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Does Nerve Impulse Activity Modulate Fast Axonal Transport?

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Contents

Abstract

The possibility that the amount of newly synthesized material made available for fast axonal transport is regulated by nerve impulse activity was examined in an in vitro preparation of bullfrog dorsal root ganglia (DRG) and sciatic nerve. Under conditions that precluded effects of impulse activity on either uptake or incorporation of precursor, patterned stimulation of the sciatic nerve (1 out of every 2 s) produced a frequency- and time-dependent decrease in the amount of radiolabeled protein accumulating at a nerve ligature. The response to patterned stimulation was significantly greater than that to continuous stimulation when the same number of stimuli were delivered. In unligated nerve preparations, patterned stimulation decreased the amplitude of the transport profile with no concomitant change in the wave front distance. Nerve stimulation produced no observable ultrastructural alterations within neuronal cell bodies of the DRG. We propose that the physiological significance of these results is not that nerve impulse activity decreases fast axonal transport, but

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that the amount of transport increases during periods of electrical quiescence. According to this hypothesis, activity-dependent macromolecules of the axolemma and nerve terminals are replenished during periods when the neuron is firing less frequently. These findings are discussed in light of reports that chronic in vivo stimulation *increases* the amount of fast-transported, radiolabeled protein (Chan et al., 1989) and that TTX-blockade of neuronal activity has *no effect* on protein transport (Edwards and Grafstein, 1984; Riccio and Matthews, 1985).

Index Entries: Fast axonal transport; action potentials; dorsal root ganglion; sciatic nerve.

Introduction

The neuronal cell body and its elongated cytoplasmic extensions (axon and dendrites) maintain a dynamic contact by two principal means: bioelectrical signaling via conduction of action potentials, and intracellular transport of structural, metabolic, and informational molecules. Although action potential propagation is a cell-surface event, whereas molecular transport occurs within the cellular interior, the question has often been raised as to whether electrical activity exerts any effect on intraaxonal or intradendritic transport (*see* Grafstein et al., 1982).

The clearest evidence that nerve impulse activity and fast axonal transport are not obligatorily coupled and can occur independently comes from studies in which axoplasm from the squid giant axon is extruded from its sheath as a cylindrical gel. On the one hand, replacement of the axoplasm with a potassium-rich salt solution enables the axolemma to conduct normal action potentials for several hours (Baker et al., 1962). On the other hand, video-enhanced light microscopy reveals a bidirectional transport of organelles in the extruded cylinder of axoplasm that continues, again for several hours, at normal rates (Brady et al., 1982,1985). These latter observations are consistent with an array of previous findings that the *rate* of fast axonal transport is little changed by nerve impulse activity or by the suppression of such activity (*see* Grafstein and Forman, 1980). For example, the fast transport velocity in peripheral sensory neurons in vivo is unchanged when these same nerve trunks are placed in vitro, cut off from their source of afferent stimulation as well as from their cell bodies (Ochs, 1972). Further, physiological-range electrical stimulation of peripheral nerve trunks has little or no consistent effect on the transport rate of either radiolabeled proteins (Ochs and Smith, 1971; Ignelzi and Nyquist, 1979) or endogenous markers (Jankowska et al., 1969; Dahlström, 1971; Garcia et al., 1974; Keen and McLean, 1974). Thus, materials undergoing fast transport within the axon appear to be little affected by the waves of action potentials passing along the axolemmal surface.

However, although transport *velocity* in the axon appears independent of nerve conduction, it is less clear whether impulse activity can alter the *amount* of material in the cell body that becomes available for fast transport. Such regulation could occur via activity-induced up- or down-regulation of protein synthesis, thereby altering the pool size of potentially transportable protein, or trains of nerve impulses could affect the transfer of newly synthesized proteins from the endoplasmic redculum through the Golgi cisternae to the kinesin-driven fast transport system (Hammerschlag and Brady, 1989). For example, antidromic stimulation of spinal motor neurons, following microinjection of [^{3}H]glycine into their cell bodies, increased the amount of fast-transported radiolabeled material appearing in the axon (Lux et al., 1970), but autoradiography also revealed an increased grain density in the cell bodies, suggesting that the altered level of transport was at least in part a secondary effect of an activity-induced effect on protein synthesis. Increases in amino acid incorporation have also been detected in isolated CNS tissue following electrical stimulation (Jones and McIlwain, 1971) and in brain in vivo following physiological stimuli, including light (Jones-Lecointe et al., 1976) and sound (Rojik and Fehér, 1980).

