Domoic acid-induced seizures in California sea lions (Zalophus californianus) are associated with neuroinflammatory brain injury\textsuperscript{*}

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\textbf{A B S T R A C T}

California sea lions (CSLs) exposed to the marine biotoxin domoic acid (DA) develop an acute or chronic toxicosis marked by seizures and act as sentinels of the disease. Experimental evidence suggests that oxidative stress and neuroinflammation are important mechanisms underlying the seizurogenic potential of environmental toxins but these pathways are relatively unstudied in CSLs. In the current study, we investigated the role of glutamate–glutamine changes and gliosis in DA-exposed CSLs to better understand the neurotoxic mechanisms occurring during DA toxicity. Sections from archived hippocampi from control and CSLs diagnosed with DA toxicosis were immunofluorescently stained for markers of gliosis, oxidative/nitrative stress and changes in glutamine synthetase (GS). Quantitative assessment revealed increasing loss of microtubule associated protein–2 positive neurons with elevations in 4-hydroxynonenal correlating with chronicity of exposure, whereas the pattern of activated glia expressing nitric oxide synthase 2 and tumor necrosis factor followed pathological severity. There was no significant change in the amount of GS positive cells but there was increased 3-nitrotyrosine in GS expressing cells and in neurons, particularly in animals with chronic DA toxicosis. These changes were consistently seen in the dentate gyrus and in the cornu ammonis (CA) sectors CA3, CA4, and CA1. The results of this study indicate that gliosis and resultant changes in GS are likely important mechanisms in DA-induced seizure that need to be further explored as potential therapies in treating exposed wildlife.

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1. Introduction

Exposure to the marine biotoxin domoic acid (DA) during harmful algal blooms (HABs) results in a syndrome known as domoic acid toxicosis marked by reproductive failure, cardiotoxicity, and most prominently neurological dysfunction (Gulland et al., 2002; Scholin et al., 2000). The California sea lion (Zalophus californianus; CSL) is the most commonly affected species with hundreds of animals standing and/or dying each year. CSLs act as sentinels of this disease and are used for predicting potential human health threats associated with deteriorating ocean conditions (Bossart, 2011; Lefebvre and Robertson, 2010). HABs and domoic acid producing diatom blooms are increasing in frequency worldwide, possibly due to agricultural runoff and climate change, thus posing an increasing threat to wildlife and human health and iterating the need to better understand how these toxins cause disease (Bossart, 2011; Erdner et al., 2008; Lefebvre and Robertson, 2010).

Exposure to DA in CSLs occurs primarily through ingestion of contaminated food sources (Lefebvre et al., 1999; Scholin et al., 2000). Once absorbed, DA acts as an excitotoxin binding to kainate/alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) glutamate receptors causing activation of neurons, which leads to seizurogenic excitations and neuronal injury in hippocampal and limbic structures (Dakshinamurti et al., 1993; Sutherland et al., 1990). The most common clinical sign in DA-exposed sea lions is the development of persistent seizures resulting in the development of an acute
toxicosis or a chronic epileptic syndrome, similar to human temporal lobe epilepsy (TLE) (Scholin et al., 2000; Silvagni et al., 2005). A significant percentage of animals diagnosed with acute toxicosis often progress to the chronic syndrome even with symptomatic in treatment or restraint as their condition worsens (Goldstein et al., 2007; Thomas and Harvey, 2010). Additionally, research in rodents indicates that prenatal or low-dose exposure to DA can have long term effects on behavior without causing overt neuronal death in the hippocampus (Pérez-Gómez and Tasker, 2013; Schwarz et al., 2014). Therefore, direct toxicity and/or seizure-induced neuronal death is insufficient to explain the progression of disease in CSLS or the cause of behavior changes in rodents, as the condition worsens even in the absence of continued exposure or when seizure is pharmacologically prevented.

