The following supplement accompanies the article

Magnetic resonance imaging quality and volumes of brain structures from live and postmortem imaging of California sea lions with clinical signs of domoic acid toxicosis

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Diseases of Aquatic Organisms 91: 243–256

Supplement. Additional tables and figures

Table S1. Brain volumes of California sea lions determined from imaging alive and postmortem-intact. Volumes are in cm^3. CSF: cerebrospinal fluid; WM: white matter; GM: gray matter

Sea lion name		Left CSF	Right CSF	Left ventricles	Right ventricles	Cerebellum and brainstem WM	Cerebellum and brainstem GM	Left cerebral GM	Right cerebral GM	Left cerebral WM	Right cerebral WM
Kirina											
Live	Mean	19.94	19.02	3.76	3.83	25.65	34.94	64.82	64.06	27.00	27.43
	SD	1.06	1.03	0.05	0.07	1.13	1.45	2.84	2.12	1.12	0.89
2 h	Mean	20.91	20.34	3.24	3.27	24.69	39.48	68.02	68.99	28.77	27.60
	SD	2.61	2.68	0.23	0.20	1.80	1.85	2.85	3.16	0.54	0.95
24 h	Mean	19.59	19.43	2.76	2.90	19.69	34.17	64.23	64.07	25.50	24.88
	SD	1.53	1.57	0.22	0.09	0.63	1.73	1.53	1.90	1.26	1.61
48 h	Mean	16.50	15.67	2.34	2.58	19.69	34.40	64.13	63.95	25.77	24.60
	SD	1.58	0.55	0.11	0.07	0.63	1.87	1.54	1.79	1.71	1.20
Barlich											
Live	Mean	21.38	18.66	3.22	3.41	31.96	44.11	80.37	78.62	33.79	33.40
	SD	1.65	1.35	0.14	0.13	0.52	0.29	0.22	0.91	1.02	0.35
2 h	Mean	27.60	23.11	3.18	3.48	30.73	42.25	80.31	78.79	32.91	34.03
	SD	0.43	0.41	0.03	0.22	0.48	0.72	0.85	0.81	0.84	0.81
24 h	Mean	25.38	24.24	3.16	3.41	30.21	39.13	83.13	81.14	31.84	31.37
	SD	0.24	0.34	0.07	0.03	0.58	0.15	0.59	0.34	0.86	0.37
48 h	Mean	24.66	20.78	2.95	3.07	29.02	41.23	84.19	82.84	31.99	31.63
	SD	0.70	0.66	0.03	0.04	0.04	0.63	0.52	0.47	0.29	0.34
Gratitude											
Live	Mean	19.42	13.22	3.44	2.61	28.83	46.75	85.77	90.94	29.65	30.48
	SD	1.24	0.59	0.17	0.15	0.72	0.04	0.50	0.43	0.10	0.17
2 h	Mean	26.31	22.33	3.00	2.23	26.86	45.38	83.35	86.08	29.52	30.15
	SD	1.76	2.14	0.06	0.02	1.64	2.54	2.24	1.47	1.05	0.49
24 h	Mean	18.78	12.67	2.90	2.02	28.22	44.61	87.06	92.91	29.95	29.80
	SD	0.72	0.14	0.27	0.26	0.34	0.37	1.43	0.33	0.98	0.17
48 h	Mean	18.67	12.73	2.28	1.53	26.36	47.78	84.30	88.90	31.15	31.88
	SD	0.93	0.10	0.08	0.27	1.43	2.01	2.62	0.58	2.03	0.78

Table S2. Volumes of brain structures associated with hippocampus in California sea lions determined from imaging alive and postmortem-intact. Volumes are in mm^3. WM: white matter; GM: gray matter

Sea lion name		Left lateral ventricle (ventral horn)	Right lateral ventricle (ventral horn)	Left hippocampal sulcus	Right hippocampal sulcus	Left para-hippocampal gyrus GM	Right para-hippocampal gyrus GM	Left para-hippocampal gyrus WM	Right para-hippocampal gyrus WM
Kirina									
Live	Mean	514.22	492.82	313.79	348.37	1112.42	1168.00	351.06	324.29
	SD	8.75	7.49	5.49	15.54	27.98	29.07	6.40	12.29
2 h	Mean	447.33	456.93	337.23	436.59	943.49	988.65	304.27	358.06
	SD	28.70	9.66	33.62	29.52	29.02	65.44	15.83	9.72
24 h	Mean	405.23	413.53	355.31	349.45	1161.75	1144.42	294.41	299.17
	Sd	6.21	23.48	3.97	19.16	30.04	11.39	9.32	21.58
48 h	Mean	342.98	345.18	331.02	297.33	1233.01	1191.88	330.41	364.83
	Sd	18.30	20.37	26.24	13.12	15.88	38.49	4.32	28.48
Barlich									
Live	Mean	310.66	322.62	300.89	347.76	1352.97	1049.15	382.06	283.07
	SD	6.39	6.39	31.59	23.65	34.18	8.46	8.63	17.78
2 h	Mean	294.80	346.68	435.42	339.72	1385.74	1106.93	286.62	249.51
	SD	23.65	31.42	57.49	64.05	26.24	18.99	13.47	10.70
24 h	Mean	300.15	368.02	297.71	273.79	1646.63	1473.91	356.67	364.60
	sd	2.24	4.66	10.88	3.97	29.35	22.96	7.60	35.73
48 h	Mean	263.91	303.22	315.43	309.93	1575.67	1351.55	237.67	232.05
	Sd	22.44	5.18	3.80	2.94	5.52	5.87	16.05	8.80
Gratitude									
Live	Mean	343.75	245.73	297.61	200.32	1305.54	1405.64	291.38	265.75
	SD	12.77	10.88	19.34	12.95	16.05	60.94	11.57	3.63
2 h	Mean	311.64	250.72	272.33	224.48	1185.39	1284.01	260.12	259.27
	SD	6.39	10.36	26.41	10.88	11.91	25.38	1.21	5.52
24 h	Mean	231.08	125.24	171.75	131.47	1337.50	1350.69	337.64	282.47
	SD	3.28	2.42	4.66	0.17	66.12	8.46	15.88	7.25
48 h	Mean	182.98	100.34	114.99	84.84	1212.63	1385.12	251.10	242.67
	SD	11.91	7.60	1.04	5.01	53.17	31.25	5.01	8.63

