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14. ABSTRACT

Fragile X syndrome (FXS) is the most common single gene cause of autism and intellectual dysfunction. It is marked by devastating alterations in cognition and behavior that originate in infancy. Many of the core symptoms of the disorder arise from altered development of the connections between neurons in the infant brain which causes the long-term changes in the ability of the brain to process information, that contributes directly to the challenges faced by FXS individuals including hyperarousal, social withdrawal and anxiety. Studies from our laboratories on a mouse model of FXS have found that early intervention with an FDA approved drug (bumetanide, targeting Cl- transporters in neurons) can reverse many of the functional problems associated with sensory information processing in the brain of the mouse model.

This project is designed to understand a critical problem in the FXS field, address important knowledge gaps, and ultimately to determine whether we can find ways to rectify the development of brain circuits that contribute to altered brain activity. The ultimate outcome will be to firmly establish a preclinical mechanism of how FXS changes brain function. The long-term benefit will be to FXS patients if we can establish that treatments targeting these mechanisms that safely and effectively rectify altered activity in the brain, and thereby address some of the most debilitating symptoms of the disorder in children with FXS.

15. SUBJECT TERMS

None listed.

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1. **Introduction:**

Fragile X syndrome (FXS), the most common inherited form of mental impairment is caused by the loss of the Fragile X Messenger ribonucleoprotein (FMRP). As a result, major disruptions of synapse maturation and experience-dependent rewiring of brain circuits are found in FXS. We found that the timing of the normal GABA polarity switch (E_{GABA}) during early development is delayed, and this is causal to synaptic and circuit phenotypes that underlie the sensory disruptions in FXS. However, the precise effects of disrupted GABA signaling on the developing cortex and particularly on the development of inhibitory interneurons is not known. Our objectives are to determine whether the development of interneurons and their signaling during early development are interconnected and have linked deficits in FXS. The project will thus address: 1) Is E_{GABA} disruption present in PV interneurons, and what are the functional consequences to the sensory microcircuit? 2) whether E_{GABA} disruption in FXS mice is present only during early development? 3) whether inhibiting the chloride (Cl^-) transporters can rectify cellular, circuit and behavior alterations in FXS mice? 4) is altered Cl^- handling by neurons the trigger for a homeostatic response in the cortical microcircuit? 5) how are altered E_{GABA} maturation and PV interneuron dysfunction connected (e.g., is one causal to the other)? We will use a combination of technical approaches to functionally analyze neurons in vitro and using in vivo imaging tools in parallel determine how these cellular properties disrupt the activity of neurons in the somatosensory cortex.

2. **Keywords:** Fragile X, Interneuron, GABA, Somatosensory, Synapse

3. **Accomplishments:**

What were the major goals of the project?

The expansion project continued the collaborative work between the Contractor lab at Northwestern and the Portera-Cailliau lab at UCLA. This successful collaboration had previously yielded new information on cellular and circuit disruptions caused by loss of FMRP. The proposal was organized as two separate Aims with the SOW devised so that Aim 1 was performed at Northwestern and Aim 2 (under a subcontract) was performed at UCLA.

Aim 1: To determine the cellular mechanisms that contribute to altered E_{GABA} and altered inhibition in sensory circuits
Is E_{GABA} disruption present in different cell types in sensory cortex:

a. Measure the mode of GABA signaling in developing PV neurons in acute slices from Fmr1 KO mice
b. Use functional measures to determine the influence of Cl^- cotransporters on Cl^- homeostasis
c. Determine whether targeting the Cl- co-transporters can permanently restore circuit E-I balance adaptation deficit is due to altered inhibition
d. Use conditional deletion of FMRP to determine whether direct of adaptive effects contribute to altered Cl- homeostasis in glutamatergic and GABAergic neurons in the FXS mouse cortex

Is E_{GABA} disruption observed in human derived neurons:

a. Measure E_{GABA} in developing human derived induced GABAergic neurons (iGNs)
b. Functionally determine the influence of Cl^- cotransporters in developing human derived neurons

Does NkCC1 inhibition rectify neuronal connectivity in Fragile X

a. Measure synaptic connectivity of human derived FMRP deficient neurons after chronic bumetanide treatment
b. Measure synaptic connectivity of mouse cortical PV neurons after chronic systemic bumetanide administered during or after the critical period

Aim 2: To determine whether E_{GABA} and/or PV interneuron dysfunction are the culprits of circuit alterations and sensory hypersensitivity in Fmr1 KO mice
Does NKCC1 reverse activity deficits in sensory cortex of FRX mice in vivo:

a. Determine whether a single dose of bumetanide can restore circuit activity in sensory cortex of Fmr1 KO mice
b. Determine whether administration of bumetanide to older mice (post critical period) reverses circuit malfunction

Is the altered EGABA causal to PV hypofunction in vivo:

a. Determine if PV activity and PV interneuron density in vivo in Fmr1 KO is restored by inhibiting NKCC1

What was accomplished?

