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# Magnetic resonance imaging reveals that brain atrophy is more severe in older California sea lions with domoic acid toxicosis

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ABSTRACT

In 1998, domoic acid (DA) toxicosis was first documented in marine mammals, when more than 400 California sea lions (*Zalophus californianus*) were determined to have been exposed to DA through contaminated prey that was linked to a bloom of toxin-producing diatoms. Over the last fifteen years, these blooms have increased in frequency and distribution, and DA toxicosis has become a more complex disease. Evidence with laboratory animals show that DA can cause epilepsy, may affect brain development, and may have synergistic effects with some pollutants. Detecting these effects in marine mammals requires quantitative methods to evaluate brain morphology in live animals. In this study, our goal was to employ magnetic resonance imaging (MRI) to examine the morphologic effects of DA toxicosis on the brain in live, wild California sea lions admitted to a rehabilitation center. We performed MRIs and volumetrics of brain structures of 53 sea lions that exhibited clinical signs of acute or chronic DA intoxication. We found that the volumes of the hippocampi and parahippocampal gyri of sea lions with chronic DA toxicosis were less than the volumes of these structures in non-DA intoxicated animals. In addition, adult sea lions with chronic DA toxicosis had more structural damage to the brain than younger animals. This pattern may be explained by one or more of the following possibilities: (i) repetitive, sublethal exposure; (ii) increased susceptibility of adults to DA compared to younger animals; and/or (iii) initial exposure to the toxin followed by the progressive effects of ongoing seizure activity. Of these three possibilities, increased susceptibility and progressive effects of ongoing seizure activity most likely explain why atrophy of the hippocampus and thinning of the parahippocampal gyrus is most severe in adults.



## 1. Introduction

Domoic acid (DA) is a naturally produced algal toxin that is structurally related to glutamate, an amino acid that acts as a major excitatory neurotransmitter in the brain. DA interacts with glutamate receptors, causing $Ca^{2+}$ influx, excitotoxic effects, and subsequent cell death (Hampson and Manalo, 1998). A high concentration of glutamate receptors in the hippocampus and parahippocampus causes these brain structures to be very sensitive to DA. The first reported event of neurotoxic illness from DA in humans occurred in 1987 in Montreal, when 107 patients exhibited gastrointestinal symptoms and neurologic abnormalities from eating contaminated mussels from Prince Edward Island (Perl et al., 1990). Neuropathological studies of three patients that died revealed neuronal necrosis of the hippocampus and amygdala (Teitelbaum et al., 1990) and patients that survived suffered short term memory loss. Thus, the damage to the hippocampus and associated memory loss resulted in domoic acid intoxication being commonly referred to as Amnesic Shellfish Poisoning (ASP) (Perl et al., 1990).

In 1991, DA toxicosis was first reported in wildlife when Brandt's cormorants (*Phalacrocorax penicillatus*) and pelicans (*Pelecanus occidentalis*) displayed central nervous system signs suggestive of acute intoxication (Work et al., 1993). DA toxicosis was then documented in marine mammals in 1998, when more than 400 California sea lions (*Zalophus californianus*) were found poisoned with contaminated prey that was linked to a bloom

*Abbreviations:* ASP, amnesic shellfish poisoning; DA, domoic acid; DDTs, dichlorodiphenyltrichloroethane; H&E, hematoxylin and eosin; MRI, magnetic resonance imaging; PD, proton density; SBB, Santa Barbara Basin; 3D, three dimensional; TMMC, The Marine Mammal Center.

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of toxin producing diatoms, *Pseudo-nitzschia australis* (Scholin et al., 2000). Sea lions that died acutely and contained detectable levels of DA in blood and urine exhibited neuronal necrosis in the dentate granule cells and cornu ammonis (CA) sectors CA4, CA1, and CA3 of the hippocampus; in some animals, necrosis extended into the olfactory bulb, pyriform lobe, and rostral thalamic nuclei (Scholin et al., 2000; Silvagni et al., 2005).

DA toxicosis has become an increasing health concern for wildlife and humans. A fifteen-year time-series (1993–2008) of sediment trap samples collected from the Santa Barbara Basin (SBB) indicated an abrupt shift towards greater frequency and a higher magnitude of *Pseudo-nitzschia* blooms and toxic DA flux events since the year 2000 (Sekula-Wood et al., 2011). Furthermore, in California sea lions, evidence suggests that DA toxicosis is more complex and that at least two clinical manifestations of DA poisoning exist: acute DA toxicosis (Scholin et al., 2000; Gulland et al., 2002), and a second syndrome characterized by epilepsy and atypical behaviors associated with sub-lethal exposure to the toxin (Goldstein et al., 2008). In addition, exposure studies with rodents and zebrafish have shown that low levels of exposure to the developing fetus and neonate, as well as co-exposure to anthropogenic pollutants such as dichlorodiphenyltrichloroethane (DDTs), may cause subtle changes in the brain and may result in long-term cognitive impairment (Ramsdell and Zabka, 2008; Tiedeken and Ramsdell, 2009, 2010).

