Association among Autism-Like Behaviors and Gut Microbiota

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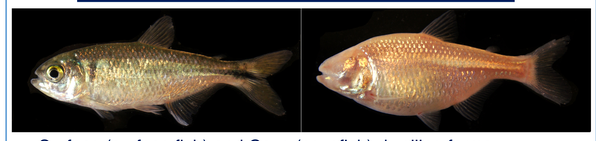
INTRODUCTION

Recent metagenomic analysis and clinical studies have shown a significant correlation between gut microbiota and Autism Spectrum Disorder (ASD) in humans[2]. However, there are a lack of suitable experimental systems to address the mechanistic relationship between ASD-related symptoms, number of autism-risk genes and gut microbiota. The cave-dwelling morph of *Astyanax mexicanus* displays many ASD-associated phenotypes: asociality, repetitive behaviors, hyperactivity, imbalanced attention, and loss of sleep. The advantage of this system is that the cave-dwelling morph has a surface-dwelling conspecific that, instead, expresses normative behaviors, allowing us to apply powerful comparative studies and genetics.

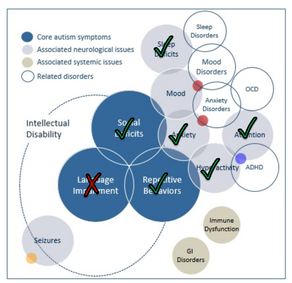
In addition to phenotypic similarity, this system has a suite of genetic and physiological similarities to humans: (1) cavefish transcriptome exhibited significantly similar directional gene-expression changes seen in the brains of ASD patients, (2) The cave morph also exhibits similar trends in overall gut microbiota diversity to ASD patients, with firmicute species depleted, and (3) Cavefish behaviors are mitigated by two FDA approved-antipsychotic drugs for ASD as they are in humans[1]. In this study, we investigate associations between gut microbiota and autism-like behaivors

A Model Organism: *A. mexicanus*[4]



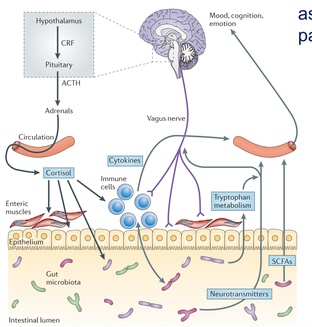
- Surface (surface fish) and Cave (cavefish) dwelling forms
- Independent evolution of cave forms in multiple caves
- Surface fish and cavefish are interfertile

Increased traits in CF	Decreased traits in CF
Imbalanced attention: • vibration attraction behavior (VAB)[5]	Sociality: • lack of schooling[6] • lack of hierarchical dominance[8]
Hyperactivity[9]	Sleep[9]
Anxiety (elevated cortisol)[7,10]	
Repetitive behavior[11]	



AUTISM SPEAKS®

Cavefish share many symptoms with human autism. In addition: (i) cavefish behaviors are mitigated by antipsychotic drugs for ASD. (ii) Cavefish genome has >90% of homologs of human ASD-risk genes, and majority of them exhibit the same directional changes of gene-expressions as seen in the brains of ASD patients[1]



Cryan et al., Nat Rev Neurosci **13**, 701–12 (2012)

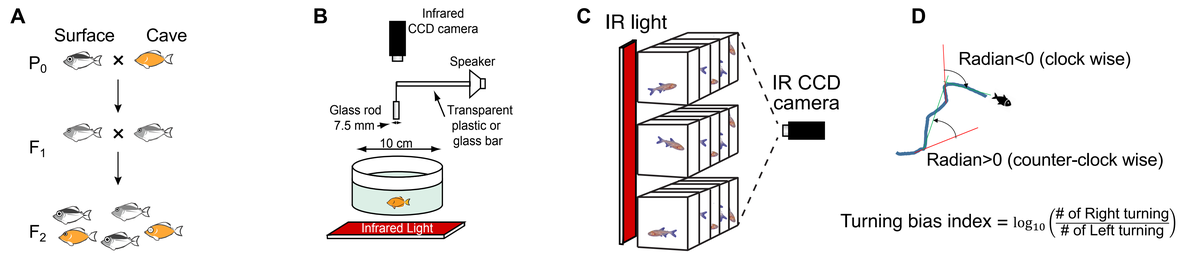
Proposed mechanism of reciprocal interaction between gut and brain.

