Table 2. Time course of effects of dibutyryl cyclic AMP (DAMP) on pineal synthesis of C^{14}-melatonin and C^{14}-serotonin. Groups of six culture tubes each containing a rat pineal gland were incubated with C^{14}-tryptophan ($10^{-4}M$) in the presence or absence of $3 \times 10^{-3}M$ DAMP for 4, 16, or 48 hours at 37°C. Results are expressed as counts per minute of C^{14} radioactivity.

DAMP (+ or 0)	Melatonin (count/min)	Serotonin (count/min)
	4 hours	
0	125 ± 36	182 ± 52
+	309 ± 57*	233 ± 35
	16 hours	
0	484 ± 43	601 ± 25
+	1770 ± 276†	1272 ± 165‡
	48 hours	
0	591 ± 52	636 ± 45
+	2424 ± 215†	1751 ± 28†

* $P < .05$. † $P < .001$, differs from controls without DAMP. ‡ $P < .01$.

cyclic AMP. This substance then works as a "second messenger" to mediate such intracellular effects of the catecholamine as stimulation of labeled serotonin and melatonin synthesis.

The enhanced accumulation of C^{14}-protein in pineals incubated with norepinephrine probably reflects an increase in the intracellular specific activity of its precursor, C^{14}-tryptophan, secondary to the increased uptake of the amino acid (*4*). In contrast, the acceleration of C^{14}-indole synthesis induced by norepinephrine is not simply a "pool effect," inasmuch as the concentrations of labeled serotonin and melatonin in the medium show a much greater proportionate increase than the concentration of labeled tryptophan in the pineal (*3, 4*).

The pattern of changes that norepinephrine induces in the synthesis of pineal indoles (that is, increases in labeled serotonin and melatonin, no change in labeled 5-hydroxyindoleacetic acid) is essentially the same as the pattern produced by adding DAMP to the medium; moreover the time courses of these changes are similar (*3*). Since norepinephrine elevates the activity of adenyl cyclase in pineal homogenates (*6*), it seems likely that the effects of DAMP are not simply pharmacological, but reproduce the actions of endogenous cyclic AMP, which is produced in response to norepinephrine or to sympathetic nervous stimulation. The precise mechanisms by which DAMP enhances the syntheses of labeled serotonin and melatonin have not yet been identified. However, the stimulation of C^{14}-melatonin synthesis probably involves an increase in the activity of hydroxyindole-*O*-methyl transferase, the enzyme that catalyzes the formation of melatonin from *N*-acetyl serotonin (*9*), inasmuch as sympathetic nervous function controls this enzyme in vivo (*1*), and norepinephrine enhances its activity in vitro (*10*).

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Oculomotor Neurons in Fish: Electrotonic Coupling and Multiple Sites of Impulse Initiation

Abstract. *Oculomotor neurons are electrotonically coupled in three teleosts. Electron microscopy revealed axosomatic synapses with close appositions of pre- and postynaptic membranes. Similar junctions are associated with electrotonic coupling in many other cases. Stimulation of the ipsilateral eighth nerve usually initiated impulses at sites distant from the cell bodies; stimulation of the ipsilateral ophthalmic nerve initiated impulses close to the cell bodies. Electrotonic coupling may synchronize impulses arising near the cell bodies to generate synchronous muscle contractions. Impulses arising distant from the cell bodies may lead to contractions of graded strength.*

Neurons in nuclei controlling synchronously acting effector organs such as electric organs are electrotonically coupled in fishes in many instances (*1–5*). The basic experimental observation is that current passed through an electrode in one cell produces a potential in a neighboring cell that is larger than can result from current passing through extracellular space surrounding the cells. The functional significance of electrotonic coupling in these systems is that it provides rapidly acting positive feedback between cells which synchronizes their firing. A cell that is relatively more depolarized advances spike initiation in neighboring cells; a cell that is relatively less depolarized retards spike initiation in neighboring cells. Thus, these electrotonic synapses are at the same time excitatory for less depolarized cells and inhibitory for more depolarized cells and have been termed synchronizing synapses (*6*). Mutually excitatory synapses that transmit chemically could not produce the highly synchronous discharges observed because of the relatively long delay associated with this mode of transmission (usually about 0.5 msec in fishes). Electrically transmitting synapses can exhibit a much briefer delay (less than 0.05 msec). Electrically mediated transmission from higher to lower level neurons is also known in a number of systems controlling escape reflexes (*7*). The functional significance in these instances appears to be a reduction in reflex latency leading to more rapid escape.

