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Supplemental Material

Prolonged, Low-Level Exposure to the Marine Toxin, Domoic Acid, and Measures of Neurotoxicity in Nonhuman Primates

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Serum Chemistry Reactions on the Beckman Coulter AU System

1. Sodium, Potassium, and Chloride
   The Beckman Coulter AU System ISE module for Na+, K+, and Cl- employs crown ether membrane electrodes for sodium and potassium; and a molecular oriented PVC membrane for chloride that are specific for each ion of interest in the sample. An electrical potential was developed according to the Nernst Equation for a specific ion. When compared to the Internal Reference Solution, this electrical potential is translated into voltage and then into the ion concentration of the sample.

2. Urea Nitrogen
   Urea was hydrolyzed enzymatically by urease to yield ammonia and carbon dioxide. The ammonia and α-oxoglutarate were converted to glutamate in a reaction catalyzed by L-glutamate dehydrogenase (GLDH). Simultaneously, a molar equivalent of reduced NADH was oxidized. Two molecules of NADH were oxidized for each molecule of urea hydrolyzed. The rate of change in absorbance at 340 nm, due to the disappearance of NADH, is directly proportional to the BUN concentration in the sample.

3. Total Protein
   Cupric ions in an alkaline solution reacted with proteins and polypeptides containing at least two peptide bonds to produce a violet-colored complex. The absorbance of the complex at 540/660 nm is directly proportional to the concentration of protein in the sample.

4. Albumin
   At pH 4.2, bromocresol green reacts with albumin to form an intense green complex. The absorbance of the albumin-BCG complex was measured bichromatically (600/800nm), proportional to the albumin concentration in the sample.

5. Total Bilirubin
   Total bilirubin in serum is composed of direct (conjugated) bilirubin and indirect (unconjugated) bilirubin. A stabilized diazonium salt, 3,5-dichlorophenyl-diazonium tetrafluoroborate (DPD), reacted with bilirubin to form azobilirubin, which absorbs maximally at 570/660 nm. Caffeine and a surfactant were used as reaction accelerators. The absorbance at 570/660 nm is proportional to the bilirubin concentration in the sample. A separate serum blank was performed to eliminate endogenous serum interferences.

6. Calcium
   Calcium ions (Ca2+) react with Arsenazo III (2,2'-[1,8-Dihydroxy-3,6-disulphonaphthylene-2,7-bisazo]-bisbenzenear-sonic acid) to form an intense purple colored complex. Magnesium does not significantly interfere in calcium determination using Arsenazo III. In this method, the absorbance of the Ca-Arsenazo III complex was measured bichromatically at 660/700 nm. The resulting increase in absorbance of the reaction mixture is directly proportional to the calcium concentration in the sample.

7. Phosphate
   Inorganic phosphate reacts with molybdate to form a heteropolyacid complex. The use of a surfactant eliminates the need to prepare a protein free filtrate. The absorbance at 340/380 nm was measured in this reaction as directly proportional to the inorganic phosphorus level in the sample.

8. Cholesterol
   Cholesterol esters in serum are hydrolyzed by cholesterol esterase (CHE), using reagents certified to meet the National Cholesterol Education Program's (NCEP) performance criteria for accuracy. The free cholesterol produced was oxidized by cholesterol oxidase (CHO) to cholest-4-en-3-one with the simultaneous production of hydrogen peroxide (H202), which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromophore. The red quinoneimine dye formed was measured spectrophotometrically at 540/600 nm as an increase in absorbance.

9. Alkaline Phosphatase
   Alkaline phosphatase activity was determined by measuring the rate of conversion of p-nitro-phenylphosphate (pNPP) in the presence of 2-amino-2-methyl-1-propanol (AMP) at pH 10.4. The rate of change in absorbance due to the formation of pNP was measured bichromatically at 410/480 nm and is directly proportional to the ALP activity in the sample.