The difficulty of establishing whether activity-related increases in amount of fast transport are secondary to alterations in synthesis also arises when examining endogenous markers of fast transport (e.g., Reis et al., 1975). An additional, potentially confounding variable is an activity-related change in precursor uptake. This possibility has been discussed in relation to findings of increased transport of radiolabeled adenosine (Grafstein et al., 1982), glucosamine (Edwards and Grafstein, 1984), and fucose (Riccio and Matthews, 1985).

As an extension of our laboratory's interest in defining the "initiation phase" of fast axonal transport—the somal steps that comprise the interface between protein synthesis and axonal transport (Hammerschlag and Stone, 1982, 1986)—we have reexamined the possibility that nerve-impulse activity modulates the amount of material reaching the anterograde transport system. For these studies, a protocol was chosen that precluded effects of impulse activity on either uptake or incorporation of radiolabeled precursor. Preparations of bullfrog DRG and peripheral nerves were utilized in vitro to facilitate addition of the protein synthesis inhibitor, cycloheximide, *following* radiolabeling of the DRG with [^{3}H]leucine, but *prior* to electrical stimulation of the sciatic nerve. The present article will describe the halting, yet tantalizing progress of these studies, much of which has appeared in abstract form (Hammerschlag et al., 1988; Hammerschlag and Bobinski, 1991).

Methods

Tissue Preparation and Fast Axonal Transport

Lumbar DRG 8 and 9, in continuity with their dorsal roots, spinal nerves, and common sciatic nerve, were dissected from adult bullfrogs (*Rana catesbeiana*), placed in Lucite incubation chambers, and immersed in frog Ringer solution. Details of DRG pulse labeling with [^{3}H]leucine, ligation of nerves, incubation procedure, and detection of fast-transported [^{3}H]protein were as previously described (Stone and Hammerschlag, 1981).

Electrophysiology

Suction electrodes fashioned from polyethylene tubing with silver wire inside were employed for simultaneous stimulation of (sciatic nerve or DRG) and recording from (dorsal root) in vitro preparations immersed in frog Ringer solution. Silver wire coiled around the outside of the suction electrode served as a reference electrode. Stimulation consisted of constant voltage, monopolar pulses, 0.5 ms in duration at a magnitude 50% greater than necessary for single maximal responses. The frequency and duration of the pulse trains were experimental variables. Compound action potentials generated by stimulation of the nerve or DRG were monitored throughout the experiments to detect any artifacts or decline in tissue viability.

Following radiolabeling of DRG, preparations were transferred to chase medium containing the protein synthesis inhibitor, cycloheximide, and stimulated electrically via sciatic nerves or DRG. The sciatic nerve was cut and stimulated approx 10 mm distal to the junction of the eighth and ninth spinal nerves. In studies where the sciatic nerve was stimulated more distally, the magnitude of the effect on transport was reduced. This was, presumably, because the number of fibers activated by sciatic nerve stimulation decreases with distance from the spinal nerve junction owing to the presence of side branches along the nerve. Modulation of axonal transport by nerve impulse activity was assessed by comparing the amount of [^{3}H]protein in the 3-mm nerve segment proximal to the ligature on stimulated vs contralateral control nerves or in sequential 3-mm nerve segments of stimulated vs control nonligated preparations.

