Science Advances

AAAS

advances.sciencemag.org/cgi/content/full/2/2/e1501733/DC1

Supplementary Materials for

Bidirectional radial Ca^{2+} activity regulates neurogenesis and migration during early cortical column formation

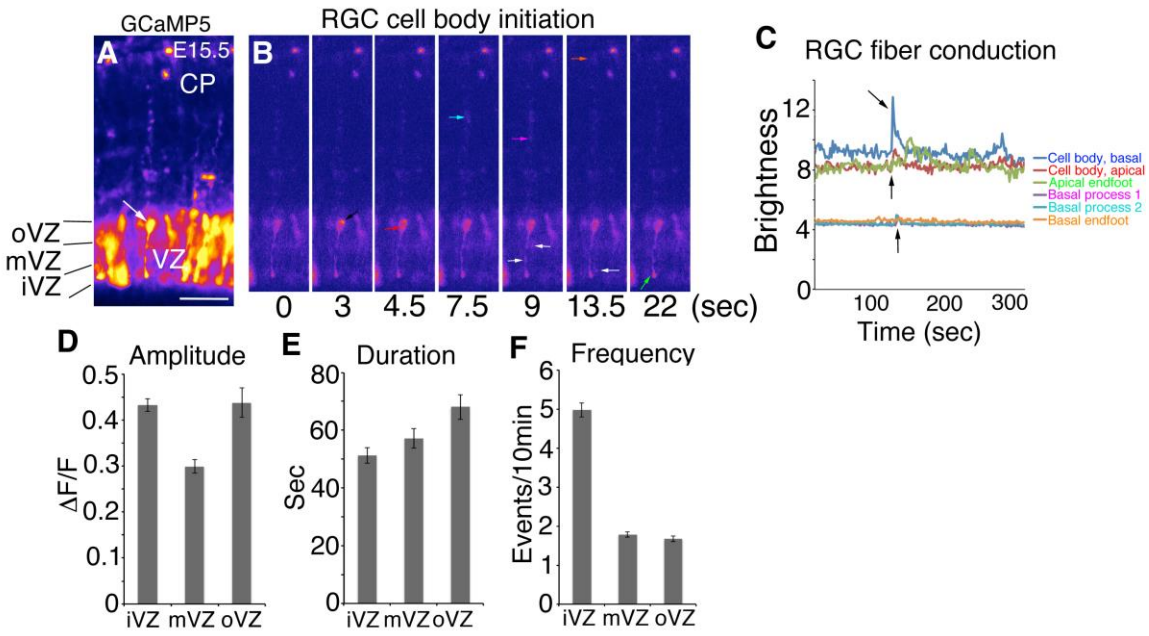
Brian G. Rash, James B. Ackman, Pasko Rakic

Published 26 February 2016, *Sci. Adv.* **2**, e1501733 (2016)
DOI: 10.1126/sciadv.1501733

