Organelle motility and metabolism in axons vs dendrites of cultured hippocampal neurons

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SUMMARY

Regional regulation of organelle transport seems likely to play an important role in establishing and maintaining distinct axonal and dendritic domains in neurons, and in managing differences in local metabolic demands. In addition, known differences in microtubule polarity and organization between axons and dendrites along with the directional selectivity of microtubule-based motor proteins suggest that patterns of organelle transport may differ in these two process types. To test this hypothesis, we compared the patterns of movement of different organelle classes in axons and dendrites along with the directional selectivity of microtubule-based motor proteins suggest that patterns of organelle transport may differ in these two process types. First, in addition to the difference in net transport direction, the large phase-dense organelles exhibited more persistent changes in direction in proximal dendrites where microtubule polarity is mixed than in axons where microtubule polarity is uniform. Second, while the net direction of mitochondrial transport was similar in both processes, twice as many mitochondria were motile in axons than in dendrites. Third, the mean excursion length of moving mitochondria was significantly longer in axons than in dendrites. To determine whether there were regional differences in metabolic activity that might account for these motility differences, we labeled mitochondria with the vital dye, JC-1, which reveals differences in mitochondrial transmembrane potential. Staining of neurons with this dye revealed a greater proportion of highly charged, more metabolically active, mitochondria in dendrites than in axons. Together, our data reveal differences in organelle motility and metabolic properties in axons and dendrites of cultured hippocampal neurons.

Key words: Organelle motility, Microtubule polarity, Axon, Dendrite, Mitochondrion

INTRODUCTION

Neurons are highly polarized cells with distinct axonal and dendritic domains whose integrity is critical to their proper functioning. A fundamental question in cellular neurobiology is how neurons establish and maintain these different domains, specifically how they achieve the necessary cytoplasmic compartmentation and how they meet regional metabolic demands. Selective and spatially regulated organelle transport seems likely to play an important role in the establishment and maintenance of the known structural, functional and biochemical differences between axons and dendrites. However, virtually nothing is known about regional differences in organelle traffic. Our knowledge of organelle transport in neurons stems largely from a rapidly growing body of biochemical and biophysical data, in vitro motility analyses, and from studies of axons. Only a few studies have addressed dendritic transport types. First, in addition to the difference in net transport direction, the large phase-dense organelles exhibited more persistent changes in direction in proximal dendrites where microtubule polarity is mixed than in axons where microtubule polarity is uniform. Second, while the net direction of mitochondrial transport was similar in both processes, twice as many mitochondria were motile in axons than in dendrites. Third, the mean excursion length of moving mitochondria was significantly longer in axons than in dendrites. To determine whether there were regional differences in metabolic activity that might account for these motility differences, we labeled mitochondria with the vital dye, JC-1, which reveals differences in mitochondrial transmembrane potential. Staining of neurons with this dye revealed a greater proportion of highly charged, more metabolically active, mitochondria in dendrites than in axons. Together, our data reveal differences in organelle motility and metabolic properties in axons and dendrites of cultured hippocampal neurons.

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and Kirkpatrick, 1972; Bray and Bunge, 1981; Abelson, 1984; Burton, 1987; Chen et al., 1992; Yu and Baas, 1994). In addition, the polarity orientation of microtubules differs in different process regions. In axons, these polar protein polymers are uniformly oriented with their plus-ends directed distally (Burton and Paige, 1981; Heidemann et al., 1981; Baas et al., 1988). In dendrites, including those of the frog mitral cells (Burton, 1988), cultured sympathetic neurons (Baas et al., 1991), and cultured hippocampal neurons (Baas et al., 1988, 1989), microtubules are of mixed orientation and exhibit changes in this mixture along their length (Baas et al., 1988). Within the proximal half of the dendrite, microtubule orientation is mixed: half of the microtubules have their plus ends directed distally, the other half their minus ends. With increasing distance from the cell body, the relative abundance of microtubules with their minus ends directed distally gradually decreases, with the most distal 10% of dendritic length containing only plus-end distal microtubules, like the axon. These known differences in microtubule organization and the unidirectional character of known microtubule-based motors (Brady, 1991) have prompted speculation about possible differences in axonal and dendritic organelle transport (Black and Baas, 1989; Peters et al., 1991).

