Altered Glutamate Protein Co-Expression Network Topology Linked to Spine Loss in the Auditory Cortex of Schizophrenia

Supplement 1

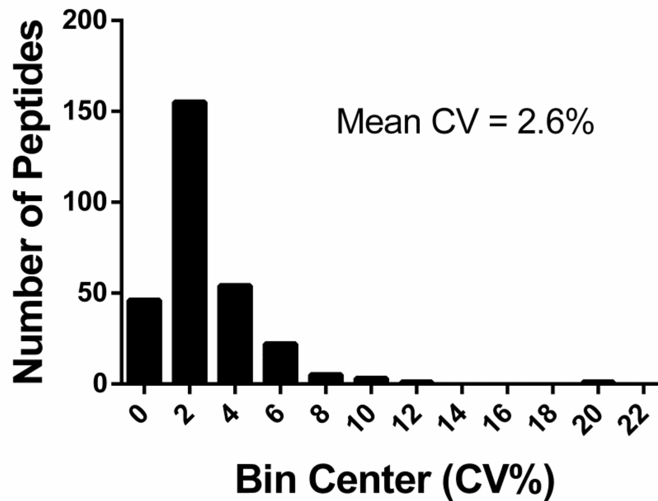

Figure S1. Assay precision. Four 10 µg aliquots from a single auditory cortex gray matter homogenate were prepared and analyzed by liquid chromatography-selected reaction monitoring/mass spectrometry. 287 peptides from 184 proteins were quantified. The distribution of peptides coefficient of variation (CV) % is shown.