There is considerable evidence that electrotonic coupling between cells is mediated by specialized junctions where cell membranes are so closely apposed as to occlude most or all of the extracellular space between them. These junctions have been found in every instance where electrotonically coupled cells have been studied at the ultrastructural level and are rare or absent where cells are not coupled (*3–5*). In a given nucleus, neurons can be coupled by dendrodendritic junctions or through presynaptic fibers that form junctions on more than one cell. Experimental treatments that disrupt and then rejoin the junctions lead to loss and then recovery of electrotonic coupling (*8*).

We undertook this study to determine if electrotonic coupling was involved in the control of more ordinary organs than the highly specialized ones cited above. The oculomotor system in fish was an attractive subject because of the requirement for synchronous activity in saccadic eye movements and in the quick phase of nystagmus, because of the technical simplicity of working with these animals, and be-

cause of the general similarity of the oculomotor system throughout the vertebrates. We report here that electrotonic coupling does occur between oculomotor neurons, that different excitatory inputs project to widely separated regions of the cells, and that impulses can arise at different sites depending on which excitatory inputs are stimulated.

For electrophysiological studies, puffers (*Spheroides maculatus*), spiny boxfish (*Chilomycterus schoepfi*), and, in a few experiments, long-horned sculpin (*Myoxocephalus octodecemspinosus*), 15 to 30 cm long, were held in a horizontal position by rods in a plexiglass box and were perfused through the mouth with seawater. Some fish were injected with curare (10 mg/kg) to prevent movement. The eyeball was exposed dorsally and pulled outward to make room for the dissection of the abducens, trochlear, and ophthalmic nerves and of the four branches of the oculomotor nerve. The nerves were severed distally so that they could be placed on a pair of wires for stimulation or recording. The brain, intracranial portion of the oculomotor and trochlear nerves, and sometimes the eighth nerve or spinal cord were then exposed. The size of antidromic volleys in the muscle nerves was monitored by a monopolar electrode placed intracranially on the nerves. Bipolar stimulating electrodes were placed on the eighth nerve or spinal cord. The central portion of the optic tectum and the valvula of the cerebellum were removed, exposing the ventricular floor which covers the oculomotor and trochlear nuclei. The brain and nerves were covered with mineral oil to prevent drying. After this procedure, animals not injected with curare could show apparently normal movements of fins and intact eye for as long as 36 hours. Glass microelectrodes filled with 3*M* KCl were used for intracellular recording and stimulating by means of a bridge circuit (*4*). Methods of display and of recording were conventional.

Cells in the midbrain tegmentum that were penetrated by microelectrodes were identified as oculomotor neurons by the short and constant latency of action potentials after antidromic stimulation of the motor nerves (Fig. 1A). The usual recording site was the cell body because large postsynaptic potentials (PSP's) could be evoked by appropriate stimulation, and in a number of instances cells were iontophoretically injected with methyl blue (*9*) or procion yellow M4RS (*10*). The oculomotor neurons are about 30 to 40 μm in diameter, and the cell bodies lie as little as 50 μm below the surface. Cells injected with procion yellow M4RS or impregnated by the Golgi technique had one large dendrite. This single large process was usually oriented perpendicular to the ventricular floor and branched into two to four long dendrites radiating into the tegmentum. The axon was observed in two instances in which it arose from the large process about 100 μm from the cell soma.

The oculomotor nuclei lie immediately posterior to a midline artery that bifurcates into lateral branches supplying the ventricular floor. The cells of the contralateral superior rectus are most anterior. Behind them lie the ipsilateral anterior and inferior rectus cells, and behind these are the contralateral trochlear cells. The ipsilateral inferior oblique cells are deep to the anterior and inferior rectus cells. The eye muscle nerves were shown by light microscopy to contain 30 to 50 large axons. There are also many small fibers which probably include some pain fibers since the fish often moved when a muscle nerve was severed.