Table S3. Volumes of brain structures in California sea lions determined from imaging alive and postmortem-fixed. The number in parentheses following formalin indicates the elapsed time from death to fixation. WM: white matter; GM: gray matter; na: not available

Sea lion name		Left cerebral GM (cm^3)	Right cerebral GM (cm^3)	Left cerebral WM (cm^3)	Right cerebral WM (cm^3)	Cerebellum and brainstem WM (cm^3)	Cerebellum and brainstem GM (cm^3)	Left para-hippocampal gyrus GM (mm^3)	Right para-hippocampal gyrus GM (mm^3)	Left para-hippocampal gyrus WM (mm^3)	Right para-hippocampal gyrus WM (mm^3)
Fairbanks											
Live	Mean	85.26	85.44	33.27	34.18	28.13	43.62	1280.93	1057.31	348.61	258.16
	SD	1.20	1.08	0.63	0.41	0.44	0.54	99.78	58.69	6.90	1.90
Formalin (2 h)	Mean	82.88	84.56	33.30	33.14	na	na	1378.80	1191.65	374.76	343.00
	SD	0.28	0.63	0.73	0.66	na	na	51.50	42.64	11.99	6.52
Tiki											
Live	Mean	82.92	84.66	31.14	32.68	25.43	39.04	1207.20	1318.52	358.01	330.06
	SD	0.16	0.99	0.60	0.41	0.25	0.19	6.56	25.89	5.70	3.45
Formalin (3 h)	Mean	77.43	78.04	31.29	32.60	na	na	1200.20	1280.45	471.73	400.93
	SD	0.20	0.86	0.43	0.10	na	na	39.90	37.17	2.28	1.37
Rupert											
Live	Mean	89.21	89.14	37.01	37.94	30.12	49.44	1350.96	1388.62	258.35	284.74
	SD	0.05	0.66	0.78	1.34	0.77	0.85	26.10	15.32	6.07	20.03
Formalin (4 h)	Mean	83.71	84.22	37.44	39.30	na	na	1387.31	1130.81	426.43	395.64
	SD	1.39	1.36	1.00	0.72	na	na	23.36	49.00	6.22	12.74
Tintoretto											
Live	Mean	98.15	97.59	39.50	40.51	31.15	43.09	1604.33	1450.17	412.43	429.64
	SD	2.74	2.91	0.75	1.06	0.28	1.18	7.60	7.77	10.88	15.19
Formalin (23 h)	Mean	90.70	88.54	41.71	42.61	28.21	37.19	1828.62	1232.32	519.71	362.64
	SD	0.68	1.56	0.17	0.85	0.09	1.11	104.09	8.50	37.33	4.85
Barlich											
Live	Mean	80.37	78.62	33.79	33.40	31.96	44.11	1352.97	1049.15	382.06	283.07
	SD	0.22	0.91	1.02	0.35	0.52	0.29	34.18	8.46	8.63	17.78
Formalin (48 h)	Mean	80.32	79.10	31.66	31.16	28.73	46.87	1401.52	1164.43	286.33	286.76
	SD	3.45	2.10	1.40	0.57	0.92	0.01	108.63	53.40	4.10	0.46
Gratitude											
Live	Mean	85.77	90.94	29.65	30.48	28.83	46.75	1305.54	1405.64	291.38	265.75
	SD	0.50	0.43	0.10	0.17	0.72	0.04	16.05	60.94	11.57	3.63
Formalin (51 h)	Mean	73.09	80.01	29.30	29.99	26.00	35.12	1207.64	1228.66	357.91	427.11
	SD	2.86	2.88	0.64	0.26	0.03	1.83	51.28	4.55	3.34	14.72

