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Chapter Ten

DEVELOPMENT OF SOMATOSENSORY SYSTEM STRUCTURES

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I INTRODUCTION

At all levels of the mammalian central somatosensory system there is a characteristic topographic map that reflects the distribution and density of receptors on the body surface. While such somatotopic organization is usually inferred from neurophysiological recording procedures, it can be directly observed with routine anatomical techniques in the somatosensory system of a number of small rodents. This relationship between the periphery and central structure was first noted by Woolsey and Van der Loos (1970), who correlated the distribution of multicellular cytoarchitectonic units in layer IV of mouse somatosensory cortex, which they called "barrels," with the distribution of mystacial vibrissae on the snout. Since the initial description of this isomorphic relationship between the periphery and central structure, a number of investigators have studied the developmental events that underlie this relationship and how it can be modified during the course of development.

The focus of the present review is on morphological events that underlie the formation of somatotopic patterns within the central nervous system. An analysis of this problem, like most problems in developmental biology, involves two complementary approaches. The first is a description of the normal developmental events related to the system under investigation. The second is the experimental manipulation of some aspect of the system under investigation during the course of its development and assaying the outcome of the manipulation on the system under investigation. The premise underlying the second approach is that the aberrant organization resulting from the experimental manipulation will shed some light on normal developmental mechanisms. The present review will lean heavily toward the first approach, as the second is treated in detail in other chapters (see Chapters 11 and 12, this volume). However, some overlap is inevitable, as experimental manipulation provides necessary verification of hypotheses based on the description of normal developmental events.

The evidence reviewed in this chapter leads to several generalizations about the development of somatotopic patterns within the central nervous system. First, somatotopic patterns are formed in a sequential order beginning at the periphery and ending in the cerebral cortex. This sequence along with supporting experimental evidence has led to the view that a pattern at the periphery provides a primary template that is replicated in sequence at each level of the somatosensory system and that the process of pattern formation at a given level of the system is dependent on the previous level. Second, the formation of somatotopic patterns is a relatively late developmental event and is best regarded as an overlay on a preexisting topographic order. Together, these generalizations suggest that the periphery plays a limited but important instructive role in the organization of the central nervous system.

Finally, it should be pointed out that while the development of the three major sensory systems represented in the cerebral neocortex (audition, vision, and somatosensory) have much in common, there may be differences between these systems. For example, the distribution of peripheral somatosensory receptors is clearly punctuate, while the retina, on the other hand, although possessing some regional variations in receptor density, is more clearly a continuous receptor surface. Further, at the cortical level, the mammalian visual system has the added task of combining projections from both sides of the neural axis to form a coherent map of visual space, while in the somatosensory system the representation of each half of the body surface is confined to one-half of the neural axis. Such differences may well be reflected in the ontogeny of a given sensory system and exploited to provide a fuller picture of the development of the brain.

II OVERVIEW OF THE LEMNISCAL PATHWAYS

Somatosensory information is processed and represented both within the brain and the spinal cord. However, the development of patterns of somatotopic organization within the cerebral cortex appears to be most closely coupled with the development of the lemniscal pathways. The evidence for this statement is the major focus of this review, but before turning to this evidence, it is necessary to briefly outline the organization of the lemniscal pathways.

A Peripheral Organization

Tactile sensation is subserved by a diverse group of mechanoreceptors that are distributed throughout the body surface in a nonrandom fashion. In small rodents, a particularly important collection of receptors are located on the snout (Woolsey et al., 1975). These are associated with the mystacial vibrissae. In the mouse and rat, these large tactile hairs are organized into five rostral–caudally organized rows of four to seven large tactile hairs (designated rows A through E from dorsal to ventral). Other species possess a varying number of rows of vibrissae; for example, there are seven rows of vibrissae on the face of the gerbil. The mystacial vibrissae are not a passive organ of touch. Each mystacial vibrissa follicle is encased in a sling of muscle tissue that allows the vibrissae to be actively moved or whisked in concert at a species-specific rate (Dorfl, 1982). The ensemble of whiskers and the accompanying musculature form the vibrissal or whisker pad, a sensitive tactile organ for the exploration of the environment (Vincent, 1912; Welker, 1964). Each mystacial vibrissa follicle is complexly innervated at two levels of the hair shaft and contains a rich complement of mechanoreceptor types including Merkel discs, Golgi-Manzoni, lanceolate, and free nerve endings (Andres, 1966; Renehan and Munger, 1986). The vibrissa follicles are innervated by the infraorbital nerve, which is the largest peripheral nerve in the

mouse and rat. In the rat, the infraorbital nerve contains approximately 33,000 nerve fibers (Jacquin et al., 1984). It is in turn a branch of the maxillary nerve, which becomes one of the three major subdivisions of the trigeminal nerve. Each vibrissa follicle is innervated by both myelinated and nonmyelinated fibers (Vincent, 1913). The number of myelinated nerve fibers innervating a given vibrissa follicle is related to the position of that follicle within a row. For example, the C-1 vibrissa, which is located in the rostral end of that row, is innervated by an average of 69 myelinated nerve fibers, while the more caudal C-6 vibrissa is innervated by 162 nerve fibers on average (Lee and Woolsey, 1975). Physiological studies indicate that each trigeminal ganglion cell is responsive to stimulation of a single vibrissa (Zucker and Welker, 1969).

Central patterns suggest that there are also discrete distributions of peripheral receptors on the glabrous surface of the forepaw and hindpaw as there are discrete representations of both the pads and digits of the distal extremities within the central nervous system (Welker, 1976; Belford and Killackey, 1978; Dawson and Killackey, 1987). The association between peripheral receptor distributions and central patterns has not been as clearly established for the limb representations as for the mystacial vibrissae. The dermal papilla of the glabrous skin of the mouse digital pads contain many digital or Meissner corpuscles (Ide, 1976). Most likely it is these receptor complexes that are associated with the primary afferents of the limbs and the corresponding central patterns. However, this needs to be more thoroughly investigated. The forepaw is innervated by the radial, ulnar, and medial nerve and the hindpaw by the sciatic and saphenous nerve. The cell bodies associated with these primary afferents are located in dorsal root ganglia found at cervical and lumbar portions of the spinal cord, respectively.

B Subcortical Organization

The primary afferents conveying tactile information from the body surface terminate in the brainstem and spinal cord. In the brainstem, afferents associated with the face terminate in the brainstem trigeminal nuclei and those associated with the forelimb and hindlimb terminate in the dorsal column nuclei (see Figure 1). On entering the brainstem, trigeminal afferents bifurcate into ascending and descending branches (Cajal, 1911). The ascending branches terminate in the principal sensory nucleus and the descending branches in the spinal trigeminal nucleus, which can be further subdivided into the subnuclei oralis, interpolaris, and caudalis. A single trigeminal primary afferent innervates all of these subdivisions of the brainstem trigeminal complex (Hayashi, 1980). These afferents terminate in discrete clusters, which form bands running rostral to caudal in the horizontal plane and in the transverse plane replicate the pattern of mystacial vibrissae and sinus hairs on the face (Belford and Killackey, 1979a,b; Ar-

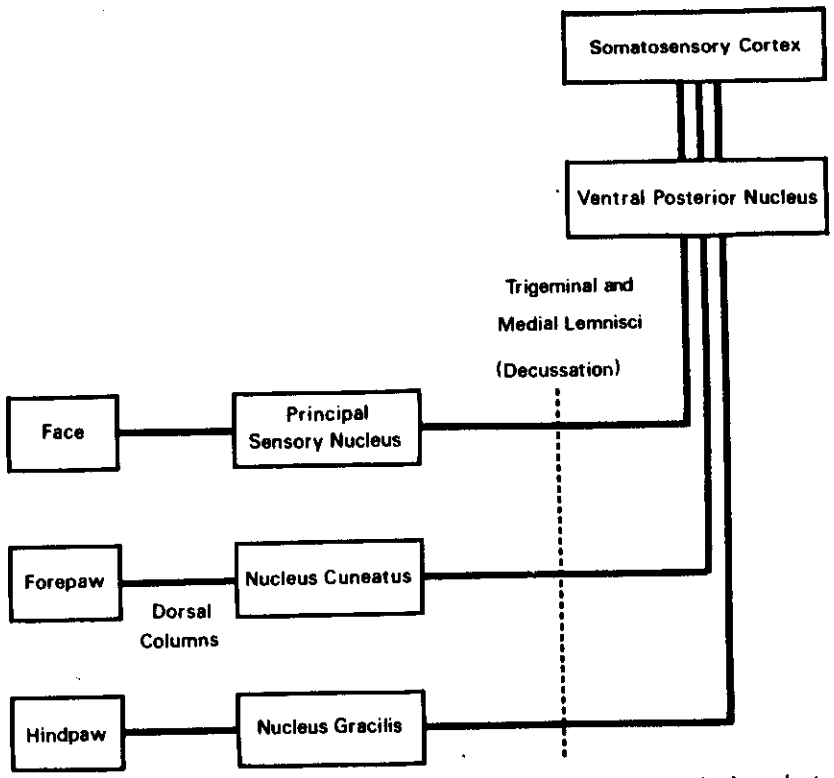

FIGURE 1. Diagrammatic representation of the somatosensory pathways in the rodent.

vidson, 1982; Bates and Killackey, 1985). This pattern is particularly obvious in the principal sensory nucleus and the subnuclei interpolaris and caudalis (Figure 2). This high degree of somatotopic organization is also demonstrable with physiological techniques (Nord, 1967).

Each of the subdivisions of the brainstem trigeminal complex projects in a unique fashion to other portions of the central nervous system. The major projections from the principal sensory nucleus are to the contralateral ventral posterior nucleus of the dorsal thalamus (Smith, 1973; Erzurumlu et al., 1980; Peschanski, 1984). The subnucleus interpolaris also projects to the contralateral ventral posterior nucleus as well as to the superior colliculus and the cerebellum (Smith, 1973; Fukushima and Kerr, 1979; Erzurumlu and Killackey, 1980; Killackey and Erzurumlu, 1981; Peschanski, 1984; Steindler, 1985; Bruce et al., 1987). The portion of subnucleus caudalis in which the vibrissae are represented projects to the lateral division of the facial nucleus, which in turn innervates the musculature of the vibrissae pad, suggesting that this is a pathway subserving reflex movements of the vibrissae (Erzu-

rumlu and Killackey, 1979). The connections of the subnucleus oralis are largely confined to the brainstem trigeminal complex itself; in particular, the subnucleus oralis projects heavily to the subnucleus caudalis (Hockfield and Gobel, 1982).

Primary afferents associated with the limbs are organized in a similar fashion. On entering the spinal cord these fibers also bifurcate, and one branch terminates within the spinal cord. The other branch travels the length of the spinal cord in the dorsal fasciculus and terminates in the dorsal column nuclei in the caudal brainstem (Basbaum and Hand, 1973). The nucleus gracilis, located most medially, is related to the hindpaw and the more lateral nucleus cuneatus is related to the forepaw. Both of these nuclei contain a pattern that can be related to their respective peripheral input (Belford and Killackey, 1978) and in turn project to the contralateral ventral posterior nucleus (Lund and Webster, 1967; McAllister and Wells, 1981). Thus, stretching across the upper part of the lower brainstem is a complete map of the body surface with the head represented laterally in the brainstem trigeminal complex and the hindpaw most medially in the nucleus gracilis.

The trigeminal lemniscus and the medial lemniscus are the fiber pathways between the brainstem somatosensory nuclei and the dorsal thalamus. The trigeminal afferents terminate in the dorsal and medial portions of the ventral posterior nucleus (ventral posterior medial, VPM), while those of the medial lemniscus terminate in more lateral and ventral portions of this nucleus (ventral posterior lateral, VPL). The VPM is the largest portion of this nucleus, and it is separated from the VPL by a clear fiber plexus. The entire nucleus is characterized by a high degree of somatotopic order that can be demonstrated with both physiological (Emmers, 1965; Waite, 1973; Rhoades et al., 1987b) and anatomical (Van der Loos, 1976; Belford and Killackey, 1979a,b; Ivy and Killackey, 1982) techniques. In this nucleus, like in the brainstem, a peripheral structure such as a vibrissa is represented by a cylinder of neural tissue that runs roughly rostral to caudal through the nucleus. In the VPM, the receptive field of the vast majority of neurons is restricted to a single vibrissa, suggesting the system possesses a high degree of spatial resolution.

One feature of the rat ventral posterior nucleus worthy of note is its relatively simple organization. In contrast to some thalamic nuclei, the rat ventral posterior nucleus appears on morphological, immunohistochemical, and functional grounds to contain no interneurons (Spacek and Lieberman, 1974; McAllister and Wells, 1981; Barbaresi et al., 1986; Harris, 1986). It consists of a relatively pure population of projection neurons. Its synaptic organization is correspondingly simple, and the three types of synaptic profiles found in the nucleus can be correlated with inputs from the lemnisci, cortex, and thalamic reticular nucleus. The major target of the ventral posterior nucleus is the primary somatosensory cortex (Killackey, 1973; Donaldson et al., 1975).

C Cortical Organization

The primary somatosensory cortex of all mammals is characterized by a map that reflects both the distribution and density of receptors on the body surface. Usually, such a somatotopic map results from determining the cortical loci of low-threshold tactile stimulation of the body surface. In small rodents, somatotopic organization is a directly observable feature of primary somatosensory cortex. This was first noted by Woolsey and Van der Loos (1970), who described the relationship between the distribution of large mystacial vibrissae on the face of the mouse and discrete groups of cells in the fourth layer of cortex, which they termed "barrels." A combined anatomical and physiological study of the rat somatosensory cortex by Welker (1976) suggested that there was a similar anatomically visible organization in other parts of somatosensory cortex as well. This has recently been confirmed by Dawson and Killackey (1987), who provided evidence that other portions of the body surface, particularly the distal extremities, are discretely represented in the cortex. Overall, this anatomical map is congruent with the physiological map obtained by other investigators (Welker, 1971, 1976; Chapin and Lin, 1984). It should also be pointed out that there are both species and strain differences in the organization of the peripheral pattern that is reflected in the cortical pattern and, presumably, the subcortical patterns as well. Different species of rodents possess varying numbers of rows of vibrissae. For example, the vibrissae of both the mouse (Figure 2) and rat are arranged in five rows, while in the gerbil they form seven rows, and this peripheral difference is reflected in the organization and number of cortical barrels (Woolsey et al., 1975). Similarly, some mice have extra vibrissae and corresponding extra cortical barrels (Yamakado and Yohro, 1979), and this trait can be selected for in a breeding program (Welker and Van der Loos, 1986).

Primary somatosensory cortex, like most other cortical areas, is composed of six layers. The anatomically discrete map of the body surface is characteristic of the fourth cortical layer. This cortical layer is composed of stellate cells, and it is the layer in which the majority of thalamic afferents from the ventral posterior nucleus terminate. Within this layer, thalamocortical afferents form synapses with the dendrites and somata of these stellate cells and, also, with the layer IV portions of dendrites of neurons whose somata are found in other layers (White, 1978). The somatotopic map is reflected in the discrete termination pattern of thalamocortical afferents (Killackey et al., 1976; Killackey and Belford, 1979; Dawson and Killackey, 1987). Indeed, individual thalamocortical afferent terminations are of the same size as the barrel in which they terminate (Jensen and Killackey, 1987a). Further, there is a variation in both the size of termination and of the corresponding barrel that is related to the position of the vibrissae on the face. Vibrissae that are caudal on the face are larger and are innervated by

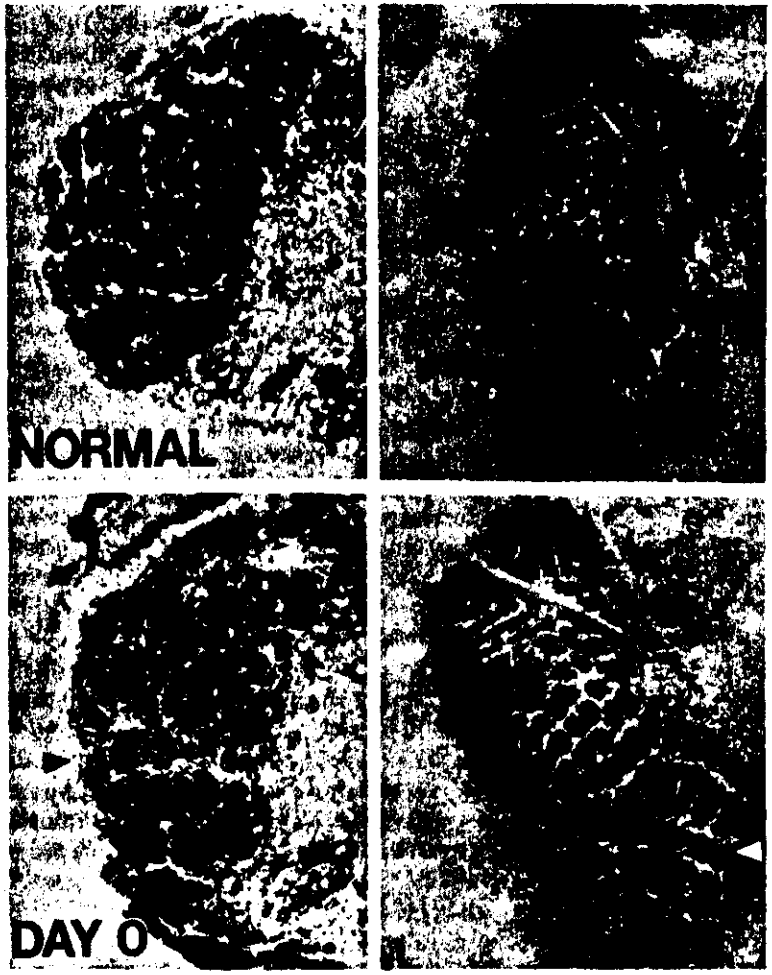

FIGURE 2. Each pair of photomicrographs shows the representations of the mystacial vibrissae at two levels of the somatosensory system of a single mouse. For each pair, the left micrograph shows the representation in the subnucleus interpolaris of the spinal trigeminal in coronal section and the right shows layer IV of the contralateral somatosensory cortex in flattened tangential section. Each animal was sacrified on postnatal day 9, and all sections were stained for succinic hydrogenase activity. *Normal:* This pair shows the normal pattern of vibrissae representation on postnatal day 9. *Day 0:* The pair in this figure and in the next two figures shows the vibrissae representation resulting from cautery of the vibrissae follicles in row C on the day shown in the label. The arrows point out the abnormal pattern related to the cauterized row of mystacial vibrissae. Note that (1) the extent of disruption of the row C representation decreases with cautery at progressively older ages and (2) for each day of cautery, the abnormal pattern in layer IV of somatosensory cortex mimics the abnormal pattern seen in the brainstem of the same mouse.

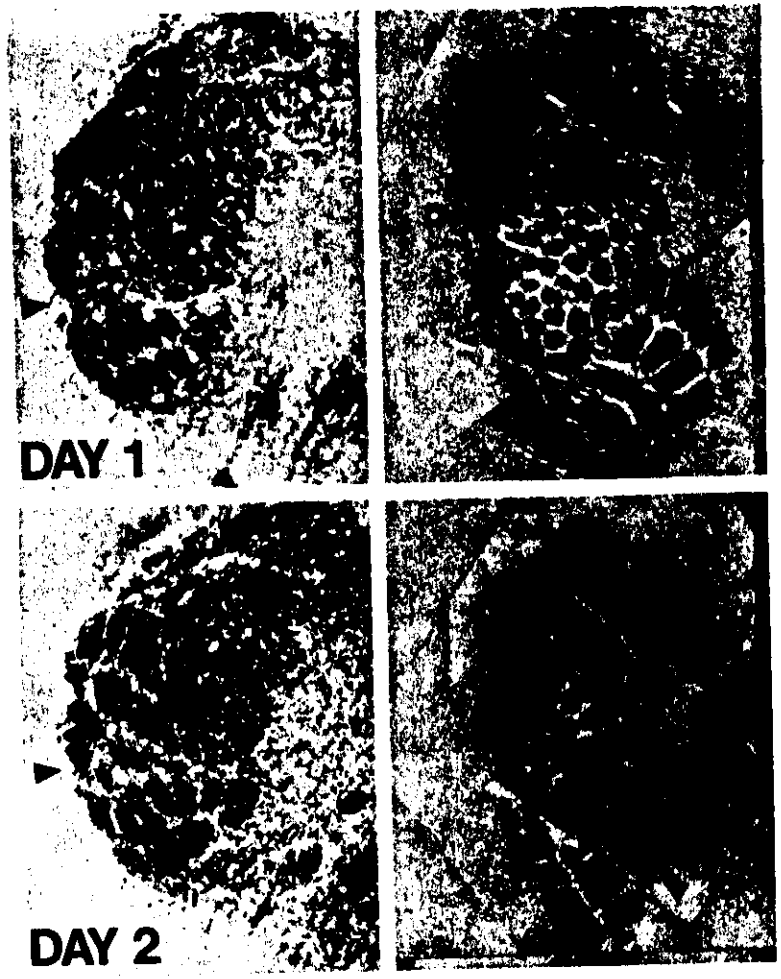

FIGURE 3. Effects of cautery of the vibrissae follicles in row C on postnatal days 1 and 2 (see Figure 2).

in the cortex by the number of cells that compose a given barrel, the volume of a barrel, and the size and branching density of the associated thalamocortical afferent (Lee and Woolsey, 1975; Welker and Van der Loos, 1986; Jensen and Killackey, 1987a). The dendrites of the barrel stellate cells are oriented toward the thalamocortical terminations. For example, the dendrites of a stellate cell on the side of a barrel are all oriented toward the center of that barrel and seldom cross the border between adjacent barrels (Lorente de Nó, 1922; Killackey and Leshin 1975; Steffen and Van der Loos,

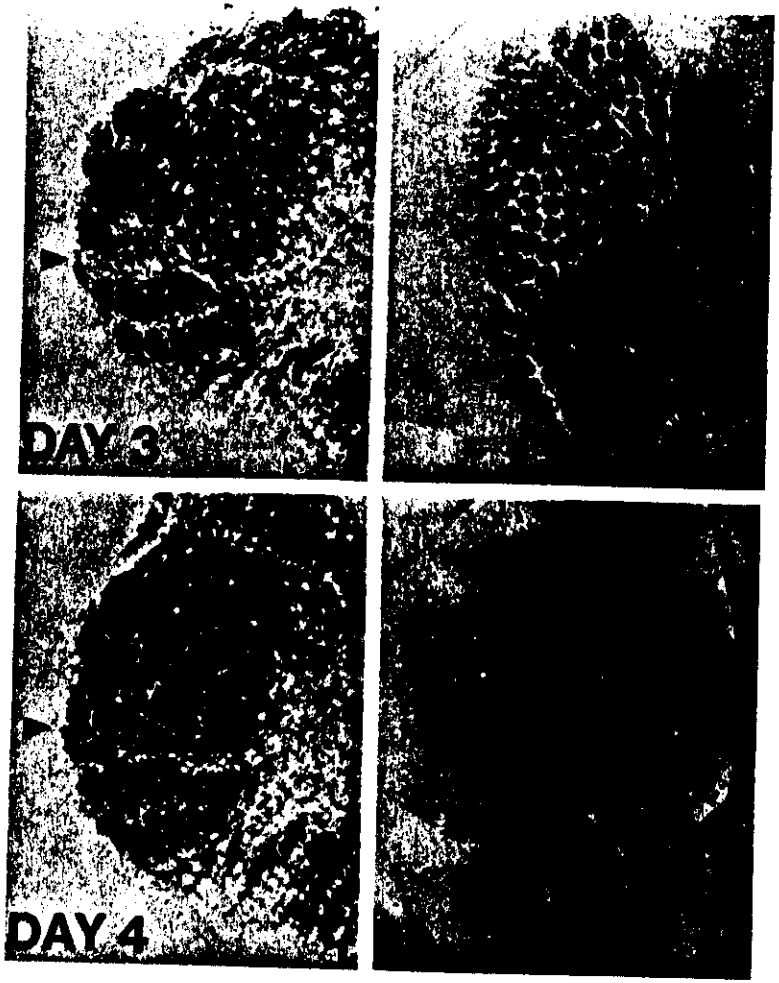

FIGURE 4. Effects of cautery of the vibrissae follicles in row C on postnatal day 3 and 4 (see Figure 3). Neural representation is less disrupted by surgical intervention on postnatal day 4 than on previous days.

