Chronic, Low-Level Oral Exposure to Marine Toxin, Domoic Acid, Alters Whole Brain Morphometry in Nonhuman Primates

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ABSTRACT

Domoic acid (DA) is an excitatory neurotoxin produced by marine algae and responsible for Amnesiac Shellfish Poisoning in humans. Current regulatory limits (~0.075-0.1 mg/kg/day) protect against acute toxicity, but recent studies suggest that the consumption of DA below the regulatory limit may still cause deficits in memory in adult humans. Sensitive groups, such as the developing fetus, children and elders, may be at the highest risk of exposure-related neurotoxicities, especially as DA-algal blooms are increasing in both severity and frequency. To better understand the chronic reproductive and neurobehavioral effects of DA at real-world exposure levels, we initiated a long-term study using a nonhuman primate model (*Macaca fascicularis*) exposed to daily, oral doses of 0.00, 0.075 or 0.15 mg/kg of DA. In this study, we found that exposed adult subjects demonstrated a noticeable, intentional tremor that increased with exposure duration. The present study explores this neurobehavioral finding, using diffusion tensor magnetic resonance imaging and spectroscopy. Diffusion tensor analyses showed that DA-related tremors were negatively correlated with fractional anisotropy, a measure of structural integrity, in the internal capsule, fornix, pons, and corpus callosum. Brain concentrations of lactate, a neurochemical closely linked with astrocytes, were weakly, but positively associated with tremors. These results provide striking evidence in a highly translational preclinical model that oral exposures to DA near the current human regulatory limit are related to structural and chemical changes in the brain.

KEYWORDS

Domoic acid; diffusion tensor imaging; magnetic resonance spectroscopy; fractional anisotropy

1. INTRODUCTION

Domoic acid (DA) is an excitatory neurotoxin produced by marine algae in the family *Pseudo-nitzschia* and found in ocean waters around the world. DA can accumulate in many types of seafood, including razor clams, scallops, oysters, mussels, anchovies, sardines, and crabs. When DA-contaminated seafoods are consumed, people may experience symptoms that include gastrointestinal distress, seizures, and the disruption of memory processes, collectively known as the clinical syndrome, Amnesic Shellfish Poisoning (Perl et al., 1990a; Perl et al., 1990b). The largest known DA human poisoning episode occurred in 1987 on Prince Edward Island, Canada, where over 150 people became ill and four died after consuming DA-contaminated mussels. Clinical T2-weighted magnetic resonance (MR) imaging shortly before the death of intoxicated subjects displayed stark atrophy of the hippocampus (Cendes et al., 1995). Post-mortem histology in affected patients showed that DA excitotoxicity leads to gross necrosis, astrocytosis, and atrophy, primarily in the limbic system and temporal lobe of the brain, including the hippocampus, amygdala, and thalamus (Carpenter, 1990). Since 1987, there have been no documented cases of human DA poisonings, but toxic algal blooms have been increasing in both severity and frequency (Smith et al., 2018a; Wells et al., 2015). This oceanographic change has been linked to many causal factors, including seasonal upwelling (Du et al., 2016; Schnetzer et al., 2013; Seubert et al., 2013; Smith et al., 2018b) and changing ocean temperatures (McCabe et al., 2016; McKibben et al., 2017; Zhu et al., 2017).

To protect human health, current regulations close beaches to shellfish harvesting when DA concentrations are at or above 20 ppm in shellfish tissue (Wekell et al., 2004). This value was derived from estimates after the 1987 mass poisoning; people showing symptoms of toxicity consumed approximately 200 µg DA, and a 10-fold uncertainty factor was added to protect sensitive populations. Follow-up studies have calculated that this regulatory limit is equivalent to approximately 0.075-0.10 mg DA/kg bodyweight (Mariën, 1996; Toyofuku, 2006).

This regulation, however, was initially established based on acute toxicity data and, in recent years, there has been a growing interest in the health effects of chronic DA exposure to levels below the regulatory limit (Lefebvre and Robertson, 2010). Laboratory studies have suggested that low-level exposure can cause short-term, recoverable deficits in cognition in chronically exposed adult mice (Lefebvre et al., 2017) and epidemiological reports from a coastal cohort of adult Native Americans in Washington state links the consumption of >15 razor clams/month (a proxy for low-level, chronic DA exposure) to decreased performance on several different memory exams (Grattan et al., 2018, 2016). These effects may be of particular concern to sensitive groups, such as the developing fetus, children and elders (Costa et al., 2010; Grant et al., 2010), as low-level DA exposure can cause lasting learning and memory effects in developing rodents (Adams et al., 2009; Shiotani et al., 2017). Together, these laboratory and epidemiological data suggest that the current regulatory limits may not adequately protect populations from DA related effects. Yet because DA exposure in humans is measured with self-reported dietary surveys and most rodent studies do not use oral exposures, it is difficult to interpret dose-response effects of DA in humans, especially in chronic exposure scenarios.

One opportunity to study the effects of chronic DA exposure on health and behavior has been in sentinel marine species, naturally exposed to DA through the consumption of contaminated seafood (Bossart, 2011). Elevated levels of DA in plasma and urine have been documented in a variety of animals (Lefebvre et al., 2016), but DA toxicity has been most well-defined in California sea lions. Many afflicted animals display symptoms that are similar to those observed in acutely poisoned humans, including changes in cognition, seizures, and, in the case of sea lions, a death rate exceeding 50 % (Gulland et al., 2002; Scholin et al., 2000). Sickened animals have signs of gliosis and neuronal necrosis in patterns similar to human DA toxicity cases, with damage primarily in the hippocampus and dentate gyrus (Silvagni et al., 2005). Recent studies have begun to examine the more subtle effects of the toxin and reported

that sea lions with DA symptomology have diminished memory scores, which are associated with the severity of hippocampal lesions (Cook et al., 2016, 2015).

