An Organizing Principle of Thalamic Plasticity

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Why should we subsidize intellectual curiosity?

RONALD REAGEN

campaign speech, 1980
There is nothing which can better deserve our patronage than the promotion of science and literature. Knowledge is in every country the surest basis of public happiness.

GEORGE WASHINGTON
address to Congress, January 8, 1790

The effort to understand the universe is one of the very few things that lifts life, a little above the level of farce, and gives it some of the grace of tragedy.

STEVEN WEINBERG
The First Three Minutes, 1977

Humanity is exalted not because we are so far above other living creatures, but because knowing them well elevates the very concept of life.

EDWARD O. WILSON
Biophilia, 1984
Abstract

Three dimensional reconstructions of thalamic (ventroposterolateral nucleus; VPL) sensory maps were used to detect somatotopic reorganization in the thalamus. Hindlimb input was removed (>60%) by nucleus gracilis lesions in adult rats (control (n=8); acute (n=6); 1-week post-lesion (n=8); 1-month post-lesion (n=8)). The removal of hindlimb input by nucleus gracilis lesions revealed a focal zone of reorganization within the VPL (300 μm thick) at times 1-week and 1-month post-lesion. Shoulder sensory maps in the thalamus were found to increase in total volume by 25% as early as 1-week post-lesion (p<0.001). From this finding the “hot spot model” was postulated, which predicted that such zones were manifested at other levels of the nervous system and independent of the form of neural trauma. In the second study, nerve transection of the hindlimb was performed to see if a similar outcome was obtained (sham (n=6); nerve-transection (n=8)). Despite numerous differences between nerve transection and nucleus gracilis lesions, the same focal zone of reorganization in the VPL was observed. This latter finding further underscored the importance of this focal zone and the validity of the hot spot model. Finally, it was asked if higher levels of brain organization contribute to plasticity at lower levels of the nervous system. Cortical aspiration of the forelimb somatosensory cortex was performed the same day as nucleus gracilis lesions (n=8). Remarkably, the removal of the forelimb somatosensory cortex blocked all thalamic somatotopic reorganization (n=7) when compared to nucleus gracilis lesioned animals (n=7; p<0.05). Cortical lesions alone had no effect on the volume of thalamic sensory maps (n=6, p>0.05). Importantly, if cortical lesions were performed after thalamic reorganization had occurred, thalamic plasticity was unaffected (n=6; p<0.05). In conclusion, there is a focal zone of somatotopic plasticity in the thalamus that appears to be independent of the form of deafferentation, which requires the presence of an intact cortex for the induction of somatotopic reorganization, but not its maintenance.
Dedication

I dedicate this work to my parents, Gary and Jill Parker. They have always been strong supporters of my academic pursuits, without which, this research would not have been possible.

Sometimes issues become so esoteric in basic research, I've known I could always count on the unassailable interest of at least one person; my mother. My father did not have the benefit of an education beyond high school - but has showed me from a very early age that solutions come from asking the right questions. In light of this I believe that a university is a place to learn for those who choose to do so, but ultimately, the engine of curiosity draws its strength from a much more modest source: the home.
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Jayson L. Parker, M. Sc.
Organization of Thesis

Chapters outlining the author’s original studies: Introduction, three papers and a Summary and Conclusion. The Introduction provides an overall framework to view the field, progressing from receptive fields to sensory maps. What follows are three chapters (2 - 4) which are self-contained papers. Because of the extensive discussions in each of the three data chapters, a brief Summary and Conclusions is offered at the end of the thesis.
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Chapter I

Introduction
Prolog

Tuthmosis III, the reigning Pharaoh in Egypt during the approximate time period 1600 BC, experienced a turbulent rule over his dominion (Grimal 1988). His rule was made difficult by the fact that, at least initially, he was co-regent with his stepmother queen Hatshepsut (Grimal 1988). Due to problems with the Pharaoh line prior to Tuthmosis III rise to power, Hatshepsut ruled Egypt as both king and queen, going so far as to wear a false beard to fulfill these roles (Pellegrino 1994).

Secular archeological analysis suggest that if the Judean-Christian figure Moses (Exodus 1000 BC) actually existed, Tuthmosis III would have had dealt with this figure (Pellegrino 1994). The Hebrew prophet Moses, through his God, commanded seven plagues on Egypt in an effort to force the release of his people (Exodus 1000 BC). Secular interpretations (Pellegrino 1994) of this period in history have suggested that the seven plagues were actually an account of events catalyzed by the most powerful volcanic eruption in the history of mankind. The volcano Thera erupted in the Aegean sending soot and tsunamis towards Egypt during the reign of Tuthmosis III (Stanley and Cheng 1987; Hughes 1988).

While Biblical texts are often evoked to describe this period in history, until recently little attention had been paid to the Egyptian version of the event. Egyptian texts, while they do not appear to mention Moses (sic), do refer to the Hebrew emigration (Grimal 1988). The prevailing conditions described by the Egyptians are: "For nine days there was no exit from the palace and no one could see the face of his fellow. ...The Sun is covered and
Introduction
does not shine to the sight of men. Ra [Sun God] has turned his face from mankind. If only it would shine even for one hour! No one knows when it is midday... The Sun in the heaven resembles the moon...” (Ipuwer papyrus). The Egyptian Ipuwer papyrus reveals events that appear to be more consistent with the secular view of a volcanic eruption than seven discrete plagues from the heavens recounted in the Old Testament.

Such a discussion has only recently been possible. The ability to translate the ancient scripts of Egypt was lost to mankind for more than a millennium. It was only the discovery of the Rosetta stone by Napoleon during his campaign in Egypt in 1799 (Champollion 1814) that has enabled the translation of ancient Egyptian texts.

The Rosetta Stone as a Metaphor in CNS Organization

The Rosetta stone is a black granite tablet containing a decree issued during the reign of Ptolemy V Epiphanes over Egypt in approximately 250 BC (Bernal 1987). It contains three passages layered one on top of the other. The top passage was written in Egyptian hieroglyphs. The middle passage was written in demotic Egyptian, a more common script of the people. The bottom passage was written in Greek, still understood today. The translation of the Greek allowed the eventual decipherment of the above two passages.

It took linguists 20 years to translate the Rosetta stone (Bernal 1987). Two assumptions appear to have been made about the passages to enable translation. First, it was assumed that Egyptian script was based on a lexicon - the use of discrete elements such as words. This was contrary to the widespread belief that hieroglyphs were above the use of alphabets or
phonetics but were instead based on some higher form of representation (Kircher 1652; Champollion 1814). Second, it was assumed that each passage communicated the same message. In summary, the translation of the Rosetta stone required linguists to make two assumptions about the passages on the tablet:

I. A discrete elementary basis to information.
II. Each level is isomorphic in content.

This author wonders whether the organization of our brains parallels that seen in the Rosetta Stone: (I) Is it possible that there is a motif of nervous system organization that exists at all levels of the neuraxis? (II) Are the changes in central representations precipitated by the removal of afferent input the same from the spinal cord to the cortex? If we provisionally accept the structure of the Rosetta Stone, with its hierarchical organization, as a metaphor for central nervous system organization (CNS), the case will be made in this thesis that neuroscientists must adopt similar assumptions in deciphering plasticity in the nervous system.

The next few sections will identify plausible analogs of these two assumptions in the nervous system. The first supposition can be loosely equated with the Mountcastle’s cortical columns (Mountcastle 1957) or more closely with the “hot spot” model of neural plasticity (Parker et al. 1998) erected in this work. The hot spot model is not formally discussed until Chapters 2 and 3. The second assumption is identified with a currently ignored model of brain function - reentrant connectivity (Edelman 1987). Reentrant connectivity is discussed in detail in Chapters 3 and 4. Therefore,
the Rosetta stone metaphor of central nervous system organization is buttressed by two theories that are analogous to the assumptions above:

I. Elementary units in the form of Columnar Organization.
II. Isomorphic content made possible by Reentrant Connectivity.

In the next four sections suppositions I and II will be examined against the corpus of literature documenting the organization and plasticity of the somatosensory system in adult animals.

Anatomical Overview of the Somatosensory System

Information pertaining to pain, temperature and tactile input all pass through the somatosensory system. Input from the periphery is relayed via the dorsal root ganglion into the dorsal horn of the spinal cord (Figure 1.1 depicts the spinal somatosensory system). Upon entering the spinal cord three basic pathways emerge that ascend the spinal cord (Gilman and Newman 1987). One pathway is the lemniscus pathway, which conveys fine tactile (low threshold) input. Sensory input from the lower trunk of the body is carried by the funiculus gracilis, while input contacting the upper body is conveyed by funiculus cuneatus. Collectively both pathways are referred to as the dorsal columns, and terminate in the dorsal column nuclei. There are actually two pathways to the dorsal column nuclei. One projection originates from a neuron in the dorsal horn that is contacted by the primary afferents. This has been referred to as the post-synaptic dorsal column pathway (Nathan
and Smith 1959). The second projection is from the dorsal root ganglion that travels with the spinal cord and terminates in the dorsal column nuclei. From the dorsal column nuclei, the lemniscal pathway decussates and terminates in the contralateral VPL. VPL neurons then project to the cortex, terminating chiefly in the granular layer and layer IIIb (Jones and Powell 1969; Landry and Deschenes 1981). Corticothalamic input to the VPL originates from the infragranular layers, V and VI (Jones 1985; Deschenes et al. 1994).

The two remaining pathways convey low threshold cutaneous stimuli, in addition to other types of input. The spinothalamic tract is chiefly involved in the transmission of high threshold mechanical and temperature input (Willis et al. 1974). A neuron in the dorsal horn receiving input from the dorsal root ganglion sends a projection that immediately decussates, and ascends the spinal cord, terminating in the thalamus and is relayed to the cortex (Getz 1952). Finally, a neuron in the dorsal horn receiving input from the dorsal root ganglion projects ipsilaterally to the lateral cervical nucleus, located just lateral in the upper cervical dorsal horn. Projection neurons from this nucleus immediately decussate and ascend to the VPL. The spinocervical pathway responds primarily to tactile inputs although some responses to noxious input have been reported (Giesler et al. 1979).

The trigeminal system, while not part of the spinal somatosensory system, conveys input from the face to the ventroposterior medial nucleus
Figure 1.1 The spinal somatosensory system. Three basic pathways course through the spinal cord, conveying cutaneous input from the periphery to the VPL: lemniscal (dorsal columns), spinothalamic tract (STT) and spinocervical tract (SCT). Weak ipsilateral projections reaching the thalamus and cortex are not shown (nor projections to the brainstem or other thalamic nuclei). See text for details. Abbreviations: dorsal root ganglion (DRG), post-synaptic dorsal column (PSDC), dorsal horn (DH), dorsal column nuclei (DCN), ventroposterior lateral nucleus (VPL) and lateral cervical nucleus (LCN).
(VPM) of the thalamus (Jones 1985). Input from the oral-facial region enters the brainstem nuclei of V, an equivalent structure to the dorsal horn. Input then ascends to the nucleus principalis of V (PrV), an equivalent structure to the dorsal column nuclei. Axons from PrV decussate in the ventral pons to join the medial lemniscus pathway before reaching the VPM (Jones 1985). This pathway conveys noxious, temperature and cutaneous input. While the focus will be on the spinal somatosensory system, literature in the trigeminal system is discussed when research performed in this pathway has no equivalent study in the spinal somatosensory system.

Receptive Fields: A Neural Lexicon?

The connectivity of a single neuron is a paragon of complexity in the nervous system. A typical neuron may have 1000 to 10,000 synapses conveying information from approximately a 1000 other neurons (Stevens 1979). If one then moves their frame of view from the neuron to the human brain, this complexity takes on added dimensions with $10^{11}$ neurons and roughly $10^{14}$ synapses (Hubel 1979). Anatomically, if one allowed two synapses in a given pathway, it is possible to show linkages between virtually any two structures in the brain, "Give me two synapses, and I'll give you the brain" (Dr. Jean Saint Cyr, Personal Communication, 1992).

Prima facie, this connectivity suggests that stimuli entering the sensory apparatus of the neuraxis could only result in widespread activation with
seizure (grand mal) like consequences. Despite the connectivity of a single neuron, research on the neuromuscular junction suggested that many synapses may be unused (Mark 1970; Marotte and Mark 1970a; Marotte and Mark 1970b; Mark and Marotte 1972; Mark et al. 1972). This research was concerned with the rapidity of reinnervation of fish eye muscles in the course of embryological development. The rate of reinnervation was considered important since "...loss of incorrect action (incorrect innervation) occurs quite suddenly and completely on the very first sign of reinnervation by the correct nerve" (Mark 1970). The author suggested that while the rate at which inappropriate action by the nerve was lost precluded degeneration, it may be possible that the action of the inappropriate nerve was blocked through repression by the appropriate nerve. Thus, some synapses may be unused and this may be achieved through inhibition (Mark 1970; Mark et al. 1972), a concept later applied to neurons in the CNS (Merrill and Wall 1972; Wall 1977).

Functionally "silent synapses" (Wall 1977) contacting a neuron are consistent with electrophysiological findings that neurons possess a receptive field. In the somatosensory system a receptive field has been defined as "a region of skin which, when stimulated with the appropriate modality, evokes impulses in the neuron at a rate which significantly exceeds the rate of spontaneous activity" (Wilson and Kitchener 1996). The receptive field of a neuron in the CNS is much smaller than would be expected from a neuron's afferent input, since input from some skin regions is functionally "silent". Small receptive fields permit greater tactile acuity, in such tests as two-point discrimination (Sinclair 1981). As one moves to more distal areas of the body surface, receptive fields decrease in size (Mountcastle 1957) consistent with
observed increases in acuity on two-point discrimination tests (Weber 1834; Sherrington 1900).

While the size of a receptive field may be a determinant of tactile acuity, other parameters play an important role since receptive fields are not homogeneous domains of sensory input. Receptive fields are often most excitable in one location, typically near their centre (Taub 1964; Brown et al. 1986; Clemo and Stein 1991; DiCarlo et al. 1998). As one moves away from the excitatory receptive field centre, evoked excitation drops off steeply (Laskin and Spencer 1979). In rarer instances, neuronal receptive fields have been reported that either have several focal sites of excitation (Johansson 1976) or are entirely uniform in their excitability (Lindblom 1965; Knibestol 1975).

Excitatory receptive fields may only be one important component governing the responsiveness of a neuron "... when neurons with receptive fields on fingerpads are stimulated with scanned, complex, spatial stimuli, almost all yield responses that are more complex than those accounted for by simple excitatory receptive fields" (DiCarlo et al. 1998). Much of this complexity may be accounted for by overlapping inhibitory receptive fields with the excitatory receptive field (Laskin and Spencer 1979; Gardner and Costanzo 1980; DiCarlo et al. 1998). In these reports, inhibitory receptive fields were explored by measuring the efficacy of skin stimulation in several different areas to block or attenuate responses evoked from subsequent stimulation of the excitatory receptive field. Stimulation within an area of the excitatory receptive field can attenuate subsequent stimulation to another area of the excitatory receptive field. Zones of maximal excitation and inhibition have been found in the same area (Iwamura and Inubushi 1974b;
Laskin and Spencer 1979). The interval for such paired stimulation effects is well outside the refractory period of neurons (Laskin and Spencer 1979).

Different spatial configurations have been described between excitatory and inhibitory receptive fields. In primate somatosensory cortex, three quarters of excitatory receptive fields were contiguous with an inhibitory zone, in half of those cases the inhibitory zone was located to one side of the excitatory receptive field (DiCarlo et al. 1998). Thus, inhibition could have lead to motion selectivity within a somatosensory neuron, as spontaneous activity was depressed when stimulation occurred in a non-preferred direction (Gardner and Costanzo 1980).

It has also been known for some time that inhibitory zones may surround the excitatory receptive field, often referred to as surround inhibition (Mountcastle 1957; Mountcastle and Powell 1959; Iwamura and Inubushi 1974b; Janig et al. 1977). There is some data that suggested that more distal body regions experienced greater surround inhibition than more proximal body regions (Iwamura and Inubushi 1974a). In shaping responses to complex stimuli inhibition may act to "funnel" (Békésy 1967) the response of a neuron toward the earlier larger stimulus component of a complex pattern at the expense of later or weaker stimulation (Laskin and Spencer 1979).

One of the most important aspects of a receptive field is its border, since factors that shape this contour can be important determinants in receptive field plasticity. Neurons have been described that had abrupt receptive field boundaries, such as those in lamina IV of the dorsal horn (Taub 1964; Brown
Increases in the excitability of lamina IV neurons by removal of descending inhibition by reversible cold block of the thoracic cord or post-tetanic stimulation, did not alter the border of the receptive field (Wall 1967; Hillman and Wall 1969). Conversely, decreases in excitability by barbiturate anesthesia (Wall 1960) or brainstem stimulation (Taub 1964) did not affect the receptive field border. In the context of the spinal cord it has been suggested that receptive field boundaries are not the product of inhibition (Merrill and Wall 1972). However, this conclusion was contradicted by studies on zones that extended beyond the receptive field border (reviewed below).

Previously it had been suggested that many synapses may be unused or silent, possibly by some form of inhibition from “correct” input (Mark 1970). These “silent” synapses (Wall 1977) were believed to convey input from skin regions beyond the excitatory receptive field (subliminal fringe) that are unable to evoke discharge in the cell (Merrill and Wall 1972). However, when these skin regions outside the excitatory receptive field were probed with electrical stimulation they evoked neuronal discharge (Merrill and Wall 1972; Brown and Fyffe 1981). It had been suggested that receptive field expansion in the context of plasticity may have occurred by the “unmasking” (Merzenich et al. 1983) of silent synapses in the subliminal zone by the removal of inhibition following deafferentation (Wall 1977; Merzenich et al. 1983; Merzenich et al. 1984). In an investigation of cuneate neurons, it was found that receptive field borders were more susceptible to inhibition (Janig et al. 1977). Additionally, in the cerebral cortex microiontophoresis of the GABA (Gamma - aminobutyric acid) antagonist bicuculline resulted in receptive field expansion (Hicks and Dykes 1983; Dykes et al. 1984), although a similar manipulation at the thalamic level had inconsistent effects on
receptive field sizes (Hicks et al. 1986). Inhibition appears to be an important factor regulating receptive field borders, but there may also be exceptions to this scheme across nervous system structures.

One would expect that the observed receptive field for a neuron must show some overlap with the receptive fields of neurons providing afferent input. This does not appear to be entirely correct based on characterization of terminal arborizations and receptive fields of primary afferents and spinocervical tract neurons (Brown and Noble 1982). On the one hand, primary afferents that synapse proximal to the cell body of spinocervical tract neurons have correspondingly centrally placed receptive fields on the skin within the receptive field of the spinocervical tract neuron. Further, primary afferents with synaptic contacts distal on the spinocervical tract neuron had receptive fields peripherally located within the receptive field of the spinocervical tract neuron. The closer the primary afferent's contact to the soma of the spinocervical tract, the more aligned their receptive field centres came to represent maximal excitatory zones. However, in the same system neurons have been described with receptive fields that do not overlap with primary afferent receptive fields (Woolf and Fitzgerald 1986). In this group of cells there appeared to be a genuine mismatch between the receptive fields of spinocervical tract neurons and primary afferent input. These authors proposed that interneurons and descending input may override the receptive field characteristics of a neuron that would otherwise be derived from its primary afferent input.

Viewing the nervous system as an assembly of receptive fields provides one elementary unit of organization. But as a lexicon we are left
with a language with approximately $10^{11}$ members. Another level of nervous system organization may yield a more manageable lexicon that encompasses receptive fields: Mountcastle's cortical columns.

**Columnar Organization: A Neural Lexicon?**

If we perceive receptive fields as elementary filters from which more complex transformations of sensory input may be achieved, the spatial organization of receptive fields provides an additional window on such computation. In this section we will consider what the properties of a columnar motif may be and its existence at multiple levels of the somatosensory pathway.

Lorente De No (1949) was the first to suggest that the vertical organization of neurons or input terminals constituted an elementary unit of the neocortex. This has been variously referred to as neuronal chains, slabs or modules (Iwamura et al. 1985). A functional demonstration of modular organization in the primary somatosensory cortex strengthened this speculation (Mountcastle 1957) bringing it to the forefront of research in neuroscience.

*Evidence From the Cerebral Cortex*

Vertical penetrations in cat primary somatosensory cortex (SI) revealed that neurons encountered along this trajectory had the following characteristics based on mechanical stimulation of the skin (Mountcastle 1957): (1) responded to the same single class of cutaneous receptor; (2) possessed almost identical peripheral receptive fields and (3) each lamina
responded at latencies that were not significantly different. The apparent uniformity of these properties for a vertical array of neurons powerfully reinforced the concept of modular organization. Property three suggested that neurons were not only grouped into columns with similar receptive fields, but such columnar organization was also the spatial unit of cortical computation. Mountcastle's (1957) research with angled electrode penetrations into the cortex suggested that the dimensions of these cortical columns were about 500 μm in diameter.

Electrophysiological organization of the cortex viewed under the rubric of columnar organization has encountered some opposition. One principal difficulty has crystallized over the years: the finding that there was receptive field overlap between adjacent electrode penetrations in the cortex (Towe 1975; Sur et al. 1980; Dykes 1983; Favorov and Whitsel 1988), which appeared to do violence to the idea of modular organization. Receptive field overlap has been observed between electrode penetrations separated by as much as 1.5 mm (Sur et al. 1980). However, as Towe's (1975) influential critique may have overlooked, Mountcastle was aware of this phenomenon as a similar distance was mentioned in his canonical work (1957). Mountcastle later contended (Mountcastle 1978) that columnar organization is a product of dynamic interactions between neurons in a network and underlying receptive field overlap is not unexpected. On the other hand, it has been reported that receptive field overlap was not observed in the granular layer (lamina IV) (Sur et al. 1984; Sur et al. 1985). These latter reports demand a closer examination of the structure of cortical column with respect to receptive field organization.
A number of investigators have reported that receptive field size varies with cortical lamina and that granular layer neuronal receptive fields are small with sharply defined boundaries (McKenna et al. 1984; Alloway and Burton 1985; Chapin 1986). In contrast, it has been found that neurons in the infra- (V, VI) and supragranular layers (I-III) have receptive fields that are much larger than granular layer neurons (IV), with less sharply defined edges (Alloway and Burton 1985; Chapin 1986). Chapin (1986) proposed an "hour glass" model of receptive field organization across cortical lamina: neurons in the supra- and infragranular possess large receptive fields, while those neurons interposed between these two layers (granular layer) have small receptive fields.