In the first year of the award, we strove to make the foundation for execution and completion of the experiments outlined in the Aims and SOW. In some cases, this required the establishment of new mouse lines that will be used in the preceding years to complete the experiments. For instance, expanding colonies of Fmr1 KO mice and starting a new colony of Fmr1 cKO mice are in process for this. Similarly establishing IPS cell lines that will be uses in Aim 1 are in progress and will be available for experiments in the next few months. Of all the outlined experiments we have made the most advance in the experiments aimed at determining whether NKCC1 inhibition with bumetanide. As these are well progressed, we present the analyzed data here (Figure 1). These experiments were proposed as part of Aim1 and required electrophysiological analysis of cortical neurons in 4 separate groups of mice (Figure 1). In a control group the mice were treated with a control diuretic that does not cross the blood brain barrier and does not inhibit the Cl- transporter NKCC1, chlorothiazide. Mice were treated twice daily starting a P4 and until recordings were made. Single cell voltage clamp recordings were made from layer II/III pyramidal neurons and direct monosynaptic excitatory currents (EPSCs) recorded by holding the neuron at the reversal potential for Cl- ($GABA_A$ receptors), -50mV. Di-synaptic inhibitory currents (IPSCs) were recorded by holding the cell at the reversal potential for cations (AMPA receptors), 0mV (see Figure 1A). In this way we are able to make a ratio of the excitatory to inhibitory transmission (E/E+I) (Figure 1D). In mice treated with a control substance we found that the E:I ratio was significantly enhance in recordings from Fmr1 KO mice (Figure 1D). This was due to a dramatic reduction in the

feedforward IPSC (Figure 1C) and no apparent difference in the EPSC (Figure 1B). This data confirms previous observations and again underlines that GABA signaling is impaired in FRX.

In the experimental group that was administered bumetanide (NKCC1 inhibitor) we found that this difference between the genotypes in the E:I ratio was completely abolished. Figure 1E shows sample traces of EPSCs and IPSCs in WT and FRX mice treated with

