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Serotonergic Modulation of Somatosensory Activity at the Level of the Thalamus of the Rat

by

Elizabeth C. Cropper

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fullfillment of the requirements for the degree of Doctor of Philosophy, the City University of New York.

1985

This manuscript has been read and accepted for the Graduate Faculty in Biomedical Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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date

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Abstract

Serotonergic Modulation of Somatosensory Activity at the Level of the Thalamus of the Rat

by

Elizabeth C. Cropper

Adviser: Dr. Joseph S. Eisenman

The purpose of this project was to determine whether there is evidence for serotonergic modulation of somatosensory activity at the level of the thalamus of the rat, and, if so, if there is a difference in modulatory effects exerted on nonnociceptive and nociceptive activity.

Serotonin immunocytochemistry, and extracellular recording techniques, were used to map the distribution of serotonin immunoreactive fibers, in somatosensory regions of the thalamus, and physiologically characterize regions, with different densities of innervation. The ventrobasal (VB) complex, the primary relay for somatotopically organized nonnociceptive information, contained few immunoreactive fibers. Posterior regions of the thalamus, where nonsomatotopically organized responses to nociceptive stimulation were more commonly recorded than responses to nonnociceptive stimulation, contained a moderate number of immunoreactive fibers.

Microiontophoretic techniques were also used to apply serotonin to units responding to nociceptive and nonnociceptive stimulation, in the VB complex, and in posterior regions of the thalamus. Continuous ratemeter plots of unit activity, averaged over 1 sec intervals, were used to monitor serotonergic effects on neuronal firing frequency. The spontaneous activity of 10/10 nonnociceptive, and 13/13 nociceptive, and the evoked activity of 16/16 nociceptive, and 6/7 nonnociceptive units was inhibited. Also using microiontophoretic techniques, the time course of responses to nonnociceptive stimulation was examined, and the effects of serotonin on different response components determined. Iontophoretic application of serotonin inhibited the phasic response components of 10/11 units tested, and the tonic response component of 5/5 units tested. Percent decreases in phasic response components were less than percent decreases in tonic components, when the effect of iontophoretic application of serotonin on components of the same response were determined.

These experiments indicate that there are serotonergic fibers in thalamic nuclei, particularly in posterior thalamic regions associated with transmission of nociceptive activity, and that serotonin can exert an inhibitory effect on somatosensory transmission.

Acknowledgement

I would like to thank my advisor Dr. Joseph Eisenman and the department of Physiology for making my time spent at the Mount Sinai Medical Center such a pleasurable enriching experience.

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I. INTRODUCTION

There is evidence suggesting that somatosensory transmission is modulated by serotonergic input, from raphe and reticular nuclei of the brainstem, as it is relayed through the dorsal horn of the spinal cord and through the trigeminal nuclear complex. It has also been suggested that there may be a difference in modulatory effects on nociceptive and nonnociceptive transmission. Specifically, nociceptive transmission may be subject to more modulatory input than nonnociceptive. The pupose of this project was to investigate whether or not there is evidence for serotonergic modulation of somatosensory activity at the next somatosensory relay, the thalamus. Another question of interest was whether there is any difference in serotonergic modulation of nociceptive and nonnociceptive activity at the thalamic level.

A. Somatosensory transmission to thalamic level

Somatosensory information from the face and head passes to the trigeminal nuclear complex. From the trigeminal complex it is then relayed through the thalamus to the cerebral cortex. Somatosensory information from the rest of the body is relayed through the spinal cord and then similarly through the thalamus and cerebral cortex.

1. SOMATOSENSORY TRANSMISSION THROUGH THE SPINAL CORD

The most satisfactory description of the nuclear groups of the spinal cord is based upon the cytoarchitectural lamination of the spi-

nal gray evident in thick sections (80-100 um) stained with cresyl violet (Rexed, '54). There are nine distinct cellular laminae, plus an area X, in most regions of the spinal gray matter. Laminae I-IV make up the dorsal horn of the spinal cord, and laminae VIII and IX constitute the ventral horn. Lamina I is also known as the posteromarginal nucleus, lamina II as the substantia gelatinosa, laminae III and IV together as the nucleus proprius, and lamina VII as the intermediate gray zone.

Central processes of cells in the spinal ganglia enter the dorsolateral aspect of the spinal cord. Larger diameter fibers, conveying impulses from large encapsulated somatic receptors, are located medially in entering rootlets (medial bundle), and smaller, thinly myelinated, or unmyelinated fibers, which represent the central processes of smaller ganglion cells related to free nerve endings, tactile, thermal, and other somatic and visceral receptors enter more laterally (lateral bundle) (Cajal, '09-'11). Upon entering the spinal cord these central processes divide into ascending and descending branches (Ranson, '13).

Some of the processes of the medial bundle ascend in the ipsilateral dorsal columns and terminate somatotopically on the dorsal column medullary relay nuclei, i.e., the nucleus gracilis and the nucleus cuneatus (Carpenter et al., '68; Shriver et al., '68). In the rat most of the processes projecting from these relay nuclei cross the midline, and turn upward as a discrete bundle, the medial lemniscus, which ascends through the medulla, pons, and midbrain and terminates somatotopically on the ventrolateral ventrobasal (VB) complex of the thalamus (Lund and Webster, '67a; Feldman and Kruger, '80). There

appear to be a few neurons in the dorsal column nuclei which project ipsilaterally to the VB complex of the rat (Feldman and Kruger, '80).

The smaller lateral bundle of the dorsal rootlets enters the spinal cord and divides into ascending and descending branches which eventually terminate in the dorsal horn. Axons of spinal cord neurons then ascend in the ventral and ventrolateral funiculi, or the zone of Lissauer and terminate in the spinal cord (propriospinal, spinocervical, or dorsal column postsynaptic tracts), in the reticular formation (spinoreticular tract), and in the thalamus (spinothalamic tract) (Kevetter and Willis, '84).

Axons of the ventral and ventrolateral spinothalamic tracts, cross the midline at spinal levels and ascend to the thalamus in two bundles; a medial bundle, the medial spinothalamic tract (M-STT), which terminates in the medial thalamus, e.g., in the intralaminar nuclei, and a lateral bundle, the lateral spinothalamic tract (L-STT), which terminates in lateral thalamic regions, e.g., VB complex (Giesler et al., '79a; Giesler et al., '81a; '81b; Kevetter and Willis, '84). HRP injections into medial thalamic structures produces labeling in the ventral areas of the dorsal horns, in the intermediate gray zone, and in the ventral horns, while injections into lateral thalamic structures produces dense labeling within the caudal extent of the dorsal column nuclei; in the marginal laminae (I and II), and nucleus proprius (Giesler et al., '79a). There are differences, therefore, in the cells of orign of the M-STT and L-STT tracts suggesting that they may differ functionally. In fact, the majority of L-STT cells respond to wide dynamic range (WDR) stimulation (as the strength of stimula-

tion increases from nonnociceptive to nociceptive intensities there is a proportional increase in unit firing frequency) and have ipsilateral excitatory receptive fields (Giesler et al., '81b). The majority of M-STT cells, however, can be classified as high threshold (they respond only to noxious or near noxious stimuli), and have excitatory receptive fields which include several limbs or even the entire surface of the body and face (Giesler et al., '81b).

Besides the spinothalamic and the medial lemniscal pathways there are other routes by which somatosensory information reaches the thalamic level. Most notably these include the spinoreticular, spinocervical, and dorsal column postsynaptic pathways. In the rat spinoreticular fibers ascend in the lateral columns of the spinal cord and terminate in reticular nuclei of the medulla and pons including the nucleus reticularis pontis, nucleus reticularis gigantocellularis, nucleus reticularis ventralis, nucleus reticularis dorsalis, and nucleus reticularis lateralis (Zemlan et al., '78). There are two components of these spinoreticular projections; a medial and a lateral tract (Chaouch et al., '83; Menetrey et al., '83). In the rat the medial spinoreticular tract originates primarily from laminae V, VII, and VIII and terminates contralaterally in medial reticular nuclei of the medulla and pons, particularly in the nucleus reticularis gigantocellularis and nucleus reticularis pontis (Chaouch et al., '83). The lateral spinoreticular tract originates primarily from the nucleus of the dorsolateral funiculus (the region of the dorsolateral funiculus containing cell bodies intercalated between fiber tracts (Gwyn and Waldron, '68)), laminae VII, VIII, and X, and the superficial laminae

of the dorsal horn (Menetrey et al., '83). This tract terminates contralaterally in the lateral reticular nucleus (Menetrey et al., '83). Reticular projections to the thalamus are not as well described as spinal afferents to reticular nuclei in the rat. It has been demonstrated, however, that the nucleus reticularis gigantocellularis projects ipsilaterally to the intralaminar nuclei of the thalamus (Zemlan et al., '84; Peschanski and Besson, '84a), and to posterior portions of the medial VB complex (Zemlan et al., '84). Menetrey et al. ('80) have found 40% of the spinoreticular units tested to be WDR, 26% to respond only to light touch and 20% to nociceptive stimulation only.

In the rat the spinocervical tract originates primarily in the nucleus proprius, ipsilateral to the lateral cervical nucleus. Axons ascend in the dorsal-most portion of the lateral funiculus and terminate in the lateral cervical nucleus, i.e., in the dorsal-most portion of the lateral funiculus within spinal segment C2 (Baker and Geisler, '84). The lateral cervical nucleus then projects contralaterally to thalamic nuclei (Baker and Giesler, '84). Giesler et al. ('79b) found all units tested in the lateral cervical nucleus of the rat responded to innocuous mechanical stimuli; 27% of these being WDR and the other 73% light touch only. They did not, however, find the lateral cervical nucleus to be somatotopically organized; receptive fields for units were often large and included two or more body quadrants.

In addition to the ascending branches of primary afferent fibers the dorsal columns contain long ascending axons which originate from

neurons in the dorsal horn and travel uninterrupted to the medulla (Uddenberg, '66; '68a; '68b). These fibers have been called dorsal column post synatpic (DCPS) afferent fibers. In the rat, DCPS fibers originate from a region subadjacent to the substantia gelatinosa of the dorsal horn (which appears to correspond to laminae III and IV) ascend primarily in the ipsilateral dorsal columns and synapse in the dorsal column nuclei (Giesler et al, '84). DCPS neurons constitute about 38% of the neurons that project to the cuneate nucleus and about 30% of the cells that project to the gracile nucleus (Giesler et al., '84). The response properties of DCPS neurons have not been as well characterized in the rat as they have in the cat. In the cat, DCPS cells respond to nonnociceptive stimuli only (25-50%), or to wide dynamic range stimulation (50-75%) (Lu et al., '83; Bennett et al., '84).

2. SOMATOSENSORY TRANSMISSION THROUGH THE TRIGEMINAL NUCLEAR COMPLEX

Some primary afferent fibers, which originate from cells of the trigeminal ganglion, enter the brain stem at upper pontine levels and either ascend and terminate somatotopically in the trigeminal main sensory nucleus, or descend in the dorsolateral brainstem as the spinal trigeminal tract, to terminate in the spinal trigeminal nucleus (Cajal, '09-'11). Other afferent fibers enter the brainstem and bifurcate to enter both ascending and descending tracts (Windle, '26; Hayashi, '80). Cytoarchitecturally, the spinal nucleus has been divided into three rostro-caudal subnuclei; an oral part extending from the main sensory nucleus to the rostral pole of the hypoglossal

nucleus (subnucleus oralis), an interpolar part extending caudally from subnucleus oralis to the level of obex (subnucleus interpolaris), and a caudal part which begins at the level of obex and extends caudally to the cervical spinal cord (subnucleus caudalis)(Olszewski, '50). The spinal subnucleus caudalis can further be divided into two superficial laminae, I and II (also known as marginal and gelatinosa) which cap a more medially located magnocellular region (the nucleus proprius) (Gobel et al. '77). The laminated pattern of organization of the subnucleus caudalis appears very much like that of the spinal cord dorsal horn and it has been suggested that the two structures are analagous (Gobel, et al., '77; Gobel, '78a; '78b; Hockfield and Gobel, '78).

Several lines of evidence suggest that the main sensory nucleus and the subnucleus interpolaris function as the "lemniscal" component of the trigeminal nuclear complex. Anatomical studies have demonstrated that both of these regions primarily project somatotopically to the contralateral medial two thirds of the ventrobasal (VB) complex of the thalamus. For example, lesions of either the main sensory nucleus (Smith, '73) or the subnucleus interpolaris (Erzurumlu and Killackey, '80) produce considerable degeneration in the VB complex. Furthermore, large ventrobasal HRP injections result in labeled cells in the contralateral main sensory nucleus (Fukushima and Kerr, '79) and in the subnucleus interpolaris (Erzurumlu et al., '80). Furthermore, electrophysiological studies have demonstrated that responses to nonnociceptive stimuli are more commonly recorded in the main sensory nucleus (Azerad et al., '82; Kirkpatrick and Kruger, '75) and subnu-

cleus interpolaris (Hayashi et al., '84) than responses to nociceptive stimuli. Moreover, it has been observed that lesions of the main sensory nucleus and rostral spinal subnuclei produce severe tactile deficits (Spiller, '15).

The sensory relay function of the spinal subnucleus oralis is less clear. Electrophysiological studies have shown that responses to nonnociceptive stimulation are more common than nociceptive in subnucleus oralis (Kirkpatrick and Kruger, '75; Azerad et al. '82). It appears, however, that this subnucleus does not project to the thalamus; it is not labeled by large thalamic HRP injections (Fukushima and Kerr, '79). Cytoarchitectonic analyses, in the rat, have indicated that subnucleus oralis can be divided into dorsomedial, intermediate, and ventrolateral subdivisions (Falls, '83). Each of these subdivisions contains large numbers of small (8-15 um) neurons (Falls, '83). Retrograde HRP studies have shown that a number of these small neurons send descending intratrigeminal projections to the subnucleus caudalis (Falls, '83). Anterograde HRP studies have indicated that neurons in the subnucleus caudalis also send axons which terminate ipsilaterally in the subnucleus oralis. Some of these axons are collaterals of ascending parent fibers, others terminate in subnucleus oralis directly (Falls, '84). Besides these intratrigeminal efferents subnucleus oralis also projects to the spinal cord and cerebellum (Matsushita et al. '82).

Lesions of the subnucleus caudalis produce loss of pain and temperature sensation and only a moderate tactile deficit (Weinberger and Grant, '42; Hamby et al., '48). It has been hypothesized, therefore,

that subnucleus caudalis is equivalent to the "spinothalamic" portion of the trigeminal system. There is evidence, however, that there are functional differences between the superficial laminae of subnucleus caudalis and the magnocellular portion. Electrophysiological studies have shown that units responding to nociceptive mechanical and thermal stimulation appear to predominate only in the superficial laminae of subnucleus caudalis (Mosso and Kruger, '73; Price et al., '76). Responses to nonnociceptive stimulation are more commonly recorded than responses to nociceptive stimulation in the magnocellular region (Mosso and Kruger, '73; Price et al. '76). Injections of HRP into the thalamus also produce different amounts of labeling in the two regions (Fukishima and Kerr, '79; Shigenaga et al., '79). The main source of projections from subnucleus caudalis to the thalamus seems to be the superficial laminae (Fukishima and Kerr, '79; Shigenaga et al., '79) which project somatotopically to the medial VB complex and nonsomatotopically to the PO complex (Shigenaga et al., '79. There are fewer projections from the magnocellular region to the thalamus (Fukushima and Kerr, '79; Shigenaga et al., '79).

In conclusion the main sensory nucleus, and the spinal subnucleus interpolaris of the trigeminal nuclear complex function primarily as relays of nonnociceptive information from the periphery to the thalamus. The superficial laminae of the spinal subnucleus caudalis seem to function as the relay for nociceptive information. The functional roles of the spinal subnucleus oralis and the magnocellular portion of the subnucleus caudalis as somatosensensory relay nuclei however are less clear.

3. SOMATOSENSORY NUCLEI OF THE THALAMUS

The somatosensory nuclei of the thalamus have been divided into the ventral posterolateral (VPL) nucleus, and the ventral posteromedial nucleus (VPM) (which together constitute the VB complex) and distinguished from the posterior (PO) complex on the basis of cytoarchitectural differences. In the monkey and cat it has long been recognized that these anatomical divisions represent functionally distinct nuclei as well (Mountcastle and Henneman, '52; Rose and Mountcastle, '52; Poggio and Mountcastle, '59; Perl and Whitlock, '61; Whitlock and Perl, '61). Specifically, within the area designated as VPM, units respond primarily to contralateral light touch (LT) of the head, within VPL they respond primarily to contralateral LT of the body, and within the PO complex they respond primarily to nociceptive stimulation applied bilaterally. In the rat, however, whether this correlation between unit response characteristics and anatomical localization exists is less clear. Previous electrophysiological studies have shown that responses to somatosensory stimulation of the head are recorded medially to those of the body (Davidson, '65; Emmers, '65), but have not attempted to correlate the localization of these responses with the cytoarchitecturally distinct VPM and VPL. Furthermore, one recent electrophysiological study has suggested that there may not be a difference in unit response characteristics between the VB and PO complexes; the percentages of nociceptive and nonnociceptive responses recorded from these two areas was reported to be the same (Guilbaud et al., '80).

Anatomical studies in the rat have indicated that VPM receives contralateral somatotopic projections from the trigeminal main sensory nucleus (Lund and Webster, '67b; Erzurumlu et al., '70; Smith, '73; Fukushima and Kerr, '79) and spinal subnucleus interpolaris (Fukushima and Kerr, '79; Euzurumlu et al., '80; Erzurumlu and Killackey, '80), while VPL receives contralateral projections from the dorsal column nuclei (Lund and Webster, '67a; Feldman and Kruger, '80) and the dorsal horn of the spinal cord (Peschanski et al., '83). The VB complex also receives projections from other sources. Large VB HRP injections produce labeling in the superficial laminae of the trigeminal subnucleus caudalis and to a lesser degree in the magnocellular portion of caudalis (Fukushima and Kerr, '79; Shigenaga et al., '79). Zemlan et al. ('84) have also found labeling in posterior regions of the medial VB complex following injections of radiolabeled amino acids into the nucleus reticularis gigantocellularis.

The PO complex of the rat receives projections from a variety of sources. HRP injections into posterior thalamic regions produce labeling in the marginal lamina of the trigeminal subnucleus caudalis (Shigenaga et al., '79). Lesions of the trigeminal subnucleus interpolaris (Erzurumlu and Killackey, '80), trigeminal main sensory nucleus (Smith, '73), dorsal columns of the spinal cord (Zemlan et al., '78), and dorsal column nuclei (Lund and Webster, '67a) produce anterograde degeneration in posterior portions of the rat thalamus. Labeling can also be seen in posterior thalamic regions following injections of radiolabeled amino acids into the dorsal column nuclei (Feldman and Kruger, '80).

B. Role of serotonin in modulating nociceptive transmission

The physiological mechanisms by which serotonin modulates nociceptive transmission are not completely understood, although a lot has been learned about how it may occur under experimental conditions. This information has come from studies originally inspired by Reynold's ('69) demonstration that analgesia can be produced by stimulation of the periaqueductal gray (PAG) region. Later, it was reported that lesions of the dorsal lateral funiculus (DLF) of the spinal cord abolished the analgesia produced by PAG stimulation (Basbaum et al., '77). Since there were no known projections from the PAG descending directly to the spinal cord in the DLF, it was hypothesized that another brainstem nucleus namely, the nucleus raphe magnus (NRM), was serving as a relay between the PAG and spinal cord (Basbaum et al., '77). Attention was focused on NRM since Ruda ('76) had demonstrated that there were projections from the PAG to the nucleus raphe magnus in the cat. The nucleus raphe magnus had also been shown to be an effective site for stimulation produced analgesia (Oleson and Liebeskind, '75). Since it was known that the nucleus raphe magnus contained serotonergic cell bodies (Dahlstrom and Fuxe, '64) and that pharmacological manipulations which decreased 5-HT levels produced hyperalgesia and those which increased 5-HT levels produced hypoalgesia (see Messing and Lytle, '77 for review) it was also hypothesized that serotonergic projections from NRM were involved in mediating analgesic effects of PAG stimulation. In summary, it was concluded that there was a descending inhibitory pathway originating in the NRM, taking the route of the DLF of the spinal cord, which mediated the

analgesic effects of PAG stimulation and that this pathway was probably serotonergic in nature (Basbaum, '77).

Since then anatomical studies have demonstrated that there are descending serotonergic projections from other nuclei of the ventral brainstem, besides NRM, to the spinal cord, i.e., nucleus raphe obscurus, nucleus raphe pallidus, and nucleus reticularis gigantocellularis (Bowker et al., '82; '83). It has also been shown that serotonergic projections from the ventral medulla do not travel exclusively in the DLF (Johanessen et al., '84). Moreover, electrophysiological studies have demonstrated that electrical stimulation of other nuclei of the brainstem, e.g., nucleus reticularis gigantocellularis, nucleus reticularis magnocellularis, and nucleus cuneiformis, is effective in inhibiting spinal responses to nociceptive stimulation (Gray and Dostrovsky, '83). These studies suggest, therefore, that other nuclei of the ventral medulla, besides the NRM, may be part of the serotonergic-pain-inhibitory system and that descending projections other than those traveling in the DLF mediate serotonergic effects. In fact, Sandkuhler and Gebhart ('84a; '84b) have examined the relative contributions of the NRM and adjacent reticular formation to the inhibition of a spinal nociceptive reflex produced by stimulation of the PAG, and concluded that descending inhibitory pathways from the PAG course laterally as well as medially through the NRM. Specifically, they found that the stimulation threshold for inhibition of the tail flick reflex was not increased significantly by injection of lidocaine into the NRM. Thresholds were not significantly increased unless lidocaine was injected into the ipsilateral reticular formation as well as into NRM.

As a result of these recent studies, therefore, emphasis is no longer placed on a specific PAG-NRM-DLF pain-inhibitory system.

In any case, there is substantial evidence indicating that serotonergic modulation of nociceptive transmission does occur at the spinal level. There is a dense innervation of the superficial laminae of the dorsal horn with 5-HT immunoreactive fibers (Steinbusch, '81). The superficial laminae receive nociceptive input (Christensen and Perl, '70). These 5-HT immunoreactive fibers most likely originate from the ventral medulla. Injection of HRP into the caudal spinal cord labels cells in the medulla also characterized by serotonin immunoreactivity (Bowker et al., '82; '83). Furthermore, Ruda et al. ('81a; '81b) have observed that the two most common types of axonal endings in dorsal horn laminae I and II labeled after exposure to exogenous [^{3}H]5-HT are similar to those labeled in the trigeminal subnucleus caudalis following injection of tritiated amino acids into the serotonin-containing raphe and reticular nuclei. There do, therefore, appear to be serotonergic projections from the ventral medulla to areas of the spinal cord receiving nociceptive information.

Electrophysiological studies have provided evidence indicating that these serotonergic medullary-spinal projections are involved in inhibiting nociceptive transmission. Stimulation studies have shown that a conditioning stimulus delivered to NRM 30 msec prior to administration of a peripheral test stimulus inhibits the responses of 88% of the wide dynamic range (WDR), and 94% of the nociceptive specific (NS) units recorded from in the dorsal horn (Gray and Dostrovsky, '83). That at least some of the medullary spinal projections which produce this inhibition act through release of 5-HT is implied by the

fact that cells in laminae I and II, whose nocieptive evoked activity is inhibited by NRM stimulation, are characterized by axo-dendritic serotonergic synapses (Miletic et al., '84). A number of investigators have also shown that iontophoresis of 5-HT inhibits nociceptive responses (Randic and Yu, '76; Headley et al., '76; Belcher et al., '78; Willcockson et al., '84), glutamate evoked activity (Willcockson et al., '84), and spontaneous activity (Randic and Yu, '76; Jordan et al., '79; Willcockson et al., '84) in the dorsal horn of the spinal cord.

Serotonergic modulation of nociceptive transmission appears to occur in the trigeminal nuclear complex as well as in the spinal cord. The superficial laminae of the subnucleus caudalis which receive nociceptive input (Mosso and Kruger, '73; Price et al., '76) are densely innervated with 5-HT immunoreactive fibers (Cropper et al., '84). These fibers probabaly originate from the ventral medulla. Terminals in these laminae which take up [^{3}H]-5-HT and degenerate following application of 5,6-DHT are morphologically similar to those labeled following injection of tritiated amino acids into the medial brain stem (Ruda and Gobel, '80; Ruda et al., '81a;'81b). Furthermore, injection of HRP into the spinal subnucleus caudalis results in labeling of medullary cells in the nucleus raphe magnus, raphe obscuris, nucleus reticularis gigantocellularis, and the nucleus reticular paragigantocellularis characterized by 5-HT immunoreactivity (Beitz, '82). There are also, therefore, serotonergic projections from the ventral medulla to areas of the trigeminal nuclear complex receiving nociceptive input.

As in the spinal cord, electrophysiological studies have indicated that these serotonergic projections probably inhibit nociceptive transmission. Lovick and Wolstencroft ('79) and Sessle et al ('81) have demonstrated that a conditioning stimulus applied to NRM 10-50 msec prior to delivery of a test stimulus produces inhibition of trigeminal subnucleus caudalis WDR and NS units which lasts from 250 to 750 msec. Sessle et al. ('81) have also demonstrated that stimulation of nucleus raphe magnus produces an inhibition, lasting about 500 msec, of the digastric reflex evoked by tooth pulp or infraorbital nerve stimulation. Furthermore, iontophoresis of 5-HT blocks nociceptive activity of trigeminal units (Burns and Haigler, '83).

In summary, it has been shown that there are serotonergic projections from raphe and reticular nuclei of the ventral medulla to nociceptive areas of the trigeminal nuclear complex and spinal cord. These projections most likely inhibit nociceptive transmission since electrical stimulation of serotonin-containing nuclei of the ventral medulla and iontophoresis of 5-HT inhibits nociceptive responses of spinal and trigeminal units.