What was unique about Chapin's (1986) study was his three-dimensional (3D) reconstructions of neuronal receptive fields, based on recordings from rat somatosensory paw cortex. For a given recording site, he mapped the spatial dimensions of the receptive field while collecting data on the magnitude of neuronal discharge to a mechanically controlled tactile stimulus to each location on the skin. These 3D reconstructions suggested that granular layer receptive fields were simpler by comparison with other lamina, with a single sharp peak of responsiveness. On the other hand, the infragranular layer demonstrated multiple peaks yielding a response profile across the body surface that was much more complex. The supragranular area also appeared to be more complex with low broad peaks in receptive field structure. It was suggested that the complexity of the infra- and supragranular layers in comparison with the granular layer may reflect input from other columns.
Edelman (1987) had taken the hour glass model one step further by suggesting that this relationship may be dynamic; an expansion of receptive fields in the granular layer would result in a corresponding contraction in receptive fields sizes in the infra- and supragranular layers. In this latter view, reciprocal inhibition may exist between lamina within the column. The issue of receptive field overlap and its consequences for modular organization in the cortex is yet unresolved. The hour glass model suggests that receptive field centres may be aligned within a column but the size of receptive fields may vary with cortical lamina.

The hour glass model did highlight the importance of the granular layer with its sharply defined receptive fields. Indeed, in the study of somatotopic plasticity in the cerebral cortex, investigators appeared to record almost exclusively from this layer (McKenna et al. 1984; Jenkins et al. 1990; Recanzone et al. 1992). The granular layer also appeared to be distinguished by the presence of alternating bands of rapidly adapting and slowly adapting input (Dykes 1983; Sur et al. 1984; Sur et al. 1985). While rapidly adapting input was found in the granular, supra- and infragranular layers, slowly adapting input was exclusively found in the granular layer (Sur et al. 1984; Sur et al. 1985). The authors suggested that there may be a double representation of the body surface in the granular layer, each representation carried by the rapidly and slowly adapting neurons respectively. Additionally, it was suggested that both bands should be included in a unit considered fundamental to cerebral cortical organization.

Recently evidence has suggested that the cortical column is not homogeneous with respect to plasticity (Diamond et al. 1994). Paired
stimulation of rat vibrissa was conducted and examined within 24 hours by comparison to stimulated unpaired whiskers in control animals. Recordings were performed in the barrel cortex. Paired stimulation resulted in a greater likelihood that neurons responsive to one whisker would respond to stimulation of either whisker. Remarkably, it was found that such changes did not occur in a uniform manner within a vertical electrode penetration in rat vibrissae cortex. Changes occurred in the infra- and supragranular layers but not the granular layer. The authors suggested that changes observed at an acute time interval in the infra- and supragranular layer were temporary, enabling similar changes in the granular layer as has been commonly reported at chronic time intervals (Merzenich et al. 1988). This study demonstrated that not all cortical layers are equally plastic, indicating that the cortical column may not be uniformly plastic along its length.

To summarize the properties of a columnar motif derived from data collected at the cortical level:

- Isomorphic receptive fields
- Isomorphic cutaneous modality
- Segregated rapidly and slowly adapting neurons
- Hour glass model of receptive field interaction
- Focal areas of plasticity

Evidence From the Other Levels of the Somatosensory Pathway

The arrangement of somatotopy in the cortex may be regarded as two-dimensional (2D) based on evidence for Mountcastle’s cortical columns (Mountcastle 1957; Mountcastle 1978). However, at the thalamic level
detailed mapping has lead some investigators (Kaas et al. 1984) to hypothesize that "...the disruptions, regions of isorepresentation and regions of gradual change result from the thickening, splitting and folding of a two dimensional representation of the skin surface to occupy a three dimensional (3D) volume." According to this latter view, 2D sensory representations at the cortical level are folded into a 3D configuration at the thalamic level. On the other hand, the same study did report lines of isorepresentation with gradual receptive field changes along the rostrocaudal and dorsoventral axis consistent with an alternative hypothesis: thalamic rods (Kosar and Hand 1981; Jones and Friedman 1982; Jones et al. 1982).

Thalamic rods are the same constructs as Mountcastle's cortical columns, except they have been rolled in the thalamus such that they are parallel to the anteroposterior axis (Jones et al. 1982). This has been referred to as rotated somatotopy (Parker et al. 1998). Somatotopic representation in the cortex is mapped with vertical electrode penetrations parallel to columnar organization, but to achieve the same relationship in the VPL one must advance the microelectrode parallel to the anteroposterior axis (Jones et al. 1982; Parker et al. 1998). Electrode penetrations following the anteroposterior axis in the thalamus encountered lines of isorepresentation over a distance of 500 - 800 μm (in monkey) followed by gradual receptive field shifts on the body surface (Jones et al. 1982). The distance for isorepresentation was likely underestimated since the VPL is crescent-shaped along the anteroposterior axis (Cajal 1911; Rainey and Jones 1981).

Reminiscent of Mountcastle's (1957) strategy for determining the width of presumptive cortical columns, angled electrode penetrations in the
VPL of the thalamus encountered rapid receptive field changes over distances as short as 100 \( \mu \text{m} \) (Jones et al. 1982). The rapidity of receptive field changes with angled penetrations would be predicted since such trajectories cut across the main axis of columnar organization (Mountcastle 1957; Jones et al. 1982).

Tract tracing studies also support columnar organization in the thalamus. Elongated injections of isotope along the anteroposterior axis of the thalamus anterogradely labelled a comparatively small portion of the primary somatosensory cortex compared with injections elongated along the mediolateral or dorsoventral axes (Jones et al. 1982). These results provided some evidence for the existence of thalamic rods. Injections spread along the anteroposterior axis would be expected to reach the same thalamic rod, consistent with the comparatively small labeling observed in the cortex. Injections spread along any other axis would be expected to label groups of thalamic rods, consistent with the larger staining pattern observed in the cortex. Further, horseradish peroxidase (HRP) injections placed in the cortex label what appear to be clusters of neurons in the VPL if viewed in the coronal plane, while such labeling is elongated along the anteroposterior axis (Kosar and Hand 1981).

There is only one other component of the columnar motif that some data at the thalamic level addresses. There does appear to be evidence for segregation of rapidly adapting and slowly adapting neurons at the thalamic level (Dykes et al. 1981). These authors did not report any obvious pattern to the segregation, such as the alternating strips observed in the granular layer of the cortex (Dykes 1983; Sur et al. 1984; Sur et al. 1985). However, since this appears to be the same group of investigators that had previously argued for a
3D organization of thalamic sensory maps (Kaas et al. 1984), perhaps their data might have revealed a pattern if viewed in the context of thalamic rods.

There has been some evidence of columnar organization in the dorsal column nuclei, largely inferred from tract tracing studies. This distribution of cervicothoracic roots in the cuneate nucleus with Fink-Heimer staining revealed what the authors described as cellular "bricks" or "slabs" (Basbaum and Wall 1976). Each brick appeared to be bound by fibers coursing vertically and horizontally through the nucleus. Bricks tended to be rectangular (50 μm by 380 μm) in the horizontal plane, and extended vertically 400 - 450 μm in the rat. It was speculated that such structures may be equivalent to a staining pattern seen in lamina IV of vibrissa cortex known as "barrels" (Woosley and Van der Loos 1970).

Such bricks may be a recurrence of the columnar motif, extending along the length of the cuneate nucleus. Interestingly, comparisons of receptive fields located at either end of the dorsal column nuclei revealed that they were larger than those observed in the middle (Gordon and Paine 1960; Gordon and Seed 1961; McComas 1963). Together this suggests that receptive fields in the middle of such bricks tends to be restricted in size relative to its dorsal and ventral neurons, reminiscent of the Chapin's (1986) hour-glass model of receptive field distribution in cortical columns.

A columnar motif at the dorsal column nuclei level is also consistent with the known somatotopy of this structure. Microelectrode mapping of cutaneous submodalities reveals that they are longer rostrocaudally than mediolaterally (Dykes et al. 1982). Injection of tracers into the digits and
forearm of a primate have revealed columnar staining patterns in the cuneate nucleus in primates (Florence et al. 1989) and raccoons (Johnson et al. 1968; Rasmusson 1988). Some investigators have reported that somatotopic representation is restricted to the caudal portion of the cuneate nucleus (Kuhn 1949; Rustioni and Macchi 1968) but the consensus that appears to have emerged is that cutaneous representation of the body surface is present at all levels of the nucleus (Kruger et al. 1961; Nord 1966; Johnson et al. 1968; Xu and Wall 1996). Successive cutaneous representations of the body surface along the length of the nucleus are similar to the successive representations encountered in the cortex as one moves dorsoventrally. Although electrode penetrations parallel to the anteroposterior axis of the dorsal column nuclei have not been done, successive sensory maps suggest lines of isorepresentation, consistent with columnar (or brick) organization of receptive fields.

It is not clear yet whether slowly and rapidly adapting input are segregated in the dorsal column nuclei. Such input has been reported to be mixed, but the authors could not comment on organization of such input in the bricks (Dykes et al. 1982). As part of the columnar motif, it should be determined if in fact such bricks contain both types of input and whether they are segregated.

The spinal cord presents some unique difficulties. As one ascends the spinal cord are there successive sensory representations suggestive of lines of isorepresentation? This is difficult to answer since new dermatomal afferents merge with the spinal cord as one ascends the neuraxis, adding
representations to the somatotopic map (Schiebel and Schiebel 1968; Brown and Fuchs 1975; Swett and Woolf 1985).

Cajal (1911) originally described "flame shaped" dendritic arbors of neurons located in the substantia gelatinosa of the dorsal horn that received input via primary afferents from hair follicles (Scheibel and Scheibel 1968; Brown et al. 1977). The primary afferents enter the spinal cord and form what appear to be sheets comprised of axons, that extend along the length of the spinal cord sometimes referred to as "ribbons" (Scheibel and Scheibel 1968). The flame shaped arbors appear to be aligned with each other as one ascends the spinal cord (Scheibel and Scheibel 1968; Brown et al. 1977). Given the relationship of dendritic arbors and receptive fields, their anatomical observations have lead some investigators to suggest that these may be columns of cells with isomorphic receptive fields (Brown and Fuchs 1975).

Summary

A columnar motif, a unit of nervous system organization that includes several properties based on data gathered at the cortical level, has been postulated to exist at multiple levels of the somatosensory pathway. In examining the somatosensory pathway, lines of isorepresentation (Mountcastle’s cortical column) are the only aspect of this motif that can be consistently addressed at each level. Other aspects such as the hour glass model, segregated rapidly adapting and slowly adapting input, and focal areas of plasticity cannot be satisfactorily answered at all levels. Also, evidence for columnar organization of receptive fields at multiple sites of the somatosensory pathway is still not a settled issue.
With respect to the properties of the Rosetta Stone, the columnar motif is tentatively described in this section as a possible lexicon of the nervous system. This conforms to the first assumption linguists made in deciphering the Rosetta Stone: each of the three passages were all based on a system of discrete elements (e.g. words). Based on the columnar motif described in this section one would expect thalamic somatotopy to present additional features other than thalamic rods, such as focal areas of plasticity. This hypothesis will be tested in Chapters 2 and 3.

**Reentrant Connectivity: Isomorphic Content Across Levels?**

The second assumption linguists made about the structure of the Rosetta Stone to enable translation was isomorphic content: each of the three passages conveyed the same information. In the CNS, one would predict that a change in somatotopic representation at one level would be mirrored by similar changes at multiple levels of the somatosensory system. If plasticity is confined to certain structures in the somatosensory pathway (e.g. cortex) in the absence of similar changes elsewhere (e.g. VPL), then information pertaining to the relative representation of body regions in each somatotopic map would be contradictory. A more general issue is whether all levels of the somatosensory pathway are plastic. For Edelman (1987), extensive projections from the cortex to upstream structures (Auer 1956; Jones and Powell 1968; Anderson et al. 1972; Deschenes et al. 1994; Coleman et al. 1997) suggests that *the neuraxis embarks on plasticity as a functionally integrated whole, not a disjointed apparatus that permits conflicting somatotopic reorganization across tiers of the somatosensory pathway.*
The view that only the developing nervous system is plastic was fostered, perhaps, by the ethologists Ninteko Tinbergen and Conrad Lorenz. Both men jointly received the Noble prize in 1973 for their research in animal behavior (along with Karl von Frisch). Tinbergen investigated the invariant character of behavioral patterns in adult animals and referred to them as "fixed action patterns" or instinct (Tinbergen 1939). Lorenz demonstrated that such patterns were modifiable, but only early in life (Lorenz 1935; Lorenz 1943). Beyond this phase of modifiability is what Lorenz canonized as a "critical period", a time horizon in the animal's life beyond which behaviours could no longer be modified.

Ethology may be responsible for the perception that the capacity for neural plasticity is strictly under the providence of the young. This was challenged by Wall and Egger's (1971) study demonstrating plasticity in the thalamus of adult animals. Ironically, while plasticity has subsequently been demonstrated at many levels of the nervous system of adult animals (Millar et al. 1976; Devor and Wall 1978; Kalaska and Pomeranz 1979), plasticity in the thalamus has remained polemical (Snow and Wilson 1991; Jaine et al. 1995).

**Cerebral Cortex**

The somatotopy of the primary somatosensory cortex has been subject to extensive study. The cortex is particularly amenable to electrophysiological mapping, since it's horizontal laminations provide the substrate for well defined 2D maps of the body surface. The cortex is divided into six basic layers: laminae I-III (supragranular); lamina IV (granular) and laminae V, VI (infragranular). Receptive fields in the granular layer have been reported to be smaller than that found in the other layers (Chapin 1986). As described
earlier, thalamocortical fibers terminate in the granular layer and lamina IIIb (Figure 1.1). This projection appeared to be more prominent in motor and association cortex. It has been long recognized that there is a second somatotopic representation of the body surface in the cortex referred to as SII (Merzenich et al. 1978). Subsequently, it had been suggested that there may be up to 10 different somatotopic representations of the body surface, for such input as rapidly adapting fibers, slowly adapting fibers, joint movement and muscle length (Dykes 1983).

Plasticity in the nervous system has been most extensively studied in the cerebral cortex. While there was an early suggestion of plasticity in the rat somatosensory cortex, data were not presented to support this claim (Wall and Egger 1971). Subsequently, Kalaska and Pomeranz (1979) examined the effects of transecting nerves serving the paw, in both kittens and cats, on the somatosensory cortex. In kittens, they reported a 52% increase in the number of neurons in the paw cortex that responded to stimulation of the forearm 10 weeks post-lesion. Natural stimulation with hand-held probes and air puffs was used. Electrical stimulation revealed a similar increase of 58%. In adult cats the increased representation of the forearm cortex was observed at 10 weeks post-lesion, but not as dramatic as seen in kittens. Only 7% of the neurons in paw cortex responded to forearm stimulation. This was accompanied by an increase in the number of neurons in which receptive fields could not be ascertained.

Plasticity in the adult primary somatosensory cortex of animals is now well established. Digit (D5) amputation in raccoons revealed unresponsive cortex at 2 weeks post-surgery, followed by the emergence of small low
threshold receptive fields on the adjacent digit (Rasmusson 1982; Rasmusson and Turnbull 1983). Transection of the median nerve in squirrel monkeys lead to the appearance of “new” inputs from the ulnar and radial nerves several months after nerve transection (Merzenich et al. 1983). Transection of the sciatic nerve in the rat, which serves 85% of the hindpaw somatosensory cortex, lead to an expansion of the cortical territory responsive to saphenous nerve stimulation (Wall and Cusick 1984). While cortical territory served by the saphenous nerve tripled in size, up to half of the denervated cortex remained silent, even when observed as late as 5 months post-lesion.

While plasticity in the adult cortex has been demonstrated, it has become apparent that there may be limits to the distances representations can advance into denervated territory. Merzenich et al. (1984) have suggested the maximal distance in which cortical representations can expand in adult animals to be in the range of 500 - 700 μm. This was based on their research with adult owl monkeys that had digit amputation that were examined 2 - 8 months later. While digits adjacent to the amputated digit expanded their cortical representations, there appeared to be limits to the distances covered by such expansion into denervated territory. These investigators suggested that such a distance reflected the divergence of common thalamocortical arbors (Rausel and Jones 1995), that may provide subthreshold input into adjacent representations that could be “unmasked” (Merzenich et al. 1983). The distance of thalamocortical arbor input could be considered greater than 700 μm when one considers that adjacent neurons in the same part of the thalamus can project to cortical targets as discrepant as 1.5 mm apart (Rausel and Jones 1995). Other factors that may limit cortical reorganization will be discussed in detail in Chapter 3.
The view has emerged that if any expansion of a sensory representation in the cortex occurs beyond the distance served by a common thalamocortical fiber, the expansion was therefore unlikely to be thalamic in origin (Pons et al. 1991; Darian-Smith and Gilbert 1995). The most dramatic example of such plasticity has been described in *Cynomolgus* monkeys that had undergone dorsal rhizotomies 12 years earlier (Pons et al. 1991). Each monkey had received some form of amputation to the upper limb. Mapping somatotopic organization revealed that the face representation had expanded 10 - 14 mm across SI cortex such that it was juxtaposed against the trunk representation. This was well beyond any upper estimate for thalamocortical arbor, and suggested that such plasticity may be cortical in origin.

Further highlighting the autochthonous character of somatosensory cortex reorganization is the recent finding of plasticity in this structure in the absence of thalamic changes (Wang et al. 1995). Tactile stimuli were delivered in a synchronous manner to the digits of adult owl monkeys. Somatotopic mapping later revealed a unitary sensory representation of these digits at the cortical level. However, electrode penetrations at the thalamic level did not reveal concomitant digit reorganization. The authors suggested that such plasticity was therefore cortical in origin.

Such findings appear to challenge the Rosetta Stone metaphor, by suggesting that some forms of cortical reorganization may be independent of events at the thalamic level. In Chapter 4 evidence will be presented underscoring the critical importance of thalamocortical loops. These data will be used to erect a different theoretical framework in which to view thalamic
contributions to cortical plasticity. In reference to research on tactile timing (Wang et al. 1995), data have been collected that has suggested (Parker et al. 1998) that the failure to see such changes at the thalamic level reflects the use of methods unlikely to detect plasticity at this level (also discussed in Chapter 2).

Thalamus (VPL)

The VPL is found laterally to its more medial counterpart, the ventroposterior medial nucleus (VPM). Afferent input that conveys sensory input from the body surface terminates in the VPL, unless it is from the face, in which case it terminates in the VPM (Jones 1985). The basic somatotopy of the VPL indicated the hindlimb is represented laterally and the forelimb medially in the rat. There has been a suggestion that there may be a second somatotopic map in the thalamus reminiscent of SII in the cortex (Emmers 1965). This hypothesis was based on investigation of the pattern of degeneration in the thalamus following lesions of SII cortex. In contrast to the strikingly horizontal lamination of the cerebral cortex, it has been suggested that corresponding somatotopic maps at the thalamic level may occupy 3D volumes (Kaas et al. 1984). The morphology of the VPL is such that it is crescent-shaped in both the horizontal and coronal planes (Paxinos and Watson 1997). This fact has made it difficult to ascertain its precise somatotopy.

Plasticity of sensory representations in the VPL of the thalamus has been difficult to demonstrate. Transection of the gracilis funiculi tracts bilaterally (T₃ to T₅) in monkey has been reported to lead to thalamic plasticity, with an increased number of microelectrode penetrations detecting
forelimb units than in controls (Pollin and Albe-Fessard 1979). In this case, investigators used the ratio of forelimb units to hindlimb units as a measure of plasticity - an analysis that has obvious problems. The mere reduction of hindlimb units alone will automatically increase this ratio in lesioned animals. Further, selected electrode tracks were compared across animals based on whether they were in anatomically similar locations in the VPL. Technically this is very difficult. If we could guarantee that two recording locations across two animals were anatomically similar, does that give us license to assume that such regions have the same functional role given the variability in somatotopic organization reported at the cortical level (Merzenich et al. 1987)?

More commonly a few electrode penetrations are made in the VPL of each animal and the results compared between groups. In this paradigm there is no attempt to fully map the thalamic somatosensory map. Further, recordings are performed in acute preparations. It was reported that in reversible cooling of the dorsal column nuclei, 15% of the cells had changed their receptive fields (Fadiga et al. 1978). Receptive fields were explored with electrical stimulation of the periphery. In another study, blockade of thalamic receptive fields with procaine injections in the paw of a cat revealed that approximately 25% of the neurons investigated changed their receptive fields (Nakahama et al. 1966). Air puff stimuli were used to explore receptive fields. In another study, lesions of the gracile nucleus resulted in plasticity of 50% of the neurons examined in the VPL (Alloway and Aaron 1996). A comparatively high number of neurons recorded from the VPL changed their receptive fields, 70%, following lidocaine administration into their receptive fields (Shin et al. 1995). In the VPL of the thalamus, it appears that the
proportion of cells displaying plasticity can range from 15 to 70%. This sort of variability in the incidence of receptive field plasticity within the VPL precludes its use as a baseline (control group) in which to test hypotheses relating to its mechanism or relevance to CNS function. Importantly, sparse sampling of the VPL with a few electrode penetrations in each animal assumes that the nucleus is homogeneous with respect to its neural plasticity, a supposition we now have evidence against (Parker et al. 1998).

Interestingly, investigators working with other thalamic nuclei, such as the VPM which receives facial input (Armstrong-James and Callahan 1991; Nicolelis et al. 1993; Diamond et al. 1994; Lee et al. 1994) or the medial geniculate body which receives auditory input (Edeline and Weinberger 1991a; Edeline and Weinberger 1991b), have demonstrated far greater reliability in their findings; they were able to pool their data across animals and show statistically significant treatment effects.

Part of the difficulty in studying plasticity of sensory representations in the VPL may be methodological. In the rat trigeminal and the auditory systems the sensory epithelium are well defined for the delineation of receptive fields. Vibrissa provide a simple grid on the body surface to compare relative position and size of receptive fields across animals. Similarly, in the auditory system the frequency range of a receptive field is quantifiable and allows one to readily determine similar receptive fields across animals (or best frequency). In the VPL, however, which serves the entire body surface save the face (Emmers 1965; Jones 1985), it is much more difficult to quantify and identify equivalent receptive fields across animals.
There has been one paradigm that possesses considerable merit in tackling plasticity in the VPL. In the raccoon thalamus there is a well-defined parallel representation of individual digits in the VPL. Rasmusson's laboratory was able to infer plasticity following digit removal by passing an electrode across the axis of the represented digits in the thalamus (Rasmusson 1996a). If he did not encounter a silent region corresponding to the deafferented digit, he concluded that the adjacent digits had expanded their respective representations. There are three minor criticisms of this approach, despite it's obvious ingenuity. First, its classification of plasticity tends to be all or none. Second, practical difficulties exist since this technique is specifically dependent on the use of the raccoon species, which can be a costly undertaking precluding large group sizes. Third, the technique cannot be used to test how generalizable the findings are in other species.

There has been a preliminary report of receptive field plasticity in humans following temporary digit deafferentation (Kiss et al. 1994). Neurons with receptive fields on the digits were blocked by the administration of lidocaine. In almost all of the single units investigated, responsiveness was found on adjacent digit or skin area that was previously unresponsive digit (6 of 7 single units). In this study investigators were able to pool data across patients and show statistically significant treatment effects.