Differences in organelle transport in axons and dendrites are also likely to be influenced by differences in local metabolic demands within neuronal cells. It is known that regional metabolic needs within cells influence organelle traffic, and that changes in such local needs result in new patterns of organelle transport and distribution (e.g. Fawcett, 1981; Morris and Hollenbeck, 1993). But how the transport and localization of a class of organelles is related to subcellular metabolic needs remains poorly understood. In two studies organelle transport has been shown to correspond to presumed subcellular metabolic needs in neurons. Mitochondria are known to be driven toward and accumulate in active axonal growth cones, which are probable regions of high ATP consumption, and to evacuate inactive growth cones (Morris and Hollenbeck, 1993). In addition, autophagic vacuoles exhibit an increased rate of formation and upregulation of net retrograde axonal transport when axon outgrowth is blocked (Hollenbeck and Bray, 1987; Hollenbeck, 1993; Hollenbeck and Weld, 1994). Before additional similar relationships can be identified, local metabolism throughout neuronal cells must be further characterized. Although the structural, biochemical, and functional differences between axons and dendrites strongly suggest that there must be metabolic differences between these two subcellular domains, such differences have not been demonstrated.

This report represents the first extensive comparative study of organelle transport and metabolism in axons and dendrites of neuronal cells. Our data clearly demonstrate differences in both general organelle traffic and individual organelle motility behaviors specific to process region as well as organelle type. Large phase-dense organelles exhibit significant net retrograde transport in axons and distal dendrites but not in proximal dendrites. Also, these organelles exhibit more persistent changes in direction in the proximal dendrites where microtubule polarity is mixed than in axons where microtubules are uniformly aligned. Although quantitative analysis of mitochondrial traffic did not reveal regional differences in their net transport direction, our data do demonstrate regional variation in individual mitochondrial motility patterns; axonal mitochondria move more frequently and move farther than dendritic mitochondria. Furthermore, our data definitively show that there are differences between axonal and dendritic metabolism; dendritic mitochondria are more metabolically active than those in axons.

**MATERIALS AND METHODS**

**Materials**

Minimum essential medium (MEM), horse serum, Hanks’ buffered saline solution (HBSS) and trypsin were purchased from Gibco (Grand Island, NY). All other cell culture reagents were purchased from Sigma (St Louis, MO). Glass coverslips were purchased from Carolina Biological Supply Co. (Burlington, NC). Rhodamine 123 was obtained from Molecular Probes, Inc. (Eugene, OR). JC-1 was a gift from Dr Lan Bo Chen.

**Cell culture**

Embryonic rat hippocampal neurons were cultured on glass coverslips using the method of Goslin and Banker (1991). Neurons were maintained in glial conditioned neuronal maintenance medium supplemented with 10 mM Hepes, pH 7.3, for all fluorescent labeling and microscopy. Axons and dendrites were distinguished based on well-established morphologic characteristics and only spatially isolated regions and unambiguous processes were selected for observation. Observations were made at previously characterized developmental stages (Dotti et al., 1988). Axons were observed in stage 4 cells (4-6 days in vitro). Dendrites were observed in stage 5 cells (8-10 days in vitro), with some observations of mitochondria made in late stage 4 cells (6 days in vitro). These developmental stages were chosen for 2 reasons: (i) At these stages, the two process types are as mature as possible without compromising spatial resolution of individual processes; and (ii) the microtubule polarity orientations are in their mature states (Baas et al., 1989). Data from different developmental stages were pooled only when there was no significant difference in results from the different stages.

**Video microscopy**

For all experiments, live neurons were observed using a Zeiss IM 35 inverted microscope equipped with a ×63 planapochromatic objective and maintained at 37°C using an air curtain stage heater. Cultured neurons were maintained in a sandwich of 2 coverslips separated by coverslip fragments, filled with culture medium and sealed with a mixture of Vaseline, lanolin and paraffin (1:1:1). Video-enhanced phase-contrast images were obtained using a Hamamatsu Newvicon camera mounted on a video port using relay lenses to obtain a field width of 47 μm. Rhodamine 123 labeled mitochondria were imaged using a Hamamatsu intensified CCD video camera mounted in the ocular position on the microscope, giving a field width of 102 μm. Images of JC-1 labeled mitochondria were collected with the same camera mounted on the video port to obtain a field width of 47 μm. All video sequences were recorded using a Panasonic AG-7300 Super-VHS video cassette recorder.

**Motility analysis**

Organelles were categorized based on morphological characteristics seen by video-enhanced phase-contrast microscopy. An organelle was identified as a mitochondrion if it was phase-dense and had a length at least twice that of its width, a large phase-dense organelle if it was phase dark with an apparent diameter of at least 0.4 μm, or a macropinosome if it were phase bright and at least 0.4 μm in diameter (Dailey and Bridgman, 1993). Organelles which did not meet any of these criteria were categorized as ‘other organelles’. Organelle movement was observed in three neuronal domains: axon shafts greater than 100 μm from cell bodies or growth cones, the proximal...
half of dendritic length, and the most distal 10% of dendritic length. Quantitative analysis was done by drawing a line across the video screen perpendicular to and bisecting the process and counting the number of organelles that crossed the line during a 4× time lapse playback of the video sequence. Anterogradely and retrogradely transported organelles were counted from separate video playbacks, as were organelles of different classes. Counts were often repeated 2 to 5 times then averaged to ensure accuracy.