1980; Harris and Woolsey, 1981). This highly specific morphological organization is also reflected in the functional properties of barrel neurons. The receptive field of individual barrel neurons is dominated by a single vibrissa (Simons, 1978). However, the tightness of this functional relationship has recently been questioned (Armstrong-James and Fox, 1987).

The discrete morphological organization of the fourth cortical layer is not obvious in the other layers of primary somatosensory cortex. The superficial cortical layers give rise to corticocortical projections as do portions of the

deep cortical layers (Akers and Killackey, 1978). Subcortical projections arise from restricted portions of the deep cortical layers (Wise and Jones, 1977; Killackey and Erzurumlu, 1981; Killackey, 1983; Bates and Killackey, 1984; Killackey et al., 1989). The surrounding cortical areas are major targets of the primary somatosensory cortex. One area of particular interest is located lateral and caudal to primary somatosensory cortex. This is the second somatosensory area. This area is somatotopically organized and receives major projections from the ipsilateral primary somatosensory cortex and projections from the opposite hemisphere via the corpus callosum (Koralelek et al., 1990).

A final point with regard to the normal organization of the rat somatosensory cortex is the distribution of interhemispheric projections. These projections, which arise and terminate in both the supragranular and infragranular layers of the rat, surround and interdigitate the primary somatosensory cortex of the rat (Akers and Killackey, 1978). Overall, they form a pattern that is complementary to the pattern of thalamocortical afferents (Olavarria et al., 1984). This high degree of organization in these projections as well as in many other portions of the somatosensory system between the periphery and cortex raises a number of intriguing questions about the development of this system.

III DEVELOPMENT OF THE LEMNISCAL PATHWAYS

A The Periphery

The characteristic patterns of the rodent somatosensory system first develop in the periphery. The sequence in this process has been best detailed in the trigeminal system. The development of the vibrissa pad is a relatively early developmental event that begins about embryonic day 10 in the mouse and a day or two later in the rat. (In general, the developmental sequence in the rat lags behind that of the mouse by about 2 days; allowing for this time lag the developmental events in the two species appear to be quite similar.) On the muzzle, which at this time can be subdivided into a nasal and maxillary process, the pattern appears first as five longitudinal ridges of epithelium separated by grooves. Superimposed on the ridges a series of domes that are the precursors of the vibrissa follicles develop in a caudal-to-rostral sequence (Yamakado and Yohro, 1979; Van Exan and Hardy, 1980). The hair follicles are present by embryonic day 12 in the mouse, and the associated mechanoreceptors (the Merkel discs in particular) develop at a still later time (English et al., 1980). These events appear to be an extrinsic property of the epithelium. Andres and Van der Loos (1982) have presented evidence that facial epithelium that is isolated before trigeminal innervation and raised in tissue culture will express a pattern of vibrissa follicles. There is also recent evidence that the facial epithelium produces a growth factor

capable of attracting the outgrowing trigeminal nerve fibers (Lumsden and Davies, 1986). This growth factor appears to be specific for trigeminal fibers and to be present for only a short time in the developmental process.

The neurons that compose the trigeminal ganglion are largely produced on embryonic days 12 and 13 in the rat (Forbes and Welt, 1981). In the mouse, peak production is on embryonic day 11 (Taber Pierce, 1970). As early as embryonic day 12 in the rat trigeminal primary neurons can be seen to be distributed in a polarized fashion with their peripheral and central processes arranged along the same axis (Erzurumlu and Killackey, 1983). This axis appears to radiate from the point at which the central processes contact the brainstem. The peripheral processes are pointed straight at their epithelial targets. During the next several days, the trigeminal ganglion differentiates into its three major components (the ophthalmic, maxillary, and mandibular subdivisions), and there is a straight outgrowth of the peripheral processes of ganglion cells to their epithelial targets. This occurs during the same time as epithelial differentiation is taking place. The epithelial events described in the preceding are first detectable in the rat on embryonic day 14. On this day, fine nerve fibers can be seen to contact mesenchymal condensations that are the first signs of follicle formation. It seems likely that the growth factor referred to in the preceding plays some role as a local signal in the final stages of the growth of the peripheral fiber toward its epithelial target. The general morphology and innervation of the vibrissa follicle at the light microscopic level resembles that of the adult around embryonic day 17.

A major unresolved question is how the epithelial-based pattern is coded in the trigeminal nerve and passed to the central nervous system. At present, it is only possible to speculate about the mechanisms involved. The pattern of fasciculation in the peripheral portion of the trigeminal ganglion fibers that is present at late prenatal stages may be a reflection of these mechanisms (Erzurumlu and Killackey, 1983). The spatial distribution of the peripheral targets of the trigeminal nerve is reflected in the fasciculation pattern, and this pattern develops after the epithelial pattern. In the mouse, approximately 50% of the original population of trigeminal ganglion neurons and fibers are eliminated during this period by naturally occurring neuronal death (Davies and Lumsden, 1984). There is also evidence that this occurs in the rat (Renehan and Rhoades, 1984). Lumsden and Davies (1986) have recently presented evidence that at early ages before an ordered pattern of fasciculation would presumably develop in the mouse some subpopulation of peripheral fibers shift fasciculi during their course. Perhaps it is this subpopulation of fibers that is eliminated. It should also be emphasized that the ordered pattern of fasciculation should be regarded as a relatively gross reflection of cellular adhesive interactions that may play a role in the transmittal of the pattern rather than as a causative agent. How the pattern in the peripheral portion of the trigeminal fibers comes to be expressed in their central processes has yet to be clearly elucidated. In this regard, it would be of extreme interest to determine the morphological organization of the central portions of primary trigeminal afferents during the course of development.

The central processes of trigeminal primary afferents reach the vicinity of their brainstem target quite early in development. In the rat they appear to have reached the brainstem by the embryonic day 12. The clustered pattern of terminations associated with these afferents develops much later, around the time of birth. This will be dealt with in the next section.

The development of primary afferents associated with the limbs have been studied in less detail. In the rat, genesis of dorsal root ganglion cells located at lower cervical and upper thoracic levels that later innervate the forelimb takes place between embryonic days 12 and 14 (Altman and Bayer, 1984). The time course of neurogenesis of dorsal root ganglion neurons located at lower lumbar levels and associated with the hindpaw is approximately 1 day later. Peripheral processes of dorsal root ganglion neurons reach the epithelium by embryonic day 16 or 17 but do not form functional connections with receptors until embryonic day 20 or later (English et al., 1980). The central processes of dorsal root ganglion neurons associated with the forepaw reach their target, the cuneate nucleus, on embryonic day 17. Hindlimb afferents reach the gracile nucleus on the following day (Altman and Bayer, 1984). Thus, by the time of birth primary somatosensory afferents have established contact with both their epithelial and brainstem targets. In addition primary afferents have also developed functional properties by this time. Fitzgerald (1987) has recently reported that primary afferents at the lumbar level are spontaneously active by embryonic day 16 and can be activated from small, well-defined peripheral receptive fields on the following day.

B The Brainstem

The neurons that compose the brainstem trigeminal nuclei are generated toward the end or after the time period during which the primary trigeminal afferent neurons are generated. In the rat, neurogenesis as determined by tritiated thymidine labeling takes place on embryonic days 13 and 14 (Altman and Bayer, 1982). The sequential order of generation of the primary and secondary neurons of the trigeminal system is continued in more central stations (see what follows), and overall, the trigeminal system is generated in a centripetal fashion.

The vibrissa-related pattern develops in the brainstem around the time of birth. On the day of birth (embryonic day 21) a pattern can only be detected in the lateral portions of the brainstem trigeminal nuclei where the most caudal vibrissae are represented. It should be noted that these are the vibrissae that develop first. Over the next day or two a pattern becomes visible in more medial portions of the trigeminal nuclei. Thus, in the [illegible] develops along the same gradient as vibrissae de-

velop on the face (Erzurumlu and Killackey, 1983). As near as can be determined, the vibrissa-related pattern develops concurrently in both the terminations of the primary afferents and in clusters of trigeminothalamic relays of the principal sensory nucleus. One would assume that the primary afferents play a causal role in the clustering of the relay neurons, but direct evidence for this is lacking, as is an understanding of the attractive interactions that would bring about the clustering of relay neurons. The indirect evidence that supports this notion is the fact that if a row of vibrissae are cauterized on the day of birth, the associated primary afferents degenerate (Bates and Killackey, 1985; Killackey, 1987) and the associated neurons in the principal sensory nucleus fail to cluster normally (Bates et al., 1982; see Figure 2 on the nucleus interpolaris).

The interaction between primary afferents and their target relay cells in the principal sensory nucleus have been hypothesized to be the primary event in pattern formation in central trigeminal structures (Belford and Killackey, 1980; Bates and Killackey, 1985). If vibrissae are damaged after the first few days of life when normal clustering has already occurred, more central patterns in the thalamus and cortex are unaffected (Figures 2–4). Thus, the system is characterized by a "sensitive" or "critical" period when peripheral damage can result in altered central organization. This period most likely coincides with the normal time course of primary afferent induction of clustering of principal sensory nucleus thalamic relay neurons. There are, however, alternate interpretations of sensitive and critical periods (see Woolsey, Chapter 12). It should also be mentioned that while vibrissa-related patterns are characteristic of three brainstem trigeminal nuclei (the principal sensory nucleus and subnuclei interpolaris and caudalis), only the principal sensory nucleus seems to play a role in pattern formation in more rostral structures. Neonatal lesions of the principal sensory nucleus result in a lack of pattern in the ventral posterior nucleus while similar lesions of the spinal trigeminal nuclei do not effect pattern formation in the ventral posterior nucleus (Killackey and Fleming, 1985).

The dorsal column nuclei are generated a day or two later than the brainstem trigeminal complex (Altman and Bayer, 1984). Forelimb primary afferents reach the cuneate nucleus on embryonic day 17; hindpaw afferents reach the gracilis nucleus on the following day. This difference in arrival time has been exploited to shed light on the role of primary afferents in specifying their targets. If a rat forelimb is amputated before primary afferent central processes have reached the cuneate nucleus, hindlimb afferents are capable of expanding their terminal territory to include portions of the cuneate as well as the gracile nucleus. Such an expansion of gracile afferents does not occur if the forelimb is removed after the forepaw afferents have reached the cuneate nucleus (Dawson and Killackey, 1986; Killackey and Dawson 1989). This suggests that once a target has been invaded by primary afferents, it is no longer capable of accepting afferents from a second source. This observation may also explain the difference between the

the hamster and rat trigeminal system to section of the infraorbital nerve on the day of birth (Rhoades et al., 1983). Following infraorbital nerve section in the newborn hamster, the mandibular afferent terminations expand into the denervated maxillary territory. Such an expansion does not occur in the newborn rat. This difference is more likely due to the relative immaturity of the hamster at birth compared to the rat and, one would assume, the incomplete innervation of the hamster's brainstem trigeminal nuclei at birth. Together, these results underscore the similarity in developmental mechanisms throughout the entire somatosensory system.

C The Thalamus

The neurons that compose the ventral posterior nucleus of the rat are generated rather abruptly on embryonic day 14 (McAllister and Das, 1977). As at other levels of the somatosensory system, this same event occurs a few days earlier in the mouse (Angevine, 1970). Brainstem lemniscal afferents grow into the ventral posterior nucleus during the late embryonic period. On embryonic day 20, lemniscal fibers can only be detected in the caudal and ventral portions of the ventral posterior nucleus. By the time of birth, they have reached the full extent of the nucleus although the density of fibers in portions of the nucleus continues to increase until postnatal day 4 (unpublished observations). During the first few postnatal days, these afferents first segregate into bands that can be related to the rat's five rows of vibrissae and then further segregate into individual discrete clusters within the bands. During this same period, the distribution of thalamocortical relay cells are undergoing a similar shift in their distribution from continuous to discrete (Ivy and Killackey, 1982a).

The other major afferent to the ventral posterior nucleus arises in the somatosensory cortex. Corticothalamic afferents invade the ventral posterior nucleus at a later time than the lemniscal afferents. One day after birth, these afferents surround rostral and lateral portions of the ventral posterior nucleus but have yet to invade it in a major way. This process occurs during the next several days. By postnatal day 4, these afferents are distributed throughout the nucleus in a latticelike pattern that is a mirror image of the vibrissa-related clusters of trigeminal afferents (Akers and Killackey, 1979). Over the next week, this latticelike pattern is obscured by the gradual encroachment of the corticothalamic afferents into the discrete clusters that are the domain of the trigeminal afferents and are not detectable in the adult (Hoogland et al., 1987). Other aspects of the vibrissa-related morphological pattern noted above also gradually become less distinct during this period. Evidence from Golgi studies (Scheibel et al., 1976) suggest that this gradual obscurement is correlated with the gradual increase in the complexity and overlap of afferent terminal arbors of these fiber systems as viewed at the light microscopic level during this period. It

crete patterns detectable during the early postnatal period is not in any sense a breakdown of the high degree of somatotopic organization that characterizes the ventral posterior nucleus.

Section of the infraorbital nerve on the day of birth results in the absence of a vibrissa-related pattern within the ventral posterior nucleus (Killackey and Shinder, 1981). The effects of more subtle peripheral manipulations such as the removal of a row of vibrissae are also clearly detectable in this nucleus. The effects of such a manipulation at birth can be detected within the ventral posterior nucleus with the succinic dehydrogenase (SDH) stain within 48 h of the time the peripheral damage is inflicted (Belford and Killackey, 1979b). Indeed, abnormal organization occurs with the same time course as normal discrete organization. This has been interpreted as evidence of the role of the periphery in guiding the formation of central somatotopic patterns. Given that the SDH stain is a reflection of the pattern of lemniscal terminations within the ventral posterior nucleus, it also suggests that it is the pattern of afferent terminations that is most closely controlled by the periphery. This point will be returned to in more detail later in the consideration of somatosensory cortex.

It is also important to emphasize the rather obvious point that the ventral posterior nucleus is further removed from the periphery than the brainstem somatosensory relay nuclei and that, consequently, the effects of a peripheral manipulation are somewhat different than at more peripheral levels. At the level of the primary afferent, peripheral manipulations such as follicle cautery (Savy et al., 1981; Bates and Killackey, 1985; Killackey, 1987) or nerve cut (Waite and Cragg, 1982) result in explicit degeneration or loss of fibers during the developmental period. The exact degree of loss, however, is difficult to determine, as primary afferents are capable of some degree of regeneration that complicates assessment (Rhoades et al., 1987b). It does seem reasonable to assume that the brainstem trigeminal targets of these afferents are relatively completely denervated for at least several days following peripheral manipulation (i.e., during the critical or sensitive period when the periphery plays its role in central pattern formation). This is supported by the finding that cortical-evoked potentials that can normally be elicited by vibrissa stimulation 2 or 3 days after birth are not elicited in nerve cut animals until at least 7 days after birth (Waite and Cragg, 1982).

At the level of the brainstem targets of the primary afferents there is some neuronal loss following complete vibrissa follicle destruction. Hamori et al. (1986) have reported an 18% cell loss and a 33% decrease in volume within the subnucleus interpolaris following such a manipulation in the mouse. This same study reported no loss of neurons within the ventral posterior nucleus in the same animals. On the contrary, they report an increased number and density of neurons in the ventral posterior nucleus in which the damaged periphery is represented, which they attribute to the failure of the normally occurring cell death process. In a previous study using a more restricted peripheral manipulation in the mouse, removal of a single row of vibrissae, Woolsey et al. (1979) report no changes in cell density between the affected region of the ventral posterior nucleus and the normal side. Whatever the basis of this discrepancy, these results do suggest that basic organizational features of the ventral posterior nucleus, such as the source and size of its afferent input and its basic cytological organization, are relatively normal in animals with neonatal peripheral trigeminal damage. The major change in the nucleus is in the pattern of afferent terminations formed in the nucleus, which in turn affects the distribution of the terminal arbors of its projection to the somatosensory cortex.

D The Neocortex

The neurons that compose the neocortex of the rat are generated over a time period that extends from embryonic day 16 to 21 (Berry and Rogers, 1965; Hicks and D'Amato, 1968). Further, as first demonstrated in the mouse (Angevine and Sidman, 1961), time of origin of cortical cells is reflected in their laminar position. Cortical neurons in the deeper cortical layers are generated before neurons in the more superficial cortical layers, resulting in an "inside-out" sequence of cortical development. Given that laminar location of a cortical neuron is also correlated with its projection target (Wise and Jones, 1977), the time of origin of cortical neurons may also be correlated with its ultimate projection target. Jensen and Killackey (1984) have provided experimental evidence for this hypothesis.

The neurons of the fourth cortical layer that compose the barrels are generated on embryonic day 18 in the rat. The first signs of the barrels in layer IV are detectable on postnatal day 3 in Nissl-stained material (Rice et al., 1985). This coincides with the arrival of thalamocortical afferents in layer IV (Wise and Jones, 1978) and the first appearance of discrete clusters of SDH staining in this layer (Killackey and Belford, 1979). At the cortical level, there is no evidence of the pattern developing along a gradient as it does in the brainstem; rather, the whole pattern appears to develop at approximately the same time (see however, Rhoades et al., 1990). This is probably attributable to the concurrent ingrowth of thalamocortical afferents into the cortical layers as opposed to the staggered growth of primary afferents into the brainstem. The effects of a peripheral manipulation such as removal of a row of vibrissae can also be detected at the same time as the normal pattern. Once again, this suggests that it is normal developmental events that are perturbed by neonatal peripheral manipulations. Several experiments provide some insight into these events at the cortical level.

At the time of birth, thalamocortical projections have reached the cortex but are located in the white matter beneath the still-forming cortical layers (Wise and Jones, 1978). Recent preliminary evidence, however, suggests that thalamocortical fibers may penetrate the cortical plate as early as postnatal day 1 (Senft and Woolsey, 1987). At the time of birth, discrete topographic relations between the thalamus and cortex can be demon-

strated by the retrograde transport of horseradish peroxidase from cortex to thalamus (Dawson and Killackey, 1985). Thus, topographic relations between the thalamus and cortex develop well before there are any hints of a vibrissa-related somatotopic pattern in either structure and even before the ventral posterior nucleus is fully innervated. This suggests that the overall topographic relations between these two structures are intrinsically determined before birth and that peripheral manipulations at birth affect events in this system that occur between birth and day 3. This is the time period during which thalamocortical afferents are growing into the fourth cortical layer and forming their terminal arbors.

It was previously noted that the afferent terminations of individual thalamocortical afferents completely fill the discrete cortical cluster with which they are associated and that cluster size can be correlated both with peripheral location and innervation density. This suggests that the major role of the periphery is in shaping the size of afferent terminations. Presumably, this process is in some way related to "activity" in the system and has occurred by between the third and fourth postnatal day when discrete clusters of SDH activity are apparent. Jensen and Killackey (1987b) have provided evidence favoring this hypothesis. Neonatal infraorbital nerve section that abolishes all evidence of a vibrissa-related pattern at the level of the brainstem and thalamus (Killackey and Shinder, 1981; Bates et al., 1982) also results in very anomalous cortical patterns that are not readily related to the periphery. Individual thalamocortical afferent terminations in adult rats subjected to neonatal nerve cut are also severely perturbed. Such terminations are much larger than normal and have a much reduced branching density. While this result directly supports the hypothesis that the role of the periphery in guiding somatotopic organization is exerted at the level of afferent terminations, two interpretations of the result are possible. First, the periphery plays a role in forming terminal arbors that are normally discrete at all stages including initial outgrowth. Second, the periphery functions in trimming back terminal arbors that are diffuse in their initial outgrowth. The short time period in which the terminal arbors are formed favors the first of these alternatives, but the question deserves further attention.

The periphery also appears to play some role in the sculpting of other aspects of cortical organization, namely, the distribution of cortical projection neurons. While this role has yet to be clearly defined, some hints of it are evident in the distribution of callosal projection neurons. As noted in the preceding, callosal projection neurons and terminations that in adult rat somatosensory cortex are located in the supra- and infragranular layers are distributed in a complementary fashion to the discrete thalamocortical afferents arising from the ventral posterior nucleus (Akers and Killackey, 1978; Olavarria et al., 1984). At birth the cells of origin of the immature callosal projection are continuously distributed in the deep layers below the cortical plate, and callosal afferents are evenly distributed [illegible]

white matter (Ivy et al., 1979; Ivy and Killackey, 1982b). The discontinuous adult pattern is established during the next 10 days or so and is accompanied by the specific ingrowth of callosal afferents into appropriate regions. The mechanism underlying this change is the elimination of neuronal processes (O'Leary et al., 1981; Ivy and Killackey, 1982b). Double labeling has indicated that many neurons in the neonatal rat somatosensory cortex project both ipsilaterally to motor cortex, and presumably to other ipsilateral targets as well, and across the corpus callosum. During development neurons located within primary somatosensory cortex lose their callosal processes, resulting in the discontinuous distribution of callosal projections characteristic of the adult. This elimination process also seems to be influenced by the periphery. The complementary organization of thalamic and callosal projections in the normal adult may be taken as indirect evidence for this assertion. More direct evidence is provided by the distribution of callosal projection neurons after neonatal infraorbital nerve section, the same manipulation that profoundly disrupts the distribution of thalamocortical afferents. This same manipulation both reduces the density of callosal projection neurons and alters their distribution in a way that reflects the changed distribution of thalamocortical afferents (Koralek and Killackey, 1990). This manipulation also produces changes in the distribution of callosal projections outside of primary somatosensory cortex. The face region of the second somatosensory area which normally receives callosal projections is now devoid of callosal connections. This suggests that the train of organizational events that begins at the periphery and has been traced in some detail up to primary somatosensory cortex may continue in other cortical areas beyond primary somatosensory cortex.

The foregoing has focused on the trigeminal system as the unique distribution of peripheral receptors, and their central representations have generated considerable interest and can be experimentally manipulated with relative ease. However, the principles derived from the trigeminal system apply equally well to other portions of the somatosensory system. Dawson and Killackey (1987) have provided evidence that neonatal limb removal or section of the afferent innervation to the limbs at birth results in an anomalous organization of the associated cortical patterns. Amputation of a forelimb at or before embryonic day 17 results in an even more surprising cortical change (Dawson and Killackey, 1986; Killackey and Dawson, 1989). As noted in a previous section, this manipulation results in what has been interpreted as a partial invasion of the "virgin" cuneate nucleus by later arriving hindlimb primary afferents at the level of the brainstem. At the cortical level, the manipulation results in a doubling of the size of the cortical representation of the hindpaw. This expansion appears to be at the expense of cortical areas outside the primary somatosensory cortex. Such results further emphasize the point that no level of the central nervous system develops in isolation of other levels. The effects [illegible]

be interpreted within the larger framework of the entire system under study.

