

**Stem Cell Reports, Volume 3**

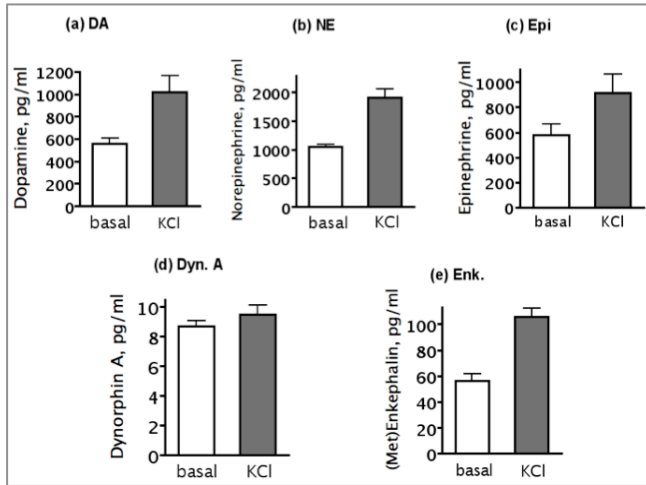
**Supplemental Information**

**Human iPSC Neurons Display Activity-Dependent  
Neurotransmitter Secretion: Aberrant Catecholamine Levels  
in Schizophrenia Neurons**

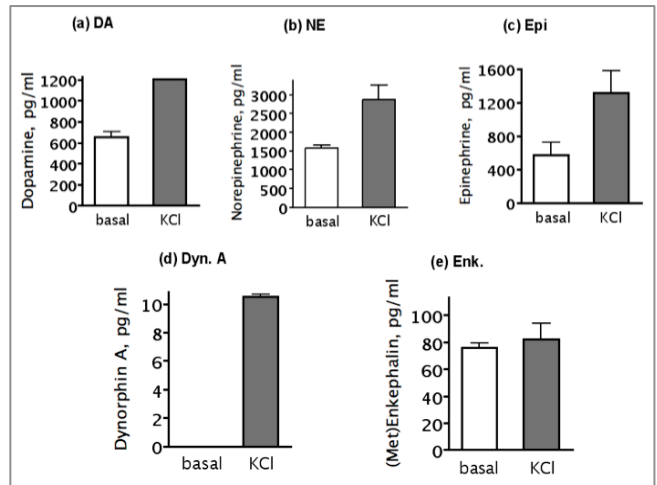
**Vivian Hook, Kristen J. Brennand, Yongsung Kim, Thomas Toneff, Lydiane Funkelstein,  
Kelly C. Lee, Michael Ziegler, and Fred H. Gage**