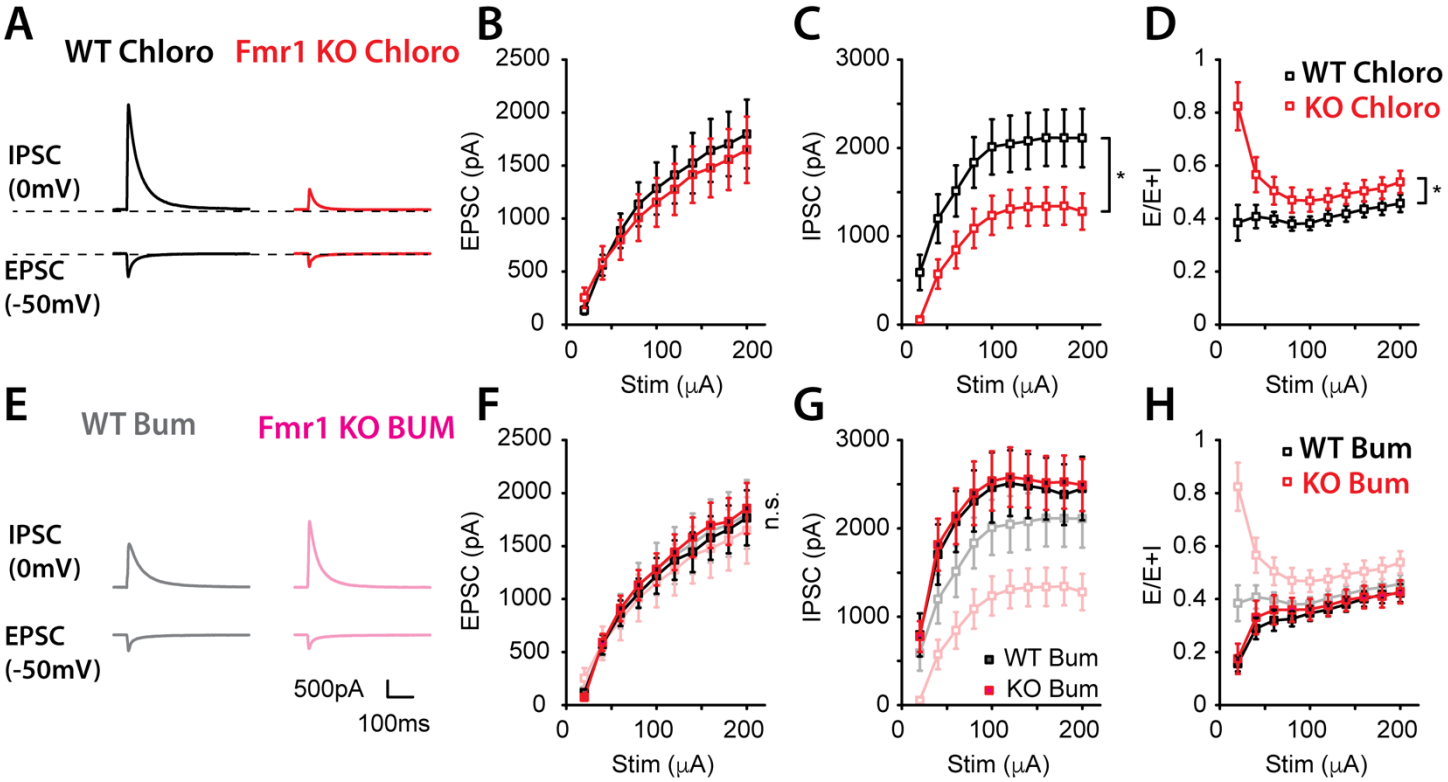

Figure 1. Altered Excitatory to Inhibitory Ratio in FRX mice is corrected by NKCC1 inhibitor
A) Example traces from WT an FRX mice treated with chlorothiazide B) ESPC recorded with increasing stimulation intensity in both genotypes C) IPSC recorded with increasing stimulation in both genotypes D) E: I ratio is increased in FXS mice (KO) when treated with control diuretic. E) Example traces in mice treated with bumetanide F) EPSC recorded with increasing stimulation in bumetanide treated mice. G) IPSC in bumetanide treated mice. No difference observed between genotypes. H) E:I ratio is not different between genotypes after treatment with bumetanide.

bumetanide (Bum). No difference was observed in either the monosynaptic EPSC (Figure 1F) or the disynaptic IPSC (Figure 1G) and also in the ratio E:I (Figure 1H). Together these results demonstrate that this major synaptic phenotype in FRX mice is rescued by the perinatal administration of a NKCC1 inhibitor.

What opportunities for training and professional development has the project provided?
Nothing to report

How were the results disseminated to communities of interest?
Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

In the next period of the award additional data acquisition will occur to further the Aims outlined above

4. **Impact:**

What was the impact on the development of the principal discipline(s) of the project?
Nothing to report

What was the impact on other disciplines?
Nothing to report

What was the impact on technology transfer?
Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. **Changes/Problems:**

Nothing to report

6. **Products**

Nothing to report

7. **Participants and Collaborating Organizations**

Name: Anis Contractor
Project Role: PI
Researcher Identifier (e.g. ORCID ID) :
Nearest person month worked: 1.2
Contribution to Project: Overall lead for the project, provides scientific direction, mentors students and postdocs, analyses data and performs administrative duties

Funding Support: None (Complete only if the funding support is provided from other than this award.)

Name: Toshihiro Nomuro
Project Role: Research Associate
Researcher Identifier (e.g. ORCID ID) :
Nearest person month worked: 4.8
Contribution to Project: Performed experiments and analyzed data

Funding Support: None (Complete only if the funding support is provided from other than this award.)

Name: John N Armstrong
Project Role: Research Assistant Professor
Researcher Identifier (e.g. ORCID ID) :
Nearest person month worked: 9.6
Contribution to Project: Performed experiments and analyzed data

Funding Support: None (Complete only if the funding support is provided from other than this award.)