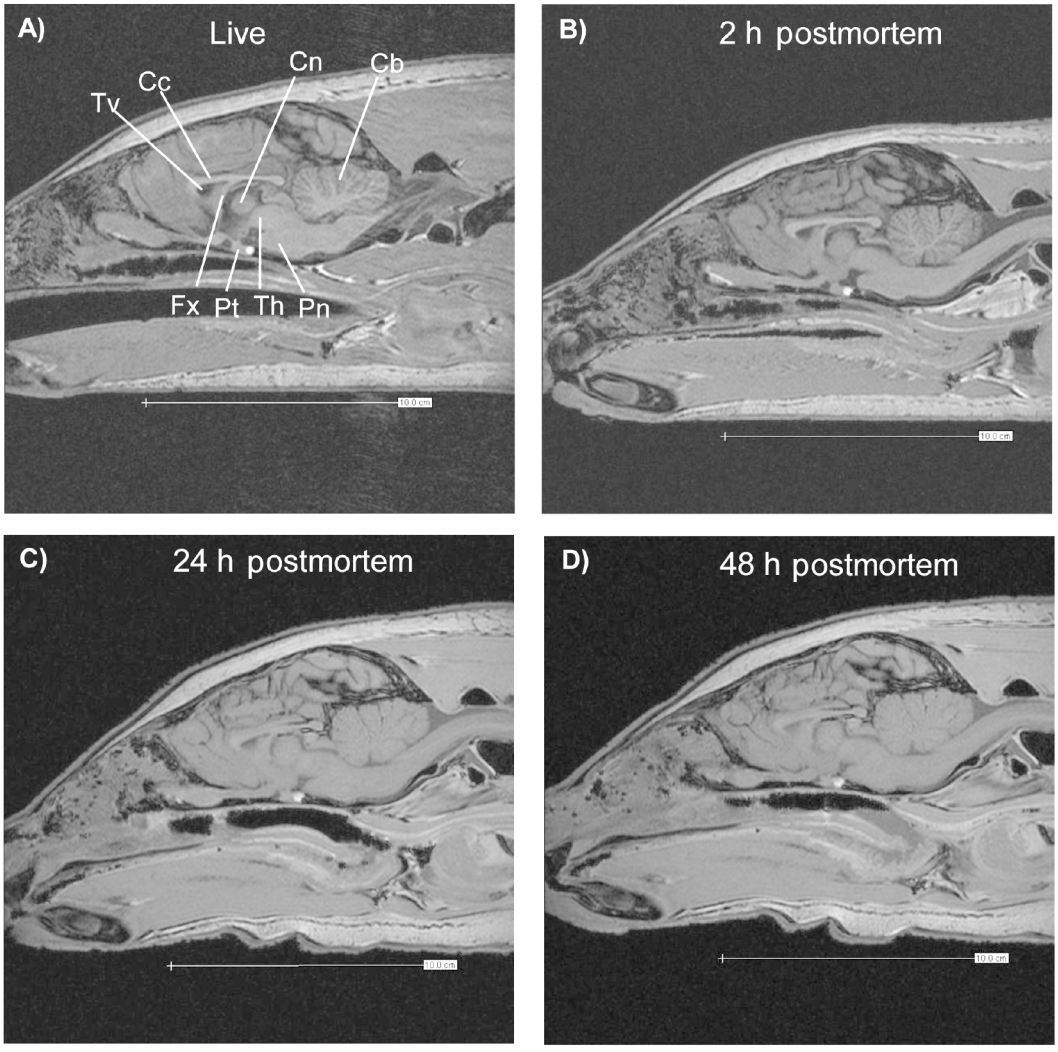

Fig. S1. *Zalophus californianus.* Magnetic resonance images acquired in the sagittal plane using the FLASH sequence of the California sea lion 'Kirina' that was imaged (A) alive and (B) 2 h, (C) 24 h and (D) 48 h postmortem-intact. This sea lion did not show any clinical signs of domoic acid toxicosis. The clarity of brain structures and the boundary of the white matter and gray matter were blurred as postmortem time increased. Thus, FLASH is not a reliable MRI sequence for postmortem evaluation of the brain. Cc :corpus callosum; Cn: caudate nucleus; Cb: cerebellum; Fx: fornix; Pt; pituitary gland; Th: thalamus; Pn: pons; Tv: third ventricle. Scale bar = 10 cm