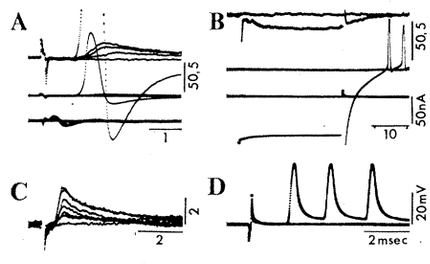

Fig. 1. Potentials in the oculomotor nucleus. (A) Antidromic responses in an oculomotor neuron innervating the anterior rectus (puffer). Upper and middle traces: recording from an oculomotor neuron at high and low gain, respectively. Lower trace: monopolar recording of the antidromic volley. Five superimposed sweeps at different stimulus strengths. The strongest stimulus evoked an antidromic spike in the penetrated cell. Three of the sweeps showed small depolarizations that began less than 0.2 msec later than the onset of the antidromic spike (upper trace). (B) Electrotonic spread between oculomotor neurons innervating the anterior rectus (spiny boxfish). Upper trace: recording from an oculomotor neuron at high gain. Middle trace: recording from a second oculomotor neuron at low gain. Lower trace: current applied in the second cell. Two superimposed sweeps, one with and one without polarizing current. Note "anode break" responses in the second cell. (C) Short latency depolarizations in a presynaptic fiber evoked by antidromic stimulation of the oculomotor nerve (sculpin). Seven superimposed sweeps at different stimulus strengths. (D) Response of the same fiber as in (C) to threshold spinal stimulation. Two superimposed sweeps at constant stimulus strength. In Figs. 1 and 3 artifacts associated with nerve stimulation are brief deflections on the left that are biphasic (except in Fig. 1C). Voltage and time calibrations are in millivolts and milliseconds, respectively.

Electron micrographs of oculomotor neurons showed that the cell bodies and proximal dendrites were contacted by numerous synaptic profiles containing vesicles and mitochondria and sometimes showing the termination of the myelin sheath (*11*). About one-sixth of the synaptic profiles had a small region where the cell membranes were closely apposed as at other electrotonic synapses (Fig. 2, A_1, A_2, and inset). Since only a small part of any synaptic profile was occupied by a region of close apposition, many synapses must have had these junctions at sites out of the plane of section. At the sites of close apposition, the overall thickness of the two membranes was less than 160 Å, indicating that there was little extracellular space between the two unit membranes. Synaptic specializations characteristic of chemically mediated transmission were also observed. At these sites presynaptic vesicles were clustered against the axonal membrane; the pre- and postsynaptic membranes formed a desmosome-like complex where the membranes were separated by about 200 Å and the cytoplasm on each side contained material of increased density. Similar desmosome-like complexes that lacked any accumulation of synaptic vesicles were also found (Fig. 2, A_1). In some synaptic profiles (Fig. 2, A_2), there were both close appositions and desmosome-like complexes with closely associated presynaptic vesicles. Other terminals formed these two types of junction with apparently different cells. Since no direct dendrodendritic synapses were observed between oculomotor neurons, it is likely that axosomatic synapses with close appositions are the sites of electrotonic coupling demonstrated below. As compared to other coupled nuclei that are morphologically similar, it is likely that these synapses are from afferent fibers and that electrotonic spread between motoneurons is by way of these fibers (*1*, *3*, *5*).

Electrophysiological studies suggest that there are chemically transmitting synapses on the cells which presumably are included among those profiles without close appositions. The presence of desmosome-like complexes with asso-

ciated presynaptic vesicles at synapses that also show close appositions suggests that both chemically and electrically mediated transmission might occur at these synapses. While insufficient data have been obtained from oculomotor neurons, PSP's generated at several morphologically similar synapses exhibit little or no chemically mediated component (*3*).

Electrotonic coupling between oculomotor neurons was demonstrated between pairs of cells that innervate the same muscle by means of electrodes placed in two neighboring cells. In about one-fifth of the pairs recorded, hyperpolarizing current applied in one cell caused a slowly rising and falling hyperpolarization in the other cell (Fig. 1B). Similar currents caused negligible potentials when the electrodes were moved to just outside the cells. The degree of coupling was not easily evaluated because the large currents passed through the bridge circuit (about 0.1 μa) prevented determination of the potential in the polarized cell. However, in different experiments where separate current and voltage electrodes were placed in the same cell, the input resistance was 1 to 2 megohms. Thus, we conclude that, at most, 2 to 5 percent of the potential in the polarized cells spread to the neighboring cells.

