RUTGERS
Robert Wood Johnson
Medical School

The autism-associated 16p11.2 microdeletion variant impacts the effects of microbiota disturbances on hippocampal development and behavior throughout the lifespan

Emanuel DiCicco-Bloom, MD
Neuroscience and Cell Biology/Pediatrics
Rutgers Robert Wood Johnson Medical School

Courtney R. McDermott, PhD
Columbia University

May 28, 2025
Autism Research Institute Webinar

Rutgers, The State University of New Jersey

Autism Spectrum Disorder has a Genetic Basis

GENES ←- - -→ ENVIRONMENT

Twin Studies

61-94% concordance rate among identical/monozygotic twins

10-17% concordance rate among dizygotic/fraternal twins

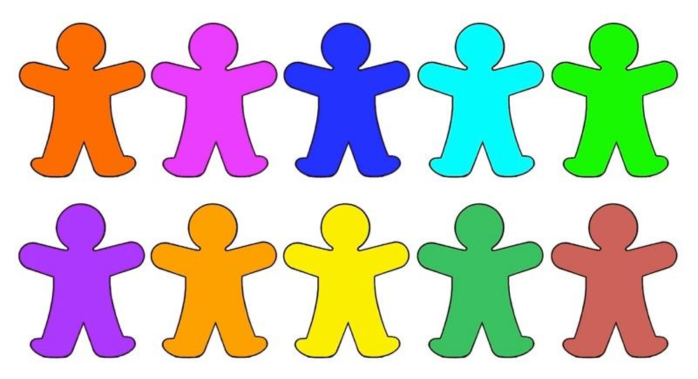
Family Studies

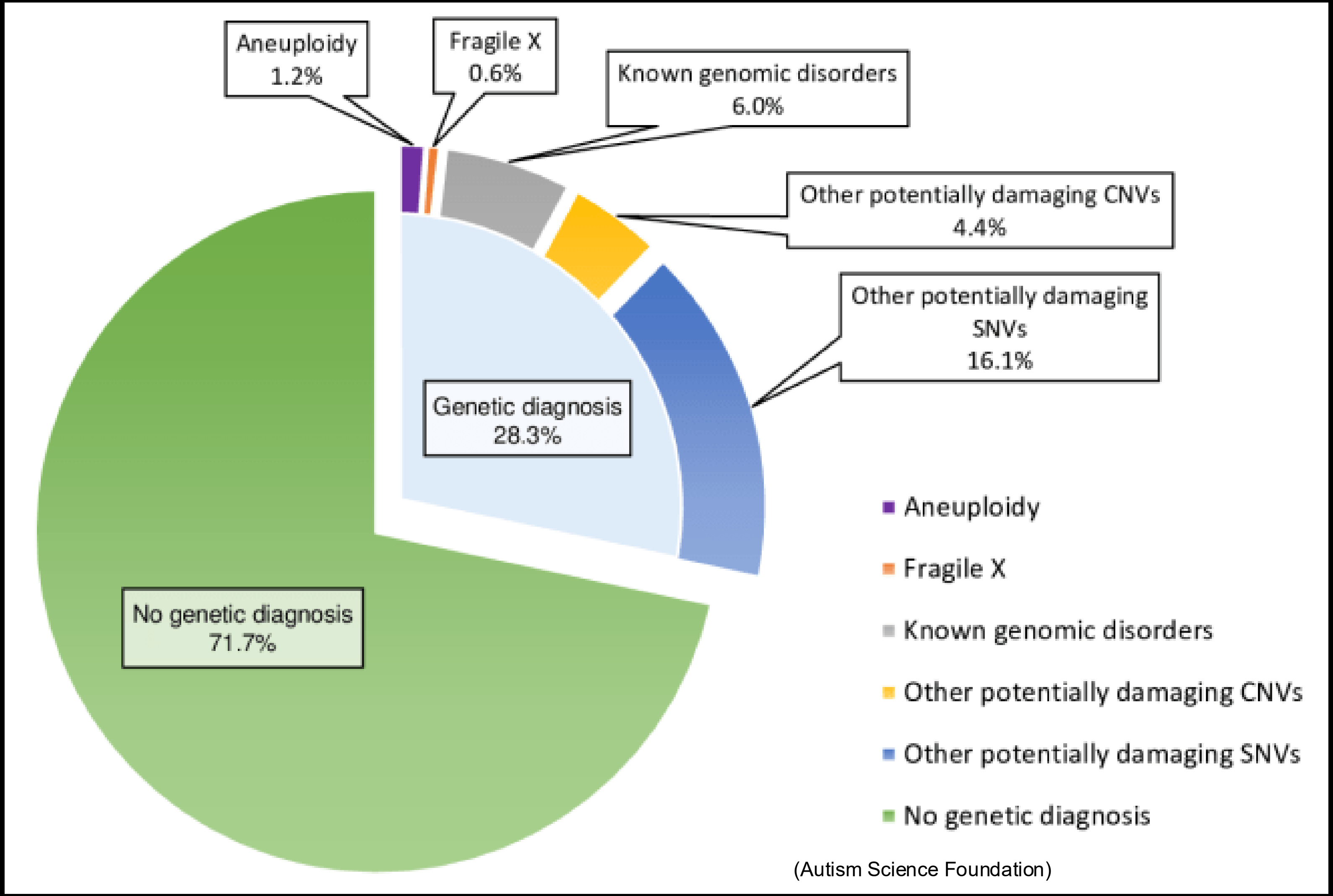
Risk of developing autism is 10 times greater for siblings of autistic individuals than for general population, occurring 10-20%

We consider the non-genetic factors to be in part environmental, that is the intrauterine environment:

Medications (Valproic acid), maternal infection, challenging delivery, very low birth weight; may be differences in placental function, maternal stress, chemicals like pesticides

There are many different genetic causes of ASD



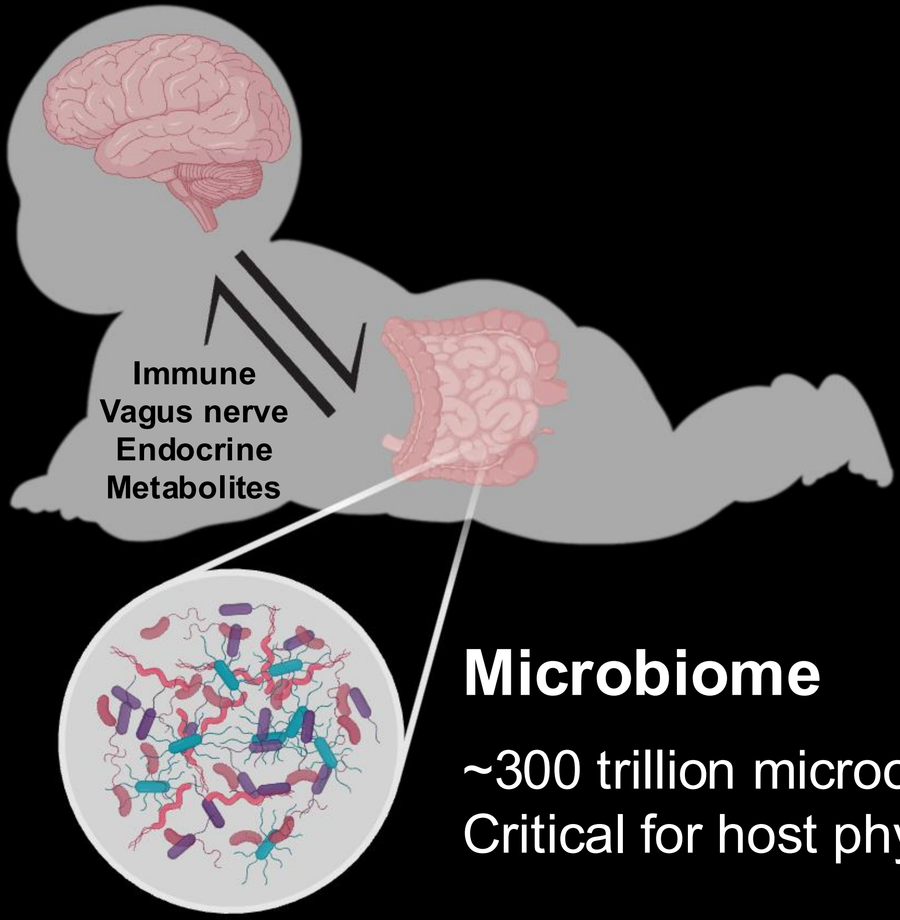


(Autism Science Foundation)

The gut microbiome and brain have heightened sensitivity to environmental factors during early life

CDC estimates that an infant will receive 2-3 courses of antibiotics

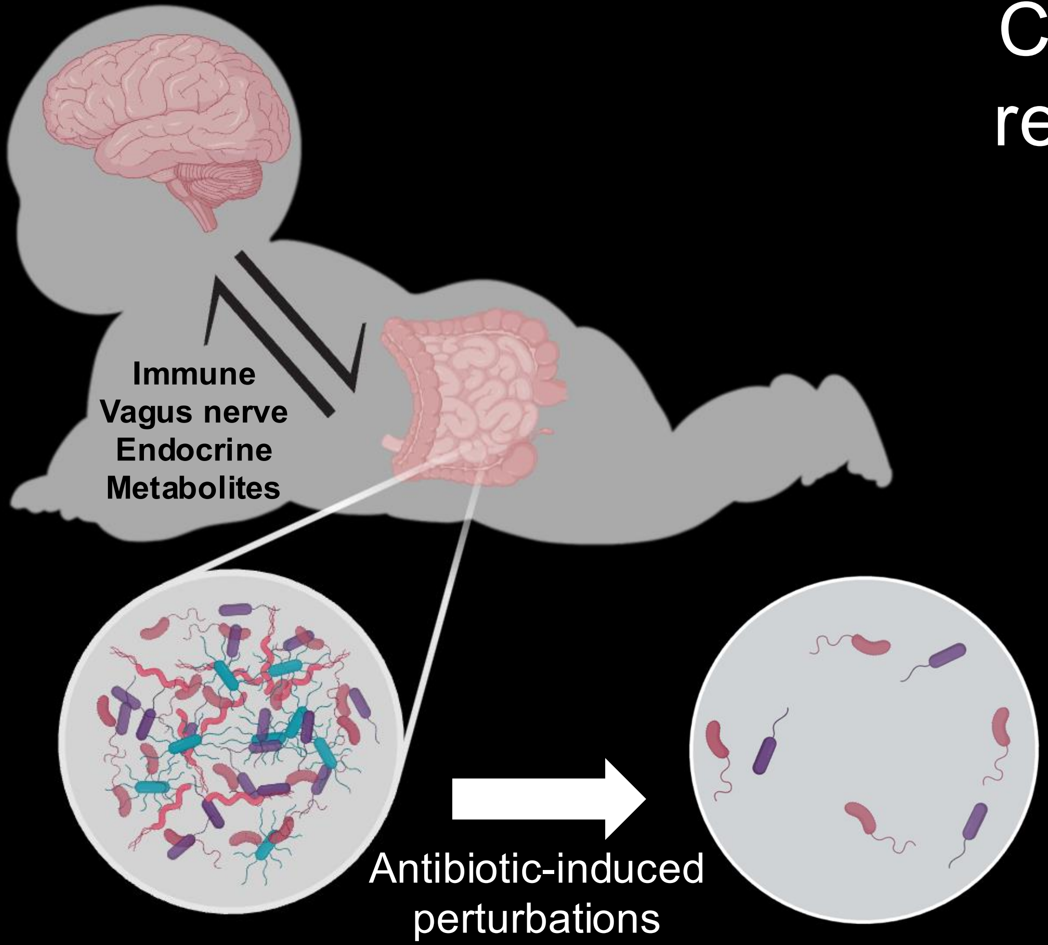
Susceptible to infections



Microbiome

~300 trillion microorganisms
Critical for host physiology and health

The gut microbiome and brain have heightened sensitivity to environmental factors during early life



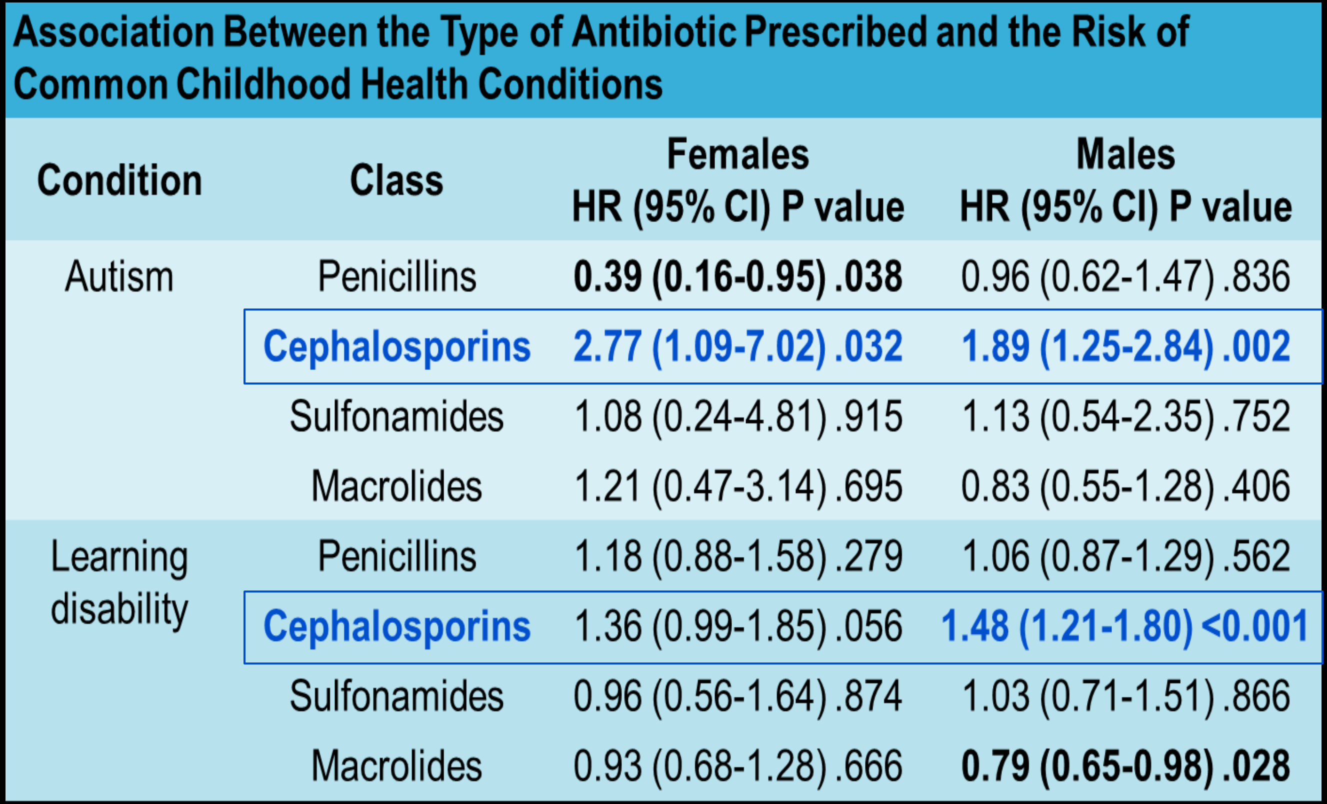
CDC estimates that an infant will receive 2-3 courses of antibiotics

Susceptible to infections

Aversa et al., (2021)
Slykerman et al., (2019)
Lavebratt et al., (2019)
Wimberley et al., (2018)

Postnatal antibiotic exposure

Infant cephalosporin antibiotic exposure is *associated* with increased autism onset



