

Alterations in a Unique Class of Cortical Chandelier Cell Axon Cartridges in Schizophrenia

Supplemental Information

Table S1. Detailed demographic and postmortem characteristics of human subjects. There were no statistical differences between schizophrenia and comparison subjects in age (45.05 ± 7.27 yrs and 45.75 ± 8.69 yrs, respectively), PMI (9.53 ± 3.39 hrs and 9.92 ± 4.04 hrs, respectively), and storage time (11.69 ± 5.29 yrs and 13.78 ± 5.28 yrs, respectively). Previously studied designates histology or protein expression studies that included the pair (1-6). Numbers designate the associated reference(s).

Healthy Comparison Subjects							Subjects with Schizophrenia											
Pair	Case	Sex/ Race	Age (yrs)	PMI ^a	Storage Time ^b	Cause of Death	Case	DSM IV diagnosis	Sex/ Race	Age (yrs)	PMI ^a	Storage Time ^b	Cause of Death	Antipsy- chotic ATOD	Antide- pressant ATOD	Benzodiazep- ine/ Anticon- vulsant ATOD	Nicotine Use ATOD	Previously studied
1	727	M/B	19	7.00	12.90	Trauma	829	Schizoaffective disorder; ADC; OAR	M/W	25	5.00	11.34	Suicide by salicylate overdose	N	N	Y	Y	1,2,3,6,7
2	852	M/W	54	8.00	10.90	Cardiac tamponade	781	Schizoaffective disorder; ADR	M/B	52	8.00	12.16	Peritonitis	Y	Y	N	Y	1,2,3,7
3	1307	M/B	32	4.80	5.20	ASCVD	10024	Paranoid schizophrenia	M/B	37	5.98	5.96	ASCVD	N	N	N	N	1,7
4	567	F/W	46	15.00	14.80	Mitral valve prolapse	537	Schizoaffective disorder	F/W	37	14.50	15.25	Suicide by hanging	N	N	N	U	1,2,6,7
5	1047	M/W	43	13.80	8.10	ASCVD	1209	Schizoaffective disorder	M/W	35	9.1	6.50	Suicide by diphenhydramine overdose	Y	N	N	N	1,7
6	739	M/W	40	15.8	13	ASCVD	933	Disorganized schizophrenia	M/W	44	8.30	9.66	Myocarditis	Y	Y	Y	N	1,3,7
7	451	M/W	48	12.00	16.30	ASCVD	317	Chronic undifferentiated schizophrenia	M/W	48	8.30	18.9	Bronchopneumonia	Y	Y	N	N	5,7
8	178	M/W	48	7.80	20.50	ASCVD	377	Chronic undifferentiated schizophrenia	M/W	52	10.00	18.1	Gastrointestinal bleeding	Y	N	N	N	4,5,7
9	452	F/W	40	14.30	16.30	ASCVD	341	Chronic undifferentiated schizophrenia	F/W	47	14.50	18.6	Suicide, chlorpromazine overdose	Y	N	N	Y	6,7
10	449	F/W	47	4.30	16.30	Accidental CO poisoning	517	Chronic disorganized schizophrenia	F/W	48	3.70	15.10	Intracerebral hemorrhage	Y	N	N	Y	4,5,7
11	681	M/W	51	11.60	14.10	Hypertrophic cardiomyopathy	234	Chronic paranoid schizophrenia	M/W	51	12.80	21.00	Cardiomyopathy	N	N	N	N	4,5,6,7
12	395	M/W	42	12.30	18.80	Pericardial tamponade	322	Chronic undifferentiated schizophrenia	M/W	40	8.50	20.20	Suicide, combined drug overdose	Y	Y	N	N	7
13	575	F/B	55	11.30	15.40	ASCVD	597	Schizoaffective disorder	F/W	46	10.10	15.1	Pneumonia	Y	Y	N	Y	6,7
14	278	M/W	50	4.50	20.40	ASCVD	640	Chronic paranoid schizophrenia	M/W	49	5.20	14.5	Pulmonary Embolism	Y	Y	N	U	7
15	1284	M/W	55	6.40	5.60	ASCVD	1105	Schizoaffective disorder	M/W	53	7.90	7.47	ASCVD	Y	N	N	Y	7
16	1122	M/W	55	15.40	7.30	Cardiac Tamponade	930	Disorganized schizophrenia; ADR; OAR	M/W	47	15.30	9.70	ASCVD	Y	N	Y	Y	7
17	250	F/W	47	5.30	19.70	ASCVD	398	Schizoaffective disorder	F/W	41	10.30	17.7	Pulmonary embolus	Y	N	Y	U	4,5,6,7
18	412	M/W	42	14.20	17.50	Aortic stenosis	422	Chronic paranoid schizophrenia	M/W	54	11.00	17.20	ASCVD	Y	N	Y	U	4,5,7
19	344	M/W	50	6.80	18.60	ASCVD	1296	Undifferentiated schizophrenia	M/W	48	7.80	5.42	Pneumonia	Y	Y	N	Y	7
20	1391	F/W	51	7.8	4	ASCVD	1189	Schizoaffective disorder; AAR	F/W	47	14.4	7	Suicide by combined drug overdose	Y	Y	Y	Y	1,7

^aPMI, postmortem interval (hours).