Electron Microscopy

Stimulated and nonstimulated DRG were fixed in 4% paraformaldehyde/2% glutaraldehyde for 24 h, postfixed in 1% OsO_4 for 1 h, dehy-

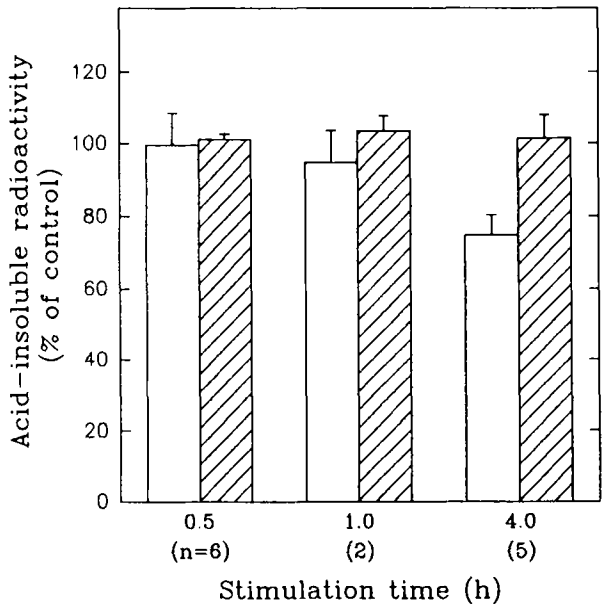

Fig. 1. Changes in amounts of fast-transported [^{3}H]protein and total [^{3}H]protein following electrical stimulation of sciatic nerve. DRG were radiolabeled in [^{3}H]leucine for 1 h, transferred to chase medium containing 100 µg/mL cycloheximide, and nerves were immediately stimulated at 10 Hz for periods of 0.5–4 h. Transported material was assayed 16 h postradiolabeling as acid-insoluble radioactivity in the 3-mm nerve segment proximal to a ligature 20 mm from the DRG. Radioactivity in the segment from the stimulated nerve was corrected for any difference in total radioactivity in stimulated vs contralateral control preparations and compared to radioactivity in the corresponding segment from the control nerve. □ Ligature; ▨ total.

drated through graded alcohols, and embedded in Epon/Araldite. Sections (70 nm) were stained with uranyl acetate and lead citrate, and viewed with a Philips CM-10 microscope.

Results and Discussion

Fast Axonal Transport Following Sciatic Nerve Stimulation

In an initial series of studies, bullfrog sciatic nerves were stimulated electrically at 10 Hz for varying periods (Fig. 1). Stimulation for 30 to 60 min produced little or no effect on fast-transported radiolabeled protein accumulating at a nerve ligature, whereas lengthening the stimulation period to 4 h decreased transport by approx 25% relative to that in nonstimulated contralateral nerves. Stimulation at 10 Hz for 4 h seemed to be at the upper limit of the range of physiologically relevant conditions, and since the effects produced did not appear great enough to warrant stimulation attempts at lower frequencies, we reconsidered whether continuous stimulation of the sciatic nerve was an effective means of altering fast transport events in the cell bodies of DRG neurons. The axons of these neurons bifurcate within a short distance from their cell bodies, providing a physical path for afferent action potentials to travel from the peripheral branch to the central branch without invading the soma. In particular, the efficacy of action potential invasion of the soma is apt to decline when the peripheral nerve is firing repetitively (Ito and Saiga, 1959). For this reason, we designed a set of studies to examine the effect on fast axonal transport of neuronal stimulation via suction electrodes placed directly on the DRG.

Fast Axonal Transport Following Stimulation of Dorsal Root Ganglion

Electrical stimulation of DRG at 10 Hz for 4 h resulted in a 58% reduction in the amount of fast-transported protein accumulating at a nerve ligature (Table 1), more than double the effect produced by stimulating the sciatic nerve under these conditions. Further, ganglionic stimulation had no significant effect on the total amount of acid-insoluble ^{3}H-material in DRG plus nerve trunk. The addition of cycloheximide ruled out the possibility that stimulation-related effects on axonal transport could be secondary to alterations in amino acid uptake or incorporation. It also appears that the stimulation-produced decrease in transported protein was not a secondary effect of a generalized enhancement of protein catabolism. However, since the fraction of total labeled protein destined for fast axonal transport is small

Table 1
Effects of Electrical Stimulation of Dorsal Root Ganglion on Fast Axonal Transport of [^{3}H]Protein

Stimulus parameters			[^{3}H]Protein proximal to nerve ligature,[a]	Total[^{3}H]protein,[b]
Frequency, Hz	Time, h	*n*	% of control	% of control
10	4	(6)	42 ± 9[c]	93 ± 10[c]
10	1	(9)	38 ± 3	89 ± 6
1	1	(6)	62 ± 4	90 ± 9
10 +TTX (10 μg/mL)	1	(6)	119 ± 13	94 ± 10

[a]Acid-insoluble radioactivity in 3-mm nerve segment proximal to ligature 20 mm from DRG.
[b]Acid-insoluble radioactivity in DRG plus nerve trunk.
[c]Values are mean ± SEM.

