

Vagal Nerve Stimulation Modifies Neuronal Activity and the Proteome of Excitatory Synapses of Amygdala/Piriform Cortex

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Supplemental Materials

Supplemental Figure Legends:

Figure S1. Assessment of reproducibility of the analytical approach employed in this study. A, Triplicate PSD fractions were generated and analyzed to determine the variation in any one protein's measured intensity across three replicate preparations. B, A histogram of the number of proteins within bins of measured variations (bins of 5% relative standard deviation) show that the sample preparation protocol is reproducible and the workflow is capable of quantitatively measuring differences in samples due to biology.

Figure S2: Three-dimensional principal component analysis (PCA) of the z-score corrected protein intensity values across control (n=4 animals, n=8 analytical injections) and VNS challenge (n=6 animals, n=12 analytical injections). For each unique biological (i.e., animal) sample, two datasets (i.e., technical duplicates) were acquired. A, The PCA analysis at the global protein level (n=436 proteins) indicated a VNS-stimulus dependent separation based on PC2. B, The PCA analysis using only those n=22 proteins classified as being differentially regulated in VNS versus Control indicated a VNS-stimulus dependent separation based on PC1.

Figure S3. A, Subcellular distribution of PSD proteins in the rat brain. Proteins (10 or 1 μ g) of each fraction isolated from rat hippocampus were analyzed by immunoblotting analysis with indicated antibodies. B, Quantification of enrichment of PSD-95 and synaptophysin in rat brain subcellular fractions. PSD-95, a marker of the postsynaptic density, was highly enriched in PSD-1T and PSD-2T fractions whereas synaptophysin, a marker of the presynaptic axon terminal, was highly enriched in presynaptic fraction and absent in PSD fractions. The enrichment of PSD-95 and absence of synaptophysin immunoreactivities in the PSD fractions validated the purification of the PSD fraction.

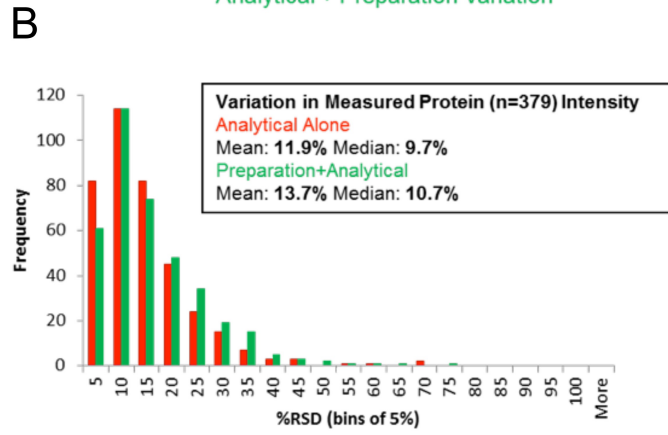
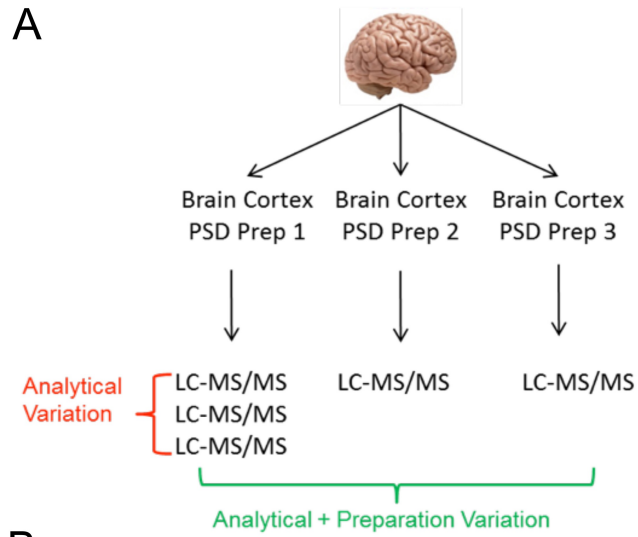


Figure S1.