These limitations in treating DA-induced seizure in CSLS parallels the difficulty in treating intractable seizure in nearly one third of human epileptic patients (Eid et al., 2008; Waldbaum and Patel, 2010). Most modern anti-epileptic only treat neuronal excitability but do not address other pathophysiological events that lead to a progressive increase in seizure frequency. Studies in rodent seizure models and human TLE have consistently identified changes in neuronal transmitters. Specifically, studies have identified alterations in the glutamate-glutamine cycle with increased extracellular levels of the excitatory neurotransmitter glutamate and decreased synthesis of the inhibitory neurotransmitter gamma-aminobutyric acid GABA; (Cavus et al., 2005; Ortinski et al., 2010). These changes are a consequence of decreased astrocytic glutamate synthase (GS) expression and activity (EID et al., 2004; Petroff et al., 2002) caused by high levels of reactive oxygen/nitrogen species and inflammatory factors that adversely affect GS (Bidmon et al., 2008; Görg et al., 2007).

Activated glia are the primary source of pro-inflammatory and pro-oxidant factors (Vezzani et al., 2011) and can be seen prior to recurrent seizures in experimental models of seizures (Losi et al., 2012; Scallet et al., 2005) and in human patients with TLE (Aronica and Cino, 2011). Production of reactive oxygen species (ROS) and resultant oxidative stress is a central mechanism in DA-induced excitotoxic cell death (Dakshinamurti et al., 1993) and is thought to underly seizure-induced neuronal loss (Devì et al., 2008; Waldbaum and Patel, 2010). Blockade of glial activation or inhibition of neuroinflammatory factors such as nitric oxide synthase 2 (NOS2) and interleukin-1 Beta (IL-1β) protects against neuronal loss and prevents or reduces the magnitude or frequency of experimentally induced seizures (Ananth et al., 2003; Cho et al., 2008; Chuang et al., 2010). These data collectively support a role for neuroinflammation in epileptogenesis; however, the mechanisms by which activated glia contribute to the etiology of DA toxicosis are not well understood.

In the current study, we investigated the role of glutamate-glutamine changes and glicosis in acute and chronic DA-exposed CSLS to better understand the neurotoxic mechanisms occurring during DA toxicity. To explore this objective, we examined brains from DA-exposed sea lions, postulating that the extent of neuronal injury would correlate with loss of GS and activation of astrocytes and microglia.

### 2. Materials and methods

#### 2.1. Source and selection of animals

This study utilized archived formalin-fixed, paraffin-embedded sea lion tissues collected by The Marine Mammal Center (MMC) in Sausalito, CA from animals that died or were humanely euthanized during veterinary care between 2000 and 2011 (Table 1) and sent to Colorado State University Diagnostic center as diagnostic samples as described in Madl et al. (2013). Animals were classified as DA intoxicated (n = 12) based on positive neurological symptoms recorded in the clinical histories and/or presence of DA consistent pathological lesions (Scholin et al., 2000; Silvagni et al., 2005). Control animals (n = 8) died or were euthanized for unrelated causes and lacked any clinical symptomology or histopathology typical of DA exposure. When available, cases were further classified based on levels of DA measured in urine by direct competitive DA enzyme-linked immunosorbent assay (ELISA) or feces by high-performance liquid chromatography (Table 1; Gulland et al., 2012; Madl et al., 2013).

DA cases were further subdivided based on chronicity (acute versus chronic) and severity of lesion as normal (n = 10), moderate (n = 5) or severe (n = 5). Acute cases (n = 5) were animals that straddled during or near a bloom in clusters, had clinical signs of ataxia, head weaving, and seizures, and had hippocampal necrosis (Scholin et al., 2000; Gulland et al., 2002; Goldstein et al., 2008). Chronic CSLS (n = 7) were animals that stranded alone, had intermittent seizures at least two weeks apart, abnormal behaviors, and had chronic pathologic changes such as hippocampal atrophy and glicosis. Severity of DA induced lesions was determined by blind evaluation of hematoxylin and eosin stained sections by board certified veterinary pathologists for extent of neuronal loss in the hippocampus.

