MECHANISMS OF RAPID RECEPTIVE FIELD REORGANIZATION

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Michael James Pettit, M.S.

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Rapid receptive field (RF) reorganization of somatosensory neurons in the cat dorsal column nuclei (DCN) was studied using electrophysiological and histological methods. Soon after denervation of the peripheral RF by lidocaine injection, every DCN neuron tested exhibited a reorganized RF. Reorganized RFs were located on new areas of the skin, and some had response properties unlike those of the original RF. These results suggest that reorganized RFs arose from the unmasking of previously ineffective inputs. To test whether reorganization occurs at the level of the DCN or is imposed by descending cortical influences, RFs were assessed in cats with the sensorimotor cortex removed before peripheral lidocaine injection. Similar results were obtained, suggesting that the neural mechanisms underlying rapid RF reorganization operate at the earliest stages of somatosensory processing.

Subcutaneous injection of capsaicin into peripheral RFs also produced rapid RF reorganization in all neurons tested. Reorganization did not require complete blockade of afferent drive from the original RF, and it was unrelated to changes in spontaneous firing rate. These results suggest that rapid RF reorganization can be produced by blockade of a subset of peripheral afferents, and are consistent with the hypothesis that tonic activity from some peripheral afferents restricts RF size and contributes to specificity of response properties.

One class of peripheral afferents that may be tonically active and that is affected by capsaicin injections, contains the neuropeptide substance P. To determine potential sites of action of these afferents in the DCN, substance P receptor binding sites were localized in the DCN of the rat, cat, monkey, and human. In each species dense binding was observed in the clusters region of the DCN, which contains neurons that respond to cutaneous inputs. The localization of substance P receptors in this region suggests that this neuropeptide may be involved in restricting RF size and producing response specificity in the DCN.

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CHAPTER I

1. INTRODUCTION

The organization of somatosensory maps in the brain can be altered by peripheral denervation, central lesions, or alteration in the amount or pattern of afferent stimulation (see reviews by Merzenich et al. 1988; Kaas 1991; Snow and Wilson 1991; Garraghty and Kaas 1992). Such reorganization in somatosensory maps demonstrates that the organization of the mature brain remains relatively dynamic and subject to environmental influence. Somatosensory maps are composed of the responses of individual neurons. Thus, the underlying basis for reorganization of somatosensory maps is the reorganization of peripheral receptive fields (RFs) of individual neurons. Somatosensory RF reorganization is a complex series of processes which can begin within minutes of a peripheral denervation and can continue for years. The general goal of this study is to examine mechanisms which may be responsible for the initial changes in RF organization of somatosensory neurons in the dorsal column nuclei (DCN) of the cat following peripheral denervation.

Rapid RF reorganization can be defined as the appearance of a new, peripheral RF within minutes after denervation of the original, peripheral RF (e.g. Nakahama et al. 1966). This new RF will be referred to as a reorganized RF. The reorganized RF occupies a new area of the skin to which the neuron was previously unresponsive, and it may have response properties which differ from those of the original RF (Nakahama et al.

1966; Metzler and Marks 1979; Turnbull and Rasmusson 1991; Kelahan and Doetsch 1984; Rasmusson et al. 1993).

Rapid RF reorganization is thought to be due to an unmasking of previously ineffective synapses (Wall 1977; Calford and Tweedale 1991b). These unmasked synapses may form a substrate for subsequent long-term changes in the somatosensory map. Thus, study of this process of rapid RF reorganization may elucidate the initial mechanisms responsible for plastic changes in the brain following injury or alteration of afferent input. Study of rapid RF reorganization is also important to advance understanding of basic brain mechanisms of processing of somatosensory information. The changes seen in RF response properties during rapid RF reorganization may provide insight into the mechanisms that produce specificity of neuronal RF response properties.

1.1 The dorsal column-medial lemniscal system

The focus of this study is rapid reorganization within the dorsal column-medial lemniscal system (hereafter referred to as the dorsal column system). In mammals, low-threshold mechanoreceptive cutaneous information (i.e., touch sensation) from the distal extremities is mediated primarily through the dorsal column system (reviewed by Mountcastle 1984; Willis and Coggeshall 1991; the following overview of dorsal column system organization is based primarily on these reviews). At every level within this system there exists an orderly somatotopic map of the body surface. The dorsal columns consist of the gracile and cuneate fasiculi which traverse the dorsal spinal cord. The first synaptic relay is the DCN in the caudal medulla. The DCN consist of the gracile and cuneate

nuclei. The gracile nucleus receives input from the hindlimbs and trunk via the gracile fasiculus. The cuneate nucleus receives input from the forelimbs and upper body via the cuneate fasiculus. Within each nucleus, neurons in the dorsal-medial region (termed the cell clusters) receive low threshold mechanoreceptive inputs mainly from primary afferents; they may also receive some input from neurons of the dorsal horn of the spinal cord (see section 1.2.1). The majority of cell cluster neurons project contralaterally to the ventroposterolateral nucleus of the thalamus (VPL) via the medial lemniscus (additional thalamic regions are described in section 1.2.4. VPL is a subdivision of the ventrobasal complex (VB) of the thalamus. The ventroposteromedial nucleus (VPM) is also part of VB. VPM receives cutaneous input from the trigeminal system). Additionally, some cell cluster neurons project to the contralateral cerebellum. VPL neurons project to the primary somatosensory cortex (SI). SI has extensive recurrent projections to both VPL and DCN. There is no recurrent projection from VPL to DCN.

1.2 Rapid RF reorganization

Rapid RF reorganization has been studied at all levels of the somatosensory system using a variety of experimental protocols.

1.2.1 Dorsal horn

Neurons of the dorsal horn of the spinal cord are a secondary source of input to the DCN and thus will be briefly discussed. The dorsal horn is the first synaptic relay of the spinothalamic system which primarily transmits nociceptive and thermoceptive

information (e.g., see Mountcastle 1984; Willis and Coggeshall 1991). In dorsal horn neurons, RF expansion occurs in response to activation of nociceptive inputs as part of the inflammatory response to injury (McMahon and Wall 1984; Hylden et al. 1984; Laird and Cervero 1989; Simone et al. 1989; Woolf and King 1990; Hoheisel et al. 1993). Thus, the mechanisms for this process are probably different from rapid RF reorganization, which results from peripheral denervation. This conclusion is strengthened by the observation that dorsal horn cells with low threshold mechanoreceptive RFs (i.e. those which might be expected to project to the DCN) do not exhibit RF expansions as a result of nociceptive stimulation (cat: Simone et al. 1989; rat: Woolf and King 1990). No studies have examined the capacity for rapid RF reorganization in dorsal horn neurons following peripheral denervation.

1.2.2 Dorsal column nuclei

Two reports have described rapid RF reorganization in the DCN. Following cooling of the dorsal columns, 11 of 40 cat gracile nucleus cells exhibited rapid RF shifts from the hindlimb to the trunk (Dostrovsky et al. 1976). These shifts were reversible with warming of the dorsal columns. In an abstract it was reported that, in recordings of single and multiple units from rat DCN, 3 of 6 units exhibited RF expansions following subcutaneous lidocaine injection into their peripheral RFs (Shin et al. 1991).

1.2.3 Somatosensory nuclei of the thalamus

Rapid RF reorganization has been reported in neurons of somatosensory nuclei of the thalamus for several species. Nakahama et al. (1966) studied rapid RF reorganization in cat VB using subcutaneous injections of procaine to inactivate peripheral RFs. Thirteen of 59 neurons exhibited new RFs which were usually proximal to, and contiguous with, the original RF. One neuron exhibited a reversal from inhibition to excitation with reorganization.

In the same study, it was noted that the reorganized RFs often persisted after the original RF regained esthesia. The authors suggested that this might result from rapid plastic changes in synaptic connections. However, procaine-type anesthetics produce a differential blockade of peripheral afferents such that unmyelinated and smaller diameter nerve fibers usually recover more slowly than larger diameter fibers (Franz and Perry 1974). Since the primary afferent drive to these neurons is from large diameter fibers, persistent reorganized RFs might be due to prolonged blockade of a subset of peripheral inputs (see sections 1.4.3 and 2.4.).

Rapid RF reorganization has also been demonstrated for neurons in rat somatosensory thalamus. Denervation of peripheral RFs by subcutaneous lidocaine injection produced spatial shifts in RF location in 30 of 55 neurons in rat VPM (Nicolelis et al. 1993). Temporal shifts from short to long latency response times were noted for 27 of these neurons. The authors proposed that the increase in response latency of the reorganized RFs occurs via unmasking of polysynaptic pathways. In single and multiple

unit recordings from rat VB, 8 of 11 units exhibited reorganized RFs after peripheral denervation by subcutaneous lidocaine injection (Shin et al. 1991).

In multiple unit recordings from raccoon VPL, rapid RF reorganization has been produced by subcutaneous lidocaine injection (n = 9), capsaicin application to peripheral nerves (n = 2), and peripheral nerve section (n = 4) (Rasmusson et al. 1993). In most raccoon VPL neurons, lidocaine injection and nerve cut produces reorganized RFs with new excitatory fields as well as new widespread inhibitory fields (lidocaine: 7 of 9 neurons; nerve section: 3 of 4 neurons). In contrast, capsaicin application produces reorganized RFs with excitatory fields only.

1.2.4 Primary somatosensory cortex

Rapid RF reorganization has been studied most extensively in SI. Metzler and Marks (1979) used epidural blocks to study RF reorganization in cat primary somatosensory cortex (SI). They found that 13 of 46 neurons exhibited reversible RF shifts from the proximal hindlimb to either the trunk or distal hindlimb. Three of these neurons had reorganized RFs that responded to deep, but not light, cutaneous stimulation. It is uncertain whether these deep reorganized RFs resulted from unmasking of deep inputs or are produced by high threshold cutaneous fields.

Rapid RF reorganization is produced in SI neurons following amputation or lidocaine denervation in flying fox (Calford and Tweedale 1988; 1991c), and macaque (Calford and Tweedale 1991a). These studies were based on both single and multiple unit recordings. Rapid RF reorganization occurred in every unit tested and was expressed as a

RF expansion proximal to the denervation site. Following peripheral lidocaine injection, the reorganized RF begins to contract as the original RF regains esthesia.

Blockade of C fiber activity by capsaicin application also produces rapid RF reorganization in SI neurons of cat and flying fox (Calford and Tweedale 1991b).

In rat SI, digit amputation as well as peripheral lidocaine injection produces rapid RF reorganization (Byrne and Calford 1991). Partial RF denervation results in faster onset of reorganization than total RF denervation, and this effect does not depend on the method of denervation. Following lidocaine denervation, the reorganized RFs do not always contract when the original RFs regain esthesia, and the original RFs do not always return to their original boundaries. Fluctuations in the size of the reorganized RF can occur. For the data reported, the recording period was less than 60 min; consequently, some of these results may have been produced by the differential effect of lidocaine on afferent fibers. As discussed previously (section 1.2.4), conduction block may have still been present in smaller diameter and unmyelinated fibers. Blockade of these fibers may contribute to reorganization. Further, since these data were derived from multiple unit recordings, instability of the recording electrode could give rise to apparent RF shifts. Shin et al. (1993) reported in an abstract that 9 of 12 units in rat SI exhibited rapid reorganization following peripheral lidocaine denervation.

Raccoon SI neurons undergo rapid RF reorganization following digit amputation (Rasmusson and Turnbull 1983; Kelahan and Doetsch 1984; Turnbull and Rasmusson 1990; Turnbull and Rasmusson 1991). In this species rapid RF reorganization results most often in unmasking of new inhibitory fields.

1.3 The site of reorganization

In order to understand the mechanisms underlying rapid RF reorganization it is necessary to identify the initial site of these changes. Most studies of cortical rapid RF reorganization in the somatosensory system have examined the forepaw representation. The existing data for DCN reorganization are from cat gracile neurons (Dostrovsky et al. 1976), while the location of the three rat DCN units was not specified (Shin et al. 1991). Thus, the ability of cuneate clusters neuron RFs to rapidly reorganize, and their potential contribution to RF reorganization in thalamus and cortex, is unknown. Further, previous data are insufficient to determine the initial site of reorganization due to lack of control for the descending connections from cortex to thalamus and DCN.

There are at least three hypotheses regarding the initial site of reorganization: 1) reorganization begins at the initial synaptic relay and is imposed upon subsequent levels; 2) reorganization takes place at each level independently, and is amplified by input from preceding levels; 3) reorganization occurs in SI and is imposed onto lower levels by feedback connections.

The DCN are a likely initial site of reorganization in the lemniscal system. The major input to the DCN cell clusters region is from primary afferents of low threshold mechanoreceptors. The dorsal horn probably plays a minor role, if any, in rapid RF reorganization of DCN cluster neurons. Although afferents from dorsal horn neurons synapse in the clusters region in the primate (Cliffer and Willis 1994) and rat (Cliffer and Giesler 1989) DCN, whether this occurs in the cat is unclear (Rustioni 1974; Pierce et al. 1990).

The reorganization of DCN neuron RFs in cat (Dostrovsky et al. 1976) suggests that RF reorganization can begin at this synaptic relay. It has been postulated that RF reorganization results from a decrease in inhibition which unmasks previously ineffective synapses (Nakahama et al. 1966; Wall 1977; Calford and Tweedale 1988). Inhibitory components are present in the RFs of cat DCN neurons (Janig et al. 1977), and alteration of inputs to these components during peripheral denervation might produce reorganized RFs. However, the cat DCN also receive descending inputs from SI (Kuypers and Tuerck 1964; Weisberg and Rustioni 1976; Cheema et al. 1983), and cortical stimulation produces primary afferent depolarization in the DCN (Andersen et al. 1964a). Thus, a decrease in cortical input might also serve to produce reorganization via unmasking.

