High Resolution Metabolite Imaging in the Hippocampus Following Neonatal Exposure to the Environmental Toxin BMAA using ToF-SIMS

Jörg Hanrieder [1,2,], Lorenz Gerber[3], Åsa Persson Sandelius[4], Eva B. Brittebo[5], Andrew G. Ewing [1,2,4], Oskar Karlsson [5,6]*

1 National Center for Imaging Mass Spectrometry, University of Gothenburg and Chalmers University of Technology, Gothenburg, Sweden

2 Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg Sweden

3 Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology Swedish University of Agricultural Sciences, Umeå, Sweden

4 Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden

5 Department of Pharmaceutical Biosciences, Drug Safety and Toxicology , Uppsala University, Uppsala, Sweden

6 Department of Environmental Toxicology, Uppsala University, Uppsala, Sweden

Content:

SI Table 1: Molecular species that were found changed in the CA1 between BMAA and control animals as revealed by OPLS DA.

positive ions Increased in BMAA m/z	I.D.[1]	Ratio BMAA/Ctrl[2]	Loading[3]
22.99	Na+	1.20	0.023
86.1	TME, C5H12N+	1.04	0.010
104.1	choline, C5H14NO+	1.09	0.025
119.87	K2CNO+	1.21	0.013
166.88	C5H13PNO3	1.07	0.001
184.09	C5H15PNO4+	1.02	0.016
369.29	cholesterol C27H45+	2.22	0.014
496.25	LPC (16:0)	1.76	0.030
520.25	LPC (18:2)	1.49	0.031
523.42	DAG(30:0-OH)	1.57	0.021
524.48	LPC (18:0)	1.78	0.027
551.28	DAG(32:0-OH)	1.66	0.031
568.09	PC (22:6)	2.47	0.033
577.38	DAG(34:1-OH)	1.81	0.032
725.23	SM(d18:1/16:0)+Na	1.64	0.031
731.41	SM(d18:1/18:0)	1.73	0.022
734.39	PC(32:0)	1.44	0.020
753.54	SM(d18:1/18:0)+Na	2.02	0.030
756.39	PC(32:0)+Na	1.94	0.032
758.35	PC(34:2)	1.76	0.014
760.4	PC(34:1)	1.22	0.003
769.41	SM(d18:1/18:0)+K	2.00	0.032
770.15	PC (32:0)+K	2.15	0.027
770.41	PC(32:1)+K	1.55	0.034
772.36	PC(32:0)+K	1.57	0.029
780.19	PC (34:2)+Na	2.12	0.021
782.38	PC(36:4)	1.55	0.010
784.39	PC(36:3)	1.52	0.021
786.39	PC(36:2)	1.63	0.015
788.42	PC(36:1)	1.51	0.008
804.31	PC(36:4)+Na	1.44	0.007
806.31	PC(38:6)	1.74	0.014
810.4	PC(38:4)	1.86	0.015
820.31	PC(36:4)+K	1.63	0.008
822.27	PC (36:3)+K	2.65	0.010
824.29	PC(36:2)+K	1.34	0.006
826.36	PC(36:1)+K	1.45	0.007
832.42	PC(38:4)+Na	1.71	0.006
834.46	PC (40:6)	2.21	0.010

negative ions

Increased in BMAA m/z	I.D.[1]	Ratio BMAA/Ctrl[2]	Loading[3]
385.33	cholesterol, C27H45O-	1.38	0.002
675.49	PA(34:0)	1.07	0.011
701.47	PA(36:1)	1.23	0.008
716.53	PE(16:0/18:1)	1.08	0.005
722.46	PE(16:0p/20:4)	1.56	0.027
726.52	PE(18:1p/18:0)	1.46	0.019
728.51	PE(18:0p/18:0)	1.19	0.006
742.52	PE(18:1/18:1)	1.24	0.008
746.48	PE(16:0p/22:6)	1.13	0.012
750.47	PE(18:0p/20:4)	1.55	0.025
762.52	PE(16:0/22:6)	1.55	0.024
764.52	PE(18:1/20:4)	1.30	0.019
766.49	PE(18:0/20:4)	1.19	0.006
774.59	PE(18:0p/22:6)	1.08	0.003
778.49	ST(d18:1/16:0)	2.45	0.034
786.54	PS(36:2)	1.32	0.006
788.51	PS(36:1)	1.45	0.011
790.49	PE(18:0/22:6)	1.28	0.013
794.5	PE(18:0/22:4)	1.82	0.032
806.55	ST(d18:1/18:0)	1.81	0.033
810.47	PS(18:0/20:4)	1.62	0.024
816.5	PS(18:0/20:1)	1.48	0.023
805.29	TAG(48:0)	1.95	0.015
829.32	TAG(50:0)	2.04	0.013
Decreased in BMAA			
78.97	PO3-	0.73	-0.034
96.99	H2PO4-	0.91	-0.020
747.45	PG(16:0/18:1)	0.89	-0.007
833.43	PI(16:0/18:2)	0.93	-0.002
837.59	PI(16:0/18:0)	0.68	-0.014