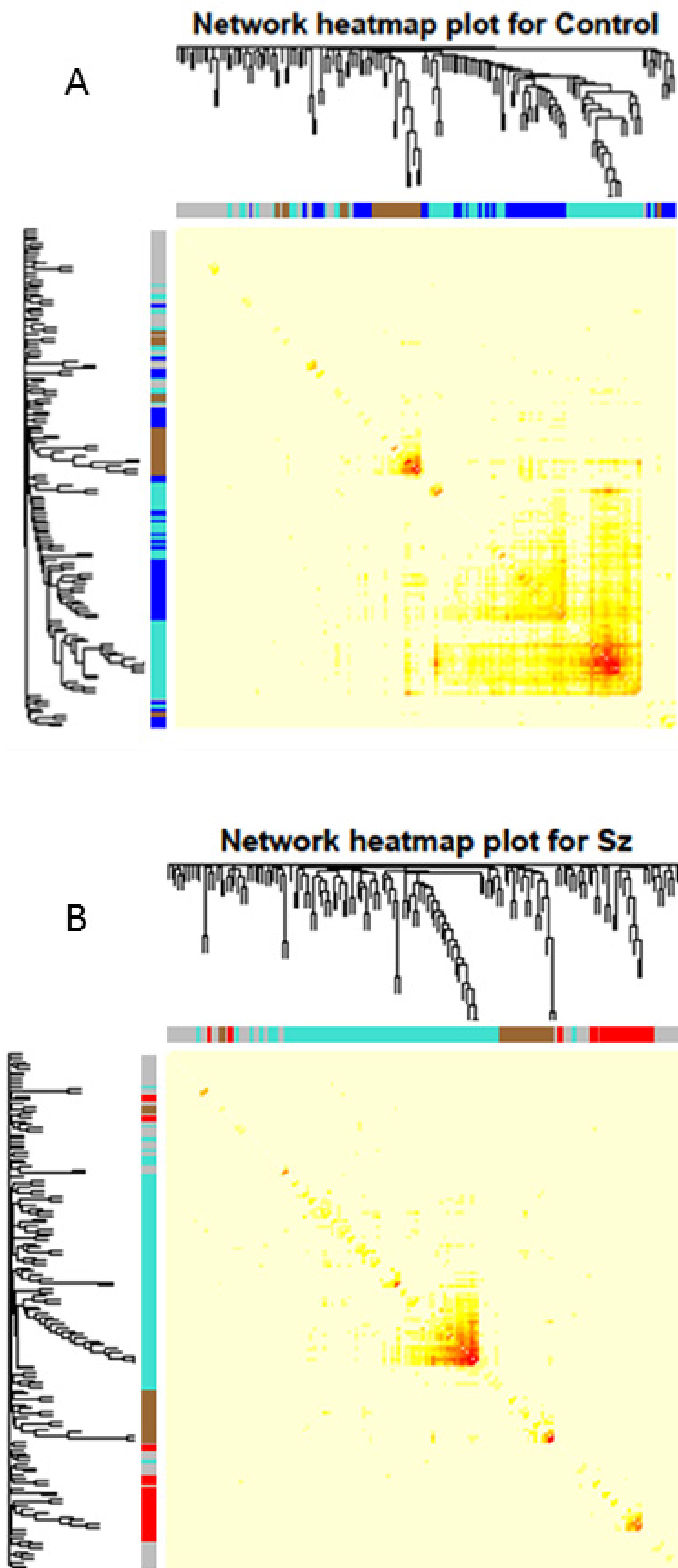

Figure S2. Altered glutamate signaling protein network in auditory cortex in (A) control and (B) schizophrenia (Sz) subjects. Heat maps of protein co-expression where red indicates greater correlation.