There are data which are compatible with a role of SI in rapid RF reorganization. Many neurons in rat VPM exhibit an increased number of long latency responses following peripheral denervation (Nicolelis et al. 1993). Temporal shifts in response latencies could result from unmasking of polysynaptic pathways. Such long latencies might arise if corticothalamic inputs impose reorganization onto thalamus. RF reorganization in SI (flying fox, Calford and Tweedale 1990; macaque, Calford and Tweedale 1991a) and VB (rat, Shin et al. 1991) occurs ipsilateral to the denervation site. It may be possible that contralateral SI imposes reorganization onto ipsilateral cortex via callosal connections, and then onto ipsilateral thalamus via corticothalamic connections. In the developing rat, SI ablation disrupts cytochrome oxidase "barrel" staining patterns in the DCN, VB, and contralateral SI (Erzurumlu and Ebner 1988). Also, in the visual system binocular retinal lesions produce RF reorganization in primary visual cortex, but not in the lateral

geniculate nucleus of the thalamus (Eysel et al. 1980; Chino et al. 1992; Gilbert and Weisel 1992).

Chapter 2 discusses experiments designed to determine if the DCN, and specifically the cuneate nucleus, is a site of rapid RF reorganization following denervation of peripheral RFs by subcutaneous lidocaine injection. Experiments were performed in cats with cortex intact and in cats with somatosensory and motor cortex removed. Two objectives of these experiments were: 1. to determine if rapid RF reorganization is exhibited by cuneate neurons following a small, peripheral denervation; and 2. to determine if rapid reorganization occurs in cuneate neurons independent of cortical inputs.

1.4 Mechanisms of rapid RF reorganization

RF reorganization can result either from plastic changes which produce new synaptic connections (e.g. sprouting) or from mechanisms which alter the efficacy of transmission across existing synapses, thereby unmasking certain inputs. Due to its rapid onset (i.e. within minutes), rapid RF reorganization almost certainly arises from an unmasking of inputs. Release from inhibition and changes in cable properties of neurons are two potential mechanisms for unmasking during rapid RF reorganization. In order to investigate the possible role of these mechanisms, a detailed examination of RF response properties and spontaneous activity rates before and after rapid RF reorganization is necessary. However, most studies of rapid RF reorganization have relied primarily on multiple unit recordings (Calford and Tweedale 1988; Byrne and Calford 1991; Calford and Tweedale 1991a,b,c; Shin et al. 1991; Rasmusson et al. 1993). Two studies of

peripheral lidocaine-induced rapid RF reorganization have been restricted to observations of single units (Nakahama et al. 1966; Nicolelis et al. 1993), yet neither study included careful analysis of RF response properties. As detailed below, a careful examination of single unit RF properties and activity patterns may provide evidence for changes in inhibition or cable properties as mechanisms responsible for rapid RF reorganization.

1.4.1 Relation of inhibition to rapid reorganization

Nakahama et al. (1966) suggested that rapid RF reorganization in the thalamus results from a release from inhibition within the original RF following denervation. Based on the observation that the anatomical extent of cat dorsal horn afferents is usually larger than the extent of physiological receptive fields, Wall (1977) suggested that rapid reorganization is the result of unmasking previously ineffective synapses via disinhibition. Anatomical and physiological studies have demonstrated similar mismatches between terminal arborizations and RFs in the cat cuneate (Fyffe et al. 1986a,b; Weinberg et al. 1990). This type of mismatch is also demonstrated by the observation that cat VB neurons can be antidromically activated from areas of SI outside the normal somatotopic RFs of the VB neurons (Snow et al. 1988). Further, electrical stimulation of peripheral afferents also reveals larger RFs than those produced by natural stimulation in cat cuneate (Rosen 1969) and SI (Towe et al. 1964).

Inhibition is present at all levels of the dorsal column system and arises from intrinsic mechanisms (Mountcastle 1984). GABAergic neurons occur at every level of this system in significant proportions (Spreafico et al. 1983; Heino and Westman 1991; Li and

Schwark 1994). Iontophoretic studies have demonstrated that inhibition functions to restrict the size of somatosensory RFs. Iontophoresis of the $GABA_A$ receptor blocker bicuculline produced RF enlargement in 12.5% of cat VB neurons tested (Hicks et al. 1986) and 60-75% of cat SI neurons tested (Dykes et al. 1984; Alloway et al. 1989). In SI of cat (Alloway et al. 1989) and monkey (Alloway et al. 1991), inhibitory blockade produced RF enlargement more commonly in rapidly adapting (RA) neurons than in slowly adapting (SA) neurons. This has lead to the suggestion that RA neurons are subject to more inhibition than SA neurons (Alloway et al. 1991). Examination of RF response properties may reveal differences in the rapid RF reorganization between DCN neurons with RA and SA peripheral RFs.

Inhibition may also function to restrict the response properties of somatosensory neurons in the dorsal column system. It has been suggested that the major role for the dorsal column system is the transmission of somatotopically- and modality-specific information to the forebrain, and that the majority of neurons at each synaptic level exhibit specific response properties (Mountcastle 1984). In particular, physiological studies demonstrate that most DCN neurons exhibit specificity for response class (e.g. hair, skin), adaptation characteristics (e.g. RA, SA), and submodality (e.g. cutaneous, deep) (Kruger et al. 1961; Gordon and Jukes 1964; Brown et al. 1974; Janig et al. 1977; Golovchinsky 1980; Cheema et al. 1983). However, physiological and anatomical studies suggest that DCN neurons receive a high degree of convergent input (Rosen 1969; Fyffe et al. 1986a,b; Weinberg et al. 1990; Pierce et al. 1990). A detailed examination of the RF response

properties before and after rapid RF reorganization may reveal whether inhibitory masking mechanisms produce response specificity in DCN neurons.

Calford and Tweedale (1991b) demonstrated that application of capsaicin to peripheral nerves produces rapid RF reorganization in SI neurons in a manner similar to peripheral lidocaine injection. Rapid RF reorganization coincided with the abolition of the C fiber component of the compound action potential from the peripheral nerve. The responsiveness of the original RF was unaffected. The authors proposed that rapid RF reorganization might arise from release of tonic inhibition transmitted via C fiber afferents within the spinothalamic system. In raccoon VPL, capsaicin application to peripheral nerves produces effects different from peripheral lidocaine injection or digit amputation (Rasmusson et al. 1993). Based on a small sample (2 capsaicin and 9 lidocaine denervations; 4 amputations), it was suggested that reorganization via capsaicin inactivation occurs by a mechanism different from that of other types of inactivation. Rasmusson et al. also speculated that the widespread inhibitory fields which appeared after lidocaine injection and digit amputation were most likely due to spinothalamic influences rather than cuneate reorganization because of the larger spatial convergence of spinothalamic neurons. A test of this hypothesis is to look for the presence of widespread inhibitory components in the reorganized RFs of DCN neurons.

Experiments in Chapter 3 were designed to investigate if rapid RF reorganization occurs in cuneate neurons following subcutaneous capsaicin injection into peripheral RFs. One objective of these experiments was to determine if capsaicin-induced reorganization can be mediated by the dorsal column system. In this chapter, possible sources of masking

inhibition in the DCN are discussed and a potential mechanism for tonic masking in the DCN is presented.

Chapters 2 and 3 present a detailed examination of RF response properties before and after reorganization. Four specific goals of these experiments were to: 1) understand if rapid RF reorganization is related to a neuron's RF adaptation characteristics or other RF response properties; 2) determine the extent to which physiological masking produces specificity of response properties in DCN neurons; 3) determine if widespread inhibitory reorganized RFs appear in DCN neurons; and 4) determine if capsaicin and lidocaine denervation produce different types of reorganized RFs.

1.4.2 Relation of spontaneous activity to rapid RF reorganization

Another possible mechanism for rapid RF reorganization is activity-dependent changes in the cable properties of neurons. A theoretical model has been proposed which suggests that background synaptic activity can influence neuronal electrotonic properties (Bernander et al. 1991). According to the model, increased synaptic activity results in an increased membrane conductance and concomitant decrease in time constant, length constant, and input resistance. The net effect of these changes is to decrease the electrotonic size of the neuron. Thus, inputs located on the outer dendrites would be rendered ineffective by background synaptic activity. Rapid RF reorganization could be produced by an increase in electrotonic size resulting from decreased background synaptic activity after denervation. In the DCN, many neurons are spontaneously active (Pubols et al. 1989). If changes in cable properties are responsible for rapid RF reorganization, then

the onset of reorganization should be preceded by, or coincident with, a decrease in spontaneous activity. The spontaneous activity of neurons before and during rapid RF reorganization is reported in Chapters 2 and 3.

1.5 Distribution of substance P receptors in DCN

Substance P (SP) may be involved in DCN rapid RF reorganization. SP is a neuropeptide which is associated with C fibers (Levine et al. 1993) and C fiber inactivation produces rapid RF reorganization of somatosensory neurons (Calford and Tweedale 1991b; Rasmussen et al. 1993).

Indirect evidence suggests that SP may be involved in RF reorganization in the dorsal horn. SP is thought to exert modulatory effects on dorsal horn interneurons (Levine et al. 1993), and is released from the rat spinal cord following noxious stimulation (Go and Yaksh 1987). Activation of C fibers by noxious stimuli produces RF expansions in rat dorsal horn neurons (McMahon and Wall 1984; Hylden et al. 1984; Laird and Cervero 1989; Simone et al. 1989; Woolf and King 1990; Hoheisel et al. 1993). Noxious stimulation of C fibers also mediates neurogenic hyperalgesia in primates (Baumann et al. 1991; Simone et al. 1991; LaMotte et al. 1991; LaMotte et al. 1992).

SP-immunoreactive terminals are present in the DCN; however, it is unclear whether SP inputs to the DCN arise from primary afferents or from dorsal horn afferents (Conti et al. 1990; Westman et al. 1984). SP results in long-lasting excitation when applied to DCN neurons (Krnjevic and Morris 1974; Fox et al. 1978). SP terminals are

found ventral to and between the cell clusters of the DCN (Conti et al. 1990; Westman et al. 1984). However, transmitter receptor mismatches have been described between afferents and receptors for a number of peptides, and it has been suggested that peptides may diffuse considerable distances to exert their effects (Herkenham and McLean 1986). Consequently, SP might play a role in rapid RF reorganization in DCN neurons. If so, then SP receptors should be present in the clusters region of the DCN.

Chapter 4 describes autoradiographic studies of SP receptor binding in rat, cat, monkey, and human DCN. The objective of these experiments was to determine the density and distribution of SP receptors in the DCN in order to determine if SP has the potential to influence RF reorganization.

1.6 Specific Objectives

In summary, the specific objectives of the studies described here are:

1. To determine if the DCN are a subcortical site for rapid RF reorganization following lidocaine denervation of peripheral RFs. Chapter 2 details experiments to test the ability of DCN neurons to undergo rapid RF reorganization following peripheral denervation. Recordings were made primarily from cuneate neurons in the cell clusters region. The majority of inputs to this region are from primary afferents and thus, any RF reorganization at this level is unlikely to be influenced by dorsal horn reorganization.

2. To determine if rapid RF reorganization of cuneate neurons occurs independent of cortical inputs. The cuneate nucleus receives inputs from somatosensory and motor cortex. It may be that rapid RF reorganization takes place first in cortex and is then imposed upon subcortical levels. To test this hypothesis, lidocaine denervation of peripheral RFs was done in animals in which motor and somatosensory cortex had been removed. These experiments are presented in Chapter 2.

3. To determine if capsaicin-induced rapid RF reorganization occurs in the dorsal column system. It has been suggested that tonic activity of C fibers within the spinothalamic tract may produce inhibition that normally masks some inputs in SI. Capsaicin application desensitizes certain types of nerve fibers, including C fibers. Chapter 3 describes experiments to test the ability of cuneate neurons to undergo rapid RF reorganization following peripheral injection of capsaicin.

4. To characterize the response properties of DCN neurons before and after rapid RF reorganization. Most studies of rapid RF reorganization have used multiple unit recordings, which can make examination of changes in response properties difficult. Most neurons in the cuneate clusters region have specific response properties. A detailed examination of RF response properties before and after RF reorganization may provide insight into: 1) mechanisms responsible for rapid RF reorganization; and 2) mechanisms which produce specificity of response

properties in cuneate neurons. Results from these observations are presented in Chapters 2 and 3.

5. To characterize the distribution of SP receptors in the DCN. SP is a neuropeptide associated with C fibers, and the release of SP from C fiber afferents is thought to produce RF expansion in dorsal horn neurons. Afferents to the DCN which contain SP have been described. Chapter 4 describes autoradiographic studies of SP receptor binding in the DCN of rat, cat, monkey, and human.

CHAPTER 2

2. RECEPTIVE FIELD REORGANIZATION IN DORSAL COLUMN NUCLEI DURING TEMPORARY DENERVATION

2.1 Summary

Altered sensory input can result in reorganization of somatosensory maps in the cerebral cortex and thalamus, but the extent to which reorganization occurs at lower levels of the somatosensory system is unknown. In the present experiments in cat dorsal column nuclei (DCN), injection of local anesthetic into the receptive fields of DCN neurons resulted in the emergence of a new receptive field in all 13 neurons studied. New receptive fields emerged rapidly (within minutes), sometimes accompanied by changes in adaptation rates and stimulus selectivity, suggesting that the new fields arose from unmasking of previously ineffective inputs. Receptive field reorganization was not imposed by descending cortical inputs to the DCN, because comparable results were obtained in 10 additional cells when somatosensory and motor cortex were removed prior to recording. These results suggest that mechanisms underlying somatotopic reorganization exist at the earliest stages of somatosensory processing. Such mechanisms may participate in adaptive responses of the nervous system to injury or continuously changing sensory stimulation.

2.2 Introduction

Sensory maps in the cerebral cortex are maintained through dynamic processes. Modification of peripheral inputs to the central nervous system results in reorganization of cortical somatosensory maps in a number of species (Merzenich et al. 1988; Kaas 1991) including humans (Ramachandran et al. 1992; Halligan et al. 1993; Mogilner et al. 1993). Identification of the mechanisms which are involved in map reorganization has clinical implications for treatment of peripheral nerve injury and phantom limb pain.