#### 2.2. Immunofluorescence

Representative paraffin-embedded 5 µm coronal sections from the hippocampus were immunolabeled with microtubule associated protein-2 (MAP-2) to assess neuronal loss, GS and 3-Nitrotyrosine (3-NT) to assess expression and nitrosylation of GS, and glial fibrillary acidic protein (GFAP) or ionized binding adaptor protein-1 (IBA-1) in combination with NOS2 or TNF-α to assess glicosis. Sections were deparaffinized in xylene and graded ethanol followed by antigen retrieval by boiling sections in 0.01 M sodium citrate buffer (pH 8.5). Sections were blocked in 2% donkey serum (Sigma; St. Louis, MO) in a 0.2% Triton-X solution made in 0.05 M Tris Buffered Saline (Tris A; pH 7.6). Sections were incubated overnight at 4 °C in the primary antibodies MAP-2 (Abcam;

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<th>Table 1: Clinical data for study population.</th>
<th>Control CSLS</th>
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*a* 5 of the 7 CSLS classified as chronic DA exposed were previously stranded.
Cambridge, MA), GS (1:250; Chemicon, Temecula, CA) and 3-NT (1:100; Millipore, Billerica, MA), or GFAP (1:250; Cell Signaling, Danvers, MA) or IBA-1 (1:250; Novus Biologicals, Littleton, CO) in combination with TNF-α (1:100; Abcam, Cambridge, MA) or NOS2 (1:100, BD Biosciences, San Jose, CA). After rising in Tris A, sections were incubated for 3 h at room temperature in Alexafluor-488, Alexafluor-555, or Alexafluor-647 conjugated secondary antibodies (1:500; Invitrogen, Carlsbad, CA). To confirm specificity of staining, primary only, secondary only, and substitution of rabbit or mouse sera for primary antibodies were performed (data not shown). Sections were mounted in media containing 4:6-diamidino-2-phenylindole dihydrochloride (DAPI) to detect cell nuclei and coverslipped. Completed sections were stored at 4 °C until imaging.

Fluorescence images were captured using 20× or 40× air plan apochromatic objectives on a Zeiss Axiovert 200 M inverted fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) equipped with a Hamamatsu ORCA-ER-cooled charge-coupled device camera (Hamamatsu Photonics, Hamamatsu City, Japan). Boundaries of specific areas of the hippocampus including the dentate gyrus (DG), subiculum (Sub), and areas 1, 2, 3, and 4 of the cornu ammonis (CA), were determined by low magnification (10×) montage imaging. Two randomly acquired images from within the boundaries of specific areas of the hippocampus were quantified blindly utilizing Slidebook software (version 5.0, Intelligent Imaging Innovations Inc, Denver, CO).

Assessment of neuronal loss, GS expression, and gliosis was determined by cell counts. The mean number of positive cells, defined as expressing the protein of interest and having a DAPI positive nucleus, were determined per region and summed for the entire hippocampus. 3-NT levels per GS positive cell were calculated by determining the sum intensity of 3-NT in areas co-localized with GS divided by the number of GS positive cells in that field.

2.3. Immunohistochemistry

Representative paraffin-embedded 5 μm coronal sections from the hippocampus were labeled with 4-hydroxynonenal (4-HNE) to assess oxidative stress. Tissue sections were prepared for immunohistochemical staining as described above. Sections were incubated overnight at 4 °C in a polyclonal rabbit 4-HNE antibody (1:500), kindly provided by Dr. Dennis Petersen, University of Colorado Denver, and then developed using horseradish peroxidase-conjugated secondary antibodies and diaminobenzidine reagents from the Vectastain ABC Kit (Vector Labs, Burlingame, CA) as described previously (Liu et al., 2006). Sections were then counterstained with hematoxylin and dehydrated in graded ethanol then xylene and coverslipped. Amount of 4-HNE was assessed for each animal by examining whole hippocampi montages obtained on low magnification (10×).