1 Tentative assignment based on accurate mass, isotope pattern and literature values. Lipid annotation is based on lipid maps (*1, 2*). Abbreviations: **TME**: trimethylethylimine; **PC**: phosphatidylcholine; **LPC**: lysophosphatidyl-choline; **DAG**: diacylglyceride; **TAG**: triacylglyceride; SM: sphingomyelin; **PA**: diacylphosphate, **FA**: fatty acids; **AA**: arachnoid acid; **PE**: phosphoethanolamine; **ST**: sulfatide; **PS**: phosphatidylserine; **PG**: phosphatidylglycerol; **PI**: phosphatidylinositol

2 average fold change in intensity in BMAA compared to Controls.

3 Loadings on component 1. Positive loadings indicate contribution to BMAA, while negative loading values indicate association with control.

SI Table 2: Molecular species that were found changed in the DG between BMAA and control animals as revealed by OPLS DA.

m/z	I.D.[1]	Ratio BMAA/Ctrl[2]	Loading[3]
positive ions			
Increased in BMAA			
22.99	Na+	1.24	0.033
38.97	K+	1.08	0.018
86.1	TME, C5H12N+	1.19	0.030
104.1	Choline, C5H14NO+	1.13	0.030
184.09	C5H15PNO4+	1.06	0.014
496.49	LPC (16:0)	1.28	0.012
520.25	LPC (18:2)	1.30	0.029
524.48	LPC (18:0)	1.46	0.023
523.42	DAG(30:0-OH)	1.46	0.020
551.28	DAG(32:0-OH)	1.52	0.029
577.38	DAG(34:1-OH)	1.82	0.037
603.29	DAG(36:2-OH)	1.52	0.032
731.41	SM(d18:1/18:0)	1.40	0.031
734.39	PC(32:0)	1.26	0.020
753.19	SM(d18:1/18:0)+Na	1.49	0.026
756.39	PC(32:0)+Na	1.64	0.035
758.35	PC(34:2)	1.60	0.022
770.41	PC(32:1)+K	1.32	0.030
772.12	PC(32:0)+K	1.54	0.032
780.19	PC (34:2+Na)	1.62	0.020
784.39	PC(36:3)	1.27	0.023
796.29	PC (34:2+K)	1.09	0.010
Decreased in BMAA			
369.29	cholesterol C27H45+	0.77	-0.011
385.27	cholesterol C27H45O+	0.54	-0.024
430.27	Vitamin E, C29H50O2+	0.33	-0.029
negative ions			
Decreased in BMAA			
26.01	CN-	0.72	-0.026
42.01	CNO-	0.66	-0.028
62.97	PO2-	0.78	-0.024
78.97	PO3-	0.83	-0.027
96.99	H2PO4-	0.84	-0.032
281.25	FA (18:1)	0.67	-0.018
283.26	FA (18:0)	0.80	-0.019
303.24	AA	0.76	-0.013

