

The BTX Current

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Phosphorylation of E3 Ligase Smurf1 Switches Its Substrate Preference in Support of Axon Development

INTRODUCTION

Here we report that protein kinase A (PKA)-dependent phosphorylation of Smad Ubiquitin Regulatory Factor 1 (Smurf1) can switch its substrate preference between two proteins of opposing actions on axon development.

METHOD

The method of *in utero* electroporation follows previously described procedures (Saito and Nakatsuji, 2001), with minor modifications. Timed-pregnant Sprague-Dawley rats were anesthetized at E18 with isoflurane, and the uterine horns were exposed by way of a laparotomy. Saline solution containing the expression plasmid of interest (2 mg/ml) together with the dye Fast Green (0.3 mg/ml; Sigma) was injected (1–2 μ l) through the uterine wall into one of the lateral ventricles of the embryos, and the embryo's head was electroporated by tweezer-type circular electrodes across the uterus wall, and five electrical pulses (50 V, 50-ms duration at 100-ms intervals) were delivered with a square-wave electroporation generator (model ECM 830, BTX, Inc.). The uterine horns were then returned into the abdominal cavity, the wall and skin were sutured, and the embryos continued their normal development.

DISCUSSION

To further determine the functional relevance of Thr306 phosphorylation of Smurf1 on neuronal development *in vivo*, we used *in utero electroporation* (Saito and Nakatsuji, 2001) to express construct encoding Smurf1T306D, Smurf1T306A, or shRNA against Smurf1 (shRNA-Smurf1) in a subpopulation of neural progenitor cells. For better observation of polarity phenotype, brain slices were obtained from rat pups at P4 when most cells had arrived at cortical plate. Cortical neurons were visualized by coexpressing the fluorescent marker protein EGFP or tdTomato. Control neurons (expressing marker protein alone) were mostly (93%) located in the cortical plate (CP) and exhibited polarized morphology (Figures 6A and 6B), with dendritic arbors oriented toward the pial surface and the axon oriented radially in CP and horizontally near the intermediate zone (IZ) and subventricular zone (SVZ). Compared to control neurons expressing the EGFP construct, cortical neurons expressing phosphorylation mimicking Smurf1T306D arrived at CP without obvious migration defect, with a high percentage of cells exhibiting complex morphology with multiple highly branched long processes (termed "multipolar"), and reduced percentage of cells exhibiting polarized morphology (unipolar or bipolar). Finally, neurons expressing shRNA-Smurf1 showed severe defects in polarization and radial migration, with most cells accumulating in IZ/SVZ and exhibiting only short processes. Thus, normal PKA-dependent Smurf1 phosphorylation at Thr306 is required for proper polarity formation and radial migration of newly generated cortical neurons, two tightly linked events during neuronal development *in vivo*.