As a final point, it is obvious from the foregoing that the authors favor the hypothesis that the periphery plays a key instructive role in the formation of the central somatosensory system. This view has been challenged (Cooper and Steindler, 1986 and Cooper et al., 1989). These authors have postulated that glial associated adhesion molecules intrinsic to the developing neocortex play a primary role in forming borders between functional units such as "barrels". Further, they postulate that this glial event "results in the formation of 'premaps' which can be further sculped during early postnatal life by more precise map-conveying afferent systems, . . .". We regard this view as a very tenuous one for two reasons. First, the earliest detectable "barrel-like" patterns in the neocortex are clearly formed by extrinsic afferent systems and not intrinsic glial elements (Rhoades et al., 1990). Second, neonatal peripheral manipulations such as those reviewed above alter the patterns of glial boundaries just as patterns of neural elements are altered (Cooper and Steindler, 1989). Thus, at present the role of glial boundaries in pattern formation does not appear to be a primary one, rather their role is secondary to that of the extrinsic afferent input to a given level of the somatosensory neural axis.

IV SUMMARY

The studies reviewed in this chapter provide evidence that the overall development of the somatosensory system takes place in a sequential fashion beginning at the periphery and progressing centrally. These studies also demonstrate that the development of the system is accomplished by a number of diverse mechanisms, some of which are confined to particular levels of the system and others that operate at all levels. One of the most prominent features of the system at each level of the neural axis, somatotopic patterns, develops relatively late and seems to be most closely related to the formation of afferent terminations. Major unresolved questions involve the molecular mechanisms that play a role in sculpting of patterns of afferent terminals and their "transmittal" along the neural axis. Finally, the studies also point out that when focusing on a particular level of the system, it is important to do so within the larger context of the entire system, keeping in mind that what occurs at one level is the result of interactions intrinsic to that level as well as events that have occurred at previous levels.

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Neocortical Expansion: An Attempt toward Relating Phylogeny and Ontogeny

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Abstract

■ The neocortex is the most characteristic feature of the human brain. On gross inspection, its convoluted surfaces can be seen to have overgrown and covered most other brain structures. In the functional sphere, it is to the neocortex that we attribute those behaviors assumed to be most uniquely human such as cognition and linguistic behavior. This essay is an attempt to understand how this structure expanded during the course of mammalian evolution. At present, any attempt must be more speculative than definitive, but it is offered in the hope that it will generate more discussion on a topic that is central to all neurobiology, as well as a number of allied disciplines. I will proceed by outlining current views on the evolution of the brain, briefly review the organization of the somatosensory cortex in several mammalian forms, and then discuss in some detail ontogenetic mechanisms that may have some bearing on neocortical phylogeny. The primary proposition put forth is that the mammalian neocortex is relatively unspecified by strict genetic means, and that this allowed the neocortex to expand and adapt to a variety of circumstances during the course of phylogeny. ■

APPROACHES TO THE EVOLUTION OF THE BRAIN

The evolution of the brain has been approached from two directions. The first is by study and measurements of endocasts, the fossil shadows of once living brains. This approach, which has been most penetratingly applied by Jerison (1973), documents the history of the overall shape of the brain and its expansion from the first appearance of vertebrates in the fossil record to the present. Under the most favorable circumstances, this approach can also provide some insight into functional localization through preserved patterns of sulci and gyri (Radinsky 1975). However, this approach, powerful as it is, is limited in that it is restricted to the overall form of the brain and its external features. It reveals little about the internal organization of the brain, that is, the pattern of distribution of neurons and the relationships among them. The second approach addresses this problem. It is that of the comparative neurologist, the study of the morphology and connectivity of the brain in existing vertebrate species. This second approach was given considerable impetus by the introduction of a number of new and powerful neuroanatomical techniques beginning in the early 1960s (see Nauta and Karten 1970).

The data from these new approaches to neuronal connectivity are still somewhat fragmentary, as the internal organization of the brain has been studied in only a small number of extant vertebrate species, and in less detail in any species than is necessary for a complete picture of neuronal organization. However, the data that have emerged from these investigations are more than enough to alter the classical view of brain organization that was built on several assumptions that have not been borne out by recent investigations. These assumptions include the olfactory dominance of the telencephalon in early vertebrates, the progressive invasion of the telencephalon by other sensory systems (namely visual, auditory, and tactile), and the supposed emergence of discretely organized neuronal systems from earlier diffusely organized systems. (See Northcutt 1981 for review of this issue.)

There is a third approach to the study of the evolution of the brain. This is the study of the development of the nervous system. For it is during the course of development that the processes that mold the mature form of the brain are operating. I will suggest that there are at least two developmental processes that are relevant to understanding the evolutionary expansion of the mammalian neocortex. These are afferent specification of target tissue and the developmental exuberance of cortical neurons. It should be emphasized that this approach is

not that of recapitulation. I am not suggesting that development be studied to reveal either ancestral organization or phyletic history. The approach is based on the premise that similar developmental mechanisms are operating in all vertebrates and that small changes in these mechanisms, or in their rate of action, can provide the basis of evolutionary diversity. This point of view has been extensively articulated by Gould (1977).

A similar approach has recently been presented by Ebbesson (1984), in what he terms a "parcellation" theory of vertebrate brain evolution. There are several basic tenets of his proposition. First, the ancestral vertebrate brain was characterized by a diffuse organization. Second, that diffuse organization is still a major characteristic of the vertebrate brain during ontogeny. Third, that during both phylogeny and ontogeny there is a selective loss of connections within daughter systems and neural aggregates that result in the formation of new neural circuits and aggregates through processes of segregation, isolation, and parcellation. The proposal that I will present differs from this thesis in several significant ways. First, its scope is much more limited. It is aimed at providing insights into the expansion of mammalian neocortex and not at explaining the evolution of the vertebrate brain as a whole. Second, given neocortical expansion, it is very difficult to envision how this expansion could occur by essentially subtractive mechanisms. As a consequence, I will focus on plausible mechanisms by which cortical areas could be added. Third, I am more impressed than is Ebbesson by the evidence for the discrete organization of the vertebrate brain in the existing forms that have been studied both as adults and during the course of their ontogeny. The occurrence during ontogeny of an excess in neurons and neural processes is not necessarily evidence of diffuse organization. These phenomena occur within definable constraints that will be detailed below. It has been my experience that the use of the term "diffuse" to characterize a neuronal system more often reflects our state of knowledge of that system than the organization of that neuronal system (for example, see the recent review by Parnevelas & Papadopoulos 1989 regarding this issue in a different context).

Comparative neurologists, as biologists in general, are often concerned with the history of a trait or character. Is the presence of a character across a group of animals the result of its presence in a common ancestor or has the trait evolved independently in several different lines in response to common functional requirements? More formally, is a character that is being compared across a group of animals homologous or homoplastic? This issue, although of obvious import, is not the primary concern here. The issue to be examined is given that the cortical neural systems that process motor and somatosensory information are organized differently in various mammalian lines, can these differences in organization be understood in terms of the same developmental mechanisms. The primary concern is not what the condition was in the common ancestor, although this is of course relevant, and assumptions will be made about the initial state of organization of these systems. It will be assumed that the simplest organization is composed of the least number of cortical areas, that is, the least differentiated. The determination of homologies between portions of these systems in extant mammals is much more difficult. Clearly, the neocortex as a morphological structure is homologous across mammals. Similarly, the somatosensory system considered as a broad structural and functional entity is most likely homologous across mammalian forms. However, an attempt to establish homologies between isolated portions of the system in extant forms is exceedingly difficult if not impossible. It is my opinion that at this level of analysis we have gone beyond the point at which the concept of homology can be fruitfully applied at the present time.

This conclusion is similar to that reached by Ulinski (1983) in his discussion of forebrain organization in reptiles and birds. He points out that two alternate strategies for comparing structures in different organisms have long been recognized by comparative biologists. The first is an approach based on homology. However, for a number of reasons discussed by Ulinski it is difficult, if not impossible, to apply the concept of homology in a meaningful sense to neural structures across different lineages. Difficulties with the use of the term homology include the very definition of the term and at what level the term is applied (e.g., the individual neuron or ensembles of neurons), the establishment of ancestral relationships between lineages of extant vertebrates, and the criteria that are to be met for establishing homologies. Such criteria usually involve listing the similarities between the structures being compared, which brings us back to the difficulty of distinguishing between homologous and homoplastic traits. The alternative to an approach based on homology is one based on what Ulinski terms "design." That is to compare and contrast the organization of what is assumed to be the same functional system in different lineages. Although such an approach is fraught with difficulties of its own, these can be minimized, by making clear the assumptions that are made in the course of the analysis. This is the course that will be followed in my analysis of the expansion of mammalian neocortex.

PREVIOUS VIEWS OF CORTICAL EVOLUTION

For most of the twentieth century, the study of neocortical organization was dominated by cytoarchitectonics, the study of the structural organization of the neocortex as revealed by the Nissl method. The basic premise of this approach, which may be regarded as a descendant of phrenology, is that areal differences in the structure of neocortex reflect functional differences. To a large

extent this premise has been borne out by more recent studies on neocortical organization that have used a wide variety of morphological and functional techniques. However, it is important to point out that cytoarchitectonics alone cannot unequivocally define a cortical area. For example, a number of different cytoarchitectonic maps of rat neocortex place different portions of the unitary body surface map (which defines the primary somatosensory cortex in this species) in different cortical areas (compare Figure 1 of Dawson & Killackey 1987 with Figures 46 and 47 of the cytoarchitectonic atlas of Zilles 1985).

Many of the early cytoarchitectonists (particularly, Brodmann 1909 and von Economo 1929) recognized that the neocortex of what they termed "primitive" mammals was characterized by fewer cytoarchitectonic subdivisions than the neocortex of more "advanced mammals." This view, however, did not go entirely unchallenged. Lashley and Clark (1946) strongly advocated the position that there was no real difference in the number of cytoarchitectonic areas between mammalian species. They state that "the total number of functionally diverse areas demonstrated in primates, even including man, does not exceed the number found in lower mammals." A similar, but less extreme position, was also put forward by von Bonin and Bailey (1961). From this point of view, neocortical expansion would simply result in larger cortical areas. Clearly, the weight of modern evidence supports the proposition that neocortical expansion is accompanied by an increase in the number of neocortical areas. In defense of Lashley and Clark (1946), it should be mentioned, that their position was a necessary counterpoint to the views of the more extreme cytoarchitectonists who subdivided neocortex into increasingly more subdivisions on increasingly more tenuous criteria.

If the classical cytoarchitectonists noted an increase in the number of neocortical areas in larger brained mammals, how did they view the expansion process? Technical limitations prevented any real investigation of these processes and by current standards their views must be regarded as speculative. In general, new cortical areas were thought to emerge through the gradual subdividing of preexisting cortical areas by selective pressures. The mechanisms by which this could be accomplished were not considered in detail. The most recent and detailed views of the expansion of neocortex based on cytoarchitectonic criteria are those of Sanides (1970).

One of the most influential views of neocortical expansion was initiated by Bishop (1959). Based on an analysis of fiber diameter size, degree of myelinozation, and central targets of differing size axons in the somatic and visual sensory pathways, Bishop concluded that thicker myelinated fibers evolved later in phylogeny. Applying this conclusion to the neocortex, Bishop reasoned that highly myelinated areas were the newest cortical areas. Since primary sensory cortical areas are the most highly myelinated, they must be the newest cortical areas, and most likely emerged from the less myelinated and presumably less precisely organized secondary cortical fields. A similar view based on somewhat different evidence has been expressed by Diamond and Hall (1969), who noted the relative paucity of cytoarchitectonic and functionally distinct neocortical areas in a "primitive" mammal, the hedgehog. They suggested that the cortical representation of each sensory system, but most particularly the visual system, could be divided into core (primary visual area) and belt (second visual area) regions, and that the belt region is the phylogenetically older area. This assertion is based on the fact that the belt receives visual input via a pathway phylogenetically older than the core, that the cytoarchitectonic organization of the belt is more primitive than the core, and that its topographic organization is less precise than the core. They conclude that the belt region is the precursor of the more discrete core. This view implies that the formation of the later evolved core region (primary visual cortex) is brought about by the invasion of a new thalamic target by the retina and the formation of a new thalamocortical pathway in mammals (the geniculostriate pathway). However, more recent evidence suggests that this view needs some modification (for example, see Hall & Ebner 1970).

The increase in the number of functional topographically organized neocortical areas in different mammalian lines has been documented by a number of investigators using a variety of modern techniques. The work of Allman and Kaas in the visual system (see Kaas 1989 for review) and Kaas and co-workers in the somatosensory system (see Kaas 1983 for review) has been particularly influential. Both Allman (1982) and Kaas (1987) have entertained similar ideas on the genesis of new cortical fields although they have drawn their metaphors from different areas of biology (Kaas from the classical experimental literature and Allman from molecular biology). Both point out that replication of existing structure is an important factor in the development of new functional capabilities during the course of evolution. Thus, a new cortical area that replicates a preexisting one could result from a change in the genetic program from one generation to another. It is also conceivable that such a replication process could operate at levels other than that of the cortical area, for example, at a subcortical level or at the level of the cortical column. In both cases, ontogenetic constraints on the developmental process could well result in two different cortical areas with each of these areas having different patterns of connectivity, and, hence, different functions.

A limitation of some of the above views is that they attempt to distinguish between what is phylogenetically new and old on criteria that are bound to be somewhat arbitrary. Also, most of these views only indirectly address the question of the plausible mechanisms by which neocortical expansion could be accomplished. Before turning to such mechanisms, it is necessary to briefly

outline several of the major organizational features of neocortex.

GENERAL FEATURES OF NEOCORTICAL ORGANIZATION

Traditionally, the neocortex is regarded as a six-layered structure. This nomenclature recognizes a varying number of sublayers in different cortical areas: for example, in the primary visual cortex of the monkey layer IV is subdivided into sublaminae a, b, and c, and layer IVc is subdivided into three zones termed alpha, beta, and gamma. The motor cortex represents the converse. This cortical area is generally regarded as lacking a fourth cortical layer. The cytoarchitectonic heterogeneity of the neocortex was noted by early neuroanatomists, and, indeed, this heterogeneity provided the first basis for regional subdivision of the neocortex. Later functional studies provided support for the subdivision of the neocortex into morphologically and functionally distinct areas along its horizontal dimension. The second major dimension of the neocortex is the vertical one. Although laminar differences in cell type and density were noted by early investigators, the full significance of laminar organization as a reflection of the segregation of both afferent inputs and efferent outputs was not fully realized until the introduction of modern neuroanatomical techniques for pathway tracing.

The increase in number of functional topographically organized neocortical areas in different mammalian lines has been previously alluded to. Before proceeding, it is necessary to document at least one example of this phenomenon, as an increase in the number of cortical areas seems to be the basic way in which the neocortex has expanded. This increase has been best documented in the mammalian visual system (see Van Essen 1985; Kaas 1989), however, I will use the somatosensory system to illustrate this point as it is the system with which I am most familiar. I will briefly describe the cortical organization of the rat, opossum, and rhesus monkey somatosensory systems. This comparison will provide an example of the diversity of organization of these functional systems in mammalian neocortex and of the increase in the number of neocortical areas that compose these systems in certain mammals. This comparison also provides the basic context within which neocortical expansion must be understood. That is, the addition of functionally and morphologically discrete areas that carry out particular subfunctions within the framework of a larger functional system.

The primary somatosensory cortex of the rat occupies roughly the middle third of the rostrocaudal extent of the cerebral hemispheres. Morphologically, it can be defined by its primary cytoarchitectonic feature, the distribution of dense granule cells, or stellate cells, found in the fourth cortical layer (Welker 1976). A second morphological distinguishing characteristic is the distribution of thalamocortical afferents that arise from the ventral posterior nucleus and terminate in dense discrete clusters in the fourth cortical layer and, to a lesser extent, in the deep portions of the third cortical layer (Killackey 1973; Jensen & Killackey 1987a). In the rat, both elements can be demonstrated to be distributed so as to form a discrete but distorted visible map of the rat's entire body surface, which reflects the differential innervation of peripheral surfaces. Thus, in the cortex there are enlarged discrete representations of those portions of the body surface that are most densely innervated, and are presumably of the greatest adaptive significance. Such morphological maps are congruent with the more conventional functionally defined maps of the rat primary somatosensory cortex, which are based on the activation of cortical neurons by light touch of the body surface.

The morphologically demonstrable primary somatosensory cortex of the rat provides a starting point for defining other areas in which somatosensory information is processed within the neocortex. Partially embedded within the primary somatosensory cortex on its rostral side is the "dysgranular" cortex, which also processes somatosensory information (Killackey 1983). The thalamic input to this cortical area arises from the posterior nucleus, which is located dorsal and medial to the ventral posterior nucleus in the thalamus (Koralek et al. 1988). Posterior to and abutting the main body of the primary somatosensory cortex is the second somatosensory cortex, which is functionally defined as that portion of the neocortex containing a second complete representation of the body surface (Koralek, Olavarria, & Killackey 1990).

Lying rostral to the primary somatosensory cortex is the final cortical area to be considered, the motor cortex. Several morphological criteria can be used to define this cortical area. First, this cortex appears to be lacking a fourth cortical layer composed of stellate cells and is thus characterized as "agranular" (Donaghue & Wise 1982). The major thalamic input to the motor cortex arises from the ventral lateral nucleus, which in turn receives input from the deep nuclei of the cerebellum. Functionally, motor cortex is defined as the cortex that elicits the lowest threshold activation of the major skeletal muscle groups. The overall topographic organization of the motor cortex is commonly represented as a map of the body surface, which represents the underlying musculature and forms a mirror image to the map in the primary somatosensory cortex. In the rat, evidence suggests that the functionally defined motor and somatic maps are not completely independent; rather the two maps partially overlap in the region of the hindpaw representation. That is the sensory representation of the hindpaw is the same cortical region from which the lowest threshold movements of the hindpaw can be obtained. Morphologically, the cortical hindpaw representation seems to receive a dual thalamic input from both the ventral posterior and ventral lateral nucleus (Donag-

hue, Kerman, & Ebner 1979). This partial overlap highlights the difficulty of defining cortical areas on the basis of a single criteria such as cytoarchitectonics. It is also the first hint of a major difference in the organization of somatosensory and motor cortex across species, which will be returned to below.

There is one very significant difference between the organization of the somatosensory and motor cortex in the opossum and the rat. In the Virginia opossum the primary somatosensory cortex and motor cortex are not separate entities; instead, they entirely overlap. This condition is very different from the rat in which there is at best a partial overlap of the hindpaw representations that involves less that 10% of the primary somatosensory cortex. The evidence for this overlap in the opossum is both physiological and morphological. Lende (1963) used physiological techniques to define what he termed a somatosensory–motor amalgam in the neocortex of the Virginia opossum. He demonstrated that the neocortex of the opossum contained a somatotopic map of the body surface, as it does in other mammalian species, and then demonstrated that the same cortical point that responded to low threshold tactile stimulation of the body surface also elicited the lowest threshold motor movements of the same body region. That is, there is no separate motor cortex in this species. This has also been demonstrated anatomically. Anterograde degeneration techniques have shown that in the opossum both the ventral posterior nucleus and the ventral lateral nucleus project to the same cortical area and that their terminal fields are coextensive, although they differ in terms of cortical layers (Killackey & Ebner 1973). In the rat, as previously noted, the cortical targets of these two cortical areas is largely separate. This complete overlap of the cortical target of two thalamic nuclei in the opossum has also been demonstrated with retrograde labeling techniques (Donaghue & Ebner 1981). There is also evidence of a similar overlap of somatosensory and motor cortical fields in other marsupial species. It should also be emphasized that this overlap of sensory and motor systems is restricted to the cortical level. Both the ventral posterior nucleus and the ventral lateral nucleus of the thalamus receive separate nonoverlapping inputs from the lemniscal systems and the deep nuclei of the cerebellum, respectively, as is the case in other mammalian species. Thus, in the Virginia opossum one cortical area, the somatosensory–motor amalgam, must perform processing functions that are associated with the largely separate primary somatosensory and motor cortex of the rat. There is firm evidence for only one other somatosensory processing area in the opossum, the second somatosensory area (Pubols 1977). This could be a true species difference in cortical organization, although it may simply reflect a lacunae in the literature. However, the close apposition of primary somatosensory cortex and visual cortex in this species seems to preclude much in the way of intervening cortical areas.

The major cortical somatosensory processing areas in the rhesus monkey are located in the postcentral gyrus. Traditionally, the primary somatosensory cortex of the rhesus monkey was regarded as occupying four distinct strip-like cytoarchitectonic areas in the postcentral gyrus. These are from rostral to caudal areas 3a, 3b, 1, and 2, which together were thought to contain a single functional map of the body surface. Kaas and co-workers have reexamined and thoroughly modified this view (see Kaas 1983 for review). They present evidence that there are two separate representations of body surface receptors, one in area 3b and another in area 1. Area 3a contains a representation of muscle spindle receptors, whereas area 2 contains a representation of both cutaneous and deep receptors. Thus, what was once regarded as a single functional area can now best be regarded as four separate functional areas. It should also be noted that although each of these areas is somatotopically organized it is not as easy in the monkey as in the rat to represent the overall map for a given area as a continuous but distorted body surface map. The distortions are simply too great, and body surface parts seem to be represented in rostrocaudally oriented strips. In Kaas' view only area 3b should be regarded as equivalent to the primary somatosensory cortex of the rat. There are also several other cortical areas directly involved in the processing of somatosensory information. In addition to the second somatosensory area, these are area 5 located caudal to area 2 on the posterior wall of the postcentral gyrus and area 7b, which is located further caudally in the inferior partial lobule.

Finally, the organization of the motor cortex in the rhesus monkey should be briefly mentioned. As in other placental mammals, the primary motor cortex (area 4) in this species lies rostral to the primary somatosensory cortex and when stimulated with low level of currents produces movement. Traditionally, this cortex has been regarded as organized in a homuncular fashion, in a rough mirror image of primary somatosensory cortex. A recent study (Gould, Cusick, Pons, & Kaas 1986) of owl monkey motor cortex provides an alternative organization of this cortex, which most likely also applies to the rhesus monkey. These authors propose that the organization of primary motor cortex is better described as a mosaic of regions producing given movements with multiple regions capable of eliciting each movement. Overall, the movement regions within the mosaic are broadly somatotopically organized, but there are a number of nonsomatotopic borders within the mosaic. Perhaps such a mosaic organization best characterizes the motor cortex in other species as well as the monkey. This would make it easier to understand how the completely overlapping somatosensory–motor cortex of marsupials could have differentiated into the separate primary somatosensory and motor areas of placental mammals, as it would not require the formation of mirror image maps in each area, and the required migration of the most

caudal portions of the map to a most rostral position as suggested by the traditional view of the topographic organization of these cortical areas.

This brief overview of the organization of the somatosensory and motor cortex in rat, opossum, and monkey provides clear evidence that each of these species possesses differing numbers of cortical areas devoted to the processing of somatosensory information. Further, I would interpret this difference as evidence that information that is processed in one cortical area in the Virginia opossum, for example, is processed in a distributed fashion over several cortical areas in other mammalian species. Before considering developmental mechanisms that potentially play a role in establishing cortical areas, the other major dimension of cortex, the vertical one, should be briefly mentioned.