Researchers have also connected DA toxicosis in sea lions to differences in the structural integrity of the brain, using diffusion tensor imaging (DTI) (Cook et al., 2018). DTI is a model used with diffusion-weighted imaging (DWI), a variation of MR imaging that measures the diffusion rate and anisotropy, or the degree of directionality, of water in tissues. These measures can be used to estimate changes in the density or integrity of axon bundles and myelin, as well as changes in glial cells or extracellular fluids. Cook and colleagues conducted a post-mortem DTI analysis of sea lions diagnosed with DA toxicosis and decreased anisotropy in the fornix, a white matter tract connecting the hippocampus and thalamus. These data demonstrate a link between oral DA exposure and changes in the microscopic architecture of the mammalian brain, but translating results from a feral sea lion model to human exposure scenarios remains difficult due to differences in neuroanatomy and the lack of quantifiable dose-response data.

The study described in this paper offers an innovative approach that links brain structure with behavioral toxicity in a nonhuman primate model of chronic, low-level, oral DA exposure. Subjects utilized in the present research were selected from a larger, longitudinal reproductive and developmental study (Burbacher et al., *under review*). In the parent study, adult female macaque monkeys were chronically exposed to 0.0, 0.075 or 0.15 mg/kg/day oral DA prior to, during, and post pregnancy. These doses were selected to mirror estimates of DA exposure in humans who consumed shellfish with elevated levels of DA below the regulatory threshold (Ferriss et al., 2017; Kumar et al., 2009). Results from our investigation yielded unanticipated signs of neurotoxicity in the adult females in the form of subtle intentional tremors during a reaching and grasping task. The aim of the present imaging study was to explore how intentional tremors in the animals chronically exposed to DA were related to changes in the in

vivo brain structure and neurochemical concentrations. Subjects were selected based on individual tremor and dose status and underwent a single, sedated MR scan with DWI to measure whole brain, voxel-wise diffusion measures. We additionally conducted MR spectroscopy to measure neurochemical concentrations of n-acetyl aspartate (NAA), choline, creatinine, glutamate/glutamine (Glx), and lactate. Results from this translational study represent the first presentation of data that describe in vivo structural changes in nonhuman primates after chronic, oral DA exposures at levels consumed in the real world.

2. MATERIAL AND METHODS

2.1 General Study Design

Thirty-two healthy, adult female *Macaca fascicularis* were enrolled in the parent reproductive and developmental study. All animals were housed in the Infant Primate Research Laboratory at the Washington National Primate Research Center, paired with a grooming contact social partner, and allowed unrestricted access to water. Monkeys were fed with Purina High Protein Monkey Diet (St. Louis, MO) biscuits twice a day and provided extensive enrichment (fresh produce, toys, movies/music, and frozen foraging treats). All animal procedure guidelines followed the Animal Welfare Act and the Guide for Care and Use of Laboratory Animals of the National Research Council and protocols were approved by the University of Washington Institutional Animal Care and Use Committee.

Methods for the longitudinal parent study are described in detail in Burbacher et al., *under review*. In brief, enrolled subjects were trained with positive reinforcement techniques to learn to drink from a syringe and complete a battery of clinical assessments to monitor toxicity (Burbacher et al., 2004). Once training was complete, every morning, blinded testers orally administered 1 ml of either 0, 0.075, or 0.15 mg/kg of DA (BioVectra, Charlottetown, PE, Canada) in 5% sugar water to trained adult females. General health was monitored daily by

clinical staff, and weights were recorded weekly.

2.2 Subject Selection and Behavioral Tremor Assessment

Blinded testers regularly assessed tremors, at least three times per week, by recording either the presence or absence of tremor when the subject reached for a small treat, offered approximately 6-8 inches from the front of the individual homecage, requiring full extension of the subject's arm. The fraction of total cumulative tremors from day 1 of exposure to the day of imaging was calculated for each individual and a Shapiro-Wilk test was used to assess the normality of the data. Tremor scores were used to select the subset of animals in the present manuscript, using cumulative tremor scores approximately 1 month before imaging to select either high tremoring, DA exposed females (n=6) or low tremoring, control females (n=6). The relationship of cumulative tremor scores at imaging and DTI and MR spectroscopy measures was evaluated using a nonparametric rank-based correlation. One control female that exhibited a high rate of tremors was also included in the study to investigate whether a different pattern of effects was observed in a non-DA exposed female exhibiting tremors (see Table 1). This female was scanned and analyzed separately from the rest of the subjects.

Subject Number	Dose (mg/kg/day)	Days Exposed	Age	Weight	Percent Tremoring Pre-Exposure	Percent Tremoring at MRI
1	0.150	363	11.58	4.80	0.08	0.32
2	0.150	546	8.08	4.30	0.00	0.25
3	0.150	554	7.94	4.10	0.00	0.65
4	0.150	346	8.27	3.05	0.15	0.79
5	0.075	381	7.96	3.95	0.00	0.26
6	0.075	321	7.52	4.40	0.05	0.39
7	0.000	0	9.24	5.12	0.00	0.02
8	0.000	0	7.91	3.59	0.00	0.03
9	0.000	0	7.93	3.99	0.00	0.01
10	0.000	0	8.43	5.23	0.03	0.09
11	0.000	0	8.14	4.36	0.00	0.04
12	0.000	0	7.44	3.34	0.00	0.08
13*	0.000	0	8.46	3.06	0.20	0.64

*Indicates high-tremoring, non-exposed control animal, scanned and analyzed separately from the rest of the subjects.

Table 1: Characteristics of enrolled subjects.