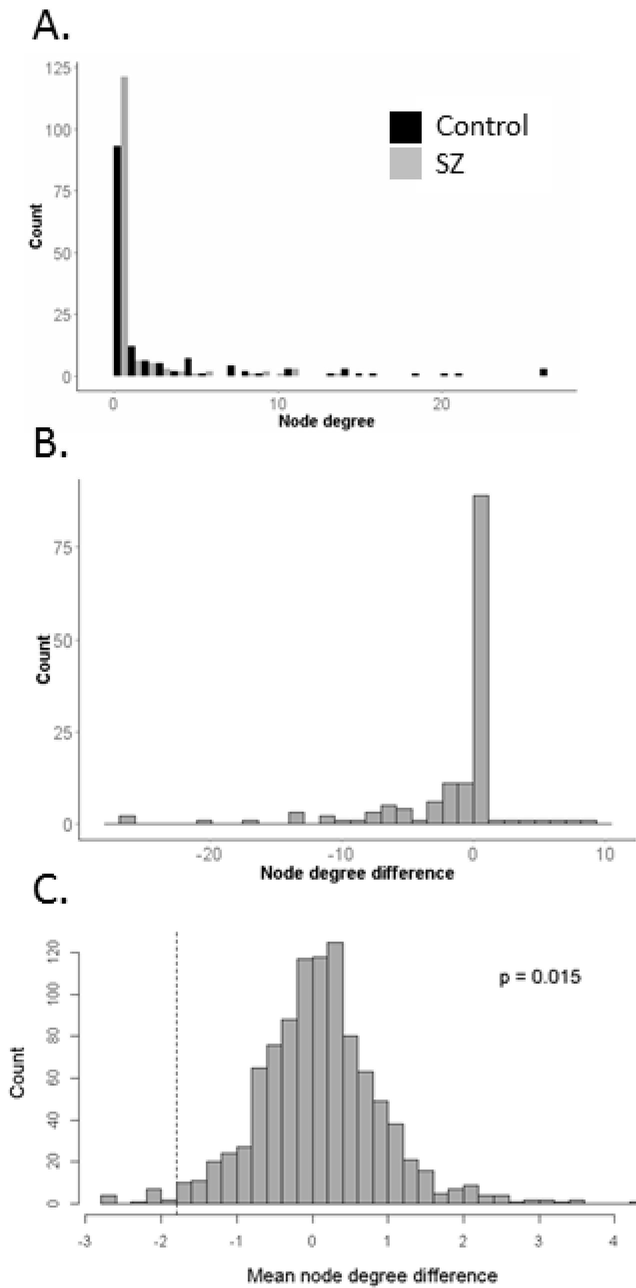

Figure S3. Statistical comparison of Control and Schizophrenia (SZ) network connectivity. (**A**) Distribution of node degree scores for all proteins quantified by liquid chromatography-selected reaction monitoring/mass spectrometry in the SZ and control cohorts. (**B**) Distribution of the node degree differences (SZ - Control) for all proteins. Mean = -1.80 indicating greater connectivity in the Control network. (**C**) Distribution of the average mean node degree differences calculated from 1000 permutations of sample diagnosis. The observed mean difference from (**B**), -1.80, is indicated by the dotted line.

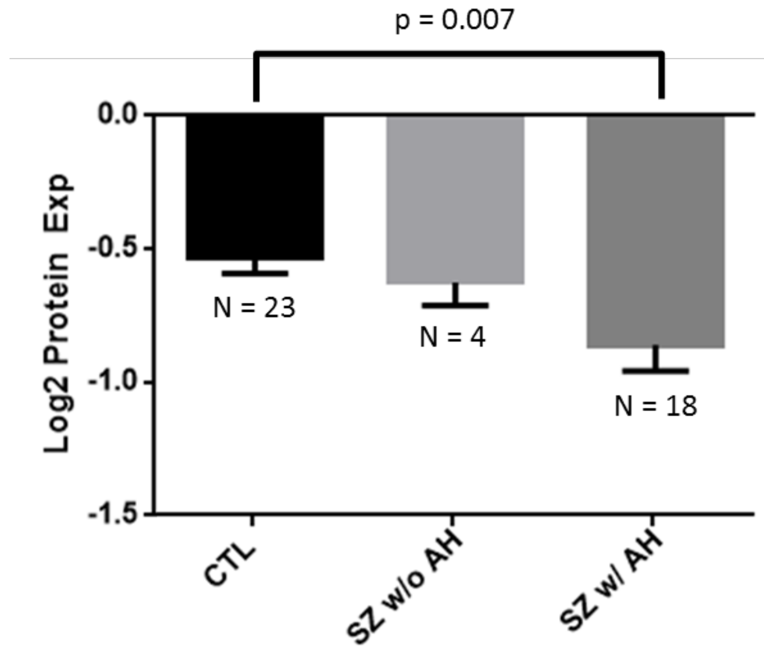

Figure S4. ATP1A3 expression is linked to auditory hallucinations (AH) in schizophrenia (SZ). ATP1A3 mutations that decrease protein expression are associated with auditory hallucinations in rapid onset dystonia parkinsonism patients. The SZ cohort was divided into those with and without auditory hallucinations. The decrease in ATP1A3 was greater, reaching significance, in SZ patients with auditory hallucinations. CTL, control.

Table S1. Subject characteristics.