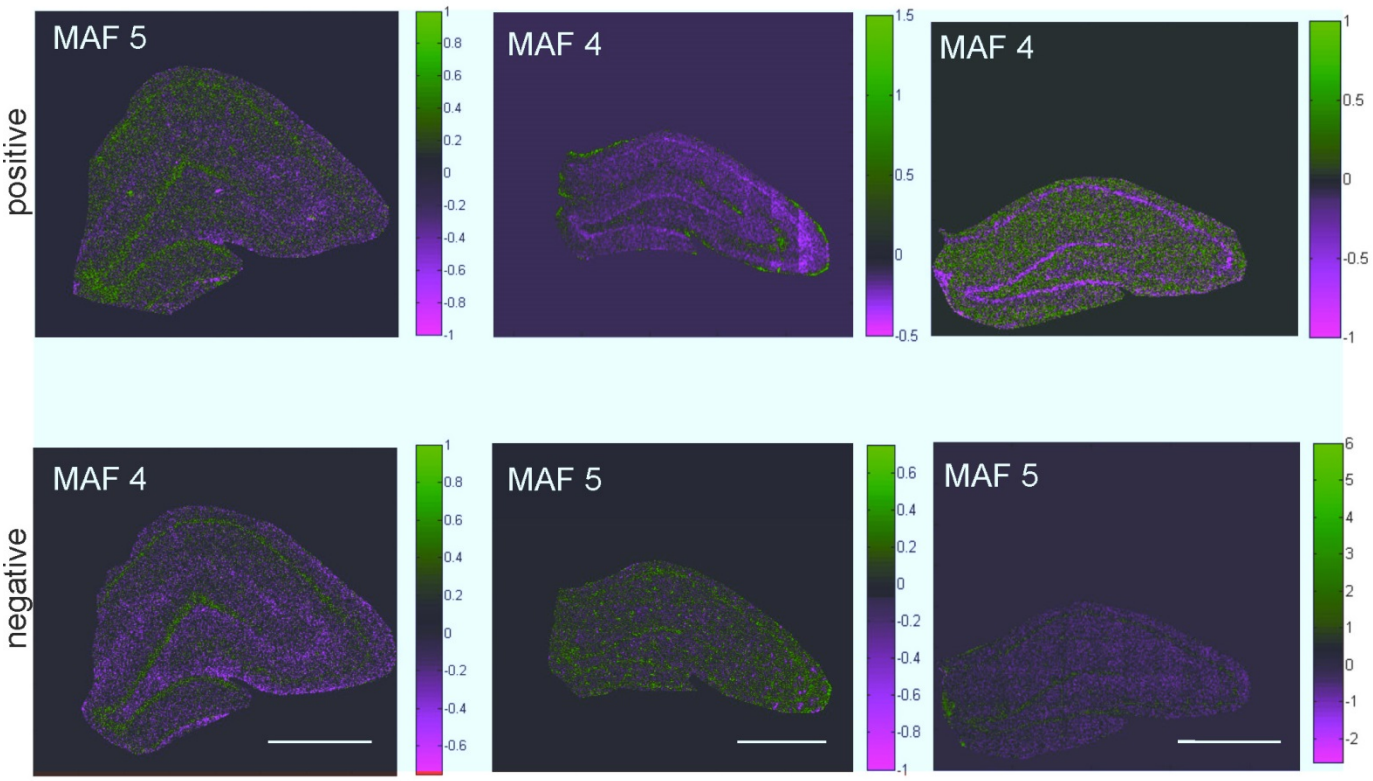
m/z	I.D.[1]	Ratio BMAA/Ctrl[2]	Loading[3]
385.33	cholesterol, C27H45O-	0.55	-0.020
429.3	Vitamin E, C29H49O2-	0.49	-0.027
722.46	PE(16:0/18:1)	0.94	-0.009
726.52	PE(16:0p/20:4)	0.94	-0.008
728.51	PE(18:1p/18:0)	0.84	-0.015
742.52	PE(18:0p/18:0)	0.84	-0.013
744.51	PE(18:1/18:0)	0.80	-0.015
746.48	PE(16:0p/22:6)	0.88	-0.013
747.45	PG(16:0/18:1)	0.88	-0.007
750.47	PE(18:0p/20:4)	0.81	-0.020
762.52	PE(16:0/22:6)	0.93	-0.007
764.52	PE(18:1/20:4)	0.90	-0.014
766.49	PE(18:0/20:4)	0.88	-0.007
770.49	PE(20:0/18:2)	0.75	-0.021
774.59	PE(18:0p/22:6)	0.74	-0.019
778.49	ST(d18:1/16:0)	1.15	0.018
786.54	PS(36:2)	0.66	-0.019
788.51	PS(36:1)	0.73	-0.018
790.49	PE(18:0/22:6)	0.79	-0.013
806.55	ST(d18:1/18:0)	0.93	-0.017
810.47	PS(18:0/20:4)	0.86	-0.013
833.43	PI(16:0/18:2)	0.76	-0.018
223.19	PI fragment	0.71	-0.032
241.25	PI fragment	0.64	-0.031
837.59	PI(16:0/18:0)	0.36	-0.029
805.51	TAG(48:0)	0.95	-0.006