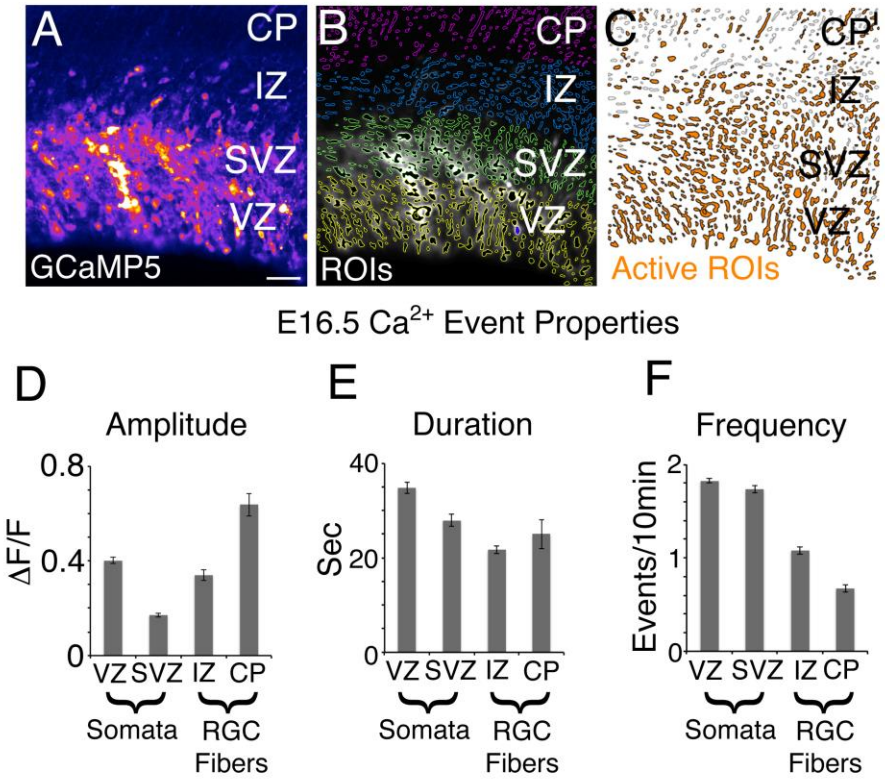
This PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at advances.sciencemag.org/cgi/content/full/2/2/e1501733/DC1)

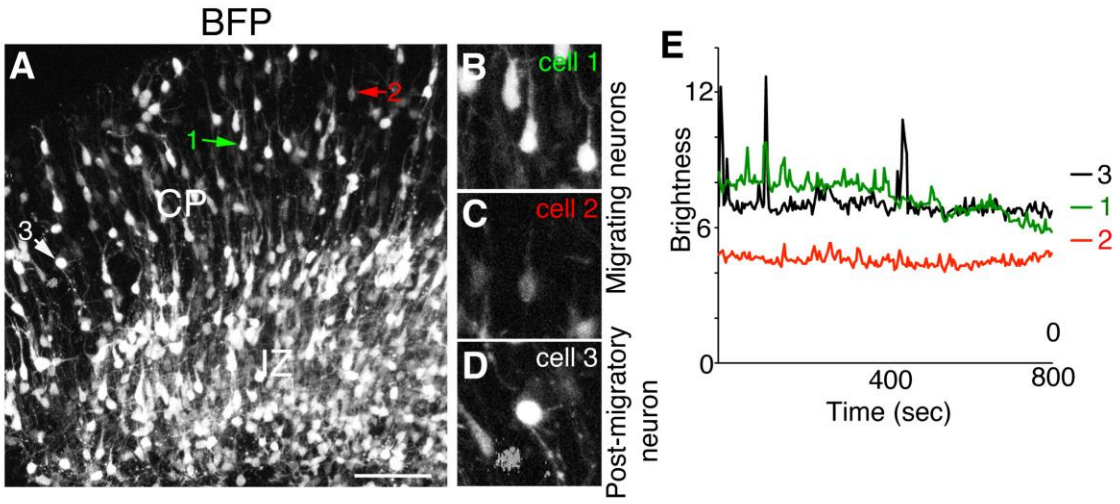
Video S11 (.avi format). Impairment of Ca^{2+} activity by 2-APB reduces RGC motility.
Video S12 (.avi format). Calcium burst activity during initial neuronal migration.
Video S13 (.avi format). En passant Ca^{2+} activity in an RGC fiber repeatedly induces a neuronal Ca^{2+} response.
Video S14 (.avi format). En passant Ca^{2+} activity in an RGC fiber induces a neuronal Ca^{2+} response.
Video S15 (.avi format). Ex vivo Ca^{2+} transient activity at E11.0.
Video S16 (.avi format). Baseline Ca^{2+} activity before FGF2 application.
Video S17 (.avi format). FGF2 induces Ca^{2+} activity in RGCs.
Video S18 (.avi format). Baseline Ca^{2+} activity before FGF2 application.
Video S19 (.avi format). Increased PCTs in RGC fibers after FGF2 exposure.
Video S20 (.avi format). Notch activation increases Ca^{2+} activity in RGCs but reduces bursting associated with neurogenesis.



