

Electronic Supplementary Information:

Cortical spheroids display oscillatory network dynamics

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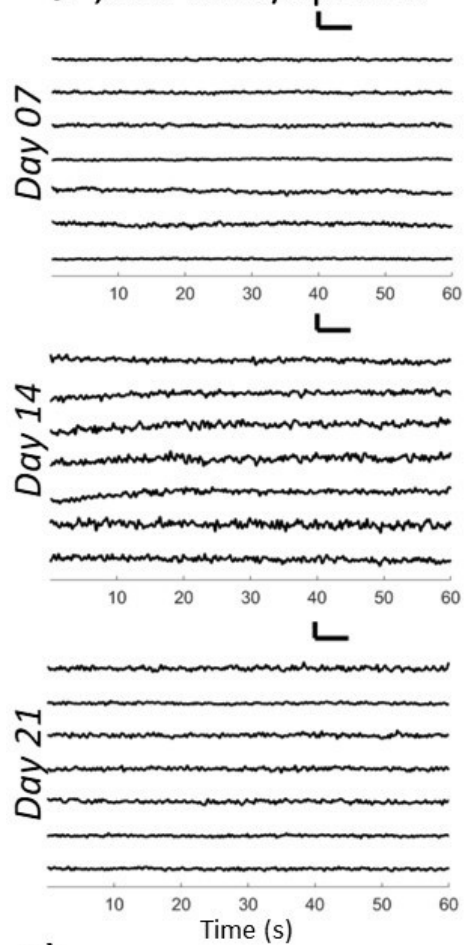
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A) 2,000 Cells/Sphere



B) 4,000 Cells/Sphere

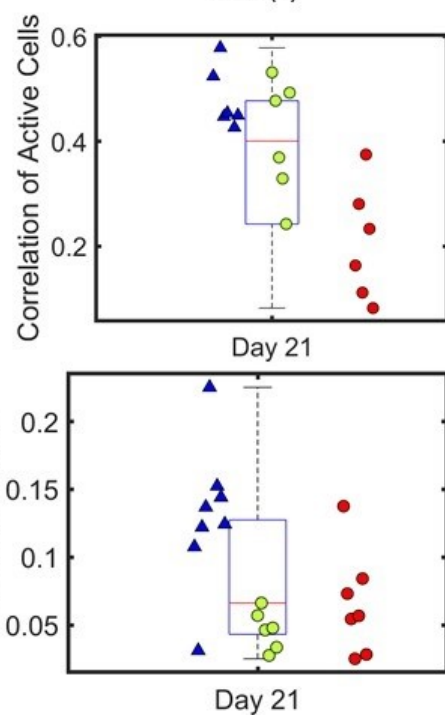
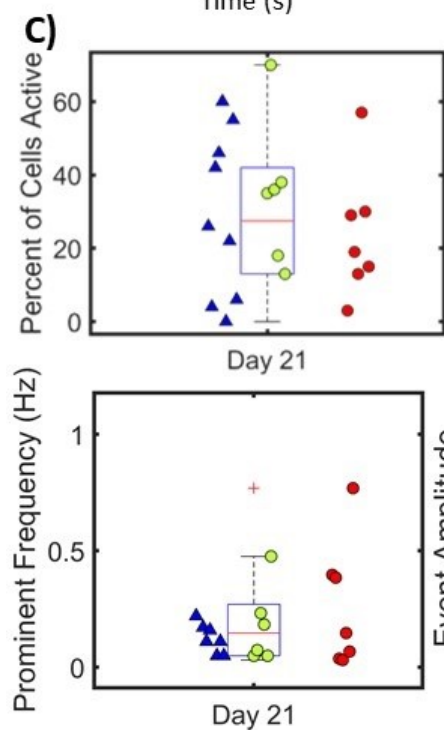
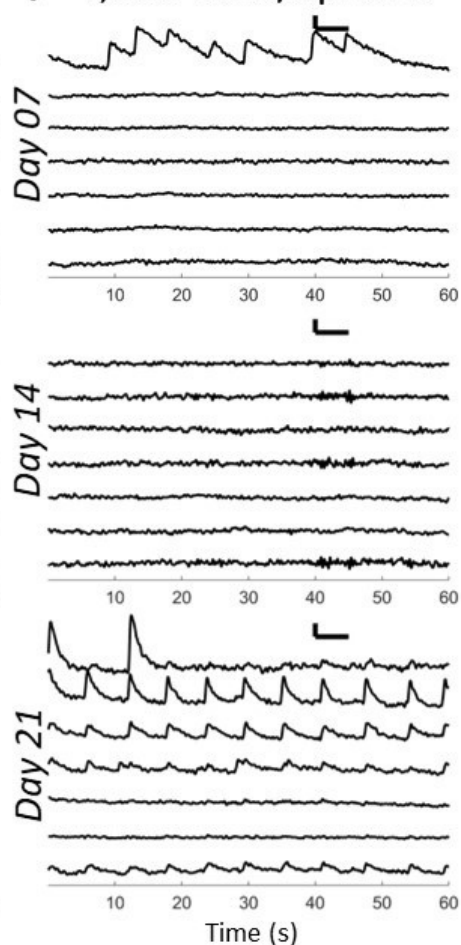