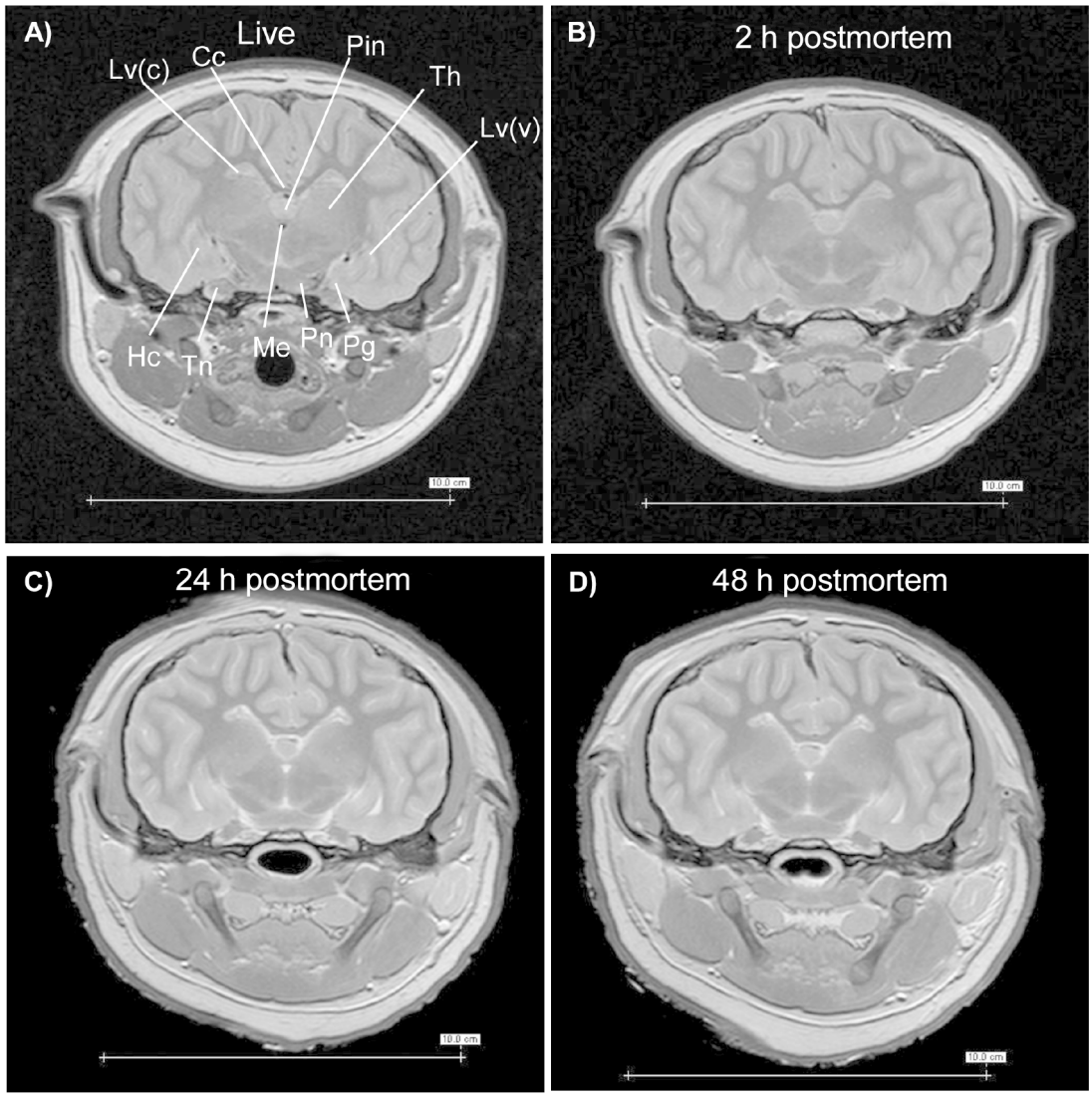

Fig. S2. *Zalophus californianus*. PD-weighted magnetic resonance images acquired in the transverse plane of the California sea lion 'Kirina' that was imaged (A) alive and (B) 2 h, (C) 24 h and (D) 48 h postmortem-intact. This sea lion did not show any clinical signs of domoic acid toxicosis. Cc: corpus callosum; Hc: hippocampus; Lv(c): lateral ventricle (central); Lv(v): lateral ventricle (ventral horn); Pin: pineal gland; Me: mesencephalic aqueduct; Pg: parahippocampal gyrus; Pn: pons; Th: thalamus; Tn: trigeminal nerve. Scale bar = 10 cm