(<1% in frog motor neurons during the first 4 h of protein labeling: McIlwain D. L., personal communication), a selective degradative effect of nerve stimulation on fast-transported (membrane-associated) proteins could not be detectable in the present study and must remain a possibility.

Reducing the stimulation period from 4 to 1 h produced an effect of equal magnitude (Table 1). However, reducing the stimulation frequency from 10 to 1 Hz (for 1 h) led to a 38% decrease in fast-transported protein, providing an indication that the effect was frequency-dependent (Table 1). Addition of tetrodotoxin (10 μg/mL) to the incubation medium prevented the stimulation-induced decrease in fast axonal transport (Table 1), suggesting that the observed effects were mediated by action potentials and not field stimulation. In response to stimulation, [^{3}H]protein accumulating proximal to a dorsal root ligature was reduced to the same extent as that at a peripheral nerve ligature, indicating a decline in the amount of fast-transported material entering both branches of primary afferent neurons (data not shown).

Although the cumulative results from ligated nerve preparations are indicative of a stimulation-induced reduction of fast axonal transport, they do not permit an inference as to whether the effect reflects a decrease in the amount or the velocity of transport. To distinguish between these possibilities, axonal transport was examined in unligated nerves of stimulated and nonstimulated preparations (Fig. 2A). At 11 h following DRG stimulation (1 Hz/1 h), the transport profiles revealed a consistent decrease in amplitude (amount of transport) as well as a decrease in wave front distance (rate of transport). Thus, a simple distinction between amount and rate was not apparent, and an independent approach was sought to understand how nerve impulse activity was affecting fast transport. A delay was imposed between radiolabeling and stimulating the DRG (Fig. 3). The magnitude of the effect varied linearly from ≈60% decrease in transported protein (no delay) to no decrease (8-h delay). This suggests that by 8 h, the major pulse of radiolabeled protein has passed the site at which nerve impulse activity can act to regulate it. Such a result is consistent with the proposal that the site(s) at which electrical stimulation affects axonal transport is relatively early in the transport process, possibly within the neuronal cell body or initial segment of the axon.

As a test of general metabolic effects of electrical activity, ATP levels were assayed in DRG and in nerve trunks directly following DRG stimulation (10 Hz/1 h). Surprisingly, ATP levels were decreased by approx 40% in DRG, although they were unaffected in nerve trunks. In its best light, this finding suggested that nerve impulse activity regulates an ATP-dependent step in the transfer of newly-synthesized proteins to the transport system. In a dimmer light, however, stimula-

