Aquatic Mammals 2018, *44*(3), 231-238, DOI 10.1578/AM.44.3.2018.231

The Evaluation of Olfaction in Stranded California Sea Lions (*Zalophus californianus*) and Its Relevance to Domoic Acid Toxicosis

Lauren M. De Maio,[1] Peter F. Cook,[2] Colleen Reichmuth,[3] and Frances M. D. Gulland[4]

[1]*Ocean Associates Inc., National Marine Fisheries Service, West Coast Region, 501 W. Ocean Beach Boulevard, Long Beach, CA 90802, USA*
E-mail: lauren.demaio@noaa.gov
[2]*New College of Florida, 5800 Bayshore Road, Sarasota, FL 34243, USA*
[3]*University of California Santa Cruz, Long Marine Laboratory, 115 McAllister Way, Santa Cruz, CA 95060, USA*
[4]*The Marine Mammal Center, 2000 Bunker Road, Fort Cronkite, Sausalito, CA 94965, USA*

Abstract

Domoic acid is an algal toxin that has caused neurologic disease and reproductive failure in California sea lions (*Zalophus californianus*). In affected sea lions, necrotic neurons have been observed in the olfactory bulb and pyriform lobe of the brain, indicating potential for disrupted olfactory capability in addition to other documented neurological effects. Sea lions use olfaction in social interactions, and deficits could lead to maladaptive interactions, including between mothers and pups. Here, to assess olfactory capability in wild California sea lions, we developed a behavioral assay for use in a clinical context. We tested 24 stranded sea lions with no apparent neurological symptoms and 22 sea lions with a clinical diagnosis of chronic domoic acid toxicosis, probing differential responses to a scented and unscented object. The neurologically healthy animals spent significantly more time with the scented object than with the unscented object, establishing this method as effective in demonstrating olfactory discrimination in California sea lions. The domoic acid toxicosis group showed a nonsignificant reduction in response to the scented stimulus. However, variability in responses suggests that olfactory sensitivity is impaired in at least some sea lions with domoic acid toxicosis.

Key Words: strandings, domoic acid, hippocampal atrophy, olfaction, naso-nasal contact, olfactory bulb, California sea lions, *Zalophus californianus*

Introduction

Domoic acid (DA), a neurotoxin produced by the diatom species *Pseudo-nitzschia australis*, bio-accumulates in the food chain and affects multiple species of marine life, especially along the California coast where sea lions (*Zalophus californianus*) have been the most visible victims (Scholin et al., 2000; Torres de la Riva et al., 2009). DA-producing algal blooms are increasing worldwide, in part due to anthropogenic effects on coastal oceans (Silver et al., 2010; Lefebvre et al., 2016). These blooms can expose marine mammals to toxic levels of DA, leading to a variety of medical complications, epilepsy, reproductive failure, and death (Gulland et al., 2002; Brodie et al., 2006; Goldstein et al., 2008).

In 1998, at least 400 California sea lions died during an observed bloom of *Pseudo-nitzschia*, with many showing evidence of neurologic disease (Scholin et al., 2000). DA has been found in northern anchovies (*Engraulis mordax*)—a prey species for sea lions—and in blood and urine samples from affected sea lions, indicating the trophic transfer of the biotoxin resulting in marine mammal mortality (Scholin et al., 2000; Goldstein et al., 2008). Histologic examination of California sea lions that stranded and died following toxic exposure to DA has revealed the hippocampus as the primary site of neuronal necrosis (Buckmaster et al., 2002, 2014; Silvagni et al., 2005; Ramsdell & Gulland, 2014).

Domoic acid is similar in structure to kainic acid and acts as a partial agonist on subtypes of inotropic glutamate receptors (Hampson & Manalo, 1998; Nanao et al., 2005), promoting glutamate-mediated excitation of N-methyl-D-aspartate glutamatergic receptors (Novelli et al., 1992), leading

to consistent observable seizure behaviors in multiple species (Tryphonas & Iverson, 1990; Tasker et al., 1991). In rodents, the expression of glutamate receptors is particularly strong in pyramidal cells of the cerebral cortex, pyramidal cells of the hippocampus, and mitral cells of the olfactory bulb (Kanai et al., 1995). A single high controlled dose has been reported to activate (as assessed by Fos histochemistry) and damage (as assessed by cupric silver histochemistry) two primary regions: (1) the olfactory bulb and (2) the Ammon's horn region of the hippocampus (Peng et al., 1994; Scallet et al., 2004).

Similarly, in some free-ranging sea lions that were exposed naturally to DA, necrotic neurons were present throughout the cortical and subcortical limbic system, including the pyriform lobe, the rostral thalamic nuclei, and the olfactory bulb (Silvagni et al., 2005). Within the hippocampus, DA appears to target granule cells in the dentate gyrus. This leads to mossy fiber sprouting and the development of an abnormal positive-feedback circuit hypothesized to play a role in the generation of temporal lobe epilepsy, with more generalized lesions in the CA portion of the hippocampus likely a result of continued epileptic insult (Buckmaster et al., 2002, 2014). Ramsdell & Gulland (2014) proposed that DA-induced seizures damage dendritic spines of olfactory granule cells, and this proximal site of DA action can trigger excitability of mitral cells and activate downstream pathways of the olfactory cortex.