Figure S1) Coordinated activity failed to develop in spheroids composed of 2,000 cells; emerged by Day 21 in 4K spheroids. A) Activity was not detected in 2K spheroids.

Representative $\Delta F/F$ traces from 2,000 cell spheroids at Day 7 (top), Day 14 (center) and Day 21 (bottom). Time series scale bars are 5 sec (horizontal) by 0.1 $\Delta F/F$ (vertical). **B) Some sporadic activity was evident in 4K spheroids. Representative $\Delta F/F$ traces from 4K spheroids at Day 7 (top), Day 14 (center) and Day 21 (bottom). Time series scale bars are 5 sec (horizontal) by 0.1 $\Delta F/F$ (vertical).** **C) Percent of cells active, correlation of active cells, amplitude of events, and frequency of events were calculated for 4K spheroids imaged at Day 21 (n = 18). Marker symbol indicates the sex of rodent the spheroid was derived from ($\blacktriangle = \text{♂}$, $\bullet = \text{♀}$). Marker color ranges from violet to maroon and indicates which data points came from the same dissections. t-tests without assuming equal variance were conducted and found no significant differences between means of male- and female-derived spheroids in any category with $\alpha=0.01$. Red + symbols indicate statistically detected outliers.**

Table S1) Individual Data for Rat, 8K, Control/Spontaneous Condition.

Date YYMMDD	Dissection #	Species	Size	Sex	Days In Vitro	Sphere #	Avg Event Amplitude	Avg Event Rise Time (s)	Avg Event 50% Decay Time (s)	Percent of Cells Active	Correlation of Active Cells	Prevalent Frequency (Hz)
180417	1	RT	8K	F	07	01	0.000	0.000	0.000	0	0.000	0.000
180417	1	RT	8K	F	07	02	0.126	1.381	0.870	2		0.330
180417	1	RT	8K	F	07	03	0.075	1.037	1.122	9	0.035	0.378
180417	1	RT	8K	F	07	04	0.104	1.633	1.410	24	0.693	0.012
180417	1	RT	8K	F	07	05	0.132	1.242	1.614	5	-0.070	0.256
180417	1	RT	8K	F	07	06	0.086	1.146	1.150	16	0.287	0.378
180417	1	RT	8K	F	07	08	0.000	0.000	0.000	0	0.000	0.000
181118	6	RT	8K	F	07	01	0.229	1.629	1.923	67	0.450	0.061
181118	6	RT	8K	F	07	02	0.185	1.466	1.859	91	0.561	0.073
181118	6	RT	8K	F	07	03	0.101	1.897	1.079	100	0.889	0.012
181118	6	RT	8K	F	07	04	0.091	1.630	1.284	92	0.456	0.073
181118	6	RT	8K	F	07	05	0.134	1.621	1.463	93	0.571	0.110
181118	6	RT	8K	F	07	06	0.136	1.894	1.606	88	0.566	0.012
181118	6	RT	8K	F	07	07	0.094	1.481	1.298	95	0.547	0.061
181118	6	RT	8K	F	07	08	0.115	1.473	1.602	95	0.639	0.085
181204	7	RT	8K	F	07	01	0.048	1.019	0.925	53	0.110	0.500
181204	7	RT	8K	F	07	02	0.039	1.110	0.872	67	0.280	0.427
181204	7	RT	8K	F	07	03	0.060	1.120	0.967	91	0.231	0.378
181204	7	RT	8K	F	07	04	0.045	0.917	0.929	75	0.369	0.439
181204	7	RT	8K	F	07	05	0.045	0.970	0.823	59	0.396	0.452
181204	7	RT	8K	F	07	06	0.051	1.209	1.088	53	0.084	0.061
181204	7	RT	8K	F	07	07	0.038	1.158	1.016	59	0.155	0.366
181204	7	RT	8K	F	07	08	0.046	1.195	1.069	44	0.106	0.171
180417	1	RT	8K	M	07	01	0.092	1.083	1.918	4	0.156	0.159
180417	1	RT	8K	M	07	02	0.118	0.964	1.226	20	0.384	0.232
180417	1	RT	8K	M	07	03	0.205	1.720	1.197	4	0.013	0.110
180417	1	RT	8K	M	07	04	0.176	1.123	1.438	14	-0.018	0.146
180417	1	RT	8K	M	07	07	0.114	1.530	1.325	11	-0.019	0.012