A critical issue which must be resolved before mechanisms of reorganization can be uncovered is the extent to which reorganization at subcortical levels contributes to changes described in the cortex. Mapping studies suggest that peripheral nerve transection can result in map reorganization in the primate ventral posterior thalamic (VPL) nucleus (Garraghty and Kaas 1992). Although such reorganization has not been found in DCN or trigeminal nuclei of mature animals (McMahon and Wall 1984; Waite 1984), it is difficult to detect reorganization in subcortical maps, which are three-dimensional and can exhibit large somatotopic shifts over relatively small distances. An alternative approach, which we have used in the present study, is to map a single neuron's receptive field (RF), inject local anesthetic into the field to temporarily silence input from the RF, and then test for the appearance of a new RF. In thalamus (Nakahama et al. 1966; Nicolelis et al. 1993) and cortex (Calford and Tweedale 1991c; Byrne and Calford 1991) this approach has demonstrated that new RFs can emerge within minutes following injection of lidocaine. Such changes in cat DCN neurons have not been investigated,

although cold block of the dorsal columns has been reported to result in the emergence of new RFs in a small proportion of nucleus gracilis neurons (Dostrovsky et al. 1976).

2.3 Methods

To study subcortical reorganization in the present experiments, recordings were made from 13 DCN neurons in six anesthetized, adult cats. Anesthesia was induced by ketamine injection and maintained by continuous intravenous infusion of sodium thiopental (3-4 mg/kg/h). Prior to recording, paralysis was induced and maintained by continuous intravenous infusion of gallamine triethiodide (8 mg/kg/h). Single unit recordings of neurons with cutaneous RFs on the forepaw or hindlimb were made with parylene-coated tungsten electrodes or glass micropipettes. Recording sites were marked by making lesions or by iontophoresis of horseradish peroxidase or pontamine sky blue. Data were collected exclusively from single neuron recordings to permit the precise definition of response properties and to preclude the possibility that instability of multiunit recordings might give rise to apparent changes in RF organization. To ensure that the same cell was recorded throughout an experiment, two stimulating electrodes placed at the caudal pole of the VPL were used to evoke action potentials during nonresponsive periods, and all action potentials were monitored on a digital storage oscilloscope triggered by the output of a window discriminator.

Original RFs were mapped using hand held stimuli over periods of 10-30 min prior to inactivation to determine the stability of RF borders. No variations were seen over these periods in RF size or neuronal response properties. Temporary denervation was

then produced by 1-3 subcutaneous injections of 2% lidocaine (4-30 μl each). Additional injections were made (for 10 of the 23 cells) if the RF was difficult to silence or was very large. The area was again mapped, and the boundaries of responsive areas were determined.

To test for potential nonspecific effects of the lidocaine injections, the RF of one neuron was injected with an equal volume of saline after the effects of an initial lidocaine injection had disappeared.

Ten neurons from four additional cats were recorded following removal of cortical inputs to the DCN. Cortical inputs to the DCN were removed by cortical aspirations that included the complete forepaw representations and most of the trunk representations of all somatosensory and motor cortical areas (Fig. 1c). The effects of lidocaine injection were tested three to 20 h after the aspirations.

2.4 Results and Discussion

Subcutaneous lidocaine injections into the original RF resulted in the rapid emergence of a new RF in every neuron tested (Table 1, Fig. 1A, B). Even in the absence of cortical inputs, subcutaneous lidocaine injections resulted in the rapid emergence of new RFs in every neuron tested (Table 1, Fig. 1C). Therefore, subcortical reorganization was not imposed by descending cortical inputs to the DCN. There were no evident differences in the RF reorganizations in these two sets of experiments, so the data have been combined for the following description.

Lidocaine injection resulted in emergence of new RFs for every DCN neuron tested, even in eight neurons where blockade of the original RF was incomplete.

In 16 of the 23 neurons, the new RFs remained throughout the entire recording period (up to 6 h), even after responsiveness in the original RFs had returned. Neither recovery of responsiveness in the original RF nor stability of the new RF were related to neuronal response properties or to the size of the new RF.

Control injection of saline had no effect on RF organization or activity. Following recovery from the effects of an initial lidocaine injection, the RF of one neuron was injected with an equal volume of saline. No changes in the RF were seen over the subsequent 29 min. A second injection of lidocaine was then made into the RF, and this injection produced a sequence of RF reorganization virtually identical (both temporally and spatially) to the first lidocaine injection.

In eight neurons the response class (hair or skin) or adaptation characteristics of the new RF differed from those of the original receptive field (Table 1, Fig. 1B). In six other neurons new inhibitory RFs emerged following lidocaine injection. The appearance of these new response characteristics, which were not evident in the original RF, suggests that the new fields arose from peripheral inputs which were normally ineffective. These neurons were located in the core and rim of the cell cluster region of the middle cuneate nucleus and in the rostral cuneate (Kuypers and Tuerk 1964).

In contrast to the neurons described above, nine neurons showed a simpler type of RF reorganization: they responded selectively to stimulation of hair or skin in the original RF, and following lidocaine injection each began to respond to the same type of

stimulation in a new field (Fig. 1A). These neurons were all located in the core of the cell cluster region.

Dynamic aspects of RF reorganization were particularly evident in three neurons in which the new RFs fluctuated in size, 44-120 min after their initial appearance. The response threshold in one of these new RFs also fluctuated; the threshold decreased from 2.35 g at 52 min after lidocaine injection to 0.22 g at 83 min, then increased to 0.44 g over the next 40 min, and by the end of the six h recording period, had returned to 2.35 g.

The present results suggest that 1) some inputs to DCN neurons are normally masked; 2) masked inputs become effective when activity in the original RF is reduced; and 3) unmasking does not depend upon cortical inputs to the DCN. The anatomical substrate of masked inputs may involve the dendritic spread of DCN neurons (up to 500 μm) and the widespread terminal arborizations of primary afferents (Fyffe et al. 1986a,b; Weinberg et al. 1990). It has been estimated that as many as 300 primary afferents overlap at any point in the middle cuneate nucleus (Fyffe et al. 1986b). The mechanisms of masking are not known. Calford and Tweedale (1991b) have suggested that tonic, inhibitory inputs might serve this function, and have shown that silencing peripheral C-fibers leads to RF expansion in cortical neurons. The influence of C-fiber inputs on RF organization in the DCN has not been investigated.

The presence in the DCN of mechanisms which underlie RF reorganization raises the question of whether such mechanisms also exist at higher levels of the somatosensory system, or whether reorganization observed at higher levels is a reflection of changes in the DCN. A potential anatomical substrate for rapid reorganization in cerebral cortex is

the widespread termination pattern of thalamocortical afferents (Landry and Deschenes 1981; Snow et al. 1988). Direct evidence for RF reorganization in sensory cortex has been found in studies of retinal lesions, which result in immediate RF expansions in cortical (Gilbert and Weisel 1992), but not thalamic (Eysel et al. 1980) neurons. Therefore, it appears that mechanisms underlying RF reorganization exist at multiple levels of sensory systems. Following injury, such mechanisms might be involved in the development of referred sensations involving phantom limbs (Ramachandran et al. 1992; Halligan et al. 1993; Mogilner et al. 1993). In normal brain function, such mechanisms might serve in adapting to a continuously changing sensory environment.

TABLE 1. Receptive field response properties before and after subcutaneous lidocaine injection

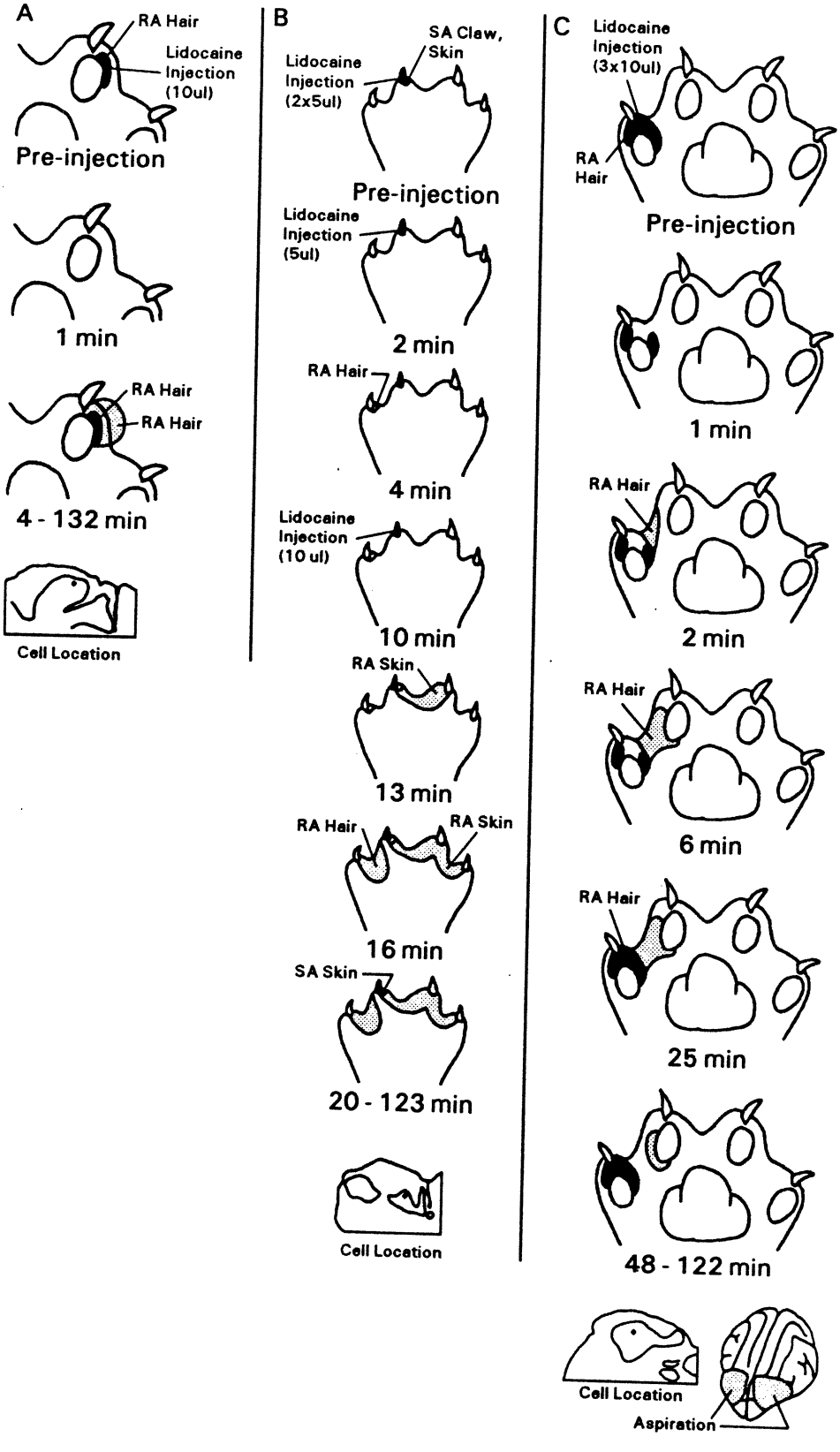
Original Receptive Field	New Receptive Field	Size of New Receptive Field	Spontaneous Activity (Hz)			Cell Location	Observation Time (min)
			Pre-Inj.	Post-Inj	Ratio**		
RA, hair	RA, hair	0.4	1	1	1	core	20
RA, hair	RA, hair	2.2	0	0	1	core	132
RA, hair	RA, hair	0.6	1	0	0	core	79
RA, hair	RA, hair	0.1	0	2	-	core	125
RA, hair Ipsi. forearm(-)***	RA, hair	0.2	32	31	1	gracilis	70
RA, skin	RA, hair	0.4	0	0	1	core	69
RA, hair, skin & hair(-)	hair(-), deep(-) Ipsi. forepaw	20.7	27	46	1.7	core	150
RA, hair & hair(-) Ipsi. hindlimb(-)	RA, skin & hair	2.5	16	5	0.3	core	134
SA, skin	SA & RA, hair	6.2	-	0	-	rim	125
SA, skin & claw	RA, skin & hair; claw	-	-	-	-	rim	123
SA, skin	SA, skin, hair & deep	1.8	89	0	0	-	117
SA, hair & skin	SA, skin & claw(-)	1.1	43	28	0.7	core	133
SA, claw & hair(-) Ipsi. & contra. forepaw(-) Ipsi. hindpaw(-)	hair(-) & deep(-)	-	13	24	1.8	core	106
Cells recorded following removal of cortical inputs to the DCN							
RA, hair	RA, hair	2.6	29	27	0.9	core	122
RA, hair	RA, hair	2.7	19	21	1.1	core	100
RA, hair	hair(-)	25.7	9	16	1.8	core	70
RA, hair & hair(-)	hair(-)	0.2	14	14	1	core	82
RA, hair	RA, skin & hair	1.4	28	-	-	rostral	364
RA, skin, hair(-) & skin(-) Ipsi. & contra. forepaw(-)	RA, skin	0.5	6	13	2.2	rim	168
RA, skin Ipsi. forepaw(-)	RA, skin	1.6	-	11	-	core	18
SA, skin	RA, skin	0.5	21	23	1.1	rim	84
SA, claw & skin	SA, skin	-	28	19	0.7	core	119
SA, skin & hair(-)	skin, skin(-) & hair(-)	1.0	14	11	0.8	core	36

* Ratio of new receptive field size to original receptive field size.

** Ratio of post-injection spontaneous activity to pre-injection spontaneous activity.

*** Inputs from other limbs are listed according to the limb from which they were elicited. No injections were made into these fields. (-) denotes inhibitory fields.

Fig. 1. Responsive areas of the original receptive field (RF) are shown as black regions, and areas which became newly responsive after lidocaine injection (new RFs) are shown as shaded regions. Each column in the figure shows the temporal sequence of RF changes for a single cell. Within a column, successive changes in RF organization are illustrated, with time after injection indicated. Recording site in the DCN is shown at the bottom of each column. **A.** A simple form of RF reorganization, in which the new RF responded to the same type of stimulus as the original field. **B.** In this cell the original, slowly adapting (SA) response was elicited only by stimulation of the skin and claw. A series of lidocaine injections silenced the skin RF and resulted in the emergence of new RFs; rapidly adapting (RA) responses were elicited by stimulation of hair in one field and skin in another. **C.** RF reorganization followed lidocaine injections even in the absence of cortical inputs to the DCN (extent of the cortical aspiration is shown at the bottom of the column). The original, RA response was elicited by stimulation of hair. Lidocaine injections silenced the central portion of the RF, and 2-6 min later a new RF with similar response properties emerged.