It is less clear, however, whether there is an ascending serotonergic modulatory system, and whether modulation occurs in the next somatosensory relay, the thalamus. The medial forebrain bundle (MFB) has been described as the major source of the serotonergic innervation of the forebrain (Ungerstedt, '71). Harvey and co-workers have found that bilateral MFB lesions produced either electrolytically (Harvey and Lints, '65) or using 5,7-dihydroxytryptamine (5,7-DHT)(Harvey and Simansky, '81) decrease telencepalic, but not brain stem serotonin content, and produce hyperalgesia. They also found that the electro-

lytically induced hyperalgesia could be reversed by systemic administration of D,L-5-HTP, while injections of either L-DOPA or D-5-HTP were without effect (Lints and Harvey, '69). On the other hand, electrolytic and 5,7-DHT lesions of either the dorsal or median raphe nuclei, which together account for about 80% of forebrain serotonin (Azmitia, '78) produce no significant change in nociceptive threshold (Hole and Lorens, '75). Harvey and Simansky ('81) suggested that lesions of the mesencepalic raphe nuclei damaged nociceptive afferents, ascending in the central gray region, which produced a partial analgesia that masked the hyperalgesia induced by the serotonin depletion. Another possibility is that lesions of the median and dorsal raphe nuclei did not produce complete destruction of ascending serotonergic fibers.

There is electrophysiological evidence, however, which indicates that the dorsal raphe nucleus (DRN) may be involved in modulating nociceptive transmission, and at the thalamic level. Besson et al. ('81) have found that the DRN is the most effective area in the feline periaqueductal gray for stimulation produced analagesia. Andersen and Dafny ('82; '83) have shown that stimulation of the DRN inhibits thalamic, i.e., parafascicular, responses to nociceptive stimulation and that intraventricular administration of 5,7-DHT decreases the percentage of units inhhibited. Moreover, Andersen and Dafny ('82) have reported that iontophoresis of 5-HT in the parafascicular nucleus also inhibits nociceptive activity.

It is also possible that the hyperalgesia produced by MFB lesions is not a result of damage to dorsal or median raphe efferents. It may

be due to damage to efferents from medullary or pontine raphe nuclei. Although the majority of serotonergic fibers innervating the forebrain originate from the mesencephalic raphe nuclei, raphe dorsalis and medianus (Azmitia, '78), Takagi et al. ('80) have identified a raphe magnus component of the MFB at the level of the preoptic area of the hypothalamus. Furthermore, Bobillier et al. ('76) and Peschanski and Besson ('84b) have described ascending projections from the medullary and pontine raphe nuclei, including raphe magnus and raphe pontis, which terminate in the intralaminar nuclei of the thalamus.

In conclusion, there is some direct evidence indicating that an ascending serotonergic system for modulation of nociceptive transmission exists; 5,7-DHT lesions of the MFB affect nociceptive threshold and stimulation of the DRN inhibits nociceptive responses in the parafascicular nucleus of the thalamus. Attempts to alter nociceptive threshold by lesions of the dorsal and median raphe nuclei have been less successful, however. There are several possibile explanations for this discrepancy; e.g., mesencephalic lesions may damage ascending nociceptive afferents, or projections from the medullary or pontine raphe nuclei may be involved in modulating nociceptive transmission.

C. Role of serotonin in modulating nonnociceptive transmission

While there is considerable evidence indicating that serotonin is involved in modulating nociceptive transmission, it is less clear whether modulation of nonnociceptive transmission occurs as well.

Anatomical studies mapping the distribution of 5-HT-IR fibers in nociceptive and nonnociceptive areas of the trigeminal nuclear complex and the spinal cord have found nociceptive regions to be more densely innervated than nonnociceptive (Steinbusch, '81; Cropper, et al., '84). For example, the superficial laminae of the trigeminal spinal subnucleus caudalis are densely innervated with 5-HT-IR fibers (Cropper et al., '84). This region has been particularly associated with nociceptive transmission (Mosso and Kruger, '73; Price et al., '76; Shigenaga et al., '79). In contrast, there are fewer 5-HT-IR fibers in the magnocellular portion of subnucleus caudalis (Cropper et al., '84), where there is more nonnociceptive than nociceptive activity (Mosso and Kruger, '73; Price et al., '76). In addition, there is little labeling in the principal sensory nucleus (Cropper et al., '84), an area particulary associated with transmission of nonnociceptive activity (Smith, '73; Kirkpatrick and Kruger, '75; Fukushima and Kerr, '79; Erzurumlu et al., '80; Azerad et al., '82). The superficial laminae are the most densely innervated part of the dorsal horn of the spinal cord (Steinbusch, '81). They receive nociceptive input (Christensen and Perl, '70). The dorsal column nuclei are only sparsely innervated (Steinbusch, '81). Anatomical data, therefore, seems to suggest that serotonergic effects on somatosensory transmission are exerted primarily on nociceptive rather than nonnociceptive activity.

An early electrophysiological study comparing the effects of electrical stimulation of the NRM on nociceptive and nonnociceptive responses of units in the trigeminal spinal subnucleus caudalis pro-

vided results supporting this hypothesis. Stimulation of nucleus raphe magnus had a weaker effect on responses to nonnoxious inputs, e.g., hair movement, than on responses to nociceptive stimuli, e.g. applied to tooth pulp (Lovick and Wolstencroft, '79).

The majority of electrophysiological studies comparing the effects of electrical stimulation of the brain stem on units responding to peripherally applied nociceptive and nonnociceptive stimuli, however, have produced different results. Dostrovsky et al. ('83) found that in the trigeminal subnucleus caudalis there was no statistical difference in the incidence of inhibition of nociceptive (including nociceptive specific (NS) and wide dynamic range (WDR)) and nonnociceptive responses produced by stimulation of nuclei of the brain stem. They also found no significant difference in the current thresholds necessary to produce this inhibition for NS and LT units. Conditioning stimuli applied to the PAG, CU, NRM, NGC, and NMC, consisting of 500 Hz, 100 ms trains of .1 ms pulses, delivered 130 msec prior to a test stimulus applied to the receptive field under study inhibited 96% of the nonnociceptive units tested, and 97% of the nociceptive units. Mean stimulus intensities necessary to produce this inhibition were also comparable, e.g., in NRM 88 $\pm$ 8 uA for nociceptive units, and 101 $\pm$ 7 uA for nonnociceptive units. Sessle et al. ('81) have reported similar results. Also in the trigeminal subnucleus caudalis, Shah and Dostrovsky ('82) have demonstrated that stimulation of NRM, NGC, PAG, and CU produces an increased latency, or blocks, antidromic responses to thalamic stimulation for all units responding to nociceptive stimulation and the majority of units responding to LT.

In the dorsal horn of the spinal cord similar electrophysiological studies have been done. Gray and Dostrovsky ('83) have demonstrated that there is no statistical difference in the incidence of brain stem inhibition of nociceptive and nonnociceptive responses; 74% of nonnociceptive units are inhibited by brain stem stimulation, 85% nociceptive. Current thresholds for inhibition for the different brain stem nuclei were also comparable for nociceptive and nonnociceptive units, e.g., for NRM 130 $\pm$ 12 uA for nonnociceptive units, 119 $\pm$ 9 uA for nociceptive units. Furthermore, Dostrovsky ('80) found that the incidence of inhibition with either PAG or NRM stimulation of nonnociceptive units in the dorsal column nuclei was similar to the incidence of inhibition of nociceptive units in the trigeminal subnucleus caudalis. Also working in the spinal cord Kajander et al. ('84) found that stimulation of PAG and NRM inhibits the majority of spinocervical cells responding to nonnoxious peripheral stimulation.

In addition to the data from stimulation studies, comparing modulatory effects on different somatosensory responses, there are some data comparing iontophoresis of 5-HT on nociceptive and LT units. Belcher et al. ('78) reported that 18 out of 20 nociceptive units in the dorsal horn of the spinal cord were inhibited by iontophoresis of serotonin, while only 5 out of 16 LT responses were affected by 5-HT; 3 of these being inhibited. Todd and Millar ('83), however, found no difference in serotonergic effects on LT, NS, or WDR units in laminae I-III of the dorsal horn of the spinal cord. Likewise, Willcockson et al. ('84) found 5-HT inhibited 2 out of 2 spinothalamic cells which responded to LT of the ipsilateral hindlimb, and 9 out of 13 spinothalamic cells which responded to pinch.

In conclusion, anatomical data suggest that the modulatory effects of serotonin on somatosensory transmission may be exerted primarily in nociceptive rather than nonnocieptive areas; the density of 5-HT-IR fibers is greater in regions where responses to peripherally applied nociceptive stimuli are more frequently recorded than are responses to nonnociceptive stimuli. The majority of electrophysiological studies, however, have failed to see a statistical differennce in modulatory effects on nociceptive and nonnociceptive units, e.g., the incidence of inhibition by electrical brain stem stimulation is similar for LT and nociceptive units and current intensities necessary to produce this inhibition appear to be the same for both kinds of units. Iontophoresis of serotonin also inhibits responses to both nociceptive and nonnociceptive stimuli.

One explanation for this apparent discrepancy may be that, except for Dostrovsky's study, the majority of electrophysiological studies comparing modulatory effects of 5-HT on somatosensory activity have been conducted in primarily nociceptive areas, e.g., in the trigeminal spinal subnucleus caudalis. In these regions, densely innervated with 5-HT-IR fibers, modulation of both nociceptive and nonnociceptive activity may be occurring. More electrophysiological studies comparing effects of 5-HT on nonnociceptive activity in primarily nonnociceptvie regions, e.g., the trigeminal main sensory nucleus, with effects of 5-HT on nociceptive activity in primarily nociceptive areas. e.g., spinal subnucleus caudalis, might find a difference in modulatory effects.

D. Experimental design

This thesis project examines the question of whether serotonergic modulation of somatosensory transmission occurs at the level of the thalamus of the rat. Another question of interest was whether there is a difference in serotonergic effects on nociceptive activity in primarily nociceptive regions of the thalamus, and effects on nonnociceptive activity in primarily nonnociceptive regions. In the first part of the project, serotonin immunocytochemistry was used to map the distribution of 5-HT-IR fibers in the thalamus of the rat. The purpose of these experiments was to determine whether there are 5-HT-IR fibers in the thalamus and to compare the density of innervation of different anatomically distinct regions. In the second set of experiments extracellular single unit recording techniques were used to localize responses to peripherally applied somatosensory stimuli and to correlate unit response characteristics with the cytoarchitectural divisions of the thalamus. The purpose of these experiments was to physiologically characterize (i.e., as primarily nociceptive or primarily nonnociceptive) different anatomical divisions of the thalamus particularly those containing different densities of serotonerigc innervation. In the last part of the project microiontophoretic techniques were used to apply serotonin to units responding to peripherally applied somatosensory stimuli in order to determine the effects of 5-HT on somatosensory units and to compare effects in physiologically distinct regions, e.g., in nociceptive and nonnociceptive areas.

1. SEROTONERGIC FIBERS IN THALAMIC NUCLEI

Investigators using formaldehyde-induced fluoresence (FIF) (Fuxe, '65; Anden et al., '66) and autoradiography, i.e., intraventricular infusion of ^{3}H-5-HT (Chan-Palay, '78; Parent et al., '81) have reported the existence of serotonergic fibers in the thalamus. Discrepencies amongst these reports exist, however. For example, in the midline nuclear group, Fuxe ('65) only found dense innervation in nucleus periventricularis. Parent ('81) also described nucleus rhomboideus as well labeled. Chan-Palay ('78) listed midline innervation as sparse with the exception of nucleus paratenialis which was reported to be well labeled. In the lateral geniculate nucleus Fuxe ('65) found dorsal innervation to be greater than ventral, while Parent et al. ('81) found ventral staining to be more intense than dorsal in this nucleus.

The discrepancies in fluorescent and autoradiographic reports of thalamic serotoninergic innervation may be related to difficulties involved with these techniques. The FIF method is subject to rapid photodecomposition and has proven to be less sensitive in detecting indoleamines than catecholamines (cf. Bjorklund et al., '75). In uptake studies in which radioactive material is infused intraventricularly more silver grains are seen in the vicinity of the ventricle than in deeper structures which are penetrated by lesser amounts of the labeled substance (Aghajanian et al., '66; Chan-Palay, '76; Parent et al., '81).

In an attempt to avoid some of these difficulties Steinbusch et al. raised an antibody to a serotonin-bovine serum albumin (BSA) con-

jugate ('78), and used it to map the distribution of serotonin immunoreactivity in the central nervous system of the rat (Steinbusch '81). This immunocytochemical procedure proved to be both sensitive and specific.

Recently, Wallace et al. ('82) modified Steinbusch's original technique by substituting an antiserum raised against a serotonin-hemocyanin (HC) conjugate for the 5-HT-BSA conjugate originally used. The HC antiserum appears to contain a higher titer of antibodies than the BSA antiserum (Wallace et al., '82). In the present study, therefore, a 5-HT-HC conjugate antiserum was used to map the distribution of serotonin immunoreactivity in the thalamus to clarify existing discrepancies.

2. PHYSIOLOGICAL CHARACTERIZATION OF THALAMIC NUCLEI

The somatosensory thalamus of the rat has been divided into the VB and PO complexes and the VB complex has been subdivided into the VPL nucleus and the VPM nucleus on the basis of cytoarchitectural differences as has been done in other mammals, e.g., the monkey and cat (McAllister and Wells, '81). In the rat, however, it is less clear that these cytoarchitectural divisions also represent functional divisions than it is in the monkey and cat.

The purpose of this part of the study, therefore, was to compare unit response characteristics of the different divisions of the somatosensory thalamus of the rat and to determine whether there is indeed a correlation between anatomical localization and response characteristics. This information in conjunction with the results of the first

part of the study would then provide information on the density of 5-HT immunoreactive fibers in functionally distinct regions of the thalamus.

3. EFFECTS OF SEROTONIN ON SOMATOSENSORY ACTIVITY AT THALAMIC LEVEL

There is considerable evidence indicating that 5-HT seems to exert an inhibitory effect on somatosensory, particularly nociceptive transmission. Pharmacological studies have shown that manipulations which decrease serotonin levels produce hyperalgesia, and those which increase 5-HT levels produce hypoalgesia (see Messing and Lytle, '77 for review). Electrophysiological studies have shown that electrical stimulation of serotonin-containing nuclei of the ventral brainstem inhibits extracellulary recorded single unit responses to peripherally applied somatosensory stimulation (Hu and Sessle, '79; Dostrovsky, '80; Shah and Dostrovsky, '82; Gray and Dostrovsky, '83; Dostrovsky et al., '83; Dostrovsky, '84; Hayashi et al., '84). It might be expected, therefore, that iontophoretic application of 5-HT onto units responding to peripherally applied somatosensory stimulation would also produce an inhibitory effect. In fact, the majority of investigators working in the trigeminal nuclear complex and in the dorsal horn of the spinal cord have shown this to be true. For example, in the dorsal horn of the spinal cord, Randic and Yu ('76), Belcher et al. ('78), and Headley et al. ('78) found that 5-HT inhibited most of the somatosensory units tested. Furthermore Willis and co-workers reported that serotonin inhibited the majority of spinothalamic tract cells tested (Jordan et al., '79; Willcockson et al., '84). In the

trigeminal spinal subnucleus caudalis, Burns and Haigler ('83) reported that there was a predominantly inhibitory effect of 5-HT on units which responded to noxious stimulation with an increase in firing frequency.

There have been some reports of predominantly excitatory effects of 5-HT on somatosensory units in the dorsal horn and in the trigeminal nuclear complex, however. Burns and Haigler ('83) found that serotonin produced an excitatory effect on units which responded to somatosensory stimuli with a decrease in firing frequency. Todd and Millar ('83;84) reported that 5-HT excited somatosensory units in the superficial laminae of the dorsal horn of the spinal cord. Belcher et al. ('78) also noted excitatory effects in the dorsal horn of the spinal cord when they iontophoretically applied 5-HT to spontaneously active units and to units activated by D,L homocysteic acid.

Although there have been a number of studies of the iontophoretic effects of 5-HT on somatosensory units of the spinal cord and trigeminal nuclear complex there have been fewer such investigations at the thalamic level particularly in the VB and PO complexes. In one study Andersen and Dafny ('82) demonstrated that iontophoretic application of 5-HT inhibited nociceptive responses of the majority of cells in the parafascicular nucleus but they did not investigate other thalamic nuclei. In another study, Andersen and Curtis ('64) found that serotonin inhibited spontaneous activity and C fiber responses to electrical stimulation of the contralateral ulnar nerve but found no effect of 5-HT on short latency responses in the VB complex. They did not, however, look at iontophoretic effects of 5-HT on VB responses to

physiological stimulation. One of the purposes of this part of the study, therefore, was to investigate iontophoretic effects of 5-HT on units responding to mechanically applied somatosensory stimulation in the VB and PO complexes.

Another question of interest was whether there is a difference in serotonergic effects on responses to nociceptive and nonnociceptive stimulation. Some iontophoretic studies at the spinal level have addressed this question but these studies have produced conflicting results. Belcher et al. ('78) have reported differences in 5-HT effects on nociceptive and nonnociceptive activity. Todd and Millar ('83;'84) and Willcockson et al. ('84), however, have not seen much of a difference between iontophoretic effects on nociceptive and nonnociceptive units.

II. MATERIALS AND METHODS

A. Immunocytochemical localization of serotonergic fibers

1. MATERIALS

a. Animals, drugs, chemicals

The animals used for immunocytochemical experiments were male albino rats (150-390 gm; Charles River, Wilmington, MA, or Zivic Miller, Allison Park, PA). They were housed 1-7 per cage in a temperature controlled room with a 12 hour light and dark cycle. Purina rat chow and H_2O were available ad libitum. The following drugs and chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO): pargyline hydrochloride, L-tryptophan, 5-hydroxytryptamine creatinine sulfate, and 3-3' diaminobenzidine tetrahydrochloride, grade 2.

b. Antisera

The sheep antirabbit (SAR) antiserum and the normal sheep serum (NSS) were purchased from the Pocono Rabbit Farm and Lab (Canadensis, PA), and the peroxidase antiperoxidase (PAP) antiserum from Miles Lab (Ekhart, IN).

The serotonin (5-HT) antiserum was a gift from the laboratory of Dr. J. Lauder where it was raised according to the method of Wallace et al. ('82). Briefly, 5-HT-creatinine sulfate (5-HT-CS) was coupled to limulus hemocyanin (an invertebrate respiratory protein) with formaldehyde. The serotonin-hemocyanin (5-HT-HC) conjugate was then

injected subcutaneously on the back of a rabbit. Blood was collected from an ear vein, the serum separated by filtration and stored frozen, or lyophilized, with sodium azide added as a preservative.

The antiserum was characterized by Lauder and co-workers using the immunoabsorption method (Lauder et al. '82; Wallace et al. '82), i.e., antiserum was preabsorbed with different concentrations of the substance to be tested for cross-reactivity, and tissue stained with this preabsorbed antiserum. Sections were examined, and a semi-quantitative scale (0-+4) used to represent the relative amount of immunostaining present. To reduce detectable staining in sections preabsorbed with dopamine, norepinephrine, and epinephrine to levels of those seen in sections preabsorbed with 5-HT, 10-50 times as much dopamine, 20-100 times as much norepinephrine, and 200 times as much epinephrine as 5-HT was necessary. A complete list of the different compounds and the concentrations at which they were used is included in Table I.

Wallace et al. ('82) also determined the specificity of the antiserum using pharmacological treatments. Parachlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase in nerve terminals, was injected, 300 mg/kg, IP, on the first day of treatment followed by 6 daily injections, 100 mg/kg, IP. This treatment resulted in almost a complete loss of terminal staining in most brain regions. Other substances were used to test for potentiating effects on 5-HT immunoreactivity. Nialamide, a monoamine oxidase inhibitor, and the serotonin precursor, tryptophan were given, 300 mg/kg, 2.5 and 2 hours prior to sacrifice respectively. This dual pretreatment increased immunoreac-

Table I. Results of immunoabsorption experiments with 5-HT-HC antiserum.

	Final Concentrations (uM) of Compound Added to Antiserum						
Compound	0	10	100	500	1000	2000	4000
5-HT	+++	++	+	0	0	0	0
5-MT	+++	+++	+	0	0	0	0
Tryptamine	+++	++	+	0	0	0	0
Dopamine	+++	+++	+++	++	+	+	+
Norepinephrine	+++	+++	-	+++	++	+	+
Epinephrine	+++	+++	+++	+++	+++	++	++
Histamine	+++	-	+++	++	++	+	+
L-tryptophan	+++	-	-	+++	+++	+++	+++
5-HTP	+++	-	-	+++	+++	+++	+++
Melatonin	+++	-	-	+++	+++	+++	++
5-HIAA	+++	-	-	+++	+++	+++	+++
Indole	+++	-	+++	+++	+++	+++	++

Scoring of intensity of immunoreactivity in 5-HT cells of the B4-B9 complex of embryonic day 15 rat brain employs semi-quantitative scale; ++++ (overstained) to 0 (no detectable staining), (-) not tested at this dilution. Abbreviations 5-MT, 5-methoxytryptamine, 5-HTP, 5-hydroxytryptophan, 5-HIAA, 5-hydroxyindole acetic acid.

(From Wallace et al., '82)

tive labeling but also produced a staining pattern not seen in control animals (i.e., nonpretreated or after either pretreatment alone). Light staining was seen in areas known to be primarily dopaminergic; specifically, in the zona compacta of the substantia nigra, and in the ventral tegmantal nucleus (SN-VTN). To determine whether this labeling was due to dopaminergic cross-reactivity or to small amounts of 5-HT in these cells, a series of experiments were done. Tissue from animals pretreated with L-DOPA, the immediate precursor of dopamine, L-DOPA and nialamide, or tyrosine, the precursor of L-DOPA, was examined for immunoreactivity in the SN-VTN. (All drugs were administered at 300 mg/kg. L-DOPA and tyrosine were given at 2 hours prior to sacrifice, nialamide 2.5.) No labeling in the SN-VTN was found, therefore, Wallace et al. ('82) concluded the immunoreactivity seen in nialamide and tryptophan pretreated animals was not due to cross-reactivity of the antiserum with dopamine. It has been hypothesized that these cells synthesize both 5-HT and dopamine but normally 5-HT levels are too low to be detected histochemically (Wallace et al. '82; Steinbusch and Verhofstad, '82).

In conclusion, the 5-HT antiserum used this study has been well characterized and it has been determined that there is little cross-reactive binding with other neurotransmitters found in the thalamus.

2. METHODS

a. Pretreatment of animals

Ten animals were used for immunocytochemical studies. To

increase intraneuronal 5-HT levels 9 of the 10 animals were pretreated with a monoamine oxidase inhibitor, pargyline (200 mg/kg, IP), and the serotonin precursor, tryptophan (200 mg/kg, IP) 1.5 and 1 hour prior to perfusion respectively. One animal was not pretreated.

All animals were anesthetized with ether, then perfused with an ice-cold 0.1 M phosphate buffer solution (pH 7.4) containing 4% paraformaldehyde, 3% sucrose, and 0.05% magnesium sulfate using a perfusion pump. Animals were decapitated and whole heads postfixed overnight in the same fixative.

Brains were cut into 50 um sections on a vibratome (Oxford). The sections were rinsed 3 times in 0.1 M phosphate buffered saline (PBS), and then 3 times in 0.1 M TRIS buffered saline (TBS) at room temperature. Each rinse lasted 10 minutes.

b. Immunocytochemical staining

A modification of Sternberger's peroxidase-antiperoxidase (PAP) technique was used to process tissue for immunocytochemical staining (Sternberger '79). Sections were incubated sequentially in the following antisera: (1) rabbit antiserotonin (1:2,000 dilution, overnight at $4^{o}C$), (2) sheep antirabbit (1:100 dilution, half an hour at room temperature), and finally, (3) rabbit peroxidase antiperoxidase (1:100 dilution, one hour at room temperature). Between each incubation the tissue was rinsed 3 times at room temperature, in TBS. Each rinse was 10 minutes. All antisera were diluted with a solution containing 1% NSS and 0.1-0.2% Triton X-100 in TBS.

Following incubation in the antisera the PAP complex was visualized by placing the tissue in a 3-3' diaminobenzidine (DAB)-TBS solution containing 0.05% DAB and 0.003% H_2O_2 for 10 minutes.

After being rinsed for 10 min in TBS the sections were mounted and counterstained with methyl green.

c. Photography

Sections were examined and photographed with a Leitz orthoplan microscope equipped with both a light- and darkfield condenser. Nuclear delineations were more apparent under lightfield illumination but immunoreactive fibers were more easily visualized with the aid of a darkfield condenser. Photographs, therefore, were all taken under darkfield illumination with a 35 mm attachment and Panatomic-X film (32 ASA, fine grain panachromatic). They were printed on Ilfospeed paper (grade 4 or 5).

B. Extracellular recordings of neuronal activity

1. ELECTRODES

a. Single barrel

Capillary tubing (A.M. Systems, Inc., Everett, WA, omega dot, 1.5 mm OD) was pulled into electrodes on a horizontal electrode puller, and electrode tips were broken back to a final diameter of about 2.5 um under microscopic observation. Electrodes were filled with 2% Niagara Sky Blue dye (Allied Chemical, Morristown, NJ) in 0.5 M sodium acetate (Hellon '71). In most cases they were pulled, filled, and used on the same day.

b. Multibarrel

In microiontophoresis experiments, electrodes were made from commercially prepared 5 barrel blanks with glass filaments in each barrel (R & D Scientific Glass, Spencerville, MD), or from 7 barrel blanks made by fusing single pieces of capillary tubing (W.P. Instruments Inc., omega dot, 1.0 mm OD). The latter were made by fixing 6 pieces of capillary tubing with flared ends around a central piece of tubing using shrink tubing and cyanoacrylic glue. All blanks were pulled on a vertical electrode puller. Homemade blanks were heated and twisted (about 180^{o}) before pulling to fuse the barrels. Tips of all electrodes were broken under microscopic observation to final tip diameters of 2.5-5.0 um; most tips were about 2.5 um.

The outer barrels of all blanks were filled with 5-HT creatinine sulfate (0.05 M, pH 4.0), or with NaCl (4.0 M). 5-HT was made up in distilled water just before use. The central barrel was filled with

Niagara Sky Blue dye (2% in 0.5 M sodium acetate) (Hellon, '71), and served as the recording barrel. All electrodes were filled with the aid of centrifugation. The flared ends of all drug barrels were coated with vaseline after the electrodes were filled to prevent formation of fluid current paths between barrels.