2.3 MR Image Acquisition

Each subject underwent a single, sedated scan. Less than 30 days before the scan, animals were required to meet health standards on a physical exam, conducted by clinical veterinary staff. MR image data were acquired on a Philips 3T Achieva (version 5.17) and a custom made 8-channel rf head coil that was developed by Dr. Cecil Hayes and optimized for the small primate head. Macaques were pre-anesthetized with ketamine (5-10 mg/kg i.m.) and atropine (0.04 mg/kg i.m.) and maintained on inhaled sevoflurane (0.8 - 2.5%) and 100% oxygen. Sedated subjects were placed in the scanner in prone position, and the coil was arranged over the head. Oxygen saturation levels and single-channel ECG were monitored with an MRI-compatible device (InvivoPrecess^{TM}) and temperature was maintained with warm packs. Diffusion weighted images were acquired with the following parameters: spin-echo echo-planar

pulse sequence with diffusion gradients, repetition time 5500 ms, echo time 77.98 ms, reconstructed matrix 128x128, number of slices 44, resolution/voxel size 0.78x0.78x1.5mm, 64 different diffusion weighted directions and one non-diffusion volume at Blip right, b value 1500, 5 different diffusion weighted directions and one non-diffusion volume at Blip left, which where compatible with FSL's topup and eddy software.

2.4 Diffusion Weighted Image Processing and Analysis

Whole brain, voxel-wise DTI measures were obtained in FSL (Jenkinson et al., 2012), using a method that is similar to tract-based spatial statistics, but allows for better alignment (Schwarz et al., 2014). Diffusion images were processed using FSL's topup software and FSL's eddy software to minimize distortion from eddy currents and head motion (Andersson et al., 2003; Smith et al., 2004), The FSL program, dtifit (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FDT), was used to reconstruct the diffusion tensor for each voxel, and the matrix was diagonalized to obtain tensor eigenvalues, L1, L2, L3. Outcomes of interest included voxel-wise fractional anisotropy (FA), a measure of the directionality of water diffusion and white matter integrity, mean diffusivity (MD, MD=(L1 + L2 + L3)/3), axial diffusivity (AD, AD=L1), and radial diffusivity (RD, RD=(L2+L3)/2). Software buildtemplate, part of Advanced Normalization Tools (ANTs) (Avants et al., 2011), was used to coregister individual FA maps to a target brain, chosen at random from among the subjects. FSL software randomise, a method that uses 500 random permutations and threshold-free cluster enhancement (TCFE) for multiple comparisons, was used to compute correlations to a design matrix of individual demeaned cumulative tremor score at the time of the scan (Table 1) (Smith and Nichols, 2009; Winkler et al., 2014). The locations of significant clusters were identified using the macaque NeuroMaps atlas (Dubach and Bowden, 2009; Rohlfing et al., 2012). Diffusion measures from voxels within significantly different clusters were then correlated to the demeaned tremor scores, using the nonparametric Spearman rank method.

2.5 MR Spectroscopy

MR spectroscopy data were acquired using the same scanner and rf coil described above with the following parameters: PRESS pulse sequence, repetition time 2000 ms, echo time 32 ms, number of FID points 2048, number of averages 48, voxel size 15x15x15 mm and voxel place centered over right thalamus (and including other brain regions) as shown in Figure 1. MR spectroscopy spar/sdat files were processed using software Lcmodel writen by Provencher (Provencher, 1993), using both water-suppressed MRS and non-water suppressed MRS files as inputs (Figure 2). Absolute concentrations of n-acetyl aspartate (NAA), choline, creatinine, glutamate/glutamine (Glx), and lactate were obtained by scaling the in vivo spectrum to the unsuppressed water peak. Concentrations were corrected for cerebral spinal fluid (CSF) volume and were correlated with the individual tremor scores in R using Spearman's correlation methods (R Core Team, 2018). Because this was a preliminary and exploratory study, we did not apply any corrections for multiple comparisons to these measures.

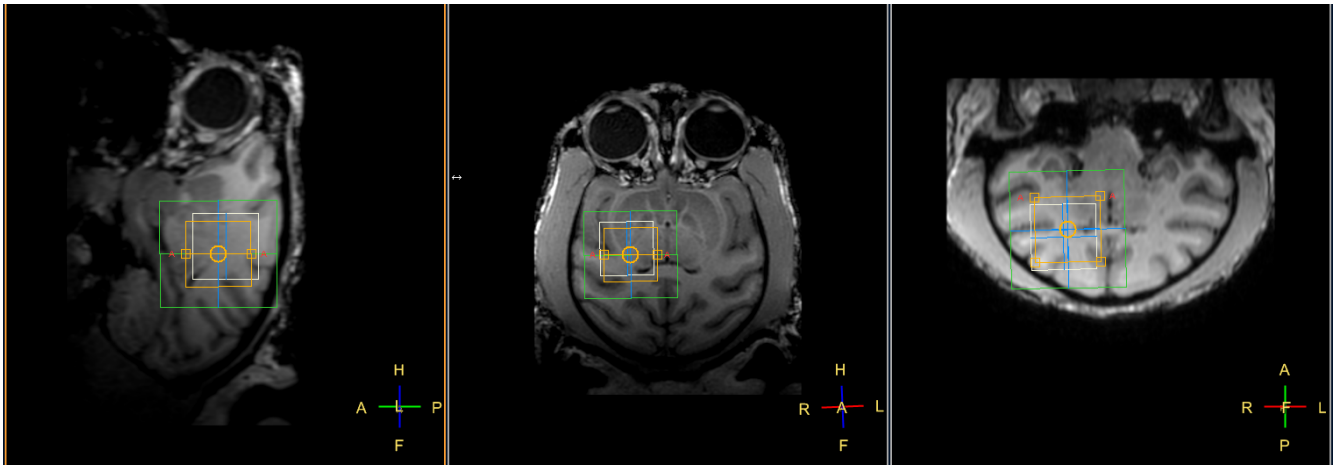


Figure 1. Placement of voxel for MR spectroscopy measurement.