Damage to brain regions supporting olfaction in sea lions could have a number of negative consequences for wild sea lions. Sea lions and fur seals (Family Otariidae) have functional peripheral and central olfactory structures (Thewissen & Nummela, 2008). In the laboratory, otariids are capable of learning differential responses to olfactory stimuli (Laska et al., 2008); and in the wild, they show behavioral evidence of olfactory function in their social interactions. Olfaction contributes to recognition of estrous females (Gentry, 1998), and naso-nasal inspection is used in mutual identification among mothers and their pups (Schusterman et al., 1992; Dobson & Jouventin, 2003). Because California sea lions have a long lactation period, and mothers regularly leave their pups to forage, individual recognition is an important factor in reunification (Melin et al., 2000; Insley et al., 2003)—for example, after initially locating her pup, the female smells the pup extensively in what appears to be a final check of identity before accepting it as her own (Trillmich, 1981; Gisiner & Schusterman, 1991; Schusterman et al., 1992). Inability to complete the olfactory stage of identification could have dire consequences. Pitcher et al. (2010) showed that wild Australian sea lion mothers preferred model pups marked with their own pup's scent and could be highly aggressive to model pups marked with the scent of an unfamiliar pup, biting, shaking, and even throwing the model. Anecdotal reports from the stranding community indicate that some sea lion mothers with DA toxicosis show atypical aggression toward their pups. This might be explained in part by olfactory dysfunction as a result of damage to olfactory regions in the brain rather than damage to the olfactory bulb.

While prior studies have linked behavioral changes in California sea lions to DA exposure generally (Cook et al., 2011; Wittmaack et al., 2015), and hippocampal damage specifically (Cook et al., 2015, 2016), no studies have examined potential changes to olfactory capability. Toward that end, we designed a simple behavioral discrimination procedure relying on spontaneous, differential exploratory response to scented and unscented objects. We tested stranded California sea lions undergoing rehabilitation, and then classified individuals into groups by diagnosis, including chronic DA toxicosis and controls (no neurological symptoms). Efficacy of the approach was assessed first in controls and then compared between control and chronic DA groups to examine potential olfactory deficits in sea lions with DA toxicosis.

Methods

Subjects

Fifty-five stranded California sea lions were evaluated in this study. The majority of subjects were tested in the summer months of 2009 to 2011 during treatment at The Marine Mammal Center (TMMC) in Sausalito, California. Sex and age classes for all subjects were determined using body length, weight, genital morphology, and stage of sagittal crest development as described by Greig et al. (2005). Age classes were assigned as yearling (1 to 2 y old), male juvenile (2 to 3 y old), female subadult (2 to 5 y old), and adult female (greater than 5 y). No subadult or adult males were tested.

Subjects were opportunistically tested as they became available during rehabilitation by an experimenter blind to their diagnoses. The only pre-criterion for inclusion in the study was that each animal tested had to be eating well and medically stable.

Clinical Assessment and Group Assignment

Two subtypes of clinical DA toxicosis have been described: (1) acute and (2) chronic (Goldstein et al., 2008). Acute DA toxicosis is a result of recent exposure to DA and presents as a range of atypical behaviors and seizures. Frequently, these animals do not show gross brain lesions when assessed with MRI or histology. Chronic DA

toxicosis is a persistent epileptic condition, characterized by intermittent seizures and gross hippocampal lesions. Importantly, these conditions are not independent; animals stranding with acute exposure effects may suffer from the chronic condition as well. Because our interest herein was in possible olfactory deficits as a result of accrued brain damage, only cases of chronic toxicosis were used in the study.

All animals were assessed clinically by TMMC veterinary staff either before or after testing, and then sorted into one of three groups: (1) animals with acute DA toxicosis, (2) animals with chronic DA toxicosis, and (3) animals with no apparent neurological symptoms ("controls") according to the criteria in Goldstein et al. (2008). In all cases, diagnosing veterinarians were blind to performance in the behavioral task, and experimenters running and interpreting the behavioral task were blind to veterinary diagnoses. Animals classified by veterinary staff as controls showed no neurological symptoms, and no signs of brain disease or abnormality; in some cases ($n = 2$), healthy brain tissue was confirmed by MRI. The comparison group comprised chronic DA cases that were classified by either MRI ($n = 14$) or histopathology ($n = 8$). Animals diagnosed with acute DA toxicosis were not included in the comparison group.