Association Between the Type of Antibiotic Prescribed and the Risk of Common Childhood Health Conditions

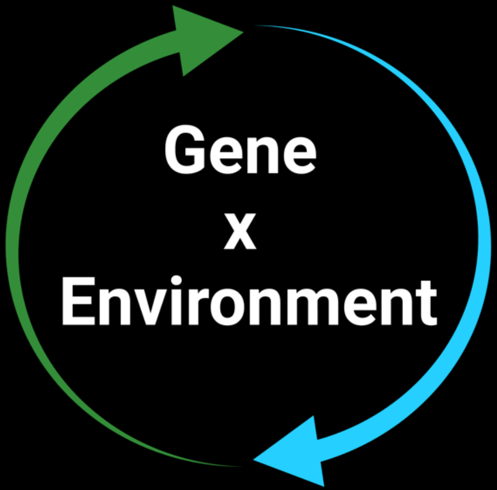
Condition	Class	Females HR (95% CI) P value	Males HR (95% CI) P value
Autism	Penicillins	**0.39 (0.16-0.95) .038**	0.96 (0.62-1.47) .836
	Cephalosporins	**2.77 (1.09-7.02) .032**	**1.89 (1.25-2.84) .002**
	Sulfonamides	1.08 (0.24-4.81) .915	1.13 (0.54-2.35) .752
	Macrolides	1.21 (0.47-3.14) .695	0.83 (0.55-1.28) .406
Learning disability	Penicillins	1.18 (0.88-1.58) .279	1.06 (0.87-1.29) .562
	Cephalosporins	1.36 (0.99-1.85) .056	**1.48 (1.21-1.80) <0.001**
	Sulfonamides	0.96 (0.56-1.64) .874	1.03 (0.71-1.51) .866
	Macrolides	0.93 (0.68-1.28) .666	**0.79 (0.65-0.98) .028**

Sample: 14,572 children (10,220 prescribed at least 1 antibiotic between birth and 2 years of age)

Aversa et al., (2021)

Not every child that is exposed to a cephalosporin antibiotic will receive an autism diagnosis

Contributing role of *genetic vulnerability*



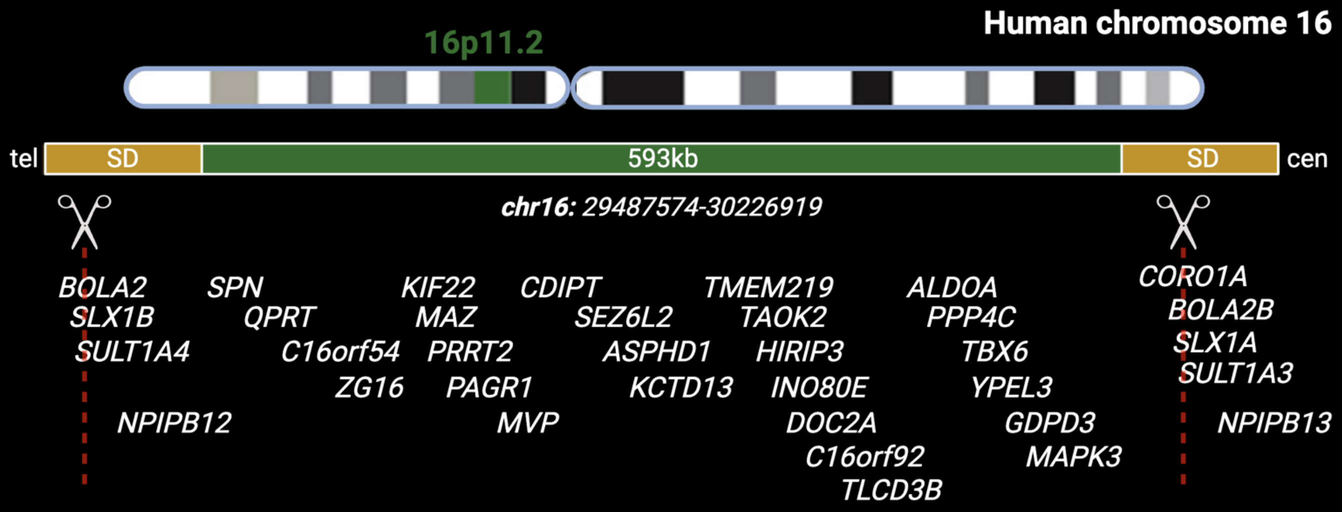
Gene
x
Environment

Modeling genetic risk: The human heterozygous 16p11.2 (16pDel) copy number variation

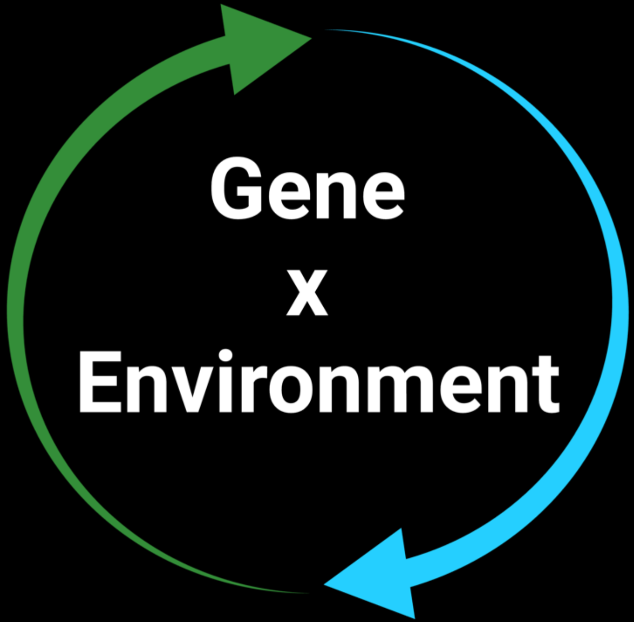
This CNV accounts for ~1% ASD cases

General population: 1-5/10,000

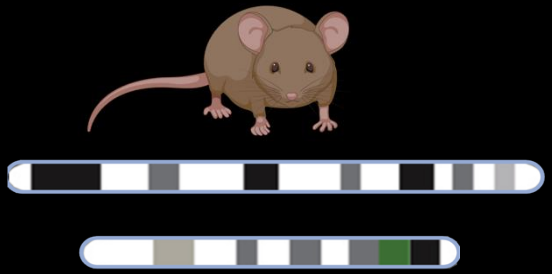
High frequencies of ASD, ADHD, anxiety & mood disorders



Taylor et al., (2021) *GeneReviews®*;
Niarchou et al., (2019); Tai et al., (2022)



Gene x Environment



Chung et al., (2021)
Niarchou et al., (2019)
Duyzend & Eichler et al., (2015)
Blumenthal et al., (2014)

16p11.2 microdeletion copy number variation

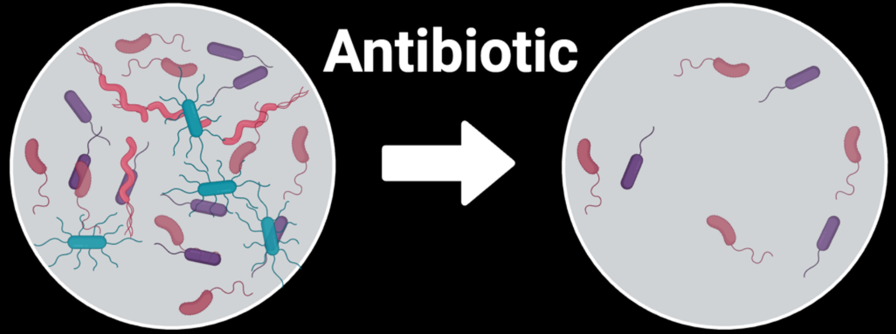
Accounts for ~1% ASD cases

Aversa et al., (2021)
Slykerman et al., (2019)
Lavebratt et al., (2019)
Wimberley et al., (2018)

Postnatal antibiotic exposure

Association with NDDs

Warrants investigation of underlying biological mechanisms

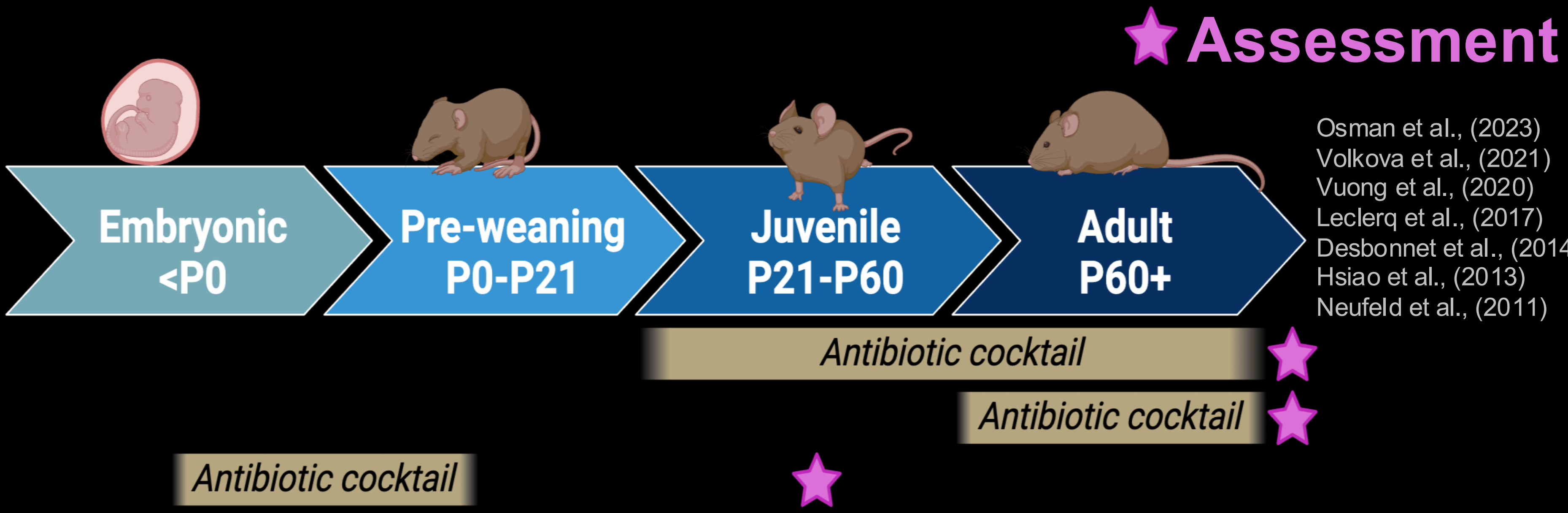


Antibiotic

Pre-exposure

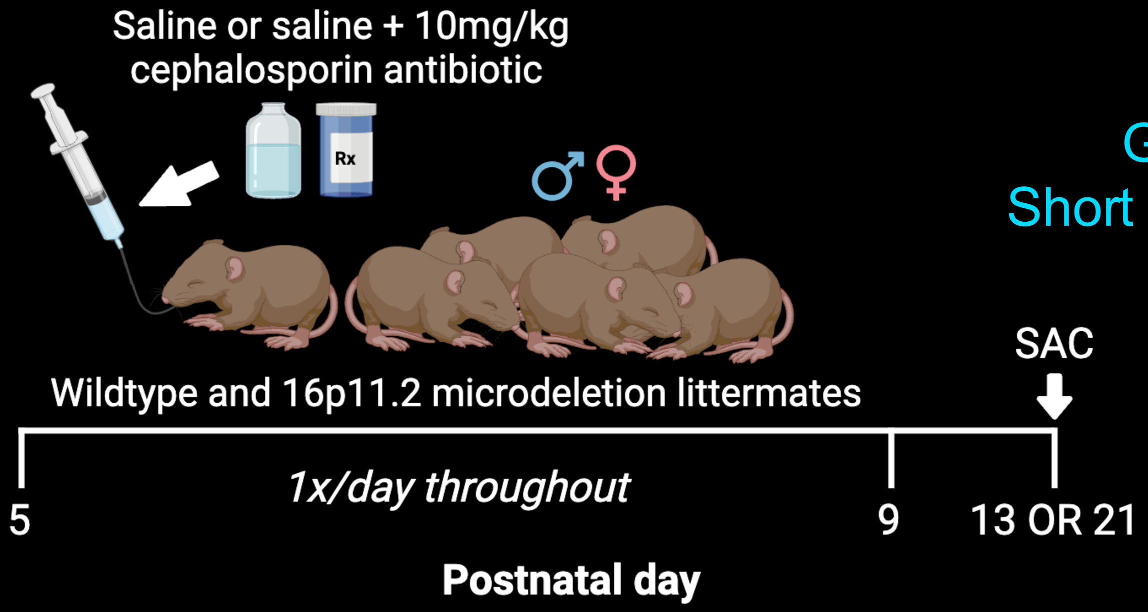
Post-exposure

Previous research



Osman et al., (2023)
Volkova et al., (2021)
Vuong et al., (2020)
Leclerq et al., (2017)
Desbonnet et al., (2014)
Hsiao et al., (2013)
Neufeld et al., (2011)

Our model

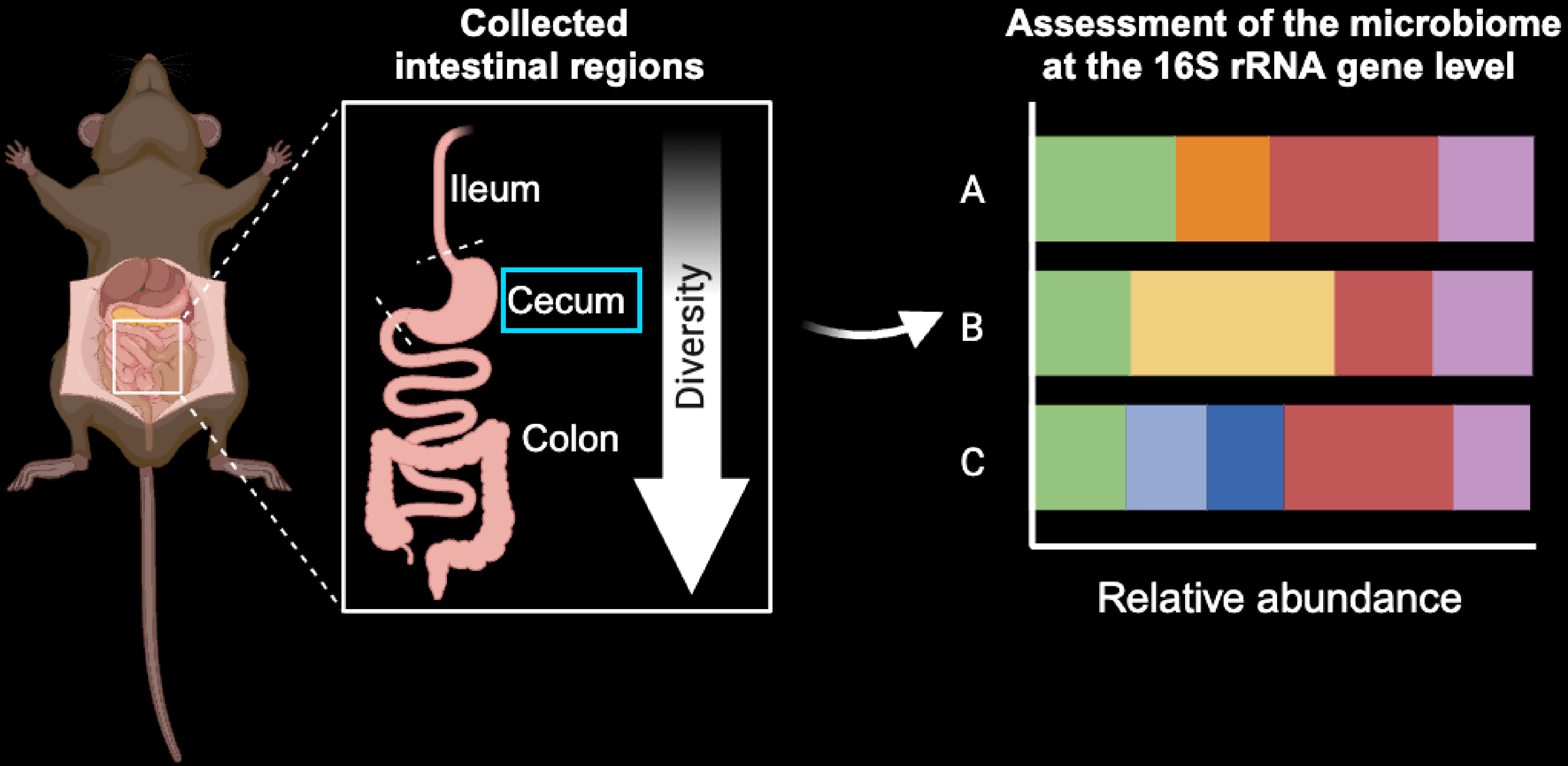


Single antibiotic
Genetic vulnerability
Short term, early life exposure

Hypothesis:

Early life antibiotic exposure will perturb the gut microbiome and *selectively* dysregulate hippocampal development and behavior in 16pDel mice

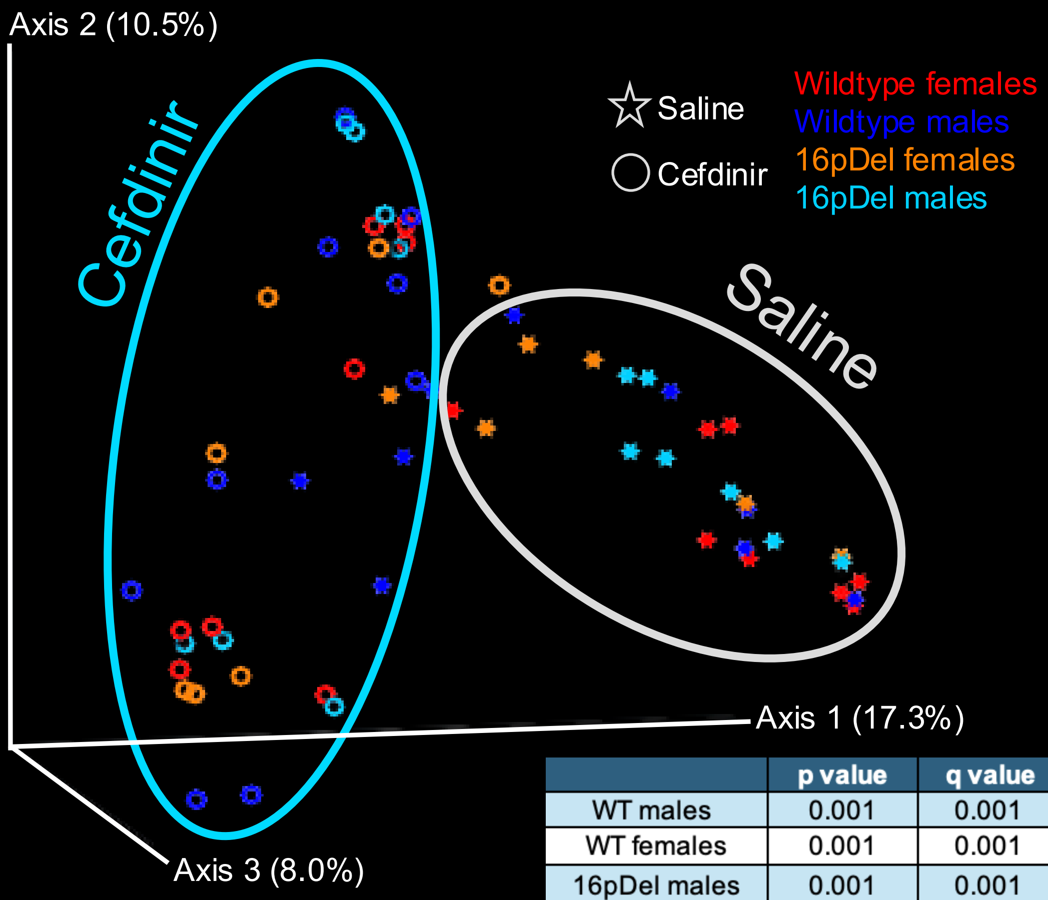
Brief overview of gut microbiome workflow



Early life cefdinir exposure successfully perturbed the gut microbiome

↓ *Streptococcus* ↑ *Enterococcus*

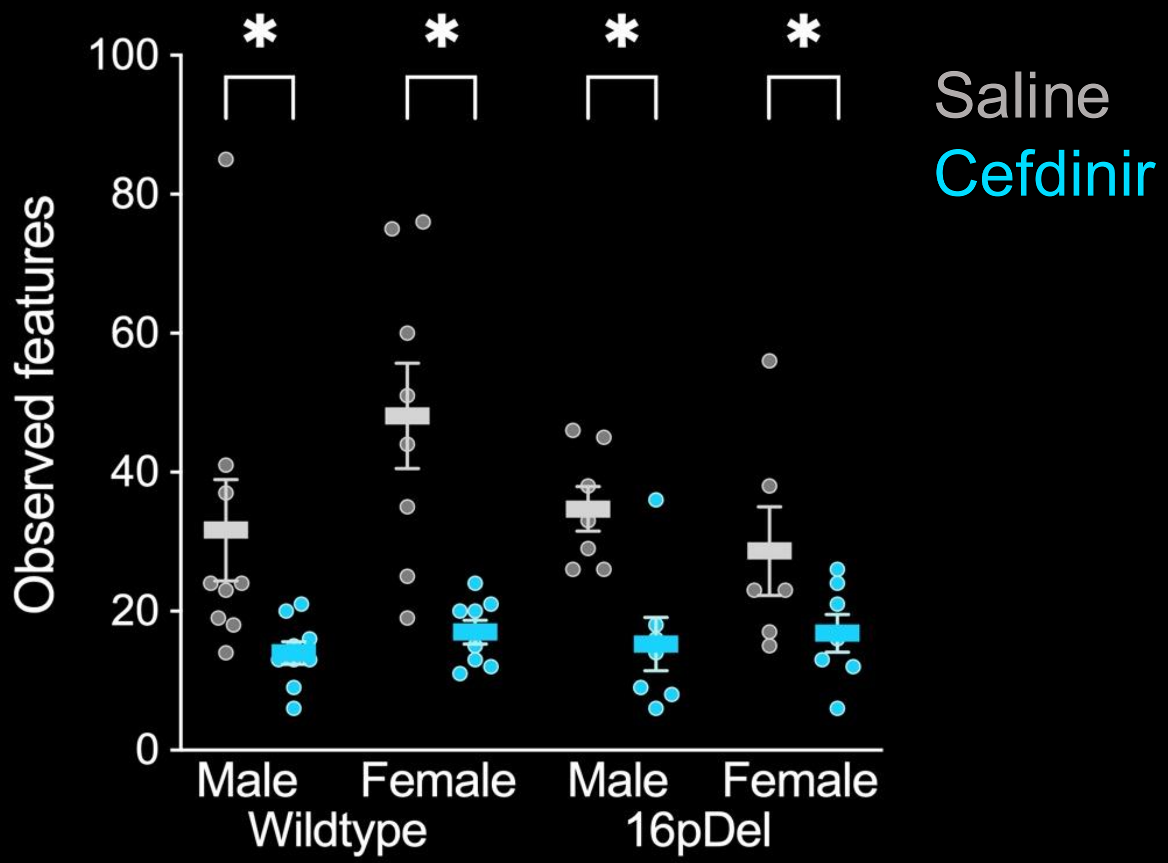
Beta diversity: Jaccard distances



	p value	q value
WT males	0.001	0.001
WT females	0.001	0.001
16pDel males	0.001	0.001
16pDel females	0.004	0.004

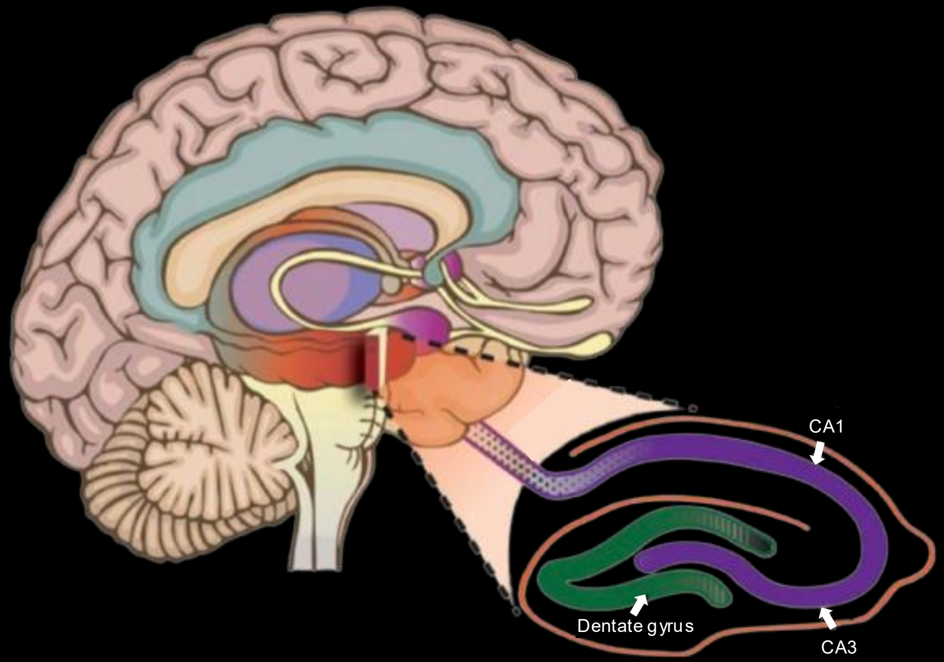
P13 cecum, 4 litters/treatment
Analysis performed by Dr. Zhan Gao, Blaser lab

Alpha diversity: Bacterial richness

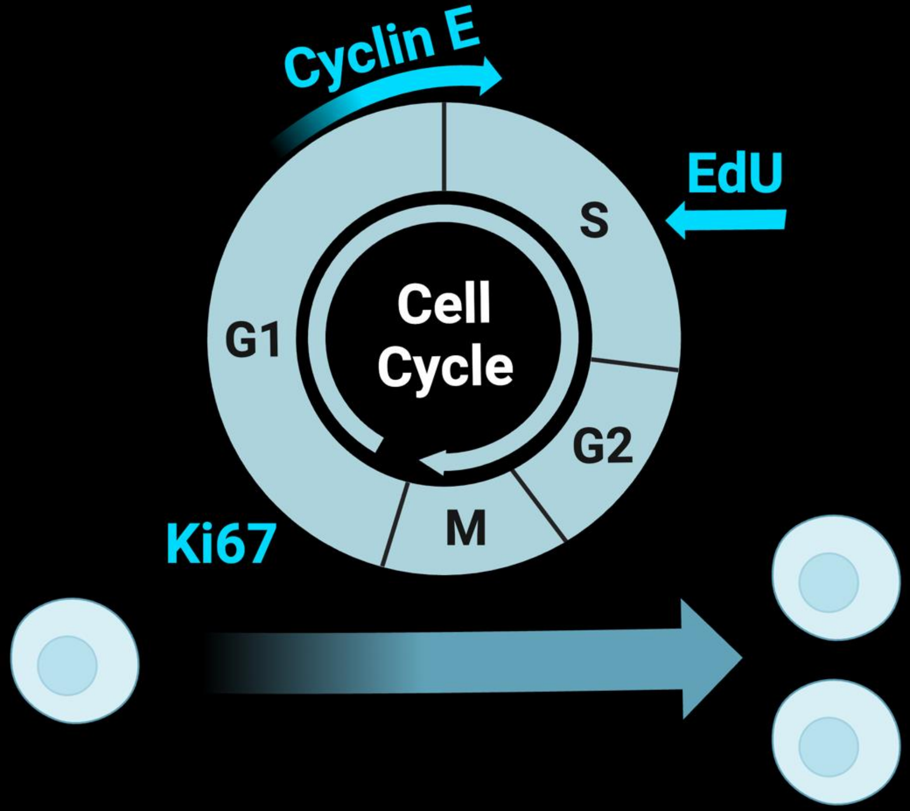


Similar results were observed in the ileum and colon

Does early life cefdinir exposure alter hippocampal proliferation?

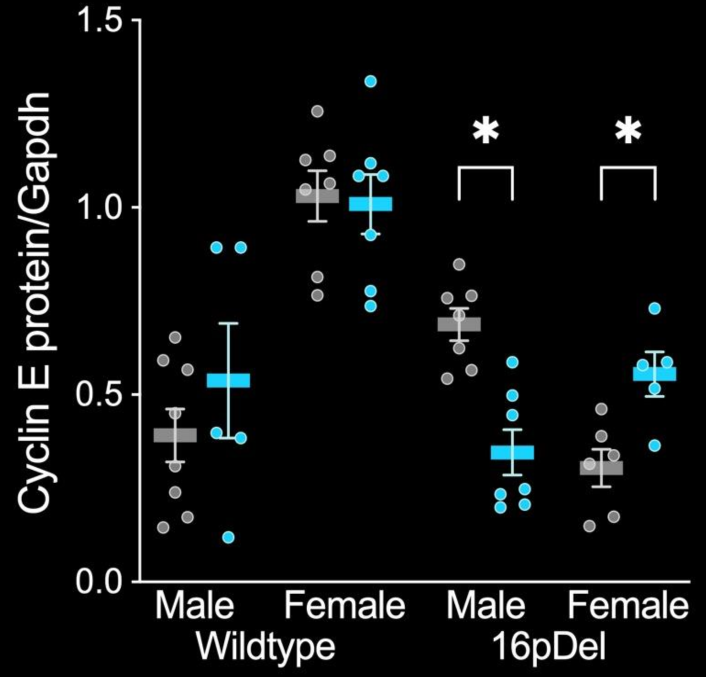


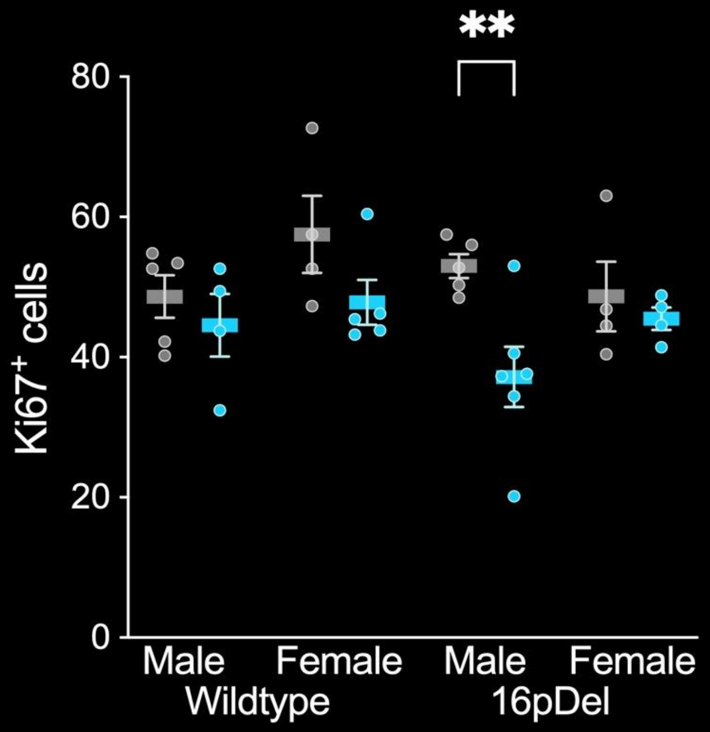
Critical for learning & memory
Implicated in NDD pathogenesis
Sensitive to genetic & environmental factors