2. PREPARATION OF ANIMALS

The animals used in extracellular single unit studies were male albino rats (199-522 gm, Zivic Miller, Allison Park, PA or Perfection Breeders). They were housed and fed in the same manner as described for immunocytochemical studies. All animals were anesthetized with urethane (Sigma Chemical Co., St. Louis, MO, 1.2-1.3 g/kg,IP). Tracheal cannulations were performed and the animals mounted, head level, in a Kopf stereotaxic instrument. A hole was drilled in the skull, the underlying dura removed under a dissecting microscope, and a drop of oil placed over the cortex.

3. RECORDING PROCEDURE

a. Materials

The signal from the recording electrode was passed to a high impedance Grass P16-DC amplifier and displayed on an oscilloscope screen. Unit firing frequencies were monitored with the aid of an audio monitor. For some units records of spontaneous firing frequency or activity evoked by mechanical stimulation were also made by counting (HP 5304 A Timer/Counter) the output of a time-window discriminator (BAK Electronics, DIS-1), and plotting it in 1 sec intervals on an

T-Y plotter. In other cases somatosensory stimuli were applied with an electromagnetic device, driven by a Grass stimulator, and peristimulus time histograms were generated using a Nicolet Signal Averager and a T-Y plotter. The size and shapes of all spikes counted were monitored using a second oscilloscope which provided a delayed (Bak Electronics, AD-3) display of the spikes passed by the window discriminator to insure that only a single unit was counted.

b. Methods

Recording sites lay 4.0-7.0 mm anterior to interaural zero, 2.0-4.0 mm lateral to midline, and from 4.0-8.0 below cortical surface. Single units were isolated and their size and shape noted. They were then tested for changes in firing frequency with the application of peripherally applied mechanical stimuli. Units were tested for responsiveness to light touch (LT), whisker movement and to nociceptive pinch. Receptive fields of each unit were mapped. In microiontophoresis experiments, the evoked and spontaneous activity of units were also tested for sensitivity to 5-HT and to current.

In each animal a series of electrode penetrations were made in the mediolateral and rostrocaudal planes. At the conclusion of experiments reference marks were made by passing 2.5-5.0 uA of cathodal current through the dye filled recording electrode for 5-10 min at selected points in these tracks.

4. SOMATOSENSORY STIMULATION

For some units nonnociceptive stimuli, e.g., light touch, stroking, tapping, or whisker movement were applied with forceps while continuous ratemeter plots were made of corresponding changes in firing frequency. For others an electromagnet was used to apply LT stimuli, and peristimulus time histograms were generated by a Nicolet Signal Averager.

Nociceptive stimuli, e.g., strong pinch, were applied with forceps, or with an alligator clip and continuous ratemeter plots of firing frequency made.

5. MICROIONTOPHORESIS OF SEROTONIN

The serotonin creatinine sulfate complex was used in microiontophoretic experiments, and serotonin was applied to neurons with cationic current. It has been demonstrated that iontophoretic application of creatinine, the other cation in this complex, has no effect on thalamic units when applied with relatively large currents, i.e. 100-200 nA (Curtis and Davis, '62).

Serotonin was iontophoretically applied with currents ranging from 20-70 nA. It is known that for a particular iontophoretic current the amount of drug actually applied can vary from electrode to electrode (Krnjevic et al., '63). Based on Krnjevic et al.'s ('63) estimation of the transport number for serotonin, which is about .15, and the assumption that:

$$m=nit/zF$$

(where m is the amount of 5-HT applied, n is the transport number for serotonin, i is current, t is time, z is valence (assumed to be 1), and F is Farraday's constant)

however, a rough approximation of the quantitiy of 5-HT applied with currents used in these experiments can be made. It is likely that about 10^{10}-10^{11} molecules/sec of 5-HT were applied with 20-70 nA of current. In all cases during periods of drug ejection an opposite balancing current was applied through the NaCl barrel. In addition, most units were also tested for sensitivity to unbalanced current passed through the NaCl or dye barrel. Units were discarded if there was not a clear difference between unbalanced current and unbalanced drug effects. In some cases a retaining current of 10 nA was applied to drug barrels.

In some microiontophoresis experiments continuous ratemeter plots of firing frequency were made by counting, and then plotting, versus time, the number of spikes per second. 5-HT was iontophoresed onto units during periods of spontaneous activity, and during application of peripheral somatosensory stimuli. Either continuous, nociceptive mechanical stimultion, e.g., continuous pinch, or rapidly repeated nonnociceptive mechanical stimultion, e.g., stroking or whisker movement was used.

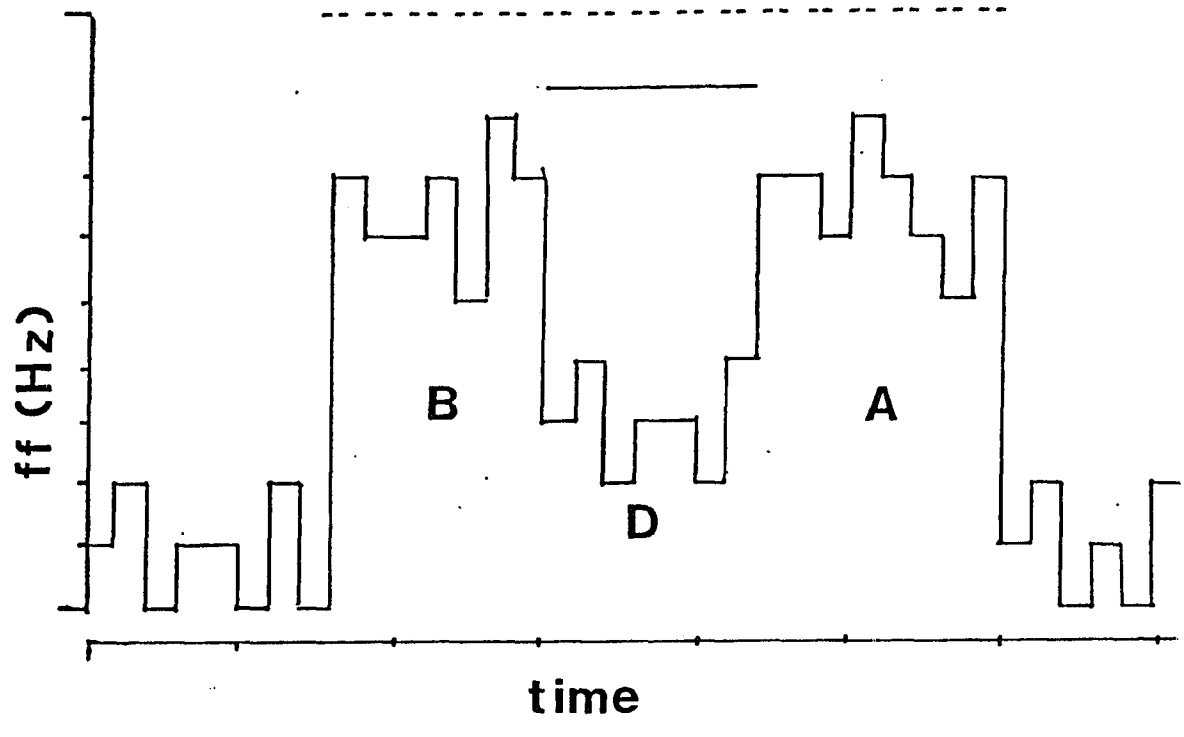
For 5-HT trials on spontaneous activity, ratemeter plots of unit activity were made before, during and after drug application. Firing frequency, in spikes/second, for 15 second intervals in each of these periods were tested for normality (Wilk-Shapiro test, critical value

alpha = .05). In most cases, unless activity levels were very low so that firing frequency alternated between 0 and 1 spike per second, the number of counts in these intervals was normally distributed about a mean. Mean firing frequencies were calculated for intervals and the percent changes induced by the drug determined for each neuron studied. T-tests were used to evaluate whether mean firing frequencies before and during iontophoresis, and mean firing frequencies before and after drug were significantly different.

In some microiontophoresis experiments continuous ratemeter plots of unit activity evoked by peripherally applied somatosensory stimulation were also made before, during, and after iontophoresis of 5-HT, as schematically illustrated in Fig 1. A record of spontaneous activity was obtained and the somatosensory stimulus applied. A record of evoked activity was made before drug application. Serotonin was iontophoretically applied, and a record of evoked activity during drug iontophoresis made. Finally, the iontophoretic current was turned off, and a record of evoked activity was made after drug application. Mean firing frequencies for evoked activity were then compared for before, during, and after drug intervals and t-tests used to evaluate the significance of changes in firing frequency as previously described. For these units, ratemeter plots of unit responses to somatosensory stimulation alone, without iontophoresis of 5-HT, were also made.

In order to study the time course of the somatosensory response, and the effect of 5-HT on early and late response components, nonnociceptive mechanical stimuli were applied to the receptive fields of

Figure 1. Schematic drawing of a continuous ratemeter plot of the effect of serotonin on activity evoked by somatosensory stimulation. The x-axis is time, the y-axis firing frequency in Hertz. B,D, and A represent intervals of time before, during, and after drug application respectively.



some units using an electromechanical device and peristimulus time histograms were generated. Histograms of unit responses to repeated application (10-15 times) of this type of stimultion were made during periods of 5-HT iontophoresis and during periods with no iontophoresis. Histograms of responses before iontophoresis were compared to histograms during iontophoretic application of 5-HT and to histograms made after iontophoresis with regard to magnitude and latencies of responses.

6. HISTOLOGICAL VERIFICATION OF RECORDING SITES

At the conclusion of an experiment animals were perfused with physiological saline followed by 10% formalin. The fixed brains were sliced in 80 um coronal sections on a freezing microtome. Sections were mounted and counterstained with neutral red. Using visible traces of electrode tracks and the Niagara Sky Blue spots for reference, units were localized on projection outline drawings of the slides. Localizations from all experiments were then plotted on a series of representative outlines spaced at 0.5 mm intervals through the somatosensory thalamus, following the atlas of Paxinos and Watson ('82).

III. RESULTS AND DISCUSSION

A. Localization of serotonin immunoreactive fibers in the thalamus

1. RESULTS

In animals pretreated with L-tryptophan and a MAO inhibitor, pargyline, the number of labeled fibers in the thalamic region was greater than in nonpretreated animals. The following data are the result of work done on pretreated animals. Preabsorption of the primary antiserum with 5-HT-creatinine sulfate (10^{-2} M) also virtually abolished thalamic labeling.

Steinbusch ('81) has divided 5-HT-immunoreactive fibers into three categories: fibers with no or few varicosities (found in fiber bundles); thinner fibers with varicosities, and; fibers without clear intervaricose connections (found in densely innervated areas). The majority of the 5-HT fibers seen in the thalamus were characterized by both varicosities and intervaricose segments. However, as observed by Steinbusch ('81) some fibers without intervaricose segments were present in very densely innervated areas such as in the midline nuclei (Fig. 2a), and in fiber bundles, such as in the stria terminalis, a few fibers without varicosities were visualized (Fig. 2b). In most nuclei fiber distribution was homogeneous; fibers were not organized in discrete clusters (Figs. 5,6,8). Any one particlar fiber also appeared to contact more than one cell. This was suggested by the observation that, except in fiber bundles, labeled fibers were often tortuous; in a particular plane of section they could be seen passing in close proximity to a number of cells partially encircling each as

it was bypassed (Fig. 2c). There did not appear to be differences in the innervation of different cell types; fibers encircled both large and small cells alike. Fibers also seemed to make multiple contacts with cells, i.e. the length of labeled fibers which encircled cells was often composed of a number of varicosities. Cells may have been partially encircled by more than one fiber although it was impossible to distinguish different fibers from branches of the same fiber.

a. Midline nuclei and nucleus medialis dorsalis

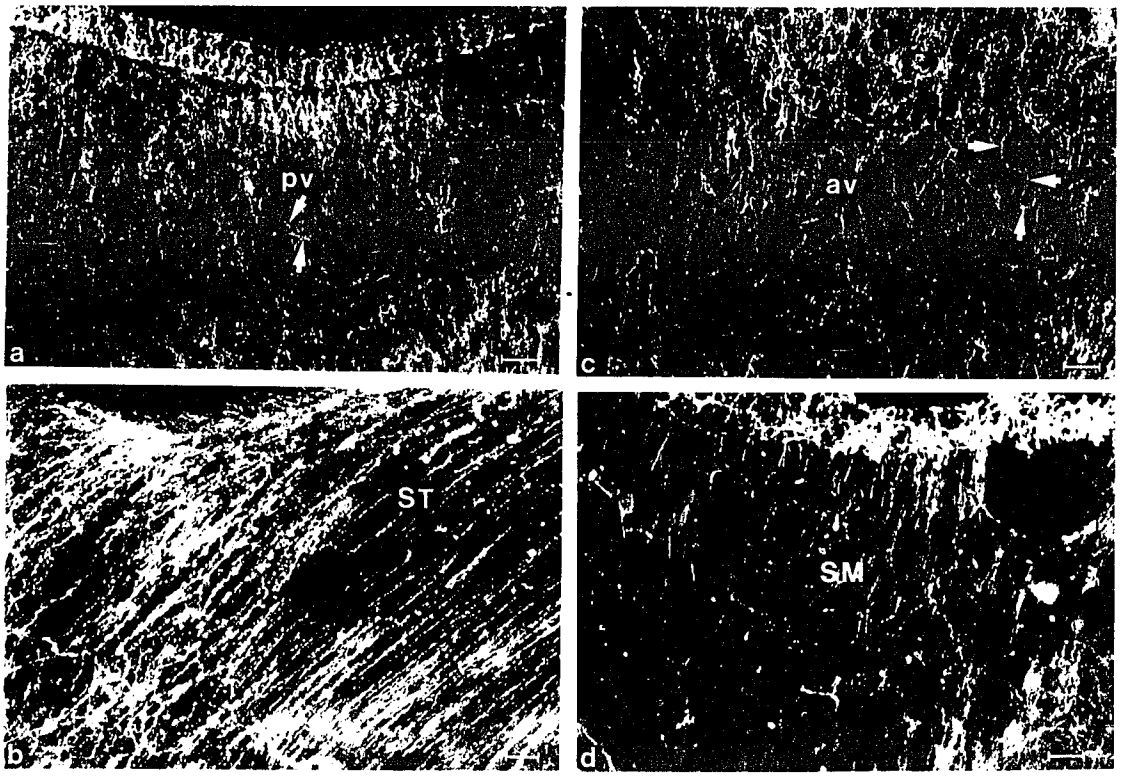
The midline nuclear group consists of nucleus periventricularis, nucleus rhomboideus, nucleus reuniens, nucleus paratenialis, and nucleus gelatinosus (Figs. 3,4). Labeling was uniformly dense in nucleus periventricularis (Figs. 5a,b; 6a,c), nucleus reuniens (Fig. 5a,b), and nucleus rhomboideus (Fig. 6a,c). Individual fibers were difficult to visualize in these nuclei; they did not extend far in the plane of section. Many of them seemed to have varicosities without intervaricose segments (Fig. 2a). A diffuse labeling, possibly due to release of serotonin from the fibers was also present in these regions. Immunoreactivity was observed in the supraependymal plexus dorsal to the nucleus periventricularis and the dorsal thalamus (Figs. 2a,d; 6a,c). These fibers consisted of very densely stained large varicosities separated by thin intervaricose segments (Fig. 2a,d).

The nucleus paratenialis (Fig. 5a,b), nucleus medialis dorsalis (Fig. 6a,c), and nucleus gelatinosus (Fig. 6a,c) contained few immunoreactive fibers.

Table II. Abbreviations for Figures 2-8

ad:	nucleus anterior dorsalis
am:	nucleus anterior medialis
av:	nucleus anterior ventralis
CC:	crus cerebri
CFV:	commissura fornicus ventralis
CI:	capsula interna
CP:	commissura posterior
cp:	nucleus caudatus putamen
dgl:	nucleus dorsalis corporis geniculati lateralis
F:	columna fornicis
FMP:	fasciculus medialis prosencephali (medial forebrain bundle)
FMT:	fasciculus mammillothalamicus
FR:	fasciculus retroflexus
g:	nucleus gelatinosus
gm:	nucleus corporis geniculati medialis
GP:	globus pallidus
HI:	hippocampus
LAMI:	lamina medullaris interna
ld:	nucleus lateralis dorsalis
lh:	nucleus lateralis habenulae
LM:	lemniscus medialis
lp:	nucleus lateralis pars posterior
md:	nucleus medialis dorsalis
mh:	nucleus medialis habenulae
p:	nucleus pretectalis
pf:	nucleus parafascicularis
pm:	nucleus posteromedianus
po:	nucleus posterior (posterior complex)
pt:	nucleus paratenialis
pv:	nucleus periventricularis
r:	nucleus reticularis
re:	nucleus reuniens
rh:	nucleus rhomboideus
SM:	stria medullaris
ST:	stria terminalis
st:	nucleus interstitialis striae terminalis
v:	nucleus ventralis
vd:	nucleus ventralis pars dorsomedialis
vgl:	nucleus ventralis corporis geniculati lateralis
vm:	nucleus ventralis medialis
ZI:	zona incerta

Figure 2. Dark field high power photomicrographs of coronal thalamic sections; scale bars 100 um. a. Photomicrograph of the nucleus periventricularis illustrating the characteristics of immunoreactive fibers found in the midline nuclei. Arrow points to a labeled fiber with varicosities and no intervaricose segments. b. Photomicrograph of the stria terminalis and dorsal thalamus. Fibers appear to run ventromedially from the stria terminalis where they have fewer varicosities to the dorsal thalamus where the number of varicosities increases. Arrow points to a labeled fiber without varicosities. c. Photomicrograph of nucleus anterior ventralis illustrating the characteristics of immunoreactive fibers found in the anterior nuclei. Arrow points to a labeled fiber with varicosities and intervaricose segments. d. Photomicrograph of the stria medullaris which contains a sparse to moderate number of labeled fibers.


pv
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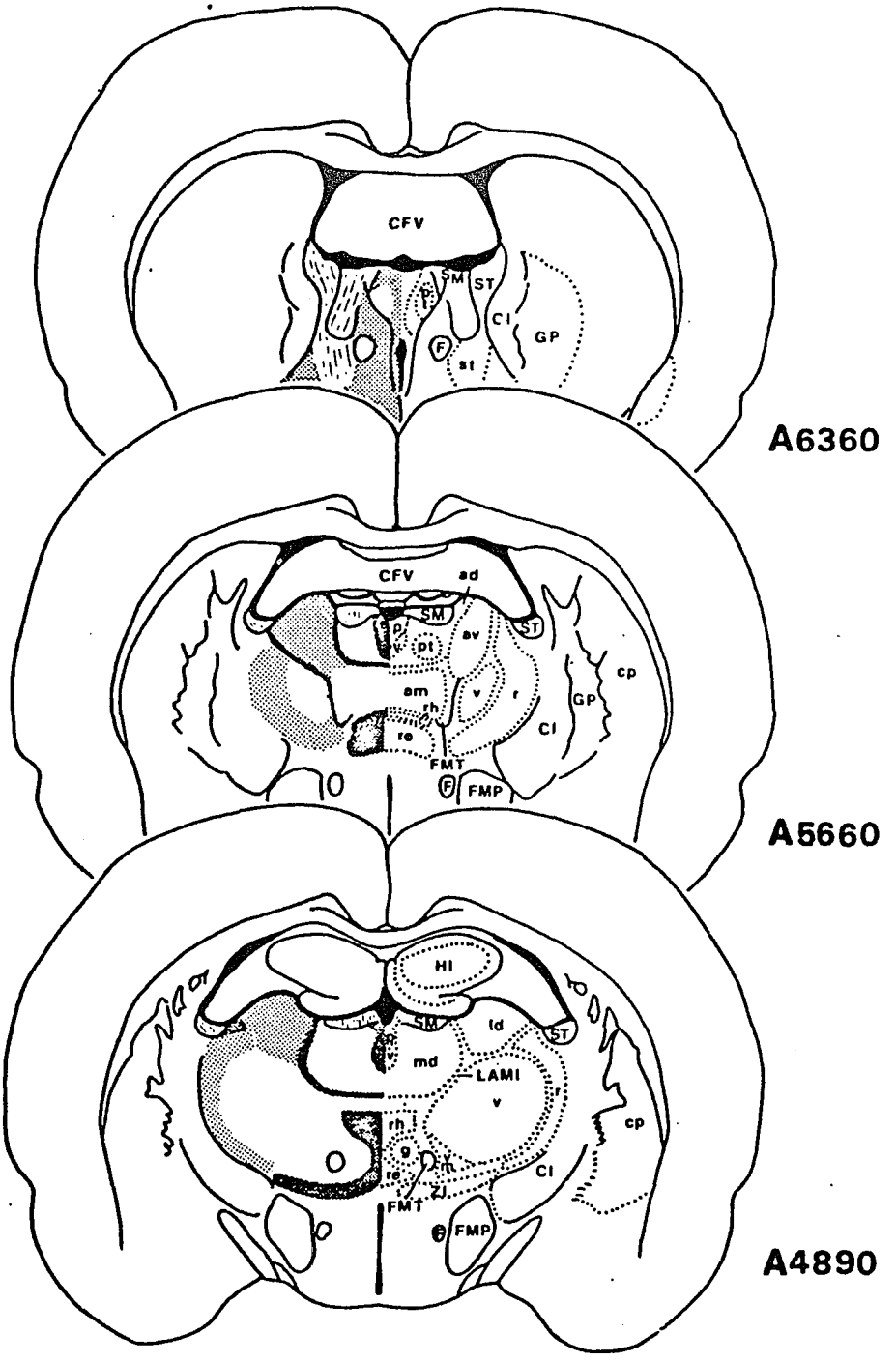
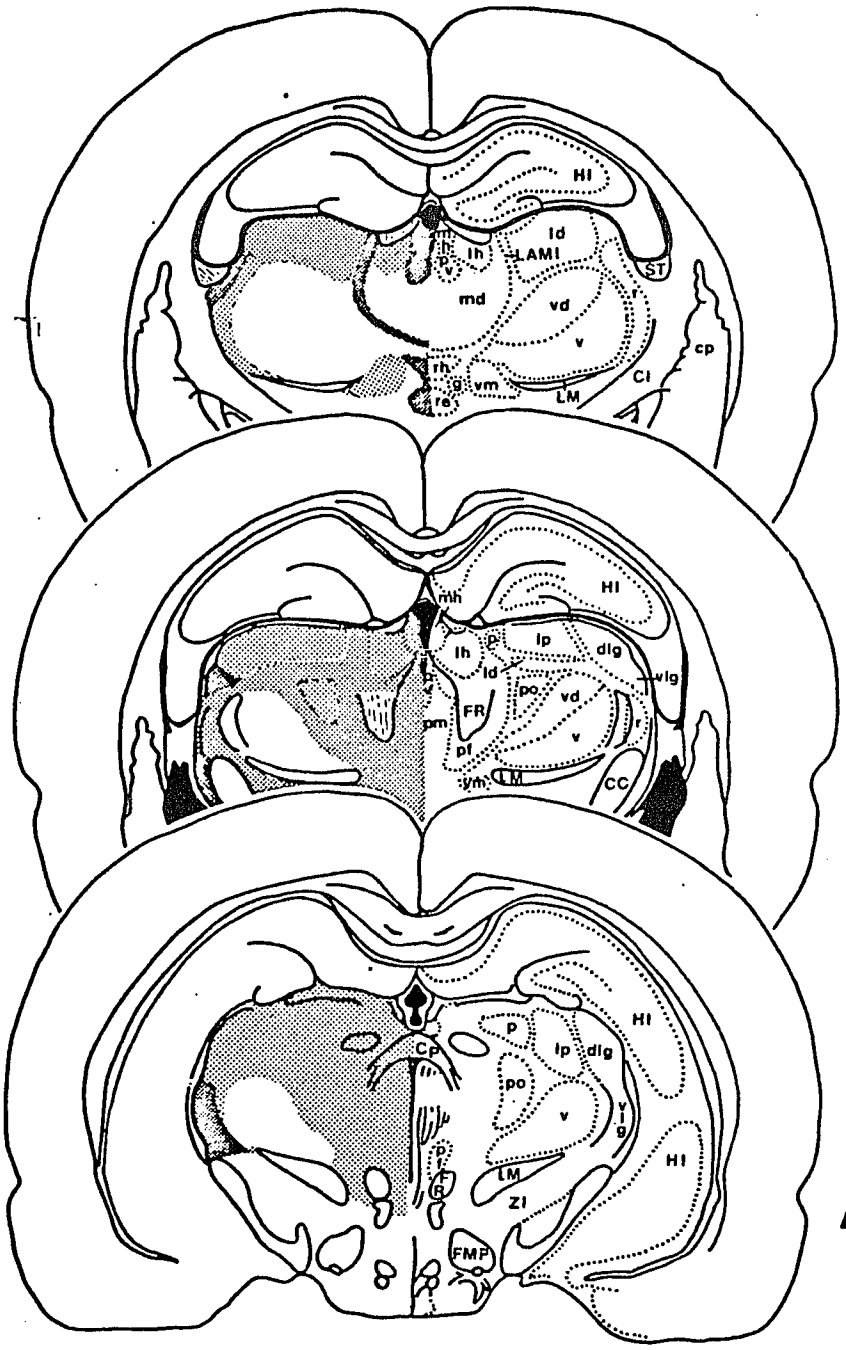
Figures 3 and 4. Schematic diagrams based on the atlas of Konig and Klippel ('70) illustrating the distribution of serotonergic fibers in the thalamus. Increasing intensity of labeling is indicated by increasing density of stippling used.

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A2790

Figure 5. Dark field photomicrographs of a coronal thalamic section; A 5780 in Konig and Klippel ('70), scale bars 400 um. a. Low power photomicrograph illustrating relative fiber distribution. The medially located nuclei are more densely innervated than those dorsally located. Few fibers are present in the ventral thalamus. b. The anterior midline nuclei showing dense innervation of the nucleus periventricularis and nucleus reuniens and sparse innervation of the nucleus paratenialis. c. The nucleus anterior ventralis, well labeled.

