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Peripheral nerve damage facilitates functional innervation of brain grafts in adult sensory cortex

(peripheral regeneration/cortical plasticity/thalamocortical projections)

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ABSTRACT / **The neural pathways that relay information from cutaneous receptors to the cortex provide the somatic sensory information needed for cortical function. The last sensory relay neurons in this pathway have cell bodies in the thalamus and axons that synapse on neurons in the somatosensory cortex. After cortical lesions that damage mature thalamocortical fibers in the somatosensory cortex, we have attempted to reestablish somatosensory cortical function by grafting embryonic neocortical cells into the lesioned area. Such grafts survive in adult host animals but are not innervated by thalamic neurons, and consequently the grafted neurons show little if any spontaneous activity and no responses to cutaneous stimuli. We have reported that transection of peripheral sensory nerves prior to grafting "conditions" or "primes" the thalamic neurons in the ventrobasal complex so that they extend axons into grafts subsequently placed in the cortical domain of the cut nerve. In this report we present evidence that the ingrowth of ventrobasal fibers leads to graft neurons that become functionally integrated into the sensory circuitry of the host brain. Specifically, the conditioning lesions made prior to grafting produce graft neurons that are spontaneously active and can be driven by natural activation of cutaneous receptors or electrical stimulation of the transected nerve after it regenerates. Furthermore, oxidative metabolism in these grafts reaches levels that are comparable to normal cortex, whereas without prior nerve cut, oxidative metabolism is abnormally low in neocortical grafts. We conclude that damage to the sensory periphery transsynaptically stimulates reorganization of sensory pathways through mechanisms that include axonal elongation and functional synaptogenesis.**

Thalamic fibers reach the neocortex during the prenatal period of development, yet their final distribution, terminal arborization, and function depend on intact peripheral nerves and sensory experience after birth. In the rat, a distorted representation or "map" of the contralateral body surface is elaborated in the somatosensory (SI) cortex of each hemisphere during the first postnatal week through a complex interplay among peripheral receptor density, genetically programmed differentiation, and activity-dependent use of sensory systems (1–3). If the signals from the body surface are perturbed by injury to a peripheral sensory nerve or the area of SI cortex is reduced by damage, then the cortical map is reorganized to reflect these changes (4–8).

At birth, thalamic and cortical neurons in rodent cortex are nearly devoid of synapses, show little sign of spontaneous activity, and are unresponsive to stimulation of cutaneous receptors (9, 10). The formation of synapses by sensory relay neurons in the thalamus initiates peripherally driven neural activity in the cortex and then, in response to use during the first few postnatal weeks, activity-dependent adjustments are made in the strength of thalamocortical synapses.

In previous experiments (11) we used the absence of peripherally elicited synaptic activity during normal development as a rationale for enhancing regenerative responses from mature thalamic sensory relay neurons after cortical injury. We reasoned that cutting sensory nerves would eliminate normal sensory activity during the period after a cortical lesion when thalamic neurons were attempting to reestablish synapses with the remaining cortical neurons. To measure the ability of thalamic neurons to organize a regenerative response, with and without their peripheral sensory inputs intact, we placed pieces of embryonic cortex into the lesion site and measured the extent of specific thalamic fiber ingrowth into the grafted tissue. There was no detectable thalamic fiber ingrowth into grafts in normal adult host mice, but transection of the topographically appropriate peripheral sensory nerve prior to grafting produced extensive ingrowth into transplants (11). The sprouted axons were of large caliber, arose from the somatic sensory relay nucleus in the thalamus [in the ventrobasal (VB) complex], and formed highly branched terminal arbors in circumscribed regions of the grafts.

If the formation of new connections in sensory pathways is facilitated by lesions of the peripheral sensory inputs, a pivotal question is whether the sprouted fibers form functional synapses with the graft neurons that are able to generate physiological responses after regeneration of the peripheral nerve. The present results show that nerve cut 2 days prior to cortical damage and grafting allows later functional innervation of graft cells, while under the same conditions graft cells in hosts with intact peripheral nerves remain unresponsive and show little, if any, spontaneous activity.

