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Spinothalamocortical inputs nonpreferentially innervate the superficial and deep cortical layers of SI

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Using a combined anterograde and retrograde tracing technique, we examined the distribution pattern of the thalamocortical cells which projected to superficial layers of the hand region of the primary somatosensory cortex (hSI), and quantitatively analyzed the retrogradely labeled cells which putatively contacted terminals of the spinothalamic tract (STT) in the squirrel monkey and the macaque. Less than 25% of the superficial hSI projecting cells were putatively contacted by terminals of cervical enlargement spinothalamic neurons. These cells were primarily located in ventroposterior lateral, ventroposterior inferior and centrolateral nuclei. Although the number of superficial hSI projecting cells numbered less than 20% of the total hSI projecting cells, their patterns of location and their proportion of overlap with STT terminals within each thalamic nucleus were similar. It is suggested that the spinothalamic nociceptive information input to the cortex equally accesses both superficial and deep SI.

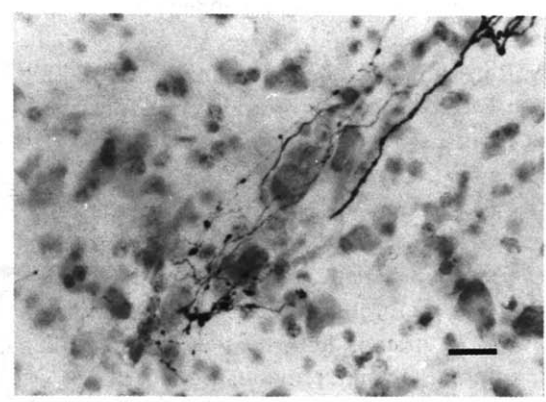
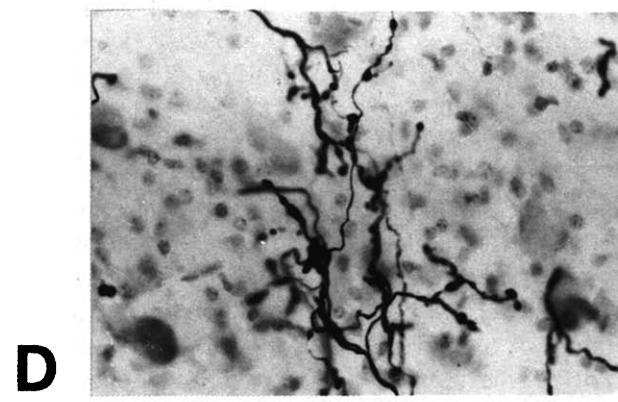
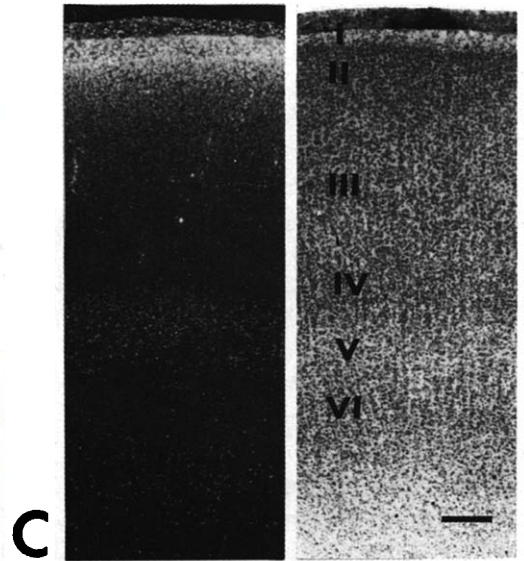
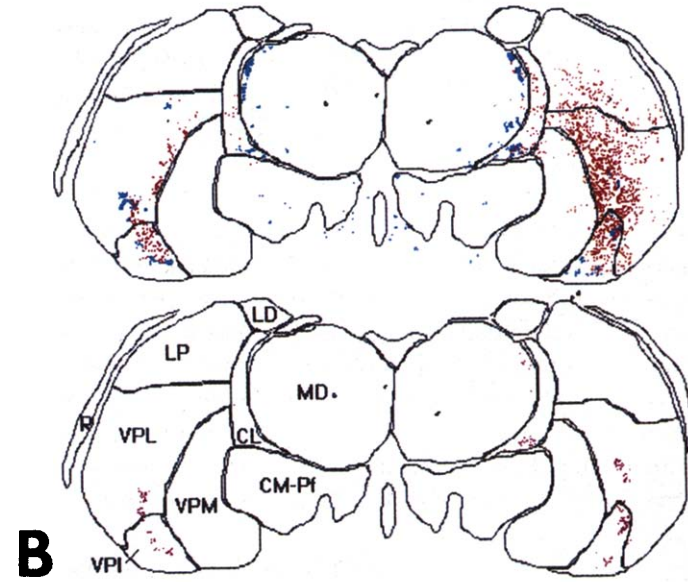
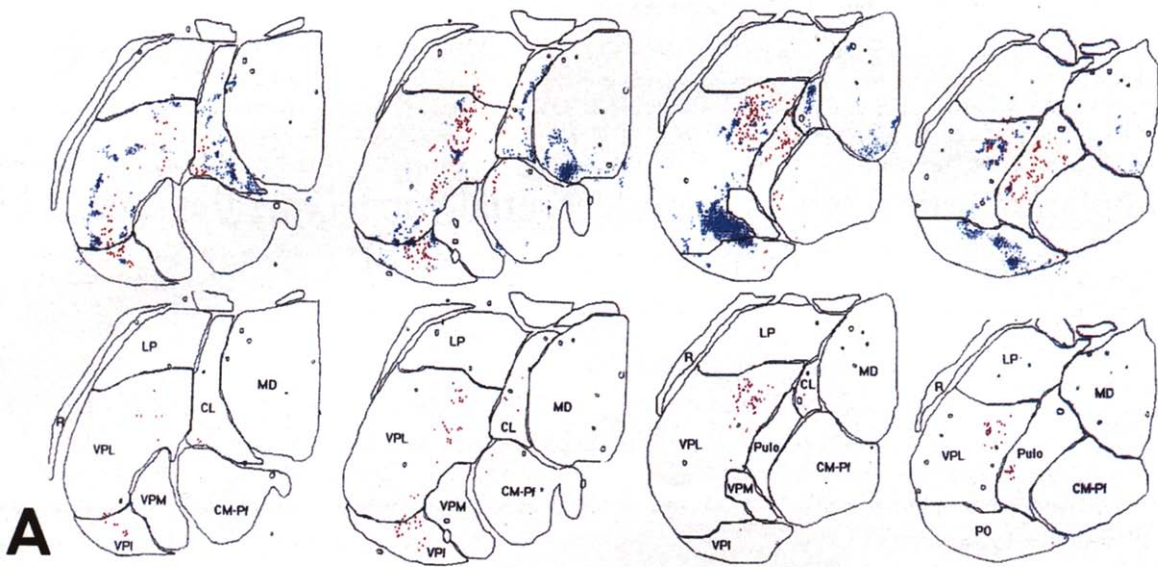
The spinothalamic tract (STT) is the major direct spinal cord pathway signaling somatic and visceral nociceptive stimuli (see refs. 10, 22). In primates, the STT terminates in ventroposterior lateral (VPL), ventroposterior inferior (VPI), centrolateral (CL), mediadorsal (MD) and the posterior (PO) nuclei as well as other thalamic nuclei [1, 9, 18]. Nociceptive neurons are found in these regions [6, 8, 15], many neurons in these nuclei project to the primary somatosensory cortex (SI), see [13], and some cortical neurons in SI do respond to nociceptive stimuli [7, 16]. Clinical evidence [4] and regional blood flow studies [2, 11, 21] also demonstrate that SI is involved in nociception in humans.