It was suggested by Lorente de No (1938) that a vertical strip of cortex could be regarded as "an elementary unit, in which, theoretically, the whole process of the transmission of impulses from the afferent fiber to the efferent axon may be accomplished." Since that suggestion the vertical or columnar functional organization of the neocortex has been well documented, most notably by Mountcastle (1978) within the somatosensory cortex of the cat and monkey. Indeed, this aspect of cortical organization has been so thoroughly discussed elsewhere (see Mountcastle 1978 for review) that there is no need to detail it further here. However, one morphological aspect of the vertical organization of neocortex is worth stressing. Rockel, Hiorns, and Powell (1980) document a remarkable uniformity in one feature of the basic organization of the neocortex. These authors report that the absolute number of neurons in a small volume of neocortex extending from the pial surface to the underlying white matter is relatively invariant across both cortical areas and species. It should be emphasized that this invariance in neuronal number is present in spite of both the cytoarchitectonic and functional differences that characterize different cortical areas and the approximately 3-fold difference in cortical thickness across species. I will report the results of this study in some detail as I believe it has major implications for our understanding of the evolution of neocortex.

The approach of these investigators was a very straightforward one. They simply counted the number of neurons in a narrow strip (30 μm) of a 25-μm-thick section throughout the depth of neocortex in mouse, rat, cat, monkey, and man in Nissl-stained material (a volume of 750 μm^3). These counts were made in a number of diverse cortical areas (motor, primary somatosensory, primary visual, frontal, parietal, and temporal) in each species and by independent observers. With the exception of primary visual cortex in primates, the sampled strip in all of these cortical areas in all species contains approximately 110 neurons. The primary visual cortex in primates was found to contain approximately 2.5 times the number of neurons in other cortical areas. The reason for this increase in neuronal number in the primary visual cortex is unclear, but undoubtedly is related to the processing requirements of the highly specialized primate visual system. However, overall, the basic constancy in neuronal number is most striking, and is interpreted by Rockel et al. (1980) as suggesting that during evolution it is the area of neocortex that increases while the number of neurons within its depths at a given point remains relatively constant.

This study has two major implications for the present discussion. First, it suggests that the fundamental unit of cortical processing is composed of roughly the same number of neurons across both species and cortical areas. Further, this basic unit is quite conservative and has remained relatively unchanged in the course of mammalian evolution. Second, it suggests that cortical expansion has occurred largely by the addition of the same basic units. Thus, a larger brain contains many more basic units than a smaller brain, but the basic organization of the two is quite similar. This is not to suggest that there has been no change in the vertical dimension of neocortex. An increase in cortical thickness of a factor or two or three may be quite significant, as it at least partially reflects increases in both dendritic length and spines and the richness of the axonal strata that contact cortical neurons.

On the basis of the foregoing evidence, I would make the following assumptions about neocortical organization and expansion. First, vertical or columnar organization is a fundamental feature of cortical organization, and small groups of cells distributed along this vertical dimension form a processing unit, or a "column." Second, a "column" has a finite processing capability, and when these capabilities are reached, there are evolutionary pressures for the addition of new processing units. Third, these new processing units are added in series with the preexisting units and they can be regarded as an extension of these units. This allows a greater processing capacity and is, perhaps, required by the development of more specialized peripheral receptor arrays. Fourth, this extension is not accomplished by an increase in the vertical dimension of the neocortex but by an addition of the new units at the border of the preexisting cortical area. Fifth, the "columns" of the new cortical area would be connected with those of the preexisting cortical area by intrahemispheric connections that are, by necessity, topographically organized. Thus, information that is processed in a single "column" in one species is distributed over several columns in different cortical areas in other species.

These assumptions raise several questions that will be the focus of the remainder of this essay. First, is there evidence that the mammalian neocortex expanded by the simple addition of basic units? Second, if such an expansion has taken place, can we understand how these basic units are formed into cortical areas with precise patterns of interconnections, within the context of mech-

anisms we know to operate during the course of neocortical development? My answer to both questions is yes. However, the answers need to be qualified by the caveat that the available evidence is far from complete and much research remains to be done. The available evidence is summarized below.

ONTOGENETIC CONSIDERATIONS

Insight into how the basic number of units that compose the neocortex may have expanded during the course of mammalian evolution may be gained by looking at the development of the neocortex. The early phases of this process are quite similar in all mammalian species studied to date. Rakic (1988) has eloquently summarized and interpreted these events in a recent article largely based on his work on the primary visual cortex of the rhesus monkey. Briefly, germinal cell precursors of neocortical neurons proliferate along the ventricular walls of the telencephalon and migrate to their final position using radial glial cells as guides. Rakic hypothesizes that the neuronal progeny of a given ventricular zone progenitor cell is a radial unit and forms an "ontogenetic" or embryonic "column." In total, this migration forms the laminated neocortex by an inside-out gradient and, in Rakic's words, "the cortex is the sum of its ontogenetic columns." The earliest generated neurons are located in the deeper cortical layers and later generated neurons are located in progressively more superficial layers. This laminar organization is evidence of some degree of early specification of the overall projection pattern of several different classes of cortical neurons. Most notably, both corticocortical projection neurons and corticosubcortical projection neurons have distinct laminar positions. Further, several experiments have provided evidence that this aspect of neuronal commitment may be determined before neurons migrate from the ventricular zone (Jensen & Killackey 1984; Yurkewicz, Valentino, Floeter, Fleshman, & Jones 1984; McConnell 1985). However, it should be emphasized that the focus of this essay is not the fate of either individual or small groups of cortical neurons, a subject that has recently received considerable attention (see Sanes 1989 or McConnell 1989 for a review of the issues involved), but on factors that influence the formation of cytoarchitectonic areas and the ultimate targets of subclasses of cortical projection neurons.

The neurons that compose the neocortex of a rat are generated over a 5-day period, roughly between embryonic days 16 and 21 (Berry & Rogers 1965). Further, in the mouse, neurogenesis proceeds in a wave-like fashion from rostrolateral portions to caudomedial areas of the neocortex (Smart 1983), suggesting that ontogenetic "columns" are added in a roughly annular fashion. Thus, one might speculate that one straightforward way for the cortex to expand would be to simply leave the proliferative machinery on for a longer time period and produce larger numbers of ontogenetic columns as suggested by Rakic (1988). In the rhesus monkey, neocortical neurons are generated over a 50-day period, roughly between embryonic days 45 and 100 (Rakic 1974). Assuming that the generative cycle time is of the same order of magnitude in the two species, the 10-fold increase in proliferation time may be a reflection of the longer time necessary to produce the greater number of neurons that compose the expanded primate neocortex. This is but one example of a phenomenon that is characteristic of primates, and particularly robust in man, the prolongation of developmental events over a longer time period.

Although the prolongation of the period of neurogenesis has an obvious bearing on the overall expansion of the neocortex it does not directly contribute to our understanding of how new neocortical areas are formed other than in the sense that more basic units provide the building blocks for new cortical areas. There is still the major question of how new discrete cytoarchitectonic functional areas with unique sets of connections are formed out of the basic units. Rakic, although noting that afferent input may play some role in this process, has suggested that such specification occurs largely at the ventricular zone before neurons migrate and form the neocortex. Indeed, he has suggested that the ventricular zone contains a "protomap" of the later formed cytoarchitectonic organization. I, on the other hand, would like to suggest that such a "protomap" may be unnecessary and focus on two candidate mechanisms that may be involved in the process of cortical specification but occur somewhat later.

Afferent Specification

The first process I call afferent specification. I will first attempt to define what I mean by this term and then discuss evidence indicating that it plays some role in determining the organization of the neocortex. My basic assumption is that the major afferent input to a neural structure is one major factor that plays a role in the ontogenetic determination of a neural target's structural organization. In terms of neocortex, the dorsal thalamus provides the major afferent input, and, hence, would be expected to play the major extrinsic role in the guidance of its organization. However, it should be kept in mind that the dorsal thalamus is only the penultimate link in a sequence of neural connections that begins with receptor surfaces at the periphery. Thus, it is ultimately the peripheral receptor surfaces acting through the immediate agency of the dorsal thalamus that under normal circumstances play a role in neocortical organization. This point of view assumes that the neocortex is initially relatively unspecified, and that thalamic input plays a role in the specification of such features of neocortical organization as the formation of cytoarchitectonic borders. This process probably occurs during the initial invasion

of the neocortex by thalamic afferents and is the net result of interactions of thalamic fibers with one another as well as with their target neocortical tissue. For example, primary somatosensory cortex is specified as that portion of neocortex into which a dense thalamic input from the ventral posterior nucleus grows. At the experimental level, this implies that the boundaries of neocortical areas can be altered by manipulations of either the thalamus or the periphery during the course of development.

Although such a process can be most readily understood in terms of the primary sensory cortical areas, it may play a role in other cortical areas as well. For example, cortical regions that receive either a less dense thalamic input or one that grows in relatively late in development may receive organizational guidance from major inputs that arise in the primary cortical areas. In this regard, afferent specification may play some role in the determination of patterns of connectivity of cortical projection neurons. This point will be discussed in detail below. However, the evidence that suggests that afferent input plays a guiding role in the organization of neocortex will be reviewed first.

The imprint of the entire body surface on the primary somatosensory cortex of the rat provides presumptive evidence of the role of afferent input in guiding the organization of target structures. This peripherally related pattern is reflected in both the cytoarchitectonic organization of the primary somatosensory cortex and the thalamic input from the ventral posterior nucleus to this cortical area. Although it is theoretically possible that this pattern is an intrinsic property of the primary somatosensory cortex, as has recently been suggested by one group of investigators (Steindler, Cooper, Faissner, & Schachner 1989), there is strong evidence that this is not the case and that this pattern is formed under peripheral guidance (see Killackey & Belford 1979; Killackey 1980; Belford & Killackey 1980; Bates & Killackey 1985 for further details). First, peripheral damage during the first few days of birth alters both the cytoarchitectonic organization of the somatosensory cortex and the distribution of thalamic afferents to this cortex. Second, the same pattern seen in cortex is also characteristic of the subcortical somatosensory relays in the brainstem and thalamus, and this pattern develops in a sequence that begins at the periphery and ends in the neocortex. Third, peripheral damage has an effect on the subcortical patterns that is similar to the effect on the cortical one, and the altered patterns develop with the same time course as the normal patterns. This suggests that the peripheral damage is simply altering the outcome of normal developmental processes.

My view of these processes at the level of the thalamus and cortex is based on several experiments performed in my laboratory. (We have also hypothesized that similar events occur at the subcortical relays but here I will focus on the thalamus and cortex.) At the time of birth afferent brainstem somatosensory fibers have reached the ventral posterior nucleus but have not formed their characteristic pattern of termination in this nucleus. At the same time, thalamocortical afferents have reached the neocortex but have not yet invaded the neocortical layers. The growing tips of these fibers are located in the white matter underneath the cortex (Wise & Jones 1976) and are organized in the same topographic order characteristic of the adult (Dawson & Killackey 1985). During the next few days, the thalamocortical afferents grow into the neocortex and their terminal arborization pattern forms on the basis of afferent input to the thalamic cells. This last statement is based on observations of the morphology of the terminal arbors of individual thalamic arbors. In the normal adult animal, arbor size can be related to the innervation density of the peripheral structure to which it is connected (Jensen & Killackey 1987a). In adult animals in which peripherally based information has been altered by neonatal peripheral nerve section, the terminal arbors of individual thalamocortical afferents are grossly abnormal. They extend over wider cortical areas and have many fewer terminal arborizations and boutons (Jensen & Killackey 1987b). Based on this evidence, I conclude that it is the formation of terminal arbors that influences the cytoarchitectonic organization of the somatosensory cortex. In the normal mouse, the size of individual cytoarchitectonic units, or "barrels," can be related to the innervation density of their peripheral structure (Welker & Van der Loos 1986b). Further, neonatal peripheral damage alters both overall cytoarchitectonic organization in the somatosensory cortex and the dendritic organization of the individual stellate cells that compose the "barrels" and are a major target of the thalamic input (Harris & Woolsey 1979; Steffen & Van der Loos 1980). The summarized evidence can be taken to indicate that at least some aspects of cortical organization are not intrinsically organized (or contain "protobarrels" as suggested by Steindler, Cooper, Faissner, & Schachner 1989) and that the periphery acting directly through the thalamus as well as through other subcortical relay stations plays a role in the organization of neocortex.

Further evidence that the thalamus is an extrinsic source of cortical organization comes from an experimental paradigm that may be regarded as the converse of those reported. Ito and Seo (1983) have introduced small lesions in the presumptive somatosensory cortex of the rat on the day of birth and shortly thereafter and at a later date examined the resultant pattern of succinic dehydrogenase and cytochrome oxidase staining in the somatosensory cortex. As noted, these stains reflect the pattern of termination of the thalamocortical afferents in the primary somatosensory cortex. They found a complete peripherally based pattern that tended to avoid the site of the lesion in animals that received such lesions before 10 days of age. In the adult animals assayed the lesion most frequently located on the border of the

overall pattern or in a minority of instances between the representation of rows. Although the experiment is difficult to interpret unequivocally, as there is no way of determining where the lesion was placed relative to the later forming pattern, it is very suggestive. It can be interpreted as evidence that particular thalamocortical afferents are not coupled to a particular site in the cortex and that the overall pattern is somewhat independent of its exact cortical locus. It also supports the notion that the pattern is imposed on the neocortex by the thalamus. Finally, direct evidence for this statement has been provided by a recent experiment in which it was demonstrated that fetal occipital cortex tissue transplanted to somatosensory cortex develops both the discrete pattern of thalamic afferents and cytoarchitectonic organization characteristic of the rat primary somatosensory cortex (Schlaggar & O'Leary 1989).

It should be pointed out that most of the experiments previously referred to can be interpreted as suggesting that the role of the thalamus in organizing neocortex takes place within the confines of intrinsically determined borders. That is, the borders of a cytoarchitectonically or functionally distinct cortical area may be intrinsically regulated and thalamic organizing guidance operates within these borders. In a recent experiment, we removed peripheral input at a much earlier stage of development and obtained evidence that the overall size of a portion of the neocortical body surface representation can be influenced by peripheral manipulations.

Forelimb removal at the time of birth in the rat results in an anomalous cortical representation of the associated limb that covers the same cortical area as the normal forepaw representation (Dawson & Killackey 1987). The remaining portions of the body representation are unchanged. This same manipulation at embryonic day 17 also produces an anomalous cortical forepaw representation of the appropriate cortical area. It also results in the expansion of the hindpaw representation. Following such a manipulation the cortical representation of the hindpaw doubles in area (Killackey & Dawson 1989). Our interpretation of this expansion is that primary hindpaw afferent terminations in the brainstem that arrive at the brainstem 1 day later than forelimb afferents are able to capture more target cells in the dorsal column nuclei when the forelimb is removed before primary afferents associated with the forelimb reach their brainstem target. Further, this recruitment of more neurons into the hindpaw representation is repeated at the thalamic and cortical level.

Several facets of this expansion deserve mention. First, the expansion appears to be restricted to the hindpaw. Other portions of the body surface representation, such as the lower lip, which are equally close to the forepaw representation, do not expand. Second, and most important, the expansion appears to be at the expense of a second cortical area, the dysgranular cortex. Normally, there is a strip of dysgranular cortex between the representations of the forepaw and hindpaw. In the early manipulated animals this strip is missing and the hindpaw representation directly abuts the anomalous forepaw representation. The dysgranular cortex does not normally receive inputs from the ventral posterior nucleus. Its thalamic input arises from the medial portion of the posterior nucleus (Koralek, Jensen, & Killackey 1988) and from the opposite hemisphere via the corpus callosum. Further, this major callosal input invades the neocortex later than thalamic input (Wise & Jones 1976). I interpret this experiment as evidence that thalamic afferents from the ventral posterior nucleus play a role in establishing the borders of the primary somatosensory cortex.

Presumptive evidence that can be interpreted in a similar fashion comes from experiments by Welker and Van der Loos (1986a, 1986b). These investigators have selectively bred mice over a number of generations for the presence of supernumary vibrissae. The primary interest of these investigators was the relationship between peripheral innervation density and the size of individual cortical "barrels," but they present data that also relate to the current topic. In four of the five strains maintained by these investigators, there is a tendency for the overall area of the "barrel" cortex to be enlarged in the peripherally "enriched" strains compared to the normal strain. If this is indeed the case, it suggests not only that the periphery plays a role in guiding the organization of neocortex but that the borders of cytoarchitectonically and functionally discrete cortical areas can be changed over the relatively brief time span of 20 or so generations.

Recent experiments on the monkey primary visual cortex also support the hypothesis that afferent input plays some role in specifying this cortical area. Rakic (1988) first reported that binocular enucleation around embryonic day 60 in the rhesus monkey reduces to about one-half the number of neurons in the lateral geniculate and results in a marked reduction in the size of the primary visual cortex. The cytoarchitectonic properties of the remaining striate cortex are remarkably normal. This basic result has recently been replicated by Dehay, Horsburgh, Berland, Killackey, and Kennedy (1989), who further suggest that cortex immediately surrounding the reduced primary visual cortex, and that normally would have been specified as primary visual cortex, has characteristics not normally associated with the primary visual cortex, namely, callosal projections. As suggested by Rakic (1988), this cortex may be regarded as either part of an expanded area 18 or as "hybrid" cortex not found in the normal animal. In either case, the results suggest that afferent input play some role in the specification of neocortex. In this context, one "natural experiment," the anopthalamic mouse, should also be mentioned. This mutant mouse strain never possesses eyes and has only three-quarters of the lateral geniculate neurons found in normal mice, yet normal topographic relations between thalamus and cortex develops (Kaiserman-Abramof,

Graybiel, & Nauta 1980). However, this should not be interpreted as evidence that thalamic afferents do not play a role in specifying the neocortex. Rather, it suggests that such specification can occur in the absence of peripheral input. This is not particularly surprising in view of the results in the monkey, and the fact that broad aspects of cortical specification, cytoarchitectonics, appear to occur before all aspects of sensory pathways are in place.

Finally, there are several other relevant experiments that demonstrate that sensory information from one sensory system (vision) can be processed in a relatively normal fashion by cortex associated with a second sensory system (somatosensory or auditory). Frost has accomplished this by ablating the major retinal targets (dorsal lateral geniculate and superior colliculus) and a portion of the afferent input into the ventral posterior nucleus in the newborn hamster, a very altricial species. This results in the formation of stable and ordered connections between the retina and the ventral posterior nucleus (Frost 1981, 1986). These projections seem to result from both the persistence of a transitory projection that is usually eliminated during the course of normal development and reactive sprouting induced by the removal of normal target tissue. Under these conditions, an orderly projection from the retina to the somatosensory cortex via the ventral posterior nucleus can be demonstrated with transneuronal anterograde transport techniques (Frost 1982). This aberrant projection to the somatosensory cortex is capable of mediating visual function. Frost and Metin (1985) have recorded visual receptive fields from the primary somatosensory cortex of such animals that were at least partially topographically ordered. Similarly, Sur and colleagues (Sur, Garraghty, & Roe 1988) demonstrated that visual projections experimentally induced into the medial geniculate of the ferret, another extremely altricial species, result in neurons in auditory cortex that have visual receptive fields. Some of these neurons are directionally sensitive or have oriented receptive fields resembling those of complex cells in the primary visual cortex. These results demonstrate at a functional level that organizational features of the neocortex are dependent on its afferent input. Further, it suggests that the organization of cortical circuitry in different cortical areas has enough in common that circuitry usually related to one sensory modality can, under the appropriate (albeit highly artificial) circumstances, process information related to another sensory modality.

The evidence presented best favors the hypothesis that the formation of cortical subdivisions is guided by thalamic afferent input. It is obviously meager in view of the large body of work that has addressed the question of the effects of neonatal peripheral damage and sensory deprivation on the organization of the central nervous system. I would submit, however, that most of this literature is not directly relevant to this question. Typically, the effects of such manipulations are studied relatively late in the course of mammalian development, after initial relations between the thalamus and cortex have been established.

Cortical Exuberance

The focus of the previous section was on the role of afferent input in determining the organization and borders of neocortical areas. The evidence summarized supports the conclusion that these borders are not intrinsically fixed but are determined by processes that involve the thalamic input to the neocortex. I would now like to review the evidence that the distribution of different classes of cortical projection neurons is also not completely intrinsically determined. Further, I will suggest that cortical projection neurons have some "choice" in the matter of their ultimate targets and that afferent specification may play some role in this determination.

A major morphogenetic feature of the development of most parts of both the peripheral and central nervous system is the process of cell death. This phenomenon has been identified in a wide variety of neuronal structures in a large number of different vertebrate species (see Cowan, Fawcett, O'Leary, & Stanfield 1984 for review). In the chick spinal cord about 40% of the motor neurons die between the fifth and ninth day of incubation. In other portions of the chick central nervous system the number of neurons eliminated during the course of development varies from approximately 15% of the neurons that initially compose the auditory relay nucleus of the brainstem to 80% of the neurons that initially compose the mesencephalic trigeminal nucleus. The pervasiveness of the phenomenon of cell death has lead to the generalization that in nearly all parts of the nervous system neurons are overproduced and later eliminated. Cell death is thought to play a role in several different developmental events, including matching the size of a population of projection neurons with their target, removal of erroneous projections, and, at least in invertebrates, controlling cell lineages.

Cell death does occur in the neocortex (Finlay & Slattery 1983) but it does not appear to be a particularly major phenomenon in this structure. Indeed, it seems intuitively counterproductive for major cell loss to occur during the development of a structure in which there appears to be evolutionary pressure to increase the size and absolute number of units that compose that structure. There is considerable evidence that a mechanism that allows the neocortex to solve the same problems that confront other neuronal structures without major cell loss has evolved and, at least at present, this mechanism seems to be unique to mammals. This mechanism can be regarded as a variant of cell death; however, rather than involving the loss of a whole neuron, only a neuronal process is eliminated. This phenomenon has been termed cortical exuberance and was first described by Innocenti, Fiore, & Caminiti (1977) in the visual cortex

of the cat. Before describing this process in detail, the context within which cortical projection neurons develop should be clearly specified.

Cortical projection neurons in the rat are distributed throughout the horizontal extent of neocortex in specific layers (Akers & Killackey 1978; Ivy & Killackey 1981; Olavarria, Van Sluyters, & Killackey 1984). Within a given cortical area, cortical projection neurons have imposed on their perikaryal distribution a topographic organization determined by the afferent input to that area. For example, cortical projection neurons in the lateral portion of primary somatosensory cortex will be related to the face whereas more medially placed neurons will be related to the limbs or trunk. In turn, cortical projection neurons must project to other cortical or subcortical targets that may be, but are not necessarily ordered, in precisely the same fashion. Thus, during development cortical projection neurons must come into register with both the source of afferent input into the cortical area in which they reside and their target. Cortical exuberance appears to be part of a unique solution to this problem.

During the course of development callosal projection neurons in rat somatosensory cortex undergo a dramatic restriction in their areal (or horizontal) distribution while their vertical or laminar distribution remains unchanged (Ivy, Akers, & Killackey 1979; Ivy & Killackey 1981). The pattern of callosal projection neurons in the somatosensory cortex of the adult rat is punctate and discontinuous. The overall pattern of callosal projection neurons and their terminations is clearly complementary to the distribution of thalamic afferents from the ventral posterior nucleus. However, at birth the cells of origin of the immature callosal projection are continuously distributed in the deeper cortical layers below the cortical plate and callosal afferents are evenly distributed in the underlying white matter. The discontinuous adult pattern is established during the early postnatal period and is accompanied by the specific ingrowth of callosal afferents into appropriate regions.

