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Natural exposure to domoic acid causes behavioral perseveration in Wild Sea lions: Neural underpinnings and diagnostic application

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ABSTRACT

Domoic acid is a naturally occurring algal toxin that causes neurological symptoms and mortality in exposed marine life. California sea lions (*Zalophus californianus*) are the most visible victims, and suffer epilepsy and progressive hippocampal atrophy. Despite its reliable neurological effects, little is known about how exposure to domoic acid alters behavior, which is critical for understanding the impact of toxic exposure on long-term survival in sea lions and other exposed animals, including humans. Better understanding of the behavioral effects may also inform veterinary diagnosis and treatment. Anecdotally, exposed sea lions have been reported to show enhanced perseverative behavior. To assess the neurobehavioral effects of domoic acid, we compared veterinary diagnoses, measures of hippocampal volume from *in vivo* MRI, and behavioral measures of habituation and dishabituation in 27 wild sea lions undergoing rehabilitation. The sample was divided post-hoc between subjects with clear veterinary diagnoses of chronic domoic acid toxicosis and those with no evidence of the disease. In the behavioral task, subjects were exposed repeatedly to sounds from two source locations, and, following a short delay, exposed again. Veterinary diagnosis of domoic acid toxicosis was associated with extent of hippocampal damage, predicted delayed habituation in phase 1, and enhanced dishabituation in phase 2. Receiver operating characteristic analysis indicated that delayed habituation in phase 1 was diagnostically predictive. Enhanced dishabituation in phase 2 was correlated with reduced right ventral hippocampal volume. Together, delayed habituation and enhanced dishabituation following domoic acid exposure indicate a clinically relevant and potentially maladaptive behavioral pattern of perseveration.

1. Introduction

Domoic acid (DA) is a neurotoxic metabolite of *Pseudo-nitzschia* algae; it propagates through ocean food chains, causing neurological insult and mortality in a range of species (Beltrán et al., 1997; Kvitek et al., 2008; Lefebvre et al., 1999; Lefebvre et al., 2002; Scholin et al., 2000). Due to environmental changes and anthropogenic influences in marine systems, the size and persistence of *Pseudo-nitszschia* blooms are increasing (Anderson et al., 2008; Lefebvre et al., 2016; Silver et al., 2010). This has increased toxic exposure in sea life that feed directly on algae and animals at higher trophic levels that bioaccumulate DA through feeding on prey that feed on the algae. There is also concern regarding repeated low dose exposure in some human populations (Jeffery et al., 2004; Lefebvre and Robertson, 2010). California sea lions, which are exposed to the toxin through filter-feeding fish prey species, are the most visible victims of DA poisoning and can serve as bellwethers for its widespread effects on other species (Bossart, 2011; Reddy et al., 2001). Prior to 1998, there were few documented neurological signs in stranded sea lions along the California coast, but since that time several thousand California sea lions have come to shore in distress (i.e., stranded) with indications of DA poisoning. Chronic exposure to DA—and resultant mortality—in North Pacific sea lion populations is now believed to be widespread (Goldstein et al., 2008). As a neurotoxin, DA alters the behavior of exposed animals. While the acute effects of intoxication can be fatal, many animals survive initial exposure. The persistent behavioral effects of DA exposure may interfere with long-term survival and need to be better understood. Improved understanding of these behavioral effects may also aid veterinary diagnosis and treatment. Managing the welfare of affected sea lions is a pressing concern for wildlife managers. In addition, due to the large number of individuals affected, wild sea lions with DA toxicosis provide an accessible, natural model for understanding the effects of DA.

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The acute and chronic neurotoxic effects of DA have been explored both in laboratory models and wild animals, and, despite some differences in disease course among species, the mechanisms are fairly well understood (Pulido, 2008). DA binds preferentially to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors inducing excitotoxic effects. AMPA and kainate receptors are distributed throughout the brain (Bahn et al., 1994; Ritter et al., 2001), and distributed lesions are sometimes seen in sea lions naturally exposed to DA (Silvagni et al., 2005) and rodents exposed in the laboratory (Colman et al., 2005). However, kainate receptors are highly concentrated in the mammalian hippocampus (Bernabeu and Sharp, 2000), and the most reliable site of gross neurological damage in DA-affected animals has been the hippocampus (Goldstein et al., 2008; Montie et al., 2012; Silvagni et al., 2005; Pulido, 2008). As a result, the bulk of neurobehavioral research on DA has been focused on the hippocampus and the behavioral and cognitive functions it supports. In both laboratory and wild animals, DA exposure leads to extensive cell death in the dentate gyrus and CA3 portion of the hippocampus, and can cause seizures (Muha and Ramsdell, 2011; Ramsdell and Gulland, 2014; Silvagni et al., 2005). Long-term sub-lethal effects include chronic epilepsy and resultant gross hippocampal atrophy (Dakshinamurti et al., 1991).

Although the molecular mechanism of DA toxicity is extensively studied, and appears generally consistent across species, dose-response relationships and patterns of gross brain damage are not consistent between laboratory rodents and wild animals exposed naturally. Relatively low doses of DA are fatal to rodents, and rodents are more likely to suffer bilateral lesions than sea lions and humans, which tend to present with unilateral left or right brain lesions (Goldstein et al., 2008; Pulido, 2008). Potential species differences in gross neurological sequelae to DA underscore the need for ecologically valid models to study the disease (Buckmaster et al., 2014; Cook et al., 2015). Not only may these better illustrate the neurological effects across species, but they will allow more confident assessments of potentially maladaptive behavioral and cognitive deficits in humans and wild animals naturally exposed to DA. However, to date, the cognitive and behavioral effects of DA have been studied predominantly in rodent models.

As noted, DA disproportionately targets the hippocampus, a structure that plays an integral role in allocentric spatial memory across species (Burgess et al., 2002; Eichenbaum et al., 2016; Squire, 1992). This has guided the bulk of behavioral research with DA exposed laboratory rodents, which has demonstrated reliable spatial memory deficits following toxic exposure (Clayton et al., 1999; Grant et al., 2010; Petrie et al., 1992; Sutherland et al., 1990). A recent neurobehavioral study with wild sea lions with DA toxicosis also found disrupted spatial working and long-term spatial memory, notably dependent on extent of right dorsal hippocampal atrophy (Cook et al., 2015). This sea lion finding is consistent with evidence that the dorsal portion of the hippocampus, and in humans the right dorsal portion, selectively contributes to spatial and event memory (Bohbot et al., 1998; Moser and Moser, 1998). However, animals with toxic DA exposure show atrophy throughout the hippocampal assembly, and spatial memory deficits are unlikely to represent the sum total of behavioral impairment from toxic exposure.

The hippocampus is also generally implicated in direction of attention (Pribram and McGuinness, 1975), arousal (Green and Arduini, 1954), emotion (LeDoux, 2003), and decision making (Johnson et al., 2007). There is some reason to believe these functions are at least partially localized to ventral portions of the hippocampus (Fanselow and Dong, 2010), which, again, are damaged in rodents with DA exposure and selectively damaged in some exposed sea lions (Cook et al., 2015; Pulido, 2008). However, very few behavioral studies have assessed the effect of DA exposure on cognitive and behavioral domains outside of memory.

One promising avenue of research is examination of DA's effect on perseverative behavior and related phenomena such as habituation and dishabituation. Perseveration, defined as a lack of behavioral flexibility or tendency to repeat behaviors without utility (Sandson and Albert, 1987), can accompany epilepsy and hippocampal damage or dysfunction (Abela et al., 2012; Kimble and Kimble, 1965; Langston et al., 2012; Sakata et al., 2013; Trent et al., 2013) and is associated with arousal and attention in humans (Fischer et al., 2005), both linked with ventral hippocampal function. As defined by Rankin et al. (2009), habituation is the process by which response weakens to a repeated stimulus, while dishabituation is the process by which a previously habituated response recovers. Hippocampal subregions have been functionally implicated in both processes (Crusio and Schwegler, 1987; Save et al., 1992; Yamaguchi et al., 2004).

In laboratory studies, rodents exposed to kainic acid, a close analog to DA, show perseverative behavior (Arkhipov et al., 2008). Increased reactivity and altered habituation have been shown in rodents exposed to DA (Zuloaga et al., 2016). Wild sea lions with DA exposure have been described in the rehabilitation setting as engaging in repetitive and seemingly non-directed behaviors, such as scratching, pacing, and chewing (Goldstein et al., 2008), and one experimental behavioral study found delayed habituation to auditory tones in wild sea lions with signs of DA toxicosis (Cook et al., 2011).

Perseverative behavior is likely maladaptive for flexible central place foragers like sea lions, which change their foraging strategies in response to changing environmental conditions (Francis et al., 1991). Specifically, perseveration could lead to persistence in ineffective foraging strategies, making wild sea lions with chronic DA toxicosis less efficient predators, which could in turn help explain death from malnutrition and dehydration (Goldstein et al., 2008). In addition, if habituation and dishabituation are reliably altered in animals with DA toxicosis, measures of these phenomena may be useful tools to enhance veterinary diagnosis.

Such enhancements of veterinary assessments are needed. To date, clinical diagnosis of DA toxicosis in wild animals has been reliant on the use of in vivo brain imaging to assess hippocampal atrophy (Goldstein et al., 2008; Gulland et al., 2002), an expensive and resource-intense process. Because DA is cleared from the blood stream quickly following exposure (Pulido, 2008; Truelove and Iverson, 1994), and exposure to low doses may not always produce neurological signs (Iverson et al., 1988; Tryphonas et al., 1990), diagnosis based on tissue, fecal, or urine sampling is unreliable. Recent diagnostic research has turned to machine learning algorithms to assess proteins and serum peptide patterns in blood (Neely et al., 2015; Neely et al., 2012). Behavior has also been assessed for diagnostic merit. An observational assessment of sea lions with DA toxicosis showed a pattern of behavioral abnormalities—including perseveration (Wittmaack et al., 2015)—with potential diagnostic efficacy, and habituation rates have also been shown predictive of diagnosis in wild sea lions with DA toxicosis (Cook et al., 2011). However, replication is needed under realistic clinical conditions, and neither of these studies assessed the relationship between specific neurological changes and behavioral abnormality.

