SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Generation and Staging of Embryos

Adult *Xenopus laevis* females were primed and injected with human chorionic gonadotropin (Sigma-Aldrich, St. Louis, MO) to induce ovulation. Eggs were fertilized *in vitro*. Embryos were grown at 22°C in 0.1x Marc's modified Ringer's solution (MMR) (1x MMR contains 100 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$, 5 mM HEPES, 0.1 mM EDTA, 2 mM $CaCl_2$, pH adjusted to 7.8) and staged according to Nieuwkoop and Faber (1967).

Plasmids and Morpholinos

The hKir2.1-pBluescript construct was a generous gift from Dr. Eduardo Marban (Johns Hopkins University, Baltimore, MD). The hKir2.1-mCherry fusion gene was subcloned into a pcDNA3.1 vector by *Nhe*I and *Not*I restriction sites, resulting in a recombinant construct hKir2.1-mCherry-pcDNA3.1. mCherry-pcDNA3.1 was constructed as a control plasmid. The BDNF-pHluorin construct was a generous gift from Dr. Mu-ming Poo (UC Berkeley). The BDNF-pHluorin gene was subcloned into a pCS2 vector by EcoRI and XbaI restriction sites. BDNF and JNK morpholinos (BDNF MO and JNK MO) were previously described (Huang et al., 2007; Yamanaka et al., 2002). The 5'-lissamine-tagged BDNF MO and JNK MO were supplied by GeneTools (BDNF MO: 5'-ATGGTCATCACTCTTCTCACCTGA-3' and JNK MO: 5'-TGCTGTCACGCTTGCTTCG GCTCAT-3'). A control 5'-lissamine-tagged MO (CMO) was used to test the specificity of the effects of the BDNF and JNK MO (5'-CCTCTTACCTCAGTTACAATTTATA-3').

Blastomere injection

hKir2.1-mCherry-pcDNA3.1 or mCherry-pcDNA3.1 (control) DNA was amplified by the Qiagen midiprep kit (QIAGEN, Venlo, The Netherlands) and dissolved in distilled water.

A range of different amounts of DNA (from 100 pg to 800 pg) was injected into single 16-cell blastulae; 200 pg DNA (0.2 mg/ml x 1 nl) were determined to be the optimal amount for labeling single neurons. hKir2.1 and BDNF-pHluorin mRNA were transcribed using the Ambion mMessage mMachine kit (Ambion, Austin, TX). 5-10 nl of a 0.01-0.1 mg/ml capped mRNA solution in nuclease-free water were co-injected with 10,000 MW Cascade Blue dextran (20 mg/ml, Invitrogen, Carlsbad, CA). Healthy embryos were transferred to Petri dishes containing 0.1× MMR after injections and incubated in the dark. The survival rate was 70-80%; fluorescence was detected by stage 15 and persisted for at least 3 days.

Calcium Imaging

A Leica SP5 confocal system (Nussloch, Germany) with a 40x water immersion objective with an argon laser line (488 nm) was used to detect Fluo-4-loaded neurons and monitor Ca^{2+} spike activity. A DPSS laser line (561 nm) was used to visualize mCherry-labeled neurons. Image movies were imported into NIH Image J for analysis and the cell bodies of neurons of interest were outlined using the tracing tools. The average pixel intensity was determined and exported to Microsoft Excel for analysis. These data were plotted against the time of image acquisition to yield fluorescence kinetics and intensity.

Immunocytochemistry

1.5 d old (stage 28) and 3 d old (stage 41) embryos were fixed in 4% paraformaldehyde (PFA) with or without 0.1% glutaraldehyde in phosphate-buffered saline (PBS) at pH 7.4 for 30 min to 2 hr at 4°C, incubated in sucrose for 2.5 h, and embedded in OCT. Cryostat sections 20 μm in thickness were made over a length of 400 μm starting from

the rostral end of the spinal cord. All sections were blocked with 1% fish gelatin (Sigma-Aldrich, St. Louis, MO) in 0.1% Triton X-100 in PBS for 0.5 hr before staining overnight at 4°C with single primary antibodies at the following concentrations: rabbit anti-vesicular glutamate transporter 1 (VGluT1; Sigma-Aldrich, St. Louis, MO) 1:5,000; rabbit anti-glutamate (Sigma-Aldrich) 1:10,000; guinea pig anti-GABA (Millipore Bioscience Research Reagents, Temecula, CA) 1:300; goat anti-TrkB (Santa Cruz Biotechnology, Santa Cruz, CA) 1:200; rabbit anti-cJun (Santa Cruz Biotechnology) 1:100; rabbit anti-P-cJun (Santa Cruz Biotechnology, Santa Cruz, CA) 1:100; rabbit anti-P-JNK (Promega, Madison, WI) 1:1000; rabbit anti-JNK (Santa Cruz Biotechnology) 1:100. Fluorescently tagged secondary antibodies were used at 1:300 for 2 hr at 22°C. Sections were mounted in Vectashield mounting medium (DAPI; Vector Laboratories, Burlingame, CA) to track cell numbers. Labeling was examined on an Axioskop with a 40x water-immersion objective or an Olympus confocal microscope (Olympus IY81, Center Valley, PA) with a 20x air objective, using the appropriate excitation and emission filters for Alexa 488, Alexa 594, and DAPI. Images were acquired and analyzed with NIH Image, Image J (W. Rasband, NIH), Axiovision (Carl Zeiss imaging) and Leica Application Suite (Leica Microsystems). Cell counts were performed based on DAPI (Vector Laboratories) or DRAQ5 (Biostatus, Leicester, UK) nuclear staining using the Image-based Tools for Counting Nuclei plugin for Image J (Center for Bio-image Informatics, Santa Barbara, CA).

