Enteric nervous system & Rett syndrome

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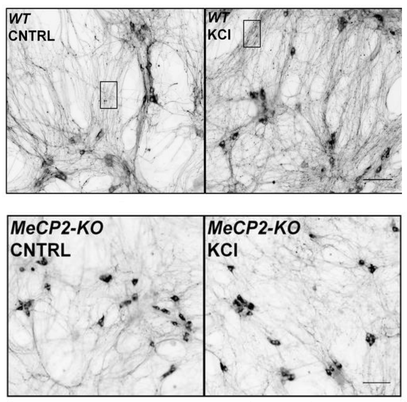
Introduction

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, caused by mutations in the MeCP2 gene, and characterized by dysregulated neuronal networks in the central and enteric nervous systems. A global imbalance of excitatory and inhibitory neural circuits in RTT give rise to symptoms such as developmental delay, seizures and gastrointestinal dysmotility[1,2,3]. Notably, enteric neurons are unable to orchestrate normal peristalsis in RTT mice, resulting in increased intestinal transit times[2]. Previous work has shown intestinal dysmotility in RTT is a function of impaired synaptic plasticity in enteric neurons[2].



Enteric neurons express MeCP2, and display homeostatic synaptic plasticity (HSP). nNOS (inhibitory marker) is upregulated following stimulation with KCl.

HSP is impaired in MeCP2 knockout enteric neurons. No significant change in nNOS levels following KCl treatment.

Fig.1: Mouse enteric neurons (WT and MeCP2-KO) treated with KCl[2]

Research Question

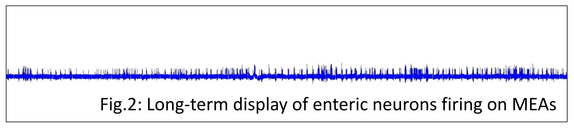


Fig.2: Long-term display of enteric neurons firing on MEAs

Mature enteric neurons can be isolated and grown in culture, and show network firing activity in vitro. Mature enteric neurons are effective for small scale culture experiments, but have limited utility on a larger scale since they do not divide.

Neurosphere-derived enteric neurons can be expanded as embryoid bodies pre-differentiation, giving a high yield of cells. Large pools of cells with the desired genotype can be easily generated and cryopreserved.

Table 1: Comparing enteric neuron sources

Enteric neuron	Mature	Neurosphere-derived
Time to functional maturity (from date of dissection)	days	weeks
Can be expanded *in vitro*	no	yes
Can be cryopreserved	no	yes
Cost to run 48 well plate experiment	High (need >20 adult mice)	Reasonable (need one or two pregnant mice)

Hypothesis

Neurosphere-derived enteric neurons can be cultured on multichannel electrode array (MEA) plates and matured until constant firing is observed *in vitro*. MeCP2 knockout enteric neurons will show dysregulation of electrical network activity compared to wild-type enteric neurons. When cells are treated with the right drug, we should see correction of the dysregulated activity in MeCP2 knockout neurons.

Multi-channel electrode array (MEA) systems are a relatively new technology that allow recording of long-term neuronal network activity *in vitro*, through electrodes embedded in cell culture dishes. This project will continue to work towards validating use of MEA in characterizing the neuronal network activity in RTT enteric neurons.

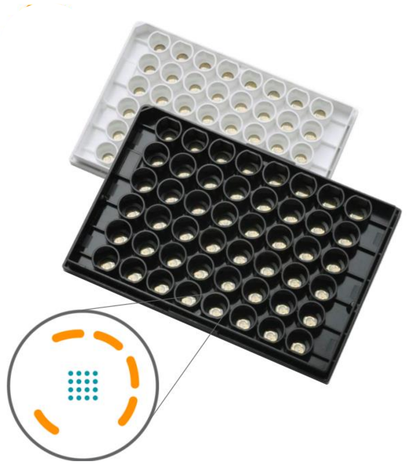


Fig.3: Multichannel electrode array 48 well culture plates, showing placement of electrodes in the wells