To clarify the relationship between perseverative behavior and neurological insult from DA in wild sea lions, and to assess the diagnostic efficacy of experimental measures of habituation and dishabituation, we evaluated 27 wild California sea lions undergoing rehabilitation. Twelve individuals presented with a primary diagnosis of chronic DA toxicosis, and 15 individuals presented with a range of health concerns, including malnutrition, infection, injury, and cancer, but none of the signs of DA toxicosis. We measured behavioral orienting responses to repeated auditory stimuli for comparison to veterinary diagnostics and morphometric analyses of regional hippocampal volume from in vivo magnetic resonance imaging (MRI). To support potential diagnostic use of the behavioral testing, all subjects were exposed to the same sequence of auditory stimuli in a presentation schedule made up of two phases: 54 repetitions of an auditory tone alternating between two specific source locations (A and B), followed 3 min later by 54 repetitions of the same auditory tone alternating between one familiar (A) and one unfamiliar (C) source location. This fixed and automated exposure schedule allowed us to objectively assess initial responsiveness

(*habituation*) in phase 1 and response recovery (*dishabituation*) in phase 2, as well as relative responsiveness to novel versus familiar locations in phase 2 (potentially associated with *spatial memory*). Neurologically healthy animals typically show differential response to objects in novel locations, and hippocampal damage can interfere with this (Eacott and Norman, 2004; Ennaceur et al., 1997; Mumby et al., 2002).

We hypothesized that, relative to control subjects, California sea lions with chronic DA toxicosis would: 1) exhibit hippocampal atrophy, 2) demonstrate delayed behavioral habituation in phase 1 (AB), and 3) demonstrate enhanced dishabituation in phase 2 (AC). We also assessed relationships between total and regional hippocampal atrophy and behavioral performance.

2. Methods

2.1. Subjects

Twenty-nine wild California sea lions undergoing rehabilitation at The Marine Mammal Center (TMMC) in Sausalito, California were evaluated (Table S1). The sea lions ranged in age from pups (<1 year old) to adults (>5 years old). Data were collected during two separate testing periods (July 2010–November 2011 and August–November 2012, described below). During both periods of data collection, subjects were selected opportunistically from the available patient population at TMMC by an experienced veterinarian tasked with providing a sample that was approximately equally balanced between sea lions with primary diagnoses of chronic DA toxicosis (Goldstein et al., 2008) and control sea lions with no apparent neurological deficits. Decisions used for subject selection were based on preliminary diagnoses, and did not inform subsequent grouping for data analysis. Because subjects were selected from a rehabilitation population, there was a range of potentially overlapping medical conditions represented among them, including malnutrition, infection, physical trauma, and DA toxicosis (see Greig et al., 2005). To be included in the study, individuals had to be generally responsive to environmental stimuli and willing to eat. Sea lions were not selected so as to balance secondary diagnoses between the preliminary DA and control groups. However, the majority of individuals had one primary diagnosis, suggesting that secondary diagnoses would be unlikely to explain apparent effects of DA toxicosis.

Experimenters were blind to preliminary veterinary evaluations during collection and analysis of all behavioral and neural data. Following completion of behavioral and MRI testing (at final disposition for each sea lion) the same veterinarian who selected subjects for the study assigned a post-hoc diagnosis that was then used for sorting sea lions into DA and control groups for data analysis (as in Cook et al., 2015; Cook et al., 2011). The deciding veterinarian was blind to the behavioral data collected in the present study and to the quantitative measures of hippocampal volume acquired from MRI, but took into account all other available information on each subject. Relevant information included any clinical results (such as eosinophilia in a blood sample, levels of DA in urine or fecal samples), observations made of the subject during rehabilitative care at TMMC (e.g., observed seizures), final outcome for each subject (e.g., successful release, death, euthanasia), post-mortem and histological information (when available), a qualitative review of the MRI images conducted by an experienced veterinary radiologist, and epidemiological data on presence of DA producing blooms at the stranding site and concurrent DA cases. The post-hoc diagnosis allowed for a discrete assessment of whether each subject was suffering from acute or chronic DA toxicosis, and it was this final diagnostic measure that was used in further analysis, rather than the initial veterinary assessment made during subject selection.

Of note, although the radiologist's qualitative assessment of hippocampal atrophy did not characterize regional specificity or gross extent of damage, and veterinarians were blind to volumetric assessment of hippocampus (described below), the inclusion of this information in the diagnosis meant that clinical diagnosis and quantitative assessment of regional hippocampal volume were not wholly independent experimental measures. However, veterinary diagnosis of DA in this population typically involves qualitative assessment of hippocampal damage from in vivo MRI or post-mortem histology, and any attempt to validly assess the relationship between typical diagnosis and morphometric measures of hippocampal volume must include all typically obtained measures.

2.1.1. Period 1

Between July 2010 and November 2011, 16 sea lions were individually tested at Long Marine Laboratory (LML) at the University of California Santa Cruz. These subjects were part of a separate study examining the neurobehavioral effects of toxic DA exposure (Cook et al., 2015). They undertook testing for the present study following 10 to 20 days of participation in behavioral procedures at LML. In brief, these procedures comprised a number of food-based spatial memory assays, and did not involve auditory stimuli or exposure to the testing enclosure used in the present study. The mean time (±std. dev) under care for each of these subjects, from their admission to TMMC to the date of testing at LML for the present study, was 37 days ± 16.6.

2.1.2. Period 2

Between August and November 2012, 13 additional subjects were tested at TMMC while undergoing rehabilitation. These subjects did not take part in the previous study described above. The mean time (±std. dev) from admission to TMMC to testing to the date of testing for these subjects was 30 days ± 20.6.

Testing conditions and procedures were the same between the two test periods and facilities, except as noted in the supplementary text. Behavioral and neural measures were compared between periods, and there was no statistical evidence that time period and testing conditions influenced results.

2.2. Behavioral testing procedure

At both sites, subjects were tested in square, cement-floored pens measuring approximately 3 × 3 m (Fig. 1). Both pens were surrounded by chain-link fence on three sides with a solid wall along the fourth side. Subjects were not exposed to the pens prior to testing. Two Advent AV570 amplified speakers were placed outside of the pen prior to the

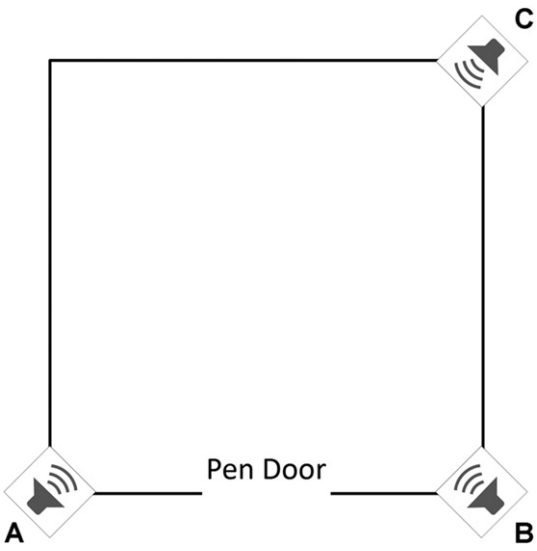

Fig. 1. Testing arrangement for auditory exposures. During phase 1 of testing, the auditory stimulus was presented repeatedly alternating between speakers in location A and B. During phase 2 of testing, the auditory stimulus was presented repeatedly alternating between speakers in location A and C. Behavioral responses were recorded to an overhead video camera.

subject's arrival. Speakers were placed at ground level, facing into the pen from the two corners at either side of the entry gate (defined as locations "A" and "B"). Testing sessions were filmed in their entirety with a Sony HD DCR-SR68 Handycam placed with full overhead view of the pen.

Subjects were transported individually to the testing pen from their living enclosure in a kennel used for transport (an enclosed box with a hinged cage front, similar to a large dog crate) and were tested between noon and 7 PM, not less than 1 h from their most recent meal. Transport in the kennel took no more than 5 min. Following transport, subjects were released into the pen and the kennel was removed. Each subject was allowed 3 min to acclimate to the enclosure prior to the start of testing. Experimenters were in a control room, out of sight, during acclimation and subsequent sound presentation.

The auditory stimulus used for this study was a "looming" tone, which has some of the auditory characteristics of a rapidly approaching object (Ghazanfar et al., 2002). The tone was 750 ms in duration, with the majority of energy centered at 1 kHz. The tone increased in amplitude from ~65 to 85 dB re 20 μPa at 1 m over its duration (unweighted). Stimulus features were selected to be salient to sea lions based on their established auditory sensitivity. As sea lion hearing threshold in air at this frequency is approximately 20 dB (Reichmuth et al., 2013; Schusterman, 1974), the equivalent maximum sensation level of the received stimuli was only 65 dB. No physiological measures were obtained, although no gross startle or clear avoidance behavior was observed in any subjects in this or a previous study (Cook et al., 2011) using the same stimuli.

Auditory cues were transmitted to the speakers via Windows Media player from an HP notebook computer. Sound presentation always followed an ABAB (or ACAC) pattern such that stimuli alternated repeatedly between the two speaker locations. There were two phases of sound presentation, constituting the two experimental phases in this study. In the first phase, the sound stimulus was presented alternately from the initial speaker locations A and B, 54 times in total (27 per location). Following this presentation phase, an experimenter physically moved one of the speakers from its original location (B) to a new location (C), diametrically opposed to location "A". The speaker at location "A" remained in the same position throughout testing. To control for stimulus enhancement from differentially interacting with the speakers, after moving the speaker from location "B" to "C", the experimenter walked over to the speaker at location "A" and crouched next to it for approximately 3 s.

After altering the speaker configuration following the first presentation phase, the experimenter retreated again to the out-of-sight control room, and a three-minute delay period began. Following the delay, the second presentation phase began. The auditory stimulus was presented 54 times in an alternating pattern switching between the familiar (A) and novel (C) speaker locations.