Efforts to replicate Wall and Egger's (1971) original finding of plasticity in VPL somatotopy have been plagued with variability. This has raised concern among some investigators who have recently questioned the veracity of Wall and Egger's (1971) original report (Snow and Wilson 1991). Snow and Wilson (1991) questioned how changes on the order of 400µm could have
been detected, when electrode penetrations at 0.5 mm intervals (sic) were used. Further, if hindlimb deafferentation precipitated forelimb expansion into the hindlimb zone at the thalamic level (Wall and Egger 1971) one would predict a similar finding cortically. At the cortical level, no shift between the hindlimb and forelimb was found following deafferentation of funiculus gracilis (Jaine et al. 1995). This latter discrepancy between cortical and thalamic plasticity with essentially the same manipulation raised doubts for these investigators regarding Wall and Egger's (1971) original findings.

The thalamic VPL would appear to challenge the metaphor of the Rosetta Stone. It is not clear that this region can display plasticity as readily as other levels of the somatosensory pathway. Further, the reported changes at the thalamic level do not appear to be mirrored at the cortical level (Jaine et al. 1995). It must be kept in mind, however, that most research on plasticity at the thalamic level has followed Wall and Egger (1971) and examined changes between hindlimb and forelimb areas (Nakahama et al. 1966; Fadiga et al. 1978; Alloway and Burton 1985; Garraghty and Kaas 1991; Panetsos et al. 1995). In Chapters 2 and 3 evidence will be presented at the thalamic level that the type of somatotopic reorganization that occurs between hindlimb and forelimb representations following deafferentation is fundamentally different from that reported with other body regions (Merzenich et al. 1983; Merzenich et al. 1984; Jenkins et al. 1990; Rasmusson 1996a). Failure to find evidence of reorganization at the cortical level following hindlimb deafferentation may reflect the fact that investigators were looking for the wrong type of plasticity (Dr. Jaine, personal communication, October 1997). We must therefore reformulate our predictions of somatotopic reorganization following hindlimb deafferentation at the cortical level, based on new evidence of the
nature of such changes at the thalamic level (Chapter 2 and 3), before concluding whether or not cortical and thalamic reorganization are similar.

**Dorsal Column Nuclei**

The dorsal column nuclei have two components: nucleus gracilis and nucleus cuneatus. Each nucleus relays sensory input from the trunk and upper body (excluding face) respectively (Gilman and Newman 1987). Within each nucleus it has been suggested that there may be two subdivisions. The rostral subdivision is considered to be less somatotopic in comparison with the caudal region, since there is much overlap between dermatomes (Hand 1966; Keller and Hand 1970). The caudal subdivision is somatotopically organized, with little segmental overlap between dermatomes (Hand 1966; Keller and Hand 1970). While both subdivision project to the VPL, the rostral region includes other targets such as the zona incerta and tectum (Lund and Webser 1967). In nucleus gracilis, the representation of the hindlimb is lateral, with the abdomen and trunk more medial (Dostrovsky et al. 1976). In nucleus cuneatus, the distal forelimb is lateral while the proximal forelimb is medial (Nord 1966).

While the number of studies directed at the plasticity of this structure is not as numerous as that seen in thalamic research, there does not appear to be any question that this structure can undergo plasticity in adult animals. Nerve transection of all roots caudal to L3 in adult rats leads to an immediate increase in the number of neurons responsive to abdominal stimulation, as well as the appearance of inhibitory receptive fields (Dostrovsky et al. 1976). In an acute manipulation, all roots caudally from L4, except S1 or L7, were severed (Millar et al. 1976). The authors noted the appearance of a number of
silent neurons, while the representation occupied by the remaining intact dorsal roots appeared to increase. Further, neurons of the severed dorsal roots displayed abnormal receptive fields. In a follow-up experiment, the same manipulation was used and animals were examined 8 months later. Neurons in nucleus gracilis showed an increase in the representation of these remaining intact roots, namely, trunk and abdomen. Reversible deafferentation has also induced plasticity in this structure (Panetsos et al. 1995; Dostrovsky et al., 1976). Lidocaine injections into the rat hindpaw precipitated new receptive fields from neurons recorded in nucleus gracilis. Similarly, reversible denervation by lidocaine injections into the receptive fields of cuneate neurons resulted in their expansion (Pettit and Schwark 1996). Several investigators favor unmasking of synapses for this plasticity, perhaps by the removal of inhibition (Panetsos et al. 1995).

Anatomical evidence of reorganization in the cuneate has been described (Florence and Kaas 1995). Three monkeys underwent therapeutic amputation of the hand. Subsequently, 1 to 13 years later, they were injected with transganglionic tracer in the forearm and sacrificed. It was found that HRP labeling in the cuneate was more extensive, extending into areas normally served by the hand, than seen in normal controls. For these investigators, such results implied an expanded representation of the forearm.

However, one study has reported that injection of lidocaine into the digits of raccoons failed to alter receptive fields of neurons located in the cuneate nucleus (Northgrave and Rasmusson 1996). At this juncture it is
difficult to explain this discrepancy, perhaps this reflects species specific difference for raccoons or sampling differences of the cuneate nucleus.

While we are concerned with changes in the adult nervous system, one developmental study should be mentioned. Removal of the forelimb in neonatal rats later revealed marked changes in the cuneate nucleus when they reached adulthood (Lane et al. 1995). Sciatic nerve afferents from the hindlimb invaded the cuneate nucleus. However, there were no signs of hindlimb expansion into the forelimb area of the cortex. Since manipulations performed in neonates have much stronger effects than those performed in adults (Kalaska and Pomeranz 1979), this study suggests that changes induced at one level may not be mirrored at other levels. This questions our second assumption from the Rosetta Stone of isomorphic content across levels in the nervous system. In Chapter 4 contrary evidence will be provided by demonstrating the interdependence of CNS levels through feedback to initiate plasticity.

Dorsal Horn

The dorsal horn was recognized as a laminated structure when Rexed published his nomenclature for this structure (Rexed 1952). While Rexed divided the spinal cord into 10 laminae, within the dorsal horn there are six laminae. Laminae I - VI receive sensory input directly from the periphery (Willis and Coggeshall 1978). Lamina II receives unmylinated cutaneous afferents which generally have small receptive fields. Lamina III possess neurons with wide dynamic range receptive fields (Willis and Coggeshall 1978). Such neurons are responsive to low threshold cutaneous input in addition to noxious input.
Wall's laboratory was the first to report somatotopic plasticity in the spinal cord (Basbaum and Wall 1976). A shift in low threshold receptive fields into the dermatome of an intact dorsal horn in the cat some weeks or months after extensive deafferentation was reported. Today, somatotopic plasticity in the spinal cord of adult animals is generally accepted (Wilson and Kitchener 1996).

In other studies in adult cats in which saphenous and sciatic nerves were cut, virtually all cells in the medial dorsal horn (lumbar segment) were left without receptive fields (Devor and Wall 1978). However, within a few weeks after nerve transection, denervated neurons acquired receptive fields on the thigh, lower back or perineum. Similarly, in the adult rat the sciatic and saphenous nerves were transected and examined 4 days post-lesion (Devor and Wall 1981). Many of these denervated neurons in the spinal cord took on receptive fields located on the thigh, lower back or perineum. In an acute variation of this manipulation, when the nerves were transected they were then immediately sutured back together. In this latter case, novel receptive fields were not observed, suggesting to the authors that spinal cord reorganization had been reversed.

There have been two reports that failed to find plasticity in the dorsal horn following deafferentation. Employing the same manipulation as Devor and Wall (1981), Pubols et al (1984) concluded there was no significant reorganization of somatotopy within the dorsal horn. The medial portion of the dorsal horn receives distal hindlimb input. While on the one hand the denervated group of animals displayed more receptive fields on the proximal
leg, the most medial electrode trajectory within the dorsal horn was not significantly different from the contralateral trajectory in the intact hemicord. One may argue that the increase of receptive fields on the proximal limb portents somatotopic reorganization, or question the accuracy of electrode track reconstruction in making such a comparison. Brown et al (1984) failed to detect any mechanical receptive fields in the medial dorsal horn, a region normally responsive to distal hindlimb, when examined up to 55 days later. It has been suggested (Kiss 1998) that differences may be accounted for by the use of noxious stimulus used by Devor and Wall (1981) to map receptive fields may have elevated the excitability of medial dorsal horn they studied. Anesthetic differences may also be responsible for differences between these latter two studies.

In another experiment, rats were subjected to sciatic nerve ligation and examined with electrical stimulation (Markus et al. 1984). Stimulation of the saphenous innervated territory evoked discharge from dorsal horn neurons within the sciatic representational territory. When examined 3 weeks later, natural stimulation of saphenous innervated skin elicited similar responses. In another study, L6 - L7 dorsal roots were cut unilaterally in the cat (Koerber and Brown 1995). When mapped more than 6 months later, mapping of the L6 - L7 segment revealed a medial shift of receptive fields (low threshold mechanical) in the lateral portion of the dorsal horn.

Some investigators have suggested that changes precipitated at the dorsal horn level are responsible for similar changes observed at higher levels of the nervous system (Pons et al. 1991; Florence and Kaas 1995). Three monkeys had been treated with therapeutic amputation of the hand several
years earlier (Florence and Kaas 1995). HRP injections into the forearm of the skin revealed a much wider zone of staining in the dorsal horn compared to a contralateral control injection. The authors (Florence and Kaas 1995) interpreted this staining pattern as reflective of similar reorganization in the dorsal horn and suggested that such changes cascade to the cortical level (Pons et al. 1991).

The observation of plasticity at the level of the spinal cord is consistent with the Rosetta Stone metaphor. The suggestion that changes cascade from the dorsal horn producing similar changes in the cortex is also consistent with the metaphor's assumption of isomorphic content across levels. However, the reentrant model, while predicting similar changes across levels of CNS organization, also suggests that such changes are bi-directional, relying on descending and ascending input. Cascade and reentrant model predictions will be tested in Chapters 3 and 4.

Summary

We asked whether the literature supported the second assumption in translating the Rosetta Stone: isomorphic content across levels. Are the somatotopic changes that accompany deafferentation similar at each level of the nervous system? The literature does not give a clear answer, but it appears to argue against this supposition. First, it is uncertain whether the VPL is capable of plasticity in adults. Second, the magnitude of reorganizational events that appear to take place at the cortical level are beyond currently accepted thalamic mechanisms and are therefore regarded as purely cortical in origin. In summary, the literature appears to suggest somatotopy at thalamic and cortical levels can be different, since plasticity in
the VPL is uncertain and examples of cortical plasticity exist without corresponding evidence for any change in the VPL.

**Paradigm**

Whether or not it is valid to view the nervous system through the metaphor of the Rosetta Stone may be best evaluated by investigating VPL thalamic plasticity. If content is isomorphic across CNS levels, made possible by reentrant connectivity, then the removal of the cerebral cortex should impair or block thalamic plasticity.

Pertinent to the Rosetta Stone Metaphor, we must first ascertain if the thalamus itself can display somatotopic reorganization. Today, plasticity in the VPL remains moored in the same waters as the time of Wall and Egger (1971). As stated above, many of the papers published since that report have attempted to induce plasticity with varying degrees of success. The difficulty is that plasticity in the VPL itself remains elusive in adult animals and is inconsistent at best.
Figure 1.2  A summary of the hypothesis and arguments that have been set out in the Introduction. The assumptions made by linguists about the properties of the Rosetta Stone to allow translation are indicated on the tablet. Analogs of these assumptions are identified in the nervous system. The columnar motif predicts many things, one of which is a focal zone of plasticity at all levels of the somatosensory system. This is tested in the thalamus. Isomorphic content across levels of the nervous system must first be answered by resolving whether or not the thalamus can undergo reorganization in adult animals. In light of the data collected in this thesis, explanations are offered to resolve apparent mismatches between thalamic and cortical plasticity. Finally, two different theories for the coordination of somatotopic reorganization across levels of CNS are tested.
Rosetta Stone

Elementary Units

Isomorphic Content Across Levels

Columnar Motif

Plasticity at Each Level of the CNS?

Predicts Focal Plasticity in Thalamus: TEST in (Ch 2, 3)

Thalamic Plasticity Uncertain: TEST in Ch 2, 3

Cortex - Thalamus Plasticity Mismatch.

By Cascade or Reentrant Mechanisms?: TEST in Ch 4

Plasticity in Thalamus but Not Cortex: Addressed in Ch 2

Plasticity in Cortex but Not Thalamus: Addressed in Ch 2, 4
In developing a paradigm for the induction and measurement of plasticity in the VPL four properties were sought:

1. **A Metric of Plasticity.** A simple, meaningful, quantified measure of plasticity so that data are not to be presented anecdotally with representative maps (Wall and Egger 1971; Calford and Tweedale 1991).

2. **Data Amenable to Statistical Analysis.** This implies attention to the variance in the data and statistical comparisons so that any change is not assumed to be significant and worth reporting (Nakahama et al. 1966; Wall and Egger 1971; Shin et al. 1995; Alloway and Aaron 1996).

3. **Control Groups.** These are necessary to make statistical comparisons possible since the use of contralateral controls make additional assumptions about the dynamics of a system and should therefore be avoided (Wall and Egger 1971; Florence et al. 1989; Garraghty and Kaas 1991).

4. **Reliability of Treatment Effects.** Observations purporting thalamic plasticity must be reliable, since this will provide a baseline condition for the final experiment (Chapter 4). Also, reliability in data is crucial to predictability (and the range reported in the literature of 15 - 70% of identified neurons in the thalamus displaying plasticity suggests that this area of research has not attained this).

In this research the rodent was chosen since that was the species originally used by Wall & Egger (1971), a starting point of this research.
In the study of somatotopic plasticity in the somatosensory cortex reviewed earlier, invariably recordings were performed in the granular layer. Possible somatotopic reorganization in the supra- and infragranular layers were ignored. This is understandable, since the experiments could not be completed if in addition to detailed mapping of somatotopy, one had to perform the same procedure for all cortical layers.

In subcortical structures there has been a similar tendency. For example, Dostrovsky et al (1976) mapped a single transverse section of the dorsal column nuclei to detect plasticity. How much error is one permitted with this technique in identifying a similar transverse section from animal to animal? The same could be asked in pooling “similar” electrode penetrations across animals from the thalamus (Albe-Fessard et al. 1983). The question of error here is not relevant if one believes that a given structure is homogeneous with respect to neuroplasticity. If it is not, such error could introduce considerable ‘noise’ into the data.

The primary somatosensory cortex can be economically mapped because of its clear lamination and the stereotaxic ease with which it can be accessed. This is not so of subcortical structures. Even if a given structure is laminated, stereotactically they all present great challenges in locating a specific lamina consistently across animals. How do we ensure that we are placing a few electrode penetrations in similar areas of the VPL, for example, for each animal in a study? This problem becomes unwieldy if it is true that somatotopic maps are folded in the VPL of the thalamus to occupy a 3D volume (Kaas et al. 1984). If this is the case, targeting a specific area in the
thalamus would be sensitive to any error in electrode placement in all three planes.

To address this issue this author started with an approach inspired by Angel and Clark (1975): a determination of the entire volume of VPL responsive to the stimulation of a given body region. Traditional mapping efforts in the thalamus have been handicapped by a demand for too much detail at each recording site. In the thalamus, if one rigorously determines the receptive field for each recording site, the entire VPL cannot be mapped in a single animal. To obtain such maps investigators must assemble partial maps from each animal to obtain a complete map (Emmers 1965; Angel and Clark 1975). This is perfectly acceptable if one’s goal is to document the somatotopy of the thalamus for a given species as a matter of anatomical record.

In the research presented here the volume of somatosensory thalamus responsive the stimulation of a given body region was used as a dependent measure. To achieve this required complete maps of the VPL from each animal. For this to be feasible the mapping procedure had to be simple and rapid. The number of body regions probed at each recording sites was small: hindlimb, forepaw and shoulder. Further, each site was classified: if a recording site was responsive to some area within the shoulder, it was categorized as “shoulder”. Detailed boundaries of the receptive fields were not mapped. The simplicity of this procedure gave the speed necessary to map the entire VPL. Typically, each animal required well over a 100 electrode penetrations with more than several hundred recording sites surveyed.
Two measures of thalamic plasticity were employed: volume and shape. With a grid of electrode penetrations (200 μm steps in each plane - the practical limit on the stereotaxic frame) the number of points responsive to a given body region yielded a volumetric estimate. For estimates of shape changes in thalamic sensory maps, these functional maps were sliced and their cross sectional areas measured (detailed in Chapter 2). This paradigm has three strengths: (1) By comparing the volumes of represented body regions in the thalamus across animals, it does not make assumptions about the equivalence of electrode placements; (2) it allowed one to work with chronic preparations; and (3) one can readily make comparisons between animals (control groups or otherwise).

In retrospect, strictly speaking this paradigm measures “hypercolumns” in the thalamus. The concept was borrowed by Sur et al (1980) from the visual system (Hubel and Wiesel 1974) and applied to the somatosensory system. By definition, a hypercolumn is a group of neurons all responsive to the same body region (Sur et al. 1980). One disadvantage of this approach is that it relied on between-subject design comparisons, which does not have the power of within-subject design comparisons that many acute preparations use (Nakahama et al. 1966; Nicolelis et al. 1993; Shin et al. 1995; Alloway and Aaron 1996). To mitigate against this, cross-sectional analysis of the functional maps provided a repeated measures component to the analysis. In other words, one is working with multiple measures from each animal for a given variable. It was a concern that while thalamic sensory maps may undergo volumetric increases, such increases could be subtle and confined to a region within the VPL. Overall volumetric comparisons might miss such a change. Comparing cross-sectional area profiles from each functional map
allowed the VPL to be parsed into small areas which permitted greater spatial resolution.

Cross-sectional area comparisons of functional maps presented theoretical difficulty. A standard frame of reference was required to ensure slice equivalence across animals. This may sound similar to the problem of guaranteeing equivalence of electrode penetrations across animals (Albe-Fessard et al. 1983). However, one advantage in this case is that the entire functional map would be sliced and at small increments. Equivalent slices across animals were identified based on their distance from either an anatomical or functional landmark. The functional landmark was the centroid of the sensory map itself. The centroid concept was borrowed from multivariate statistics. Loosely speaking, it can be thought of as the moment of inertia or centre of mass of an object, in this case a functional map. A tangential issue was whether these two different landmarking systems would produce discrepant results in cross sectional analysis, since they make different assumptions about the placement of sensory maps in the thalamus across animals.

There were two concerns: would individual variability in the volume and shape of thalamic sensory maps across animals preclude the use of this metric for the investigation of plasticity? Pilot data in normal animals confirmed consistency of sensory map volumes across animals, with a level of variance that did not disqualify it’s use as a metric. Several normal rats (approximately 10) had their thalamic somatotopy mapped in an effort to determine the size of each sensory map, the time required to map them, consistent mapping rules and the variance in the volume of these sensory
maps across normal animals. The comparatively small size of the standard error bars relative to the mean volumes of the sensory maps suggested that the variance was manageable. The second concern was more serious: was such a crude mapping procedure sensitive enough to detect plasticity in the thalamus? As one colleague had pointed out (Dr. Hallet, personal communication, October 1997), perhaps the resolution of this grid of electrode penetrations would be too coarse to detect any change. An answer to this latter concern could only be determined through an application of the paradigm.

The first paper followed Wall and Egger's (1971) manipulation, with the intent of testing the sensitivity of the paradigm:

**Chapter 2**

**Question 1:** Will the removal of hindlimb input by nucleus gracilis lesions result in a volumetric increase of the forelimb sensory map in the thalamus?

**Question 2:** Does a characteristic topological transformation accompany the volumetric expansion of the forelimb sensory map in the thalamus?

A number of surprising findings came from Chapter 2 (Parker et al, 1998). Since dorsal column nuclei lesions could be considered an artificial and incomplete lesion (Dr. Sessle and Dr. Harrison, personal communication October 1997), nerve transection was tested next to gauge the generality of the findings:

**Chapter 3**

**Question 1:** Will a different form of neural trauma, such as nerve transection of the hindpaw, result in volumetric expansion of the shoulder sensory map in the thalamus?
Question 2: Is the expansion of the shoulder functional map achieved by topographic overlap with that of the neighboring forepaw representation?

Question 3: Will volumetric increases occur within a restricted zone of the shoulder sensory map located towards the rostral pole of the VPL?

The final paper was the climax of this thesis: a test of Edelman's (1987) reentrant connectivity model. Pursuing these hypotheses was only possible after the above was in place:

Chapter 4

Question 1: Will the chronic removal of the forelimb somatosensory cortex affect the corresponding representation at the thalamic level with respect to volume or shape?

Question 2: Can the removal of the forelimb somatosensory map at the cortical level block thalamic plasticity?

Question 3: Is the somatosensory cortex necessary for induction or expression of thalamic plasticity?
Chapter II

A Focal Zone of Thalamic Plasticity
Abstract

In this study, sensory maps in the thalamic tactile relay, the VPL, were investigated by examining their volume and shape. The forelimb representation in adult rats following the removal of hindlimb input by nucleus gracilis lesions was determined. Three-dimensional reconstructions of thalamic sensory maps were obtained from a grid of electrode penetrations. It was found that the volume of the shoulder sensory map contracted more than 50% at an acute time interval (n=6), followed by a robust volumetric sensory map expansion of 25% at 1-week (n=8) and 1-month (n=8) post-lesion relative to controls (n=8). The topology of the volumetric increase was scrutinized by slicing functional maps in the coronal, sagittal and horizontal planes. The equivalence of such slices from each animal was established by virtue of their distance from either a functional or neuroanatomical landmark. Surprisingly, all of the volumetric increase unequivocally occurred in a circumscribed coronal slice 300 μm thick. This focal zone was located towards the rostral pole of the VPL. Analysis in the sagittal plane revealed that, unexpectedly, the shoulder map volume expanded by superimposing its representation on that of the forepaw, via an advancement of the shoulder representation by 0.6 mm medially. A “hot spot” hypothesis is proposed in which focal zones of plasticity may not be specific to the thalamus, but may have manifestations elsewhere in the nervous system such as the cerebral cortex or dorsal column nuclei.
Introduction

Plasticity of somatosensory maps in adult animals was first reported at the thalamic level (Nakahama et al. 1966; Wall and Egger 1971; Fadiga et al. 1978; Pollin and Albe-Fessard 1979). Plasticity of somatotopic maps has subsequently been discovered at other levels of the neuraxis in adult animals. With respect to cutaneous pathways, plasticity in somatotopic maps in adult animals has been demonstrated in the dorsal horn (Basbaum and Wall 1976; Devor and Wall 1978), dorsal column nuclei (Dostrovsky et al. 1976; Millar et al. 1976) cerebral cortex (Kalaska and Pomeranz 1979; Merzenich et al. 1983) and the VPM (Rhoades et al. 1987; Nicolelis et al. 1993). The principle that has emerged from this research is that once a brain region is denervated, adjacent representations expand into the deafferented zone.

Plasticity in the VPL is difficult to detect reliably and has remained polemical. Wall and Egger's (1971) report of thalamic plasticity has been questioned, in part due to the sparse data presentation given in the paper (Snow and Wilson 1991; Jaine et al. 1995) and the small magnitude of the forelimb border shift relative to the spacing of electrode penetrations.

