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Intracellular fibril formation, calcification and enrichment of chaperones, cytoskeletal and intermediate filament proteins in the adult hippocampus CA1 following neonatal exposure to the non-protein amino acid BMAA

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Supplementary Material

Table 1 Details of primary and secondary antibodies, and detection systems used in the immunohistochemistry

Primary Antibody/ dilution	Manufacturer/ product number	Antigen retrieval	Secondary antibody/ dilution	Detection system
Alpha-synuclein 1/500	Abcam/ ab87599	NA	OmniMap Rb (Roche)/ prediluted	Chromo-Map DAB kit (Ventana/ Roche)
GFAP 1/2000	DAKO/ Z0334	Protease 1, 8 min	Goat anti Rabbit biotin immunoglobulin (prod no E0432 Dako)/ 1/150	DAB-Map kit (Ventana/ Roche)
Isolectin B4 Biotin conjugated 1/100	Vector/ B-1205	Mw 10 min pH6 citrate buffer	NA	Streptavidin HRP (Dako)
TARDBP (TDP-43) 1/1000	Abcam/ ab42474	Protease 2, 8 min	OmniMap Rb (Roche)/ prediluted	Chromo-Map DAB kit (Ventana/ Roche)
Tau protein 1/2000	Invitrogen/ AHB0042	SCC1	Goat anti Mouse biotin immunoglobulin (prod no E0433 Dako)/ 1/250	DAB-Map kit (Ventana/ Roche)
Tubulin	Abcam/ ab6046	SCC2	OmniMap Rb (Roche)/ prediluted	Chromo-Map DAB kit (Ventana/ Roche)
Ubiquitin 1/100	Abcam/ ab7780	SCC1	OmniMap Rb (Roche)/ prediluted	Chromo-Map DAB kit (Ventana/ Roche)

Supplemental Table 2: Proteins enriched in of histologically altered hippocampal CA1 area*

Accession	Proteins	MW [kDa]	Control ¹	Sample ¹	Abundance ratio
G6S3A5	Plectin 1 [G6S3A5_RAT]	519.7	2	48	24.0
P31000	Vimentin [VIME_RAT]	53.7	3	68	22.7
A1E252	Glial fibrillary acidic protein (GFAP) epsilon, Fragment [A1E252_RAT]	18.5	1	16	16.0
D3ZE14	Uncharacterized protein [D3ZE14_RAT]	46.0	1	10	10.0
P15865	Histone H1.4 [H14_RAT]	22.0	1	9	9.0
Q00715	Histone H2B type 1 [H2B1_RAT]	14.0	2	17	8.5
P47819	Glial fibrillary acidic protein [GFAP_RAT]	49.9	17	133	7.8
P02650	Apolipoprotein E [APOE_RAT]	35.7	1	7	7.0
P37397	Calponin-3 [CNN3_RAT]	36.4	1	6	6.0
Q66HD0	Endoplasmic [GRP94] [ENPL_RAT]	92.7	1	6	6.0
P08050	Gap junction alpha-1 protein (connexin 43) [CXA1_RAT]	43.0	1	6	6.0
D3ZJ08	Histone H3 [D3ZJ08_RAT]	15.4	1	6	6.0
P04764	Alpha-enolase [ENOA_RAT]	47.1	2	11	5.5
O08651	D-3-phosphoglycerate dehydrogenase [SERA_RAT]	56.5	1	5	5.0
P06761	78 kDa glucose-regulated protein [GRP78_RAT]	72.3	2	8	4.0
D3ZH77	Microtubule-associated protein (Map4) [D3ZH77_RAT]	95.0	2	8	4.0
Q6IFZ4	Type II keratin Kb15 [Q6IFZ4_RAT]	57.6	1	4	4.0