In both experimental phases, inter-stimulus intervals were between 0.2 and 7.5 s, semi-randomized such that the mean interval across each phase was 4 s. Stimulus presentation schedule was held constant across subjects. Both presentation phases were begun based on time elapsed (from subject entry to the testing pen in phase 1 and from the return of the experimenter to the testing room following speaker rearrangement in phase 2), and were not dependent on subject behavior, which the experimenter did not monitor during testing. Following the second testing phase, the subject returned to its home pen.

2.3. Behavioral data collection

Behavioral response data were coded post-hoc from video recordings by an individual who was blind to subject identity, veterinary assessment, and imaging results. A second independent coder, also blind to subject identity, veterinary assessment, and imaging results, recorded responses for a subset of ten subjects to obtain a measure of interobserver reliability. Both coders employed the same criteria for determining response, as originally defined and implemented in Cook et al. (2011). Following each individual sound presentation, a decision was made by the coder regarding whether the sea lion had produced an orienting response to the stimulus or had not. This was defined as a binary choice—because either "response" or "no-response" had to be coded for each stimulus presentation, the coder was allowed to watch each stimulus presentation event as many times as necessary to make a decision. A response was defined as a noticeable change in head orientation (>5°) in the vertical or horizontal plane toward the location of the speaker presenting the stimulus. The change in head orientation had to begin after the onset of the stimulus and before 0.5 s had elapsed following stimulus offset. The subject's head was not required to move into direct alignment with the speaker presenting each stimulus for a response to be counted; rather, it merely needed to move to closer angular alignment with the relevant speaker location than it had begun. Head movement away from the speaker was not counted. Further, other apparent behavioral markers of response, such as body startle, were not considered. Examples of an orienting response and of no response can be seen in Videos S1 and S2. Notably, responses were recorded from a wide-angle camera placed above the pen looking down. Therefore, ability to see reorientation in the vertical plane was likely limited. However, quite small alterations of head orientation were clearly visible in the horizontal plane.

To maximize potential ease of future veterinary implementation, and due to the discrete nature of the behavioral coding, Phase 1 (AB) and Phase 2 (AC) responsiveness was measured simply as the total number of coded positive responses for each subject. Thus, more responses in phase 1 was taken as a mark of delayed habituation, while more responses in phase 2 was taken as a mark of enhanced dishabituation. Relative response to the novel (C) versus familiar (A) location in Phase 2 was coded as the ratio of total responses to C versus total responses to A. These values were used in all subsequent behavioral analyses.

2.4. MRI data collection

Within a few days following behavioral testing, each subject underwent structural MRI evaluation. Subjects were imaged at AnimalScan Advanced Veterinary Imaging in Redwood City, CA, on a 1.5 T Siemens Magnetom Symphony scanner. Subjects were imaged in vivo under isoflurane anaesthesia with veterinary supervision as described by Cook et al. (2015). Subjects' heads were placed in a CP extremity coil, selected to optimize signal-to-noise ratio. The primary use of the MRI data was to obtain quantitative, volumetric brain measurements. These measurements were computed from manual tracings on the output images from Turbo Spin Echo (TSE) T2-weighted scans obtained in an oblique plane perpendicular to the long axis of the hippocampus as in Cook et al. (2015). This imaging orientation contributes to the ease of manual sectioning of the hippocampus in California sea lions (Goldstein et al., 2008; Montie et al., 2009).

Scans obtained from subjects in data acquisition period 1 (July 2010–November 2011) were acquired with the following imaging parameters: TR = 5470 ms, TE = 14 ms, FOV = 160 × 160 mm, slice thickness = 2.0 mm, voxel size = 0.625 mm × 0.695 mm × 2 mm. Subjects included in the second data acquisition period (August–November 2012) were being imaged opportunistically during a separate experimental brain imaging protocol. As a result, time constraints mandated increasing slice thickness on the oblique imaging sequence so as to limit total scanning time to under 1 h, above which extended anaesthesia can lead to increasing health complications. The oblique scans obtained from subjects included in data acquisition period 2 (August–November 2012) were acquired with the following parameters: TR = 3950 ms, TE = 98 ms, FOV = 160 × 160 mm, slice thickness = 3.0 mm, voxel size = 0.625 mm × 0.695 mm × 3 mm. Possible confounds in volumetric measurements due to increased slice thickness

in data acquisition period 2 were evaluated and determined to be minimal, as discussed in the supplemental text.

2.4.1. Hippocampal morphometrics

Manual tracing of MRI images was conducted by an experienced individual who was blind to veterinary assessment, behavioral results, and subject identity. A second, independent tracer, also blind to veterinary assessment, behavioral results, and subject identity, evaluated a subset of ten brains to allow for a measure of inter-observer reliability. Tracing was conducted using Quanta2 software (UC Davis IDEA Lab, Alzheimer's Disease Center grant, NIH P30 AG010129). For each subject, the right and left dorsal and ventral hippocampus, and total brain (minus the cerebellum) were traced (Fig. 2). Criteria for hippocampal tracing were the same as those described in Cook et al. (2015), and were informed by prior hippocampal morphometry with sea lions (Montie et al., 2012; Montie et al., 2009). Importantly, although relative brain volume is a related measure to extent of hippocampal atrophy from DA exposure, regional brain volume cannot be taken solely as a measure of possible atrophy—relative hippocampal volume will vary even among healthy animals.

In brief, the cornu ammonis (CA), dentate gyrus, alveus, and a portion of the subiculum were traced along the longitudinal axis of the hippocampus. Termination criteria for the septal and temporal hippocampal boundaries were conservatively selected to maximize reliability of tracing across subjects. Hippocampus was traced ventral to the splenium of the corpus callosum and dorsal to the interpeduncular fossa as presenting in the oblique imaging orientation. Lateral and medial boundaries were predominately defined by fluid filled structures surrounding the hippocampus. To correct for variation in hippocampal size due to natural variability in total brain size, relative hippocampal volumes were computed for each sea lion by dividing the absolute volumes of the hippocampal regions by total brain volume. These relative volume measurements, expressed as percentages, were then used in further analyses, and are referred to subsequently in this manuscript simply as “hippocampal volume.” To obtain regional measures of ventral and dorsal right and left hippocampal volume, tracings were split evenly along the longitudinal axis of the hippocampus such that there was an equal number of slices in the dorsal and ventral portion, and the volume from each portion calculated. For hippocampi traced across an odd number of slices, the volume of the center slice was halved and apportioned equally between the dorsal and ventral volumes.

2.5. Statistical analyses

Intra-class correlation coefficients (Shrout and Fleiss, 1979) were computed to assess reliability between the two volumetric hippocampal tracers. Cohen's Kappa (Cohen, 1968) was computed to assess reliability between the two individuals coding behavioral orienting responses.

To determine if DA subjects in the current study had gross hippocampal atrophy, a standard *t*-test was used to compare total hippocampal volume between control and DA subjects (as in Cook et al., 2015). To determine if lesions were regionally specific, a within-subjects ANOVA was then computed with DA status as the independent variable and regional hippocampal volume for each subject as the dependent variable. As noted above, a diagnosis of DA toxicosis can be supported by qualitative assessment of hippocampal atrophy from MRI. Quantitative and qualitative measures of hippocampal volume are not wholly independent, meaning that assigned DA status and hippocampal volume in the current study are not wholly independent. However, this relationship was not of primary experimental interest, but was rather assessed to confirm in the current sample prior evidence of a link between DA status and hippocampal atrophy.

DA toxicosis is most commonly seen in adult female sea lions. This made it difficult to balance the DA group for sex and age. The control group showed wide variability in sex and age (Table S1), and to assess potential effects of sex and age on responsiveness, a MANOVA was used to probe for a relationship between sex and age and phase 1 and 2 responsiveness in the control group.

As in Cook et al. (2011), phase 1 responsiveness was compared between DA and control subjects with a standard t-test. This was the primary analysis in the current study. To determine if DA status also predicted Phase 2 responsiveness, Phase 2 responsiveness was used as the dependent variable with diagnosis as the independent variable and Phase 1 responsiveness as nuisance variable in an ANCOVA analysis. Controlling for variance associated with number of responses in phase 1 was an important consideration, as it has been clearly demonstrated that repeated stimulation after apparent behavioral habituation can delay the onset of response recovery (Rankin et al., 2009).

As in Cook et al. (2011), a receiver operating characteristic (ROC) curve (cf., Metz, 1978) was computed for phase 1 responsiveness, and the area under the curve was used as a measure of diagnostic efficacy.