Cell Culture

Embryos were injected at the one-cell stage with hKir2.1 mRNA and 10,000 MW Cascade Blue dextran (20 mg/ml, Invitrogen, Carlsbad, CA) or with 10,000 MW

Cascade Blue dextran alone. Cells were plated in 35 mm tissue culture-treated plastic dishes (Corning, NY, USA) with 2 ml of saline (with or without 2 mM $CaCl_2$) referred to as 2 mM or 0 mM Ca^{2+} medium. Each dish contained cells from four embryos in order to obtain higher density cultures. All cultures were grown at room temperature (21-23°C). Medium from each dish was collected at different times for ELISA assay (1, 3, 5, 7, 9 and 14 hr *in vitro*). Cells were collected at 9 hr *in vitro* for western blotting.

Whole Mount In Situ Hybridization

Albino embryos of chosen stages were fixed in MEMPFA (0.1 M MOPS pH 7.4, 2 mM EGTA, 1 mM $MgSO_4$, 3.7% PFA) and stored in methanol at -20°C until use. They were then rehydrated, washed with PBST (0.1% Tween-100 in PBS), and hybridized in hybridization buffer (50% formamide, 5x SSC, 1 mg/ml Torula RNA, 100 μg/ml heparin, 1x Denhart's, 0.1% Tween-20, 0.1% CHAPS, 5 mM EDTA) at 60°C overnight. Embryos were washed with maleic acid buffer (MAB), incubated in 20% heat-inactivated lamb serum for 2 hr at 22°C, and then incubated overnight with anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche, Basel, Switzerland) at 1:2,000. After the antibody solution was removed and embryos were washed in MAB 5 times for 1 hr each at 22°C, the chromogenic reaction was started by replacing MAB with BM Purple AP substrate (Roche, Basel, Switzerland). Embryos were examined periodically and the chromogenic reaction was stopped by replacing the staining solution with MEMPFA. Embryos were dehydrated and mounted with 2:1 benzyl benzoate/benzyl alcohol for observation.

BDNF Immunoassay

Standards (in duplicate) and undiluted samples of cell-conditioned culture medium (in duplicate or triplicate) were incubated in 96-well ELISA plates precoated with anti-BDNF monoclonal antibody. The captured BDNF was bound by a second specific antibody, which was detected using a species-specific antibody conjugated to horseradish peroxidase as a tertiary reactant. All unbound conjugates were removed by subsequent wash steps. After incubation with chromagenic substrate, color change was measured in an ELISA plate reader (Bioteck, Winooski, VT) at 450 nm. BDNF concentrations were calculated from regression analysis of BDNF standard curves run in each assay.

Western Blotting

Cells were cultured in a 2 mM or 0 mM Ca^{2+} medium for 9 h. Cultures were treated with 10 µM of different kinase inhibitors as indicated. Cells were homogenized in lysis buffer (20 mM Tris HCl, pH 7.8, 1 mM EDTA, 20 mM NaCl, 1 mM EGTA, 1% Triton X-100, 0.5 mM Na_3VO_4, 0.5 mM PMSF, 1 mM NaF, 1 µg/ml leupeptin, 1 µg/ml aprotinin and 1 µg/ml pepstatin). Homogenates were subjected to 12.5% SDS-PAGE and transferred overnight to PVDF membranes. Membranes were blocked with Tris-buffered saline (TBS)/Tween 20 (TTBS) buffer (100 mM Tris-HCl, 150 mM NaCl and 0.1% Tween, pH 7.5) containing 5% nonfat dry milk for 1 hr at room temperature and incubated overnight with the primary antibody. Antibodies used were as follows: 1:100 rabbit anti-cJun (Santa Cruz Biotechnology, Santa Cruz, CA), 1:100 rabbit anti-P-cJun (Santa Cruz Biotechnology, Santa Cruz, CA). Blots were then incubated with peroxidase-conjugated anti-mouse 1:10,000 for 1 hr at room temperature. Bands were visualized using HRP chemiluminescence (HyGLO Quick Spray; Denville Scientific, Metuchen, NJ) according to the manufacturer's recommendations and exposed to Kodak BioMaxLightFilm.

SUPPLEMENTAL REFERENCES

Nieuwkoop, P. D. and Faber, J. (1967). Normal Table of *Xenopus laevis* (Daudin) (Second edition ed.). Amsterdam: North Holland Publishing Company.