Thrane [*Analyst* **103**, 1195 (1978)]. In brief, phosphine was extracted from tissue culture medium containing cells with toluene and injected with a gastight syringe into a Varian 3700 gas chromatograph equipped with a nitrogen and phosphorus detector and a Poropak Q column. The detector temperature was set at 250°C. Phosphine standards were prepared from zinc phosphide [D. Hill, in *Analytic Methods for Pesticides and Growth Regulators*, G. Zweig and J. Sherma, Eds. (Academic Press, New York, 1986), p. 145]. The extracted phosphine concentration was determined by comparison to prepared standard concentration curves.

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Electrophysiologic Responses in Hamster Superior Colliculus Evoked by Regenerating Retinal Axons

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Autologous peripheral nerve grafts were used to permit and direct the regrowth of retinal ganglion cell axons from the eye to the ipsilateral superior colliculus of adult hamsters in which the optic nerves had been transected within the orbit. Extracellular recordings in the superior colliculus 15 to 18 weeks after graft insertion revealed excitatory and inhibitory postsynaptic responses to visual stimulation. The finding of light-induced responses in neurons in the superficial layers of the superior colliculus close to the graft indicates that axons regenerating from axotomized retinal ganglion cells can establish electrophysiologically functional synapses with neurons in the superior colliculus of these adult mammals.

THE FAILURE OF CUT AXONS TO REgenerate in the central nervous system (CNS) of adult mammals causes a permanent functional disconnection between the axotomized neurons and their targets. However, when transplanted segments of peripheral nerve (PN) are used in vivo to test and influence the regenerative capacities of axotomized CNS neurons, nerve cells in various regions of the CNS can regrow lengthy axons (*1*). When the optic nerve of adult rodents is replaced with a PN graft joining the eye and the superior colliculus (SC), the regenerating axons of some axotomized retinal ganglion cells (RGCs) extend for several centimeters through the grafts, penetrate the SC for short distances, and form well-differentiated synapses in the superficial layers of the SC (*2, 3*). At least some of the RGCs that regrow axons into PN grafts can respond to visual stimuli and must therefore have afferent connections from other neurons within the retina (*4*). There has been no proof, however, that the regenerating axons of these or any other adult mammalian CNS neurons can transsynaptically excite or inhibit neurons in the reinnervated area of the CNS.

To investigate the possibility that contacts between regenerated RGC axons and SC neurons could mediate synaptic transmission, we recorded unitary responses to light in the SC of adult hamsters with cut optic nerves and PN grafts linking one retina and the ipsilateral SC (*5*). Transection of both optic nerves ensured that any responses to light recorded in the SC would be mediated by RGC axons that had regenerated across the PN graft. In addition, we took care to distinguish postsynaptic responses of SC neurons from responses in RGC axons that had penetrated the SC. These electrophysiologic experiments were performed 15 to 18 weeks after the grafts were implanted in the SC.

Extracellular recordings with glass-insulated carbon fiber electrodes (*6*) were made in the right SC of eight animals with PN grafts while a high-intensity, 10-μs flash of light was delivered every 3.1 s by a Grass photic stimulator 8 to 10 cm from the eye with the graft (*7*). For purposes of comparison, responses to the flash of light were recorded directly from regenerated axons of RGCs in one hamster. The caudal end of the graft was removed from the SC 14 weeks after insertion, and small bundles of axons were teased from the graft and placed on a silver wire electrode with a nearby ground as a reference (*4*).

Excitatory or inhibitory responses to the flash of light were recorded from single units in the SC of all eight animals. Responsive units tended to be clustered together. When a unitary response to the flash was obtained (Fig. 1A), the following procedure was used in an attempt to establish that the response was recorded from an SC neuron rather than from a regenerated RGC axon that had grown into the SC. Units that responded to light were identified as transsynaptically activated SC neurons if they also responded to electrical stimulation of the graft at a variable latency with an ill-defined threshold (Fig. $1B_1$) and if stimulation of the graft with double (or in some cases triple) pulses separated by 2 to 4 ms evoked one or more spikes more consistently than single pulses at the same intensity (Fig. $1B_2$). Sequential subthreshold postsynaptic potentials of appropriate timing

