ. This allowed us to follow the trajectory of the trigeminal nerve and its relationship to the vibrissae on one hand, and to the brain stem on the other. The histological procedures were as follows. For the silver stain, the heads of embryos and postnatal Sprague–Dawley rats were first fixed in Bouin’s fluid, cleared with methyl benzoate and embedded in paraffin. Serial sections were cut in the coronal and parasagittal plane at a thickness of 10 μm and were stained with Unicover's silver stain. For the SDH assay, E20 and older embryos and postnatal day 0–8 rats were perfused intracardially with 10% glycerol–0.5% formaldehyde and their heads were removed and immersed in cold isopentane (–40 °C). The heads of these animals were then sectioned in a cryostat in the coronal or parasagittal plane at a thickness of 40 μm and mounted on gelatin coated slides. The sections were then processed for SDH histochemistry as described by Killackey and Belford.

The results of the present study are illustrated in Figs. 1 and 2. A parasagittal section through the vibrissae pad of an E16 rat is shown in Fig. 1A. After emerging from the infraorbital foramen, the infraorbital branch of the maxillary division of the trigeminal nerve fans out towards the 5 rows of vibrissae. In addition, the nerve is fasciculated, and these fascicles innervate specific rows of vibrissae in an orderly fashion as they run through the grooves between the vibrissae rows. Caudally, inside the infraorbital foramen, axons innervating individual rows of vibrissae are organized into distinct fascicles and can be traced back to the trigeminal ganglion (Fig. 1B). The central processes of the bipolar ganglion cells retain the order exhibited by their peripheral counterparts (Fig. 1C). They, however, are more abundantly fasciculated as they run towards the pontine flexure where they enter the pons ventrolaterally (Fig. 1C). The order within the trigeminal nerve, in relation to rows of vibrissae, is more apparent in the coronal plane, as illustrated in Fig. 1D–G. Individual fascicles are seen to form a vertical row in close association with and parallel to the 5 rows of vibrissae (Fig. 1D). Caudally, these fascicles move close together and merge into larger bundles (Fig. 1E and F). Further caudally, the ventral fascicles move laterally and dorsally, forming a circular pattern of 5 or 6 distinct bundles (Fig. 1G). At this level the nerve is organized so that the fascicles which innervate dorsal rows of vibrissae on the face are found medially and those which innervate the ventral rows and the buccal pad laterally. As can be seen most clearly in Fig. 1G, there are separate and distinct bundles of fasciculated fibers related to the rows of vibrissae and the buccal pad.

The organization within the infraorbital nerve is but a subcomponent of the overall topography of the trigeminal nerve. The maxillary division of the trigeminal nerve is first joined by the ophthalmic division, which innervates the brow, and later by the mandibular division, which innervates the lower jaw. Within the cranium the mandibular division lies lateral to the maxillary division while the ophthalmic division lies medial to it (Fig. 1F and G). Thus, at this level of the trigeminal nerve there is a topographic relationship in which the dorsoventral axis of the face has shifted 90°. This spatial relationship is maintained in the central processes of the trigeminal ganglion cells. The medial portion of the trigeminal nerve enters the pons ventrally, whereas the lateral portion enters dorsally. This results in another 90° rotation of the topography in the trigeminal nerve. Upon entering the pons, the trigeminal afferents
Fig. 1. A–C: parasagittal sections through the head of an 18-day-old rat embryo, showing the course of the trigeminal nerve. In A, the 5 rows of vibrissae are clearly seen, and rows A and B are indicated. Note the fasciculated fibers which fan out towards these 5 rows. B illustrates the path of the nerve between the infraorbital foramen and the trigeminal ganglion, and C illustrates the central processes of the ganglion cells as they enter the pons. D–G: coronal sections through the head of an 18-day-old embryo. These sections are at progressively caudal levels as indicated by the arrows in A–C. The location of rows A and B and their associated fibers are indicated. In F and G, the mandibular (mn) and ophthalmic (o) divisions of the trigeminal nerve are also labeled. In D–G, lateral is to the left and medial to the right. All scale bars equal 0.1 mm; B and C are the same scale as A.

form the trigeminal tract, which occupies a crescent-shaped area along the lateral convexity of the brain stem. Within the trigeminal tract, the fibers which were medially situated in the nerve occupy a ventral position, while the fibers which were laterally situated in the nerve occupy a dorsal position. The trigeminal tract encapsulates the sensory nuclei of the trigeminal nerve and along its route fibers turn off perpendicularly to enter various divisions of the sensory trigeminal nuclei in parallel arrays. Thus, a 180° rotated map of the face is conveyed to the brain stem; the mandibular nerve representation is most dorsal, the ophthalmic nerve representation is most ventral and the maxillary nerve representation is in between. The termination pattern of the vibrissae-related afferents provides the most distinct example of this reversed map. In the brain stem, the most dorsally situated row of vibrissae on the face is represented most ventrally and the most ventrally situated row of vibrissae on the face most dorsally. We have experimentally verified this topography by selectively sectioning the branches of the infraorbital nerve innervating different rows of vibrissae. Following
this procedure, fascicles related to the cut branch can be identified and traced as they obviously contain a reduced number of fibers, thus confirming the topographic relationship between the periphery and the fasciculation within the trigeminal nerve.