Methods

1. Dissection of embryonic tissue

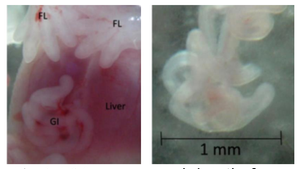


Fig.4: E14 mouse, and detail of embryonic gut

E14 mice were dissected from pregnant dams in a semi-sterile environment. Care was taken to avoid breaking the amniotic sac during removal of the embryos. Intestinal tissue was removed, triturated by flame polished pipette, and plated on uncoated cultureware at a density of 320,000 cells/cm^2

2. Tissue culture

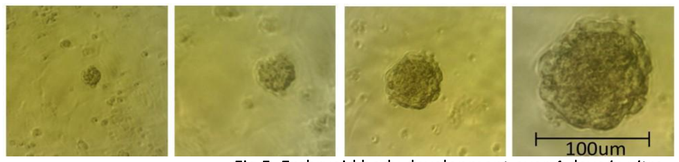


Fig.5: Embryoid body development over 4 days *in vitro*

Embryoid bodies were maintained in egf/fgf medium until colonies >100um could be observed. Colonies were manually picked by micropipette under a compound microscope, and plated onto pre-coated wells.

3. Plating on the MEA

Currently in troubleshooting phase, trials are in progress for different coatings and plating densities

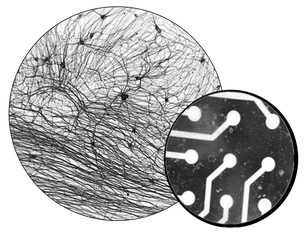


Fig.6: Differentiated enteric neurons stained with Tuj, and detail of a MEA well

Neurosphere overview:

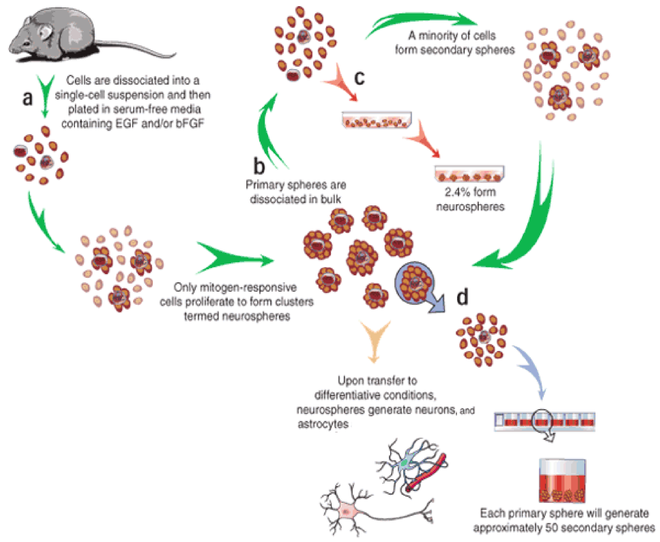


Fig.7: Generation, passaging and expansion of neurospheres[4]

Future directions

Protocols are still being established for plating neurosphere-derived enteric neurons on the MEA. Once in place, experiments using WT and MeCP2-KO cells can be performed.
Pools of embryoid bodies will be generated from pregnant WT and MeCP2-KO dams:

- Embryos will be individually dissected and genotyped for MeCP2 status, if applicable (KO or Het from MeCP2-KO dams)
- Colonies will be picked and expanded as embryoid bodies, keeping genotypes separate
- Cells will be differentiated on MEA plates, or cryopreserved in liquid nitrogen for future experiments

Ultimately, RTT enteric neurons will be treated with a small FDA approved drug library. A shift in activity towards control values will be considered evidence of treatment efficacy. Success will assist in discovery of novel, patient-specific treatments for neurodevelopmental disorders.

Acknowledgements

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References:
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