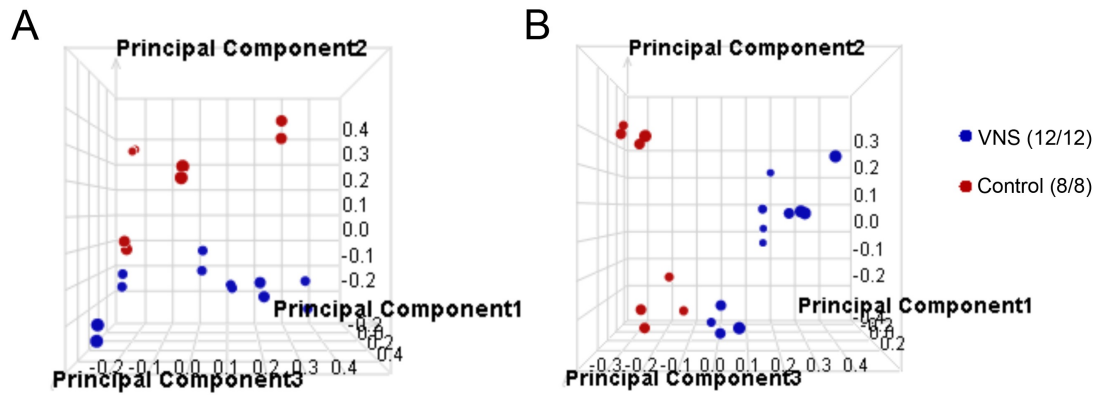


Figure S2.

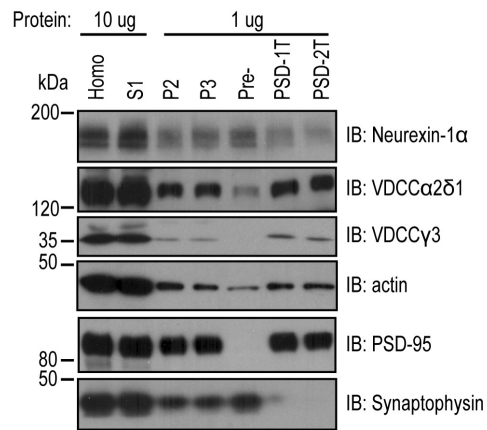
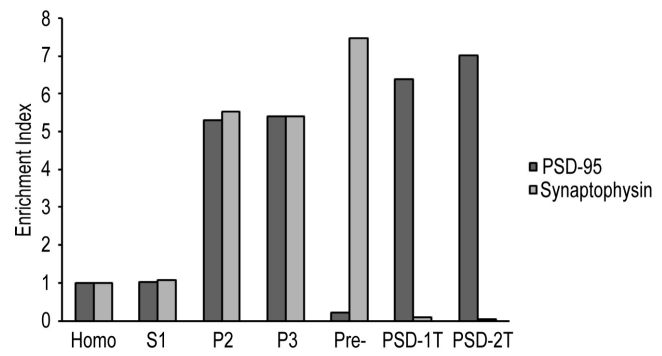
A**B**

Figure S3.