Multivariate regression was used to look for relationships between regional hippocampal atrophy and behavioral measures. Phase 1 responsiveness, phase 2 responsiveness, and relative response to novel

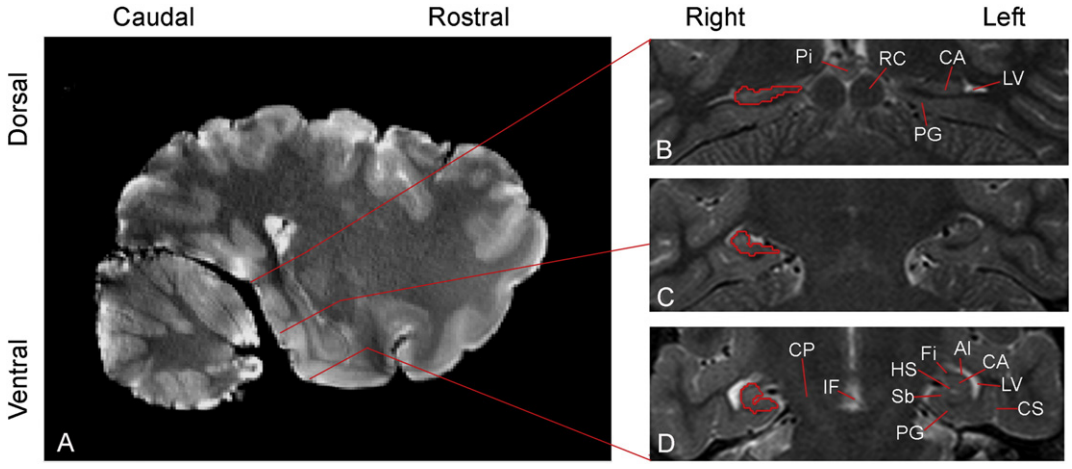

Fig. 2. Hippocampal Tracing. Three representative T2-weighted image slices obtained along the transect indicated by the red lines in (A). A right hippocampal tracing—represented on the left side of the images—is shown for the caudodorsal-most slice (B), a center slice (C), and the rostroventral-most slice (D). White signal next to the traced hippocampus is cerebrospinal fluid in the lateral ventricle, which is visibly enlarged in (D) as a result of right hippocampal atrophy. Abbreviations: Al: alveus CA: cornu ammonis CP: cerebral peduncle CS: collateral sulcus Fi: fimbria IF: interpeduncular fossa HS: hippocampal sulcus LV: lateral ventricle of temporal horn PG: parahippocampal gyrus Pi: pineal gland RC: rostral colliculus Sb: subiculum.

vs familiar location in phase 2 were all used as dependent variables against dorsal and ventral left and right hippocampal volumes as independent variables in three separate regression analyses. The regression with phase 2 responsiveness also included phase 1 responsiveness as a nuisance variable, as in the ANCOVA comparing DA diagnosis to phase 2 responsiveness. Significance of model *p* values were assessed using a Bonferroni correction for multiple comparisons. In models showing significance, individual variables (i.e., regional hippocampal volumes) were assessed for their conditional contribution to the model, that is, whether each made a significant contribution to the model holding all other variables steady (as in Cook et al., 2015).

All statistics were computed in the software package R. All P values reported were two-sided, except for that in the comparison of total hippocampal volume to DA status—given the strongly established directional link between these measures, a one-sided test was deemed appropriate. For significant results, measures of effect size were computed: Cohen's d for *t*-tests, partial eta squared for ANOVA, and Cohen's f^2 for local effect size in multiple regression.

3. Results

3.1. Diagnosis

Twelve sea lions received a post-hoc diagnosis of chronic DA toxicosis and one diagnosis of acute DA toxicosis. Again, the diagnoses used in analysis were provided by an experienced veterinarian at final disposition for each subject, taking into account all available clinical information, but blind to study findings. The acute subject was excluded from analysis to focus interpretation on subjects presenting with chronic DA toxicosis. The other 15 sea lions received a variety of diagnoses but had no neurological signs save for one subject with restricted cerebellar lesions attributed to pneumocerebellar disease (see Van Bonn et al., 2011). Reliable MRI data could not be obtained from two other subjects due to motion during scanning that produced artifacts interfering with image quality, and behavioral data from one of these subjects was compromised by equipment failure during testing—this subject was completely excluded from analysis. Thus the final sample of subjects for behavioral analysis was 27, for MRI analysis 26, and for combined behavioral and brain analysis, 25.

3.2. Reliability of behavioral and neurological findings

Behavioral and MRI measures were highly reliable between independent raters. Interobserver reliability between the two independent coders of behavioral orienting response data indicated reliable criteria and implementation for determining response (Kappa + SE = 0.66 + 0.22, 83.2% agreement). Intra-class correlations between the two tracers for volumetric measurements of the hippocampus from MRI showed high reliability in tracing criteria as in Cook et al. (2015) (Right hippocampus: $r = 0.838$, $P < 0.0001$, Left hippocampus: $r = 0.904$, $P < 0.0001$).

3.3. Validity of findings given rehabilitation population

DA and control groups were not optimally balanced to control for effects of age and sex. We therefore assessed potential effects of these factors on behavioral response in the control group. Neither age nor sex predicted phase 1 and 2 responsiveness in a MANOVA (Age: $F_6 = 1.60$, $P = 0.20$; Sex: $F_2 = 1.98$, $P = 0.20$). This is consistent with a prior auditory response task with DA and control sea lions in a rehabilitation setting, where age and sex were not predictors of performance (Cook et al., 2011). This suggests that neurobehavioral differences between the control and DA group are likely not attributable to biases in subject selection.

3.4. DA status and hippocampal atrophy

To determine whether our measure of hippocampal volume was related to assigned DA status, we assessed relationships between total hippocampal volume and diagnosis and between regional hippocampal volume and diagnosis. Subjects with DA toxicosis had lower relative total hippocampal volume compared to control subjects, as found previously in Cook et al., 2015, and consistent with extensive prior evidence demonstrating hippocampal atrophy in animals with DA exposure (Goldstein et al., 2008; Montie et al., 2012; Silvagni et al., 2005) (Fig. 3). Although relative hippocampal volume may vary aside from DA status, this finding suggests that lower hippocampal volumes in the current study are indicative of damage from DA toxicosis. There was no regional specificity of hippocampal volume between DA and control subjects, showing a pattern of variable damage in all four regions of interest for the study.

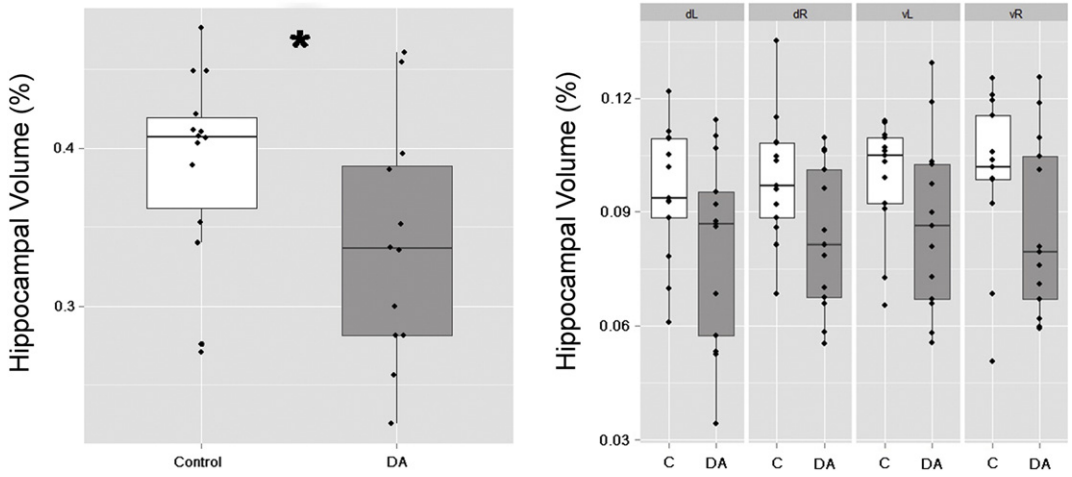

Fig. 3. Reduced hippocampal volume in sea lions with DA toxicosis. Total (summing left and right volume, left panel) and regional (right panel) hippocampal volumes as percentage of total brain volume are shown for control subjects and those with DA toxicosis. Plots show mean, 25th and 75th percentile within each box, with whiskers extending to the highest and lowest values within 1.5 * the inter-quartile range. Total hippocampal volume was lower in DA (0.339%) than control (0.393%) subjects (*t* test: $t_{24} = 1.93$, $P = 0.03$, one-tailed, Cohen's d = 0.88). There was no significant interaction between diagnosis and regional hippocampal volume (ANOVA: $F_3 = 0.69$, $P = 0.56$). C = control subjects, DA = subjects with DA toxicosis, dL = dorsal left hippocampus, dR = dorsal right hippocampus, vL = ventral left hippocampus, vR = ventral right hippocampus.

Although hippocampal atrophy is reliably associated with DA toxicosis and was observed in the current study, the hippocampus is not the only brain region affected by DA toxicosis. An experienced veterinary radiologist did note a range of other neurological signs in the DA subjects, including increased signal intensity and signs of inflammation in cortical regions. However, aside from hippocampal changes, there was no consistent sign of neuropathology across the majority of subjects. Areas of particular interest in regards to auditory responsiveness may be brain regions subserving auditory and vibrissal sensory processing. There were no gross signs of damage to the trigeminal or vestibulocochlear nerves or consistent signs of lesions in dorsal temporal or trigeminal cortex in any of the subjects.

3.5. DA status and behavioral performance

To determine whether subjects with DA toxicosis had altered patterns of habituation and dishabituation, and whether these might be useful for diagnosis, we compared number of responses in phase 1 and phase 2, and responses to novel versus familiar locations in phase 2, between DA and control sea lions. DA status was a strong predictor of delayed habituation in phase 1 (Fig. 4), with DA subjects showing nearly double the responsiveness. In addition, an ROC analysis of phase 1 responsiveness against post-hoc diagnosis suggested that phase 1 responsiveness was a useful diagnostic measure of chronic DA toxicosis (Fig. 5). Together, this constitutes a robust replication of the findings in Cook et al. (2011). DA status was also a marginally significant predictor of enhanced dishabituation in phase 2 (Fig. 4), linking a diagnosis of DA toxicosis in the present study with hippocampal atrophy, decreased habituation, and increased dishabituation.

3.6. Hippocampal volume and behavioral performance

Next we assessed potential relationships between hippocampal volume—indicative of extent of DA-related hippocampal atrophy—and the behavioral measures. Although DA status predicted hippocampal atrophy and phase 1 responsiveness, neither total hippocampal volume nor regional hippocampal volumes predicted habituation rate in phase 1 or relative response to the novel versus familiar sound source location in phase 2. However, while total hippocampal volume did not predict phase 2 responsivness, ventral right hippocampal volume *did* predict total phase 2 responsiveness (Fig. 6). Smaller ventral hippocampal volume was correlated with increased responsiveness when controlling for phase 1 responsiveness, indicating that ventral right hippocampal atrophy is linked with enhanced dishabituation.