P07335	Creatine kinase B-type [KCRB_RAT]	42.7	9	33	3.7
F42123	L-lactate dehydrogenase B chain [LDHB_RAT]	36.6	2	7	3.5
P16617	Phosphoglycerate kinase 1 [PGK1_RAT]	44.5	2	7	3.5
Q6P750	Pyruvate kinase [Q6P750_RAT]	52.9	6	19	3.2
P35213	14-3-3 protein beta/alpha (Ywhab) [1433B_RAT]	28.0	1	3	3.0
P61983	14-3-3 protein gamma (Ywhag) [1433G_RAT]	28.3	1	3	3.0
P38650	Cytoplasmic dynein 1 heavy chain [DYHC1_RAT]	531.9	1	3	3.0
D3ZC88	Elongation factor 1-alpha [D3ZC88_RAT]	50.1	4	12	3.0
P05197	Elongation factor 2 [EF2_RAT]	95.2	1	3	3.0
P61980	Heterogeneous nuclear ribonucleoprotein K [HNRPK_RAT]	50.9	1	3	3.0
F1LMU0	Myosin-4 [F1LMU0_RAT]	222.7	2	6	3.0
B1WBUB9	Phosphorylase [B1WBUB9_RAT]	97.3	1	3	3.0
P67779	Prohibitin [PHB_RAT]	29.8	1	3	3.0
B0K023	Rpl7 protein, Fragment [B0K023_RAT]	26.2	1	3	3.0
Q8CFD0	Sideroflexin-5 [SFXN5_RAT]	37.3	1	3	3.0
Q3ZB99	Tight junction protein 2 [Q3ZB99_RAT]	131.3	1	3	3.0
D3ZTF3	Uncharacterized protein [D3ZTF3_RAT]	32.9	1	3	3.0
O88767	Protein DJ-1 (Park7) [PARK7_RAT]	20.0	2	5	2.5
P09117	Fructose-bisphosphate aldolase C [ALDOC_RAT]	39.3	7	17	2.4
P11980	Pyruvate kinase isozymes M1/M2 [KPYM_RAT]	57.8	9	21	2.3
P04797	Glyceraldehyde-3-phosphate dehydrogenase [G3P_RAT]	35.8	12	26	2.2
P30835	6-phosphofructokinase [K6PL_RAT]	85.3	2	4	2.0
D3Z299	Alpha-adducin [D3Z299_RAT]	73.1	1	2	2.0
F1LPM4	Amine oxidase [flavin-containing] B (Maob) [F1LPM4_RAT]	56.9	1	2	2.0
G3V936	Citrate synthase [G3V936_RAT]	51.8	1	2	2.0
Q7M0E3	Actin-depolymerizing factor (Destrin) [DEST_RAT]	18.5	2	4	2.0
Q62952	Dihydropyrimidinase-related protein 3 [DPYL3_RAT]	61.9	2	4	2.0
Q8JHL4	Drebrin-like protein [DBNL_RAT]	48.6	1	2	2.0
G3V6R2	Excitatory amino acid transporter (EAT2) 2 [G3V6R2_RAT]	60.9	3	6	2.0
B4F772	Heat shock 70 kDa protein [B4F772_RAT]	94.2	1	2	2.0
P10111	Peptidyl-prolyl cis-trans isomerase A [PIPA_RAT]	17.9	1	2	2.0
P35704	Peroxiredoxin-2 [PRDX2_RAT]	21.8	1	2	2.0
F1LRX5	Protein Sptbn1, Fragment [F1LRX5_RAT]	267.7	5	10	2.0
P52873	Pyruvate carboxylase, mitochondrial [PYC_RAT]	129.7	2	4	2.0
Q64548	Reticulon-1 [RTN1_RAT]	83.0	1	2	2.0
Q64428	Trifunctional enzyme subunit alpha, mitochondrial [ECHA_RAT]	82.6	1	2	2.0
D4A786	Uncharacterized protein [D4A786_RAT]	6.4	1	2	2.0
P60711	Actin, cytoplasmic 1 [ACTB_RAT]	41.7	34	67	2.0
P68035	Actin, alpha cardiac muscle 1 [ACTC_RAT]	42.0	26	47	1.8
P62250	40S ribosomal protein S16 [RS16_RAT]	16.4	2	2	Exclusively detected
P62909	40S ribosomal protein S3 [RS3_RAT]	26.7	3	3	Exclusively detected
P62755	40S ribosomal protein S6 [RS6_RAT]	28.7	2	2	Exclusively detected
