Mitochondrial transport in processes of cortical neurons is independent of intracellular calcium

Luis Beltran-Parrazal,1,* Héctor E. López-Valdés,1,* K. C. Brennan,1 Mauricio Díaz-Muñoz,2 Jean de Vellis,2 and Andrew C. Charles1

1Department of Neurology, David Geffen School of Medicine and 2Mental Retardation Research Center, University of California at Los Angeles, Los Angeles, California; 3Department of Cellular and Molecular Neurobiology, Universidad Nacional Autónoma de México-Juriquilla, Querétaro, México

Submitted 1 May 2006; accepted in final form 24 July 2006

Beltran-Parrazal, Luis, Héctor E. López-Valdés, K. C. Brennan, Mauricio Díaz-Muñoz, Jean de Vellis, and Andrew C. Charles. Mitochondrial transport in processes of cortical neurons is independent of intracellular calcium. Am J Physiol Cell Physiol 291: C1193–C1197, 2006. First published August 2, 2006; doi:10.1152/ajpcell.00230.2006.—Mitochondria show extensive movement along neuronal processes, but the mechanisms and function of this movement are not clearly understood. We have used high-resolution confocal microscopy to simultaneously monitor movement of mitochondria and changes in intracellular [Ca2+]i in rat cortical neurons. A significant percentage (27%) of the total mitochondria in cortical neuronal processes showed movement over distances of >2 μm. The average velocity was 0.52 μm/s. The velocity, direction, and pattern of mitochondrial movement were not affected by transient increases in [Ca2+]i, associated with spontaneous firing of action potentials. Stimulation of Ca2+ transients with forskolin (10 μM) or bicuculline (10 μM), or sustained elevations of [Ca2+]i, evoked by glutamate (10 μM) also had no effect on mitochondrial transit. Neither removal of extracellular Ca2+, depletion of intracellular Ca2+ stores with thapsigargin, or inhibition of synaptic activity with TTX (1 μM) affected mitochondrial movement. These results indicate that movement of mitochondria along processes is a fundamental activity in neurons that occurs independently of physiological changes in [Ca2+]i, associated with action potential firing, synaptic activity, or release of Ca2+ from intracellular stores.

MITOCHONDRIA play a crucial role in the aerobic production of ATP and also participate in the homeostasis of intracellular Ca2+ concentration ([Ca2+]i) (16, 17). Other important roles for mitochondria include the compartmentalization of metabolic pathways, the generation of reactive oxygen species, and contributions to the process of apoptotic cell death (6). Mitochondria exist as tubular structures of variable size that fuse, divide, and branch in a highly dynamic reticular network (11, 23). It is well recognized that mitochondria are flexible and dynamic organelles capable of movement throughout the cellular topology (9, 10). Mitochondrial movements occur by means of molecular motors, including kinesin, dynein, and myosin, and several linkers and anchors that enable transport along microtubules (12) and actin filaments (10). Recent evidence suggests that mitochondria need to be strategically positioned at particular subcellular sites to provide a localized energy supply and to participate in region-specific intracellular signaling (18). Mitochondrial movement can result in rearrangement of the spatial pattern of ATP production and Ca2+ buffering. Ca2+ plays an important role in regulating a great variety of neuronal processes and its cytosolic levels are in constant interplay with extracellular and intracellular sources (3). Despite significant differences in the energy metabolism and Ca2+ dynamics of axons and dendrites, mitochondrial transport has been reported to be similar in both types of compartments (14–16, 19, 22). Mitochondrial movement is bidirectional and alternates between stops and runs. In hippocampal neurons, for example, ~33% of mitochondria in axons and dendrites were observed to be in motion at any time, primarily in the anterograde direction, with mean velocities of 0.1–0.2 μm/s (14).

In axons of a variety of neurons, stimuli such as depolarization or activation of ligand-gated receptors increase the levels of intracellular Ca2+ and have been reported to inhibit mitochondrial movement (20, 24). Other studies (4, 5) indicate that external manipulation of cytoplasmic [Ca2+]i does not influence mitochondrial movement. However, there have been no reports of simultaneous observation of mitochondrial transit and cytoplasmic Ca2+ events in any region of intact neurons. We have used high-resolution confocal microscopy to directly investigate the relationship between Ca2+ signaling and mitochondrial motility in cortical neurons under physiological conditions. The commonly used fluorescent mitochondrial label MitoTracker Green and Ca2+ indicator fluo-4 have similar excitation and emission spectra. However, because of their different distributions within the cell and quantum yields, we have found that we are able to simultaneously image both indicators with excellent temporal and spatial resolution. Using this approach, we show that mitochondrial movement in processes of cortical neurons occurs independently of spontaneous or evoked Ca2+ signaling.

