Selective serotonin reuptake inhibitor exposure *in utero* and during breastfeeding results in abnormalities in enteric nervous system development and gastrointestinal function

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Introduction

- Depression during pregnancy occurs in 14-23% of women
- Selective serotonin reuptake inhibitors (SSRIs) are first-line treatment
  - Antenatal SSRI use has increased from 1.5% to 6.4% nationally
- Good safety profile
- SSRIs cross the placenta
  - two-fold increased risk of congenital malformations
    - Alter central nervous system development
    - Alter brain circuitry
    - Maladaptive behaviors that persist into adulthood
- SSRIs inhibit the serotonin reuptake transporter (SERT)
  - Increase in serotonergic neurotransmission

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[1]Reefhuis J et al. BMJ. 2015 Jul 8;351.
[2]Gentile S. J Affect Disord. 2015 Aug 15;182:132-7.
[3]Weisskopf E et al. Expert Opin Drug Saf. 2015 Mar;14(3):413-27.

Introduction

- Serotonin plays critical roles in:
  - ENS development
  - GI motility
  - Intestinal epithelial proliferation
- Little known about the effects of ante- and post-natal SSRI exposure on subsequent ENS or GI function
  - Children exposed *in utero* to SSRIs & tricyclic antidepressants require laxatives 10-fold more often
    - SERT inhibition during development may lead to abnormal ENS development and disturbed GI motility

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[1]Gross-Margolis et al. Gut. 2014 Jun;63(6):928-37.
[2]Gross et al. Gastroenterology. 2012 Aug;143(2):408-17.
[3]Li Z, Gross-Margolis et al. J Neurosci. 2011 Jun 15;31(24):8998-9009
[4]Li Z, Gross-Margolis et al. J Neurosci. 2010 Dec 8;30(49):16730-40.
[5]Bakker, M.K. et al. *Pharmacoepidemiol Drug Saf* 19, 808-813 (2010).

Hypothesis

- Administration of an SSRI (fluoxetine) from gestation through weaning will inhibit SERT and thus enhance serotonin-mediated effects to alter ENS development.
  - The resulting ENS abnormality will lead to long-lasting changes in:
    - GI motility
    - Intestinal epithelial homeostasis

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Will fluoxetine alter ENS development and GI function?

- Dams given fluoxetine or water by oral gavage daily during pregnancy and breastfeeding
- No exposure to Fluoxetine for 3-5 weeks
- Fluoxetine-exposed and control pups examined at 6-8 weeks
  - ENS development
  - Motility
  - Intestinal epithelial homeostasis
- Concurrent experiments done with SERTKO mice
  - Rule out off-target drug effects

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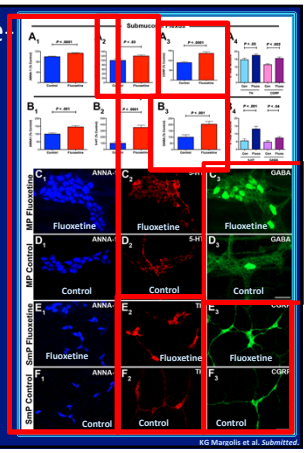
The ENS of fluoxetine-treated mice is hyperplastic

- Hyperplasia of:
  - Total neurons
  - serotonin-dependent (late-born) neurons
    - Submucosal
      - Dopaminergic
      - CGRP-expressing
    - Myenteric
      - GABAergic



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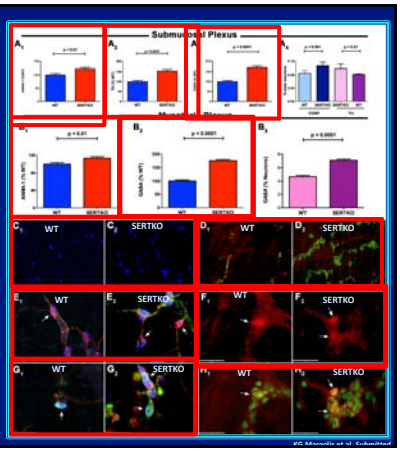
    - Total
    - GABAergic



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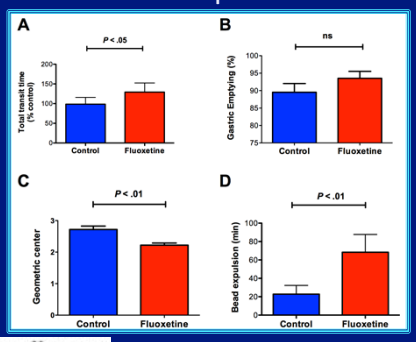
In vivo intestinal transit is slower in fluoxetine-exposed mice



