Bissonnette Supplemental Methods

Media Formulations [All percentages are (vol/vol)]

hESC Media: 50% DMEM-F12 [+L-Glutamine], 1% Non-essential amino acids, 16.66% Knockout Serum Replacement [Invitrogen], 100µM ß-mercaptoethanol
Neurosphere Media: 50% DMEM-F12 [+L-Glutamine],1% amino acids,1% N2 supplement,100µM ß-mercaptoethanol, 8 µg ml^{-1} Heparin, 20 ng ml^{-1} bFGF, 20 ng ml^{-1} EGF
Neuron Media 1: 30% DMEM-F12 [+L-glutamine], 2% B-27 Supplement, 100 ng ml^{-1} NGF
Neuron Media 2: Neurobasal Media, 1% B-27 Supplement, 0.5% Glutamax, 0.5% Glutamine, 20 ng ml^{-1} bFGF, 100 ng ml^{-1} NGF

siRNA Studies. We used 100pg (5µl of 20nM stock) of Dharmacon ON-TARGETplus SMARTpool siRNA specific to human *Lhx8* for each replicate siRNA nucleofection, while control replicates received an equal amount of scrambled non-specific ON-TARGETplus Non-Targeting Pool. To determine siRNA nucleofection efficiency, an equivalent amount of siGLO Red Transfection Indicator siRNA was used in separate experiments. To show the efficacy of the *Lhx8* siRNA, dissociated neurospheres were nucleofected with either *Lhx8*-specific or non-targeting siRNA, plated for 6 hours, then treated with BMP9 for 6 hours before harvesting the cells for RNA.

Cell Analysis. RNA was extracted with the RNAqueous-4PCR kit [Ambion], treated with TurboDNAse [Ambion] for 30 minutes, then reverse transcribed with Thermoscript for 90 minutes [Invitrogen]. qRT-PCR was performed using an Eppendorf Realplex thermocycler and SybrGreen mastermix [Applied Biosystems]. Before harvesting, all neurons were grown without NGF for 48hrs and then again with NGF for the last 24 hrs; because ChAT is a 2.1 kb [kilo base pair] cDNA comprising 15 exons spliced from a 56 kb locus it is very stable and thus has low basal expression levels: this transient NGF withdrawal allows for accurate determination of gene expression levels. The sequences for all qRT-PCR primers are provided [Supplemental Table 2 online]. Cells for immunohistochemistry were rinsed in 4% (wt/vol) PFA [paraformaldehyde] then fixed in 4% PFA for 20 minutes followed by 30 minutes of permeabilization with 0.10% (vol/vol) Triton X-100 [Sigma]. After blocking in 10% (vol/vol) goat serum for 30 minutes, primary antibodies [ChAT 1:666 Aves Labs, Map2 1:500 Abcam (both rabbit and mouse IgG2a), p75 1:666 Abcam, VChAT 1:250 SYSY, VGlut 1:3000 SYSY, HB9 1:1000 Abcam, GFAP 1:1000 Sigma, GFP 1:500 Abcam, MBP 1:666 Sternberger Monoclonals Inc., Synapsin 1:500 SYSY, FORSE1 1:75 Developmental Studies Hybridoma Bank, Nestin 1:500 Abcam, SV2A 1:200 SYSY, Bassoon 1:500 SYSY, Densin160 1:500 SYSY, Synaptotagmin1 1:1000 SYSY, Synaptophysin1 1:500 SYSY, FoxG1 1:500 Abcam, Nkx2.1 1:500 Abcam, ChAT 1:500 Chemicon (Figure 1h,i only),] were added for 60 minutes at room temperature. Appropriate fluorescent secondary antibodies [Aves Labs (FITC)-conjugated anti-Chicken IgY 1:500 for ChAT, Molecular Probes 1:1000 for all others] were added for 45 minutes. All photomicroscopy and counts of ChAT, p75, Map2, synapsin1, α-bungarotoxin, and HB9, Synaptotagmin1, Synaptophysin1, SV2A, Bassoon, Densin160, FoxG1, and Nkx2.1 were performed using a Zeiss LSM 510UV META laser scanning confocal microscope while VChat, VGlut, GFAP, MBP, and ChAT (Figure 1h,i only) analysis used a Zeiss epifluorescence microscope. Acetylcholine levels in cultured neurons were determined with an AmplexRed detection kit [Invitrogen] and correlated with protein levels determined using the FluoroProfile Protein Quantification Kit [Sigma]. For α-bungarotoxin labeling, live murine cortical cultures with engrafted BFCN were rinsed with HBSS, incubated with 3 mls 0.05 mg ml^{-1} AlexaFluor594-conjugated α-bungarotoxin [Invitrogen] for 30 minutes in final neuron media, rinsed in HBSS, rinsed in 0.1% Triton X-100 in PBS, then fixed in 4% PFA for 30 minutes.