10. Alanine Aminotransferase
    This method utilizes a methodology recommended by the International Federation of Clinical Chemistry (IFCC). ALT transfers the amino group from alanine to α-oxoglutarate to form pyruvate and glutamate. The pyruvate enters a lactate dehydrogenase (LD) catalyzed reaction with NADH to produce lactate and NAD+. The decrease in absorbance due to the consumption of NADH was measured at 340nm and is proportional to the ALT activity in the sample.

11. Aspartate Aminotransferase
    This method uses methodology recommended by the IFCC. In this method, aspartate aminotransferase (AST) catalyzes the transamination of aspartate and α-oxoglutarate, forming L-glutamate and oxalacetate. The oxalacetate is then reduced to L-malate by malate dehydrogenase, while NADH is simultaneously converted to NAD+. The decrease in absorbance due to the consumption of NADH was measured at 340 nm and is proportional to the AST activity in the sample.

12. Gamma Glutamyl Transferase
    GGT catalyzes the transfer of the gamma-glutamyl group from the substrate, gamma-glutamyl-3-carboxy-4-nitroanilide, to glycylglycine, yielding 5-amino-2-nitrobenzoate. The change in absorbance at 410/480 nm is due to the formation of 5-amino-2-nitrobenzoate and is directly proportional to the GGT activity in the sample.

Table S1: Animal Characteristics

Animal Number	**Dose** (mg/kg/day)	**Weight** (kg)	**Age** (years)	**Total Dosing Time** (days)	**Tremor Score** (% of total behavioral sessions tremoring)	**Conceived and Carried Pregnancy?**	**Stopped DA Exposure?**	**Experimental Assays**				
								CBC + SC	Cyto+Chemokine	MR Imaging	Histopathology	Transcriptomics
Vehicle Control Animals												
A15236	0	3.98	7.3	724	0.02	✓		✓	✓	✓	✓	✓
A15238	0	3.24	6.4	678	0.02	✓		✓	✓	✓	✓	✓
A15247	0	3.58	6.4	653	0.03	✓			✓	✓	✓	✓
A16112	0	3.58	7.0	454	0.04	✓		✓	✓	✓	✓	✓
A16103	0	3.38	7.2	448	0.06	✓		✓	✓	✓	✓	✓
A16117	0	3.12	6.2	477	0.06	✓		✓	✓	✓	✓	
A15248	0	3.62	5.9	717	0.11			✓*	✓		✓	✓
A15239	0	3.58	6.2	254	0.02	✓		✓				
A15250	0	3.80	6.6	249	0.09	✓		✓				
0.075 mg/kg/d Animals												
A16113	0.075	3.41	6.7	420	0.26	✓		✓	✓	✓	✓	✓
A16115	0.075	3.11	6.3	385	0.4	✓		✓	✓	✓	✓	
A16108	0.075	3.19	7.1	436	0.37	✓		✓	✓		✓	
A14400	0.075	3.8	11.1	371	0.29	✓		✓			✓	✓
A15249	0.075	4.83	6.4	604	0.18	✓		✓			✓	
A16107	0.075	3.33	7.1	392	0			✓			✓	
A16116	0.075	3.08	5.9	321	0.18	✓		✓			✓	
A15237	0.075	3.03	6.0	322	0.35	✓	✓	✓			✓	
A15240	0.075	3.05	6.5	268	0.09	✓	✓	✓			✓	
A15241	0.075	4.69	6.7	259	0.04	✓	✓	✓			✓	
0.15 mg/kg/d Animals												
A14392	0.15	4.00	10.4	413	0.3	✓		✓*	✓	✓	✓	✓
A15252	0.15	3.77	6.4	612	0.68	✓		✓	✓	✓	✓	
A15242	0.15	3.46	6.5	598	0.25	✓			✓	✓	✓	
A16106	0.15	2.96	7.1	392	0.8	✓		✓	✓	✓	✓	
A15244	0.15	4.22	5.5	294	0.61	✓	✓	✓	✓		✓	✓
A15234	0.15	3.56	7.7	268	0.69	✓	✓	✓	✓		✓	✓
A16114	0.15	3.72	6.5	428	0.11	✓		✓			✓	
A16105	0.15	3.26	7.1	371	0.01	✓		✓			✓	
A16110	0.15	3.06	7.0	343	0.03	✓		✓			✓	
A15246	0.15	3.35	6.4	350	0.02	✓	✓	✓				
A15251	0.15	3.83	6.5	248	0.13	✓	✓	✓				

