Function-based screening of botulinum neurotoxin countermeasures with multi-well microelectrode arrays

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Introduction

Botulinum neurotoxins (BoNTs) are the most potent substances known, with estimated lethal doses as low as 1–2 ng/kg. Expressed by anaerobic bacteria of the *Clostridum* genus, BoNTs have the potential to be converted into aerosols and utilized as agents of bioterrorism (Arnon et al., 2001). BoNTs induce synaptic blockade through the intracellular cleavage of SNARE (Soluble NSF Attachment Protein REceptor) proteins essential for neurotransmission, resulting in the inhibition of neuromuscular communication. The resulting flaccid paralysis can cause death by asphyxiation if respiratory muscles are paralyzed. Although the mechanism of the toxin has been known for decades, no treatments currently exist that can reverse the symptoms of BoNT poisoning. A factor contributing to the absence of effective BoNT countermeasures has been the lack of physiologically relevant high-throughput *in vitro* models of BoNT intoxication for use in candidate drug screening. Traditionally, BoNT countermeasure screening has occurred through either highly scalable yet barely relevant *in vitro* cell lines, or through cost-ineffective and resource intensive *in vivo* studies. To address the need for novel *in vitro* models for BoNT countermeasure screening, the potential utility of a multi-well microelectrode array (mwMEA; Axion Biosystems) was explored. This research had two objectives; first was to characterize the capacity of the technology to functionally measure the effect of BoNTs on blocking neuronal communication, thereby replicating the pathophysiology responsible for the manifestations of clinical disease. Second was to assess whether the mwMEA could be used to screen novel BoNT countermeasures for efficacy.

Materials and Methods

The mwMEA system reads plates of 48-wells (figure 1), each well containing 16 electrodes that record extracellular voltage changes that occur during the firing of action potentials. Beyond passively recording spontaneous activity of primary rat cortical and hippocampal neuronal cultures, the mwMEA has the ability to evoke neuronal network responses through voltage stimulation (Hales, Rolston, & Potter 2010).

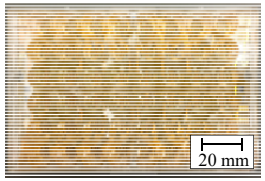


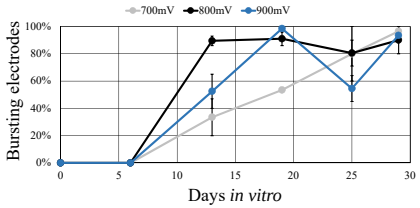
Figure 1: A 48-well plate on which neuronal networks were plated, cultured, and recorded. There are 16 electrodes at the bottom of each individual well that detect environmental changes in voltage and send data to the mwMEA.

Due to the high variability of spontaneous network activity within each well, the development of consistent voltage stimulation is optimal for reproducible activity analysis. Pilot studies were used to longitudinally characterize neuronal maturation, network emergence, and neuronal responses to external stimuli. Next, to assess the utility of this platform for BoNT studies, neuronal activity was measured following intoxication with BoNT/A (100 pM) or BoNT/E (500 pM), two serotypes of BoNT commonly associated with human disease. Lastly, the capacity of the mwMEA to evaluate lead botulinum countermeasures was tested by treating BoNT/A intoxicated cultures with 3,4-diaminepyridine (3,4-DAP) and GV-58 and tested for recovery of network activity.

Materials and Methods (cont.)

Results

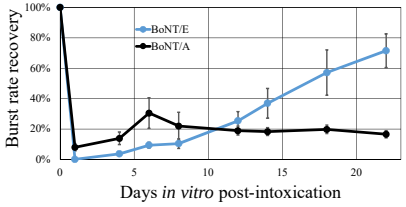
Evoked behavior during network maturation and emergence



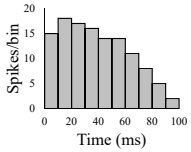
Graph 1 (left): The average percent of electrodes that exhibited bursting behavior stabilized after two weeks of growth. A stimulation voltage of 800 mV elicited the most consistent responses, and was selected for the stimulation protocol. Error bars represent the standard error from the mean of individual wells.

Graph 2 (right): Network activity was measured through the burst rate of each well and normalized to the pre-intoxication average rate. After two weeks, cultures exposed to BoNT/E began to exhibit signs of synaptic communication, while networks intoxicated with BoNT/A showed no recovery. Error bars represent standard error from the mean of burst rates in individual wells.

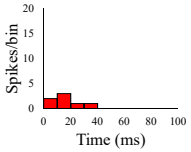
Longitudinal characterization of evoked activity post-BoNT intoxication



Pre-intoxication



Post-intoxication



Post-treatment

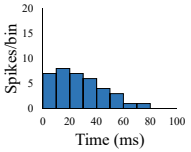
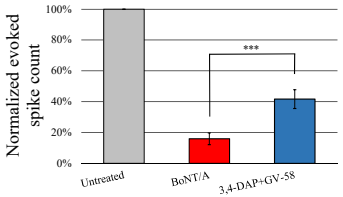


Figure 2 (a, b, c): Histograms showing evoked responses in an individual well following stimulation under three conditions: (a) pre-intoxication, (b) 1 day post-BoNT intoxication, and (c) 1 day post-treatment. The stimulation occurred at time zero and action potentials (spikes counts) were broken into 10 ms bins to show burst intensity over time.

Results (cont.)

Candidate drug analysis



Graph 3: Evoked spike counts after BoNT intoxication and following addition of 3,4-DAP and GV-58 were normalized to pre-intoxication levels. The recovery observed after drug introduction is displayed. A paired *t*-test determined the significance of the recovery to be below 0.001, signified by three asterisks ($n = 18$). Error represents standard error from the mean of each treatment.

Neuronal network activity was recovered through the addition of 3,4-DAP and GV-58, suggesting the drug treatment has at least some capacity to mitigate the synaptic blockade induced by BoNT/A.

Discussion

The purpose of this study was to develop and optimize the mwMEA as a high-throughput platform for modeling synaptic activity *in vitro* for the purpose of screening therapeutic drugs against neurotoxin exposure. Consistent evoked responses were observed following a minimum of two weeks of network growth, thereby establishing reproducible time-points for use in future studies. Cultures were then intoxicated in parallel with either BoNT/A or BoNT/E. Recovery of evoked responses was observed only in those wells intoxicated with BoNT/E, replicating the accelerated recovery observed in clinical cases of BoNT/E poisoning. When 3,4-DAP and GV-58 were simultaneously added to BoNT/A intoxicated neuronal networks, recovery of evoked responses was observed, mirroring results reported from other, lower-throughput *in vitro* and *ex vivo* models. Collectively, the results suggest that the mwMEA is a physiologically relevant method for studying toxins and drugs that alter synaptic communication. This technology allows us to functionally measure the effect of BoNTs on neuronal signaling, thereby replicating the pathophysiology responsible for the manifestations of clinical disease. Furthermore, these studies suggest that the mwMEA may provide a useful approach to not only screen candidate drugs for their efficacy as neurotoxin countermeasures, but also as a novel model from which neuronal network growth and changes can be studied.

References

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Hales, C. M., Rolston, J. D., & Potter, S. M. (2010). How to culture, record and stimulate neuronal networks on micro-electrode arrays (MEAs). *JoVE (Journal of Visualized Experiments)*, *39*, e2056-e2056.