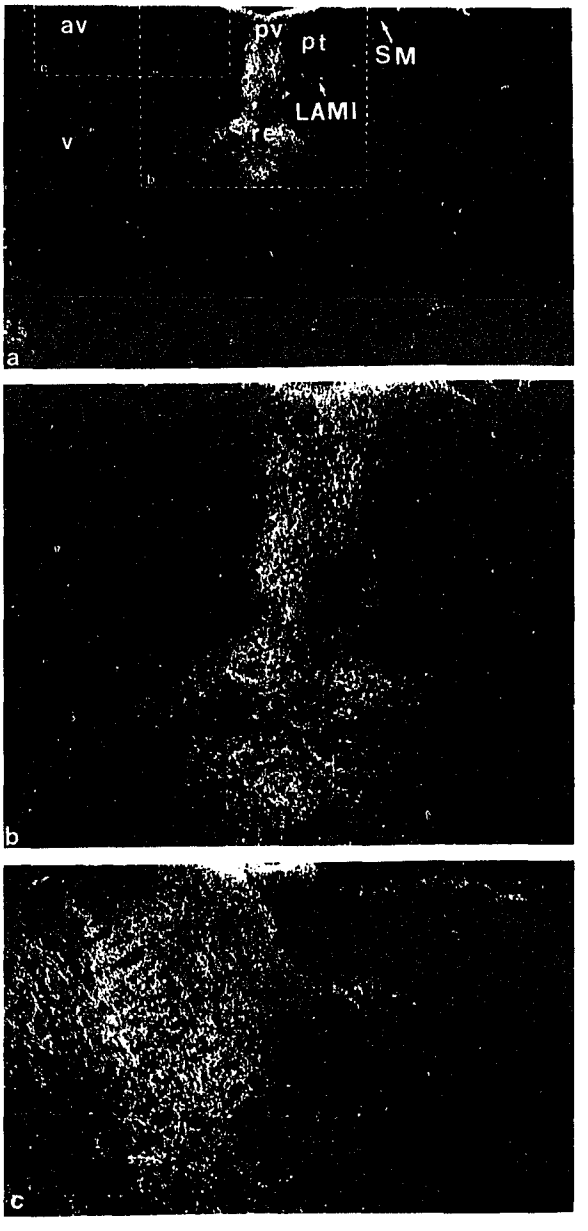
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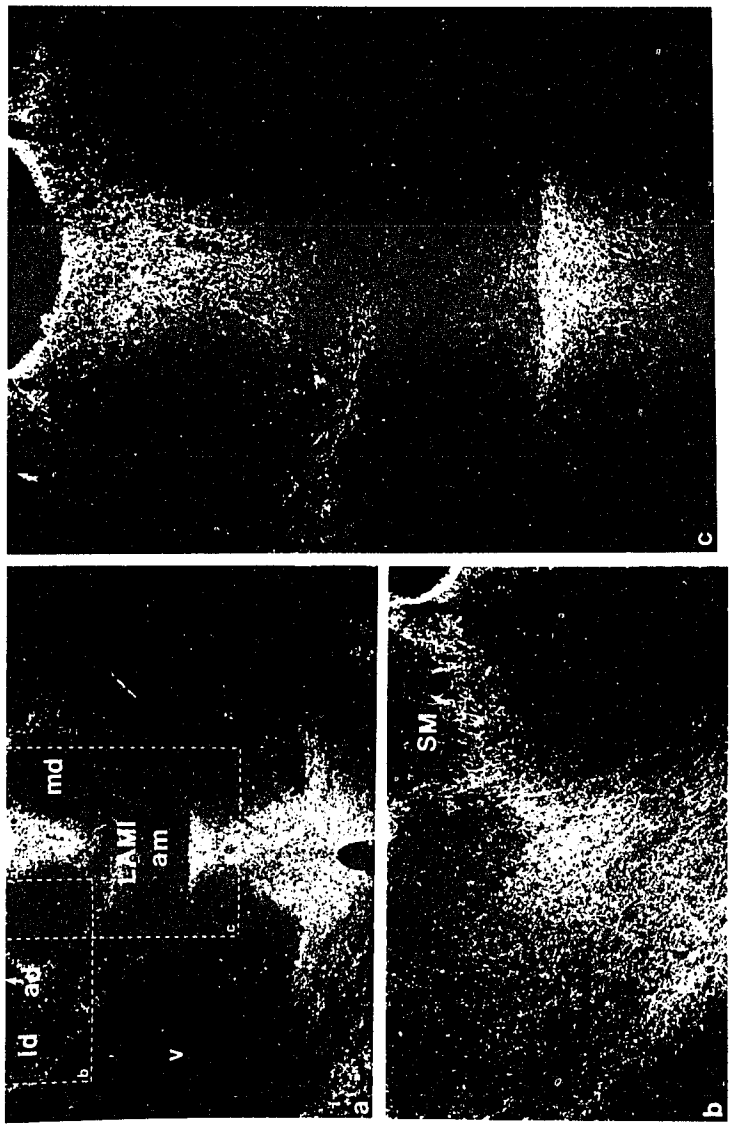
Figure 6. Dark field photomicrographs of a coronal thalamic section; A 5150 in Konig and Klippel ('70), scale bars 400 um. a. Low power photomicrograph. The midline nuclei are the most densely innervated. Also well labeled is the the dorsal thalamus, i.e., nucleus lateralis dorsalis. Few immunoreactive fibers are found in the ventral thalamus. b. Nucleus lateralis dorsalis and nucleus anterior dorsalis. Lateralis dorsalis is well innervated although not as uniformly as the midline nuclei. Nucleus anterior dorsalis contains a moderate amount of label. c. Uniformly dense innervation of the midline nuclei, nucleus periventricularis, and nucleus rhomboideus, sparse innervation of nucleus medialis dorsalis.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b. Intralaminar nuclei

At anterior levels the internal medullary lamina, including nucleus centralis lateralis, nucleus paracentralis, and nucleus centromedianus, was well labeled, although not quite as densely as was the midline nuclei (Figs. 3,4,5a,b; 6a,c). At more posterior levels, however, i.e. at the level of the nucleus corporis geniculati lateralis, internal medullary lamina labeling was less dense. The nucleus parafascicularis was fairly well innervated, although the distribution of immunoreactive fibers in this nucleus was not as homogeneous as in the midline nuclei (Fig. 7c). Parafascicular fibers were similar to those found in the anterior midline nuclei, they appeared to be fine and not to extend far in the dorsoventral plane.

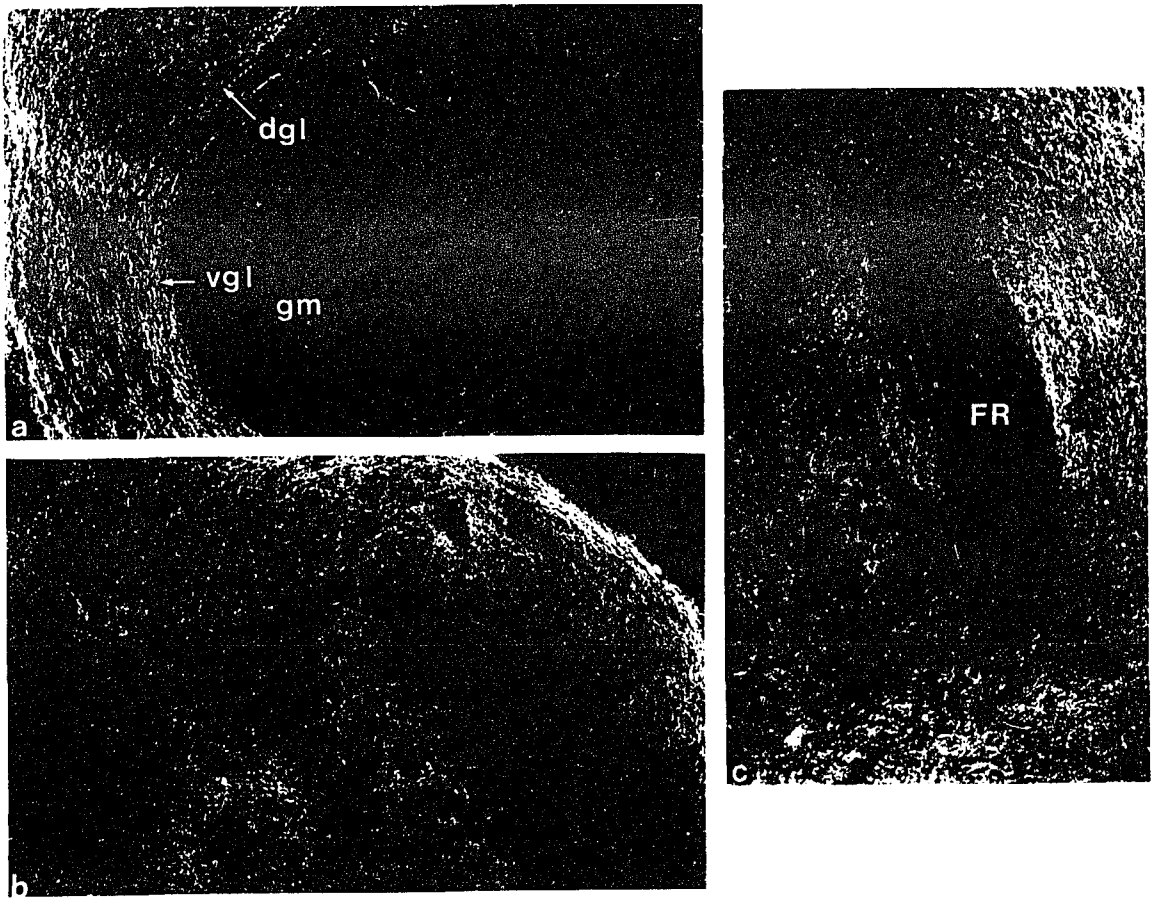
c. Anterior nuclei

The anterior nuclei consist of nucleus anterior ventralis, nucleus anterior dorsalis, and nucleus anterior medialis (Fig. 3). Nucleus anterior ventralis was well innervated, and labeling was uniform (Fig. 5a,c). Fewer immunoreactive fibers were found in nucleus anterior medialis, or in nucleus anterior dorsalis (Fig. 6a,b). Fibers in the anterior nuclei coursed for fairly long distances in the dorsoventral plane. They were usually characterized by both varicosities and intervaricose segments (Fig. 2c).

d. Lateral nuclei

The lateral nuclei include nucleus lateralis dorsalis, and nucleus lateralis pars posterior (Figs. 3,4). Nucleus lateralis dor-

Figure 7. Dark field photomicrographs of coronal thalamic sections; scale bars 400 um. a. The nucleus corporis geniculati medialis and nucleus corporis geniculati lateralis. The nucleus corporis geniculati lateralis is well innervated, the ventral half more so than the dorsal half. The nucleus corporis geniculati medialis contains few labeled fibers. b. The nucleus lateralis pars posterior, moderately well labeled, although fiber distribution is not uniform. c. The nucleus parafascicularis and fasciculus retroflexus. The nucleus parafascicularis is well labeled. Again fiber distribution is not uniform. Immunoreactive fibers running dorsoventrally can also be seen in the fasciculus retroflexus.



salis was well innervated (Fig. 6a,b), while innervatation in the nucleus lateralis pars posterior was only moderate (Fig. 7b). Innervation in the lateral nuclei was not as homogeneous as that found in the midline or anterior nuclei. Ventral parts of nucleus lateralis dorsalis appeared to be more densely innervated than dorsal parts (Fig. 6b). In the nucleus lateralis pars posterior the dorsal parts of the nucleus contained more immunoreactive fibers than the ventral parts (Fig. 7b). Fibers in lateralis dorsalis appeared to be similar to those found in the anterior nuclei. In lateralis pars posterior, however, fibers were smaller and coursed for shorter distances dorsoventrally.

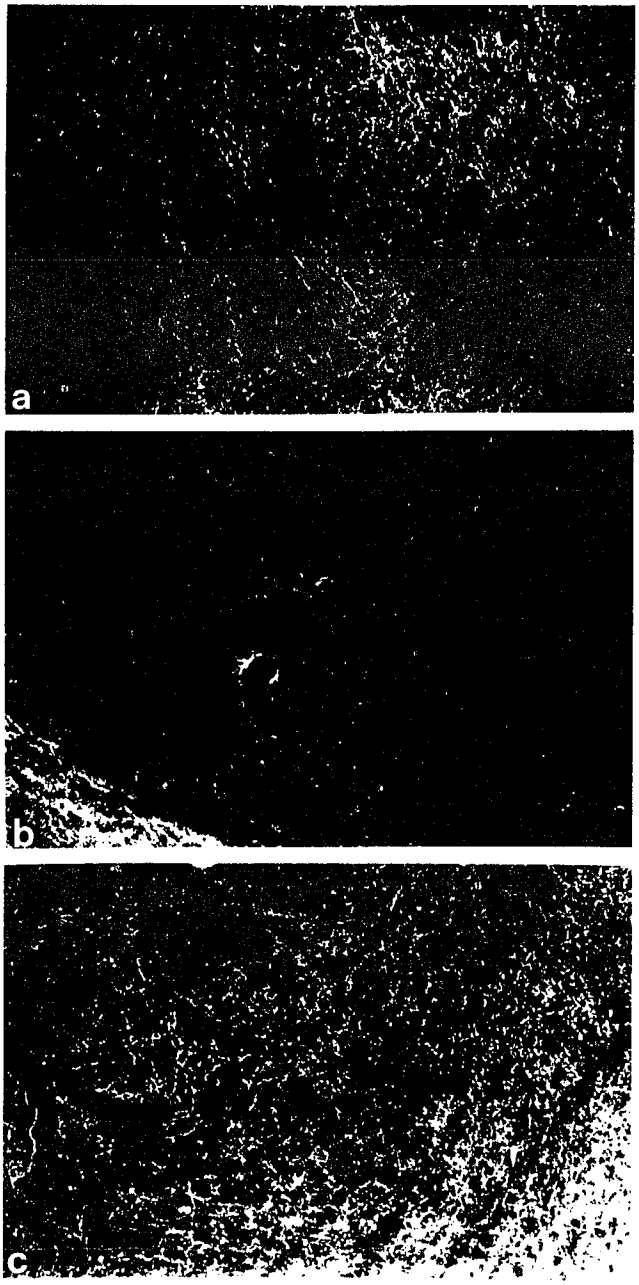
e. Nucleus reticularis, ventral nuclei, and posterior complex

At the anterior pole of the thalamus the nucleus reticularis was moderately well innervated (Fig. 8a). Except for one area, which appeared to correspond to Gurdjian's ('27) ventralis medialis and was moderately innervated, the ventral thalamus, including the VB complex and the part of the PO complex dorsomedial to the VB complex contained few immunoreactive fibers. Areas posterior to the VB complex were moderately well labeled. Fibers in these nuclei appeared to be similar to those found in the anterior nuclei.

f. Metathalamus

The metathalamus consists of nucleus ventralis corporis geniculati lateralis, nucleus dorsalis corporis geniculati lateralis, and nucleus corporis geniculati medialis. The ventral part of nucleus

Figure 8. Dark field photomicroghraphs of coronal thalamic sections; scale bars 400 um. a. The nucleus reticularis; A 5910 in Konig and Klippel ('70), moderately well labeled. b. The VB complex; A 4110, sparsely innervated. c. The posterior complex posterior to the VB complex (A 2790), also moderately well labeled.


a
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corporis geniculati lateralis was densely innervated while the dorsal part was only moderately so. Particularly striking was a band of dense innervation which occured at the division between the dorsal and ventral parts of this nucleus (Fig. 7a). The nucleus corporis geniculati medialis contained few labeled fibers (Fig. 7a).

g. Fiber bundles in thalamic region

Fiber bundles that have previously been described as providing either serotonergic or raphe input to thalamic nuclei were examined for 5-HT immunoreactivity. These include the fasciculus retroflexus, the fasciculus mamillothalamicus, the stria terminalis, and the stria medullaris.

The fasciculus retroflexus contained a few immunoreactive fibers which, for the most part, ran for short distances in the dorsoventral plane (Fig. 7c). These fibers had both varicosities and intervaricose segments. The fasciculus mamillothalamicus also contained 5-HT immunoreactive fibers with varicosities and intervaricose segments which could be seen coursing for fairly long distances in the plane of section particularly at anterior levels. A sparse-to-moderate number of immunoreactive fibers could be seen in the stria medullaris (Fig. 2d,4d,6b). The stria terminalis contained a greater number of labeled fibers. Most of these fibers ran parallel to each other. They appeared to run ventromedially from the stria terminalis, where they tended to be non-varicose or have few varicosities, to the dorsal thalamus, where the number of varicosities increased (Fig. 2b).

2. DISCUSSION

In the present study a recently developed immunocytochemical technique for the visualization of serotonin-containing neurons was used to map the fiber distribution in the thalamus of the rat.

Previous investigators using histochemical fluorescence (Fuxe, '65), autoradiography, i.e. intraventricular infusion of ^{3}H-5-HT (Chan-Palay, '78; Parent, '81), and immunocytochemistry (Steinbusch, '81) have described serotonergic fibers in nuclei of the rat thalamus. In most cases nuclei previously described as containing labeled fibers were also characterized by serotonin immunoreactivity in the present study. There were differences in the density of innervation observed, however. In the midline nuclei Fuxe ('65), Chan-Palay ('78), Parent ('81), and Steinbusch ('81) all found dense innervation in nucleus periventricularis, which is in agreement with these results. In the present study, however, nucleus rhomboideus and nucleus reuniens were also well labeled. Parent ('81) and Steinbusch ('81) reported innervation in these nuclei but not as dense as that described in this study. In the present study the more densely labeled nuclei of the anterior and lateral nuclear groups, nucleus anterior ventralis and nucleus lateralis dorsalis, were also found to contain a greater number of labeled fibers than previously indicated (cf. Fuxe ('65), Chan-Palay ('78), Steinbusch ('81)). At more caudal levels of the thalamus, Steinbusch ('81) and Fuxe ('65) observed sparse innervation in the posterior complex. The present study, on the other hand, found this area to be moderately well labeled.

The disagreements between the results of the present study and those of previous investigators who used fluorescent and autoradiographic techniques are probably due to the differences in these techniques. Fuxe ('65) used a formaldehyde induced fluorescence method to visualize serotonergic neurons. This technique is not as sensitive for serotonin as it is for catecholamines; the transformation of 5-HT into fluorescent molecules is low under standard conditions, and serotonin fluorophores have a high photodecomposition rate (cf., Bjorklund et al., '75). Chan-Palay ('78) and Parent ('81) infused ^{3}H-5-HT intraventriculaly and mapped the resulting distribution of radiolabeled fibers. With this method labeling is found to be more dense in periventricular regions than in deeper structures, suggesting that penetration of the radioactive substrate into the tissue may be a problem (Aghajanian et al., '66; Chan-Palay, '76; Parent et al., '81).

The results of the present study are also somewhat at variance with those of Steinbusch ('81). As previously mentioned, there are differences in the density of innervation reported in some nuclei. Furthermore, maximal innervation in this study, found in the nucleus periventricularis for example, appears to be greater than Steinbusch's maximal innervation (compare nucleus periventricularis in Fig. 5a,b; 6a,c to Steinbusch's Fig. 19a,b). This difference is probably due in part to the difference in the immunocytochemical method used. Steinbusch used the indirect fluorescent method for labeling the primary antiserum. In the present study the PAP method, which has been described as being more sensitive, was used (cf., Sternberger, '79). In addition, Steinbusch only pretreated some animals, and in those

cases used colchicine to aid in the visualization of cell bodies. In this study 5-HT levels were elevated by pretreatment with both tryptophan and a MAO inhibitor. This type of pretreatment increases immunoreactivity in both terminals and cell bodies (Frankfurt et al., '81; Wallace et al., '82).

a. Origins of serotonergic projections to the thalamus

The results of this study do not provide any information as to the origin of serotonergic input to nuclei of the thalamus, but this subject has been dealt with by previous investigators. The nucleus raphe dorsalis is most likely to be one source of thalamic afferents. HRP injected into the posterior thalamus labels cells in the ipsilateral lateral wing of the nucleus raphe dorsalis in the rat (Kevetter et al., '82), and in medial parts of the nucleus raphe dorsalis in the cat (Comans and Snow, '81). HRP-labeled cells can also be seen in this nucleus following localized HRP injection into the nucleus corporis geniculati lateralis (Mackay-Sim et al., '83). Furthermore, dorsal raphe injections of radioactive amino acids or wheat-germ agglutinin-HRP result in labeled terminals in the midline, medial, anterior, lateral, and intralaminar nuclear groups,in the nucleus corporis geniculati lateralis and in the pretectal area (Bobillier et al., '76; Taber Pierce et al., '76; Azmitia and Segal, '78; Peschanski and Besson, '84b). Moreover, electrolytic lesions of nucleus raphe dorsalis and nucleus raphe medianus produce degeneration in the thalamic nuclei medialis dorsalis and periventricularis (Conrad et al., '74).

Raphe nuclei other than nucleus raphe dorsalis undoubtedly also project to the thalamus. Injections of radioactive amino acids into nucleus raphe medianus (Azmitia and Segal, '78), nucleus raphe centralis superior (Bobillier et al., '76), nucleus raphe pontis (Bobillier et al., '76), and nucleus raphe magnus (Bobillier et al., '76) result in labeling of thalamic nuclei. Peschanski and Besson ('84b) used the anterograde transport of wheat-germ agglutinin-HRP to demonstrate that the nucleus raphe magnus projects to anterior intralaminar nuclei (nucleus paracentralis and nucleus centralis lateralis), and to midline nuclei (nucleus paraventricularis), and that the nucleus raphe medianus projects to the nucleus reticularis, nucleus medialis dorsalis, and the VB complex.

b. Ascending fiber pathways

Most of the forebrain 5-HT is supplied via the medial forebrain bundle (MFB) (Azmitia and Segal, '78). Previous investigators have observed that serotonergic fibers appear to leave the MFB and take several routes to innervate nuclei of the thalamus. At caudal levels of the thalamus serotonergic fibers have been described as passing from the MFB into the fasciculus retroflexus (Parent et al., '81). Results of the present study are in agreement with this observation. Fasiculus retroflexus immunoreactive fibers could be seen coursing in the dorsoventral plane. Raphe projections have been identified which take this route and terminate in the nucleus parafascicularis and midline nuclei (Bobillier et al., '76). Others appear to ascend with the internal medullary lamina and extend to the rostral pole of the thala-

mus to innervate the dorsal thalamus (Moore et al., '78). Azmitia and Segal ('78) have found labeling in the fasciculus retroflexus following injection of ^{3}H-proline in either the nucleus raphe dorsalis or nucleus raphe medianus. Fibers originating from nucleus raphe dorsalis and taking this route appeared to be particularly important in innervating nucleus lateralis dorsalis (Azmitia and Segal, '78). Other fibers taking this course may originate from nucleus raphe centralis superior and innervate the midline nuclei and the nucleus parafascicularis (Bobillier et al., '76).

At a somewhat more rostral level, raphe projections leave the MFB and follow the path of the fasciculus mamillothalamicus (FMT) (Conrad et al. '74; Azmitia and Segal, '78; Moore et al., '78;). Results of the present study indicate that at least some of these raphe projections are likely to be serotonergic. Particularly at anterior levels, where the FMT travels for longer distances in the dorsoventral plane, 5-HT immunoreactive fibers could be seen in this fiber bundle. Azmitia and Segal ('78) have found that raphe projections taking this course may originate from either the nucleus raphe dorsalis or the nucleus raphe medianus and may innervate the anterior nuclei. Bobillier et al. ('76) have also described nucleus raphe centralis superior projections ascending with the fasciculus mamillothalamicus. Other raphe projections ascending in the MFB have been observed to turn laterally, join the ansa peduncularis-ventral amygdaloid bundle and terminate in the ventral thalamus (Moore et al., '78).

At rostral levels of the thalamus serotonergic fibers have been described traveling dorsally from the MFB and dividing into medial and

lateral components which feed into the stria medullaris and stria terminalis, respectively (Conrad et al., '74; Parent et al., '81). Moore et al. ('78) have observed that as the stria medullaris runs from the rostral to the caudal pole of the thalamus the number of fibers labeled after raphe injections of ^{3}H proline progressively decreases. This suggests that raphe projections may be taking this course and innervating the dorsal thalamus, i.e. the anterior and lateral nuclei. This hypothesis is supported by our results. Immunoreactive fibers running parallel to the coronal plane of section, i.e. in the dorsoventral plane, are seen in the stria medullaris. The present study also suggests that the dorsal nuclei of the thalamus may be receiving innervation from MFB serotonergic fibers which take the route of the stria terminalis. Fibers can be seen which appear to be running ventromedially from the stria terminalis to the dorsal thalamus.

Not all of the serotonergic fibers innervating nuclei of the thalamus take the route of the MFB. In an autoradiographic tracing study, Azmitia and Segal ('78) identified six ascending fiber tracts from the nucleus raphe dorsalis and the nucleus raphe medianus, four of these lying outside the MFB. Two non-MFB tracts were found to innervate thalamic nuclei: the raphe dorsalis periventricular tract, innervating nucleus periventricularis of the thalamus; and, the nucleus raphe dorsalis arcuate tract, innervating the nucleus ventralis corporis geniculata lateralis. In another autoradiographic tracing study, Bobillier et al. ('76) found that thalamic nuclei receive projections from nucleus raphe pontis and nucleus raphe magnus. Fibers from these nuclei were found to ascend ventrolaterally and the nucleus raphe pontis was found to innervate the intralaminar

nuclei, nucleus reticularis, and nucleus anterior dorsalis, while nucleus raphe magnus innervated the intralaminar nuclei.

c. Functional implications

Serotonin has been described as a modulatory neurotransmitter, not specifically necessary for the execution of any one particular neurophysiological function. Rather, it is involved in influencing activity levels in a number of systems. The serotonergic innervation pattern found in the thalamus seems to fit well with what would be expected of such a modulatory role. Serotonergic input is found in functionally diverse nuclei of the thalamus. Within a particular nucleus there also does not appear to be any distinct organization of immunoreactive fibers. In most nuclei fiber distribution is homogeneous. Different cell types appear to be equally likely to receive serotonergic input.