MATERIALS AND METHODS

Animals. Pregnant female Sprague-Dawley rats (Charles River) were housed according to guidelines set by the National Institutes of Health, and experiments were conducted in accordance with the University of California Los Angeles Chancellor’s Animal Research Committee and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

* L. Beltran-Parrazal and H. E. López-Valdés contributed equally to this work.

Address for reprint requests and other correspondence: A. C. Charles, Dept. of Neurology, 635 Charles Young Dr., Los Angeles, CA 90095 (e-mail: acharles@ucla.edu).

http://www.ajpcell.org 0363-6143/06 $8.00 Copyright © 2006 the American Physiological Society C1193

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
**Primary rat cortical neuronal culture.** Cultures were prepared as previously described (8) with minor modifications. Embryonic cerebral cortices were removed from embryonic day 16 rats, combined in DMEM/F-12 (Invitrogen Life Technologies), 10% fetal calf serum (Atlanta Biologicals), and mechanically dissociated for 2 min with the use of a sample processor (Stomacher 80 Biomaster, Seward). The dissociated cells were filtered through 140 μm and 230 μm sieves (Celllector, E-C Apparatus) to remove large clusters. Sieves were washed sequentially with DMEM/F-12 and pure calf serum. Cells were collected by centrifugation, the supernatant was discarded, and the cell pellet was resuspended in a neuronal specification medium, TII (8), supplemented with bovine FGF (10 ng/ml), B7 (1:50), and creatine (2 mg/ml). The cells were plated onto poly-o-lysine-coated glass coverslips and grown in culture for 10 days in TII medium. The composition of the cultures was determined by immunolabeling in addition to physiological characterization. The majority of cells (90–95%) were positive for neuronal markers (Map2, TuJ1, or NeuN), whereas <5% of cells showed immunolabeling for the astrocytic marker glial fibrillary acidic protein or the oligodendrocyte marker CNPase.

**Image acquisition.** Confocal microscopy was used to measure intracellular Ca\(^{2+}\) and mitochondrial movement in cortical neurons 10 days after plating. Neurons were loaded with 2.5 μM fluo-4 AM, together with Pluronic F127 acid (20% wt/vol) solution in DMSO (all from Molecular Probes, Eugene, OR) to visualize mitochondria. After 1 min of incubation with MitoTracker, the coverslips were washed extensively for 5 min to ensure that excess dye was cleared. The coverslip was used as the bottom of an open superfusion chamber that was mounted on the stage of an inverted microscope (Diaphot T200; Nikon). The chamber volume was 200 μl. During the experiments, the neurons were continuously superfused with the control solution at a flow rate of 2–3 ml/min at 31.5°C. The drugs were applied with the use of an automatic superfusion system, by switching from drug-free control solution to a drug-containing solution.

Fluorescence imaging was performed using a custom-built videorate confocal microscope, based on modifications of the design of Sanderson and Parker (21). In brief, cells were excited with a 475-nm diode laser and imaged with a ×60 (1.4 numerical aperture) oil-immersion objective. The resulting fluorescence was band-pass filtered at 510 nm and detected by a photomultiplier tube (Hamamatsu). Excitation and emission were scanned with two oscillating mirrors (GSI Lumonics). Images were captured with a Raven board (Bit Flow) using Video Savant software. Maximum image resolution was 980 × 730 at 15 frames per second.

**Imaging analysis.** Recording was typically performed at a rate of 2 frames per second for 8–10 min. Recordings at higher frame rates (up to 30 frames per second) were also performed, and produced results that were qualitatively similar to those at the lower rate. NIH Image J version 3.1 software was used for image analysis. Three investigators independently analyzed mitochondrial movements.

Only processes with a clear point of origin from the soma and a tapering diameter characteristic of dendrites were chosen for analysis. We assume that the majority of these processes are dendrites, although we cannot exclude the possibility that some axons were analyzed.