A variety of laboratories have investigated VPL thalamic plasticity following either deafferentation or the reversible blockade of a receptive field, and examined the consequences with a manifold of dependent measures.
A Focal Zone of Thalamic Plasticity

(Nakahama et al. 1966; Fadiga et al. 1978; Pollin and Albe-Fessard 1979; Garraghty and Kaas 1991; Shin et al. 1995; Alloway and Aaron 1996; Rasmusson 1996a). Comparisons of map border relationships between control and lesioned animals have been difficult, given the three-dimensional (3-D) character of thalamic sensory maps (Pollin and Albe-Fessard 1979). The proportion of neurons reported to respond to new input following blockade of their original receptive field has ranged from 15% to 70% (Nakahama et al. 1966; Fadiga et al. 1978; Shin et al. 1995; Alloway and Aaron 1996). Such variation makes it difficult to use this plasticity as a baseline in which to test hypotheses relating to its mechanism or role in nervous system function.

This study used a protocol that quantifies thalamic plasticity based on a single measure: the volume of somatosensory thalamus responsive to a given body region. It is assumed that increased volume of a thalamic sensory map corresponding to a specific body region, in lesioned animals relative to controls, reflects a change in the receptive field of the neurons sampled. Following Wall and Egger (1971), hindlimb input was removed with lesions of nucleus gracilis. Histology was used to verify electrode placements and obtain length estimates of certain thalamic nuclei. All volumetric estimates and cross sectional analysis were performed on a computer displaying the three dimensional distribution of recording sites for each body region throughout the VPL.
It was asked whether the forelimb, composed of shoulder and forepaw sensory maps, would increase in volume following nucleus gracilis lesions compared to intact controls at various times post-lesion. Second, it was asked whether 3-D reconstructions of thalamic sensory maps would reveal any characteristic topological feature behind the volumetric expansion of the forelimb representation. Comparisons of shape were obtained by sectioning functional maps in each plane, and comparing the profile of their cross-sectional area.

Methods

Subjects and Anesthesia: 30 male Wistar rats with weights ranging from 250 to 350 grams were used. The approximate age of these rats upon arrival was 40 to 50 days (Dr. Gwen Ivy, personal communication, 1999), which typically acclimated in the animal facilities for 2 to 3 weeks, bringing their age to 60 to 70 days. Rats are considered fully mature and adult by 60 days (Dr. Gwen Ivy, personal communication, 1999). Four groups of rats were mapped at various times following nucleus gracilis lesions: unoperated controls (n = 8), acute group (n = 6), 1 week post-lesion (n = 8) and 1 month post-lesion (n = 8). Rats in the acute group were mapped immediately following their gracile lesion, without recovery from anesthesia. Since mapping takes at least 12 hours to complete, the acute condition covered this span of time immediately
following gracile lesions. All experimental protocols were approved by the University of Toronto's animal care committee and conducted in accordance with Canadian Council on Animal Care (CCAC) guidelines.

**Nucleus Gracilis Lesions:** Rats were anesthetized with Ketamine hydrochloride xylazine (i.p.). The rat's head was tilted forward in the stereotaxic frame and the obex was exposed surgically. Under a dissecting microscope, lesions were made of the gracile nucleus with jeweler's forceps. Pilot work had established a clear correspondence between the morphological boundary separating nucleus cuneatus and gracilis, with the perimeter of the hindlimb responsive zone as delineated by multiunit recordings. The nucleus was completely macerated with jeweler's forceps down its length. Animals appeared fully recovered from this procedure within an hour. At no time post-lesion was discomfort observed.

**Surgery:** Animals were water-deprived overnight prior to mapping of the thalamus. Five minutes prior to anesthesia, atropine sulphate (300 mg/kg, s.c.) was injected. Five minutes later, ketamine hydrochloride (200 mg/kg, i.p.) and xylazine (50 mg/.kg, i.p.) were administered. Maintenance doses of ketamine hydrochloride and xylazine were administered every hour at one quarter the size of the animal's respective loading dose. Anesthetic level was maintained by ensuring that there was no response to tail pinch, while gentle touch to the eye yielded a weak corneal reflex. Atropine sulphate was
administered every two hours (300 mg/kg, s.c.). Body temperature was monitored with a rectal thermometer and kept at approximately 37°C by a thermostatically controlled heating pad underneath the animal. A hole in the trachea was cut and periodic cleaning with paper tissue followed for at least 20 minutes until secretions stopped accumulating. A polyethylene tube was then inserted into the trachea. A unilateral craniotomy contralateral to the side of the gracile lesion was made, extending to all suture lines and the rhinal fissure. The dura was subsequently removed with forceps. Throughout the experiment, the exposed cortex (approximately 1.5 cm²) was covered in mineral oil.

**Recordings:** Multiunit recordings were conducted with glass-coated tungsten microelectrodes with a tip diameter of 35 μm, with an impedance between 2.5-3.0 MΩ at 1000 Hz. Low- and high-pass filters with cut-off frequencies of 6000 Hz and 300 Hz respectively were used. The amplifiers, filters and oscilloscope had the same settings across recording sessions for every animal. The initial electrode penetrations of each session were performed at AP-0.35 cm and ML±0.33 cm corresponding to the VPL (Paxinos and Watson, 1997). The electrode was lowered 0.45 cm relative to the surface of the cortex. Electrode advancement was manually operated. Activity was monitored by the operator based on auditory discrimination of whether or not neuron(s) in the recording location in question demonstrated detectable discharge contingent upon stimulation of a specific body region. Normally, two or
three penetrations were required to locate sites responsive to stimulation of the forelimb or hindlimb (and subsequently confirmed histologically). Mapping was carried out by making successive penetrations in a two-dimensional grid of electrode penetrations in the rostral-caudal and mediolateral planes in 0.2 mm increments, with successive recordings in the dorsal-ventral plane in the same increments. To reduce the mapping time, a restricted number of body regions was examined. For each recording site (Figure 2.1) we tested whether the units discharged following the application of brush stimuli to either the forepaw (from elbow to paw), shoulder (from elbow to upper extremity of the arm) or hindlimb (thigh or foot). The term forelimb refers to forepaw and shoulder body regions. If a site failed to respond to stimulation of one of these body regions, it was classified as "blank". If a site responded to more than one body region, each one was indicated. At the end of the mapping session, two electrolytic lesions were placed (one caudal, one anterior, at the same depth) and the coordinates recorded. The rat was then sacrificed by anesthetic overdose.

**Mapping Rules:** A mapping protocol was developed that produced consistent sensory-map data across pilot animals with respect to volume and shape. The rules were: (1) successive penetrations along the medial-lateral axis were terminated with two contiguous blank penetrations; (2) successive rows of penetrations along the rostral-caudal axis were terminated if an additional row of tracks was blank at each recording site, including one additional
penetration medially and laterally for each track; (3) recording commenced in a new track 0.6 mm above the most dorsal positively identified site obtained in any adjacent electrode track; (4) if no units were encountered within an electrode track, the penetration continued until it was 0.6 mm below the most ventral positively-identified site in any adjacent electrode track. Such rules generated an aggregate map in the VPL that represented all three body regions examined required 80-100 penetrations per animal and contained approximately 400 positively identified sites per animal. On average, from tracheotomy to perfusion, the procedure required 12-14 hours per animal.

Sensory Maps: The forepaw sensory map includes any site responsive to forepaw stimulation, regardless of whether the same site was also responsive to other body regions (see Figure 2.1). Likewise, the shoulder sensory map includes all sites responsive to shoulder stimulation, ignoring the fact that some sites may have been responsive also to other body regions. However, the zone of overlap between shoulder and forepaw includes those sites responsive to both shoulder and forepaw stimulation, regardless of its responsiveness to the stimulation of other body regions. On average, per sham animal, there were 200 recording sites responsive to forepaw stimulation, 140 sites responsive to shoulder stimulation and 90 sites responsive to hindlimb stimulation.
Histology: Animals were perfused transcardially with saline followed by 4% paraformaldehyde. Brains were removed, placed in a 30% sucrose solution of 4% paraformaldehyde, and sectioned in the horizontal plane with a slice thickness of 0.1 mm. This plane of sectioning allowed us to discern the entire pattern of electrode tracks. Dorsal column nuclei were sectioned coronally with a slice thickness of 40 μm. All tissue was Nissl-stained and coverslipped.

Volumetric and Spatial Analysis: Sensory maps were rendered and visualized with Interactive Data Language (IDL™ Boulder, Colorado) on a Sun Ultra model 170 Sparcstation (Sun Microsystems, Mountain View, California) from data recorded onto a 3-D spreadsheet. The volume of each sensory map was calculated from the number of recording sites obtained per body region multiplied by the volume element (0.008 m³) of the grid. Volumetric analysis allowed us to ascertain which body representations underwent expansion. Spatial analysis was then conducted on these sensory maps to explore for any characteristic topological features mediating the volumetric changes. Profiles of successive cross-sectional areas of the maps in the coronal, sagittal and horizontal planes were compared between groups. Equivalent slices of a given sensory map across animals were identified by virtue of their distance from neuroanatomical or functional landmarks. The neuroanatomical landmark was the midpoint of the length of the Ventrobasal complex (VB) along the anterior-posterior axis. The functional landmark was the centroid of the sensory-map image. For a single axis, the
centroid is simply the average coordinate of recording sites (non-blank) along that axis. In 3-D space the centroid is a vector with x, y and z coordinates. For reasons that will become clear in the results section, the centroid reference point was used for most of the spatial analysis.

**Statistics:** If the requirement of homogeneity of variance was unsatisfied for a given ANOVA, a correction was performed to the number of degrees of freedom with Huynh-Feldt Epsilon, making the required level of significance more stringent. If normality was violated a non-parametric test was used. If the results of the non-parametric test were the same as an ANOVA, the ANOVA was reported. All statistics were performed with SPSS (SPSS Inc., version 6.1 for Macintosh, Chicago, Illinois). Post-hoc unpaired t-test (two-tailed) comparisons that followed ANOVA models are reported as being less than or greater than a 0.05 significance level.

**Results**

Volumetric analysis of each sensory compartment is reported first. Sensory maps that exhibited volumetric changes, were analyzed in the topology of their expansion. In the topological analysis, the decrease of hindlimb input was examined in detail.

**Volumetric Analysis**

Three thalamic sensory map volumes are depicted in Figure 2.2. The shoulder-forepaw representational overlap refers to any recording site that
responded to brush stimuli of both shoulder and forepaw. For each sensory map, all treatment groups are included. An overall multivariate analysis of variance of all three sensory map volumes (Figure 2.2) revealed a main effect of group (Pillias(9,78)=0.99, p<0.001). Then the effect of group was examined on each individual sensory map volume with a 1-factor ANOVA.

The shoulder sensory map showed an initial contraction in volume relative to controls, followed by a robust volumetric expansion at 1-week and 1-month post-lesion. An ANOVA on shoulder sensory map volumes by group revealed a clear treatment effect of lesion (F(3, 29)=9.78, p<0.001). Post-hoc unpaired t-test comparisons indicated that acute, 1-week and 1-month post-lesion groups all had significantly different volumes compared to controls (all p's<0.05). Post-hoc t-test comparisons did not reveal any significant difference between 1-week and 1-month post-lesion shoulder map volumes (p > 0.05).

The data for forepaw sensory map volumes suggests some contraction following lesions of the gracile nucleus. However, this trend was not statistically significant (ANOVA, F(3, 29)= 2.03, p>0.1). Therefore, the forepaw sensory map did not show any significant change in volume as a function of time post-lesion.
The pattern of results suggested by the volume of representational overlap between the forepaw and shoulder sensory maps appeared to mirror the time course of the shoulder sensory map volumetric changes. Accordingly, an ANOVA revealed a main effect of group ($F(3, 29)=15.1, p<0.001$) on representational overlap. Post-hoc t-test comparisons indicated that the 1-week and 1-month post-lesion groups had significantly elevated volumes compared to controls, while the acute group significantly decreased in volume relative to controls (post-hoc t-test). Again, post-hoc t-tests did not show any significant difference between the volumes obtained at 1-week and 1-month post-lesion.

In summary, the shoulder sensory map volume, following an initial period of contraction at an acute time interval, expanded in a robust and consistent manner at 1-week and 1-month post-lesion. The volume of sensory map overlap between forepaw and shoulder displayed the same time-dependent changes as the shoulder sensory map following nucleus gracilis lesions. These findings suggest that the shoulder representation may be increasing its volume by expanding medially and overlapping with the forepaw representation.
Figure 2.1 Sample data from a control (top) and lesioned (bottom) animal mapped with vertical electrode penetrations. This is a coronal view of a functional map, depicting classifications of recording sites for all body regions examined (H-hindlimb; F-forepaw; S-shoulder). The forepaw centroid in this plane is the cell with a bold border. For clarity, "blank" recording site designations are omitted. The diagram that follows provides a schematic view of the boundaries for each sensory map in a coronal view of the VPL in a control animal.
Coronal View of Shoulder, Forepaw and Hindlimb Sensory Maps in the VPL of a Normal Rat
Figure 2.2  Volume of three thalamic sensory maps at different times following nucleus gracilis lesions. A different group of animals was used for each time point. The figurine depicts the recording sites included in the calculation of each sensory map. Asterisks indicate significant differences between a given treatment group compared to controls. Bars are the Mean ± Standard Error (SE).
Figure 2.3a Coronal plane area of the shoulder sensory map as a function of anterior-posterior distance. Maps from each animal are aligned with respect to the anatomically determined anterior-posterior midpoint of the VB (anatomical registration). Lateral view of a rat brain indicates the plane of sectioning used on functional shoulder maps. The bar indicates a "focal zone" of robust change common to both 1-week and 1-month post-lesion groups. Asterisks indicate that both 1-week and 1-month post-lesion groups are significantly different from controls. Bars are the Mean ± SE.

Figure 2.3b Coronal plane area of the shoulder sensory map as a function of the anterior posterior distance. Maps from each animal are aligned with respect to the centroid of the forepaw (functional registration). Asterisks indicate that both 1-week and 1-month post-lesioned groups are significantly different from controls. Compared to anatomical alignment of coronal slices (Figure 3a), differences emerge more clearly with the functional alignment. All subsequent planar analysis is on coronal slices corresponding to the focal zone. Bars are the Mean ± SE.
Topological Analysis of Volumetric Changes

The cross-sectional profile of the shoulder sensory map in the coronal, sagittal and horizontal planes was examined in this section. A circumscribed region was discovered along the rostral-caudal axis in which all coronal cross-sectional area increases occurred and designated this region a “focal zone”. Subsequent analysis in sagittal and horizontal planes focused on this region of interest. Data from the coronal plane were used to compare two different forms of slice alignment from sensory maps: neuroanatomical and functional.

It was asked whether the volumetric increase in the shoulder sensory map reflected uniform increases in the cross-sectional area of the map along the anterior-posterior axis (Figure 2.3a). Since it was not always possible to discern the border between the VPM and the VPL by histology, the boundaries of the ventrobasal complex (VB; which includes VPM and VPL) were used for anatomical measurements. Anatomically, VPM and VPL occupy similar rostral-caudal borders. Each coronal slice from a rat's functional map was referenced based on its distance from the VB midpoint. In horizontal histological sections the midpoint of VB was identified between the most rostral border of VB with the reticuler nucleus and caudally with its border against the superior thalamic radiation (Paxinos and Watson 1997). On the abscissa, zero refers to the midpoint of VB. Negative spatial offsets are caudal to the VB midpoint while positive distances are anterior. Using this
alignment, the average coronal area of all slices from animals at a given distance from the VB midpoint within a treatment group was obtained.

The increases in coronal area at 1-week and 1-month post-lesion occurred almost exclusively at the rostral pole of the shoulder sensory map (Figure 2.3a). A 2-factor repeated measures ANOVA of group by distance revealed a significant effect of group (F(3, 26) = 10.4, p < 0.001), distance (F(6.47, 168.2) = 41.3, p < 0.001) and an interaction (F(19.41, 168.2) = 2.9, p < 0.001). Post-hoc t-test comparisons revealed that both 1-week and 1-month post-lesion groups at 0.6 and 0.8 mm from the VB midpoint were significantly different from the control group. At a rostral distance of 0.4 mm, a post-hoc t-test comparison between 1-week post-lesion and control was significant but 1-month post-lesion versus control was not. Post-hoc t-test comparisons between control and 1-week or 1-month post-lesion were not significant at any other distance. The acute group displays a trend towards decreased area at almost all distances from the VB midpoint compared to controls, but with post-hoc t-tests this was only significant at a distance of -0.6 mm relative to VB.

The same type of comparisons were conducted in the coronal plane again with one difference: the identity of a coronal slice and the alignment of shoulder sensory maps was achieved by reference to the centroid of the forepaw representation (Figure 2.3b). Since it appears that the forepaw
representation is unaffected by nucleus gracilis lesions at the time intervals under inspection, its centroid was used as an alternative reference point.

For each animal, slices of the shoulder sensory map were identified by virtue of their anterior-posterior distance from the centroid of the forepaw (Figure 2.3b). For each animal within a treatment group, coronal slices of the same distance from the forepaw centroid were averaged. These coronal areas were plotted as a function of their distance from the forepaw centroid. With functional alignment, differences in this graph are consistent with differences detected with neuroanatomical alignment (Figure 2.3a), but emerged more clearly.

A 2-factor repeated measures ANOVA of the data depicted in Figure 2.3b revealed a significant effect of group (F(3, 26)=9.8, p<0.001) distance (F(7.6, 196.5)=47.4, p<0.001) and an interaction (F(22.7, 196.5)=4.5, p<0.001). When 1-week and 1-month post-lesion groups were compared with controls, they were significantly larger at distances of 0.2 and 0.4 mm rostral to the forepaw centroid (post-hoc t-tests). At a distance of 0.6 mm, the 1-month post-lesion group was significantly different from control while the 1-week post-lesion was not (post-hoc t-test). At the midpoint, only the 1-week post-lesion group was significantly different from controls (post-hoc t-test). Finally, significant differences emerged between acute and control groups from 0.2 mm rostral to 1.0 mm caudal to the centroid (except at the midpoint, with post-hoc t-tests).
At no point was there a significant difference between 1-week and 1-month post-lesion (post-hoc t-tests).

Displayed in Figure 2.4 are data from a control and 1-week post-lesion animal for the shoulder sensory map. Each cube represents a recording site that was responsive to shoulder stimulation. The control map may upon first inspection appear as two separate representations. However, minor discontinuities in sensory maps were not uncommon across animals, and did not display any spatial consistency in their manifestation. If one averaged representations across animals, the composite map would appear solid in its entirety.

Functional alignment generated the same pattern of results as neuroanatomical registration of coronal slices, but differences appeared clearer. For this reason, and the relative ease with which a functional reference point permits comparisons in other planes, functional alignment was used for the remainder of the analysis. The region of interest with focal changes common to both 1-week and 1-month post-lesion occurred at distances of 0.2 and 0.4 mm rostral to the forepaw centroid (Figure 2.3b). I identify this region of interest as a 'focal zone' of reorganization.

Perhaps, however, the focal zone of expansion of the shoulder representation near the rostral pole is related to a massive loss of hindlimb
input compared to more caudal segments of the map. In Figure 2.5, hindlimb data are plotted in the same manner as in Figure 2.3b using functional alignment. An extensive loss of hindlimb input occurred in regions rostral and caudal to the forepaw centroid.

Statistical comparisons confirmed the extensive loss of hindlimb input rostrally and caudally to the forepaw centroid (Figure 2.5). A 2-factor repeated measures ANOVA revealed a main effect of group (F(3, 26)=32.9, p<0.001) distance (F(9.1, 237)= 28.2, p<0.001) and an interaction (F(27.3, 237)=5.5, p<0.001). The interaction suggests that the loss of hindlimb input was not constant with distance from the centroid. This result is largely due to the fact that relatively more hindlimb input remained intact at the caudal part of the map. The loss of hindlimb input at distances corresponding to the focal zone (0.2 and 0.4 mm anterior to centroid indicated by bar in Figure 2.5), was similar to that observed (e.g. >60% loss) at more caudal distances of -0.2 mm to -0.6 mm. Yet, at these caudal positions, from -0.2 to -0.6 mm, extensive loss of hindlimb responses did not produce an expansion of the shoulder sensory map at corresponding coronal planes (as in Figure 2.3b). Overall, post-hoc t-test comparisons revealed a significant reduction of hindlimb input for all groups from 0.6 mm to -1.2 mm.

It was asked how strong a predictor the reduction of hindlimb input would be of the ensuing shoulder expansion in the designated focal zone.
Two coronal slices were extracted from each animal's functional shoulder map corresponding to the focal zone of expansion (0.2 mm and 0.4 mm from the forepaw centroid - refer to Figure 2.3b). For each animal the counts from these two slices were averaged, and in turn those values were averaged across animals within a group for further planar analysis.

Whether residual hindlimb volume in this focal zone (0.2 mm to 0.4 mm) could predict the degree of shoulder expansion was addressed. A linear regression, which combined 1-week and 1-month post-lesion focal zones of each rat into a group large enough for this analysis, failed to reach significance ($F(1, 14)=3.6, p>0.08$). In this model the extent of hindlimb deafferentation was used as a predictor of ensuing shoulder expansion within each rat. The same analysis was repeated by including residual hindlimb input in the entire functional map as a predictor of shoulder expansion in the focal zone, also failed to reach significance ($F(1, 14)=0.35, p>0.5$). Thus, the level of hindlimb loss did not predict the extent of shoulder expansion.

The spatial distribution of recording sites that were responsive to hindlimb stimulation is depicted in Figure 2.6. The intact hindlimb map in the control is restricted along the medial-lateral axis, but extends some distance in the dorsal-ventral direction. The extant hindlimb representation following a gracilis lesion (Figure 2.6) indicated a number of punctate zones
where input appears to have remained intact, but overall the map is considerably reduced (>60%) in size.

For the remainder of the planar analysis the two coronal slices corresponding to the focal zone of shoulder expansion at 0.2mm and 0.4mm were used (Figure 2.3b). Figure 2.7 depicts a profile of sagittal slices of the shoulder representation in this focal zone, taken along the medial-lateral axis. Sample mapping data from a control animal from which Figure 6 was derived are displayed in Figure 2.1. The coronal slice taken in Figure 2.1 was taken at a distance from the forepaw centroid corresponding to the focal zone seen in lesioned animals. The identified unit corresponding to the centroid of the forepaw is enclosed in a box. To the left of the centroid (medially) are forepaw recording sites and to the right of the centroid, are shoulder and hindlimb recording sites.

Visual inspection of Figure 2.7 suggests that expansion of the shoulder representation at 1-week and 1-month post-lesion progressed medially with reference to the forepaw centroid. A 2-factor repeated measure ANOVA revealed a significant effect of group \( (F(3, 26)=16.4, p<0.001) \), distance \( (F(6, 155.5)=39.7, p<0.001) \) and an interaction \( (F(17.9, 155.5)=4.1, p<0.001) \). Compared to controls, 1-week and 1-month post-lesion groups were significantly elevated, in zones progressing from the centroid (zero) to 0.4 mm medially (post-hoc t-tests). At 0.6 mm the 1-month post-lesion group
was significantly increased (post-hoc t-test). The acute group did not reveal any significant contraction or expansion (post-hoc t-tests). It appears that along the medial-lateral axis, expansion of the shoulder sensory map extended its border up to 0.6 mm medially. The small lateral expansion to the right of the forepaw centroid appears to be confined within the zone of the forepaw representation (Figure 2.1).