*Shows the individual level animal data for all animals enrolled. Age and weight were at the beginning of the study (Baseline Period). Stopped DA exposure indicates animals that were followed on the study, but not receiving domoic acid postpartum. *Denotes missing CBC due to sample clotting. Abbv: DA- domoic acid; CBC – complete blood count; MR – magnetic resonance; SC – serum chemistry*

Table S2: Curated Gene Lists from Literature, Used in GSEA

Neuronal Health Gene List	Excitotoxicity Gene List	Inflammation/Glial Gene List	White Matter, Myelin, Axons Gene List
APOE	*ADAM17*	*AIF1*	*ACTB*
APP	*APEX1*	*BDNF*	*C11ORF9*
ATP5D	*CD63*	*Cd14*	*CDC42*
ATP5F1	*CKAMP52 (SHISA6)*	*CDC73*	*CLDN11*
CX3CL1	*CNN3*	*CX3CL1*	*CNP*
DRD2	*CORNICHON 3 (CNIH3)*	*DCAF1*	*EIF2AK3*
FGF2	*CRHBP*	*EOMES*	*GAL*
HOXA10	*DGK*	*GDNF*	*GAL3ST1*
HOXD3	*DHPS*	*GFAP*	*GAP43*
HRAS	*ERK1/2*	*IFNG*	*ILK*
HTT	*FLT1*	*IL1*	*KLK6*
MAOA	*FOS*	*IL11RA*	*MAG*
NDUFV2	*FOSB*	*IL13*	*MAL*
RAF1	*FXYD7*	*IL17A*	*MBP*
RASSF1	*GFAP*	*IL1A*	*MOG*
SLC1A1	*GRIA1*	*IL1B*	*MTOR*
SLC6A3	*GRIA3*	*IL1R1*	*NRG1*
TGFB1	*GRIA4*	*IL1RN*	*OLIG2*
VEGFD	*GRIK3*	*IL2*	*OMG*
WNT3A	*GRIK4*	*IL4*	*PLLP*
	GRIK5	*IL6*	*PLP1*
	GRIN2B	*IL6R*	*POU3F1*
	GRIN3A	*LHX2*	*PTEN*
	GRM1	*Ly96*	*RAC1*
	GRM7	*Myd88*	*RTN1*
	GRM8	*NCR2*	*SARM1*
	GSG1L	*NOTCH2*	*SOCS3*
	GSTO1	*NR3C1*	*STMN1*
	IGFBP2	*PRL*	*STMN2*
	IL1B	*RCAN1*	*TF*
	JUN	*S100A10*	*TSC2*
	JUND	*S100A8*	*TSPAN2*
	LXN	*SAA3*	*TUBA1A*
	MAP2K1	*SEMA7A*	*TUBB2A*
	MAP3K1	*Slc1a3*	
	MAPK1	*Spp1*	
	MAPK3	*STAT3*	
	MUSTN	*TCF7*	
	NPY	*TGFB1*	
	P38	*TIMp1*	
	PD98059	*TNFAIP3*	
	PDPN	*TNFα*	
	PEA15	*TNFβ (LTA)*	
	PKNOX2	*TREM2*	
	PLCB1	*VIM*	
	PRPN5	*TAF4B*	
	PTK2B		
	RECS1		
	S100A10		
	SB203580		
	SCN4B		
	SHISA9 (CKAMP44)		
	SOX2		
	SOX4		
	SP1		
	SP3		
	TARP-GAMMA2 (CACNG2)		
	TARP-GAMMA3 (CACNG3)		
	TGM2		
	TNFα		
	TNFβ		
	TP73		
	TSC2		
	UPB216		
	WDR5		
	VGLL3		

Neuronal Health, Inflammation/Glial, and White Matter, Myelin, and Axons genes are based off of results from Cahoy et al., 2008. Excitotoxicity genes are based off of results from Pappas et al., 2012.