Date YYMMDD	Dissection #	Species	Size	Sex	Days In Vitro	Sphere #	Avg Event Amplitude	Avg Event Rise Time (s)	Avg Event 50% Decay Time (s)	Percent of Cells Active	Correlation of Active Cells	Prevalent Frequency (Hz)
180417	1	RT	8K	M	07	08	0.172	1.234	2.116	13	-0.030	0.159
181118	6	RT	8K	M	07	01	0.061	1.134	0.876	89	0.479	0.293
181118	6	RT	8K	M	07	02	0.082	1.150	1.011	65	0.418	0.256
181118	6	RT	8K	M	07	03	0.072	1.168	1.078	88	0.445	0.244
181118	6	RT	8K	M	07	04	0.062	1.321	1.044	91	0.552	0.183
181118	6	RT	8K	M	07	05	0.077	1.323	1.058	82	0.357	0.195
181118	6	RT	8K	M	07	06	0.097	1.239	1.044	92	0.612	0.208
190212	8	RT	8K	M	07	03	0.043	1.199	1.040	42	0.258	0.378
190212	8	RT	8K	M	07	04	0.048	0.965	0.757	40	0.091	0.391
190212	8	RT	8K	M	07	05	0.053	1.055	0.861	68	0.615	0.305
190212	8	RT	8K	M	07	06	0.043	1.150	0.903	47	0.314	0.317
190212	8	RT	8K	M	07	07	0.055	1.157	0.937	73	0.295	0.305
180420	1	RT	8K	F	09	01	0.031	0.804	0.714	3		0.146
180420	1	RT	8K	F	09	02	0.156	1.244	1.057	61	0.660	0.220
180420	1	RT	8K	F	09	03	0.075	0.903	0.824	9	0.150	0.525
180420	1	RT	8K	F	09	04	0.198	1.342	1.229	88	0.570	0.134
180420	1	RT	8K	F	09	05	0.183	1.183	1.092	64	0.665	0.195
180420	1	RT	8K	F	09	06	0.148	1.391	1.320	80	0.641	0.110
180420	1	RT	8K	F	09	07	0.109	1.262	1.341	72	0.635	0.195
180420	1	RT	8K	F	09	08	0.108	1.310	1.349	71	0.650	0.195
180913	3	RT	8K	F	09	01	0.079	0.870	0.710	86	0.533	0.513
180913	3	RT	8K	F	09	01	0.079	0.870	0.710	86	0.533	0.513
180913	3	RT	8K	F	09	02	0.141	0.955	0.818	94	0.720	0.293
180913	3	RT	8K	F	09	03	0.093	1.001	0.838	93	0.554	0.366
180913	3	RT	8K	F	09	04	0.121	0.915	0.707	94	0.773	0.391
180913	3	RT	8K	F	09	05	0.156	0.984	0.771	100	0.787	0.305
180913	3	RT	8K	F	09	06	0.062	0.982	0.670	94	0.687	0.366
180913	3	RT	8K	F	09	07	0.075	0.994	0.694	100	0.750	0.354
180913	3	RT	8K	F	09	08	0.067	0.904	0.723	100	0.790	0.366

