Supporting Information

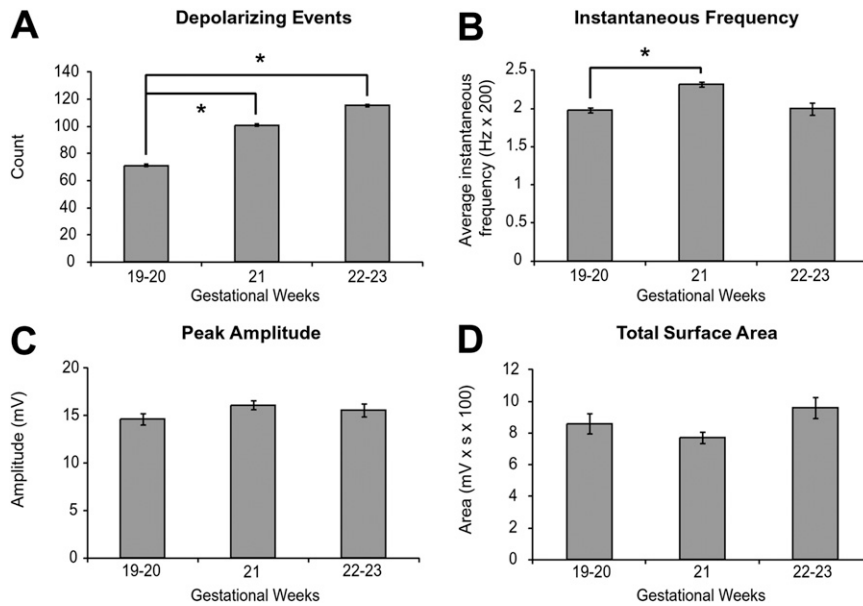## Moore et al. 10.1073/pnas.1405253111

Fig. S1. Spontaneous electrical activity across three gestational ages. (*A*) The number of depolarizing events (threshold set at 5 mV; see *Methods*) detected during a 5-min recording session. (*B*) Average instantaneous frequency of depolarizing events during the 5-min recording session. (*C*) Average peak amplitude of all depolarizing events, including action potentials. (*D*) Sum of the measured surface area of the data trace. *$P < 0.05$. The number of subplate (SP) neurons included in age groups 19–20, 21, and 22–23 gestational weeks (gw) was $n = 44$, 45, and 29, respectively.
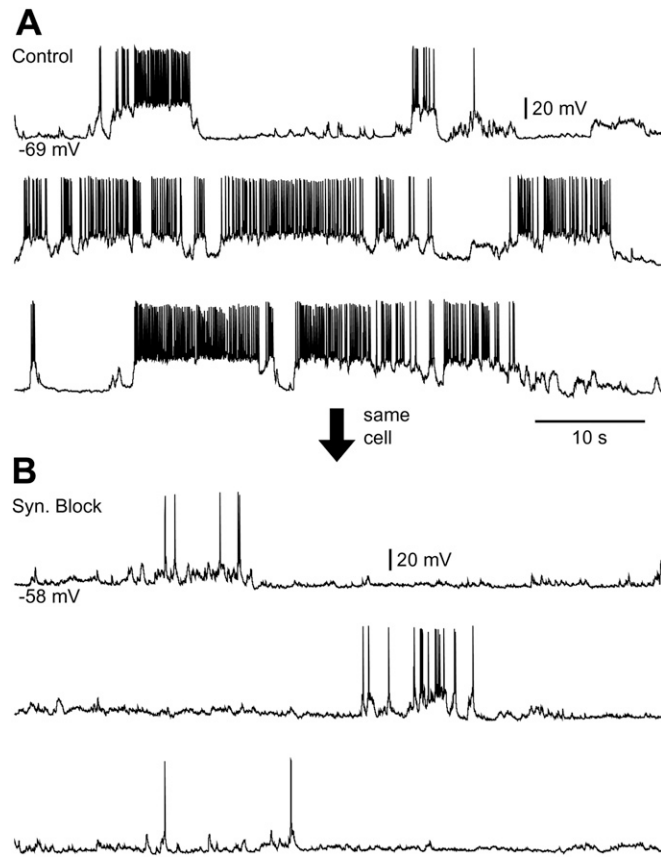
Fig. S2. Synaptic blockers reduced but did not eliminate spontaneous electrical activity. (*A*) Spontaneous activity in human SP neuron at 21 gw. (*B*) Same cell as in *A* is bath perfused with a mixture of synaptic blockers including 6,7-dinitroquinoxaline-2,3(1H,4H)-dione (DNQX; 20 μM), DL-2-amino-5-phosphonovaleric acid (APV; 20 μM), bicuculline (20 μM), and strychnine (20 μM).