			Peptide 1	Peptide 2	Peptide 3	Peptide 4	Peptide 5	Peptide 6	Peptide 7	Peptide 8	Peptide 9	Peptide 10	Peptide 11	Peptide 12	Peptide 13	Peptide 14	Peptide 15	Peptide 16	Peptide 17	Peptide 18	Peptide 19	Peptide 20																	
PGAM5_RAT	Serine/threonine-protein phosphatase PGAM5, mitochondrial	1	AVETTDISK																																				
SKP1_RAT	S-phase kinase-associated protein 1	1	NDFTEEEEAQVR																																				
SRCN1_RAT	SRC kinase signaling inhibitor 1	8	THPGPTPTPDHKPKK	SSGATPVSGPPPPA	SLSTYSAALOSDL	VVTDTLAQIR	LGGAPTSQGVSPSPSAILER	STGASTAGAPPELPPGPE	LQLQNQESVR	AGAGGPLYDGGYG																													
Membrane Trafficking																																							
AP2A2_MOUSE	AP-2 complex subunit alpha-2	5	KGPSTVTDLEETKR	DDVQGYAAK	NADVELOQR	ALLLSTYIK	ATIQDVL																																
AP2B1_MOUSE	AP-2 complex subunit beta-1	6	LQNNVYTIK	NINLVQK	LAPPLVTLISGEPE	LLSTDPVQYVALR	TAK	FLELLPK	IOPGNPYTLCLK																														
AP2M1_MOUSE	AP-2 complex subunit mu-1	5	LNYSDDHVIK	SGIYETR	DIILPFR	TFITQQGIK	ISEENIK																																
DYN1_MOUSE	Dynamain-1	4	VPVGDQPPDIEFQIR	LQDAFSAIQONADL	DLQPIAVVVGQSSA	SSVLENFVGR	GISPVPINLR																																
NPC1_MOUSE	Niemann-Pick C1 protein	1	GTERRLLNF																																				
SYN1_RAT	Synapsin-1	15	VLLVIDEHPDWDWAK	LPSPATAAQSSQATPMTQGGQR	GSHSQT	PSPGALP	GSHSQT	ASTAAPV	ASRAAPS	PGSSGG	FYATAEP	EM[147.0354]LSSTYPVVVK				SQSLTNA	FNLPEA	PPRPSLS	GGPPQ	GGPPQ	GGPPQ	GGPPQ																	
SYN2_RAT	Synapsin-2	6	FPLIEQTYYPNHR	EMLTLPFPVVVK	QTAASAGLVDAPAPSAASR	QLITDLVSK	TPALSPQ	TPALSPQ	RPLTTQQ	QSGTLK	EPDSSK																												
SNP25_MOUSE	Synaptosomal-associated protein 25	5	RADQLADESLESTRR	ADQLADESLESTRR	EQMAISGFIR	AWGNNGDGVVASK	MLQVEE																																
SYT1_MOUSE	Synaptotagmin-1	9	MDVGGSLDPYVK	NTLNPFY	NESFSFE	VPFEIQK	LTVVILEA	DLQSAEK	EEQEK	VFVGYNS	TGAELR	VPSELG	TLNPFVNEQFTFK	LGDI[16.0307]FS	VFLLPDK																								
SYT7_MOUSE	Synaptotagmin-7	2	NLNPIFNESFADFIPTEK	NDPIGEVSIPLNK																																			
SYTL3_MOUSE	Synaptotagmin-like protein 3	1	ALGLLLNR																																				
SNPH_RAT	Syntaxin-1	8	HSELDLHP	SGPR	VGQAAGC[160.0307]SSVLK	EEGTGES	EAPEPT	QAGDPS	ADSGFVA	ADDTLSR	NTILEK	AIGTDFV	QYQPD	LAL																									
STX1A_RAT	Syntaxin-1A	3	SIEQSIEQEGLNR	IAENVEE	QALSEI	TR																																	
STX1B_MOUSE	Syntaxin-1B	4	QLEITGR	QALNEI	LAIFTDI	K	AIEQSIEQEGLNR																																
STXB1_MOUSE	Syntaxin-binding protein 1	8	YETSGIGEAR	EPLPSLE	AVYLTPS	EK	DNALLAQ	LIQDK	SSASFST	TAVSAR	HIAEVSG	EVTR	ISEQTYQ	LSR	SQLLILDR	LNKTDEE	ISS																						
Metabolism																																							
CN37_RAT	2',3'-cyclic-nucleotide 3'-phosphodiesterase	17	AGQVFLLELGNHK	ADFSEYK	LSISALF	TPK	LDC[160.0307]JAQLK	LDEDLAG	YC[160.0307]R	AIFTGYY	GK		TAGAOLV	LNEQELQ	LWPSLDD	KPSSSES	LFPPGR	HFISGDE	GGSQGE	EVGELPR	RPPGVLH	C[160.0307]TJK	MVSADAY	K	ADFSEYK	HFISGDE	PK	ATGAEY	AQDDV	R	VLLDDT	NHER	QWQLS	LDDLK	LDLVSF	GK			
THIL_RAT	Acetyl-CoA acetyltransferase, mitochondrial	4	FANEITPITISVK	IAAFADAA	VDPIDFP	LAPAYAV	PK	LGTIAIQ	AIEK	LEDLIVK																													
ADH1_YEAST	Alcohol dehydrogenase 1	8	ANELLINKV	ANGTTVLVGMPAG	YVVDTSK	EALDFFA	K	DIVGAVL	K			C[160.0307]C[160.0307]SDVFNQVVK	VVGLSTL	PEIYEK	SISVSGY	VGNR																							
AOFA_RAT	Amine oxidase [flavin-containing] A	7	EVLNALGK	DIWVEEP	ESK	EIPVDAP	WQAR	WVDVGG	AYVGP	QTNR	INVLVLEA	R	YVISAIPPI	LTAK	IFSVTNG	SGER																							
AATM_MOUSE	Aspartate aminotransferase, mitochondrial	1	IAATILTPDLR																																				
KCRU_MOUSE	Creatine kinase, ubiquitous mitochondrial	7	TVGMVAG	DEETYEVE	AELFDVPIQ	ER	TVGM147	0354]VAG	GTGGVD	TAATGSV	FDISNLD	R	VVVDALS	GLK	LGYLTC	[160.0307]	PSNLGTG	LYPPSAE	YPDLR	SGYFDER																			
QCR1_RAT	Cytochrome b-c1 complex subunit 1, mitochondrial	8	IEEVDAQM	VR	EVESIGA	HLNAYSTR	LC[160.0307]TSATE	SEVTR	TDLTDYL	SR	VWELLADI	VONISLE	DSQIEK	MVLAAG	GVK	FTGSEIR	IPLAWE	SR																					
QCR2_RAT	Cytochrome b-c1 complex subunit 2, mitochondrial	12	AVAFQNPQ	TR	LASTLTK	AVAGGNL	SSADVOA	AK	LASTLTK	AVAGGNL	SSADVOA	AK	TSAPGGV	PLOPQEL	EFTK	SMTASG	NLGHTEP	LDEL	IENLHDV	AYK	NALANPL	YC[160.0307]PDYR	EVAEQFL	NIR	RGNNTTS	LLSQSVA	K	GNNTTSL	LSQSVAK	WEVAALRL	LSR	LPNGLVIA	SLENYAF						