Recently, Rausell et al. [20] and Rausell and Jones [19] hypothesize that the superficial SI preferentially receives nociceptive inputs. These authors subdivided the macaque VPL and the ventroposterior medial nucleus (VPM) into two domains, based on staining patterns of cytochrome oxidase (CO) and calcium binding proteins. They found that many thalamocortical cells projecting to the superficial SI were located in the CO-weak domain of VPM and VPL. In different animals, they observed that STT and trigeminothalamic terminals were also located

in the CO-weak domain. Based on these results, Rausell and Jones propose that nociceptive inputs from the STT preferentially gain access to thalamocortical cells projecting to the superficial layers of the SI cortex. In the present study we directly test this proposition by examining the distribution pattern of the superficial hSI projecting thalamocortical cells and by quantitatively analyzing the overlap of these cells with STT terminals in the same tissue.

Four squirrel monkeys and one macaque monkey were used. Animals were anesthetized with either ketamine chloride (25 mg/kg, i.m.) and Nembutal (20 mg/kg, i.v.) or a gas mixture of 1/3 NO₂, 2/3 O₂ and 0.5–2% halothane. Under sterile conditions, a parietal craniotomy was performed and the hSI on the left hemisphere mapped physiologically. In the squirrel monkeys, the hSI surface was pretreated with a 0.1 M acetate buffer (pH 4.5), then a filter paper soaked in 2% diamidino yellow (DY) was placed on hSI for 15–40 min (for details see ref. 3). Following the cortical injection, the brain was closed, and the cervical enlargement exposed through a laminectomy. A total of 3–5 μ l of 2% WGA-HRP, or 3 μ l biotinylated dextran (BD, in one squirrel monkey), was injected into 10 sites along the right side of the cervical enlargement (C₆–T₁). In the macaque monkey, 4% DY was placed on the surface of the mapped hSI on the left side following pretreatment with 0.1 M acetate buffer

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(pH 3.0). Additionally, the contralateral hSI was injected intracortically (2% DY), and 5 μ l of 2% WGA-HRP was injected bilaterally in the cervical enlargement.

Following appropriate survival periods (4 days for WGA-HRP, 5 weeks for BD), the animals were overdosed with Nembutal and perfused transcardially with

Fig. 1. Thalamic label in a squirrel monkey and a macaque. A: thalamic label in a squirrel monkey following a surface injection of DY on hand SI cortex (shown in C) and WGA-HRP in the contralateral cervical enlargement. Top panels show retrogradely labeled thalamocortical cells (red dots) and anterogradely labeled spinothalamic terminals (blue dots). Bottom panels show the labeled cells which overlapped with spinothalamic terminals. B: a single thalamic section in the macaque which had bilateral WGA-HRP injections in the spinal cord and DY injected on the surface (left) and within the cortex (right) in hand SI. Top panel shows labeled cells (red dots) and spinothalamic terminals (blue dots). Bottom panel is the overlapped cells. C: left panel is a fluorescent micrograph of DY deposited on the surface of hand SI penetrating only layers I and II of the cortex. Right panel is the same cortical section stained with cresyl violet, the cortical layers are labeled. Bar=200 μ m. D: photomicrographs of BD-labeled spinothalamic terminals in Cresyl Violet stained tissue. Both panels are the same magnification. Left panel: large terminal boutons connected to large terminal axons within VPL. Right panel: small terminal boutons and axons within CL. Bar=20 μ m. CL, centrolateral n.; CM-Pf, centromedian-parafascicular n.; LP, lateral posterior n.; MD, mediodorsal n.; PO, posterior n.; PULO, pulvinar oralis n.; R, thalamic reticular n.; VPI, ventral posterior inferior n.; VPL, ventral posterior lateral n.; VPM, ventral posterior medial n.

2.5% paraformaldehyde. The brain and spinal cord were sectioned (50–75 μ m thick). In animals with WGA-HRP injections, sections were reacted with tetramethylbenzidine [17]. To visualize BD labeling, sections were reacted with the Vectastain Standard kit and nickel-enhanced diaminobenzidine [5]. Alternate sections were processed for CO staining.