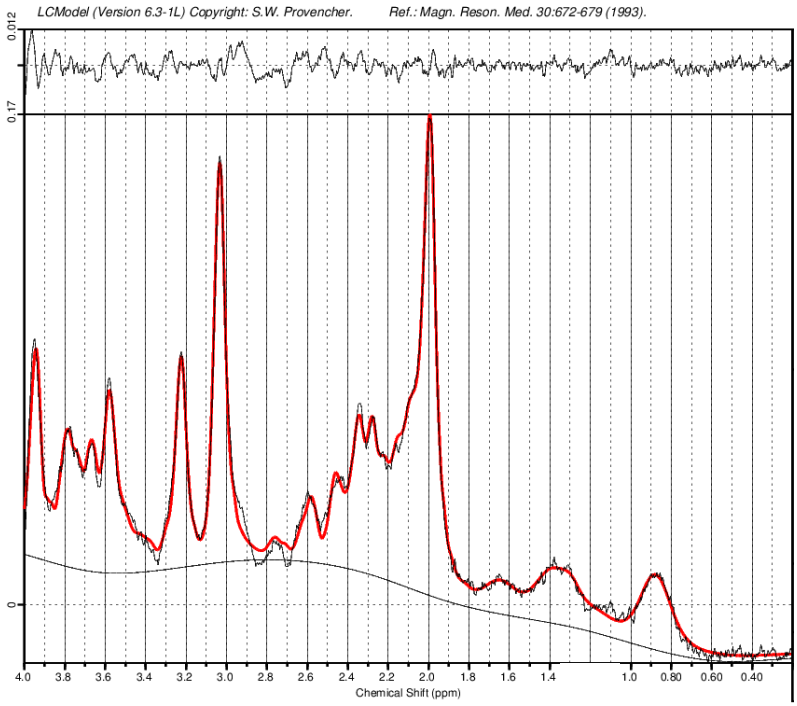


Figure 2: LCmodel fitting of the spectrum from the voxel placement shown in Figure 1.

3. RESULTS

3.1 Behavioral Tremors

Individual cumulative tremor scores were calculated from observations by blinded testers during the baseline period and from day 1 of DA exposure to the day of MR scanning (Figure 3). In general, DA exposed animals had a marked increase in intentional tremors in comparison to the control animals. A Shapiro-Wilk normality test showed that cumulative tremor scores at MR scan date were not normally distributed (W= 0.845, p=0.031), thus nonparametric Spearman's correlations were used for the analysis of MR measures. For DTI measures, the individual difference from the mean of all 12 cumulative tremor scores was used for correlative analyses. MR spectroscopy measures were correlated to the raw, cumulative individual tremor score.

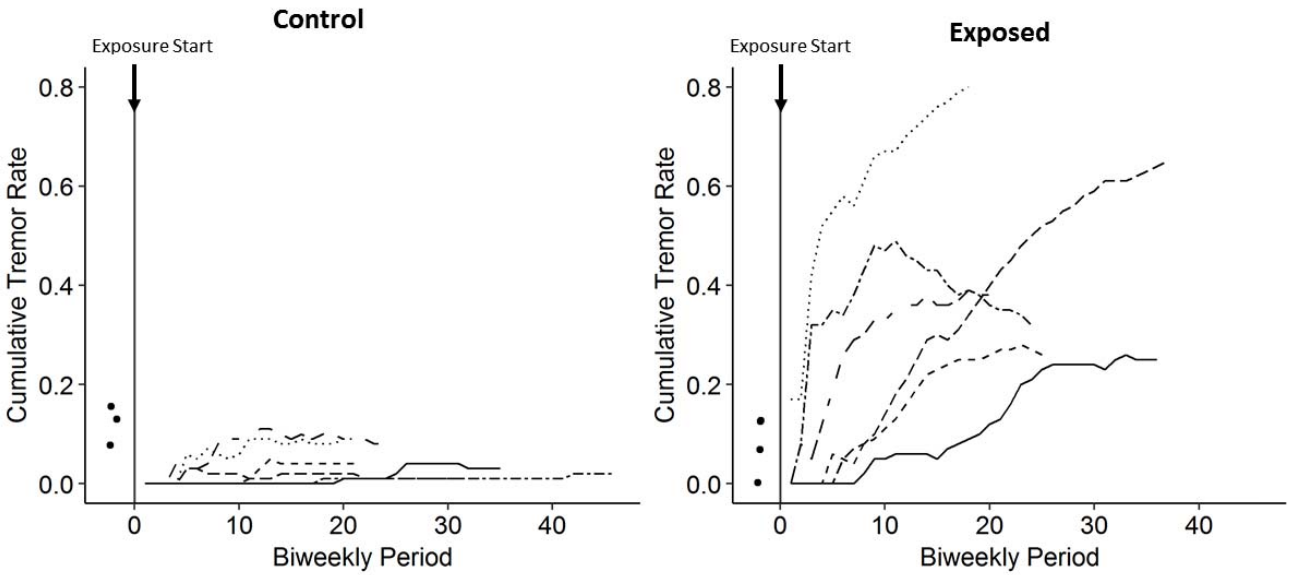


Figure 3: Shows fraction of total behavioral sessions tremoring for the 6 control subjects (left) and 6 exposed subjects (right) selected for the study, up until imaging day. Cumulative baseline tremor values are represented with a single point.