A
RA Hair
Lidocaine Injection (10ul)
Pre-injection
1 min
RA Hair
RA Hair
4 - 132 min
Cell Location
B
SA Claw, Skin
Lidocaine Injection (2x5ul)
Pre-injection
Lidocaine Injection (5ul)
2 min
RA Hair
4 min
Lidocaine Injection (10 ul)
10 min
RA Skin
13 min
RA Hair
RA Skin
16 min
SA Skin
20 - 123 min
Cell Location
C
Lidocaine Injection (3x10ul)
RA Hair
Pre-injection
1 min
RA Hair
2 min
RA Hair
6 min
RA Hair
25 min
48 - 122 min
Cell Location
Aspiration

CHAPTER 3

3. CAPSAICIN-INDUCED RAPID RECEPTIVE FIELD REORGANIZATION

3.1 Summary

1. Subcutaneous capsaicin injection was used to study rapid receptive field (RF) reorganization of cuneate neurons in anesthetized cats. Peripheral RFs of single neurons were mapped, RF response properties were characterized, and spontaneous activity rates were measured. These values were determined before and after capsaicin injection.

2. Subcutaneous injection of capsaicin (10% dissolved in 70% ethanol) into the peripheral RF produced rapid RF reorganization in all neurons tested (n = 20). RF reorganization did not require complete blockade of the afferent drive from the original RF. The onset of RF reorganization was not related to changes in spontaneous activity.

3. Following capsaicin-induced RF reorganization, subcutaneous injections of lidocaine were made into the original peripheral RFs of 8 neurons. These lidocaine injections produced no additional RF reorganization in 7 of 8 neurons, even though in every neuron lidocaine injection produced a partial blockade of the response from the original RF.

4. In approximately half of the neurons (9 of 20), the reorganized RFs exhibited response characteristics different from those of the original RF. This result suggests the presence of a physiological masking of inputs which restricts RF size and can increase the specificity of RF response properties in cuneate neurons.

5. The results of these experiments provide evidence that rapid RF reorganization in cuneate neurons can be produced by blockade of a subset of peripheral afferents. The present results also demonstrate that capsaicin-induced RF reorganization, which has been reported previously in VPL and SI, can arise within the dorsal column-medial lemniscal system at the level of the dorsal column nuclei.

3.2 Introduction

Alteration of peripheral inputs by denervation or changes in afferent stimulation can result in the reorganization of somatosensory maps in the brain (Snow and Wilson 1991; Garraghty and Kaas 1991). Somatosensory map reorganization is a complex process which can begin within minutes of a peripheral lesion and continue for years. Somatopic map reorganization results from the reorganization of the peripheral receptive fields (RFs) of individual neurons. Study of the mechanisms which produce RF reorganization in neurons following peripheral denervation may reveal the processes which produce plastic changes in the brain following injury or alteration of afferent input.

Many somatosensory neurons have peripheral RFs which are modality-specific and restricted in size (Mountcastle 1984; Willis and Coggeshall 1991). A detailed examination of RF response properties before and after peripheral denervation may elucidate basic mechanisms which contribute to specificity of RF response properties.

Somatosensory neurons can respond to new skin areas following denervation of their peripheral receptive fields (RFs) (e.g. Nakahama et al. 1966; Calford and Tweedale 1988, Pettit and Schwark 1993). This reorganization of the RF happens within minutes of

denervation. Within the somatosensory system, rapid RF reorganization occurs in a number of species and at multiple levels: the DCN (cat: Dostrovsky et al. 1976; Pettit and Schwark 1993); thalamus (cat: Nakahama et al. 1966; raccoon Rasmusson et al. 1993; rat: Nicolelis et al. 1993); and primary somatosensory cortex (SI) (monkey: Calford and Tweedale 1991a; flying fox: Calford and Tweedale 1988, 1991b,c; rat: Byrne and Calford 1991; cat: Metzler and Marks 1979, Calford and Tweedale 1991c; raccoon: Kelahan and Doetsch 1984; Turnbull and Rasmusson 1990).

Denervation of peripheral RFs by lidocaine injection produced rapid RF reorganization in every cat DCN neuron tested (Pettit and Schwark 1993). Reorganization in the DCN is not the result of cortical rapid RF reorganization, because rapid RF reorganization also occurs in cat DCN neurons when motor and somatosensory cortex are removed before recording (Pettit and Schwark 1993). This suggests that neural mechanisms underlying rapid RF reorganization exist at the earliest stages of somatosensory processing.

It has been suggested that rapid RF reorganization results from unmasking of previously ineffective synapses following denervation (Wall 1977; Calford and Tweedale 1991b). An unmasking hypothesis is supported by the observation that the reorganized RFs of DCN neurons often have response properties from the original RFs (Pettit and Schwark 1993). Calford and Tweedale (1991c) observed that peripheral C fiber blockade produces rapid appearance of new, excitatory RFs in SI neurons of cat and flying fox. They proposed that C fiber inputs within the spinothalamic tract could be the source of this masking inhibition in SI. The rapid RF reorganizations seen in cortical neurons

following capsaicin are comparable to those which appear following amputation or peripheral lidocaine injection (Calford and Tweedale 1991c). However, in raccoon ventroposterolateral thalamus (VPL), denervation by nerve cut or peripheral lidocaine results in large, new RFs which are inhibitory; whereas peripheral capsaicin injection produces new smaller RFs which are excitatory (Rasmusson et al. 1993). From these observations, the authors concluded that capsaicin and lidocaine injections produce rapid RF reorganization by different mechanisms.

In the present study we used capsaicin injection into the peripheral RFs of cuneate neurons in order to begin to understand the mechanisms which underlie rapid reorganization. The methods used were similar to Calford et al. (1991c) and Rasmusson et al. (1993) to facilitate comparisons across these studies. A second objective of the present study was to determine if rapid RF reorganization resulting from capsaicin-induced blockade differed from rapid RF reorganization which follows lidocaine blockade. Capsaicin produced rapid RF reorganization in every neuron tested. The reorganized RFs produced by capsaicin and lidocaine were similar. Further, the results suggest that blockade of a subset of peripheral inputs is sufficient to produce RF reorganization of cuneate neurons.

3.3 Methods

Experiments were performed on ten adult cats. Each cat was injected with ketamine (30 mg/kg, i.m.) and atropine (0.04 mg/kg, i.m.), and cannulae were inserted into a femoral vein and artery. Anesthesia was induced with sodium thiopental (20 mg/kg,

i.v.) and maintained throughout the experiment by continuous infusion (3-4 mg/kg-h). A tracheostomy was performed and the cat was mounted in a stereotaxic frame. Throughout the experiment blood pressure, heart rate, and body temperature were monitored and maintained within normal physiological levels. The depth of anesthesia was monitored by continuous recording of the EEG, blood pressure, and heart rate.

A craniotomy was performed and a pair of stimulating electrodes (monopolar, .5 μm exposed tips) were positioned in the medial lemniscus at the caudal pole of the ventroposterolateral thalamic nucleus (VPL). A small portion of the occipital region of the skull was removed and the cisterna magna was opened to access the DCN. Recording electrodes were introduced into the DCN in a horizontal plane from the rear. The craniotomy and DCN recording site were covered with 4% agarose. Prior to recording from the DCN, the cat was paralyzed with gallamine triethiodide (8 mg/kg-hr, i.v.) and artificial ventilation was begun. Expired CO_2 was monitored and maintained at 4%.

Single unit recordings were made from the DCN using parylene- or glass-coated tungsten electrodes (5-15 μm tips). When a single neuron was isolated, its RF was carefully mapped with hand-held stimuli (wooden probes, fine brushes, air puff, tuning fork). To test for slowly-adapting responses, a fixed stimulus was used and the paw was positioned so that it was isolated from movement caused by the cat's respiration. If tactile stimulation of a skin area inhibited the cell's ongoing spontaneous activity, it was defined as an inhibitory RF.

The cell's response to VPL stimulation was tested. Responses that had a fixed latency, fixed threshold, and that could follow a train of three spikes at frequencies of > 500 Hz were classified as antidromic.

The stability of RF boundaries was tested by remapping the field over a period of 30 min. The RF was drawn on a map of the limb and, in most cases, marked directly on the limb.

Action potentials from single units were identified and monitored throughout the experiment using a digital oscilloscope that was triggered by the output of a window discriminator. Spontaneous activity was periodically sampled by a computer which calculated the average firing rate over periods of 20 sec. Spontaneous activity was sampled (20 sec intervals) for one to two minutes immediately following an injection into a RF. In later experiments spontaneous activity was continuously monitored by a computer program which calculated average firing frequency every five sec.

Application of capsaicin (10% dissolved in 70% ethanol) to peripheral RFs was administered using one to three subcutaneous injections (10-30 µl/injection). In three cases additional capsaicin injections were made.

Following injection, the RF was mapped repeatedly until the reorganized RF became stable, after which the RF was mapped at regular intervals of 10-15 min. To test for effects of the vehicle, in nine experiments a subcutaneous injection of 70% ethanol was made prior to the capsaicin injection. This injection was made at the same site and with the same volume as the subsequent capsaicin injection. The RF was then mapped for at least 20 min prior to capsaicin injection. In eight experiments, additional injections of 2%

lidocaine were made subsequent to the capsaicin injections. In all but one cell, these injections were made at least 20 min after the reorganized RF had stabilized. The volume and location of the lidocaine injections were the same as the capsaicin injections which preceded them. In one cell, the lidocaine injection was repeated to produce further blockade of the original RF.

In one experiment a solution of 1% capsaicin dissolved in 1:1 dimethyl sulfoxide (DMSO) and ethanol (EtOH) was applied topically to a RF. Capsaicin applied in this manner is an irritant which produces sensitization, but not desensitization, of nociceptive afferents (Baumann et al. 1991).

Comparisons were made between the size of injections, injection substance, changes in spontaneous activity, original RF block, reorganized RF size, original RF properties, and reorganized RF properties using nonparametric statistical tests.

Each recording site was marked by making an electrolytic lesion. At the conclusion of each experiment, a lethal overdose (60 mg/kg) of thiopental was rapidly administered and the cat was perfused with normal saline followed by a solution of paraformaldehyde and glutaraldehyde. After cryoprotection in 30% sucrose, the DCN was sectioned (50 μm, coronal) on a freezing microtome and sections were stained with thionin.

3.4 Results

All results are from recordings of single cells. This approach permitted a detailed examination of RF properties before and after RF reorganization, and it also eliminated the

potential difficulty of instability of multiple unit recordings which may give rise to apparent RF shifts. Twenty-two neurons were recorded. All were located in the cluster region of the middle cuneate.

Twenty cells were tested with subcutaneous capsaicin injection, and in every cell this produced rapid RF reorganization (Table 2; e.g., Fig. 2). The RF reorganization began 5 - 16 min after capsaicin injection for all but one cell. The volume of capsaicin initially injected ranged from 10 μl (1 injection site) to 90 μl (3 injection sites, each 30 μl). Additional capsaicin injections were made for three cells. In two of these, the additional injections resulted in further expansion of the existing reorganized RF. The third cell did not exhibit RF reorganization until the additional capsaicin injection was administered. Nevertheless, there was no relation between the volume of capsaicin injected, the size of the reorganized RF, and the type of reorganization.

3.4.1 Effects of capsaicin and lidocaine injections on the original receptive field

Intradermal capsaicin injection has been reported to produce desensitization of unmyelinated and thinly myelinated fibers at the injection site within minutes (Baumann et al. 1991; LaMotte et al. 1992). Such injections can also raise the thresholds of some myelinated fibers, especially Type II slowly-adapting (SA) fibers (Baumann et al. 1991), and bath application of capsaicin to cat sciatic nerve can produce a nonspecific conduction block (Such and Jancso 1986). Based on the results of these and many other studies, it appears that low doses of capsaicin injected subcutaneously will tend to silence unmyelinated and thinly-myelinated peripheral afferents. Consequently, we determined if

capsaicin-induced blockade of the original RF in DCN cells was related to either the original RF properties, the reorganized RF properties, or both.

Capsaicin injection produced no changes in the responses from the original RF in 10 cells (3 SA, 7 rapidly adapting (RA)). In the remaining 10 cells, capsaicin injection either transiently blocked the original RF (3 cells; 1 (SA), 2 (RA)) or resulted in decreased responsiveness to stimulation (7 cells; 1 SA, 6 RA). In six of these cells, responsiveness of the original RF recovered completely (n = 4) or partially (n = 2) by the time the reorganized RF appeared. In the remaining four cells, the original RF did not begin to recover until after the onset of RF reorganization. Recovery of responsiveness in the original RF never corresponded to a decrease in responsiveness in the reorganized RF.

Following RF reorganization, the original RFs of eight cells were injected with 2% lidocaine. The lidocaine injections blocked at least part of the original RF in each of the cells. In seven cells no additional RF reorganization occurred (Figs. 1, 2). In the remaining cell further RF reorganization was observed. As esthesia returned to the original RF of this cell, the lidocaine-induced reorganized RF persisted and even increased slightly in size, suggesting that this new RF was not simply the result of blockade of low-threshold afferent input.

In half the cells tested, capsaicin injection produced RF reorganization without blockade of low-threshold inputs to the cells. Loss of responsiveness from the original RF was not related to the injection volume of capsaicin. Also, there was no relation between blockade of the original RF and the size of the reorganized RF, the type of RF reorganization produced (see below), or change in spontaneous firing rate. These data

suggest that blockade of the major afferent drive (i.e. low-threshold inputs) to DCN neurons is not a necessary condition to produce RF reorganization.

