MODELING FROM SINGLE CELL ELECTROPHYSIOLOGY TO NEURONAL NETWORK INTERACTIONS IN HUMAN INDUCED PLURIPOTENT STEM CELL DERIVED RETT SYNDROME AND ISOGENIC CONTROLS

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Introduction

Rett Syndrome (RTT) is a rare neurodevelopmental disorder caused by a single heterozygous loss-of-function mutation in the gene methyl-CpG-binding protein 2 (*MECP2*) found on the X-chromosome.

- MeCP2 protein is most abundant in neurons.
- Acts as an activity-dependent global transcriptional regulator.

Morphological and functional hypoconnectivity

IPSC-derived cortical neurons with the *MECP2* mutation have smaller cell bodies, shorter dendrites with less branching, and decrease in frequency of spontaneous excitatory post-synaptic currents.

Hypothesis

Hypoconnectivity between *MECP2*-mutant neurons will result in altered network development and function. Furthermore, the mechanisms driving the differences will be investigable through computational modelling of the networks.

Methodology

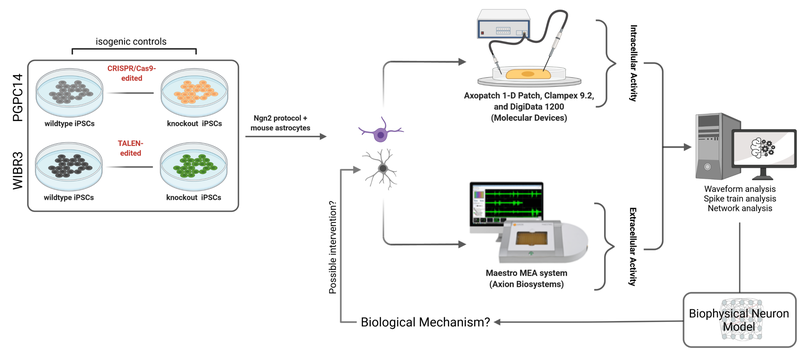

Figure 1. Two unaffected females were used to generate 2 pairs of isogenic lines. Each culture was differentiated using an *Ngn2* differentiation protocol and plated on top of mouse astrocytes. Single cell and population activity was measured and analyzed to inform a computational biophysical neuron model to probe potential biological mechanisms that may be underlying the *in vitro* phenotypes observed.

MECP2 mutant neurons exhibit altered firing rate

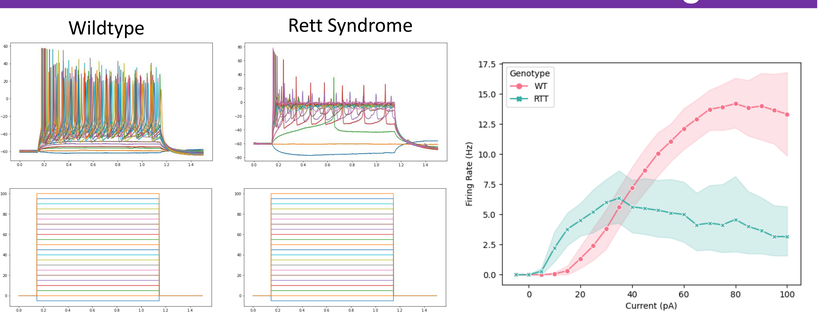

Figure 2. *MECP2*-mutant (Rett Syndrome) single neurons are more excitable, and exhibit altered firing rate patterns compared to their isogenic pair.

Visualizing Network Activity

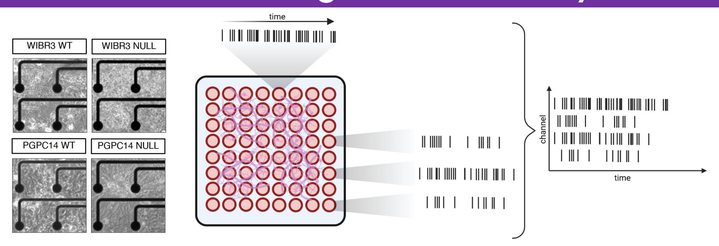

Figure 3. Activity of the developing neuronal network was measured using Axion Biosystems Maestro Multielectrode Array (MEA) system. Neuron cultures, with a density of 100k, were plated over top a grid of 64 electrodes. Using spike detection algorithms, multi-unit action potentials were identified and plotted together as a raster plot.