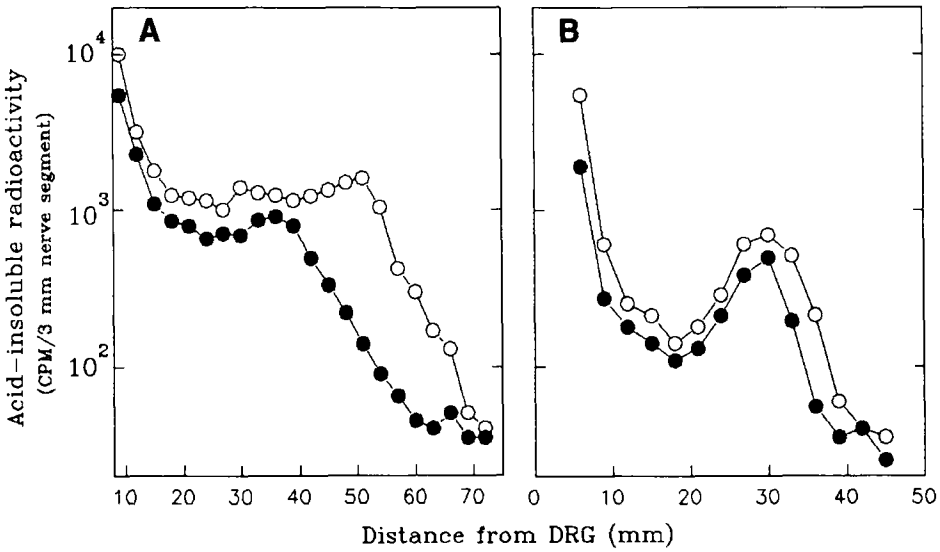

Fig. 2. Profiles of fast-transported [^{3}H]protein in nonligated spinal and sciatic nerves following electrical stimulation of DRG (A) or sciatic nerve (B). DRG were radiolabeled in [^{3}H]leucine for 1 h and transferred to chase medium containing 100 µg/mL cycloheximide. Stimulation was at 10 Hz for 60 min (DRG) or at 10 Hz, 30 min, 1 s on/1 s rest (sciatic nerve). Incubation was for 11 h following DRG stimulation and for 7 h following sciatic nerve stimulation; the shorter time in the latter experiment was necessary to allow detection of the wave front within the spinal nerve proximal to the site of stimulation (*see* Methods). Following incubation, acid-insoluble radioactivity was assessed in successive 3-mm nerve segments of control (O-O) and stimulated (●-●) preparations as previously described (Stone and Hammerschlag, 1981). Each figure is representative of three experiments.

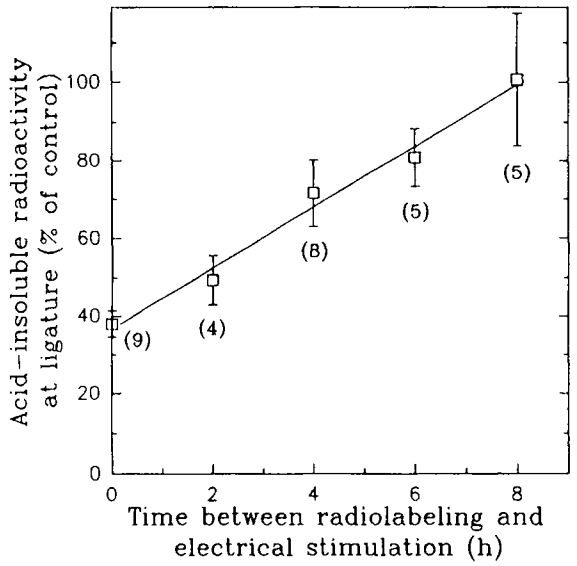

Fig. 3. Change in amount of fast-transported [^{3}H]protein produced by electrically stimulating DRG at varying times after radiolabeling of DRG. Fast axonal transport was assayed as described in legend to Fig. 1. Stimulation was 10 Hz/60 min.

tion of the DRG might cause a nonspecific metabolic insult with transport being a casualty. The answer was provided from electron micrographs of neuronal cell bodies within the stimulated DRG (Fig. 4A). Disrupted Golgi cisternae, distorted mitochondria, and vacuoles were apparent in these cells, but not in cells from nonstimulated DRG. At this stage in our studies, the physiological significance of all, or at least a portion, of the apparent coupling between stimulation and transport was called into doubt.

Fast Axonal Transport Following Patterned Stimulation of Nerve

Our interest in the relation between nerve impulse activity and axonal transport revived when we became aware of a study in which the diurnal activity of the pineal enzyme, serotonin *N*-acetyltransferase, was found to be regulated