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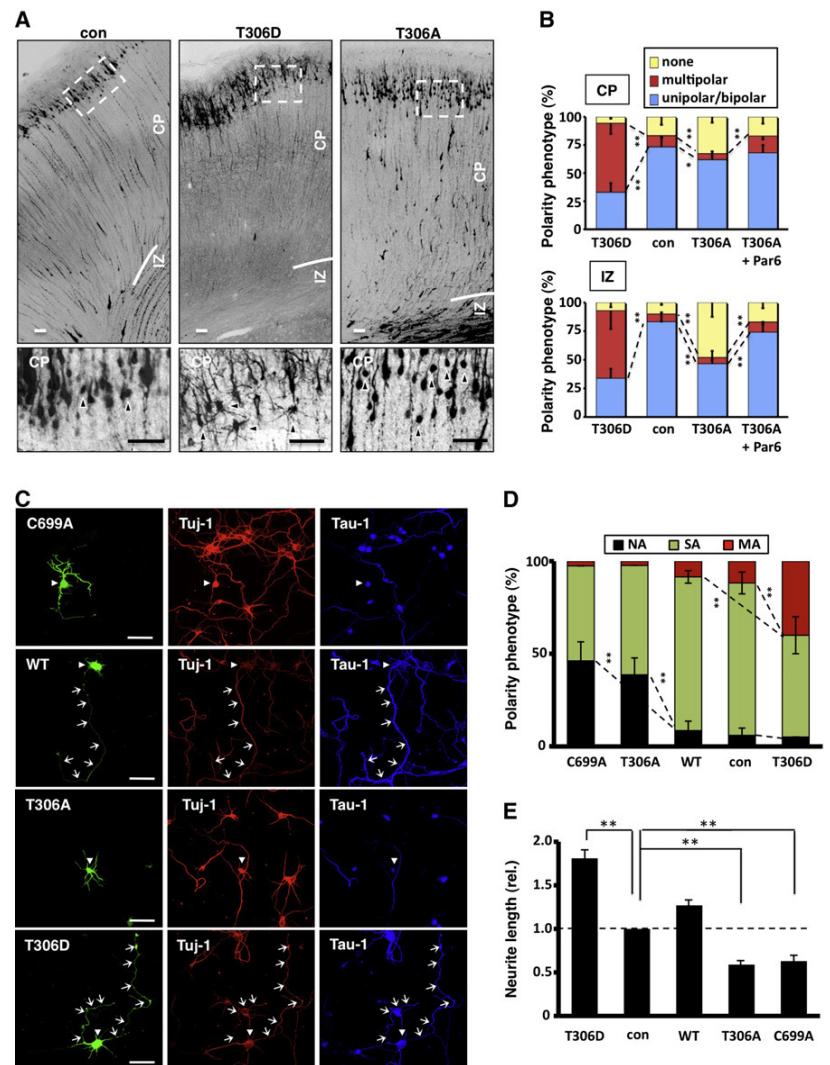


Figure 5. RhoA Degradation Is Required for Spontaneous and BDNF-Induced Axon Initiation (A) RhoA inhibition promoted spontaneous axon formation. (A1) Immunostaining with axon-specific Tau-1 and dendrite-specific MAP-2 showed a 5-DIV cultured hippocampal neuron that was exposed to the ROCK inhibitor Y-27632 (5 mM) since 4 hr after cell plating. (A2 and A3) Similar to that in (A1), except that the neuron was transfected with a constitutively active RhoA-CA (A2) or Smurf1-resistant RhoAk6,7R (A3), showing no axon formation or a short axon (marked by asterisk). Bar, 25 μ m. (A4) Summary graph showing polarization phenotypes as in A1–A3. Data represent mean \pm SEM (n = 3, 100 cell each; *p < 0.01; **p < 0.001, Tukey test). (B) RhoA inhibition is sufficient to initiate axon formation, and its stabilization prevented BDNF-induced axon initiation. (B1 and B2) Images of hippocampal neurons with the axon initiated on the stripe coated with Y-27632 (B1), and off the BDNF-coated stripe for neurons expressing RhoAk6,7R (B2), immunostained on 3 DIV for axon identification. Stripe width, 50 μ m. (B3) Summary graphs showing the percentage of axons that were initiated on or off the stripes coated with Y-27632 or BDNF. Only neurons with the soma located at the stripe boundary were counted. Data represent as mean \pm SEM (n = 3, 90 cells each; **p < 0.01, Tukey test).