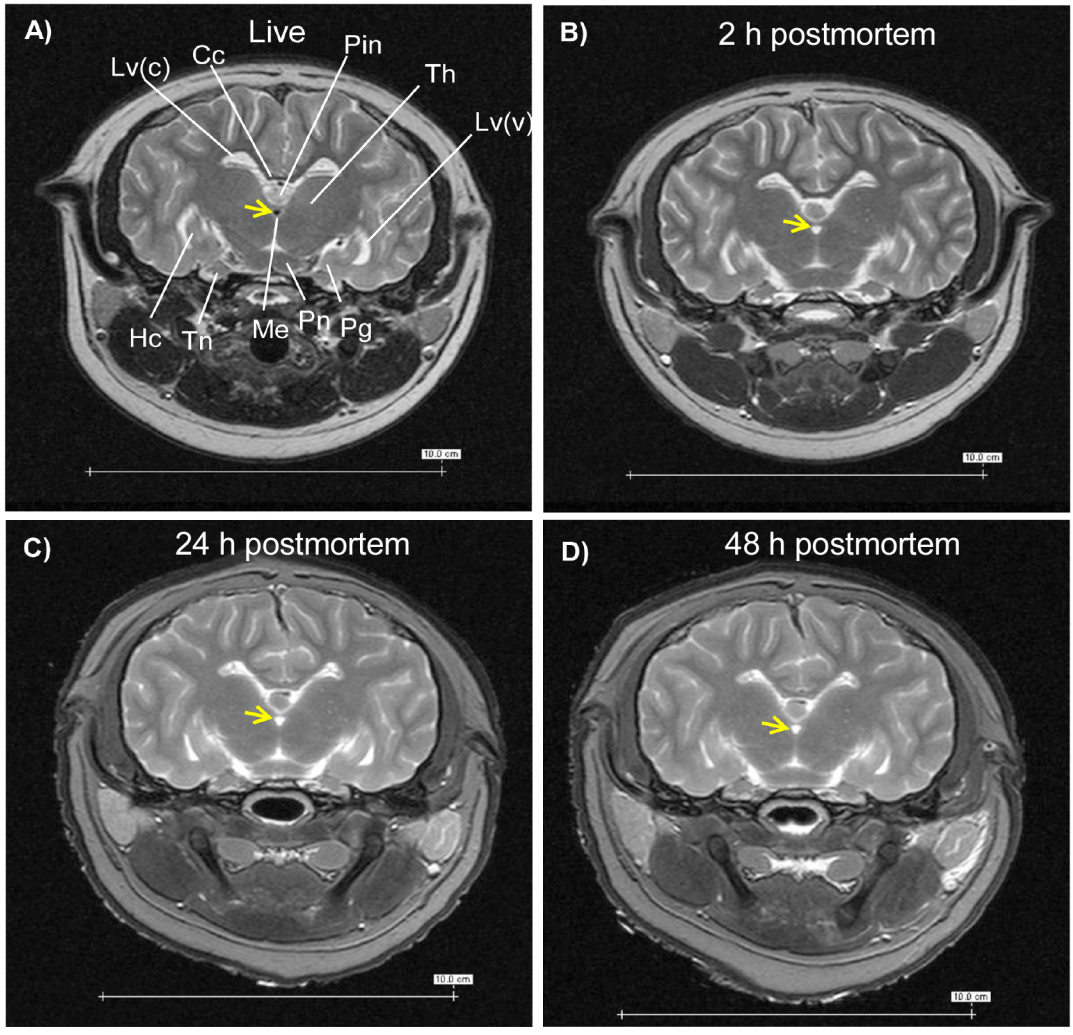

Fig. S3. *Zalophus californianus.* T2-weighted magnetic resonance images, acquired in the transverse plane of the California sea lion 'Kirina' that was imaged (A) alive and (B) 2 h, (C) 24 h and (D) 48 h postmortem-intact. This sea lion did not show any clinical signs of domoic acid toxicosis. The diffusion artifact caused by cerebrospinal fluid (CSF) flow in the mesencephalic aqueduct was present (indicated by a yellow arrow) in the live images but not in postmortem images. Cc: corpus callosum; Hc: hippocampus; Lv(c): lateral ventricle (central); Lv(v): lateral ventricle (ventral horn); Pin: pineal gland; Me: mesencephalic aqueduct; Pg: parahippocampal gyrus; Pn: pons; Th: thalamus; Tn: trigeminal nerve. Scale bar = 10 cm

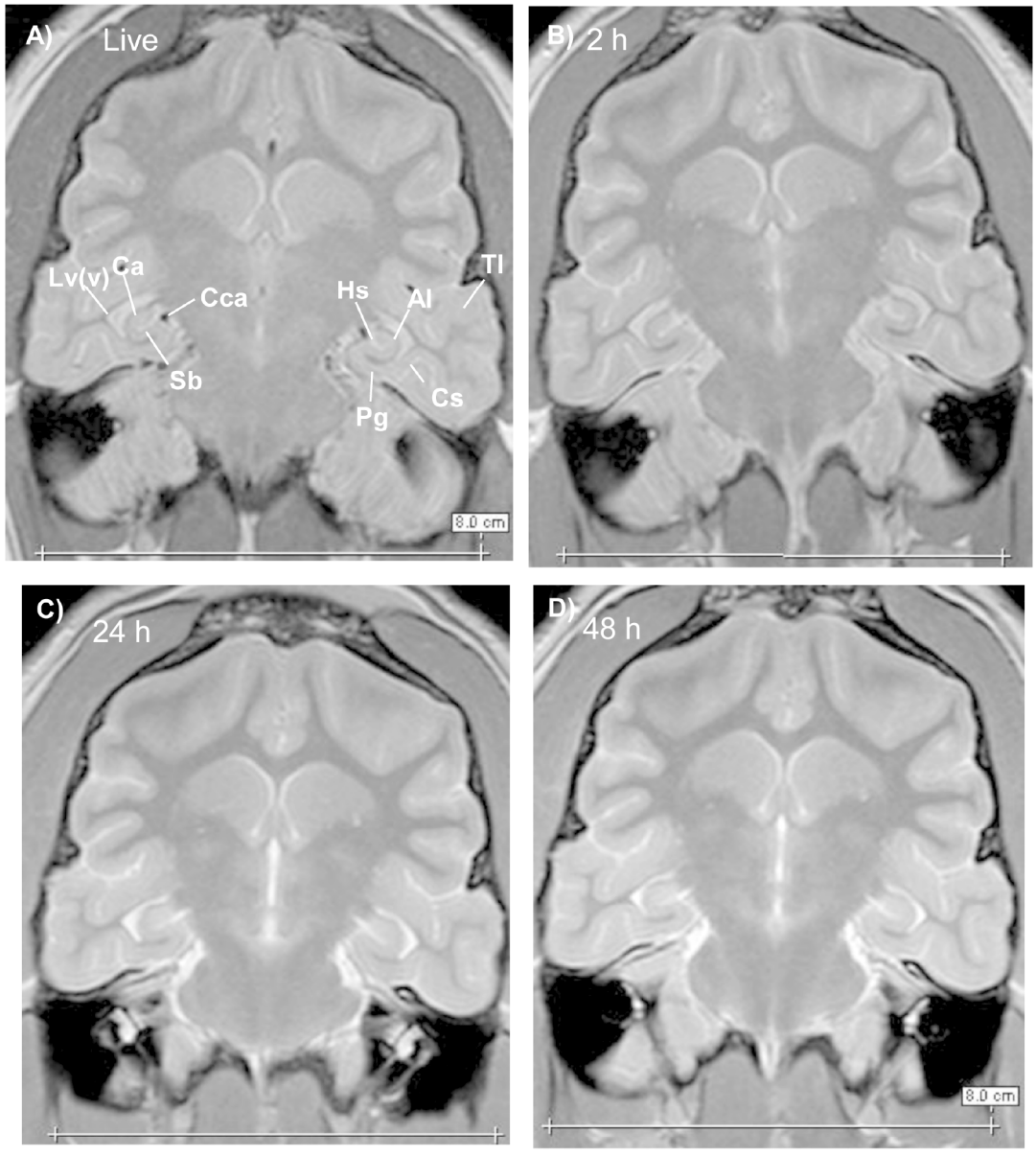

Fig. S4. *Zalophus californianus.* PD-weighted magnetic resonance images, acquired in the oblique plane of the California sea lion 'Kirina' that was imaged (A) alive and (B) 2 h, (C) 24 h and (D) 48 h postmortem-intact. This sea lion did not show any clinical signs of domoic acid toxicosis. Al: alveus; Ca: cornu ammonis; Cca: caudal cerebral artery; Cs: collateral sulcus; Hs: hippocampal sulcus; Lv(v): lateral ventricle (ventral horn); Pg: parahippocampal gyrus; Sb: subiculum; Tl: temporal lobe. Scale bar = 8.0 cm