This same phenomenon occurs in the somatosensory cortex of the rhesus monkey during the last trimester of fetal life. In the adult rhesus monkey there is a gradient of callosal projections across the postcentral gyrus: they are most dense caudally in area 2, less dense in area 1, and least dense rostrally in area 3b (Killackey, Gould, Cusick, Pons, & Kaas 1983). Within a given area, callosal projections are least dense in the hand and foot representations (regions that may be presumed to receive the densest thalamocortical projections) and denser in the regions representing the limbs, trunk, and much of the head. At embryonic day 108 (approximately 2 months before birth) callosal projection neurons are located in appropriate cortical layers but are distributed in a uniform band across the postcentral gyrus. The mature distribution pattern of callosal projection neurons is discernible by embryonic day 133, approximately 1 month before birth (Killackey & Chalupa 1986).

This same phenomenon has also been described in the somatosensory cortex of the cat (Innocenti & Caminiti 1980) and Virginia opossum (Cabana & Martin 1985). Further, it is a general feature of cortical development and not restricted to the somatosensory cortex. The phenomenon was first described in the visual system of the cat and has since been reported to occur in the auditory cortex of the cat as well as the visual cortex of several rodent species. The occurrence of this phenomenon across a wide variety of mammalian species suggests that it is a general feature of neocortical ontogeny (see Innocenti 1986 for review). [It should be noted that at present there is one exception to the generality of this phenomenon. Both Dehay, Kennedy, Bullier, and Berland (1988) and Chalupa, Killackey, Snider, & Lia (1989) have reported that the primary visual cortex of the fetal rhesus monkey is essentially free of callosal projection neurons as it is in the adult. One puzzling aspect of this finding is that the previously discussed experiment of Dehay et al. (1989) suggests that the appropriate neurons of the monkey primate visual cortex have the potential to form callosal projections, but that normally this potential is suppressed at a very early time. Perhaps this is related to the high degree of specificity that characterizes the entire visual system in this species.]

The generality of the phenomenon is also supported by the fact that it occurs in a second major class of neocortical projection neuron (Stanfield, O'Leary, & Fricks 1982; Bates & Killackey 1984). These are the neurons of neocortical layer Vb, which in the adult are areally segregated but project to a variety of targets caudal to the diencephalon including the mesencephalon, pons, brainstem trigeminal nuclei, and spinal cord. In the adult rat, injections of retrograde tracers into the spinal cord result in the restricted labeling of layer Vb neurons in portions of neocortex related to the somatosensory and motor cortex. In the neonatal rat, similar injections of retrograde tracers into the spinal cord result in the labeling of layer Vb neurons throughout all areas of the neocortex including the visual cortex. Injections of tracers into the superior colliculus results in the same widespread pattern of labeled layer Vb neurons in the neonatal rat; in the adult the same injections result in more limited and discrete patterns of labeled cells. The adult distribution of corticospinal projection neurons is achieved only during the course of the second postnatal week. This same phenomenon has also been reported to occur in species other than the rat, namely, the hamster, cat, ferret, and Virginia opossum (see O'Leary & Stanfield 1989 for a recent review).

This major ontogenetic change in two classes of cortical projection neuron is achieved in the same manner. Neocortical projection neurons initially send processes to multiple target areas and later eliminate processes rather than eliminate whole cells as in the case of cell death (Ivy & Killackey 1982; Chalupa & Killackey 1989). In the case of callosal projection neurons processes are

extended to the area of potential targets within the ipsilateral hemisphere, as well as across the corpus callosum. Similarly, neurons of layer Vb send processes to multiple targets such as the superior colliculus and the spinal cord.

Given the phenomenon of exuberance of cortical projection neurons, the question of what guides the ultimate distribution and connectivity of cortical projection neurons naturally arises. It has been most clearly demonstrated in the visual system that the adult pattern of callosal projection neurons is subject to modification. This was first reported by Shatz (1977) in the Siamese cat, in which callosal projection neurons are distributed over an abnormally wide area of visual cortex. Shatz attributed this to the altered representation of the visual fields in visual cortex in this strain, which is a consequence of an aberrant retinogeniculate pathway. The results of experiments that have manipulated visual input during the early postnatal period support the notion that callosal projections can be modified during development. Dark rearing or eyelid suture in cats has been reported to decrease both callosal terminations (Lund & Mitchell 1979a) and projection neurons (Innocenti & Frost 1980; Innocenti, Frost, & Illes 1985) without altering the distribution pattern in visual cortex. Studies in cats that have altered visual alignment have yielded conflicting results (Lund, Mitchell, & Henry 1978; Lund & Mitchell 1979b; Innocenti & Frost 1979; Berman & Payne 1983). In binocularly enucleated cats and rodents callosal projection neurons are more widely distributed and fewer in number (Innocenti and Frost 1980; Rhoades and Fish 1983; Olavarria and Van Sluyters 1984). Similar effects are detectable in the visual cortex contralateral to a monocular enucleation in rodents (Cusick and Lund 1982; Rhoades and Dellacroce 1980; Rothblat and Hayes 1982). Overall, these manipulations seem to result in both the failure of misplaced neurons to retract a process (widespread distribution) and the loss of other seemingly appropriately placed neurons (drop in number of labeled neurons). Although most investigators have not directly assessed the effect of their peripheral manipulations on thalamic projections, they interpret their results as suggesting that some factor associated with thalamic afferents, usually "activity," plays a role in determining the distribution of callosal projections.

We have recently demonstrated that the distribution of callosal projection neurons in the somatosensory cortex of the rat can be altered by neonatal section of the infraorbital nerve, the vibrissae afferents (Koralek & Killackey 1990). This same manipulation alters the distribution of thalamocortical afferents to primary somatosensory cortex (Jensen & Killackey 1987b). In normal primary somatosensory cortex callosal projections are distributed in a complementary fashion to the thalamic afferents. After the manipulation, callosal projections in primary somatosensory cortex seem to be altered in a way that reflects the changed distribution of thalamocortical afferents. This same manipulation also produces major changes in the distribution of callosal projections in somatosensory areas outside the primary somatosensory cortex. The face region of the second somatosensory area, which usually contains dense callosal projections, is relatively devoid of such connections in both hemispheres after the manipulation. We assume that these changes are secondary to changes in the projection pattern of ipsilateral corticocortical projections from primary somatosensory cortex. Further, we have provided direct evidence that thalamic neonatal removal results in an extremely aberrant distribution of callosal projections in the associated neocortex.

A recent experiment on the ultimate projections of transplanted corticosubcortical projection neurons is also relevant to the present issue. O'Leary and Stanfield (1989) provided evidence that the tangential position of such a neuron in cortex is a critical factor in determining whether or not an initially extended axonal process will be eliminated or maintained. Initially, as noted above, layer Vb neurons throughout all areas of neocortex including the occipital cortex project to the spinal cord. O'Leary and Stanfield (1989) first labeled and then either transplanted embryonic day 17 occipital cortex tissue to a rostral cortical region, or, conversely, rostral cortex tissue to occipital cortex as well as the appropriate control transplants. They then assayed the transient and permanent connections of these transplants. Most importantly, they found that occipital subcortical projection neurons transplanted rostrally were maintained while such projection neurons transplanted to visual cortex from either the rostral cortex or occipital cortex did not maintain spinal cord projections. Their interpretation of these results suggests that any ventricular zone "protomap" is relatively unfixed and the early cortical mantle can be regarded as composed of equivalent proliferative units that are fixed by later occurring events. This interpretation is similar to the point of view taken in the current essay. However, it should be noted that in a recent review of these issues O'Leary (1989) has added yet another "proto"-term to the forementioned "protomap" and "protobarrel", namely, "protocortex". Given that this term simply refers to the less differentiated developing neocortex and nothing else, I would submit that this term like the other "proto"-terms is superfluous.

In total, the above experiments provide evidence that the distribution of cortical projection neurons is not fixed, but, similar to other aspects of cortical organization, is at least partially determined by ontogenetic events involving afferent thalamic input to the neocortex.

TENTATIVE CONCLUSIONS

I would now like to consider how afferent specification and cortical exuberance contribute to the organization of neocortex during ontogeny and how they could play

a role in the phylogenetic expansion of neocortex and the formation of new cortical areas.

The evidence previously reviewed leads me to assume that the initial state of the neocortical mantle is relatively undifferentiated. It is composed of a large number of vertically oriented basic units, or "columns," that have the same potential processing capabilities but are not functionally prespecified in terms of the ultimate modality or system in which they will function. The number of such units in any species is related to the length of the proliferation period and is open to modification by selection pressures. The overall projection from the dorsal thalamus embraces all of the neocortex. Particular portions of the dorsal thalamus establish relations with particular parts of the neocortex early in ontogeny (rostral thalamus to rostral cortex, etc.) and do so within the confines of a topographic order that is intrinsic to the overall thalamic projection. In other words, thalamic fibers in the course of their growth toward the neocortex tend to maintain near neighbor spatial relations. The alternate to this ordered spatial outgrowth in this or any other neuronal system is diffuse outgrowth. This seems unlikely for several reasons. First, close study of most developing neuronal systems including the thalamocortical has yielded evidence of ordered spatial growth. Second, ordered spatial growth is probably the simplest solution in terms of resources available to and mechanical constraints on a developing system. It also has the advantage of providing intrinsic cues for further organization. This established overall relationship between the thalamus and neocortex occurs early in ontogeny before the development of specific systems and provides the framework for the subdivision of the neocortex.

The somewhat later development of labeled line processing systems from both the periphery and other major neuronal structures, such as the superior collicus and cerebellum, to the neocortex via the dorsal thalamus takes place within this framework. With the ingrowth of thalamic afferents into the neocortical layers, perhaps in a temporally staggered fashion such that thalamic fibers associated with the major sensory modalities do so first, a subset of cortical ontogenetic "columns" is colonized by a particular subset of thalamic afferents and forms a particular cortical area. This assumes that ontogenetically the primary sensory areas of cortex are the first to form. It also suggests a rather plausible mechanism by which the size of a cortical area can be regulated in relationship to peripheral receptor density and distribution as the size of a cortical area would be directly related to the size of the related thalamic input, which in turn is related to its input. At the subcortical level a number of mechanisms including cell death are presumably involved in the shaping of these relationships.

In general, thalamocortical projections are densest in those cortical areas that can be related to labeled line processing systems. Thus, I would assume that thalamocortical afferents play their major role in these areas, the best examples of which are the primary sensory areas. On the basis of the evidence presented I also assume that in these primary areas thalamocortical afferents influence the feature of cortical projection neurons termed cortical exuberance. One form of this interaction is the determination of whether a given cortical projection neuron projects ipsilaterally or contralaterally. A dense thalamic input to an area seems to be correlated with a preponderance of ipsilateral cortical projection neurons and it is reasonable to assume that thalamic input plays a role in this. These ipsilateral cortical projection neurons may in turn provide the dominant input that colonizes and specifies cortical areas surrounding primary sensory corticies not invaded by labeled line thalamic input. A set of such projection neurons originating from a common primary cortical area would possess an ordered spatial topographic order that it would then impress on its cortical target tissue. If such a set of neurons issuing from one cortical area colonizes an adjacent cortical region with the added constraint that each neuron contacts the closest available target space, the mirror image topographic relationships that normally exist between primary and secondary cortical representations would result. Such processes would also explain the differential distribution of callosal projection neurons.

Finally, how are these assumed ontogenetic events related to the phylogenetic expansion of the neocortex. I would suggest that this is relatively straightforward. A host of environmental pressures that put a premium on both more complex sensory organs for abstraction of information from the environment and central processing mechanisms and that allow greater efficiency in the processing of this information and more flexibility in behavioral response obviously played a role. *The answer to these pressures was the uncoupling of a portion of the vertebrate telencephalon, the neocortex, from strict genetic determination.* This uncoupling allowed the mammalian neocortex, the telencephalic target of the major sensory systems and executor of motor control, to expand and adapt to a variety of circumstances. It is basically an organ composed of a single but expandable building unit that has subdivisions imposed on it by intrinsic sources. From this perspective, the varying number of cortical area devoted to the processing of information within the same sensory system is readily interpretable. In forms such as the Virginia opossum, the majority of necessary processing can take place within a single column. The areal overlap of thalamic inputs in this species should not be interpreted as evidence of diffuse organization. There is a clear separation of inputs to different cortical layers even in this species, which suggests some segregation of sensory and motor processing systems. However, if more cortical space becomes available through the production of more basic units, as is the case in placental mammals, each thalamic nucleus becomes associated with separate cortical territories. The data from this species can also be interpreted

as indicating that complete segregation of separate labeled line systems occurs at the subcortical level first.

This same general notion can be applied to species with multiple cortical representations of the same sensory surface such as the rhesus monkey. This is probably, at least, a two-part process. First, there may be a tendency for separation within a given sensory system along such lines as submodality processing, for example, cutaneous versus deep peripheral somatic receptors, which tend to produce separate processing centers along the length of the neural axis including separate areas within the neocortex. Second, and coupled with this, is increased processing requirements within and across a sensory modality. Such requirements can be most effectively met by an increase in the number of basic cortical units. At the ontogenetic level, differing systems and subsystems are perhaps segregated by differing patterns of activity between systems or subsystems, and increases in the density of thalamic projection within a given system. With such increases, additional cortical units could be recruited into new cortical areas organized on the basis of ipsilateral corticocortical projections from already established areas. Further, such a process could potentially be repeated in serial fashion several times.

I would close by noting that in my opinion the evidence reviewed forms a reasonable case for the proposition that neocortex is initially relatively unspecified and that afferent input plays an important role in the specification process. Further, these ontogenetic events bear on the phylogenetic consideration of the expansion of the neocortex. However, much remains to be done and the present interpretation is provisional in the sense that it must be tested by what is to come. Future research aimed at elucidating patterns of connectivity early in ontogeny utilizing newly available tracing techniques coupled with classical embryological experimental manipulations will undoubtedly clarify these events. The powerful techniques of molecular biology are also just being brought to bear on the question of cortical specification. Both of these approaches will hopefully add a wealth of exciting and novel data to the ontogenetic side of these issues.

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Expansion of the Central Hindpaw Representation Following Fetal Forelimb Removal in the Rat

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Key words: development, neocortex, gracile nucleus, cuneate nucleus

Abstract

We provide evidence that prenatal removal of a rat forelimb results in both a disruption of the anatomical representation which would normally correspond to the forepaw and in an enlargement of the adjacent hindpaw representation in the brainstem and cortex. This enlargement, which in some cases is as much as 100%, only occurs following complete forelimb amputation on embryonic day 17 (E17) or earlier. This coincides with the age at which forepaw afferents first arrive in the brainstem, suggesting to us that the expansion is permitted in part because ingrowing hindpaw afferents are in the presence of cuneate cells which have never been previously innervated; in animals older than E17, the expansion is prohibited by either an intrinsic age-dependent change in the cuneate cells, or a change imposed upon them by forelimb afferents.

The number of cells in dorsal root ganglia subserving the expanded hindpaw areas does not differ from normal suggesting that the expansion of hindpaw territory within the brainstem reflects an increased terminal arborization by a normal complement of primary hindpaw afferents.

We interpret the cortical enlargement to be an upstream reflection of the brainstem events. In cortex, the enlargement seems to result from an invasion of the dysgranular cortex by thalamic afferents arising from the ventral posterior nucleus.

Introduction

The rodent somatosensory system possesses a discrete topographical organization which is demonstrable with standard electrophysiological mapping techniques (Nord, 1967; Waite, 1973; Welker, 1971,1976; Chapin and Lin, 1984; Nussbaumer and Van Der Loos, 1985; Rhoades et al., 1987), as well as a stereotype morphological pattern of parcellation which can be visualized with routine anatomical procedures (Woolsey and Van Der Loos, 1970; Belford and Killackey, 1979,1980; Dawson and Killackey, 1987). Both the physiological and the anatomical data support the notion that tactile representations of the various body surfaces are topographically organized and sequestered in discrete regions of the brain at each level of the neural axis from the brainstem to the somatosensory cortex.

Furthermore, the developing somatosensory system is labile, in the sense that early peripheral damage results in an alteration of the associated central morphological patterns. This feature of the system, first demonstrated by Van Der Loos and Woolsey (1973), has been most extensively exploited in studies of the representation of the mystacial vibrissae. For example, in the rat cauterization of a specific row of vibrissae on the day of birth (PND0) results in a fused band of succinic dehydrogenase (SDH) activity centrally which corresponds to the damaged row of vibrissae; normally, a row of vibrissae is represented in the brain by a line of discrete SDH clusters. This change in the SDH pattern is visible in somatosensory representations at the level of the brainstem, thalamus, and cortex, but only if the damage occurs during a specific developmental period. Damage later than PND5 does not affect the thalamic or cortical pattern, although the brainstem pattern is affected because the primary afferents are directly damaged (Belford and Killackey, 1980; Bates and Killackey, 1985). Similarly, we have recently demonstrated that amputation or nerve transection of a limb on the day of birth disrupts the SDH pattern within the cortical representation of that limb (Dawson and Killackey, 1987). Further, this manipulation is ineffective in altering the cortical pattern if the surgery occurs after PND6. This suggests that the entire rat somatosensory cortex requires normal peripheral inputs during the early postnatal period in order to develop the normal pattern of SDH activity which we hypothesize to be a direct reflection of the distribution of afferent inputs (see Discussion).

A substantial portion of the rat somatosensory system develops prenatally. Afferents from the limbs invade the brainstem as early as E17 and E18 (Altman and Bayer, 1984), and there is a pattern of SDH activity in their brainstem targets, the dorsal column nuclei, on the day of birth. We therefore decided to investigate the effects of prenatal

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limb damage on these SDH patterns, when the damage is inflicted before (or coincides with) the arrival of the first forelimb afferents in the brainstem. We had reason to suspect that the effects of damage at earlier ages might be fundamentally different from those observed following postnatal damage. Johnson et al. (1972) have reported that when Virginia opossums were subjected to removal of a single hindpaw soon after birth, the ipsilateral nucleus gracilis (the target of many hindlimb primary afferents) was completely absent. The magnitude of this effect is probably due in part to the extremely altricial state of the newborn opossum, which corresponds in many ways to a prenatal stage of the rat (Hartman, 1928; Reynolds, 1952).

With this in mind, the present study was designed to address the following questions: does prenatal limb removal in the rat produce changes in the brainstem and cortex which are different from those which we observed following postnatal limb removal and if so, how do they differ, and what are the temporal constraints upon these changes?

Materials and methods

Fetal rats employed in this study were the result of timed matings. Adult rats were maintained on a 24 h light cycle consisting of 16 h of light and 8 h of darkness. Male and female Sprague–Dawley rats were paired during their 8 h dark cycle, and vaginal smears taken the following morning. The first morning that sperm was apparent in the smear was designated as embryonic day 1 of gestation (E1).

On E16, 17, or 18, pregnant females were anaesthetized with an intraperitoneal injection of Ketamine (0.1 cc/100 g) and Xylazine (0.1 cc/100 g), at which time the ventrum was shaved and rinsed with 70% alcohol. A midline incision was made beginning 1 cm anterior to the vaginal opening and extending rostrally 3 or 4 cm and fat bodies moved aside. The uterine horns were exposed, drawn from the body, and positioned in packs of saline-soaked gauze. Illumination from behind the uterus (utilizing a Dyonics fibre optic unit) enabled us to clearly visualize individual fetuses through the translucent uterine wall. The E16 rat is depicted in Figure 1. After the position of the forelimb was ascertained, a small (1–2 mm) incision was made in the uterine wall, parallel to the limb and centred over the wrist. Care was taken not to violate the amniotic sac or damage adjacent blood vessels. A purse-string suture of 6-0 silk was incorporated into the perimeter of the uterine incision, and the amniotic sac was punctured at the centre of the incision with a 25 gauge syringe needle. The tiny quantities of fluid which leaked through the hole created a vacuum which drew the limb out and rendered it accessible to experimental manipulation. The limb was pinched off tightly with a pair of fine forceps, and the distal portion of the limb removed. A fine-tipped cautery unit (National Statham Instruments) was utilized whenever necessary to prevent excessive loss of blood from the proximal stump. Following these procedures, the stump was carefully reinserted into the uterus, and the purse string suture was drawn tightly and tied to close the incision. No more than three pups per litter were operated on. In most cases, one pup was operated on in each of the distal uterine horns, and one near the cervix. Following surgery, the uterus was gently replaced into the body cavity, and fat bodies were repositioned to cover the incision sites. The abdominal muscle and fascia were closed with 6-0 silk suture, and the skin was fastened with autoclips.

Pups were born naturally on E22 or 23. The day of birth is designated as postnatal day 0 (PDN0). Roughly 70% of the pups survived the surgery and were born alive. However, the majority of these were smaller than normal, and often appeared pale or grayish instead of a healthy pink. Although they were very animated, many failed to nurse, and died within a few hours of birth. Approximately 15% of all pups operated on survived beyond the day of birth, with the mortality rate significantly higher for pups operated on E16 than E17 or 18.

FIG. 1. Appearance of the rat on embryonic day (E16). Scale at left has increments of 1 mm.