Date YYMMDD	Dissection #	Species	Size	Sex	Days In Vitro	Sphere #	Avg Event Amplitude	Avg Event Rise Time (s)	Avg Event 50% Decay Time (s)	Percent of Cells Active	Correlation of Active Cells	Prevalent Frequency (Hz)
180927	4	RT	8K	F	09	02	0.117	0.874	0.695	56	0.490	0.427
180927	4	RT	8K	F	09	04	0.234	1.453	1.006	97	0.703	0.171
180927	4	RT	8K	F	09	05	0.072	0.917	0.809	75	0.464	0.476
180927	4	RT	8K	F	09	11	0.130	0.874	0.798	87	0.617	0.464
180927	4	RT	8K	F	09	13	0.069	0.889	0.717	76	0.489	0.452
180927	4	RT	8K	F	09	15	0.075	0.964	0.677	83	0.568	0.452
181120	6	RT	8K	F	09	01	0.046	0.905	0.638	92	0.537	0.549
181120	6	RT	8K	F	09	02	0.048	0.799	0.573	73	0.585	0.635
181120	6	RT	8K	F	09	03	0.051	0.861	0.579	96	0.583	0.562
181120	6	RT	8K	F	09	04	0.041	0.879	0.633	89	0.461	0.623
181120	6	RT	8K	F	09	06	0.048	0.928	0.642	80	0.531	0.537
190427	9	RT	8K	F	09	01	0.034	1.061	0.497	5	0.286	0.940
190427	9	RT	8K	F	09	02	0.050	1.026	0.580	33	0.270	0.659
190427	9	RT	8K	F	09	03	0.044	1.161	0.602	24	0.323	0.610
190427	9	RT	8K	F	09	04	0.046	1.130	0.672	45	0.351	0.574
190427	9	RT	8K	F	09	05	0.045	0.982	0.606	38	0.546	0.549
190427	9	RT	8K	F	09	06	0.044	1.006	0.576	40	0.429	0.549
190427	9	RT	8K	F	09	08	0.047	1.035	0.579	49	0.465	0.537
190815	11	RT	8K	F	09	01	0.077	0.914	0.642	47	0.497	0.415
190815	11	RT	8K	F	09	04	0.086	1.036	0.747	52	0.434	0.073
190815	11	RT	8K	F	09	05	0.064	0.774	0.527	56	0.736	0.525
190815	11	RT	8K	F	09	06	0.068	0.946	0.614	58	0.553	0.500
190815	11	RT	8K	F	09	02	0.132	0.766	0.704	30	0.679	0.415
190815	11	RT	8K	F	09	03	0.088	0.807	0.580	81	0.719	0.415
180420	1	RT	8K	M	09	02	0.186	0.970	1.012	38	0.332	0.330
180420	1	RT	8K	M	09	03	0.126	0.863	0.816	44	0.493	0.378
180420	1	RT	8K	M	09	04	0.118	1.216	1.018	33	0.351	0.269
180420	1	RT	8K	M	09	05	0.103	1.219	1.124	32	-0.019	0.012
180420	1	RT	8K	M	09	06	0.144	1.213	1.491	52	0.501	0.122