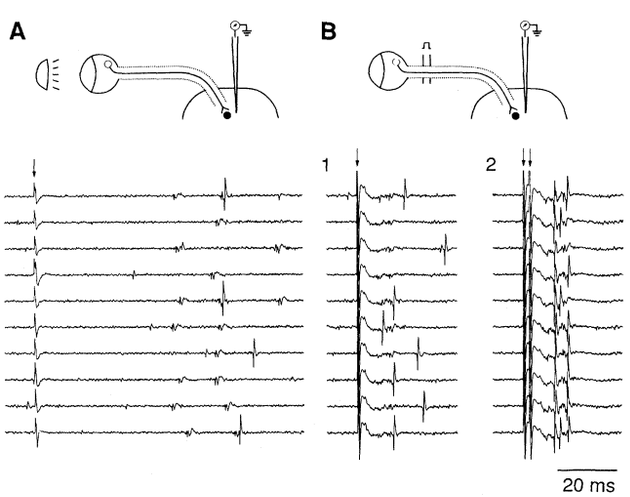

Fig. 1. (**A**) Ten successive responses to light flash (at arrow) recorded at a depth of 250 μm in the SC. The large unit responds with a single spike on four of ten iterations. (**B**) The same unit responds erratically with inconstant latency to (traces 1) single electrical stimuli (arrow) delivered to the graft and responds at a more constant latency, often with multiple spikes, to (traces 2) paired electrical stimuli (arrows) of the same intensity. This pattern of response is inconsistent with that anticipated from an RGC axon and thus identifies this unit as an SC neuron. All recordings were filtered (band pass 500 Hz to 5 kHz) and stored on magnetic tape.

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and magnitude, evoked by impulses in one or more afferent axons, can be expected to sum to bring a postsynaptic neuron to firing threshold. Conversely, repeated subthreshold stimuli would not be expected to activate a RGC axon (*8, 9*).

Using the electrophysiological criteria described above, we demonstrated transsynaptic activation for 25 light-responsive units in the SC of six of the eight animals. The responses to light of the SC neurons were generally weaker than the responses recorded from RGC axons in the graft (Fig. 2, A, B, D, and E). Many of the SC neurons responded intermittently to flash, with at most only one or two spikes; the average number of spikes per flash for most (19 of 25) of the SC neurons was less than one (range 0.2 to 3 spikes per flash). In contrast, most (18 of 20) of the RGCs with regenerated axons responded vigorously to the flash, with bursts of three or more spikes (range 0.6 to 19 spikes per flash). The depth of the postsynaptic units, as estimated by the vertical excursion of the microdrive from the surface of the SC, ranged from 12 to 450 μm.

We attempted to define receptive fields for SC units that responded to flash by moving spots of light of various sizes across a translucent screen placed 30 cm from the eye with the graft. Receptive fields were found for 4 of the 20 light-responsive SC neurons recorded long enough for testing. Because these units responded only intermittently to spots of light, the borders of the fields could not be precisely defined. Of the cells with demonstrable receptive fields, two responded to the onset of illumination (ON units) and two to the offset of illumination (OFF units). In contrast, receptive fields 4° to 11° in diameter were found for almost all (18 of 20) of the light-responsive RGCs whose regenerated axons were teased from the graft. The RGCs with receptive fields were either ON ($n = 4$) or OFF ($n = 14$) units. None of the RGCs or the light-responsive SC neurons displayed directional selectivity.

A large number of units in the SC had excitatory responses to light but did not fulfill the criteria for identification of postsynaptic neurons. These units responded to electrical stimulation of the graft at a well-defined threshold with a single spike at fixed latency and did not respond to paired subthreshold electrical stimuli. The responses to flash of these units were similar to those recorded from the teased RGC axons (0.7 to 5.6 spikes per flash) (Fig. 2C), and most of those tested had receptive fields. It is likely that most, if not all, of these units were RGC axons that had penetrated the SC (*10*).

Seven units recorded within 300 μm of the surface of the SC of two hamsters responded to flash with a 50- to 300-ms inhibition of their ongoing activity (Fig. 3A) at latencies of 50 to 190 ms. In the four units that were held long enough, electrical stimulation of the graft also elicited a decrease in ongoing activity lasting 60 to 120 ms (Fig. 3B). Furthermore, short latency spikes were not evoked in any of these units by the electrical stimulation. Such units, that were inhibited by both electrical and visual stimulation, could not have been RGC axons and thus must have been SC neurons influenced transsynaptically, perhaps via one or more interneurons (*11*). In all eight animals in which recordings were made from the SC, the position of the graft tip in the lateral aspect of the SC was confirmed histologically (*12*).