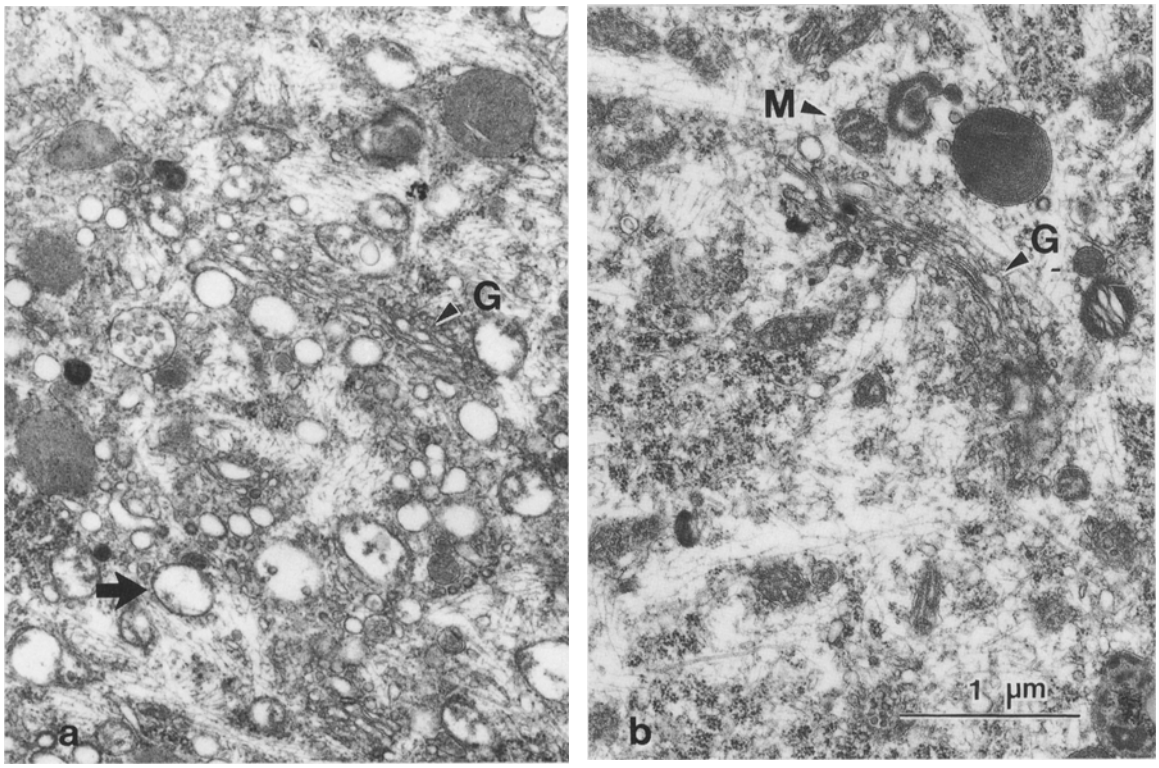

Fig. 4. Cytoplasm of neuronal cell bodies in the dorsal root ganglion after electrical stimulation of the ganglion (a) or the sciatic nerve (b). Note that ganglionic stimulation (10 Hz/30 min, continuous trains) resulted in disrupted Golgi apparatus (G), and increased numbers of vacuoles, many of which appear as presumptive abnormal mitochondria (arrow). These effects were not detected following sciatic nerve stimulation (10 Hz/30 min, 1 s on/1 s rest) (*see* Golgi apparatus [G] and mitochondria [M]), or in nonstimulated preparations (not shown). Bar = 1 μm in both micrographs.

by sympathetic nerve stimulation (Bowers and Zigmond, 1982). An intriguing finding was that changing the *pattern* of nerve impulses, without altering the total number of stimuli, could markedly affect enzyme activity. For example, over the same time period, stimulation at 10 Hz for 2 out of every 20 s was almost three times as effective in increasing enzyme activity as was constant stimulation at 1 Hz, although the same number of stimuli were delivered in both conditions. When applied to our bullfrog nerve preparation, patterned stimulation of the *sciatic nerve trunk* also proved more effective than continuous stimulation, resulting in a frequency- and time-dependent inhibition of axonal transport (Table 2). Whereas continuous stimulation of the nerve at 10 Hz for 30 min had produced little or no change in transport, the same number of stimuli applied discontinuously, alternating 1 s on with 1 s of rest, markedly depressed accumulation of [^{3}H]protein at the ligature. In response to this patterned stimulation at either 10 Hz for 60 min or 20 Hz for 30 min, transport was reduced by approx 60% (Table 2). With this stimulation pattern applied for 30 min, the inhibition of transport was linear over the range of 5–20 Hz (Table 2). The effect of patterned stimulation, moreover, was reflected solely by a decreased amount of transport; the amplitude of the transport profile was decreased with no accompanying change in the wave front distance (Fig. 2B). This finding suggests that impulse activity is without effect on either the time of release of material from the cell body or on the velocity of fast transport

