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"Pattern Formation in the Trigeminal System of the Rat"

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Pattern formation in the trigeminal system of the rat

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At several levels of the trigeminal system of the rat, the terminations of afferent fibers are organized into a pattern which replicates the distribution of receptors on the face of the rat. Neonatal manipulations of this facial pattern result in the formation of altered central patterns. Study of pattern formation in this system may add to our understanding of the formation of ordered neuronal topographies.

One of the major goals of developmental neurobiology is to determine the processes by which ordered topographic nervous connections are formed. To date, our understanding of these phenomena is largely based on experiments which manipulate some aspect of the visual system, more specifically, the retinotectal projections, of adult fish or amphibia. While this approach has been a very fruitful one, it is unlikely that study of any single system will provide a complete understanding of a process as complex as the formation of topographic neuronal projections.

An alternate system which holds some promise for adding to our understanding of how ordered arrays of neuronal connections are formed is the trigeminal system of the rat. The advantages of this system are several. First, the mystacial vibrissae which collectively form an exquisite organ of touch are arranged in a punctate fashion on the face of the rat, as are their correlates within the central nervous system. This is an important contrast to the visual system, where the retina and its central projections are, for all practical purposes, continuous rather than punctate surfaces, making it difficult to draw point to point correlations between the two surfaces. Second, the mystacial vibrissae are present at birth and are readily manipulated. Fortunately, this is during the period when discrete organization is initially forming within the central nervous system, and consequently one can assay the effects of various manipulations on the initial development of the trigeminal system rather than on a later regenerating and perhaps prespecified system. Third, discrete punctate organization within the trigeminal system can be detected at the level of the primary, secondary and tertiary afferents, allowing one to determine the effect of a peripheral manipulation on sequential levels of the neural axis. Finally, the punctate organization within the trigeminal system of the rat is discernible with routine anatomical techniques.

Cytoarchitectonic organization

The high degree of anatomical specificity within the rodent trigeminal system was first noted by Woolsey and Van der Loos[19]. These authors called attention to the unique cytoarchitectonic organization of portions of the fourth layer of the mouse somatosensory cortex. They pointed out that the cells of this layer were not uniformly distributed, rather these neurons were aggregated into hollow multicellular units which Woolsey and Van der Loos termed 'barrels'. Further, they demonstrated, by preparing tangential sections of layer IV, that the total distribution of 'barrels' in the face portion of the somatosensory cortex replicated the distribution of sinus hairs and vibrissae follicles on the face of the mouse; a correlation which was strengthened by a physiological study which reported that the neurons of a given 'barrel' were only activated by stimulation of a single vibrissa[12]. This remarkable cytoarchitectonic organization might have been viewed simply as an anatomical curiosity, or at best an example of an extreme cytoarchitectonic specialization, except for the finding of Van der Loos and Woolsey[11] that cautery of a row of vibrissae follicles in the newborn mouse resulted in a marked change in the cortical cytoarchitectonics of the adult mouse (the row of discrete 'barrels' was replaced by a single narrow band of cells). This finding raised a number of interesting questions, such as, how is this effect transmitted through multiple synapses between periphery and cortex, and what is the role of the periphery in the formation of the central patterns? This study and additional evidence provided by 'experiments of nature' suggests that the periphery plays a role in central pattern formation. This additional evidence includes the findings that species variation in vibrissae patterns[16], as well as genetic or developmental anomalies in the vibrissae pattern[15], are reflected in cortical cytoarchitecture.

Afferent organization

The neurons of cortical layer IV are not the only elements of the trigeminal system which are discretely organized with respect to the vibrissae. Indeed, the hollow cores of the 'barrels' of layer IV are filled with the terminals of the thalamocortical projections, the tertiary afferents in the trigeminal system. These axonal terminals exhibit the same discrete organization as the cells to which they project[8]. Further, cautery of a row of vibrissae follicles in the neonatal rat, a procedure which damages the peripheral tips of the primary trigeminal neurons, results in an anomalous organization of the thalamocortical projections in the adult rat[8]. Following cautery of the middle of the five rows of vibrissae follicles in the neonatal rat, the associated thalamocortical projections are distributed as a thin, fused band rather than as a row of discrete clusters. This is an effect which is very similar to that of vibrissae cautery on cortical cytoarchitectonics, and it suggests that the cortical changes may be mediated through the afferent input. This suggestion is also supported by the finding that both the terminals of the trigeminothalamic fibers in the thalamus, the secondary afferents, and the endings of the trigeminal nerve, the primary afferents in the brainstem, are distributed in a similar discrete fashion in the normal young rat and exhibit similar anomalous changes after vibrissae cautery[1]. It should be pointed out that simple vibrissae removal does not produce any detectable changes and that, while the changes which can be detected in the thalamus and cortex are remarkably similar, they differ slightly from those in the brainstem where there is a coalescence of adjacent clusters into a band but no narrowing of the band. In addition, in the brainstem there is a reduction in staining density which is not detectable at the other levels. This is probably attributable to the direct damage of the trigeminal ganglion fibers. In summary, there is a discrete afferent organization which can be affected by peripheral manipulation at each synaptic station between the face and cerebral cortex of the rat. This is illustrated in Fig. 1.