This study has shown that regenerated axons from RGCs of adult mammals can make functional synapses in the SC. Postsynaptic responses were recorded within the superficial 450 μm of the SC within the region where regenerated synapses have been demonstrated anatomically in similarly prepared animals (*3*) and where RGC axons make synapses (*13, 14*) in normal hamsters.

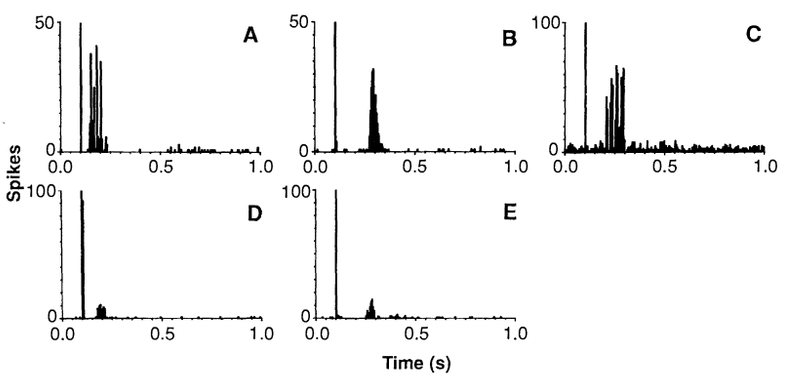

Fig. 2. (**A** and **B**) Responses to light flash recorded from regenerated RGC axons teased from PN grafts. Each histogram represents 50 sweeps at 5 ms per bin, with light flash at 100 ms. (A) ON unit and (B) OFF unit. (**C**) Response to flash of a presumed RGC axon recorded in the SC. (**D** and **E**) Excitatory responses to flash in postsynaptic units recorded at depths of 100 and 160 μm, respectively, below the surface of the SC. Histograms (C to E) represent 100 sweeps. Unitary spikes were digitized, and responses to light were analyzed on- and off-line.

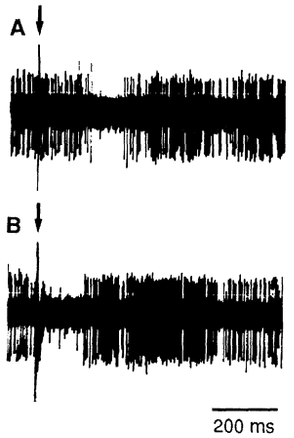

Fig. 3. (**A**) Inhibition of ongoing activity of an SC unit by light flash. (**B**) Inhibition of spontaneous activity of the same unit after electrical stimulation of the PN graft. (Each trace represents 25 superimposed sweeps.) Stimuli were delivered at arrows.

The responses to flashes of light that we recorded from reinnervated SC neurons were generally less vigorous than those recorded from regenerated RGC axons. Although individual synaptic contacts in the reinnervated SC resemble those in the normal SC at the ultrastructural level (*3*), the synaptic connections we studied may not yet have attained mature physiological characteristics. Indeed, most of the postsynaptic responses we observed resembled those recorded in the developing mammalian visual system where synaptic transmission is also characterized by erratic latencies and a low frequency of discharge in response to visual and electrical stimuli (*8, 15*). In the rat (*2*) and in the hamster (*16*), fewer than 5% of the axotomized RGCs in most PN-grafted retinas regenerate axons into the grafts, and it is thus unlikely that a normal pattern of convergent input could have been reproduced on individual reinnervated SC neurons in the hamsters we studied. Furthermore, because both optic nerves were cut and the occipital lobe and the SC itself were damaged during the grafting procedure, retinocollicular connections were established in these experiments in the absence of much of the normal visual circuitry. For all of these reasons, it is perhaps not surprising that for most of the SC neurons that responded to flash, we were unable to demonstrate responses to moving or stationary spots of light in discrete parts of the visual field, stimuli that are highly effective in eliciting discharges from SC neurons in the intact hamster (*14*).

Functional synaptic connections can be

restored in the retinotectal system (*17*) and in other regions of the CNS (*18*) of anamiotes after injury. Synaptic interactions have also been shown in mammals between grafted fetal or neonatal neurons and nerve cells in various regions of the host CNS including the tectum (*19*). We have now shown that adult mammalian CNS neurons have the capability to form functioning synapses with neurons in distant CNS regions after regeneration of their cut axons. Whether or not such synapses can be formed in sufficient density and with appropriate specificity to subserve behavioral function can now be explored.

