Supplemental materials

Myers et al., http://www.jcb.org/cgi/content/full/jcb.200702074/DC1

Immunological techniques

Neuronal cultures were fixed by incubation for 15 min at room temperature with 1× PEM (80 mM PIPES, 5 mM EGTA, and 1 mM $MgCl_2$, pH 6.8) containing 0.3% glutaraldehyde. Cells were then rinsed extensively in PBS, pH 7.4, further permeabilized by incubation with 0.2% Triton X-100 in PBS for 15 min, and treated twice with 10 mg/ml sodium borohydride in PBS, 15 min each time, to quench glutaraldehyde-related autofluorescence. Samples were blocked by incubation for 30 min with 10 mg/ml BSA + 10% normal goat serum in PBS and incubated with primary antibodies (rabbit anti-GFP at 1:2,000), diluted in PBS, kept overnight at 4°C, rinsed extensively with PBS, and reblocked for 60 min before being incubated in secondary antibodies (goat anti–rabbit Alexa-488 at 1:200; Invitrogen) and diluted in PBS for 1 h at 37°C. After extensive rinsing in PBS, samples were mounted in a medium that reduces photobleaching (0.212% N-propylgallate in 90% glycerol and 10% PBS).

All imaging studies were performed (and images were acquired) using equipment previously described (Hasaka, T.P., K.A. Myers, and P.W. Baas. 2004. *J. Neurosci*. 24:11291–11301) with either a Plan Apochromat or Fluor 100×/1.30 oil objective (Carl Zeiss MicroImaging, Inc.). Fluorescence intensity and surface area were calculated using the "measure/outline" application of Axiovision 4.5 or Axiovision LE (Carl Zeiss MicroImaging, Inc.).

Western blot analysis

Cultured neuronal lysate was collected in 1.5 ml of ice-cold PBS (1×), dissolved in sample buffer, resolved in 7.5% (for kinesin-5) and 15% (for GAPDH) SDS-PAGE gels, and transferred onto nitrocellulose membranes overnight at 4°C (25–30 mA). Transfers were probed for kinesin-5 (Kress Eg5 antibody at 1:500; secondary HRP at 1:2,000 [Pierce Chemical Co.]) and GAPDH (anti-GAPDH Ab at 1:500 [Ambion]; secondary HRP at 1:1000 [Pierce Chemical Co.]). Antibody chemiluminescence detection used ECL reagents (Pierce Chemical Co.).

Vesicle and mitochondrion transport assays

To analyze vesicle transport, rhodamine-dextran (~10,000 mol wt; Sigma-Aldrich) was added into culture medium at 1 mg/ml the night before imaging. 15 min prior to imaging, cultures were rinsed and replaced with L15 medium supplemented with 5% FBS. A total of 300 time-lapse images were taken at 400-ms exposure at 1-s intervals for each axon. Each dish was imaged for no longer than 60 min. 200 sequence frames were analyzed using the "measure/length" application in Axiovision 4.5 for calculating distance moved for each successive movie frame.

For mitochondrion transport analysis, MitoTracker FM (Invitrogen) was added onto culture medium at 5 nM final concentration for 45 min. Cells were then rinsed and replaced with L15 medium supplemented with 5% FBS. A total of 211 time-lapse images were taken at 75–120-ms exposure at 2-s intervals. Data analysis was performed as described for the vesicle transport assay.

Statistical analysis and image processing

All data analysis, statistical comparisons, and graphs were generated using Excel software (Microsoft Corp.). Data comparison was performed using a two-tailed t test assuming unequal variances for axonal morphology experiments and χ^2 analysis was used for statistical comparison of frequency data as well as for changes in stepwise data distribution. For all statistical analysis, significance was determined to 95% confidence interval ($P < 0.05$). Image processing was performed using Axiovision 4.0 and final figure processing was performed with Photoshop 7.0 (Adobe).