	Peptide 65	Peptide 66	Peptide 67	Peptide 68	Peptide 69	Peptide 70
Cytoskeleton-Actin						
ACTG_RAT						
ARPC4_MOUSE						
ACTN1_RAT						
AINX_RAT						
ANK2_MOUSE						
E41L1_RAT						
E41L3_MOUSE						
COR1A_RAT						
COR1C_MOUSE						
DREB_RAT						
CAZA2_MOUSE						
SPTA2_RAT	DVDETIG WIK	DLAALED K	LQVASDE NYKDPTN LQGK	LFGAAEV QR	EAALTSE EVGADLE QVEVLQK	LLEATEL K
SPTB2_MOUSE						
SPTN2_RAT						
SYNPO_RAT						
Cytoskeleton-Other						
GFAP_RAT						
K1C10_MOUSE						
K2C1_MOUSE						
K2C6A_RAT						
MAP1A_RAT						
MAP1B_RAT						
MAP2_RAT						
MAP4_MOUSE						
MAP6_RAT						
TAU_RAT						
MLP3B_RAT						
NFH_RAT						
NFL_MOUSE						
NFM_RAT						
SEPT3_RAT						
PLEC1_RAT						
SEP11_RAT						
SEP14_MOUSE						
SEPT2_RAT						

Supplemental Table 2. Ingenuity Pathway Analysis of 56 Proteins Significantly Changed by VNS ($p < 0.05$), as detected by LC-MS/MS.

Top Canonical Pathways

Name	p-value	Overlap
Glutamate Receptor Signaling	5.46E-09	10.5 % 6/57
Amyotrophic Lateral Sclerosis Signaling	7.83E-06	4.5 % 5/111
RhoGDI Signaling	6.63E-05	2.9 % 5/173
Signaling by Rho Family GTPases	3.49E-04	2.0 % 5/247
phagosome maturation	4.36E-04	2.8 % 4/144

Top Networks

ID	Associated Network Functions	Score
1	Cell-To-Cell Signaling and Interaction, Nervous System Development and Function, Amino Acid Metabolism	50
2	Cell-To-Cell Signaling and Interaction, Hair and Skin Development and Function, Cellular Movement	32
3	Connective Tissue Disorders, Inflammatory Disease, Inflammatory Response	22
4	Cell Morphology, Nervous System Development and Function, Tissue Morphology	6
5	Cancer, Hematological Disease, Immunological Disease	3

Top Diseases and Bio Functions

Diseases and Disorders

Name	p-value	#Molecules
Neurological Disease	7.37E-03 - 1.24E-08	38
Organismal Injury and Abnormalities	7.37E-03 - 3.31E-08	46
Psychological Disorders	7.37E-03 - 3.31E-08	22
Skeletal and Muscular Disorders	7.37E-03 - 2.14E-07	24
Hereditary Disorder	7.37E-03 - 2.38E-06	26

Molecular and Cellular Functions

Name	p-value	#Molecules
Cellular Assembly and Organization	7.37E-03 - 6.50E-11	30
Cellular Function and Maintenance	7.37E-03 - 6.50E-11	33
Cell Morphology	7.37E-03 - 5.25E-10	29
Cell-To-Cell Signaling and Interaction	7.37E-03 - 4.48E-09	28
Cellular Development	7.37E-03 - 6.89E-09	27

Physiological System Development and Function

Name	p-value	#Molecules
Behavior	7.37E-03 - 4.46E-10	20
Nervous System Development and Function	7.37E-03 - 5.25E-10	28
Tissue Morphology	7.37E-03 - 5.25E-10	22
Tissue Development	7.37E-03 - 6.89E-09	27
Embryonic Development	7.37E-03 - 3.85E-06	17