To measure the velocity of moving mitochondria, a linear region of interest was drawn along the direction of mitochondrial movement. A kymograph (spatio-temporal map) of the image sequence was generated by quantification of grayscale values for this linear region of interest (ROI) for each frame of the time series. The kymograph shows the fluorescence along the length of the ROI (y-axis) over time (x-axis). Movement of fluorescence along the ROI is seen as change in grayscale along both the x- and y-axes. The velocity of mitochondrial movement was calculated based on the angle of this x/y change. Ca\(^{2+}\) transients appeared as a change in grayscale along the entire length of the ROI. Kymographs shown in the figures represent the fluorescence changes with an inverse grayscale.

Changes in [Ca\(^{2+}\)], were analyzed as previously described (2) Briefly, the fluorescence data were analyzed off-line with custom software written in LabVIEW. Ca\(^{2+}\) transients were defined as events where fluorescence was at least 2 times the standard deviation (SD) of background fluorescence for at least 4 s. Ca\(^{2+}\) events were normalized to fluorescence baseline \(\Delta F/F_0 = (F_t - F_0)/F_0\), where \(F_t\) is the measured fluorescence at any time after the event, and \(F_0\) is the basal fluorescence. This ratio gives an appropriate correction providing that...
Mitochondria show both anterograde and retrograde movement along processes. Kymograph of a linear region of interest along a neuronal process with grayscale representing inverse of Mitotracker fluorescence. Movement of mitochondria is seen as an angled line due to change in distance (y-axis) over time (x-axis). Static mitochondria ($\Delta t = 0$) generate a simple horizontal line. Continuous velocities were obtained by calculating the tangent, which is the point velocity ($\alpha = \Delta y/\Delta t$) of a mitochondrion at a given point in time and space. Note that the mitochondrion shown moved in both anterograde and retrograde directions.

there is no change in the fluo-4 concentration during the course of the measurement.

Statistical analysis. All parameters of mitochondrial movement over time in neuronal processes were compared by an ANOVA with Dunnett’s post hoc multiple-comparison test (Origin 6.0, Microcal Software, Northampton, MA).

RESULTS

Mitochondrial movement and changes in intracellular Ca$^{2+}$ in processes of cortical neurons were monitored simultaneously with high resolution imaging of Mitotracker Green and fluo-4 fluorescence. Brief loading with low concentrations of Mitotracker (500 nM for <1 min) resulted in clearly visible mitochondria that were easily distinguishable from the fluorescence of fluo-4 in the cytosol (Figs. 1 and 4; this paper also has online data supplements). Parallel experiments were performed under multiple conditions in cells loaded only with Mitotracker. No differences were observed in any parameter of mitochondrial movement in the presence vs. absence of fluo-4 loading.

Two approaches were used to quantify the mitochondrial movement: (1) comparison of the mitochondria-associated labeling in the first and the last frames of a given experiment (usually an 8-min interval), to evaluate the percentage of mobile and static mitochondria (Table 1) and (2) generation of kymographs for the calculation of the velocity and distance of mitochondrial movement during or after the depletion of intracellular Ca$^{2+}$ or during recovery from Ca$^{2+}$ depletion (Fig. 4 and supplemental material; this article has an online data supplement). Removal of extracellular Ca$^{2+}$ abolished spontaneous Ca$^{2+}$ transients associated with action potential firing, as did the voltage-gated Na$^+$ channel antagonist TTX (1 µM). Neither 0 Ca$^{2+}$ nor TTX altered mitochondrial movement (Table 1). Exposure to the endoplasmic reticulum Ca$^{2+}$ pump inhibitor thapsigargin (10 µM) resulted in transient elevation of baseline intracellular [Ca$^{2+}$], with an increase in the frequency of Ca$^{2+}$ transients, followed by recovery to baseline [Ca$^{2+}$] and spontaneous Ca$^{2+}$ transients. There was no significant change in the movement of mitochondria during or after the depletion of intracellular Ca$^{2+}$ stores with thapsigargin (Table 1). The GABAA receptor antagonist bicuculline increased the frequency of Ca$^{2+}$ transients in the majority of neurons, and also increased the synchronization of firing between cells (Fig. 3). Activation of adenyl cyclase with 10 µM forskolin also increased the frequency of neuronal Ca$^{2+}$ transients (Fig. 3, as well as continuous; an average of 2 “stops” were detected during each recording under control conditions.