In the horizontal plane, changes in cross-sectional area along the dorsal-ventral axis reveal that expansion occurs essentially at all depths in the focal zone (Figure 2.8). This analysis examined the same two slices containing the focal zone that were scrutinized in the sagittal plane described above. A 2-factor repeated measure ANOVA revealed a main effect of group (F(3, 26)=11.2, p<0.001), distance (F(5.9, 154.4)=29.1, p<0.001) and an interaction (F(17.8, 154.4)=4.8, p<0.001). Both 1-week and 1-month post-lesion groups were significantly elevated compared to controls in the majority of horizontal planes, from 0.4 mm dorsal to the centroid to -0.4 mm ventral (post-hoc t-tests). At 0.6 mm dorsal to the centroid, 1-week post-lesion was significant, while 1-month post-lesion was not (post-hoc t-tests). There was no significant difference between 1-week and 1-month post-lesion at any horizontal level (post-hoc t-tests). With regard to the acute group, there was a significant depression at the centroid when compared to controls (post-hoc t-test).
Figure 2.4 Spatial distribution of recording sites responsive to shoulder stimulation from a control and 1-week post-lesion animal. This is a posterior perspective of the sensory map. Lesion-induced changes are most marked at the rostral pole, towards the anterior end of the display. Much of the shoulder expansion appears to occur along the medial-lateral axis following gracilis lesions.
Figure 2.5  Plot of residual hindlimb input as a function of the anterior-posterior axis. Abscissa coordinates are the same as those in Figure 3b. The area of hindlimb input corresponding to the focal zone relative to the forepaw centroid is indicated by the bar. Asterisks indicate significant differences between all treatment groups compared to control values. Bars are the Mean ± SE.
Figure 2.6  Control and 1-week post-lesioned animals. Spatial distribution of recording sites that were responsive to hindlimb stimulation depicted from the posterior vantage point.
Control

Lesioned
**Figure 2.7** A cross sectional area profile of shoulder representation. The focal zone is represented in the figurine as a dark band in the coronal plane, which was cut sagittally with reference to the intact rat brain. All asterisks indicate significant differences between control versus 1-week and 1-month groups post-lesion. As far as 0.6 mm medial to the forepaw centroid, 1-month post-lesion was significantly elevated compared to control. Bars are the Mean ± SE.
Figure 2.8 A profile of shoulder sensory representation, revealed by cross-sectional slices in the horizontal plane. The figurine indicates the focal zone as a dark coronal band, sectioned with reference to the intact rat brain. Asterisks indicate significant differences for both 1-week and 1-month post-lesion groups versus control. Bars are the Mean ± SE.
Figure 2.9 A photomicrograph depicting a coronal view of the dorsal column nuclei stained with Nissl one week post-lesion. The damage observed in the gracile nucleus existed along the length of the nucleus. The accompanying diagram is a schematic rendering of the photomicrograph, barring tissue processing artifacts. The zone of gliosis is indicated by the stippled area. Abbreviations: cu fasc. (cuneatus fasciculus), Cu (cuneate nucleus), Gr (gracile nucleus).
Figure 2.10 A photomicrograph of a 1-month post-lesion rat brain, sectioned in the horizontal plane of the VPL and stained with Cresyl Violet. Arrows are interposed between two rows of electrode penetrations corresponding to the focal zone.
Histology

Figure 2.9 is a representative photomicrograph of the dorsal column nuclei one week post-lesion and shows that the damage to the gracile is very extensive. The cuneate nucleus on the right side is very close to the medial edge of the left gracile nucleus with a small bar of gliotic tissue in between. Such damage was present along the length of the nucleus. There was also some suggestion of gliosis in the cuneate nucleus, although this appeared weak.

Figure 2.10 presents a representative photomicrograph of a Nissl-stained section of the thalamus in the horizontal plane of a lesioned animal (1-month post-lesion). Brains sectioned this way allowed visualization of the entire pattern of electrode penetrations. Because the VPL is crescent-shaped in the coronal plane, penetrations at the presented slice level that appear too medial actually contact the VPL at more ventral levels. Consistently, the shoulder expansion appeared to occur at medial electrode penetrations, at the rostral pole of the VPL.

Discussion

Volumetric analysis indicated that expansion of the forelimb representation was confined to the shoulder region. However, such expansion in sensory map volume observed at 1-week and 1-month post-
lesion occurred after an unexpected transient contraction in volume at an acute time interval. Measures of representational overlap between forepaw and shoulder demonstrated the same temporal changes in volume as the shoulder sensory map. This latter data set suggested that the shoulder expanded by overlapping the forepaw representation, rather than expanding laterally into the denervated hindlimb zone.

Thalamic representation changes illustrated by volumetric data presaged similar findings in a detailed topological analysis. Remarkably, a focal zone of shoulder expansion was common to both 1-week and 1-month post-lesion at approximately 0.2-0.4 mm rostral to the forepaw centroid. Surprisingly, analysis of this focal zone in the sagittal plane clearly indicated that expansion progressed medially, overlaying the forepaw representation. There was no gradient or zone of hindlimb denervation along the anterior-posterior axis that corresponded uniquely to the focal zone of shoulder expansion.

A schematic summary of the somatotopic changes occurring in the identified focal zone at 1-week and 1-month post-lesion is presented (Figure 2.11). The medial progression of the shoulder forepaw sensory map is derived from the volumetric data of compartmental overlap between shoulder and forepaw representations that increased after an acute time interval (Figure 2.2). Additionally, when the focal zone was sectioned in the
sagittal plane (Figure 2.7) described above, a clear medial progression of the shoulder sensory map into the forepaw representation was revealed. Qualitatively this is consistent with what I observed in my raw data from the focal zone (Figure 2.1b).

It is unlikely that the results are a product of the type of anesthetic employed, or subcortical bleeding since all groups were exposed to the same mapping protocol. Therefore, such an explanation fails to explain the different results obtained for each time point with respect to control. Further, such an artifact is not consistent with the observed changes localized to the rostral pole of the VPL, rather than occurring throughout the length of the nucleus. Finally, the fact that the acute group showed the opposite changes to later time points also suggests that the results are not the product of mapping-induced neuronal injury or changes in anesthesia level.

It was not necessary to control for possible morphological differences in the size of thalamic nuclei across animals. While such differences may exist, there is no reason to believe that there are systematic differences between groups. If one argued for lesion-induced atrophy in the size of thalamic nuclei producing such differences, one would have to postulate increases in size of the VPL, since the absolute dimensions of the shoulder sensory map enlarged with time.
It may be possible that damage was caused to the shoulder input, as reflected in the contraction in its sensory map volume at an acute time interval. Histology did reveal some gliosis in the nucleus cuneatus. However, no statistically significant difference was seen between control and any of the lesioned groups for the forepaw sensory map volume. One would have expected damage to the forepaw sensory map if this hypothesis was correct, since it is interposed between the gracile nucleus and the shoulder functional map (Nord 1966). Additionally, coronal slices of the shoulder sensory map outside the focal zone at one and four weeks post-lesion have exactly the same cross-sectional area as controls, suggesting that the contraction observed at an acute time interval is not a product of damage.

A study of the thalamus following nerve transection (sciatic and saphenous) at 1-week post-lesion is presented in detail in Chapter 3. In that study, changes observed following nerve transection were similar to those described in this study. This further argues that possible damage to nucleus cuneatus caused by gracile lesions or residual hindlimb input to VPL is not a likely explanation for the pattern of reorganization described herein.

Concordant with Wall and Egger (1971), lesions of nucleus gracilis in this study failed to block hindlimb input completely. It is likely that the extant hindlimb sensory map was receiving input from pathways independent of the dorsal column nuclei. The spinocervical tract can convey
cutaneous information, and does so independently of the dorsal column nuclei (Morin 1955; Giesler et al. 1979). In addition, in the rat it appears that many spinothalamic tract neurons respond to innocuous inputs, and at least some of these neurons terminate in the VPL (Dado et al. 1994). Either one of these pathways may have been responsible for mediating the residual hindlimb input observed in this study.

There were also clear differences in this report with that of Wall and Egger (1971). In essence, they reported that within 1-week post-lesion following nucleus gracilis lesions that the forelimb representation had expanded into the denervated hindlimb zone of the VPL. The most poignant difference is that shoulder expansion did not appear to progress into the denervated hindlimb zone, but rather annexed territory by superimposing itself on the existing forepaw representation. Importantly, this change was contained in a 300 µm coronal slice towards the rostral pole of the VPL. Such discrepancies may be related to differences in the choice of the control group. Wall and Egger (1971) used the hemisphere ipsilateral to their gracile lesion as a within subject control. However, it is now known that there are ipsilateral consequences from a lesion (before its decussation), such as somatotopic plasticity (Calford and Tweedale 1990) and changes in acetylcholine binding in the cortex (Hanisch et al. 1992). In comparing my treatment groups against a separate group of non-lesioned animals, this study has avoided possible misinterpretation in using the contralateral hemisphere as a control.
One mechanism that may account for the contraction of the shoulder functional volume is the emergence of inhibitory receptive fields which are known to result from deafferentation (Dostrovsky et al. 1976; Rasmusson et al. 1993). Following digit amputation in the raccoon, neurons that have suffered loss of input acquire inhibitory receptive fields on adjacent intact digits ("off-focus inhibition"), which depress spontaneous activity when stimulated (Northgrave and Rasmusson 1996). Consistent with the transient character of the shoulder map volume contraction, inhibitory receptive fields are observable at acute time intervals, but appear to be absent in chronic preparations (Dostrovsky et al. 1976; Northgrave and Rasmusson 1996; Rasmusson 1996b).

A decrease in shoulder map volume at an acute time interval may be the product of off-focus inhibition by neurons that previously had receptive fields for shoulder and hindlimb. Off focus skin regions are adjacent but outside a neuron's receptive field. However, stimulation of off focus areas can have inhibitory effects on a neuron. Single unit characterization of VPL neurons has encountered neurons with large receptive fields that can cover virtually half the body surface of the rat (Sherman et al. 1996). In mapping acutely lesioned animals, off-focus inhibition generated by a few neurons with receptive fields covering the shoulder and hindlimb would cause a decrease in spontaneous excitability of neurons responsive to shoulder
stimulation at the recording site. Off-focus inhibition generated by a few units reduces the number of recording sites responsive to shoulder stimulation, which in this paradigm may have been reflected as an overall decrease in the shoulder map volume.

An issue not addressed in this study is whether the hindlimb denervated zone in the VPL is occupied by the expansion of another body representation. Other body regions outside the forelimb could be involved such as the face or the abdominal area. This report does not claim that the denervated hindlimb zone remains silent, only that the forelimb representation does not advance into this zone.

An overview of the literature might suggest that the direction of the shoulder expansion is inconsistent with the maxim that increased somatotopic representation occurs via border extensions into an adjacent deafferented zone (Wall and Egger 1971; Kalaska and Pomeranz 1979; Merzenich et al. 1983; Wall and Cusick 1984; Merzenich et al. 1987). However, there may be an unusual relationship between forelimb and hindlimb somatotopic maps that may warrant the suspension of this maxim in this case. Research at the somatosensory cortical level that has examined changes in somatotopy following fasciculus gracilis lesions failed to find any expansion of the forelimb into the hindlimb representation (Jaine et al. 1995). Also, investigations with optical imaging of the cerebral cortex in rats have
found considerable topographic overlap between different skin regions within either the forelimb or hindlimb representations (Godde et al. 1995). In contrast, the authors found no overlap between the forelimb and hindlimb representations, leading to the speculation that these two somatotopic domains may be functionally insulated from each other in the context of plasticity. One might argue that cortical and thalamic somatotopic changes may be independent (Wang et al. 1995) or question whether the currency of somatotopic territory can be freely exchanged between hindlimb and forelimb.

If we provisionally accept that border shifts between forelimb and hindlimb representations do not occur, it is not immediately obvious why the loss of hindlimb input would induce topographic overlap between shoulder and forepaw. There is good evidence supporting the view (Merrill and Wall 1972) that inhibition is important in restricting receptive field size and this has been confirmed at the cortical (Hicks and Dykes 1983; Dykes et al. 1984) and thalamic levels (Lee et al. 1994). Interestingly, recordings from the VPL revealed mutual inhibition between forelimb and hindlimb units in 45% of the neurons tested in response to electrical stimulation (Roberts and Wells 1990). Perhaps the loss of the hindlimb representation removed inhibition acting on units in the forepaw representation of the VPL, leading to an increase in their receptive field size enabling these units to respond to shoulder stimulation.
There are some limitations in this study that should be addressed in future investigations. First, since purely low-threshold input has been characterized, these findings may not generalize to other cutaneous inputs such as high-threshold input or temperature sensation. Second, based on the evidence presented here, partial removal of hindlimb input by nucleus gracilis lesions could be responsible for the form of somatotopic organization observed. Thirdly, unoperated controls have been used rather than sham animals. Finally, the work has not been conducted under experimentally ‘blind conditions’. Although this last criticism is mitigated by the fact that the exact placement of the focal zone relative to the forepaw centroid is essentially blind, since such a determination can only be achieved with analysis after the recording session is complete.
Figure 2.11 A schematic of the topographic overlap between shoulder and forepaw in the focal zone at 1-week and 1-month post-lesion. This is a coronal view of forepaw, shoulder and hindlimb sensory maps in the VPL. The plasticity depicted is derived from data on the volume of shoulder-forepaw overlap (Figure 2) and planar analysis (Figures 7 and 8).
Figure 2.12 The hot spot model of neural plasticity. A depiction of the identified focal zone or hot spot of reorganization in the thalamus. It is proposed that hot spots exist at other levels of the somatosensory pathway such as the cortex. Cortically, the hot spot is a slice oriented orthogonally to Mountcastle’s cortical columns (Mountcastle 1957), in the same way the thalamic hot spot is orthogonally oriented with respect to Jones’s thalamic rods (Jones et al. 1979; Jones and Friedman 1982; Jones et al. 1982). The somatotopy of the thalamus is rotated compared to that of the cortex, given the difference in the orientation of thalamic rods and cortical columns. See text.
**Hot Spot Model**

The plastic focal zone described in the thalamus following nucleus gracilis lesions appears to have four properties: (1) it is a subregion of a sensory map defined by low-threshold mechanical input that appears to display somatotopic reorganization; (2) it reveals reorganizational events that occur over chronic rather than acute time intervals; (3) reorganizational events take place in a zone that is a slice; (4) this slice appears to be oriented orthogonally to columns of cells possessing the same receptive fields.

Property four is partly an inference drawn from the literature. Jones has built a persuasive case that the columnar organization of the cerebral cortex (Mountcastle 1957) is also found in the VB of the thalamus (Jones et al. 1979; Jones and Friedman 1982; Jones et al. 1982) in the primate. These "thalamic rods" are analogous to the columnar organization of the cortex since within a rod cells possessed the same receptive field properties (Jones et al. 1982). Since studies of thalamic rods have been conducted in primates it is assumed here that they also exist in rats. Further, these rods were proposed to be oriented along the anterior-posterior axis in the thalamus. If thalamic rods exist, they intersect this slice of plasticity at roughly a ninety degree angle (Figure 2.12).

This focal zone may be a "hot spot" that displays thalamic reorganization in general and may have manifestations elsewhere in the
nervous system. This 'hot spot' hypothesis has two predictions. First, the thalamic hot spot is not specific to nucleus gracilis lesions, but may also be important in displaying somatotopic reorganization following other types of neural trauma (e.g. Chapter 3). Second, the existence of a hot spot of plasticity is not a feature specific to the thalamus, but may also exist in other structures such as the cerebral cortex or dorsal column nuclei.

If a hot spot exists in the cerebral cortex, properties three and four would predict the orientation of the slice that reveals reorganization. The existence of columns of cells with isomorphic receptive field properties is organized vertically in the cortex (Mountcastle 1957), opposite to the orientation of thalamic rods (Figure 2.12). According to property four of the hot spot description, the slice would be oriented horizontally in the cortex, orthogonal to Mountcastle's cortical columns.

Properties one and two of the hot spot applied to the cortex would predict the existence of a circumscribed zone displaying plasticity over chronic time intervals. While a consensus may not exist with respect to the role of each lamina in mediating plasticity, it is clear that over time specific lamina seem to be particularly modifiable (Diamond et al. 1994; Kirkwood and Bear 1994; Johnson and Alloway 1996). In terms of the hot spot hypothesis, a slice displaying plasticity may include one or a subset of the six laminae in the cortex (property 1). For illustrative purposes layer IV is a possibility (Figure
A Focal Zone of Thalamic Plasticity

2.12), which seems to undergo plasticity over chronic rather than acute time intervals (Jenkins et al. 1990; Recanzone et al. 1992; Diamond et al. 1994), consistent with property two of the hot spot.

Differences in the orientation of hot spots at each level of the neuraxis can determine how easily they are detected with standard vertical electrode penetrations. Statistically, a large number of electrode penetrations would be required before the thalamic hot spot was detected, while in the cortex, every vertical electrode penetration traverses this plastic slice (Figure 2.12). This difference may explain why learning-induced changes in cortical somatotopy were not detected in the thalamus (Wang et al. 1995). A detailed 3-D reconstruction of the entire somatotopic map in the thalamus would be necessary to detect such a zone.

In sum, the hot spot hypothesis can make predictions about the time course and orientation of a slice of plasticity at different levels of the nervous system. This author does not venture to speculate on what the inter-relationships may be between hot spots at different levels of the neuraxis, if they in fact exist outside the thalamus.

Conclusions

To this author's knowledge, the contraction of sensory maps at acute time intervals post-lesion, followed by increased topographic overlap
represent new properties of thalamic plasticity. Further, such topographic overlap mediating shoulder sensory map expansion occurred within a narrow coronal slice of the VPL that has been referred to as a focal zone. Thus, it appears that thalamic sensory maps defined by low-threshold mechanical input are not homogeneous with respect to their potential for plasticity. It remains to be demonstrated whether this phenomena is a property of other areas in the CNS as well as different sensory and neurotransmitter systems.
Chapter III

A Focal Zone of Thalamic Plasticity: Independence of the Form of Deafferentation?
Abstract

In this study thalamic plasticity was induced by transection of the sciatic and saphenous nerves in the hindlimb of adult rats. Previously we described a focal zone of plasticity within the VPL precipitated by dorsal column lesions. In this study we asked whether a focal zone of plasticity would emerge within the VPL following nerve transection. Mapping was performed in adult rats 2 weeks following nerve transection (n = 8) and was compared with sham-operated controls (n = 6). Animals had their sciatic and saphenous nerves cut and ligated. With a dense array of electrode penetrations, 3D reconstructions of sensory maps in the thalamus are presented. A focal zone of plasticity was found within the VPL as a result of nerve transection. Within this focal zone, the shoulder representation increased in volume. This provides further evidence that a focal zone, located towards the rostral pole of the VPL, may have fundamental importance in somatotopic reorganization. Finally, the similarity in the magnitude in reorganization between nerve transection and dorsal column lesions suggests the level of the lesion in the nervous system does not determine the extent of reorganization observed at higher levels of the neuraxis.
Introduction

A central issue in neuroscience is understanding the sequela of changes initiated in the brain following nerve injury. While literature documenting cortical changes has now reached leviathan dimensions (Kalaska and Pomeranz 1979; Merzenich et al. 1983; Merzenich et al. 1984; Wall and Cusick 1984; Pons et al. 1991; Webster et al. 1991; Recanzone et al. 1992; Wang et al. 1995), there have also been reports of plasticity at lower levels of the nervous system. In adult animals, receptive field changes in cutaneous pathways have been discovered in the dorsal horn (Brown and Fuchs 1975; Basbaum and Wall 1976; Devor and Wall 1978; Devor and Wall 1981), dorsal column nuclei (Dostrovsky et al. 1976; Millar et al. 1976; Pettit and Schwark 1993), trigeminal nucleus (Rhoades et al. 1987; Nicolelis et al. 1993) and VPL (Nakahama et al. 1966; Wall and Egger 1971; Rasmusson 1996a) following the disruption of peripheral input.

It has been argued that changes initially induced at the level of the spinal cord propagate to each successive level of the nervous system inducing similar changes in somatotopic reorganization (Pons et al. 1991; Florence and Kaas 1995). At the level of somatosensory cortex, a circumscribed change at the spinal cord level has been greatly amplified through the divergence of axonal arbors in sensory transmission. This process will be referred to as a 'cascade'.
Previously, changes in thalamic somatotopic organization following lesions of the dorsal column nuclei were documented (Chapter 2). Such lesions partially removed low-threshold cutaneous input from the hindlimb. 3D reconstructions of the forelimb sensory map from multiple electrode penetrations enabled visualization of changes in the thalamic sensory maps. It was found that there was an overall volumetric increase in the shoulder sensory map. Importantly, topological analysis revealed that this volumetric expansion occurred in a circumscribed coronal slice towards the rostral pole of the VPL that was referred to as a focal zone. A hot spot model which had been framed that predicted that this area may display somatotopic reorganization exclusively within the VPL (Chapter 2). Similar hot spots, were also predicted to exist at other levels of the neuraxis (dorsal column nuclei etc.), but this latter postulate is for future research.

This paper tests some of the predictions of the cascade and hot spot models. The cascade hypothesis would predict that somatotopic changes initiated as the spinal cord level, initiated by nerve transection, should be greater in magnitude than those observed following nucleus gracilis lesions. Not only does nerve transection remove sensory input at a lower level of the neuraxis, it completely deaффerents all modalities from the hindlimb, unlike nucleus gracilis lesions. Based on such differences, the cascade model would predict more dramatic reorganization at the thalamic level compared to
nucleus gracilis lesions. Because of such differences between nucleus gracilis lesions and nerve transection, it would be interesting to see whether the same region of the thalamus, the "focal zone", is exclusively involved in displaying somatotopic reorganization at the thalamic level. Thus, the present study involved transection of the sciatic and saphenous nerves in adult rats and 2 weeks post-lesion sensory maps in the VPL were examined.

**Methods**

Unless described below, all other aspects of experimental protocol were the same as those described earlier (Chapter 2).

