Gut instincts: enteric nervous system homeostatic synaptic plasticity in Rett syndrome

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Introduction

Rett syndrome

Rett syndrome is a severe cognitive deficit disorder caused by a mutation in the X-linked MeCP2 gene. Each case is a de novo mutation.

It affects 1/13 000 girls and is the second most common cause of severe mental retardation in females (after Down syndrome).

MeCP2 is involved in maintaining the synaptic plasticity between neurons which forms the basis for learning and memory. This includes both synaptic potentiation and homeostatic synaptic plasticity (HSP).

http://www.autismunderstanding.com.au/resources/2-general-information/62-rett-syndrome

Symptoms of Rett

- slowed growth
- loss of purposeful hand movements
- GI dysfunction
- seizures
- social and language skills decline
- Lack of muscle co-ordination

Homeostatic synaptic plasticity

Homeostasis: tendency of biological systems to maintain a steady state for optimal bodily function.

Homeostatic synaptic plasticity (HSP) is a negative feedback system used by the nervous system when adjusting to excessive inhibitory or excitatory input to maintain homeostasis.

We suggest that HSP occurs not only in the central nervous system (CNS) but the enteric nervous system (ENS) as well.

Enteric nervous system

"Second brain of the body"

Controls GI activity including motility and secretion of acid, bile, and enzymes.

The neurotransmitters of the ENS are similar to those found in the CNS.

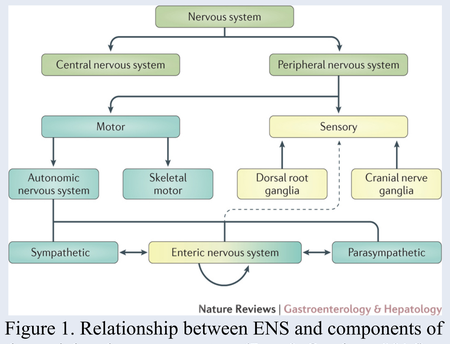

Figure 1. Relationship between ENS and components of the peripheral nervous system (Rao & Gershon, 2016).

Objectives

Purpose of this project was to document differences in enteric HSP between KO and WT mice, both *in vivo* and *in vitro*.

http://www.ozgene.com/services/knock-in-mice

KO mice : gene of interest inactivated WT mice: normal

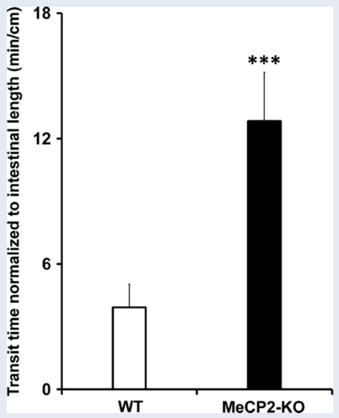

Figure 2. Transmit time (a measure of gut motility) of WT and MeCP2 KO mice, normalized to intestinal length (Wabha et al. 2016).

Enteric dysmotility in KO mirrors Rett Syndrome

- Why study the ENS?
  - MeCP2 expressed throughout ENS
  - MeCP2 dependent HSP observed in ENS
  - ENS is **100x** more pharmacologic-ally accessible than the CNS

Methods

In vitro (WT mice)

In vitro culture of dissociated ENS neurons

1. Isolate neurons from adult mouse gut
2. Plate and culture in a 96 well plate
3. Excite with 50 mM KCl
4. Stain with nNOS and image

In vivo (KO & WT mice)

Sectioning

1. Gut tissue extraction
2. Tissue fixation using 4% paraformaldehyde
3. Sectioning to obtain Myenteric plexus
4. Stain with nNOS and image

RT- PCR

1. RNA extraction
2. RT-PCR to amplify cDNA of interest (loading normalized to a control)
   - vAChT: vesicular transporter of acetylcholine
   - CalR: type of calcium binding protein
   - nNOS: nitric oxide synthase
3. Gel electrophoresis to visualize and compare amplicons