The stability of the reorganized RFs varied. In half the cells, the reorganized RFs remained unchanged throughout the remainder of the recording period (range of observation times: 20-109 min; Figs. 3, 4). In the remaining cells, in one case the reorganized RF disappeared after 45 min, in two cases the reorganized RFs diminished in responsiveness (observation times: 56 & 111 min), in four cases the reorganized RFs fluctuated in size or responsiveness (range of observation times: 33-85 min; Fig. 2), and in three cases the reorganized RFs underwent further expansions (range of observation times: 75-96 min). The stability of the reorganized RF was not related to the degree of blockade of the original RF, capsaicin injection volume, original RF properties, or reorganized RF properties. There was also no apparent relation between recovery of responsiveness from the original RF and changes in reorganized RFs.

3.4.2 Control experiments

Control injections of ethanol vehicle produced partial RF blockade in two of nine cells (both RA) but never resulted in RF reorganization (Table 2). Seven cells were subsequently tested with capsaicin, and these injections resulted in the appearance of reorganized RFs. These data suggest that injection of ethanol alone was not sufficient to produce RF reorganization.

Topical application of 1% capsaicin (in 1:1 DMSO and EtOH) to the peripheral RF of one cuneate neuron produced no change in RF size or properties over 24 min. At 25 min capsaicin was injected into the RF and resulted in RF reorganization.

3.4.3 Properties of reorganized receptive fields

The reorganized RFs appeared as either contiguous expansions of the original RF or as new, non-contiguous fields. Some of the reorganized RFs had response properties which differed from the original RF, including some which contained new, inhibitory fields.

3.4.3.1 Simple receptive field reorganization

In 11 cells, the response properties of the reorganized RF and the original RF were the same (Table 2). Seven of these cells had single, excitatory, RA, hair fields. Simple RF reorganizations were also observed in four cells with other RF properties (Table 2). For example, capsaicin injection into the RF of a cuneate cell with a single, excitatory, SA, RF on hairy skin produced a simple RF expansion which appeared at 5 min post-injection (Fig 2). The expanded RF reached its largest extent at 8 min; thereafter, the expansion persisted but its responsiveness fluctuated. Lidocaine injection at 33 min blocked the responsiveness of parts of the original RF and the expanded RF, but produced no further reorganization over the remainder of the recording period.

3.4.3.2 Changes in response class

Reorganized RFs with response classes (e.g. hair, skin, claw) which differed from the original RF were present in six cells (Table 2). In four cells the original RFs were RA hair fields and the reorganized RFs were either RA hairy skin (n = 3) or RA glabrous skin (n = 1; plus, a new inhibitory field; see below). In another case, the original RF was RA hairy skin and the reorganized RF was RA claw and hairy skin. The original RF of the remaining cell was RA glabrous skin on toepads four and five (Fig 3). Five min after capsaicin injection into both toepads, the cell exhibited a new RA field on hairy skin distal to toepad four. At 16 min (post-injection time) the new field had expanded in size and included the distal skin of toes three, four, and five. Further expansion onto the glabrous skin of the central footpad occurred at 20 min, and this expansion was not contiguous with the previous expansion on hairy skin. At 40 min lidocaine injections were made into toepads four and five. These injections resulted in decreased responsiveness from toepad four. Lidocaine injections were repeated 6 min later, and resulted in blockade of responsiveness from most of toepad four and decreased responsiveness from toepad five. No additional RF reorganization was produced as a result of the lidocaine injections (observation time: 40 min).

3.4.3.3 New inhibitory fields

Three cells developed new, inhibitory fields following capsaicin injection (Table 2). These cells did not have inhibitory components in their original RFs. In two cells the original RF was hair (1 RA, 1 SA) and the reorganized RF contained inhibitory hair fields.

The original RF of the third cell was RA, glabrous skin of toepad three (Fig. 4). Seven min after capsaicin injection, stimulation of toepads two and four inhibited ongoing spontaneous activity. At nine min, stimulation of toepad five produced an inhibitory response that was weaker than the response from the other two fields. At 15 min, stimulation of the central footpad produced very weak inhibitory modulation of spontaneous activity. All of these inhibitory fields were confined to glabrous skin and were discontinuous with each other and with the original RF. No further changes were noted (observation time: 35 min).

3.4.4 Spontaneous activity

Spontaneous firing rates were analyzed in nine cells injected with ethanol and in nineteen cells injected with capsaicin (seven cells were in both groups: see Table 2). Following ethanol injection, the spontaneous firing rate increased in four cells, decreased in two cells, and was unchanged in three cells (Mann-Whitney U test, alpha = 0.05). Following capsaicin injection, the spontaneous firing rate increased in 13 cells, decreased in four cells, and was unchanged in two cells (Mann-Whitney U test, alpha = 0.05). For the seven cells which were included in both the ethanol and capsaicin test groups, the spontaneous firing rate of five cells responded similarly to both treatments, spontaneous firing rate of one cell decreased after ethanol and increased after capsaicin, and spontaneous firing rate of one cell was unaffected by ethanol and increased by capsaicin. Thus in this small sample, ethanol and capsaicin injection had similar effects on spontaneous activity rates.

A normalization procedure was used to compare the relative magnitude of the change in spontaneous activity firing rate without regard to the direction of change. If injection of ethanol or capsaicin produced a significant increase in a cell's spontaneous firing rate, the post-injection rate was divided by the pre-injection rate (two cells in the capsaicin injection group had initial spontaneous firing rates of zero and were excluded). If injection produced a significant decrease in spontaneous firing rate, the pre-injection rate was divided by the post-injection rate. Thus, decreases and increases in spontaneous firing rate were scaled the same. Capsaicin injection produced a significantly greater change in the normalized rate of spontaneous activity than ethanol injection (Mann-Whitney U test, $p < 0.02$).

For most of the cells, the changes in spontaneous activity produced by ethanol and capsaicin were transitory. The greatest changes in spontaneous firing rate took place immediately after injection and then began to decline within minutes. In all but one cell, spontaneous activity returned to near pre-injection levels by the end of the recording period (EtOH: 6 of 6; capsaicin: 15 of 16). Changes in spontaneous firing rate were not related to injection volume or RF reorganization events.

Sixteen cells were tested by electrical stimulation of the medial lemniscus. Eleven cells were driven antidromically, four cells were driven synaptically, and one cell was not driven. There were no significant differences in spontaneous firing rates or RF reorganization between these groups.

3.5 Discussion

The results of the present study suggest that in the cat, cuneate neurons with cutaneous peripheral RFs undergo rapid RF reorganization following subcutaneous injection of capsaicin. This RF reorganization occurred even when the original RF remained responsive. All neurons tested with capsaicin exhibited RF reorganization. The absence of RF reorganization following subcutaneous injection of the ethanol vehicle suggests that rapid RF reorganization occurred as a specific response to capsaicin. These results are similar to those of Calford and Tweedale (1991c) for neurons in SI of cat and flying fox. Capsaicin-induced rapid RF reorganization has also been demonstrated for raccoon VPL neurons (Rasmusson et al. 1993). Although it has been suggested that capsaicin-induced rapid RF reorganization of SI neurons may be mediated by the spinothalamic system (Calford and Tweedale 1991c), the present data suggest that such reorganization can also arise within the dorsal column-medial lemniscal system.

We found no differences between capsaicin-induced RF reorganization of cuneate neurons in the present study and lidocaine-induced RF reorganization of cuneate neurons (Pettit and Schwark 1993). These results are consistent with the idea that RF reorganization is produced by the same mechanism following either type of injection.

3.5.1 Mode of action of capsaicin

The effects of capsaicin on primary afferents are complex, and vary with dosage and route of administration (Holzer, 1991). C and A delta fibers appear to be most susceptible to capsaicin, although it has been shown that bath application can also block

larger myelinated fibers (Such and Jancso, 1986). Delivered by intradermal injection, 0.1 mg of capsaicin initially excites C and A delta afferents, but within minutes results in hypoalgesia and desensitization of these afferents as well as elevated response thresholds in some low-threshold mechanoreceptor afferents, especially slowly adapting type II (SA II) (Simone et al. 1989; Baumann et al. 1991; LaMotte et al. 1992). Topical application of capsaicin produces excitation of C and A delta afferents without subsequent desensitization (Baumann et al. 1991; LaMotte et al. 1992).

In the present study subcutaneous injections of capsaicin were made into the RF. The dose range of initial injections was 1-9 mg. The sizes of these injections were comparable to those used in a previous study of SI reorganization (Calford and Tweedale 1991c).

The present data suggest that rapid RF reorganization of cuneate neurons was produced by blockade of a subset of peripheral afferent inputs. In half of the cells, there was no apparent loss of cutaneous responsiveness in the original RF following capsaicin injection. In the remaining cells, capsaicin injection produced at least a partial blockade of low-threshold, mechanoreceptive afferents; however, in 60% of these cells RF reorganization did not begin until the original RF had begun to recover. Also, recovery of responsiveness in the original RF never coincided with disappearance of the reorganized RF. Lidocaine injection, administered after capsaicin-induced reorganization, blocked low-threshold afferents from the original RF but produced further RF reorganization in only one cell. Thus, blockade of low-threshold mechanoreceptors from the original RF was not necessary to produce RF reorganization. This is consistent with the observation

that RF reorganization temporally corresponds with the abolition of the C fiber component of the compound action potential during application of capsaicin to the radial nerve (Calford and Tweedale 1991c).

It is unlikely that RF reorganization was produced by activation of nociceptors as occurs in the dorsal horn (McMahon and Wall 1984; Cook et al. 1987; Hylden et al. 1989; Laird and Cervero 1989; Simone et al. 1989; Woolf and King 1990; Dougherty and Willis 1992; Hoheisel et al. 1993). Excitation of C and A delta afferents by topical application of capsaicin to a peripheral RF failed to produce RF reorganization. Also, rapid RF reorganization of DCN neurons occurs following block of peripheral RFs by subcutaneous lidocaine injections which probably do not excite nociceptors (Pettit and Schwark 1993). Calford and Tweedale (1991c) noted that neither topical capsaicin application nor electrical stimulation of C fibers results in RF reorganization of SI neurons.

3.5.2 Unmasking of inputs in reorganized receptive fields

The present results suggest that the specificity of RF response properties exhibited by cuneate neurons derives in part from physiological mechanisms which normally mask some afferent inputs. Previous single-unit studies in cat have indicated that most DCN neurons exhibit specificity for response class (i.e., hair, skin), adaptation characteristics (i.e., RA, SA), and submodality (i.e., cutaneous, deep) (Kruger et al. 1961; Gordon and Jukes 1964; Brown et al. 1974; Janig et al. 1977; Golovchinsky 1980; Cheema et al. 1983). The reported proportions of cat DCN neurons with convergent RF properties ranges from 1% to 18% (Gordon and Jukes 1964; Brown et al. 1974; Golovchinsky 1980;

Saade et al. 1982). In the present study, two of 20 neurons had convergent RF properties. After RF reorganization, an additional six neurons exhibited convergent RF properties. In a previous study of lidocaine-induced reorganization, there was a similar increase in the number of neurons with convergent response class properties (3 of 23 before injection, 7 additional neurons after reorganization; Pettit and Schwark 1993). Cell clusters in the middle cuneate of the cat are approximately 200-300 μm in size, and appear to constitute functional units in which cells have similar response properties (Kuypers and Tuerk 1964; Dykes et al. 1982). However, the arborization of primary afferents in this region is widespread, and the dendritic spread of DCN neurons can reach 500 μm (Fyffe et al 1986a,b; Weinberg et al. 1990). Thus, physiological masking of widespread inputs might serve to produce both small RFs and specificity of RF response properties in cuneate cluster neurons.

3.5.3 Inhibition in reorganized receptive fields

New inhibitory fields appeared in the reorganized RFs of cuneate neurons following capsaicin-induced reorganization. These results agree with earlier observations, based on the results of lidocaine injections, that some cuneate neurons have inhibitory inputs which are normally masked (Pettit and Schwark 1993).

The nature of the inhibitory changes which we observed differ from those reported in raccoon SI and VPL (Rasmusson and Turnbull 1983; Kelahan and Doetsch 1984; Turnbull and Rasmusson 1990; Rasmusson et al. 1993). In raccoon, inhibitory fields arise following denervation by lidocaine, but not capsaicin, and their size and appearance

depend upon whether the RF is located on hairy or glabrous skin (Rasmusson et al. 1993). The present results demonstrate that widespread, inhibitory, reorganized RFs occur in the dorsal column-medial lemniscal system. Further, elimination of all afferent drive from the original RF is not required to produce such RFs. In cat cuneate neurons, either capsaicin or lidocaine injection (Pettit and Schwark 1993) can produce large, inhibitory, reorganized RFs spanning several digits. These new, inhibitory fields occur in neurons with original RFs on either hairy or glabrous skin, and are not related to the degree of blockade of the original RF.

3.5.4 Mechanisms of rapid receptive field reorganization in cuneate neurons

Rapid RF reorganization occurs following the loss of input from the peripheral RF. It has been proposed that inhibition arises from the peripheral RF and masks some inputs (Wall 1977; Calford and Tweedale 1991b). According to this hypothesis, rapid RF reorganization is produced following RF denervation due to the loss of masking inhibition. In a simulation study, it has been suggested that moderate levels of background synaptic activity can decrease the electrotonic size of a neuron (Bernander et al. 1991). As a consequence, inputs located on distal dendrites would be rendered ineffective by background synaptic activity. Thus, normal background synaptic activity arising from afferent input would produce masking inhibition of some inputs. If this model is accurate, rapid RF reorganization could arise by an increase in electrotonic size resulting from decreased background synaptic activity produced by denervation.

The present data do not appear to support this model. Changes in spontaneous firing rate were not to related to rapid RF reorganization. Thus, decreases in masking inhibition which may produce rapid RF reorganization were not reflected by corresponding changes in spontaneous firing rate.

