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Chronic deafferentation in monkeys differentially affects nociceptive and nonnociceptive pathways distinguished by specific calcium-binding proteins and down-regulates γ-aminobutyric acid type A receptors at thalamic levels

(calcium binding proteins/γ-aminobutyric acid receptors/central pain/neural plasticity)

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ABSTRACT **Chronic deafferentation of skin and peripheral tissues is associated with plasticity of representational maps in cerebral cortex and with perturbations of sensory experience that include severe "central" pain. This study shows that in normal monkeys the nonnociceptive, lemniscal component of the somatosensory pathways at spinal, brainstem, and thalamic levels is distinguished by cells and fibers immunoreactive for the calcium-binding protein parvalbumin, whereas cells of the nociceptive component at these levels are distinguished by immunoreactivity for 28-kDa calbindin. Long-term dorsal rhizotomies in monkeys lead to transneuronal degeneration of parvalbumin cells at brainstem and thalamic sites accompanied in the thalamus by a down-regulation of γ-aminobutyric acid type A receptors and an apparent increase in activity of calbindin cells preferentially innervated by central pain pathways. Release from inhibition and imbalance in patterns of somatosensory inputs from thalamus to cerebral cortex may constitute subcortical mechanisms for inducing changes in representational maps and perturbations of sensory perception, including central pain.**

Long-standing denervation of skin and other peripheral tissues is associated with modifications of cerebral cortical representational maps of the body surface in experimental animals (1, 2) and in humans with abnormal sensations that can include severe pain (3, 4). Central mechanisms that underlie plasticity of cortical maps and phenomena of central or deafferentation pain are poorly understood but are commonly believed to derive from an imbalance in the inputs to higher centers from nociceptive and nonnociceptive components of the ascending somatosensory pathways (5). These pathways include the dorsal column–lemniscal and spinothalamic systems, which, to a large extent, reflect the division of the somatosensory system into nonnociceptive and nociceptive components, respectively. Mechanisms of representational plasticity and perturbed sensory perceptions, such as central pain, may involve unmasking of previously silent synaptic connections (6), up- or down-regulation of neurotransmitter systems (7), sprouting of axons, and formation of new synapses (8); these mechanisms may operate at cortical or subcortical levels or both (9). The present investigation shows the differential expression of two calcium-binding proteins in the somatosensory pathways and the effects wrought by massive loss of afferent input upon the thalamus, the key structure in relay of sensory information to cerebral cortex (10).

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METHODS

Twelve *Macaca fascicularis* and three *Macaca fuscata* monkeys were used in these investigations. Nine of the former and all three of the latter species were normal. Three *M. fascicularis* monkeys at 3–4 yr had been subjected in another laboratory to unilateral or bilateral section of all dorsal roots of the spinal cord from the second cervical to the fourth thoracic segments and permitted to survive for 12 or more years (11). These animals, housed at the Delta Regional Primate Center, were reported in 1987 to show shortening of the affected limb, wrist deformities, and cervical spinal fusion. Several had demonstrated repeated, self-inflicted injuries of the limb consistent with the existence of "phantom" or deafferentation pain. Before sacrifice, the part of the postcentral gyrus in which the deafferented upper limb would normally be represented was mapped electrophysiologically (12). This area was excitable and contained an expanded representation of the lower part of the face, especially of the lower jaw region. In both normal and deafferented animals, the brain and spinal cord were fixed by perfusion with 2% paraformaldehyde/0.2% glutaraldehyde/0.1 M phosphate buffer. Serial frozen sections alternating at 15 and 30 μm were cut in the frontal plane from the thalamus and in a plane transverse to the long axis of the brainstem and upper spinal cord. The thicker sections were stained histochemically for cytochrome oxidase (13), a marker of neuronal oxidative metabolism, or with thionin. The thinner sections were stained immunocytochemically for the inhibitory transmitter, γ-aminobutyric acid (GABA) with a mouse monoclonal antibody (14), for GABA type A ($GABA_A$) receptors using a mouse monoclonal antibody that recognizes β_2/β_3 subunits (15), and for the calcium-binding proteins parvalbumin and 28-kDa calbindin with polyclonal antisera (supplied by P. C. Emson, Cambridge, U.K.). Bound antibodies were visualized by the ABC-peroxidase method with Vectastain kits. The immunocytochemical methods, including procedural controls, are described in detail elsewhere (16).