2.4. Statistical analysis

Data were analyzed using a one-way analysis of variance using a Tukey-Kramer post hoc analysis test with Prism software (version 6, Graphpad Inc., San Diego, CA). For all analyses, a p < 0.05 was considered significant with *indicating a p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 as compared to control or normal. All data are presented as mean±SEM.

3. Results

3.1. Neuronal injury and oxidative stress

The extent of neuronal injury was determined by counting the number of MAP-2 positive neurons in the DG, CA4, CA3, CA2, CA1, and subiculum of DA intoxicated animals in comparison to control cases. Representative photomicrographs are shown in Fig. 1B, H and E depicting the pattern of MAP-2 immunolabeling of neurons in the hippocampus between control, acute DA, and chronic DA animals. The loss of MAP-2 positive neurons occurred in an increasing trend based on chronicity with significant loss seen in chronic cases at the total hippocampal level (Fig. 1J; p < 0.05) and in the DG (p < 0.05) and CA1 (Fig. 1K; p < 0.05). Acute DA cases showed loss of MAP2 immunoreactivity at the total hippocampal level and in the DG with significant loss of immunolabeling occurring in the CA2 region (p < 0.05). The CA3, CA1, and subiculum areas also showed significant loss of MAP-2 positive neurons in animals with lesions classified as severe (data not shown).

To explore the role of oxidative stress in DA toxicosis, we labeled hippocampal sections from control and DA-exposed animals with 4-HNE, an indicator of lipid peroxidation. 4-HNE staining also followed a pattern based on chronicity with the most intense staining appearing in CSls classified as chronic. High levels of immunoreactivity appeared in neurons of the CA3, CA4, and DG, classified in order of higher intensity, and with weaker intensity in the CA1 and subiculum in chronic CSls (Fig. 1I). Acute cases (Fig. 1H) showed occasional 4-HNE positive neurons in the DG, CA4, and CA3 with low 4-HNE detected in the CA2, CA1, and subiculum regions. Few of the control cases (Fig. 1G), especially in the DG, CA4, and CA3 regions, had weak 4-HNE detectable neurons whereas the other areas lacked any observable staining. 4-HNE immunoreactivity did not appear to correlate with the severity of lesioning as many animals with minimal hippocampal histopathology had high levels of 4-HNE staining whereas other animals classified as severe had low detectable 4-HNE reactivity.

3.2. Loss and changes in glutamine synthetase

The number of GS expressing cells was similarly examined by counting the number of GS+ cells in control versus DA-exposed CSls by immunofluorescence (Fig. 2). In control animals (Fig. 2A), GS positive cells showed characteristic glial morphology with about 50% of GS+ cells co-labeling with the astrocytic marker GFAP. The pattern of GS labeling appeared similar in acute DA animals (Fig. 2B); however, in 2/7 chronic DA cases, glial and neuronal type cells were found expressing GS in primarily CA2, CA1, and subiculum. Neuronal expression of GS was confirmed by co-localizing the GS+ cells with the neuronal marker MAP-2 (not shown). Counts revealed a significant decrease in GS reactive cells only in the DG of chronic cases (Fig. 2E; p < 0.05) whereas total levels remained unchanged (Fig. 2D). When GS levels were examined by severity of DA induced lesion, animals with mild lesions had significant decreases in GS expression, but severe cases were not different from controls (not shown).

The extent at which GS activity may be impaired due to nitration was assessed by co-immunofluorescence of GS with the nitration marker 3-NT. Hippocampi of control animals showed low overall 3-NT reactivity and low GS nitration (Fig. 2F) as measured by total immunofluorescence intensity per GS positive cell. Animals classified as having acute DA toxicity showed increased levels of GS nitration (Fig. 2G); however, only DA chronic CSls (Fig. 2H) had significant increases over control animals at the whole hippocampus level (Fig. 2I; p < 0.05) and in the DG (p < 0.05) and CA4 regions (Fig. 2J; p < 0.05). The amount of nitrated GS also appeared to increase based on severity but was not significant (not shown). In acute and chronic cases, 3-NT positive neurons were also identified especially in the CA3, CA4, and CA1 regions. Protein nitration in neurons was similarly more intense in chronic than acute or control animals.
3.3. Astrocytosis and microgliosis