4. Discussion

We found that wild sea lions with chronic DA toxicosis had hippocampal lesions, delayed habituation of behavioral orienting to a repeated auditory cue and enhanced dishabituation of behavioral orienting to that same cue following a brief recovery period. In addition, extent of right ventral hippocampal atrophy predicted enhancement of dishabituation following the short delay. Dissociable features of DA toxicosis may delay habituation and enhance dishabituation in sea lions. ROC analysis indicated that a simple responsiveness measure has diagnostic efficacy, replicating and extending prior research with wild sea lions (Cook et al., 2011). These findings demonstrate a clinically relevant pattern of perseverative behavior in sea lions with DA toxicosis that may directly affect survival in the wild.

4.1. Study validity

Because this study used wild animals in a rehabilitation setting, it was impossible to control for DA dose and to fully control for secondary health factors that may have affected habituation and dishabituation. However, the results have the benefit of ecological validity, being measured directly in the species of interest. Wild sea lions with DA toxicosis present with a range of possible neurological signs in addition to specific hippocampal lesions. These may affect behavior as well. However, these signs have not been consistently localized across subjects in prior research or the current study. DA subjects in the current study showed no consistent extra-hippocampal neurological signs that could easily explain altered habituation and dishabituation behavior.

Also of note, although DA status has been repeatedly and reliably linked with hippocampal atrophy in sea lions both by histology and volumetry (Cook et al., 2015; Goldstein et al., 2008; Montie et al., 2012; Silvagni et al., 2005), and there was an apparent statistical relationship between relative hippocampal volume and DA status in the current study, relative hippocampal volume is not a direct analog for hippocampal atrophy. Hippocampal volume will vary between animals for a wide range of reasons aside from specific insult from DA. Therefore, relationships between behavioral impairment and hippocampal volume in the current study may be only partly indicative of the specific effects of DA-related atrophy.

4.2. Negative finding for memory impairment

Despite evidence of impaired discrimination of spatial novelty with hippocampal damage in rodents (Eacott and Norman, 2004), and despite our prior findings of impaired spatial memory correlated with

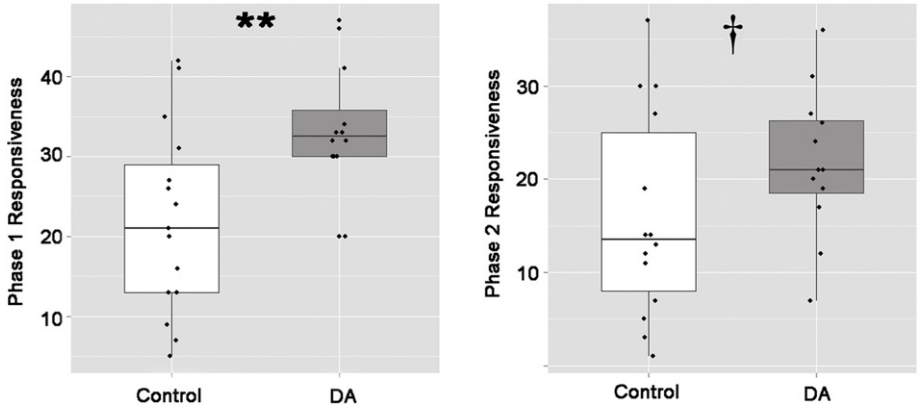

Fig. 4. Diagnosis and behavioral response plots show responsiveness during phase 1 between subjects diagnosed with DA toxicosis and controls. Total measured orienting responses for each subject to the 54 stimulus presentations are shown on the y axis. Boxes show mean, 25th and 75th percentile data, with whiskers extending to the highest and lowest values within 1.5 * the inter-quartile range. (Left panel) In phase 1, DA subjects were far more responsive than controls indicating delayed habituation (*t* test: $t_{25} = 3.06$, $P = 0.005$, two-tailed, Cohen's $d = 1.17$, Fig. 4, Table S2). (Right panel) Responsiveness in Phase 2 was also greater in DA subjects than controls when correcting for total Phase 1 responsiveness, albeit with marginal significance, indicating enhanced dishabituation (ANCOVA: $F_{23} = 3.97$, $P = 0.06$, two-tailed, eta squared = 0.09). ** = $P < 0.01$; dagger = $P < 0.1$.

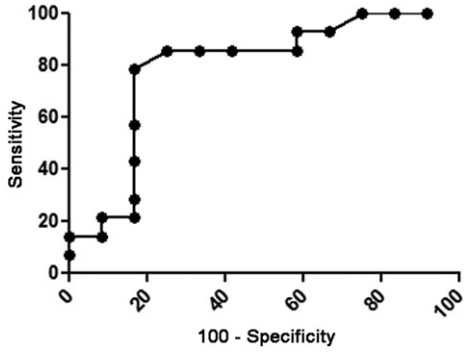

Fig. 5. Diagnostic efficacy of Phase 1 responsiveness. ROC curve shows diagnostic efficacy of measures of total responsiveness in phase 1. Sensitivity (proportion correct positive diagnoses) is on the y axis versus 100 – specificity (proportion of incorrect positive diagnoses) on the x axis, and points on the curve represent responsiveness thresholds for diagnosis, beginning with higher thresholds to the left and progressing to lower thresholds to the right. An optimal diagnostic test has 100% sensitivity (no missed positive diagnoses) with 100% specificity (no false positive diagnoses). With the present data, a correct positive diagnosis rate of 79% would be achievable with a false positive diagnosis rate of 29% at a response threshold of 28 (area under the curve = 0.735, $P < 0.05$, two-tailed, Table S4).

hippocampal damage in sea lions with DA toxicosis (Cook et al., 2015), hippocampal volume did not predict relative response to the novel versus familiar sound source for the sea lions in the current study. This may be attributable to stimulus modality—most laboratory studies have used exploration of objects, not passive presentation of auditory stimuli, to assess sensitivity to spatial novelty. Notably, because the salience of an auditory stimulus varies strongly with proximity to sound source, relative response to the two sound sources during phase 2 of the current experiment may have been confounded by variability in subject location during stimulus presentations. Subjects may have been more likely to respond to a proximal speaker than a distal one, irrespective of which location was novel and which familiar. Future examination of relative responsiveness to sound location in this population will need to take potential positional biases into account.

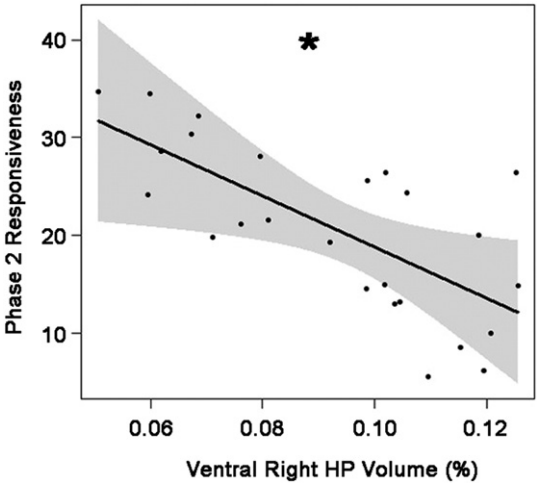

Fig. 6. Ventral hippocampal volume predicts dishabituation. Mean responsiveness in phase 2 is plotted conditionally (i.e., after regressing out the other independent variables, in this case volumes of the other hippocampal regions and phase 1 responsiveness) against ventral right hippocampal volume as a percentage of total brain volume. A multivariate regression model including dorsal and ventral right and left hippocampal volumes as independent variables, with phase 2 responsiveness as the dependent variable and phase 1 responsiveness as a nuisance variable, was a strong predictor of phase 2 responsiveness ($F_{19} = 6.75$, $R^2 = 0.64$, $P < 0.001$, two-tailed) and ventral right hippocampal volume predicted phase 2 responsiveness ($t_{19} = -2.5$, $P = 0.02$, two-tailed, Cohen's $f^2 = 0.22$). Total hippocampal volume did not predict phase 1 responsiveness, phase 2 responsiveness with phase 1 responsiveness as a nuisance variable, nor relative response to novel versus familiar location in phase 2 (Phase 1: $F_{24} = 1.79$, $R2 = 0.09$, $P = 0.09$; Phase 2: $t_{22} = 0.91$, $P = 0.37$; Novel versus familiar in Phase 2: $t_{22} = 0.64$, $P = 0.53$). Regional hippocampal volumes did not predict responsiveness during phase 1 (not pictured)($F_{21} = 0.80$, $R^2 = 0.13$, $P = 0.54$, two-tailed). Nor did regional hippocampal volumes predict relative novel to familiar responsiveness in phase 2 (not pictured) ($F_{20} = 0.76$, $R^2 = 0.13$, $P = 0.56$, two-tailed). * = $P < 0.05$.

4.3. Dissociation of habituation and dishabituation

DA status was associated with reduced hippocampal volume, decreased habituation and enhanced dishabituation. However, hippocampal volume predicted only dishabituation, not habituation. This could indicate a dissociation between different features of DA toxicosis and different behavioral sequalae. A neurobehavioral dissociation of habituation and dishabituation is consistent with the general literature on these phenomena (Carew et al., 1971; Groves and Thompson, 1970; Rankin et al., 2009; Thompson and Spencer, 1966). Because extent of hippocampal damage did not predict extent of decreased habituation in the current study featuring subjects with chronic DA toxicosis, nor in a previous study featuring subjects with acute DA toxicosis (Cook et al., 2011), decreased habituation is likely driven by some feature of DA toxicosis unrelated to disease progression or severity. One candidate is insult to the dentate gyrus that may occur during initial exposure to the toxin.