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180420	1	RT	8K	M	09	07	0.106	1.341	0.993	48	0.558	0.281
180420	1	RT	8K	M	09	08	0.117	1.151	0.987	62	0.513	0.281
180913	3	RT	8K	M	09	01	0.077	0.858	0.710	52	0.474	0.574
180913	3	RT	8K	M	09	02	0.082	0.885	0.639	97	0.729	0.500
180913	3	RT	8K	M	09	03	0.044	0.691	0.686	36	0.247	0.574
180913	3	RT	8K	M	09	04	0.051	0.969	0.702	34	0.225	0.647
180913	3	RT	8K	M	09	05	0.061	1.017	0.800	98	0.715	0.305
180913	3	RT	8K	M	09	06	0.060	0.951	0.706	84	0.606	0.403
180913	3	RT	8K	M	09	07	0.067	0.969	0.829	87	0.734	0.293
180913	3	RT	8K	M	09	08	0.044	1.046	0.784	83	0.525	0.378
190927	12	RT	8K	M	09	07	0.246	1.334	1.157	75	0.685	0.244
190927	12	RT	8K	M	09	08	0.103	1.184	0.834	69	0.593	0.256
190927	12	RT	8K	M	09	09	0.117	1.038	0.860	80	0.750	0.256
190927	12	RT	8K	M	09	10	0.112	1.160	0.879	76	0.657	0.220
190215	8	RT	8K	F	10	01	0.046	1.108	0.915	42	0.328	0.391
190215	8	RT	8K	F	10	02	0.052	1.111	0.890	23	0.213	0.391
190215	8	RT	8K	F	10	03	0.047	1.220	0.923	51	0.318	0.317
190215	8	RT	8K	F	10	04	0.059	1.091	1.006	48	0.212	0.305
190215	8	RT	8K	F	10	05	0.048	0.966	0.816	57	0.499	0.330
190215	8	RT	8K	F	10	07	0.052	1.025	0.974	62	0.330	0.342
180817	2	RT	8K	M	10	01	0.092	0.615	0.672	9	0.537	0.745
180817	2	RT	8K	M	10	09	0.068	0.884	0.875	21	0.128	0.586
180817	2	RT	8K	M	10	10	0.093	0.830	0.808	27	0.277	0.586
180817	2	RT	8K	M	10	11	0.076	0.735	0.556	15	0.396	0.610
180817	2	RT	8K	M	10	12	0.075	0.782	0.901	36	0.213	0.525
180817	2	RT	8K	M	10	13	0.082	0.772	0.791	32	0.425	0.623
180817	2	RT	8K	M	10	14	0.061	0.802	0.648	25	0.431	0.806
180817	2	RT	8K	M	10	15	0.065	0.815	1.126	33	0.243	0.549
180817	2	RT	8K	M	10	16	0.082	0.675	0.728	40	0.686	0.500
190215	8	RT	8K	M	10	01	0.039	1.056	0.872	64	0.293	0.488

Date YYMMDD	Dissection #	Species	Size	Sex	Days In Vitro	Sphere #	Avg Event Amplitude	Avg Event Rise Time (s)	Avg Event 50% Decay Time (s)	Percent of Cells Active	Correlation of Active Cells	Prevalent Frequency (Hz)
190215	8	RT	8K	M	10	05	0.048	1.116	0.862	70	0.527	0.354
190215	8	RT	8K	M	10	07	0.040	0.998	0.908	77	0.358	0.476
180424	1	RT	8K	F	14	01	0.130	1.280	0.786	46	0.508	0.159
180424	1	RT	8K	F	14	02	0.132	1.374	0.704	30	0.279	0.122
180424	1	RT	8K	F	14	03	0.122	0.957	0.748	21	0.082	0.500
180424	1	RT	8K	F	14	04	0.141	1.199	0.678	28	0.314	0.171
180424	1	RT	8K	F	14	05	0.118	1.134	0.573	17	0.246	0.586
180424	1	RT	8K	F	14	06	0.125	1.308	1.121	27	0.418	0.256
180424	1	RT	8K	F	14	07	0.081	0.888	0.571	36	0.541	0.598
180424	1	RT	8K	F	14	08	0.106	1.002	0.575	28	0.140	0.452
181113	5	RT	8K	F	14	01	0.101	0.803	0.714	5	-0.019	0.659
181113	5	RT	8K	F	14	03	0.077	0.521	0.438	5	0.023	0.610
181113	5	RT	8K	F	14	04	0.072	0.762	1.078	9	0.000	0.061
181113	5	RT	8K	F	14	05	0.097	0.643	0.752	5	0.111	0.549
181113	5	RT	8K	F	14	06	0.111	1.639	2.328	76	0.941	0.256
181113	5	RT	8K	F	14	07	0.043	0.679	0.823	5	0.059	0.378
181113	5	RT	8K	F	14	08	0.105	0.774	0.795	12	0.029	0.464
181211	7	RT	8K	F	14	01	0.179	0.852	1.147	88	0.792	0.244
181211	7	RT	8K	F	14	02	0.181	0.898	1.101	87	0.733	0.232
181211	7	RT	8K	F	14	03	0.099	0.893	1.120	85	0.713	0.281
181211	7	RT	8K	F	14	04	0.051	0.918	0.704	94	0.470	0.549
181211	7	RT	8K	F	14	05	0.069	0.897	0.677	78	0.515	0.464
181211	7	RT	8K	F	14	06	0.069	1.004	1.010	94	0.574	0.256
181211	7	RT	8K	F	14	07	0.069	1.032	0.808	96	0.486	0.293
181211	7	RT	8K	F	14	08	0.049	1.091	0.803	100	0.336	0.500
190626	10	RT	8K	F	14	01	0.219	0.959	1.164	6	-0.340	0.403
190626	10	RT	8K	F	14	04	0.085	1.250	0.567	7	-0.014	0.427
190626	10	RT	8K	F	14	06	0.099	1.175	0.656	49	0.459	0.476
190626	10	RT	8K	F	14	07	0.117	1.001	0.781	32	0.463	0.562