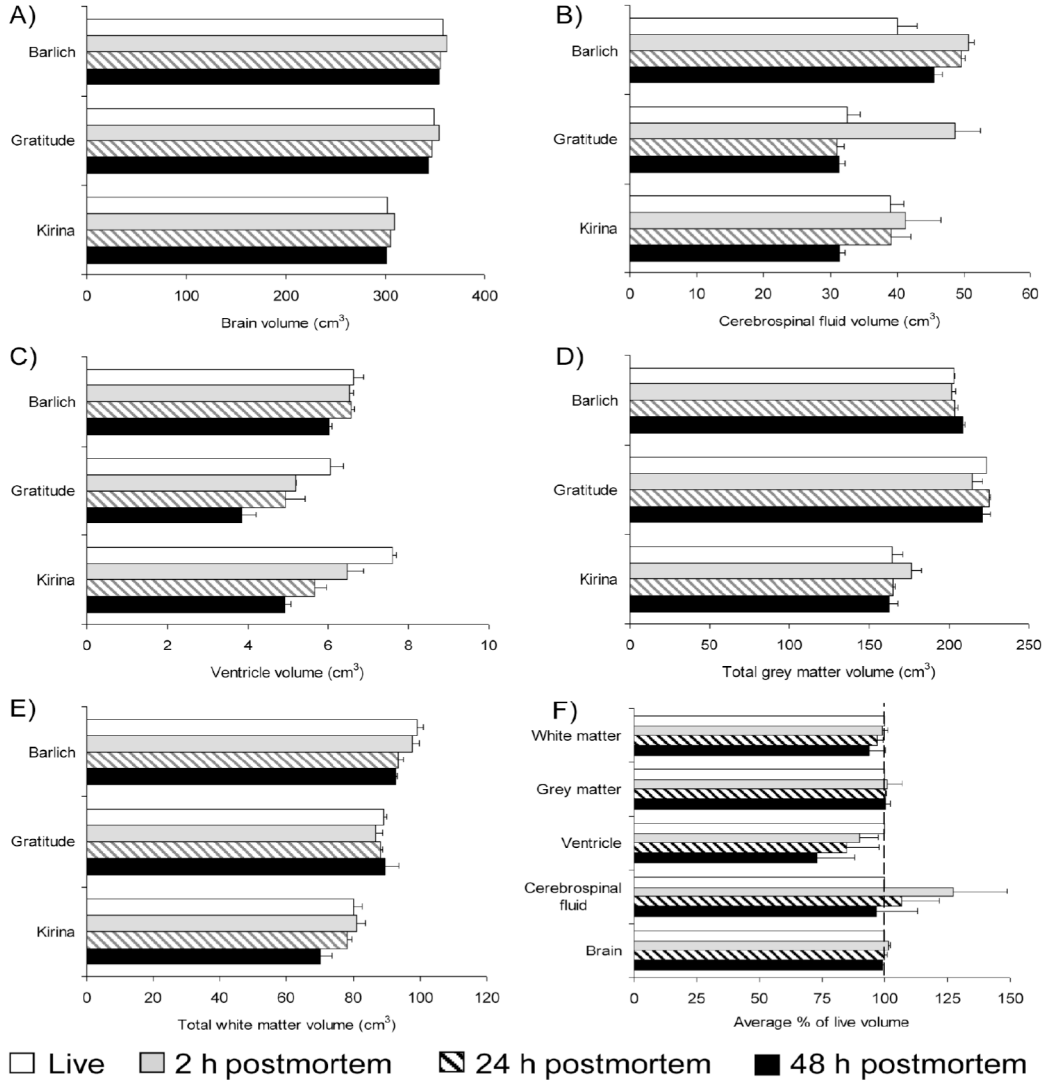

Fig. S5. *Zalophus californianus.* Volumes (cm^3) of brain structures from images acquired alive and postmortem-intact of California sea lions 'Kirina', 'Barlich' and 'Gratitude'. (A) Total brain volume. (B) Total cerebrospinal fluid volume. (C) Ventricular volume. (D) Total gray matter volume. (E) Total white matter volume. (F) Average percent (%) of live volume; this value was calculated by dividing the postmortem volume by the live volume and multiplying by 100% for 'Kirina', 'Barlich' and 'Gratitude'. The mean and SD values for these 3 sea lions were reported. Structural volumes were determined twice, which provided the source of the error in the error bars