4.3.1. Dentate gyrus and habituation

Cells in the dentate gyrus are primary targets of DA, and animals show death of hilar neurons in this region following initial neurotoxic exposure (Pulido, 2008). Of note, the sea lion dentate gyrus is thinner than that observed in primates and rodents (Buckmaster et al., 2014), and sea lions with DA toxicosis show markedly more cell loss in dentate gyrus than is typically observed in exposed laboratory rodents (Silvagni et al., 2005). Dentate disruption has been implicated in delayed habituation seen in laboratory rats exposed to DA (Zuloaga et al., 2016), and targeted disruption of dentate gyrus function interferes with sensorimotor habituation in rats (Caine et al., 1991). In a normally functioning brain, the dentate gyrus is believed to act as a sensory filter between entorhinal cortex and the CA portion of the hippocampus (Hsu, 2007), and is implicated in the function of hippocampal networks that drive attentional processes and behavioral orienting responses (Fanselow and Dong, 2010; Friedman et al., 2009; Monaco et al., 2014; Sokolov, 1990). Evidence of the role the dentate gyrus may play in habituation in a healthy brain is supported by studies using cellular recording and in vitro methods. The dentate shows progressive response decrement with repeated stimulation in a pattern consistent with behavioral habituation (Krug et al., 1989; Mays and Best, 1975; Rausche et al., 1989; Teyler and Alger, 1976). Importantly, this property is not consistent across hippocampal subregions – CA does not show the same pattern of response decrement in parallel studies. Together, these observations suggest that initial insult and cell death in the dentate gyrus from the acute effects of DA toxicosis may be enough to significantly disrupt habituation of behavioral orienting responses in affected sea lions, prior to manifestation of gross hippocampal atrophy driven by epilepsy.

Of note, epilepsy has also been shown to alter responsiveness and habituation in humans (Trent et al., 2013). All DA subjects in the current study were believed to have chronic epilepsy, but we had no way to quantify severity of epileptic disease for comparison with habituation and dishabituation.

4.3.2. Hippocampal volume and dishabituation

While hippocampal volume did not predict altered habituation in the current study, ventral right hippocampal volume was correlated with enhanced dishabituation such that animals with greater ventral hippocampal atrophy showed increased phase 2 responsiveness. Laboratory experiments with DA exposed animals have not focused on specific deficits potentially related to ventral hippocampal damage. However, general neurobehavioral studies using lesions and cellular

recording have attempted to determine the specific function of ventral hippocampus. The ventral hippocampus contributes to response inhibition (Bickford-Wimer et al., 1990; Chudasama et al., 2012; Daenen et al., 2003), and though hippocampal damage to the dorsal or ventral portion does not reliably impair behavioral habituation (Gray and McNaughton, 1983; Kant et al., 1984; Köhler, 1976; Leaton, 1981; Save et al., 1992), disruption of ventral hippocampal function can lead to hypersensitivity and hyperactivity (Bast et al., 2001; Chambers and Self, 2002; Clark et al., 1992; Daenen et al., 2001; Kamback, 1967; Sams-Dodd et al., 1997; Sanwald et al., 1970). Both hypersensitivity and hyperactivity are associated with dishabituation in the behavioral literature (Hochner et al., 1986). While some evidence indicates that dishabituation and sensitization are themselves dissociable, both processes are independent of habituation (Marcus et al., 1988). In the present study, it is possible that ventral hippocampal disruption related to DA led to disinhibition of attentional behavior or sensory hypersensitivity, which then contributed to enhanced behavioral dishabituation in phase 2.

4.4. Lateralization

The right lateralization of brain atrophy related to dishabituation in the current study is also of interest. Lateralization of hippocampal function has been shown clearly in humans (Burgess et al., 2002), predominately related to memory performance. Hippocampal lateralization of memory functions has also been shown in some birds (Bingman and Gagliardo, 2006; Jonckers et al., 2015), but has been debated in rodents (Spasojevic et al., 2013; Fenton and Bures, 1993). There is evidence for lateralization of other functions in the rodent brain (Costa et al., 2016). We previously found evidence for right lateralization of hippocampal memory processes in wild sea lions (Cook et al., 2015), and sea lions do show evidence of behavioral lateralization (Böye et al., 2005; Wells et al., 2006), as well as some hemispheric lateralization during sleep (Lyamin et al., 2008; Lyamin and Chetyrbok, 1992). This evidence suggests a general propensity to lateralization of neurobehavioral function in sea lions that may explain the predictive relationship of right hippocampal atrophy to behavioral dishabituation in the current study.

4.5. Diagnosis

In addition to illuminating the neurobiological underpinnings of behavioral change with DA toxicosis, the present findings also have direct clinical application. The finding of delayed habituation in the sea lions with chronic DA toxicosis in the present study is a robust replication of an earlier finding of delayed habituation in sea lions with acute and chronic DA toxicosis (Cook et al., 2011), supporting the reliability of auditory responsiveness as diagnostic. The current procedure was highly efficient and optimized for use in a wildlife rehabilitation setting, employing a fixed presentation schedule and post-hoc behavioral tally of orienting responses. Because delayed habituation does not track with extent of hippocampal atrophy, which is linked with disease progression, a basic auditory habituation assay may effectively identify sea lions with DA toxicosis, irrespective of disease progression.

4.6. Conclusions

Together, delayed habituation and enhanced dishabituation suggest a behavioral pattern of hyperresponsiveness and perseveration that emerges in sea lions with initial insult from DA and may worsen with progressive regional hippocampal damage. This is in line with recent work showing behavioral perseveration as a result of both hippocampal damage and epilepsy in rodents and humans (Abela et al., 2012; Langston et al., 2012; Sakata et al., 2013; Trent et al., 2013). Because sea lions are flexible, central-place foragers who rely on adaptive hunting strategies to meet changing ocean conditions and prey availability (Costa, 1991; Francis et al., 1991; Villegas-Amtmann et al., 2008; Weise et al., 2006), behavioral perseveration emerging with DA toxicosis could interfere with optimal foraging behavior with energetic consequences (Costa et al., 2004; Costa and Gales, 2003). Behavioral perseveration could also have a compounding relationship with previously demonstrated spatial memory deficits in these sea lions (Cook et al., 2015). In addition, the present findings support the use of diagnostic assays relying on behavioral sequelae to neurotoxic exposure.

The ability to conduct controlled neurobehavioral assays on wild animals in a rehabilitation setting represents an ecologically valid parallel approach to laboratory study of domoic acid's damaging effects. While there is a tradeoff with experimental control, wild sea lions are accessible, experimentally tractable, big-brained, long-lived mammals, and as such may serve as ideal models for exploring the effects of environmental ocean toxins, and for predicting their impact on other naturally exposed animals, including humans.

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Transparency document

The Transparency document associated with this article can be found, in online version.

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References

Abela, A.R., Dougherty, S.D., Fagen, E.D., Hill, C.J., Chudasama, Y., 2012. Inhibitory control deficits in rats with ventral hippocampal lesions. Cereb. Cortex bhs121.

Anderson, D.M., Burkholder, J.M., Cochlan, W.P., Glibert, P.M., Gobler, C.J., Heil, C.A., ... Vargo, G.A., 2008. Harmful algal blooms and eutrophication: examining linkages from selected coastal regions of the United States. Harmful Algae 8 (1), 39–53.

Arkhipov, V., Kulesskaja, N., Lebedev, D., 2008. Behavioral perseveration and impairment of long-term memory in rats after intrahippocampal injection of kainic acid in subconvulsive dose. Pharmacol. Biochem. Behav. 88 (3), 299–305.

Bahn, S., Volk, B., Wisden, W., 1994. Kainate receptor gene expression in the developing rat brain. J. Neurosci. 14 (9), 5525–5547.

Bast, T., Zhang, W.N., Feldon, J., 2001. Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABAA receptor antagonist picrotoxin. Psychopharmacology 156 (2–3), 225–233.

Beltrán, A.S., Palafox-Uribe, M., Grajales-Montiel, J., Cruz-Villacorta, A., Ochoa, J.L., 1997. Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. Toxicon 35 (3), 447–453.

Bernabeu, R., Sharp, F.R., 2000. NMDA and AMPA/kainate glutamate receptors modulate dentate neurogenesis and CA3 synapsin-I in normal and ischemic hippocampus. J. Cereb. Blood Flow Metab. 20 (12), 1669–1680.

Bickford-Wimer, P.C., Nagamoto, H., Johnson, R., Adler, L.E., Egan, M., Rose, G.M., Freedman, R., 1990. Auditory sensory gating in hippocampal neurons: a model system in the rat. Biol. Psychiatry 27 (2), 183–192.

Bingman, V.P., Gagliardo, A., 2006. Of birds and men: convergent evolution in hippocampal lateralization and spatial cognition. Cortex 42 (1), 99–100.

Bohbot, V.D., Kalina, M., Stepankova, K., Spackova, N., Petrides, M., Nadel, L.Y.N.N., 1998. Spatial memory deficits in patients with lesions to the right hippocampus and to the right parahippocampal cortex. Neuropsychologia *36* (11), 1217–1238.

Bossart, G.D., 2011. Marine mammals as sentinel species for oceans and human health. Vet. Pathol. Online 48 (3), 676–690.

Böye, M., Güntürkün, O., Vauclair, J., 2005. Right ear advantage for conspecific calls in adults and subadults, but not infant, California sea lions (*Zalophus californianus*): hemispheric specialization for communication? Eur. J. Neurosci. 21 (6), 1727–1732.

Buckmaster, P.S., Wen, X., Toyoda, I., Gulland, F., Van Bonn, W., 2014. Hippocampal neuropathology of domoic acid–induced epilepsy in California sea lions (*Zalophus californianus*). J. Comp. Neurol. 522 (7), 1691–1706.

Burgess, N., Maguire, E.A., O'Keefe, J., 2002. The human hippocampus and spatial and episodic memory. Neuron 35 (4), 625–641.

Caine, S.B., Geyer, M.A., Swerdlow, N.R., 1991. Carbachol infusion into the dentate gyrus disrupts sensorimotor gating of startle in the rat. Psychopharmacology 105 (3), 347–354.

Carew, T.J., Castellucci, V.F., Kandel, E.R., 1971. An analysis of dishabituation and sensitization of the gill-withdrawal reflex in aplysia. Int. J. Neurosci. 2 (2), 79–98.

Chambers, R.A., Self, D.W., 2002. Motivational responses to natural and drug rewards in rats with neonatal ventral hippocampal lesions: an animal model of dual diagnosis schizophrenia. Neuropsychopharmacology 27 (6), 889–905.