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5. In nine 90- to 120-day-old hamsters (*Mesocricetus aureatus*), the right optic nerve was transected intraorbitally, and one end of a segment of autologous peroneal nerve approximately 2 cm long was anastomosed to the ocular stump (*2, 3*). After 7 to 8 weeks, the right (ipsilateral) SC was exposed by aspiration of some of the overlying occipital lobe and the distal end of the graft was inserted into the lateral aspect of the SC (*3*). Two to three weeks later, the left optic nerve was transected in an attempt to vacate synaptic sites on neurons in the right SC at the time when the regenerating RGC axons reached the SC (*2, 3*). All of these surgical procedures were done under pentobarbital anesthesia (35 mg/kg, intraperitoneal). For the terminal electrophysiological experiments, animals were anesthetized with urethane (1.25 g/kg, intraperitoneal) and the right SC was reexposed by aspiration of overlying cerebral cortex and scar tissue. The graft was exposed as it emerged from the orbit and desheathed to permit electrical stimulation. The animal's head was fixed to an animal frame by an aluminum bar attached to the occipital bone with low-melting-point dental wax (Moyco, Philadelphia). This arrangement avoided any obstruction of the visual field of the grafted eye. The right pupil was dilated with topical atropine and the cornea protected with a noncorrective contact lens. The animal's temperature was maintained at 37°C.
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7. Microelectrode penetrations were made systematically through the superficial layers of the SC to a depth of 600 μm (inferred from the reading on the hydraulic micromanipulator) at 50- to 200-μm increments in the anteroposterior and mediolateral planes. In animals in which the insertion site of the graft could be seen, recordings were begun in this region.
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10. Our criteria for identification of SC neurons are criteria of exclusion, SC neurons being identified as units that are not RGC axons. These criteria could fail to identify postsynaptic SC neurons that responded consistently and at fixed latency to electrical stimulation of the graft.
11. Electrical stimulation of PN grafts inserted into the rat retina gives rise to inhibition of RGC discharge by antidromic invasion of the retina, but this inhibition lasts no more than 30 ms (S. A. Keirstead and M. Rasminsky, unpublished results).
12. At the end of each experiment, the microelectrode was moved a measured distance medial to the last recording site, lowered into the brain, and broken off to serve as a positional reference. The animal was perfused through the heart with 4% paraformaldehyde, the brain was removed, and coronal sections were cut on a cryostat. Because the axons in the grafts were not labeled, we were not able to identify the extent of penetration or arborization of RGC axons within the SC. However, we estimate that all recordings from light-responsive SC neurons were made within 1 mm of the graft.
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Protection of Dentate Hilar Cells from Prolonged Stimulation by Intracellular Calcium Chelation

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Prolonged afferent stimulation of the rat dentate gyrus in vivo leads to degeneration only of those cells that lack immunoreactivity for the calcium binding proteins parvalbumin and calbindin. In order to test the hypothesis that calcium binding proteins protect against the effects of prolonged stimulation, intracellular recordings were made in hippocampal slices from cells that lack immunoreactivity for calcium binding proteins. Calcium binding protein–negative cells showed electrophysiological signs of deterioration during prolonged stimulation; cells containing calcium binding protein did not. When neurons without calcium binding proteins were impaled with microelectrodes containing the calcium chelator BAPTA, and BAPTA was allowed to diffuse into the cells, these cells showed no deterioration. These results indicate that, in a complex tissue of the central nervous system, an activity-induced increase in intracellular calcium can trigger processes leading to cell deterioration, and that increasing the calcium binding capacity of a cell decreases its vulnerability to damage.

CALCIUM-MEDIATED PROCESSES INside neurons are critical to a variety of cell functions (*1*). Intracellular free Ca^{2+} is normally maintained at a low level, but the level may increase significantly by events that release intracellular stores of bound Ca^{2+} or that allow Ca^{2+} flux across the membrane. Large increases of intracellular Ca^{2+} are thought to occur during periods of excessive neuronal excitation and trigger processes that lead to cell death (*2*). Specific cell types appear to be especially vulnerable to damage under conditions that facilitate Ca^{2+} influx via voltage- or neurotransmitter-gated channels. Intracellular Ca^{2+} binding proteins are important in regulating free Ca^{2+} (*3*), and their capacity to buffer intracellular Ca^{2+} may be important in determining cell vulnerability under conditions associated with Ca^{2+} influx.

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