# The Brain-Gut Axis: Implications in Developmental Neurogastroenterology and the Critical Roles of Serotonin

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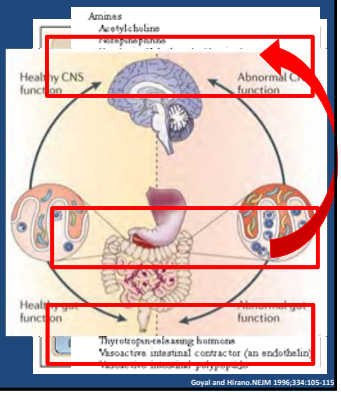
Columbia University Medical Center

## Introduction

- Similarities between the enteric nervous system and central nervous system
  - Link between brain and gut in brain-gut axis disorders
- Disorders of the brain-gut axis
  - Stress during development
  - Serotonin signaling abnormalities during development
    - Irritable bowel syndrome ( IBS)
    - Autism

## ENS: "The Brain in the Gut"

- Similar to CNS:
  - complex integrated circuits
    - Several hundred million neurons
    - reflexes
  - Same neurotransmitters
- Factors that affect CNS affect ENS
  - Genetics
  - Environment
  - Interact



# Brain-Gut Axis and Defecation Disorders

J Pediatr. 2015 Jun;166(6):1482-7.e1. doi: 10.1016/j.jpeds.2015.03.016.

**A multicenter study on childhood constipation and fecal incontinence: effects on quality of life.**

Kovacic K[1], Sood MR[1], Mugie S[2], Di Lorenzo C[2], Nurko S[3], Heinz N[3], Ponnambalam A[4], Beesley C[1], Sanghavi R[5], Silverman AH[1].

⊕ Author information

J Pediatr Psychol. 2015 Sep;40(8):814-24. doi: 10.1093/jpepsy/jsv028. Epub 2015 Apr 2.

**Measuring Health-Related Quality of Life With the Parental Opinions of Pediatric Constipation Questionnaire.**

Silverman AH[1], Berlin KS[2], Di Lorenzo C[3], Nurko S[4], Kamody RC[5], Ponnambalam A[6], Mugie S[3], Gorges C[7], Sanghavi R[8], Sood MR[7].

⊕ Author information

J Pediatr Gastroenterol Nutr. 2015 Oct;61(4):384-92. doi: 10.1097/MPG.0000000000000882.

**Stressful Life Events in Children With Functional Defecation Disorders.**

Philips EM[1], Peeters B, Teeuw AH, Leenders AG, Boluyt N, Brilleslijper-Kater SN, Benninga MA.

# Brain-Gut Axis, Functional Disorders and IBS

J Acad Nutr Diet. 2014 Mar;114(3):403-13. doi: 10.1016/j.jand.2013.10.013. Epub 2013 Dec 19.

**Child and parent perceived food-induced gastrointestinal symptoms and quality of life in children with functional gastrointestinal disorders.**

Carlson MJ, Moore CE, Tsai CM, Shulman RJ, Chumpitazi BP.

J Pediatr Gastroenterol Nutr. 2015 Sep;61(3):323-9. doi: 10.1097/MPG.0000000000000795.

**Symptom Profiles in Patients With Irritable Bowel Syndrome or Functional Abdominal Pain Compared With Healthy Controls.**

Varni JW[1], Shulman RJ, Self MM, Nurko S, Saps M, Saeed SA, Bendo CB, Patel AS, Dark CV, Zacur GM, Pohl JF; Pediatric Quality of Life Inventory Gastrointestinal Symptoms Module Testing Study Consortium.

⊕ Author information

J Pediatr. 2014 Jul;165(1):85-91.e1. doi: 10.1016/j.jpeds.2014.02.063. Epub 2014 Apr 14.

**Relationship of gastrointestinal symptoms and psychosocial distress to gastric retention in children.**

Wong GK[1], Shulman RJ[2], Malaty HM[3], Czyzewski D[1], Seghers VJ[1], Thompson D[4], Chumpitazi BP[5].