3.1 Diffusion Weighted Images

None of the females had noticeable lesions or abnormalities. Using a TCFE based analysis, we found that demeaned cumulative tremor scores from the 12 subjects were negatively correlated with FA (p=0.048, Figure 4). Clusters of FA that were significantly related to tremors were observed bilaterally in the anterior internal capsule and fornix. Correlations revealed strong relationships in these regions, as well as with smaller clusters observed in the pons and corpus callosum (Figure 5). Axial (p=0.178), radial (p=0.218), mean diffusivity (p=0.232) were neither positively nor negatively correlated with tremor scores.

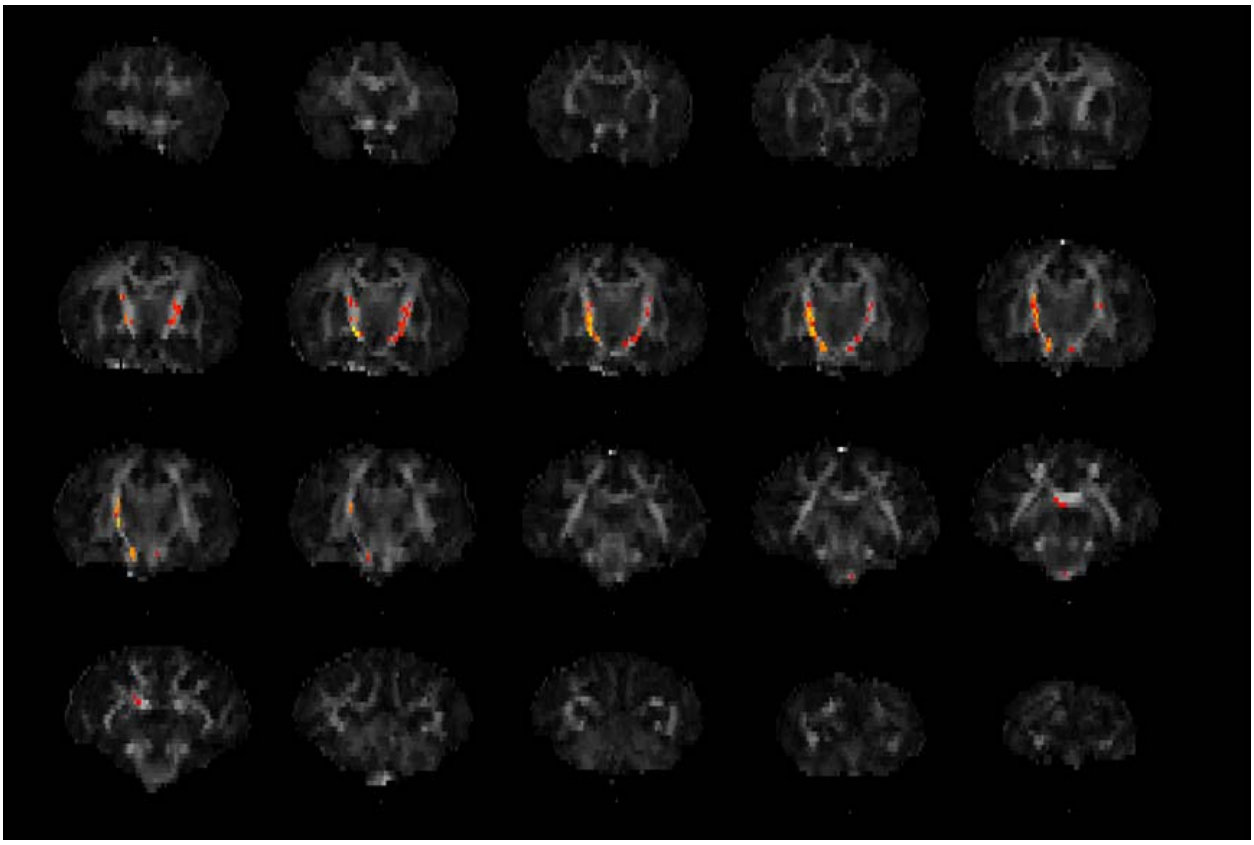
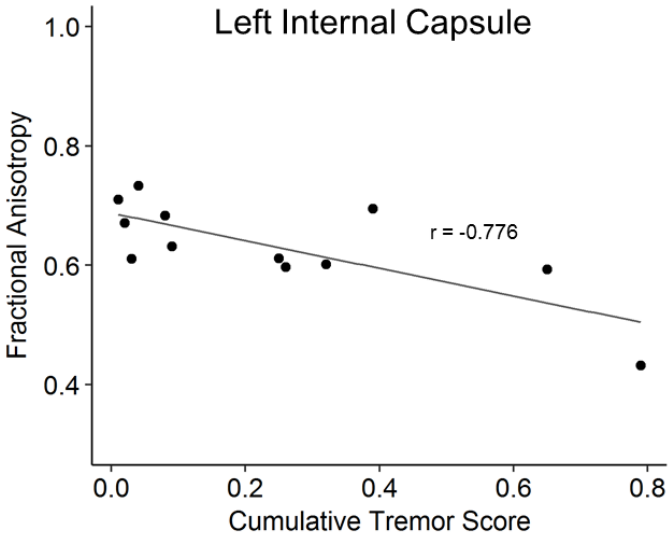
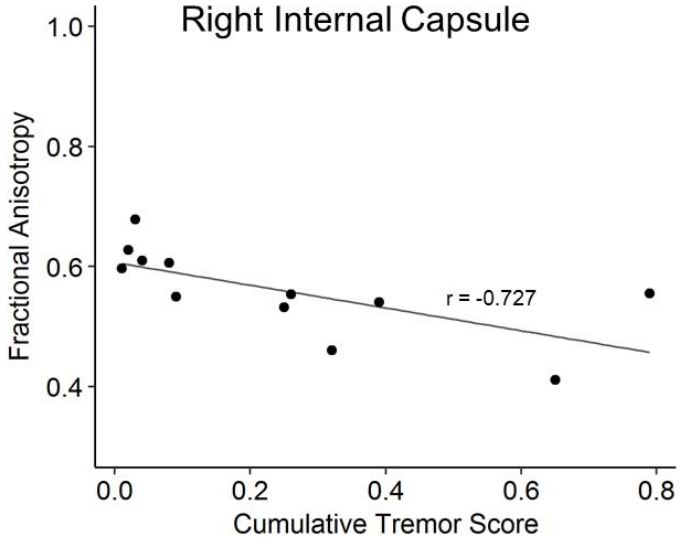
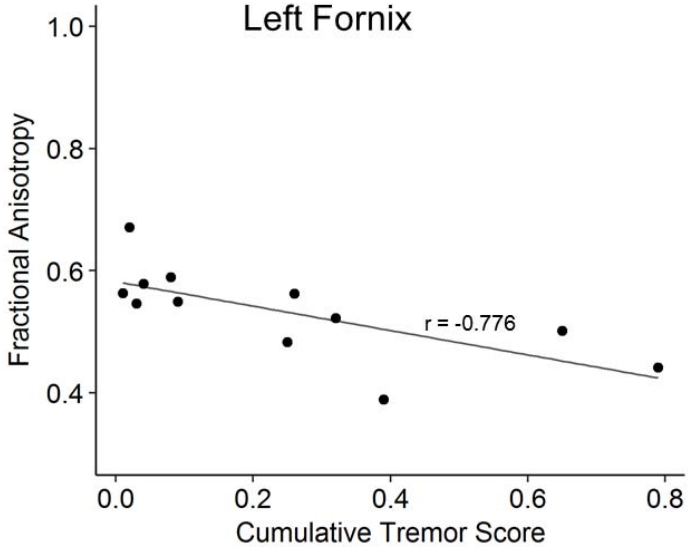
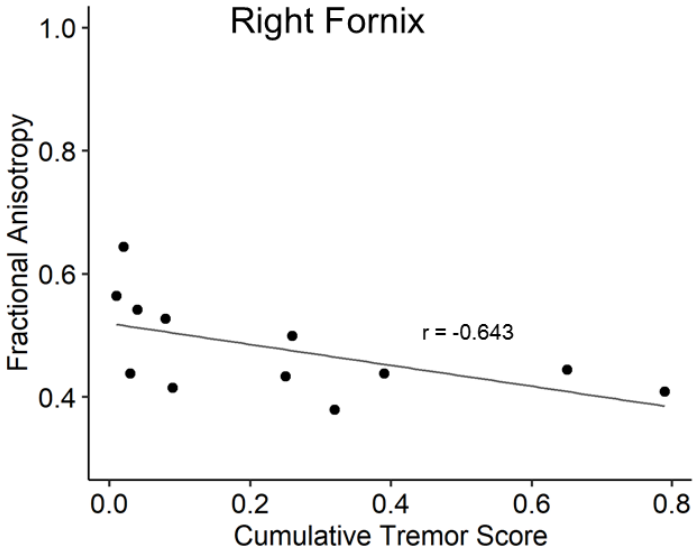