Discussions of the functional implications of the serotonergic innervation of the thalamus have previously been confined to the nonspecific nuclei, i.e. to the midline nuclei and nucleus parafascicularis (Azmitia, '78). It is now apparent, however, that other thalamic nuclear groups receive serotonergic input. The anterior nuclear group contains 5-HT immunoreactivity. These nuclei are associated with limbic functions, receiving afferents from the mammillary bodies and limbic cortex (Kaitz and Robertson, '81) and sending efferents back to the limbic cortex (Robertson and Kaitz, '81). 5-HT immunoreactive fibers were also seen in the lateral nuclei, which relay visual information from the superficial laminae of the superior collicu-

lus and the nuclei of the pretectum to the cortical areas adjacent to the striate area (Takahashi, '82). The serotonergic system, therefore, seems to be involved in modulating activity in a number of systems at the thalamic level.

B. Localization of responses in somatosensory nuclei in the thalamus

1. RESULTS

Nuclear delineations within the thalamic somatosensory relay system were based on descriptions of the cytoarchitecture of the different thalamic regions receiving somatosensory input (Cajal, '27; Gurdjian, '27; Jones and Leavitt, '74; Feldman and Kruger, '80; McAllister and Wells, '81; Bold et al., '84). Specifically, the ventrolateral area, characterized by loosely packed, discoid somata (Feldman and Kruger, '80) arranged in concentric laminae which run parallel to the external medullary lamina (McAllister and Wells, '81), and which are pierced by coarse thalamo-cortical bundles (Feldman and Kruger, '80), was designated as VPL (McAllister and Wells, '81)(Fig. 9A-B). The dorsomedial area, characterized by large (12-13 um), round, densely packed somata (Feldman and Kruger, '80; Bold et al., '84) which appear to be almost linearly arranged (Gurdjian, '27), was designated as VPM (McAllister and Wells, '81) (Fig. 8A-B). The ventromedial nucleus was not included as part of VPM since it appears to be functionally involved with other systems e.g., the extra pyramidal motor system (Herkenham, '79). Together VPM and VPL constitute the VB complex. The PO complex, which does not seem to be a discrete nucleus (Feldman and Kruger, '80) was considered to include the area ventral to the lateral nuclei and dorsomedial to VPM. This is equivalent to Gurdjian's nucleus ventralis pars dorsomedialis (Gurdjian, '27) or Lund and Webster's posterolateral complex pars lateralis (Lund and Webster, '67a; '67b) (Fig. 9A-B). This region was easily distinguished from

VPM. Cells were less densely packed (Bold et al., '84), had no special arrangement, and small cells (5-6 um) were more common. Also included in the PO complex were regions posterior to the VB complex and medial to the medial geniculate nucleus (Bold et al., '84) (e.g., Lund and Webster's medial geniculate pars medialis (Lund and Webster, '67a; '67b), or Scheibel and Scheibel's ('66) magnocellular area medial to the medial geniculate) (Fig. 9C). The more dorsally located region medial to the medial geniculate was considered separately since, although some authors have refered to this region as the posterior complex (Cajal, '27; Gurdjian, '27), others have considered it to be distinct from the PO complex, designating it the anterior pretectal area (Lund and Webster, '67a; '67b) or pretectal region (Bold et al., '84) (Fig. 9C).

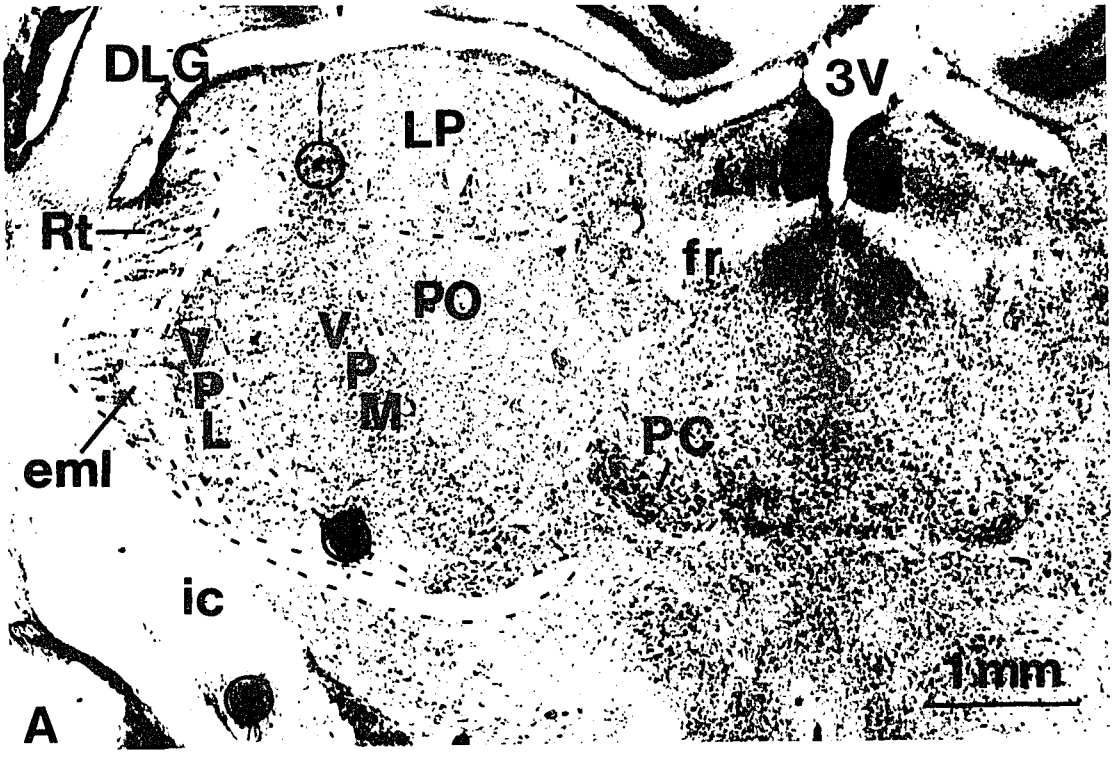
Recordings were made from 413 units. Most thalamic spikes were biphasic (positive-negative), had amplitudes between 100-400 uV, and durations between 1.3 and 2.5 msec. The majority of thalamic units were spontaneously active; spontaneous firing frequencies fluctuated from 0-5 Hz for some units and up to 10-40 Hz for others. There was generally more spontaneous activity in the VB complex and the part of the PO complex dorsomedial to VPM than in posterior regions, including the medial geniculate nucleus, anterior pretectal area, and the PO complex posterior to the VB complex.

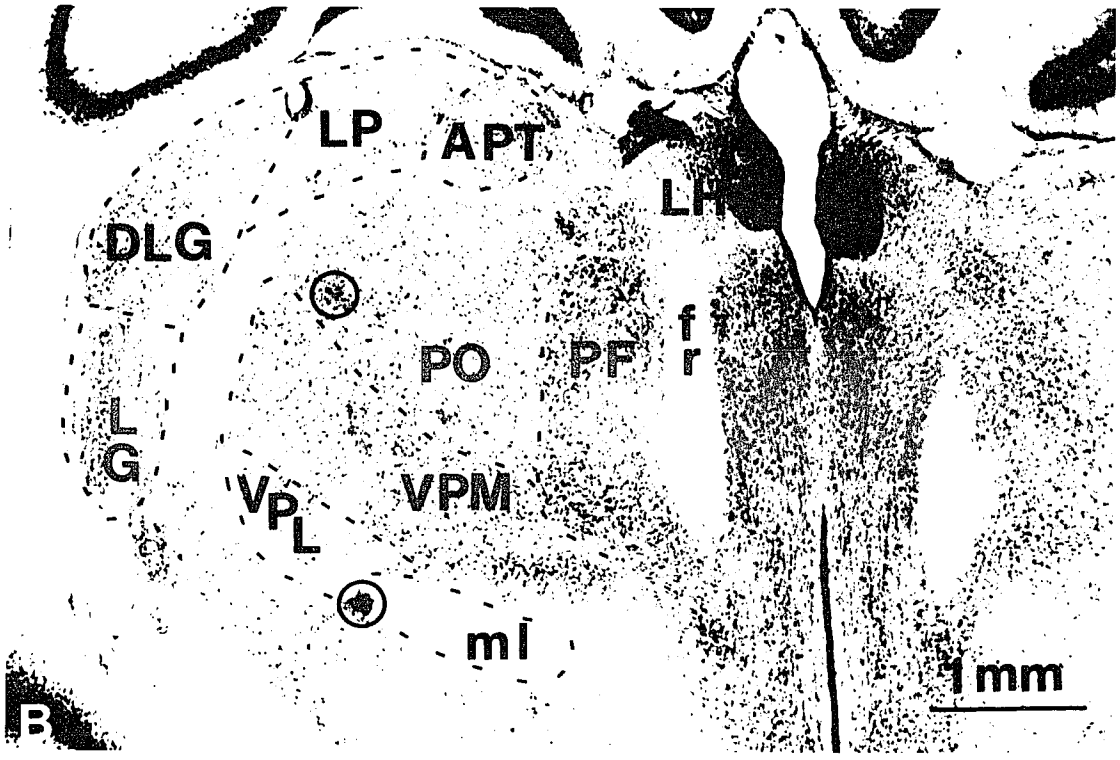
Some units responded to LT stimulation applied in small (e.g., single whisker, single digit, or 3 X 2 cm trunk), contralateral receptive fields, with an increase or, in a few cases, a decrease in firing frequency. Other units responded only to nociceptive pinch in large,

Table III. Abbreviations for Figures 9-12

3V	third ventricle
APT	anterior pretectal area
Aq	cerebral aqueduct
CG	central gray
CL	central lateral thalamic nucleus
CM	centromedian thalamic nucleus
DLG	dorsal lateral geniculate nucleus
eml	external medullary lamina
f	fornix
fr	fasciculus retroflexus
G	gelatinosus thalamic nucleus
hbc	habenular commissure
ic	internal capsule
LD	lateral dorsal thalamic nucleus
LH	lateral habenular nucleus
LP	lateral posterior thalamic nucleus
MD	mediodorsal thalamic nucleus
MG	medial geniculate nucleus
MH	medial habenular nucleus
ml	medial lemniscus
mt	mammillothalamic tract
opt	optic tract
OT	nucleus optic tract
PC	paracentral thalamic nucleus
pc	posterior commissure
PF	parafascicular thalamic nucleus
PO	posterior complex
PV	paraventricular thalamic nucleus
PVP	paraventricular thalamic nucleus, posterior
Re	reuniens thalamic nucleus
Rh	rhomboid thalamic nucleus
Rt	reticular thalamic nucleus
SC	superior colliculus
sm	stria medullaris thalamus
SNR	substantia nigra, reticular
st	stria terminalis
str	superior thalamic radiation
VL	ventral lateral thalamic nucleus
VLG	ventral lateral geniculate nucleus
VPL	ventral posterolateral thalamic nucleus
VPM	ventral posteromedial thalamic nucleus
ZI	zona incerta

Figure 9A-C. Low power photomicrographs of 80 um coronal thalamic sections, stained with neutral red, illustrating the anatomical divisions of the somatosensory thalamus of the rat. Spots made by iontophoresis of Niagara Sky Blue dye are circled. A. illustrates VPM and VPL and is a little anterior to 5.2 mm from interaural zero in the Paxinos and Watson ('82) stereotaxic system. B. is a little anterior to 4.7 mm from interaural zero. It illustrates posterior VPM and VPL. C. illustrates the regions posterior to the VB complex and is about 4.2 mm anterior to interaural zero.

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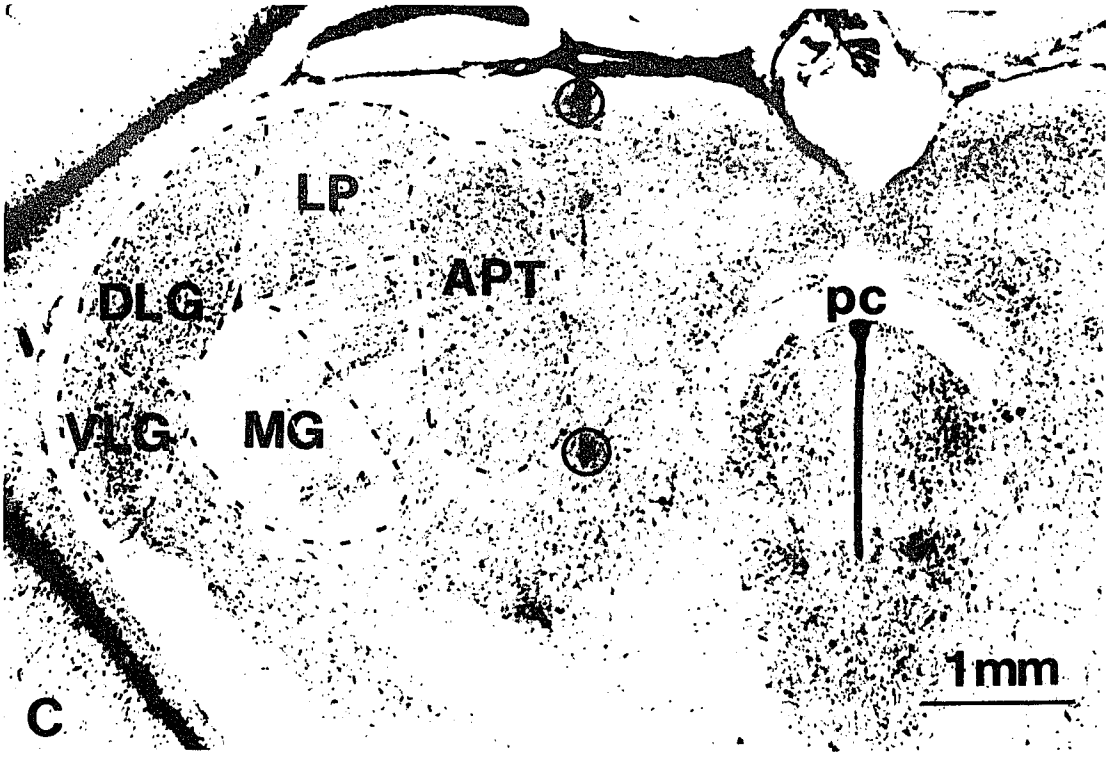
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bilateral receptive fields, often including the whole body, with either an increase or decrease in firing frequency. Still others responded to LT in small contralateral receptive fields and nociceptive pinch in large bilateral receptive fields.

a. VPM

VPM was designated as the densely cellular dorsomedial portion of the VB complex (Fig. 9A-B; Fig. 10B-E). Recordings were made from 112 units in this area (Table IV); 46 (41%) responded to nociceptive stimulation in large bilateral receptive fields, all with an increase in firing frequency; 41 (37%) responded to contralateral LT, or LT and nociceptive stimulation, within a small receptive field, 40 with an increase in firing frequency, 1 with a decrease; 6 (5%) responded to both contralateral LT and bilateral nociceptive stimulation with an increase in firing frequency; and 19 (17%) did not respond to any mechanical somatosensory stimulation applied. Of the VPM units responding to LT or to both LT and nociceptive stimulation, 43 (91%) had whisker receptive fields, 4 (9%) had receptive fields located elsewhere on the head (Table V). Most vibrissal units had single whisker receptive fields although some responded to stimulation in larger receptive fields e.g., whole rows of whiskers. A somatotopic organization of whisker responses was observed; whiskers in the most ventral horizontal rows were represented in the thalamus at levels rostral to whiskers in dorsal rows and, at a particular level in the rostrocaudal plane, caudal whiskers were dorsal to rostral whiskers. In other words, the head was represented in the thalamus, with the jaw

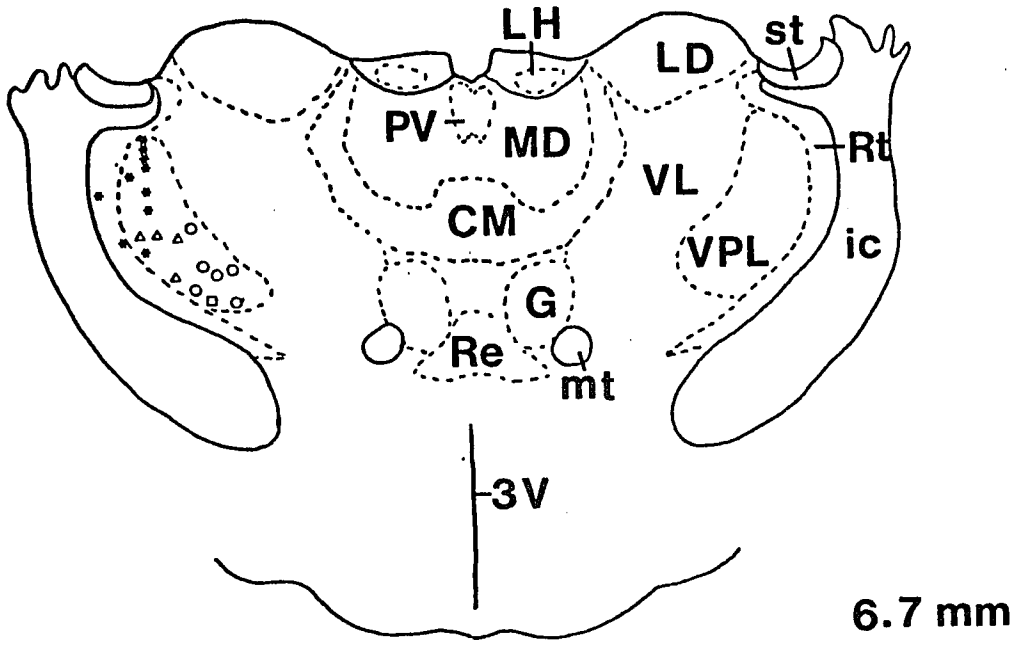
rostrally placed and the nose down. There did not appear to be any somatotopic organization of nociceptive responses. LT or LT/nociceptive units with receptive fields located on the forefeet, hindfeet, or trunk were not found in VPM (Table V).

The ratios of LT to nociceptive and LT to nonresponsive units were greater at anterior levels of VPM than at more posterior levels (Fig. 10B,C vs 10D,E). Units with single whisker receptive fields also appeared to be more common rostrally. This decrease in the proportion of LT responses was correlated with a change in cytoarchitecture. At posterior levels of VPM, cells became less densely packed, more loosely arranged and smaller (5-6 um) cells were more common (Fig. 9B).

b. VPL

VPL was designated as the ventrolateral region of the VB complex characterized by loosely packed, discoid, somata arranged in laminae parallel to the external medullary lamina (Fig. 9A-B; Fig. 10B-E). Recordings were made from 81 units in this area (Table IV). Of these units, 28 (35%) responded to nociceptive stimulation in large bilateral receptive fields, 27 with an increase in firing frequency, 1 with a decrease; 45 (56%) responded to LT or LT and nociceptive stimulation within small contralateral receptive fields, 44 with an increase in firing frequency, 1 with a decrease; 6 (7%) responded to both contralateral LT and bilateral nociceptive stimulation; and 2 (2%) did not respond to applied stimuli. Of the 51 units responding to LT and LT/nociceptive stimulation, 8 (16%) responded to stimulation on the head,

Figure 10. A-H. Line drawings after Paxinos and Watson ('82) illustrating the anatomical localization of units responding to somatosensory stimulation. Numbers in lower right represent distance from interaural zero. ● ; units responding to LT or LT/nociceptive stimulation in small contralateral receptive fields on the head. ○; units responding to LT or LT/nociceptive stimulation in small contralateral receptive fields on the forepaw. △;units responding to LT or LT/nociceptive stimulation in small contralateral receptive fields on the hindpaw. □; units responding to LT or LT/nociceptive stimulation in small contralateral receptive fields on the trunk. *; units responding to nociceptive stimulation in large bilateral receptive fields.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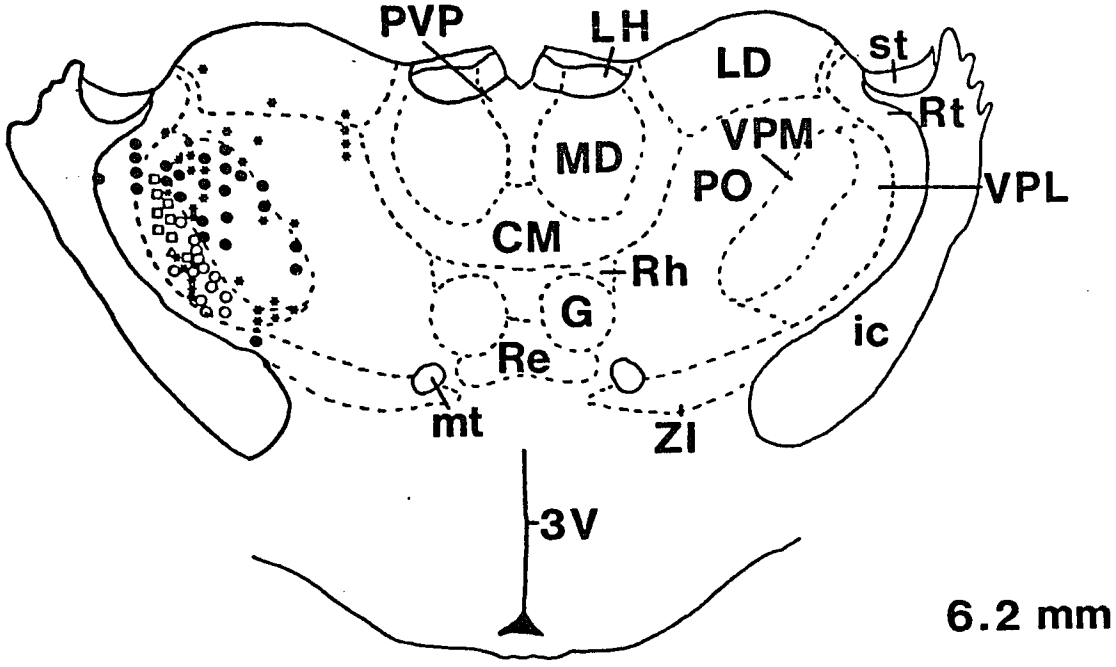
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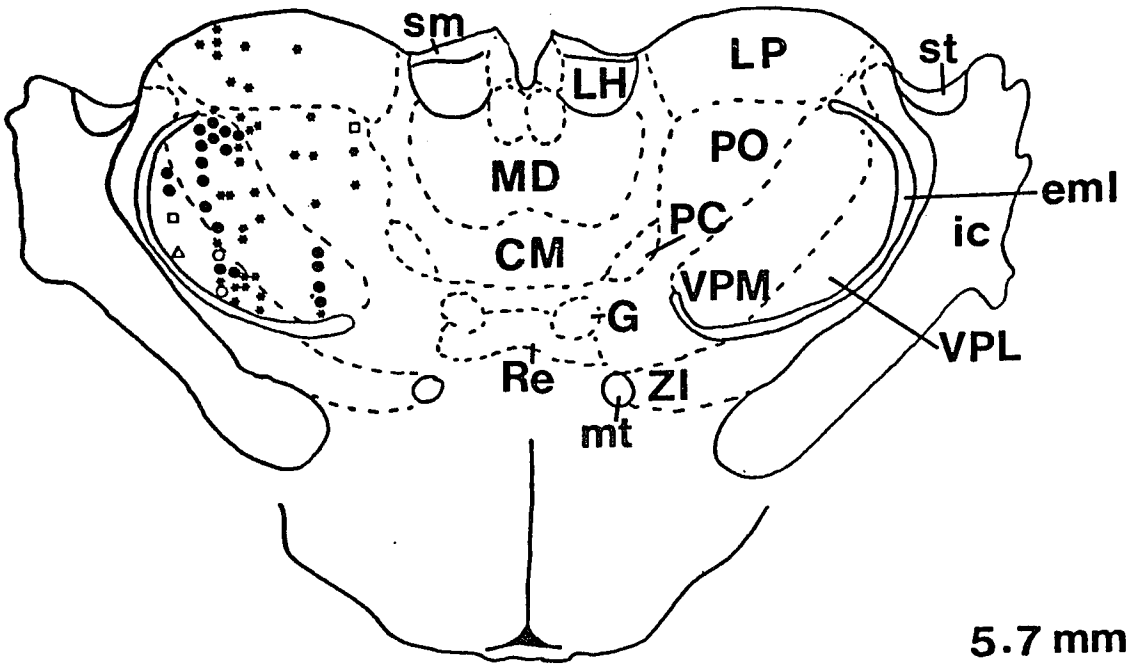
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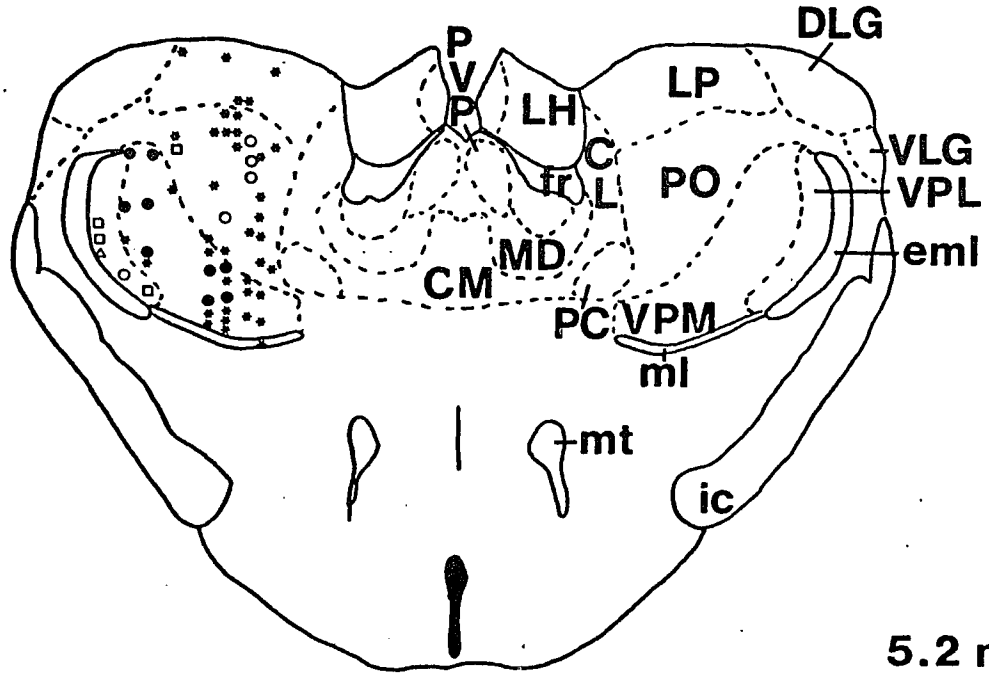
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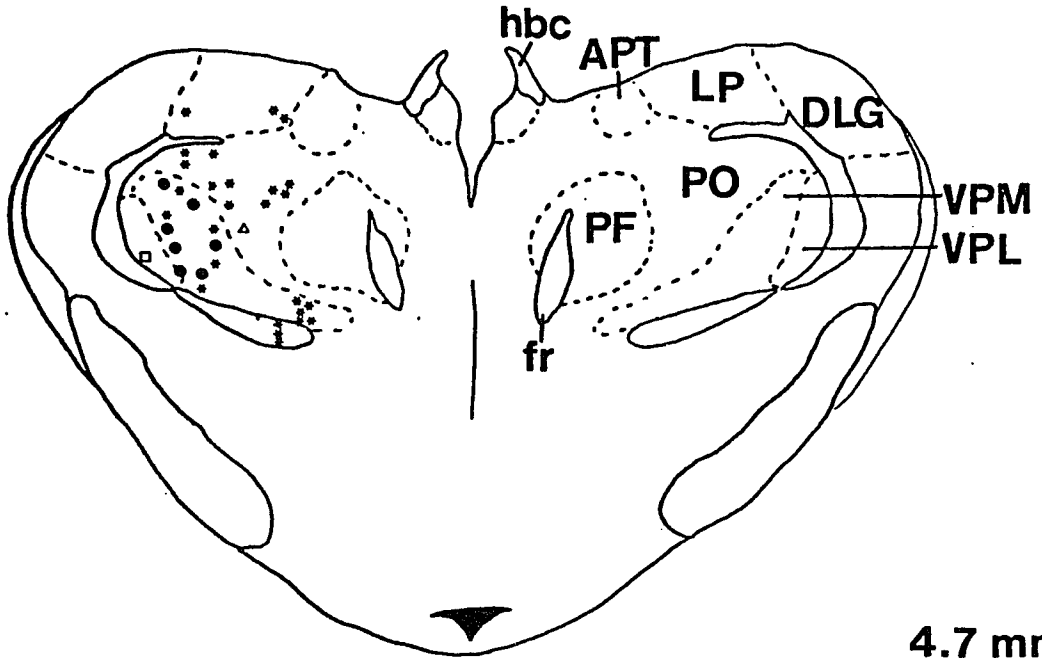
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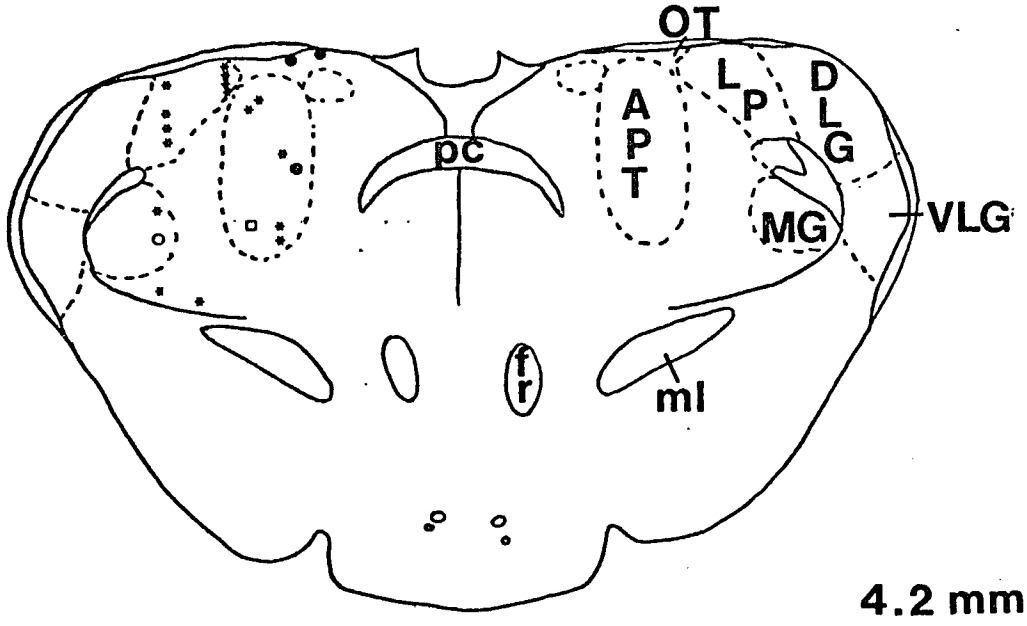
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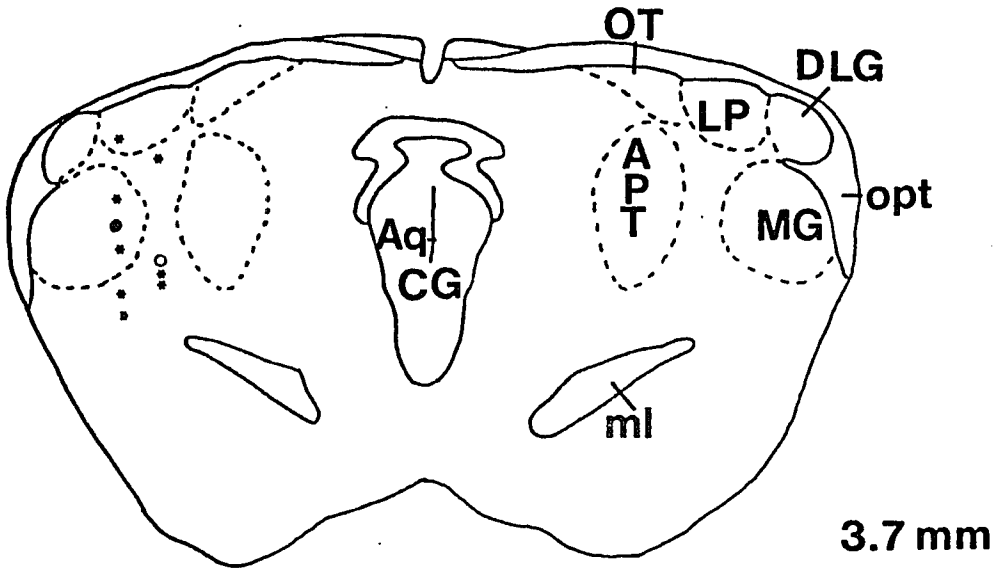