⊕ Author information

*The American Journal of Gastroenterology* **109**, 1350-1365 (September 2014)

**Effect of Antidepressants and Psychological Therapies, Including Hypnotherapy, in Irritable Bowel Syndrome: Systematic Review and Meta-Analysis**

Alexander C Ford, Eamonn M M Quigley, Brian E Lacy, Anthony J Lembo, Yuri A Saito, Lawrence R Schiller, Edy E Soffer, Brennan M R Spiegel and Paul Moayyedi

J Pediatr Gastroenterol Nutr. 2014 Sep;59(3):280-7. doi: 10.1097/MPG.0000000000000445.

**Psychotropic medications for pediatric functional gastrointestinal disorders.**

Hussain SZ[1], Hyman PE.

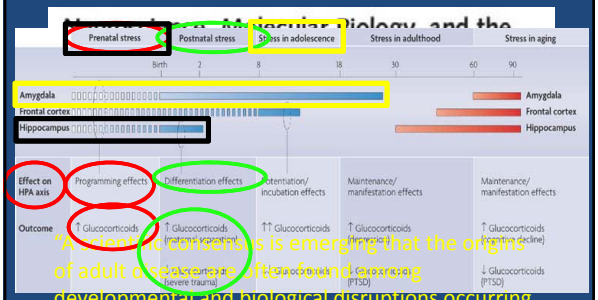
⊕ Author information

Am J Clin Hypn. 2015;58(1):5-21. doi: 10.1080/00029157.2015.1012705.

**Hypnosis and Guided Imagery Treatment for Gastrointestinal Disorders: Experience With Scripted Protocols Developed at the University of North Carolina.**

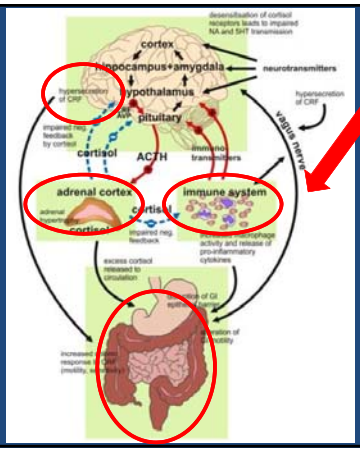
Palsson OS[1], van Tilburg M.

## Developmental Impact




"A coherent framework is emerging that the origins of adult disease and ill-health are found in developmental and biological disruptions occurring in the early years of life "

*Lupien S et al. Nature Reviews Neuroscience* **10**, 434-445 *JAMA*. 2009;301(21):2252-2259.




## Stress Alters the Brain-Gut Axis

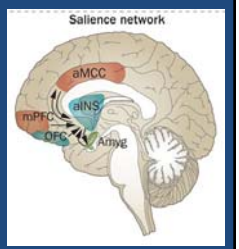
## Altered neural circuity in the gut-brain axis in IBS

Nat Rev Gastroenterol Hepatol. 2015 Oct;12(10):592-605. doi: 10.1038/nrgastro.2015.121. Epub 2015 Aug

**Towards a systems view of IBS.**

Mayer EA[1], Labus JS[1], Tillisch K[2], Cole SW[1], Baldi P[3].

- Neuroimaging studies identify key areas in CNS in IBS
- Salience network
  - Intrinsic brain network
    - Brain: anxiety, poor coping
    - Gut: chronic abdominal pain, visceral hypersensitivity, altered bowel habits
    - Extensive connections to hypothalamus (amongst others)



## fMRI & the Salience Network in Children

**Excessive coupling of the salience network with intrinsic neurocognitive brain networks during rectal distension in adolescents with irritable bowel syndrome: a preliminary report**

Xiaolin Liu,[1] Alan Silverman,[2] Mark Kern,[3] B. Douglas Ward,[1] Shi-Jiang Li,[1] Reza Shaker,[3] Manu R. Sood[2]

- Brain responses to rectal distension similar between adolescents and adults with IBS
- Excessive coupling of the salience network with the major networks
  - emotion and pain perception

**Reduced Functional Connectivity Between the Hypothalamus and Cortex in Pediatric Patients with Irritable Bowel Syndrome**

Manu R. Sood, Xiaolin Liu[1], Gisela Chelimsky, Douglas Ward[1], Shi-Jiang Li[1], Reza Shaker[2]

- IBS patients demonstrate reduced hypothalamus connectivity
- Disrupted hypothalamus functioning may contribute to IBS

## Stress Impacts Brain Development Similarly in Mice and Humans

Neuroimage. 2014 Feb 15;87:403-15. doi: 10.1016/j.neuroimage.2013.09.050. Epub 2013 Sep 29.

