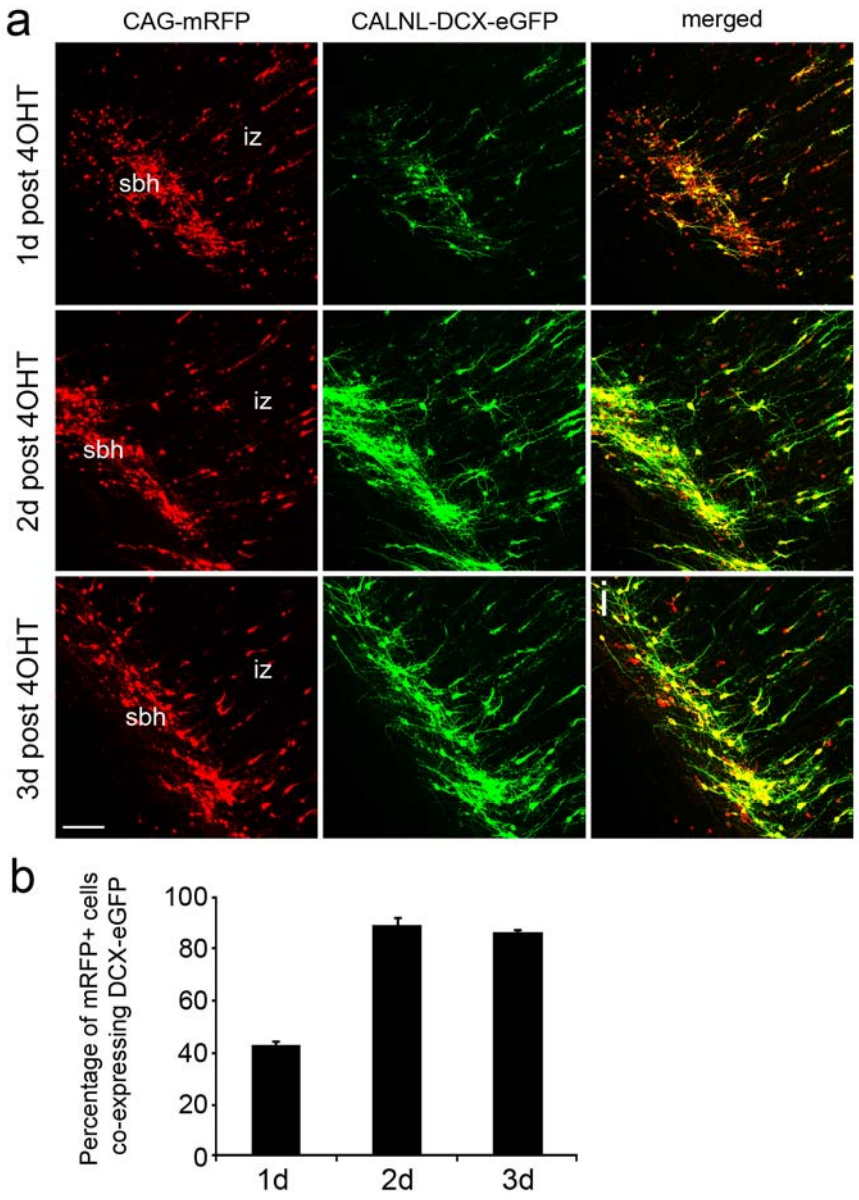
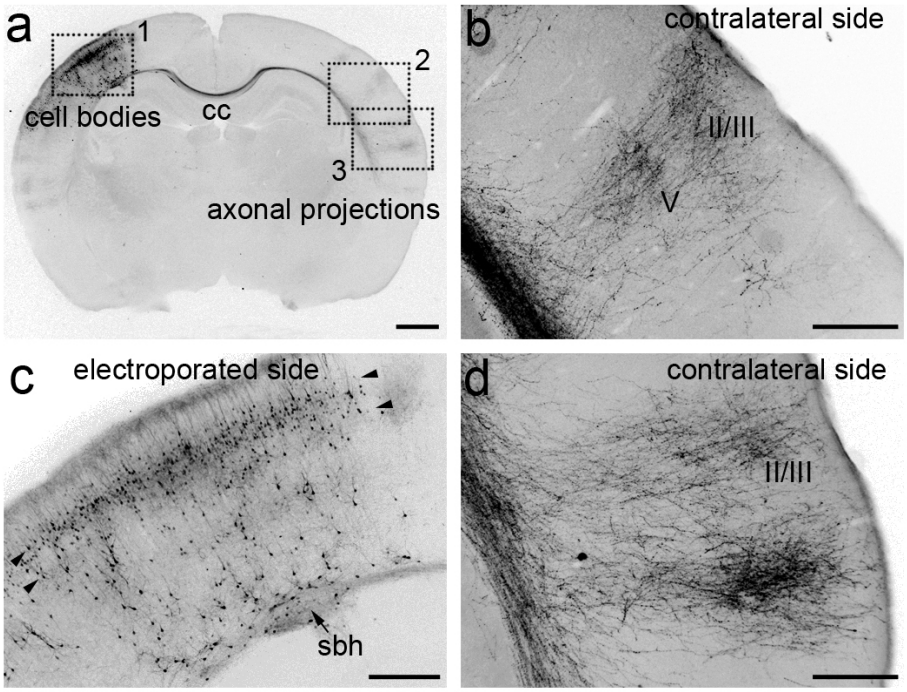
SUPPLEMENTARY INFORMATION