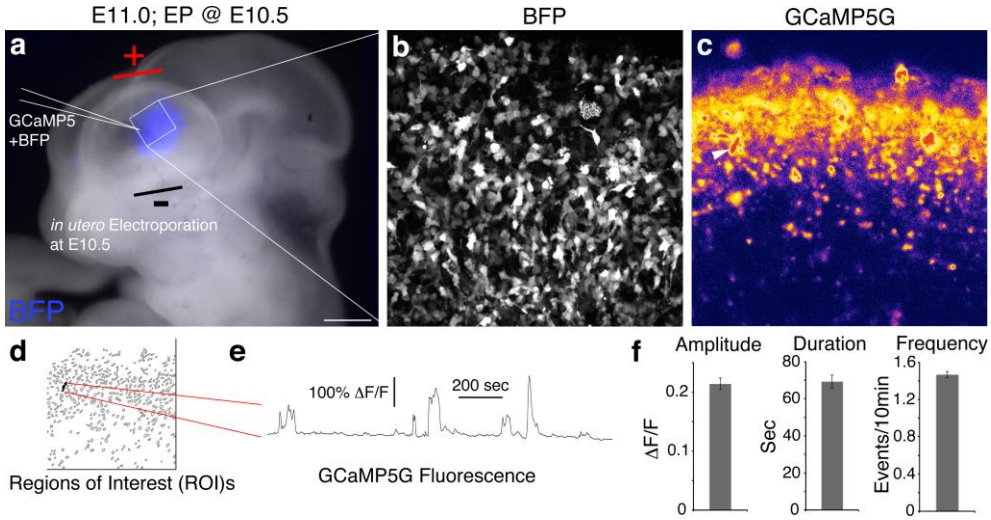
Supplementary Fig. 1: **Conduction of a RGC cell body-initiated calcium transient to apical and pial endfeet**. **(A)** An isolated RGC with visible apical and basal fibers and endfeet (arrow). **(B)** Time-lapse series showing initiation of the Ca^{2+} transient in the RGC cell body followed by responses along the RGC fiber. **(C)** Calcium activity traces at each point. A short interval of approximately 10 sec separated the cell body event from distant fiber events. **(D** to **F)** The VZ was divided into three zones (Inner, iVZ; Middle, mVZ; and Outer, oVZ) and Ca^{2+} event properties from each zone were extracted in Matlab and summarized. The iVZ contains some mitotic RGC cell bodies as well as apical endfeet, and was the most active zone in the cortical wall. *$p < 0.05$; **$p < 0.001$. Error bars, mean ± s.e.m. Scale bar: 50 μm.



