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Supplemental Information

NEUROD1 Instructs Neuronal Conversion in Non-Reactive Astrocytes

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Experimental Procedures (continued)

In vitro Astrocyte Cell Culture

Primary astrocytes were isolated and cultured as previously published (Matsuda T. *et al.*, 2015). Briefly, primary astrocyte cultures were prepared from P1 mouse brains and cultured on uncoated dishes in 10% FBS DMEM media. After 7 days the cultures were shaken at 200 rpm for 1 hr to remove contaminating cells, and then treated with AraC (5uM) to kill proliferating cells for a total of 2 days. Cultures were shaken once more, and then infected with a lentivirus expressing *NEUROD1* together with GFP (lenti-CAG-*NEUROD1*-UbiC-GFP). Approximately 1 day later the culture media was changed to N2/B27 and the culture was allowed to continue for an additional 6 days before cells were fixed.

Virus Production

For in vivo studies, the mouse cDNA for *NEUROD1* was cloned into an AAV9 vector backbone (pZac) obtained from Dr. Mauro Giacca. Expression of the protein was confirmed in 293T HEK cells via western blot analysis and the construct was sent for AAV9 custom packaging (SAB Tech) to obtain a viral titer of at least 1e13vg/ml. AAV9-GFP virus was purchased from SAB Tech to use as control in these studies. For in vitro studies, the cDNA for *NEUROD1* was cloned into the lentiviral vector (FUW; Addgene) to produce lenti-CAG-*NEUROD1*-UbiC-GFP. The control lentivirus for all experiments was lenti-CAG-UbiC-GFP. To prepare lentivirus, HEK293T cells were co-transfected with each of these constructs and lentiviral packaging vectors (pCAG-HIVgp and pCMV-VSV-G-RSV-Rev) using polyethylenimine (Polysciences). The culture supernatants were collected 48 hrs after transfection, and the virus was introduced into primary cultured astrocytes by adding the supernatants to the culture medium. The viral titer was between 10^7-10^8 viral particles per ml.

Immunohistochemistry

Mice were anesthetized and perfused transcardially with cold 4% paraformaldehyde (PFA) in 0.1 M PBS. Brains were removed and post-fixed in 4% PFA overnight, then cryoprotected in 30% sucrose in 0.1 M PBS. Brains were bisected and half-brains were coronally sectioned 30 um thick on a freezing microtome (Leica, SM 2000R). Immunohistochemistry was performed with

either tissue mounted on charged slides or free-floating tissue sections. Slides underwent antigen retrieval using 0.01 M citric acid, pH 6.0 at 100 °C for 15 min, followed by 12 min in 1x TBS at room temperature. Staining with free-floating tissue sections was the same except for the antigen retrieval step which was omitted. For Tyramide Plus signal amplification, we removed endogenous peroxidase activity by incubating sections with 0.3% H2O2 for 30 min at room temperature. Nonspecific binding was blocked with 3% normal donkey serum and 0.3% Triton-X-100 or 3% normal donkey serum and 1% Triton X-100 in 1x TBS for 1 hr at room temperature. Primary antibodies used in this study were as follows: goat anti-*NEUROD1* (1:500, Santa Cruz Biotechnology sc-1084), chicken anti-GFP (1:1,000 for free floating sections, 1:8,000 for Tyramide Plus Amplification Aves Lab GFP-1020), mouse anti-GFAP (1:500, Millipore Clone GA5 MAB3609), goat anti-DCX (1:1,000, Santa Cruz Biotechnology sc-8066), guinea pig anti-DCX (1:1,000, Millipore AB2253), mouse anti-NeuN (1:1,000, Millipore MAB377), mouse anti-S100β (1:1000 Sigma Clone SH-B1 #S2532), Rabbit anti-NG2 (1:250 Millipore #AB5320), rabbit anti-OLIG2 (1:1000 Millipore #AB9610), rabbit anti-IBA1 (1:500 Wako #019-19741), and rabbit anti-Ki67 (1:250 Thermo Scientific #RM-9106) For double or triple labeling, primary antibodies were simultaneously incubated and further processed for each antibody. For GFP, GFAP, Dcx, S100β, OLIG2, IBA1, Ki67, and NeuN, a fluorescent-tagged secondary antibody was used (1:100-1:500, Jackson ImmunoResearch). For GFP, *NEUROD1*, and NG2 (slide mounted), primary antibody incubation was followed with an appropriate biotin-tagged secondary antibody (1:200, Jackson ImmunoResearch) for 1 hr at room temperature, followed by ABC (Vector Laboratories PK-6100) for 1 h, and Tyramide-Plus signal amplification (1:50, PerkinElmer NEL701001KT) for 1-3 min. Sections were counterstained with DAPI (4,6-diamidino-2-phenylindole; 1:5,000, Roche 236276). Fluorescence stained sections were mounted in a 2.5% PVA-DABCO Media (PVA Sigma #D2522, DABCO: Sigma #D2522).

Microscopic analysis and quantification

Quantification of cell number was performed by a user blinded to the experimental groups. In fluorescence labeled sections of the cortex and striatum quantification was performed using an upright microscope (BX60; Olympus), or a confocal microscope (LSM700/LSM710; Carl Zeiss Microscopy). Approximately 500 cells across each region (cortex and striatum) over a total of 3

representative sections was quantified. The numbers counted from each section were added and multiplied by 24 to estimate the total number of cells in one mouse brain.

Statistics

All of the data are expressed at mean ± S.E.M. Experimental groups were assigned by simple randomization. No statistical methods were used to pre-determine the sample size in each group, however the sample sizes are similar to those reported in previous publications (Cho K. *et al.*, 2015; Parent J.M. *et al.*, 1997; Pun R. *et al.*, 2012). Data that passed selection criteria were collected blind. Prism was used to perform statistical analysis (Version 6.0g, Graphpad Software, Inc.). Statistical differences were analyzed using two-tailed Student's t-test for the data with equal variances or Student's t-test with Satterwaite's correction for the data with unequal variance. Values of $P<0.05$ were considered statistically significant.