Given the large sample size, imaging and histopathology were obtained opportunistically when they had been ordered by veterinary staff. In the cases where MRI images were obtained ($n = 16$), data were reviewed and interpreted by Eric Montie (PhD neurobiologist), Sophie Dennison (DVM radiologist), or Jerome Barakos (MD neuroradiologist) without knowledge of the sea lions' clinical status. MRI scans have previously been used to support diagnoses of chronic DA toxicosis in wild sea lions (Goldstein et al., 2008). Herein, the scans were compared to an MRI-based atlas of the normal California sea lion brain (Montie et al., 2009). Imaging was used to support diagnosis of chronic DA toxicosis; this involved visual assessment of the distribution and variety of brain damage, including atrophy of the hippocampus and parahippocampus as in Thomas et al. (2010) and Cook et al. (2015). In cases where histopathology was used to support diagnosis, evidence of neuronal necrosis was used to indicate chronic DA toxicosis similar to Goldstein et al. (2008).

Behavioral Assay

Subjects were tested in an isolated, fenced, 2.5 m × 2.5 m pen with a cement surface. Two physically identical objects were placed in the test enclosure before the animal was moved in. The objects were large, capped PVC pipes, 20 cm high with 2.5 cm holes drilled at the top (Figure 1). One object was unmarked for control scent (S-), and the second object (S+) was marked with 0.75 kg of thawed herring (*Clupea pallasii*) hidden within the PVC container. The odor stimulus was positioned in the S+ and was not visible to the test subjects. Two stimulus positions (A and B), placed 1.5 m apart, were used in this assay; the position of the S+ and S- were randomized across subjects. At the start of the trial, a sea lion subject was brought to the testing enclosure in a transport crate. The S+ and S- were equidistant from the enclosure doorway, and the crate was placed equidistant from the two objects (Figure 1). Once the crate door was opened, the sea lion was free to explore the enclosure and the objects. Animals had no visual access to humans during the testing interval, and behavior was recorded with a fixed position high-definition video.

The video recording of each session was later reviewed by an observer to measure exploration time within a strict 300-s time interval that began with the sea lions' release into the enclosure. A subject was classified as attending to either the S+ or S- when the tip of his or her nose was within 10 cm of one object or the other. A calibrated length marking visible on each object was used to enforce the 10 cm criterion when reviewing the video. A stopwatch was used to measure the total time the sea lion spent exploring each object. Each video was reviewed several times to ensure accuracy of scoring.

Data Analysis

Two primary analyses were conducted using the metric of time spent with the S+ and S- during the

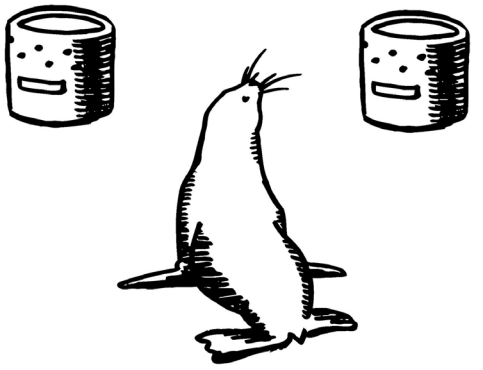

Figure 1. A sketch illustration of the experiment. Each sea lion was released equidistant from the two objects—one marked with fish scent (S+) and one without (S-). Position of the S+ and S- were randomized across subjects. White markings on the objects provided a 10 cm scale used to determine distance of the subject's head from the objects.

300-s trial interval. To assess the general efficacy of the test, the proportion of total time spent with the S+ vs the S- was examined within the control group. To probe potential olfactory impairment in animals with chronic DA toxicosis, proportion of time spent with the S+ vs the S- was compared between the control group and the comparison group. Both primary assessments used a one sample *t* test with a 95% confidence interval and *post-hoc* correction for repeated sampling. To further assess potential differences between the two groups, an *F* test for equal variance was conducted. An alpha level of 0.05 was used for all statistical tests.

Chronic DA toxicosis is more common among older female sea lions than in young animals, potentially due to differences in the feeding behavior and distribution of sea lions of different sex and age classes along the California coast (Gulland et al., 2002). To determine whether subject variables (sex, age) needed to be accounted for in the comparison of olfactory responses, we used additional *t* tests (with Bonferroni corrections for multiple comparisons) to determine within the control group (1) whether there was a difference in exploration time between males and females, and (2) whether there was a difference in exploration time between younger animals (< 3 y) and older animals (≥ 3 y). Data were arcsine square root-transformed to meet the assumptions of normality for equal variances prior to evaluation.

Results

Clinical Assessment

Of the 55 animals tested, 24 were subsequently classified as controls through the independent clinical assessment, and 31 were classified as suffering from either acute or chronic DA toxicosis. Table 1 shows subject data, performance on the olfactory assessment, and disposition for all subjects tested. Nine acute domoic acid cases were removed from further analysis of this study; however, we provide complete results for the 55 stranded sea lions in the table. Of the 24 control animals tested, there were 15 males, nine females, 18 younger individuals (< 3 y old), and six older individuals (≥ 3 y old) in the group. There were 22 chronic cases in the DA-exposed group and nine acute cases, and there were five males, 26 females, six younger individuals, and 25 older individuals.