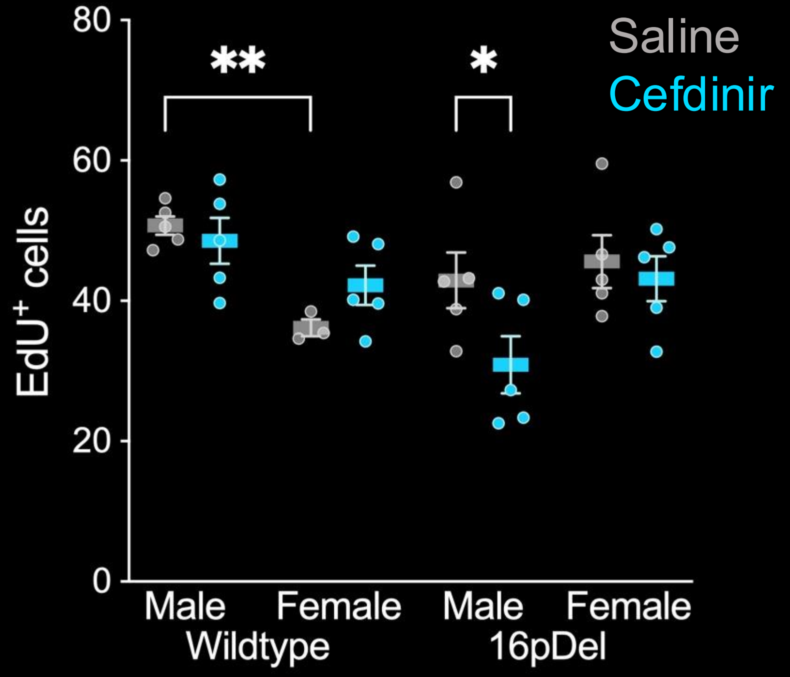


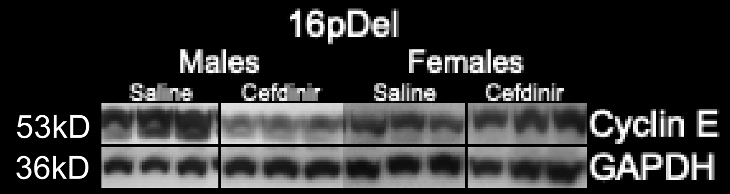
DiCicco-Bloom & McDermott (2024); Willsey et al., (2021)
Obiorah et al., (2015); Wegiel et al., (2011); Plessen et al., (2006)

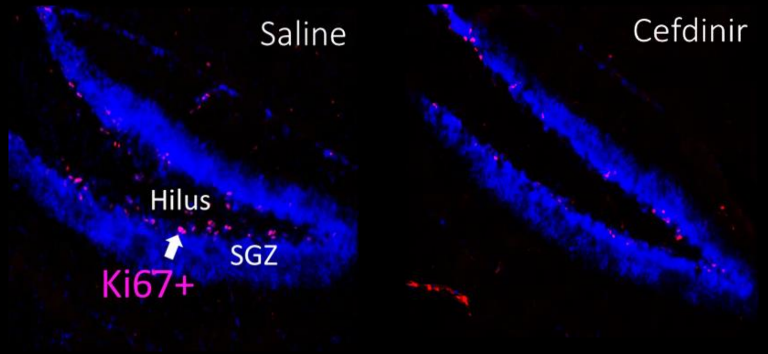
Hippocampal proliferation is selectively reduced in cefdinir-exposed 16pDel mice

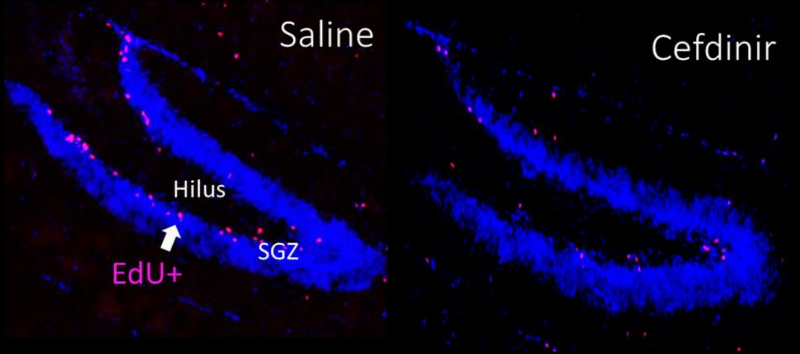






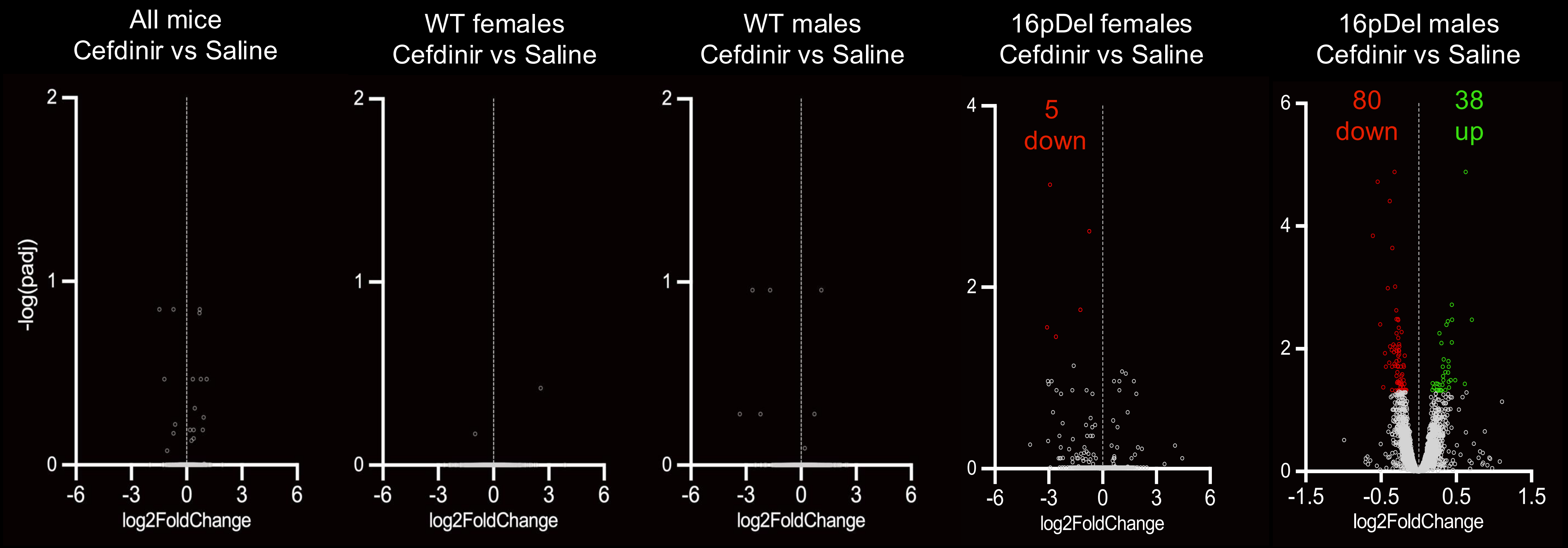






P13 hippocampus, 4 litters/treatment
Anya Mirmajlesi and Katherine Kimbark

Hippocampal gene expression is altered in cefdinir-exposed 16pDel mice


All mice
Cefdinir vs Saline

WT females
Cefdinir vs Saline

WT males
Cefdinir vs Saline

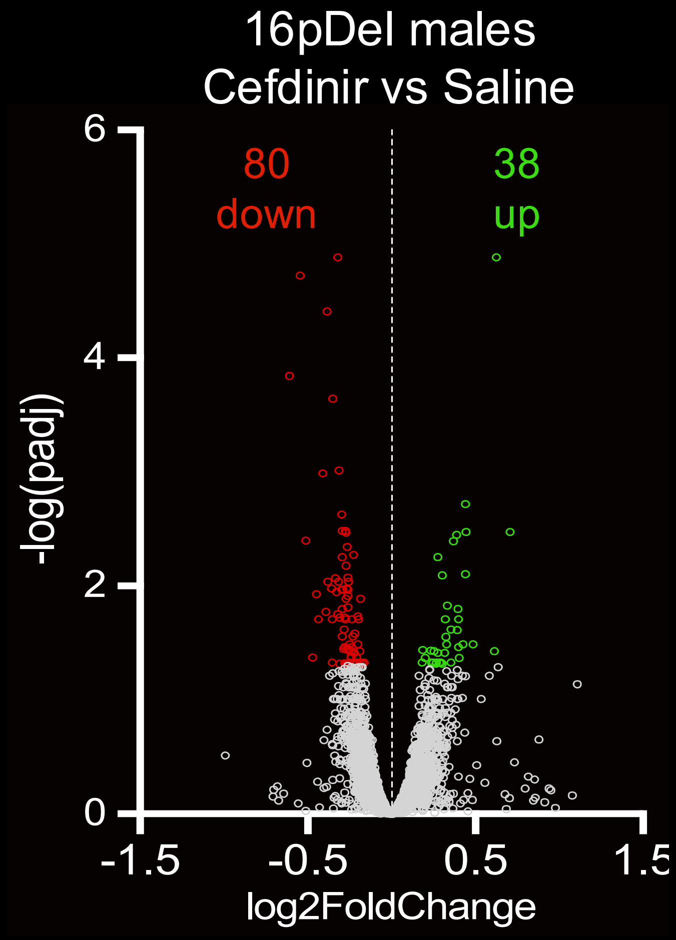
16pDel females
Cefdinir vs Saline

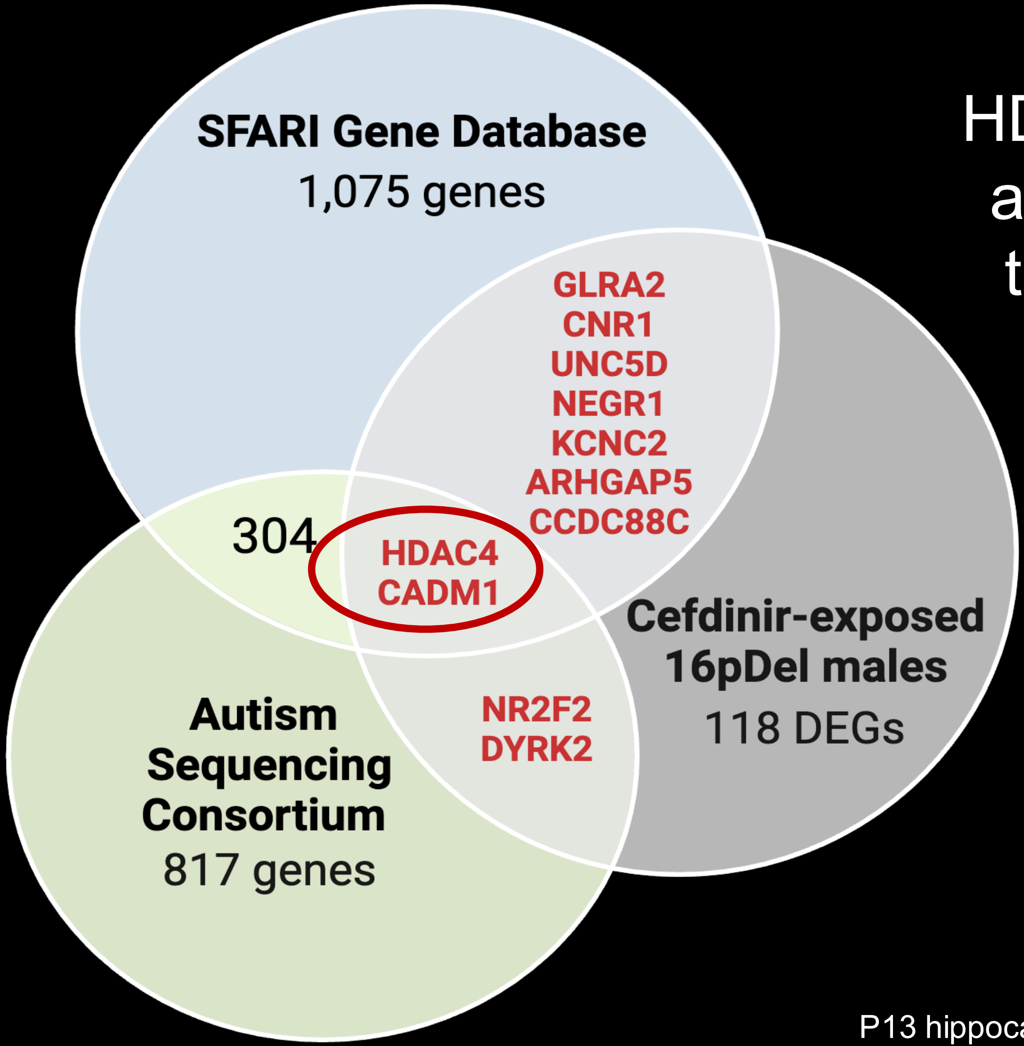
16pDel males
Cefdinir vs Saline

Overlap with high-risk genes implicated in NDDs?

P13 hippocampus; n=3/treatment, across 3 different litters
In collaboration with Dr. Jinchuan Xing, Rutgers University
Bulk RNAseq analysis performed by Azenta Life Sciences

Transcriptional changes in P13 cefdinir-exposed 16pDel males overlap with autism-associated genes





HDAC4 & CADM1 play a fundamental role in the mitotic cell cycle

P13 hippocampus; n=3/treatment, across 3 different litters
In collaboration with Dr. Jinchuan Xing, Rutgers University
Bulk RNAseq analysis performed by Azenta Life Sciences
ASC dataset: Fu et al, (2022) PMID: 3598216.