Case	Sex	Race	Age	PMI	pH	COD	Hand	Diagnosis
1326	M	W	58	16.4	6.7	ASCVD	R	None
1086	M	W	51	24.2	6.8	ASCVD	R	None
10005	M	W	42	23.5	6.7	Trauma	R	None
1201	F	W	52	16.4	6.2	ASCVD	R	None
1255	M	B	37	22	5.9	Pulmonary embolism	R	None
1119	M	W	57	20.2	6.8	ASCVD	R	None
1317	M	W	56	22.9	6.5	ASCVD	L	None
1307	M	B	32	4.8	6.7	ASCVD	R	None
1067	M	W	49	6	6.6	ASCVD	R	None
1196	F	W	36	14.5	6.4	Asphyxiation	R	None
806	M	W	57	24	6.9	Pulmonary embolism	R	None
739	M	W	40	15.8	6.9	ASCVD	R	None
822	M	B	28	25.3	7	ASCVD	L	None
727	M	B	19	7	7.2	Trauma	R	None
659	M	O	46	22.2	6.9	Peritonitis	R	None
852	M	W	54	8	6.8	Cardiac tamponade	R	None
685	M	W	56	14.5	6.6	Hypoplastic coronary artery	R	None
686	F	W	52	22.6	7	ASCVD	R	None
1092	F	B	40	16.6	6.8	Mitral valve prolapse	R	None
1488	M	B	39	21.5	6.4	Pulmonary embolism	R	None
1047	M	W	43	13.8	6.6	ASCVD	R	None
700	M	W	42	26.1	7	ASCVD	R	None
818	F	W	67	24	7.1	Anaphylactic reaction	R	None
1453	M	W	62	11.1	6.4	Trauma	R	Paranoid schizophrenia
1256	M	W	34	27.4	6.4	Hanging	R	Undifferentiated schizophrenia
1189	F	W	47	14.4	6.4	Combined drug overdose	R	Schizoaffective disorder
10020	M	W	38	28.8	6.6	Salicylate overdose	R	Paranoid schizophrenia
1263	M	W	62	22.7	7.1	Asphyxiation	R	Undifferentiated schizophrenia
1173	M	W	62	22.9	6.4	ASCVD	R	Disorganized schizophrenia
1361	M	W	63	23.2	6.4	Cardiomyopathy	U	Schizoaffective disorder
10024	M	B	37	6	6.1	ASCVD	L	Paranoid schizophrenia
10023	F	B	25	20.1	6.7	Drowning	R	Disorganized schizophrenia
665	M	B	59	28.1	6.9	Intestinal hemorrhage	R	Paranoid schizophrenia
1088	M	W	49	21.5	6.5	Combined drug overdose	R	Undifferentiated schizophrenia
787	M	B	27	19.2	6.7	Gunshot	L	Schizoaffective disorder
829	M	W	25	5	6.8	Salicylate overdose	U	Schizoaffective disorder
930	M	W	47	15.3	6.2	ASCVD	R	Disorganized schizophrenia
722	M	B	45	9.1	6.7	Upper GI bleed	R	Undifferentiated schizophrenia
1105	M	W	53	7.9	6.2	ASCVD	R	Schizoaffective disorder
802	F	W	63	29	6.4	Right ventricular dysplasia	A	Schizoaffective disorder
1010	F	B	44	18.7	6.2	Sudden unexpected death	L	Undifferentiated schizophrenia
1222	M	W	32	30.8	6.4	Combined drug overdose	R	Undifferentiated schizophrenia
933	M	W	44	8.3	5.9	Myocarditis	U	Disorganized schizophrenia
625	M	B	49	23.5	7.3	ASCVD	R	Disorganized schizophrenia
917	F	W	71	23.8	6.8	ASCVD	U	Undifferentiated schizophrenia

F, female; M, male; W, white; B, black; COD, cause of death; ASCVD, atherosclerotic cardiovascular disease; GI, gastrointestinal; L, left-handed; R, right-handed; A, ambidextrous; U, unknown; PMI, postmortem interval.

Table S2. Differentially expressed peptides in auditory cortex gray matter homogenates, as measured by LC-SRM/MS. The difference in average expression values and *p*-values for the peptides used to quantify the proteins reported in Table 2 are reported here. Bolded proteins are members of the glutamate signaling pathway.