**Distributed BOLD and CBV-weighted resting-state networks in the mouse brain.**

Sforazzini F[1], Schwarz AJ[2], Galbusera A[3], Bifone A[3], Gozzi A[4].

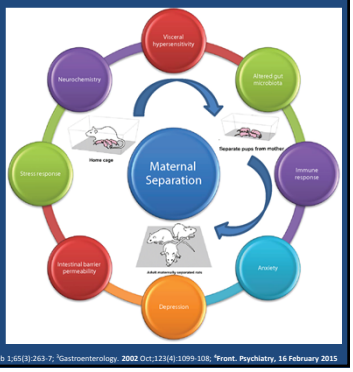
Neuroscience. 2014 May 16;267:252-62. doi: 10.1016/j.neuroscience.2014.01.064. Epub 2014 Feb 8.

**Differential activation of the prefrontal cortex and amygdala following psychological stress and colorectal distension in the maternally separated rat.**

Felice VD[1], Gibney SM[2], Gosselin RD[2], Dinan TG[3], O'Mahony SM[4], Cryan JF[1].

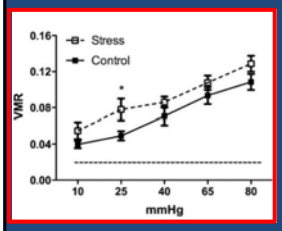
## Maternal Separation Impacts IBS

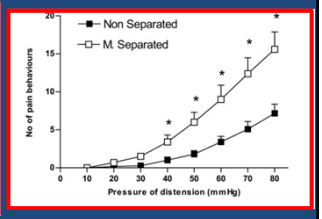
- Altered brain signaling
  - Enhanced stress response
- Altered gut function
  - Colonic transit
  - Intestinal permeability
  - Visceral hypersensitivity




[1]Neuroimmunomodulation. **2006**;13(2):82-8; [2]Biol Psychiatry. 2009 Feb 1;65(3):263-7; [3]Gastroenterology. **2002** Oct;123(4):1099-108; [4]**Front. Psychiatry, 16 February 2015**

## Maternal Separation Results in Visceral Hypersensitivity



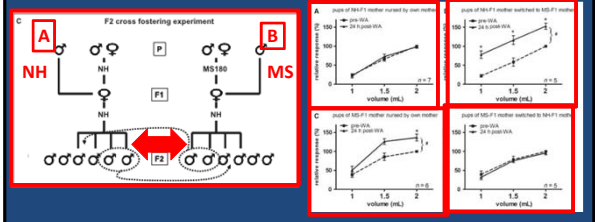

Neurosci Lett. 2012 Mar 23;512(2):99-102.

## Environmental Stress and Development

Neurogastroenterol Motil. 2013 Dec;25(12):e780-90. doi: 10.1111/nmo.12202. Epub 2013 Aug 21.

**Susceptibility to stress induced visceral hypersensitivity in maternally separated rats is transferred across generations.**

van den Wijngaard RM[1], Stanisor OI, van Diest SA, Welting O, Wouters MM, Cailotto C, de Jonge WJ, Boeckxstaens GE.




- Stress-induced visceral hypersensitivity can be transmitted across generations without further exposure
- Maternal care crucial

## Autism Spectrum Disorder (ASD)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Review Article

Gastrointestinal Symptoms in Autism Spectrum Disorder: A Meta-analysis

Barbara O. McElhanon, MD[a], Courtney McCracken, PhD[a], Saul Karpen, MD, PhD[a], and William G. Sharp, PhD[a,b]

- Huge number of GI disorders!