Early life antibiotic exposure → Perturb the gut microbiome → Dysregulate hippocampal neurogenesis → Consequential outcomes on postmitotic cells → Altered behavioral outcomes

✓ **All groups**

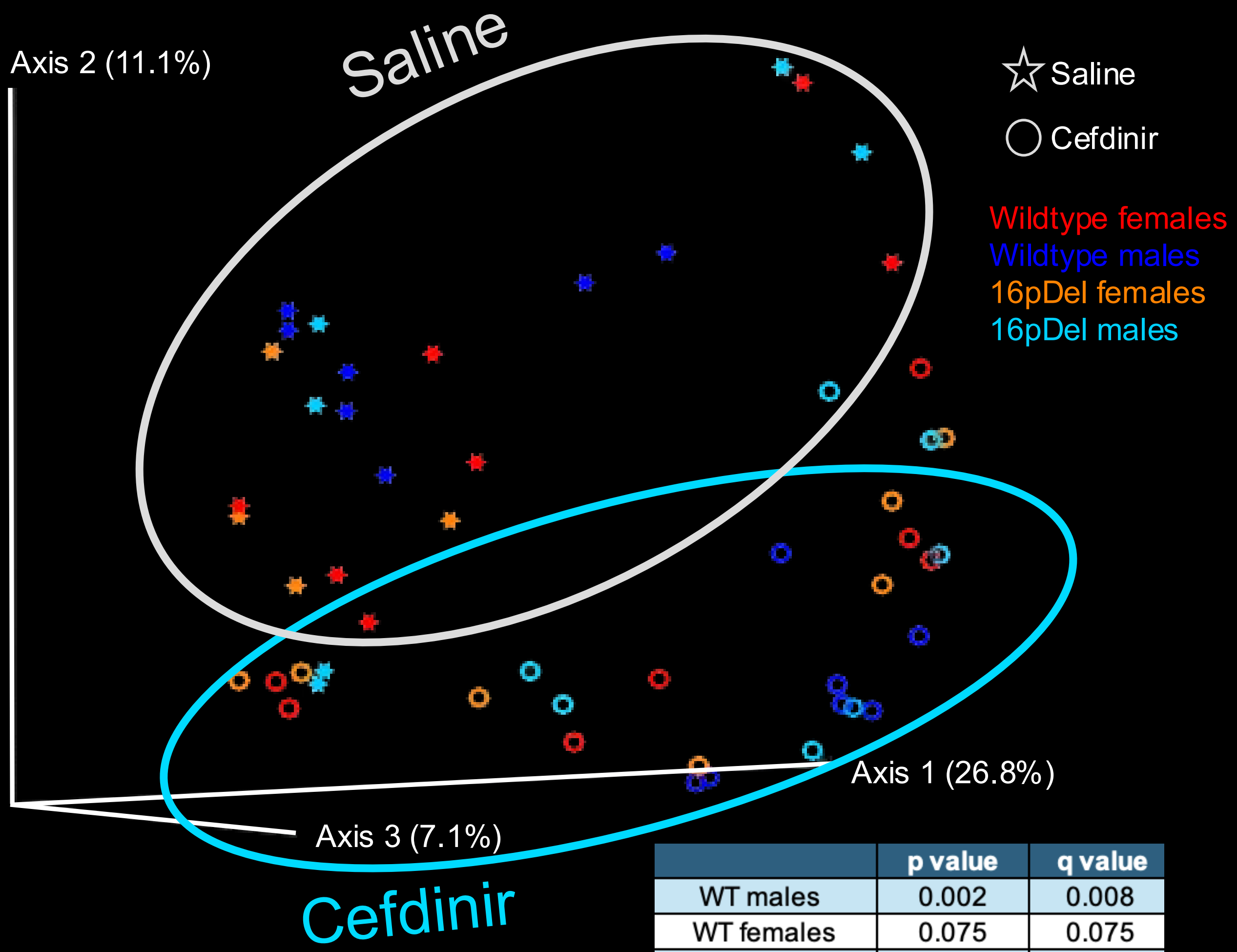
GxE effect

?

* Microbial composition
* Dentate gyrus cell numbers

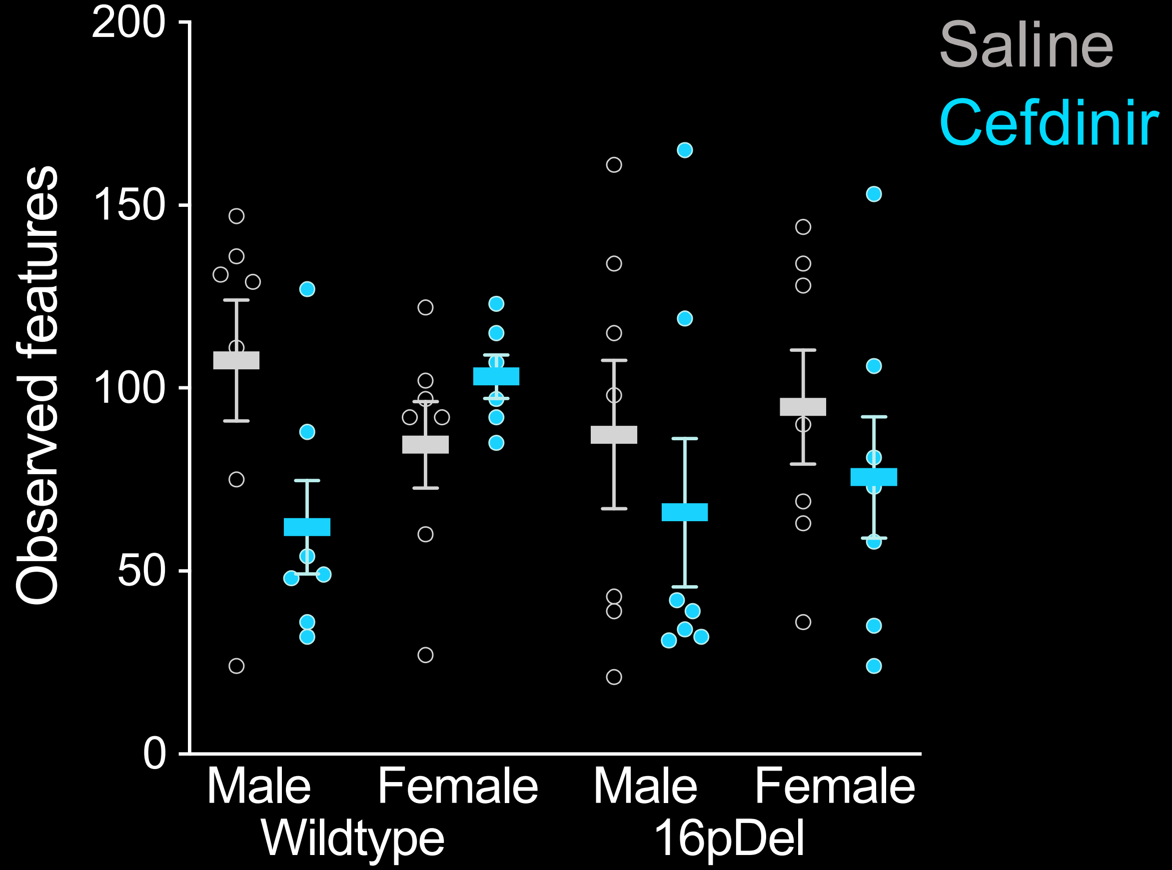
The cefdinir-induced microbiome begins to normalize by P21

Beta diversity: Jaccard distances



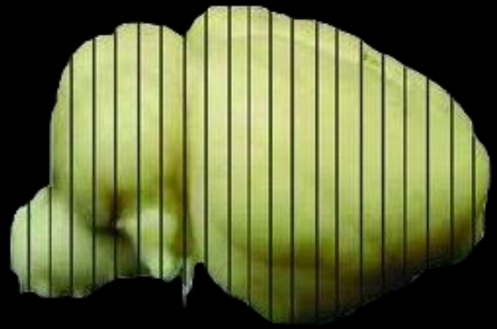
	p value	q value
WT males	0.002	0.008
WT females	0.075	0.075
16pDel males	0.015	0.028
16pDel females	0.021	0.028

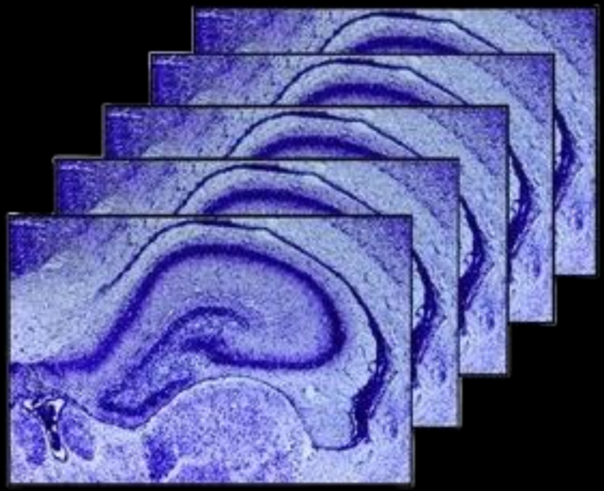
Alpha diversity: Bacterial richness

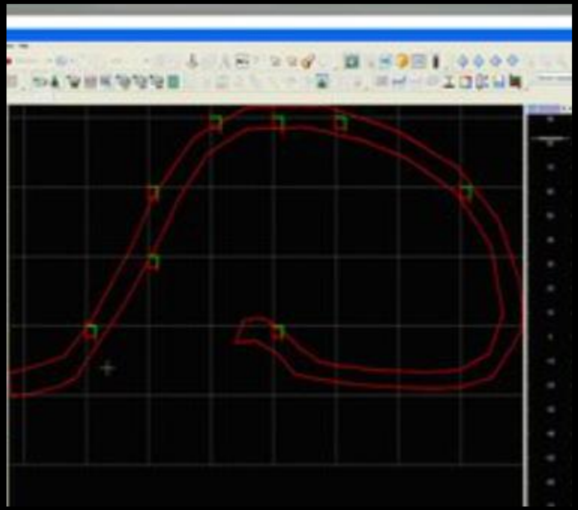


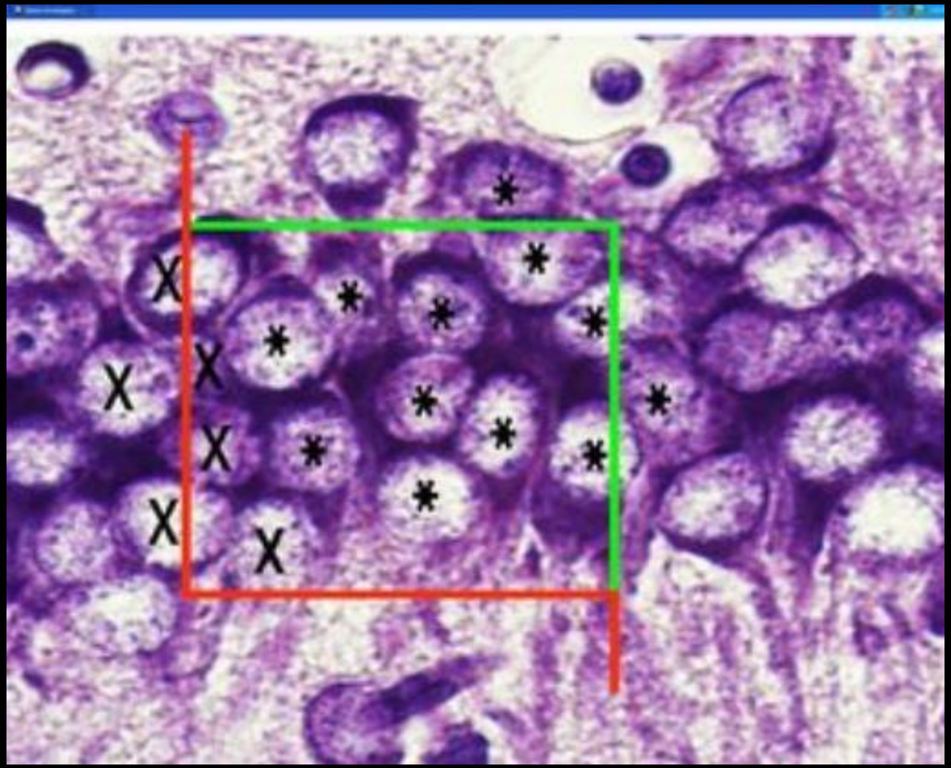
P21 cecum, 4 litters/treatment
Analysis performed by Dr. Zhan Gao, Blaser lab

Brief overview of stereology

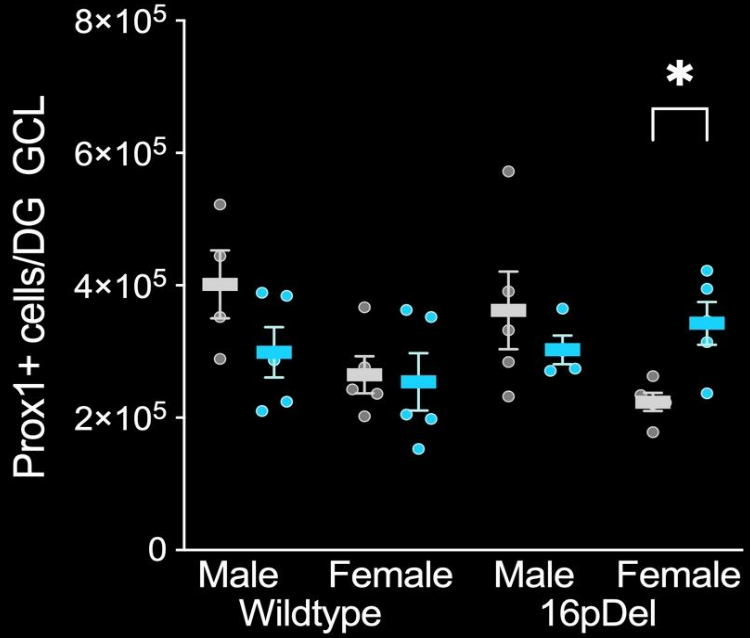


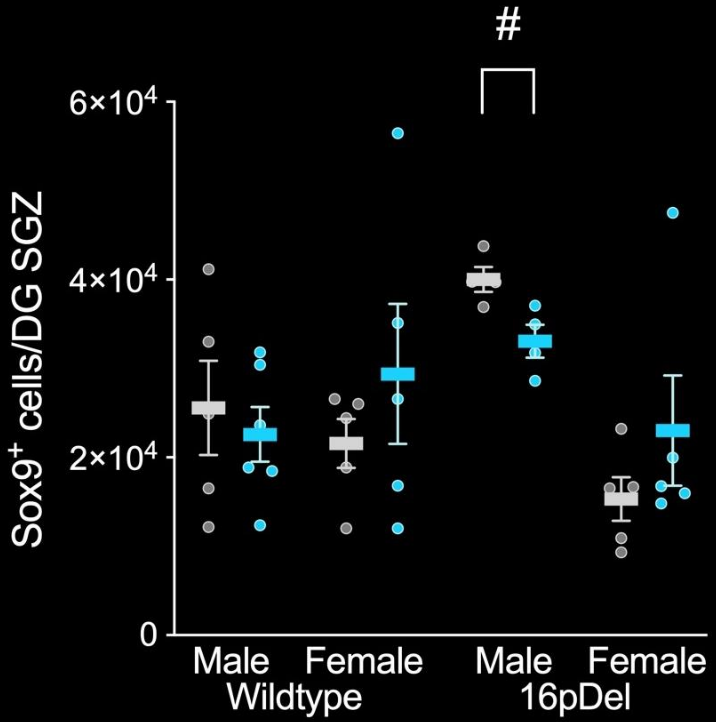




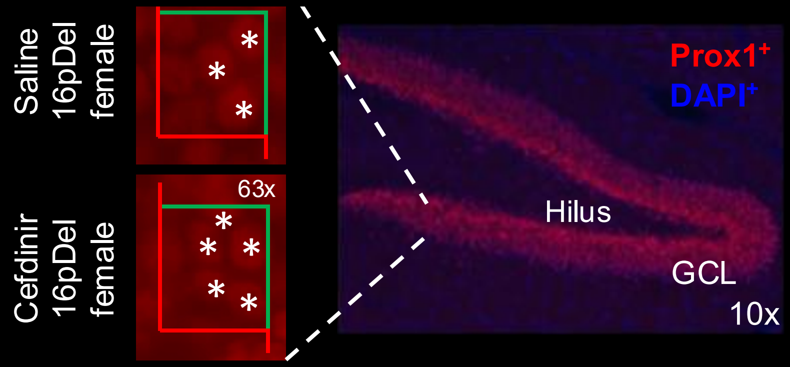


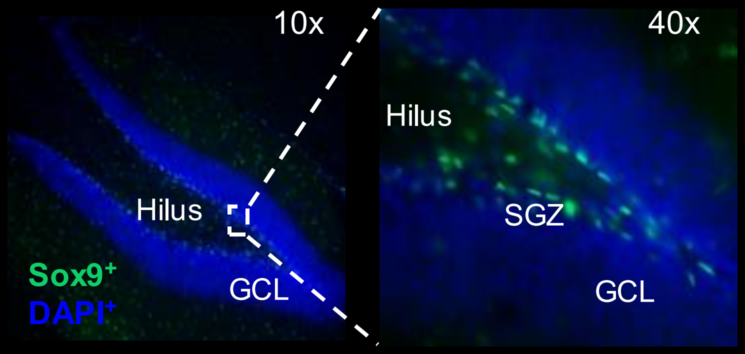
Dentate gyrus postmitotic cell numbers are distinctly altered in cefdinir-exposed 16pDel mice