Chudasama, Y., Doobay, V.M., Liu, Y., 2012. Hippocampal-prefrontal cortical circuit mediates inhibitory response control in the rat. J. Neurosci. 32 (32), 10915–10924.

Clark, A.J.M., Feldon, J., Rawlins, J.N.P., 1992. Aspiration lesions of rat ventral hippocampus disinhibit responding in conditioned suppression or extinction, but spare latent inhibition and the partial reinforcement extinction effect. Neuroscience 48 (4), 821–829.

Clayton, E.C., Peng, Y.G., Means, L.W., Ramsdell, J.S., 1999. Working memory deficits induced by single but not repeated exposures to domoic acid. Toxicon 37 (7), 1025–1039.

Cohen, J., 1968. Weighted kappa: nominal scale agreement provision for scaled disagreement or partial credit. Psychol. Bull. 70 (4), 213.

Colman, J.R., Nowocin, K.J., Switzer, R.C., Trusk, T.C., Ramsdell, J.S., 2005. Mapping and reconstruction of domoic acid-induced neurodegeneration in the mouse brain. Neurotoxicol. Teratol. 27 (5), 753–767.

Cook, P., Reichmuth, C., Gulland, F., 2011. Rapid behavioral diagnosis of domoic acid toxicosis in California sea lions. Biol. Lett. 7, 536–538.

Cook, P.F., Reichmuth, C., Rouse, A.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 (6267), 1545–1547.

Costa, D.P., 1991. Reproductive and foraging energetics of high latitude penguins, albatrosses and pinnipeds: implications for life history patterns. Am. Zool. 31 (1), 111–130.

Costa, D.P., Gales, N.J., 2003. Energetics of a benthic diver: seasonal foraging ecology of the Australian sea lion, *Neophoca cinerea*. Ecol. Monogr. 73 (1), 27–43.

Costa, D.P., Kuhn, C.E., Weise, M.J., Shaffer, S.A., Arnould, J.P., 2004, December. When Does Physiology Limit the Foraging Behavior of freely Diving Mammals? International Congress Series Vol. 1275. Elsevier, pp. 359–366

Costa, N.S., Vicente, M.A., Cipriano, A.C., Miguel, T.T., Nunes-de-Souza, R.L., 2016. Functional lateralization of the medial prefrontal cortex in the modulation of anxiety in mice: left or right? Neuropharmacology.

Crusio, W.E., Schwegler, H., 1987. Hippocampal mossy fiber distribution covaries with open-field habituation in the mouse. Behav. Brain Res. 26 (2–3), 153–158.

Daenen, E.W.P.M., Van der Heyden, J.A., Kruse, C.G., Wolterink, G., Van Ree, J.M., 2001. Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus. Brain Res. 918 (1), 153–165.

Daenen, E.W., Wolterink, G., Van Der Heyden, J.A., Kruse, C.G., Van Ree, J.M., 2003. Neonatal lesions in the amygdala or ventral hippocampus disrupt prepulse inhibition of the acoustic startle response; implications for an animal model of neurodevelopmental disorders like schizophrenia. Eur. Neuropsychopharm. 13 (3), 187–197.

Dakshinamurti, K., Sharma, S.K., Sundaram, M., 1991. Domoic acid induced seizure activity in rats. Neurosci. Lett. 127 (2), 193–197.

Eacott, M., Norman, G., 2004. Integrated memory for object, place and context in rats: a possible model of object-location and object-context recognition. J. Neurosci. 24, 1948–1953.

Eichenbaum, H., Komorowski, R., MacDonald, C.J., Kraus, B.J., Robitsek, J., 2016. How does the hippocampus support the spatial and temporal attributes of memory? The Neurobiological Basis of Memory. Springer International Publishing, pp. 39–57

Ennaceur, A., Aggleton, J., Fray, P., 1997. Delayed non-matching to sample in a novel automated visual memory apparatus using mixed retention intervals. Neurosci. Res. Commun. 20, 103–111.

Fanselow, M.S., Dong, H.W., 2010. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65 (1), 7–19.

Fenton, A.A., Bures, J., 1993. Place navigation in rats with unilateral tetrodotoxin inactivation of the dorsal hippocampus: place but not procedural learning can be lateralized to one hippocampus. Behav. Neurosci. 107 (4), 552.

Fischer, M., Barkley, R.A., Smallish, L., Fletcher, K., 2005. Executive functioning in hyperactive children as young adults: attention, inhibition, response perseveration, and the impact of comorbidity. Dev. Neuropsychol. 27 (1), 107–133.

Francis, M., Lowry, M.S., Yochem, P.K., Stewart, B.S., Heath, C.B., 1991. Seasonal and annual variability in the diet of California sea lions, *Zalophus californianus*, at San Nicolas Island, California, 1981–86. Fish. Bull. 89, 331–336.

Friedman, D., Goldman, R., Stern, Y., Brown, T., 2009. The brain's orienting response: an event-related functional magnetic resonance imaging investigation. Hum. Brain Mapp. 30, 1144–1154.

Ghazanfar, A., Neuhoff, J., Logothetis, N., 2002. Auditory looming perception in rhesus monkeys. Proc. Natl. Acad. Sci. 99, 15755–15757.

Goldstein, T., Mazet, J., Zabka, T., Langlois, G., Colegrove, K., Silver, M., Bargu, S., Van Dolah, F., Leighfield, T., Conrad, P., Barakos, J., Williams, D., Dennison, S., Haulena, M., Gulland, F., 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. Proc. R. Soc. Biol. Sci. 7, 267–276.

Grant, K.S., Burbacher, T.M., Faustman, E.M., Gratttan, L., 2010. Domoic acid: neurobehavioral consequences of exposure to a prevalent marine biotoxin. Neurotoxicol. Teratol. 32 (2), 132–141.

Gray, J.A., McNaughton, N., 1983. Comparison between the behavioral effects of septal and hippocampal lesions: a review. Neurosci. Biobehav. Rev. 7 (2), 119–188.

Green, J.D., Arduini, A.A., 1954. Hippocampal electrical activity in arousal. J. Neurophysiol.

Greig, D.J., Gulland, F.M., Kreuder, C., 2005. A decade of live California sea lion (*Zalophus californianus*) strandings along the Central California coast: causes and trends, 199–2000. Aquat. Mamm. 31 (1), 11–22.

Groves, P.M., Thompson, R.F., 1970. Habituation: a dual-process theory. Psychol. Rev. 77 (5), 419.

Gulland, F., Haulena, M., Fauquier, D., Langlois, G., Lander, M., Zabka, T., Duerr, R., 2002. Domoic acid toxicity in California sea lions (*Zalophus californianus*): clinical signs, treatment and survival. Vet. Rec. 150, 475–480.

Hochner, B., Klein, M., Schacher, S., Kandel, E.R., 1986. Additional component in the cellular mechanism of presynaptic facilitation contributes to behavioral dishabituation in Aplysia. Proc. Natl. Acad. Sci. 83 (22), 8794–8798.

Hsu, D., 2007. The dentate gyrus as a filter or gate: a look back and a look ahead. Prog. Brain Res. 163, 601–613.

Iverson, F., Truelove, J., Nera, E., Tryphonas, L., Campbell, J., Lok, E., 1988. Domoic acid poisoning and mussel associated intoxication. Preliminary investigations into the response of mice and rats to toxic mussel extract. Food Chem. Toxicol. 27, 377–384.

Jeffery, B., Barlow, T., Moizer, K., Paul, S., Boyle, C., 2004. Amnesic shellfish poison. Food Chem. Toxicol. 42, 545–557.

Johnson, A., van der Meer, M.A., Redish, A.D., 2007. Integrating hippocampus and striatum in decision-making. Curr. Opin. Neurobiol. 17 (6), 692–697.

Jonckers, E., Güntürkün, O., De Groof, G., Van der Linden, A., Bingman, V.P., 2015. Network structure of functional hippocampal lateralization in birds. Hippocampus 25 (11), 1418–1428.

Kamback, M., 1967. Effect of hippocampal lesions and food deprivation on response for stimulus change. J. Comp. Physiol. Psychol. 63, 231–235.

Kant, G.J., Meyerhoff, J.L., Jarrard, L.E., 1984. Biochemical indices of reactivity and habituation in rats with hippocampal lesions. Pharmacol. Biochem. Behav. 20 (5), 793–797.

Kimble, D.P., Kimble, R.J., 1965. Hippocampectomy and response perseveration in the rat. J. Comp. Physiol. Psychol. 60 (3), 474.

Köhler, C., 1976. Habituation after dorsal hippocampal lesions: a test dependent phenomenon. Behav. Biol. 18 (1), 89–110.

Krug, M., Wagner, M., Brödemann, R., Matthies, H., 1989. Habituation of monosynaptic field potentials in the dentate gyrus of freely moving rats interferes with LTP. Brain Res. 476 (1), 163–166.

Kvitek, R.G., Goldberg, J.D., Smith, G.J., Doucette, G.J., Silver, M.W., 2008. Domoic acid contamination within eight representative species from the benthic food web of Monterey Bay, California, USA. Mar. Ecol. Prog. Ser. 367, 35–47.

Langston, J.L., Wright, L.K., Connis, N., Lumley, L.A., 2012. Characterizing the behavioral effects of nerve agent-induced seizure activity in rats: increased startle reactivity and perseverative behavior. Pharmacol. Biochem. Behav. 100 (3), 382–391.

Leaton, R.N., 1981. Habituation of startle response, lick suppression, and exploratory behavior in rats with hippocampal lesions. J. Comp. Physiol. Psychol. 95 (5), 813.

LeDoux, J., 2003. Emotion Circuits in the Brain.

Lefebvre, K.A., Robertson, A., 2010. Domoic acid and human exposure risks: a review. Toxicon 56 (2), 218–230.

Lefebvre, K.A., Powell, C.L., Busman, M., Doucette, G.J., Moeller, P.D., Silver, J.B., ... Tjeerdema, R.S., 1999. Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. Nat. Toxins 7 (3), 85–92.