Evidence for electrotonic coupling was also obtained by grading the strength of antidromic stimuli. In most cells with higher threshold axons, graded depolarizations up to 4 mv in amplitude were evoked by stimuli below threshold for the axon of the penetrated cell. Focal potentials recorded extracellularly were negative going during the graded antidromic depolarizations, so that the transmembrane potentials were somewhat greater than the intracellularly recorded potentials. These depolarizations are ascribable to electrotonic spread of antidromic spikes from excited to unexcited cells because (i) the depolarizations often arose within 0.2 msec of the onset of the antidromic spikes, which is a latency too short to allow for chemically mediated transmission by afferents or recurrent collaterals (Fig. 1A); (ii) the size of the depolarizations increased with the size of the antidromic volleys; and (iii) there were few if any large afferent fibers in the oculomotor nerves that could be activated by mechanical stimulation of the muscle. Testing for graded antidromic depolarizations indicated that coupling did not occur between neurons innervating different muscles.

Antidromic stimulation sometimes evoked short latency, graded potentials in presynaptic fibers. These fibers were identified by absence of spikes in response to antidromic stimuli (Fig. 1C), absence of PSP's preceding spontaneous or evoked discharges (Fig. 1D), and lack of effect on spontaneous or evoked discharges of applied hyperpolarizing current. The first property demonstrates that the response was not from a motoneuron; the last two indicate that the recording site was distant from the site of impulse initiation. The finding of presynaptic fibers coupled to a number of motoneurons is further evidence that the neurons are coupled by way of presynaptic fibers.

Stimulation of ipsilateral eighth cranial and ophthalmic nerves initiated impulses at different sites in the oculomotor neurons (Fig. 3). In most cells, eighth nerve stimulation evoked spikes that, recorded in the soma, arose from a nearly level base line (Fig. 3, A_1). Large hyperpolarizations were required to delay or block these spikes, and when they were blocked very little PSP remained (Fig. 3, A_2). These properties indicate that the spikes arose at a site distant from the cell body. Eighth nerve stimulation below threshold for spikes in the oculomotor neurons did not produce large PSP's in the cell bodies, although slow depolarizations of 1 or 2 mv that must have been larger at the distant site of impulse initiation were recorded. Sometimes small spike-like potentials remained when hyperpolarization blocked the main component of spikes evoked by eighth nerve stimulation. Further hyperpolarization delayed and finally blocked these potentials, which suggests that they represented spike activity in dendrites. These small potentials appear similar to those ascribed to dendritic spikes in hippocampal, ventral thalamic, and immature neocortical neurons (*12*).

Responses recorded in the soma to ipsilateral ophthalmic nerve stimulation were usually quite different from re-