Dcx Re-expression Reduces Subcortical Band Heterotopia and Seizure Threshold in an Animal Model of Neuronal Migration Disorder

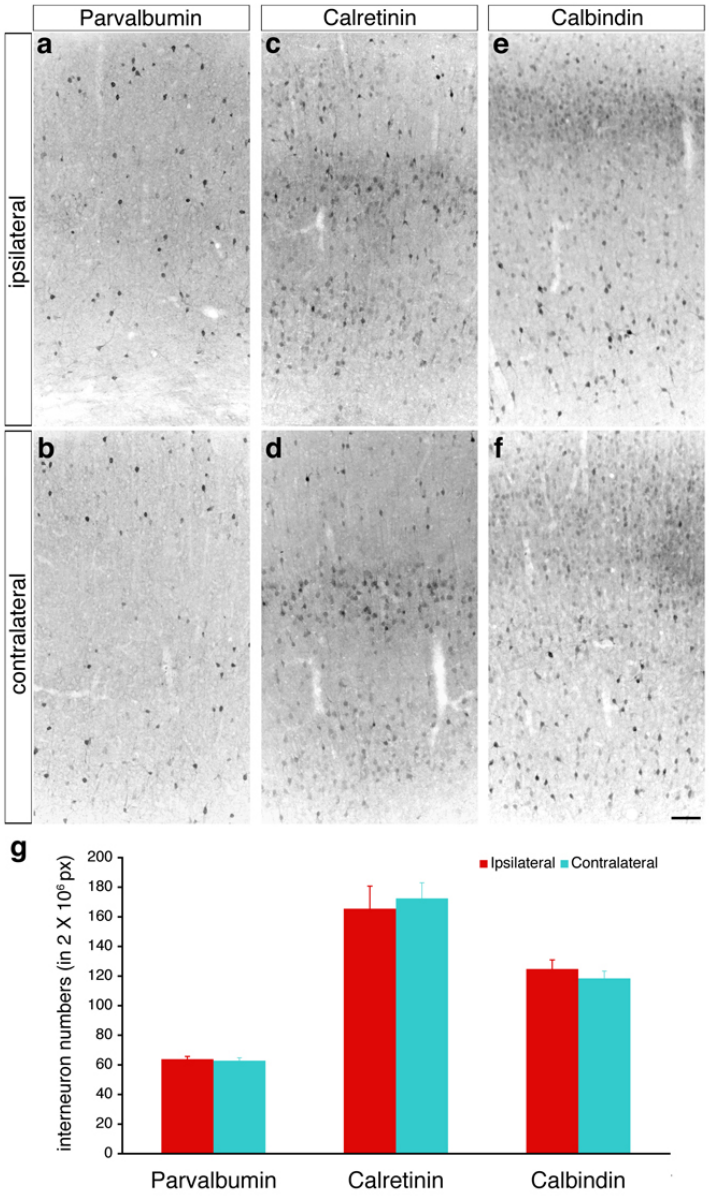
Jean-Bernard Manent, Yu Wang, YoonJeung Chang, Murugan Paramasivam, and Joseph J Loturco