Because indicators of oxidative and nitrative stress were increased in DA-exposed animals, we investigated the presence of reactive glia expressing the proto-typical neuroinflammatory markers TNF-α and NOS2 in the hippocampus and subiculum of DA-exposed CSLs. Activation of astrocytes (Figs. 3 and 4) and microglia (Figs. 5 and 6) were significantly above control with increasing lesion severity than with chronicity (except in the DG, CA4, and CA3 regions). Animals with no significant pathology (classified as...
Fig. 2. DA-exposed sea lions have progressive alterations in glutamine synthetase that is predicted by chronicity of exposure. (A) DG of a control CSL reveal normal GS immunolabeling of cell bodies and astrocytic processes. (B) Acutely exposed animals have slight loss of GS immunolabeling; DG. (C) Number of GS+ cells is decreased with expression primarily only seen in cell bodies and sometimes within neurons (arrow) in chronically exposed CSLs; DG. Quantitative counts of total summation (D) and average number (E) of GS+ cells within the dentate gyrus = DG and cornu ammonis = CA regions 1–4 in control (n = 8; Cn), acute DA (n = 5; Ac), and chronic DA (n = 7; Chr) animals. GS+ cells, red, of the CA4 region in control CSLs (F) lack 3-NT immunolabeling, green whereas acutely exposed (G) and chronically exposed CSLs (H) have large increases in 3-NT. Quantitative analysis of 3-NT intensity over the total hippocampus (I) and individual regions (J) in control, acute DA, and chronic DA sea lions. * Indicates a p < 0.05 as compared to control. (For interpretation of the references to color in this text, the reader is referred to the web version of the article.)

normal) showed limited numbers of GFAP- (Fig. 3A) or IBA-1-positive cells (Fig. 5A). Astrocytes present in these animals showed characteristic fibrous morphology typical of a resting phenotype whereas astrocytes in mild (Fig. 3B) and severely (Fig. 3C) lesioned animals had a thickened and hypertrophic appearance. Quantitative counts revealed significant increases in the number of GFAP positive cells over the entire hippocampus (Fig. 4A; p < 0.05) as well as in the DG (p < 0.05), CA3 (p < 0.05), and CA1 (p < 0.01) in only
severely lesioned CSLs (Fig. 4B). Expression of NOS2 in astrocytes increased significantly in animals with mild lesions especially in CA3 ($p < 0.01$), CA1 ($p < 0.05$), and subiculum (Fig. 4D and E; $p < 0.05$) whereas the number of TNF-α positive astrocytes was highest in CSLs with severe lesions with significant changes seen in the DG ($p < 0.05$), CA4 ($p < 0.001$), and CA3 ($p < 0.05$) regions (Fig. 4F and G). Representative images from the CA3 region showing astrocytic expression of NOS2 and TNF-α for normal, mild, and severely lesioned animals are shown in Fig. 3D–I.

The degree of microgliosis was assessed in a similar manner and revealed a pattern of activation also based on severity of DA-induced histopathology (see, Fig. 5). Microglia were present in low numbers in CSLs with normal (Fig. 5A) and mild lesions (Fig. 5B) whereas microglia numbers significantly elevated in animals with severe lesions (Fig. 5C). Microglia present in severe animals had an obvious morphological change to a reactive phenotype with increased numbers of rod and amoeboid type microglia present. Unlike the graded increase in the number of GFAP positive cells, quantitative counts of IBA-1 positive glia demonstrated that only CSLs with severe lesions had any demonstrable increase in the number of microglia. This increase was significant in all areas of the hippocampus (Fig. 6A and B; $p < 0.05$). A similar trend was seen when evaluating the number of microglia expressing NOS2 (Fig. 6C and D; $p < 0.05$) or TNF-α (Fig. 6E and F, $p < 0.05$) as represented by images from the CA3 region (Fig. 5D–I).