P62243	40S ribosomal protein S8 [RS8_RAT]	24.2	2	2	Exclusively detected
D3ZD02	60S ribosomal protein L13 [D3ZD02_RAT]	24.0	3	3	Exclusively detected
P50878	60S ribosomal protein L4 [RL4_RAT]	47.2	2	2	Exclusively detected
F2Z3S2	60S ribosomal protein L7a, Fragment [F2Z3S2_RAT]	29.8	3	3	Exclusively detected
O70371	Annexin, Fragment (Anxa5) [O70371_RAT]	33.9	3	3	Exclusively detected
P07150	Annexin A1 [ANXA1_RAT]	38.8	2	2	Exclusively detected
Q07936	Annexin A2 [ANXA2_RAT]	38.7	4	4	Exclusively detected
P14669	Annexin A3 [ANXA3_RAT]	36.3	11	11	Exclusively detected
P55260	Annexin A4 [ANXA4_RAT]	35.8	4	4	Exclusively detected
P15429	Beta-enolase [ENOB_RAT]	47.0	7	7	Exclusively detected
P09456	cAMP-dependent protein kinase regulatory subunit (Prkar1a) [KAP0_RAT]	43.1	4	4	Exclusively detected
G3V7K3	Ceruloplasmin [G3V7K3_RAT]	120.6	2	2	Exclusively detected
D4ACB8	Chaperonin subunit 8 (Cct8) [D4ACB8_RAT]	59.6	3	3	Exclusively detected
G3V836	Clusterin (apolipoprotein J) [G3V836_RAT]	51.4	3	3	Exclusively detected
P47875	Cysteine and glycine-rich protein 1 [CSR1_RAT]	20.6	2	2	Exclusively detected
P36201	Cysteine-rich protein 2 [CRIP2_RAT]	22.7	2	2	Exclusively detected
Q68FS4	Cytosol aminopeptidase [AMPL_RAT]	56.1	3	3	Exclusively detected
Q9J166	Electrogenic sodium bicarbonate cotransporter 1 [S4A4_RAT]	121.3	2	2	Exclusively detected
G3V732	Elongation factor 1-delta [G3V732_RAT]	28.7	2	2	Exclusively detected
G3V846	Excitatory amino acid transporter 1 [G3V846_RAT]	59.6	2	2	Exclusively detected
P55051	Fatty acid-binding protein, brain [FABP7_RAT]	14.9	6	6	Exclusively detected
D4A3F5	G protein-coupled receptor (Gprc5b) [D4A3F5_RAT]	44.0	6	6	Exclusively detected
P04904	Glutathione S-transferase alpha-3 [GSTA3_RAT]	25.3	5	5	Exclusively detected
B6DYQ1	Glutathione S-transferase mu 1 [B6DYQ1_RAT]	26.0	6	6	Exclusively detected
D3ZNT4	Heat shock protein beta-1 (Hsp27) [D3ZNT4_RAT]	21.7	12	12	Exclusively detected
Q4V8K6	Heterogeneous nuclear ribonucleoprotein C (C1/C2) [Q4V8K6_RAT]	32.8	2	2	Exclusively detected
G3V8L3	Lamin A, isoform CRA_b [G3V8L3_RAT]	74.3	3	3	Exclusively detected
Q63016	Large neutral amino acids transporter small subunit 1 [LAT1_RAT]	55.9	2	2	Exclusively detected
Q6P9U7	L-lactate dehydrogenase (Ldh) [Q6P9U7_RAT]	29.8	2	2	Exclusively detected
Q9ZXP0	L-xylulose reductase [DCXR_RAT]	25.7	3	3	Exclusively detected
Q6AYC4	Macrophage-capping protein [CAPG_RAT]	38.8	3	3	Exclusively detected
B2GV33	Maoa protein, Fragment (Maoa) [B2GV33_RAT]	58.8	3	3	Exclusively detected