On PND15, animals were placed under deep ether anesthesia and perfused through the heart with a 10% glycerol solution in distilled water. The brains were removed from the skull and the neocortical layers were carefully separated from the underlying white matter. The cortical tissue was flattened between two glass microscope slides and immersed in isopentane (−55°C) for 2 min, then separated from the slides and immediately placed in a cryostat (−15°C). The tissue was sectioned tangentially to the cortical surface at a thickness of 80 microns, and was reacted for the enzyme succinic dehydrogenase. In a number of cases, the brainstems were also processed. Coronal sections were taken at a thickness of 32 microns, and alternate sections were reacted for SDH or stained with cresyl violet acetate. In several animals, the dorsal root ganglia (DRG) at the level of L5 (one of the principal sources of sciatic nerve afferents) were dissected on PND15, post-fixed in Bouin's fixative and sunk in 20% sucrose, and sectioned orthogonal to their cranio-caudal axis at a thickness of 10 microns. These were stained with cresyl violet acetate, and cell counts were made. Cells which contained a nucleolus were tailed in alternate sections, and this

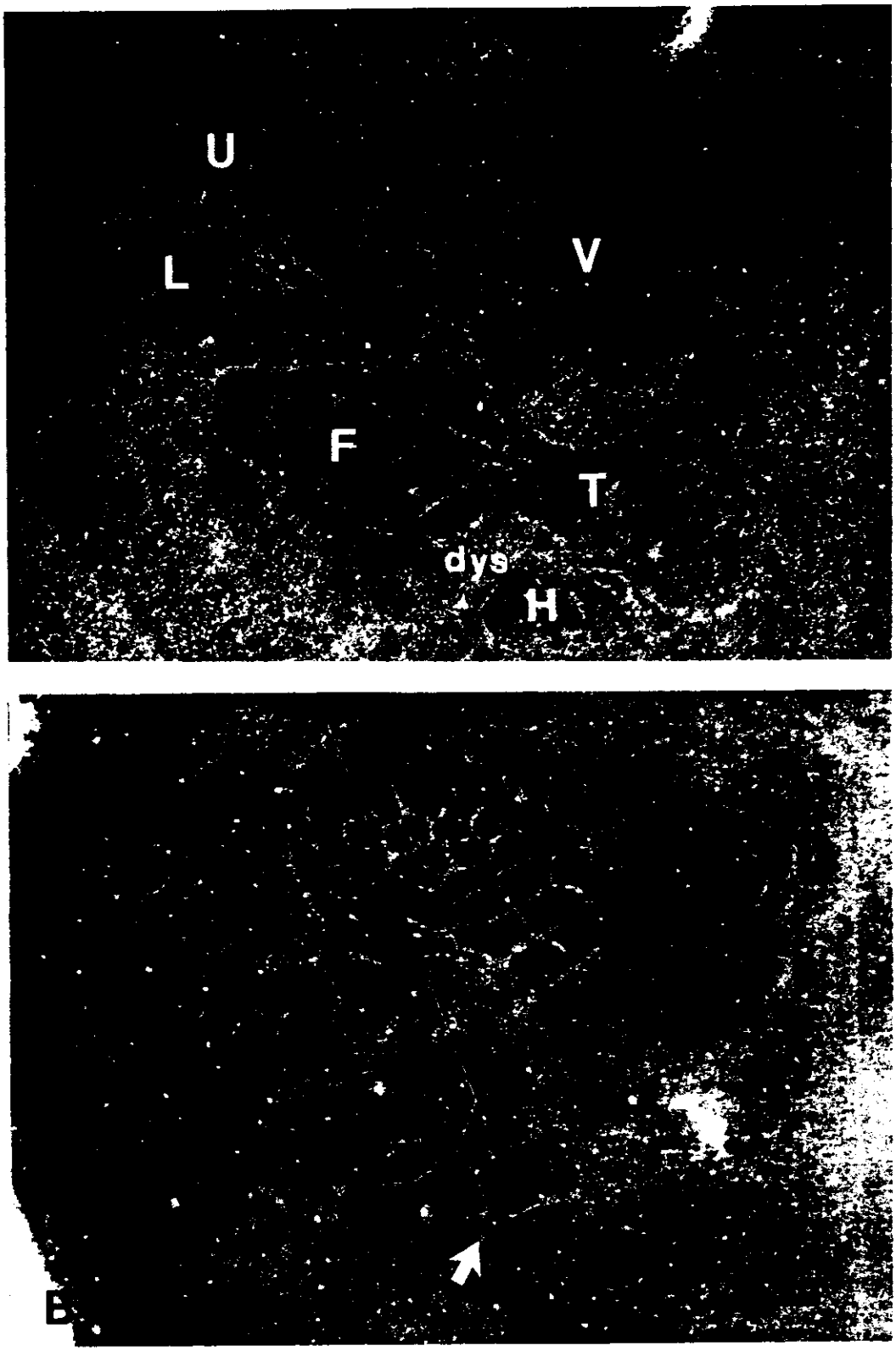

FIG. 2. (A) Photomicrograph of the normal pattern of SDH activity in layer IV of the rat primary somatosensory cortex. The cortex has been flattened and sectioned tangentially. This is the right cerebral cortex of this animal. To facilitate comparison, all cortical photomicrographs are oriented in this fashion regardless of which forelimb was amputated. The representations of the vibrissae (V), buccal pad (U), lower jaw (L), forepaw (F), hindpaw (H), and trunk (T). The dysgranular cortex between the forepaw and hindpaw representations is labelled dys.

(B) Similar pattern of SDH activity in an animal which underwent removal of the contralateral forelimb on E16 of gestation. Note the increase in the size of the hindpaw region at the lower right. The white arrow indicates the normal location of the dysgranular cortex. A = Anterior, M = Medial.

Calibration bar is 1 mm.

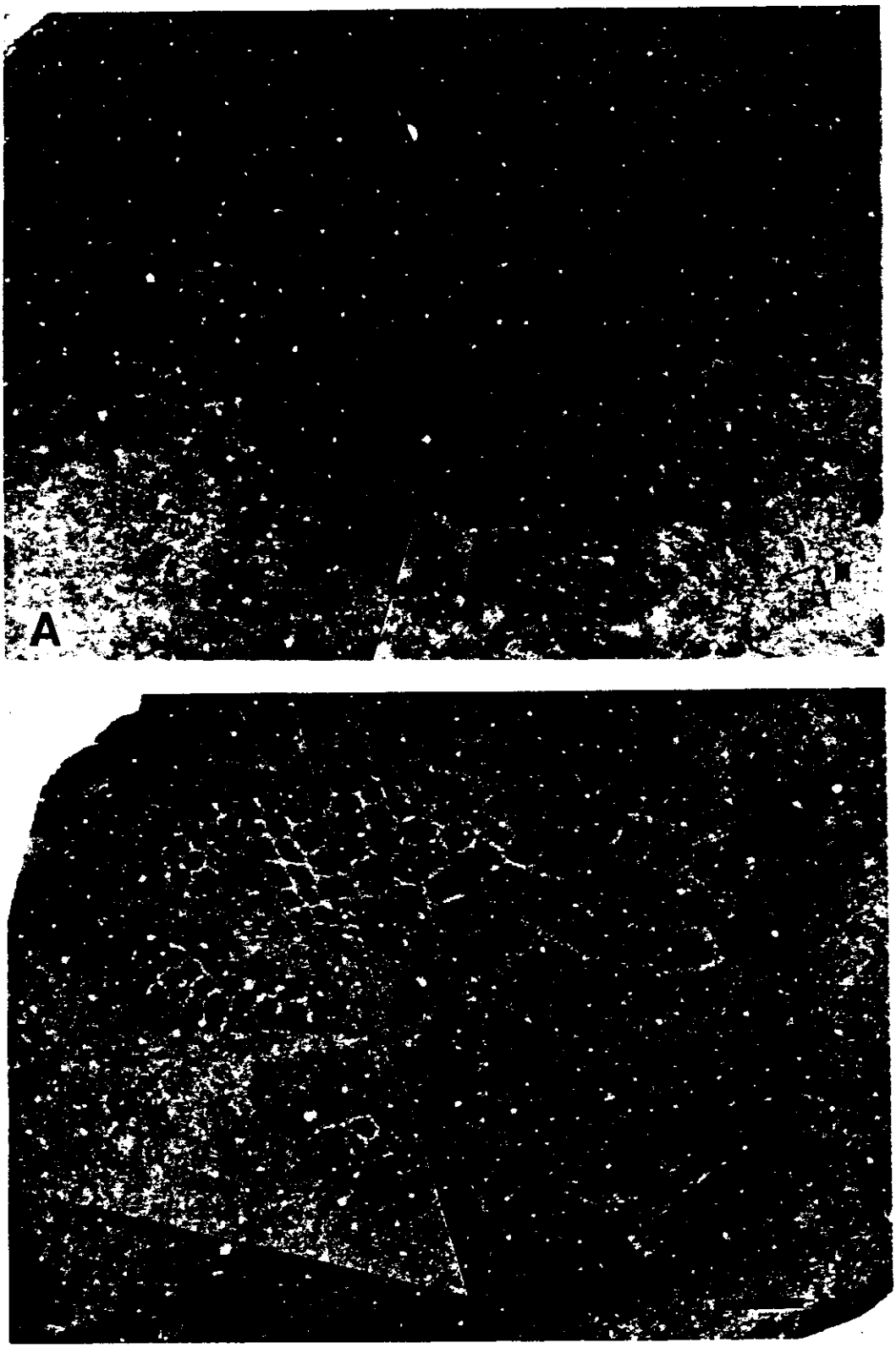

FIG. 3. Photomicrographs of the pattern of SDH activity in rat somatosensory cortex following prenatal forelimb amputations at different ages.
(A) Complete forelimb removal on E18.
(B) Complete forelimb removal on E17. A = Anterior, M = Medial.
Calibration bar is 1 mm.

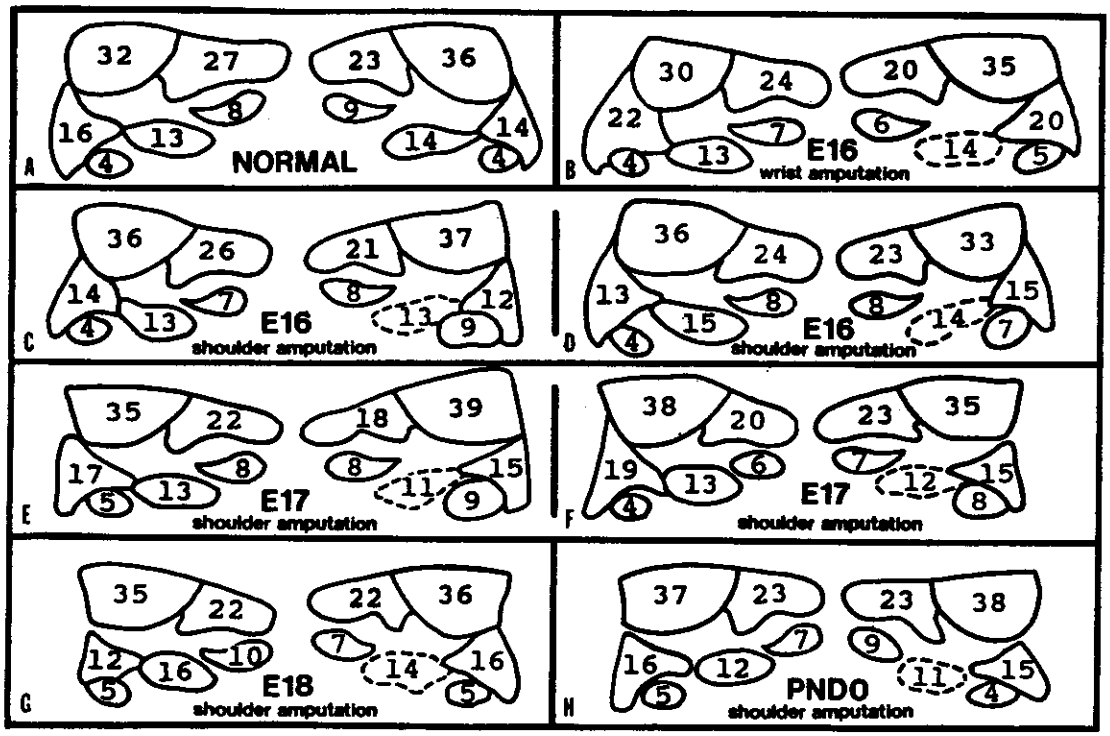

FIG. 4. The relative areas of specific subregions of the SDH pattern are depicted for animals under a variety of experimental conditions. The number within each region corresponds to the percentage of the total SDH map that the region occupies. In B–H, the normal hemisphere of the animal is depicted on the left, and the hemisphere contralateral to the peripheral manipulation is on the right. Dashed lines represent the forepaw region in denervated hemispheres.

(A) Normal animal.
(B) Forepaw amputation at the level of the wrist on E16.
(C and D) Complete forelimb amputation on E16.
(E and F) Complete forelimb amputation on E17.
(G) Complete forelimb amputation on E18.
(H) Complete forelimb amputation on PND0.

figure was doubled. It has been demonstrated that a number of cells in the dorsal root ganglia possess multiple nucleoli. To correct for this factor within a section, all cells observed to possess more than one nucleolus were counted only once. To correct for multiple nucleoli across sections, representative adjacent sections from the dorsal root ganglia of several normal and operated animals were traced at high magnification (350×) and 150–200 cells containing nucleoli were delineated in each case. The tracings of adjacent sections were then positioned atop one another, and precisely aligned with the aid of a light box, in order to determine how many cell profiles possessed nucleoli in both sections. A similar method has been put forth previously by Coggeshall et al. (1984). In the present study, it was observed that 3% of the cells counted in adjacent sections had nucleoli in both sections. Thus, the cell counts we made were multiplied by a correction factor of 0.97 to arrive at estimates of actual cell numbers.

Both the relative and absolute areas of specific regions of SDH activity in the cortical patterns within intact hemispheres and hemispheres related to a removed forelimb were calculated. The relative areas of subfields within the somatotopic map were assayed by the 'cut and weigh' procedure. Sections reacted for SDH were viewed under a light microscope, and the outlines of the SDH fields were traced onto paper of uniform thickness with the aid of a drawing tube attachment, enlarged 32 times. Individual fields were cut out and weighed on an analytical balance. These figures were then converted to percentages of the total SDH area for a given map. The absolute areas were calculated in a similar fashion. After individual regions had been weighed, a Bausch and Lomb microscope slide containing a 1 mm scale was enlarged to the same magnification as the tracings of the SDH fields. This scale was then utilized to generate a square with an area of 1 mm^2 at the appropriate magnification. The square was reproduced onto paper of uniform thickness, cut out, and weighed as before. The weights of the SDH tracings were then divided by the weight of the square, to give an estimate of absolute area.

Results

The major findings of the present study are that damage to the forelimb on E16 and E17 results not only in an anomalous cortical pattern associated with the damaged forelimb, but also in an expansion of the cortical hindlimb pattern. The expansion appears to occur in a specific cortical area, and seems to depend upon both the severity of the damage to the forelimb and the age at which the damage is inflicted. Further, there are corresponding changes in the dorsal column nuclei of the brainstem.

Figure 2A depicts the pattern of SDH activity in the primary somatosensory cortex of a normal rat. The large clusters in the posterior and medial region of the pattern represent the large mystacial vibrissae of the face (V), while the smaller clusters anterior and lateral represent hairs on the upper lip and furry buccal pad (U). More medially, a paisley shaped region of clusters represents hairs on the lower lip (L). Further medial to this region is an area containing several thick bands of SDH activity, which correspond to the digits and palm pads of the forepaw (F). Posterio-medial to the forepaw region is a small group of SDH clusters which is a representation of the hindpaw (H). Finally, a hazy

TABLE 1. Absolute area of specific subregions of the SDH representation in primary somatosensory cortex in forelimb-amputated and contralateral control hemispheres (in mm^2).

	Forelimb-denervated (age = PND 15)								Contralateral							
Day of forelimb removal	Mys. vib.	Ant. vib.	Low. lip	Tot. face	Fore-paw	Hind-paw	Torso	Total	Mys. vib.	Ant. vib.	Low. lip	Tot. face	Fore-paw	Hind-paw	Torso	Total
	7.7	4.2	1.5	13.4	2.7	1.7	2.5	20.3	7.7	5.2	1.5	13.9	2.5	0.7	2.7	19.8
E16	7.0	5.0	1.7	13.7	3.0	1.5	3.0	21.2	6.7	4.5	1.5	12.7	2.7	0.7	2.0	18.1
	7.5	4.7	1.7	13.9	2.7	1.5	2.5	20.6	7.7	5.5	1.7	14.9	3.7	1.0	3.2	22.8
$\bar{X}$ =	7.4	4.6	1.6	13.6	2.8	1.6	2.7	20.7	7.4	5.1	1.6	13.8	3.0	0.8	2.6	20.2
% =	36	22	8	66	14	8	13	100	37	25	8	68	15	4	13	100
	8.2	3.8	1.6	13.6	2.4	2.0	3.2	21.2	6.6	4.2	1.6	12.4	2.6	1.0	3.4	19.4
E17	6.6	4.2	1.4	12.2	2.0	1.4	2.8	18.4	7.2	3.8	1.2	12.2	2.6	0.8	3.8	19.4
	6.6	5.0	1.8	13.4	2.4	1.2	2.2	19.2	8.0	5.0	1.8	14.8	2.8	0.8	2.6	21.0
$\bar{X}$ =	7.1	4.3	1.6	13.0	2.3	1.5	2.9	19.6	7.3	4.3	1.5	13.1	2.7	0.9	3.3	19.9
% =	36	22	8	66	12	8	15	100	37	22	8	66	14	5	17	100
	7.5	4.3	1.3	13.1	2.1	0.8	2.6	18.6	7.6	4.6	1.5	13.7	2.6	0.6	2.3	19.2
E18	7.0	4.1	1.5	12.6	3.0	1.0	3.5	20.1	7.0	3.8	1.5	12.3	2.6	0.8	3.5	19.2
	6.8	3.6	1.8	12.2	2.5	0.8	2.8	18.3	7.0	3.8	1.6	12.4	2.6	0.8	3.6	19.4
	7.0	4.6	1.3	12.9	2.1	1.0	3.0	19.0	6.8	3.8	1.3	11.9	2.1	0.8	3.6	18.4
$\bar{X}$ =	7.1	4.1	1.5	12.7	2.4	0.9	3.0	19.0	7.1	4.0	1.5	12.6	2.5	0.8	3.2	19.0
% =	37	22	8	67	17	5	16	100	37	21	8	66	13	4	17	100
	7.8	4.7	1.6	14.1	2.3	0.8	2.2	19.4	7.0	4.1	1.6	12.7	1.8	0.7	1.8	17.0
PND0	7.3	4.0	1.2	12.5	1.8	0.6	2.5	17.4	6.6	4.5	1.6	12.7	2.2	0.7	2.0	17.6
	6.8	4.2	1.5	12.5	1.8	0.7	2.7	17.7	7.6	4.6	1.2	13.4	2.3	1.0	3.3	20.0
$\bar{X}$ =	7.3	4.3	1.4	13.0	1.9	0.7	2.5	18.1	7.1	4.4	1.5	12.9	2.1	0.8	2.4	18.2
% =	40	24	8	72	10	4	14	100	39	24	8	71	12	4	13	100
	Normal adults (age = 120 days)															
	8.3	4.7	1.7	14.7	2.3	1.0	4.0	22.0								
	9.7	5.1	2.3	17.1	2.7	1.0	3.4	24.2								
	10.0	5.0	1.6	16.6	2.5	0.9	3.9	23.9								
	8.3	4.9	2.0	15.2	2.3	0.9	3.4	21.8								
	9.1	4.9	1.9	15.9	2.5	0.9	3.7	23.0								
$\bar{X}$ =	39	21	8	68	11	4	16	100								

triangle of intermediate SDH activity which connects the paw representations to that of the mystacial vibrissae is the representation of the torso (T). This identification is based on a previous anatomical study (Dawson and Killackey, 1987) which both assayed the effect of neonatal peripheral damage on portions of the SDH pattern and made a detailed comparison between the SDH pattern and somatotopy in rat primary somatosensory cortex as revealed by physiological methods (Welker, 1971, 1976; Wall and Cusick, 1984).

Figure 2B depicts a similar pattern of activity in the cortex of an animal which underwent complete amputation of the forelimb on E16. The normal pattern of SDH activity in the region corresponding to the forepaw representation (see Fig. 2A) is clearly disrupted in this animal. None of the characteristic bands are apparent, and the boundaries of the region are less distinct than normal. We have previously reported a similar effect in animals which underwent forelimb removal or nerve transection on the day of birth (Dawson and Killackey, 1987).

A second, more striking effect of the prenatal limb removal is also apparent in Figure 2B. The size of the region devoted to the representation of the hindpaw in this animal was expanded. It now occupies 1.5 mm^2 and accounts for 7% of the total SDH pattern. A normal hindpaw representation, such as that pictured in Figure 2A, occupies only 0.8 mm^2 (3–5% of the total pattern). The SDH clusters which make up the cortical hindpaw representation appear to accomplish this expansion by extending into the zone between the forepaw and hindpaw regions, as the expanded hindpaw representation directly abuts the anomalous forepaw representation. Normally, a zone which stains very lightly for SDH separates the forepaw and hindpaw representation (see Fig. 2A).

Figure 3A depicts the pattern of SDH activity observed in response to removal of the forelimb on E18. As mentioned previously, the fine details of the forepaw pattern are clearly disrupted. The configuration and areal extent of the hindpaw representation, however, is essentially normal, and occupies 1.0 mm^2 (5%) of the total pattern. For comparison purposes, Figure 3B is a photomicrograph of the SDH pattern obtained from another animal whose forepaw was removed on E17. In this case, the hindpaw representation has enlarged and occupies 2.0 mm^2 (9%) of the total body map.

Data from a number of subjects are summarized in Figure 4 (relative areas) and Table 1 (absolute areas). Briefly, we found that complete amputation of the forelimb on E16 and E17 was effective in producing a significant enlargement of the adjacent hindpaw representation relative to the size of that representation in the contralateral hemisphere (see Fig. 4C–F and Table 1). This effect is statistically significant (Mann–Whitney Test, N1 and N2=6, U=0, P=.001 (Siegel, 1956)). Animals which were manipulated on E18 or later did not show such an enlargement (Fig. 4G–H and Table 1) and there is no difference in the size of the hindpaw representation between the two hemispheres (Mann–Whitney Test, N1 and N2=7, U=27). Furthermore, there

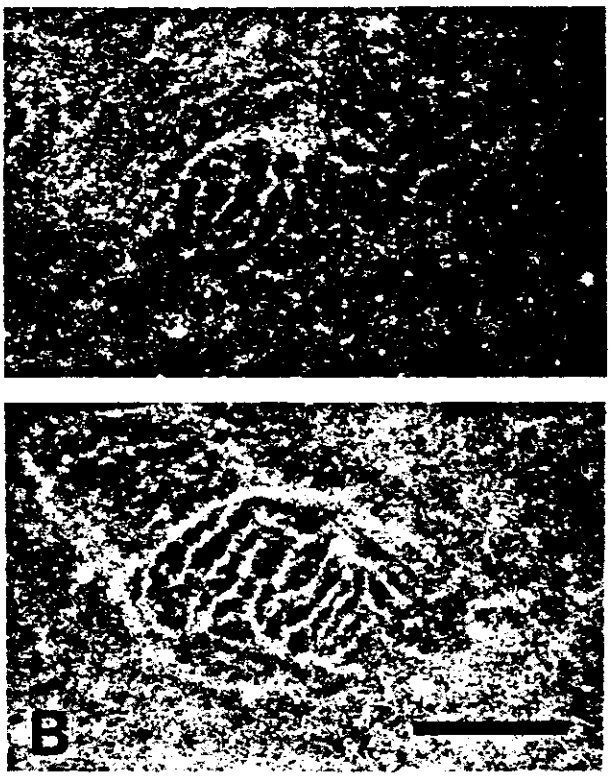

FIG. 5. Photomicrographs of the pattern of SDH activity in the cortical hindpaw representations of an animal which underwent forelimb removal on E17. The normal hemisphere is illustrated in (A) and the hemisphere contralateral to the forepaw removal is illustrated in (B). (Note: in reality the patterns in the two hemispheres would be oriented as mirrow images of each other. these photographs have been printed so that both have the same orientation.) A = Anterior, M = Medical.
Calibration bar is 1 mm.

is a statistically significant difference in the size of the hindpaw representation in the rats amputated on or before E17 compared to those amputated later (Mann−Whitney Test, N1 = 6, N2 = 7, U = 0, P = .001). It should also be noted that only a complete amputation (transection of the arm above the elbow joint) on E17 or earlier resulted in an enlargement in the hindpaw pattern. Animals which underwent amputation at the wrist, or amputation of the digits only, did not possess larger than normal hindpaw representations (Fig. 4B).

The internal structure of the enlarged hindpaw representation was examined, to determine whether the increase in size was a result of greater numbers of SDH clusters, or expansions in the size of existing ones. Figure 5 depicts normal and enlarged hindpaw rrepresentations for opposite hemispheres of the same animal (age E17 at the time of surgery). The hindpaw representation of the hemisphere opposite to the amputated forepaw (Fig. 5B) possesses an internal organization that is similar to the unoperated side (Fig. 5A), and to the normal pattern which has been described previously (Dawson and Killackey, 1987). The pattern consists of four elongated clusters at the anterior extent of the hindpaw field, bordered posteriorly by a number of smaller clusters.