Individual motility behaviors of the large phase-dark organelles were analyzed to characterize changes in direction in axons and proximal dendrites. To eliminate bias, all large phase-dark organelles visible in each video sequence were followed for the duration of the sequence, until they moved out of the plane of focus, or until they moved out of the video field. An organelle was considered stopped if it remained stationary for at least 1 second, and a movement was counted only if the displacement was at least 0.5 μm. All observed stops were scored, and the direction of resumed movement was recorded as either the same or changed. A change in direction was considered persistent if the organelle moved continuously in the new direction for at least 3 μm or if it continued movement in this new direction even after one or more subsequent stops. The data from all observed organelles was pooled to get a total number of stops observed, and numbers of stops followed: (i) by resumed movement in the original direction; (ii) by any change in direction; and (iii) by a persistent change in direction.

Observation of rhodamine 123 labeled mitochondria
To more clearly visualize mitochondria for analysis of individual organelle motility, neurons were labeled with the mitochondrial vital dye rhodamine 123 (0.2 μg/ml) for 30 minutes at 37°C, washed once with HBSS, then returned to culture medium for immediate observation. For fluorescent images, a 50 W mercury lamp and a 1.0 neutral density filter were used. For phase images, a tungsten lamp and 1.5 neutral density filter was used. Live sequences were recorded as running averages of 8 video frames using Image 1 image processing software (Universal Imaging Corporation). A process was recorded for as long as its fluorescent image remained in focus. Brief phase images were also recorded so that the type of process and the direction to the cell body could be determined.

To quantify individual mitochondrial motility behaviors, one minute video sequences of axons and proximal dendrites were analyzed. To eliminate bias, the first minute of each video sequence throughout which all mitochondria remained in focus was used for analysis, and every mitochondrion in the process was individually tracked for the full minute. A fluorescent organelle was counted only if more than one end was visible. For example, a cluster of mitochondria with three visible ends was counted as only one mitochondrion, and one with four ends as two mitochondria. For quantitative analysis, three motility characteristics were recorded for each mitochondrion observed: (i) whether or not it moved; (ii) its net direction of movement (anterograde or retrograde); and (iii) its excursion length (net displacement). Net displacement was measured in μm using a calibrated ruler taped to the video monitor.

JC-1 labeling and analysis
To label mitochondria for metabolic analysis, neurons were incubated in medium containing freshly prepared JC-1 (2 μg/ml) for 3 minutes at 37°C, washed 2 times in HBSS for 1 minute each, then returned to normal medium. Cells were observed immediately following labeling and for no more than 45 minutes. Fluorescent mitochondria were visualized using shuttered tungsten illumination to minimize bleaching. The fluorescent green and red JC-1-loaded mitochondria were viewed separately using standard fluorescein and Texas Red filter sets, respectively. MetaMorph imaging software (Universal Imaging Corporation) was used for all digital image collection and image processing. Separate images of fluorescent green and red mitochondria were acquired as digital grey scale averages of 16 video frames. For each field, the red image was obtained immediately after the green image to minimize mitochondrial movements between channels.

For analysis, green/red image pairs were colorized and overlaid, yielding single images containing green and red mitochondria. Each labeled mitochondrion was categorized as either green, red or both green and red. If greater than 90% of a mitochondrion’s area was green or red, it was counted as green or red, respectively. All other mitochondria were categorized as both green and red.

Statistical analysis
Organelles observed by phase-contrast microscopy were evaluated for net transport in one direction using a chi-square test. Data was compared to an expected distribution of 50:50 for anterograde:retrograde movement, and statistically significant differences reflected net transport in one direction. Comparisons of mean excursion lengths and of mitochondrial metabolic categories were done using an unpaired two-tailed t-test. For all other data, the proportions from different data sets were compared assuming normal distributions and a null hypothesis of equality. In all cases, differences were considered statistically significant where P<0.05.