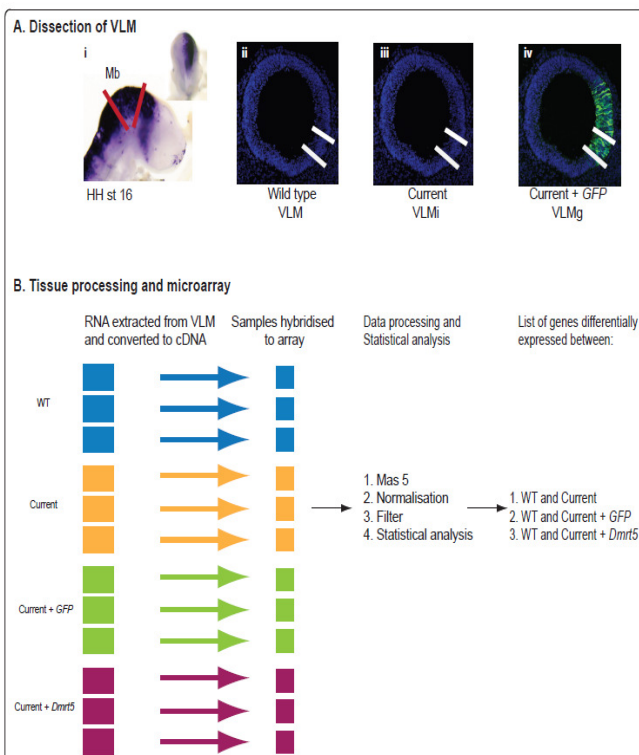


Figure 1 Experimental strategy. (A) Dissection of the ventral lateral midbrain (VLM) region. (i) *In situ* sagittal view of a HH st16 electroporated embryo expressing pCAB-IRES-Dmrt5 construct (dorsal view in the inset). Red lines indicate the midbrain (Mb) region, which was dissected out. (ii–iv) Coronal sections of midbrain. White lines mark the VLM region. This region was isolated from (ii) control embryos (VLM), (iii) VLM exposed to current (VLMi), (iv) VLM exposed to current + GFP (VLMg), and VLM exposed to current + Dmrt5 (VLMd; image not shown) for investigation of transcriptional profiles by microarray analysis. (B) Tissue processing and microarray analysis. Six VLM tissues were pooled for each biological replicate, and three biological replicates were used for each condition. cDNA was isolated from these pools and hybridized to the Affymetrix Chicken Genome Array. Following MAS5, normalization and filtering, genes whose expression differed significantly between the wild type (WT; VLM) and VLMi, VLMg and VLMd were identified by one-way ANOVA.

450489, 450488—5 & 7 mm Platinum Tweezertrodes

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Effects of *in ovo* electroporation on endogenous gene expression: genome-wide analysis

Performed with ECM 830 & Tweezertrodes

INTRODUCTION

In ovo electroporation has been used to study development in mouse embryos [12] along with other vertebrates, including *Danio rerio* [13] and *Xenopus laevis* [13,14] and non-vertebrates such as *Drosophila melanogaster* [15] and *Ascidacea* [16] (for reviews, see [17,18]). However, *in ovo* electroporation has been most widely applied to avian embryos because the avian embryo develops *in ovo* and has a planar topology. These characteristics greatly facilitate injection of the DNA construct, placement of electrodes and incubation of the electroporated embryo.

METHOD

HH st10 embryos were windowed and electrodes placed on either side of the developing head. A solution containing 7 μ g/ μ l DNA construct pCAB-IRES-GFP or pCAB-IRES-Dmrt5, 2% polyvinyl alcohol, 0.05% Fast Green in water was injected into the developing midbrain. Five 12 V square wave pulses of 50 ms duration with an interval of 100 ms were applied across the electrodes using an ECM830 Electro-S Square Porator (BTX Inc.). Following exposure to current, embryos were incubated at 37°C for 24 hours; embryos were collected at HH st16.

DISCUSSION

In ovo electroporation is an extremely powerful technique to investigate the function of genes and regulatory regions during development. With advances in methods used to analyze electroporated embryos, such as microarray analysis and next generation sequencing, a better understanding of the effects of this technique are necessary. To this end we carried out a genome-wide analysis of the effect of this technique on endogenous gene expression. Our analysis has established that the electric current used during electroporation (5 \times 50-ms pulses of 12 V) has a minimal affect on gene expression, causing a change in expression of only 21 endogenous genes. The upregulation of Hsp25, which encodes a heat shock protein known to be upregulated 24 hours after heat shock in response to protein aggregation [37], combined with changes in two members of the ubiquitination pathway indicate that some protein denaturation may have occurred upon exposure to current.