The enlargement of the region of cortex devoted to the hindpaw is a specific effect, in the sense that the hindpaw representation is the only cortical SDH field which expands following forelimb removal. The small vibrissae of the lower lip are represented by a region of SDH clusters adjacent to the anterior border of the forepaw cortical field (refer to Fig. 2A). The lip representation and the hindpaw representation are at roughly equal distances for the forepaw representation, but only the hindpaw region expands following the prenatal amputations (refer to Fig. 4C−F and Table 1). Thus, we would infer that the observed expansion is a result of specific interactions between the forepaw and hindpaw afferents, rather than a generalized response to forepaw deafferentation by all surrounding cortical areas within a given radius. Similarly, we measured the absolute distance from a common landmark (the SDH cluster representing vibrissa E3 which is the third cluster from the right in the lowest row of virissae related clusters illustrated in Figs. 2 and 3) to the lateral and medial borders of the cortical hindpaw representation in normal and operated animals. In those animals denervated at E17 or earlier, the lateral border of the hindpaw area was closer to E3 (ranging from 1.8 to 2.3 mm) than in animals operated on at later ages (2.4 and 2.6 mm) or normal animals (2.6 mm). In all animals, the distance from E3 to the medial border of the hindpaw region was similar (3.3 or 3.4 mm). This would suggest to us that the *outer* border of the hindpaw representation has retained its normal relationship to other parts of the primary somatosensory cortex body representation, but that the *inner* border of the hindpaw representation has reorganized at the expense of dysgranular cortex.

In order to determine if the expansion was unique to the cortex, or was instead a reflection of events at a lower level of the nervous system, coronal sections were taken through the brainstem at the level of the dorsal column nuclei (the synaptic targets of many primary limb afferents). Figure 6 depicts SDH and nissl stained sections from two animals which underwent prenatal amputation of an entire forelimb. The animal depicted in Figure 6A and B underwent amputation of the right forelimb on E17. On the left side of the brainstem, the cuneate nucleus is seen to contain the normal, characteristic pattern of SDH segmentation, and is clearly separated from the gracile nucleus by a band of low SDH activity (arrow in Fig. 6a). This band of low activity is absent on the operated right side, and instead, there is a continuous pattern of SDH activity encompassing both nuclei. The adjacent section (Fig. 6B) was stained for nissl substance. On the right side of the brainstem, the entire region from the medial border of the gracile nucleus through the altered cuneate nucleus is densely and continuously populated with cells. This contrasts with the normal side of the animal, in which the cuneate and gracile nuclei are visible as two distinct nuclei separated by a region containing fewer, smaller cells.

Figure 6C depicts the SDH pattern in the brainstem of an animal which underwent forelimb removal on E18. The most striking difference between this animal and the one depicted in Figure 6A is the presence of a zone of low SDH activity intruding between the cuneate and gracile nuclei on the operated side (arrow). This is similar to the pale band which is observed between the cuneate and gracile nuclei on the normal side of each brainstem in Figure 6A and C. Furthermore, the adjacent nissl section (Fig. 6D) reveals that although the denervated cuneate nucleus is smaller than its counterpart on the normal contralateral side, it is nevertheless clearly discernable as a nucleus, and is separated from the gracile nucleus by a region containing fewer cells. This contrast with Figure 6B, in which a continuous band of cells is apparent between the midline and the cuneate nucleus.

Finally, cells were counted in the dorsal root ganglia at the level of L5 in animals which had undergone forelimb amputation on E17. These results are summarized in Table 2, and representative sections from ganglia on the operated and the normal side of one rat are presented in Figure 7. Briefly, there was not significant difference

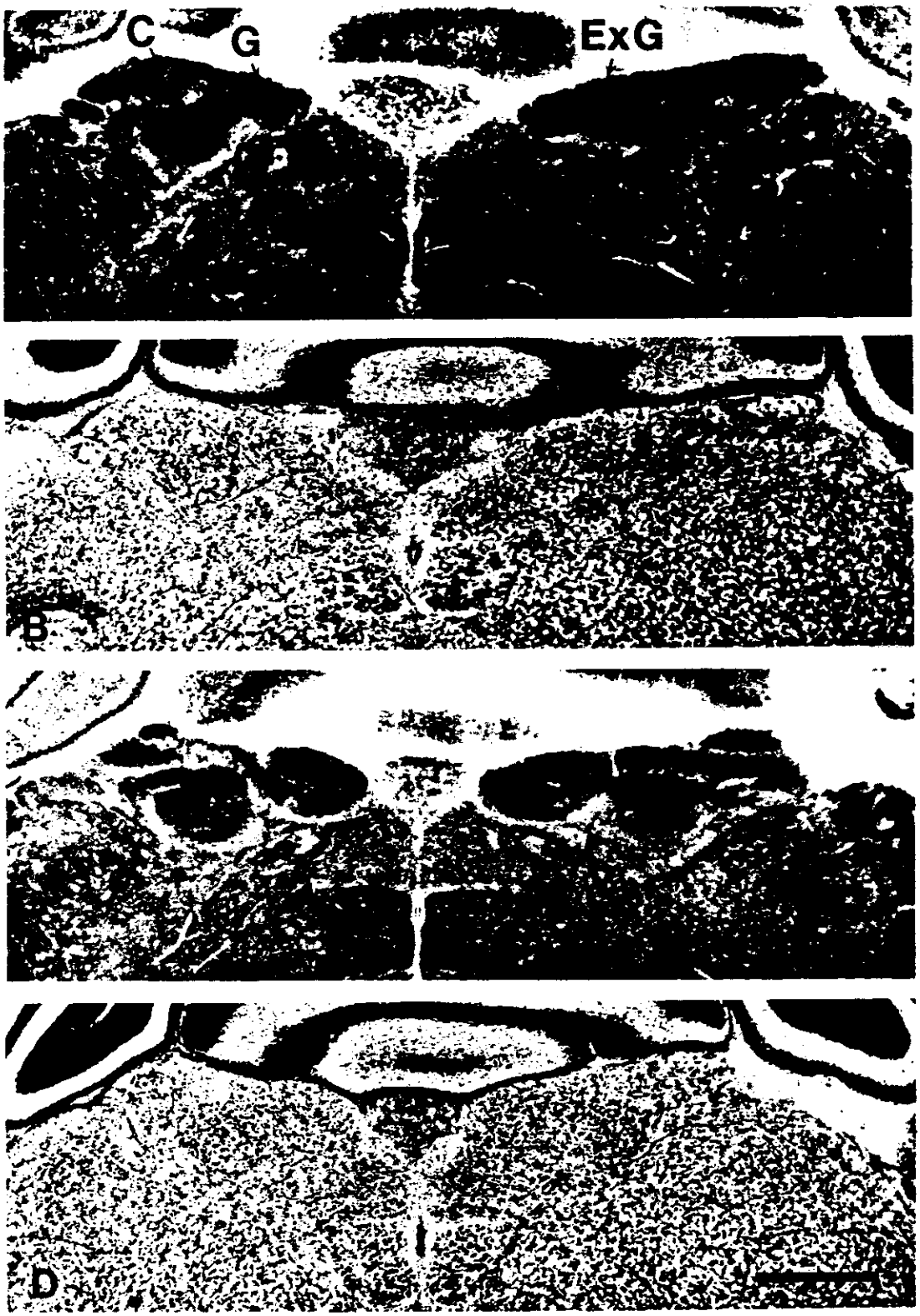

FIG. 6. Photomicrographs of coronal sections through the brainstem of rats which underwent complete right forelimb removal at E17 and E18.

(A) Pattern of SDH activity in the dorsal column nuclei following E17 amputation of the forelimb. Note the continuous SDH staining in the dorsal column nuclei on the right side and the band of low SDH activity separating the cuneate and gracile nuclei on the left (arrow).

(B) Adjacent section stained with cresyl violet acetate.

(C) Pattern of SDH activity following E18 amputation of the right forelimb. Note the band of low SDH activity (arrow) intruding between the denervated cuneate nucleus and gracile nucleus on the right.

(D) Adjacent section stained with cresyl violet acetate. C=Cuneate, G=Gracile, ExG=Expanded Gracile, D=Dorsal.

Calibration bar is 1 mm.

TABLE 2. Number of cells in L5 dorsal root ganglia subserving normal and expanded hindpaw representations.

	Normal side	Amputated side
	8226	8034
	8057	8810
	8045	7244
	9048	8538
X =	8344	8156

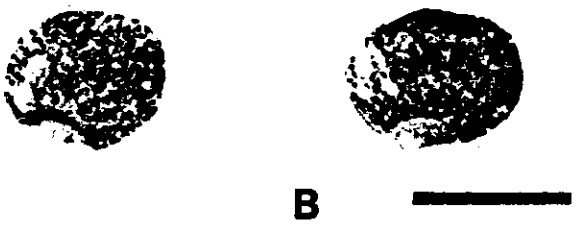

FIG. 7. Top: Dissection of the dorsal root ganglia in an animal which underwent amputation of a forelimb on E17. Arrow indicates L5 of the amputated side.
Bottom: Representative sections through L5 dorsal root ganglia from the operated (A) and normal (B) sides of an animal which was forelimb deafferented on E17.
Calibration bars are 1 mm.

between the number of cells within L5 DRGs subserving expanded hindpaw representations and those innervating normal-sized hindpaw regions.

Discussion

The major finding of the present study is that one portion of primary somatosensory cortex (the hindlimb representation) expands into an adjacent cortical field (dysgranular cortex) after damage to the periphery (forelimb removal). The expansion only occurs when peripheral damage is inflicted prior to E18. This result raises two major topics for discussion. The first is the nature of the subcortical events which underlie this phenomenon. The second is why a second cortical area is invaded rather than the anomalously organized portion of the primary somatosensory cortex (forepaw representation).

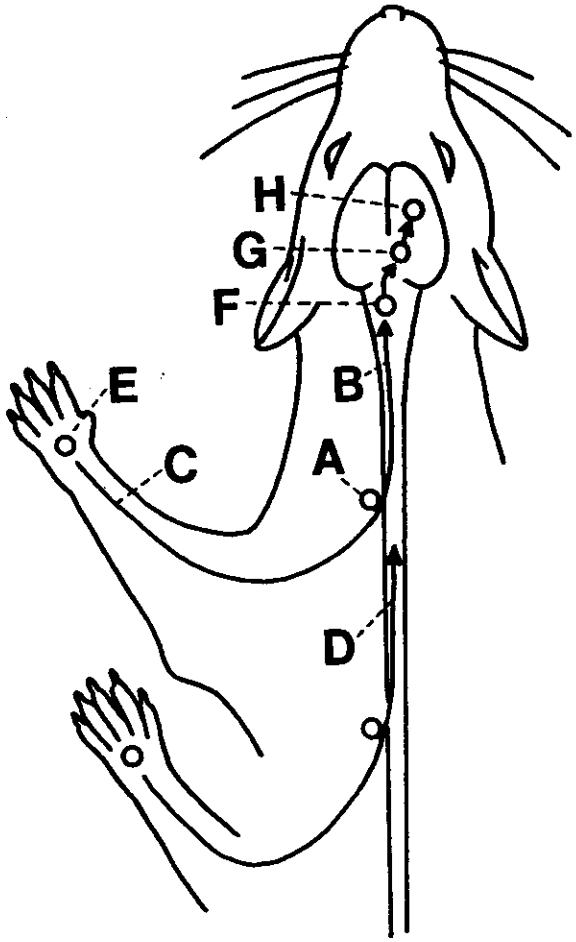

FIG. 8. Summary of the sequence of events in the development of normal limb innervation: forelimb dorsal root ganglion cells differentiate on E12–14 (A) (Altman and Bayer, 1984), primary afferents invade the brainstem (B) and reach the periphery (C) on E17 (Altman and Bayer, 1984; English et al. (1980), hindlimb primary afferents invade the brainstem one day later (D) on E18 (Altman and Bayer, 1984), forelimb primary afferents are spontaneously active on E16 and can be activated from the periphery (E) on E17 (Fitzgerald, 1987) and SDH patterns become apparent in the brainstem (F) on PDN0 or earlier, in the thalamus (G) on PND2–3 (Belford and Killackey, 1979,1980) and in the cortex (H) on PND3–4 (Killackey and Belford, 1979).

Development of the lemniscal pathway

The time course of events in the development of the lemniscal system is summarized in Figure 8 and provides the context within which our experimental manipulations are to be interpreted. Cells of the dorsal root ganglia at cervical levels 7 and 8 and thoracic level 1 which innervate the forelimb differentiate on E12 through E14 (A), peaking on E14 (Altman and Bayer, 1984). Projections from these cells grow in two directions; centrally, to enter the spinal cord and peripherally to innervate the forelimb. Central processes growing into the cuneate

nucleus (B) reach their destination on E17 as well, coinciding with the day upon which cells in the cuneate nucleus undergo their final mitosis (Altman and Bayer, 1984). Outgrowing processes (C) arrive in the periphery on E16 (English et al., 1980). Primary forepaw afferents are spontaneously active on E16 and can be driven from the periphery (E) on E17 (Fitzgerald, 1987). The cells and processes associated with the hindlimb (D) undergo a similar developmental course which takes place approximately one day later. Thus, the hindlimb afferents reach the nucleus gracilis on E18.

A distinct pattern of SDH activity is apparent in the cuneate nucleus on the day of birth (F), and perhaps earlier (unpublished observations). Subsequently, SDH patterns are apparent in the ventral posterior nucleus of the thalamus (G) beginning on PND2−3, and in the forepaw region of somatosensory cortex (H) on PND3−4 (Belford and Killackey, 1979,1980). At both the thalamic and cortical level, the total SDH pattern (including trigeminal portions) appears to develop in synchrony. There is no evidence for the development of the pattern in a gradient-like fashion as would be expected in the dorsal column nuclei from the staggered arrival of afferents and as has been demonstrated in the brainstem trigeminal nuclei (Erzurumlu and Killackey, 1983). We regard the SDH pattern to be a direct reflection of the pattern of afferent terminals. This has been well established within the rat somatosensory system (see Killackey and Belford, 1979; Bates and Killackey, 1985; Dawson and Killackey, 1987; Jensen and Killackey, 1987a for evidence and discussion of this point). Thus, we regard changes in the SDH pattern as reflecting changes in the distribution of afferent inputs.

At the level of the brainstem, the major difference between complete forelimb removal on E17 or earlier and removal at a later date is the continuous pattern of SDH staining and cell distribution across the dorsal column nuclei. This suggests to us that removal of cuneate afferents before they establish a definitive relationship with their target cells allows gracile afferents to recruit and maintain the territory between the cuneate and the gracile nuclei, and possibly to take over cells of the cuneate itself. Forelimb removal probably results in a loss of associated cuneate afferents similar to the loss of afferents observed in the rat trigeminal system following peripheral damage (Waite and Cragg, 1982; Bates and Killackey, 1985). Further, in the trigeminal system of the newborn hamster mandibular afferents expand into infraorbital nerve territory after the latter is severed on the day of birth. This effect is not observed when the same procedure is applied to the slightly more precocial PND0 rat (Jacquin and Rhoades, 1985). Several mechanisms have been hypothesized which might account for the expansions of afferent territory such as those observed in the hamster trigeminal and rat lemniscal systems. First, individual afferents may develop larger terminal fields. Second, a larger number of projection neurons associated with the intact system may survive the normally occurring process of cell death (Davies and Lumsden, 1984; Oppenheim, 1981). In the present study, we found no evidence of an increase in the number of cells in L5 DRGs in animals which possessed expanded hindpaw representations. The numbers of cells in the L5 DRGs of both normal and forelimb denervated rats were comparable to figures reported by Jacobs et al. (1981) for normal rats. Thus, we would infer that the expansion in this case is a result of normal complement of DRG cells which produce an increase number of afferent terminals in the gracile nucleus and its surroundings. This hypothesis is illustrated in Figure 9.

Given an increase in the terminal territory of the gracile afferents in the brainstem a recruitment of more cells into the dorsal column nuclei hindpaw representation is a likely consequence. This expanded hindpaw representation would then serve as a template for pattern

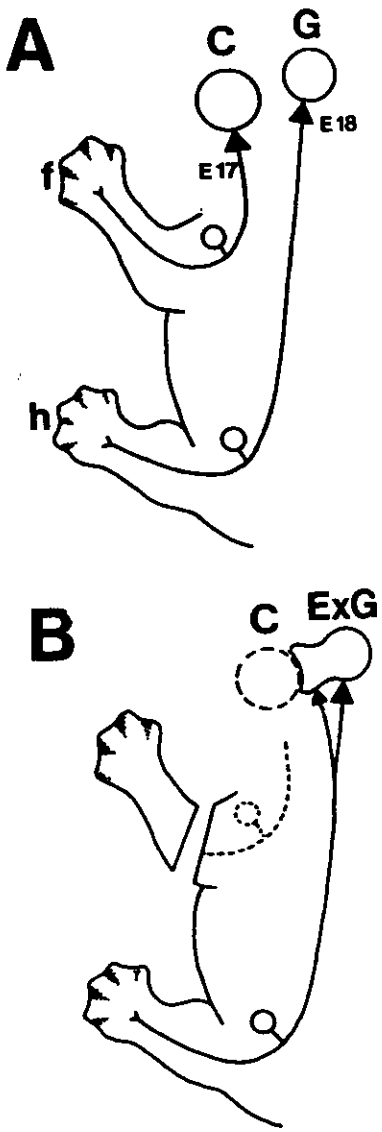

FIG. 9. Normal series of events in the development of connections between limb afferents and cells of the dorsal column nuclei (A) and hypothesized expansion of gracile fibres into adjacent territory following E17 forelimb denervation (B). C=Cuneate, G=Gracile, ExG=Expanded Gracile, F=Forepaw, H=Hindpaw.

formation in more rostral portions of the neuraxis in the same manner as suggested by Bates and Killackey (1985) for the trigeminal system. The net result of this process would be that more territory would presumably be allocated to the hindpaw representation in the ventral posterior nucleus, and (as the present results demonstrate) in the somatosensory cortex.

The failure of incomplete forepaw amputation to produce a larger cortical hindpaw representation supports the expansion and recruitment hypothesis. In the cuneate nucleus, distal structures (digits) are represented most laterally, and proximal structures more medially (Nord, 1967). Thus, incomplete amputation would spare the medial cuneate and provide an effective barrier to the expansion of the still more medially placed gracile fibres. The lack of expansion following complete forelimb removal on E18 or later suggests that neurons become committed to a given fate at the time of arrival of their major afferent source.

The expanded cortical hindpaw representation

A second question raised by our results is whether the expansion of the hindpaw region of cortex occurs at the expense of other portions of primary somatosensory cortex or at the expense of other cortical areas. The data given in Table 1 and Figure 4 suggest to us that there is no systematic change in the absolute or relative size of primary somatosensory regions surrounding the hindpaw region which could account for the expansion. In the case of the absolute area measurements, the total size of the somatosensory region in E16 and E17 amputees was greater than the contralateral side in some cases, and smaller in others. Comparisons of normal and expanded cortical areas within the same animal, as well as comparisons of the mean areas of forepaw, hindpaw, and torso from normal and expanded hindpaw animals suggests that the expansion cannot be entirely accounted for by reductions of adjacent somatosensory regions. Although in some cases the forepaw region is slightly decreased in size following denervation, it is similar in relative size. (Admittedly, it is more difficult to define the exact borders of the forelimb representation on the manipulated side.) While the unchanged relative size of the anomalous forepaw representation may seem at first glance odd, it is in agreement with the finding that thalamocortical fibres in rats which had undergone early infraorbital nerve transection still project to the appropriate portion of somatosensory cortex, albeit anomalously (Jensen and Killackey, 1987b). We would suggest that the same is probably true of thalamocortical afferents which would have been associated with the forepaw in the present case. It should also be noted that the expansion of the hindpaw representation following early amputation differs considerably from the expansion reported in other studies of the rodent somatosensory system. Several previous studies (Woolsey and Wann, 1976; Jeanmonod et al., 1981) have reported that the central representation of rows of vibrissae flanking a damaged row can expand into the central territory usually associated with that row following neonatal peripheral damage. In this case, both the peripheral receptors and their central representations are in close proximity both at the periphery and at each level of the neuraxis. In the present case, neither the peripheral receptor surfaces (forelimb and hindlimb), nor their cortical representations are directly adjacent. Adjoining central representations of these peripheral surfaces only occur at brainstem and thalamic levels. Further, the previously reported expansions of the vibrissae representation occurred within the confines of primary somatosensory while the present expansion of the hindpaw representation appears to occur at the border between primary somatosensory cortex and another cortical area.

One facet of the data which supports the hypothesis that the hindlimb representation expands at the expense of another cortical area and not the primary somatosensory cortex is the close abutment of the expanded hindpaw representation and the anomalous forepaw representation in the early manipulation cases. Normally, there is an intervening cortical area between the forepaw and hindpaw representations. In preparations reacted for SDH, this area is very lightly stained. In nissl stained material, layer IV in this area is not particularly well developed. Consequently, this area has been termed the dysgranular area, in contrast with the granular primary somatosensory cortex (Killackey, 1983). Functionally, it was first suggested that this area could not be activated by stimulation of the body surface (C. Welker, 1976). More recently, it has been determined that the dysgranular region can be activated by tactile and deep cutaneous stimulation although receptive fields tend to be larger, and require stimuli of greater intensity than those capable of activating primary somatosensory cortex (W. Welker et al., 1984). The dysgranular area does not receive inputs from the vental posterior nucleus. It is innervated by the medial portion of the posterior nucleus of the thalamus (Koralek et al., 1988) and has dense reciprocal connections with the oppostite hemisphere via the corpus callosum (Akers and Killackey, 1978; Olavarria et al., 1984).

While the development of the dysgranular area has not been well characterized, it has been determined that callosal projections develop later than thalamocortical projections in the somatosensory system (Wise and Jones, 1978) and that the mature pattern of callosal projection neurons develop after the formation of the SDH pattern which characterizes primary somatosensory cortex (Ivy and Killackey, 1981). On this basis, we would tentatively hypothesize that thalamic afferents arising from the portion of the ventral posterior nucleus associated with the hindpaw representation can expand into the dysgranular zone after early limb removal because of the relative immaturity of the dysgranular cortex. This interpretation implies that thalamic afferents of the major relay nuclei play some developmental role in specifying cortical areas and that the boundaries beteen cortical areas are not completely predetermined by genetic factors. A similar suggestion has recently been made by both Rakic (1988) and Dehay et al. (1989) in interpreting the effects of early binocular enucleation in the rhesus monkey on the organization of areas 17 and 18 and on the boundary between them. Together, these experiments expand the potential role of the periphery in the regulation of cortical organization and provide a tentative opening to understanding the factors which contribute to the cytoarchitectonic subdivisions of the neocortex.

