Supporting Information

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Fig. S1. hESC-derived forebrain cultures contain significant numbers of GABAergic neurons. (*A*) Confocal images of 6-wk-old human cultures alone (*Left*) and mouse–human cultures (*Right*) that display GABA⁺ neurons (green). Note that in cocultures, GABA⁺ cells (arrows) and GABA⁻ cells (arrowheads) express the human-specific nuclear antigen (blue). (*B*) Pooled data demonstrate that hESC-derived cultures are comprised of ~40% GABAergic neurons, which is not affected by coculture with mouse cortical cells.



Fig. S2. Spontaneous bursting is maintained in mouse cortical networks for at least 2 mo. (*A*) Representative whole cell recordings (–70-mV holding potential) from mouse cortical neurons at 1 wk (upper trace) and 8 wk (lower trace) in vitro. (*B*) Representative traces from mouse (*Upper*) and human (*Lower*) that illustrate "superbursts" in each cell type.



Fig. S3. Bursting amplitudes in hESC-derived neurons are significantly smaller than those in mouse neurons. (*A*) Representative voltage-clamp traces of individual bursts in hESC-derived neurons at 2 wk (*i*), 4 wk (*ii*), and 8 wk (*iii*) in coculture or bursts in a mouse neurons (*iv*). (*B*) Pooled raw data demonstrate a significantly smaller mean bursting amplitude in hESC-derived neurons compared with mouse cortical neurons (P < 0.001 for all time points; n = 4). (*C*) Pooled data normalized to cell size demonstrate a significantly smaller bursting amplitude in hESC-derived neurons at 2, 4, and 8 wk (*P < 0.05; n = 4). Data are means \pm SEM.



Fig. 54. Mouse cortical neurons display simultaneous bursting. Representative current-clamp recordings from two mouse cortical neurons at 6 wk in vitro.



Fig. S5. Multiple postsynaptic currents are triggered in individual mouse neurons during light stimulation. (*A*) Whole-cell recording from a mouse cortical neuron during brief (10-ms) light stimulations. Lower trace reveals multicomponent, large-amplitude postsynaptic responses between ~10 and 100 ms following an individual light stimulation. (*B*) Whole-cell patch-clamp recordings from two hESC-derived neurons in coculture with mouse neurons for 8 wk illustrates simultaneous AP generation in both cells upon light stimulation.



Fig. S6. Spontaneous postsynaptic currents make light-induced currents difficult to detect in some neurons. Representative traces from two different mouse cortical neurons during light stimulation after 6 wk in coculture with ChR2-expressing human neurons.



Fig. S7. Morphology of transplanted hESC-derived neurons. (A) Compressed confocal z-stack of a transplanted hESC-derived neuron expressing both ChR2mCherry and the human nuclear antigen. (B) 3D reconstruction of the neuron in A demonstrating multipolar morphology. Scale bars, 50 μ m.

 Table S1. Basic physiological properties of hESC-derived neurons in coculture with mouse cortical cultures

	Cap (pF)	Rin (GΩ)	RMP (mV)
2 wk			
(M)	85.6 ± 5.3	0.23 ± 0.05	-70.9 ± 2.4
(H)	22.6 ± 2.4	1.5 ± 0.1	-46.3 ± 1.7
4 wk			
(M)	87.6 ± 6.6	0.19 ± 0.04	-68.4 ± 4.9
(H)	26.9 ± 2.5	1.1 ± 0.1	-57.1 ± 2.1
6 wk			
(M)	92.1 ± 7.2	0.22 ± 0.04	-72.3 ± 2.9
(H)	30.9 ± 3.1	1.0 ± 0.2	-60.2 ± 3.7
8 wk:			
(M)	90.1 ± 6.3	0.19 ± 0.03	-71.4 ± 3.2
(H)	34.4 ± 4.1	0.9 ± 0.1	-61.7 ± 4.2

H, human; M, mouse.

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