MATERIALS AND METHODS

Sixteen normal adult (300–350 g) Long–Evans (hooded) rats were used in these studies to determine the physiological effect of nerve transection on thalamic fiber innervation of grafted embryonic cortical neurons. The following four procedures were performed on the experimental group: (*i*) transection of the infraorbital (IO) branch of the trigeminal nerve, (*ii*) cortical lesion followed immediately by implantation of embryonic neocortical tissue, (*iii*) physiological single-unit recording from graft and host cortical neurons, and (*iv*) analysis of thalamocortical fiber ingrowth by using horseradish peroxidase (HRP) and of metabolic levels by

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Abbreviations: SI, somatosensory; VB, ventrobasal; IO, infraorbital; HRP, horseradish peroxidase.

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using cytochrome oxidase histochemistry. For the nerve cut, each experimental animal (n = 10) was anesthetized with sodium pentobarbital (50 mg/kg) and the IO branch of the trigeminal nerve was sectioned where it emerged from the skull into a subcutaneous position anterior to the orbit. This procedure effectively eliminates the normal sensory inputs from the large mystacial vibrissae ("whiskers") and the sinus hairs on the snout of the rat that project to the posteromedial barrel subfield ("barrel field") region of the SI cortex through the synapses in the brainstem trigeminal nuclear complex and the VB nucleus of the thalamus. Two days later a lesion was made in the contralateral barrel field cortex into which a graft of embryonic cortical tissue was placed. The transplantation procedure has been described in detail (12). Briefly, rectangular solids of presumed barrel field cortex from rats of the same Long–Evans strain at gestational day 17–20 were dissected into modified Eagle's medium and suctioned into a 0.8-mm square capillary by hydraulic pressure. The capillary was then inserted into the host barrel field cortex parallel to the pial surface. The control group consisted of six adult host animals that received similar transplants without prior sensory nerve transection (i.e., the contralateral IO nerve was intact).

Approximately 35 days after grafting (range = 30–45 days), all animals were anesthetized with urethane (1.5 g/kg, i.p., supplemented as necessary) and prepared for physiological recording of unit activity in the transplant, in the surrounding host cortex (ipsilateral), and in the opposite barrel field cortex (contralateral) with either single- or three-barreled carbon-fiber electrodes (1–5 MΩ) (32). Rectal temperature was maintained at 37°C by a self-regulating heating pad. Cisternal drainage was routinely performed to reduce cortical pulsations.

To expose the hemisphere containing the graft, the skin was retracted before carefully removing the thick connective tissue covering that had formed over the barrel field cortex since the transplant surgery. The anterior and posterior extents of the transplant were apparent where the cortical surface was damaged during grafting. The exposed area was covered with 4% agar in saline to stabilize the hemisphere and prevent drying of the cortical surface. Recording sites were marked with either electrolytic microlesions (1 μA for 30 sec) or iontophoretic injection of 20% (wt/vol) HRP (1 μA for 2 min) and histologically localized to determine whether they were in- or outside of the graft. These marks were typically 75–100 μm in diameter and only those clearly within or outside of the transplant–host interface were included in the analysis. Neuronal responses to IO nerve stimulation (0.5 mA for 200 μsec) or whisker movement (500-μm deflection of 1-msec duration) were collected on-line by a data acquisition system (Modular Instruments, Southeastern, PA) interfaced with a computer. In two experimental cases the barrel field cortex contralateral to the graft was also exposed for detailed comparison of the cortical responses to whisker movement or electrical stimulation of the uncut IO nerve.

After physiological analysis 0.02 μl of 20% (wt/vol) HRP (Boehringer Mannheim grade I) was pressure injected into the VB nucleus by using stereotaxic coordinates to label thalamic fibers and their terminals in and around the grafts by anterograde axonal transport of the enzyme. The animals were perfused 18–20 hr later with a solution of 1.0% paraformaldehyde and 1.25% (vol/vol) glutaraldehyde in 0.1 M sodium phosphate (pH 7.4). Coronal 75-μm sections were cut with a freezing microtome and were reacted with diaminobenzidine by the glucose oxidase method (13). The relative levels of oxidative metabolism in the graft and host cortex were compared in both experimental and control cases by reacting a series of sections for cytochrome oxidase histochemistry according to the method of Wong-Riley (14).

RESULTS

Table 1 summarizes the number and responsiveness of neurons analyzed in the three categories of cells isolated for this study. SI neurons in the hemisphere contralateral to the graft were indistinguishable in latency and response characteristics from those previously reported for normal SI cortex by similar techniques (15, 16). Neurons in layers IV and V of normal barrel field cortex responded to 500-μm step movements of their principal whiskers with a short latency (7–10 msec) and with a high probability of response to a single peripheral stimulus (approaching 1.0) (Fig. 1*A*). In addition, many neurons in these layers were spontaneously active, often in organized bursts of action potentials typical of SI cortex under light urethane anesthesia (17).