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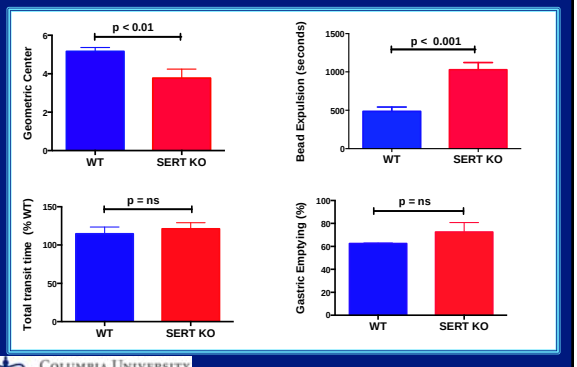
In vivo motility is slower in SERTKO mice



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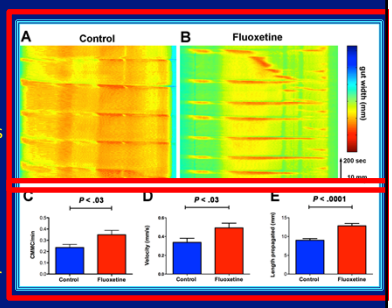
CMMCs are enhanced in fluoxetine-treated mice

- Peristaltic reflex (CMMC) measured in vitro
  - Independent of extrinsic neuronal influence
  - ENS-dependent
- Spatio-temporal maps constructed with video imaging
  - Frequency, velocity , and length of CMMCs greater in fluoxetine-treated mice
- *In vivo* motility slower
- *In vitro* motility faster



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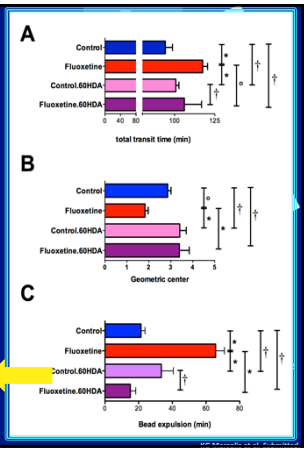
Chemical sympathectomy reverses fluoxetine-induced slow GI transit

- Exposure of mice to fluoxetine during development affects ENS and CNS.
  - Measurements of GI motility in vivo are stressful
    - likely to increase sympathetic nerve activity.
  - Sympathectomy to determine whether sympathetic nerve-mediated slowing of the gut responsible for slow transit in fluoxetine treated mice.
    - 6-OHDA administered to sympathectomize mice prior to measuring GI motility.
- Sympathectomy eliminates fluoxetine-induced slow transit.
  - Fluoxetine treatment increases sympathetic nerve activity
  - Likely a central effect

Total intestinal transit
Small intestinal motility
Colonic motility



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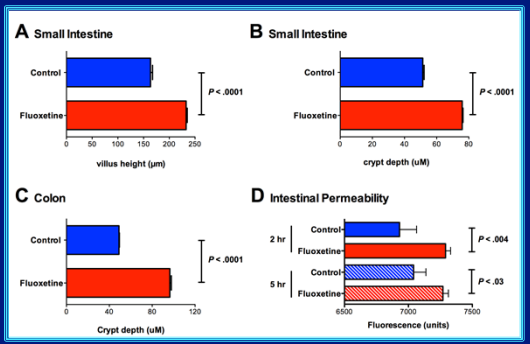
Fluoxetine treatment enhances mucosal growth and permeability



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Conclusions

- We tested the hypothesis that SERT inhibition with an SSRI (fluoxetine) during development potentiates serotonin and alters the ENS to cause long-lasting changes in GI function.
  - Fluoxetine-treatment from gestation to weaning:
    - Neuronal hyperplasia
    - Slow in vivo GI transit due to increased sympathetic discharge
    - Enhanced CMMCs in isolated bowel
    - Enhanced mucosal growth and permeability
- Similar findings in SERTKO mice
- *The coincidence of effects of fluoxetine treatment and SERTKO support the idea that serotonin and SERT are critical regulators of ENS development*

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Conclusions

- Potential effects of SSRI exposure on the developing ENS should be further investigated
- The exquisite sensitivity of ENS development to SERT activity may underlie the pathophysiology of gut-brain axis disorders