Behavioral Assay

Controls—Out of the 24 control animals, 23 showed some exploration of stimuli (95%) (Figure 2). There was no significant difference between excess time spent with S+ vs S- stimuli between females (35.8 s ± 29.9) and males (58.3 s ± 56.2) ($t = 1.3$, $df = 23$, $p = 0.21$) or between younger (57.2 s ± 49.7) and older (27.8 s ± 40.8) animals ($t = 1.1$, $df = 23$, $p = 0.28$). In addition, there was no significant difference with time proportionally spent with the scented object between females (0.66 ± 0.47) and males (0.75 ± 0.43) ($t = 0.48$, $df = 23$, $p = 0.68$) or between younger (0.73 ± 0.44) and older (0.65 ± 0.48) animals ($t = 0.38$, $df = 23$, $p = 0.64$). These findings indicate that sex and age are not likely to drive differences in olfactory exploration in sea lions.

Overall, the control sea lions showed significantly more time proportionally with the scented object than with the unscented object (0.71 ± 0.24 vs 0.5; $t = 4.0$, $df = 23$, $p < 0.001$), demonstrating the efficacy of the assay in showing olfactory capability in wild California sea lions in a rehabilitation setting.

Chronic—Of the 22 individuals in the chronic group, 20 showed some exploration of the stimuli (90%) (Figure 2). The sea lions affected with chronic DA toxicosis did not spend significantly more time with the scented object than the unscented object (0.57 ± 0.49 vs 0.5; $t = 0.6$, $df = 21$, $p > 0.001$).

Comparison of Chronic vs Control Groups—The mean proportion of time with the S+ out of total exploration time was marginally lower in the chronic DA than in the control group (0.61 ± 0.37 vs 0.71 ± 0.25; $t = -1.58$, $df = 44$, $p = 0.06$ [one-sided]).

The chronic DA animals showed significantly higher variance in proportion of time with S+ vs S- (0.14 vs 0.06; $F = 2.3$, $p < 0.05$ [one-sided]; Figure 3).

Discussion

The results indicate that a simple behavioral discrimination can be used to assess gross olfactory sensitivity in untrained California sea lions in a rehabilitation setting; and further, that the test is effective in females of all ages and in juvenile males. Subjects with chronic DA toxicosis showed a nonsignificant trend toward spending less time with scented objects. In addition, sea lions with chronic DA toxicosis showed significantly higher response variance than did controls. The increased variance suggests that some DA sea lions may show altered and possibly reduced response to olfactory stimuli. Importantly, not all animals with chronic DA toxicosis show damage to olfactory brain regions (Silvagni et al., 2005; Ramsdell & Gulland, 2014), and only a portion of DA animals in the current study were examined by histology. Therefore, it is possible that the high variance in response in the chronic DA cases was due in part to an uneven distribution of olfactory lesions in the sample.

Table 1. This table provides the identification number of each subject; strand date; test date; sex (M = male, F = female); age class (Y = yearling [1 to 2 y], J = juvenile [male 2 to 4 y], SA = subadult [female 3 to 5 y; male 4 to 8 y], or A = adult [female 5+ y; male 8+ y]); behavioral score = absence (0) or presence (1) of stereotypical abnormal characteristic behavior of DA; diagnosis = either acute DA (A), chronic DA (C), or neurologically normal (control or "CN"); proportion of time spent with S+ and S- during 300-s trial interval; and disposition ($N = 55$).

