The difference between activity and function: Utilizing mouse models and hiPSCs for elucidating the electrophysiology of bipolar disorder

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Abstract

Bipolar disorder (BD) is a neuropsychiatric disease that impacts 2.6% of the adult population, and is characterized by oscillations in depressive and manic behavior. BD is the most fatal psychiatric disease due to a high suicide rate, and little is known regarding its underlying pathology. Currently there is no therapy that is both safe and efficacious for treating BD, which is a critical unmet need. Recent discoveries utilizing transgenic mouse models have demonstrated collapsin response mediator protein-2 (CRMP2) plays an integral role in BD's molecular pathology, but how CRMP2 mediates BD has yet to be elucidated[1]. Employing CRMP2 transgenic mice as models for BD, we have discovered CRMP2 activity impacts neuronal electrophysiology, structure, and proteomics. Interestingly, many of the aberrations found in the transgenic CRMP2 neurons superficially appear counter-intuitive, but under further examination expose the complexity of how neuronal circuits function. Specifically, BD-like transgenic CRMP2 neurons appear to have hyperactive calcium activity, while having less neuronal-network signaling. Collectively, these works begin to illuminate long sought-after insights in BD pathology, and offer new targets for future BD therapeutics to be designed for.

CRMP2 Overview

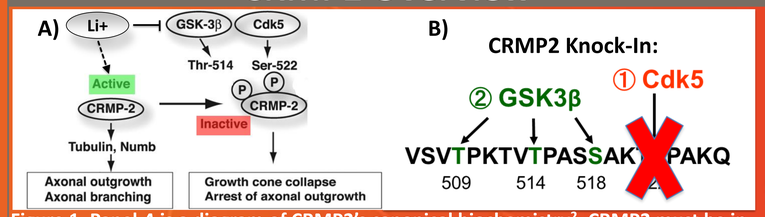


Figure 1. Panel *A* is a diagram of CRMP2's canonical biochemistry[2]. CRMP2 must be in its unphosphorylated form to be active, allowing it to bind to cytoskeletal elements like tubulin and promote polymerization. CRMP2 is inactivated via sequential phosphorylation first by CDK5 and then GSK3b. Recent work by Tobe *et al* discovered the main BD therapeutic, lithium, inhibits GSK3b which leads to higher levels of unphosphorylated CRMP2 in neurons[1]. *B* is a diagram of a mutation to make a constitutively active CRMP2 mouse, where all CRMP2 proteins are in their active state.

CRMP2 Activity Impacts BD Behavior

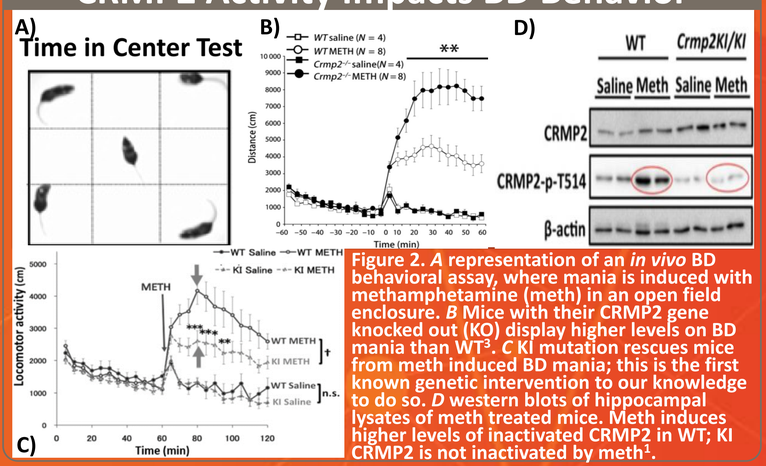


Figure 2. *A* representation of an *in vivo* BD behavioral assay, where mania is induced with methamphetamine (meth) in an open field enclosure. *B* Mice with their CRMP2 gene knocked out (KO) display higher levels on BD mania than WT[3]. *C* KI mutation rescues mice from meth induced BD mania; this is the first known genetic intervention to our knowledge to do so. *D* western blots of hippocampal lysates of meth treated mice. Meth induces higher levels of inactivated CRMP2 in WT; KI CRMP2 is not inactivated by meth[1].