RESULTS

In the normal monkeys immunoreactivity for the calcium-binding proteins selectively stains the two components of the ascending somatosensory pathways. The fibers of the normal dorsal columns and the cells of the dorsal column nuclei on which they end are strongly immunoreactive for parvalbu-

Abbreviations: GABA, γ-aminobutyric acid; $GABA_A$, GABA type A; SG, substantia gelatinosa; VPL, ventral posterior lateral nucleus; VPM, ventral posterior medial nucleus.

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min, whereas the marginal layer and substantia gelatinosa (SG) of the spinal cord and caudal nucleus of the spinal trigeminal complex are strongly immunoreactive for calbindin (Fig. 1). The dorsal column nuclei contain no calbindin-positive cells. In the spinal gray matter, a few small parvalbumin-immunoreactive cells are present in the neck of the dorsal horn (primarily lamina IV), and a moderate number of large immunoreactive cells and axonal ramifications fill the intermediomedial nucleus at all levels (Fig. 2) and Clarke's column in the thoraco–lumbar region. In parts of the spinal gray matter outside the marginal layer and SG a very small number of calbindin-positive cells is seen in every layer except the neck of the dorsal horn. All these cells are small and weakly immunoreactive, except for a few larger neurons in the intermediate zone (lamina VII) that have well-stained processes. Many calbindin-immunoreactive fibers are concentrated deep to the SG but cannot be traced into any tracts

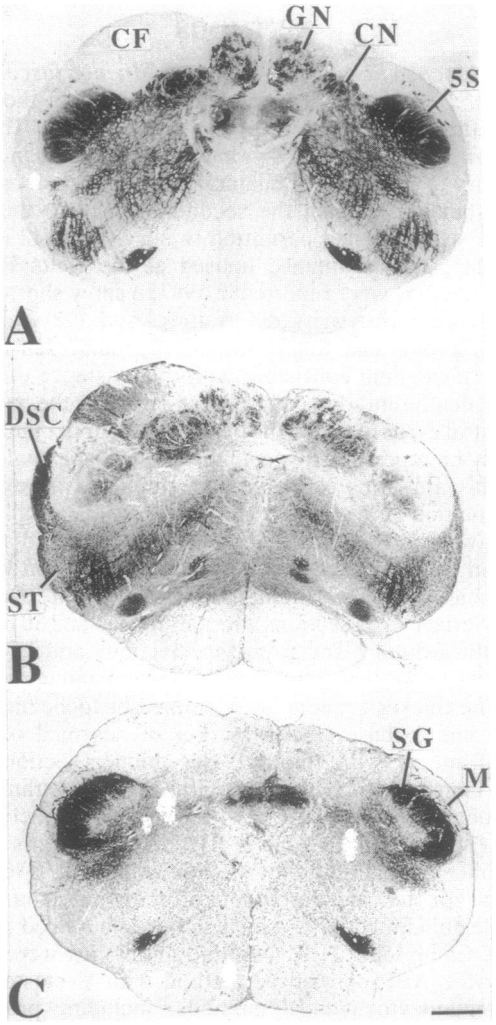

FIG. 1. Adjacent sections through caudal medulla oblongata of a rhizotomized animal, stained histochemically for cytochrome oxidase (*A*) and immunocytochemically for parvalbumin (*B*) or calbindin (*C*). Left side shows appearances that are the same as in normal animals. Note reduction in size of cuneate nucleus (CN) and overlying cuneate fasciculus (CF) with loss of parvalbumin fibers on side of rhizotomies (right) in comparison with normal side and lack of compensatory hypertrophy of adjacent gracile (GN) and caudal spinal trigeminal (5S) nuclei. Normal parvalbumin-positive dorsal spinocerebellar tract (DSC) is present on left side but is absent on side of rhizotomies. Position of crossed, affected spinothalamic tract (ST) is indicated contralateral to rhizotomies (*B*). SG and marginal zone of 5S, like those of the spinal cord dorsal horn (Fig. 2), are normally strongly immunoreactive for calbindin (*C*). (Bar = 1 mm.)

of the white matter. In the pons, the principal trigeminal nucleus (Fig. 2) shows a high concentration of parvalbumin-immunoreactive cells and fibers arranged in the lobular formations that characterize this nucleus in the primate (17). Calbindin-immunoreactive cells are also present but lie mainly at the perimeter of the nucleus, although a few intrude into its center between the parvalbumin-positive lobules. The calbindin-positive cells occupy a similar position in the oral and interpolar components of the spinal nucleus. The medial lemniscus is strongly immunoreactive for parvalbumin throughout its length and can be traced into the thalamus. Calbindin fiber staining in the brainstem is far less, and the spinothalamic tracts are not delineated.

