Two-photon imaging of excitatory/inhibitory cortical activity in mosaic Mecp2 female animal model

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Rett syndrome (RTT) is engendered by deficits in neuronal circuit formation, refinement and Excitatory/Inhibitory (E/I) imbalance. RTT female patients, due to the X-Chromosome inactivation (XCI), are a mosaic that may range from a salt-and-pepper distribution to entire regions being mutated5. Indeed, affected subjects exhibit a wide variability in severity and progression of the disorder. How exactly neuronal circuit activity changes in mutated and not- mutated neurons before, during and after the regression of the disorder remains an open and controversial question. An additional difficulty in establishing changes in excitation and inhibition and the overall effect in balancing and shaping network dynamics is that E and I are highly interconnected in a recurrent way, and thus all the changes to their property are inextricably linked. Overall, these factors make very challenging to develop a targeted and effective intervention6.

The visual system represents an ideal model for dissecting and analyzing neuronal circuit processing since a strong foundational body of knowledge exists regarding its experience-dependent development and plasticity in early postnatal life. Importantly, cortical sensory processing deficits have been demonstrated both in MeCP2 mutant mice as well in RTT patients. At the cellular level, excitatory cortical circuits exhibit a dramatic loss of activity and structural suffering while inhibitory circuits are dynamically misregulated - ranging from hyperconnectivity and earlier maturation of parvalbumin-positive circuits to an overall down-regulation of inhibition as disorder progresses. Importantly, how E/I imbalance across cortical networks unfold over the progression of RTT or in response to treatment in the very same freely moving mosaic animal has not been studied yet.

Our overarching goal is to determine how neuronal activity of excitatory or inhibitory cortical circuits is impacted by the expression or absence of MeCP2 during regression and recovery in visual cortex of Mecp2 Het female mice, a close model to RTT patients. By utilizing genetically modified mouse lines to visualize both XCI and cell types in combination with in vivo 2-photon microscopy in awake moving mutant and control female mice, we will be able to quantify neuronal activity either in excitatory pyramidal or inhibitory cortical cells before, during and after regression of visual cortical circuits and in response to pharmacological intervention9. Specifically, we will address the following aims:

Aim 1: In-vivo longitudinal analysis of neuronal activity by Calcium transients in Rett Syndrome. We will measure network activity in layers 2/3 pyramidal or inhibitory visual cortical neurons by using two-photon microscopy and targeted expression of genetically encoded Ca++ sensor. Response to visual stimuli will be recorded every two weeks starting around the onset of RTT phenotype. Wild-type littermates will be used as controls.

Aim 2: Rescue of sensory system deficits via chronic pharmacological treatment in Rett Syndrome. Chronic treatment with pan-NMDAR antagonist ketamine in Mecp2 mutant male mice is sufficient to significantly enhance cortical activity and slow visual regression9. Here, we will perform a similar pre-clinical study but in MeCp2 female mutant and control mice while monitoring neuronal circuit activity as in Aim 1.

Together the results will yield a comprehensive understanding of how MeCP2 mosaic affects neuronal activity and sensory processing at the single cell- and circuit-level during the progression of the disorder and in response to intervention.