Date YYMMDD	Dissection #	Species	Size	Sex	Days In Vitro	Sphere #	Avg Event Amplitude	Avg Event Rise Time (s)	Avg Event 50% Decay Time (s)	Percent of Cells Active	Correlation of Active Cells	Prevalent Frequency (Hz)
180821	2	RT	8K	M	14	01	0.090	0.614	0.523	2	0.000	0.049
180821	2	RT	8K	M	14	02	0.103	1.148	1.097	6	-0.091	0.049
180821	2	RT	8K	M	14	03	0.224	2.858	1.406	13	-0.087	0.049
180821	2	RT	8K	M	14	04	0.035	0.859	0.644	6	-0.004	0.061
180821	2	RT	8K	M	14	05	0.041	0.879	0.766	19	0.015	0.110
180821	2	RT	8K	M	14	06	0.077	2.169	1.585	13	0.022	0.061
180821	2	RT	8K	M	14	07	0.043	0.858	0.782	9	0.007	0.110
180821	2	RT	8K	M	14	08	0.059	0.965	0.673	10	0.004	0.159
180821	2	RT	8K	M	14	10	0.094	1.262	0.992	15	0.109	0.134

Table S2) Individual Data for Mouse, 8K, Control/GCaMP condition and OBG, DMSO conditions

<i>Date</i> <i>YYMMDD</i>	<i>Species</i>	<i>Size</i>	<i>Sex</i>	<i>Days In Vitro</i>	<i>Sphere #</i>	<i>Condition</i>	<i>Avg Event Amplitude</i>	<i>Avg Event Rise Time (s)</i>	<i>Avg Event 50% Decay Time (s)</i>	<i>Percent of Cells Active</i>	<i>Correlation of Active Cells</i>	<i>Prominent Frequency</i>
191126	MS	8K	M	08	01	GCAMP	0.748	1.577	0.890	100	0.710	0.146
191126	MS	8K	M	08	02	GCAMP	1.029	5.361	1.088	95	0.867	0.073
191126	MS	8K	M	08	04	GCAMP	0.503	1.121	0.777	93	0.498	0.232
191126	MS	8K	M	08	05	GCAMP	0.870	1.577	0.936	100	0.488	0.122
191126	MS	8K	M	08	06	GCAMP	0.744	1.610	0.835	94	0.645	0.012
191126	MS	8K	M	08	01	DMSOF127	0.864	1.230	0.671	92	0.347	0.110
191126	MS	8K	M	08	02	DMSOF127	0.540	1.515	0.636	100	0.268	0.159
191126	MS	8K	M	08	03	DMSOF127	0.360	1.242	0.528	95	0.187	0.085
191126	MS	8K	M	08	04	DMSOF127	0.131	1.409	0.739	93	0.516	0.073
191126	MS	8K	M	08	05	DMSOF127	0.753	0.906	0.593	100	0.919	0.256
191126	MS	8K	M	08	01	OGB	0.117	0.978	0.586	96	0.513	0.415
191126	MS	8K	M	08	02	OGB	0.068	1.087	0.712	91	0.470	0.342
191126	MS	8K	M	08	03	OGB	0.066	1.068	0.667	76	0.367	0.403
191201	MS	8K	M	13	03	GCAMP	0.605	1.438	0.770	100	0.587	0.171
191201	MX	8K	M	13	05	GCAMP	0.534	0.886	0.575	94	0.738	0.073
191201	MS	8K	M	13	06	GCAMP	0.861	1.469	0.894	100	0.857	0.122
191201	MS	8K	M	13	07	GCAMP	1.099	1.026	0.903	100	0.956	0.183
191201	MS	8K	M	13	08	GCAMP	0.772	1.809	1.384	92	0.856	0.110
191201	MS	8K	M	13	14	GCAMP	1.447	2.350	0.840	100	0.883	0.098
191201	MS	8K	M	13	15	GCAMP	0.677	1.770	0.515	100	0.559	0.183
191201	MS	8K	M	13	16	GCAMP	0.601	1.490	0.672	100	0.524	0.208
191201	MS	8K	M	13	17	GCAMP	0.580	1.269	0.627	83	0.544	0.061