To better understand and address this rising health concern, it is imperative to develop quantitative techniques that are able to detect subtle changes in brain morphology due to varying DA exposure levels and scenarios in live California sea lions. Most studies to date have used histological methods to investigate the effects of DA on the brain (Tryphonas et al., 1990a,b; Silvagni et al., 2005). However, histological methods cannot be used to assess the neurological effects of DA on live animals, whereas magnetic resonance imaging (MRI) offers a means to detect "gross" structural change (i.e., atrophy). MRI, coupled with image analysis, can accurately determine the volumes of brain structures (i.e., volumetric neuroimaging), providing a quantitative tool to measure the degree and extent of brain damage in live animals. This technique has been used in marine mammals, specifically California sea lions and Atlantic white-sided dolphins (*Lagenorhynchus acutus*) (Montie et al., 2007, 2008, 2009, 2010).

Our goal in this study was to employ MRI and volumetric neuroimaging to examine the brains of California sea lions ($N = 53$) that exhibited clinical signs of acute or chronic DA toxicosis. Specifically, the objectives were to: (1) determine the volumes of the hippocampus and parahippocampal gyrus of male and female California sea lions of varying age classes that did not exhibit any clinical signs of DA toxicosis; (2) compare the volumes of the hippocampus and parahippocampal gyrus of animals that were diagnosed with acute or chronic DA toxicosis to the volumes of presumed normal animals; (3) examine the volume loss of the hippocampus and parahippocampal gyrus in sea lions with chronic DA intoxication; and (4) determine if the number of brain pathologies and volume loss in chronic DA intoxicated animals was correlated with age. The hippocampus and parahippocampal gyrus, brain regions that are very important in learning and memory, were chosen for analysis because of the well known toxic effects of DA on these brain structures.

## 2. Methods

### 2.1. Animal information and sample processing

California sea lions used in this study stranded live along the California coast from Marin to San Luis Obispo Counties, between 2004 and 2010. Sea lions were transported to The Marine Mammal Center (TMMC), Sausalito, CA for clinical assessment, treatment, and rehabilitation. The sex of the animal was determined based on genital morphology, while age class determination was based on body length, tooth size, and stage of sagittal crest development (Greig et al., 2005). Serum, urine, and feces samples were collected and analyzed for domoic acid by direct competitive DA ELISA (Biosense Laboratories, Bergen, Norway). Animals were transported from TMMC to Animal Scan, Redwood City, CA for MRI of the entire brain under general anesthesia. Following scanning, some brains of euthanized animals were removed, fixed in 10% formalin, and sectioned for histology in the oblique plane perpendicular to the long axis of the sylvian fissure and temporal lobe. Tissues were processed routinely, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (H&E). All studies were approved by TMMC Institutional Animal Care and Use Committee protocol number 2007-2, and sea lions were collected under a Letter of Authorization from the National Marine Fisheries Service to TMMC.

DA toxicosis was classified into two separate clinical syndromes: acute DA toxicosis as described by Scholin et al. (2000) and Gulland et al. (2002); and a chronic epileptic syndrome indicated by behavioral changes and seizures as documented by Goldstein et al. (2008). The two syndromes were distinguished using the criteria of Goldstein et al. (2008). Acute cases presented with clinical signs that included ataxia, head weaving, seizures, or coma, varied in severity and were continuous during the interval of toxicosis, which lasted about one week and was followed by recovery, if treated, or death. These animals also stranded in clusters with at least one animal in the cluster having DA in body fluids. Chronic cases were characterized as animals that developed intermittent seizures at least two weeks apart or at least two weeks following admission to TMMC, but showed no signs between seizures and stranded individually (Goldstein et al., 2008). Animals classified as normal were animals with no neurological signs that stranded due to trauma, leptospirosis, or malnutrition.

### 2.2. Magnetic resonance data acquisition

Radiographs of the head and thorax of each animal were obtained prior to MRI to rule out the presence of metallic objects (e.g., bullets) that would result in image artifacts and/or injure the sea lion during MRI acquisition. Live animals anesthetized with isoflurane ($N = 47$) and postmortem specimens ($N = 6$) were imaged with a 1.5-T Siemens Magnetom Symphony scanner (Siemens, Munich, Germany) equipped with a CP Extremity Coil. Previous studies have shown that proton density (PD)- and T2-weighted images of postmortem-intact brains, up to 48 h after death, provided similar quality to images acquired from live sea lions (Montie et al., 2010). MRI data acquisition followed similar methods previously described by Montie et al. (2009, 2010).

### 2.3. Volume analysis of brain structures

Anatomical structures were identified using the MRI-based, brain atlas of the California sea lion (Montie et al., 2009). The initial evaluation of MR images occurred at the MRI unit. Post-processing, segmentation (i.e., assigning pixels to particular structures), three-dimensional (3D) reconstructions, and volume analyses were performed using the software program AMIRA 4.1.1 (Mercury Computer Systems, San Diego, CA) as described by Montie et al. (2009, 2010). The volumes of cerebral gray matter (GM), cerebral white matter (WM), cerebellum and brainstem GM, cerebellum and brainstem WM, hippocampus, and parahippocampal gyrus were determined. Other structures of the limbic system including the amygdala, anterior thalamic nuclei, septum, limbic cortex, and