Results

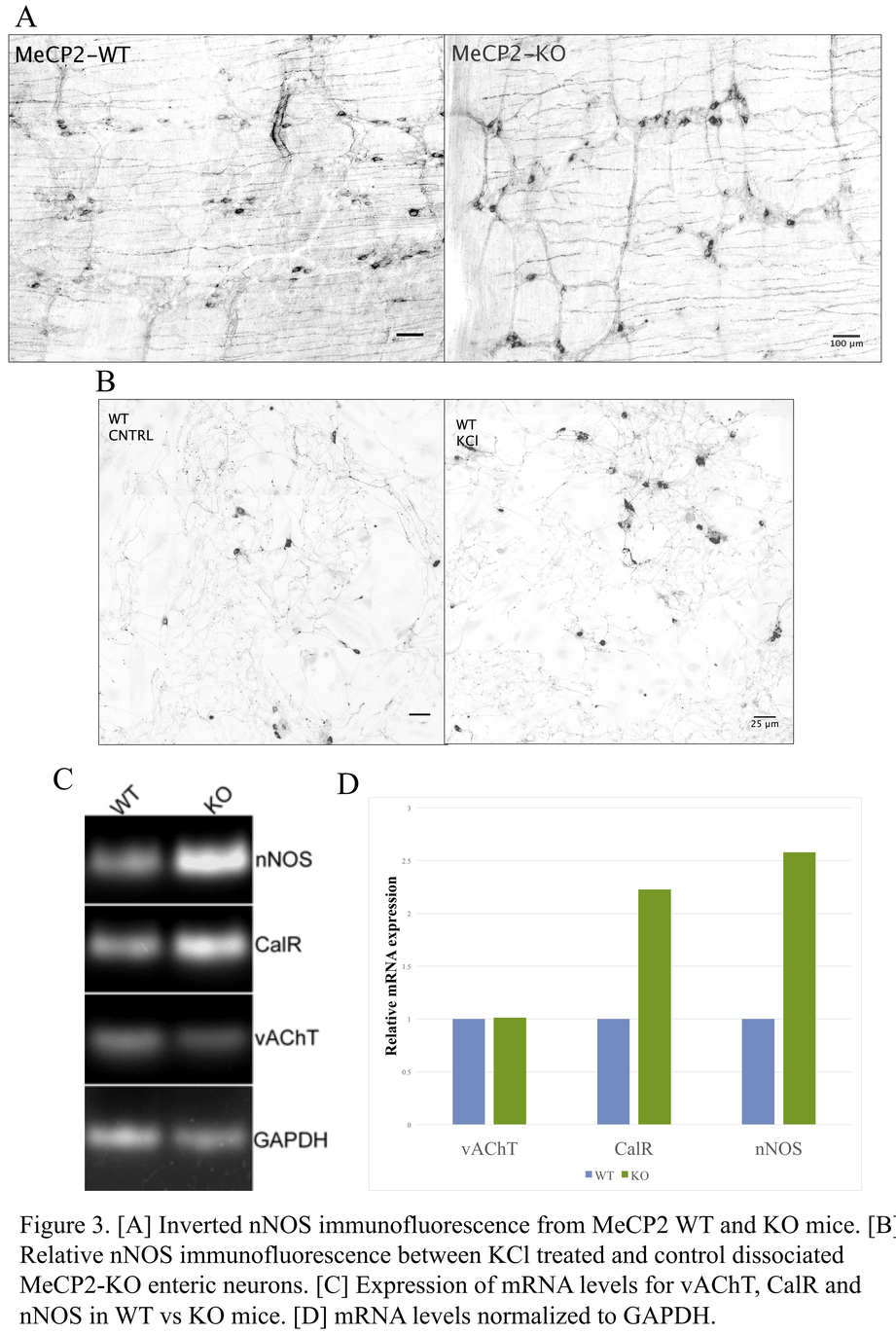

Figure 3. [A] Inverted nNOS immunofluorescence from MeCP2 WT and KO mice. [B] Relative nNOS immunofluorescence between KCl treated and control dissociated MeCP2-KO enteric neurons. [C] Expression of mRNA levels for vAChT, CalR and nNOS in WT vs KO mice. [D] mRNA levels normalized to GAPDH.

Discussion

Finding	Implication
MeCP2 KO shows relatively higher nNOS immunofluorescence as compared to WT.	This confirms previous findings from our lab where expression of nNOS was relatively higher in ENS tissue in MeCP2 KO mice as compared to WT.
Dissociated ENS neurons *in vitro* show relatively higher levels of nNOS immunofluorescence following excitation by KCl.	This supports the theory that the ENS is capable of HSP as an increase in excitatory input induced by KCl was followed by an increase in expression of nNOS which synthesizes the major enteric inhibitory neurotransmitter nitric oxide (NO).
MeCP2 KO tissue shows increased levels of mRNA for CalR and nNOS but relatively similar levels of vAChT.	This also supports the theory that changes in neurotransmitter levels in the gut of MeCP2 KO mice are responsible for the GI dysmotility seen in Rett patients.

Conclusion

These findings add to the evidence that the ENS displays altered HSP (nNOS changes following excitation) and neurotransmitter levels in MeCP2 KO tissue which might be responsible for the GI dysfunction experienced by Rett patients. Future studies to further analyze and quantify data for statistical significance can be used to eventually pave the way towards finding pharmacological treatments targeting the ENS.

Acknowledgments

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References & Contact

Amir, R. E., Van den Veyver, I. B., Wan, M., Tran, C. Q., Francke, U., & Zoghbi, H. Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nature genetics, 23(2), 185-188.

Ito-Ishida, A., Ure, K., Chen, H., Swann, J. W., & Zoghbi, H. Y. (2015). Loss of MeCP2 in parvalbumin-and somatostatin-expressing neurons in mice leads to distinct rett syndrome-like phenotypes. Neuron, 88(4), 651-658.

Rao, M., & Gershon, M. D. (2016). The bowel and beyond: the enteric nervous system in neurological disorders. *Nat Rev Gastroenterol Hepatol*, *13*(9), 517–528. Retrieved from http://dx.doi.org/10.1038/nrgastro.2016.107

Wahba, G., Schock, S. C., Claridge, E., Bettolli, M., Grynspan, D., Humphreys, P., & Staines, W. A. (2015). MeCP2 in the enteric nervous system. Neurogastroenterology & Motility, 27(8), 1156-1161.

Wahba, G., Schock, S. C., Cudd, S., Grynspan, D., Humphreys, P., & Staines, W. A. (2016). Activity and MeCP2-dependent regulation of nNOS levels in enteric neurons. Neurogastroenterology & Motility, 28(11), 1723-1730.

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