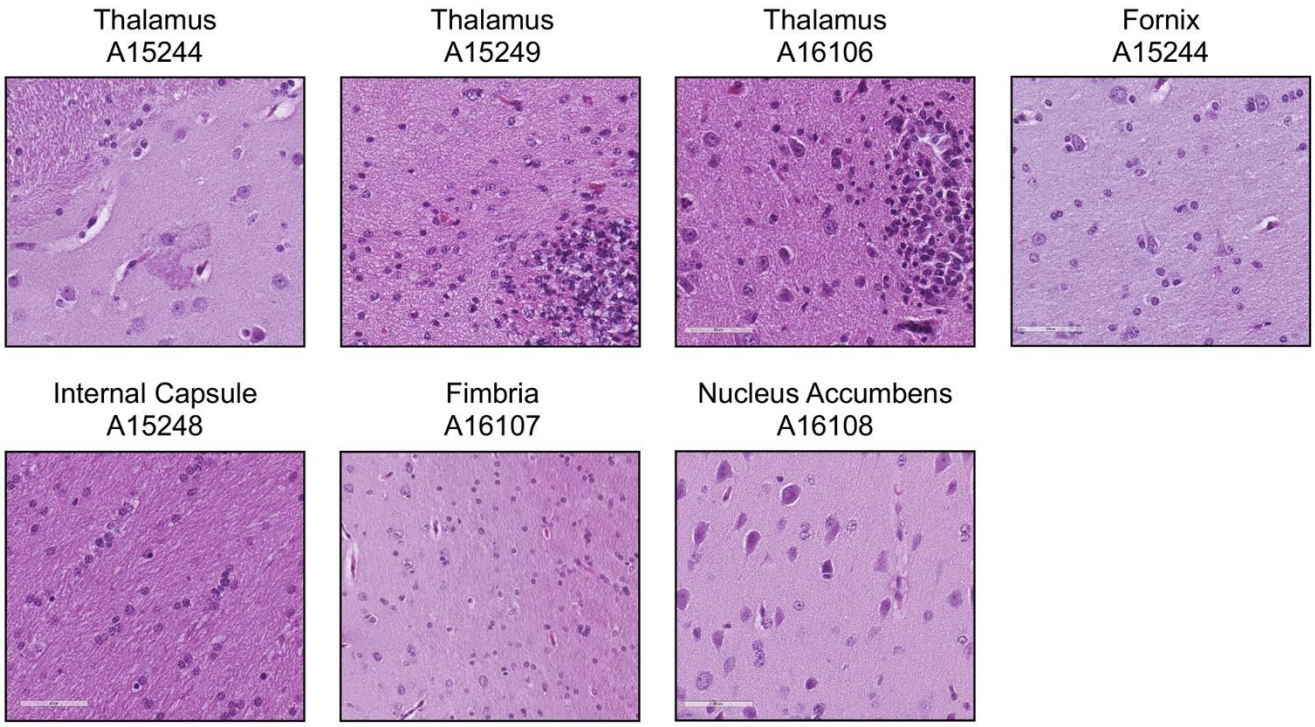

Figure S1: Matching H&E staining for focal sites of microglia reactivity. Representative images of H&E staining in 10% formalin-fixed, paraffin-embedded, 10 µm sections at focal sites of reactivity in the thalamus, fornix, fimbria, internal capsule, and nucleus accumbens of female *Macaca fascicularis* following prolonged exposed to domoic acid (0.15 mg/kg/d) or vehicle (5% sucrose). Numbers correspond to Animal Numbers in Table S1. A15244 and A16106 were in the 0.15 mg/kg/d group, A15249, A16107, and A16106 were in the 0.075 mg/kg/d group, and A15428 was in the control group. Scale bar = 60 µm.