CSL ID	Strand date (d/mo/y)	Test date (d/mo/y)	Sex	Age	Behavioral score	Diagnosis	S+	S-	Disposition
8739	17/7/2009	21/7/2009	F	A	1	A	0.00	0.00	Died
8268	4/6/2009	16/6/2009	F	SA	1	A	0.67	0.33	Released
8999	25/8/2009	12/9/2009	F	A	1	A	0.86	0.14	Euthanized
9517	21/5/2010	20/6/2010	F	A	1	A	0.14	0.86	Released
9543	25/5/2010	8/6/2010	F	SA	0	A	0.79	0.21	Released
9560	27/5/2010	21/6/2010	F	Y	0	A	0.25	0.75	Released
9666	13/6/2010	27/6/2010	M	Y	0	A	0.80	0.20	Released
9672	14/6/2010	25/6/2010	M	Y	0	A	0.77	0.23	Released
9715	23/6/2010	28/6/2010	M	J	1	A	0.83	0.17	Euthanized
8105	3/5/2009	12/5/2009	F	SA	1	C	0.79	0.21	Died
8107	4/5/2009	12/5/2009	F	A	1	C	0.00	1.00	Released
8176	23/5/2009	29/5/2009	M	J	1	C	0.97	0.03	Died
8622	4/7/2009	10/7/2009	F	A	1	C	1.00	0.00	Died
8673	8/7/2009	21/7/2009	F	A	1	C	0.87	0.13	Euthanized
8883	4/8/2009	16/8/2009	F	SA	1	C	0.89	0.11	Died
8890	4/8/2009	16/8/2009	F	A	1	C	0.80	0.20	Died
8898	6/8/2009	16/8/2009	F	A	1	C	0.95	0.05	Released
9325	23/12/2009	5/2/2010	F	A	1	C	0.00	0.00	Euthanized
9356	21/6/2010	27/6/2010	F	A	1	C	0.25	0.75	Euthanized
9587	31/5/2010	11/6/2010	F	A	1	C	0.00	0.00	Released
9597	2/6/2010	11/6/2010	F	A	0	C	0.14	0.86	Euthanized
9679	16/6/2010	20/6/2010	F	SA	1	C	0.15	0.85	Euthanized
9690	18/6/2012	20/6/2010	F	SA	0	C	0.90	0.10	Released
9881	17/10/2010	11/12/2010	F	A	1	C	0.79	0.21	Euthanized
9931	26/3/2011	4/5/2011	F	A	1	C	0.63	0.37	Euthanized
9933	17/4/2011	29/4/2011	F	A	1	C	0.38	0.62	Euthanized
9949	24/5/2011	24/6/2011	F	SA	1	C	0.79	0.21	Euthanized
9990	3/7/2011	17/7/2011	F	A	1	C	0.52	0.48	Euthanized
10091	30/8/2011	13/9/2011	F	A	1	C	0.71	0.29	Euthanized
10121	12/9/2011	2/11/2011	F	A	1	C	0.94	0.06	Released
10187	10/10/2011	16/11/2011	M	J	1	C	0.00	1.00	Euthanized
8166	21/5/2009	28/7/2009	F	Y	0	CN	0.41	0.59	Released
8202	26/5/2009	9/6/2009	M	J	0	CN	1.00	0.00	Released
8415	17/6/2009	24/7/2009	M	Y	0	CN	0.61	0.39	Released
8530	27/6/2009	24/7/2009	M	Y	0	CN	0.83	0.17	Released
8544	27/6/2009	28/7/2009	M	Y	0	CN	0.42	0.58	Released
8641	5/7/2009	24/7/2009	M	Y	0	CN	0.43	0.57	Released
8655	6/7/2009	24/7/2009	F	Y	0	CN	0.77	0.23	Released
8684	10/7/2009	16/7/2009	F	A	1	CN	0.00	0.00	Released
8690	11/7/2009	28/7/2009	M	Y	0	CN	0.80	0.20	Released
9364	19/3/2010	8/4/2010	M	J	0	CN	0.50	0.50	Released
9432	15/6/2010	25/6/2010	M	Y	0	CN	0.47	0.53	Released
9459	12/5/2010	10/6/2010	M	Y	0	CN	0.88	0.12	Released
9484	16/5/2010	27/6/2010	M	Y	0	CN	0.87	0.13	Released
9516	22/5/2010	10/6/2010	M	Y	0	CN	0.96	0.04	Released
9526	22/5/2010	27/6/2010	M	Y	0	CN	0.84	0.16	Released
9527	23/5/2010	25/6/2010	F	Y	0	CN	0.58	0.42	Released
9528	23/5/2010	9/6/2010	F	SA	0	CN	0.88	0.22	Released
9558	27/5/2010	9/6/2010	F	SA	0	CN	0.60	0.40	Released
9562	27/5/2010	21/6/2010	M	Y	0	CN	0.75	0.25	Released
9590	31/5/2010	9/6/2010	F	Y	0	CN	0.73	0.27	Released
9656	11/6/2010	27/6/2010	F	Y	0	CN	1.00	0.00	Released
9657	11/6/2010	20/6/2010	F	SA	0	CN	0.93	0.07	Released
9665	13/6/2010	21/6/2010	M	Y	0	CN	0.96	0.04	Released
9692	18/6/2010	25/6/2010	M	Y	0	CN	0.91	0.09	Released

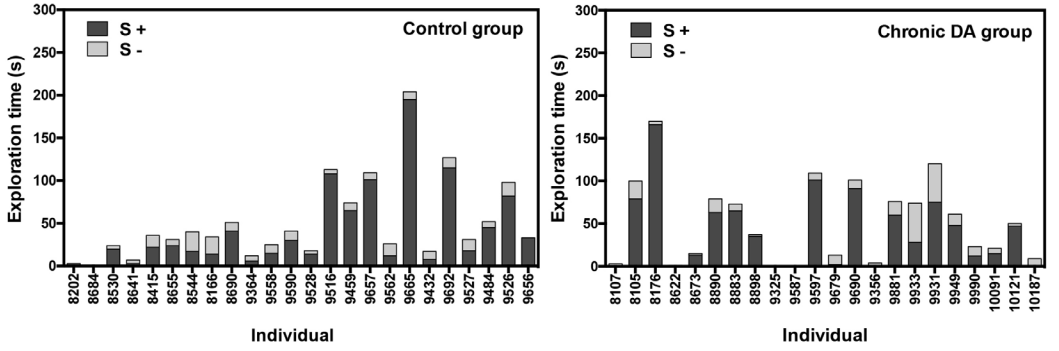

Figure 2. *(Control, Panel Left)* Total exploration time during the 300-s test interval for control subjects. Each subject is shown in order of testing date (oldest to most recent). Dark (lower bar) is time (s) with the scented object (S+), and light (upper bar) is time (s) with the unscented object (S-). *(Chronic DA, Panel Right)* Total exploration time in a 300-s test interval for chronic DA subjects. Each subject is shown in order of testing date (oldest to most recent). Dark (lower bar) is time (s) with the scented object (S+), and light (upper bar) is time (s) with the unscented object (S-).