*Subjects and Nerve transection:* 14 male Wistar rats with weights ranging from 250 to 350 grams were used. Rats were anesthetized with Ketamine hydrochloride (200 mg/kg, i.p.) and xylazine (50 mg/.kg, i.p.). Sham-animals (N = 6) received surgical exposure of the saphenous and sciatic nerves. Nerve transection animals (N = 8) had their saphenous and sciatic nerves sectioned, with a 5 mm length of the nerve removed and a ligature applied to both cut nerve ends. Animals had their thalamus mapped 2-weeks post-nerve transection. Autotomy was not observed during this experiment.
Results

Volumetric Analysis

Volumes are shown for three sensory map compartments for sham and nerve transection groups (Figure 3.1). An omnibus multivariate comparison is unnecessary, since the previous study (Chapter 2) gives exact predictions with respect to the direction of change for each sensory compartment. Accordingly, univariate tests are presented for each sensory compartment. The shoulder representation exhibited a robust volumetric expansion relative to controls \((t(12) = 2.6, p < 0.02)\). However, the forepaw sensory compartment was not significantly different relative to controls \((t(12) = 1.67, p > 0.1)\). Finally, the volume of overlap between shoulder and forepaw sensory maps was significantly different from controls \((t(8.21) = 2.94, p < 0.01)\). There was no residual hindlimb input detected in any of the nerve transected rats.

Topological Analysis

In this section the shoulder functional map was analyzed in the coronal, sagittal and axial planes. A functional map was constructed by a 3D reconstruction of the spatial distribution of recording sites in the VPL responsive to the stimulation of a specific body region. Volumetric analysis indicated that the shoulder functional map was of key interest. This sensory map was examined in detail.
Representative shoulder functional maps are depicted in Figure 3.2. It can be readily seen that the nerve transection animal had experienced an overall volumetric expansion of the shoulder functional map. These functional shoulder maps were sliced in each plane. Equivalent slices across animals were identified by virtue of their distance from the forepaw centroid. The forepaw centroid appears reliable as its functional map does not undergo volumetric changes following hindlimb deafferentation (section above) and yields very similar results to the anatomical alignment of functional maps.

Shoulder functional maps were sectioned in the coronal plane and the cross-sectional area profile compared between control and nerve transection groups (Figure 3.3). There was a main effect of Group (2-way ANOVA; F(1,12) = 5.22, p < 0.05; Power 0.56) and a Slice by Group interaction (F(11, 283.2) = 11.14, p < 0.001; Power 0.92). Thus, nerve transection significantly increased the cross-sectional area of the shoulder functional map relative to controls and this varied significantly as a function of the anteroposterior distance from the forepaw centroid. Post-hoc t-test comparisons revealed a circumscribed area of expansion towards the rostral pole of the VPL. It is referred to as a focal zone.

For the analysis that follows this focal zone was sliced in the sagittal and horizontal planes. The cross-sectional profile of the focal zone from the
shoulder functional map (Figure 3.4) suggests a clear medial advancement of
the inner border towards the midline of the rat brain. A 2-way ANOVA
revealed a significant effect of Group (F(1,12) = 10.9, p < 0.01; Power 0.856) and
a Slice by Group interaction (F(3.82, 45.89) = 2.62, p < 0.05; Power 0.83). Post-
hoc t-test comparisons revealed significant differences between the control
and nerve transection groups as far as 0.6mm toward the midline of the brain.

Finally, the focal zone as sliced in the horizontal plane and the cross
sectional areas of control and nerve transection groups is compared (Figure
3.5). The expansion appears to be essentially uniform along the dorsoventral
axis. A 2-way ANOVA revealed a significant effect of Group (F(1, 12) = 12.4, p
< 0.01; Power 0.90) and Slice by Group interaction (F(3.95, 47.4) = 2.88, p < 0.05;
Power 0.97). While the expansion may have been uniform, the tapering of
the map dorsally and ventrally was likely responsible for the interaction.

**Histology**

Figure 3.6 presents a photomicrograph of a Nissl-stained section of the
thalamus in the horizontal plane of a lesioned animal (2-weeks post-
transection). Because the VPL is crescent-shaped in the coronal plane,
penetrations at the presented slice level that appear too medial actually
contact the VPL at more ventral levels. Consistently, the shoulder expansion
appeared to occur at medial electrode penetrations, at the rostral pole of the
VPL.
Figure 3.1. Volumes of three thalamic sensory maps. Sham (n=6) and nerve transection (n = 8) groups are depicted. Both the shoulder sensory map, and the region of overlap between shoulder and forepaw displayed significant increases in volume relative to shams. Columns indicate mean; bars indicate ± SE.
Thalamic Sensory Map Volumes Following Nerve Transection of the Hindlimb
**Figure 3.2** Representative 3D reconstructions of the shoulder sensory map for sham and nerve transection groups. The reconstruction represents the spatial distribution of recording sites responsive to shoulder stimulation. Such a reconstruction is referred to as a ‘functional map’ which was sectioned in several planes.
Figure 3.3 The cross-sectional area profile of the shoulder functional map along the anteroposterior axis. Equivalent slices across animals were identified by virtue of their distance from the forepaw centroid. Functional maps sliced in this manner revealed that the cross-sectional area increases were localized to the rostral pole of the VPL. Asterisks indicate significant differences between nerve transection and shams. Error bars indicate ± SE.
Coronal Profile of the Shoulder Functional Map Following Hindlimb Transection
Figure 3.4  The focal zone has been sectioned in the sagittal plane yielding a cross-sectional area profile along the mediolateral axis. The figurine shows the sectioning of the focal zone relative to the intact rat brain. Asterisks indicate significant differences between nerve transection and sham groups. Error bars indicate ± SE.
Sagittal Profile of the Shoulder Functional Map in the Focal Zone Following Nerve Transection

![Graph showing the number of positively identified shoulder sites vs. distance (mm) from forepaw centroid. The graph compares sham and nerve transection conditions with error bars. There is an inset image of a sagittal plane with a cross indicating the focal zone.](image-url)
Figure 3.5 The focal zone is sectioned in the horizontal plane yielding a cross-sectional area profile along the dorsoventral axis. The expansion observed in the nerve transection group appeared to be uniform along the dorsoventral axis. Asterisks indicate significant differences between nerve transection and sham groups. Error bars indicate ± SE.
Horizontal Cross Sectional Area Profile of the Shoulder Sensory Map Following Nerve Transection of the Hindlimb

Number of Positively Identified Shoulder Sites

Distance (mm) from Forepaw Centroid

Dorsal

Ventral

Focal Zone

Horizontal Plane
Figure 3.6 A photomicrograph of a rat brain 2 weeks post-nerve transection. Tissue was sectioned in the horizontal plane and Nissl-stained. Arrows are interposed between two rows of electrode penetrations that correspond to the focal zone.
Discussion

Somatotopic reorganization of the forepaw and shoulder sensory maps in the thalamus following hindlimb deafferentation displayed four principal characteristics: (1) a volumetric expansion of the shoulder representation; (2) a medial progression of the shoulder sensory map away from the lateral denervated zone of the hindlimb; (3) increased topographic overlap between forelimb and shoulder sensory maps, and most importantly, (4) all reorganization was confined to a coronal slice located towards the rostral pole of the shoulder sensory map.

Nerve transection and nucleus gracilis lesions differ in several fundamental ways: interruptions of polysynaptic vs. monosynaptic pathways to the VPL; complete vs. partial block of low-threshold cutaneous input; deafferentation of all cutaneous modalities vs. low-threshold cutaneous input and a smaller deafferented body surface vs. a comparatively large surface area. The implications for the cascade and hot spot model are considered in turn.

Cascade Model

The results did not appear to support the cascade hypothesis (Pons et al. 1991; Florence and Kaas 1995). The magnitude of plasticity reported in this study, surprisingly, was exactly the same as observed following nucleus
gracilis lesions in an earlier study (Chapter 2). The volume of the shoulder representation (1.06 ± SE 0.08 mm³) following nerve transection, while significantly greater than controls, was quite close to that found following nucleus gracilis lesions at either 1-week post-lesion (1.04 ± SE 0.11 mm³) or 1-month post-lesion (0.95 ± SE 0.10mm³). Note that control values were also quite close in magnitude between this study and the earlier report with nucleus gracilis lesions. The cascade model predicted that nerve transection should have precipitated a greater volumetric increase; reorganization initiated at lower levels of the nervous system should have undergone greater amplification at the thalamic level than that observed after nucleus gracilis lesions. This prediction of the cascade model was not supported by the volumetric data.

The pattern of results reported following nerve transection in this study were very similar to that following nucleus gracilis lesions (Chapter 2). While the cascade model may not predict qualitatively different reorganization at the thalamic level between nucleus gracilis lesions and nerve transection, is does predict differences in magnitude. The medial progression of the shoulder sensory map in the focal zone in this study was 0.6mm, a little greater than that observed at 1-week post-lesion (0.4mm) but the same as 1-month post-lesion (0.6mm) following nucleus gracilis lesions. The failure to observe clear differences in magnitude speaks against the cascade hypothesis.
One may argue that nerve transection, while invoking a polysynaptic pathway to the thalamus, requires a longer time interval for changes at the thalamic level compared to nucleus gracilis lesions. One might predict, therefore, that changes at the thalamic level at early time points should actually be smaller than those observed following nucleus gracilis lesions. But given the close agreement numerically between changes reported here and nucleus gracilis lesions at 1-week and 1-month post-lesion mitigates against both predictions. Further, changes following nucleus gracilis lesions, while not manifest at an acute time interval, were manifest at 1-week post-lesion and did not appear to change significantly compared to 1-month post-lesion (Chapter 2). Thus, for nucleus gracilis lesions plasticity appeared to plateau by 1-week post-lesion. The fact that the magnitude of change of nerve transection described here fell almost exactly within this range suggested that nerve transection changes may have also plateaued. Whether or not either form of lesion yields additional changes beyond 1-month post-lesion is not known, however, the evidence within the 1-month time frame suggested that no quantitative difference between these groups occurred.

The focal zone described here was a little larger than that reported following nucleus gracilis lesions (Chapter 2). The nerve transection focal zone was up to 0.6 mm in coronal thickness, while nucleus gracilis lesioned animals had focal zones spanning 0.3 mm approximately. This difference
may appear to support the cascade prediction. However, such a difference may be due to slightly greater variability in the location of the focal zone across animals in the nerve transection group since overall volumetric changes were no different between nerve transection and nucleus gracilis lesions for the shoulder sensory map.

The data presented here should not be marshaled, however, to deny the importance of each level of the neuraxis in adapting to neural trauma. Edelman's (1987) reentrant model proposes that all levels of the somatosensory pathway interact simultaneously in determining reorganizational events in any structure along the somatosensory pathway. This coordination is achieved through corticofugal input to each level of the neuraxis (Coleman et al. 1997).

The cascade model is different from the reentrant model because it is temporally serial in nature with changes traversing uni-directionally along the neuraxis (Florence and Kaas 1995). Each change is the product of lower level events that act as a template. In the reentrant model, corticofugal input allows simultaneous and bi-directional contributions of all levels of the neuraxis. The importance of corticofugal input is highlighted by the recent finding that such input is necessary for the induction of thalamic plasticity (Chapter 4). In sum, the reentrant model would predict that the level of the
Nerve Transection

lesion is irrelevant to reorganizational processes, consistent with findings discussed here.

**Hot Spot Model**

The results provide strong support for one of the tenents of the hot spot model (Chapter 2). In the context of this study, the hot spot model predicted that the focal zone was not specific to nucleus gracilis lesions, but would exclusively display reorganization for other types of neural trauma over chronic time intervals. Nerve transection could have resulted in plasticity throughout the length of the VPL. Alternatively, a focal area of change could have again emerged, but in a different area of the VPL. Spatially, a number of differences in thalamic reorganization could have emerged between nerve transection and nucleus gracilis lesions.

It is fascinating that neither one of these possibilities occurred. The focal zone reported here spanned from 0 to 0.4mm rostral to the forepaw centroid, while the earlier report with nucleus gracilis lesions indicated a focal zone 0.2 to 0.4mm rostral to the forepaw centroid. The focal zone following nerve transection does appear to be slightly larger, but otherwise occurs in the same location of the VPL.

The strong concordance in the spatial dimensions of the focal zone and in its placement along the length of the VPL, between nucleus gracilis lesions
and nerve transection groups is unlikely due to chance. Sensory maps in the VPL extend at least 2.5 mm rostrocaudally (Emmers 1965; Parker et al. 1998), while the focal zone has an upward estimate in coronal thickness of only 0.6 mm. Thus, with respect to the rostrocaudal axis, there are other locations in the VPL where a focal zone could have appeared that would have violated the hot spot prediction. In summary, nerve transection produced reorganization that was both spatially restricted and confined to a very similar region of the shoulder sensory map as seen with nucleus gracilis lesions, consistent with the prediction of the hot spot model.

Somatotopic Fracture Lines

It is curious that changes following the removal of hindlimb input should manifest as an expansion of the shoulder sensory map medially away from the more lateral denervated hindlimb zone. The border with the hindlimb appears unchanged, but there is increased topographic overlap by the shoulder with the forepaw as it moves medially. This progression appears to contradict the findings describing advancement of adjacent maps into a denervated zone of the nervous system (Merzenich et al. 1988). Experiments involving hindlimb and forelimb appear to yield a different pattern of results. Cortically, no map border shift is found between hindlimb and forelimb representations following the removal of hindlimb input (Jaine et al. 1995). However, these investigators did not examine the internal somatotopy of the forelimb representation in any detail (Dr. Jaine, personal
communication 1997). The research presented here confirms such a barrier between hindlimb and forelimb representations at the thalamic level.

There may be "somatotopic fracture lines" that limit plasticity. It has long been proposed that an overlap of axonal arbors from two body regions must exist for potential map-border shifts to occur between such represented body regions following deafferentation (Merzenich et al. 1983; Merzenich et al. 1984). Cortically, overlapping arbors do not appear to exist between hand and face (Manger et al. 1997) or forelimb and hindlimb (Godde et al. 1995). In essence a somatotopic fracture line exists between these body regions that may preclude the exchange of somatotopic territory (Figure 3.7).

Somatotopic fracture lines, however, do not explain why plasticity occurs within the forelimb representation following the removal of hindlimb input. It was previously suggested that, given evidence for mutual inhibition between hindlimb and forelimb (Roberts and Wells 1990), the deafferentation of the hindlimb may allow receptive field expansion of the forelimb (Chapter 2). It has been suggested that such mutual inhibition may be mediated by GABAergic input from the thalamic reticular nucleus (Roberts and Wells 1990) as some corticothalamic (Jones 1975) projections are relayed through this area before reaching the VPL. A further possibility may be the activation of the thalamic reticular nucleus from ascending input from the dorsal column nuclei, via the VPL (Jones 1985). While the exchange of
representational territory may not occur across a somatotopic fracture line, reorganizational changes (e.g. increased topographic overlap) across this divide may be mediated by the removal of inhibition from the thalamic reticular nucleus.

The possible basis for plasticity between hindlimb and forelimb representations raises the concern of paradigm specificity. While one may accept the logical basis for somatotopic fracture lines, why should mutual inhibition exist between hindlimb and forelimb sensory maps? Does mutual inhibition also exist between hand and face representations? In other words, since hand and face are also separated by a somatotopic fracture line, does that mean that mutual inhibition also exists between these two representations? Would the removal of the hand representation result in plasticity, possibly increased topographic overlap, within the face representation? No border shift has been found between cortical hand/face representations following amputation of the hand in monkeys, but the internal somatotopy of the face has not been examined in detail (Garraghty et al. 1994). One study examining the effects of therapeutic amputation of the arm in primates 13 years later appears to be an exception to this, but such long time scales may involve additional mechanisms (Pons et al. 1991).
Figure 3.7 A schematic depiction of somatotopic fracture lines and resulting domains. Map border shifts cannot occur between representations in separate somatotopic domains. Map border shifts can occur between representations within the same somatotopic domain, since there is overlap among axonal arbors (e.g. thalamocortical). The central representation could be any level of the neuraxis. The axons are afferents carrying cutaneous input. While map border shifts cannot occur across somatotopic fracture lines, plasticity can still be induced. The removal of input in one domain (e.g. hindlimb - gracile pathway) can remove inhibition, possibly via the thalamic reticular nucleus (TRN), acting on an adjacent representation located in a separate somatotopic domain (e.g. forelimb - cuneatus pathway). Thus, increased topographic overlap among represented regions of the forelimb occurs with the removal of hindlimb input, due to reduced inhibition. It is suggested that mutual inhibition may also exist between the trigeminal and cuneatus pathways. Arm amputation (cuneatus pathway) in this model predicts increased topographic overlap among sub-representations of the face (trigeminal pathway).
Somatotopic Fracture Lines

Axonal Arbors

Fasciculus Gracilis
(Domain)

Fasciculus Cuneatus
(Domain)

Trigeminal Pathway
(Domain)

Central Representation
While this study constitutes an important test for the hot spot model, much research remains to be done. Digit amputation is a good example of a test of the hot spot model. An investigation of digit representation in the thalamus occurs within the same “somatotopic domain” (Figure 3.7), that is both the denervated and adjacent representations are within the same anatomical pathway and possess some overlap in their thalamocortical (Jones and Powell 1969) and intracortical (Manger et al. 1997) arbors. Therefore, digits are not separated by a somatotopic fracture line. Is the focal zone identified from forelimb reorganization precipitated by hindlimb deafferentation also important in digit expansion? Is it possible that digit reorganization is a uniform event throughout the VPL? If such reorganization is spatially restricted within the VPL, does such an area fall in the rostral pole of the VPL? Is there a hot spot for other sensations, such as temperature or high-threshold input? Addressing such questions in the future would provide a much more thorough evaluation of the hot spot model’s limitations.

Conclusion

The data support the hot spot model and suggest that a focal zone within the thalamus exists that may be exclusively important in displaying somatotopic reorganization. Comparisons with earlier research with nucleus gracilis lesions suggest that, contrary to the cascade model, the level of lesion
along the neuraxis may not be important in determining the magnitude of somatotopic reorganization observed at higher levels of the nervous system.
Chapter IV

Cortical Control of Thalamic Plasticity
Abstract

The thalamus is a gateway through which sensory input ascends to the cerebral cortex. Nerve injury causes a reorganization of somatosensory representations that is thought to cascade from the periphery to the thalamus, before reaching the cortex. Cortical reorganization is regarded as a process that uses changes at the thalamic level as a template. Previously, thalamic reorganization was detected as an increase in the volume of the shoulder sensory map following the removal of hindlimb input. This study examined the role of the cortex in the plasticity of thalamic sensory maps with respect to low-threshold cutaneous input. Unilateral somatosensory cortex lesions were performed on the same day as the removal of hindlimb input by unilateral nucleus gracilis lesions. Somatosensory cortex lesions blocked all thalamic reorganization in this paradigm. However, somatosensory cortex lesions alone had no effect on sensory maps in the thalamus. Remarkably, if reorganization was allowed to occur at the thalamic level following the removal of hindlimb input, subsequent somatosensory cortex lesions 1-week later did not disrupt plasticity. Thus, the importance of the cortex in the induction of thalamic plasticity suggests, for the first time, that higher levels of the nervous system play a crucial role, by permitting reorganization, at lower levels of the brain.
Introduction

The thalamus and cerebral cortex represent two principal levels of nervous system organization. A major step in understanding how the nervous system orchestrates its reorganization in response to neural trauma can be achieved by elucidating the interplay between cortex and thalamus.

Anatomists have long recognized the existence of descending cortical feedback to the thalamus (Auer 1956; Jones and Powell 1968). This intriguing projection has been the focus of much speculation (Edelman 1987; Koch 1987; Steriade et al. 1993; Sherman and Guillery 1996). Research on the role of the cortex in thalamic function has suggested varied effects such as excitation (Anderson et al. 1972; Albe-Fessard et al. 1983; Yuan et al. 1985), inhibition (Heránandez-Peón and Hagbarth 1955; Shimazu et al. 1965; Sessle and Dubner 1971), receptive field size maintenance (Jacquin et al. 1990), oscillatory modulation (Timofeev and Steriade 1996), increasing signal to noise ratio (McClurkin et al. 1994) or sharpening of receptive fields (Zhang et al. 1997). However, no published studies to date have examined the potential role of the cortex in thalamic plasticity.

One view of plasticity in the nervous system holds that sensory map reorganization at higher levels of the nervous system is a consequence of similar changes at lower levels of the neuraxis (Edeline and Weinberger 1991a; Edeline and Weinberger 1991b; Pons et al. 1991; Florence and Kaas 1995; Steriade and Timofeev 1997). Others maintain that cortical plasticity is an autochthonous event, independent of subcortical reorganization (Armstrong-
One other view of the relationship between sensory maps at multiple levels of the nervous system is the "reentrant connectivity" model (Edelman 1987; Finkel and Edelman 1989). This framework proposes that cortical feedback to multiple levels of the nervous system (Coleman et al. 1997) couples sensory map reorganization across brain structures. Thus, the reentrant model postulates that plasticity in any given sensory map of the neuraxis reflects contributions from sensory maps at multiple levels of the nervous system. The coordination of sensory maps along the neuraxis is important, since there are differences in overall somatotopic map structure across brain structures (Xu and Wall 1996). A denervated body representation may have different centrally represented body regions as neighbours, such that the adjacent map that expands into the deafferented territory may be different from the spinal cord, dorsal column nuclei and cerebral cortex.

Is an intact somatosensory cortex necessary for VPL thalamic plasticity? Previously a system (Chapter 2) for the investigation of thalamic plasticity was described that was inspired by Wall and Egger’s (1971) early research. Hindlimb input was removed by nucleus gracilis lesions in adult rats and a volumetric increase in the thalamic shoulder sensory map at 1-week post-lesion was observed. Detailed investigation of the shape of the sensory maps revealed that all of this reorganization occurred exclusively at the rostral pole of the thalamic tactile relay, the VPL. This rostral region of the VPL was referred to as a "focal zone".

In this study, topological and volumetric measures of the shoulder sensory map under normal and plastic conditions were examined in the context of somatosensory cortex lesions of the forelimb representation. The forelimb representation was chosen for aspiration since it is this body region that undergoes increased representation in the thalamus following nucleus gracilis lesions (Parker et al., 1998). A separate group involving cortical aspiration of the hindlimb zone only was not possible, since the cerebral vasculature in this area traverses laterally to feed the forelimb representation as well.

The role of the cortex in both the induction and expression of thalamic plasticity was examined. This dissociation of roles by isolating induction and expression comes from research with long-term potentiation (Brown et al., 1988; Cormier et al., 1993) and behavior (Helmstetter, 1992). In this system, if the cortex is necessary for the initiation of reorganization of somatotopy in the VPL, this was referred to as induction. If the cortex is necessary to maintain plasticity of somatotopy in the VPL, this was referred to as expression. A central control condition to either of these experiments was determining the effects, if any, of cortical forelimb sensory map aspiration on sensory maps in the VPL in the absence of any other lesion.

**Methods**

Unless otherwise specified, all procedures are the same as those stated in Chapter 2.
**Subjects and Groups:** 30 male Wistar rats with weights ranging from 250 to 350 grams were used. Five treatment groups of adult rats were used: sham nucleus gracilis and sham cortical lesions (Sham; N=6); nucleus gracilis lesions (Gr; N=7); somatosensory cortex lesions (CTX; N=6); nucleus gracilis and immediate somatosensory cortex lesions (Gr&CTX; N=8) and nucleus gracilis lesions followed by somatosensory cortex lesions 1-week later (Expression; N=6). The Expression group tests whether prior reorganization at the thalamic level requires an intact cortex to be maintained.