Acknowledgements

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Abbreviations

DRG dorsal root ganglia
E1 embryonic day 1
PND0 postnatal day 0
SDH succinic dehydrogenase

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Callosal projections in rat somatosensory cortex are altered by early removal of afferent input

(development/corpus callosum/thalamic ablation/nerve section)

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ABSTRACT **During the first postnatal week, the distribution of callosal projection neurons in the rat somatosensory cortex changes from a uniform to a discontinuous pattern. To determine if this change is influenced by afferent inputs to the somatosensory cortex, the effect of both early unilateral infraorbital nerve section and unilateral removal of the dorsal thalamus on the distribution of callosal projections in rat somatosensory cortex was examined. One month after either of the above manipulations at birth, the tangential distribution of callosal projections in the somatosensory cortex was examined using the combined retrograde and anterograde transport of horseradish peroxidase. Both manipulations alter the distribution of callosal projection neurons and terminations in the somatosensory cortex. After infraorbital nerve section, the distribution of callosal projections is altered in the contralateral primary somatosensory cortex. The abnormalities observed are consistent with the altered distribution of thalamocortical projections. In addition, consistent abnormalities were observed in the pattern of callosal projections of the second somatosensory area of both hemispheres. Most notably, they are absent in a portion of the region that contains the representation of the mystacial vibrissae and sinus hairs in this area. Thalamic ablation resulted in highly aberrant patterns of callosal projections in the somatosensory cortex on the operated side, where abnormal bands and clusters of callosal projections were observed in apparently random locations. These results are interpreted as evidence that both peripheral and central inputs influence the maturational changes in the distribution of callosal projection neurons.**

In the rat somatosensory system, early peripheral and more central damage has a profound effect on neuronal organization at succeeding levels of the neural axis up to and including primary somatosensory cortex (1–5). Similar effects have also been reported in the visual system (for review, see refs. 6 and 7) but in this case an additional effect of early damage on the distribution of interhemispheric projections that interconnect cortical visual areas by way of the corpus callosum has also been reported (8–12). The one study (13) that examined the effect of neonatal damage to afferent cortical input in the rat somatosensory system reported that direct thalamic damage in the neonatal rat has no effect on the distribution of callosal projections in somatosensory cortex. This result is somewhat surprising for two reasons. (*i*) In most other respects the central effects of peripheral injury in the somatosensory system are very similar to those in the visual system. (*ii*) The early distribution of callosal projection neurons is very widespread throughout all of the somatosensory cortex, and during the first postnatal week or so they are largely eliminated from regions of primary somatosensory cortex that receive dense thalamic input from the ventral posterior nucleus (14, 15). In light of this, we decided to further assess the effect of early peripheral injury and thalamic damage on the distribution of callosal projections in rat somatosensory cortex.

MATERIALS AND METHODS

Thirteen litters of Sprague–Dawley or of Long–Evans rats were used in this study. All neonatal surgery was performed under cryogenic anesthesia, after which rats were revived on a heating pad and returned to their dams. Rats from 4 litters were subjected to unilateral section of the infraorbital nerve on the day of birth. Rats from 7 litters had their dorsal thalami removed unilaterally by aspiration through a small-diameter glass pipette that was inserted into the cranium from a posterior approach. Rats from 2 additional litters were used as age-matched controls. The pattern of callosal projections as determined by the combined retrograde and anterograde transport of horseradish peroxidase and patterns of staining for succinic dehydrogenase of all rats were examined when they were 1 month old. In addition, the distribution of horseradish peroxidase label in the thalamus ipsilateral to the cortical injections was examined to verify the uniformity of tracer uptake in the cortex (see Fig. 2*D*). In this context, both the absence of the ipsilateral thalamus for verifying cortical injections and the difficulty of combining complete thalamic ablations with large horseradish peroxidase injections for labeling the entire callosal pathway precluded study of the effects of thalamic ablation in the hemisphere contralateral to thalamic removal.

Under ketamine/xylazine anesthesia, rats received multiple injections of horseradish peroxidase [50% (wt/vol); Sigma, type VI] evenly distributed over the surface of the cortex. After a 24- to 36-hr survival period, rats were deeply anesthetized and perfused transcardially with saline followed by a 1.25% (vol/vol) glutaraldehyde/1% paraformaldehyde buffered fixative solution. The brains were removed, the cortices were detached, and the noninjected cortex was held flattened between glass slides during post-fixation and sucrose infiltration. The thalami were sectioned in the coronal plane and the cortices were sectioned in the tangential plane on a freezing microtome. Sections were processed for horseradish peroxidase histochemistry according to the protocol of Mesulam (16), mounted on gelatin-coated slides, and air-dried before being covered with a coverslip. Sections through the thalami were counterstained with neutral red.

One rat from each litter was used to determine cortical patterns of succinic dehydrogenase staining. These rats were deeply anesthetized and perfused transcardially with 10% (vol/vol) glycerol. The brains were removed and the cortices were detached and held flattened between glass slides as they were immersed in isopentane cooled to −40°C. Tangential sections were then cut in a cryostat, collected on gelatin-

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coated slides, and reacted for succinic dehydrogenase histochemistry as described by Killackey and Belford (17).

RESULTS

In layer IV of rat primary somatosensory cortex (SI), the distribution of afferent terminations arising from the ventral posterior nucleus is reflected in the pattern of succinic dehydrogenase activity (17, 18), as in Fig. 1*A*. Overall, this pattern is a caricature map of the body surface of the rat. Note the exaggerated representation of densely innervated portions of the head such as the mystacial vibrissae and smaller sinus hairs on the muzzle. Lateral to SI, there is a second cortical area that also contains a representation of the body surface of the rat. Although this representation is not detectable with succinic dehydrogenase histochemistry, it can be demonstrated with physiological (19, 20) and anatomical (ref. 21; unpublished observations) techniques. In this area (SII), the map is organized as a rough mirror image of the first representation. Thus, there are two adjoining representations of the vibrissae and upper jaw sinus hairs in the rat somatosensory cortex. The infraorbital nerve innervates both the vibrissae and sinus hairs of the upper jaw of the rat. If this nerve is sectioned at birth, as shown in Fig. 1*B*, the regions in SI in which these structures are normally represented are highly abnormal. Instead of rows of punctate clusters in SI, there are bands as well as areas of irregular staining in which there is no discernible pattern. This altered succinic dehydrogenase pattern reflects the altered distribution of thalamocortical afferents (5). Other portions of the map (e.g., lower jaw, forelimb, and hindlimb representation) are normally organized and there is no detectable change in enzyme staining in SII.

The normal pattern of callosal projections to rat SI cortex is largely complementary to the pattern described above (22). Labeled axons and somata surround and interdigitate with the regions that receive dense thalamic input from the ventral posterior nucleus (see Fig. 1*C*). In the region of the head representation, labeled callosal neurons and terminations tend to outline the representations of individual vibrissae and sinus hairs. There is also a dense band of callosal projections that overlaps the border between SI and SII. Adjoining this band within SII, there is a caudomedial to rostrolateral alignment of "holes" that contain very sparse callosal projections separated by labeled "bridges" of callosal cells and terminations. Such bridges are continuous with a further lateral and caudal band of labeled callosal projections in SII.

Manipulations of the peripheral input to the somatosensory cortex result in profound modifications of the pattern of callosal projections. In rats whose infraorbital nerves were sectioned at birth, the band of densely labeled callosal cells and terminations that straddles the border of SI and SII is disrupted (see Fig. 1 *D–F*). The major anomaly is a large gap in this band of callosal projections in a constant location largely within SII. In addition, smaller more variable gaps in the band are seen further caudally and medially in SII. These anomalies in the pattern of callosal projections are seen in both hemispheres although they tend to be more pronounced in the hemisphere contralateral to the infraorbital nerve section. Further, they are found in the portion of SII where the vibrissae and sinus hairs are represented. In the hemisphere contralateral to the nerve section (see Fig. 1 *D* and *F*), the pattern of callosal projections within SI is also perturbed. Abnormal clusters of label are sometimes found within the region in which the mystacial vibrissae and sinus hairs would normally be represented. In portions of SI in which the succinic dehydrogenase pattern was not affected normal patterns of callosal connectivity were observed. Note in particular the normal pattern of callosal projections in the cortex associated with the lower jaw in all the cases illustrated.

Manipulation of the thalamic input to somatosensory cortex affects the distribution of callosal projections even more profoundly. The cortices of three rats in which the thalamus was removed at birth as well as a coronal section through the thalamus from one of these rats, indicating the extent of the lesions, are illustrated in Fig. 2. In these cases, aberrant patterns of callosal projections are found throughout the neocortex on the side of the lesion. In most cases, abnormal bands, patches, and clusters of labeled axons and somata are seen in presumptive somatosensory cortex. The location of this label varies from rat to rat and the apparently random patterns formed bear no resemblance to any portion of the patterns observed either in normal rats or in rats subjected to infraorbital nerve section at birth. In some rats (which are not illustrated), the label is more diffuse and, in yet others, large regions of the neocortex are devoid of callosal projections. This change in the distribution of callosal projections appeared to be contingent upon complete lesions of the ventral posterior nucleus. In cases in which a portion of the ventral posterior nucleus was left intact callosal projections were largely normal.

DISCUSSION

The present results provide evidence that the pattern of callosal projections in somatosensory cortex is dramatically altered by removal of thalamic afferents to this cortex and is more subtly altered by section of the nerve innervating the periphery represented in a portion of this cortex. These results are best discussed in terms of the effect of these experimental manipulations on the different cortical areas composing the rat somatosensory cortex, namely, SI and SII, and in the context of developmental change in the distribution of callosal projection neurons. Initially, callosal projection neurons are uniformly distributed throughout somatosensory cortex although located in their correct laminar position (14, 15). At approximately the end of the first postnatal week, the mature disjunctive pattern of callosal projections is achieved by a process that apparently involves the retraction, or elimination, of the axonal processes of inappropriately located neurons rather than cell death (23, 24). In the rodent visual cortex, a similar change in the distribution of callosal projection neurons occurs (25) and various early manipulations have been found to result in callosal projections within primary visual cortex in areas from which they are normally eliminated (9–11). On this basis, it has been suggested that peripheral input acting through the thalamus plays some role in the maturation of the callosal pattern. The altered pattern of callosal projections in SI after infraorbital nerve section can be interpreted in a similar fashion. Infraorbital nerve section at birth results in alterations in neuronal projection patterns at each succeeding level of the neural axis (5, 26, 27). Thus, the periphery can be regarded as providing an extrinsic template that is passed from a given level of the neuroaxis to the next within the confines of a preexisting topographic order (28). At the cortical level, the chief effect of infraorbital nerve section seems to be on the terminal arbors of the thalamocortical projection fibers (5). After infraorbital nerve section, terminal arbors are more variable and larger in size. They also tend to be more elongate in form and the extend of overlap between adjacent arbors is much greater than normal. The net result of this is a "smearing" of the normally punctate somatotopic map (compare the face representation in Fig. 1 *A* and *B*). In this context, the altered distribution of callosal projections in SI after infraorbital nerve section is a direct consequence of the altered distribution of thalamocortical projections in somatosensory cortex that in turn results from an altered extrinsic template. Thus, in SI the distribu-

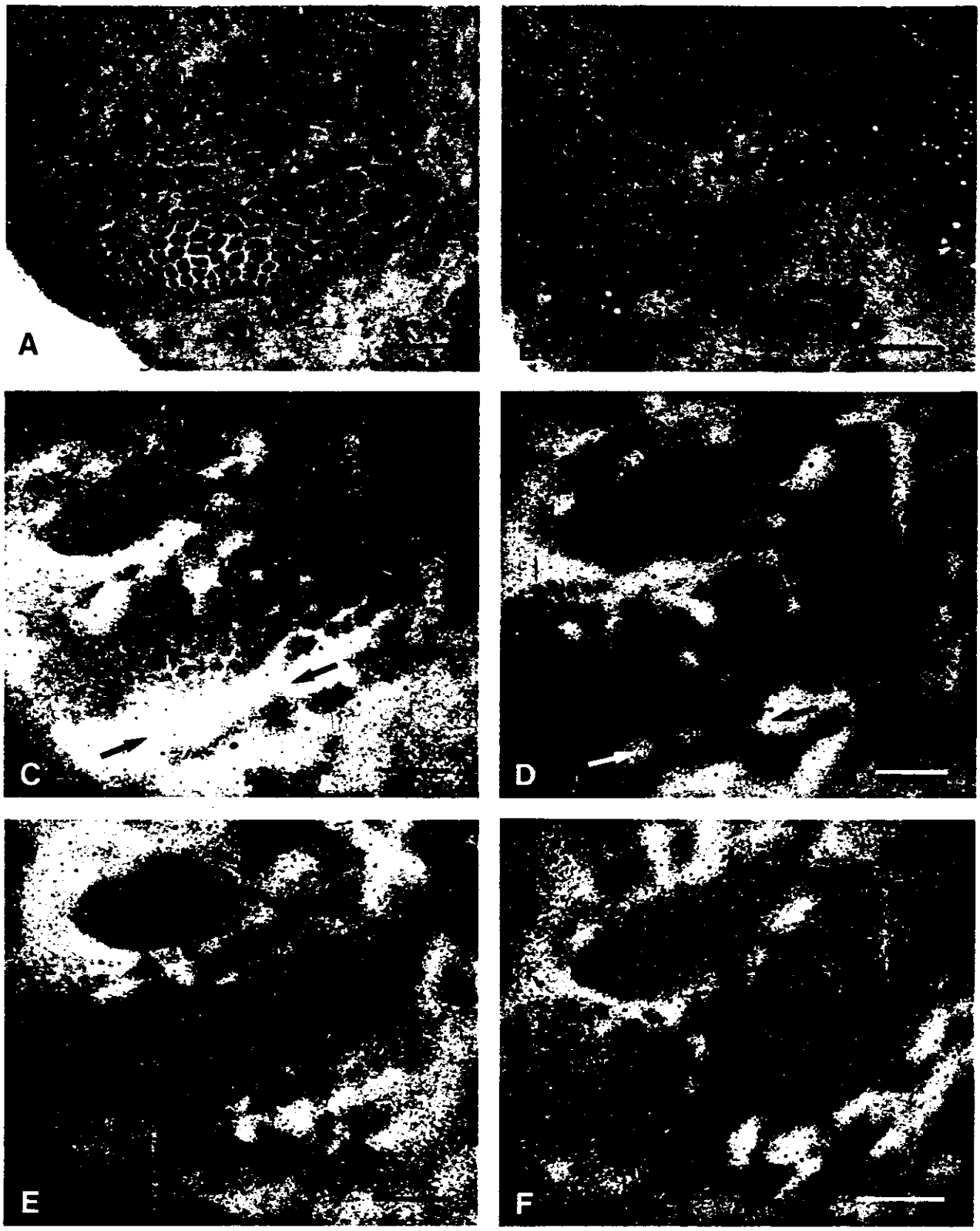

FIG. 1. Photomicrographs of tangential sections through the flattened somatosensory cortex. (*A*) Pattern of succinic dehydrogenase staining in a normal rat. The representation of various portions of the body surface (F, forepaw; H, hindpaw; L, lower jaw; T, trunk; U, upper jaw; V, vibrissae) in the primary somatosensory cortex and the location of the second somatosensory area (SII) are indicated. (*B*) Pattern of succinic dehydrogenase staining in somatosensory cortex contralateral to the side on which the infraorbital nerve was sectioned at birth. Note the anomalous pattern of staining in the portions of SI in which the vibrissae and upper jaw are represented but not in the area of the representation of the lower jaw or limbs. (*C*) Dark-field photomicrograph illustrating the distribution of horseradish peroxidase-labeled callosal neurons and terminations in the somatosensory cortex of a normal rat. The pattern of callosal projections in the somatosensory cortex contralateral (*D* and *F*) and ipsilateral (*E*) to the neonatal infraorbital nerve section. The arrows in *C* and *D* point out the normal pattern of callosal projections in a portion of the face region at the SI/SII border and the altered pattern after infraorbital nerve section, respectively. (Bars = 1 mm.)

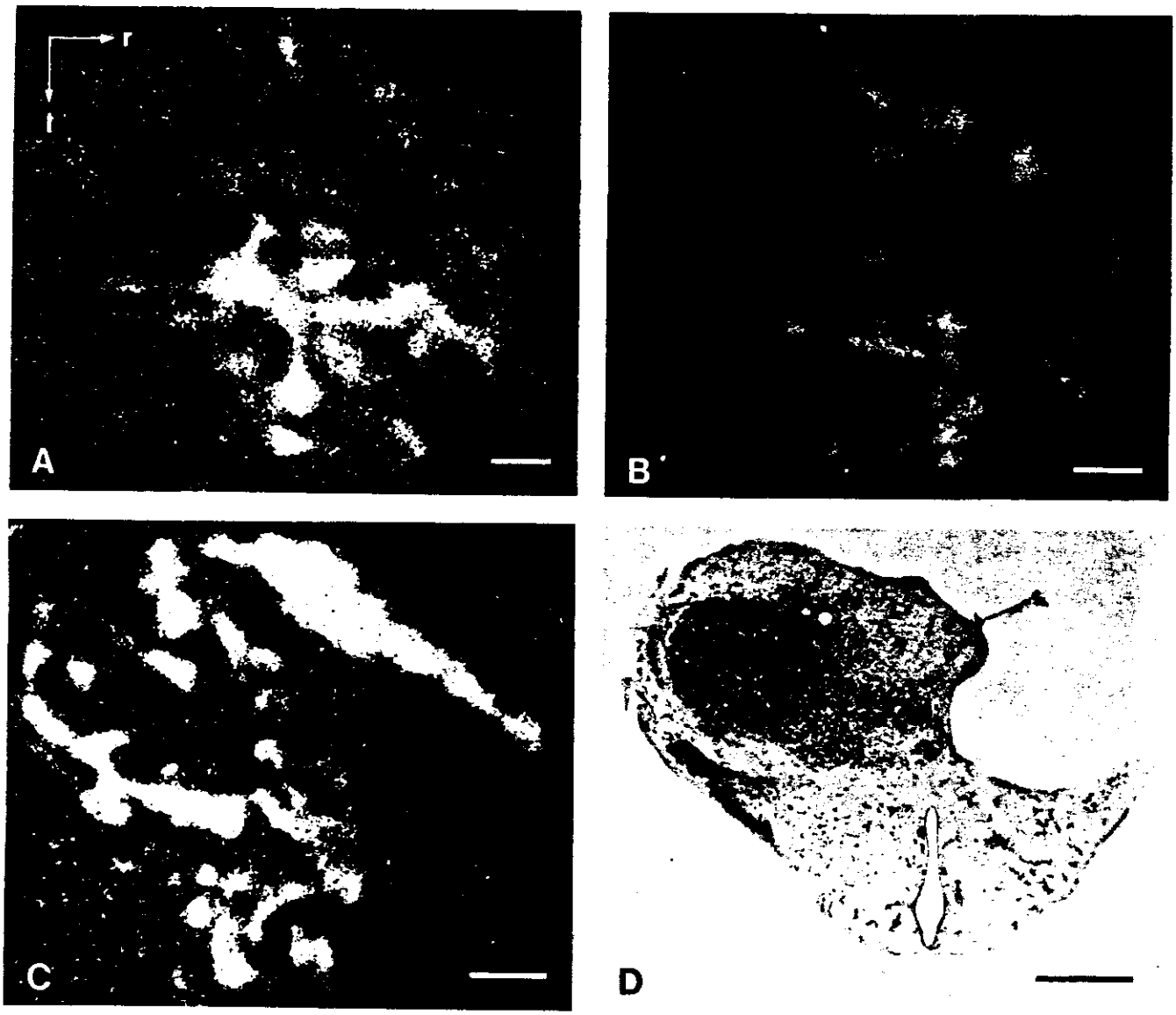

FIG. 2. (*A–C*)Dark-field photomicrographs of tangential sections through the flattened cortex after the removal of the ipsilateral dorsal thalamus at birth. The distribution of callosal projections in all three rats bears no resemblance to either the normal pattern or altered patterns illustrated in Fig. 1. (*D*) A coronal section through the thalamus of the rat whose cortex is shown in *C*, illustrating the extent of the ipsilateral lesion. (Bars = 1 mm.)

tion of callosal projections after nerve section are determined by the same mechanism(s) that operates in the normal animal; however, the extrinsic cues provided to this mechanism are different.

The pattern of callosal projections after thalamic ablation, on the other hand, can be interpreted as a result of an expression of the more intrinsic organizational properties of these projections. In this context, thalamic ablation may be regarded as the ultimate way of removing organizing influences that are extrinsic to the neocortex. If an extrinsic template such as alluded to above was the only source of organization available to the maturing callosal projections, one would have expected this manipulation to result in a maintenance of the initial widespread and uniform distribution of callosal projection neurons. This is clearly not the case in the somatosensory cortex (both SI and SII). Rather, the clustered nature of callosal projections in these cases demonstrates a residual tendency toward aggregation. Similarly, the lack of a uniform distribution suggests that some process elimination is occurring. The factors guiding this aggregation and elimination may be intrinsic to callosal neurons or related to remaining inputs to the cortex—most notably, callosal projections originating in the opposite hemisphere. Another point to consider in relation to SI is the apparent discrepancy between the current results and the report of Wise and Jones (13) that neonatal thalamic ablation does not alter the pattern of callosal projections in somatosensory cortex. These authors focused on the laminar pattern of callosal terminations that is best viewed in the coronal plane. They reported that the laminar pattern of terminations is unchanged and that these terminations form discrete patches in the less granular portions of somatosensory cortex as in the normal rat. This is not inconsistent with the present results as we also observe discrete patches of callosal label in somatosensory cortex. However, the distribution of these patches is radically altered. While this is readily apparent in the tangential plane of section, it is less obvious in the coronal plane. Finally, it should also be mentioned that thalamic ablation is a relatively massive manipulation that may have other effects on the neocortex in addition to altering the distribution of callosal projection neurons. For example, it has been reported (29) that lesions of the thalamic input nucleus result in increased

cell death in the visual cortex. This effect, however, seems to be largely focused on cortical layers IV and VI, which directly receive thalamic input, and not on the layers in which callosal projection neurons and their terminations are located. Similarly, although the present thalamic ablations also severed the subcortical projections emanating from the neocortex, there is evidence that the response of callosal projections and subcortical projections to peripheral manipulations are independent and not interactive. Rhoades and Fish (30) have reported that whereas bilateral enucleation alters the distribution of callosal projection neurons, this same manipulation does not alter the distribution of either corticotectal or corticogeniculate projections.

In SII, the pattern of callosal projections is also altered by neonatal infraorbital nerve section. The major effect of this manipulation is a lack of callosal projections in the somatotopically appropriate part of SII, portions of which are normally heavily interconnected by the corpus callosum. Further, this effect is also detectable in SII in the hemisphere ipsilateral to the nerve section. In relation to the hypothesized role of the periphery in establishing central patterns of neural organization, the result suggests that this role extends beyond primary sensory areas of cortex into cortical areas less directly associated with receptor surfaces, perhaps even into the other hemisphere. A second aspect of the result is that the nerve section resulted in an absence of normally occurring callosal projections suggesting that either abnormal process elimination or cell death has occurred. Most studies in the rodent visual cortex (9–11) generally report a more widespread distribution of callosal projections after peripheral manipulation. This difference may be attributable to the fact that these studies focused on primary visual cortex, which may be considered to be more closely related to its respective receptor surface than is SII, and the fact that primary visual cortex receives a bilateral retinal input. In any case, the present results establish that a peripheral manipulation can result in both abnormal distributions of callosal projection neurons in SI and a failure to maintain callosal projections in SII. The presence of an effect in the hemisphere ipsilateral to the lesion that receives normal peripheral input from the contralateral body surface suggests that peripheral input *per se* is not sufficient to maintain callosal projections in SII. In this area, the failure to maintain callosal projection neurons is most likely attributable to anomalies in the contralateral SII. Is this failure due to callosal processes from this hemisphere finding an abnormal target contralaterally or, conversely, is it due to the inappropriate withdrawal of callosal processes originating in the anomalous hemisphere, their failure to provide the appropriate signal for maintenance, or both? This conundrum illustrates the basic problem in understanding the maturational changes in the distribution of callosal projection neurons. At present, it is unclear whether the mechanism(s) that underlies process elimination operates by maintaining projections in "appropriate" locations, by eliminating them from "inappropriate" locations, or by both.

In summary, the present experiments provide evidence that the mature distribution of callosal projections is shaped by multiple influences. One such influence is the periphery acting by way of thalamic afferents to the cortex. In addition, reciprocal interactions between the hemispheres appear to play some role in these maturational events. The role of such reciprocal interactions is more obvious in cortical areas further removed from the periphery.

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