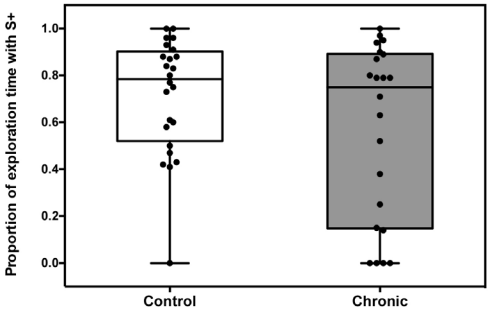

Figure 3. Box and whiskers plot showing proportion of active search time spent with S+ for each subject: 25%, 50% (median), and 75% quartiles are shown by each box; whiskers represent group 95% confidence intervals. Subjects with chronic DA toxicosis showed higher variability in relative time with S+ and a marginally lower mean.

There are unavoidable limitations with studies utilizing opportunistic recruitment of wild animals. Chronic DA toxicosis is far more common in adult females than juveniles and males (Gulland et al., 2002; Montie et al., 2012), leading to unbalanced sample sizes of control and treatment groups. We addressed this by assessing performance within control subjects on the basis of sex and age—we found none, suggesting that potential behavioral differences between the chronic DA group and the control group were not predominantly driven by sex and age of samples. In addition, imaging and histopathology were obtained opportunistically when they had been ordered by veterinary staff. It would have been beneficial to have imaging and histopathology on all available animals, and future work examining more subtle effects of DA on the olfactory system would benefit from more imaging and histopathology reports.

Our simple behavioral test was effective in demonstrating olfactory discrimination in control animals. However, this approach did not assess olfactory abilities across a range of ecologically valid contexts, including mother–offspring recognition. It is possible that many chronically diseased animals do not have gross olfactory malfunction but might still be impaired at more subtle discriminations. Seventeen of the 22 animals in our sample suffering from chronic neurological effects had confirmed hippocampal atrophy via brain imaging and/or histology. Although the hippocampus is not directly involved in olfactory function, hippocampal damage might be a marker for severity of DA-related brain damage, which might, in turn, be a predictor of olfactory damage. Further work is needed to distinguish direct DA-induced neuronal injury in the olfactory bulb from injury secondary to chronic seizure activity. Also, a more detailed histological analysis of olfactory neuronal necrosis is needed to understand the extent of damage on olfactory tracts of sea lions exposed to DA.

The findings from this study enhance our understanding of olfactory function in California sea lions. Although there were no significant mean differences in relative response to scent in the control and DA-exposed sea lions, DA-exposed sea lions showed significantly higher group variance in response, suggesting some of these animals likely do have olfactory impairment. Because olfaction plays a large role in otariid social behavior, olfactory impairment in California sea lions exposed to harmful levels of DA could have severe consequences for survival in the wild, particularly for nursing pups that may be

rejected by mothers with olfactory impairment. More precise olfactory function tests for use in stranded California sea lions are needed to characterize and assess the effects of impaired olfaction on this population across a range of ecologically relevant contexts.

Acknowledgments

Funding for this work was provided by the John H. Prescott Marine Mammal Rescue Assistance Grant and private contributions from individual foundations and corporate sponsorships to The Marine Mammal Center (TMMC). The research was conducted with the permission of the National Marine Fisheries Service to the Marine Mammal Health and Stranding Response Program (MMHSRP) under Permit Number 932-19-5/MA-009526, with approval from the Institutional Animal Care and Use Committees of the University of California Santa Cruz (UCSC) and TMMC. We thank the veterinary staff and dedicated volunteers at TMMC for their assistance. We thank Eric Montie at University of South Carolina Beaufort for providing MRI interpretation and advice, Katie Finch for the sketch illustration, and David Thompson at University of St Andrews for his continued encouragement and support.

Literature Cited

Brodie, E. C., Gulland, F. M. D., Greig, D. J., Hunter, M., Jaakola, J., St. Leger, J., . . . Van Dolah, F. M. (2006). Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). *Marine Mammal Science*, *22*(3), 700-707. https://doi.org/10.1111/j.1748-7692.2006.00045.x

Buckmaster, P. S., Zhang, G. F., & Yamawaki, R. (2002). Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *The Journal of Neuroscience*, *22*(15), 6650-6658.

Buckmaster, P. S., Wen, X., Toyoda, I., Gulland, F. M. D., & Van Bonn, B. (2014). Hippocampal neuropathology of domoic acid-induced epilepsy in California sea lions (*Zalophus californianus*). *Journal of Comparative Neurology*, *522*, 1691-1706.