The time course of afferent organization

These changes in afferent organization following peripheral manipulation are of particular import as they can be demonstrated to occur during the initial development of the trigeminal system rather than occurring later from atrophy or disuse, a possibility when the effects of a manipulation are not assayed until sometime after their production. This is a problem which is

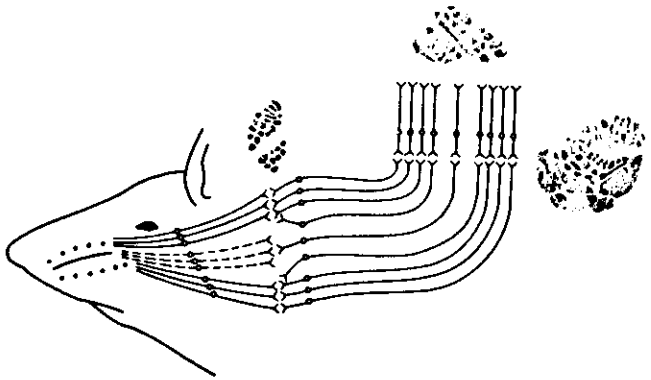

Fig. 1. Summary of the actual and postulated effects of vibrissae damage on pattern formation in the trigeminal system. Following removal of the middle row (only three of the five rows are illustrated in the schematic) changes are detected in the terminations of the primary neurons in the brainstem, secondary neurons in the thalamus and tertiary neurons in the cortex. At all three levels the effect is a band rather than discrete clusters. However, in the brainstem the terminal territory associated with the damaged row is unchanged while the thalamic and cortical bands are narrower than usual. Conversely, there is a reduction in staining density in the brainstem but no detectable density changes in the thalamus or cortex. The neuronal circuit diagram illustrates a hypothesis which accounts for these effects. See text for details.

particularly acute in anatomical experiments on developing systems if they involve tracer methods requiring surgical intervention and waiting a time period for either transport or degeneration to take place. However, we are fortunate to have at our disposal an anatomical method (succinic dehydrogenase histochemistry) which both delineates the afferent organization in the neonatal rat trigeminal system particularly well and requires neither surgical intervention nor a survival period[2]. Utilizing this method it was possible to ascertain that the normal discrete organization of the rat trigeminal system develops sequentially from brainstem to cortex commencing at about the time of birth. The central correlates of the vibrissae representation are present in the brainstem at the time of birth although they do become more distinct during the following two days (in fact, there are three separate vibrissae representations in the brainstem, although for this review they can be treated as a single entity). The vibrissae representations appear in the thalamus approximately forty-eight hours after birth and in cortex after a further twenty-four hours[6,7]. In a separate series of experiments, a row of vibrissae follicles was cauterized at birth and its central representations were assayed for the initial appearance of the anomalous organization[6,7]. From these experiments we conclude that the anomalies in the central representations arise during the initial formation of the discrete segmentation rather than at a later time.

These experiments also suggest that the brainstem vibrissae representations may serve as a template for the formation of patterns in thalamus and cortex. This is supported by an experiment which determined the period during which the central representations are sensitive to peripheral manipulations[8]. Following vibrissae cauterization at various times after birth, the effect of this manipulation was assayed at each level of the neural axis (this was accomplished within the same animals to minimize interanimal variation). From this experiment, we concluded that there is one sensitive period for the entire trigeminal system, and that this period coincides with the period during which damage produces an anomalous organization in the brainstem (a contention which is supported by physiological evidence that the functional changes in cortex after vibrissae cautery are the same as the changes in the brainstem [P. Waite, personal communication]). Thus, it is the pattern which is formed in the brainstem which is replicated in the more rostral portions of the neural axis. Currently, our view of this process is as follows (see Fig. 1). The pattern in the terminals of the primary trigeminal afferents within the brainstem is replicated along the neural axis by processes which involve an attraction of the dendrites of the secondary neurons to the central tips of the primary neurons. After damage more dendritic processes of secondary neurons are attracted to the central processes of healthy, undamaged primary afferents than to the central processes of primary neurons whose peripheral processes were damaged. This dendritic distribution may then direct the central distribution of the secondary afferents by a process of intracellular communication, and the narrow band of secondary afferents in the thalamus which results from follicle damage reflects the decrease in number of secondary brainstem neurons contacting the central processes of the peripherally damaged ganglion cells. At the cortical level, in the tertiary afferents, the pattern is a simple replica of the preceding one, hence, the marked similarities between the thalamic and cortical patterns. This view is based on several assumptions. First, dendritic processes are attracted by normal neuronal activity during development. Second, neurons receiving similar input congregate or fasciculate. Third, the entire neuron, including axonal and dendritic processes, is involved in the establishment of the pattern by a process of 'intracellular communication'. This view also points to the periphery as providing the master template for central pattern formation. Thus, to understand pattern formation in the trigeminal system, it is essential to determine how the initial pattern is established in the peripheral processes of trigeminal ganglion fibers.