Figure S1

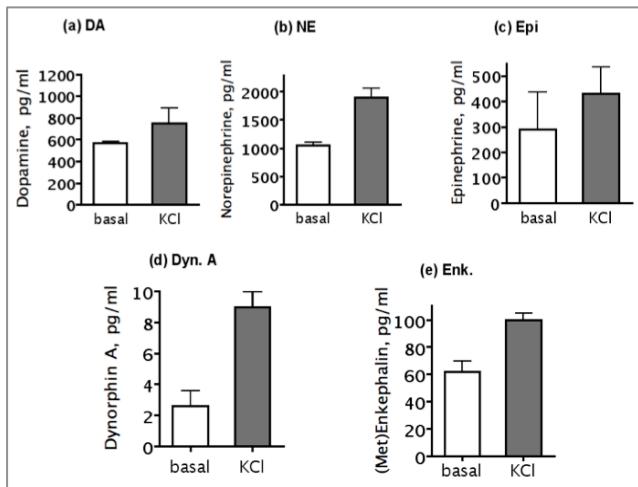
**(a) Control 92293A**



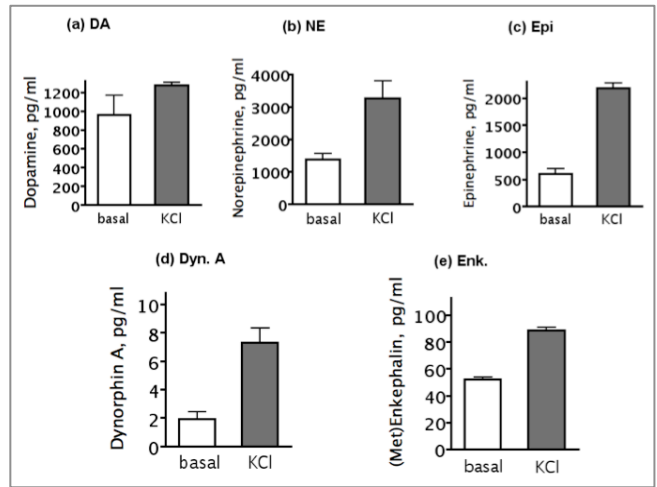
**(d) SZ 1792B**



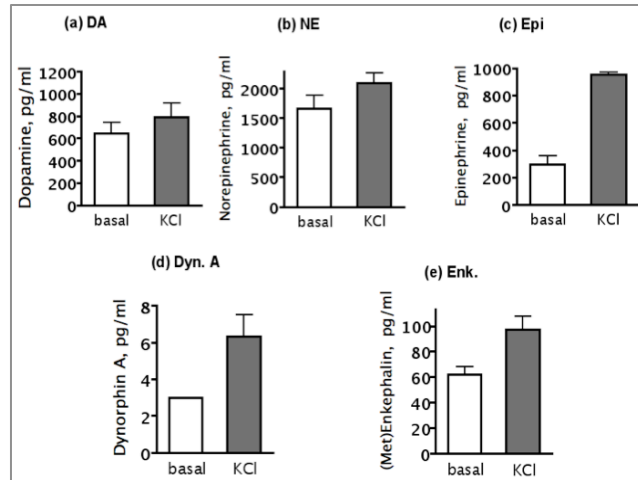
**(b) Control BJ3E**



**(e) SZ 2038B**



**(c) Control 4506CB**



**(f) SZ 2497B**

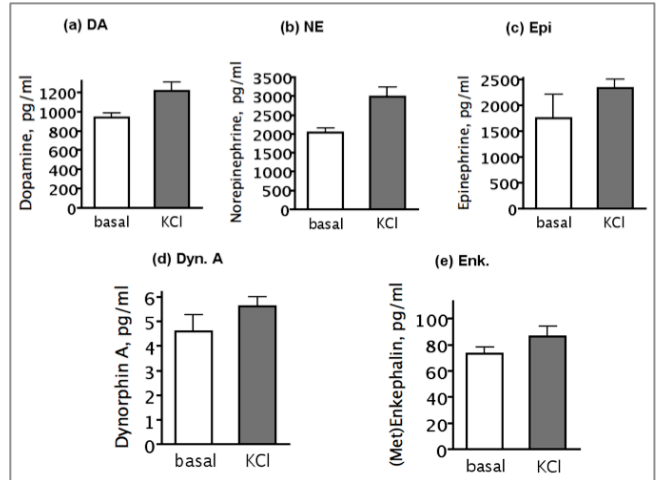


Fig. S1 shows neurotransmitters secreted in basal and KCl conditions, from individual hiPSC neuronal cell lines, derived from 3 control (a-c) and three SZ (d-f) cases (patient numbers shown). Data show the mean  $\pm$  s.e.m. of triplicate cell culture samples (and each sample assayed for the designated neurotransmitter in triplicate).

Figure S2

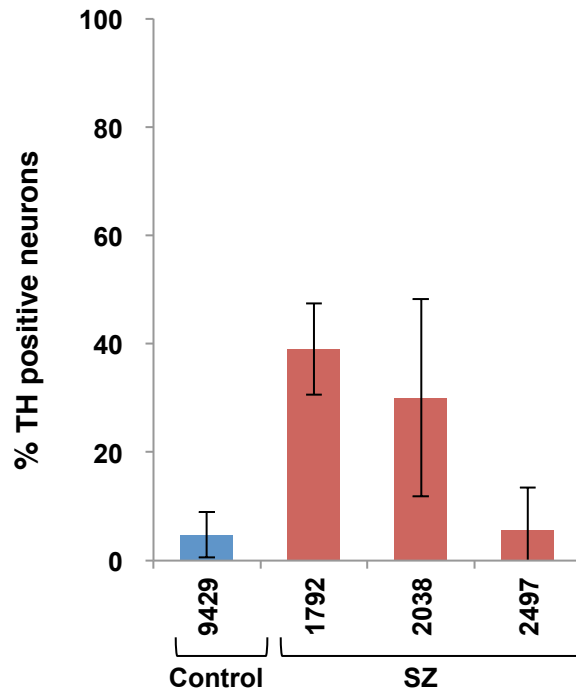


Figure S2 shows quantitation of the percent TH-positive neurons in cultures of hiPSC neuronal cell lines derived from three SZ cases, and compared to control hiPSC neurons from one control case. Three experiments were conducted for each neuronal cell line, in triplicate. Data are shown as the mean  $\pm$  s.d. (standard deviation).