In control cases (grafts in the cortex of host animals with bilaterally intact IO nerves, n = 6), graft neurons are silent after maturing for 4–7 weeks after transplant surgery. The presence of graft neurons was detected mainly by injury discharges as the electrode advanced through the transplant. The interface between graft and host tissue was characterized by the sudden onset of spontaneous activity as the electrode crossed the transplant–host interface. In the rare instances in which the presence of spontaneous neuronal activity could be ascertained, the cells could not be activated by movement of the contralateral whiskers or by electrical stimulation of the contralateral (normal) IO nerve. In contrast, when the electrode track was medial or lateral to the slight elevation in the hemisphere surface created by healthy grafts, spontaneous activity was present consisting of the usual mixture of spindle cluster, burst–pause, and unpatterned activity, and a high percentage of the cells could be briskly driven by vibrissae movement and electrical stimulation of the IO nerve. Later histological examination of the brains showed that these recording sites were always in the host cortex surrounding the transplant (Fig. 2*B*).

Graft neurons in animals with a cut IO nerve (96 cells in 10 animals) were immediately observed to be different from the controls by the presence of spontaneous activity (Fig. 1*D*). The spontaneous discharges were unusual in that they sounded unpatterned through the audiomonitor and did not include the expected spindle cluster and burst–pause activity characteristic of normal and host cortex under urethane anesthesia. Spontaneously active neurons could be isolated in such transplants and tested for responsiveness to whisker movement or electrical stimulation of the regenerated IO nerve. Twenty-two percent of the spontaneously active cells were responsive to whisker movement and/or to electrical stimulation of the IO nerve. However, their responses to peripheral stimulation showed a more variable latency and a lower than normal response to repeated stimulation (Fig. 1*C*). All of the responsive neurons were activated at short latency (7–10 msec) by movement of usually one or two vibrissae. The graft cells also produced below normal rates of spontaneous activity; almost all of it was unpatterned (Fig. 1*D*).

Since the topography of the whisker representation appeared markedly disorganized even outside of the graft in the

Table 1. Number and proportion of neurons analyzed in response to electrical stimulation of the regenerated and normal IO nerve for the experimental group

	Neurons				
	Responsive		Unresponsive		Probability of response*
	n	%	n	%	
Graft	22	22.9	74	77.1	0.3
Ipsilateral	48	84.2	9	15.8	0.6
Contralateral	39	83.0	8	17.0	0.9

*Probability of a neuron responding to a given stimulus.