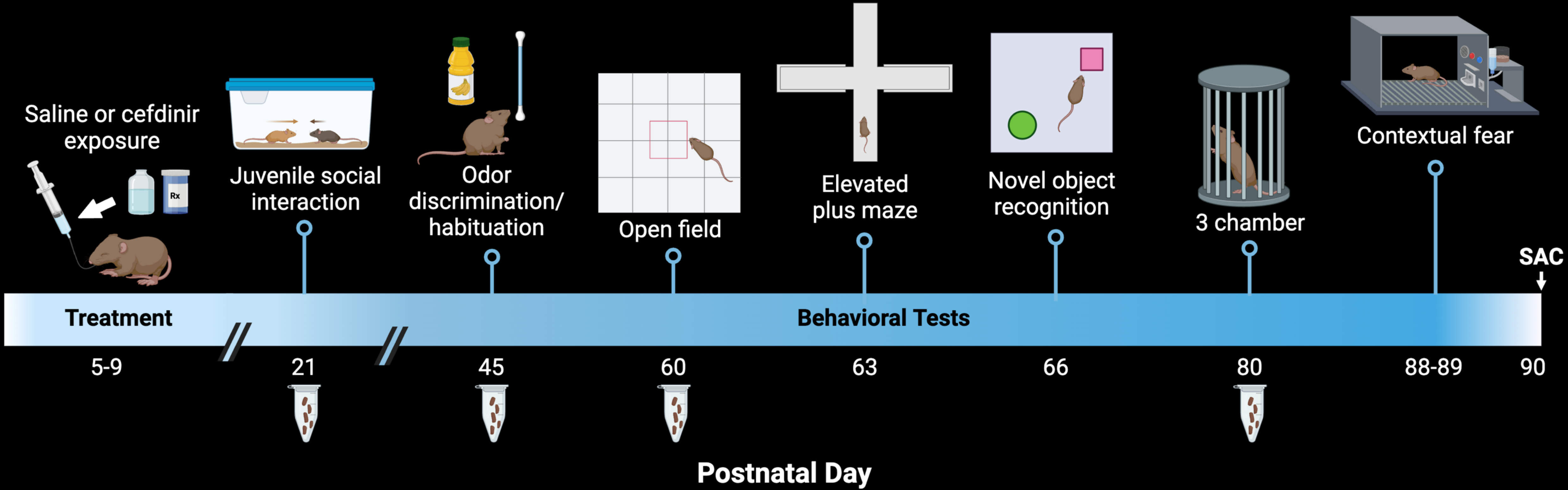
Saline
Cefdinir





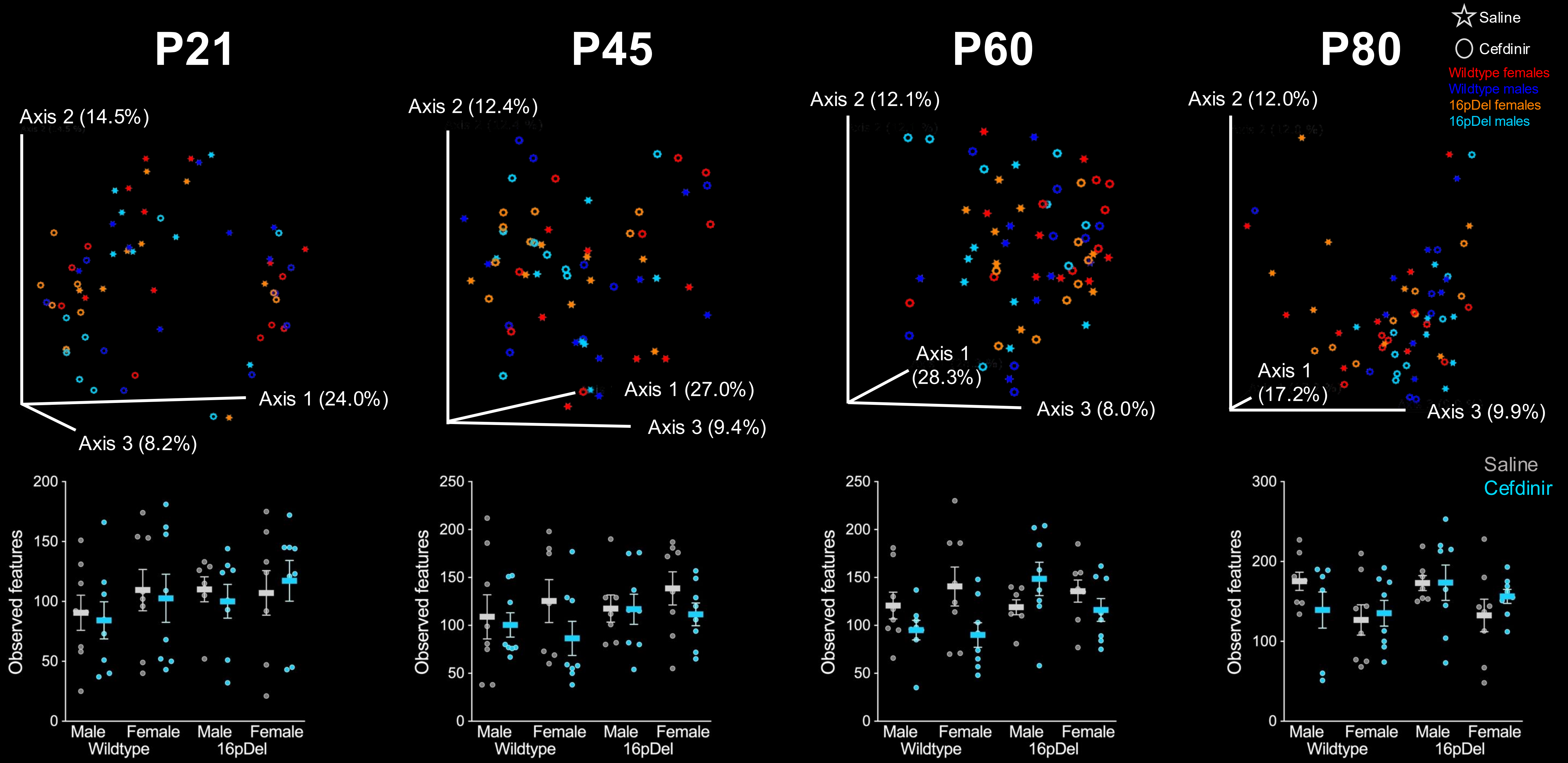
P21 hippocampus, 4 litters/treatment
Anya Mirmajlesi, Christiana Ntim,
Divya Thomas, Alisa Halchenko