References

1. Tobe et al. *Probing the lithium-response pathway in hiPSCs implicates the phosphoregulatory set-point for a cytoskeletal modulator in bipolar .* PNAS, 2017.
2. Uchida et al. *Semaphorin3A signaling is mediated via sequential Cdk5 and GSK3beta phosphorylation of CRMP2: implication of common phosphorylating mechanism underlying axon guidance and Alzheimer's disease.* Genes Cells, 2005.
3. Nakamura et al. *Comprehensive behavioral study and proteomic analyses of CRMP2-deficient mice.* Genes Cells, 2016

CRMP2 Impacts Neurite Composition

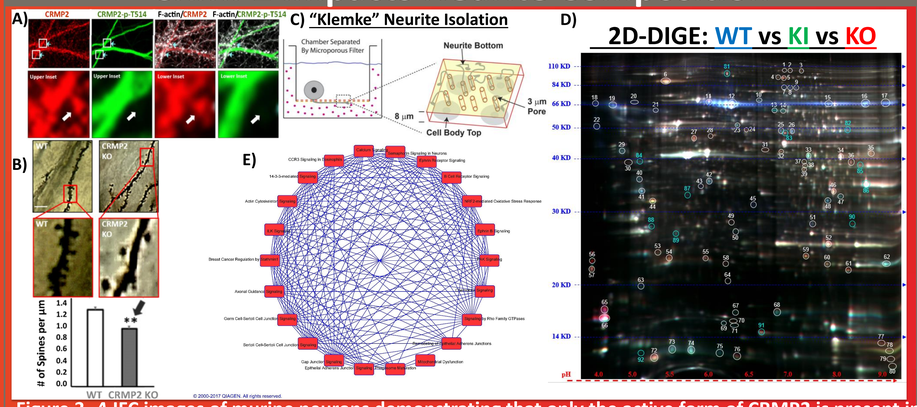


Figure 3. *A* IFC images of murine neurons demonstrating that only the active form of CRMP2 is present in synaptic spines. *B* Golgi staining of hippocampi to visualize spines; CRMP2 KO neurons have decreased spine density[1]. *C* Diagram of Klemke neurite isolation method. *D* 2-dimensional differential in gel electrophoresis comparing the neurite proteomes of CRMP2 KI, CRMP2 KO, and WT hippocampal neurites. Circles are proteins that are differentially present or post translationally modified (PTM) between the different groups; circled spots were isolated and mass spectrometry was performed to identify the proteins and their respective PTMs. *E* Ingenuity IPA pathway analysis of the proteins identified in the 2D-DIGE, interestingly calcium signaling was identified as one of the top pathways.

CRMP2's Role in Neuronal Calcium Activity

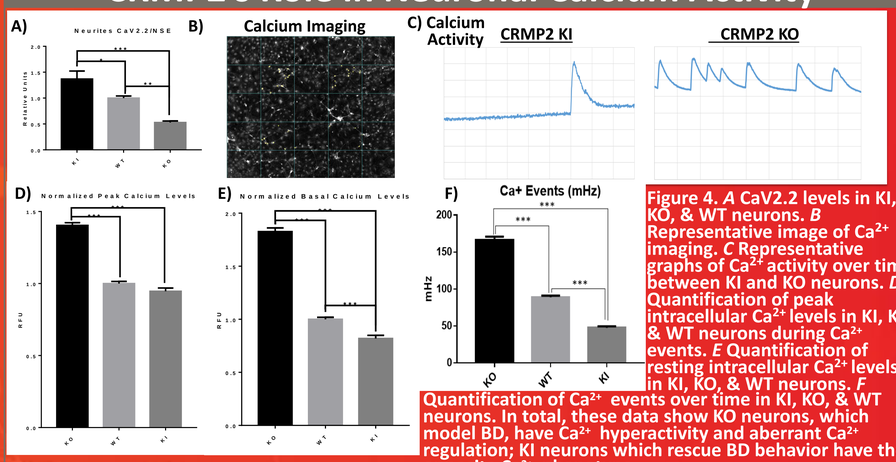


Figure 4. *A* CaV2.2 levels in KI, KO, & WT neurons. *B* Representative image of Ca^{2+} imaging. *C* Representative graphs of Ca^{2+} activity over time between KI and KO neurons. *D* Quantification of peak intracellular Ca^{2+} levels in KI, KO, & WT neurons during Ca^{2+} events. *E* Quantification of resting intracellular Ca^{2+} levels in KI, KO, & WT neurons. *F* Quantification of Ca^{2+} events over time in KI, KO, & WT neurons. In total, these data show KO neurons, which model BD, have Ca^{2+} hyperactivity and aberrant Ca^{2+} regulation; KI neurons which rescue BD behavior have the opposite Ca^{2+} phenotype.