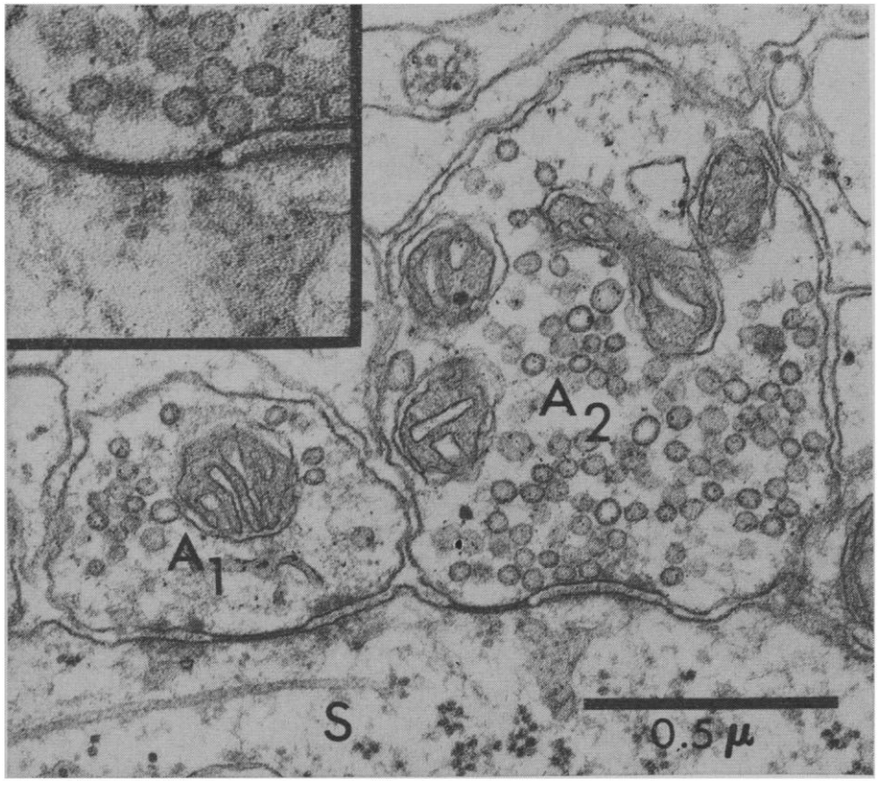

Fig. 2. Electron micrograph from the oculomotor nucleus (spiny boxfish). Two profiles (A_1, A_2), presumably axonal, synapse on the soma (*S*) of an oculomotor neuron. Both endings form specialized junctions with the soma, at which pre- and postsynaptic membranes are closely apposed. The close apposition at profile A_2 is enlarged in the inset. Profile A_2 contains numerous vesicles. To the right of the close apposition is a desmosome-like complex with closely associated presynaptic vesicles. Profile A_1 contains few vesicles. On each side of the close apposition, pre- and postsynaptic membranes form desmosome-like complexes without associated vesicles. Fixed with glutaraldehyde and OsO_4. ($\times$ 70,000; inset $\times$ 140,000)

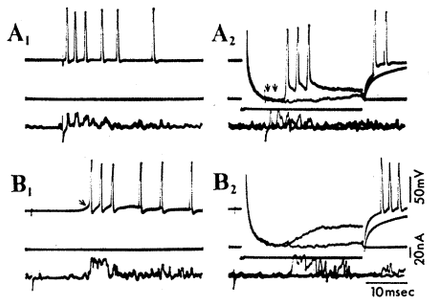

Fig. 3. Different impulse initiating sites in an oculomotor neuron (puffer). Upper trace: recording from an anterior rectus motoneuron. Middle trace: polarizing current. Lower trace: efferent activity in the anterior rectus nerve. (A_1, A_2) Ipsilateral eighth nerve stimulation at a fixed strength. (A_1) The impulses arise abruptly from a nearly level base line. (A_2) Hyperpolarization delays the first response and reduces the number of spikes, but there is little PSP recorded at the times (arrows) that the first and second spikes arise in A_1. Two superimposed sweeps with and without eighth nerve stimulation. (B_1, B_2) Stimulation of the ipsilateral ophthalmic nerve at a fixed strength. (B_1) The first impulse arises from a slowly rising PSP (arrow). Later impulses arise from successively lower levels of depolarization. (B_2) Impulses are blocked during a hyperpolarizing pulse, revealing a large depolarizing PSP. Two superimposed sweeps with and without nerve stimulation. Calibrations in B_2.

sponses to eighth nerve stimulation. A slowly rising PSP preceded the first spike in an evoked burst (Fig. $3B_1$, arrow). Spikes were also easily blocked by hyperpolarization, leaving large PSP's (Fig. $3B_2$). Thus, these impulses must have arisen near the soma. The first spike usually arose when the PSP was close to the firing level measured by direct stimulation. However, the level at which the spikes arose often changed gradually during a response; later spikes could arise abruptly from the base line, as did those evoked by eighth nerve stimulation (Fig. $3B_1$, last spike). This finding indicates that the site of impulse initiation can shift during the repetitive discharge, but at least the initial impulses evoked by stimulation of the two nerves arise at different sites (*13*).

The location of the different sites of impulse initiation cannot be decided at this time. Since the axon can branch off from a dendrite at some distance from the soma, the spikes evoked by eighth nerve stimulation could arise either in a dendrite or in the initial segment. In the latter case the initial spikes evoked by ophthalmic nerve stimulation would arise in the cell body or proximal dendrite. The gradual variations in the potential at which the spikes apparently arose may mean that the site of impulse initiation can gradually shift along the cell and that there are no discrete, low threshold "trigger zones."

The pathways mediating vestibular and ophthalmic nerve inputs were not investigated. The minimum latency of the efferent nerve responses to eighth nerve stimulation was 1.0 to 1.5 msec, and the overall conduction distance was about 2 cm. Since conduction in the efferent pathway took about 0.4 msec, the vestibulo-oculomotor pathway is likely to be monosynaptic. The latency of the motor nerve responses to ophthalmic nerve stimulation was variable and often exceeded 15 msec, which indicates that complex pathways were involved. The slow time course of ophthalmic nerve evoked PSP's suggests that there may be chemically mediated transmission at some of the synapses on the oculomotor neurons. Furthermore, hyperpolarizing potentials, which were probably chemically mediated inhibitory PSP's, were sometimes evoked by stimulation of the contralateral ophthalmic nerve.