Labeled terminals and cells were collected with a computerized plotting system. Following data collection, the sections were counterstained with Cresyl Violet. Cytoarchitectural boundaries of the thalamic nuclei were traced on the data plots using blood vessels as landmarks for alignment (see ref. 9). Retrogradely labeled cells located within 100 μ m of STT terminals were considered 'overlapping cells', i.e. putatively contacting STT terminals. Data from our previous study [9] were used for comparison.

In 3 of 4 squirrel monkeys and the macaque monkey, DY deposited on hSI surface was found to be restricted to the very superficial layers of hSI (Fig. 1C). In the other squirrel monkey which had survived 5 weeks, DY spread deeper. The intracortically injected DY in hSI on the other side of the macaque brain was found in all layers.

In all animals, more than 90% of the retrogradely labeled thalamocortical cells from superficial hSI were found in ipsilateral VPL (forelimb portion), VPI, pulvinar oralis (Pulo) and CL. The remaining superficial hSI projecting cells were distributed in other thalamic regions (see top panels of Fig. 1A and 1B). There is similarity in the pattern of thalamocortical label seen from the superficial hSI projecting cells and that seen from total hSI injections reported in the previous study [9]. However, the number of superficial projecting cells was much smaller than that of total hSI projecting cells. The average number of superficial hSI projecting cells within VPL, VPI and CL of 3 squirrel monkeys was 11.5% of the total hSI projecting cells found in the previous study (196 \pm 107 per 100 μ m section vs. 1695 cells per 100 μ m section). In the macaque monkey, the number of the superficial hSI projecting cells was about 20% of the total

hSI projecting cells seen on the opposite side of the same animal (2569 vs. 12456 cells per 100 μ m section). These results indicate that only 10–20% of the thalamocortical cells which project to hSI send afferents to superficial hSI and that the overall pattern of location of these cells is not different than that of the total hSI projecting cells.

WGA-HRP injection in the cervical enlargement resulted in anterogradely labeled STT terminals primarily in contralateral VPL, VPI, PO, CL, MD of the squirrel monkey and macaque (top panels of Fig. 1A and 1B). The distribution of BD-labeled STT terminals in one squirrel monkey was similar to that of the WGA-HRP labeled terminals in the other squirrel monkeys, although more restricted. The BD-labeled terminal boutons were very easily distinguished from their fibers: Some STT terminals had large boutons (diameters 3–7 μ m) attached to rough fibers (diameter 1.4–2.0 μ m, left panel of Fig. 1D); other STT terminal boutons were small (diameter 1.4–2.9 μ m) and were attached to fine fibers (diameter 0.4–1.0 μ m, right panel of Fig. 1D); both types could be found in many thalamic regions, in separate and overlapping patterns.

Of all superficial hSI projecting cells in the 4 squirrel monkeys, 23.3% (\pm 9.9%) were near STT terminals within 100 μ m and were, therefore, considered overlapping cells. More than 90% of the cells overlapping with STT terminals were located within VPL, VPI and CL. The number of superficial hSI projecting overlapped cells were less than the overlapped cells from the total hSI (decreased by 90.3% in the squirrel monkeys and by 67.7% in the macaque). The distribution of the overlapping cells was similar to the locations of those overlapping cells which projected to total hSI [9] (bottom panels of Fig. 1A and 1B). On average, of the three squirrel monkeys with WGA-HRP spinal injections, the overlapping cells in VPL, VPI and CL were 28.4% (\pm 10.5%), 38.8% (\pm 10.1%) and 40.3% (\pm 19.3%) of the superficial hSI projecting cells in each of these nuclei, respectively. In the previous study [9], the overlapping cells in VPL, VPI and CL were 27.4%, 42.7% and 34.3% of the total