CRMP2's Role in Neuronal Network Signaling

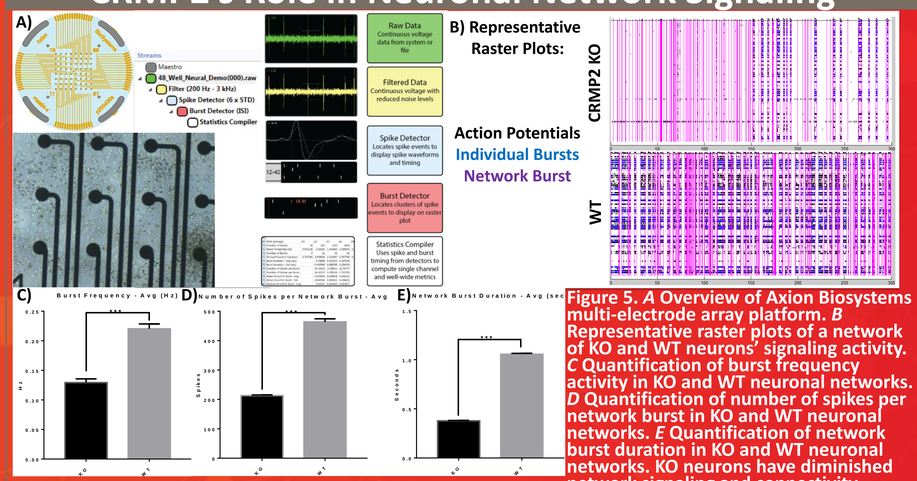
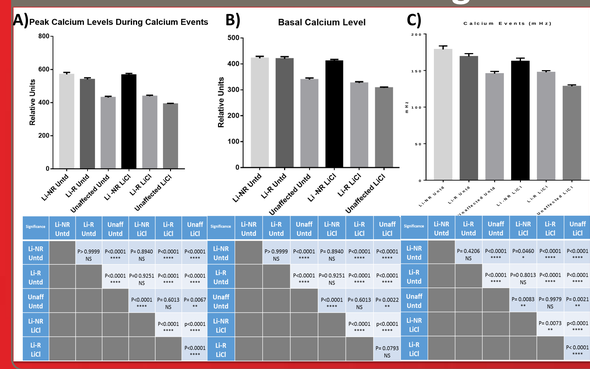


Figure 5. *A* Overview of Axion Biosystems multi-electrode array platform. *B* Representative raster plots of a network of KO and WT neurons' signaling activity. *C* Quantification of burst frequency activity in KO and WT neuronal networks. *D* Quantification of number of spikes per network burst in KO and WT neuronal networks. *E* Quantification of network burst duration in KO and WT neuronal networks. KO neurons have diminished network signaling and connectivity.

Bipolar Disorder Neuronal Calcium Kinetics Mirrors CRMP2 Transgenic Neurons



Significance	Li-NR Untd	Li-R Untd	Unaff Untd	Li-NR LiCl	Li-R LiCl	Unaff LiCl
Li-NR Untd		P= 0.9999 NS	P<0.0001 ****	P= 0.8940 NS	P<0.0001 ****	P<0.0001 ****
Li-R Untd			P<0.0001 ****	P= 0.9251 NS	P<0.0001 ****	P<0.0001 ****
Unaff Untd				P<0.0001 ****	P= 0.6013 NS	P= 0.0067 **
Li-NR LiCl					P<0.0001 ****	p<0.0001 ****
Li-R LiCl						P<0.0001 ****