Our data allow tentative assignment of functional significance to the occurrence of electrotonic coupling and multiple impulse initiation sites in the oculomotor neurons. Coupling between cell bodies synchronizes cell firing in eye withdrawal evoked by ophthalmic nerve afferents and perhaps in saccadic eye movements as well. In contrast, vestibular inputs produce volleys that are smoothly graded in amplitude to move the eye in compensation for head movements. This activity arises at the distant sites of impulse initiation where coupling of the motoneurons is probably not present, for it would tend to synchronize cell firing. The coupling of the cell bodies is not sufficiently close for impulses arising in the dendrites to involve other cells in the absence of depolarization of the cell bodies. These mechanisms may have relevance to other systems such as the hippocampus and thalamus (*14*) where neurons can fire either synchronously or independently of one another.

We believe that coupling tends to synchronize cell firing when synapses near the sites of coupling activate the cells, and synchronous muscle contractions result. Synapses initiating impulses far from the sites of coupling can generate nerve volleys smoothly graded in amplitude. Whatever the reflex significance of our results, the concept of the vertebrate neuron derived from study of the ventral horn cell is being extended by these data and related findings in other more rostral neurons (*15*). Finally, electrotonic coupling has now been found in a fast effector system that has many similar properties throughout the vertebrates.

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11. Material for electron microscopy was obtained by perfusion of puffers and spiny boxfish through the conus arteriosus for about 15 minutes, after which the region containing the oculomotor nuclei was removed for further treatment. Similar results were obtained whether fixation was by (i) 1 percent OsO_4 in Millonig's buffer, *p*H 7.3, for 90 minutes overall [G. Millonig, *Proc. Int. Conf. Elect. Microscop. 5th* 2, 8 (1962)] or (ii) *2.5*

percent glutaraldehyde in Sorenson's buffer, *p*H 7.3, for 2 hours, followed by washing in Sorenson's buffer overnight, followed by 90 minutes post fixation in 1 percent OsO_4 in Sorenson's buffer. Tissue was dehydrated in graded ethanol solutions and embedded in Epon 812. Thin sections were stained with lead citrate for 2 to 4 minu → [E. Reynolds, *J. Cell Biol.* **17**, 208 (1963)] followed by 20 percent uranyl acetate in methyl alcohol for 10 to 15 minutes. Tissue was examined with a Phillips 200 electron microscope operating at 60 kv.

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13. It might be argued that all impulses arose at a distance from the soma and that ophthalmic nerve stimuli merely produced smaller PSP's at the distant impulse initiation site. This possibility appears to be excluded because bursts of spikes of equally high frequency were evoked by both modes of stimulation, and the PSP's at a hypothetical single site would have to be of about the same amplitude.
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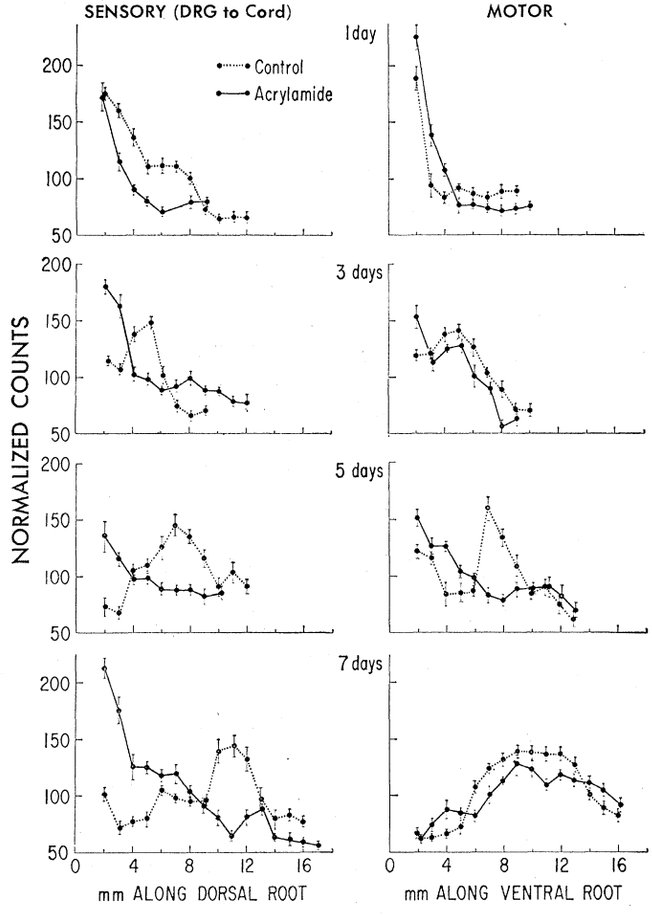
Axonal Transport of Proteins in Experimental Neuropathies