Protein	SZ/CTL	*p* (Protein)	Peptide	*p* (Peptide)
GRIA4	0.69	0.0004	QTEIAYGTLDSGSTK	0.0064
			GSSLGNAVNLAVLK	0.0236
GRIA3	0.72	0.0139	GSALGTPVNLAVLK	0.0139
ATP1A3	0.93	0.0189	CIELSSGSVK	0.0180
			GGQDNIPVLK	0.0191
PHB2	0.95	0.0323	IVQAEGEAEAAK	0.0323
UCHL1	1.1	0.0412	FSAVALCK	0.0412
VIM	0.9	0.0425	EYQDLLNVK	0.0425
HSP90B	0.89	0.0576	NPDDITQEEYGEFYK	0.0576
PSMA1	1.1	0.0594	LVSLIGSK	0.0594
GAD65	0.9	0.0598	HYDLSYDTGDK	0.0598
GNAQ	1.2	0.0611	VSAFENPYVDAIK	0.0611
NDUFV1	0.93	0.0662	GGAGFPTGLK	0.0662
SYN1	0.88	0.0696	SLKPDFVLIR	0.0456
			TYATAEPFIDAK	0.3024
PRKCA	0.86	0.0792	LTDFNFLMVLGK	0.1246
			STLNPQWNESFTFK	0.2512
PC	0.77	0.0831	GANAVGYTNYPDNVVFK	0.0831
DNM1	1.13	0.0899	NLVDSYMAIVNK	0.4664
			DMLMQFVTK	0.5083
CTNNB1	1.12	0.092	SGGIPALVK	0.0920
ATP5A1	0.93	0.0923	HALIIYDDLSK	0.0561
			VVDALGNAIDGK	0.0766
			TSIAIDTIINQK	0.0302

CTL, control; SZ, schizophrenia; LC-SRM/MS, liquid chromatography-selected reaction monitoring/mass spectrometry.

Table S3. Composition of protein co-expression networks. Proteins composing the co-expression network modules (blue, brown, turquoise, and red) visualized in Figure 1 are reported for control (CTL) and schizophrenia (SZ) subjects.

CTL	Protein	SZ
brown	ATP5A1	brown
brown	PPP2A	brown
brown	ATP1B1	brown
brown	CKB	
brown	RHOA	
brown	TUBB	
brown	STXBP1	
brown	HSPD1	turquoise
	YWHAZ	turquoise
	NDUFA10	turquoise
	HSPA5	turquoise
	PRDX1	turquoise
	SLC25A3	turquoise
turquoise	UCHL1	turquoise
turquoise	YWHAE	turquoise
turquoise	RAC1	turquoise
turquoise	ATPO	turquoise
turquoise	GLUD1	turquoise
turquoise	NSF	turquoise
turquoise	STIP1	turquoise
turquoise	VDAC1	turquoise
turquoise	GNAZ	turquoise
turquoise	GRIA2	turquoise
turquoise	ARF1	
turquoise	ENO2	
turquoise	GAPDH	
turquoise	HSPA4	
turquoise	YWHAQ	
turquoise	ACO1	
turquoise	IDH3A	
turquoise	NDUFV1	
turquoise	VDAC2	
turquoise	VDAC3	
turquoise	AP1B1	
turquoise	AP2A2	
turquoise	CNP	
turquoise	ATP1A1	
turquoise	ATP2A2	
turquoise	SYT1	
turquoise	GNAI1	
blue	GNA1	
blue	GNB1	
blue	CLTC	
blue	NRXN3	
blue	ATP1A3	
blue	NCAM2	
blue	RAB3A	
blue	SEPT3	
blue	VGLUT3	
blue	ATP6V1A	
blue	RAB10	
blue	HK1	
blue	SPTB	
blue	AP2M1	
blue	SYN1	
blue	SPTA1	red
blue	SYNJ1	red
	ANK1	red
	ANK2	red

Table S4. Proteins that differ significantly in connectivity between the control (CTL) and schizophrenia (SZ) networks.

Protein	Node Degree CTL	Node Degree SZ	Delta Node Degree	*p*
		Significantly Reduced Node Degree		
ARF1	8	0	-8	0.005
ATP2A2	6	0	-6	0.02
CNP	6	0	-6	0.008
ENO2	26	0	-26	0.001
GAPDH	34	0	-34	< 0.001
GNAZ	17	2	-15	0.001
GNB1	30	0	-30	< 0.001
HK1	15	0	-15	0.006
HSPA4	4	0	-4	0.02
IDH3A	10	0	-10	0.002
NCAM2	8	0	-8	0.018
NDUFV1	21	0	-21	0.012
NSF	28	9	-19	0.012
SEPT3	6	0	-6	0.005
SPTA1	7	3	-4	< 0.001
SPTB	14	0	-14	0.004
SYT1	9	0	-9	0.02
VDAC1	26	13	-13	0.008
		Significantly Increased Node Degree		
ANK1	0	2	2	0.016
ANK2	0	3	3	0.007
NDUFA10	0	9	9	0.016

