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A multi-laboratory evaluation of microelectrode array-based measurements of neural network activity for acute neurotoxicity testing☆

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ABSTRACT

There is a need for methods to screen and prioritize chemicals for potential hazard, including neurotoxicity. Microelectrode array (MEA) systems enable simultaneous extracellular recordings from multiple sites in neural networks in real time and thereby provide a robust measure of network activity. In this study, spontaneous activity measurements from primary neuronal cultures treated with three neurotoxic or three non-neurotoxic compounds was evaluated across four different laboratories. All four individual laboratories correctly identifed the neurotoxic compounds chlorpyrifos oxon (an organophosphate insecticide), deltamethrin (a pyrethroid insecticide) and domoic acid (an excitotoxicant). By contrast, the other three compounds (glyphosate, dimethyl phthalate and acetaminophen) considered to be non-neurotoxic ("negative controls"), produced only sporadic changes of the measured parameters. The results were consistent across the different laboratories, as all three neurotoxic compounds caused concentration-dependent inhibition of mean firing rate (MFR). Further, MFR appeared to be the most sensitive parameter for effects of neurotoxic compounds, as changes in electrical activity measured by mean frequency intra burst (MFIB), and mean burst duration (MBD) did not result in concentration-response relationships for some of the positive compounds, or required higher concentrations for an effect to be observed. However, greater numbers of compounds need to be tested to confirm this. The results obtained indicate that measurement of spontaneous electrical activity using MEAs provides a robust assessment of compound effects on neural network function.

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1. Introduction

Interest in developing medium- and high-throughput screening approaches for predictive toxicity testing has been increasing since the publication of the National Academy's report on *Toxicity Testing in the 21st Century* (NRC, 2007) and the implementation of the regulation concerning the Registration, Evaluation, Authorisation and Restriction of CHemicals (REACH) (European Union, 2006) in

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the European Union. These and other reports (Judson et al., 2008; Kavlock et al., 2009) highlighted the lack of toxicity information on thousands of chemicals and proposed that higher throughput, *in silico* and *in vitro* models based on human biology would be required in order to generate toxicity information on these chemicals in a timely manner. The present neurotoxicity regulatory guidelines (OECD Test Guidelines TG418, TG419, TG424 and TG426 and EPA Guidelines for Neurotoxicity Risk Assessment: FRL 6011-3) are costly and low throughput. These standard testing approaches for adult and developmental neurotoxicity evaluation are based on animal models and the neurotoxic potency of compounds is mainly determined by neurobehavioral and neuropathological effects *in vivo*. Using these *in vivo* approaches, only a small fraction of chemicals have been adequately evaluated for neurotoxicity. Further, effects observed in animals often provide little mechanistic information and are not always predictive of human toxicity, which were among the reasons underlying the proposal of a new toxicity testing paradigm (NRC, 2007).

Since the publication of the International Program on Chemical Safety document on "Principles and Methods for the Assessment of Neurotoxicity Associated with Exposure to Chemicals," (WHO, 1986) basic research in neurobiology has significantly improved the ability to assess how chemicals may adversely affect the nervous system. Cell cultures derived from nervous tissue have proven to be powerful tools for elucidating cellular and molecular mechanisms of nervous system development and function (Bal-Price et al., 2012), and the throughput needed for screening large numbers of chemicals can be achieved using *in vitro* approaches.

Neuronal electrical activity is a fundamental function of the nervous system and, for this reason, its analysis could be used to evaluate the potential neurotoxic effects of test substances. Primary cultures of neurons and glia plated on grids of planar microelectrodes (i.e. Microelectrode Arrays (MEAs)) form networks of interconnected neurons in culture that exhibit spontaneous electrical activity. MEAs allow for the extracellular recording of this spontaneous electrical activity in the form of action potential 'spikes' and groups of spikes ("bursts"; for review, see Pine 2006; Nam and Wheeler, 2011). The use of neural networks on MEAs has been proposed as a screening approach for identification of potential neuroactive or neurotoxic effects of test substances (Johnstone et al., 2010; Defranchi et al., 2011; McConnell et al., 2012; Schultz et al., 2015). Moreover, recent reports have proposed that the use of multi-well MEA plates could enhance the throughput of the assay (Valdivia et al., 2014; Nicolas et al., 2014). Compared to conventional, *in vivo* assays, electrophysiological evaluation could provide an early functional readout for in vitro neurotoxicity screening.