In cuneate neurons, RF reorganization apparently does not result from a disruption of primary afferent depolarization (PAD). There are at least two inhibitory processes in the DCN: postsynaptic and presynaptic (PAD). PAD is a powerful source of presynaptic inhibition in the cat DCN, and arises from stimulation of peripheral afferents or sensorimotor cortex. PAD is mediated through inhibitory cuneate interneurons (Anderson et al. 1964a,b,c; Jabbur and Banna 1968; Anderson et al. 1970; Putnam and Whitehorn 1973; Bromberg et al. 1975; Bystrzycka et al. 1977). Two observations suggest that PAD is not involved in rapid RF reorganization. First, myelinated afferents from the original RF can remain active during either capsaicin- or lidocaine-induced rapid RF reorganization (Pettit and Schwark 1993). Thus, rapid RF reorganization does not depend upon interruption of PAD from this source. Second, there was no effect of SI removal on the range of sizes for original RFs or reorganized RFs of cuneate neurons (Pettit and Schwark 1993). Thus, the abolition of cortical PAD does not produce RF reorganization, nor does it enhance the size of reorganized RFs following peripheral denervation.

It has been suggested that tonic activity in peripheral C fibers produces inhibition which functions to restrict RF size (Calford and Tweedale 1991c). There are few classes of primary afferents which are tonically active. Spontaneous activity is present in cold and warm receptors in the skin of the cat's foot and, at least in the foot, these receptors are

innervated primarily by C fibers (Stolwijk and Wexler, 1971; Handwerker and Neher, 1976). Spontaneous activity is also present in SA II mechanoreceptors in the cat (Burgess et al. 1968; Amassian and Giblin 1974). Both of these classes of afferents are susceptible to capsaicin blockade, and thus, are potential sources for tonic inhibition of RF size in cat cuneate neurons.

Evidence that C and A delta fibers provide direct input to the DCN is limited. Unmyelinated fibers constitute 29% of the primary afferents in the sacral dorsal columns of the cat. The role of C fibers in the cat DCN is largely unknown, although some of the cells in the middle region of the cat gracile nucleus respond to noxious thermal and mechanical stimuli (Cliffer et al. 1992). Presumably, some of these afferents could serve as a source of tonic inhibition to the DCN. Another source of C fiber input to the cat DCN may be postsynaptic dorsal column afferents which originate in the dorsal horn (Rustioni 1974).

It has been suggested that tonic masking inhibition might be produced by release of a neuropeptide from C fibers (Byrne and Calford 1991). If C fibers reach the DCN, a possible mechanism for tonic masking inhibition is the tonic release of a neuropeptide from these fibers onto DCN inhibitory interneurons. The most likely candidate neuropeptides are substance P (SP) and calcitonin gene-related peptide (CGRP). SP and CGRP afferents are present in the cat DCN (Westman et al. 1984; Conti et al. 1990; Fabri and Conti 1990). The distribution of CGRP terminals and receptors in DCN is not known, but SP terminals are found between the cuneate cell clusters (Westman et al. 1984), and SP receptors are present throughout the clusters region (Chapter 4). Similar mismatches

between peptide afferents and receptors have been described in other brain regions, and it has been suggested that peptides may diffuse considerable distances to exert their effects (Herkenham and McLean 1986). Iontophoresis of SP onto cat cuneate neurons produces slow excitation which can last for several minutes following cessation of application (Krnjevic and Morris 1974). Approximately 25% of the neurons in the DCN are inhibitory, as marked by gamma-aminobutyric acid (Heino and Westman 1991) or glutamic acid decarboxylase (Rustioni et al. 1984).

Tonic release of a peptide such as SP could produce slow excitation of inhibitory interneurons which, in turn, could produce long-lasting presynaptic inhibition of primary afferent terminals and therefore mask some afferent inputs. Such masking inhibition, while presynaptic, would be different than PAD. Masking would result from ongoing activity in a subset of RF inputs, whereas PAD occurs in response to stimulation of myelinated RF afferents. Such a slow time course of action is consistent with the observation that even when inputs from the original RF are blocked by lidocaine injection, RF reorganization does not begin for several minutes (e.g., Fig. 1A, Chapter 2; Byrne and Calford 1991). As has been suggested (Byrne and Calford 1991), a slow time course implies that a relatively low rate of tonic activity would be sufficient to produce masking inhibition.

In summary, the present data suggest that rapid RF reorganization in cat cuneate neurons is produced following blockade of a subset of peripheral afferents. Rapid RF reorganization did not require complete blockade of the main afferent drive from the RF. These data are consistent with the hypothesis that tonic activity from a subset of peripheral afferents can influence the activity of inhibitory interneurons, and thus modulate RF size

(Calford and Tweedale 1991c). The source of this tonic inhibition may be C fibers; however, other possible sources are A delta fibers, SA II fibers, or some combination of these. Although a role for the spinothalamic system in rapid RF reorganization has been suggested (Calford and Tweedale 1991c; Rasmusson et al. 1993), the present results suggest that capsaicin-induced RF reorganization can occur within the lemniscal system as well.

TABLE 2. Receptive field response properties before and after testing with ethanol, capsaicin, and lidocaine.

Original RF	EtOH effect	EtOH Obs Time (min)	Capsaicin dose# (ul)	Capsaicin ORF effect	New RF	Size of New RF (RRF/ORF)	Obs Time (min)	Lidocaine Effect	Lidocaine Obs Time (min)
EtOH Only									
RA, hairy skin	p/b	23	-	-	-	-	-	-	-
SA, glab skin	n/c	22	-	-	-	-	-	-	-
Simple Reorganization									
SA, h skin	-	-	10	n/c	SA, h skin	1.9	33	p/b	45
SA, h & g skin	n/c	24	10	n/c	SA, h & g skin	1.75	63	p/b	30
RA, hair	-	-	2x10	p/b	RA,hair	0.17	70	-	-
RA, (tap?) g skin	-	-	3x30	p/b	tap, g skin	0.37	20	-	-
RA, hair	-	-	10	p/b	RA, hair	1.25	71	p/b	32
RA, hair & SA, h skin	-	-	2x10; 40	p/b	RA, hair & h skin	0.66	89	-	-
RA, hair	n/c	27	3x30	n/c	RA, hair	0.64	96	-	-
RA, hair	n/c	34	3x30	n/c	RA, hair	0.67	57	-	-
RA, hair	n/c	30	2x30;50;25	p/b	RA, hair	0.36	111	-	-
RA, hair	n/c	30	3x30;2x50	p/b	RA, hair	0.06	109	p/b	33
RA, hair; (-) all limbs	-	-	10	n/c	RA, hair	0.63	56	p/b	30

TABLE 2. (Continued)

Original RF	EtOH effect	EtOH Obs Time (min)	Capsaicin dose# (ul)	Capsaicin ORF effect	New RF	Size of New RF (RRF/ORF)	Obs Time (min)	Lidocaine Effect	Lidocaine Obs Time (min)
New Response Prop									
RA, g skin	-	-	2x20	n/c	RA, h skin	15.25	40	n/c*	44
SA, h skin	-	-	10	p/b	SA, claw, g & h skin	-	75	-	-
RA, hair	-	-	2x10	p/b	RA, h skin	0.37	60		
RA, hair; (-) hair (-) wrist joint	n/c	24	2x10	n/c	RA, h skin	0.48	61	p/b	32
RA, hair	p/b	75	3x30	n/c	RA, hair & h skin	0.35	74	p/b**	64
RA, hair***	-	-	2x10	p/b	RA, g skin; (-) hair	1.38	76	-	-
SA, hair	-	-	3x30	n/c	RA, hair	0.68	85	-	-
New Inhibitory Fields									
SA, hair	-	-	2x10	p/b	(-) hair	0.81	45	-	-
RA, g skin	-	-	2x10	n/c	(-) g skin	3	35	-	-

RRF = reorganized receptive field, ORF = original receptive field

h skin = hairy skin, g skin = glabrous skin, (-) = inhibitory field, p/b = partial blockade, n/c = no change

same dose was used for EtOH and lidocaine when given

* this RF was injected twice with lidocaine

** this neuron had a small additional RF expansion after lidocaine injection

*** this neuron had both new excitatory and inhibitory fields

Figure 2. For figures 2, 3 and 4: Each column shows the temporal sequence of RF changes after injection of capsaicin. The original RF is shaded black and the new RF is stippled. The first figure shows the original RF and capsaicin injection volume. Time after injection of capsaicin is indicated at the bottom of each figure. The camera lucida drawing indicates the recording site. Figure 2 shows a simple form of receptive field (RF) reorganization, in which the new RF responded to the same type of stimulus as did the original RF. Injection of capsaicin produced no loss of responsiveness from the original slowly adapting (SA) RF. RF reorganization began 5 min after injection and the new RF was SA. A further expansion appeared at 8 min. Fluctuation in size and responsiveness of the new RF was noted at 17 and 27 min (weaker responding areas are shaded gray). Injection of lidocaine at 34 min silenced part of the original RF and part of the new RF. No further reorganization in RF properties was noted for the remainder of the recording period.

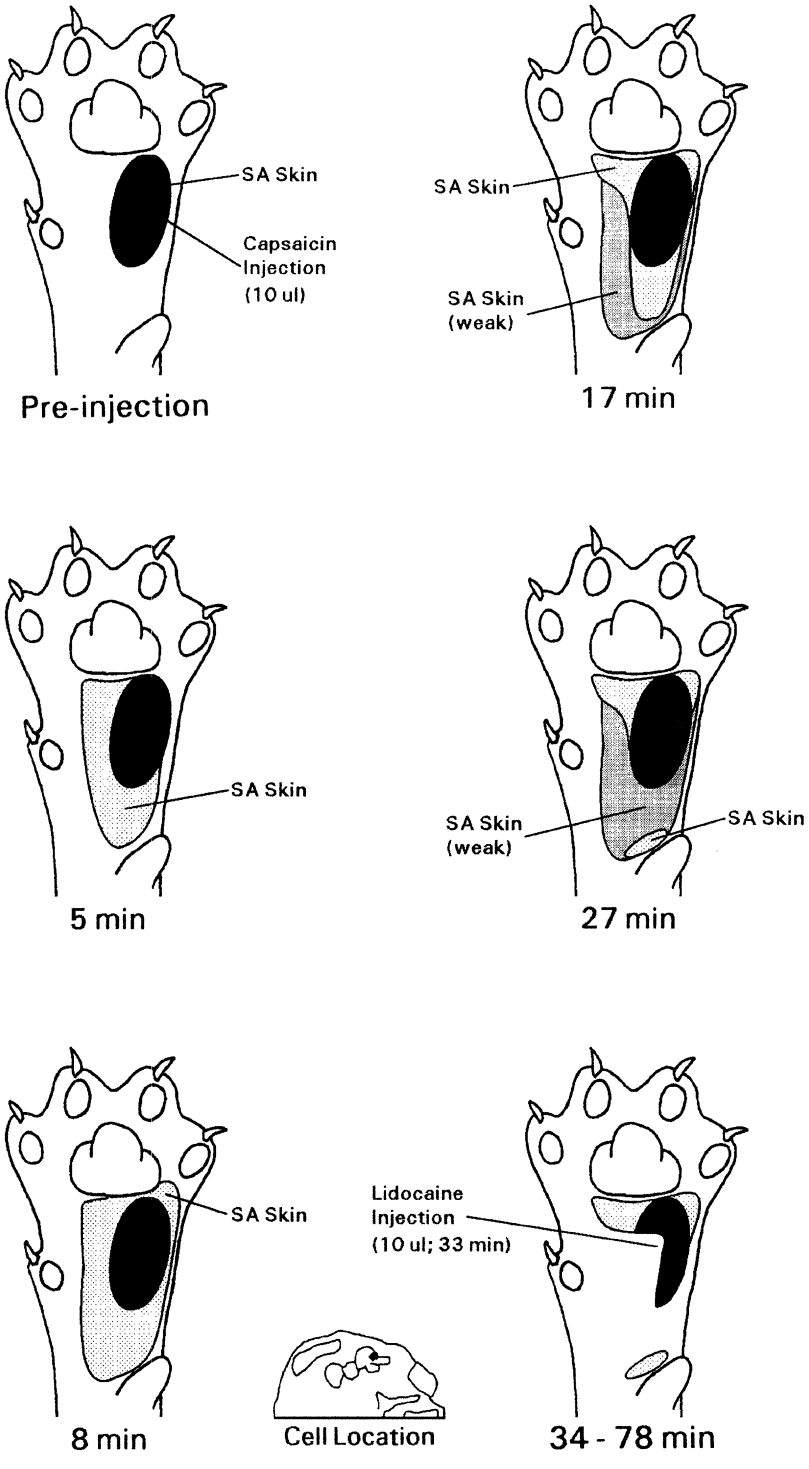

SA Skin
Capsaicin Injection (10 ul)
Pre-injection
SA Skin
SA Skin (weak)
17 min
SA Skin
5 min
SA Skin (weak)
SA Skin
27 min
SA Skin
8 min
Cell Location
Lidocaine Injection (10 ul; 33 min)
34 - 78 min

Figure 3. In this neuron the original RF responded to stimulation of glabrous skin on toepads 4 and 5 and the new RF responded to stimulation of both glabrous and hairy skin. Injections of capsaicin produced no loss of responsiveness from the original RF. At 5 min a new RF appeared on hairy skin distal to toepad 5. This new RF continued to expand until 20 min after injection. At this time the glabrous skin of the central footpad also became responsive. Lidocaine injections at 43 and 49 min produced complete blockade of some of the original RF and decreased responsiveness from the rest of the original RF (gray shading). These injections produced no changes in the new RF. The original RF had recovered responsiveness by the end of the recording period (84 min).