Significance	Li-NR Untd	Li-R Untd	Unaff Untd	Li-NR LiCl	Li-R LiCl	Unaff LiCl
Li-NR Untd		P= 0.9999 NS	P<0.0001 ****	P= 0.8940 NS	P<0.0001 ****	P<0.0001 ****
Li-R Untd			P<0.0001 ****	P= 0.9251 NS	P<0.0001 ****	P<0.0001 ****
Unaff Untd				P<0.0001 ****	P= 0.6013 NS	P= 0.0022 **
Li-NR LiCl					P<0.0001 ****	p<0.0001 ****
Li-R LiCl						P= 0.0793 NS

Significance	Li-NR Untd	Li-R Untd	Unaff Untd	Li-NR LiCl	Li-R LiCl	Unaff LiCl
Li-NR Untd		P= 0.4206 NS	P<0.0001 ****	P=0.0460 *	P<0.0001 ****	P<0.0001 ****
Li-R Untd			P<0.0001 ****	P=0.8013 NS	P<0.0001 ****	P<0.0001 ****
Unaff Untd				P= 0.0083 **	P= 0.9979 NS	P= 0.0021 **
Li-NR LiCl					P= 0.0073 **	p<0.0001 ****
Li-R LiCl						P<0.0001 ****

Figure 6. *A* Peak Ca^{2+} levels in human neurons. *B* Resting Ca^{2+} levels in human neurons. *C* Ca^{2+} event frequency in human neurons. In total, these data mirror the transgenic CRMP2 neurons' Ca^{2+} phenotypes. Ca^{2+} signaling activity is aberrant in BD neurons, but is rescued with Li+. LiR and LiNR BD neurons have distinct differences in their Ca^{2+} signaling, which might be their pathology difference.

Identifying Calcium "Synchrony" Aberrations

Utilizing a yet to be published algorithm we developed for measuring the "synchrony" of calcium signaling within a network of neurons, we measured the "functionality" of different neuronal networks. Calcium signaling synchronization is a hallmark for seizures in epilepsy, and is considered an dysfunctional in healthy tissue.

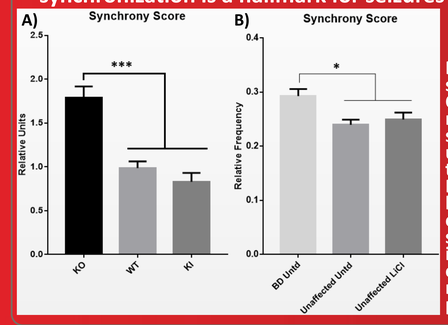


Figure 7. *A* Quantification of the Ca^{2+} signaling synchrony in transgenic CRMP2 neuronal networks. KO networks have increased Ca^{2+} synchrony, a characteristic of unhealthy tissue. *B* Quantification of the Ca^{2+} signaling synchrony in human BD neuronal networks. BD networks have increased Ca^{2+} synchrony, a characteristic of epilepsy. BD neurons' synchrony phenotype is also modeled in KO neurons, providing further evidence that CRMP2 regulation of network connectivity is central to BD pathology.

Summary

- CRMP2 inactivity (KO) plays a causative role in BD associated manic behavior, & transgenic CRMP2 KI mice model rescues BD behavior.
- CRMP2 regulates global neuronal morphology as well as local structures such as synaptic spines.
- Changes in CRMP2 activity changes the proteomic profiles of neurites and spines. These changes impact major biochemical pathways in neurites, specifically calcium signaling.
- The levels of calcium channel protein CaV2.2 is altered by CRMP2 activity.
- CRMP2 activity alters multiple important calcium signaling parameters in neurons, with CRMP2 KO neurons displaying calcium hyperactivity.
- Counter intuitively to the hyperactivity observed in CRMP2 calcium signaling, CRMP2 KO neurons have decreased complexity in their neuronal network signaling dynamics, implying neurons with decreased CRMP2 activity (i.e. BD) create less functional signaling networks.
- Human BD neurons' calcium kinetics mirror those of CRMP2 KO neurons, and CRMP2 KI neurons recapitulate human neurons treated with lithium.
- We identified that lack of CRMP2 activity can lead to unhealthy synchronized calcium signaling in networks, and that this phenotype is also found in BD neuronal networks.

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