Abstract. *Axoplasmic flow of proteins is interrupted in cats with neuropathy induced by acrylamide, but it is not interrupted in normal cats and in those with neuropathy induced by tri-orthocresyl phosphate. The proteins move from lumbosacral motor neurons along the ventral roots and from ganglion cells toward the spinal cord along the dorsal roots at about 1½ millimeters per day.*

Widespread axonal destruction resembling Wallerian degeneration without the death of nerve cell bodies is seen in the neuropathies associated with thiamine deficiency, isoniazid administration, and exposure to acrylamide, tri-orthocresyl phosphate (TOCP), and other toxins (*1*). The pathogenesis of this selective axonal lesion is unknown. Transport of intracellular proteins from site of synthesis to site of function is accentuated in neurons by the extreme length of their axonal processes (*2*). Interruption of transport could result in breakdown of the axon. To test this hypothesis, flow rates of newly synthesized proteins within sensory and motor axons of normal cats were compared with those in cats with toxic neuropathies.

Neuropathies were induced by administration of acrylamide (20 mg per kilogram of body weight; orally 5 days per week) or TOCP (0.25 ml per kilogram of body weight; intramuscularly every 2 weeks). Foot drop and an unsteady, wide-based gait appeared 2 or more weeks after the beginning of treatment with either agent, but perception of a pinprick was not impaired. Axonal degeneration, predominantly distal, was found in hind limb nerves of both groups after neurologic signs had appeared. No alterations in the cell bodies of motor or sensory neurons were evident in sections stained for Nissl substance.

Flow rates of axonal protein in motor and sensory nerves were determined in 9 cats with acrylamide neuropathy, in 6



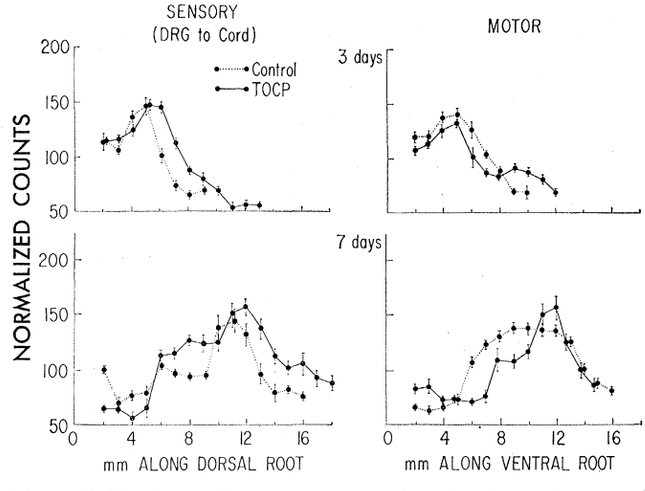


Fig. 1 (left). Autoradiograms were used in the determination of the distribution of H^3 in dorsal (sensory) and ventral (motor) roots of normal cats and cats made neuropathic by treatment with acrylamide at intervals after intraperitoneal injection of H^3-L-leucine. Results in representative cats 1, 3, 5, or 7 days after receiving the tracer are given. Maximum radioactivity was displaced away from the center of the dorsal root ganglion or anterior horn at about 1½ mm/day in normal cats but not in most cats treated with acrylamide. Points represent grain counts per field (0.01 mm^2) within a 1-mm segment of root (mean of eight determinations in each case) expressed as percentage of mean counts per 0.01 mm^2 in the entire root. Vertical lines represent ±1 standard error of the mean. DRG, dorsal root ganglion. Fig. 2 (right). Effect of TOCP on distribution of radioactivity along ventral and dorsal roots of cat neural tissue. Results are expressed as in Fig. 1. No significant difference in distribution of radioactivity between normal and TOCP-treated cats was observed.