To investigate possible modulation of mitochondrial traffic by intracellular Ca$^{2+}$, we quantified mitochondrial kinetic parameters during spontaneous neuronal firing, as well as under a variety of conditions that alter Ca$^{2+}$ dynamics (Fig. 3). Characteristic spontaneous Ca$^{2+}$ transients resulted from firing of 1–5 action potentials as measured by simultaneous Ca$^{2+}$ imaging and either cell-attached or current-clamp recordings (not shown). Ca$^{2+}$ transients associated with action potential firing occurred with an average frequency of 0.65 Hz, and were mediated by influx of Ca$^{2+}$ through voltage-gated Ca$^{2+}$ channels, as evidenced by their inhibition by removal of extracellular Ca$^{2+}$ or by Ca$^{2+}$ channel blockers (not shown). Individual Ca$^{2+}$ transients associated with action potential firing had no effect on any parameter of mitochondrial movement (direction, velocity, or stops) (Fig. 4 and supplemental material; this article has an online data supplement). Removal of extracellular Ca$^{2+}$ abolished spontaneous Ca$^{2+}$ transients associated with action potential firing, as did the voltage-gated Na$^+$ channel antagonist TTX (1 µM). Neither 0 Ca$^{2+}$ nor TTX altered mitochondrial movement (Table 1). Exposure to the endoplasmic reticulum Ca$^{2+}$ pump inhibitor thapsigargin (10 µM) resulted in transient elevation of baseline intracellular [Ca$^{2+}$], with an increase in the frequency of Ca$^{2+}$ transients, followed by recovery to baseline [Ca$^{2+}$] and spontaneous Ca$^{2+}$ transients. There was no significant change in the movement of mitochondria during or after the depletion of intracellular Ca$^{2+}$ stores with thapsigargin (Table 1). The GABAA receptor antagonist bicuculline increased the frequency of Ca$^{2+}$ transients in the majority of neurons, and also increased the synchronization of firing between cells (Fig. 3). Activation of adenyl cyclase with 10 µM forskolin also increased the frequency of neuronal Ca$^{2+}$ transients (Fig. 3, as well as continuous; an average of 2 “stops” were detected during each recording under control conditions.

To investigate possible modulation of mitochondrial traffic by intracellular Ca$^{2+}$, we quantified mitochondrial kinetic parameters during spontaneous neuronal firing, as well as under a variety of conditions that alter Ca$^{2+}$ dynamics (Fig. 3). Characteristic spontaneous Ca$^{2+}$ transients resulted from firing of 1–5 action potentials as measured by simultaneous Ca$^{2+}$ imaging and either cell-attached or current-clamp recordings (not shown). Ca$^{2+}$ transients associated with action potential firing occurred with an average frequency of 0.65 Hz, and were mediated by influx of Ca$^{2+}$ through voltage-gated Ca$^{2+}$ channels, as evidenced by their inhibition by removal of extracellular Ca$^{2+}$ or by Ca$^{2+}$ channel blockers (not shown). Individual Ca$^{2+}$ transients associated with action potential firing had no effect on any parameter of mitochondrial movement (direction, velocity, or stops) (Fig. 4 and supplemental material; this article has an online data supplement). Removal of extracellular Ca$^{2+}$ abolished spontaneous Ca$^{2+}$ transients associated with action potential firing, as did the voltage-gated Na$^+$ channel antagonist TTX (1 µM). Neither 0 Ca$^{2+}$ nor TTX altered mitochondrial movement (Table 1). Exposure to the endoplasmic reticulum Ca$^{2+}$ pump inhibitor thapsigargin (10 µM) resulted in transient elevation of baseline intracellular [Ca$^{2+}$], with an increase in the frequency of Ca$^{2+}$ transients, followed by recovery to baseline [Ca$^{2+}$] and spontaneous Ca$^{2+}$ transients. There was no significant change in the movement of mitochondria during or after the depletion of intracellular Ca$^{2+}$ stores with thapsigargin (Table 1). The GABAA receptor antagonist bicuculline increased the frequency of Ca$^{2+}$ transients in the majority of neurons, and also increased the synchronization of firing between cells (Fig. 3). Activation of adenyl cyclase with 10 µM forskolin also increased the frequency of neuronal Ca$^{2+}$ transients (Fig. 3, as well as continuous; an average of 2 “stops” were detected during each recording under control conditions.