A brain-gut axis disorder???

## Brain-Gut Links in ASD

- Genetic
  - c-Met
  - CHD8
- Serotonin
  - Genetic
    - SERT G56A (genetic defect in serotonin reuptake)
  - Environmental
    - Selective Serotonin Reuptake Inhibitors (SSRIs)

[1]8 McElhanon et al. Pediatrics 2014;133;872; [2]Mekinian A et al. Ann Rheum Dis 2013 Feb;72(2):217-22.[3]Hsiao EY et al. Cell 2013 Dec 19;155(7):1451-63;[4]Finegold SM et al. Anaerobe.2010;16(4):444-453. [5]Finegold SM et al. Med Hypotheses. 2011 Aug;77(2):270-4. [6]Williams BL et al. MBio. 2012 Jan 10;3(1). [7]Heijtz RD et al. PNAS 2011; 108: 3047-52. [8]Bravo JA et al. Proc Natl Acad Sci U S A 2011; 108: 16050–5. [9]Williams BL et al. PLoS One. 2011;6(9):e24585. [10]Kushak RI et al. Autism. 2011 May;15(3):285-94.

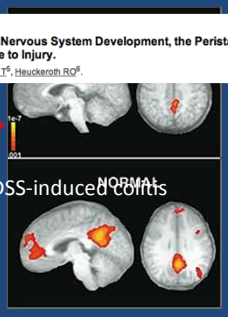
## c-MET as a Brain-Gut Connection in Autism

J Neurosci. 2015 Aug 19;35(33):11543-58. doi: 10.1523/JNEUROSCI.5267-14.2015.

**Hepatocyte Growth Factor and MET Support Mouse Enteric Nervous System Development, the Peristaltic Response, and Intestinal Epithelial Proliferation in Response to Injury.**

Avetisyan M[1], Wang H[1], Schill EM[1], Bery S[2], Grider JR[3], Hassell JA[4], Stappenbeck T[5], Heuckeroth RO[6].

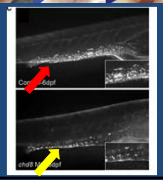
- MET variants more common in ASD
- Expression altered in the brains of people with autism
- Altered c-Met
- *c-Met* promoter variant rs1858830[7]
  - Altered peristalsis
  - Increased susceptibility to DSS-induced colitis
  - single nucleotide polymorphism that increases the risk for ASD
    - distinctively associated with individuals with ASD & GI dysfunction




[1] Rudie J.D. *et al. Neuron* **75**, 904-915 (2012); [2] Campbell D.B. *et al. Proc. Natl. Acad. Sci. U. S. A.* **103**, 16834-16839 (2006); [3]Campbell DB et al. Pediatrics 2009; 123: 1018–24; [4] Campbell D.B. *et al. Ann. Neurol.* **62**, 243-250 (2007) [7] Avetisyan M et al. J Neurosci. 2015 Aug 19;35(33):11543-58.

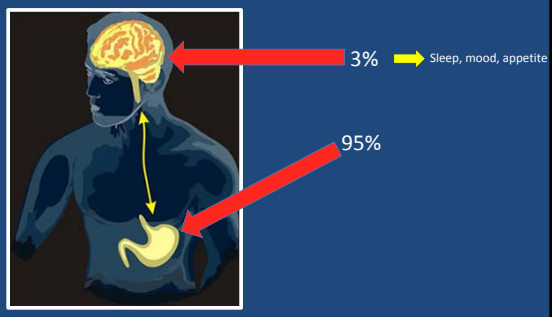
## CHD8 Mutations Define a Subtype of Autism in Early Development

- Chromodomain Helicase DNA Binding Protein 8
  - Vertebrate early development
- First mutation to demonstrate direct link with autism subtype
- 6,176 children with ASD
- 15 had a CHD8 mutation
  - All had similar characteristics in appearance
    - Large heads and wide-set eyes
- Interviewed families
  - Gastrointestinal problems
- Disrupted CHD8 gene in zebrafish
  - Developed large heads & wide set eyes
  - Fewer enteric neurons
  - Constipated



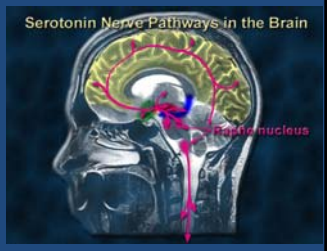
Bernier a et al. Cell. 2014 Jul 17;158(2):263-76.