RESULTS
There are differences in overall organelle traffic in axons vs dendrites
To begin to characterize organelle transport in different process regions of neurons, we examined general organelle traffic using quantitative video-enhanced phase contrast light microscopy. Using this technique, we were able to distinguish three distinct classes of organelles based on morphologic criteria (Fig. 1). First, elongated phase dense organelles were identified as mitochondria. Second, large phase-dense vesicular organelles were a distinct class and probably represent autophagic vacuoles since their light microscopic characteristics and axonal transport properties are similar to those previously described for neuronal autophagic vacuoles (Hollenbeck and Bray, 1987; Hollenbeck, 1993). Third,
macropinosomes were identified by their relatively large size and their characteristic phase-bright properties. All remaining organelles were too small to resolve clearly and were thus categorized as other organelles.

To identify regional differences in organelle traffic, the relative frequencies of anterogradely and retrogradely moving organelles were determined for each organelle class in different axonal and dendritic domains. To test the hypothesis that the organization of the microtubule tracks might influence organelle transport, this motility was quantified in three process regions with characteristic microtubule arrangements: (i) axon shafts; (ii) the distal 10% of dendrites where microtubules are uniformly aligned with all plus ends directed distally; and (iii) the proximal half of dendrites where microtubule polarity orientation is evenly mixed (Baas et al., 1988). Our data suggest that the traffic patterns of most organelle classes are similar in the three process regions (Fig. 2). There was neither a detectable net anterograde nor net retrograde flow of the total organelle population in any of the observed domains. Observations of individual organelle classes revealed that mitochondria were transported equally in both directions and macropinosomes exhibited retrograde flow in every process region examined.

In contrast, other organelle classes did exhibit regional differences in trafficking patterns. In axons, the large phase-dense vesicular organelles exhibit significant net retrograde transport as shown previously for autophagic vacuoles in axons of sympathetic neurons, with more than twice as many organelles moving retrogradely as moved anterogradely. A similar pattern was observed in distal dendrites, where the microtubule polarity orientation is uniform as it is in axons. In contrast, a different pattern was observed in proximal dendrites, where the microtubule polarity orientation is mixed. In this process region, the large phase-dense vesicular organelles were transported equally in both directions, with no significant difference in the proportions of anterogradely and retrogradely moving organelles. Another regional transport difference was observed for organelles categorized as other. These organelles were transported equally in both directions in both dendritic regions examined, but exhibited significant net anterograde transport in axons. Although the precise identity of these organelles cannot be determined using this technique, it is likely that they represent small carrier vesicles conveying membrane and protein to and from the distal ends of the processes, such as endosomes and synaptic vesicle precursors. In summary, these data demonstrate that there are indeed regional differences in organelle transport that are specific to process region and organelle class.

**Dendritic organelles exhibit more persistent changes in direction than axonal organelles**

To determine whether or not there were any regional differences in individual organelle motility behaviors, we first quantified the patterns of transport of the large phase-dense organelles in axons and proximal dendrites, where we had observed differences in gross traffic patterns for this organelle. Because the mixed polarity orientation of the dendritic microtubules might facilitate directional changes, and because dendritic organelles exhibited no net anterograde or retrograde flow in contrast to the net retrograde transport in axons, we specifically addressed the following question: do organelles change direction more frequently in dendrites than in axons?

To address this question, we tracked every large phase dense organelle in our axonal and dendritic video sequences. Two distinct classes of directional changes were observed (Fig. 3). First, there were temporary changes, in which an organelle traversed less than 3 μm in the new direction after a stop before

![Fig. 2. The net direction of transport of some organelles differs in axons vs dendrites. Organelle transport was visualized using video-enhanced phase-contrast light microscopy. The data indicate the percentage of organelles in each class moving in each direction past a fixed point within the process. Axonal transport data was obtained from central regions of axon shafts (n=7), at least 100 μm from either cell bodies or growth cones. Dendritic transport was characterized in two dendritic regions: within the proximal 50% of dendritic length (n=7), where microtubules are mixed in their polarity orientation, and in the most distal 10% of dendritic length (n=5), where microtubules are aligned uniformly with their plus ends distal. The large phase-dense vesicular organelles exhibit regional differences in traffic patterns: net transport is retrograde in axons (P=0.0001) and distal dendrites (P=0.0390), but not in proximal dendrites (P=0.7180). The other small vesicular organelles exhibit significant net anterograde transport in axons (P=0.0012), but not in proximal (P=0.1156) or distal (P=1.0000) dendrites. Macropinosomes exhibit retrograde flow in all three process regions, though they are observed so rarely that these data yield strong statistical significance only in axons (P=0.0082 in axons, P=0.0588 in proximal dendrites, P=0.1573 in distal dendrites). Neither mitochondria nor the entire population of moving organelles show a net directional transport in any of the three process regions examined. The total number of organelles counted in each category is indicated on the right. Asterisks indicate a sample size too small to have statistical significance.
stopping and changing direction again, thus finally continuing movement in the original direction (Fig. 3A). The other class of directional changes was that of persistent changes, in which the organelle exhibited sustained movement in the new direction (Fig. 3B). These organelles were either carried a long distance, further than 3 \( \mu m \), in the new direction after a stop, or stopped one or more times, each time resuming movement in the same new direction.