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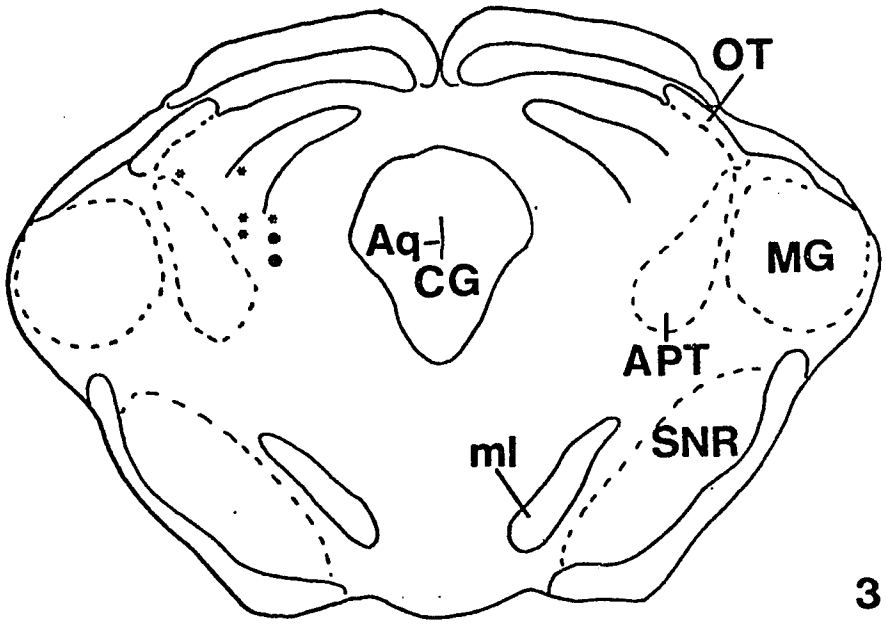


H

Table IV. Unit response characteristics in VPL, VPM, and PO Complexes

	Light Touch			Nociceptive			LT/ Noci	Nonresponsive	Total
	inc ff*	dec ff#	total	inc ff	dec ff	total	inc ff		
VPM	40	1	41	46	0	46	6	19	112
VPL	44	1	45	27	1	28	6	2	81
VB Complex			86(45%)**			74(38%)	12(6%)	21(11%)	193
PO Complex	8	1	9(9%)	50	4	54(55%)	3(3%)	32(33%)	98
Total			95			128	15	53	291

* increase in firing frequency
\# decrease in firing frequency
** percentage of total units

22 (43%) to forepaw stimulation, 7 (14%) hindpaw stimulation, and 14 (27%) to stimulation on the trunk (Table V).

Within VPL, units with forepaw receptive fields tended to be located medial to those with hindpaw and trunk receptive fields (Fig. 10A-D). In lateral parts of VPL a vertical somatotopic organization of responses to trunk and hindpaw stimulation was observed; receptive field on the trunk shifted from rostral to caudal as the electrode was lowered from dorsal to ventral in VPL (Fig. 11). Units with receptive fields on the head tended to be located either along the dorsomedial edge of VPL, just ventral to VPM, or dorsolaterally; i.e., ventral to the reticular nucleus (Fig. 10B,D). There did not appear to be a somatotopic organization of units responding to nociceptive stimulation in VPL.

In summary, 193 units were recorded from in the VB complex; 86 (45%) responded to LT, 74 (38%) responded to nociceptive stimulation, and 12 (6%) to both LT and nociceptive stimulation. Twenty one (11%) did not respond to any kind of mechanical somatosensory stimulation (Table IV). For the most part units responding to different types of stimulation were interspersed throughout the VB complex.

c. PO Complex

The area included in the PO complex consists of the region dorsomedial to VPM and the region medial to the medial geniculate nucleus, excluding the anterior pretectal area (Fig. 9A-C; Fig. 10B-H). Recordings were made from 98 units in this area (Table IV); 9 (9%) responded to LT stimulation, 8 with an increase in firing fre-

Figure 11. Line drawing after Paxinos and Watson ('82) illustrating the vertical orientation of somatotopic localization found in lateral VPL. □; units responding to LT stimulation of the trunk encountered in a single track. As the electrode was lowered the receptive field of units encountered shifted from the rostral to the caudal trunk.

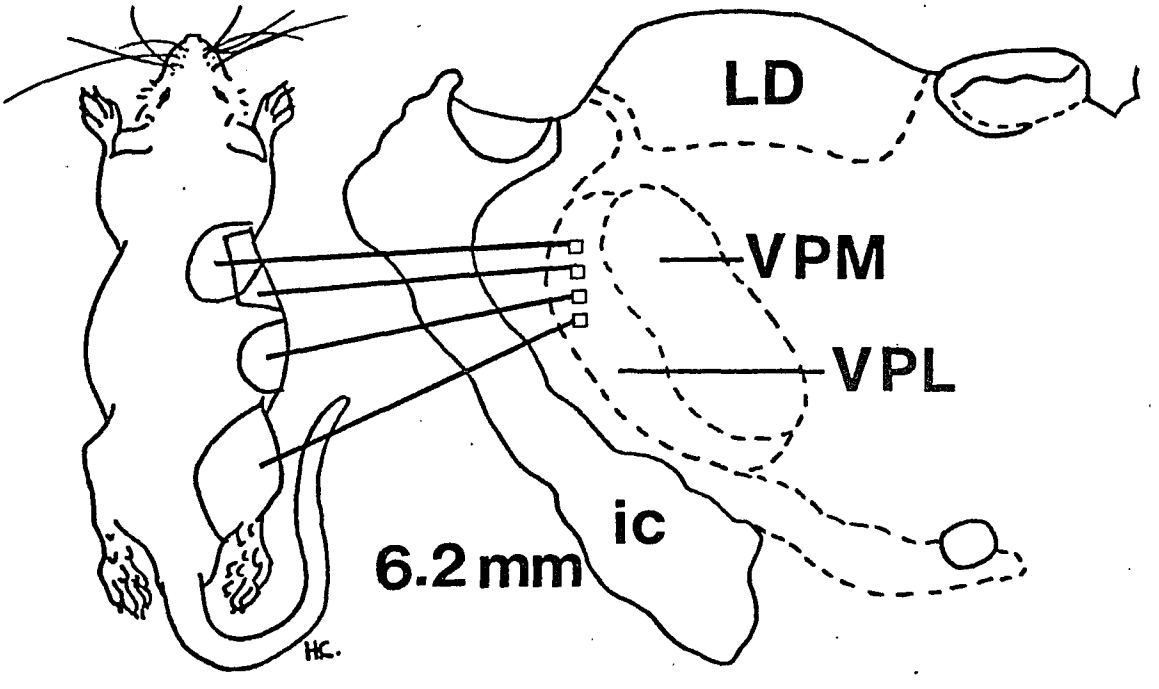


Table V. Receptive fields of LT and LT/Nociceptive units

	whi-sker	rest head	total head	fore-paw	hind-paw	trunk	total body	total units
VPM	43	4	47	0	0	0	0	47
VPL	6	2	8	22	7	14	43	51
PO Complex	2	3	5	4	0	3	7	12
Total	51	9	60	26	7	17	50	110

quency, 1 with a decrease; 54 (55%) responded to nociceptive stimulation, 50 with an increase in firing frequency, 4 with a decrease; 3 (3%) units responded to LT and nociceptive stimuation with an increase in firing frequency; and 32 (33%) of the units did not respond.

Within the PO complex no somatotopic organization of either LT or nociceptive units was observed. Some units responding to LT had receptive fields on the head, others on the body (Table V).

In all, recordings were made from 291 units in the VB and PO complexes; 238 (82%) responded to the applied mechanical somatosensory stimuli. Of the 110 units responding to LT or LT/noci stimulation in these two areas, 51 (46%) had vibrissal receptive fields, 9 (8%) had receptive fields located elsewhere on the head, 26 (24%) had forepaw receptive fields, 7 (6%) had hindpaw receptive fields, and 17 (15%) had receptive fields on the trunk (Table V).

d. Other Nuclei

Other nuclei and regions from which responses to somatosensory stimulation were observed include the medial lemniscus, the lateral dorsal thalamic nucleus, the lateral posterior thalamic nucleus, the zona incerta, the reticular thalamic nucleus, the anterior pretectal area, the superior colliculus, and the medial geniculate nucleus. Recordings were made from 122 units in these areas; 57 (47%) responded to somatosensory stimulation, 8 responded to LT, 47 to nociceptive stimulation, and 2 to LT and nociceptive stimulation (Table VI). 53% of the units tested did not respond to any kind of somatosensory stimulation tested.

In these other nuclei, therefore, the percentage of nonresponsive units was greater than in either the VB or PO complexes (11% and 33% respectively). Most (82%) of the units that did respond to somatosensory stimulation in these other regions were nociceptive, and were located in the lateral posterior nucleus.

Table VI. Response characteristics of units outside the VB and PO Complexes

	LT	Noci	LT/ noci	Total somato-sensory	Nonres-ponsive	Total
APT	2	6	0	8	4	12
LD	0	3	0	3	3	6
LP	0	25	0	25	27	52
MG	0	4	1	5	2	7
ml	0	4	0	4	1	5
ONT	2	0	0	2	5	7
Rt	2	3	1	6	4	10
SC	1	1	0	2	12	14
ZI	1	1	0	2	7	9
Total	8	47	2	57(47%)*	65(53%)	122

* percentage of total units

2. DISCUSSION

Anatomical studies have subdivided the VB complex into two nuclei, VPM and VPL, on the basis of cytoarchitectural differences (McAllister and Wells, '81). In the present study a definite correlation between the receptive field locations of LT units and their anatomical localization in these two nuclei was seen. In the dorsomedial portion of the VB complex (VPM), characterized by large, round, densely packed somata, all LT units had receptive fields on the head (Fig. 10B-E). In the ventrolateral VB complex (VPL), characterized by loosely packed, discoid somata arranged in laminae, most (84%) units had receptive fields on the body (Fig. 10A-E). Some of the VPL units that responded to LT of the head were located on the dorsomedial edge of VPL, just ventral to VPM (Fig. 10D). These recordings may actually have been made from VPM rather than VPL units. The results indicate that the anatomical subdivisions of the VB complex, VPM and VPL, do, in fact, represent functional, somatotopically organized nuclei.

These results are in general agreement with those of Emmers' ('65) who mapped multiunit responses to peripherally applied mechanical stimulation in the rat. Although Emmers did not include cytoarchitectural descriptions of regions from which recordings were made, recording sites were indicated in his photomicrographs. From his Figs. 2-7 it appears that areas from which responses to stimulation of the contralateral head were obtained lay within VPM, while responses to contralateral stimulation of the fore or hindpaw lay within VPL. The results of the present study differ from Emmers', most notably, in that responses to stimuli applied in large bilateral receptive fields

were interspersed with responses to stimulation applied within small contralateral receptive fields throughout the VB complex. Emmers found bilateral responses only in a restricted lateral area of the VB complex, which he called SII, from which no responses to stimuli applied in small contralateral receptive fields were recorded. In the present study a trunk representation was included in the somatotopic map of VPL; in the Emmers' study no trunk representation was included. It appears that, anatomically, the Emmers' SII and the trunk representation area in the present study are the same; compare Fig. 10B-E to Emmers' Fig. 3-5. Furthermore, Emmers described his SII as having a vertical somatotopic orientation; i.e., responses to stimuli applied rostrally were dorsal to those applied caudally, as opposed to the mediolateral somatotopic orientation found in the rest of the VB complex; e.g., the forepaw was medial to the hindpaw. In the present study the same vertical somatotopic orientation was observed in the trunk representation area.

Since most other electrophysiological studies do not provide information on the cytoarchitecture of the regions from which recordings were made, it is difficult to compare the results of this study with theirs, except to make a few general comments. One consistent finding, which agrees with these results, is that the representation of the body is placed ventrolateral to the representation of the head (Davidson, '65; Emmers, '65; Waite, '73) In the present study, of the units responding to LT stimulation, those with vibrissal receptive fields were the most common (46%), those with forepaw receptive fields the second most numerous (24%), and more units had forepaw receptive

fields than hindpaw (6%). This agrees with Davidson ('65), who noted that vibrissal responses were more common than those from both the fore and hindlimbs combined, and Angel and Clark ('75) who noted that the forepaw representation is larger than the hindpaw. The results of the present study disagree with Angel and Clark, however, in indicating that the trunk representation is larger than the hindpaw. They also disagree with others in that in the present study responses to LT of the body extended further rostrally than responses to LT of the head, while Davidson ('65) and Waite ('73) reported responses to whisker stimulation throughout the entire extent of the VB complex.

Some of the classes of units reported in this study, i.e. those responding to LT or nociceptive stimulation with a decrease in firing frequency, were not encountered very frequently. They have also not been described at any length by other investigators. Units responding to peripheral stimulation with a decrease in firing frequency are harder to detect than those units responding with an increase in firing frequency, particularly for units with very low spontaneous firing frequencies. In fact, most required analysis of ratemeter plots for their positive identification.

The somatotopic organization of the VB complex into two functionally discrete nuclei is supported by anatomical studies which indicate that projections from the trigeminal nuclear complex terminate in the dorsomedial, densely cellular, VB complex, and projections from the dorsal column nuclei terminate in the ventrolateral, laminated VB complex (Feldman and Kruger, '80).

Responses to LT and nociceptive pinch were recorded in both the VB and PO complexes but there was a difference in the frequency of their occurence. In the VB complex units responding to LT were more common than in the PO complex (45% vs 9%). Responses to nociceptive stimulation were more frequently recorded in the PO than in the VB complex (55% vs 38%). Another difference between the VB and PO complexes was the finding of a somatotopic organization of LT units in the VB complex and the lack of such organization in the PO complex. These results, therefore, indicate that the VB and PO complexes are also functionally distinct regions. The VB complex is involved in relaying somatotopically organized LT inputs while the PO complex is not. In the present study units that did not respond to the somatosensory stimuli were also more numerous in the PO than in the VB complex (33% vs 11%).

These findings are in direct contrast to that of Guilbaud et al. ('80) who found no difference in the percentage of LT or nociceptive units in the VB vs the PO complex, and concluded that the two regions were not functionally distinct. One reason for this discrepancy may be the difference in the regions included in the designation PO complex. Guilbaud et al. recorded from a few units in the area dorsomedial to the VB complex, but did not systematically explore this region because they did not observe it to be an area containing a clear population of units affected by somatosensory stimulation. In the present study, however, the majority (77%) of these units did respond to somatosensory stimulation, most of them being nociceptive, and therefore, it was included as part of the PO complex.

In conclusion, the results of this study indicate that there is a definite correlation between unit response characteristics and anatomical localization in the somatosensory thalamus of the rat, and imply that the anatomically distinct regions are, in fact, functionally distinct.

C. Iontophoretic application of serotonin on somatosensory units in the thalamus

1. RESULTS

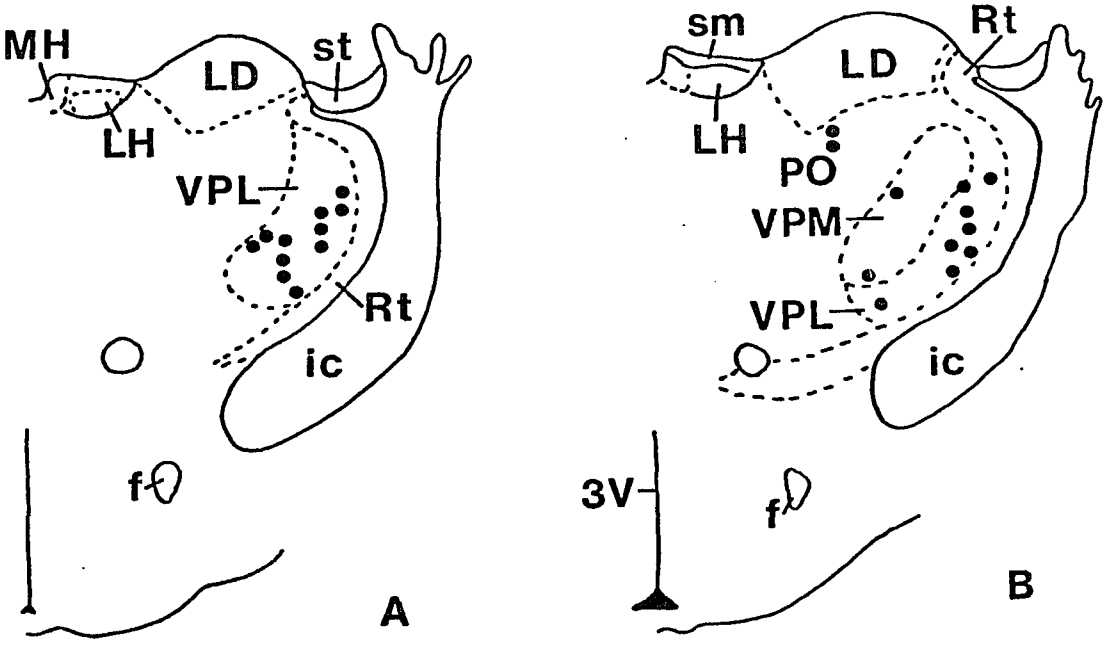
a. Serotonergic effects on nociceptive versus nonnociceptive units in VB and PO complexes

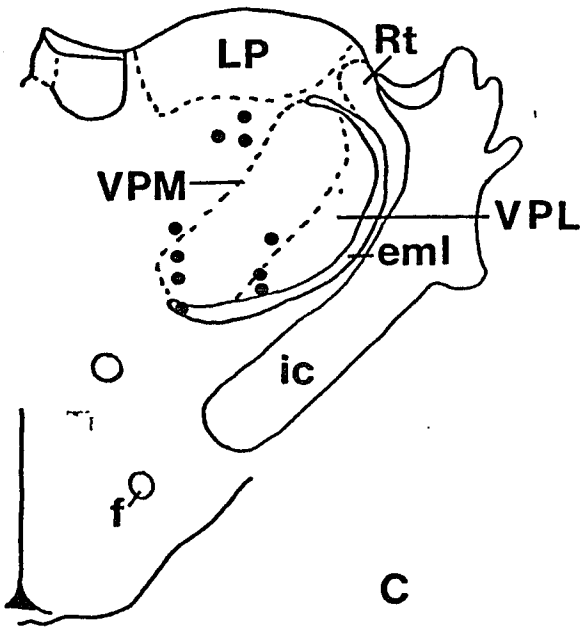
Serotonin was iontophoretically applied to spontaneously active units, and to evoked activity of units responding to nociceptive and nonnociceptive stimulation in the VB and PO complexes of the rat. Nuclear divisions were made as previously described (Fig. 12).

The spontaneous firing frequency of all units (n=23) tested was inhibited by 5-HT; the firing frequency during 5-HT application was significantly lower ($P<.05$) than the firing frequency before 5-HT (Table VII; Fig. 13). For most units inhibited by 5-HT, the firing frequency after drug application returned to predrug level within a few seconds (n=15) (Fig. 13A). For 4 units, however, the firing frequency after 5-HT application was significantly lower than before drug for a period of time, e.g., for 30 sec, before returning to predrug levels (Fig. 13B). Four units had firing frequencies significantly higher after 5-HT than before; for 1 of these units the firing frequency returned to before drug levels in about 30 sec, for the other 3 firing frequency remained elevated for the duration of the recording (i.e., 60-130 sec) (Fig. 13C).

