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Supplemental materials and methods

Reporter gene expression

GAL4 fusion protein activity was measured as previously described (Coffey et al., 2000). Cerebellar granule neurons at 6 d in vitro were transfected with a firefly luciferase reporter plasmid driven by five GAL4 elements in tandem with pGL3-G5E4Δ38, GAL4-Jun(5–105), and pRL-CMV (Promega) as an internal standard against which signals were normalized. In addition, cells were transfected with pEGFP-NES-JBD (50%) or pEGFP (50%). 20 h after transfection, cells were transferred to low (5 mM) KCl-containing medium (*trophic withdrawal*) for 4 h and then lysed. Firefly (reporter) and Renilla (internal standard) luciferase activities were assayed with the dual luciferase assay kit (Promega).

Immunofluorescence staining

Immunocytochemical staining was performed as previously described (Coffey et al., 2000). Coverslips of cortical neurons were fixed in 4% paraformaldehyde (20 min) and permeabilized in PBS/Triton X-100 (1%) for 3 min. After washing with PBS, samples were blocked with 10% serum containing 0.2% Tween-20 in PBS for 1 h. Incubation with primary antibodies was overnight at 4°C using 1:1,000 α-SCG10, 1:1,000 α–P-SCG10, 1:150 α-tubulin (KMX-1; Cedarlane Laboratories), or 1:100 α-PJNK (Cell Signaling Technology). Tubulin immunoreactivity was detected with 1:500 α-mouse Alexa-568 (Invitrogen) and SCG10 and PJNK with 1:500 α-rabbit Alexa-488 (Invitrogen). P-SCG10 immunoreactivity was amplified using 1:800 goat α-rabbit biotin (Sigma-Aldrich) and 1:1,500 streptavidin Alexa-488 (Invitrogen). Slides were scanned with an argon 488 and HeNe 543 laser using a laser-scanning confocal microscope (META 510; Carl Zeiss MicroImaging, Inc.).

Immunohistochemistry

Day 15.5 mouse embryos were fixed for 24 h in 4% paraformaldehyde before paraffin embedding. Slide-mounted sections (6 μm) were blocked with Vectastain blocking reagent and stained with JNK1 (G151-333; 1:500; CLONTECH Laboratories, Inc.), PJNK (1:500; Promega), SCG10 (1:5,000), GFAP (1:1,000), corresponding preimmune serum (1:5,000) or P-SCG10 (1:1,000). GFAP/peptide block JNK1 and P-SCG10 immunoreactivity was amplified using biotin-conjugated tyramide (1:100) and SCG10 and P-JNK with biotin anti-rabbit (1:800; Sigma-Aldrich). Immunoreactivity was detected with streptavidin-conjugated horseradish peroxidase and 3,3′-diaminobenzedine tetrahydrochloride (Sigma-Aldrich) as substrate. Sections were counterstained with Meyer's hematoxylin (Sigma-Aldrich). Sections were examined using a microscope (BX60; Olympus), and images of telencephalon were taken using a colored charge-coupled device camera (U-CMAD-2; Olympus). Staining specificity was tested by immunoblotting of 25 microdissected sections (30 &microm thickness) of mouse embryonic day 15.5 cortical plate, intermediate zone, and ventricular zone.

FRAP

Cortical neurons at 2 d in vitro were transfected with pVenus–α-tubulin, pECFP-SCG10AA, pCDNA3-Flag-JBD, or pECFP as shown. 48 h after transfection, cells were incubated in a CO_2, humidity-regulated chamber and imaged using a laser-scanning microscope at 37°C. 10 prebleach scans were followed by photobleaching of a 20-&microm^2 fixed area of the neurite using 200 iterations of a 488-nm laser set at 100% transmission and maximum power. After bleaching, images were scanned using a 4% transmission 514-nm laser and a 0.1% transmission 488-nm laser and Venus fluorescence was detected at 529–593 nm using the META detector. Maximum scan speed, 512 x 512 resolution, and a 40x oil objective (NA 1.3) were used. For each experiment, a background region and a region encompassing almost the entire image were scanned in addition to the bleached region. These regions were used to calculate background intensity and fading using a linear regression. Corrected data is shown. Kinetic plots were created by fitting the corrected raw data to the equation y = a(1 - exp[kt]) as previously described (Phair and Misteli, 2001). Mobile fractions were calculated as described previously (Beaudouin et al., 2002).

Subcellular fractionation

Postnatal day 7 rat forebrain was homogenized using a homogenizer (Potter-Elvehjem) in buffer containing 320 mM sucrose; 20 mM Tricine-NaOH, pH 7.8; 1 mM EDTA; 2 mM $MgCl_2$; 1 &microg/ml leupeptin, pepstatin, and aprotinin; 100 &microg/ml PMSF; and 50 mM &beta-glycerophosphate. Homogenate was precleared by spinning at 800 *g* for 10 min at 4°C to yield P1. The remaining supernatant (S1) was centrifuged at 16,000 *g* for 30 min at 4°C to yield P2 and S2. S2 was further centrifuged at 200,000 *g* for 20 min at 4°C to yield P3. Equal proportions of homogenate, S1, P1, S2, P2, S3, and P3 were analyzed by SDS-PAGE.

References

Beaudouin, J., D. Gerlich, N. Daigle, R. Eils, and J. Ellenberg. 2002. Nuclear envelope breakdown proceeds by microtubule-induced tearing of the lamina. *Cell.* 108: 83–96.

Phair, R.D., and T. Misteli. 2001. Kinetic modelling approaches to in vivo imaging. *Nat. Rev. Mol. Cell Biol.* 2:898–907.