For quantitative analysis, each time an organelle stopped, the direction of its resumed movement was recorded as either the same or changed, and persistent changes of direction were noted. When the two classes of directional changes were considered together, there was no significant difference in the frequency of directional changes between the axonal and proximal dendritic domains (Fig. 4A). However, separation of the two classes and comparison of the relative abundance of

![Fig. 3.](image)
each type of directional change in axons and proximal dendrites did reveal a striking difference in motility between these two neuronal domains (Fig. 4B). Nearly 20% of the observed changes in direction in dendrites were persistent, whereas none of those in axons were. In summary, these data demonstrate that while large phase dense vesicular organelles change direction with similar frequency when they stop and resume movement in axons and dendrites, the proportion of these changes that result in sustained or persistent transport in the new direction is significantly greater in dendrites where microtubule polarity orientation is mixed than in axons where it is uniform.

**Axonal mitochondria move more of the time and greater distances than those in dendrites**

Individual motility behaviors of axonal and dendritic mitochondria were next characterized to see whether or not there were any regional differences despite the similarity in general trafficking. To facilitate observation of mitochondrial motility, mitochondria were specifically labeled with the fluorescent lipophilic cationic vital dye, rhodamine 123 (Johnson et al., 1981). Epifluorescence images of mitochondria in axons and proximal dendrites of live neurons were captured on video tape for motility analysis.

To characterize mitochondrial transport in the different neuronal domains, three parameters of motility were quantified: duty cycle, net transport direction, and mean excursion length. For each mitochondrion observed in all video sequences analyzed, we recorded whether or not it moved, its direction of net movement, and its excursion length (net displacement) during the one-minute observation period. In agreement with the video-enhanced phase-contrast analysis (above), the observed mitochondria, although motile, exhibited no significant net anterograde or retrograde transport in axons or dendrites (not shown). Comparison of the proportions of mitochondria that moved in axons and proximal dendrites revealed significant regional differences in the mitochondrial duty cycle; more axonal than dendritic mitochondria moved in a defined period of time (Fig. 5A). In addition, the mean excursion length of mitochondria moving in either direction

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**Fig. 4.** Relative frequency of persistent changes of direction differ in axons vs dendrites. Large phase-dense vesicular organelles were individually tracked throughout video sequences of axons and proximal dendrites, and the total number of stops and the number of stops followed by a change in direction were counted. (A) The proportion of stops that resulted in any change of direction was calculated for axons and for proximal dendrites. There was no significant difference in the frequencies of direction changes in axons vs dendrites ($P=0.2758$). (B) The proportion of reversals of organelle transport direction that resulted in sustained movement in the new direction was calculated for axons and proximal dendrites. Dendritic organelles exhibited a significantly higher frequency of persistent changes of direction than did axonal organelles ($P=0.0005$).

**Fig. 5.** Mitochondrial transport in axons vs dendrites differs in its duty cycle and excursion length. Mitochondrial movements were quantified from one-minute video sequences of rhodamine 123-labeled hippocampal neurons. (A) The population duty cycles were calculated by counting the mitochondria which exhibit movement and the total number of mitochondria observed in axons ($n=197$) and dendrites ($n=96$). The percentage of mitochondria that exhibit movement is significantly greater in axons than in proximal dendrites ($P=0.0014$). (B) The excursion lengths of moving mitochondria in axons ($n=41$) and proximal dendrites ($n=24$) were measured then averaged for anterogradely, retrogradely, and all moving mitochondria. There was no significant difference between anterograde and retrograde movements in axons ($P=0.7759$) or in proximal dendrites ($P=0.2105$). However, axonal mitochondria traverse significantly greater distances than dendritic mitochondria, whether moving anterogradely ($P=0.0262$), retrogradely ($P=0.0186$), or either direction ($P=0.0004$). Error bars = 1 s.e.m.
was significantly longer in axons than in dendrites (Fig. 5B). Furthermore, we observed a nearly four-fold difference in the maximum excursion lengths of 25 µm in axons and 7 µm in dendrites. These data demonstrate that although the net direction of flow is similar in axons and dendrites, mitochondria do exhibit significant regional motility differences. Dendritic mitochondria are more likely to remain stationary, and when they move they are transported shorter distances than axonal mitochondria.