The only units excited by 5-HT (n=10) were characterized by a particular type of response suggesting a nonspecific effect (Fig. 14). Upon application of 5-HT firing frequency increased dramatically (e.g., as much as an order of magnitude) and the unit's shape changed.

Figure 12. A-E. Line drawings after Paxinos and Watson ('82) illustrating the anatomical localization of units tested for iontophoretic effects of serotonin.

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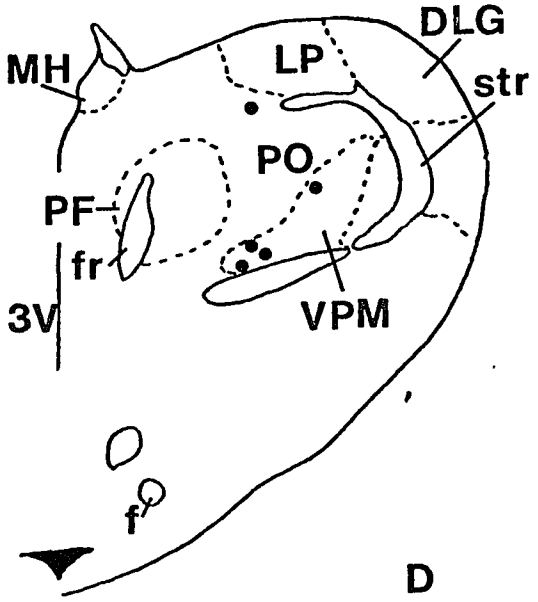
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f
D

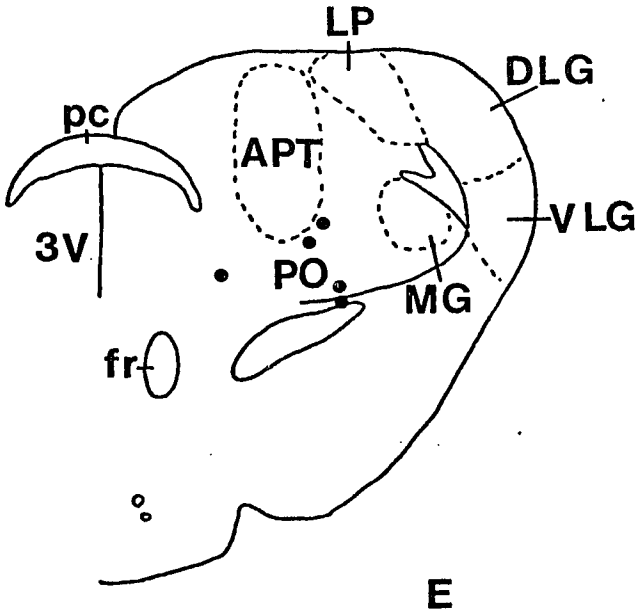
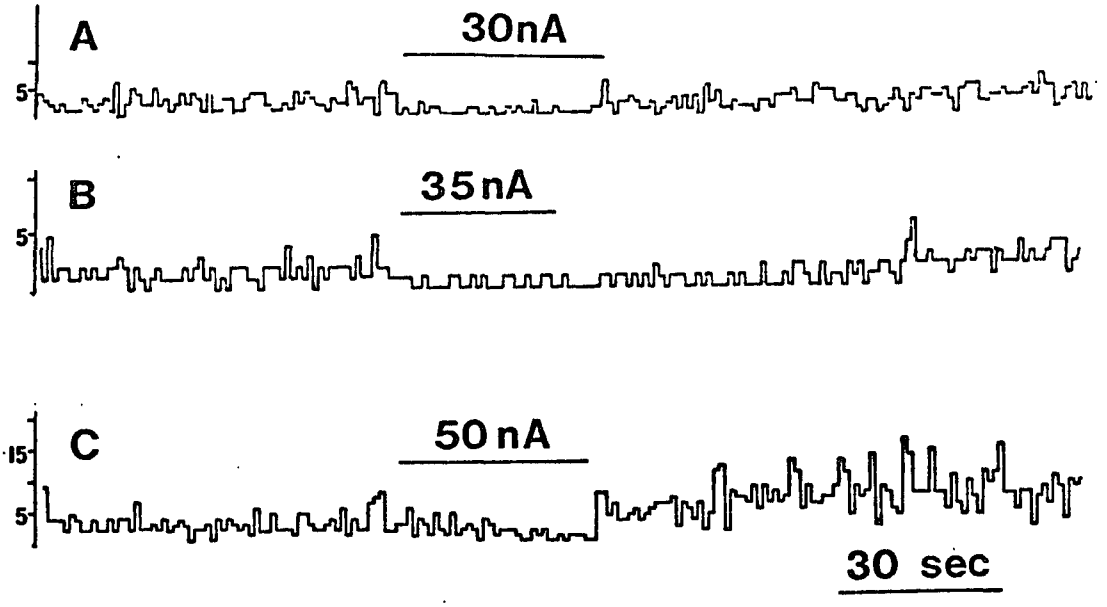

LP
DLG
pc
APT
VLG
3V
PO
MG
fr
E

Figure 13. A-C are continuous ratemeter plots, which illustrate inhibitory effects of 5-HT on spontaneous activity. On the x-axis is time (1 mm = 1 sec); on the y-axis is firing frequency in Hertz. Serotonin was iontophoretically applied with the currents indicated. A. For this unit the before serotonin firing frequency was equal to the firing frequency after iontophoresis of 5-HT. This response pattern was observed for 15 out of 23 units tested. B. For this unit the after drug firing frequency was less than than the firing frequency before drug. This response pattern was observed for 4 out of 23 units tested. C. For this unit the firing frequency after serotonin was greater than the firing frequency before iontophoresis. This response pattern was also observed for 4 out of 23 units tested.



It also appeared that there was no relationship between dose of iontophoretic current and magnitude of response. Large increases in firing frequency were observed even with reductions of iontophoretic current to very low levels. If tested for current sensitivity these units tested postively; i.e., unbalanced current passed through the NaCl or dye barrel produced similar dramatic increases in firing frequency. Often this kind of unit was lost after one iontophoretic drug trial, and could not be tested for current effects (n =4). It was concluded, therefore, that these excitatory responses were nonspecific and that no excitatory effect of serotonin iontophoresis was observed.

No difference was observed in the incidence of serotonergic inhibition of the spontaneous activity of units responding to nociceptive or nonnociceptive stimuli (Table VII). The spontaneous activity of all nonnocieptive units (n=10) and nocieptive units (n=13) tested was inhibited by serotonin. There was also no difference in the incidence of inhibition of spontaneous activity in different anatomical regions of the thalamus. The spontaneous activity of all units tested in the VB complex (n=16) was inhibited by 5-HT, as was that of all units tested in the PO complex (n=7) (Table VII).

In the first series of experiments the iontophoretic effects of 5-HT on evoked activity were determined by making continuous ratemeter plots of unit activity (Fig. 15). Mean firing frequencies of evoked activity before, during, and after drug were compared. Both nociceptive (n=16) or nonnociceptive (n=6) units were tested. The evoked activity of all nociceptive units tested was inhibited by 5-HT; unit firing frequencies during 5-HT application were significantly lower than unit firing frequencies before 5-HT (Fig. 15D). In most cases

Figure 14. A-B are continuous ratemeter plots; time is on the x-axis (1 mm = 1 sec), firing frequency, in Hertz, on the y-axis. A. illustrates an excitatory response of a unit to 50 nA of current passed through the serotonin barrel. When this unit was tested for current sensitivity it tested positively. 50 nA of current passed through the NaCl barrel also produced an excitatory effect (B).

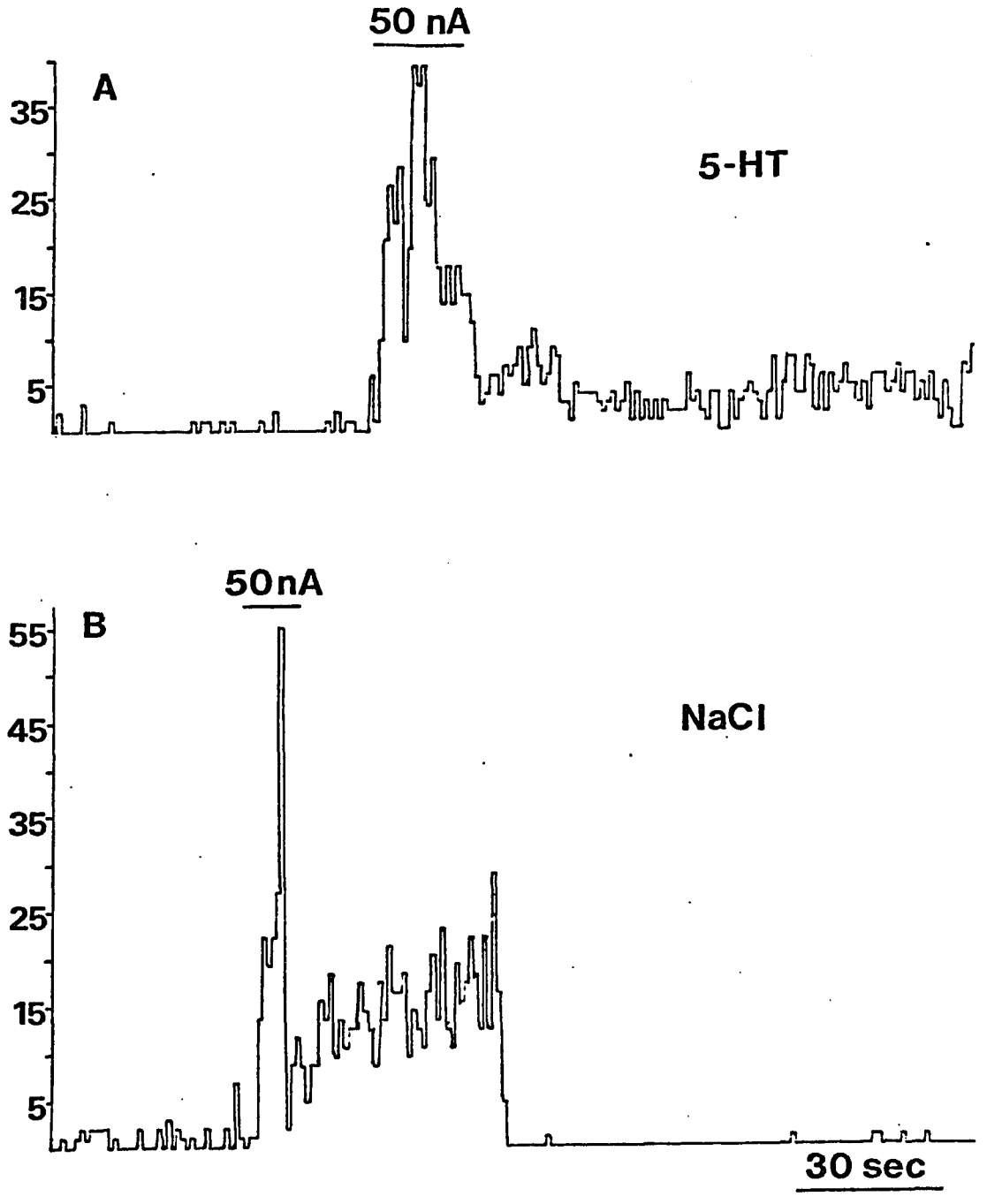

50 nA
A
35
25
15
5
5-HT
50nA
B
55
45
35
25
15
5
NaCl
30 sec

Table VII. Effects of 5-HT iontophoresis on spontaneous or evoked activity of VB and PO units.

	Spontaneous		Evoked		Spont and Evoked	
	Inhib	NA*	Inhib	NA	Inhib	NA
Nonnociceptive VB Complex	10	0	6	1	3	1
Nociceptive						
VB Complex	6	0	7	0	5	0
PO Complex	7	0	9	0	4	0
Total Nociceptive	13	0	16	0	9	0

* not affected

there was also a steady decrease in the unit's response to somatosensory stimulation alone (i.e., without 5-HT) (Fig. 15C) A comparison of firing frequency before, during, and after 5-HT iontophoresis was not adequate to describe drug effects on these units; i.e., the decrease in firing frequency during iontophoresis was partially due to a decline in the somatosensory response. Comparisons were made, therefore, between before, during, and after drug intervals and comparable time intervals during control responses to somatosensory stimulation without drug application. In all cases there was a greater decrease in firing frequency during drug application than during the comparable time interval of the somatosensory response alone. Three units had post 5-HT firing frequencies that were significantly lower than predrug but, within 25 sec, firing frequency had returned to before 5-HT levels (Fig. 15B). For two units, the firing frequency after 5-HT was nonsignificantly different than before 5-HT (Fig. 15A). For 1 unit, firing frequency immediately after drug was significanly higher than before 5-HT, but returned to predrug levels within 13 sec.

Most (6/7) nonnociceptive units for which continuous ratemeter plots were used to compare mean firing frequencies before, during, and after 5-HT iontophoresis were also inhibited by 5-HT (Fig. 15A; Table VII). Three of these units showed no significant difference between mean firing frequency before and after drug (Fig. 15A), 1 unit showed a temporary (15 sec) excitation following which firing frequency returned to predrug activity levels. Two nonnociceptive units inhibited by serotonin, were characterized by a lower firing frequency after drug application than before. One of these units also had a

decreasing somatosensory response. The evoked activity of one nonnociceptive unit was not effected by serotonin; the mean firing frequency before 5-HT was not significantly different from the mean firing frequency during drug application for the range of drug doses tested (20-70 nA).

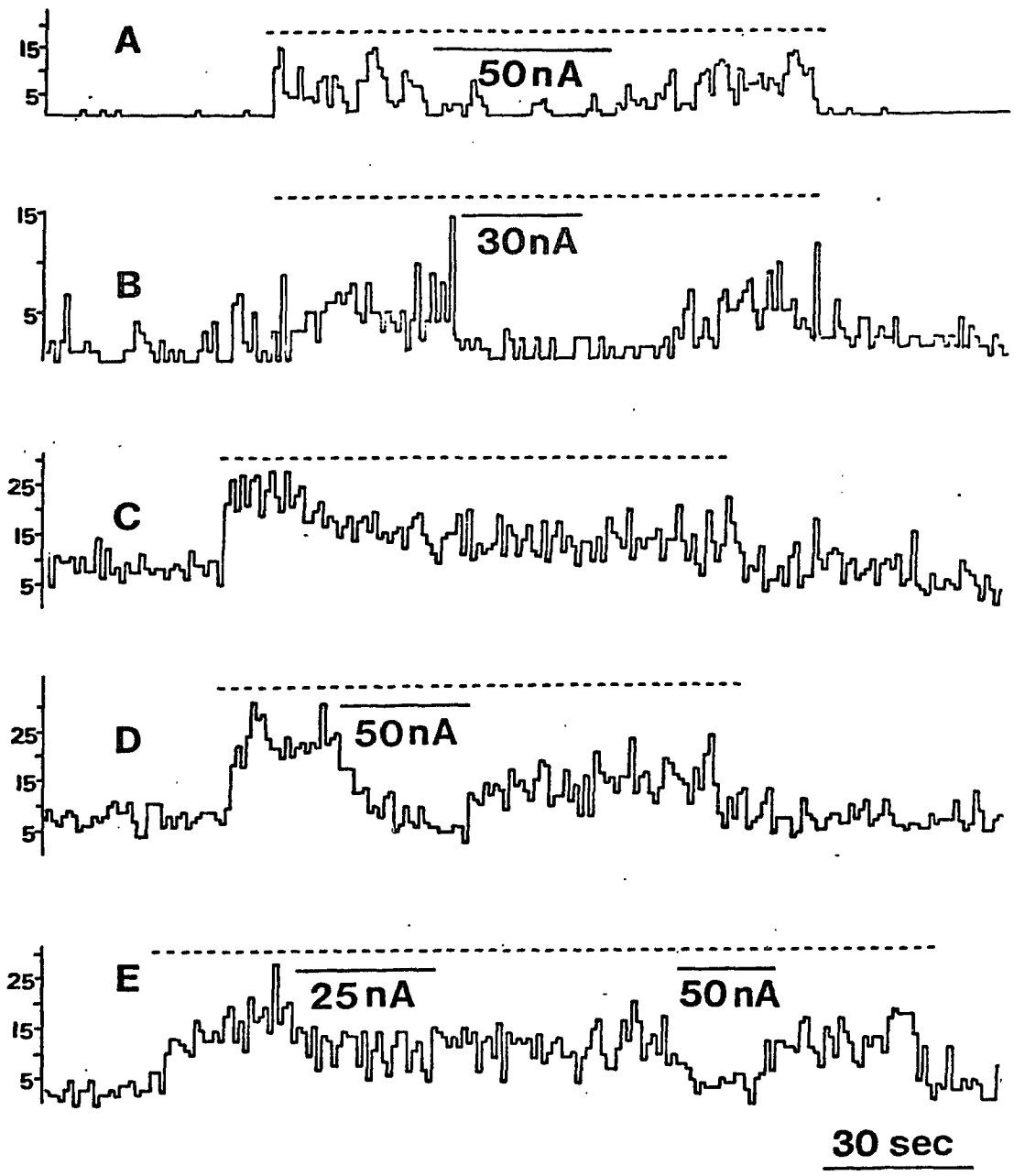
Doses of serotonin, measured as the magnitude of iontophoretic current used, ranged from 20-70 nA. Larger doses of drug generally produced greater percent decreases in firing frequency (Fig. 15E). 20-25 nA of current produced an average decrease of 39% in firing frequency (number of trials=4), 30-35 nA an average decrease of 56% (number of trials=3), 40-45 nA an average decrease of 56% (number of trials=2), and 50-55 nA an average decrease of 71% (number of trials=9).

In 13 cases the effect of 5-HT iontophoresis was tested on both the spontaneous and evoked activity of the same unit; 9 of these units responded to peripherally applied nociceptive stimulation, 4 to nonnocieptive (Table VII). Both the spontaneous and evoked activity of all of the nociceptive and 3 out of 4 of the nonnociceptive units were inhibited by 5-HT. The evoked activity of 1 nonnociceptive unit was not effected by 5-HT but spontaneous activity was inhibited.

b. Effects of serotonin on components of nonnociceptive responses

In order to study the time course of the somatosensory response and to determine the effects of 5-HT on early and late components of responses another series of experiments was done. The effects of 5-HT on evoked activity were investigated by using an electromechanical device to deliver a nonnociceptive light touch stimulus. Unit respon-

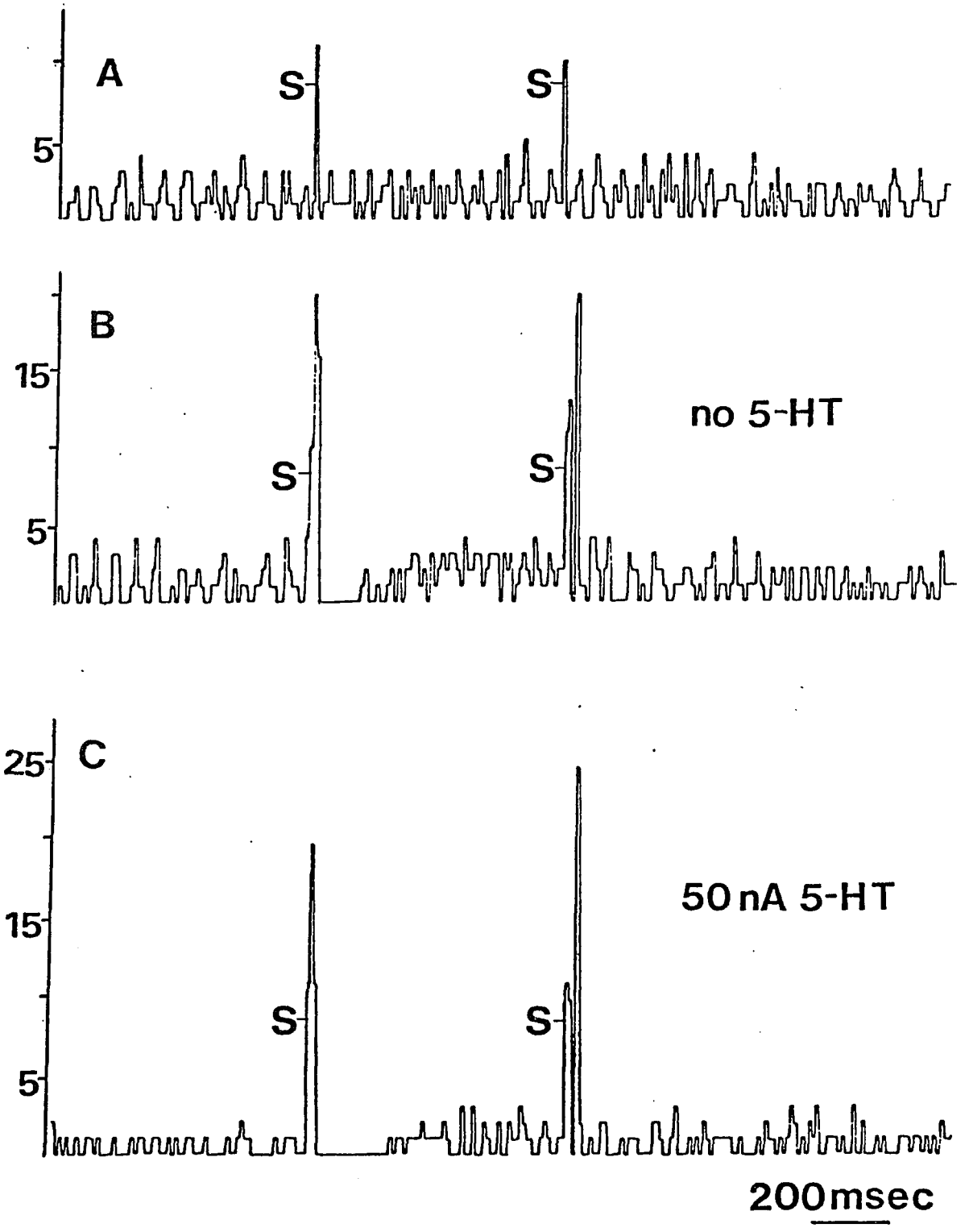
Figure 15. A-E are continuous ratemeter plots which illustrate inhbitory effects of 5-HT on nociceptive (B,D) and nonnociceptive activity (A). Serotonin was iontophoretically applied with the currents indicated. The dotted lines indicate periods of time in which somatosensory stimulation was applied. On the x-axis is time (1 mm = 1 sec), on the y-axis is firing frequency in Hertz. A. For this unit after 5-HT firing frequency was the same as before. This type of response was observed for 2 out of 16 nociceptive units tested and 3 out of 6 nonnociceptive units tested. B. For this unit the after 5-HT firing frequency was less than the before drug firing frequency immediately following iontophoresis but firing frequency returned to predrug levels with time. This response pattern was observed for 3 out of 16 units tested. C and D. are records made from the same unit. C. This unit was characterized by a decreasing response to somatosensory stimulation alone. D. The after 5-HT firing frequency, therefore, was lower than the before drug for the duration of the response. This was the response pattern observed for most nociceptive units tested (10/16), and for 1 out of 6 nonnociceptive unit. E. illustrates the effects of different doses of 5-HT on evoked activity. For this unit the effects of two doses of serotonin were tested on its response to nociceptive stimulation. 50 nA of 5-HT produced a greater decrease in firing frequency than 25 nA.


A
15
5
50nA
B
15
5
30nA
C
25
15
5
D
25
15
5
50nA
E
25
15
5
25nA
50nA
30 sec

ses to this stimulation were recorded as peristimulus time histograms averaging 10 or 15 sweeps. In other words, plots of number of spikes per bin, versus time before and after application of the stimulus, were made for 10-15 repetitions of stimulus application. In some cases histograms were made with bin widths of 10 msec to examine effects of 5-HT on both phasic and tonic components of the same unit (Fig. 16). In others, histograms were made with bin widths of 1 msec to examine the effects of 5-HT on the latency of the phasic response to nonnociceptive stimulation (n=8) (Fig. 17). Nonnociceptive stimulation produced responses which could be divided into phasic and tonic components (Fig. 16B-C). Phasic components typically had short latencies (e.g., 20 msec) and durations (e.g., 10-20 msec), and were followed by periods of inhibition or decreased activity, and then by longer duration, lower amplitude tonic responses. Repeated application of somatosensory stimuli without iontophoresis in most cases produced constant phasic responses and more variable tonic responses. The magnitude of the phasic component of nonnociceptive responses was, however, very sensitive to position of the probe of the electromechanical stimulator within the unit's receptive field.

The effects of 5-HT iontophoresis were determined on both phasic and tonic components of nonnociceptive responses by comparing histograms of unit responses obtained with and without 5-HT iontophoresis. Histograms of unit responses to somatosensory stimulation without 5-HT were made first, then histograms of unit responses with 5-HT, and, finally, histograms of unit responses without 5-HT again. Some units tested in this manner could be classified as WDR, others as LT. All of these recordings were made in the VB complex.