1 Tentative assignment based on accurate mass, isotope pattern and literature values. Lipid annotation is based on lipid maps (*1, 2*). Abbreviations: **TME**: trimethylethylimine; **PC**: phosphatidylcholine; **LPC**: lysophosphatidyl-choline; **DAG**: diacylglyceride; **TAG**: triacylglyceride; **SM**: sphingomyelin; **PA**: diacylphosphate, **FA**: fatty acids; **AA**: arachnoid acid; **PE**: phosphoethanolamine; **ST**: sulfatide; **PS**: phosphatidylserine; **PG**: phosphatidylglycerol; **PI**: phosphatidylinositol

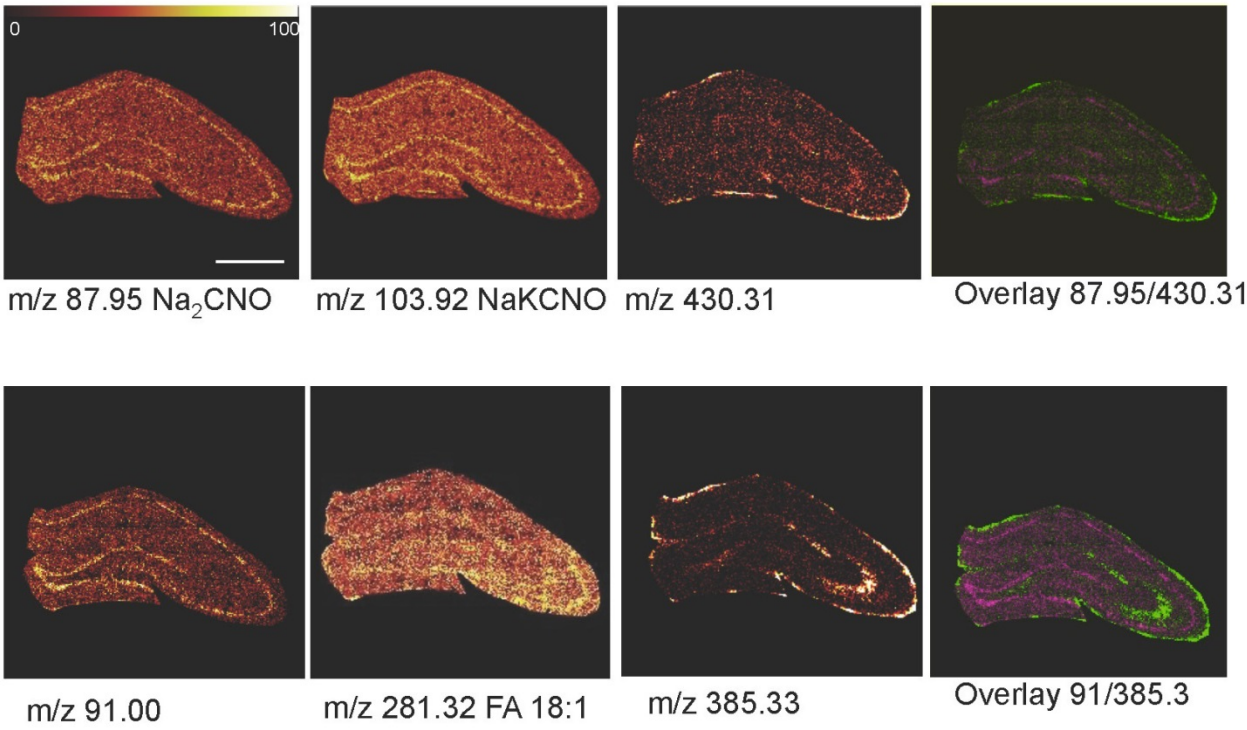
2 average fold change in intensity in BMAA compared to Controls.

3 Loadings on component 1. Positive loadings indicate contribution to BMAA, while negative loading values indicate association with control.