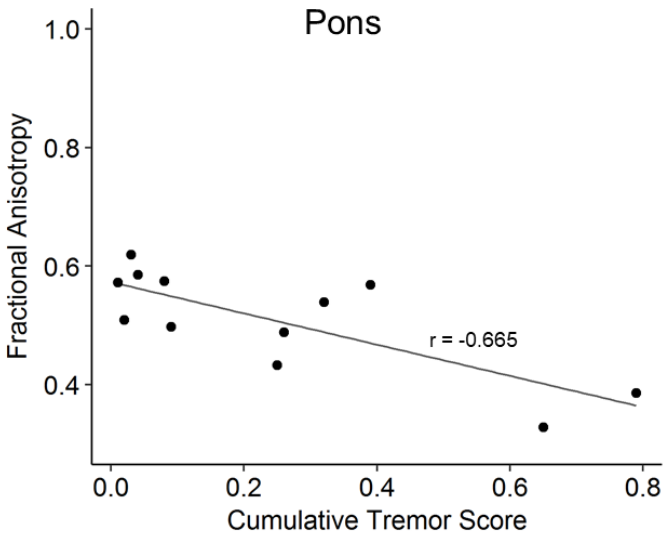
Figure 4: Sequential coronal slices of averaged FA from anterior to posterior, superimposed with significant clusters ($p<0.05$) in red-yellow.











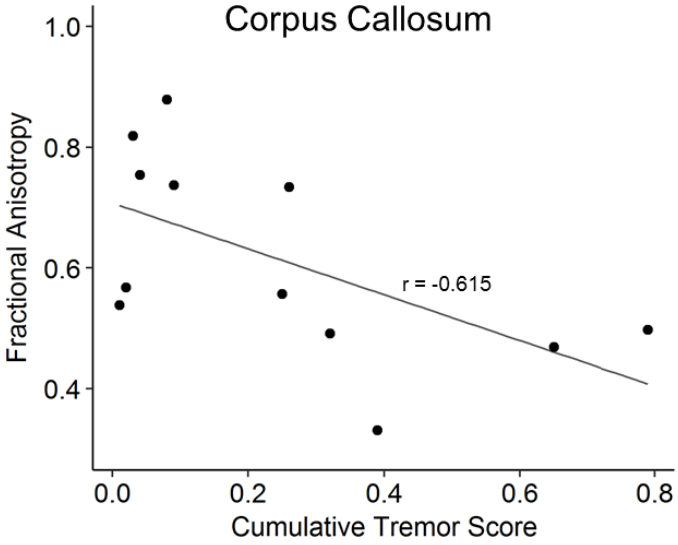


Figure 5: Spearman correlations of FA and tremors in significantly different clusters.