In the ventral posterior nucleus of the thalamus, as described (16), projection neurons are either parvalbumin or calbindin immunoreactive but rarely immunoreactive for both. There is a far greater density of parvalbumin than calbindin cells in both the ventral posterior lateral (VPL) and ventral posterior medial (VPM) subnuclei. Normally, calbindin immunoreactivity is found in small or medium cells (10–20 μm in diameter), is confined to cytochrome oxidase-weak and parvalbumin-deficient patches that intervene between larger, cytochrome oxidase-rich and parvalbumin-positive domains in VPL and VPM, forms a shell at the perimeter of the VPM, and fills the adjacent anterior pulvinar nucleus (18–20).

On the side of the rhizotomies in the operated animals, the cuneate fasciculus is markedly thinned and deficient in parvalbumin fibers (Fig. 1). The cuneate nucleus on that side is also markedly reduced in size and contains many fewer neurons than normal in the Nissl- and parvalbumin-stained sections. The remaining neurons and surrounding neuropil show a normal density of cytochrome oxidase staining and of parvalbumin immunoreactivity. There is no compensatory hypertrophy of or change in cytochrome oxidase activity or immunoreactivity in the adjacent gracile and spinal trigeminal nuclei, which receive inputs from spinal segments below the sixth thoracic segment and the face, respectively (21). The principal trigeminal nucleus receiving "lemniscal" inputs from the face shows no obvious change in parvalbumin or calbindin immunoreactivity. The marginal layer and SG of the spinal trigeminal nucleus also show the normal high density of calbindin immunoreactivity on both sides. Staining of the spinothalamic tracts does not obviously change on either side (Fig. 1), but the dorsal spinocerebellar tract (22, 23) on the lesioned side is reduced in size and in parvalbumin immunoreactivity (Fig. 1).

In the ventral posterior nucleus of the thalamus contralateral to the rhizotomies and to the atrophic cuneate nucleus, the medial half of the VPL subnucleus representing the upper limb (24–26) shows a loss of large cells (20–40 μm in diameter). Their density is reduced to 0–0.5 ± 0.25 per 100 μm^2 in comparison with 2–3 ± 1 per 100 μm^2 in the comparable region of the opposite side; the difference is statistically significant (t test, $P < 0.05$). There is an associated reduction in cytochrome oxidase activity (Fig. 3*A*) to levels considerably below that in the rest of VPL and in the adjoining VPM subnucleus that represents the face. The zone of reduced cytochrome oxidase activity contains some higher density patches but extends anteroposteriorly throughout VPL. In adjacent sections, this zone is exactly matched by an almost complete loss of parvalbumin-immunoreactive cell staining (Fig. 3*C*), a reduction in $GABA_A$ receptor immunoreactivity to background levels (Fig. 3*B*), and a slight but statistically insignificant reduction in the number of neurons immunoreactive for GABA, in comparison with adjacent regions of VPL and VPM (data not shown). By contrast, immunoreactivity for calbindin is considerably increased in dorsal parts of the matching zone and in three or four ventral patches (Fig. 3*D*). The increase in density of calbindin immunoreactivity