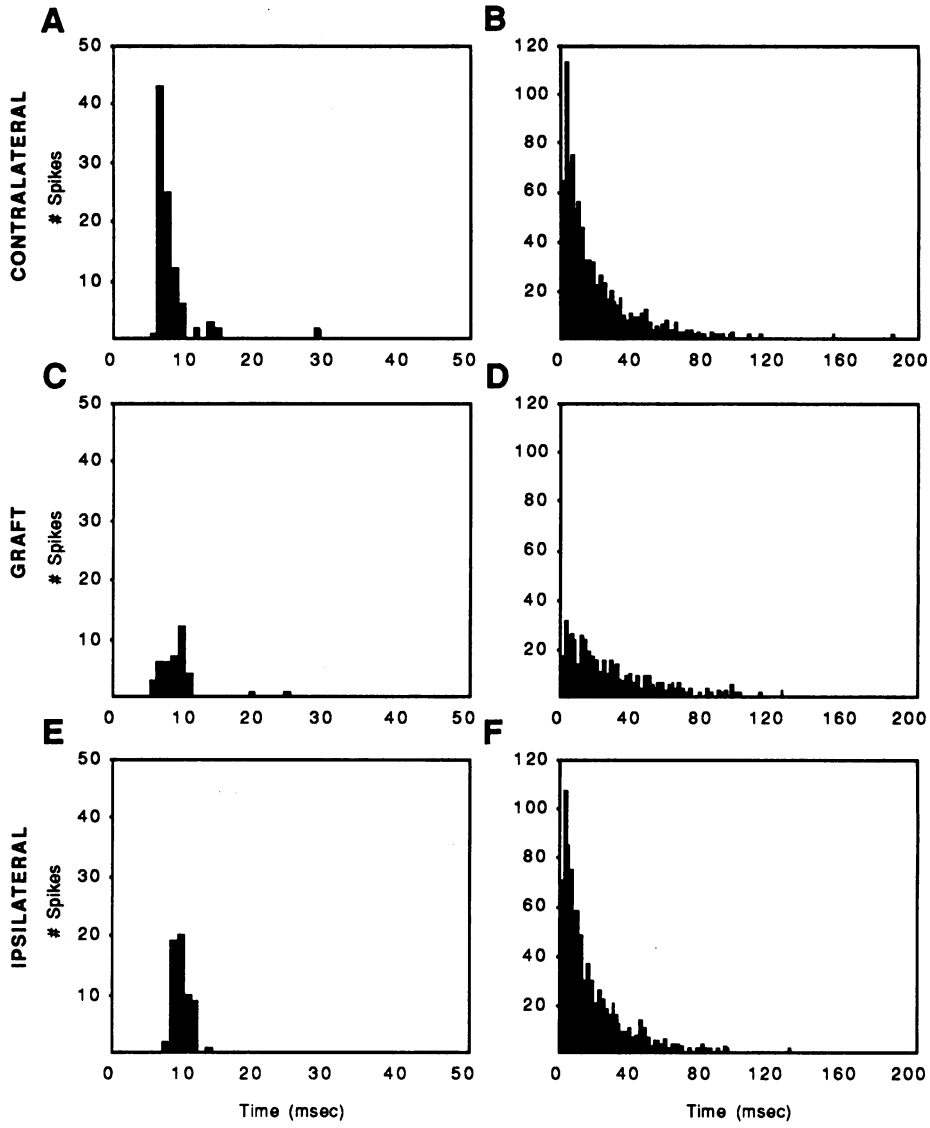

FIG. 1. Representative poststimulus time histograms for single units isolated in layers (IV + V) of the hemisphere contralateral to the graft (*A*), in the graft (*C*), and in the hemisphere around the graft (*E*) in response to peripheral stimulation. Each poststimulus time histogram represents the sum of the spikes generated in response to 50 stimulus presentations at 1 Hz. The latency of the graft neuron response is normal, but the probability of response is reduced compared to cells in the host brain. Spontaneous activity of the same cells is shown in interspike interval histograms on the right. Spontaneous activity of the graft cell (*D*) is less than cells in either the ipsilateral (*F*) or the contralateral (*B*) host cortex.

hemisphere contralateral to the regenerated IO nerve, the IO nerve alone in four animals was transectioned and the animals were allowed to survive for 4–6 weeks to determine the effect of IO nerve regeneration on the cortical map of the reinnervated whiskers. In every animal the pattern of cortical representation of the contralateral whiskers was very abnormal. Almost all responsive cells were driven only by one or two vibrissae, a result similar to the recordings from the transected IO nerve in the host cortex surrounding the graft (18). The probability of response of these neurons to a single stimulus dropped to <0.5 (Fig. 1*E*), although the spontaneous activity as measured by interspike histograms remained comparable to that of control cortices (Fig. 1*F*). This degree of disorganization in cutaneous representation reveals an unacceptable side effect of IO nerve transection and has led to studies to compare the effect of nerve transection with that of nerve crush, freeze, or conduction block, which have led to better regeneration of peripheral nerves in other studies (19–22).

The density of cytochrome oxidase staining in the host cortex around the graft and in the contralateral hemisphere showed comparable densities to normal cortex in all cases, although the cytochrome oxidase-dense bands were not as distinct in the layers of host cortex around the grafts. In contrast, the level of cytochrome oxidase staining in the grafts of control cases was consistently less dense than the surrounding cortex (Fig. 2*C*). The cytochrome oxidase staining density in the transplants of the experimental cases, on the other hand, was strikingly similar to that of the host cortex (Fig. 2*D*).

After HRP injections into the VB nucleus in our experimental cases, large caliber axons from the underlying white matter were seen crossing the host–transplant interface and forming characteristic VB terminal arbors (Fig. 3). In addition, HRP-labeled axons from the deeper layers of the adjacent cortex could be seen entering the grafts along the medial and lateral interface zones. These findings confirmed our previous conclusion that cutting the IO nerve enhanced thalamic fiber innervation of cortical grafts in adult hosts.