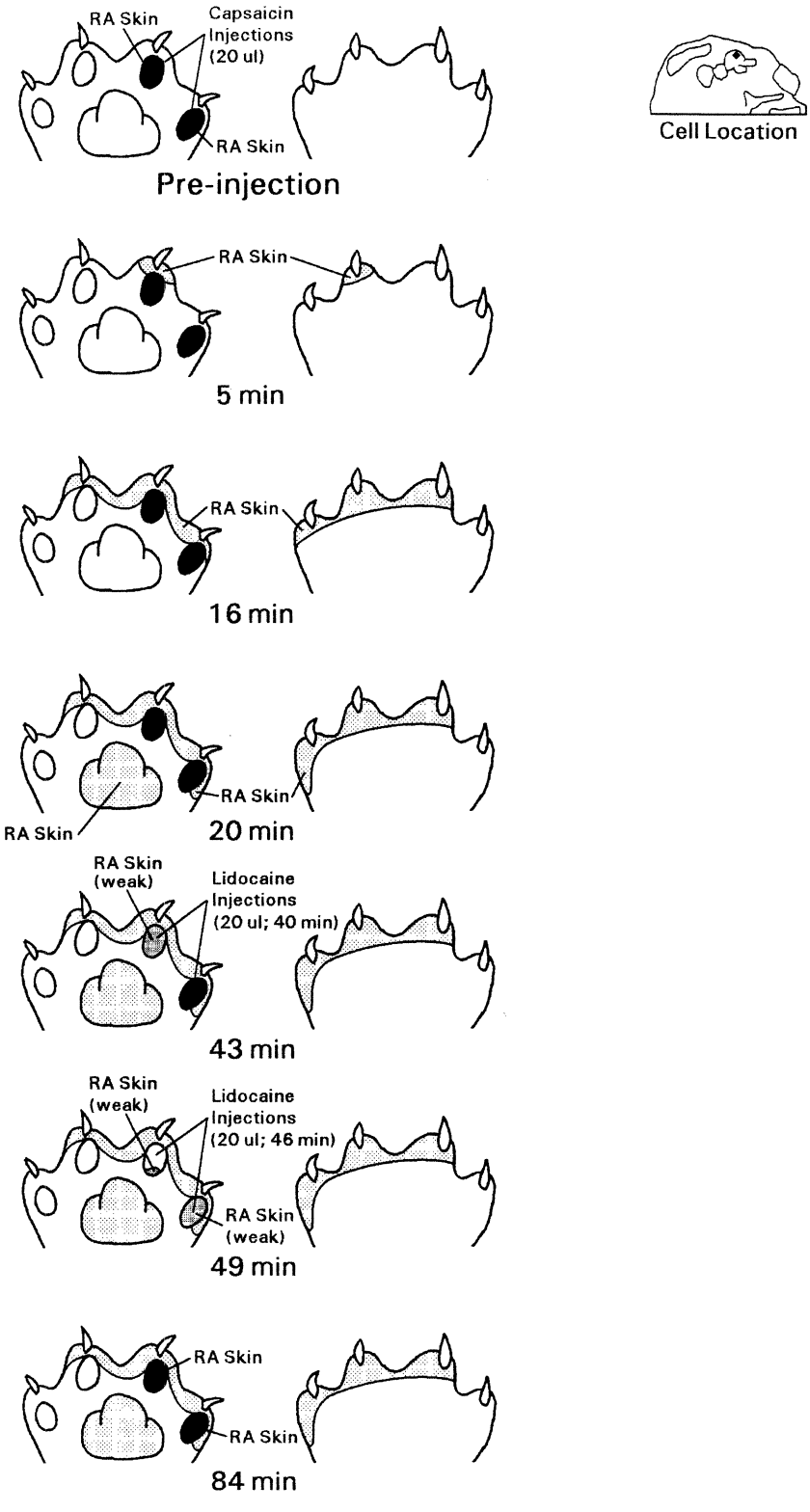
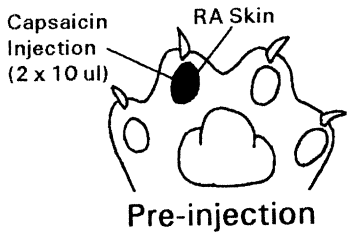

RA Skin
Capsaicin Injections (20 ul)
RA Skin
Pre-injection
Cell Location
RA Skin
5 min
RA Skin
16 min
RA Skin
RA Skin
20 min
RA Skin (weak)
Lidocaine Injections (20 ul; 40 min)
43 min
RA Skin (weak)
Lidocaine Injections (20 ul; 46 min)
RA Skin (weak)
49 min
RA Skin
RA Skin
84 min

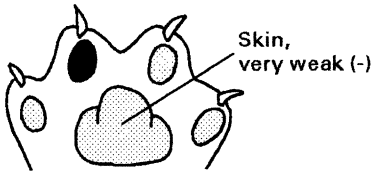
Figure 4. RF reorganization that resulted in new inhibitory RFs. The original RF of this neuron responded to stimulation of glabrous of toepad 3. Capsaicin injection produced no loss of responsiveness from the original RF. At 7 min stimulation of toepads 2 and 3 produced inhibition of spontaneous activity. Stimulation of toepad 5 became weakly inhibitory to spontaneous activity at 9 min. After 15 min, stimulation of the central pad produced an inhibitory modulation of spontaneous activity. No further changes in RF properties were noted for the remainder of the recording period (35 min).



Pre-injection

7 min

9 min



15 - 35 min

Cell Location

CHAPTER 4

4. DISTRIBUTION OF SUBSTANCE P RECEPTOR BINDING SITES IN RAT, CAT, MONKEY AND HUMAN DORSAL COLUMN NUCLEI

4.1 Summary

Substance P receptor binding sites were labeled in the dorsal column nuclei (DCN) of the rat, cat, monkey, and human using film autoradiographic procedures. Binding sites of Bolton-Hunter-labeled [^{125}I]substance P were distributed most densely in the core region of n. cuneatus in each species. Within this region, the binding pattern had a parcellated appearance due to high levels of binding in the cell clusters of the core region. The receptor binding patterns were similar to the patterns of cytochrome oxidase staining. In every species, a parcellated pattern of cytochrome oxidase staining was apparent.

4.2 Introduction

Substance P is a neuropeptide which is found in neurons throughout the nervous system (Otsuka and Yoshioka 1993). Within the somatosensory system, substance P is found in small myelinated and unmyelinated primary afferents (McCarthy and Lawson 1989), where it appears to function as a modulator of neurotransmission (Henry 1976; Randic and Miletic 1977). The localization of substance P in these afferents, and the high numbers of substance P receptors found in the dorsal horn (Charlton and Helke 1985; Mantyh et al. 1989; Yashpal et al. 1990), suggest that substance P plays a role in the

sensation of pain. Data consistent with this idea come from physiological studies describing a role of substance P in central mechanisms of hyperalgesia (e.g., Dougherty et al. 1994).

Functional roles for substance P at higher levels of the somatosensory system are less well defined. A small number of substance P-immunoreactive axons have been localized to specific regions in the dorsal column nuclei (DCN, which include n. cuneatus and n. gracilis) of the rat and cat (Conti et al. 1990). The cytoarchitectonic organization of the DCN have been described in greatest detail for the cat. The nuclei can be divided into three rostrocaudal divisions, and within the middle division a dorsal "clusters" or "core" region can be distinguished from a ventral reticular region (e.g., Berkley et al. 1986; Cheema et al. 1983; Hand 1966; Kuypers and Tuerk 1964). A somewhat simpler scheme, which will be used in the present report, divides the DCN into a core region and a reticular region (Berkley et al. 1986). The DCN of the rat and primate can also be divided into core and reticular regions (Boivie 1978; Maslany et al. 1992; Rustioni et al. 1979). Neurons in the core region are clustered into "cell nests" or clusters (Kuypers and Tuerk 1964; Berkley, et al. 1986). These neurons receive input from large, myelinated primary afferents, and project to the thalamus (e.g., Berkley et al. 1986). Outside the core region, small to medium sized neurons which lie in the reticular region of the nuclei receive input mostly from non-primary afferents traveling in the dorsal funiculi and dorsolateral funiculi (Gordon and Grant 1982; Rustioni 1974; Rustioni and Dekker 1974), and project to the cerebellum and spinal cord (Berkley et al. 1986).

The substance P-immunoreactive fibers and terminals which have been described in the cat DCN terminate primarily within the reticular region of the DCN, and avoid the core region (Conti et al. 1990; Westman et al. 1984). In the rat DCN, substance P-immunoreactive terminals are distributed more homogeneously throughout the nuclei (Conti et al. 1990). Potential cells of origin of the substance P-immunoreactive fibers in the DCN which are retrogradely labeled in double-labeling experiments are located in the dorsal root ganglia as well as in the dorsal horn (Conti et al. 1990).

A functional role for substance P in the DCN has not been described in detail. Iontophoretic application of substance P results in a long-lasting excitation of about 50% of the cat DCN neurons tested, presumably including neurons in the core region (Krnjevic and Morris 1974). Thus, substance P might act to bias the responses of DCN neurons to cutaneous inputs. However, the distribution of substance P effects does not appear to match the distribution of substance P-immunoreactive fibers in the DCN (Conti et al. 1990). It has recently been suggested that some neurotransmitters might act on postsynaptic receptors which are located some distance from the release sites (Herkenham and McLean 1986)), and there is evidence that this may be true for substance P synapses in the dorsal horn (Liu et al. 1994). In order to determine where substance P acts within the DCN, receptor autoradiographic methods were used in the present study to label substance P receptor binding sites. The results revealed that large numbers of substance P binding sites are located in the core of the DCN in a variety of mammalian species: the rat, cat, monkey, and human.

4.3 Methods

Substance P receptors were labeled by film autoradiography using Bolton-Hunter-labeled [^{125}I]substance P ([^{125}I]BH-SP). The rats (n=2) and cats (n=2) were killed by decapitation and the DCNs were rapidly removed and frozen in -30°C isopentane. The monkey (*Macaca nemestrina*) DCN was obtained from the Regional Primate Center at the University of Washington. The animal was anesthetized with ketamine and sodium pentobarbital before the brain was removed and rapidly frozen. The human DCN was obtained from the National Neurological Research Specimen Bank. The DCNs were sectioned at 20μm on a cryostat and thaw-mounted onto gelatin-subbed slides. Alternate series of sections were used for ligand binding, cytochrome oxidase histochemistry, and thionin staining.

To label substance P receptors, sections were brought to room temperature and preincubated for 10 min in 50mM Tris-HCl (pH 7.4) and 0.02% BSA, followed by incubation for 90 min in a solution containing 50pM [^{125}I]BH-SP (Amersham), 50mM Tris-HCl (pH 7.4), 3mM $MnCl_2$, 0.02% BSA, 4 mg/ml leupeptin, 40 mg/ml bacitracin, and 2 mg/ml chymostatin. The sections were then rinsed four times (1 min each) in 4°C buffer-BSA alone, briefly dipped in 4°C dH_2O, and dried in a stream of air. Nonspecific binding was determined by incubating sections as above, but with 1mM substance P added to the incubation solution.

Brain sections and ^{125}I Microscale standards (Amersham) were apposed to tritium-sensitive Hyperfilm (Amersham) for seven days. The films were developed in Kodak D-19 and processed further according to the manufacturer's instructions. Following preparation

of the autoradiographs, the sections were stained with thionin. Density profiles through the DCN were constructed using a video-based image analysis system (MCID, Imaging Research, St. Catherines, Ontario, Canada) and compared to landmarks in the sections stained for cytochrome oxidase and Nissl substance.

4.4 Results

In each of the species, cytochrome oxidase staining was very dense in the core of n. cuneatus (Fig 5). Within the core the staining pattern had a parcellated appearance that corresponded precisely with the cell nests found in this region. The areas surrounding the cell nests were more lightly stained. By comparison, the density of cytochrome oxidase staining in the reticular regions of the DCN was much lower. Levels of cytochrome oxidase staining in n. gracilis were intermediate to the core and reticular regions, and were more homogeneous than in n. cuneatus.

Binding patterns of [^{125}I]BH-SP were very similar to the patterns of cytochrome oxidase staining, especially in the cat (Fig 5). In each species, the highest binding levels were located in the core of the DCN. Outside the core regions, binding levels were much lower. Average binding levels, expressed as disintegrations per minute, for the species were (expressed as core:reticular): rat - 15228:2483; cat - 11269:2861; monkey - 12323:3350; human - 6136:1798. There were negligible levels of non-specific binding for each species.

Within the core region, differentiation of the cell clusters in the [^{125}I]BH-SP binding pattern was most distinct in the cat. The clusters were less distinct in the monkey

and human. Although clusters were visible in the core of the rat DCN in cytochrome oxidase-stained sections, they were not clearly differentiated by [^{125}I]BH-SP binding.

4.5 Discussion

Cytochrome oxidase staining revealed a parcellated staining pattern in n. cuneatus in all of the species examined in the present study. Such parcellated distributions have been described previously in the rat (Crockett et al. 1993) and monkey (Noriega and Wall 1991), but this is the first report of a similar organization in the cat and human. Except in the rat, the dense patches of cytochrome oxidase staining which gives rise to the patchy appearance correspond precisely to clusters of neurons which can be seen in Nissl-stained sections. In the rat, cytochrome oxidase-rich patches are obvious, but there is no obvious clustering of neurons in Nissl-stained sections (Crockett et al. 1993).

In the cat, the cell clusters which underlie the parcellated pattern apparently correspond to groups of neurons with similar submodality specificites (Dykes et al. 1982). In the rat, cytochrome oxidase-rich patches correspond to inputs from individual digits of the forepaw (Crockett et al. 1993). Neurons in the core region receive inputs from large, myelinated fibers which carry cutaneous information, and then relay this information to the thalamus. The increased density of cytochrome oxidase staining in these cell clusters may be due to increased packing density of the cells in these regions or to higher levels of metabolic activity.

Substance P receptor binding patterns were similar to the patterns of cytochrome oxidase staining. This result was somewhat surprising because, at least in the cat,

substance P-immunoreactive afferents to the DCN appear to avoid the cell clusters of the core region (Conti et al. 1990). Substance P-immunoreactive axon terminals localized by electron microscopy are found in highest numbers ventral to the core region (Conti et al. 1990; Westman et al. 1984). The pattern of receptor binding was much different, and corresponded more closely to the cytochrome oxidase patterns, which in turn may reflect levels of metabolic activity (Wong-Riley 1989). Thus, substance P may act at receptors that are located throughout the DCN, including neurons in the clusters which respond to cutaneous inputs.

