Variations in Neuron:Glia Ratio May Alter Sensitivity to Detect Seizurogenic Potential of Drugs

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Introduction

Firing rate, network burst behavior and synchrony measures create a profile for detecting drug-induced seizure liability using the multi-electrode array (MEA). Normally, cryopreserved dissociated rat brain cortex (RCX-500, Lonza) batches will yield a culture with baseline activity (Phenotype 1) showing evidence of network connectivity with bursting and synchrony indicators at Day 11 that stabilize during Days 14-17 (start of culture is Day 0). This phenotype makes them suitable for drug testing as GABA antagonism (seizurogenic) produces increased burst, network and synchrony behaviors. Conversely, batches with higher ratio of glia to neurons produce a uniquely different baseline activity (Phenotype 2), whereby network bursting and synchrony have an earlier onset and are elevated during the pre-defined testing window. When treated with GABA antagonists, an increase in firing is observed, rather than the burst, network and synchrony endpoints. To investigate the underlying cause of this shift, we hypothesized that reducing the astrocyte population would normalize the baseline phenotype and restore sensitivity to assess drug-induced seizurogenic properties. For this study, Phenotype 2 culture was supplemented with CultureOne (Gibco) as it is known to limit astrocyte proliferation without affecting number or morphology of neurons.

Materials and Methods

MEA assay

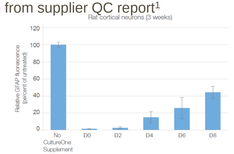
- Cryopreserved rat cortex (E18.5; QBM Biosciences, sourced from Lonza).
- Maestro multi-electrode array (MEA) system (Axion BioSystems; 48-well plate).
- Assay performed after cells are maintained 14-17 days in serum-free culture medium.
- 15 min recording of neuronal network activity taken immediately prior to and at 1 hr post compound addition .

Rat Brain Cortex (neuron:astrocyte)

- Lot A: ~1.5; yields Phenotype 1 culture
- Lot B: ~0.7; yields Phenotype 2 culture
- MAP2 and GFAP staining using ELISA; taken from supplier QC report[1]

CultureOne Supplement

- CultureOne has been shown to control level of glial population in rat cortical neuron cultures[2].



CultureOne Protocol

- Phenotype 2 culture was supplemented with CultureOne to test if inhibiting astrocyte proliferation could restore the baseline and pharmacological properties for Lot B cells.
- A 48-well MEA plate (Accuspot; Axion Biosystems) was divided into 4 groups varying by onset of this chronic treatment: Day 0, Day 4, Day 8 and no treatment control.
- Baseline culture properties were monitored daily starting at Day 6; and on Day 14, the groups were dosed with picrotoxin and gabazine (3 µM).

Background

Figure 1. Lot difference observed in response to GABA A antagonists

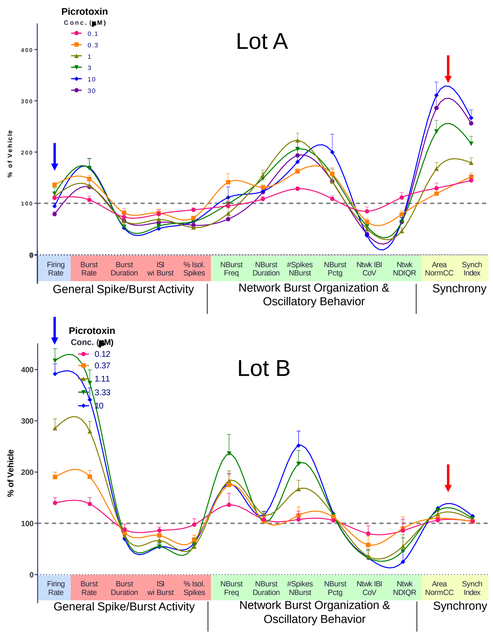


Figure 1: Composite plots for multiple MEA data end points indicated contrasting effects for Lot A and Lot B cells, mainly for the spike/burst activity and the synchrony (blue and red arrows). The two lots significantly differed in their baseline culture properties (Figure 2). With these observations, we hypothesized that the phenotypic differences (baseline and pharmacology) could be due the differences in neuron:astrocyte ratios between the lots.

Results

Figure 2. Baseline culture properties

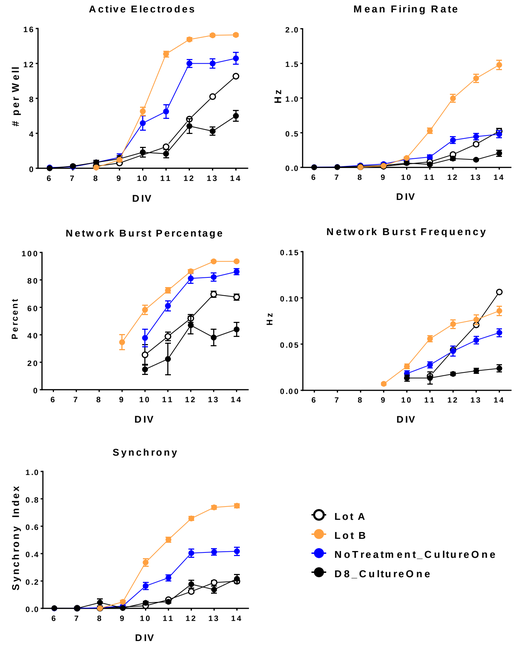


Figure 2: The baseline culture properties for the Day 8 group were similar to corresponding properties of the cells that produced Phenotype-1. The Day 0 and Day 4 groups lost baseline activity and were not included in Day 14 pharmacology testing.

Figure 3. Phenotype 2 –like response to GABA A block persists

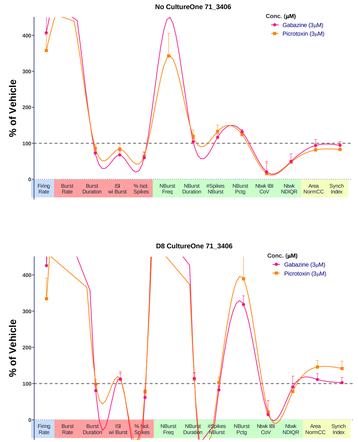


Figure 3: There were no significant differences in drug-induced seizure profile between the control (no treatment) and Day 8 groups

Summary and Conclusions

- Rat cortical neurons sensitively identify seizurogenic potential of drugs- specifically GABA antagonists
- Normalizing baseline maturity indicators does not positively impact sensitivity to detect seizurogenic potential
- We hypothesize that the astrocyte to glia ratio, impacts cortical neuron network phenotype and suggest further studies
- Reducing astrocytes produced promising baseline culture properties, but failed to restore sensitivity to detect seizurogenic potential normally observed in Phenotype 1.
- Further investigation of this phenomenon is planned using iPS cells whereby ratios of neuronal and major glial (astrocyte) populations can be controlled.

References

1. QBM Cell Science, Ottawa, Ontario, Canada
2. Application note: Control of glial cell outgrowth with the B-27 Plus Neuronal Culture System and CultureOne Supplement. Thermo Fisher Scientific, Pub. No. COL22174