Table 1. Mitochondrial kinetic parameters

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>% Movement</th>
<th>No. of Stops</th>
<th>Mean Velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>186</td>
<td>26.99±16.63</td>
<td>2.06±2.38</td>
<td>0.52±0.24</td>
</tr>
<tr>
<td>Forskolin</td>
<td>86</td>
<td>31.37±9.10</td>
<td>2.11±2.65</td>
<td>0.47±0.29</td>
</tr>
<tr>
<td>Bicuculline</td>
<td>79</td>
<td>30.13±14.33</td>
<td>2.17±2.17</td>
<td>0.51±0.23</td>
</tr>
<tr>
<td>Glutamate</td>
<td>53</td>
<td>32.38±12.67</td>
<td>2.18±2.18</td>
<td>0.41±0.27</td>
</tr>
<tr>
<td>TTX</td>
<td>59</td>
<td>15.59±3.31</td>
<td>1.73±0.96</td>
<td>0.46±0.15</td>
</tr>
<tr>
<td>CNQX + MK301</td>
<td>60</td>
<td>21.07±17.96</td>
<td>2.27±0.88</td>
<td>0.52±0.34</td>
</tr>
<tr>
<td>Thapsigargin</td>
<td>67</td>
<td>32.98±11.90</td>
<td>1.8±1.14</td>
<td>0.38±0.13</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of neurons. Summary of parameters of mitochondrial transport under different conditions. All drugs were used at 10 µM, except for TTX (1 µM). The percentage of mitochondria showing significant movement (>2 µm) was determined by selecting up to 10 mitochondria per dendrite at random, and quantifying change in position over an 8-min recording. Velocity of mitochondrial movement was calculated based on kymographs (Figs. 2 and 4). Statistical significance between treatment groups was determined by ANOVA with an α-set to 0.05, and Dunnett’s post hoc multiple-comparison test.

Fig. 2. Mitochondria show both anterograde and retrograde movement along processes. Kymograph of a linear region of interest along a neuronal process with grayscale representing inverse of Mitotracker fluorescence. Movement of mitochondria is seen as an angled line due to change in distance (y-axis) over time (x-axis). Static mitochondria ($\Delta t = 0$) generate a simple horizontal line. Continuous velocities were obtained by calculating the tangent, which is the point velocity ($\alpha = \Delta y/\Delta t$) of a mitochondrion at a given point in time and space. Note that the mitochondrion shown moved in both anterograde and retrograde directions.

Fig. 3. Calcium signaling in processes of cortical neurons. Representative traces of fluo-4 $\Delta F/F$ from individual dendrites under the following conditions (from left to right): spontaneous activity, 10 µM forskolin, 10 µM bicuculline, and 10 µM glutamate. Forskolin and bicuculline increase the frequency of Ca$^{2+}$ transients and the synchronization of neurons in the field (not shown). Glutamate (10 µM) evokes a large, sustained increase in intracellular [Ca$^{2+}$] ([Ca$^{2+}$]) (n > 100 neurons per experimental group).
presumably increasing levels of cAMP). Neither of these treatments had any effect on mitochondrial movement (Table 1). Exposure to glutamate (10 μM) resulted in a large, sustained increase in intracellular Ca^{2+} in the majority of neurons (Fig. 3). This response was associated with a slight decrease in the average velocity of mitochondrial movement. The mitochondrion then becomes static, and subsequent 7 Ca^{2+} transients are not associated with any change in its position. Only prolonged exposure to very high concentrations of glutamate (100 μM for >10 min) inhibited mitochondrial movement. These conditions were also associated with swelling and beading of processes consistent with toxicity (n = 3 experiments, data not shown). In contrast to glutamate, which stimulated Ca^{2+} signaling, exposure to a cocktail of the glutamate receptor antagonists CNQX (10 μM) and MK801 (10 μM) abolished spontaneous Ca^{2+} transients. This treatment did not change any parameter of mitochondrial movement (Table 1), nor did inhibition of synaptic activity with 0 Ca^{2+} medium or TTX as described above. The percentage of mitochondria showing anterograde vs. retrograde movement in a given dendrite under control conditions was highly variable from dendrite to dendrite (average 34.85% anterograde vs. 65.15% retrograde ± 39.06% SD for each value, n = 60). There was no statistically significant difference in anterograde vs. retrograde movement under control conditions. Similarly, there was no statistically significant difference between the percentage of mitochondria showing anterograde vs. retrograde movement when any of the treatments groups described above were compared (n = 60 for each group).