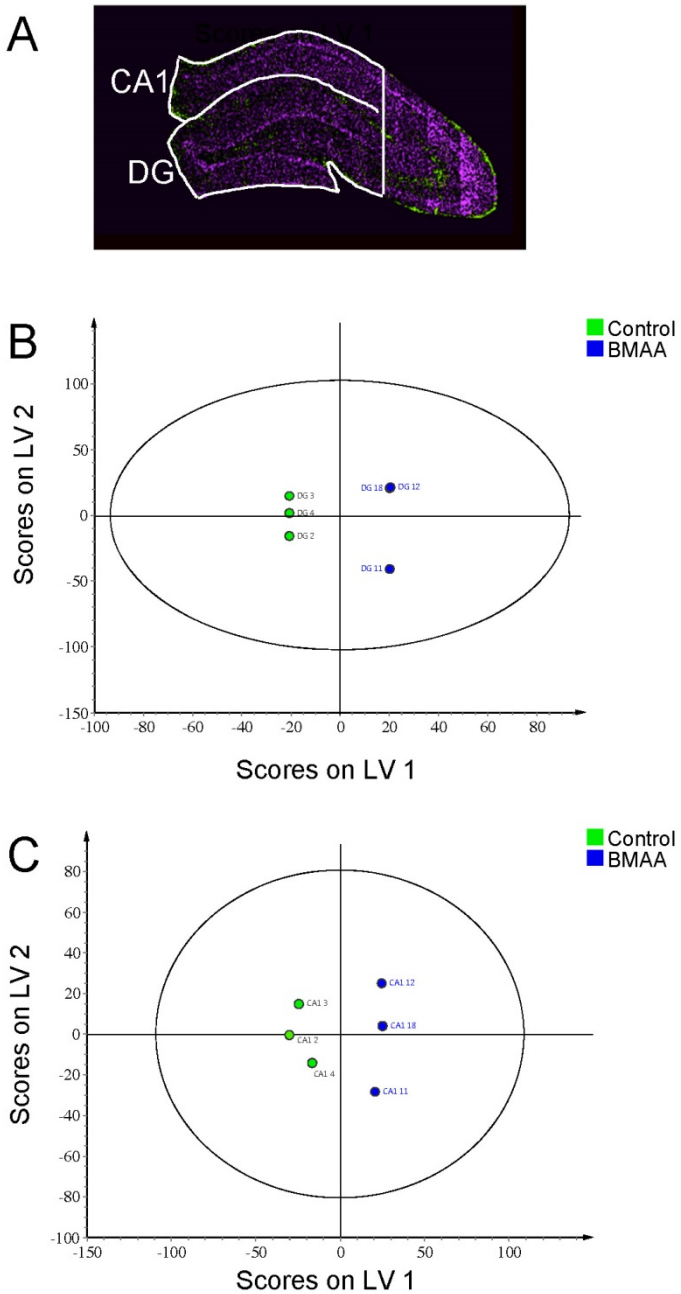
1. Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R., Shimizu, T., Spener, F., van Meer, G., Wakelam, M. J., and Dennis, E. A. (2009) Update of the LIPID MAPS comprehensive classification system for lipids, *Journal of lipid research 50 Suppl*, S9-14.
2. Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Jr., Murphy, R. C., Raetz, C. R., Russell, D. W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M. S., White, S. H., Witztum, J. L., and Dennis, E. A. (2005) A comprehensive classification system for lipids, *Journal of lipid research 46*, 839-861.