P08494	Matrix Gla protein [MGP_RAT]	12.0	2	2	Exclusively detected
D4ABB2	Mic1 [D4ABB2_RAT]	44.3	2	2	Exclusively detected
F1M047	Mitogen-activated protein kinase 3, Fragment (Mapk3) [F1M047_RAT]	42.0	3	3	Exclusively detected
Q63716	Peroxiredoxin-1 [PRDX1_RAT]	22.1	4	4	Exclusively detected
Q35244	Peroxiredoxin-6 [PRDX6_RAT]	24.8	2	2	Exclusively detected
D3ZU02	Phosphoglucosyltransferase 3 (Predicted), isoform CRA_d [D3ZU02_RAT]	55.2	2	2	Exclusively detected
G3V8L9	Polymerase I and transcript release factor (Predicted) [G3V8L9_RAT]	43.9	2	2	Exclusively detected
D4AC23	Protein Cct7 [D4AC23_RAT]	59.6	2	2	Exclusively detected
P11598	Protein disulfide-isomerase A3 [PDIA3_RAT]	56.6	2	2	Exclusively detected
F1M5V2	Protein Glipr2, Fragment (GAPR-1) [F1M5V2_RAT]	16.7	6	6	Exclusively detected
F1LV13	Protein Hnrpm [F1LV13_RAT]	73.7	4	4	Exclusively detected
D3ZS68	Protein Pcbp1 [D3ZS68_RAT]	37.5	3	3	Exclusively detected
D3ZHJ1	Protein Psat1 [D3ZHJ1_RAT]	40.2	3	3	Exclusively detected
F1LQW3	Protein Slpq, Fragment [F1LQW3_RAT]	65.7	3	3	Exclusively detected
D3ZGP5	Protein Tardbp (TDP-43) [D3ZGP5_RAT]	44.5	2	2	Exclusively detected
P50399	Rab GDP dissociation inhibitor beta [GDIB_RAT]	50.5	4	4	Exclusively detected
Q5PQK5	Radixin [Q5PQK5_RAT]	68.5	2	2	Exclusively detected
Q5X173	Rho GDP-dissociation inhibitor 1 [GDIR1_RAT]	23.4	2	2	Exclusively detected
B0BN81	Ribosomal protein S5, isoform CRA_b [B0BN81_RAT]	22.9	2	2	Exclusively detected
P29457	Serpin H1 [SERPH_RAT]	46.5	2	2	Exclusively detected
Q63555	SF120 (Hnmpu) [Q63555_RAT]	87.7	3	3	Exclusively detected
Q6LDS4	Superoxide dismutase [Cu-Zn] (Sod1) [Q6LDS4_RAT]	15.7	2	2	Exclusively detected
Q7TFB1	T-complex protein 1 subunit delta (Cot4) [TCPD_RAT]	58.1	2	2	Exclusively detected
Q06F00	T-complex protein 1 subunit epsilon (Cot5) [TCPE_RAT]	59.5	2	2	Exclusively detected
Q2LAP6	Testin [TES_RAT]	47.6	2	2	Exclusively detected
Q498D4	Tin1 protein [Q498D4_RAT]	161.9	2	2	Exclusively detected
P37805	Transgelin-3 [TAGL3_RAT]	22.5	2	2	Exclusively detected
P50137	Transketolase [TKT_RAT]	67.6	2	2	Exclusively detected
F1LP60	Uncharacterized protein, Fragment [F1LP60_RAT]	67.6	2	2	Exclusively detected

* A neonatal rat on PND 9-10 was injected sc with BMAA (460 mg/kg) and sacrificed 6 months later. Cryo-sections were used for laser capture microdissection and LC-MS/MS of the hippocampal CA1. The CA1 sample containing birefringent material was compared with an adjacent normal tissue. ¹Number of different peptides detected for each protein (indicative of protein abundance).