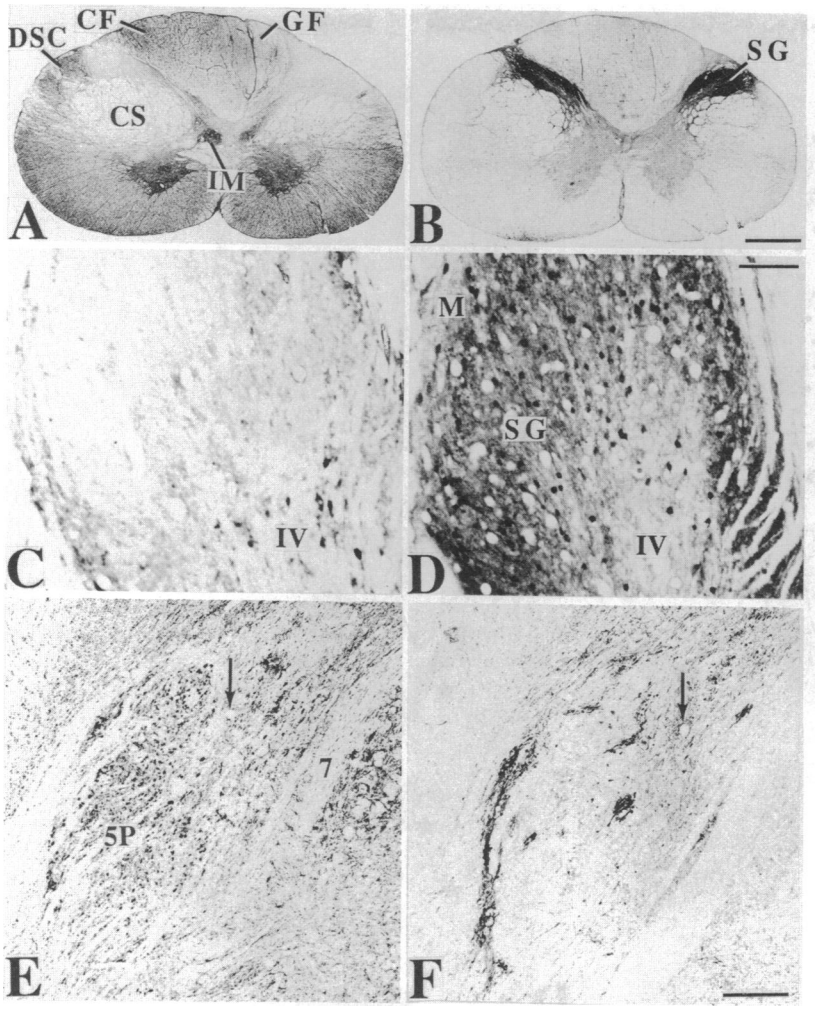

FIG. 2. Transverse sections of cervical spinal cord (*A* and *B*) from a rhizotomized animal showing reciprocal staining patterns for parvalbumin (*A*) and calbindin (*B*) in dorsal horn and parvalbumin immunoreactivity in dorsal, lateral, and ventral column pathways, except corticospinal tracts (CS). Right side shows shrinkage of cuneate fasciculus with loss of parvalbumin immunoreactivity. (*C* and *D*) Higher magnification views of parvalbumin (*C*) and calbindin (*D*) immunoreactivity in dorsal horn of a normal monkey at fourth cervical level. (*E* and *F*) Reciprocal parvalbumin (*E*) and calbindin-immunoreactive staining patterns in principal sensory trigeminal nucleus (5P) of a normal monkey. [Bars = 1 mm (*A* and *B*) or 100 μm (*C–F*)].

affects small-to-medium (10–20 μm) neurons and the intervening neuropil, both of which stain more heavily than in other parts of VPL or VPM in normal or affected monkeys (Fig. 4). Calbindin-positive cells increase from 4–5 ± 1 per 100 μm^2 in the symmetrical region of the normal side to 5–7 ± 1 per 100 μm^2 on the affected side; this increase is statistically significant (*t* test, $P < 0.05$). The zone of increased immunoreactivity is continuous with the normal calbindin-dense shell of VPM and with the calbindin-dense anterior pulvinar nucleus (17, 18).

DISCUSSION

These results show that the cells of origin and fibers of the lemniscal component of the somatosensory pathways are characterized by parvalbumin immunoreactivity, whereas cells in regions that give rise to a major element of the spinothalamic component—namely, the marginal layer and SG—are characterized by calbindin immunoreactivity. The lemniscal pathway constitutes the principal route for transmission of light tactile and proprioceptive information to the thalamus, whereas marginal and SG cells are the origins of the major pathway for transmission of noxious mechanical and thermal information (21, 23, 27–29). Whether the calbindin cells of the dorsal horn are spino–thalamic projection neurons or local circuit neurons, however, is not yet clear.

Dissociation of the lemniscal and spinothalamic components on the basis of differential calcium-binding protein immunoreactivity appears to be continued at thalamic levels. In normal monkeys, small calbindin-positive cells of VPL, VPM, and anterior pulvinar nuclei lie in cytochrome oxidase-weak domains (18–20) and receive terminations of spinothalamic and caudal trigeminothalamic fibers that carry both nociceptive and nonnoxious impulses to the thalamus (21, 29). These calbindin cells send axons preferentially to layer I of somatosensory cortex (19, 20). Parvalbumin-positive large and medium cells of VPL and VPM lie in cytochrome oxidase-rich domains and receive dorsal column–lemniscal and principal trigeminal fibers that carry impulses from nonnociceptive afferents and project preferentially to middle layers (III and IV) of somatosensory cortex (19, 20). There, thus, appears to be a fundamental dissociation of the two pathways right up to the cerebral cortex, although synaptic interactions occur at a sub-light microscopic level at all relay stations (21, 27).