Table 2
Effects of Sciatic Nerve Stimulation on Fast Axonal Transport of [^{3}H]Protein

Stimulus parameters				[^{3}H]protein proximal to nerve ligature,[a]	Total [^{3}H]protein,[b]
Frequency, Hz	Time, h	Stimulation pattern	n	% of control	% of control
10	0.5	Continuous	(7)	99.5 ± 9.0[c]	101.0 ± 1.5[c]
10	1.0	1/2[d]	(6)	33.2 ± 3.5	103.5 ± 8.5
20	0.5	1/2	(4)	39.3 ± 3.2	93.8 ± 4.6
10	0.5	1/2	(7)	58.6 ± 4.5	94.3 ± 16.2
5	0.5	1/2	(4)	78.4 ± 4.4	104.1 ± 4.9

[a]Acid-insoluble radioactivity in 3-mm nerve segment proximal to ligature 20 mm from DRG.
[b]Acid-insoluble radioactivity in DRG plus nerve trunk.
[c]Values are mean ± SEM.
[d]Stimulation was for 1 out of every 2 s.

within the axon. Of additional interest, patterned stimulation of the nerve trunk produced no apparent ultrastructural alterations within neuronal cell bodies of the DRG (Fig. 4B).

Working Hypothesis

Rather than interpreting our results to suggest that nerve impulse activity decreases the amount of fast axonal transport, it seems plausible to adopt the view that, in vivo, fast transport increases during periods of relative electrical quiescence. The physiological significance, according to this hypothesis, would be that activity-depleted macromolecules of the axolemma and nerve terminals are replenished during periods when the neuron is firing less frequently. A corollary of the hypothesis is that the supply of metabolic energy in the cell body is utilized more efficiently when peak demands for initiating action potentials are temporally separated from the demands of initiating fast axonal transport, e.g., of translocating organelles to the transport system. The present findings also suggest that the quantity of fast-transported protein reaching a given point in the axon is regulated not by varying transport velocity, which would have to be controlled at numerous points along the axon, but by an adjustment in the cell body of the amount of material delivered to the fast transport system.

Loss of Response to Patterned Stimulation of Sciatic Nerve

The last group of studies, characterizing the effects of patterned electrical stimulation of sciatic nerve on fast axonal transport, were carried out over a period of 6 mo, from April through October, 1990. At this point, for reasons that we have yet to discover, the effect could no longer be demonstrated. In stimulation experiments attempted every 2 wk over the course of the following 12 mo, the response varied from total absence to approximately half of that previously observed. Moreover, there was no time of year during which the response was consistently stronger, indicating that seasonal variation—a phenomenon often encountered in biological studies of amphibia (Mizell, 1965; Csáky and Gallucci, 1977)—could not be invoked as a causal variable. Despite the loss of response, there was no significant change either in the magnitude of DRG's ability to incorporate radiolabeled amino acid into TCA-insoluble material or in the amount of fast-transported radiolabeled material accumulating at a nerve ligature. In addition, the compound action potentials recorded from the dorsal root during sciatic nerve stimulation did not appear to be compromised.

A possible explanation for the loss of coupling between nerve conduction and axonal transport was that the efficiency of action potential invasion of the cell body had greatly diminished. As a test of this "failure to invade" idea, nerves were stimulated following bath application to the DRG of the potassium channel blockers, tetraethylammonium ion (TEA: Hille, 1967; Hermann and Gorman, 1981) and 4-aminopyridine (4AP: Le Meignan et al., 1969; Thompson, 1977). These agents promote somal invasion by increasing action potential duration. In the presence of 2 m*M* TEA and 0.1 m*M* 4AP, fast transport was depressed approx 20% in both stimulated and nonstimulated preparations. The possibility remained that efficiency of action potential invasion was unaltered, but that the coupling mechanism(s) had become dysfunctional. Unfortunately, the loss of response had occurred prior to the onset of studies designed to identify the putative site of coupling. Finally, the response was not recoverable using bullfrogs obtained from a supplier in New Jersey instead of Northern California, nor was it detectable in nerve preparations dissected from *Xenopus laevis*.

