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Imaging and recording subventricular zone progenitor cells in live tissue of postnatal mice

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IN VIVO ELECTROPORATION IN NEONATES

A pCAG plasmid is diluted in PBS containing 0.1% fast green as a tracer to a concentration of 0.5–3 μg/μl. 0.5–1 μl of plasmid solution is injected into the lateral ventricle using a pulled glass pipette (beveled to a <50 μm diameter) in pups anesthetized on ice. Positive pressure for plasmid injection can be applied by expiration or using a pressure application device (Picospritzer). After plasmid injection, tweezer-type electrodes (model 520, BTX) were placed on the heads of the P0-P1 pups, and 5 square-pulses of 50 ms duration with 950 ms intervals at 150 V were applied using a pulse ECM830 BTX generator. Animals are then be warmed on a heating pad and under light, then placed back in the cage with the mother. Electroporation does not require removing the skin overlying the skull.

SLICE AND WHOLE-MOUNT PREPARATION FOR LIVE RECORDING (PATCH CLAMP OR IMAGING)

Mice are anesthetized using pentobarbital (50 mg/kg) and decapitated. The brain is quickly removed and chilled (0–4°C) in 95%O_2/5%CO_2 saturated artificial cerebrospinal fluid (aCSF) containing (in mM): NaCl 125; KCl 2.5; $CaCl_2$ 2; $MgCl_2$ 2; $NaHCO_3$ 25; glucose 10 or in a sucrose-based aCSF in which all or two-thirds of the NaCl was replaced by isotonic sucrose. The tissue was glued to the stage of a vibratome and 250–300 μm thick slices that contained the region of interest were cut in cold oxygenated ACSF. After a recovery period of >1 h in ACSF, slices are placed in a flow-through chamber, held in position by a nylon mesh glued to a U-shaped platinum wire and continuously superfused with oxygenated ACSF at room temperature. The chamber is mounted on the stage of an upright microscope (Olympus BX50WI) equipped with a 60× water immersion objective and infra-red optics.

For preparing whole-mounts, the entire brain is placed in cold, oxygenated high sucrose-aCSF under a dissecting scope. After removal of the cerebellum, a medial incision is performed between the two hemispheres. The lateral ventricle is opened through its dorso-medial part and by peeling away the corpus callosum and cortex. The tissue located ventrally to the lateral ventricle as well as the anterior and posterior ends are dissected out. The hippocampus is then removed making the entire lateral ventricle visible. The choroid plexus, cortex and corpus callosum are dissected out.

WHOLE-CELL RECORDINGS AND CELL FILLING

Patch pipettes are pulled from thin-walled borosilicate glass (o.d., 1.55 mm; i.d., 1.2 mm; WPI, TW150F-40) on a PP-83 puller or a Sutter P-90 puller. Pipettes had resistances of 6–8 MQ when filled with one of the following solutions (in mM): (1) KCl 140; $CaCl_2$ 1.0; $MgCl_2$ 2.0; ethylene glycol-bis(-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA) 10; HEPES 10; pH adjusted to 7.2 with Tris-base, (2) Solution #1 with addition of 4 mM MgATP or Na_2ATP plus 0.4 mM MgGTP, (3) Solution #1 with addition of an ATP regenerating solution that included 4 mM K_2ATP 4, 20 mM K_2-phosphocreatine, 50 U/ml creatine phosphokinase and 6 mM $MgCl_2$; KCl was reduced to 110 mM. We highly recommend using a regenerative solution or perforated patch clamp (see below) for neuroblasts which are small and fragile cells. The osmolarities of the intracellular and extracellular solutions are adjusted to 295–300 mOsm and 305–310 mOsm, respectively. For morphological identification, 0.1% Lucifer Yellow (LY, dilithium salt) or Alexa dyes are routinely added to the pipette solution.

In our case, whole-cell recordings are performed using an Axopatch-200B amplifier (Axon Instruments). Current signals are low-pass filtered at 2–5 kHz and digitized on-line at 5–20 kHz using a Digidata 1320 digitizing board (Axon Instruments) interfaced with an IBM-compatible computer system. Data acquisition, storage and analysis are performed using PClamp version 8.0.2 (Axon Instruments). Correction of junction potentials should be performed either on-line or off-line. Images of cells visually chosen for recordings and LY fills can taken on-line and archived using various modes of acquisition.

PERFORATED PATCH-CLAMP RECORDINGS

The cation-selective ionophore gramicidin is used for perforated patch recordings to prevent interference with the intracellular Cl– concentration ([Cl^-]i). The gramicidin-containing pipette solution is prepared fresh for each experiment from a stock solution (5 mg/ml in DMSO, stored at −20°C) diluted in prefiltered intracellular solution (solution #1 above) to yield a final concentration of 5 μg/ml. To ensure full efficacy of the gramicidin, the solution needs to be protected from light and renewed every 2 h. Patch pipettes are expected to have a resistance of 5–8 MΩ when filled with the intracellular solution. Two precautions are necessary to minimize gramicidin ejection from the patch pipette when approaching the cells: positive pressure is avoided and the pipette tip is filled with the normal intracellular solution by 10–15 s dipping (the gramicidin-containing solution was added by regular filling). Stable perforated recordings (≤10 MΩ series resistances) are obtained in 12–15 min or sometimes faster depending on how long the pipette tip was dipped into normal intracellular solution.

CALCIUM IMAGING

The SVZ cells are loaded with Fluo 4-AM by bath application (45 min in 10 μM) or pressure application (250 μM, 2–4 min) either on the surface or deep inside the tissue. The frequency of cytosolic Ca^{2+} increases is calculated using CalSignal. Images are acquired every 1.16–3 s with FluoView acquisition software. F_0 (i.e. baseline) is the mean fluorescence intensity measured throughout all the regions of interest, and F is the mean fluorescence intensity in a single cell. A change in fluorescence is considered to be a Ca^{2+} increase if >15% F/F_0. Ca^{2+} data were collected in >3 slices from >3 mice.

IMAGING CELL MIGRATION

Migration movies are acquired at 37°C on the Olympus confocal microscope with a Super 20× dry objective (N.A. 0.95) in high glucose-DMEM using whole-mounts of the lateral ventricle or acute slices. For each drug treatment, the migration speed is measured in >3 whole-mounts or slices from three mice. At least six movies are acquired per drug: three movies under control conditions followed by a 30-min drug wash-in period, and three movies with the drug. Each movie is at least 1 h long and contained 12 image-stacks spaced by 5 min. Each image-stack is a series of 2 μm-spaced Z-sections over 30–80 μm. Greater than 10 neuroblasts in chains are analyzed per movie. In electroporated mice, the same neuroblasts can be tracked over time. Image-stacks are realigned and YFP-fluorescent cells were tracked using ImageJ plug-ins (Stackreg, Thevenaz et al., 1998, and MTrackJ written by Dr. E. Meijering, Biomedical Imaging Group Rotterdam, respectively).

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