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Evaluation of microelectrode array data using Bayesian modeling as an approach to screening and prioritization for neurotoxicity testing☆

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ABSTRACT

The need to assess large numbers of chemicals for their potential toxicities has resulted in increased emphasis on medium- and high-throughput in vitro screening approaches. For such approaches to be useful, efficient and reliable data analysis and hit detection methods are also required. Assessment of chemical effects on neuronal network activity using microelectrode arrays (MEAs) has been proposed as a screening tool for neurotoxicity. The current study examined a Bayesian data analysis approach for assessing effects of a 30 chemical training set on activity of primary cortical neurons grown in multi-well MEA plates. Each well of the MEA plate contained 64 microelectrodes and the data set contains the number of electrical spikes registered by each electrode over the course of each experiment. A Bayesian data analysis approach was developed and then applied to several different parsings of the data set to produce probability determinations for hit selection and ranking. This methodology results in an approach that is approximately 74% sensitive in detecting chemicals in the training set known to alter neuronal function (23 expected positives) while being 100% specific in detecting chemicals expected to have no effect (7 expected negatives). Additionally, this manuscript demonstrates that the Bayesian approach may be combined with a previously published weighted mean firing rate approach in order to produce a more robust hit detection method. In particular, when combined with the weighted mean firing rate approach, the joint analysis produces a sensitivity of approximately 96% and a specificity of 100%. These results demonstrate the utility of a novel approach to analysis of MEA data and support the use of neuronal networks grown on MEAs as a for neurotoxicity screening approach.

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1. Introduction

The National Academies report on Toxicity Testing in the 21st Century highlighted the need to characterize the toxicity of thousands of chemicals present in the environment (NRC, 2007) to provide adequate protection of human health. As a result, there has been a substantial effort to develop rapid, cost-efficient methods to screen thousands of chemicals for their potential to cause toxicity. This effort includes new approaches to characterizing the potential for chemicals to disrupt function of the nervous system, following both acute (Novellino et al., 2011; Defranchi et al., 2011; McConnell et al., 2012), and developmental exposure (Breier et al., 2008; Radio et al., 2008; Robinette et al., 2011; Hogberg et al., 2011).

One approach that has been proposed as a screening method for neurotoxicity is the use of microelectrode array (MEA) recordings from primary cultures of neurons (for review, see Johnstone et al., 2010). Recently, several studies have demonstrated that detection of chemical effects on of neuronal network function is reproducible across different laboratories (Novellino et al., 2011) and that MEA-based assays have high specificity and selectivity (Defranchi et al., 2011; McConnell et al., 2012). These results with small sets of chemicals (20–30 compounds), indicate that testing larger numbers of chemicals using MEAs is feasible. As larger libraries of compounds are examined, particularly those where the potential actions on neuronal network activity are unknown, it will be necessary to have unbiased approaches to determine which

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chemicals alter activity (“hit” detection) and prioritize them for additional testing.

To date, detection of chemical effects using MEAs has been based on changes in the mean firing rate (MFR) of the network of neurons in each array (Defranchi et al., 2011; McConnell et al., 2012). In doing so, the data that are obtained from the typically 60–64 electrodes in the array are averaged to a single value for each concentration of compound that is examined. From a pathophysiological standpoint, while changes in network firing rates may be an important indicator of neuroactivity or neurotoxicity, patterns and distributions of activity in neural networks are extremely important to physiological processes such as network formation during development, plasticity, and information sharing and distribution (Crumiller et al., 2011; Uhlhaas et al., 2009; Banerjee and Ellender, 2009). Therefore, detection of changes in distributions of activity across a network may also be important terms of screening compounds for potential neurotoxicity. One of the advantages of MEA approaches is that they allow the opportunity to record from multiple individual neurons in a network simultaneously. Thus, the practice of averaging data across all electrodes does not fully utilize the high content aspect of the data collected from MEAs and more importantly may not detect changes in other significant functional parameters.