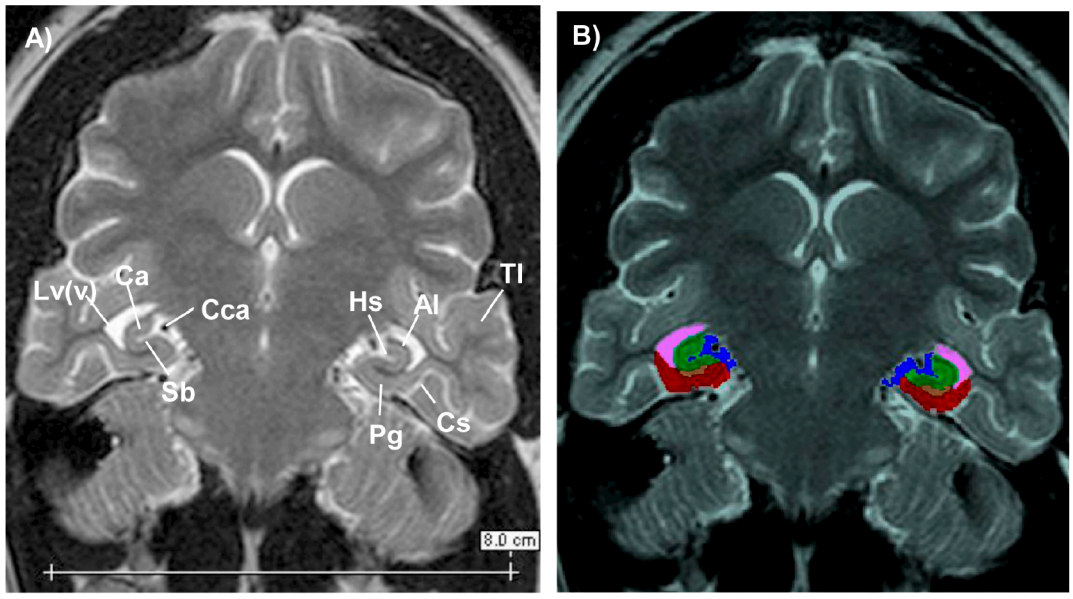

Fig. S6. *Zalophus californianus.* (A) T2-weighted magnetic resonance images acquired in the oblique plane of the California sea lion 'Kirina' that was imaged alive. Al: alveus; Ca: cornu ammonis; Cca: caudal cerebral artery; Cs: collateral sulcus; Hs: hippocampal sulcus; Lv(v): lateral ventricle (ventral horn); Pg: parahippocampal gyrus; Sb: subiculum; Tl: temporal lobe. Scale bar = 8.0 cm. (B) Label map of the image displayed in panel A illustrating the boundaries of the hippocampus and associated structures. This sea lion did not show any clinical signs of domoic acid toxicosis. In panel B, the ventral horns of the lateral ventricles are shown as pink, the hippocampal sulci are blue, the hippocampi are green, the gray matter of the parahippocampal gyri are red and the white matter of the parahippocampal gyri are brown.