A

Experiment 1

Sample:	Ganglionic Eminence	Cortical Mantle 1	Cortical Mantle 2	Subventricular zone (SVZ)	Cortical Plate (CP)	Human Adult Brain Total RNA
Age:	17 gw	17 gw	17 gw	19 gw	19 gw	commercial sample
Cxn26	negative	positive	positive	positive	positive	positive
Cxn32 (Var1)	negative	negative	negative	negative	negative	negative
Cxn32 (Var2)	negative	negative	negative	negative	negative	positive
Cxn36	negative	negative	negative	positive	positive	negative
Cxn43 (Primer 1)	positive	negative	negative	negative	negative	negative
Cxn45	positive	positive	positive	positive	positive	negative
Pxn1 (Primer 1)	positive	negative	negative	negative	negative	negative
Beta Actin	positive	positive	positive	positive	positive	positive

B

Experiment 2

Sample:	Cortical Plate 1	Cortical Plate 2	Cortical Mantle	Cortical Mantle	Isolated Cells	Human Fetal Brain Total RNA
Age:	17 gw	17 gw	18 gw	20 gw	22 gw	commercial sample
Cxn26 (Primer 1)	negative	positive	negative	(black)	(black)	positive
Cxn26 (Primer 2)	negative	negative	negative	(black)	(black)	negative
Cxn32 (Var1)	negative	negative	negative	negative	negative	negative
Cxn32 (Var2)	negative	negative	negative	negative	negative	negative
Cxn36 (Primer 1)	negative	negative	negative	negative	negative	negative
Cxn36 (Primer 2)	positive	positive	negative	(black)	(black)	positive
Cxn43 (Primer 1)	negative	negative	negative	(black)	(black)	negative
Cxn43 (Primer 2)	positive	positive	negative	(black)	(black)	negative
Cxn45	negative	negative	negative	(black)	(black)	negative
Cxn47	negative	negative	negative	(black)	(black)	negative
Pxn1 (Primer 1)	positive	positive	positive	negative	negative	positive
Pxn1 (Primer 2)	negative	negative	negative	negative	negative	negative
Beta Actin	positive	positive	positive	positive	positive	positive

Fig. S3. Gene expression analysis in human fetal cortex during the second trimester of gestation. (*A*) This table corresponds with the PCR data shown in Fig. 5*A*. Green boxes indicate that the expression of a gene was positively detected (positive). White boxes signify that tissue was tested but the expression of that gene was not detected (negative). (*B*) A second experiment was performed by using: (*i*) new tissue samples; (*ii*) the same primers as used in experiment 1 (primer 1); as well as (*iii*) newly designed primers (primer 2). Black boxes mean that the PCR was not attempted due to a low RNA yield in these two samples. Because of limited availability, we had to prioritize the genes that were screened. As a consequence, only five genes in two human samples, 22 gw and Isolated cells, were tested. Isolated cells means that human fetal brain tissue was dissociated, and the resulting cells were cultured. We used isolated cells because we could not obtain fresh tissue samples at older age (22 gw) suitable for PCR analysis. Cortical plate marks human fetal brain slices in which the subventricular zone (SVZ) was removed. "Cortical Mantle" marks human fetal brain slices encompassing the entire cortical wall, including the ventricular zone and SVZ. The following primers were used to perform RT-PCR:

ACTB: F 5′-CCTCGCCTTTGCCGATCC, R 5′-GATGCCGTGCTCGATGGGGT;

Cx 26: F 5′-TCCCGACGCAGAGC AAAC, R 5′-AGGGTGTTGCAGACAAAGTC;

Cx 30: F 5′-CTTCACCCGAAACCCGACG, R 5′-CCACTGGAGTGCCTAGA AGC;

Cx31.1: F 5′-TTCCCCGCTTCTGGATATGAAA, R 5′-GGCGAGTATTGCAGTCGAAG;

Cx32: F 5′-GACATGAGAC CATAGGGGAC, R 5′-GTGTCCCTGAGATGTGGACC;

Cx36: F 5′-ACTCCACTATGATCGGAAGGATCC, R 5′-CTGCAGG GTGTTGCACAC;

Cx43: F 5′-TGAGTGCCTGAACTTGCCTT, R 5′-TGACACCATCAGTTTGGGCA;

Panx1: F 5′-ATTCA TATTGCTGGGCGGCT, R 5′-GCAGCCTTAATTGCACGGTT.

Fig. S4. Octanol diminishes spontaneous electrical activity of human SP neurons in acute brain slices. (*A*) Acute brain slice harvested from human fetal cortex: gw 19. Whole-cell recording from a positively identified SP neuron (*Results* and Fig. 1). Spontaneous activity recorded (in voltage monitoring mode) before (Control) and during (Octanol, 1 mM) drug application. Dashed line, resting membrane potential (V_R). (*B*) Parameters of spontaneous electrical activity quantified off-line by using a Clampfit 10.2 feature called Threshold Search. Depol. Events, average number of depolarizing events per 5 min recording time. Peak amplitude, average amplitude of depolarizing events; duration, average half-width of depolarizing events; surface area, average area under the curve. Control, white; octanol, gray. *$P < 0.05$.