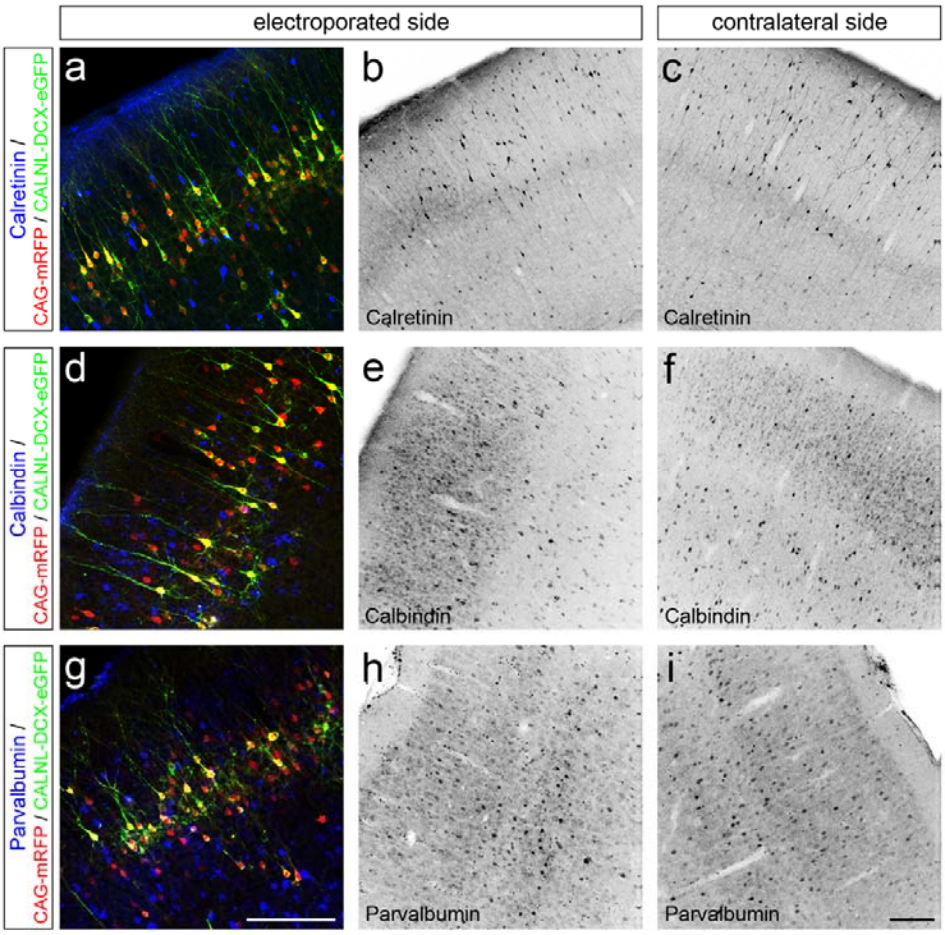
Supplementary Figure 1. Time-course of Dcx re-expression following 4-OHT.
(**a**) Photomicrographs illustrating the time course of DCX-eGFP expression 1, 2 and 3 days after 4OHT injection at birth. As shown in the bar graph (**b**), about 40% of mRFP expressing cells also co-express DCX-eGFP 1 day after 4OHT and more than 80% later on day 2. Scale bar, 100 μm.



Supplementary Figure 2. Callosal projections in the contralateral hemisphere of a P0 rescue animal at P15.
Rescued neurons located in layers 2-3 (box 1 enlarged in **c**) extend axons across the corpus callosum (cc) and project to appropriate territories and layers (mainly II/III and V) in the contralateral hemisphere (box 2-3 enlarged in **b,d**). Scale bars, 500 μm.



Supplementary Figure 3. SBH induction does not change the number of interneurons in normotopic cortex above the SBH.
Labelling and comparison of three interneuron subtypes, Parvalbumin (**a,b**), Calretinin (**c,d**), and Calbindin (**e,f**) in sections of P21 brains in which a SBH was induced with DCX RNAi in one hemisphere (ipsilateral side, **a,c,e**) and the contralateral hemisphere (**b,d,f**) served as an unmanipulated control. The SBH in each case is not shown in the images but is just below the region of ipsilateral normatopic cortex. The comparison of cell number (**g**) was between the similar region of contralateral and ipsilateral side of transfection. The number of CB+, CR+ and PV+ interneurons was determined using Image J. 2-3 brains and 3 sections per brain were used for quantification. Student-t test was used to test for statistically significant changes in number of each cell type. There were no significant differences in the number of any of the three interneuron cell types compared between ipsilateral and contralateral hemispheres (N=8, $p>0.05$, ns). Scale bar, 100 µm.



Supplementary Figure 4. Distribution of interneuron subtypes in P0 rescue animals. Photomicrographs illustrating neurons immuno-positive for Calretinin (**a-c**), Calbindin (**d-f**) and Parvalbumin (**g-h**) in the electroporated, ipsilateral, cortex (**a, b, d, e, g, h**) and the contralateral cortex (**c, f, i**). Triple labellings (**a, d, g**) do not reveal any mRFP (red) and DCX-eGFP (green) transfected cells immuno-positive for interneuron markers (in blue) consistent with interneurons not being directly electroporated by transfections directed to the pallial VZ surface. (**b-i)** shows that as with SBH containing brains there is no noticeable difference in the numbers or densities of interneurons in P0 rescue or contralateral hemisphere. Scale bars, 200 μm