Preparation of Murine Organotypic Hippocampal Slice Culture. P5-P6 CD1 mice were chilled on ice and sacrificed by decapitation; brains were removed under aseptic conditions followed by the separation of the hippocampus and enthorinal cortex (HEC) from the two hemispheres as previously described [50]. The HEC tissue blocks were cut using a McIlwain tissue chopper into 350 µm-thick coronal slices. Slices were placed on semiporous membrane inserts [Millicell-CM, 0.4 µm] and transferred to six-well culture plates with 1.2 ml of MEM supplemented with 25% (vol/vol) horse serum, 6.5 mg ml^{-1} D-glucose, and 0.5 mM L-

glutamine. After 3–4d in culture, the medium was changed to final neuron media supplemented with 4% knockout serum replacement, and the media changed every 72 hrs.

Micrografting of BFCN Derived from hESC into Murine Slice Cultures. Presumptive BFCN generated through *Lhx8*/*Gbx1* nucleofection were FACS-purified at 48 hours and subsequently grown in culture for 5–7 days. To maintain fluorescent signal intensity beyond that from the transient nucleofection, purified cells were permanently labeled with an adenovirus encoding eGFP. Cultures were dissociated using accutase, counted, and loaded on the tip of a 0.5 µl Hamilton syringe mounted on a micromanipulator. After the slices had been transferred into the serum-supplemented final neuron media, suspensions of these FACS-purified cells (3–4,000 cells/0.2 µl) were seeded locally onto the murine hippocampal cultures in the area of the dentate gyrus (DG). After 2–3d in culture, grafted cells were continuously monitored for GFP fluorescence using live confocal microscopy and processed for electrophysiological recording and eventual immunohistochemistry.

Electrophysiological Recordings of BFCN Derived from hESC into Murine Slice cultures. Organotypic slice cultures 2–19 days old were incubated for 30 min at 37°C in oxygenated standard artificial CSF [ACSF] containing [in mM]: 130 NaCl, 24 NaHCO3, 3.5 KCl, 1.25 NaH2PO4, 1.5 CaCl2, 1 MgSO4, and 10 glucose, saturated with 95% O_2 and 5% CO_2 at pH 7.4 and then maintained at room temperature until being transferred to the recording chamber in oxygenated standard ACSF. The eGFP-positive engrafted cells were observed with the aid of a fluorescence microscope [BX-50WI; Olympus] and visualized with a chilled charge-coupled device video camera [Dage-MTI] with a 40x water-immersion differential interference contrast objective. Whole cell patch clamp recordings were performed either from eGFP-expressing cells or neighbouring murine cells [not eGFP-expressing] located in the subgranular zone of the dentate gyrus. For whole cell voltage clamp recordings, patch electrodes with a resistance of 5–7 MΩ were pulled from borosilicate capillaries [World Precision Instruments; PG52165-glass]. Patch pipettes were filled with a solution of [in mM] 150 KCl, 10 HEPES, 4 Mg2ATP, 0.5 NaGTP, and 10 phosphocreatine. The pH was adjusted to 7.3 with KOH. For whole cell current clamp experiments the pipette solution was potassium gluconate, 100; EGTA, 10; MgCl2, 5; Hepes, 40; ATP, 3; GTP, 0.3; pH adjusted to 7.2 with KOH and osmolarity to 295 mOsm with sucrose. Whole-cell voltage clamped recordings were obtained from the fluorescence-labeled cells using an Axopatch 200B patch-clamp amplifier [Molecular Devices] and the data were captured with pClamp 9.0 software [Molecular Devices]. Data analysis Data were filtered at 2 kHz and digitized at 10 kHz using a Digidata 1322A analog-to-digital board. Analysis was performed using the pClamp 9.0 [Molecular Devices], MiniAnalysis [Synaptosoft],Sigmaplot or Igor. For the detection and measurement of PSCs, all PSCs were detected in 1–3 min recording segments under the appropriate experimental configuration [baseline control, drug application]. Event frequency and amplitude were determined by MiniAnalysis software [Synaptosoft]. Drugs used: Bicuculline methiodide, 6-cyano-7-nitroquinoxaline-2,3-dione [CNQX], di-hydro-β-erythroidine HBr (DHβE), methyllycaconnitine [MLA], 4-aminopyridine [4-AP] [all Sigma] were applied by either focal or bath application. All experiments performed here were performed in accordance with animal experimentation protocols approved by the National Institutes of Health and institutional protocols.

Statistical Analysis. The RNA data [Figs. 1–3] were analyzed for statistical significance using the Analyses of Variance method (ANOVA) with version 9.1 of SAS. For the counts of ChAT-positive neurons [Fig. 4], the ratios violated the assumptions of parametric hypothesis tests with respect to normal distributions and homogeneity of variance, consequently non-parametric alternative tests were carried out. The Kruskal-Wallis test was used to test the global null hypothesis that there was no significant difference between the median ratios with respect to the four groups. Mann-Whitney U tests were used to test the null hypotheses that there were no significant differences between the median ratios when pairs of treatments were compared. The decision rule was to reject the null hypothesis if the p value of each of the Kruskal-Wallis or the Mann-Whitney U tests was less than the prescribed level of significance of $\alpha = 0.05$. The hypothesis tests were performed using SPSS version 17.0. Paired and Non-paired Student's T-Tests were used to determine the significance of the changes in electrophyiological response following specific receptor antagonist treatment [Fig 5h,i].