The calcium-binding proteins, parvalbumin and 28-kDa calbindin, are widely distributed in the central nervous system, usually being found in different cell types (16, 30, 31, 48). They have been postulated to play a role in abbreviating the duration of individual action potentials, leading to so-called "fast spiking" behavior, but no specifically different function has ever been found. Although commonly found in GABA cells in the cerebral cortex (32, 33) and in certain other sites in nonprimate species (32), in the subcortical somatosensory centers the calcium-binding proteins are evidently in long-projection neurons (16).

The effects of chronic dorsal rhizotomy were most severe on the lemniscal (parvalbumin-positive) component of the somatosensory pathways at all levels up to and including the thalamus. (The previous opening of the subarachnoid space over somatosensory cortex and the introduction of electrodes into it precluded extending the investigation to cortical levels.) Except at thalamic levels, the effect on the calbindin-

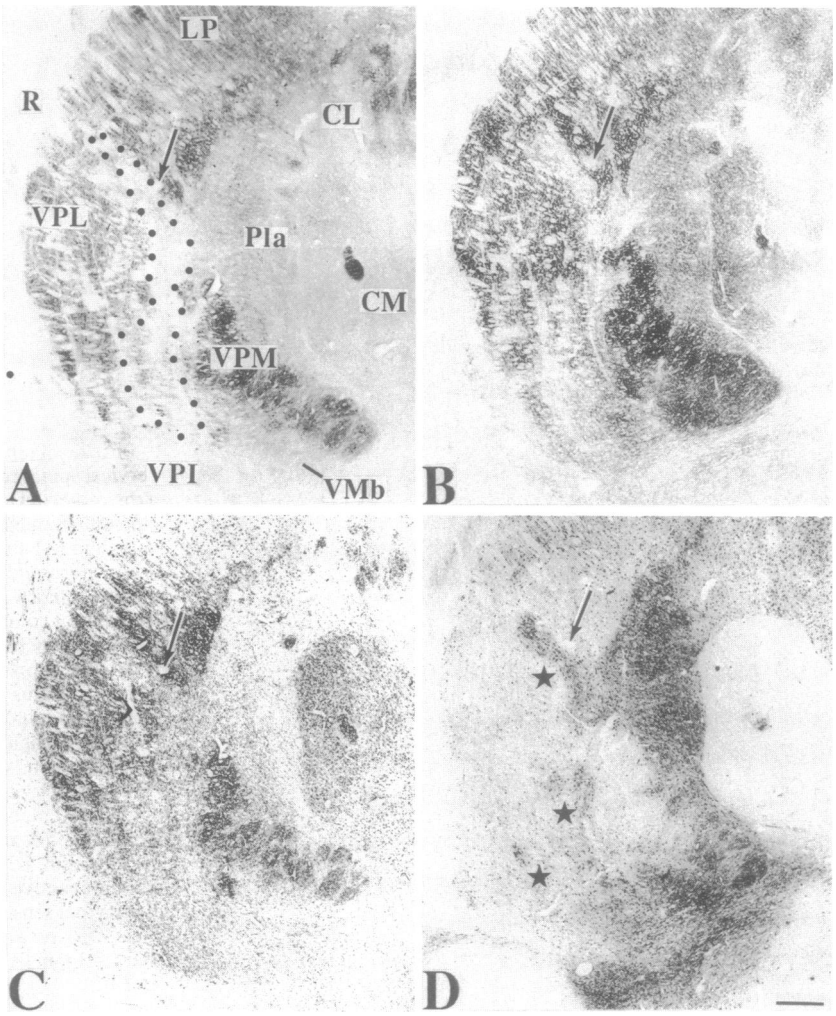

FIG. 3. Adjacent sections through ventral posterior and adjacent nuclei of thalamus contralateral to rhizotomies. Section is stained for cytochrome oxidase (*A*). Loss or reduction of enzyme activity in area (outlined by dots) corresponds to representation of most of the upper limb. (*B–D*) Section is stained immunocytochemically for $GABA_A$ receptors (*B*), parvalbumin (*C*), or calbindin (*D*) and shows loss of receptor and parvalbumin immunoreactivity and increase in calbindin immunoreactivity in the same region (cf. Fig. 4). Arrows indicate position of the same blood vessel. A large part of affected region is continuous with regions of enhanced calbindin immunoreactivity in shell of VPM and in anterior pulvinar nucleus. Patches of enhanced calbindin immunoreactivity in affected zone of VPL are indicated by stars. CL, central lateral nucleus; CM, centre médian nucleus; LP, lateral posterior nucleus; Pla, anterior pulvinar nucleus; R, reticular nucleus; VMb, basal ventral medial nucleus; VPI, ventral posterior inferior nucleus. (Bar = 1 mm.)

positive, spinothalamic component was not detectable with the methods used.