We have also established that the current and expression of exogenous DNA, in the form of GFP, has a small but statistically significant effect on gene expression, with no toxicity pathways significantly activated. The expression of exogenous DNA has a greater affect on endogenous gene expression than exposure to current alone, with 111 genes affected by exposure to current + GFP.

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The BTX Current

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Targeted electroporation of defined lateral ventricular walls: a novel and rapid method to study fate specification during postnatal forebrain neurogenesis

Performed with
ECM 830

Background

Postnatal olfactory bulb (OB) neurogenesis involves the generation of granule and periglomerular cells by neural stem cells (NSCs) located in the walls of the lateral ventricle (LV). Recent studies show that NSCs located in different regions of the LV give rise to different types of OB neurons. However, the molecular mechanisms governing neuronal specification remain largely unknown and new methods to approach these questions are needed.

Discussion

In this study, we refine electroporation of the postnatal forebrain as a technique to perform precise and accurate delivery of transgenes to NSCs located in distinct walls of the LV in the mouse. Using this method, we confirm and expand previous studies showing that NSCs in distinct walls of the LV produce neurons that invade different layers of the OB. Fate mapping of the progeny of radial glial cells located in these distinct LV walls reveals their specification into defined subtypes of granule and periglomerular neurons.

Conclusions

Our results provide a baseline with which future studies aiming at investigating the role of factors in postnatal forebrain neuronal specification can be compared. Targeted electroporation of defined LV NSC populations will prove valuable to study the genetic factors involved in forebrain neuronal specification.

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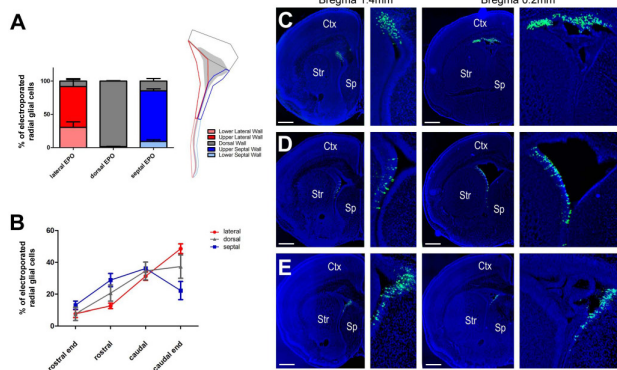


Figure 3. Targeted electroporation of defined lateral ventricle walls. (A) Quantification of the percentage of GFP+ RGCs in subregions of the LV following lateral, dorsal and septal electroporation. Note the efficient targeting of distinct LV walls with different positioning of the electrodes. The most ventral regions of the lateral and septal LV walls, however, show lower numbers of electroporated cells. Error bars represent standard error of the mean. (B) Quantification of the percentage of GFP+ RGCs at defined rostro-caudal levels of the LV. (C-E) Representative overviews of the distribution of electroporated (GFP+, green) cells at two rostro-caudal levels of the LV (that is, Bregma 1.4 mm and 0.2 mm). DAPI (blue) was used as a nuclear counterstain. Scale bars: 1 mm. Ctx, cortex; EPO, electroperoration; Sp, septum; Str, striatum.

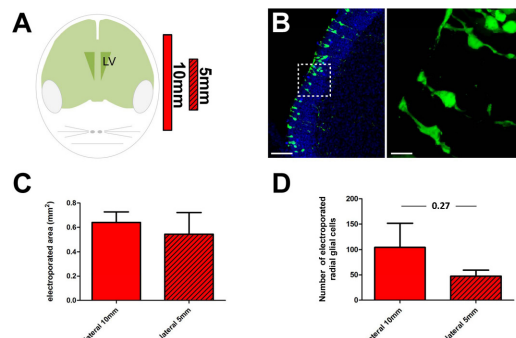
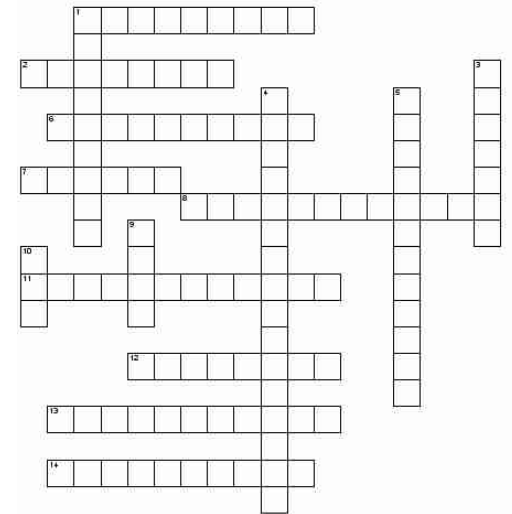