iPSC cultures exhibit bursting patterns

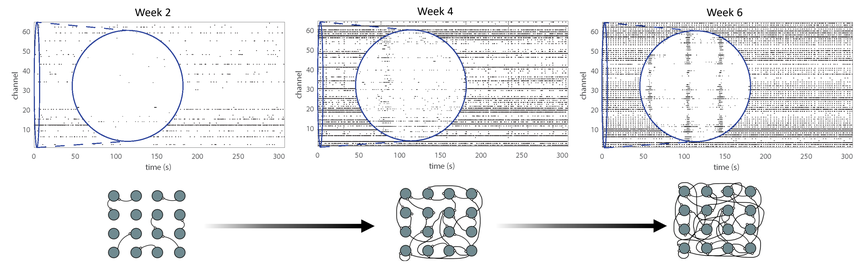

Figure 4. IPSC-derived cultures spontaneously fire and exhibit high firing rate or bursting patterns – but not all throughout development. Represented in this raster plots, neuronal cultures go from sparse firing, to asynchronous bursts, to synchronous bursting. Cultures that are bursting, are trying to wire, from discrete micro-networks to macro-networks. By quantifying bursting activity, you can make inferences on the development of functional connectivity.

MECP2 mutants have different burst frequencies

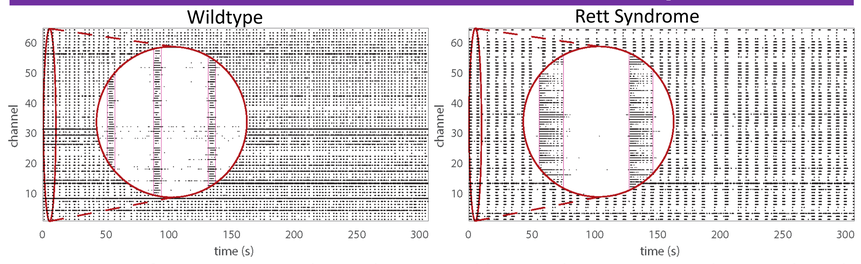

Figure 5. Burst frequencies were distinct between wildtype and *MECP2*-mutant (Rett syndrome) networks. Wildtype networks burst faster (shorter inter-burst interval) than wildtype networks.

MECP2 mutant neurons exhibit altered bursts

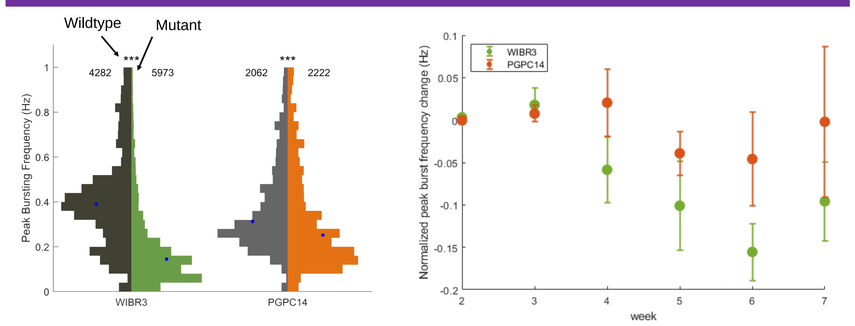

Figure 6. Power spectral density estimations enable us to identify strong frequencies contained within a time series signal. Synchronous regular bursting is capable of being captured. Burst frequencies across all electrodes and timepoints were plotted together (left) and the difference between isogenic pairs across each time point (right). Null mutants (WIBR3 and PGPC14) exhibited a decrease in burst frequencies.

Adaptive Leaky Integrate-And-Fire (ALIF) Model

$$C\frac{dV}{dt} = \overbrace{g_L(E_L - V)}^{\text{Membrane leakage}} + \overbrace{g_e(E_e - V)}^{\text{Excitatory inputs}} - \overbrace{w}^{\text{Adaptation}}$$

$$\tau_w \frac{dw}{dt} = a(V - E_L) - w \quad \underline{\text{Adaptation}} \text{ conductance}$$

$$\tau_e \frac{dg_e}{dt} = -g_e \quad \underline{\text{Excitatory}} \text{ synaptic conductance}$$

$$V(t_{spike}) = V_{spike}$$

Reset rules

$$\text{At } t = t_{spike}, \quad w \rightarrow w + b$$

Figure 7. Each neuron is represented by an equation that captures the following: capacitance, input resistance, spike triggered adaptation, subthreshold adaptation, leak conductance, synaptic conductance, reversal potential, spike threshold, time constants, synaptic weight, connection probability.