A first step toward the potential application of the MEA data for neurotoxicity assessment is demonstration of the robustness of results across different laboratories. Previously, a multi-laboratory study demonstrated that assessments of the potency of three pharmacological agents were remarkably consistent across six different laboratories (Novellino et al., 2011). However, this previous study only examined neuroactive pharmaceutical agents (fluoxetine, verapamil and muscimol) and did not include any compounds that were not expected to disrupt neural network function (e.g. "negative controls"). The present study was therefore designed as a follow-on to the initial cross-laboratory study. Six test compounds were selected for evaluation by four different laboratories. Three of the compounds were well characterized neurotoxicants; chlorpyrifos oxon (CHO; an organophosphate insecticide), deltamethrin (DEL; a pyrethroid insecticide) and domoic acid (DA; a marine excitotoxicant). By contrast, the other three compounds (glyphosate (GLY), dimethyl phthalate (DMP), acetaminophen (ACE)) are generally recognized to not be neurotoxic, and thus were expected to not cause significant effects on neural network function ("negative controls"). Each of the participating laboratories evaluated the concentration-response relationship of these six compounds and provided the data to a common laboratory for analysis and curve fitting. The focus of these initial studies was the ability of the participating laboratories to identify and separate the neurotoxic and non-neurotoxic compounds.

2. Materials and methods

2.1. Cell culture

All experimental procedures utilizing animals were approved by the institutional animal use board of the respective institutions

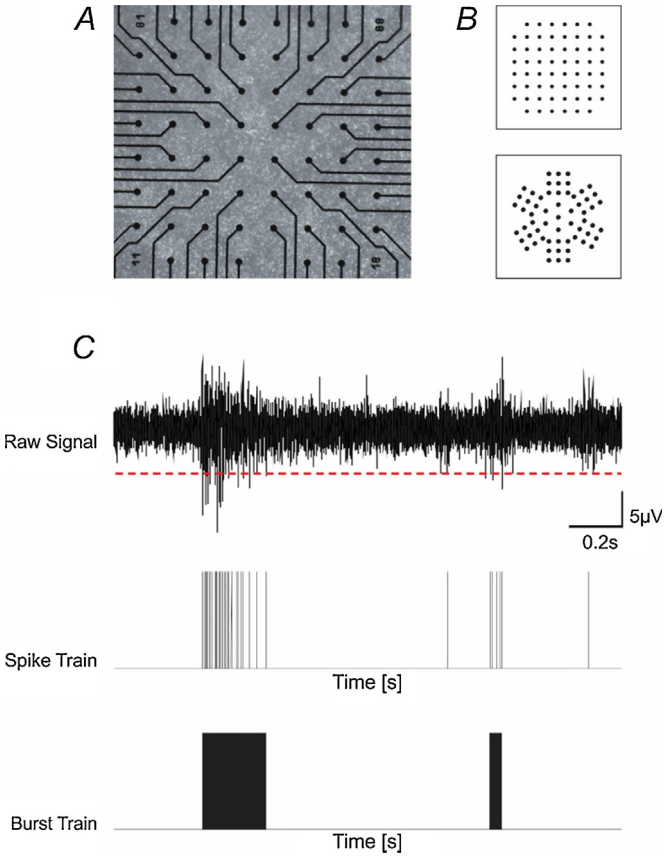

Fig. 1. Primary rat cortical cell cultures plated on MEAs exhibit spontaneous electrical activity. A 21 day in vitro (DIV) culture of cortical primary neurons on top of a planar 60-electrode array (from Lab 3). B Layouts of the two MEAs used in this study: a standard array (top panel) and a 6-well array (bottom panel). A standard MEA device has 60 electrodes arranged over an 8 by 8 square grid, with the four corners missing. One of the electrodes can be replaced by one ground reference, allowing recording from the remaining 59 electrodes. A 6-well MEA device is constituted by six independent culture chambers, divided by a makrolon separator. Inside each well, nine electrodes and one internal reference electrode allow recording of electrophysiological activity from a dissociated neural culture. C Sample trace recorded from a single microelectrode. The top panel illustrates a typical raw cortical signal characterized by the presence of spiking and bursting activity. The red dotted line constitutes a typical threshold for detecting spikes (calculated as -5σ, where σ represents the standard deviation of the basal noise). The middle panel shows the result of the spike detection procedure obtained with the red threshold depicted in the upper panel: the "Spike Train" provides a record of the temporal pattern of spikes without reference to the amplitude of those events. For this reason the peak amplitude is equal to 1. The lower panel depicts the result of the burst detection. As with the spike trains, the burst train is a temporal measure and does not consider amplitude. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).