DISCUSSION

The results show that it is possible to activate grafted embryonic neurons through inputs from the host brain after

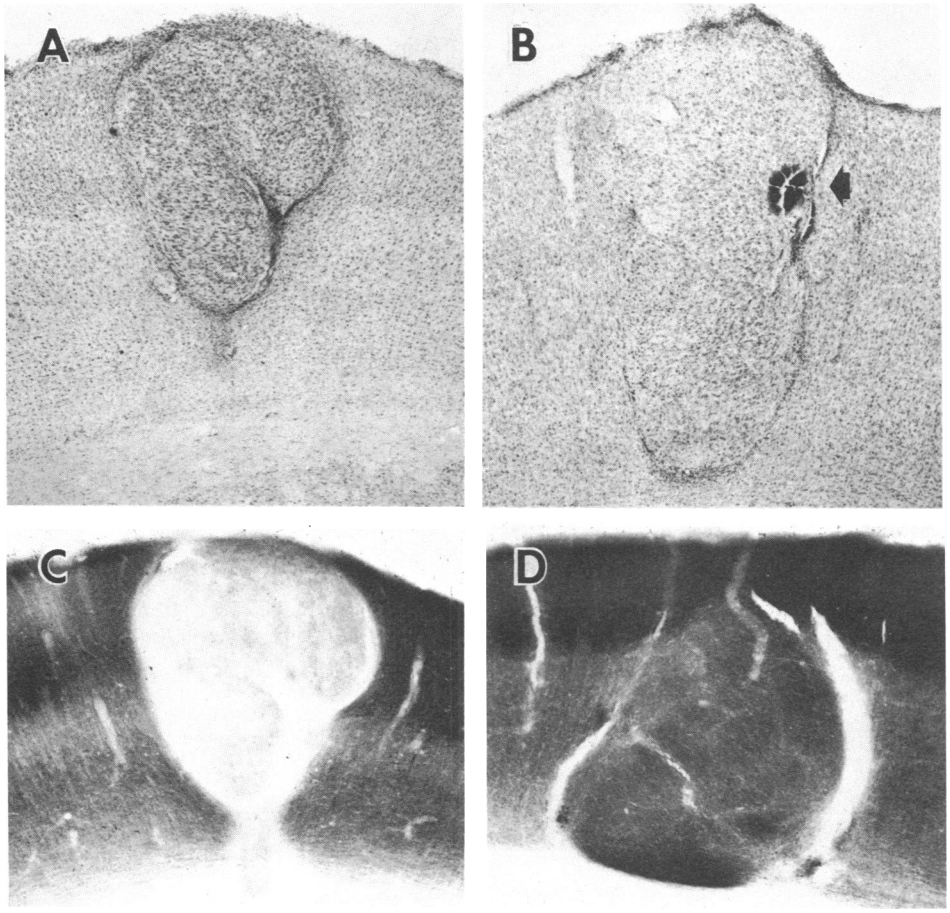

FIG. 2. Coronal histological sections through the hemisphere containing a graft with the contralateral IO nerve intact (*A* and *C*) and transected (*B* and *D*). The Nissl-stained sections demonstrate the closer cell packing always seen in normal host transplants (*A*) when compared to the more normal cell to neuropil ratio in the animals with an IO nerve sectioned (*B*). In *B*, the dark circular area (arrow) is HRP marking the location of the neuron whose responses are shown in Fig. 1 *C* and *D*. The section in *C* shows the characteristically pale reaction for cytochrome oxidase seen in grafts in normal host animals (section is from same animal as *A*). In contrast, cytochrome oxidase staining equivalent to the host brain is seen in animals with an IO nerve cut (*D*). The sections shown in *C* and *D* were treated identically according to the method of Wong-Riley (14).

the grafts differentiate in mature neocortex under certain conditions. Using our techniques, the necessary conditions include a conditioning lesion of the peripheral nerve that provides the sensory inputs to the area of cortex containing the graft. These results raise the question of how damage to a peripheral nerve can affect the regenerative capacity of a neuron several synapses away in the central nervous system. One of the simplest explanations for this effect is that the marked decrease in impulse activity along the trigeminal nerve, induced by disconnecting the peripheral receptors, provides more favorable conditions for distant thalamic neurons to sprout into the transplant territory. This interpretation of thalamic fiber ingrowth into neocortical grafts implies that peripherally evoked sensory activity along the mature trigeminal system prevents VB axons from elongating into the transplants. Hence, by blocking activity required for normal sensory function, one feature of the developmental period during which axons form terminal arbors may be recaptured, at least transiently. A straightforward test of this interpretation would be to block the sensory activity along the intact IO nerve by the application of a conduction blocking agent, such as tetrodotoxin.