3.2 MR Spectroscopy

MR spectroscopy concentrations were obtained from each individual and CSF-corrected measures for NAA, choline, creatinine, and Glx were not significantly correlated with tremors. Lactate concentration, however, was positively correlated with individual cumulative tremors, but measurements were highly variable (Figure 6, p=0.048).

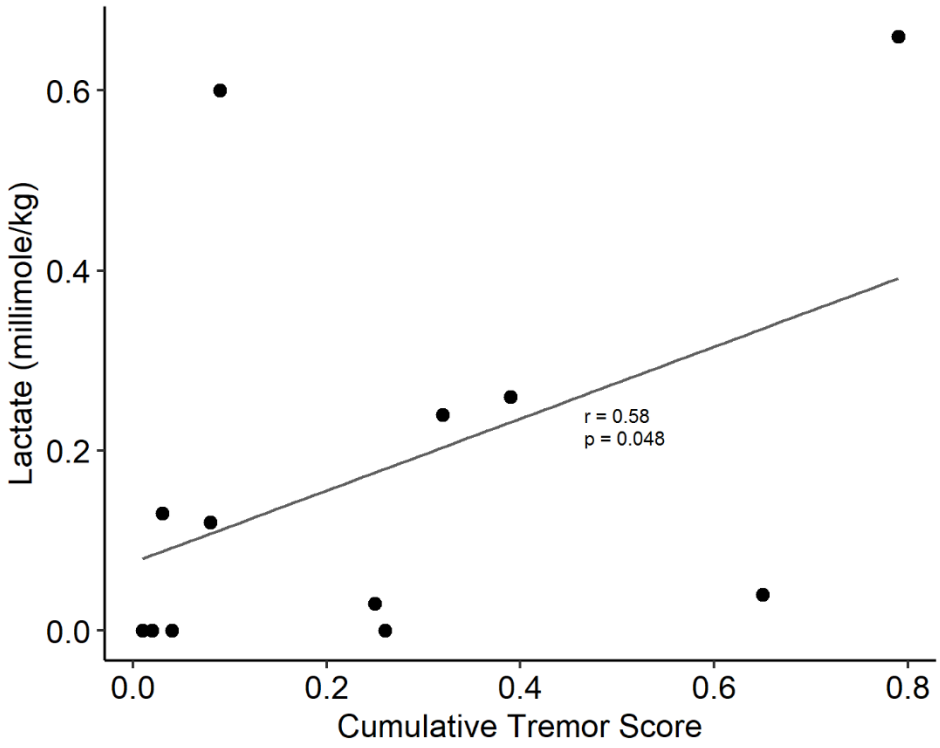


Figure 6: Spearman correlation of CSF-corrected lactate concentration and individual cumulative tremor score of the 12 subjects.

4. DISCUSSION

This study aimed to explore how intentional tremors in adult nonhuman primates chronically exposed to low-levels of DA were related to changes in brain structure and

neurochemical concentrations. Results in this report are the first from whole brain DTI-MR imaging after chronic DA exposure at levels near the current human tolerable limit. In our study, in vivo, whole brain analyses of diffusion measures describe the microstructural changes to neuronal fibers associated with chronic, low-level DA exposure. We used a TFCE approach to detect clusters of significance, a method which has been shown to have increased sensitivity over other voxel-based analysis methods (Smith and Nichols, 2009) and was untargeted and unbiased. While this approach lowered our ability to detect smaller changes in DTI measures, it also allowed us to visualize other structural changes in areas not previously known to be affected by DA. Within the sample of 6 macaque monkeys chronically exposed to low-levels of DA and 6 non-exposed controls, decreased FA, a measure of axonal and myelin integrity, was significantly correlated with increased intentional tremors, but no other diffusion measure was changed. Clusters of significantly correlated FA were primarily found bilaterally in the internal capsule and fornix, and smaller clusters of significance were observed in the pons and corpus callosum. Notably, an additional analysis of a high tremoring, control animal (see Table 1) showed that FA in the same regions more closely matched FA in other non-tremoring, control macaques and was not similar to FA in tremoring, DA-exposed individuals. This pattern of effects suggests that chronic exposure to levels of DA near the human tolerable limit can cause structural neurological damage in an adult nonhuman primate model.

FA is a measure of the directionality of water in the brain and ranges in values from 0 (no directionality or equally restricted in all directions) to 1 (fully restricted in one direction). Especially in white matter tracts, FA is typically high and reflects overall axonal integrity (Beaulieu, 2002). Lower FA scores, as found in our DA exposed macaques, have been suggested to be indicative of either direct damage to the myelin/axonal tracts or the replacement of axonal bundles with other cells (i.e. gliosis) (Alba-Ferrara and de Erausquin, 2013; Budde et al., 2011; Garcia-Lazaro et al., 2016; Smith et al., 2006). Significant clusters of

decreased FA were observed in both the right and left anterior internal capsule and fornix, and smaller clusters were found in the brainstem and the corpus callosum. The internal capsule is a complex bundle of fibers that are essential to motor function (Morecraft et al., 2002), and these fibers include projections that connect the thalamus to the prefrontal cortex, projections from the basal ganglia, and frontopontine fibers that connect the frontal cortex and brain stem (Schmahmann et al., 2004). The pons of the brain stem was also found to have small clusters of decreased FA, possibly in relation to the neurological damage observed in the internal capsule fibers. Clusters of decreased FA were also observed in the fornix, the white matter tract that connects to the hippocampus, the limbic structure responsible for memory and the primary target structure of DA toxicity (Jeffery et al., 2004), and the corpus callosum, the major white matter structure that connects the left and right hemispheres of the brain and is integral to processing stimulation from a multitude of senses (Fabri, 2014). In our study, no other diffusion measures, including axial, radial, and mean diffusivity, were changed in relation to tremors. Previous imaging studies have suggested that axial diffusivity reflects acute axonal damage, such as beading (Budde and Frank, 2010) or swelling (Dickson et al., 2007), whereas changes in radial diffusivity are symptomatic of demyelination (Song et al., 2002). Other studies have implicated that when FA is decreased, but mean diffusivity is unchanged, there may be other types of neuronal damage, such as axonal degeneration or an associated glial response, as a cause (Werring et al., 2000). Our results suggest that there may be axonal degeneration or an increased glial cell response, but not acute axonal damage in primates chronically exposed to low-levels of this neurotoxin.