^bYears stored in 30% glycerin/30% ethylene glycol solution at -30°C.

ASCVD, arteriosclerotic cardiovascular disease; ATOD, at time of death; U, unknown; M, male; F, female; W, white; B, black.

Table S1 References

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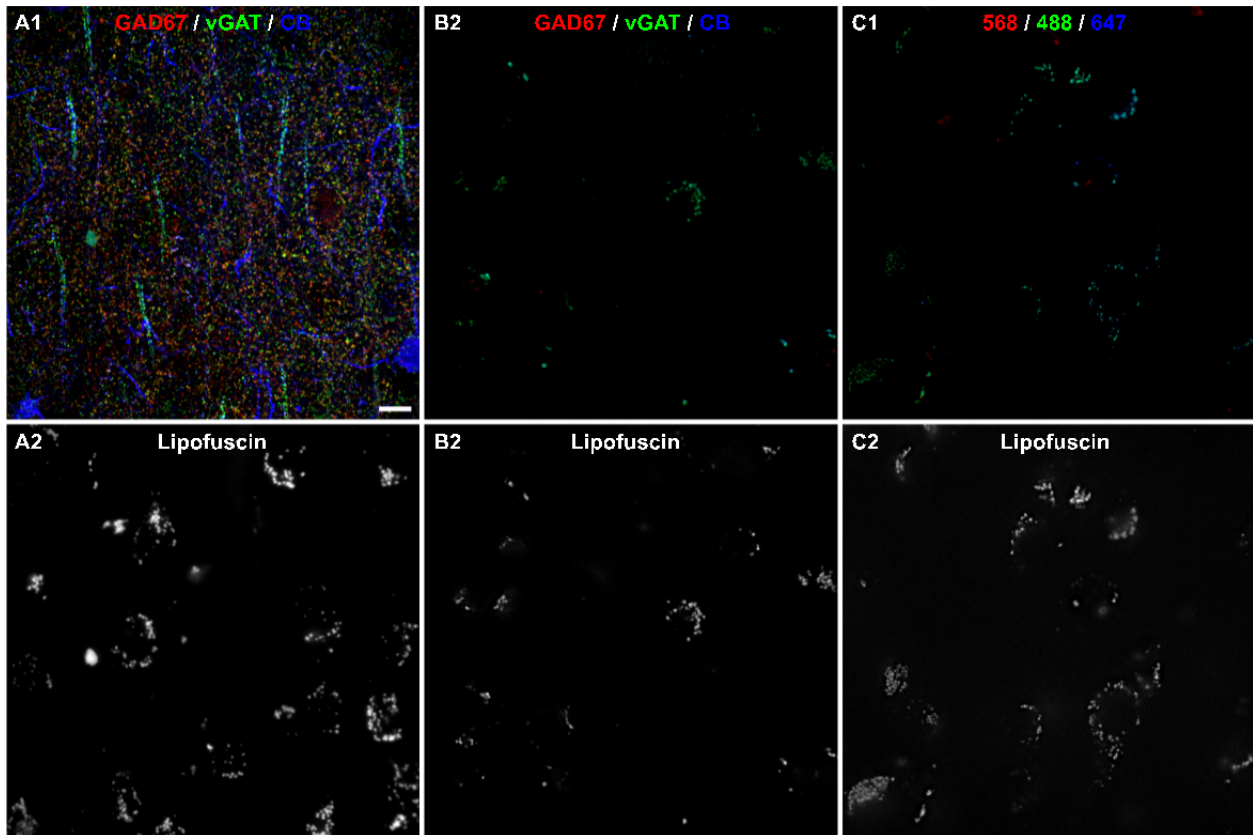


Figure S1. Specificity of GAD67, vGAT, and CB antibodies in human PFC. (A1-C1) Maximum intensity z-plane projection images of human PFC tissue sections (subject 10024). The sections were immunolabeled with (A) GAD67, vGAT, and CB antibodies; (B) GAD67, vGAT, and CB antibodies preabsorbed to their respective antigens; or (C) only Alexa 568, Alexa 488, and Alexa 647 secondary antibodies. (A2-C2) Single channel images showing the lipofuscin autofluorescence in A1-C1. Bar = 10 μ m.