Cook, P., Reichmuth, C., & Gulland, F. M. D. (2011). Rapid behavioural diagnosis of domoic acid toxicosis in California sea lions. *Biology Letters*, *7*, 536-538. https://doi.org/10.1098/rsbl.2011.0127

Cook, P. F., Reichmuth, C., Rouse, A., Dennison, S., Van Bonn, B., & Gulland, F. M. D. (2016). Natural exposure to domoic acid causes behavioral perseveration in wild sea lions: Neural underpinnings and diagnostic application. *Neurotoxicology and Teratology*, *57*, 95-105. https://doi.org/10.1016/j.ntt.2016.08.001

Cook, P. F., Reichmuth, C., Rouse, A., Libby, L. A., Dennison, S. E., Carmichael, O. T, . . . Ranganath, C. (2015). Algal toxin impairs sea lion memory and hippocampal connectivity, with implications for strandings. *Science*, *350*, 1545-1547. https://doi.org/10.1126/science.aac5675

Dobson, S. F., & Jouventin, P. (2003). How mothers find their pups in a colony of Antarctic fur seals. *Behavioural Processes*, *61*, 77-85. https://doi.org/10.1016/S0376-6357(02)00164-X

Gentry, R. L. (1998). *Behavior and ecology of the northern fur seal*. Princeton, NJ: Princeton University Press.

Gisiner, R., & Schusterman, R. (1991). California sea lion pups play an active role in reunions with their mothers. *Animal Behaviour*, *41*, 364-366.

Goldstein, T., Mazet, J. A. K., Zabka, T. S., Langlois, G., Colegrove, K. M., Silver, M., . . . Gulland, F. M. D. (2008). Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): An increasing risk to marine mammal health. *Proceedings of the Royal Society B: Biological Sciences*, *1632*, 267-276. https://doi.org/10.1098/rspb.2007.1221

Greig, D. J., Gulland, F. M. D., & Kreuder, C. (2005). A decade of live California sea lion (*Zalophus californianus*) strandings along the central California coast: Causes and trends, 1991-2000. *Aquatic Mammals*, *31*(1), 11-22. https://doi.org/10.1578/AM.31.1.2005.11

Gulland, F. M. D., Haulena, M., Fauquier, D., Langlois, G., Lander, M. E., Zabka, T., & Duerr, R. (2002). Domoic acid toxicity in California sea lions (*Zalophus californianus*): Clinical signs, treatment, and survival. *The Veterinary Record*, *150*, 475-480.

Hampson, D. R., & Manalo, J. L. (1998). The activation of glutamate receptors by kainic acid and domoic acid. *Natural Toxins*, *6*(3-4), 153-158. https://doi.org/10.1002/(SICI)1522-7189(199805/08)6:3/4<153::AID-NT16>3.0.CO;2-1

Insley, S., Phillips, A. V., & Charrier, I. (2003). A review of social recognition in pinnipeds. *Aquatic Mammals*, *29*(2), 181-201.

Kanai, Y., Bhide, P. G., DiFiglia, M., & Hediger, M. A. (1995). Neuronal high-affinity glutamate transport in the rat central nervous system. *NeuroReport*, *6*(17), 2357-2362.

Laska, M., Svelander, M., & Amundin, M. (2008). Successful acquisition of an olfactory discrimination paradigm by South African fur seals, *Arctocephalus pusillus*. *Physiology & Behavior*, *93*, 1033-1038. https://doi.org/10.1016/j.physbeh.2008.01.019

Lefebvre, K. A., Quakenbush, L., Frame, E., Huntington, K. B., Sheffield, G., Stimmelmayr, R., . . . Snyder, J. A. (2016). Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment. *Harmful Algae*, *55*, 13-24. https://doi.org/10.1016/j.hal.2016.01.007

Melin, S. R., DeLong, R. L., & Thomason, J. R. (2000). Attendance patterns of California sea lion (*Zalophus californianus*) females and pups during the non-breeding season at San Miguel Island. *Marine Mammal Science*, *16*(1), 169-185. https://doi.org/10.1111/j.1748-7692.2000.tb00911

Montie, E. W., Wheeler, E., Pussini, N., Battey, T. W., Van Bonn, W., & Gulland, F. M. D. (2012). Magnetic resonance imaging reveals that brain atrophy is more severe in older California sea lions with domoic acid toxicosis. *Harmful Algae*, *20*, 19-29. https://doi.org/10.1016/j.hal.2012.07.004

Montie, E. W., Pussini, N., Schneider, G. E., Battey, T. W. K., Dennison, S., Barakos, J., & Gulland, F. M. D. (2009). Neuroanatomy and volumes of brain structures of a live California sea lion (*Zalophus californianus*) from magnetic resonance images. *The Anatomical Record*, *292*, 1523-1547. https://doi.org/10.1002/ar.20937

Nanao, M. H., Green, T., Stern-Bach, Y., Heinemann, S. F., & Choe, S. (2005). Structure of the kainate receptor subunit GluR6 agonist-binding domain complexed with domoic acid. *Proceedings of the National Academy of Sciences USA*, *102*(5), 1708-1713. https://doi.org/10.1073/pnas.0409573102