**Nucleus Gracilis and Somatosensory Cortex Lesions:** Rats were anesthetized with Ketamine hydrochloride xylazine (i.p.). The rat's head was tilted forward in the stereotaxic frame and the obex was exposed surgically. Under a dissecting microscope, lesions were made of the gracile nucleus with jeweler's forceps. The nucleus was completely macerated with jeweler's forceps down its length. Cortical lesions involved only a small portion of the somatosensory map (approximately 5 mm\(^2\)), following the boundaries of forelimb responsive cortex. Such lesions were achieved by aspiration of the forelimb cortical sensory map guided by crude electrode mapping (e.g. recordings were made at intervals of 400 \(\mu\)m). A simple figurine of the cortex indicating areas responsive to contralateral forelimb stimulation with brush stimuli is shown in Figure 4.1a. Aspirative lesions were implemented with a Pasteur pipette mounted on a stereotaxic arm once the cortex had been mapped. Lesions followed the boundaries of the forelimb cortical area using vasculature landmarks for the mediolateral and anteroposterior extent of the representation. It is unlikely that these lesions included SII cortex, which is located on the lateral, rather than dorsal, aspect of the rat brain (Chapin and Chia-Sheng 1990). All lesions were on the dorsal aspect of the brain. The
depth of lesions was visually guided, so as to avoid damage to the underlaying hippocampus. All animals received the ‘sham’ version of the surgical protocol if they were not to be lesioned. For example, the Gr group did not have cortical lesions per se, but did have a unilateral craniotomy involving the removal of dura on the same day as the nucleus gracilis lesion. Also, rats in the CTX group, while their nucleus gracilis was intact, did have their obex surgically exposed. Sham animals had both a craniotomy and surgical exposure of the obex and removal of the dura at both sites.

**Lesion History:** The Expression group did not receive cortical lesions until 1-week post-lesion of nucleus gracilis. This allowed plasticity induced by gracile lesions to occur before CTX treatment. Therefore, the Expression group was mapped 2 weeks post-gracile lesion. All other groups were mapped at 1 week post-gracile lesion. It is known from a previous study (Chapter 2) that volumetric increases in the shoulder representation are complete by 1-week post-lesion and have the same magnitude when examined 1-month later. The placement of these lesions is shown in a simple schematic in Figure 4.1b for the CTX, Gr and Gr&CTX groups.

**Histology:** Animals were perfused transcardially with saline followed by 4% paraformaldehyde. Brains were removed, placed in a 30% sucrose solution of 4% paraformaldehyde. The brains of half the rats were sectioned in the horizontal plane with a slice thickness of 0.1mm. This plane of sectioning allowed us to discern the entire pattern of electrode tracks. Dorsal column nuclei were sectioned coronally with a slice thickness of 40μm. In the other half of the rats brains were sectioned coronally at 40μm to assess cortical
lesions, to ensure that damage did not extend below the depth of the cortex. All tissue was Nissl-stained.

**Results**

**Volumetric Analysis**

In essence there are four dependent measures, each a measurement of the volume corresponding to: the shoulder sensory map; the forepaw sensory map; the shoulder-forepaw sensory map overlap and the hindlimb sensory map. An omnibus comparison among all groups for all body regions in a multivariate statistical model revealed a significant main effect of group (Pillias (3.67, 112) = 1.38; p < 0.001) with a Power of 1.0 and an overall effect size of 0.344. Univariate ANOVA analyses are presented for each sensory map in turn. The Power of each ANOVA reported is above 0.95, with the exception of the forepaw analysis, which had a Power of 0.31.

A representative 3D reconstruction of the shoulder sensory map in the thalamus is presented (Figure 4.2) for each of three treatment conditions. Each voxel element of the display represents a recording site that was responsive to shoulder stimulation. Sensory maps are viewed from a caudal perspective. Visual inspection suggests that the volumetric expansion in the shoulder sensory map precipitated by nucleus gracilis lesions (Gr) relative to controls (Sham) is blocked by cortical lesions (Gr&CTX). The Gr&CTX group sensory map actually appears smaller than the Sham group sensory map, but this trend was not supported in the quantitative analysis below.

Volumetric analysis of the shoulder sensory map (Figure 4.3) supported many of the trends observable in the three dimensional
reconstructions (Figure 4.2). Simultaneous lesions of the cortex and nucleus gracilis (Gr&CTX group) blocked thalamic plasticity of the shoulder sensory map normally observed with nucleus gracilis lesions alone (Gr group) (Figure 4.3; 1-way ANOVA F(4, 28)=7.27, P<0.001). Relative to the Sham condition, the CTX group was not significantly different (post hoc t-test). The Gr group resulted in a robust volumetric increase of the shoulder sensory map relative to the Sham group (post hoc t-test), consistent with what has been reported previously (Chapter 2). The volume of the shoulder sensory map in the Gr&CTX group was not different from Sham controls and was significantly depressed relative to the Gr group (post hoc t-tests). Further, the volume of the shoulder sensory map in the Expression group was similar to that in the Gr group, and was significantly elevated relative to those in the Sham, CTX and Gr&CTX groups (post hoc t-tests).

In contrast, the forepaw sensory map in the thalamus did not change in volume in any of the treatment conditions (Figure 4.4). A 1-way ANOVA did not reveal any treatment effect (F(4, 28) = 1.85; p = 0.364). With the possible exception of the Gr&CTX group, the volume of this compartment appears consistent when compared across treatment groups.

The pattern of results in the volume of sensory map overlap between shoulder and forepaw (Figure 4.5) is similar to that obtained with the shoulder sensory map alone, albeit with some differences (Figure 4.3). A 1-way ANOVA revealed an overall treatment effect (F(4, 28) = 6.4, p < 0.001). The volume of overlap between the forepaw and shoulder sensory maps expanded in a robust manner following nucleus gracilis lesions (Gr) relative to Sham and CTX groups (post-hoc t-tests). However, this expansion was
blocked by cortical lesions (Gr&CTX) and was not significantly different from CTX and Sham groups (post-hoc t-tests). The Expression group was significantly elevated relative to the Gr&CTX group but not CTX or the Sham group. Further, the volume of overlap in the expression group was significantly lower than the volume of that in the Gr group (post-hoc t-tests).

Residual hindlimb input to the VPL was analyzed (Figure 4.6) and a main effect of nucleus gracilis lesions was found (1-way ANOVA, F(4, 28) = 16.1, p < 0.0001). All three groups receiving nucleus gracilis lesions (Gr, Gr&CTX and Expression) were significantly depressed in the volume of their hindlimb functional maps relative to controls (Sham and CTX; post-hoc t-tests). Roughly 50% of the hindlimb functional map volume appeared to be lost in the group that received nucleus gracilis lesions. Relative to the Gr&CTX group, the expression group was significantly elevated, but not with respect to the Gr group (post-hoc t-tests).

Topology of Plasticity

Finally, the topology of the volumetric change in the thalamic shoulder sensory map was examined by sectioning these functional maps in the coronal plane (Figure 4.7; 2-way ANOVA; effect of Group F(4, 28) = 6.75, p < 0.002; and Slice by Group interaction F(48, 248.57) = 35.51, p < 0.001). Power was above 0.95 and the effect size was 0.50. As expected, all the expansion was localized to a focal zone located towards the rostral pole of the sensory map. Groups that had a cortical lesion showed the same cross-sectional profile as Sham controls at all anterior-posterior levels of the VPL. In contrast, the Expression group had the same profile as the Gr group, as it was significantly elevated relative to all other groups in the focal zone (post-hoc t-tests).
Figure 4.1 Simple figurines depicting relative lesion placements and combinations of treatments. Nucleus cuneatus lesions and cortical lesions are shown in different combinations.
Lesion Placements with Reference to the Intact Rat Brain

CTX
Gr
Gr&CTX
Figure 4.2 Representative 3D reconstructions of the spatial distribution of thalamic (VPL) recording sites that responded to shoulder stimulation. Shown are the sensory maps for sham operated animals, nucleus gracilis lesioned animals (Gr) and nucleus gracilis and somatosensory cortex lesioned animals (Gr & CTX). The volumetric expansion that occurs in the shoulder sensory map as a result of nucleus gracilis lesions is blocked by lesions of the forelimb somatosensory area.
Sample 3D Reconstructions of the Shoulder Sensory Map in the Thalamus
Figure 4.3 Volumes of the shoulder sensory map for each group of animals in the study: Shams, somatosensory cortex lesions alone (CTX), nucleus gracilis lesions alone (Gr), nucleus gracilis and somatosensory cortex lesions (Gr&CTX) and nucleus gracilis lesions followed 1-week later by somatosensory cortex lesions (Expression). Relative to Sham, CTX and Gr&CTX, both Gr and Expression are significantly elevated. Bars are standard error of the mean.
Cortical Effects on the Volume of the Shoulder Representation in the Thalamus

![Bar chart showing the shoulder volume in mm^3 for different conditions: Shams, CTX, Gr, Gr&CTX, and Expression. The bar for Gr is significantly higher than the others, marked with an asterisk (*) indicating statistical significance.](image-url)
Figure 4.4  Volumes of the forepaw sensory map for each group of animals in the study. No significant volumetric change in the sensory map was detected.
Cortical Effects on the Volume of the Forepaw Sensory Map in the Thalamus

![Graph showing the volume of the forepaw sensory map in the thalamus for different conditions: Shams, CTX, Gr, Gr&CTX, and Expression. The graph includes error bars for each condition.](image-url)
Figure 4.5 Volumes of the topographic overlap between forepaw and shoulder. The pattern is similar to what is seen in Figure 4.3 for the shoulder volumes. However, the elevation in the expression group is not statistically significant. The Gr&CTX group indicates a depression, but it is not significant.
Figure 4.6 Volumes of the hindlimb sensory map. The representation of this body region drops dramatically following nucleus cuneatus lesions, but is not completely deafferented. Input from possible sources such as the spinothalamic tract or anterolateral system probably contribute to the residual volumes.
Figure 4.7 A cross-sectional area profile of the shoulder sensory map as a function of the anterior-posterior distance. Coronal slices were taken. Each slice was identified by its distance from the midpoint of the ventrobasal complex (VB) along the anterior-posterior plane. In both Gr and Expression groups there was a significant increase at the rostral pole of the VPL relative to controls (CTX, Sham) corresponding to a previously described focal zone. Bars are standard error of the mean.
Cortical Effects on the Topology of the Shoulder Sensory Map in the Thalamus

Distance from Midpoint of VB (mm)

Coronal Area (mm²)

Focal Zone

Sham
CTX
Gr
Gr&CTX
Expression

Anterior
0.8 0.6 0.4 0.2 0 0.2 0.4 0.6 0.8 1
Posterior
-1 -1.2 -1.4

* Significant difference
Figure 4.8 Aspirative lesions of the cerebral cortex in coronal view that has been Nissl-stained. The zone of damage was guided by electrode mapping of tissue responsive to contralateral forelimb stimulation with brush stimuli. The lateral aspect of the lesion stopped at the rhinal fissure. The lesion spared the underlaying hippocampus and thalamus.
Figure 4.9 Reconstructions of thalamic electrode penetrations for a Sham and Gr animal are presented. Tissue was sectioned in the horizontal plane and Nissl-stained. An electrolytic lesion ("X") at the top left of each schematic acted as a reference point along the dorsoventral axis. The focal zone of plasticity of the VPL is indicated (ellipse). Shown are all electrode tracks that encountered sites responsive to shoulder stimulation are indicated by filled circles. Abbreviations are from Paxinos and Watson (1997).
Histology

Figure 4.8 depicts the representative depth of damage to the cortex in a coronal view following aspirative lesions. As can be seen, damage was confined to the cortex, both the VPL and the overlaying hippocampus were undamaged. In a coronal view, some electrode tracks are discernible from mapping the VPL. The site of damage is well removed from the SII cortical representations, which is found on the lateral aspect of the rat brain (Chapin and Chia-Sheng 1990). Figure 4.9 presents a reconstruction of electrode recording sites in the VPL based on histology sectioned horizontally. The view is in two dimensions, with control (Sham) and lesioned (Gr group) animals. The focal zone of plasticity is indicated in the lesioned animal and the corresponding region for such an area in the control animal.

Discussion

The data presented here provide strong support for the hitherto unknown role of corticothalamic input in thalamic plasticity. This dynamic was best illustrated by the thalamic shoulder sensory map. My previous study established that the shoulder sensory map demonstrated a clear volumetric expansion following nucleus gracilis lesions that occurred within a focal zone located towards the rostral pole of the VPL (Chapter 2). In this study, somatosensory cortex lesions blocked this volumetric increase in the thalamic shoulder sensory map when performed on the same day as nucleus gracilis lesions. Surprisingly, if plasticity was allowed to occur at the thalamic level, somatosensory cortex lesions performed 1-week post nucleus gracilis lesions had no apparent effect, since the shoulder sensory map volume remained elevated. These data indicate that an intact cortex is necessary for the
induction of thalamic plasticity. However, once plasticity had occurred at the thalamic level, subsequent damage to the somatosensory cortex did not disrupt somatotopic reorganization. This latter finding indicates that the cortex is not necessary for the expression of thalamic plasticity.

The topology of the volumetric change of the shoulder sensory map also revealed a great deal of specificity regarding the effect of somatosensory lesions. Somatosensory cortex lesions alone had no effect on the volume or shape of the shoulder sensory map. When somatosensory cortex lesions were performed the same day as gracile lesions, these animals had identical topological profiles as controls. Thus, somatosensory cortex lesions blocked the volumetric increase of the shoulder representation in the focal zone of the VPL, but did not affect any other segment of the shoulder sensory map.

Interestingly, somatosensory cortex lesions alone had no effect on volume or topology of any of the thalamic sensory maps investigated in this report for the body regions investigated. Considerable controversy exists in the literature regarding what the effect is of cortical inactivation has on thalamic neurons (Sherman and Guillery 1996). Investigators have reported facilitation of evoked activity following cortical inactivation while others have reported the opposite (Kalil and Chase 1970; Baker and Malpelli 1977; Albe-Fessard et al. 1983; Yuan et al. 1985; McClurkin et al. 1994; Sherman and Guillery 1996). These studies involved acute preparations, in contrast to the chronic time scales permitted by the paradigm employed here. There are no detailed investigations of receptive field size in the VPL following cortical inactivation, but one group did indicate that it failed to see any change (Yuan
et al. 1985). This author cannot comment directly on receptive field structure, however, the absence of any change in any of the sensory maps with respect to volume and shape following cortical lesions alone (CTX group) is consistent with unaltered receptive fields across neurons.

The blockade of thalamic plasticity is selective. One might argue that the removal of the cortex has allowed a depression of thalamic neurons to cutaneous stimuli (Albe-Fessard et al. 1983), manifesting as a block in plasticity. The data collected in this study is derived from the number of thalamic recording sites that discharge in response to brush stimuli applied to a specific body region. A general shift in thalamic excitability would alter the size of these sensory maps. Yet, comparable sensory map volumes between Sham and CTX groups suggests that the general excitability of thalamic neurons is unchanged by cortical lesions. Further, in contrast to nucleus gracilis and cortical lesions performed the same day (Gr&CTX group), cortical lesions performed 1-week after nucleus gracilis lesions (Expression group) did not block thalamic plasticity. Finally, the blockade of thalamic plasticity in the Gr&CTX group had identical cross-sectional area profile to controls, including the focal zone. Thus, several controls (CTX and Expression groups; examination of the focal zone) suggest that the blockade of thalamic plasticity by cortical lesions is not mediated through a general depression of thalamic neurons to cutaneous stimuli.
Cortical Control of Thalamic Plasticity

**Corticothalamic Interactions: Is the Cortex the Driver?**

The reciprocal relationship between the cortex and thalamus has led to two main factions within the neuroscience community. The first group takes the position that subcortical changes are a critical determinant of plasticity at the cortical level. This view is referred to as the “cascade hypothesis”. Alternatively, many argue that plastic events at the cortical level occur irrespective of subcortical events. This view is referred to as the “cortical impunity” hypothesis.

In the cascade hypothesis, small changes induced at the spinal cord level are hypothesized to amplify as one ascends the nervous system (Florence and Kaas 1995). In the spinal cord nerve transection has been reported to induce sprouting and receptive field expansion (Basbaum and Wall 1976; Devor and Wall 1978; Devor and Wall 1981; Molander et al. 1988; Woolf et al. 1992). These newly changed spinal cord neurons, each with their own axonal arbor, interact with a vast number of neurons at the next relay in the nervous system. Thus, plasticity involving a small group of neurons is not simply translated to higher levels of the nervous system following a one to one mapping rule. Changes initiated at the spinal cord cascade up the nervous system, by recruiting proportionally more neurons at the next successive relay along the neuraxis through the bifurcation of their axonal arbors. Some investigators do not deny the possibility there may be some plastic changes independently elaborated at the cortical level (Edeline and Weinberger 1991b), but assign a pivotal role to subcortical events (Edeline and Weinberger 1991a; Florence and Kaas 1995; Steriade and Timofeev 1997).
In the cortical impunity hypothesis, supporters have published a number of examples of cortical plasticity that they argue are beyond the scope of a thalamic template (Armstrong-James and Callahan 1991; Armstrong-James et al. 1991; Pons et al. 1991; Darian-Smith and Gilbert 1995; Das and Gilbert 1995). This position relies on three primary lines of argument: (1) thalamocortical arbor dimensions; (2) cortical lesions and (3) response latency data.

According to the cascade hypothesis, if the thalamus is to act as a template for cortical change, then such changes are restricted to the maximal size of a thalamocortical arbor, where the largest reported estimate is 600μm (Rausel and Jones 1995). Yet many investigators have detected map border shifts or changes between pairs of neurons separated by distances as great as 15 mm (Pons et al. 1991; Darian-Smith and Gilbert 1995; Das and Gilbert 1995). Thus, a thalamic template whose influence is limited by the size of its thalamocortical arbors does not appear to possess the means to explain magnitude of spatial changes at the cortical level.

Some investigators have argued that the plastic component of vibrissae receptive field structure in the cortex is purely dependent on intracortical mechanisms (Armstrong-James and Callahan 1991; Armstrong-James et al. 1991). Each cortical barrel responds to deflection of a central whisker (center receptive field or CRF) and weakly to surrounding whiskers. These surrounding whiskers define the surrounding receptive field (SRF) (Armstrong-James and Fox 1987). SRF are believed to form the domain of receptive field plasticity for a cortical barrel (Armstrong-James 1975; Nussbaumer and Wall 1985). Electrolytic lesions of the D2 cortical barrel in
lamina IV removed this whisker from the SRF of the adjacent digit D1 (Armstrong-James et al. 1991). This result suggested that SRFs were independent of the thalamus, and generated through intracortical mechanisms.

Finally, the difference in response latency between thalamic barreloids and cortical barrels following stimulation of vibrissae in the SRF was several times longer than that observed following stimulation of the central whisker (Armstrong-James and Callahan 1991). It was suggested that this “extra time” indicated by the long cortical SRF response latency was used for barrel-to-barrel communication via intracortical routes. Thus, this laboratory suggests that in the trigeminal system the sensitivity of SRFs to discrete cortical lesions and the long latencies of these receptive fields from thalamus to cortex indicate dependence on intracortical pathways.

_Prima facie_, these data appear to support a view of plasticity in the nervous system as a phenomenon that falls under the providence of cortical function. This would augment the widespread conviction in cortical impunity. A model is presented in the next section that incorporates the above pieces of evidence against subcortical contributions to cortical plasticity in a revised reentrant model.

**Edelman’s Reentrant Model: A Modification**

A revision of the reentrant model is presented with two primary changes: (1) the thalamic template is not constrained by estimates of thalamocortical arbor dimensions and (2) corticothalamic loop recruitment via Hebb’s learning rule (1949). The reentrant model (Edelman 1987) was
originally constrained by the edict that 500 - 700µm was the maximal plastic change the adult nervous system could display (Merzenich et al. 1984). This concept is revisited first.

Cutaneous input from a body region, other than orofacial, eventually reaches the VPL and is then relayed to the cortex where it primarily terminates in the granular layer (lamina IV) of the cortex (Jones and Powell 1969; Herkenham 1980; Landry and Deschenes 1981), an area where thalamocortical interactions appear to be strongest (Johnson and Alloway 1996). Thalamocortical afferents possess arbor dimensions with an upper limit of 500µm (Landry and Deschenes 1981) or 600µm (Rausel and Jones 1995). Corticothalamic input to the VPL originates from the infragranular layers, namely, lamina VI (Jacobson and Trojanowski 1975), and descends to the VPL with some collaterals relayed through the reticular nucleus (Jones 1985).

Classically, cortical plasticity was thought to be limited by the size of thalamic arbors that overlap the border between two represented body regions (Merzenich et al. 1983). Input ascending through this input passes through all collaterals, providing stimulation to neurons resident to both sensory maps. In this model, most of the input from the thalamocortical afferent is subthreshold. Collaterals providing subthreshold input are referred to as suppressed or “masked” synapses (Merzenich et al. 1983; Merzenich et al. 1984). Few collaterals are successful in evoking discharge of their target neuron. Neurons that are successfully depolarized are considered members of the same sensory map.
A loss of input from a body region changes the balance of inhibitory and excitatory interactions between neurons in a somatotopic map. The removal of input and the concomitant changes in network interactions may enable previously subthreshold or silent synapses evoke discharge ("unmasking") of the target neuron (Merzenich et al. 1983). A neuron acquires membership in an adjacent sensory representation by responding to the same stimuli. This occurs if the neuron in the denervated zone shares input from the same thalamocortical neuron projecting to the adjacent sensory representation (Merzenich et al. 1983). Cells recruited into a sensory representation at distances beyond a thalamocortical arbor are thought to do so by non-thalamic mechanisms (Pons et al. 1991; Darian-Smith and Gilbert 1995; Das and Gilbert 1995). In this case non-thalamic mechanisms could be purely cortical in origin (Darian-Smith and Gilbert 1995; Das and Gilbert 1995) or events initiated at the spinal cord that cascade upward (Pons et al. 1991). Either way, the thalamus by itself does not appear to have the capacity to support massive reorganization at the cortical level.

The data in this study highlight the importance of corticofugal input in thalamic plasticity. How large is the corticothalamic arbor? Importantly, this arbor has the opportunity to recruit thalamocortical neurons that were not previously activated (Figure 4.10). Corticothalamic activity could, combined with subthreshold input to VB neurons, enable previously ineffectual cuneothalamic input to successfully depolarize thalamic neurons (spatial summation). Any recruitment of thalamocortical neurons involves an entirely new loop, in which additional corticothalamic input is secured. While we have always considered the potential of thalamocortical arbors to recruit cortical neurons, we have not seriously considered the same process
mirrored at the thalamic level: recruitment of new thalamocortical neurons and their associated arbors in the cortex by the activity of descending corticothalamic input. This reasoning could easily be extended to other levels, such as descending recruitment of cortically projecting neurons at the level of the dorsal column nuclei (corticobulbar) or dorsal horn (corticospinal).

