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Domoic acid disrupts the activity and connectivity of neuronal networks in organotypic brain slice cultures

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ABSTRACT

Domoic acid is a neurotoxin produced by algae and is found in seafood during harmful algal blooms. As a glutamate agonist, domoic acid inappropriately stimulates excitatory activity in neurons. At high doses, this leads to seizures and brain lesions, but it is unclear how lower, asymptomatic exposures disrupt neuronal activity. Domoic acid has been detected in an increasing variety of species across a greater geographical range than ever before, making it critical to understand the potential health impacts of low-level exposure on vulnerable marine mammal and human populations. To determine whether prolonged domoic acid exposure altered neuronal activity in hippocampal networks, we used a custom-made 512 multi-electrode array with high spatial and temporal resolution to record extracellular potentials (spikes) in mouse organotypic brain slice cultures. We identified individual neurons based on spike waveform and location, and measured the activity and functional connectivity within the neuronal networks of brain slice cultures. Domoic acid exposure significantly altered neuronal spiking activity patterns, and increased functional connectivity within exposed cultures, in the absence of overt cellular or neuronal toxicity. While the overall spiking activity of neurons in domoic acid-exposed cultures was comparable to controls, exposed neurons spiked significantly more often in bursts. We also identified a subset of neurons that were electrophysiologically silenced in exposed cultures, and putatively identified those neurons as fast-spiking inhibitory neurons. These results provide evidence that domoic acid affects neuronal activity in the absence of cytotoxicity, and suggest that neurodevelopmental exposure to domoic acid may alter neurological function in the absence of clinical symptoms.

1. Introduction

Domoic acid is a neurotoxin produced by algae and is found in seafood during harmful algal blooms. It can harm marine wildlife and humans by causing excitotoxicity in the central nervous system through over-stimulation of glutamate receptors (AMPA and kainate receptors) (Perl et al., 1990; Scholin et al., 2000; Stewart et al., 1990). Acute domoic acid exposure at high doses is well-known to cause seizures and neuronal death (Teitelbaum et al., 1990; Tryphonas et al., 1990), but the neurological impacts of lower-dose, sub-clinical exposure are not well-known. As domoic acid is becoming increasingly prevalent in the marine environment due to increases in harmful algal bloom frequency over an expanding geographical range (Anderson et al., 2012; Hallegraeff, 1993; Lefebvre et al., 2016; Moore et al., 2008), marine wildlife and humans are at heightened risk for domoic acid exposure. Recently, an unprecedented domoic acid-producing harmful algal bloom caused by a large warm-water anomaly persisted through all of summer 2015 along the Pacific coast of North America (NOAA, 2015). This bloom severely poisoned hundreds of sea lions and led to the closing of several important fisheries in California, Oregon, and Washington. California sea lions regularly ingest domoic acid at concentrations reaching mg/kg levels during harmful algal blooms (Bejarano et al., 2007). Multiple marine species will likely face increasing exposure risk in the future as ocean temperatures continue to rise.

The developing fetus of pregnant mammals may be at particular risk because domoic acid crosses the placenta, is retained in amniotic fluid, and enters the fetal brain (Maucher and Ramsdell, 2007), and neonatal domoic acid exposure can also occur through

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breast milk (Maucher and Ramsdell, 2005, 2007). This is especially concerning because domoic acid's disruption to the developing glutamate system, even at exposures below those that cause seizures, can produce neurological effects lasting into adulthood (Costa et al., 2010). For example, neonatal rats exposed to sub-clinical doses of domoic acid daily over postnatal days 8–14 exhibit a number of neurological and hippocampal alterations as adults. These include increased axon sprouting, increased neurotrophic factor/receptor expression (Bernard et al., 2007; Doucette et al., 2004), reduction in a GABAergic subpopulation of neurons (Gill et al., 2010), novelty-induced seizures (Doucette et al., 2004), modified stress response, increased perseveration, and altered search strategy (Gill et al., 2012). While these studies provide clear evidence of cellular/molecular and behavioral responses to developmental domoic acid exposure, there is little information about how neuronal activity and functional connectivity is affected, or how changes in hippocampal circuit development may contribute to these reported functional deficits.

Electrophysiological studies of neuronal activity can help bridge the findings of sub-clinical domoic acid exposure's cellular/molecular and behavioral impacts. For example, recording the activity of hundreds of neurons from within the same brain region can give insight into the functional networks these neurons form, how they communicate with one another, and observe what changes arise from treatment with domoic acid. In contrast, recordings of individual neurons' activity can answer questions about a compound's affects on the function and physiology of an individual cell, but these findings are difficult to extrapolate into effects on circuits of neurons that mediate regional function. Similarly, larger-scale imaging of activity in the functioning brain (*e.g.*, functional MRI) lacks the resolution to reflect cellular details, leaving a gap in our ability to understand what happens in a population of individual neurons. Gaining a better understanding of how domoic acid influences the electrophysiological activity and connectivity of neural networks could provide insight into how cellular effects translate into behavioral outcomes.