Figure 1. Electroporation efficiency using electrodes of different sizes. (A) Schematic representation of the position and size (10 mm and 5 mm) of the electrodes used in the first part of this study. Only the positive pole is represented. (B) Representative example of electroporated radial glial cells (RGCs) expressing high levels of GFP at 1 day post-electroporation. The right panel shows a higher magnification of the region surrounded by the dotted box. Only cells with clear RGC morphology, that is, with an end foot contacting the ventricle surface and a main apical process, were counted. DAPI (blue) was used as a nuclear counterstain. (C,D) Measurements of the electroporated area (C) and of the number of electroporated RGCs (D) when 10-mm (filled bar) or 5-mm (hatched bars) diameter electrodes were used. Please refer to Materials and methods and Additional file 1 for experimental details. Note that the use of smaller electrodes does not improve the precision of electroporation (that is, did not decrease the electroporated area size), but tend to decrease the number of electroporated cells. Error bars represent standard error of the mean. Scale bars: 50 µm and 10 µm in (B) (left and right panels, respectively). LV, lateral ventricle.

BTX CROSSWORD

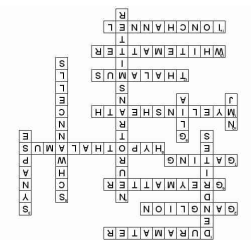


Across

- 1 Is a thick tough membrane lying close to the cranium and vertebrae. It is separated from the arachnoid membrane by the subdural space.
- 2 Refers to a group of functionally related cell bodies in the PNS.
- 6 Is that part of the nervous system that contains neuronal cell bodies.
- 7 Is the active transition of an ion channel from an open to a closed state.
- 8 Is part of the diencephalon. It lies just above the pituitary gland and has a number of important neural and endocrine functions.
- 11 Is the fatty layer of insulation that is wrapped around most large nerve fibers and which allows for rapid, unattenuated conduction of the action potential.
- 12 Is part of the diencephalon and is important not only in relaying information to the cerebral cortex but in controlling the degree of arousal and attention within CNS neural systems.
- 13 Are those parts of the CNS that primarily contain nerve fibers and glial cells.
- 14 Is a transmembrane pore that allows ions to flow across a membrane. It exists in at least an open and closed state and is regulated by either a change in membrane potential (voltage-gated channels) or the binding of a specific neurotransmitter or chemical substance (ligand-gated or chemically activated channels)

Down

- 1 The neuronal cell processes that taper from the soma outwards, branch profusely and are responsible for conveying information to the neuron.
- 3 Is the specialized site of communication between two cells, typically neurons.
- 4 A chemical substance released by a presynaptic nerve terminal that diffuses and binds to specific postsynaptic receptors which leads to an alteration in current flow in that postsynaptic cell.
- 5 Are found in the PNS and are responsible for providing the myelin sheath to axons in peripheral nerves.
- 9 Form the other major cell class in the nervous system with neurons.
- 10 Is the point of communication between the lower motoneuron axon nerve terminal and the muscle fiber it innervates.



BTX NEWS

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BTX, the Electroporation Experts, are proud to announce the launch of their new and improved website. www.BTXonline.com. The new website is completely revamped and now includes many new features and tools designed to keep users abreast of all the latest in today's research climate. New Features Include:

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