Lefebvre, K.A., Bargu, S., Kieckhefer, T., Silver, M.W., 2002. From sanddabs to blue whales: the pervasiveness of domoic acid. Toxicon 40 (7), 971–977.

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.

Lyamin, O.I., Chetyrbok, I.S., 1992. Unilateral EEG activation during sleep in the Cape fur seal, *Arctocehalus pusillus*. Neurosci. Lett. 143 (1), 263–266.

Lyamin, O.I., Manger, P.R., Ridgway, S.H., Mukhametov, L.M., Siegel, J.M., 2008. Cetacean sleep: an unusual form of mammalian sleep. Neurosci. Biobehav. Rev. 32 (8), 1451–1484.

Marcus, E.A., Nolen, T.G., Rankin, C.H., Carew, T.J., 1988. Behavioral dissociation of dishabituation, sensitization, and inhibition in Aplysia. Science 241 (4862), 210–213.

Mays, L.E., Best, P.J., 1975. Hippocampal unit activity to tonal stimuli during arousal from sleep and in awake rats. Exp. Neurol. 47 (2), 268–279.

Metz, C.E., 1978, October. Basic principles of ROC analysis. Seminars in Nuclear Medicine Vol. 8, No. 4 pp. 283–298.

Monaco, J.D., Rao, G., Roth, E.D., Knierim, J.J., 2014. Attentive scanning behavior drives one-trial potentiation of hippocampal place fields. Nat. Neurosci. 17 (5), 725–731.

Montie, E., Pussini, N., Schneider, G., Battey, T., Dennison, S., Barakos, J., Gulland, F., 2009. Neuroanatomy and volumes of brain structures of a live California sea lion (*Zalophus californianus*) from magnetic resonance imaging. Anat. Rec. Adv. Integr. Anat. Evol. Biol. 292, 1523–1547.

Montie, E., Wheeler, E., Pussini, N., Battey, T., Van Bonn, W., Gulland, F., 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.

Moser, M.B., Moser, E.I., 1998. Functional differentiation in the hippocampus. Hippocampus 8 (6), 608–619.

Muha, N., Ramsdell, J.S., 2011. Domoic acid induced seizures progress to a chronic state of epilepsy in rats. Toxicon 57 (1), 168–171.

Mumby, D., Gaskin, S., Glenn, S., Schramek, T., Lehmann, H., 2002. Hippocampal damage and exploratory preferences in rats: memory for objects, places and contexts. Learn. Mem. 9, 49–57.

Neely, B., Soper, J., Greig, D., Carlin, K., Favre, E., Gulland, F., Almeida, J., Janech, M., 2012. Serum profiling by MALDI-TOF mass spectrometry as a diagnostic tool for domoic acid toxicosis in California sea lions. Proteome Sci. 10. http://dx.doi.org/10.1186/1477-5956-10-18.

Neely, B.A., Ferrante, J.A., Chaves, J.M., Soper, J.L., Almeida, J.S., Arthur, J.M., ... Janech, M.G., 2015. Proteomic analysis of plasma from California sea lions (*Zalophus californianus*) reveals Apolipoprotein E as a candidate biomarker of chronic domoic acid toxicosis. PLoS One 10 (4), e0123295. http://dx.doi.org/10.1371/journal.pone.0123295.

Petrie, B.F., Pinsky, C., Standish, N.M., Bose, R., Glavin, G.B., 1992. Parenteral domoic acid impairs spatial learning in mice. Pharmacol. Biochem. Behav. 41 (1), 211–214.

Pribram, K.H., McGuinness, D., 1975. Arousal, activation, and effort in the control of attention. Psychol. Rev. 82 (2), 116.

Pulido, O.M., 2008. Domoic acid toxicologic pathology: a review. Mar. Drugs 6 (2), 180–219.

Ramsdell, J.S., Gulland, F.M., 2014. Domoic acid epileptic disease. Mar. Drugs 12 (3), 1185–1207.

Rankin, C.H., Abrams, T., Barry, R.J., Bhatnagar, S., Clayton, D.F., Colombo, J., ... Thompson, R.F., 2009. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. Neurobiol. Learn. Mem. 92 (2), 135–138.

Rausche, G., Sarvey, J.M., Heinemann, U., 1989. Slow synaptic inhibition in relation to frequency habituation in dentate granule cells of rat hippocampal slices. Exp. Brain Res. 78 (2), 233–242.

Reddy, L.M., Dierauf, L.A., Gulland, F.M., 2001. Marine mammals as sentinels of ocean health. CRC Handbook of Marine Mammal Medicine: Health, Disease and Rehabilitation, pp. 3–13.

Reichmuth, C., Holt, M.M., Mulsow, J., Sills, J.M., Southall, B.L., 2013. Comparative assessment of amphibious hearing in pinnipeds. J. Comp. Phys. A 199 (6), 491–507.

Ritter, L.M., Unis, A.S., Meador-Woodruff, J.H., 2001. Ontogeny of ionotropic glutamate receptor expression in human fetal brain. Dev. Brain Res. 127 (2), 123–133. http://dx.doi.org/10.1016/s0165-3806(01)00126-2.

Sakata, K., Martinowich, K., Woo, N.H., Schloesser, R.J., Jimenez, D.V., Ji, Y., ... Lu, B., 2013. Role of activity-dependent BDNF expression in hippocampal–prefrontal cortical regulation of behavioral perseverance. Proc. Natl. Acad. Sci. 110 (37), 15103–15108.

Sams-Dodd, F., Lipska, B.K., Weinberger, D.R., 1997. Neonatal lesions of the rat ventral hippocampus result in hyperlocomotion and deficits in social behavior in adulthood. Psychopharmacology 132 (3), 303–310.

Sandson, J., Albert, M.L., 1987. Perseveration in behavioral neurology. Neurology 37 (11), 1736.

Sanwald, J.C., Porzio, N.R., Deane, G.E., Donovick, P.J., 1970. The effects of septal and dorsal hippocampal lesions on the cardiac component of the orienting response. Physiol. Behav. 5 (8), 883–888.

Save, E., Poucet, B., Foreman, N., Buhot, M.C., 1992. Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. Behav. Neurosci. 106 (3), 447.

Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., ... Haulena, M., 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403 (6765), 80–84.

Schusterman, R., 1974. Auditory sensitivity of a California sea lion to airborne sound. J. Acoust. Soc. Am. 56, 1248–1251.

Shrout, P.E., Fleiss, J.L., 1979. Intraclass correlations: uses in assessing rater reliability. Psychol. Bull. 86 (2), 420.

Silvagni, P., Lowenstine, L., Spraker, T., Lipscomb, T., Gulland, F., 2005. Pathology of domoic acid toxicity in CSLs (*Zalophus californianus*). Vet. Pathol. 42, 184–191.

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. Proc. Natl. Acad. Sci. 107 (48), 20762–20767.

Sokolov, E., 1990. The orienting response, and future directions of its development. Integr. Psychol. Behav. 25, 142–150.

Spasojevic, N., Jovanovic, P., Dronjak, S., 2013. Molecular basis of chronic stress-induced hippocampal lateral asymmetry in rats and impact on learning and memory. Acta Physiol. Hung. 100 (4), 388–394.

Squire, L.R., 1992. Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. Psychol. Rev. 99 (2), 195.

Sutherland, R.J., Hoesing, J.M., Whishaw, I.Q., 1990. Domoic acid, an environmental toxin, produces hippocampal damage and severe memory impairment. Neurosci. Lett. 120 (2), 221–223.

Teyler, T.J., Alger, B.E., 1976. Monosynaptic habituation in the vertebrate forebrain: the dentate gyrus examined in vitro. Brain Res. 115 (3), 413–425.

Thompson, R.F., Spencer, W.A., 1966. Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol. Rev. 73 (1), 16.

Trent, S., Dean, R., Veit, B., Cassano, T., Bedse, G., Ojarikre, O.A., ... Davies, W., 2013. Biological mechanisms associated with increased perseveration and hyperactivity in a genetic mouse model of neurodevelopmental disorder. Psychoneuroendocrinology 38 (8), 1370–1380.

Truelove, J., Iverson, F., 1994. Serum domoic acid clearance and clinical observations in the cynomolgus monkey and Sprague-Dawley rat following a single IV dose. Bull. Environ. Contam. Toxicol. 52, 479–486.

Tryphonas, L., Truelove, J., Iverson, F., 1990. Acute parenteral neurotoxicity of domoic acid in cynomolgus monkeys (*M. fascicularis*). Toxicol. Pathol. 18, 297–303.

Van Bonn, W., Montie, E., Dennison, S., Pussini, N., Cook, P., Greig, D., ... Gulland, F., 2011. Evidence of injury caused by gas bubbles in a live marine mammal: barotrauma in a California sea lion *Zalophus californianus*. Dis. Aquat. Org. 96 (2), 89–96.

Villegas-Amtmann, S., Costa, D.P., Tremblay, Y., Salazar, S., Aurioles-Gamboa, D., 2008. Multiple foraging strategies in a marine apex predator, the Galapagos sea lion *Zalophus wollebaeki*. Mar. Ecol. Prog. Ser. 363, 299–309.

Weise, M.J., Costa, D.P., Kudela, R.M., 2006. Movement and diving behavior of male California sea lion (*Zalophus californianus*) during anomalous oceanographic conditions of 2005 compared to those of 2004. Geophys. Res. Lett. 33 (22).

Wells, D.L., Irwin, R.M., Hepper, P.G., 2006. Lateralised swimming behavior in the California sea lion. Behav. Process. 73 (1), 121–123.

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 Biol.

Yamaguchi, S., Hale, L.A., D'esposito, M., Knight, R.T., 2004. Rapid prefrontal-hippocampal habituation to novel events. J. Neurosci. 24 (23), 5356–5363.

Zuloaga, D.G., Lahvis, G.P., Mills, B., Pearce, H.L., Turner, J., Raber, J., 2016. Fetal domoic acid exposure affects lateral amygdala neurons, diminishes social investigation and alters sensory-motor gating. Neurotoxicology.