4. Discussion

HABs are increasing worldwide and the long term affects of these blooms on marine and human health is currently unknown (Bargu et al., 2011). Sea lions act as sentinels of DA blooms, but despite the number of sea lions affected each year, little is known about the pathogenesis that results in seizure (Ramsdell, 2010). The objective of the current study was to investigate potential mechanisms in the development of seizures in DA-exposed CSLs, postulating that alterations in glutamate–glutamine cycling through neuroinflammatory activation of glia would correlate significantly with severity of disease. To examine this hypothesis, we...
used immunohistochemical and immunofluorescent evaluation of archived brain tissue from CSLs classified clinically into control, DA acute, or DA chronic.

The most commonly affected region of the brain in DA toxicity is the hippocampal formation with neuronal loss and unspecified gliosis occurring most prominently in the CA3, CA4, CA1, and dentate gyrus, areas of the brain involved in learning, memory, and spatial navigation (Goldstein et al., 2007; Silvagni et al., 2005). Pathological examination of the CSL cases in this study revealed similar results with the most consistent pathological changes seen in the DG and CA3 regions. Involvement of the CA1 was variable whereas the CA2 region was more affected than reported in previous studies. The relatively more severe effects reported here may be due to the highly sensitive immunofluorescent markers of pathology used in addition to traditional HE staining. Specifically, MAP-2 fluorescence is a sensitive indicator of neuronal injury with loss of antigenicity seen prior to neuronal loss (Huh et al., 2003). Although a significant trend was not seen when comparing MAP-2 loss with the classification of lesion severity, there was a measurable difference based on chronicity (Fig. 1J and K) potentially indicating that even without a more substantial lesion, chronic animals neurons are more severely stressed.

The role of oxidative stress in DA toxicity was reported by Madl et al. (2013) and we sought to expand these findings by measuring levels of another oxidative stress marker and lipid peroxidation marker, 4-HNE. Experimental seizure models have shown excessive ROS production and increased nitric oxide (NO) and peroxynitrite (ONOO−) generation at time points preceding neuronal death in susceptible brain regions that is alleviated through the use of antioxidants such as N-tert-butyl-alpha-phenylnitrone (PBN) and melatonin (Waldbaum and Patel, 2010). In our study, neurons in all areas of the hippocampus in DA-exposed CSLs showed elevated 4-HNE immunoreactivity with more extensive staining seen in chronic versus acute CSLs (Fig. 1G and H). The amount of staining did not correlate with the extent of neuronal loss in the animal, which may indicate that oxidative stress may be more relevant in terms of epileptogenesis than explicit neuronal loss.

Glutamate synthetase is a key enzyme in the glutamate–glutamine cycle and is sensitive to reactive oxygen and nitrogen species, which reduce enzymatic activity and expression.
in astrocytes that causes damaging elevations in extracellular glutamate (Bidmon et al., 2008; Castegna et al., 2011; Görg et al., 2007; Petroff et al., 2002). Specific impairment of GS using the inhibitor methionine sulfoximine (MSO) leads to spontaneous seizures in rodents (Wang et al., 2009) and inhibition has shown to be detrimental to glutamate cycling as well as GABA synthesis (Ortinski et al., 2010), thus indicating that GS is important role in epileptogenesis. Loss of GS is consistently reported in patients with TLE (EID et al., 2004) and is also decreased DA-exposed in CSLs (Madl et al., 2013). However, decreases in GS staining in DA intoxicated sea lions were only noted in the DG of chronic animals (Fig. 2) but, interestingly, there was neuronal expression of GS in several chronic cases especially in the CA2, CA1, and subiculum areas. More importantly, there was significant elevation in the amount of nitrated GS, as assessed by co-immunofluorescence of 3NT and GS, that was correlated closely with chronicity (Fig. 2F–J) that most likely indicates reduced GS activity. Because GS activity could not be directly assessed, use of fresh sea lion tissue to perform GS activity assay would be needed to verify the loss of activity that is more often seen in KA models of seizure than actual loss of GS immunoreactivity (Bidmon et al., 2008). Additionally, the sensitivity of the assays employed may somewhat underestimate the extent of GS changes compared to previous studies (Bidmon et al., 2008; Ortinski et al., 2010).