We investigated the effect of sub-cytotoxic domoic acid exposure on the electrophysiological activity and connectivity of neural networks in organotypic hippocampal slice cultures, using a custom-made 512 multi-electrode array that provided very fine spatial and temporal resolution (Litke et al., 2004). We hypothesized that domoic acid exposure, in the absence of overt cytotoxicity, would increase the activity and connectivity of neuronal networks due to elevated glutamatergic activity. Specifically, we quantified both neuron-level activity outcomes – including spike rate and burst rate – and network-level parameters (*e.g.*, connectivity density) to address the knowledge gap between known domoic acid-induced cellular/molecular changes and changes in whole-brain function and behavior.

2. Methods

2.1. Organotypic brain slice cultures & domoic acid exposure

Cortico-hippocampal organotypic cultures (n = 37) were prepared from seven mouse pups (age postnatal day 6; five-six slice cultures per brain) following the methods described by Stoppini et al. (1991). Briefly, brains were extracted, blocked into a cube (~5 mm per side) containing the hippocampus, and sectioned at 400 μM with a vibrating blade microtome (Leica VT1000 S). The sections were trimmed to include only the hippocampus and overlying cortex, and placed on circular filter paper (hydrophile membrane PTFE ~6 mm diameter, 0.4 μM pore size; BioCell Interface, Switzerland). Cultures were grown in culture media (1 mL per culture: 50% minimum essential medium (MEM), 25% horse serum, 25% Hank's balanced salt solution, 5 mg/mL D-glucose, 1 mm L-glutamine, and 5 U/mL penicillin-streptomycin) and maintained in an incubator for 16–17 days at 37 °C and 5% CO_2 (see Ito et al. (2014) for more details). All animal care and treatments were approved by the institutional IACUC and adhered to NIH guidelines set forth in the Guide for the Care and Use of Laboratory Animals (NRC, 2011).

Half-volume (500 μL) culture media changes were done every 3 days, beginning the day after culture preparation (day 1 *in vitro*, DIV1). Cultures were pseudo-randomly assigned to control (n = 18) and domoic acid (n = 19) treatment groups; assignments were balanced across cultures that originated from the same mouse. Exposure to 0.1 μM domoic acid began on DIV4, using culture media containing domoic acid, and continued through DIV16-17.

This domoic acid dose was selected to be below those shown to cause overt cytotoxicity in organotypic cultures: our dose is 10–15-fold lower than the ≥1 to 5 μM levels shown to cause reduced cell viability in the CA1, CA3 and dentate gyrus in hippocampal brain slice cultures (Pérez-Gómez and Tasker, 2012). Domoic acid concentrations in culture media aliquots collected prior to and following organotypic culture exposures were determined by ELISA (Biosense Laboratories, Bergen, Norway) and found to be 0.12 ± 0.019 μM (mean ± SD, n = 18), which was not measurably different from the expected 0.1 μM target dose.

2.2. Multi-electrode array recording & spike-sorting

Electrophysiological activity from brain slice cultures (n = 14 control, n = 15 domoic acid) was recorded on DIV16 or 17 using a custom-made 512-electrode array system (Litke et al., 2004). This array features flat electrodes 5 μM in diameter and spaced 60 μM apart in a hexagonal lattice over a 0.9 mm × 1.9 mm rectangular area. Cultured brain tissues and adherent filter paper were gently placed on the electrode array, tissue-side down, with the hippocampal region centered on the array. A small, circular weight (~1.3 g) with fine mesh (160 mm pore size) was placed on the filter paper on top of the tissue to maintain even contact between the tissue and the array. The recording chamber (~1.8 mL volume) housing the array and mounted tissue was filled with fresh culture media (no domoic acid), and was kept perfused with culture medium (95% O_2, 5% CO_2, 37 °C) at a flow rate of 3 mL/min.

Prior to electrophysiological recording, tissues were equilibrated in the recording chamber for 30 min to avoid any transient activity that could have been caused by temperature differences from moving the culture from the incubator to the chamber. Extracellular signals from neuronal action potentials (spikes) were recorded for 90 min on each of the 512 electrode channels at a sampling rate of 20 kHz (*i.e.*, 50 μs temporal resolution). Raw waveforms were then spike-sorted using methods developed by Litke et al. (2004). Briefly, spike-sorting uses the electrophysiological activity recorded by each electrode to identify individual neurons based on the timing, waveform, and location of spiking activity on the array (see Supplemental Information for more details). This method significantly extends the information gained by simply assessing electrophysiological activity at each electrode, because more than one neuron may be contributing to the activity detected by a single electrode. Our spike-sorting analyses allowed us to evaluate individual neurons' electrophysiological activity.

2.3. Identification of hippocampal neurons in electrophysiological recordings

In general, the hippocampi of mouse organotypic brain slice cultures were slightly smaller than the array, covering ~70–80% of the recording area (Fig. S1). Because some activity from cortical neurons was occasionally included at the margins of the array, we identified and selected hippocampal neurons for analysis. This was