Model reproduces WT and mutant burst frequencies

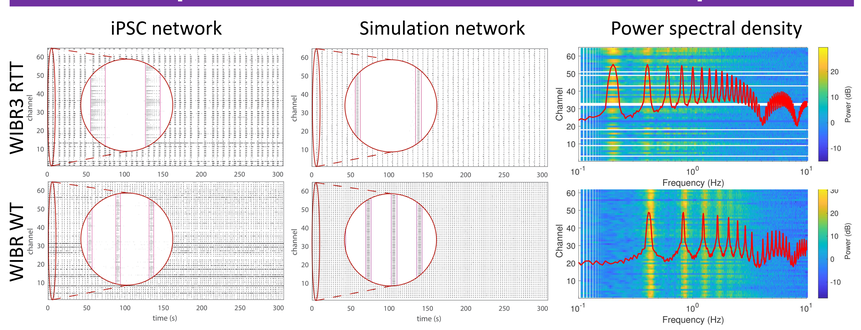

Figure 8. Network modeling of WT and RTT networks through the modulation of the adaptation conductance (equation seen above) was capable of reproducing the network bursting phenotype observed in the *in vitro* iPSC cultured networks. This is evident in the raster plots (4 plots on the left), as well as power spectral density plots of the cultured data, with the mean power spectral density across simulated neurons overlayed (2 plots on the right). This was reliably reproduced in not just WIBR3 cultures, but also PGPC14.

Channel currents, not membrane properties

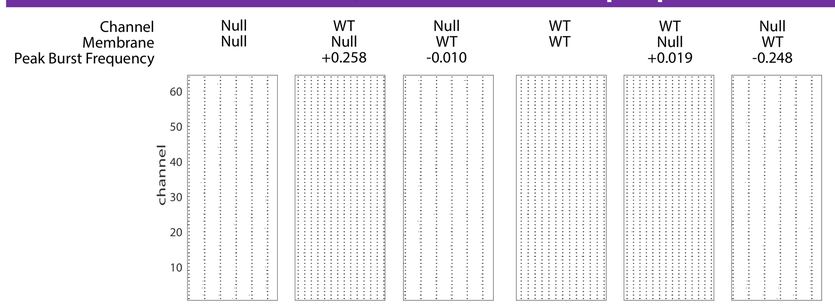

Figure 9. Computational drop out simulations to explore the contributions of adaptation channel currents relative to membrane properties (e.g., capacitance and resting membrane potential). Replaced WIBR3 null values with their isogenic WT values. When WT adaptation channel currents were replaced with Null values, burst frequency decreased. In contrast, when WT membrane properties were replaced with Null membrane properties, burst frequency did not change. Reciprocal simulations were also performed.

Adaptation channel currents or connectivity?

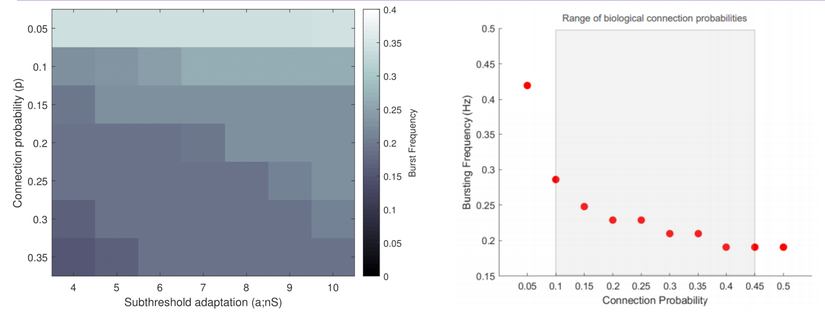

Figure 10. We studied the WIBR network model over a range of subthreshold adaptation values and connection probabilities. Over this biologically plausible range, network burst frequency decreased with the decreased connection probability typically observed in RTT neuronal networks, while the value of subthreshold adaptation had a smaller affect.

Conclusions

MECP2- mutant neurons exhibited an altered network developmental trajectory, measured through burst frequency, that grew increasingly more different through development.

Using an Adaptive Leaky Integrate-And-Fire (ALIF) neuron network model we provided insight into the mechanisms driving different bursting frequencies. These differences were mediated by neuronal adaptation and not connection probability or membrane properties. Adaptation currents include inactivation of depolarizing sodium currents and activity-dependent activation of slow hyperpolarizing potassium channels. Based on this, various subunits and regulators of channels such as BK channels, KCC2 (SLC12A5) may be implicated as downstream targets of MeCP2, permitting potential intervention.

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