Formation of the peripheral master pattern

Indirect evidence on the nature of the peripheral pattern has been obtained from experiments which have manipulated the pattern of peripheral vibrissae damage and determined the central consequences of these manipulations. Doing so, we have determined that the boundaries between rows are much more refractory to manipulation than the boundaries within a row. For example, while removal of an entire row of vibrissae results in a fused band

Fig. 2. Scanning electron micrograph of the face of a 14 day old rat fetus. In particular note the five nascent ridges which are separated by deep grooves and the individual domes on these ridges.

rather than in discrete clusters of afferents centrally, the effect of removing an arc composed of the same number of vibrissae across rows is much less severe[6]. Following such a procedure, there is some tendency for a fusion of afferent clusters across rows, but for the most part individual clusters maintain their integrity. Similarly, removal of all five rows of vibrissae results in five fused bands of afferents within the central nervous system and no evidence of the breakdown of between-row boundaries[6,7]. The normal development of the afferent segmentation in the thalamus, where it is the easiest to follow, also suggests that there may be differences in the formation of within-row versus across-row boundaries. The segmentation pattern first appears as bands related to entire rows of vibrissae, and it is only twenty-four hours or more later than within-row segmentation becomes discernible[8]. Similarly, sectioning the trigeminal nerve at the time of birth results in the complete absence of a central pattern (note that this is an effect which is greater than that of removing all the vibrissae at the same age), while nerve section twenty-four hours later results in a pattern characterized by across-row boundaries and the absence of within-row boundaries (Killackey, unpublished observations). Still later nerve section (seventy-two hours after birth) does not influence initial pattern formation. These experiments suggest that factors responsible for the formation of the borders between a row of vibrissae and those within a row may differ in terms of their mechanical and temporal properties.

From this evidence and several other anatomical studies, a hypothetical outline of the development of the initial pattern on the face of the rat can be constructed. *In utero*, the development of the vibrissae pad is a relatively early developmental event which either precedes or occurs concurrently with the genesis of the trigeminal ganglion neurons[9]. On the muzzle the pattern appears first as five longitudinal ridges of epithelium on which individual vibrissae are represented by domes which develop in a gradient from posterior to anterior[10]. This is illustrated in Fig. 2. These ridges are separated by grooves which are deeper and more clearly delineated than the spaces between the domes of a given ridge. Further, it should be noted that the trigeminal nerve fibers emerge from the skull in close proximity to the posterior ends of the ridges where the initial domes form, a position from where emerging fibers can be guided into the ridges and attracted to individual domes. A candidate source for such an attractant is the Merkel cell. These specialized cells are a prominent constituent of the vibrissae follicle[10] and have been demonstrated to evoke directional growth of nerve fibers in amphibian epithelium[4]. Further, this attractant ability is apparently lost once a nerve fiber contacts a Merkel cell. Thus, the establishment of the initial pattern of vibrissae representation may be hypothesized to be the result of a combination of mechanical, chemical and temporal factors. The emerging and exploring trigeminal ganglion fibers are channeled into ridges by the deep grooves. Initial fibers are attracted by the Merkel cells of the first forming domes located in a posterior position near the point where the fibers emerge through the skull. As the initial domes are innervated, their ability to attract nerve fibers is lost, and later emerging fibers continue anteriorly, guided by the grooves and attracted to appropriate target sites by Merkel cells of later developing domes. The above hypothesis serves as a preliminary model for understanding the formation of a master pattern in the periphery of the rodent trigeminal system and hopefully will stimulate further research both in my own and other laboratories. However, it should be noted that this hypothesis only points to, rather than answers, what must be regarded as the major question in central pattern formation. Simply put, this question is how does the peripheral process of a neuron tell its central analogue where it is located in space? Presumably, it is by the same mechanisms of 'intracellular communication' in central neurons and their processes referred to above. However, at present it is much easier (at least for this author) to conceive how the left hand lets the right hand know what it is doing than how a dendrite communicates spatial information to the axon of the same cell.

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