In seeking to place our positive results in the context of previous findings on neuronal activity-induced changes in axonal transport, we make the assumption that our results, although unreproducible at present, are valid. We trust that our continuing efforts to establish the methodological or biological cause of the loss of response will ultimately be successful, enabling a resumption of our studies of the basic phenomenon.

Relevance to Previous Studies of Coupling Between Nerve Conduction and Axonal Transport

Several paradigms have been employed for in vivo comparisons of fast axonal transport in active vs quiescent nerve trunks. Monocular injection of tetrodotoxin (TTX), to block optic nerve activity, reduced the amount of fast-transported material labeled with [^{3}H]adenosine (Grafstein et al, 1982), [^{3}H]glucosamine (Edwards and Grafstein, 1984), or [^{3}H]fucose (Riccio and Matthews, 1985) relative to transport in the nerve of the vehicle-injected eye. Thus, nerves with normal as opposed to suppressed levels of impulse conduction appear to transport greater amounts of material. Since such findings would contradict the present results with fast-transported [^{3}H]protein in bullfrog nerve, it is of interest that in each of the optic nerve studies, TTX had *no* effect on the amount of fast-transported protein when radiolabeled amino acid was injected as a precursor. This suggests that administration of TTX may lead to an inhibition in uptake of adenosine as well as an inhibition in uptake and/or incorporation of glucosamine and fucose, but not amino acids. An apparent decrease in the level of fast-transported adenosine or carbohydrate-labeled glycoprotein could then be explainable by a reduction in either specific activity or posttranslational modification of transported material. A decreased incorporation of carbohydrate residues might well be occurring, at least in retinal ganglion cells of long-term, TTX-treated eyes, since the Golgi cisternae in such cells show ultrastructural abnormalities (Riccio and Matthews, 1985).

The above explanations do not resolve why the amount of fast-transported protein is *unaffected* in TTX-"silenced" optic nerves (relative to their physiologically active counterparts), but is markedly *increased* in quiescent, in vitro sciatic nerves (relative to their electrically stimulated mates). At present, it is unclear whether the two types of imposed electrical silence (treating with TTX in vivo and silencing a normally active nerve by placing it in vitro) have equivalent effects on initiation of fast axonal transport. It is also uncertain whether the two modes and differential patterns of neuronal firing (physiologically driven activity in vivo vs electrical stimulation in vitro) exert comparable effects.

To confound matters further, a third type of effect of neuronal activity on fast-transported radiolabeled protein has been reported (Chan et al., 1989). Chronic in vivo stimulation of the trigeminal ganglion (biweekly, 4 × 15 s trains at 10 Hz for 4–5 wk), produced no effect on incor-

poration of [^{3}H]proline or on the rate of [^{3}H]protein transport, but the amount of transported radiolabeled protein was as much as five times that in nerves from sham-stimulated animals. Long-term effects on fast transport have also been examined following axotomy-suppressed activity (*see* Grafstein and McQuarrie, 1978; Benowitz, 1987). Such axotomy-induced changes in amount of transported materials, however, are not directly relevant to the present discussion, since they may again be secondary to alterations in protein synthesis; they may also be responses to specific signals that program the neuron for regeneration.

In summary, as a result of using three distinct experimental designs—acute in vitro stimulation, TTX blockade in vivo, and chronic in vivo stimulation—the question of whether the amount of material destined for fast axonal transport can be modulated by nerve impulse activity has received three different answers: It can be decreased, unaffected, or increased, respectively. Thus, it seems that the question is not so much one of "whether," but of "which"; namely, which set(s) of conditions that alters the amount of transport is reflecting the physiological state. As a start, protocols should be redesigned so that each of the means of comparing active and quiescent neurons can be examined using a single preparation. Second, it would be of value to record the normal in vivo firing patterns of the particular neurons under study. This would allow the patterns to be reproduced under in vitro conditions where such variables as precursor uptake and protein synthesis can be readily controlled for, and where the mechanisms underlying the putative coupling of neuronal activity and onset of fast transport can more easily be probed.

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