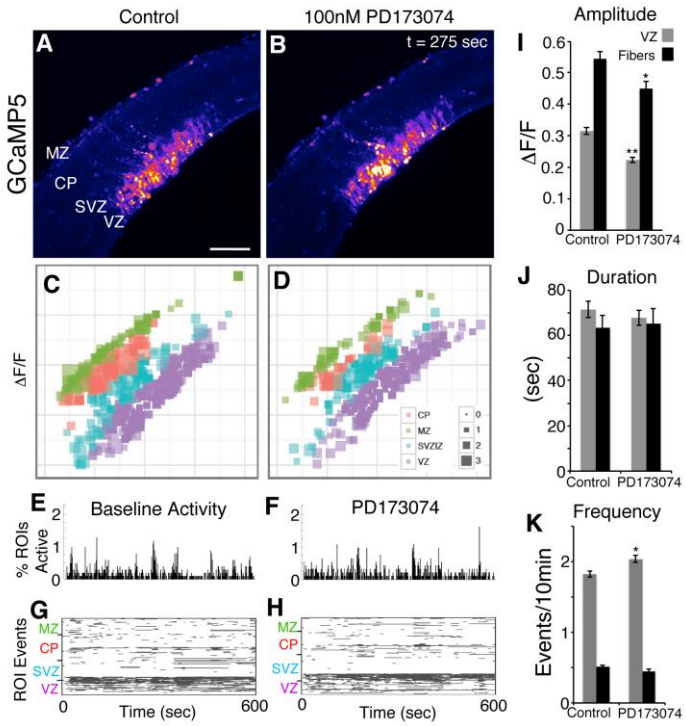
Supplementary Fig. 2. **An inverse amplitude/frequency relationship in RGC fibers**. **(A)** GCaMP5 fluorescence in a cortical slice at E16.5 after E14.5 IUE. **(B)** Corresponding Matlab ROIs. **(C)** Active ROIs **(D to F)** Population-level comparison showing amplitude, duration, and frequency Ca^{2+} event properties as a function of VZ/SVZ somata or IZ or CP fiber ROIs. Note that amplitude increases towards the pial surface while frequency decreases. n=1254 events (VZ); n=573 (SVZ); n=265 (IZ); n=161 (CP). Error bars, mean ± s.e.m. Scale bar: 20 μm.



Supplementary Fig. 3. **Neurons exhibit decreased calcium transient activity at later stages of migration**. **(A)** BFP Z-stack of an E17.5 cortical slice electroporated with BFP/GCaMP5 at E14.5 showing a sample of migrating neurons at E17.5. Many migrating neurons did not exhibit detectable Ca^{2+} transients during the recording period at this age. **(B** to **D)** High magnification images of two migrating neurons **(B** and **C)** and one putative post-migratory neuron **(D)**. **(E)** Representative traces from these cells indicated that migratory neurons (red and green traces) in the cortical plate do not show frequent, high amplitude Ca^{2+} transients. In contrast, post-migratory neurons were more active (black trace). Scale bar: 100μm.



Supplementary Fig. 4. Calcium transients in cortical progenitors at E11.0 ex vivo. **(A)** An intact E11.0 embryo co-electroporated with pCAG-BFP (blue color) and pCMV-GCaMP5G at E10.5 was removed from its mother, placed in 37°C ACSF and imaged by confocal videomicroscopy. **(B)** BFP Z-stack of boxed region in **(A)**. **(C)** GCaMP5G image of a single focal plane. Only cortical progenitor cells were labeled by electroporation from the telencephalic ventricles. Presence of a heartbeat confirmed survival of the embryo during the imaging period. Confocal GCaMP5G movies (see **Supplementary Video S1**) were obtained by imaging through the overlying ectoderm and mesenchyme and showed changes in GCaMP5G fluorescence intensity. **(D)** Regions of interest (ROI)s drawn automatically in Matlab showing individual cell outlines of the image in **(C)**. **(E)** Example of a ΔF/F fluorescence trace automatically extracted using Matlab for the cortical progenitor in **(C)** (arrowhead). The population average amplitude, duration and frequency of Ca^{2+} events are plotted in **(F)** from 719 calcium events in 405 ROIs during a 26 min recording. Scale bar: 300 μm **(A)**; 50 μm **(B** and **C)**.



Supplementary Fig. 5. Calcium activity reduced by the FGFR antagonist, PD173074. (A and B) GCaMP5 signal in control and PD173074 conditions. **(C and D)** ΔF/F amplitude of active ROIs plotted spatially; square size represents relative amplitude. **(F** to **H)**. **(E and F)** Percent ROIs active. **(G and H)** Rasterplots of all Ca^{2+} events detected, binned according to embryonic zone (VZ, SVZ, CP, MZ). **(I, J and K)** Calcium event properties of VZ ROIs compared with fiber ROIs, including ΔF/F amplitude, duration and frequency. *$p < 0.05$; **$p < 0.001$, t-test. Error bars, mean ± s.e.m. Scale bar: 100 μm.