Longitudinal behavioral paradigm

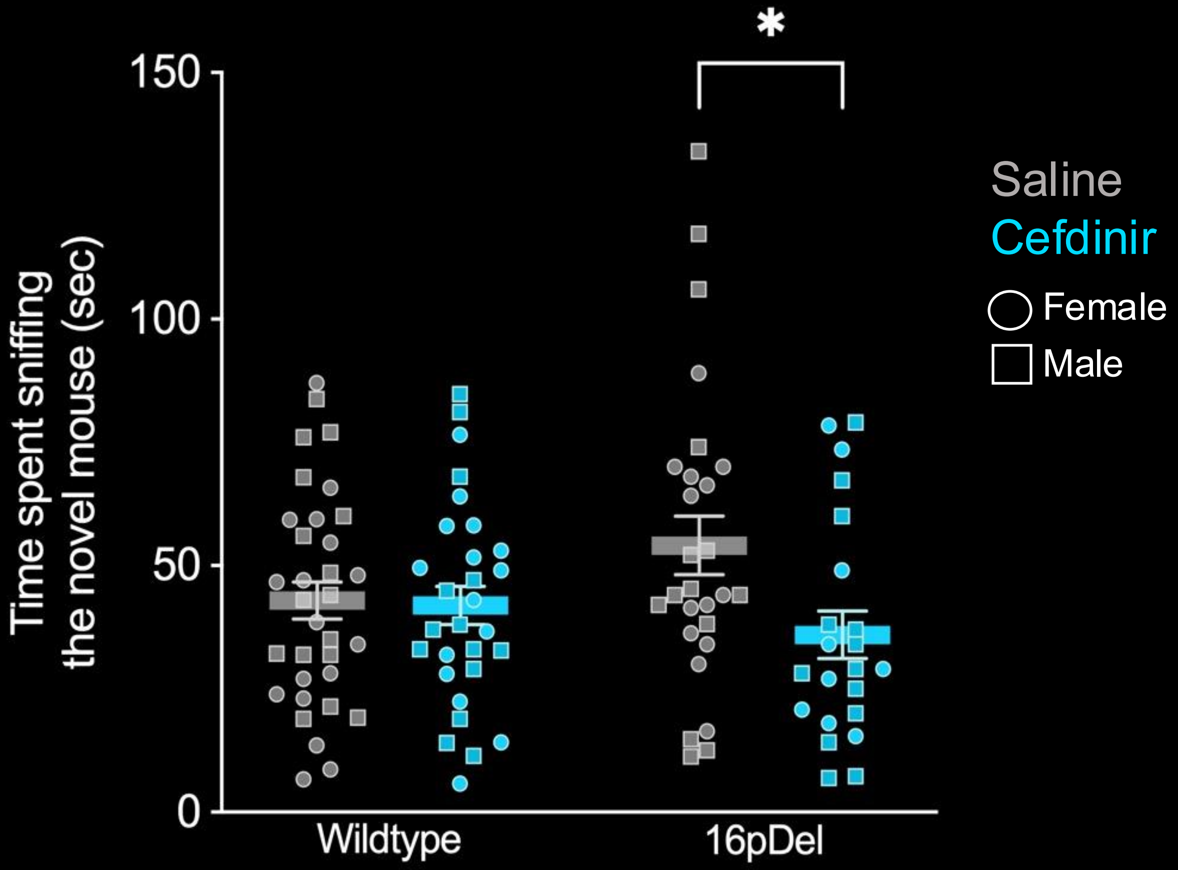


3 cohorts of mice: n ~15/group → ~120 mice total

The cefdinir-exposed microbiome normalizes by adulthood

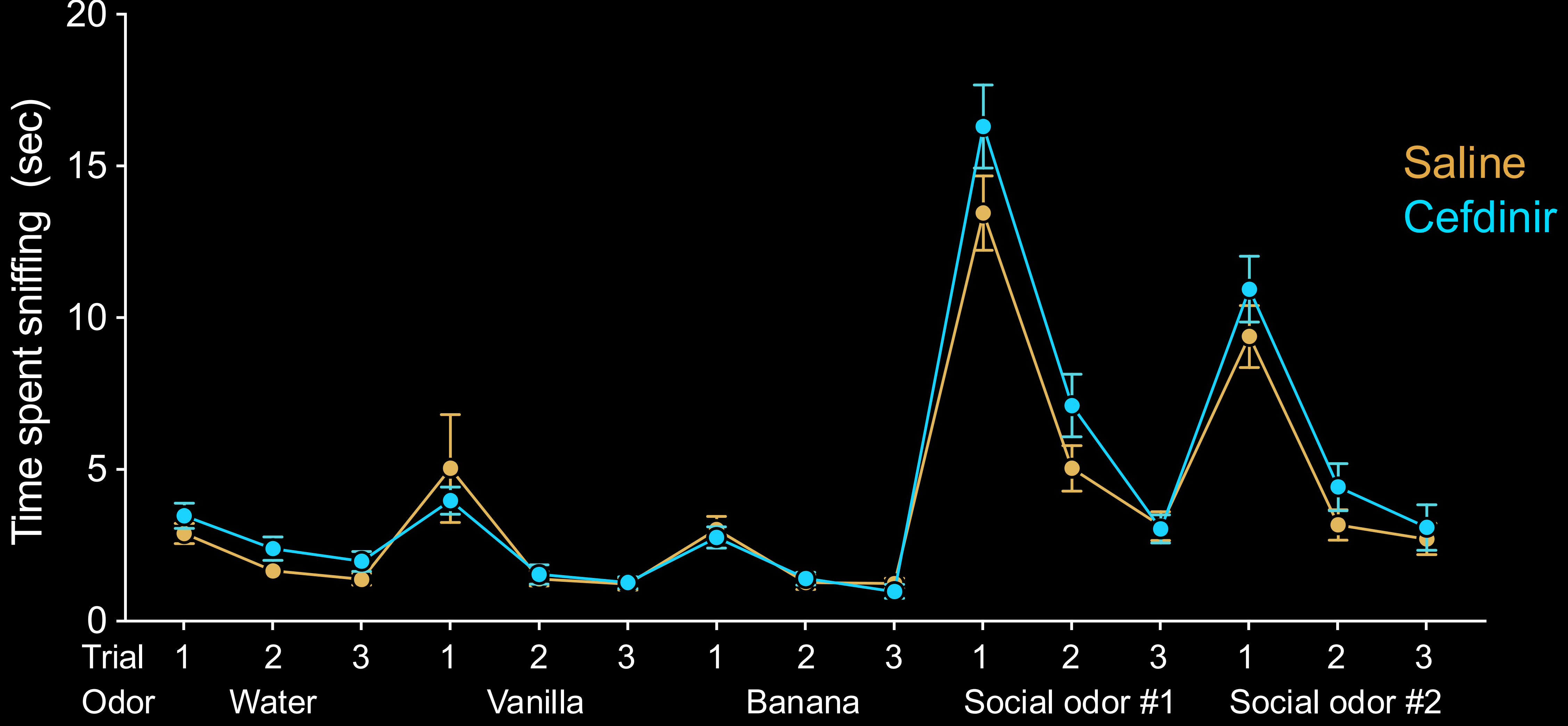


Juvenile sociability is reduced in cefdinir-exposed 16pDel mice

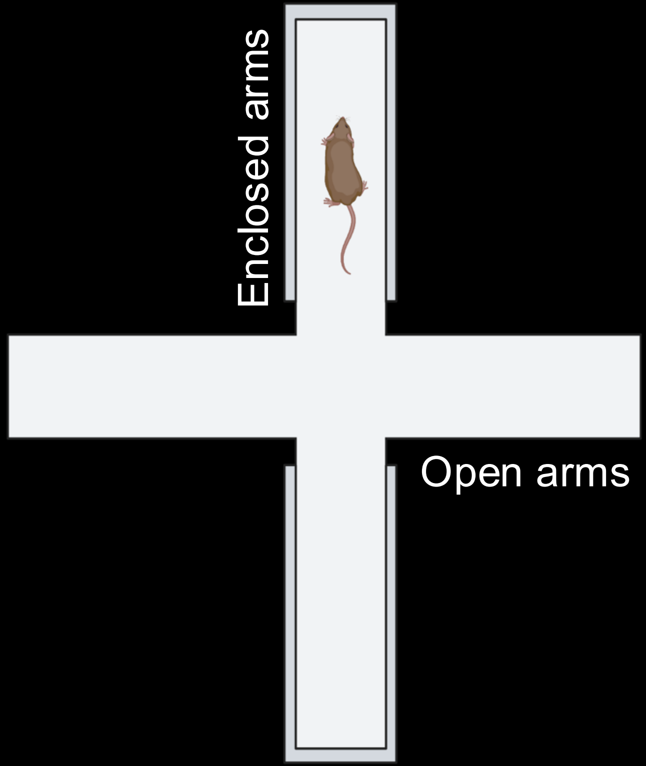


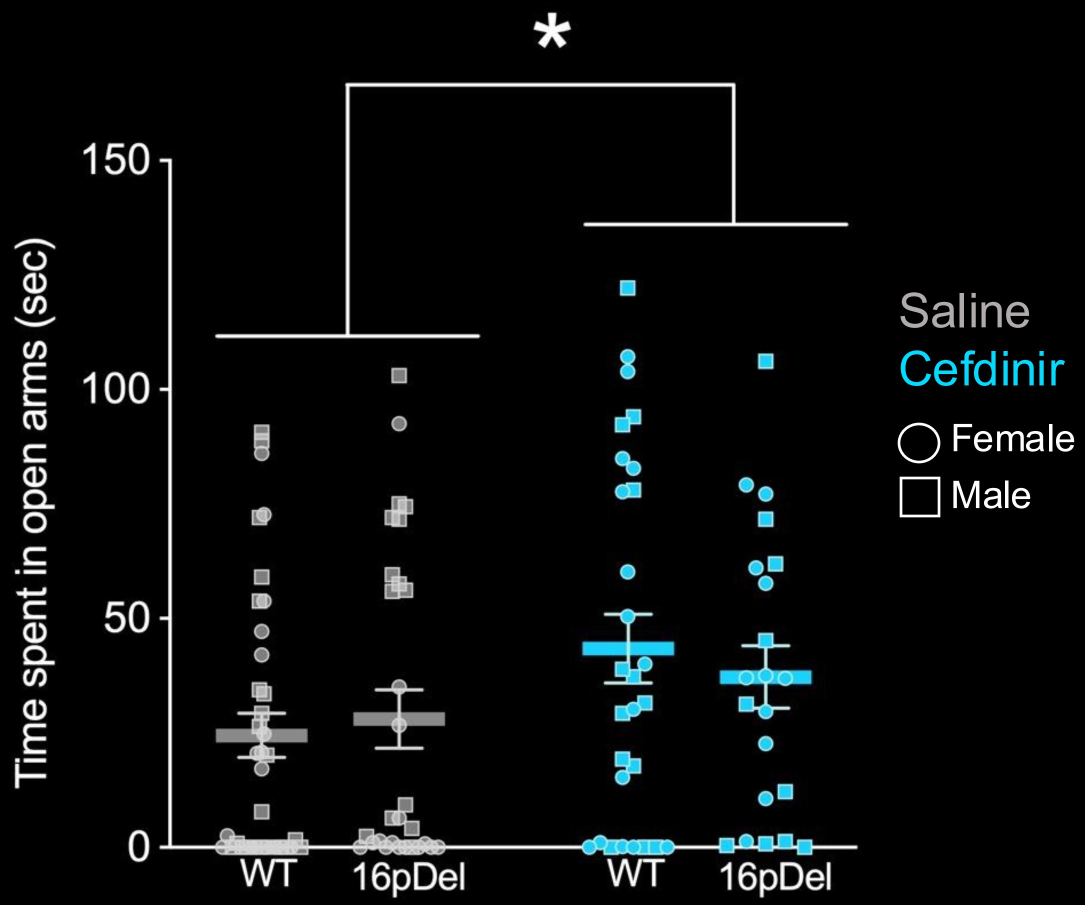
P21, 9 litters saline; 7 litters cefdinir
Anya Mirmajlesi, Christiana Ntim, Divya Thomas

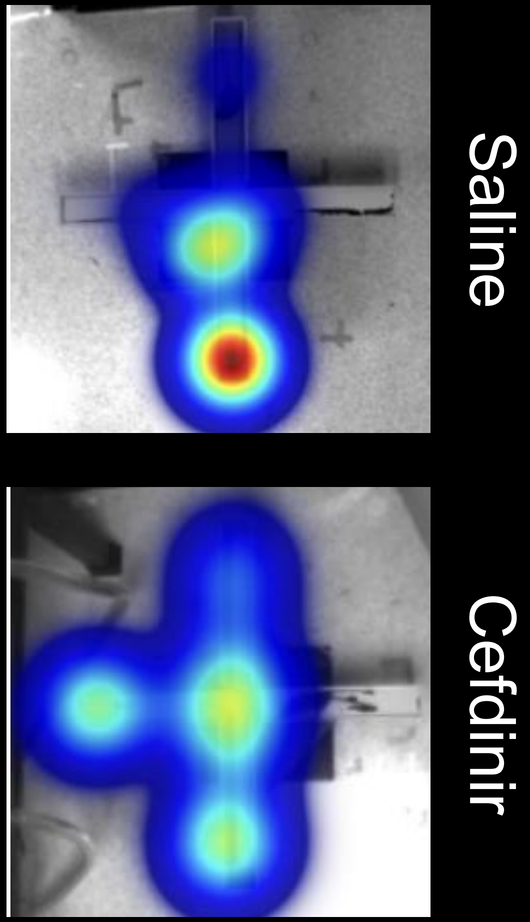
The ability to detect social odors is not impaired in cefdinir-exposed mice



Cefdinir-exposed mice have compromised risk-aversion behaviors at P63

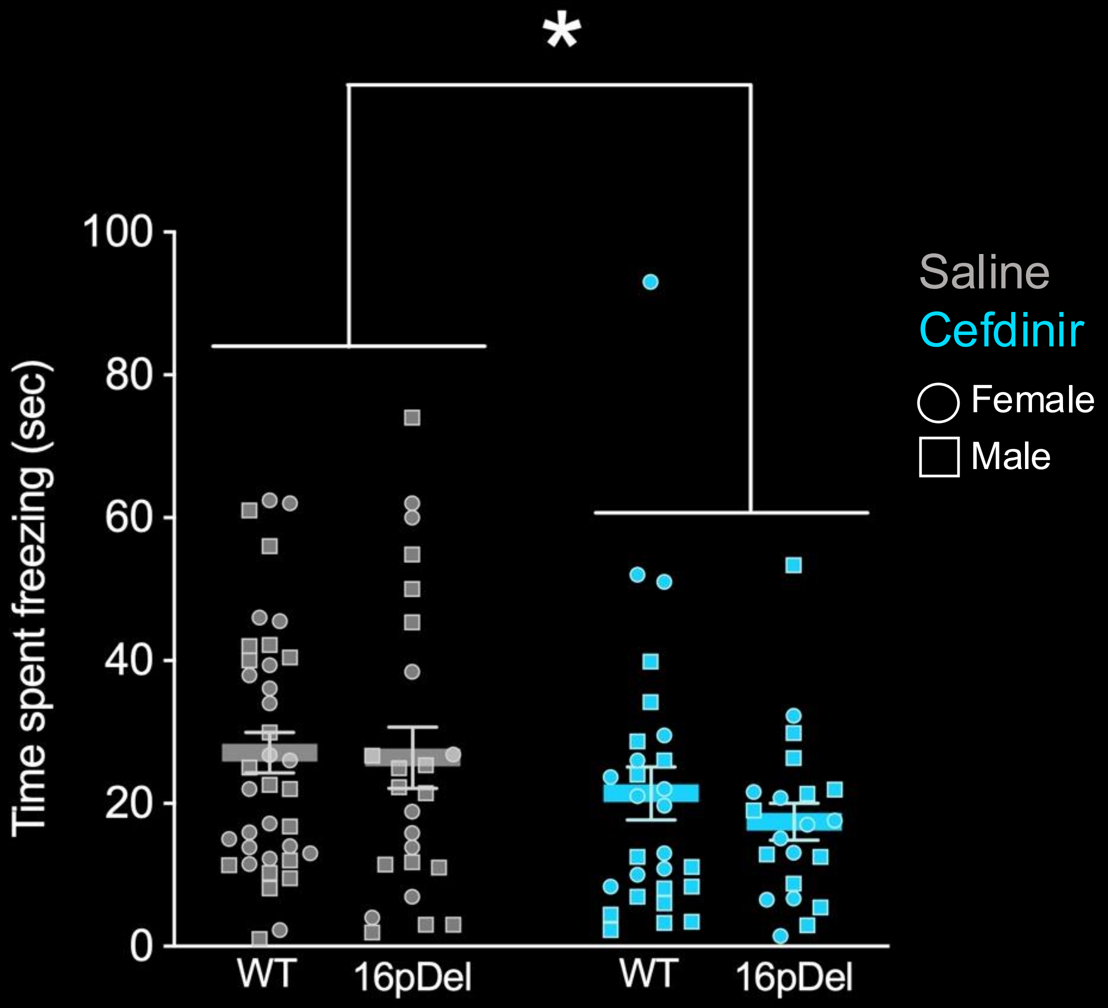






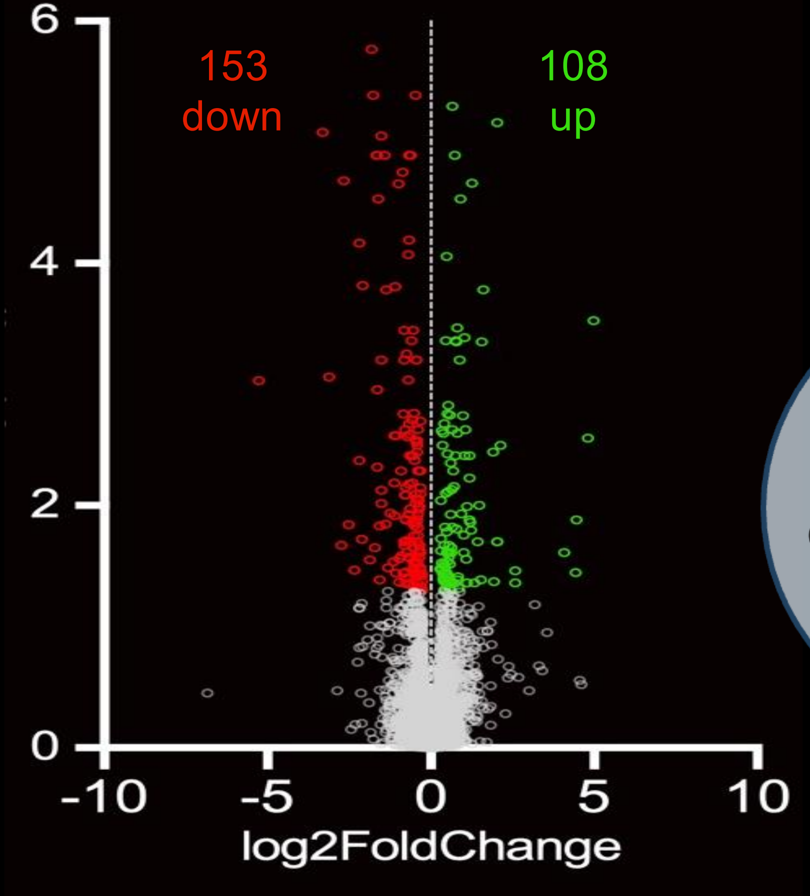
P63, 9 litters saline; 7 litters cefdinir
Tracking and analysis with *Ethovision*

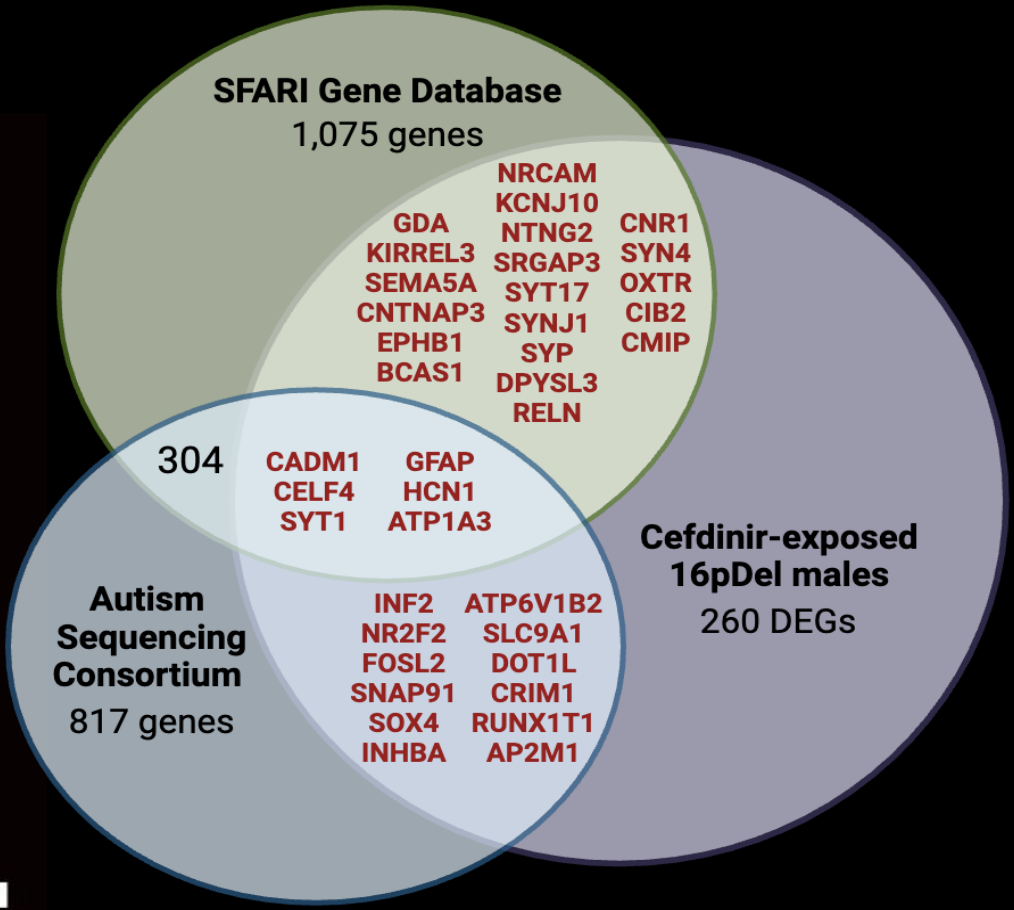
Cefdinir-exposed mice have impaired associative learning at P89



P89, 9 litters saline; 7 litters cefdinir
Anya Mirmajlesi, Christiana Ntim, Divya Thomas

Gene alterations in cefdinir-exposed 16pDel males expand and continue to overlap with human autism databases at P21


16pDel males
Cefdinir vs Saline


SFARI Gene Database
1,075 genes

NRCAM
KCNJ10
NTNG2
SRGAP3
SYT17
SYNJ1
SYP
DPYSL3
RELN

GDA
KIRREL3
SEMA5A
CNTNAP3
EPHB1
BCAS1

CNR1
SYN4
OXTR
CIB2
CMIP

304

CADM1
CELF4
SYT1

GFAP
HCN1
ATP1A3

Autism Sequencing Consortium
817 genes

INF2
NR2F2
FOSL2
SNAP91
SOX4
INHBA

ATP6V1B2
SLC9A1
DOT1L
CRIM1
RUNX1T1
AP2M1

Cefdinir-exposed 16pDel males
260 DEGs

Only the 260 DEG set in 16pDel males yielded GO terms at the FDR<0.05 cutoff value

Myelination
Gliogenesis
Neurogenesis
Axogenesis
Cell-cell adhesion
Vesicle-mediated transport

P21 hippocampus; n=3/treatment, across 3 different litters
In collaboration with Dr. Jinchuan Xing, Rutgers University
Bulk RNAseq analysis performed by Azenta Life Sciences
ASC dataset: Fu et al, (2022) PMID: 3598216.