The goal of the present study was to examine alternative approaches to analyze MEA data for hit detection and chemical prioritization. As an alternative to averaging all the data from one well into a single measure of activity (e.g. MFR), Bayesian approaches considering data from individual electrodes were examined. By utilizing Bayesian techniques, this larger, electrode-based data set was used to build firing-rate distributions by electrode for each chemical tested. Then, on a distributional basis, comparisons can be made between effects of control and chemical treatment on network activity. The resultant output may still be simplified to one or few metrics in order to simplify hit detection and prioritization, but the basis for the output is a more detailed descriptor which is derived and examined in the process. This will allow researchers to immediately extract firing rate characteristics for chemicals of interest that would have been otherwise hidden by simpler tests. To illustrate the utility of this approach with a simple example, consider the following theoretical effect of a chemical on firing rate. In the control condition, the firing rate across all electrodes in the array is represented by a Gaussian distribution (Fig. 1). Following chemical treatment, firing rates on some electrodes decrease, while it increases on other electrodes, such that the distribution across all electrodes becomes bi-modal. However, if the increases in activity offset the decreases, then it is certainly possible that the MFR of the control and treated distributions are the same. As a consequence, an automated approach that evaluates only the MFR to determine chemical “hits” would miss the differences between the distributions and hence the chemical effect, resulting in a false negative. With the suggested Bayesian approach and metric outlined below, the difference between the two distributions would be evident and easily detected.

The data set for the present analysis comes from a previous study which examined the ability of MEAs to detect changes in network function following exposure to a single concentration of 30 different chemicals (McConnell et al., 2012). The design of the experiment consisted of recording 30 min of control data followed by 30 min of data in the presence of each chemical using multi-well MEAs. Each of the 30 chemicals was assessed a minimum of three times. Hits were detected based on the ability of individual chemicals to alter the weighted mean firing rate in comparison to the vehicle control, but hits were not prioritized for further screening tests (McConnell et al., 2012).

2. Materials and methods

2.1. Data collection

A multi-well MEA system (Axion Biosystems Maestro system) was utilized to determine the ability of a training set of 30

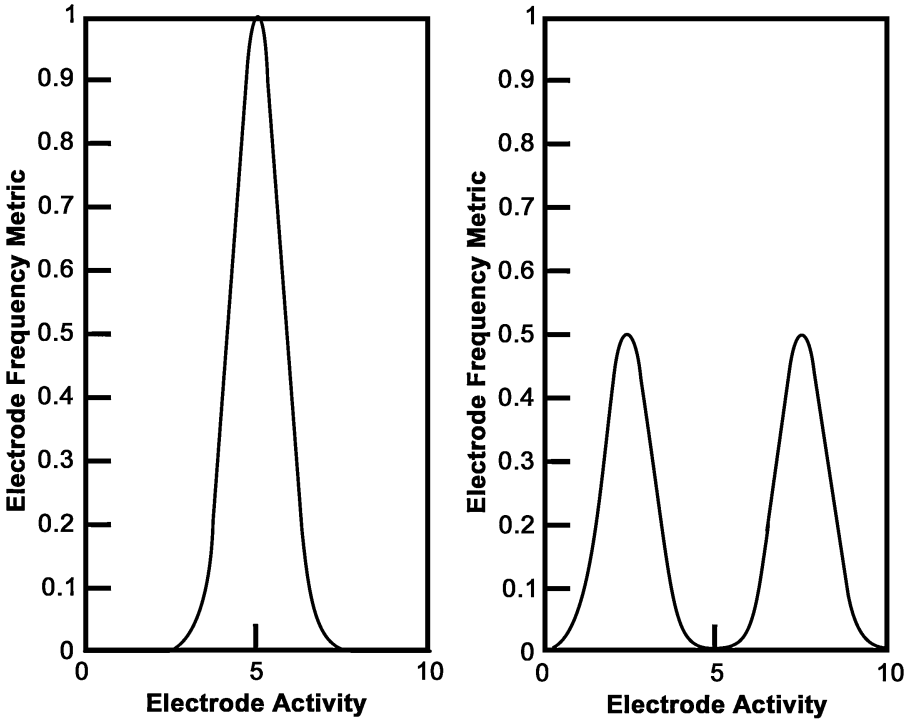

Fig. 1. These plots display two different distributions which have the same mean. The Bayesian approach will differentiate between these behaviors when simple averaging approaches will not.