The atrophy of neurons in the dorsal column nuclei is a form of *transneuronal degeneration* that commonly follows cutting afferent fibers to a nucleus. Death of neurons by this phenomenon may result in *secondary transneuronal degeneration* of neurons postsynaptic to them and so on, along a chain of synaptically linked neurons (34). For dorsal rhizotomies or dorsal root disease, transneuronal degeneration has been reported in Clarke's nucleus, the dorsal column nuclei, and the somatosensory cortex but not in the thalamus (35, 36). The atrophy of the dorsal spinocerebellar tract in the present cases may also be due to transneuronal degeneration, but direct compression could not be ruled out.

A likely explanation of the increased calbindin immunoreactivity in the thalamus is that it reflects enhanced neural activity in these cells. Levels of a number of proteins can change under activity-dependent conditions in many peripheral and central neuronal systems (e.g., refs. 37–41). Because there was no evidence of transneuronal degeneration of the calbindin cells in the deafferented dorsal horns, major inputs to the thalamic calbindin cells should have been preserved in the rhizotomized animals.

The reduction in $GABA_A$ receptors that occurs in the deprived thalamic zone, even in the absence of a major loss of GABA neurons (42, 43), may represent a reduction in pre- or postsynaptic receptors or both and in either case should result in disinhibition and increased activity of the remaining cells. The enhanced calbindin staining may, therefore, also reflect this. Excessive activation of calbindin cells selectively innervated by intact spinothalamic afferents, with the concomitant loss of lemniscal inputs caused by loss of parvalbumin fibers in the dorsal columns, would disturb the normal balance of inputs to somatosensory cortex and potentially lead to perturbations of sensory perception. Such an imbalance is one mechanism postulated for genesis of central pain (3–5) and for plasticity of receptive-field organization (6–8). Disinhibition of remaining cells in and at the margins of the deafferented zone would also potentially uncover previously masked inputs to these thalamic neurons (44), which would then be relayed to the cortex. VPL thalamic neurons subjected to blockade of $GABA_A$ receptors by iontophoresis of bicuculline, often show enlargements of their receptive fields into adjacent areas of skin (45). Thus, the expansion of the lower jaw representation into the deafferented upper limb representation of the postcentral gyrus in these animals (12)

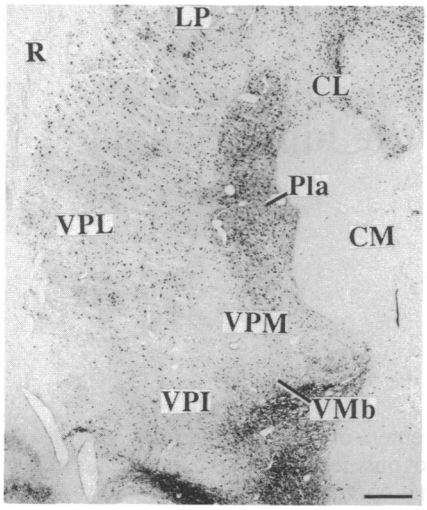

FIG. 4. Calbindin immunoreactivity in a section taken from a normal monkey thalamus at a level approximately equivalent to that of Fig. 3*D*. See Fig. 3 legend for abbreviations. (Bar = 1 mm.)

could result from an unmasking of inputs from the skin of the lower jaw on thalamic neurons remaining in the adjacent thalamic upper limb representation. The failure of the trunk representation to expand into the deactivated upper limb representation in these animals (12) remains unexplained by this hypothesis. However, skin over the lower jaw is innervated by the mandibular division of the trigeminal nerve and by the great auricular and transverse cervical nerves, which are formed by cervical spinal nerves C_2 and C_3 and represented adjacent to the lower jaw in the thalamus (19, 46, 47). Large shifts in the thalamic representation occur after acute lesions of the dorsal columns and may be due to unmasking of previously ineffective synapses or to terminal sprouting (44). Coupled with the apparent overactivity of the spinothalamic-innervated calbindin cells, this unmasking would predispose to an expansion of the lower jaw representation at the expense of the adjacent arm representation, even in the absence of axonal sprouting.

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