## Serotonin Connects the Brain and Gut in Autism




## Serotonin is Critical for Brain Development

- One of the most widely distributed & earliest systems to develop
  - Innervates almost all areas of the brain
  - Serotonergic neurons in human brain from fifth gestational week




[1]*Azmitia EC et al. J Clin Psychiatr* 1991, 52(Suppl):4-16. [2] Sundstrom E et al.*Brain Res Dev* 1993, 75:1-12 [3] Lauder JM et al. *Dev Neurosci* 1978, 1:15-30. [4] Gaspar P et al. *Nat Rev Neurosci* 2003, 4:1002-1012.

## Serotonin Signaling is Abnormal in the CNS in Autism

- Important for pre- and postnatal human brain development
  - Changes in serotonergic signaling associated with ASD
    - Increased # serotonin axon branching in temporal cortex
- PET scans show diminished serotonin synthesis


[1]Chugani DC. *Mol Psychiatr* 2002, 7(Suppl 2):S16-S17. [2]Chandana SR et al. *Int J Dev Neurosci* 2005, 23:171- 182.
[3] Chugani DC et al. *Ann Neurol* 1999, 45:287-295 [4] Rosemeyer R. *Molecular Autism* 2013, 4:37

## Serotonin is Critical for Gut Function!

- Serotonin inactivation is S
  - 95% of the body's serotonin is located in the intestines!
- Similar critical role in gut & brain
  - Early development
  - Throughout lifespan
  - Critical mediator
    - Motility
    - Secretion
    - Epithelial permeability
    - Epithelial maintenance
- Once serotonin is released and performs its functions it must be inactivated
  - Taken up by the serotonin reuptake transporter (SERT)

SERT 5-HT

- Increases in SERT activ… the effects of serotoni…
- Decreases in SERT acti… the effects of serotoni…


## SERT Variants are Overexpressed in ASD

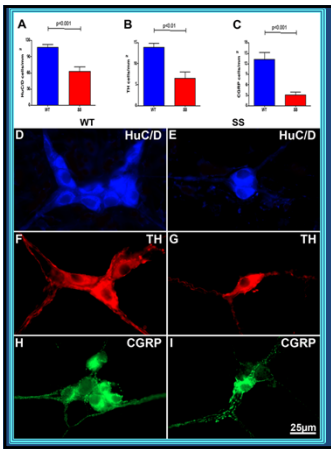
- Serotonin plays critical roles in brain & gut development and function
- GI problems 4-fold more common in children with ASD
- *Does abnormal serotonin homeostasis cause brain-gut dysfunction in ASD?*
  - GWAS: SERT variants overexpressed in ASD
    - All result in overactive serotonin transporter activity
- Most common coding variant: G56A
  - G56A transgenic mouse
    - Most common gain-of-function SERT variant in children with ASD
      - Core autism-related behavioral abnormalities
        - » Altered social function & communication, repetitive behaviors
      - Altered serotonin-related brain abnormalities

[1]Campbell DB et al. Pediatrics 2009; 123: 1018–24; [2]Russo AJ et al. Biomark Insights 2009; 4: 181–90; [3]Campbell DB et al. Ann Neurol 2007; 62: 243–50.