**Dendritic mitochondria are more metabolically active than axonal mitochondria**

To experimentally demonstrate regional metabolic differences and to explore the possibility that they might account for at least some of the observed organelle transport properties in different subcellular domains, mitochondrial activity was examined. Mitochondrial energy states in axons and dendrites were examined and compared by incubating neurons in the fluorescent mitochondrial vital dye, JC-1, an indicator of relative mitochondrial transmembrane potentials and activity (Smiley et al., 1991; Reers et al., 1991). This molecule, like rhodamine 123, is a lipophilic cationic dye that becomes concentrated inside mitochondria in proportion to their transmembrane potentials; more dye accumulates in mitochondria with greater transmembrane potentials, thus in those with greater ATP generative capacities. At lower concentrations, JC-1 emits a green fluorescence (527 nm), whereas at higher concentrations this molecule forms J-aggregates and shifts to a red fluorescence with an emission peak of 590 nm (Smiley et al., 1991). Thus, the fluorescence of JC-1 can be used as an indicator of relative mitochondrial energy state. The least active mitochondria have relatively low transmembrane potentials, thus accumulate less dye and appear green. In contrast, the more active mitochondria have higher transmembrane potentials, leading to higher intramitochondrial dye concentrations and red fluorescence.

Fluorescence images of the labeled cells reveal heterogeneous populations of mitochondria in all neuronal domains, with a greater number of highly charged red mitochondria in dendrites than in axons (Fig. 6). To quantify this observation, the proportions of relatively inactive (green), active (red), and intermediate (red and green) mitochondria in axons and dendrites were determined (Fig. 7). The data show that dendrites contain nearly four times as many highly active mitochondria and approximately half as many relatively inactive mitochondria as axons. These results demonstrate that the dendritic mitochondrial population is more metabolically active than the axonal population.

![Fig. 6. Mitochondria in cultured hippocampal neurons labeled with JC-1. Cultured hippocampal neurons were labeled with the mitochondrial vital dye JC-1, which reveals relative differences in mitochondrial energy state. Low-energy green (A), high-energy red (B) and mixed red and green (arrow in C) mitochondria were observed. Both axons (D) and dendrites (E) exhibited mixed mitochondrial populations, but in different proportions. (D) Axons contained more low-energy green mitochondria, and often contained red mitochondria in the growth cone region (arrow). (E) Dendrites exhibited more high-energy red mitochondria. Bars: 5 µm (A–C); 20 µm (D,E).]

![Fig. 7. Relative frequency of mitochondria in different energy states in axons vs dendrites. Cultured hippocampal neurons were labeled with JC-1 and the numbers of low-energy (green), high energy (red) and mixed (both green and red) mitochondria in axons and dendrites were counted then converted to proportions of the total mitochondrial population. Totals of 263 mitochondria in 55 axons and 482 mitochondria in 43 dendrites were observed. Data from all processes observed on single coverslips were pooled. The relative frequencies calculated for the three mitochondrial populations from 3 axonal and 4 dendritic coverslips were averaged. Dendritic mitochondria were more active than axonal mitochondria. Dendrites contained significantly fewer green ($P=0.0031$), and more red ($P=0.0057$) mitochondria than axons. Although there were consistently more mixed red and green mitochondria in dendrites than in axons, the data were not significantly different ($P=0.1908$). Error bars = 1 s.e.m.]

*Fig. 6.* Mitochondria in cultured hippocampal neurons labeled with JC-1. Cultured hippocampal neurons were labeled with the mitochondrial vital dye JC-1, which reveals relative differences in mitochondrial energy state. Low-energy green (A), high-energy red (B) and mixed red and green (arrow in C) mitochondria were observed. Both axons (D) and dendrites (E) exhibited mixed mitochondrial populations, but in different proportions. (D) Axons contained more low-energy green mitochondria, and often contained red mitochondria in the growth cone region (arrow). (E) Dendrites exhibited more high-energy red mitochondria. Bars: 5 µm (A–C); 20 µm (D,E).
The morphological and cytoplasmic asymmetry of neuronal cells critically underlies their functions and must be achieved at least in part by an elaborate and tightly controlled system for efficiently transporting and positioning organelles within the cell. The different metabolic demands and requirements for macromolecules likely necessitate different organelle trafficking patterns in axons and dendrites. Furthermore, the differences in the organization of the microtubule tracks in these two process types (Baas et al., 1988), in conjunction with the directional selectivity of microtubule based motor proteins (Brady, 1991), have prompted speculation about their role in selective organelle transport in the different neuronal domains (Black and Baas, 1989; Peters et al., 1991). Despite the extensive and rapidly growing literature characterizing axonal transport, dendritic transport has only briefly been examined (Kiss, 1977; Gorenstein et al., 1985; Gorenstein and Ribak, 1985; Parton et al., 1992). The data presented here represent the first direct and quantitative comparative study of organelle traffic and metabolic properties in different process regions of live neurons. In this report, we demonstrate quantitative differences in overall organelle traffic, individual organelle motility characteristics and metabolic state between axons and dendrites of developmental stage 4 and stage 5 (Dotti et al., 1988) cultured embryonic rat hippocampal neurons, respectively.