In regard to the rostrocaudal axis of topography on the face, we have found that the punctate segmentation of afferent terminals related to individual vibrissae in a given row takes place in a sequential fashion around the time of birth. Fig. 2A shows the principal sensory nucleus on E21. At this time, the SDH activity is uniform in its distribution. On the next day (PND 0), we can detect rows and individual clusters of SDH activity in the lateral portion of the nucleus (Fig. 2B). These clusters represent the vibrissae which are caudally situated on the face. By PND 3 a complete pattern of row formation and segmentation is present (Fig. 2C). Thus, the rostrocaudal organization of vibrissae in a given row is replicated in the brain stem in a lateral to medial direction over time.

The distribution of mystacial vibrissae on the face and their representation within the brain stem can be considered as 2-dimensional. Our results provide direct evidence that cues about one of these dimensions: the dorsoventral (across-row) dimension is preserved within the trigeminal nerve by the pattern of fasciculation. In addition, we have obtained indirect evidence that information about the second dimension: the rostrocaudal (within-row) dimension may be contained in the sequence of afferent terminal distribution. In this context, it should be noted that the sequence of pattern formation in the brain stem mirrors that of the formation of vibrissae follicles on the face19,22.

The formation of specific neuronal topographies has been most intensely studied

Fig. 2. Photomicrographs of the distribution of SDH activity. In the principal sensory nucleus of an E21 (A), PND 0 (B) and PND 3 (C) rat. Note the initial appearance of segmentation in the ventrolateral part of the nucleus (B). Representation of the two dorsal rows (A and B) is indicated. In all 3 sections, lateral is to the left and dorsal is towards the top of the page. Scale bars equal 0.1 mm.
in the retinotectal projections of non-mammalian vertebrates. At present, there is no agreement as to the relative contributions of the differing mechanisms which have been proposed to account for the topography in this system. The chemospecificity hypothesis originally proposed by Sperry has been discounted by a number of investigators, while others have taken a more middle ground and suggested a combination of factors. Our results do not rule out a role for chemotaxic factors in the innervation of the periphery and the brain stem, but they suggest that the establishment of the pattern itself can be understood largely in terms of spatial and temporal factors. We would hypothesize that this process takes place in the following manner: the initial fibers make contact with the caudal vibrissae, which are both closest to the point that the nerve emerges from the skull and the earliest to develop. Later fibers are perhaps attracted to and guided by their predecessors. The net result of this process is a fasciculation within the nerve related to rows. When the processes of later developing neurons reach the vicinity of their targets, the initial terminal sites are found to be occupied and processes travel further rostrally on the face (or medially within the brain stem), giving rise to the organization within the row. This hypothesis provides a basis for understanding the effect of neonatal vibrissae damage on pattern formation in the central nervous system. Neonatal removal of a row of vibrissae results in a fused band of associated afferents rather than the normal punctate rows of SDH activity. Further, boundaries between rows are more resistant to peripheral manipulation than are within-row boundaries. The basis for this resistance is the maintenance of across-row organization by fasciculation within the nerve. This fasciculation is not severely interrupted by follicle damage at birth and can serve as a guide for later arriving fibers. However, the temporal gradient with which these fibers usually arrive, as well as their target tissue, would be seriously disturbed.

The present results provide evidence for the formation of specific neuronal topographies by spatial and temporal cues. Recently, it has been hypothesized that these same factors may play a major role in determining the topography in the projections of the retina onto the tectum of the chick. Further, recent studies have indicated that the optic nerve is characterized by a high degree of order which is at least partially brought about by temporal and spatial factors. Together, these results from two different sensory systems and several diverse vertebrate species provide evidence that both order within a nerve and the sequence of its development play a role in the formation of specific neuronal connections.

Research supported by NSF Grant GB 41294.

2 Dawnay, N. A. H., ‘Chronotopic’ organization of goldfish optic pathway, J. Physiol. (Lond.), 296 (1979) 13P--14P.
7 Killackey, H. P., Anatomical evidence for cortical subdivisions based on vertically discrete thalamic projections from the ventral posterior nucleus to cortical barrels in the rat, Brain Res., 51 (1973) 326-331.
17 Ungewitter, L. H., A urea silver nitrate method for nerve fibers and nerve endings, Stain Tech., 26 (1951) 73-76.
22 Yamakado, M. and Yohro, T., Subdivision of mouse vibrissae on an embryological basis, with descriptions of variations in the number and arrangement of sinus hairs and cortical barrels in BALB/c (nu/nu: nude, nu/nu) and hairless (hr/hr) strains, Amer. J. Anat., 155 (1979) 153-174.