Table S3) Individual Data for Rat, 4K, Day 21, Control/Spontaneous Condition.

<i>Date</i> <i>YYMMDD</i>	<i>Diss- ection #</i>	<i>Species</i>	<i>Size</i>	<i>Sex</i>	<i>Days In Vitro</i>	<i>Sphere #</i>	<i>Recording Duration (s)</i>	<i>Avg Event Amplitude</i>	<i>Avg Event Rise</i>	<i>Avg Event 50% Decay</i>	<i>Percent of Cells Active</i>	<i>Correlation of Active Cells</i>	<i>Prominent Frequency</i>
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									<i>Time (s)</i>	<i>Time (s)</i>			
180501	1	RT	4K	M	21	01	60	0.108	1.245	1.107	26	0.524	0.220
180501	1	RT	4K	M	21	02	60	0.031	1.071	0.928	4		7.556
180501	1	RT	4K	M	21	03	60	0.122	1.407	1.186	42	0.578	0.171
180501	1	RT	4K	M	21	04	60	0.137	1.744	1.468	46	0.447	0.110
180501	1	RT	4K	M	21	04	60	0.225	1.433	1.284	60	0.454	0.159
180501	1	RT	4K	M	21	05	60				0		
180501	1	RT	4K	M	21	06	60	0.152	1.136	0.703	22	0.426	0.049
180501	1	RT	4K	M	21	07	60	0.144	1.423	0.946	55	0.449	0.110
180501	1	RT	4K	M	21	08	60	0.124	1.203	0.760	6		0.049
191120	13	RT	4K	F	21	05	60	0.057	1.372	0.750	35	0.532	0.049
191120	13	RT	4K	F	21	06	60	0.066	1.253	0.773	70	0.477	0.073
191120	13	RT	4K	F	21	07	60	0.046	1.172	0.706	36	0.369	0.232
191120	13	RT	4K	F	21	08	60	0.028	1.258	0.796	18	0.329	0.183
191120	13	RT	4K	F	21	09	60	0.048	1.850	1.053	38	0.242	0.049
191120	13	RT	4K	F	21	10	60	0.034	1.067	0.864	13	0.493	0.476
200106	14	RT	4K	F	21	01	120	0.138	0.849	0.405	3		0.397
200106	14	RT	4K	F	21	02	120	0.073	1.121	0.552	29	0.163	0.385
200106	14	RT	4K	F	21	03	120	0.054	1.273	0.708	19	0.280	0.037
200106	14	RT	4K	F	21	03	120	0.025	0.984	0.654	13	0.111	0.031
200106	14	RT	4K	F	21	04	120	0.057	1.557	0.771	57	0.375	0.146
200106	14	RT	4K	F	21	04	120	0.084	1.476	0.825	30	0.233	0.067
200106	14	RT	4K	F	21	06	120	0.028	1.007	0.426	15	0.082	0.769