**Influence of microtubule organization on general organelle trafficking**

The discovery of the regional differences in microtubule polarity orientation in neuronal cells has prompted speculation about a mechanistic role for microtubule polarity organization in selective organelle transport and cytoplasmic compartmentation in neurons (Black and Baas, 1989; Peters et al., 1991). The combination of unidirectional transport along microtubules and microtubule organization is known to play an important role in directed motility and positioning of specific organelles within cells. For example, there is evidence for minus-end specific transport of the Golgi apparatus in various cell types (Wehland et al., 1983; Rogalski and Singer, 1984; Ho et al., 1989; Corhésy-Theulaz et al., 1992), and of ribosomes in insect ovarian nutritive tubes (Stebbings et al., 1991). There is also evidence for plus end directed movement of Golgi elements (Lippincott-Schwartz et al., 1995) and bidirectional movement of endoplasmic reticulum (Terasaki et al., 1984, 1986; Lee et al., 1989), lysosomes (Herman and Albertini, 1984; Matteoni and Kreis, 1987; Hollenbeck and Swanson, 1990; Swanson et al., 1992), pigment granules (Rozdzial and Haimo, 1986a,b; Lynch et al., 1986a,b), mitochondria (Morris and Hollenbeck, 1993), and autophagic vacuoles (Hollenbeck and Bray, 1987; Hollenbeck, 1993). Although the regulation of the motors and their association with organelles is not clearly understood, there is also some evidence for the association of specific motors with specific organelles (e.g. synaptic vesicles: Leopold et al., 1992; Okada et al., 1995); mitochondria (Leopold et al., 1992; Nangaku et al., 1994); lysosomes (Hollenbeck and Swanson, 1990; Swanson et al., 1992); Golgi elements (Schmitz et al., 1994; Marks et al., 1994; Lippincott-Schwartz et al., 1995); pigment granules (Rodionov et al., 1991).

Our quantitative analysis of the net transport direction of four classes of organelles in three neuronal regions, axon shafts, proximal dendrites and distal dendrites, revealed one organelle type, the large phase-dense organelles, which exhibited regional differences in trafficking consistent with regional differences in microtubule organization. The size, morphology, and net retrograde axonal transport properties of these organelles strongly suggest that they are autophagic vacuoles, a predegradative compartment rigorously characterized in cultured sympathetic neurons (Hollenbeck and Bray, 1987; Hollenbeck, 1993). While these organelles exhibit net retrograde transport in both axons and the most distal 10% of dendritic length, where microtubules are uniformly aligned with their plus ends directed distally, they did not exhibit significant net transport in either the anterograde or the retrograde direction in proximal dendrites where the microtubule polarity orientation is mixed. This difference might be driven merely by regional differences in subcellular needs. However, because the similarities and differences correlate with cytoskeletal architecture rather than with process type, it is also likely that these regional variations in organelle traffic are related to the known differences in the organization of the microtubule tracks.

**Individual organelle motility characteristics and microtubule polarity orientation**

In addition to influencing general organelle traffic patterns, the differences in microtubule polarity orientation in axons and proximal dendrites likely affect the motility characteristics of individual organelles. It is well known that many organelles of many types move along microtubules in a saltatory manner and often change their direction of movement, as discussed above. We hypothesized that organelles in proximal dendrites where microtubule polarity is mixed might change direction more frequently than axonal organelles. Because of the mixed microtubule polarity orientation in dendrites, reversal of direction could be achieved by changing motor activation or association, as is most likely in the axon, or could merely require the organelle to switch to another microtubule, a phenomenon that has been observed in vitro (Smith and Forman, 1988; Brady et al., 1982). Our data demonstrate that large phase-dense organelles exhibit significantly more frequent persistent changes in direction in proximal dendrites than in axons. This increased bidirectional transport of individual large phase-dense organelles in proximal dendrites also might account for the observed regional differences in net transport direction and is consistent with the hypothesis that microtubule organization influences both general organelle trafficking patterns and individual organelle motility behaviors. Furthermore, in contrast to suggestions that microtubules in only one orientation can support organelle transport (Sheetz et al., 1989; Lopez and Sheetz, 1993), our data are consistent with dendritic microtubules in both polarity orientations supporting organelle transport.