## Hypothesis

Genetic abnormalities in the serotonin transporter (SERT), of the kind found in autism, also cause abnormalities in gut development & function

*Could the G56A mutation be a brain-gut link in ASD?*



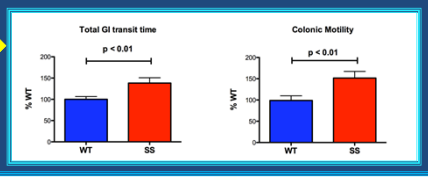

## Total and late-born enteric neurons are deficient in G56A (SuperSERT) mice

- Total neurons
  - HuC/D
- Late born neurons
  - CGRP and TH

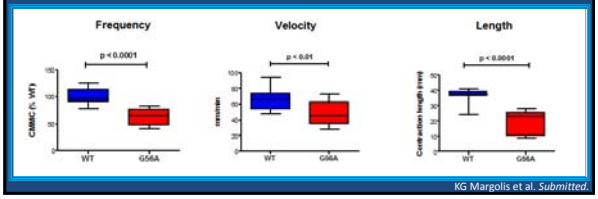
KG Margolis et al. *Submitted.*

## Motility is Diminished in G56A Mice

In Vivo



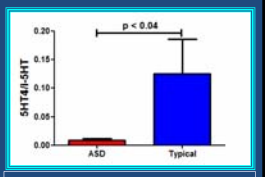

In Vitro




KG Margolis et al. *Submitted.*

## Gut Dysfunction is Preventable in the G56A Mice

- G56A mouse model represents a brain-gut link in autism:
  - ASD-associated features
  - Defects in ENS development
  - Abnormal gut movement
- Is there a way to prevent the ENS & motility defects?
- 5-$HT_4$ is a major receptor for intestinal serotonin
  - $5HT_4$ stimulation increases the number of neurons in cell culture[1]
    - G56A mice have less neurons
  - Increases gastrointestinal movement
    - G56A mice have slower GI transit
  - Treatment for chronic constipation

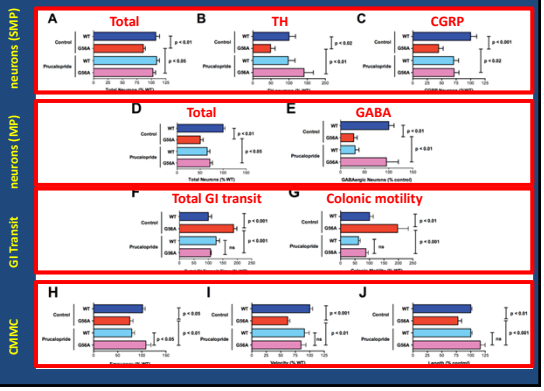



5-HT4 levels lower in children with ASD & GI dysfunction

Inflamm Bowel Dis. 2015 Apr;21(4):870-8. doi: 10.1097/MIB.0000000000000326.
**Colitis induces enteric neurogenesis through a 5-HT4-dependent mechanism.**
Belkind-Gerson J[1], Hotta R, Nagy N, Thomas AR, Graham H, Cheng L, Solorzano J, Nguyen D, Kamionek M, Dietrich J, Cherayil BJ, Goldstein AM.

[1]Liu MT et al. J Neurosci. 2009 Aug 5;29(31):9683-99.

## Prucalopride rescues G56A mice




## Antenatal SSRI Exposure

- Selective serotonin reuptake inhibitors (SSRIs):
- SSRIs inhibit SERT
  - Increase serotonergic neurotransmission
- Used to treat depression during pregnancy
  - 6%
- 2-fold risk of congenital malformations
  - Alter CNS development
  - ? increased risk of autism in males exposed
- Effects of antenatal SSRI exposure on GI unknown
  - Children exposed *in utero* to SSRIs require laxatives 10-fold more than non-exposed
    - Exposure may lead to abnormal ENS development and GI motility

PROZAC fluoxetine hydrochloride
serzone nefazodone HCl
zoloft (sertraline HCl)
Cymbalta duloxetine HCl
LUVOX fluvoxamine maleate
Lexapro escitalopram oxalate

Reefhuis J et al. BMJ. 2015 Jul 8;351; Gentile S. J Affect Disord. 2015 Aug 15;182:132-7; Weisskopf E et al. Expert Opin Drug Saf. 2015 Mar;14(3):413-27; Gross-Margolis et al. Gut. 2014 Jun;63(6):928-37; Gross et al. Gastroenterology. 2012 Aug;143(2):408-17; Li Z, Gross-Margolis et al. J Neurosci. 2011 Jun 15;31(24):8998-9009; Li Z, Gross-Margolis et al. J Neurosci. 2010 Dec 8;30(49):16730-40; Bakker, M.K. et al. *Pharmacoepidemiol Drug Saf* 19, 808-813 (2010).