**Table S1. Neurotransmitter Secretion from SZ compared to Control hiPSC Neurons, Averaged from Three Cases Each of SZ and Control hiPSC Cell Lines**

Neurotransmitter	Control			SZ		
	Basal pg/ml	KCl pg/ml	Ratio	Basal pg/ml	KCl pg/ml	Ratio
DA	590 + 27	853 + 82	1.45	928 + 90 <sup>+</sup>	1231 + 24*	1.33
NE	1340 + 180	2002 + 55	1.49	1708 + 73 <sup>+</sup>	2869 + 47*	1.68
Epi	388 + 54	767 + 169	1.98	952 + 138 <sup>+</sup>	1763 + 289*	1.85
Dyn. A	4.8 + 2.0	8.3 + 1.0	1.73	4.4 + 0.8	6.1 + 0.4	1.39
(Met)Enk.	60.1 + 1.9	101 + 2.6	1.68	63.3 + 4.1	94.3 + 4.4	1.49

Basal secretion media was collected after incubating cells in culture media for 30 min., and the same cells were then subjected to activity-dependent secretion stimulated by incubation in KCl-containing media (50 mM KCl) for 30 min. and collection of media. Basal media and KCl media were analyzed for levels of the catecholamine neurotransmitters DA, NE, and Epi, as well as the peptide neurotransmitters dynorphin A (Dyn. A) and (Met)enkephalin ((Met)Enk.). These experiments were conducted for hiPSC neuronal cell lines derived from three different SZ cases and three different control cases. Cellular assays were conducted in triplicate and neurotransmitter assays for each cellular replicate was conducted in triplicate. Data are expressed as the mean neurotransmitter levels in the secretion media (pg/ml) with standard error of the mean (s.e.m.), averaged for the three cases of each group (SZ and control). Levels of neurotransmitters secreted in the presence of KCl were compared for SZ and normal hiPSC neuronal cell lines (each group derived from three cases). \*Significant difference between KCl-stimulated secretion in SZ compared to control hiPSC neurons ( $p < 0.05$ , student's t-test). <sup>+</sup>Significant difference between basal secretion of SZ compared to control hiPSC neurons ( $p < 0.05$ ). The 'Ratio' of KCl compared to basal secretion (K/B) is also shown.

**Table S2. Individual Control and SZ hiPSC Neuronal Cell Lines: Neurotransmitter Secretion**

<b>Control 94293A:</b>			
Neurotransmitter	Basal pg/ml	KCl pg/ml	Ratio K/B
DA	560 ± 47	1015 ± 146	1.81
NE	1303 ± 31	2009 ± 6.0	1.54
Epi	577 ± 89	915 ± 152	1.59
Dyn. A	8.7 ± 0.35	9.5 ± 0.6	1.09
(Met)Enk.	56.3 ± 5.2	106 ± 6.1	1.88
<b>Control BJ3E:</b>			
Neurotransmitter	Basal pg/ml	KCl pg/ml	Ratio K/B
DA	644 ± 100	794 ± 120	1.23
NE	1669 ± 212	2094 ± 172	1.25
Epi	297 ± 63	956 ± 14	3.22
Dyn. A	3.0	6.3 ± 1.2	2.10
(Met)Enk.	62.0 ± 6.1	97.0 ± 11.0	1.56
<b>Control 4506CB:</b>			
Neurotransmitter	Basal pg/ml	KCl pg/ml	Ratio K/B
DA	567 ± 17	749 ± 140	1.32
NE	1049 ± 49	1904 ± 151	1.82
Epi	291 ± 145	431 ± 105	1.48
Dyn. A	2.6 ± 0.99	9.0 ± 1.0	3.46
(Met)Enk.	62.0 ± 7.4	100 ± 5.0	1.61
<b>SZ 1792B:</b>			
Neurotransmitter	Basal pg/ml	KCl pg/ml	Ratio K/B
DA	654 ± 50	1204 ± 2.1	1.84
NE	1573 ± 80	2876 ± 375	1.83
Epi	571 ± 153	1319 ± 262	2.31
Dyn. A	not detected	10.5 ± 0.2	--
(Met)Enk.	75.7 ± 3.5	82.0 ± 12.0	1.08
<b>SZ 2038B:</b>			
Neurotransmitter	Basal pg/ml	KCl pg/ml	Ratio K/B
DA	964 ± 203	1275 ± 33	1.32
NE	1376 ± 180	3253 ± 523	2.37
Epi	609 ± 82	2190 ± 89	3.60
Dyn. A	1.93 ± 0.5	7.3 ± 1.0	3.78
(Met)Enk.	52.0 ± 1.7	86.6 ± 2.3	1.67
<b>SZ 2497B:</b>			
Neurotransmitter	Basal pg/ml	KCl pg/ml	Ratio K/B
DA	938 ± 43	1213 ± 88	1.29
NE	2028 ± 125	2978 ± 262	1.47
Epi	1753 ± 454	2328 ± 173	1.33
Dyn. A	4.6 ± 0.66	5.6 ± 0.4	1.22
(Met)Enk.	73.0 ± 5.0	86.6 ± 7.2	1.19

Data shows neurotransmitter secretion from three different control and three different SZ hiPSC neuronal cell lines, each derived from individual patient cases (indicated by patient numbers). Values are shown as the mean ± s.e.m. (n=3 for triplicate cell samples, and each sample was assayed for the designated neurotransmitter in triplicate). Basal secretion and KCl-stimulated secretion of neurotransmitters are compared, indicated by the 'ratio' of KCl to basal (K/B) secretion.