Table S5. Association of clinical factors with differentially expressed proteins. Table shows the impact and significance of the potential confounding factors suicide, substance abuse, schizoaffective disorder, antipsychotic medication, and auditory hallucinations on expression of the differentially expressed proteins reported in Table 2.

	Suicide (Y vs N)		Substance (Y vs N)		Schizoaffective (Y vs N)		Antipsychotic (Y vs N)		Auditory Hallucination (Y vs N)	
Protein	**Fold change**	***p*-value**	**Fold change**	***p*-value**	**Fold change**	***p*-value**	**Fold change**	***p*-value**	**Fold change**	***p*-value**
GRIA4	1.15	0.337	0.87	0.346	1.19	0.266	0.90	0.615	1.13	0.507
GRIA3	1.18	0.397	0.94	0.724	1.11	0.598	0.92	0.725	0.79	0.286
ATP1A3	1.02	0.742	1.02	0.724	0.97	0.601	0.95	0.406	0.95	0.327
PHB2	1.01	0.689	1.00	0.975	1.02	0.573	0.92	0.082	0.94	0.165
UCHL1	1.08	0.583	1.25	0.089	1.14	0.352	0.72	0.069	0.94	0.691
VIM	0.91	0.21	0.99	0.912	0.86	0.073	0.95	0.648	1.01	0.906
HSP90B	0.95	0.577	0.96	0.64	0.96	0.679	0.94	0.594	1.01	0.947
PSMA1	1.03	0.677	0.96	0.586	1.05	0.544	0.88	0.185	0.89	0.202
GAD65	1.04	0.66	1.00	0.968	0.98	0.77	1.01	0.928	1.23	0.047
GNAQ	0.95	0.743	0.91	0.513	1.04	0.807	0.83	0.333	0.57	0.002
NDUFV1	1.02	0.741	1.01	0.919	0.95	0.533	0.83	0.048	1.07	0.463
SYN1	0.93	0.553	1.05	0.658	1.14	0.301	0.76	0.076	0.95	0.701
PRKCA	1.05	0.693	0.93	0.554	0.99	0.915	0.93	0.663	1.21	0.181
PC	1.15	0.455	0.85	0.482	0.88	0.488	1.18	0.414	1.26	0.337
DNM1	1.02	0.867	1.12	0.285	1.07	0.553	0.87	0.321	0.99	0.914
CTNNB1	0.87	0.16	0.86	0.112	1.09	0.405	0.97	0.798	0.80	0.053
ATP5A1	1.11	0.159	1.09	0.224	1.05	0.529	0.86	0.133	0.93	0.429

N, no; Y, yes.

Supplemental Methods

Liquid Chromatography - Selected Reaction Monitoring/Mass Spectrometry (LC-SRM/MS) of Human Heschl's Gyrus Homogenates

LC-SRM/MS analyses were conducted on a TSQ Quantum triple stage quadrupole mass spectrometer (ThermoFisher Scientific) with an Ultimate 3000 HPLC (Dionex). 5 µl (~2.5 µg protein) of the sample was loaded on to a Magic C18 column (Michrom) at 1 µl/min for 12 min, and eluted at 750 nl/min over a 25 min gradient from 3-35% mobile phase B (acetonitrile containing 0.1% formic acid). SRM transitions were timed using 1 – 1.5 min retention windows, depending on the number of SRMs to be assayed. Transitions were monitored, allowing for a cycle time of 1 sec, resulting in a dynamic dwell time, never falling below 10 msec. The MS instrument parameters were as follows: capillary temperature 275°C, spray voltage 1100 V, and a collision gas of 1.4 mTorr (argon). The resolving power of the instrument was set to 0.7 Da (full width half maximum (FWHM)) for the first and third quadrupole. Data were acquired using a Chrom filter peak width of 4.0 sec.