Neuroinflammatory activation of astrocytes and microglia is the most commonly increased biological marker during epileptogenesis in humans with TLE (Aronica et al., 2012; Losi et al., 2012; Vezzani et al., 2011). Glia are the primary source of NO and inflammatory factors such as IL-1β and TNF-α which have been shown to inhibit GS and glutamate receptor expression in astrocytes leading to impaired glutamate clearance (Ananth et al., 2003; Gras et al., 2006; Losi et al., 2012). Moreover, glia are known targets for DA toxicity undergoing vacuolation and necrosis in models of toxicity and direct application of DA on cultured astrocytes can dose dependently decrease glutamate uptake and lead to release of inflammatory factors by astrocytes and microglia (Gill et al., 2008; Pulido, 2008). In this study, we saw substantial amounts of neuroinflammatory activation of astrocytes (Fig. 3) and microglia (Fig. 5).
by co-immunofluorescence that was dependent on the severity of lesion. Interestingly, activation of astrocytes correlated more closely with severity rather than chronicity, with increased number of astrocytes observed in mildly and severely lesioned animals (Fig. 4). On the other hand, microglia were only activated in more severely lesioned animals and covered a greater expanse of the hippocampus and subiculum (Fig. 6). Activation of microglia also tracked with chronicity, indicating that microgliosis may be more important in epileptogenic changes in CSLs whereas astrocytosis may be more closely associated with neuronal injury. This finding is consistent with other models of epilepsy where pharmacological and genetic inhibition of microglia protects against seizure and reduces gliosis (Cho et al., 2008; Foresti et al., 2011) and with other neurodegenerative models indicating that microglia are an important regulator of astrocyte activation (Glass et al., 2010).

Astrocytes and microglia DA-exposed CSLs had increased expression of NOS2 and TNF-α; however, these inflammatory markers correlated more closely with severity whereas protein nitration of GS and neurons was more observable based on chronicity. The discord between these two observations could be a result of a limited number of animals examined and use of tissue from animals whose disease warranted euthanasia. Additionally, although inducible NOS (iNOS/NOS2) is most often implicated in the source of large levels of NO and specific inhibition using aminoguanadine can protect against seizures (Rehni et al., 2009), other studies demonstrate that neuronal NOS (nNOS) may also play important roles in epileptogenesis (Kovacs et al., 2009). A more thorough examination of NOS expression is therefore needed in future studies.

As with any wildlife study, the life history of the 20 sea lions included in this study is primarily unknown. Additionally, the times of exposure, age of exposure, and how often these sea lions were exposed are largely unknown and could be playing a role in the results observed.

5. Conclusions

Examination of glial involvement in DA toxicity in CSLs has been primarily limited to pathological descriptions of gliosis (Goldstein et al., 2007; Gulland et al., 2002; Silvagni et al., 2005) and few
researchers have examined pathogenic signaling mechanisms in DA-induced seizurogenesis. Given the failure rate of current treatments in exposed animals (40–60%), further study is required to understand mechanisms underlying DA-induced epileptogenesis. The results of this study indicate that gliosis and resultant changes in G5 are likely important mechanisms in DA induced seizure and in the subsequent loss of neurons that occurs as the disease progresses. California sea lions act as sentinels of the disease but are not the only affected species, with reports of intoxication occurring in dolphins, gray whales, northern seals, otters, and marine birds such as the brown pelican (Gulland, 2000; la Riva de et al., 2009). Understanding mechanisms of DA-induced seizure in CSLS may also be relevant to human epilepsy, given data indicating that DA intoxication may pose a risk for certain types of childhood epilepsy (Stewart, 2010). With the increasing frequency of toxic algal blooms that threaten human and marine health, understanding of the pathology can only aid in our ability to control and treat exposed populations.

References