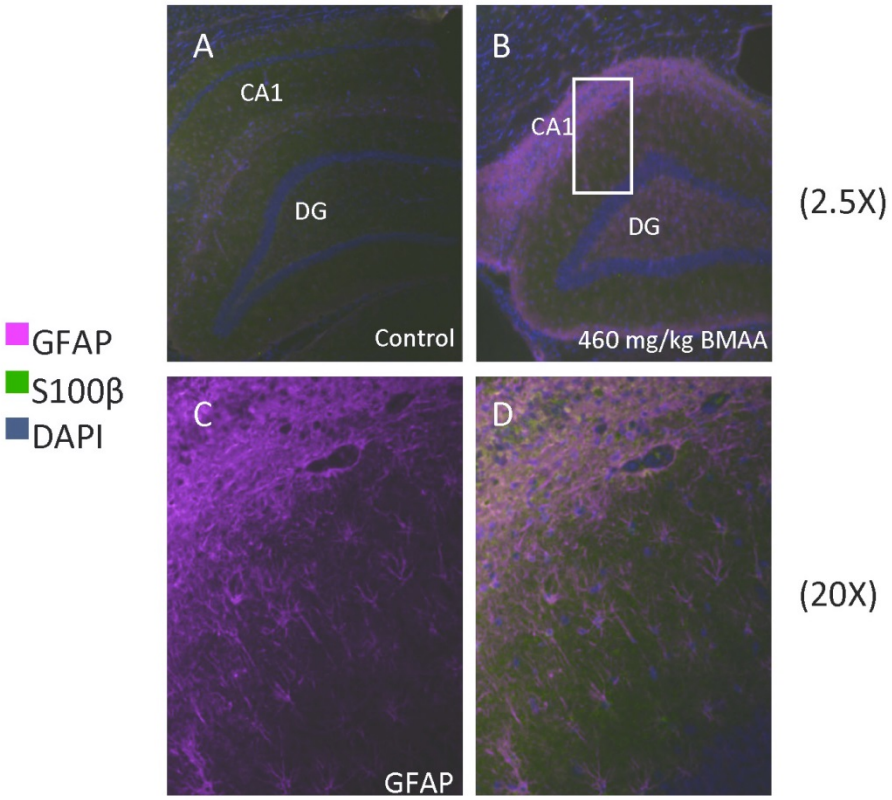
SI Figure 1. Multivariate image analysis for identification of anatomical regions of interest. ToF-SIMS imaging data of the hippocampus of 6-month old control animals (n=3) were acquired in positive and negative ion mode and analyzed by means of maximum autocorrelation factor (MAF) analysis. The composition images depict individual scores (eigenvalues) for each pixel (spectrum) in the corresponding factors (MAF 3-5). The factors capture the variances over the tissue section and allow for an unbiased annotation of regions in the hippocampus based on chemical differences (scale bar = 1 mm).



SI Figure 2. Single ion images of biochemical species that show region specific distributions. Positive and negative ion species show characteristic localization patterns that are well in line with anatomical regions of the hippocampus of 6-month old control animals. Protein specific fragments (pos: NaKCNO, Na_2CNO) were found in highest levels in the CA1-4 and the granular cell layer of the DG. Similar observations were made for a yet unidentified species at m/z 91.00 in negative mode. Vitamin E (Vit.E, $[M+H]^+$ 430.31), fatty acids (FA 18:1, $[M-H]^-$ 281.32) and cholesterol (Chol., $[M-H]^-$ 385.33) were localized to the molecular layer of the DG as well as mossy fibers projecting from the DG to the CA3. Overlay: 87.95: cyan, 430.31 green; 91.00: cyan, 385.3 green. (scale bar = 1mm)



SI Figure 3. Multivariate analysis of spectral data reveal BMAA-induced hippocampal changes. MAF analysis was used to segment the hippocampal images into regions representing CA1 and DG **(A).** OPLS-DA models were calculated for both regions, clearly separating the control group from the BMAA group. **(B)** The analysis resulted in a 1+3 (1 predictive- + 3 orthogonal components) model for DG**.** The model explained 86% of the variation in the dataset (R2X cumulative) with a predictive power of 0.96 (Q2 cumulative). **(C)** For the CA1 a 1+1 (1 predictive- + 3 orthogonal components) model where 53% variation was explained at 0.61 predictive power (Q2 cumulative).



SI Figure 4. Immunohistochemistry of hippocampus. Double antigen staining was performed against GFAP and S100 beta on hippocampal sections of control **(A)** and BMAA exposed animals **(B)**. The data show massive gliosis and reactive astrocyte species localizing to the histopathologically altered region in the hippocampus of BMAA-treated rats. This was indicated by increased GFAP immunoreactivity (cyan) in the CA1 of BMAA exposed animals **(B)** compared to controls **(A)**. **(C)** Magnifcation of outlined area indicated in **(B)**. GFAP positive cells **(C)** were also found to stain positively for S100 beta (white) serving as additional astrocyte marker **(D).** Magnifications. A-B lens x 10; C-D lens x 20.