**Mitochondrial motility and subcellular metabolism**

Although no significant regional variation in net transport direction of mitochondria was detected, they, like the large phase-dense organelles, exhibited significant regional differences at the level of individual organelle motility behaviors. Our data demonstrate that mitochondrial movement is more extensive in axons than in proximal dendrites. At any point in
time, a greater proportion of mitochondria in axons are moving, and when they move, they move significantly farther that they do in dendrites. The observed difference in excursion lengths could reflect either differences in the microtubule arrays or metabolic demands in axons and dendrites. Serial reconstructions of microtubules from electron micrographs of axons (Bray and Bunge, 1981) and dendrites (Fletcher et al., 1994) revealed that axonal microtubules are significantly longer than dendritic microtubules. The average length of axonal microtubules was calculated to be 108 µm whereas the longest observed dendritic microtubule was only 11 µm. Thus, the longer mean excursion lengths in axons could merely be related to the longer microtubules. Alternatively, the shorter dendritic excursion lengths could result from more closely spaced regions requiring high ATP consumption; while energy-dependent processes are concentrated in growth cones and synaptic terminals of axons, they are more evenly distributed along the lengths of dendrites.

The observed regional variation in mitochondrial duty cycle is most likely related to regional differences in subcellular metabolism. Our data demonstrate that dendritic mitochondria remain stationary more of the time than axonal mitochondria. The differential transport and targeting of mitochondria to regions of high ATP consumption is well known in both neuronal and non-neuronal cells (Fawcett, 1981; Morris and Hollenbeck, 1993). The decreased mitochondrial motility in dendrites could reflect greater ATP demands along their length than along axon shafts. Characterization of relative differences in mitochondrial transmembrane potentials in axons and dendrites using the lipophilic cationic mitochondrial vital dye, JC-1, yielded data consistent with this hypothesis. Dendritic mitochondria exhibited higher transmembrane potentials, thus were more metabolically active, than axonal mitochondria.

Although we cannot rule out developmental influences on organelle motility, such influences were minimized by comparing developmental stage 4 axons with stage 5 dendrites. Our experimental observations were made in axons and dendrites at these different stages of neuronal development for several reasons. First, it is known that in this and other primary neuronal culture systems, axons grow and mature earlier in neuronal development than dendrites. They initiate outgrowth, achieve their characteristic morphology (Dotti et al., 1988), and become competent to form synapses (Fletcher et al., 1994) before dendrites. Second, the chosen stages represent similar stages in the growth of these two process types, after initial outgrowth when growth is continuing slowly (Dotti et al., 1988). Third, at these stages, the axons and dendrites have matured as much as possible without sacrificing their spatial resolution for these microscopy studies. With additional growth, the axons and dendrites become a tangled meshwork and cannot be traced to either end. At the stages observed, the processes have the characteristics of older, mature processes. Stage 4 axons have achieved their mature morphology (Dotti et al., 1988), lost characteristic somatodendritic markers such as MAP2 (Matus et al., 1981) and high levels of mRNA (Kleiman et al., 1994), and are capable of making synapses (Fletcher et al., 1994). Stage 5 dendrites exhibit the mature tapered and branched morphology (Dotti et al., 1988) and are competent to form synapses (Fletcher et al., 1994). Perhaps most important for this study, the mature microtubule organization is established in dendrites of stage 5 cells (Baas et al., 1989). Specific cases of organelle motility have been shown to change with process growth state, but these changes are most pronounced near the site of active elongation (Hollenbeck and Bray, 1987; Hollenbeck, 1993; Hollenbeck and Weld, 1994; Morris and Hollenbeck, 1993). By matching the growth states of axons and dendrites, maximizing process maturity, and examining organelle motility primarily in more proximal process regions, we minimized the influence of specific developmental demands on our comparisons. Furthermore, by characterizing organelle transport in axons and dendrites of stage 4 and stage 5 neurons, respectively, we can be certain of the microtubule organization and thus can consider transport differences in terms of differences in the organization of the tracks.

The data in this report represent a first step toward an understanding of the role of organelle traffic in the establishment and maintenance of neuronal polarity and specialized subcellular domains. Our data definitively show that there are indeed regional differences in both organelle motility and metabolism. These observations are entirely consistent with the hypothesis that organelle transport properties within living cells are influenced both by the organization of the microtubule tracks and by local metabolic demands.

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