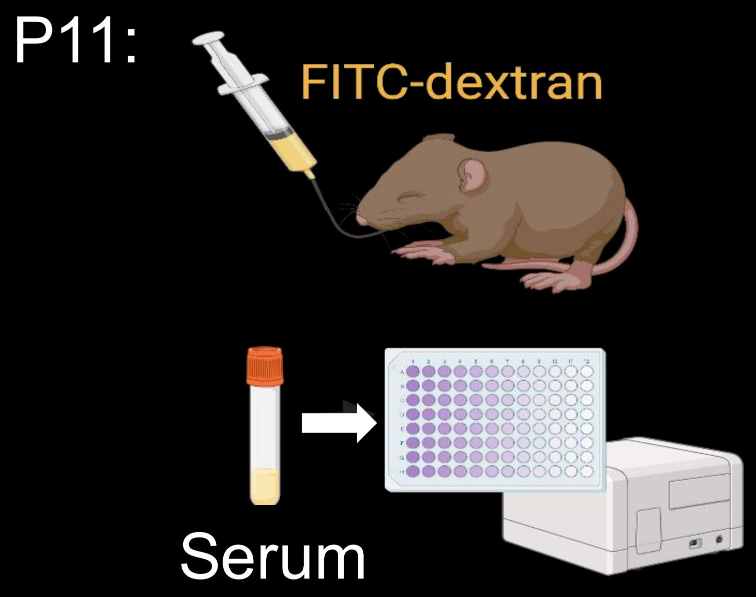
Early life antibiotic exposure → Perturb the gut microbiome → Dysregulate hippocampal neurogenesis → Consequential outcomes on postmitotic cells → Altered behavioral outcomes

✓ **All groups**

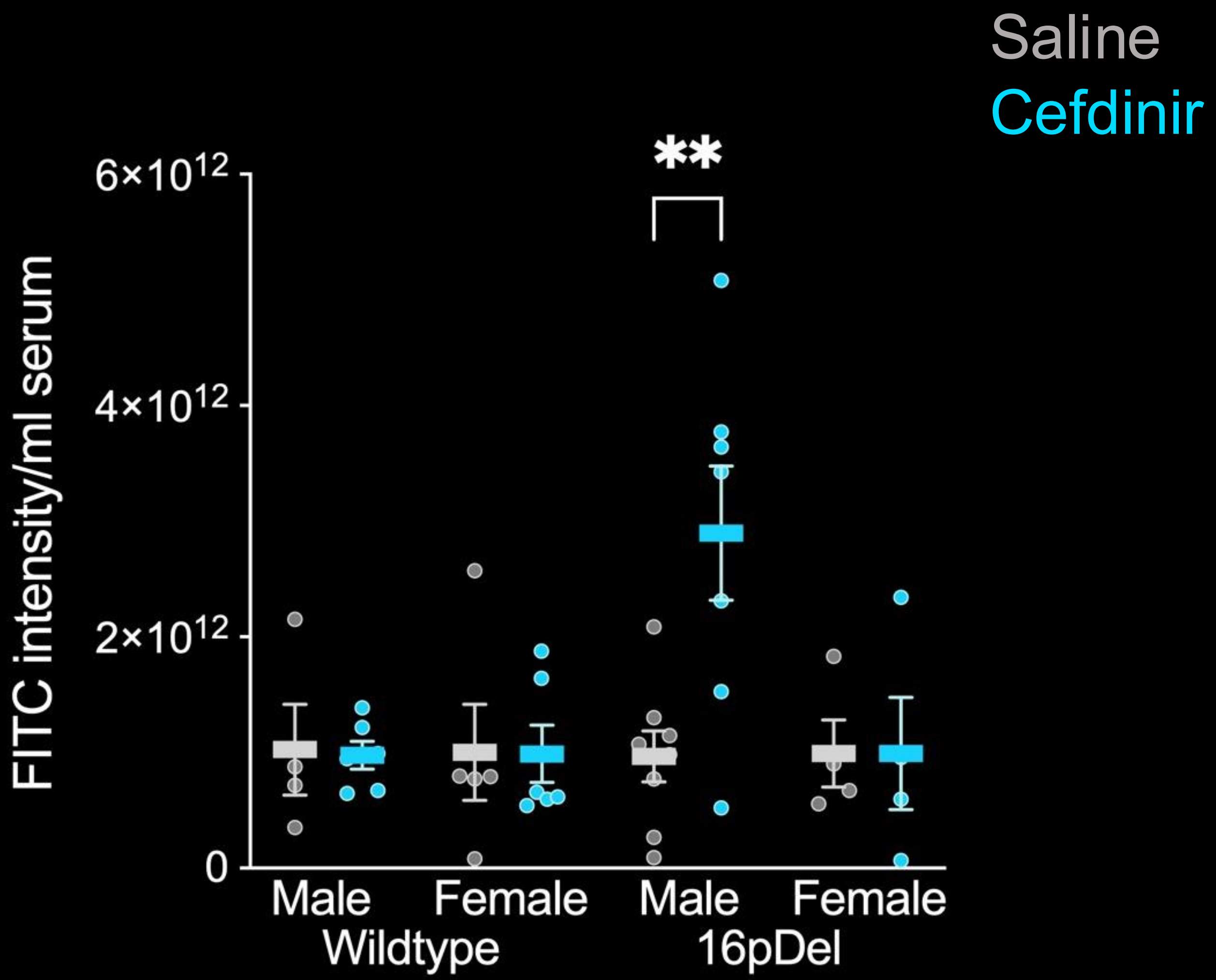
Impacted by the 16pDel

What are potential mechanisms underlying this effect?

Cefdinir-exposed 16pDel males have a compromised intestinal barrier

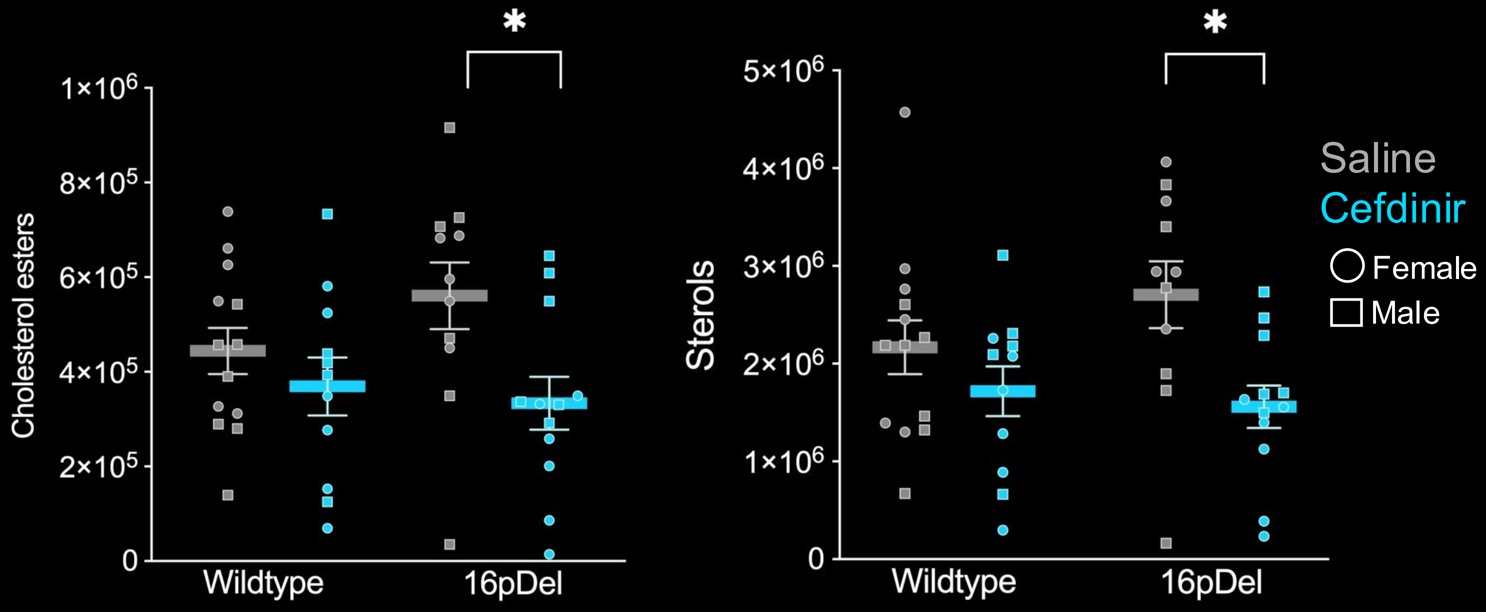


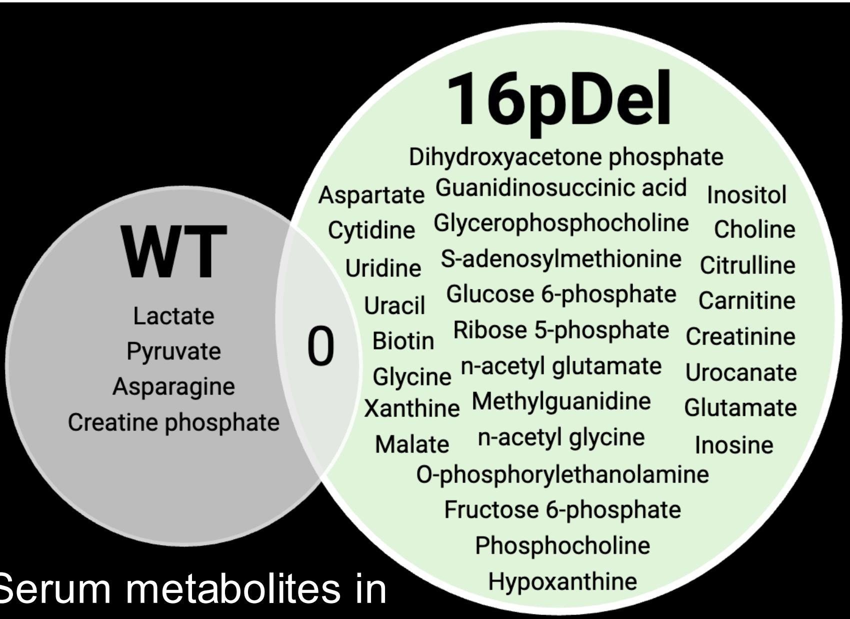
Elevated FITC in serum indicates a leaky gut



Alisa Halchenko

Lipids and metabolites are primarily altered in cefdinir-exposed 16pDel mice





WT

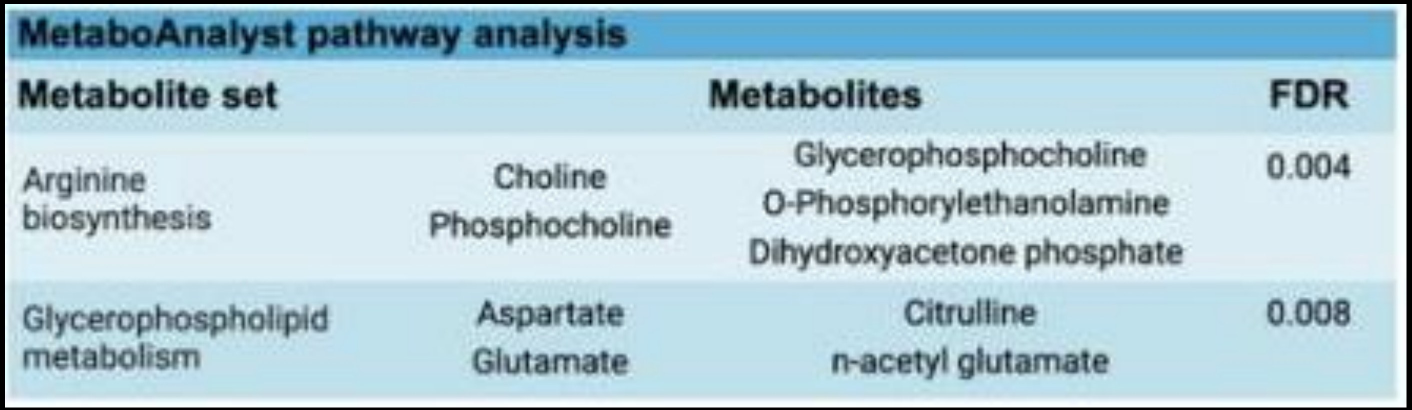
Lactate
Pyruvate
Asparagine
Creatine phosphate

0

16pDel

Dihydroxyacetone phosphate
Aspartate
Guanidinosuccinic acid
Inositol
Cytidine
Glycerophosphocholine
Choline
Uridine
S-adenosylmethionine
Citrulline
Uracil
Glucose 6-phosphate
Carnitine
Biotin
Ribose 5-phosphate
Creatinine
Glycine
n-acetyl glutamate
Urocanate
Xanthine
Methylguanidine
Glutamate
Malate
n-acetyl glycine
Inosine
O-phosphorylethanolamine
Fructose 6-phosphate
Phosphocholine
Hypoxanthine

Serum metabolites in cefdinir-exposed mice



MetaboAnalyst pathway analysis

Metabolite set	Metabolites		FDR
Arginine biosynthesis	Choline Phosphocholine	Glycerophosphocholine O-Phosphorylethanolamine Dihydroxyacetone phosphate	0.004
Glycerophospholipid metabolism	Aspartate Glutamate	Citrulline n-acetyl glutamate	0.008

P13 serum, 4 litters/treatment

Summary & Conclusions

Gene x Environment model

16p11.2 Deletion

Implications in human NDDs

Cephalosporin antibiotic exposure

Clinically-relevant: dose, duration, single

Only cefdinir-exposed 16pDel mice had changes in hippocampal stem cell proliferation, subsequent adolescent cell numbers, and gene expression compared to other groups, demonstrating that genetic predisposition can modulate the effects of early-life antibiotic exposure on neurodevelopment.

- Sufficient to perturb the gut microbiome by P13, with recolonization ~P21
- Genetic constitution impacts the effects of a cefdinir-induced perturbed gut microbiome on hippocampal development
- Cefdinir-exposed mice are less social and exhibit compromised risk-aversion and associative learning
- These distinct alterations may be mediated by gastrointestinal disturbances, as cefdinir-exposed 16pDel male mice had increased intestinal permeability and alterations in metabolites associated glycolysis and phospholipid metabolism

Collaborators & Support

DiCicco-Bloom Lab
Manny DiCicco-Bloom, MD
Xiaofeng Zhou, MA
Anya Mirmajlesi
Katherine Kimbark
Christiana Ntim
Daniel Popov
Divya Thomas
Zain Mughal
Alisa Halchenko
Jake Lessing, PhD

Blaser Lab
Martin J. Blaser, MD
Zhan Gao, MD
Xuesong Zhang, PhD

Collaborators
Jinchuan Xing, PhD
Xiaoyang Su, PhD
Eric Chiles
Maria Elena Diaz Rubio, PhD

Funding
NIH TL1TR003019, NJGOV CAUT22AFP011,
NIH NINDS 1F99NS130926 to CRM

NIH Blueprint for Neuroscience Research

Autism Research Institute, NJHF PC 124-22, Busch Biomedical Grant, Lurie Family Foundation, Mindworks Charitable Lead Trust to EDB

Emch Fund for Microbial Diversity, C&D Fund to MJB

Data figures created in Graphpad PRISM

Illustrations created in BioRender