What could account for this difference between receptor binding patterns and immunoreactive fiber distribution? It may be that the binding sites labeled in the present experiments are only a minor subset of substance P binding sites. Substance P binds to three tachykinin binding sites (NK_1, NK_2 and NK_3) but has highest affinity for the NK_1 sites (Otsuka and Yoshioka 1993). With the methods used in the present study, the binding sites revealed are the NK_1 sites. An alternative explanation is that the antibodies used to characterize substance P-immunoreactive fibers only recognized a subset of such fibers. Although control experiments were performed in these studies, it is difficult to prove specificity of an antibody. A recent report describing the distribution of an antibody directed against substance P receptors described a number of differences with the distribution revealed by ligand binding (Nakaya et al. 1994). This study of the rat revealed virtually no labeling in the DCN. A final possibility to account for the difference between binding patterns and immunoreactivity is the possibility that there is a mismatch between presynaptic release sites and postsynaptic receptors. Such a mismatch has been described

for substance P synapses in the dorsal horn of the spinal cord (Liu et al. 1994), but in this case the mismatch is at the level of individual synapses rather than across distances of many micrometers. Direct evidence for longer-distance mismatches is less convincing (reviewed by Herkenham and Morris 1986). Most of these studies relay on immunocytochemical procedures, which raises the problem of antibody specificity (see above). Also, in many cases levels of neurotransmitter in the presynaptic fibers may be below the level of detectability. Consequently, immunocytochemical procedures would reveal only cell bodies. Given the long-distance projections of many neurons, this observation could lead to the mistaken interpretation that a mismatch existed.

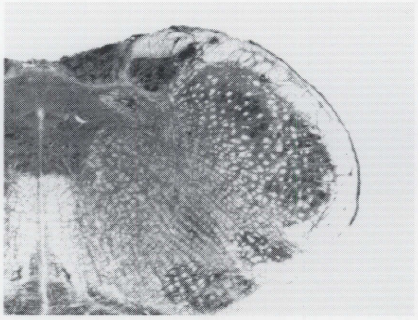
Because substance P has been associated with unmyelinated and thinly myelinated peripheral afferents, which carry nociceptive and thermoceptive information, it was surprising to find substance P binding sites densely distributed within the a cutaneous representation in the DCN. The neurons which give rise to substance P-immunoreactive fibers in the DCN appear to be located in the dorsal root ganglia and dorsal horn of the spinal cord (Conti et al. 1990). It is therefore possible that these fibers relay nociceptive or thermoceptive information to cutaneous neurons of the DCN. Nociceptive responses have been recorded in the rat n. gracilis (Cliffer et al. 1992). It may be that substance P acts as a modulator of neurotransmission in the DCN. In this manner, nociceptive or thermoceptive inputs could modulate the responses of DCN neurons to cutaneous stimulation. Psychophysical evidence consistent with such an idea has appeared recently (Apkarian et al 1994).

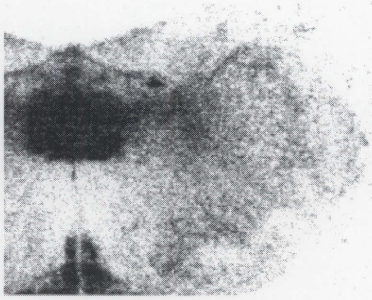
Figure 5. Bolton-Hunter-labeled [125I] substance P binding (right column) and cytochrome oxidase staining in adjacent sections (left column) through the DCN of four species. The magnification for is the same for all species.

Cytochrome Oxidase

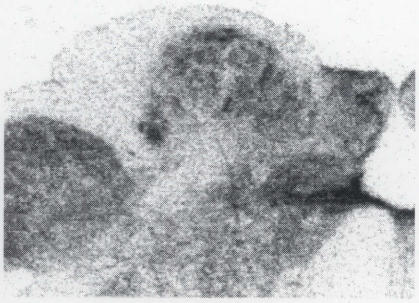
Substance P

RAT

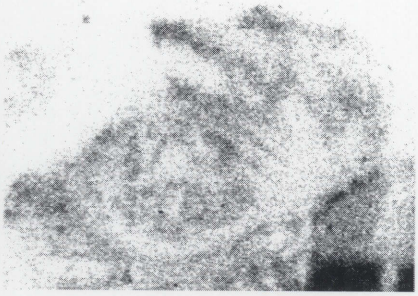




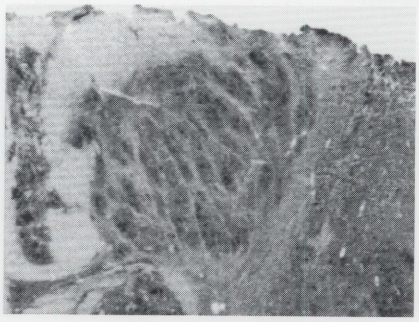
CAT

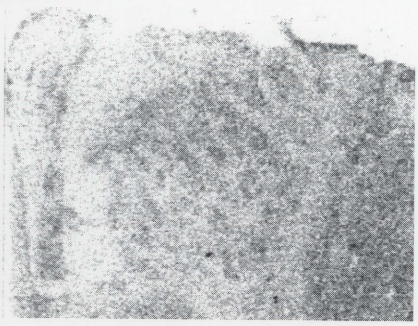


MONKEY



HUMAN





CHAPTER 5

5. CONCLUSIONS

The broad goal of this dissertation was to examine mechanisms which produce rapid receptive field (RF) reorganization in somatosensory neurons following a peripheral denervation. Because the dorsal column nuclei (DCN) are the first relay in the dorsal column-medial lemniscal system, experiments were designed to examine rapid RF reorganization of neurons at this level.

5.1 The site of reorganization

Experiments in Chapter 2 demonstrate that rapid RF reorganization occurs in DCN neurons following lidocaine denervation of peripheral RFs. The experiments in cats with somatosensory and motor cortex removed revealed that rapid RF reorganization in cuneate neurons can occur independent of cortical inputs to the DCN. In both sets of experiments, all neurons tested exhibited new RFs within minutes of denervation. Therefore, it appears that somatotopic reorganization begins at the earliest stages of somatosensory processing.

An unresolved question is whether the mechanisms which underlie RF reorganization also exist at higher levels of the somatosensory system, or whether reorganization at higher levels simply reflects changes in the DCN. As described in Chapters 1 and 2, there is anatomical and physiological evidence which suggests that RF

reorganization may occur within each level of the somatosensory system. If this is true, then the following general scheme can be proposed: 1) peripheral denervation results in reorganized RFs in DCN; 2) the altered patterns of output from the DCN produces further RF reorganization in the thalamus, which amplifies the extent of RF reorganization; and 3) the altered pattern of output from the thalamus produces further RF reorganization in primary somatosensory cortex (SI) and further amplification of reorganization.

This scheme suggests that the spatial extent of the reorganized RFs should increase at higher levels of the somatosensory system. Unfortunately, this hypothesis cannot be evaluated from existing data due to the differing approaches used by various investigators. An experiment to test this hypothesis would require simultaneous examination of rapid RF reorganization in neurons which have RFs at the same peripheral location but which are located at different somatosensory levels.

Another unresolved question is whether rapid RF reorganization following peripheral denervation occurs at the level of the spinal dorsal horn. While the DCN are most likely an initial site for RF reorganization, rapid RF reorganization should be examined in the dorsal horn to determine if these neurons might play a role in this process.

5.2 Mechanisms for rapid RF reorganization

As described in Chapters 2 and 3, rapid RF reorganization may result from a loss of masking inhibition arising from the peripheral RF. It has been proposed that normal background synaptic activity could decrease neuronal electrotonic size, thereby masking

some peripheral inputs (Bernander et al. 1991). Results of the experiments described in Chapters 2 and 3 demonstrate that rapid RF reorganization is not related to changes in spontaneous firing. These results suggest that masking inhibition does not result from activity-dependent changes in the neuronal cable properties of DCN neurons.

It has been hypothesized that masking inhibition in SI neurons arises from tonic activity of C afferents within the spinothalamic system (Calford and Tweedale 1991b). Experiments in Chapter 2 demonstrate that capsaicin-induced RF reorganization occurs in cuneate neurons. Rapid RF reorganization in cat cuneate neurons can be produced by blockade of a subset of peripheral afferents. Further, rapid RF reorganization in these neurons does not require complete blockade of the main afferent drive from the peripheral RF. These observations are consistent with a tonic masking hypothesis. However, these experiments also demonstrate that capsaicin-induced RF reorganization can arise within the dorsal column-medial lemniscal system. Thus, it is unnecessary to propose a role for the spinothalamic system in this process.

In addition to C afferents, other classes of peripheral afferents might contribute to tonic masking inhibition. As described in Chapter 3, type II slowly adapting afferents (SA II) and A delta afferents are potential sources of tonic masking inhibition in the DCN.

Indirect evidence from experiments in Chapters 2 and 3 suggests that SA II afferents do not play an exclusive role in rapid RF reorganization. Afferents from SA II receptors project directly at least to high cervical levels in the dorsal columns (Petit and Burgess 1968), and cells with SA II RF properties have been described in the DCN (e.g. Golovchinsky 1980). SA II receptors are differentially distributed in the skin of the cat:

they are rarely found in the glabrous skin (Janig 1971); they constitute 10% of the afferents from hairy skin (Burgess et al. 1968); and they are often situated at the base of claws and mediate responses to claw displacement (Brown et al. 1981). In the present studies, no apparent differences were found in the range of sizes or types of reorganized RFs exhibited by neurons with original RFs on glabrous skin versus neurons with original RFs on hairy skin. In the study of lidocaine-induced RF reorganization (Chapter 2), one SA II neuron with a RF on hairy skin was tested, as well as three neurons that were driven by claw movement. The main afferent drive to these neurons was thus presumably from SA II afferents. Although denervation of one neuron with a claw RF produced the largest excitatory reorganized RF in the study (Fig 1B, Chapter 2), the range of reorganized RF sizes in these cells did not differ from the rest of the sample. Similarly, there were no differences in the types of reorganized RFs exhibited by these four neurons and the rest of the sample. Nevertheless, due to the small sample size, these data must be regarded as preliminary. Further work is needed to clarify the role of SA II, and other classes of peripheral afferents, in rapid RF reorganization.

In Chapter 3, a mechanism for tonic masking inhibition in the DCN was presented. It was proposed that masking inhibition in the DCN might arise from tonic release of a neuropeptide from primary afferent terminals onto inhibitory interneurons of the DCN. DCN inhibitory interneurons could, in turn, synapse onto primary afferent terminals or other DCN neurons. In this manner, slow, peptidergic excitation of inhibitory interneurons would result in masking inhibition of some inputs. A slow time course is

consistent with the observation that rapid RF reorganization does not begin until several minutes after peripheral denervation (Byrne and Calford 1991).

As described in Chapter 3, the neuropeptides substance P and calcitonin gene-related peptide (CGRP), are present in the DCN. Either substance P, CGRP, or both might mediate masking inhibition in the DCN. Experiments in Chapter 4 demonstrate that substance P receptors are present throughout the DCN of several mammalian species. The distribution of CGRP receptors in the DCN is not known. Investigation of CGRP receptor distribution in the DCN would be useful to evaluate which areas of the DCN are potentially influenced by CGRP. Further investigations are also needed to identify which classes of DCN afferents contain substance P and CGRP, and it must be determined if substance P afferents originate from primary afferents or dorsal horn secondary afferents. If substance P or CGRP produce masking inhibition, these anatomical studies would provide evidence for the sources of such inhibition.

Tests of the role substance P and CGRP in rapid RF reorganization might be performed by iontophoresis experiments on DCN neurons. If substance P or CGRP mediate masking inhibition as proposed, then iontophoresis of the appropriate antagonists might result in RF reorganization of the targeted neurons. Similarly, iontophoresis of substance P or CGRP might reverse RF reorganization produced by a peripheral denervation.

5.3 Receptive field response properties before and after reorganization

Prior to the present experiments, there have been no studies of rapid RF reorganization that presented a detailed analysis of the RF response properties. In approximately half of the DCN neurons tested, the reorganized RF had the same response properties as the original RF response. In the remaining DCN neurons, the reorganized RF had either a new response class (e.g., hair, skin), new adaptation characteristics (e.g., rapidly adapting, slowly adapting), new inhibitory fields, or some combination of new response properties. These data suggest that at least some convergent input to DCN neurons is normally masked. Thus, physiological masking mechanisms restrict RF size and contribute to the specificity of response properties of DCN neurons.

Neither the type of RF reorganization nor the size of the reorganized RF were related to the original RF response properties of DCN neurons. From studies of neurons in SI and ventrobasal thalamus of cat, it has been suggested that neurons with rapidly adapting RFs are subject to greater inhibition than those with slowly adapting RFs (Dykes et al. 1984). The present data suggest that these differences are not reflected as differences in the reorganized RFs of rapidly adapting and slowly adapting DCN neurons.

Detailed studies of the RF properties of somatosensory neurons before and after reorganization need to be performed at the level of thalamus and cortex. Such studies would reveal the role of physiological masking in shaping RF properties, and would contribute to understanding how information is processed at each level of the somatosensory system.

It is possible that plastic changes in synaptic efficacy may quickly ensue after the onset of rapid RF reorganization. Previous studies have demonstrated the persistence of reorganized RFs after recovery of responsiveness from the original RF (Nakahama et al. 1966; Byrne and Calford 1991; Calford et al. 1991c). However, due to short observation times and the use of multiple unit recordings, it is difficult to interpret these data. The experiments described in Chapter 2 demonstrate that the reorganized RFs of some DCN neurons can persist for hours after recovery of responsiveness from the original RF. It is unlikely that these reorganized RFs are produced by persistent anesthetic blockade of smaller afferents, as proposed in Chapter 1. Rather, reorganized RFs may persist due to plastic changes in synaptic efficacy which result from the unmasking of previously ineffective synapses. Thus, once unmasked, previously ineffective synapses may initiate plastic changes which lead to a persistent increase in their effectiveness. It is likely that persistent reorganized RFs would disappear if observed for a sufficiently long time. Following digit amputation, reorganized RFs are initially large but shrink over a period of about a week (Calford and Tweedale 1988). The studies of rapid RF reorganization suggest that masking of inputs is a dynamic process which is subject to modification in response to changing patterns of afferent activity. This process may contribute to recovery from peripheral nerve injury, and may also serve to continuously shape RF properties in response to changes in sensory stimulation.

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