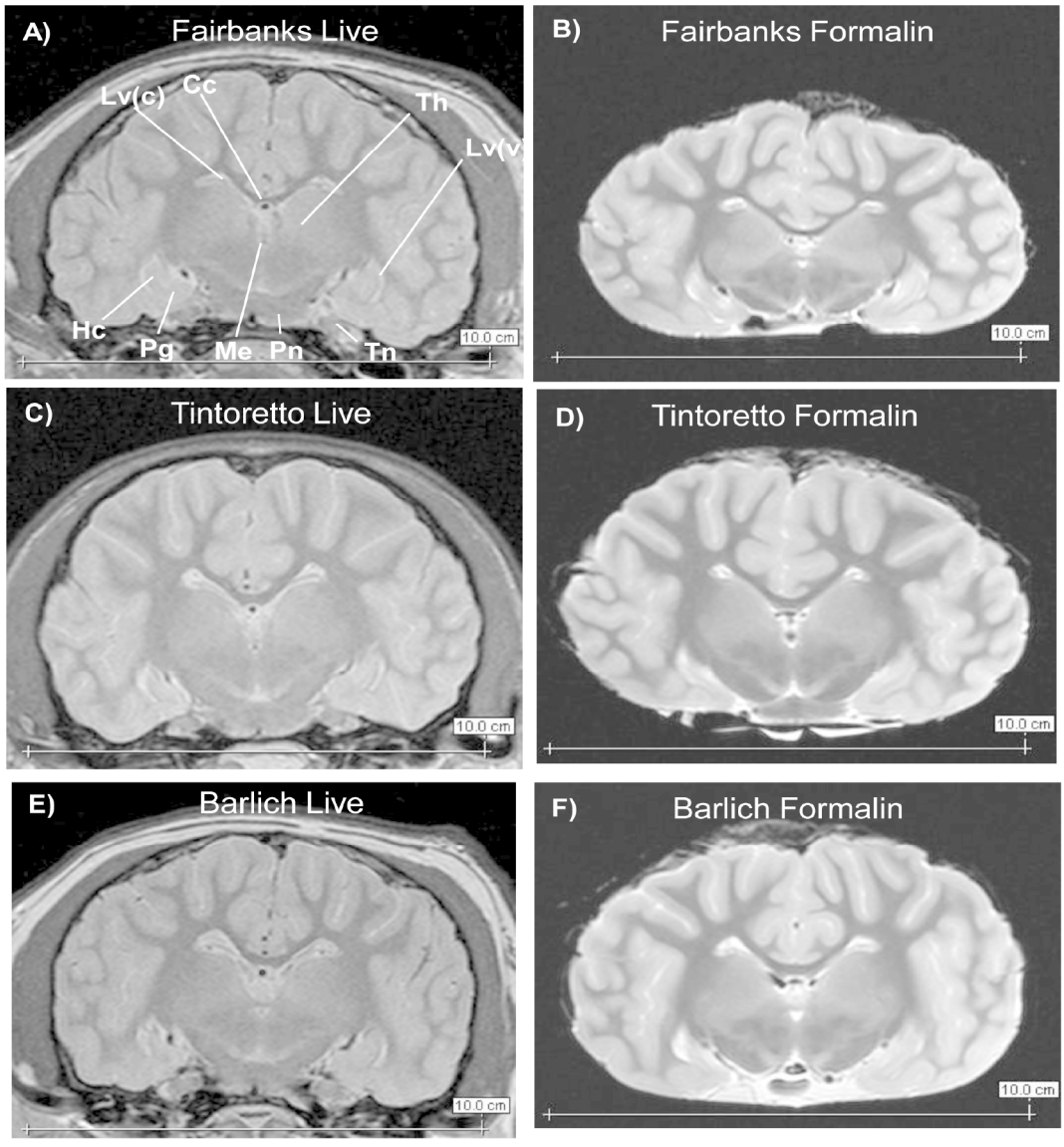

Fig. S7. *Zalophus californianus*. PD-weighted magnetic resonance images of the California sea lion brain acquired in the transverse plane imaged live and formalin fixed: (A,B) 'Fairbanks', (C,D) 'Tintoretto' and (E,F) 'Barlich'. Fixation of the brain occurred at 2 h after death for 'Fairbanks', 23 h after death for 'Tintoretto' and 48 h after death for 'Barlich'. Cc; corpus callosum; Hc: hippocampus; Lv(c): lateral ventricle (central); Lv(v): lateral ventricle (ventral horn); Me: mesencephalic aqueduct; Pg: parahippocampal gyrus; Pn: pons; Th: thalamus; Tn: trigeminal nerve. Scale bars = 10 cm

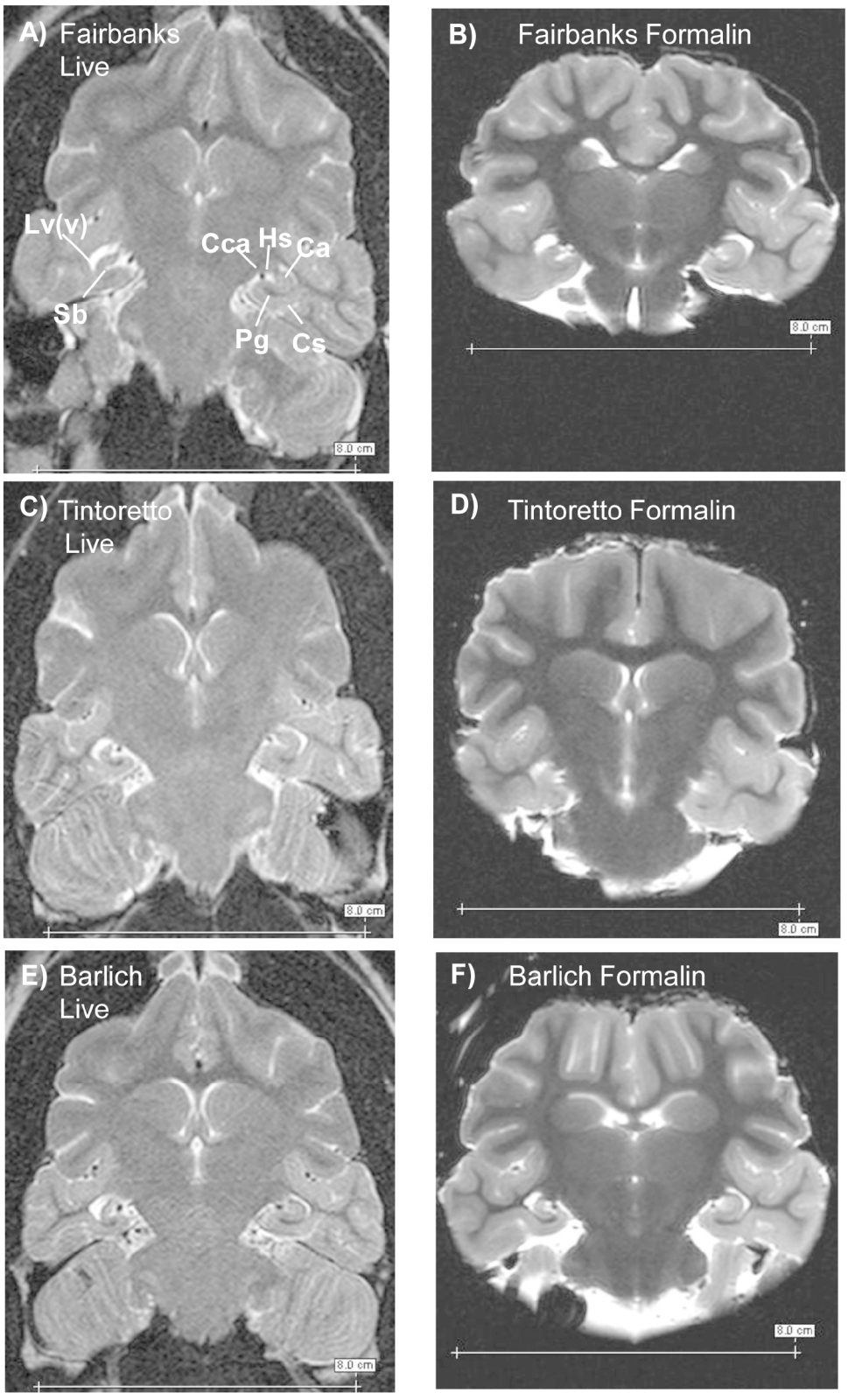

Fig. S8. *Zalophus californianus.* T2-weighted magnetic resonance images of the California sea lion brain acquired in the oblique plane imaged live and formalin fixed: (A,B) 'Fairbanks', (C,D) 'Tintoretto' and (E,F) 'Barlich'. Fixation of the brain occurred at 2 h after death for 'Fairbanks', 23 h after death for 'Tintoretto' and 48 h after death for 'Barlich'. Ca: cornu ammonis; Cca: caudal cerebral artery; Cs: collateral sulcus; Hs: hippocampal sulcus; Lv(v): lateral ventricle (ventral horn); Pg: parahippocampal gyrus; Sb: subiculum. Scale bars = 8.0 cm