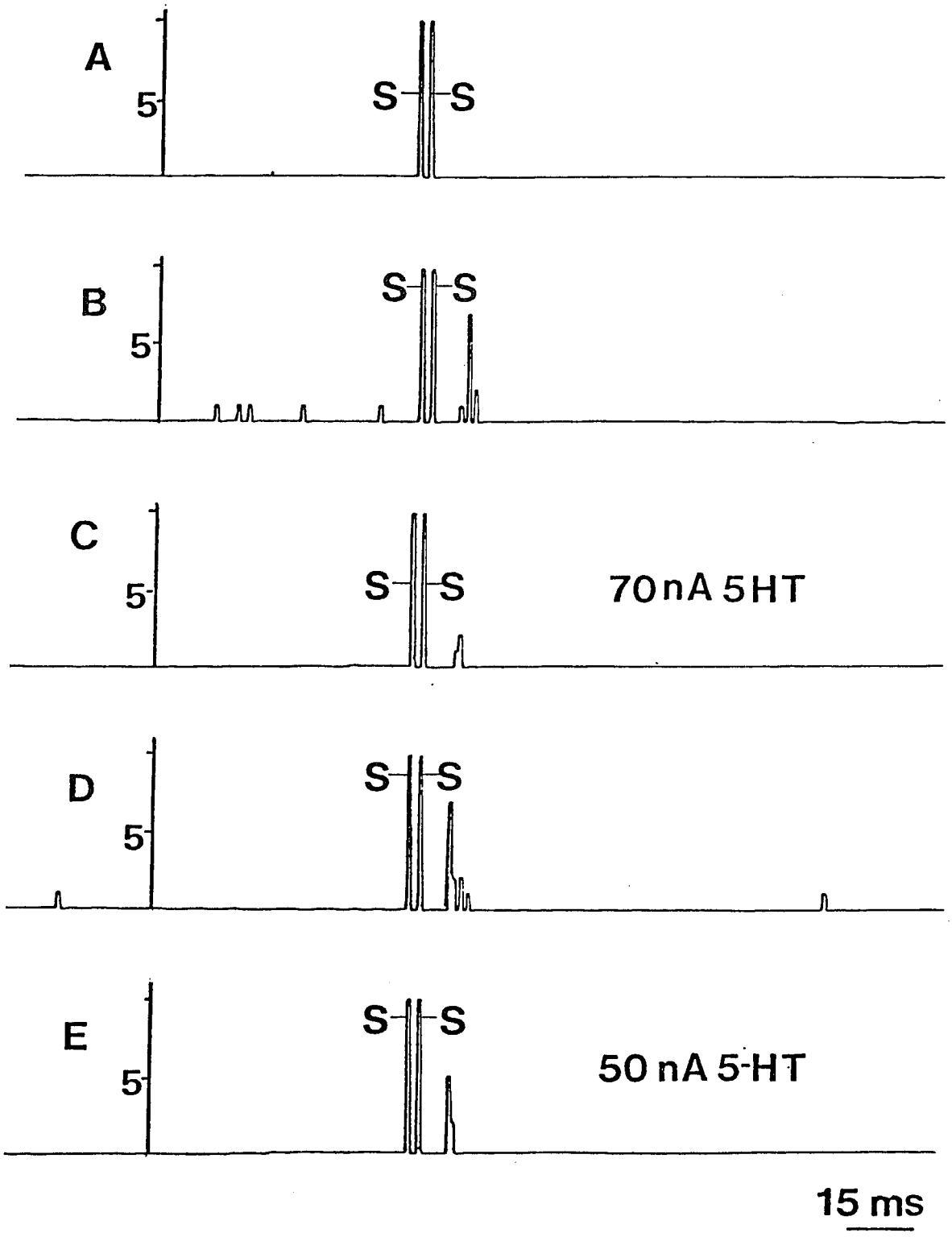
Figure 16. A-C are peristimulus time histograms of one unit's responses to nonnociceptive stimulation. On the x-axis is time (bin width = 10 msec), on the y-axis is number of counts/bin. A. is a record of the stimulus artifact; stimulation was applied outside the unit's receptive field (S-stimulus artifact). B. is a record of stimulation (10 times) within the unit's receptive field (hindpaw) without iontophoresis of 5-HT. It illustrates the phasic and tonic components of the response. C. is a record of stimulation (10 times) within the unit's receptive field with iontoporesis of 50 nA of 5-HT. There has been a decrease in both the phasic (16%) and tonic (50%) components of the response,with the greatest decrease being in the tonic component.


A
S
S
5
B
15
S
S
5
no 5-HT
C
25
15
S
S
5
50 nA 5-HT
200 msec

The effect of serotonin iontophoresis on phasic responses was studied for 11 units. In 10 cases the magnitude of the phasic component of the somatosensory response during iontophoresis was less than without (Table VIII). Decreases in phasic responses ranged from 6-100% depending on dose of 5-HT (Table VIII). In most cases, larger doses of drug produced greater percent decreases in the magnitude of the response (Fig. 17). Iontophoresis of 25 nA of 5-HT produced a 6% decrease in response, 30-35 nA produced an average response decrease of 15% (number of trials=2), 40-45 nA produced an average decrease of 40% (number of trials=2), 50-55 nA an average decrease of 41% (number of trials=6), and 70-75 nA an average decrease of 59% (number of trials=2). One unit showed a slight (20%) increase in the phasic response with iontophoresis of 5-HT at 50 nA. This unit, however, also showed an increase of 10% in its phasic response with iontophoretic application of 50 nA of current through the NaCl barrel.

Comparisons could be made between before and after drug histograms for seven units for which the effects of 5-HT on phasic responses were determined. For 4 of these units the magnitude of the response after 5-HT was the same as before; for 2 units the magnitude after 5-HT was less than before, and for 1 unit the magnitude after 5-HT was greater than before. In 2 cases, units were lost before completion of the trials, but 2 consecutive records of somatosensory stimulation without 5-HT iontophoresis were obtained. In these cases there was less of a difference between the two responses to somatosensory stimulation before iontophoresis than between unit responses to somatosensory stimulation with and without 5-HT.

Figure 17. A-E. are peristimulus time histograms of one unit's responses to nonnociceptive stimulation. On the x-axis is time (bin width = 1 msec), on the y-axis is counts/bin. A. is a record of spontaneous activity and the stimulus artifact; stimulation was applied outside the unit's receptive field (S-stimulus artifact). B. is a record of stimulation (10 times) within the unit's receptive field without iontophoresis of 5-HT. C. is a record of stimulation (10 times) within the unit's receptive field with iontophoresis of 70 nA of 5-HT. D. is a record of stimulation (10 times) within the unit's receptive field with no iontophoresis of 5-HT made after C. E. is a record of stimulation (10 times) within the unit's receptive field and iontophoresis of 50 nA of 5-HT.


A
5
S
S
B
5
S
S
C
5
S
S
70nA 5HT
D
5
S
S
E
5
S
S
50 nA 5-HT
15 ms

The effects of 5-HT on the tonic components of nonnociceptive units were determined for 5 units. All tonic responses were inhibited by 5-HT; the total count per tonic response was lower with iontophoresis of 5-HT than without (Fig 16B vs 16C) (Table VIII). For all units tested, serotonin application also increased the length of the inhibitory period between phasic and tonic components of the responses (Fig. 16B vs 16C). Doses of serotonin tested on tonic responses were: 25 nA, which produced a 45% decrease in response; 40 nA which produced an average decrease of 76% (number of trials=2), 50 nA which produced an average decrease of 74% (number of trials=2); and, 70 nA which produced an average decrease of 63% (number of trials=2).

Tonic responses to somatosensory stimulation after iontophoresis were less than before for 2 units. In both cases the difference between responses before drug application and responses during drug application were greater than differences between before drug and after drug responses (i.e., there was a 45% decrease in the tonic response during iontophoresis and only a 17% difference in pre and post drug responses for one unit and an 88% decrease in response with 5-HT and a a 15% difference in pre and post drug responsese). For one unit the tonic response was the same after iontophoresis as before. For another unit the postdrug response after 5-HT was less than before with iontophoresis of 40 nA of 5-HT, and greater than before with iontophoresis of 70 nA of 5-HT. In this case the percent decrease during iontophoresis was also greater than the percent decrease after iontophoresis (88% vs 11%).

In 8 cases histograms with short bin widths were used to examine effects of 5-HT on changes in latency of phasic responses (Table VIII). In 3 of these cases there was an increase of 1-2 msec in the latency of the phasic response with doses of 5-HT of 35, 50, and 50 nA. For one unit there was no effect of 5-HT on response latency at doses of 40 and 50 nA but there was an increase in latency of 1 msec with 70 nA of 5-HT (Fig. 17). The 3 units where latency was not effected by 5-HT were tested at 35, 50, and 50 nA respectively but were not tested at higher doses. One unit showed 100% inhibition of the phasic response with 50 nA of 5-HT and was not tested at other doses, therefore, changes in latency could not be observed.

In 4 cases large bin widths were used and iontophoretic effects of 5-HT on phasic and tonic components of the same unit determined (Table VIII). In 3 of these cases there was a decrease in the magnitude of both response types, but the decrease in the tonic component of the response was greater than the decrease in the phasic response component with comparable iontophoretic currents, i.e., 6% vs 45%, 36% vs 88%, 16% vs 50% (Fig. 16C). In one case the decrease in response was the same for the two components.

Table VIII. Effects of 5-HT on phasic and tonic components of nonnociceptive neurons in the VB complex. Doses of 5-HT are expressed as the magnitude of the iontophoretic current used; changes in responses are expressed as fractional decreases or increases in total number of spikes relative to control responses. Percent changes are indicated in parenthesis.

		Phasic Response			Tonic Response	
Unit-Trial #	# of stim-uli	Dose 5-HT (nA)	Change Response	Latency Change(msec)	Dose 5-HT (nA)	Change Response
1-1	15	25	3/31(-6)	-	25	42/93(-45)
2-1	15	70	7/15(-47)	-	40	26/41(-63)
2-2	15				70	35/74(-47)
3-1	15				70	21/27(-78)
3-2	15	40	5/14(-36)	-	40	28/32(-88)
4-1	10	40	8/18(-44)	0		
4-2	10	70	7/10(-70)	1		
4-3	10	50	5/12(-42)	0		
5-1	10				50	29/30(-97)
6-1	10	35	4/21(-19)	2		
6-2	10	50	3/19(-16)	-	50	64/127(-50)
7-1	10	35	1/10(-10)	0		
8-1	10	50	5/10(-50)	1		
9-1	10	50	4/20(+20)*	0		
10-1	10	50	9/9(-100)	-		
11-1	10	50	1/10(-10)	0		
12-1	10	50	5/20(-25)	1		

* represents an increase in response

2. DISCUSSION

In the first series of experiments of this part of the study, 5-HT was iontophoretically applied to spontaneously active units, and to units responding to nociceptive and nonnociceptive stimulation in the VB and PO complexes of the rat. Changes in activity levels were recorded by making continuous ratemeter plots of firing frequency with relatively long bin widths, i.e. 1 sec. Iontophoresis of 5-HT produced an inhibitory effect on spontaneous activity of both nociceptive and nonnociceptive units in the VB and PO complexes. An inhibitory effect of 5-HT on the evoked activity of all thalamic units responding to peripherally applied nociceptive stimulation and on most (6/7) units responding to nonnociceptive stimulation was also observed.

This inhibitory effect of 5-HT appeared to be specifically related to drug application. In all cases, units were tested for sensitivity to equal magnitudes of unbalanced current passed through the NaCl or dye barrels and there was a clear difference between the effect of unbalanced current on the unit and the effect of unbalanced drug. In most cases where 5-HT was tested on spontaneous activity, post drug firing frequencies were nonsignificantly different than pre-drug within a few seconds after drug application. Furthermore, in most cases there was a relationship between dose of drug, measured as the magnitude of the iontophoretic current, and the percent decrease in firing frequency. As the dose increased the inhibitory effect increased.

The demonstration that 5-HT produces a predominantly inhibitory effect on somatosensory units in the thalamus is in agreement with the

majority of other studies done in somatosensory nuclei. For example, Andersen and Dafney ('82) investigated serotonergic effects of 5-HT on responses to mechanically applied nociceptive stimulation in the parafascicular nucleus of the thalamus and observed a predominantly inhibitory effect. In the dorsal horn of the spinal cord, Randic and Yu ('76) found that 5-HT inhibited noxious activity of 70% of the cells tested and Headley et al. ('78) found that 78% of the cells tested in laminae IV and V were inhibited with iontophoretic application of serotonin. Also working in the dorsal horn, Belcher et al. ('78) reported that 5-HT inhibited 90% of the activity evoked by nociceptive stimulation. Moreover, Jordon et al. ('79) reported that 86% of the WDR spinothalamic tract (STT) cells and 64% of the high threshold STT cells tested were inhibited by 5-HT. Willcockson et al. ('84) found that 5-HT inhibited 53 out of 58 STT cells excited by glutamate pulses, 5 out of 5 spontaneously active STT cells and the evoked activity of 11 out of 15 STT cells. In the trigeminal subnucleus caudalis, Burns and Haigler ('83) reported that there was a predominantly inhibitory effect of 5-HT on units which responded to face pinch and noxious heat with an increase in firing frequency. 55% of the face pinch and 78% of the noxious heat units tested were inhibited.

In some cases predominantly excitatory effects of 5-HT on somatosensory units have been reported, however. Belcher et al. ('78) found that 5-HT produced excitation of the spontaneous activity of 51% of the nociceptive and 60% of the nonnociceptive units tested and produced facilitation of D,L-homocysteic evoked activity of 57% of the nociceptive and 63% of the nonnociceptive units tested. This excita-

tion was described as a large increase in firing frequency associated with a change in spike size. Todd and Millar ('83) also reported excitatory effects of 5-HT on 68% of the dorsal horn units tested. It is somewhat difficult to compare Todd and Millar's results with those of other iontophoretic studies since Todd and Millar described drug effects in terms of changes in firing frequency after drug application, instead of in terms of changes in firing frequency during iontophoresis, as is usually done. They provided no information on firing frequencies during drug application. Specifically, in their study firing frequency increased after a period of 5-HT iontophoresis, therefore, the unit was classified as excited by serotonin. In some cases we saw an inhibitory effect of 5-HT during iontophoresis, and a post drug increase in firing frequency. In this study these units were classified as inhibited by 5-HT. It is possible, therefore, that units classified as excited by Todd and Millar might have been classified as inhibited by other investigators. It can be noted, however, that Todd and Millar also described the excitatory response to drug application as a dramatic increase in firing frequency.

In this study excitatory responses to iontophoresis, consisting of dramatic increases in firing frequency, displayed a number of other characteristics suggesting that they might be nonspecific effects. Increases in firing frequency were large, and there did not appear to be a relationship between dose of drug and magnitude of response; large increases in firing frequency were seen even when iontophoretic currents were reduced to very low levels. Furthermore, there were often changes in unit shape associated with excitation, and when tested for current sensitivity these units tested positively, i.e.,

unbalanced current produced the same dramatic increases in firing frequency as drug iontophoresis. It is possible, therefore, that similar excitatory responses to 5-HT iontophoresis described by Belcher et al. ('78) and Todd and Millar ('83) were nonspecific as well . Belcher et al. ('78), however, found that excitatory responses could be antagonized by methysergide, and in a later study Todd and Millar ('84) again reported excitatory effects of 5-HT in the dorsal horn, and also found that excitation could be antagonized by methysergide.

Excitatory effects of 5-HT have been described in other regions of the brain but the nature of these effects is very different than that described by Todd and Millar ('83) and Belcher et al. ('78). Aghajanian and co-workers found that in the facial motor nucleus 5-HT exerted a facilitory effect. Specifically, McCall and Aghajanian ('79) showed that application of 10-200 nA pulses of 5-HT lasting from 1-10 min failed to excite silent facial motorneurons. However, small amounts of 5-HT (20 nA), applied to facial motorneurons excited by glutamate pulses, electrical stimulation of the motor cortex or electrical stimulation of the red nucleus enhanced both threshold and subthreshold excitations. This facilatory effect of 5-HT was associated with a slow subthreshold depolarization accompanied by an increase in excitability and input resistance (VanderMaelen and Aghajanian, '80). Other investigators (Parry and Roberts, '80; White and Neuman, '80) have observed the same facilatory effect of 5-HT on spinal motorneurons. These facilatory effects of 5-HT are clearly different than the large increases in firing frequency described by Todd and Millar ('83) and Belcher et al. ('78).

In any case, the demonstration that 5-HT produced a predominantly inhibitory effect on somatosensory units is in agreement with the majority of studies done in other somatosensory nuclei. In this study there was also not much of a difference in the incidence of serotonergic inhibition of nociceptive and nonnociceptive units. Other studies comparing iontophoretic effects of serotonin on different types of somatosensory activity have produced conflicting results. For example, Willcockson et al. ('84) found 2 out of 2 STT cells activated by brushing the ipsilateral hindlimb were inhibited by 5-HT and 9 out of 13 cells activated by pinch were inhibited. When Belcher et al. ('78) tested the effect of 5-HT on evoked activity of nonnociceptive units, however, they found that 5-HT produced a decrease in activity of only 19% (n=16) of the units tested, as opposed to the 90% (n=20) inhibited when 5-HT was iontophoretically applied to nociceptive evoked activity. Todd and Millar ('83) found no difference in serotonergic effects on LT, NS, or WDR units in spinal laminae I-III. It is difficult to know what to conclude from these results. That serotonin would inhibit all classes of units tested in densely innervated regions might be expected. Therefore, that Todd and Millar, who were working exclusively in the superficial laminae of the dorsal horn, an area densely innervated with 5-HT immunoreactive fibers, saw no difference in serotonergic effects on different classes of units might be expected. When comparing effects of iontophoresis of 5-HT in regions with different densities of innervation, however, it seems likely that differences would be seen. Willcockson et al., however, ('84) specifically noted that not only did they not see a difference in inhibition

of different classes of units, but they did not see a difference in 5-HT effect on neurons tested in different laminae of the spinal cord. Some of the units they recorded from were in the superficial laminae, which are more densely innervated with serotonergic fibers than the deeper laminae, where other unit recordings were made. In the present study there was also not much of a difference between inhibition of nonnociceptive units in the sparsely innervated VB complex, and inhibition of nociceptive units in more densely innervated posterior regions of the thalamus. It has been noted that density of endogenous serotonin and densitiy of serotonin receptors does not always correspond (Nelson et al., '78). This may be the explanation for why differences in iontophoretic effects of serotonin exerted in regions with different densities of innervation were not seen.

To examine serotonergic effects on nonnocieptive responses more closely, another type of experiment was done. As is common in responses of low threshold mechano-sensitive units, two response components to nonnocieptive stimulation were distinguishable; a phasic and a tonic. Both of these types of responses were tested for 5-HT sensitivity. There was an inhibitory effect of 5-HT on all but one of the phasic responses, and on all of the tonic responses tested. However, when 5-HT was iontophoretically applied to phasic and tonic components of the same response, in most cases there was a greater decrease in the magnitude of the tonic response than in the magnitude of the phasic response.

These inhibitory effects of 5-HT on the different components of nonnociceptive responses also appeared to be specifically related to

drug application. In most cases there was a relationship between dose of drug and the percent decrease in response. As the dose of drug increased so did the decrease in the magnitude of the response component. For most phasic responses, post drug histograms were the same as predrug, indicating that there was no decrease in the magnitude of the somatosensory response with repeated application. In 2 cases however, post drug responses were less than predrug. The decrease in the somatosensory response during serotonin iontophoresis in these cases may have been due to the effect of repeated stimulation and not to a specific drug effect. It is also possible, however, that there was a long term effect of the 5-HT iontophoresis. In the process of making histograms, 5-HT was iontophoretically applied for relatively long periods of time (i.e., 50 sec). Tonic responses were more variable than phasic when applied repeatedly without iontophoresis, so it is more difficult to determine the significance of the differences between pre and post drug histograms. In all cases where the magnitude of the tonic response after drug application was less than the response before drug, however, the percent decrease in response during iontophoresis was greater than the difference between pre and post drug responses.

Besides the inhibitory effect of 5-HT on the magnitude of phasic and tonic components of nonnociceptive responses, 4 out of 7 units were characterized by an increase in the latency of the phasic response. In addition, all units tested were characterized by an increase in the duration of the inhibitory period between phasic and tonic response components.

To our knowledge no other studies of the iontophoretic effects of 5-HT on different components of responses to physiolgical stimulation have been done in somatosensory nuclei. Andersen and Curtis ('64), however have looked at iontophoretic effects of 5-HT on different components of responses to contralateral ulnar nerve stimulation in the VB complex. They found that 5-HT produced no effect on short latency responses to neural stimulation but that spontaneous activity and later burst responses were depressed. They also found that intervening positive potentials were inhibited by 5-HT. These findings, using electrical stimulation of a peripheral nerve, are similar to ours, using mechanical stimulation to examine 5-HT effects on response components.

In conclusion, it appears that serotonin can exert an inhibitory effect on somatosensory transmission at the level of the thalamus. Whether or not there is a difference in modulatory effects of serotonin on nociceptive and nonnociceptive transmission is less clear. It does seem, however, that at the thalamic level there may be some selectivity in modulatory effects on different components of nonnociceptive activity; phasic components are less sensitive to serotonin than tonic.

IV. GENERAL DISCUSSION

The purpose of this thesis project was to determine whether there is evidence for serotonergic modulation of somatosensory activity at the level of the thalamus of the rat, and, if so, whether there is any difference in modulation of nociceptive and nonnociceptive activity. The density of 5-HT immunoreactive fibers was mapped in primarily nociceptive and in primarily nonnociceptive regions. The greatest density of serotonin immunoreactive fibers was seen in posterior, thalamic regions where responses to nociceptive stimulation were more common than responses to nonnociceptive stimulation. This might suggest that serotonergic modulation of somatosensory transmission at the thalamic level is exerted primarily on nociceptive activity. In posterior regions, with greater numbers of immunoreactive fibers, there may be more serotonergic synapses and more 5-HT receptors, and therefore, neurons would receive more serotonergic input. It has been noted, however, that the density of endogenous 5-HT and the density of serotonin receptors does not always correspond (Nelson et al., '78). For example, the hippocampus seems to have a relatively low 5-HT content, and a high receptor density (Biegon et al. '82). It is possible, therefore, that density of serotonergic innervation does not always correlate with responsiveness to serotonin.

Indeed, the results of the first microiontophoresis experiments might seem to indicate that this is so. Serotonin was iontophoretically applied to nociceptive and nonnociceptive units in the VB complex and in posterior regions of the thalamus. A primarily inhibitory effect of 5-HT was seen in both areas, and on both nociceptive and

nonnociceptive units. There did not appear to be much difference between the effect of 5-HT on units in the more densely innervated posterior regions of the thalamus, and the effect of 5-HT on units in the less densely innervated VB complex. This might suggest that, in somatosensory regions of the thalamus, density of immunoreactive fibers does not correlate with number of 5-HT receptors, and, therefore, with responsiveness to serotonin.

It should be possible to compare densities of serotonin receptors in the two regions by examining results of ligand binding studies. These studies have indicated that there are at least 2 distinct serotonin binding sites in the mammalian brain (Peroutka and Snyder, '79; '81; '82). One site, designated as 5-HT_1, may be selectively labeled by ^{3}H-serotonin, the other site, designated as 5-HT_2, may be selectively labeled by ^{3}H-spiperone (Peroutka and Snyder, '79; Peroutka, '84). 5-HT_2 receptors display a greater affinity for classical 5-HT antagonists than do 5-HT_1 receptors (Peroutka, '84). Peroutka and Snyder ('82) have postulated that 5-HT_1 receptors represent the sites which mediate inhibitory effects of serotonin, and 5-HT_2 receptors mediate excitatory effects.

This hypothesis is mainly supported by the fact that, in regions of the brain where iontophoretic application of 5-HT produces an excitatory effect, classical serotonin antagonists are effective in blocking 5-HT effects, and, in regions where 5-HT-induced inhibition can be seen, classical serotonin antagonists are less effective. For example, Aghajanian and co-workers have found that 5-HT produces a facilatory effect in the facial motor nucleus, which is antagonized by

methysergide (McCall and Aghajanian, '79; '80; VanderMaelen and Aghajanian, '80), cyproheptadine (McCall and Aghajanian, '80), cinanserin (McCall and Aghajanian, '80), and metergoline (McCall and Aghajanian, '80). A facilitory effect of 5-HT has also been observed in the ventral horn of the spinal cord (Perry and Roberts, '80; White and Neuman, '80), and White and Neuman ('80) have shown that, it too, can be antagonized by methysergide. In regions of the brain where 5-HT produces an inhibitory effect, however, the classical serotonin antagonists seem to be ineffective. Haigler and Aghajanian ('74) found that cinanserin, methysergide, methiothepin, metergoline, and cyproheptadine were ineffective in antagonizing 5-HT-induced inhibition, in the ventral lateral geniculate, amygdala, optic tectum, and reticular formation. Wang and Aghajanian ('77) also reported that cyproheptadine and methysergide were ineffective in blocking inhibitory responses to serotonin in the amygdala, and Rogawski and Aghajanian ('80) found that methysergide was ineffective as a serotonin antagonist in the ventral lateral geniculate.

If the hypothesis that 5-HT_1 receptors mediate inhibitory effects of serotonin and 5-HT_2 receptors mediate excitatory effects is true, then it would be desirable to compare density of 5-HT_1 receptors in the PO complex with the density of 5-HT_1 recptors in the VB complex, since it is an inhibitory effect of 5-HT that was seen. At the present time, using available published data, however, it difficult to make that kind of comparison. The first radiolabeled ligand binding studies were done biochemically; brain regions were dissected, homogenized, incubated with radiolabeled ligand, and samples counted for radioactivity. The anatomical resolution of this type of technique is

not very high. More recently, ligand binding studies have been done with autoradiographic techniques; intact tissue slices are frozen, incubated with radiolabeled ligands, then exposed to film. Several of these studies have been done mapping the distribution of 5-HT_1 receptors in the rat brain (Biegon et al., '82; Deshmukh et al., '83; Marankiewisz et al., '84). It is still difficult to compare densties of serotonin receptors in different regions of the thalamus, however. Illustrated brain sections are very far apart, i.e. 2-4 mm apart, and tables of relative binding of ^{3}H5-HT in different nuclei do not differentiate between nuclei of the VB complex, or between the VB and PO complex.

In conclusion, this study has produced evidence indicating that there are serotonergic fibers in regions of the thalamus, particularly in posterior areas related to transmission of nociceptive activity. These fibers may exert an inhibitory effect on somatosensory transmission; local application of serotonin to somatosensory units in the VB and PO complexes inhibited responses to nociceptive and nonnociceptive stimulation. There may be some selectivity in these modulatory effects, at least on different components of nonnociceptive responses. Phasic responses to somatosensory stimulation were less sensitive to iontophoretic application of serotonin than tonic.

It is possible that serotonin produces these inhibitory effects through a mechanism similar to that which has been described for serotonergic inhibition of hippocampal neurons. Specifically, Segal ('80) has shown that serotonin applied to hippocampal neurons in slice preparations produces a hyperpolarization and a decrease in input resistance. Segal ('80) has hypothesized that this effect is produced by

changes in potassium conductance. It is possible that iontophoretic application of serotonin to thalamic units also produces hyperpolarization, and consequently a decrease in firing frequency.

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