Novelli, A., Kispert, J., Fernandez-Sanchez, T. M., Torreblanca, A., & Zitko, V. (1992). Domoic acid-containing toxic mussels produce neurotoxicity in neuronal cultures through a synergism between excitatory amino acids. *Brain Research*, *577*(1), 41-48. https://doi.org/10.1016/0006-8993(92)90535-H

Peng, Y. G., Taylor, T. B., Finch, R. E., Switzer, R. C., & Ramsdell, J. S. (1994). Neuroexcitatory and neurotoxic actions of the amnesic shellfish poison, domoic acid. *NeuroReport*, *5*(8), 981-985. https://doi.org/10.1097/00001756-199404000-00032

Pitcher, B. J., Harcourt, R. G., Schaal, B., & Charrier, I. (2010). Social olfaction in marine mammals: Wild female Australian sea lions can identify their pup's scent. *Biology Letters*, *7*(1), 60-62. https://doi.org/10.1098/rsbl.2010.0569

Ramsdell, J. S., & Gulland, F. M. D. (2014). Domoic acid epileptic disease. *Marine Drugs*, *12*(3), 1185-1207. https://doi.org/10.3390/md12031185

Scallet, A. C., Schmued, L. C., Slikker, W., Grunberg, N., Faustino, P. J., Davis, H., . . . Hanig, J. P. (2004). Developmental neurotoxicity of ketamine: Morphometric confirmation, exposure parameters, and multiple fluorescent labeling of apoptotic neurons. *Toxicological Sciences*, *81*(2), 364-370. https://doi.org/10.1093/toxsci/kfh224

Scholin, C. A., Gulland, F. M. D., Douchette, G., Benson, S., Busman, M., Chavez, F. P., . . . Van Dolah, F. M. (2000). Mortality of sea lions along the central California coast linked to a toxic diatom bloom. *Nature*, *403*, 80-84. https://doi.org/10.1038/47481

Schusterman, R. J., Hanggi, E., & Gisiner, R. (1992). Acoustic signaling in mother-pup reunions, interspecies bonding, and affiliation by kinship in California sea lions (*Zalophus californianus*). In J. A. Thomas, R. A. Kastelein, & A. Ya. Supin (Eds.), *Marine mammal sensory systems* (pp. 533-551). New York: Springer Publishing. https://doi.org/10.1007/978-1-4615-3406-8_34

Silvagni, P. A., Lowenstine, L. J., Spraker, T., Lipscomb, T. P., & Gulland, F. M. D. (2005). Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). *Veterinary Pathology*, *42*, 184-191. https://doi.org/10.1354/vp.42-2-184

Silver, M. W., Bargu, S., Coale, S. L., Benitez-Nelson, C. R., Garcia, A. C., Roberts, K. J., . . . Coale, K. H. (2010). Toxic diatoms and domoic acid in natural and iron enriched waters of the oceanic Pacific. *Proceedings of the National Academy of Sciences USA*, *107*, 20762-20767. https://doi.org/10.1073/pnas.1006968107

Tasker, R. A. R., Connell, B. J., & Strain, S. M. (1991). Pharmacology of systemically administered domoic acid in mice. *Canadian Journal of Physiology and Pharmacology*, *69*(3), 378-382. https://doi.org/10.1139/y91-057

Thewissen, J. G. M., & Nummela, S. (Eds.). (2008). *Sensory evolution of the threshold: Adaptations in secondarily aquatic vertebrates*. Berkeley: University of California Press. Retrieved from www.jstor.org/stable/10.1525/j.ctt1pppx4

Thomas, K., Harvey, J., Goldstein, T., Barakos, J., & Gulland, F. M. D. (2010). Movement, dive behavior, and survival of California sea lions (*Zalophus californianus*) post treatment for domoic acid toxicosis. *Marine Mammal Science*, *26*(1), 36-52. https://doi.org/10.1111/j.1748-7692.2009.00314.x

Torres de la Riva, G., Johnson, C. K., Gulland, F. M. D., Langlois, G. W., Heyning, J. E., Rowles, T. K., & Mazet, J. A. K. (2009). Associations of an unusual marine mammal mortality event with *Pseudo-nitzschia* spp. blooms along the southern California coastline. *Journal of Wildlife Diseases*, *45*(1), 109-121. https://doi.org/10.7589/0090-3558-45.1.109

Trillmich, F. (1981). Mutual mother-pup recognition in Galapagos fur seals and sea lions: Cues used and functional significance. *Behaviour*, *78*(1), 21-42. https://doi.org/10.1163/156853981X00248

Tryphonas, L., & Iverson, F. (1990). Neuropathology of excitatory neurotoxins: The domoic acid model. *Toxicologic Pathology*, *18*, 165-169.

Wittmaack, C., Lahvis, G. P., Keith, E. O., & Self-Sullivan, C. (2015). Diagnosing domoic acid toxicosis in the California sea lion (*Zalophus californianus*) using behavioral criteria: A novel approach. *Zoo Biology*, *34*(4), 314-320. https://doi.org/10.1002/zoo.21217