Although there are currently no other whole brain DTI analyses in any animal or human exposed to DA, Cook and colleagues reported on a post-mortem targeted diffusion analyses in the brains of sea lions that were chronically afflicted with symptoms of DA poisoning (2018). In this DTI study, authors chose to target a restricted number of brain regions and found that FA in

the fornix, hippocampus, and tracts connecting the hippocampus and thalamus was decreased in DA afflicted animals. These results are similar to those observed in the fornix of our model but were obtained from sea lions demonstrating frank neurotoxicity, more severe than the subtle tremors observed in our study subjects. Effects in the fornix are consistent with the published literature, as DA is known to primarily target the hippocampus, resulting in diminished memory. While the present research did not include any examination of cognition, other DTI studies in humans have connected decreases in FA to reduced working memory and cognitive performance (Nusbaum et al., 2001; Schulze et al., 2011; Takeuchi et al., 2011).

Our observed increases in intentional tremors have only been documented in our model, possibly due to the highly limited number of chronic, oral DA exposure studies. Outcomes of any kind after controlled, chronic oral exposure to 0.075-0.15 mg/kg DA have never before been studied in any oral laboratory or natural exposure scenario. The only published manuscript to examine chronic oral exposures in a preclinical model used exposure levels of 0.5 and 0.75 mg/kg in macaque monkeys and did not report any significant behavioral or physiological effects after 30 days of repeated exposure (Truelove et al., 1997). It should be noted that standardized observations such as those included in the current study were not utilized in Truelove et al. Other short-term observational and histopathological studies demonstrate that higher levels of oral exposure (5-10 mg/kg in monkeys and 30-80 mg/kg in rodents) are typically associated with acute symptomology (i.e. scratching, vomiting, shaking/seizures, death) and severe neuronal damage and gliosis primarily in the hippocampus (Iverson et al., 1989; Tryphonas et al., 1990), outcomes not observed in our model. Our results suggest that chronic, low-level oral exposure below levels previously shown to be asymptomatic are related to behavioral tremors and decreased structural integrity in several areas of the nonhuman primate brain.

Our spectroscopy analysis calculated concentrations of several neurochemicals in a

voxel placed over the thalamus and adjacent areas of the brain. These data showed that concentrations of NAA, choline, creatinine, and Glx were unchanged in relation to the tremors, but lactate was significantly increased with increased tremoring in our cohort. Lactate is an important chemical in the brain, with several multifaceted roles including as: fuel for the brain (Boumezbeur et al., 2010; Smith et al., 2003); signaling for red-ox cycling and gene expression (Brooks, 2009); and conducting normal astrocyte and myelinating oligodendrocyte functions (Rinholm and Bergersen, 2014). It should be noted, however, that our lactate data were highly variable and not strongly correlated with tremors ($r = 0.58$, $p=0.048$). While there may be a connection between intentional tremors, chronic DA exposure, and increased lactate levels in the brain, further investigations are needed to confirm this.

The neurological damage observed in this study revealed new brain areas that are potential targets of DA, but it should be noted that the present study is exploratory and the first of its kind. Additional research should be conducted in other preclinical models, using both male and female animals, to verify these results and better understand the biochemical and cellular mechanisms underlying the observed changes in FA and lactate. Future research may also be directed at investigating the relationship between FA and DA-related deficits in memory. These results, however, still have compelling implications for humans who are regularly exposed to DA. Our nonhuman primate model is highly translatable to humans, sharing close similarities in brain structure, connectivity, and function (Passingham, 2009). In addition to our model, we also chose to give exposures orally and near the current regulatory limits (Mariën, 1996; Wekell et al., 2004), to bring strong environmental relevance to the study. These results may be particularly significant to already vulnerable communities that have close cultural connections to various types of seafood, such as some coastal Native American Tribes, where up to 84% of people regularly consume razor clams at high risk for contamination (Boushey et al., 2016). As DA algal blooms continue to increase in frequency and severity around the globe, it is

imperative that we continue to advance our understanding of the chronic, low-level effects of this marine toxin after oral exposure.

5. CONCLUSION

DA is a known neurotoxin, but few studies have analyzed the chronic, low-level effects in any model. We completed the first diffusion tensor imaging study and whole brain analysis in a nonhuman primate model chronically exposed to oral levels of DA near the current human regulatory limit. The results from our study revealed that exposed macaque monkeys expressing DA-related intentional tremors showed decreased fractional anisotropy in the brain. Our analysis showed that while the hippocampus, the primary target of DA, was affected, there were also other areas of the brain that showed significantly changed diffusion measures, including the internal capsule, brainstem, and corpus callosum. Additionally, we found a significant, but variable correlation between tremors and increased lactate in the thalamus. These data collectively show that adult nonhuman primates exposed to chronic, oral, low-levels of DA have neurological damage that can be observed through changes in behavior, neurochemical concentrations, and neurological structural integrity.

CONFLICT OF INTEREST

None.

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