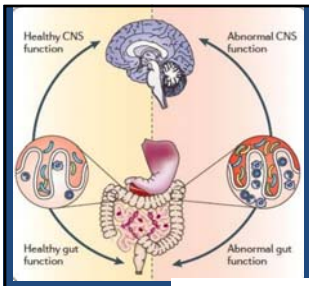
## Will Fluoxetine alter ENS development and GI function?

- Hyperplasia of the ENS
- Slower *in vivo* motility
- Faster *in vitro* motility
  - Excess sympathetic activation from the brain

## Conclusions

- Bidirectional communication between the brain & the gut
  - Starts in utero
- Developmental insults lead to brain-gut disorders
  - Present at all stages of life
    - Autism, constipation, IBS
    - Parkinson's, Alzheimers
- Serotonin plays critical roles in brain and gut development
  - Genetics, environmental
  - Critical to disorders of the brain-gut axis
- Can the brain be fixed via the gut?
  - Gut-focused target therapies

## Future Opportunities

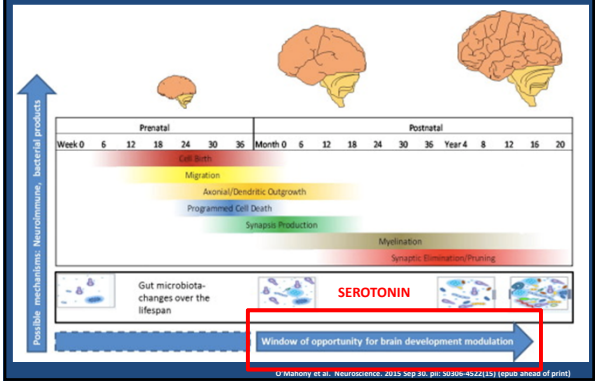



## Microbiota-Gut-Brain Axis


ORIGINAL ARTICLE
The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner


Normal gut microbiota modulates brain development and behavior

## Temporal Opportunities for Therapy




O'Mahony et al. Neuroscience. 2015 Sep 30. pii: S0306-4522(15) (epub ahead of print)


Pharmacol Biochem Behav. 2014 May;120:117-23. doi: 10.1016/j.pbb.2014.02.016. Epub 2014 Feb 26.

**Retinoids as potential targets for Alzheimer's disease.**

Sodhi RK[1], Singh N[2].

Author information

## Glia

Glia. 2015 Jun 29. doi: 10.1002/glia.22876. [Epub ahead of print]

**Enteric glia express proteolipid protein 1 and are a transcriptionally unique population of glia in the mammalian nervous system.**

Rao M[1,2,3], Nelms BD[2], Dong L[3], Salinas-Rios V[1], Rutlin M[4], Gershon MD[5], Corfas G[1,6,7].

Gut. 2010 Dec;59(12):1643-51. doi: 10.1136/gut.2010.222620.

**The brain to gut pathway: a possible route of prion transmission.**

Lawson VA[1], Furness JB, Klemm HM, Pontell L, Chan E, Hill AF, Chiocchetti R.

Mov Disord. 2015 Apr;30(4):494-8. doi: 10.1002/mds.25979. Epub 2014 Aug 7.

**Enteric glial cells: new players in Parkinson's disease?**

Clairembault T[1], Leclair-Visonneau L, Neunlist M, Derkinderen P.

## Spinal Neurons Play a Role in Brain-Gut Communication

European Journal of Pharmacology 744 (2014) 28–35

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Neuropharmacology and analgesia

NMDA receptor mediates chronic visceral pain induced by neonatal noxious somatic stimulation

Adrian Miranda [a,*], Aaron Mickle [a], Mitchell Bruckert [a], Pradeep Kannampalli [b], Banani Banerjee [b], Jyoti N. Sengupta [a,b]

## Thank You

- Gershon Laboratory
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