**DISCUSSION**

Mitochondria show extensive movement along processes of cortical neurons. By simultaneously monitoring mitochondrial movement and [Ca^{2+}]_{i}, we have shown that this movement is independent of spontaneous or evoked Ca^{2+} signaling. The absence of any effect of TTX also indicates that the firing of action potentials does not modulate neuronal mitochondrial movement, whereas the absence of an effect of forskolin suggests that acute elevation of cAMP levels also has no role. Even a large, sustained, increase in [Ca^{2+}]_{i}, evoked by glutamate did not have a significant effect on mitochondrial movement.

Squid axons have been used as a model system for investigating movement of mitochondria and other organelles (1).
Consistent with our results, some previous studies (4, 5) using extruded cytoplasm from this preparation have found that manipulation of external [Ca\(^{2+}\)] to mimic changes in [Ca\(^{2+}\)]\(_i\) in the physiological range do not influence the movement of mitochondria and other organelles. Other studies (13, 20, 24), by contrast, have reported that [Ca\(^{2+}\)]\(_i\) does have a role in mitochondrial movement. There are multiple possible explanations for the differences between these results and the results presented here. First, there are significant differences in the techniques employed in the different studies. In other reports, changes in [Ca\(^{2+}\)]\(_i\) and mitochondrial movement have been examined in parallel experiments. Here we have directly observed both simultaneously, with a time resolution of 2 frames per second (and up to 15 frames per second). In addition, the spatial resolution of our imaging system allows for definitive quantification of movement of individual mitochondria, as opposed to mitochondrial mass as observed in studies by other investigators (20, 24). Another difference is that our system allows for visualization of spontaneous Ca\(^{2+}\) signaling in intact neurons under physiological conditions, whereas other studies have used external manipulation of [Ca\(^{2+}\)]\(_i\) (24, 25), or potentially toxic concentrations of receptor ligands (20) to investigate their role in mitochondrial movement. Finally, other studies have focused on mitochondrial movement in axons, whereas the majority of processes we examined had morphological characteristics consistent with dendrites. Different mechanisms could exist in different neuronal compartments.

Microdomains of [Ca\(^{2+}\)]\(_i\) occur within neurons, and it is possible that localized changes in [Ca\(^{2+}\)]\(_i\) could be occurring that are not detected by our imaging system. However, our system has the capability of measuring highly localized (~200 nm), rapid (<10 ms) Ca\(^{2+}\) transients (not shown), such that it is unlikely that we are missing Ca\(^{2+}\) signaling events that are modulating mitochondrial movement. In addition, the absence of effects of treatments that are clearly influencing global cellular Ca\(^{2+}\) would not be consistent with modulation of mitochondrial movement by localized Ca\(^{2+}\) signaling.

Transport of mitochondria and other organelles has been associated with several motor proteins, including kinesin, dynein, and myosin (10). The independence of mitochondrial movement from Ca\(^{2+}\) signaling and action potential firing shown in our study indicates that the machinery of mitochondrial motility in processes is not modulated by Ca\(^{2+}\) or by normal physiological activity of the neuron. Since Ca\(^{2+}\) signaling reflects spontaneous and neurotransmitter-evoked depolarization, our results indicate that membrane potential changes and spontaneous excitatory synaptic activity also do not modulate the movement of mitochondria. Signaling pathways that have been implicated in mitochondrial movement include CDK5/GSK3, NGF/phosphatidylinositol 3-kinase and MAPK, Abi/Ena/VASP, mitochondrial inner membrane potential, and cytosolic Zn\(^{2+}\) (10). Our studies indicate that under conditions of physiological neuronal activity, these pathways are not activated to an extent that affects mitochondrial transport in processes. Rather, it is likely that these pathways require activation by specific trophic factors, such as NGF (7), or by toxic conditions (20, 25) to influence mitochondrial motility.

GRANTS

This work was supported by National Institutes of Health Grant RO1 NS39961 and National Institute on Drug Abuse Grant DA05010 (to A. C. Charles).

REFERENCES