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Thank You

Columbia University Medical Center
- Michael Gershon, MD
- Sam Li, MD, PhD
- Narek Israelyan, M.S.
- Virginia Saurman
- Korey Stevanovic

- GI Division
- Joel Lavine, MD, PhD

Yale Child Study Center
- George Anderson, PhD

Grant Support
- NIH KO8
- Autism Research Institute
- Meade – Johnson
- Einhorn Charitable Trust

- NASPGHAN
- AGA
- John Driscoll Fund
- Louis Gerstner Fund
- Columbia CTC

Vanderbilt University
- Randy Blakely, MD
- Jeremy Veenstra-Vanderweele

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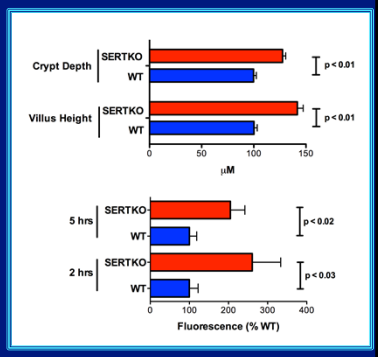
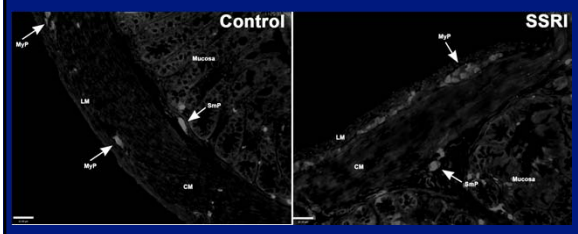
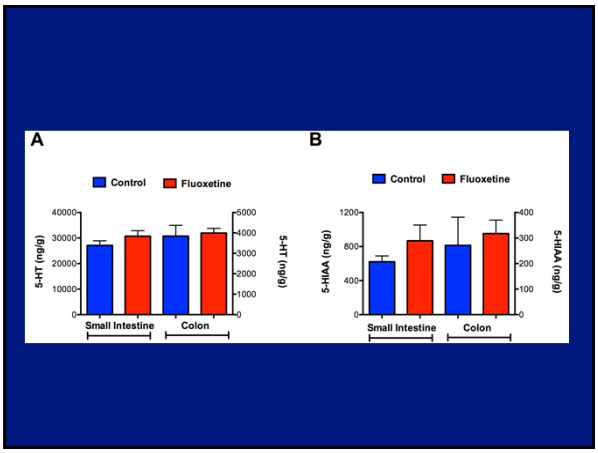
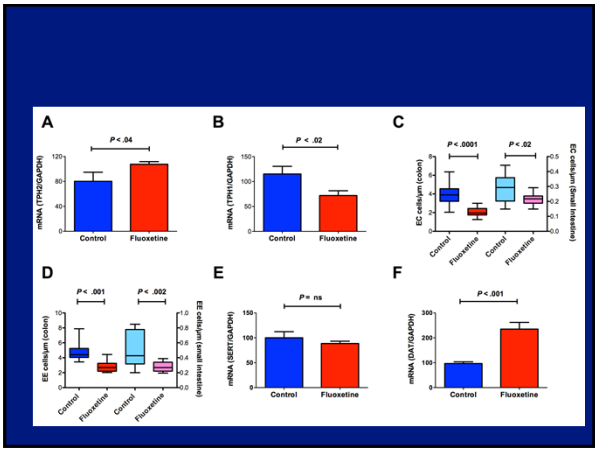
Villus height, crypt depth, and intestinal epithelial permeability are greater in SERTKO mice than WT






A
Control
Fluoxetine
5-HT (ng/g)
5-HT (ng/g)
Small Intestine
Colon
B
Control
Fluoxetine
5-HIAA (ng/g)
5-HIAA (ng/g)
Small Intestine
Colon


A
P < .04
mRNA (TPH2/GAPDH)
Control
Fluoxetine
B
P < .02
mRNA (TPH1/GAPDH)
Control
Fluoxetine
C
P < .0001
P < .02
EC cells/µm (colon)
EC cells/µm (Small Intestine)
Control
Fluoxetine
Control
Fluoxetine
D
P < .001
P < .002
EE cells/µm (colon)
EE cells/µm (small intestine)
Control
Fluoxetine
Control
Fluoxetine
E
P = ns
mRNA (SERT/GAPDH)
Control
Fluoxetine
F
P < .001
mRNA (DAT/GAPDH)
Control
Fluoxetine