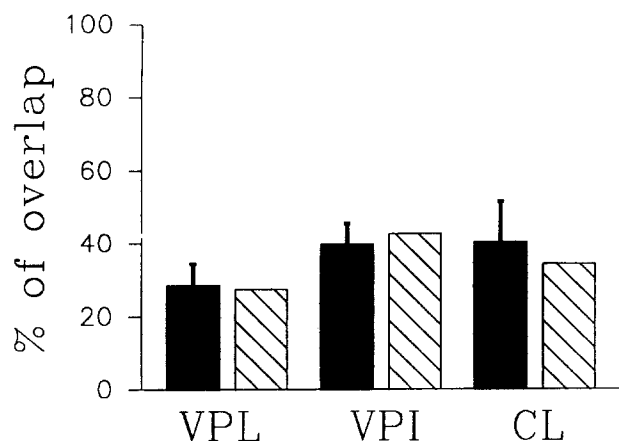


Fig. 2. Comparison of overlap between superficial hSI projecting cells and total hSI projecting cells in squirrel monkeys. Filled bars are the % overlapped cells (relative to all labeled cells) of superficial hSI projecting cells; slashed bars are the % overlapped cells of total hSI projecting cells, for each nucleus indicated.

hSI projecting cells in each of these nuclei, respectively (Fig. 2). Therefore, the probability of contacting STT terminals seems the same irrespective of the cortical target (deep vs superficial) of the hSI projecting cells, in the squirrel monkey. The majority of the overlapping cells in the macaque monkey were also located in VPL, VPI and CL. The percentages of overlapped cells in VPL, VPI and CL were respectively 23%, 18%, and, 31% from superficial hSI and 12.7%, 27.1%, and 34.2% from total hSI. The increase in percent overlap in VPL in this animal was not due to preferential contact between STT and superficial hSI projecting cells, but rather due to a larger number of retrogradely labeled cells in the VPL on the side of the total hSI injection because of spread of the injection to the hindlimb region of SI. Therefore, in the macaque, as in the squirrel monkey, the probability of contacting STT terminals seems independent of the cortical target.

The data indicate that: (i) Thalamocortical cells projecting to the superficial hSI are located primarily in VPL, VPI, Pulo and CL. The pattern of distribution of these cells resembles that of total hSI projecting cells. (ii) The number of superficial hSI projecting cells is 10–20% of total hSI projecting cells. (iii) Less than 25% of the superficial hSI projecting cells putatively contact STT terminals and the majority of these overlapping cells are located in VPL, VPI and CL. (iv) The hSI surface injections produced a pattern of label similar to that of the complete hSI injections in relation to location, proximity to STT terminals and proportion of overlap from each nucleus. Therefore, it is concluded that STT inputs most likely distribute nonpreferentially to terminate in both superficial and deep SI. The latter implies that STT no-

ciceptive information accesses superficial and deep SI, which is consistent with reports of nociceptive cells located in multiple layers of SI in primates [16].

Recent reports in primate have shown retrogradely labeled thalamic cells located in VPL, VPM, Pulo and PO following SI surface injections [3, 19, 20] (see also refs. 12, 14). Our study closely agrees with these observations. Additionally, our study shows that the superficial hSI projecting cells in VPL were located only in its hand representation portion indicating that the superficial SI also receives topographic thalamocortical projections.

The thalamic termination sites for STT are well known [1, 9, 18, 19] (see ref. 22) and agree with the results of the present study. Here, we also observed that most of the STT terminals segregated in CO-weak regions, consistent with the earlier report [19]. The locations of overlapping superficial hSI projecting cells were also evaluated for a preferential positioning within CO-rich or CO-weak regions. Numerous overlapping cells were located within CO-weak regions, such as the laminar regions of VPL and VPI; as well as in CO-rich regions, such as the core of VPL.

It needs to be emphasized that the conclusion drawn from these results is based on probabilistic measures of synaptic contacts. These measures make the assumption that thalamocortical neurons projecting to superficial or deeper cortex have essentially similar morphologies and, more importantly, their connectivity rules with STT, at the synaptic level, are not different from each other. The extent of the validity of these assumptions is unknown and needs to be determined. For this purpose, a project is currently underway in our laboratory to combine retrograde and anterograde studies with intracellular filling of identified 'overlapping' thalamocortical cells. This technique should provide a deterministic measure of STT cortical connectivity.

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