This scheme is discussed in the context of continuous somatosensory input. It takes at least 6 msec for corticothalamic input to reach thalamic neurons, following the initial activity of thalamocortical neurons in the vibrissae system (Armstrong-James and Callahan 1991). In the context of continuous vibrissa stimulation (>6 msec), however, the arrival of corticothalamic input would arrive in the presence of subthreshold input to the thalamus from sustained vibrissa stimulation (or any body region for that matter). However, corticothalamic input could not act as a factor to recruit new thalamocortical neurons if the tactile stimulus was less than 6 msec in duration—an event that seems quite unlikely. Since stimulus events contacting the body surface are likely to be longer than 6 msec duration, this suggests that most tactile stimulation reaching the thalamus does so concurrently with corticothalamic stimulation.

The lesion study (Armstrong-James et al. 1991) that reported cortical damage to a barrel appears to remove it from the SRF of an adjacent cortical barrel no longer implies that a disruption of intracortical mechanisms alone was responsible. In the model described here, such a lesion may have disrupted corticofugal activation which is an important link in thalamocortical loop recruitment. In the same set of studies, the “extra time”
associated with cortical SRFs may reflect intracortical activation, or corticothalamic loop recruitment.

In summary, the reentrant model has been revised by proposing that new corticothalamic loops can be recruited through the arbors of corticothalamic input. Each recruited loop has two new axonal arbors, anchored cortically and thalamically. Thus, one cannot preclude thalamic involvement because cortical changes extend beyond the arbor of a single thalamocortical neuron. Thalamocortical loop recruitment does raise the spectre of spreading activation, but this problem is also a feature of any cortically based recruitment model. Spreading activation has remained a perennial problem in neuroscience since the first description of the cell assembly (Hebb 1949).

*Corticothalamic Input: A Mechanism of Cortically Dependent Thalamic Plasticity*

This author speculates that corticothalamic augmentation of subthreshold cuneothalamic input from shoulder stimulation to VPL neurons (Figure 4.10) is the basis for cortical dependence during the induction period of thalamic plasticity.

In this speculation, the reorganization observed in the focal zone in the Gr group is occasioned by the unmasking of subthreshold cutaneous input by corticothalamic input (Figure 4.11). While the corticothalamic input occurs at a longer latency, this whole pathway is activated continuously (e.g. for periods greater than 6 msec). Once subthreshold shoulder input to VPL neurons has
Figure 4.10 Thalamocortical loop recruitment. Somatotopic map changes in the cerebral cortex following deafferentation of the hindlimb. Toe and hindpaw cortical territories are denervated. A dorsal view of cortical maps with thalamocortical arbors (TC) and corticothalamic arbors (CT) is indicated to the right of the figure. Neurons (bold dots) in the hindpaw and toe somatotopic maps are recruited into the tail sensory map. The toe and hindpaw neurons are recruited by thalamic mechanisms despite the fact they fall well outside the distance of a thalamocortical arbor (TC1). Neurons beyond the 600μm distance are recruited indirectly through a second thalamocortical loop (TC2). The second thalamocortical loop is recruited through the activation of CT projection to the VPL, by TC1. The arbor of CT in the VPL is able to unmask and activate TC2 to previously subthreshold cutaneous input to the VPL. The precipitating event to this shift in excitation and recruitment is the loss of afferent input into hindpaw and toe cortical territories. In this example, only one CT loop is recruited to reach a cortical distance of 1000μm. Neurons located at further cortical distances can acquire membership in the tail somatotopic map with the recruitment of additional corticothalamic loops.
**Figure 4.11** Time course of thalamic reorganization and hypothetical synaptic events in the focal zone. The speculation is that the corticothalamic loop recruitment enabling cortical reorganization (Figure 4.10) also mediates reorganization in the thalamic focal zone. At 1-day post nucleus gracilis lesion no somatotopic map reorganization has been observed in the thalamic focal zone, and by 1-week post-lesion it appears complete (Chapter 2). Strengthening of subthreshold cutaneous input in the VPL (unmasking phase) occurs through the action of corticothalamic input via the Hebb learning rule (spatial summation). This period is cortically dependent, since corticothalamic input is activated at the cortical level by thalamocortical input. Thus, cutaneous stimuli evoke responses in the thalamic focal zone at longer latencies than is the case in a non-lesioned state. Cortical dependence ends when cutaneous input is strong enough to activate VPL neurons alone. Because corticothalamic input is no longer necessary, evoked responses in the VPL now occur at shorter (normal) latencies. Since cutaneously evoked responses now occur at shorter latencies, recruited corticothalamic loops and their associated corticofugal input to the focal zone is no longer involved. It is for this latter reason that the recruited corticothalamic loop number is shown to decrease. At 1-week post-lesion, cutaneous responses occur at normal latencies, no longer in the context of corticofugal input, which corresponds to the Expression group results of cortically independent thalamic plasticity.
Time Course of Thalamic Somatotopic Map
Reorganization in the Focal Zone and Hypothetical
Cellular Events

Cortex dependent (long latencies)

1 Day (no reorganization)
X Days (reorganization begins)
7 Days (reorganization complete)

Time Post-Gracile Lesion

N Corticothalamic Loops
Synaptic efficacy
been sufficiently strengthened by the activity of corticothalamic input (this occurs gradually during the induction period), these thalamic neurons can now discharge in response to shoulder stimulation at shorter latencies (same latencies of focal zone neurons as unoperated animals). This is possible because the activity of the corticothalamic pathway is no longer necessary (e.g. the Expression group results). This would explain the involvement of the cortex in the induction of plasticity in the thalamus, while the cortex is not needed for the expression of thalamic plasticity.

At the single unit level, one can make predictions about the response latencies of neurons in the focal zone of nucleus gracilis lesioned animals. A summary of the time course and possible synaptic predictions is given in Figure 4.11 (this is speculation upon speculation). Response latencies in the focal zone to shoulder stimulation should be longer during the induction period (less than 1 week post-lesion) as compared with latencies collected from the focal zone in animals 1-week post-lesion or control animals. As synaptic strength increases among subthreshold shoulder input to the thalamus, a point is eventually reached where VPL neurons begin to discharge (unmasking phase in Figure 4.11).

This prediction is complicated by the fact that this author does not have detailed knowledge defining the time window for the induction period. At 12 hours post-lesion, no reorganization is observed in the focal zone (Parker et al. 1998). So at this acute time interval, new corticothalamic loops have not yet been recruited, therefore, the prediction of a longer latency does not hold at this time period. However, as a harbinger of corticothalamic loop recruitment, one may expect to see increased synaptic efficacy of cutaneous
input, on average, for neurons in the focal zone of lesioned animals at an acute time interval compared to controls. Increased synaptic efficacy could be measured by examining the probability of discharge throughout the focal zone following stimulation of the body surface.

**Future Directions**

Some argue that thalamic and cortical plasticity are independent of each other (Darian-Smith and Gilbert 1995; Wang et al. 1995). However, in line with the concept of reentrant connectivity (Edelman 1987; Finkel and Edelman 1989), this author suspects that the cortex and thalamus are mutually dependent upon each other as they are sculpted by neural trauma. The current results support this hypothesis.

The cascade hypothesis (Pons et al. 1991; Florence and Kaas 1995) implies that reorganization is a wave of change that slowly propagates up to the cortex, reaching each level of nervous system organization in a stepwise manner. On the other hand, the reentrant model of brain function suggests that changes in somatotopic maps in any given area of the nervous system reflect the simultaneous engagement of multiple levels of the neuraxis (Edelman 1987). This data is consistent with the reentrant model.

Previously this author described a “hot spot” model in which focal zones of plasticity exist at multiple levels of the somatosensory pathway (Chapter 2). I suggested that the equivalent to the identified focal zone in the thalamus was the granular layer in the cortex. At that time this author refrained from speculating on the relationship between cortical and thalamic hot spots. The data presented here suggests that either the cortical hot spot
controls the thalamic hot spot (consistent with cortical impunity) or that they are interdependent (consistent with the reentrant model).

It has been suggested that the necessity of an intact cortex for plasticity, while essential for the thalamus, is unlikely to hold for the dorsal column nuclei or spinal cord (Dr. Rasmussen, personal communication 1997). This position is based on the existence of topographic corticofugal input. While topographic corticofugal input exists for the thalamus, this topography deteriorates markedly at lower levels of the neuraxis (Coleman et al. 1997). Thus, it would be interesting to see if lower levels of the neuraxis, which receive corticofugal input that is not topographically organized, display the same dependence on the cortex for plasticity.

There are several limitations to this study. As with previous research by this author, all sensory maps were defined by low-threshold mechanical input. Whether the findings reported herein are relevant for temperature sensation or high-threshold input is yet to be determined. Lesions of the cortex have also been crude. It is not known for certain, for example, whether part of SII cortex has been included. Further, it is also not known whether aspiration of the cortical supragranular layer would be sufficient to block thalamic plasticity. It may also be interesting to ascertain whether thalamic plasticity induced by nerve transection of the hindlimb, could be blocked by cortical lesions. Pilot experiments were abandoned on this latter point, as 2 of 3 rats that received both nerve transection of hindlimb and cortical lesions, demonstrated severe self mutilation of the denervated limb.
Future research will determine the merits of the reentrant and cortical impurity models respectively. While cortical lesions block thalamic plasticity, it may be that blockade of thalamic plasticity immediately following neural trauma would similarly impair cortical plasticity. Accordingly, I predict that lesions of the focal zone of the VPL would block cortical plasticity. The control condition would involve lesions placed outside the focal zone in the VPL to ensure that cortical consequences do not reflect a simple disruption in the cutaneous relay properties of the thalamus.

Conclusion

It has been suggested that plasticity in the thalamus, a structure that directs sensory traffic to the cortex, may serve as a template for changes at the cortical level (Edeline and Weinberger 1991a; Edeline and Weinberger 1991b; Pons et al. 1991; Steriade and Timofeev 1997). However, data demonstrating the role of the cortex in the induction of thalamic plasticity in this study suggests the opposite view. Interestingly, as time passes following injury thalamic plasticity appears to become independent of cortical influence. In sum, reorganization in the thalamus may not simply be a reflection of a cascade of changes at lower levels of the nervous system such as the spinal cord (Florence and Kaas 1995), but higher levels of central nervous system organization as well.
Chapter V

Summary & Conclusions
Coda

It has been a tendency of research in this field to restrict its Magellanic voyages to a single level of the nervous system at a time. Within this tradition the thalamus is ideal for study, since it represents a nexus of sensory input that ascends to the cortical level. Is the thalamus an inert relay of altered sensory input or is it dynamically modified? Its unique position in the direction of sensory traffic to the cortex make this issue a significant one.

Chapter 2 unequivocally established that the thalamus (VPL) is indeed capable of somatotopic reorganization in the adult nervous system. Surprisingly, the plasticity was confined to a focal zone within the VPL. This may explain the variability in the success of previous investigators that have studied this structure to date. If one expects to detect receptive field plasticity, one must record from this focal zone. While plasticity in the VPL may be displayed exclusively in the focal zone, this cannot be interpreted as its origin. It is unclear whether the theatre of activity in the thalamus is a simple retelling of events that have been scripted by other levels of the nervous system. While descending cortical input appears to be important, it may be that such input affects lower level structures such as the dorsal horn, which is later reflected in the thalamus.

Importantly, somatotopic reorganization in the thalamus assumed a form that has not been previously described before in any brain structure: increased somatotopic overlap between two sensory representations. Chapters 2 and 3 discussed the hypothesis that such reorganization reflected the removal of inhibition. Since hindlimb and forelimb appear to
reciprocally inhibit one another, the removal of one would naturally precipitate receptive field expansion of the remaining neurons receiving intact sensory input. The advancement of the forelimb representation into the denervated territory of the hindlimb is unlikely, since there appeared to be little evidence of overlap in dendritic arbors between these two somatotopic domains (discussed in Chapter 3). Recognition of this type of plasticity gives us new predictions to test at the cortical level, in the context of hindlimb deafferentation, in assessing whether cortical changes mirror thalamic changes.

Chapter 2 laid the foundation for the hot spot model. This is a restatement of the same hypothesis that was part of the columnar motif detailed in the Introduction. The unit of CNS organization described in the Introduction was a column of neurons, isomorphic in receptive fields, that was not uniform in its neural plasticity (in addition to other properties drawn from research on the cortex). This author suggested that the granular layer, in particular, may be different from other lamina in the cortex with respect to neural plasticity. Considering that somatotopy is rotated in the thalamus relative to the cortex (Chapter 2), the corresponding layer now rests vertically, rather than horizontally. This configuration was revealed by 3D sensory map reconstructions. In the hot spot model, such a zone is postulated to exist at all levels of the somatosensory pathway.

The hot spot model postulated a second property: if such a zone is special, it will be manifest regardless the form of neural trauma. This was tested by nerve transection of the hindlimb, which has a number of distinguishing differences from nucleus gracilis lesions (Chapter 3). Would
plasticity be observed at all? Would reorganization be observed throughout the entire VPL, in the absence of any focal changes? If there were focal changes, would it occur in the same position relative to the forepaw centroid? Nerve transection provided strong support for the hot spot model by revealing almost identical changes in the VPL.

The utility of the hot spot model does not rest upon the conviction that it is ultimately correct. Future research may show that hot spots of plasticity may be confined to the thalamus. If that's true it naturally raises a second question, why are focal zones of plasticity confined to the thalamus? Is the thalamus special? The importance of the hot spot model is that it provides a framework in which pose new questions about CNS organization.

The Rosetta Stone metaphor predicted that content should be isomorphic across brain structures. In other words, the reorganizational events from the spinal cord to cortex should be the same. Contrary evidence against this supposition was discussed in detail, based on the evidence collected above. Reports of plasticity in the thalamus but not cortex have been looking for the wrong type of reorganization (Chapter 2). Reports of cortical plasticity in the absence of thalamic plasticity have either been using inappropriate recording practices at the thalamic level (Chapter 2) or myopic models of thalamocortical interactions (Chapter 4).

Isomorphic content across levels does imply tight integration of changes between sensory maps at each level of the neuraxis. This was tested directly. Such integration could be achieved by either the cascade or reentrant connectivity models. The cascade model implied unidirectional
communication between levels of the somatosensory pathway. Changes initiated at the spinal cord level were subsequently translated and amplified at each relay of the somatosensory system before reaching the cerebral cortex. The reentrant model postulates that plasticity at any given level of the neuraxis, spinal cord or cortex, is not an autochnous event. Rather, somatotopic reorganization reflects the activity of multiple sensory maps that via reciprocal innervation, have held congress to determine the sequelae of events observed. In this scheme, communication between brain structures is bidirectional.

The reentrant model was tested in Chapter 4 with decisive results. Higher levels of CNS organization appear to play a critical role in enabling plasticity in the thalamus. Decortication alone appeared to have no effect on thalamic sensory maps. However, there was a selective blockade of plasticity induced by nucleus gracilis lesions. More intriguing, was the pattern further suggested by the data that once thalamic reorganization had been allowed to occur, subsequent cortical lesions did not reverse such plasticity. The cortex therefore appeared to be involved in induction, but not the expression, of plasticity in the thalamus. This finding demands a fundamental paradigm shift (Kuhn 1962) in our views of plasticity in the nervous system. Brain structures are not isolated islands of change, and higher levels of CNS organization may be as important as changes in the spinal cord in shaping plasticity in the somatosensory pathway.

Additional evidence for reentrant connectivity came indirectly in the study of nerve transection (Chapter 3). If the cascade model were correct, one would have likely observed changes in the thalamus that were different in
magnitude than that observed following nucleus gracilis lesions. This was not the case. Nucleus gracilis lesions and nerve transection lead to reorganization in the thalamus that was almost identical in magnitude. As discussed (Chapter 3), time course manipulations may reveal deeper differences. Taken together with cortical aspiration experiments, this body of evidence provides strong support for importance of reentrant connectivity. If this author were to divine the future, it is only a matter of time before the reentrant model displaces the cascade hypothesis into the necropolis of repudiated models in neuroscience.

*Prima facie,* the cerebral cortex appears to act like Shakespeare’s Tempest of the thalamic domain (Chapter 4). It was suggested, however, that the Tempest in this case does not command such waters with impunity. Clear evidence of the importance of corticofugal input in thalamic plasticity provides impetus for a new range of models in which to conceptualize thalamocortical interactions. One of out of a constellation of possibilities were outlined: the recruitment of thalamocortical loops by corticofugal input. In this hypothesis, large changes in cortical maps no longer precludes thalamic mechanisms by deference to reported sizes of a single thalamocortical arbor.

In response to the obvious interests of colleagues, this author outlined mechanisms that may account for the role of the cortex in induction, but not maintenance, of thalamic plasticity (Chapter 4). But the experiments designed here were not intended to speak to such issues at that level of analysis. The experiments executed in this research program have focused on a level of analysis both uncommon and in some senses antithetical to practises in this
field. In an effort to document changes systematically at the level of an entire 3D map, considerable detail was sacrificed in recording at each location in the thalamus. In doing so, in some senses we now have a better understanding of thalamic plasticity than cortical plasticity. To this author's knowledge, no one has ever attempted a full 3D reconstruction of somatotopic plasticity at the cortical level, by sampling all layers of the cortex in constructing sensory maps.

**Future Studies**

Broadly speaking additional research can proceed on three paths: deploying 3D reconstructions to investigate plasticity in other brain structures; testing the hot spot model; and testing mechanisms of thalamocortical interactions. These are:

- **Thalamic control of cortical plasticity.** Can lesions of the focal zone specifically block cortical plasticity? This would be tested by comparing the effects of such lesions against similar lesions placed outside the focal zone in the thalamus. Lesions outside the focal zone should not impair cortical plasticity.

- **Cortical control of dorsal horn plasticity.** An audacious experiment; can cortical aspiration block forelimb plasticity in the spinal cord and dorsal column nuclei? Cortical control of thalamic plasticity was an unexpected finding, a demonstration of a similar phenomenon with a remote structure such as the spinal cord would be a decisive test of the reentrant connectivity model (or dorsal column nuclei etc.).

- **3D reconstructions of cortical somatotopic maps.** The same paradigm employed here, except at the cortical level. It is predicted that, consistent
with the hour glass model (Introduction), the granular layer is specifically modifiable 1 week post-lesion but not 1 day. Conversely, the supra- and infragranular layers are modified at 1 day post-lesion.

♦ *A hot spot in the dorsal column nuclei?* 3D reconstructions of somatotopic maps in the dorsal column nuclei need to be performed. The somatotopy of this structure and its plasticity are still poorly understood. The hot spot model would predict a focal zone of plasticity in this structure.

♦ *Nerve transection and sensory map contraction.* The nature of the sensory map contraction observed at 1 day post-lesion in gracilis lesioned animals was never adequately explained here. If such an outcome is the product of edema by the adjacent nucleus gracilis lesions, nerve transection should not lead to contraction of sensory maps at an acute time interval.

♦ *Generality of observed plasticity.* Are focal zones of plasticity present in the thalamus with manipulations of other body regions, such as digit amputation?

♦ *Detailed staining of the VPL.* Of the range of possible markers that may currently be in use to detect plasticity in the nervous system, would staining of the VPL reveal their preferential binding to the focal zone of lesioned animals? Conversely, could this region be used in which to screen new stains or antibodies for their ability to detect plasticity in the nervous system?
Criticisms

This author has two concerns that in the future will continue to be an albatross to this line of research. The first is paradigm specificity. While it is that station of any scientist to generalize beyond their immediate system, this is an academic exercise, not a testimony. This investigator has generalized greatly in this thesis and in print (Parker et al, 1998). Is it possible that a focal zone is simply an anomaly of the body regions, sensory system or species chosen as tools in this research? Even within these parameters issues remain; are the findings valid for other sensory sub-modalities such as high-threshold input?

The second trepidation is theoretical. Of what significance is increased somatotopic overlap? Tacit in the notion of plasticity is that it represents adaptation by a system to a changed circumstance. We differentiate this, in other words, from simple alterations in phenotype induced by trauma (e.g. a broken bone that fails to heal). Increased topographic overlap can only retard spatial acuity, an observation confirmed with perceptual (Stevens and Choo 1996) and electrophysiological studies (Spengler et al. 1995). What this work describes is a change induced by injury, but its benefit, if any, is an open question. In the formal sense, it may be an sophisticated change in the phenotype of an animal, but in the absence of evidence that it is adaptive, it may not be an example of ‘plasticity’.

Other minor concerns about this research:

Since Ketamine hydrochloride was used in these studies, which is known to be an NMDA antagonist, this research cannot speak to plasticity
involving glutaminergic mechanisms. Presumably, the plasticity described in this research may be non-glutaminergic in nature. However, animals were acutely exposed to anesthetic; once to be lesioned and once for the final recording session. Since thalamic reorganization takes up to 1 week complete, glutaminergic mechanisms could still play a role during this period.

One limitation of the volumetric dependent measure is that there is no measure of density. In other words, the number of shoulder responsive neurons has not been quantified during each recording site. It is possible there may be a decrease in the density of sensory maps, without a contraction of the perimeter of the sensory map in question. While it may lengthen procedures prohibitively to collect data on the density of units, this missing dimension in this research is a limitation.

Finally, there are some minor methodological points. While brush stimuli was used, it was not standardized. Further, the criterion for the detection of neuronal discharge in response to shoulder stimulation was not standardized. While the comparatively low variance in the data suggests that these factors were not significant contributors of noise in the data set, ideally one would like to control these parameters with greater precision in the future. Further, the experiments were not conducted under 'blind' conditions. This criticism is mitigated by the fact the experimenter never knows during recording where the focal zone of change is in the thalamus. Such a determination is made during off-line data analysis. Nonetheless, the lack of blind conditions in these studies should be articulated. The age of the rats used in this research can be considered fully adult, based on their ages.
mentioned in the Methods (Chapter 2). The CNS of immature animals is more plastic than that found in adult animals (Kalaska and Pomeranz, 1979). If clearly juvenile animals were used in this research, the findings would be more relevant to developmental plasticity than conditions found in the brains of fully mature animals.

**Conclusion**

This research has discovered that the thalamus (VPL) is not homogeneous in its capacity for neural plasticity, a finding that has fundamental implications for recording practices in the past. The focal zone identified appears to be independent of the form of neural trauma, further highlighting its candidacy for future study. Finally, the manifestation of plasticity in the focal zone is dependent on the presence of an intact cortex for induction, but not expression. These findings lead to a plethora of testable predictions and tools in which to conduct future research. To this end, the importance of the hot spot model is not derived from whether or not it is correct, but rather as a framework to begin asking questions about plasticity in the CNS we have not explored before. If the future refutes the hot spot model, the knowledge gained to do this is still important.

Views of thalamocortical interactions appear quite limited, with the thalamus relaying information to the cortex, with little emphasis on the descending limb of this exchange in shaping plasticity at either level (Merzenich et al. 1983; Merzenich et al. 1984). To borrow the rhetorical canons of Herodotus (400 BC), "Many are the proofs whereby any one capable of reasoning on the subject may be convinced that it is most unlikely that this should be the case."
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