Rhesus Monkey Tissue Preparation and LC-SRM/MS

The ventral bank of the principal sulcus was dissected as a sample of dorsolateral prefrontal cortex (Brodmann area 46), as Heschl's gyrus tissue was unavailable, from which 100 mg gray matter was dissected. 50 mg gray matter was homogenized in .5 ml 0.32 M sucrose solution (1 mM MgCl2 and 0.1 mM CaCl2 with protease and phosphatase inhibitors) with a Teflon pestle. Approximately 45 µl of the homogenate was aliquoted, solubilized with 1% SDS and clarified by centrifugation. Protein concentration was measured by micro BCA™ assay in duplicate (Pierce). 20 µg of this homogenate were mixed with 20 µg of the $^{13}C_6$STD and processed for LC-SRM/MS analysis by on-gel trypsin digestion as previously described (1). LC-SRM/MS analyses were conducted on a TSQ Vantage triple stage quadrupole mass

spectrometer (ThermoFisher Scientific) with an Eksigent 2Dnano LC (Eksigent) and a CaptiveSpray source (Michrom). 5 µl (~2.5 µg protein) of the sample was loaded on to a Magic C18 column (Michrom) at 1 µl/min for 12 min, and eluted at 750 nl/min over a 25 min gradient from 3-35% mobile phase B (ACN containing .1% formic acid). SRM transitions were timed using 1 – 1.5 min retention windows, depending on the number of SRMs to be assayed. Transitions were monitored, allowing for a cycle time of 1 sec, resulting in a dynamic dwell time, never falling below 10 msec. The MS instrument parameters were as follows: capillary temperature 275°C, spray voltage 1100 V, and a collision gas of 1.4 mTorr (argon). The resolving power of the instrument was set to 0.7 Da (FWHM) for Q1 and Q3. Data were acquired using a Chrom filter peak width of 4.0 sec.

Peptide/Protein Selection

The selection process for the proteins included in this SRM assay has been extensively described (1). Briefly: Proteins were chosen from previous discovery proteomics analyses of human postmortem cortex pre- and post-synaptic enrichments (1, 2) to interrogate glutamate signaling, and thus include glutamate receptors, signaling proteins and scaffolds as well as other synaptic proteins. Non-redundant peptides from synaptic proteins, including glutamate receptors, kinases, phosphatases and those with roles in vesicular fusion, energy and amino acid metabolism, protein trafficking, cytoskeleton and scaffolding were selected from previously published spectral libraries (1-3). The peptides were then filtered by the following criteria: 1) the presence of a lysine, the amino acid labeled in the $^{13}C_6$STD, 2) non-redundant to a selected protein or protein group (determined by BLAST search) and 3) 100% homology across mouse and human sequences (determined by BLAST search).

Data Processing

Peak areas and area ratios were calculated for each peptide within Skyline (4). Raw files generated by LC-SRM/MS analysis were loaded into Skyline files containing target proteins/peptides/transitions. All individual SRM transitions and integration areas were manually inspected. Transitions for which the signal-to-noise ratio was below 3 were excluded from analysis. The ratios of the integrated areas for "light" endogenous peptides and "heavy" $^{13}C_6$STD peptides were calculated to obtain peptide measures using multiple transitions per peptide. For the proteins with multiple peptides, peptide ratios (light/heavy, before log2 transformation) within the same protein were summed up to obtain the protein level ratios (5). All the ratios were then log2 transformed for the following statistical analysis.

Supplemental References

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5. Carrillo B, Yanofsky C, Laboissiere S, Nadon R, Kearney RE (2010): Methods for combining peptide intensities to estimate relative protein abundance. *Bioinformatics*. 26:98-103.