Previous studies on the formation of topographical connections between the sensory periphery and central nervous system structures have shown that during normal development spontaneous activity along the sensory nerves plays an important role in the morphological differentiation of related central nervous system areas (23). When the representation of the body surface is charted in the cortex after normal rearing, the map is distorted into a pattern in which greater area is allocated to parts of the body that contain a greater density of cutaneous receptors. Receptor density alone, however, is not sufficient to fix the map; activity-dependent processes, most easily demonstrable during the early postnatal "critical" period, help define the area of cortex devoted to each component of a sensory representation (24, 31). However, even in adult animals, lesions of the sensory periphery—e.g., by amputation of a digit or a limb or transection of a peripheral nerve—lead to a detectable rearrangement of the cortical map. Likewise in the adult cat visual cortex the ocular dominance profiles can be altered under special conditions (25, 26). The cortical area allotted to the silenced inputs is usually decreased in size, whereas the surrounding areas that continue to receive active inputs are enlarged (27, 28). The mechanisms that support this type of map rearrangement in adult cortex have been the subject of considerable investigation, but remain unspecified. There are two currently preferred explanations for this type of central plasticity: (*i*) "unmasking" of existing cortical or subcortical synapses and (*ii*) formation of new connections through axonal sprouting into "deafferented zones" (29). Rapid changes in cortical representations are thought to occur by modification of the effectiveness of already existing connections, since the formation of new synapses in the central nervous system after damage is thought to take days (30). Longer-term changes generally have been attributed to axon

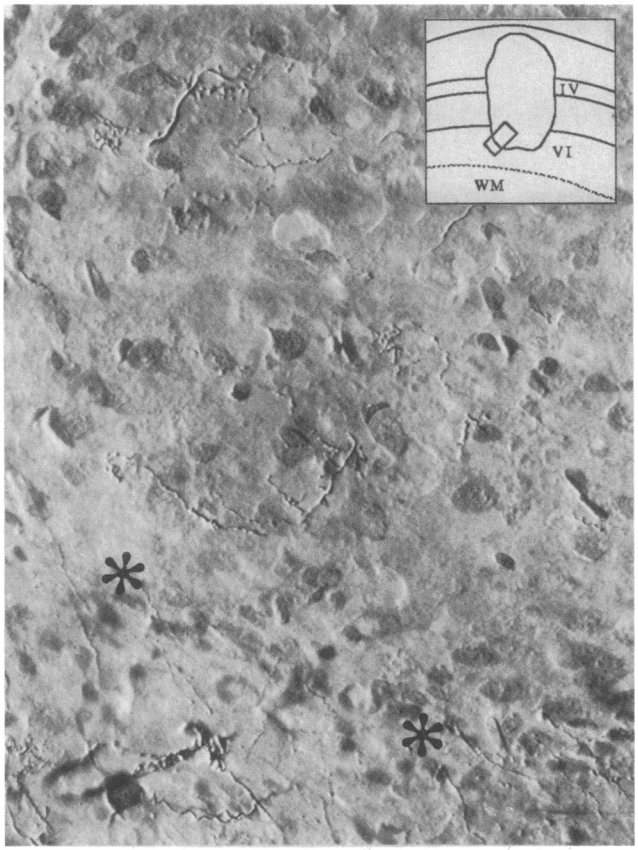

FIG. 3. Section treated with diaminobenzidine and glucose oxidase (interference–contrast optics) showing labeled thalamic fibers (wavy lines) and presumed terminals (dilatations and knobs) in a graft after IO nerve transection prior to grafting. Asterisks mark the border between the graft (above) and host (below) tissue. (*Inset*) Area of the transplant that was photographed.

growth despite the paucity of direct evidence for this effect, especially in the mammalian neocortex. The present results show that sprouting of central axons can occur after peripheral sensory nerve damage and suggest the possibility that the sprouting of axons could contribute directly to map rearrangement in cortex.

Although the enhancement of central regenerative processes induced by peripheral nerve damage acts through two synapses to alter the properties of thalamic axons in cortex, there is no evidence at the present time that the effect extends through interconnected areas of cortex. When the mechanisms supporting cortical map rearrangements and recovery of function after injury are understood, techniques can be envisioned that may facilitate the recovery of cognitive as well as sensory functions after nervous system injury.

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