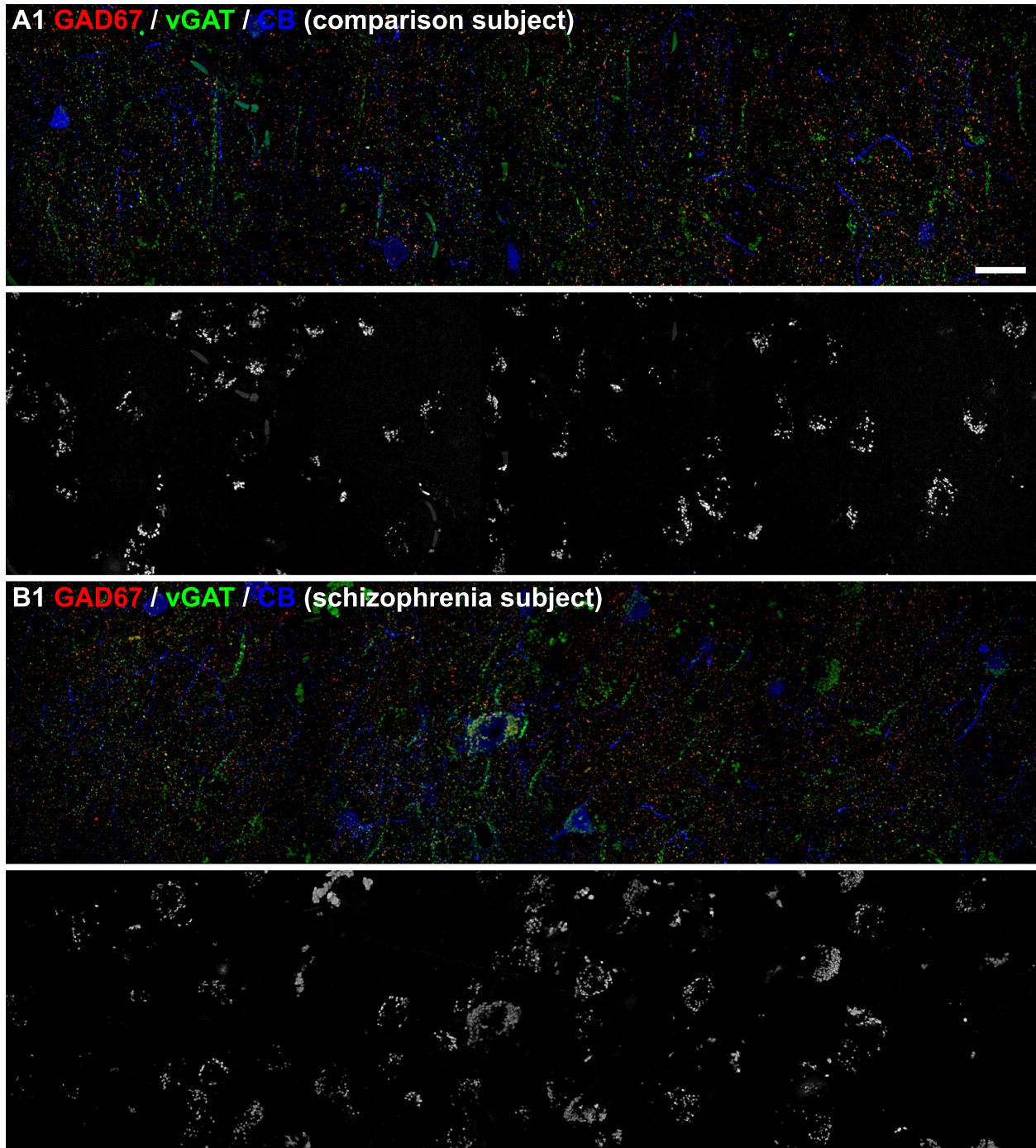


Figure S2. High magnification, maximum intensity z-plane projection montage images. (A1) Human PFC tissue section (comparison subject 452, pair 9) immunolabeled for GAD67, vGAT, and CB. (A2) Corresponding lipofuscin autofluorescence channel for montage in A1. (B1) Human PFC tissue section (schizophrenia subject 341, pair 9) immunolabeled for GAD67, vGAT, and CB. (B2) Corresponding lipofuscin autofluorescence channel for montage in B1. Bar = 20 μ m.

Supplemental Methods

Microscopy

TetraSpeck microspheres (fluorescent blue/green/orange/dark red; Invitrogen) were used to confirm the absence of alignment issues between wavelengths.

Lipofuscin Autofluorescence

A potential confound of quantitative fluorescence measures in human cortex is lipofuscin autofluorescence, which is detectable across a broad range of wavelengths. To exclude this potential confound, lipofuscin was imaged using a fourth channel at a constant exposure time across all sections. Lipofuscin autofluorescence was masked using a single optimal threshold value for each image stack, and vGAT, GAD67, and CB object masks that overlapped a lipofuscin mask were eliminated from analyses. Importantly, lipofuscin fluorescence intensity did not differ between schizophrenia (335 ± 89 a.u.) and unaffected comparison (342 ± 79 a.u.) subjects (paired $F_{1, 19} = 0.246$, $p = 0.626$; unpaired $F_{1, 37} = 0.175$, $p = 0.678$).