Neurotransmitter and short chain-fatty acids (SCFA) are proposed to mediate 'gut → brain' interaction. **Firmicute** and Bacteroides, Parabacteroids may be involved in this process by supplying SCFAs, 5-aminovaleric acid and/or taurine[2,3]

OBJECTIVE

Resolve the genetic relationship between ASD-like behaviors and gut microbiota in cavefish

EXPERIMENTAL DESIGN



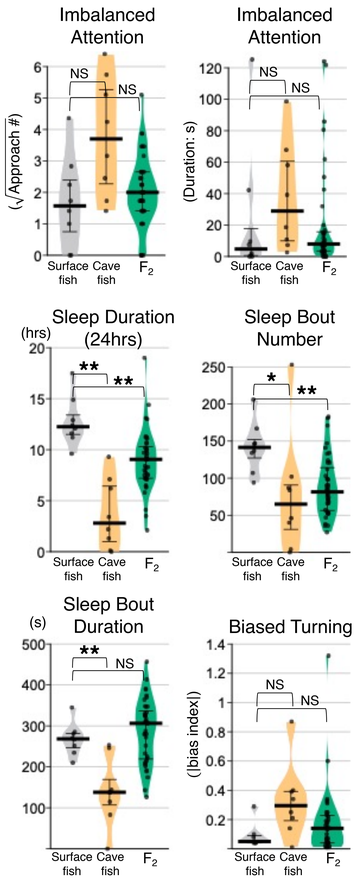
Turning bias index = $\log_{10}\left(\frac{\#\text{ of Right turning}}{\#\text{ of Left turning}}\right)$

A) 40 2nd generation hybrids (F_2) derived from a pair of surface fish and cave fish were raised to provide us with enough statistical power to map quantitative trait loci (QTL). **B)** Imbalanced attention assay characterized by adherence to a vibration stimulus (within 2cm radius from the vibrating rod at 40 Hz). **C)** Sleep and hyperactivity assay characterized by sleep duration and swimming speed. **D)** Repetitive behavior assay characterized by turning repetition and turning preference.

RESULTS

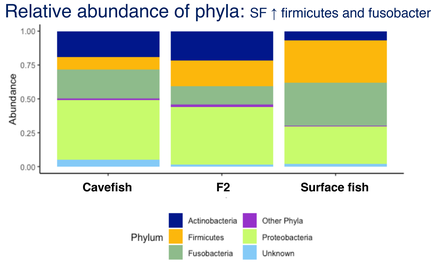
- F2 generation covered phenotypic ranges of surface ("SF") and cavefish ("CF")
- F2 generation's phenotypes were close to those of surface fish or intermediate of those of surface fish and cavefish
- The composition of cavefish's gut microbiota was distinct from that of surface fish, and F2s' overlap these; the surface fish gut microbiome was generally less diverse those of cavefish and F2 but with a higher relative contribution of Firmicutes.

Behavioral phenotypes

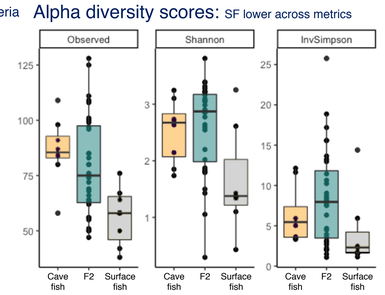


Gut bacteria OTUs based on 16S rRNA gene analyses

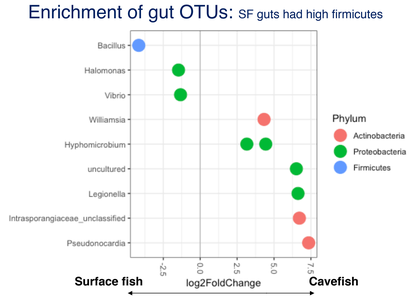
Relative abundance of phyla: SF ↑ firmicutes and fusobacteria

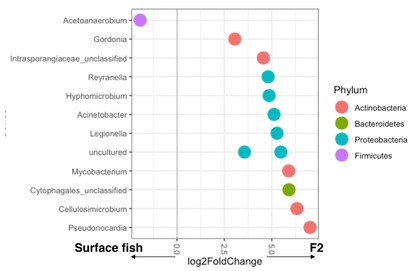


Alpha diversity scores: SF lower across metrics

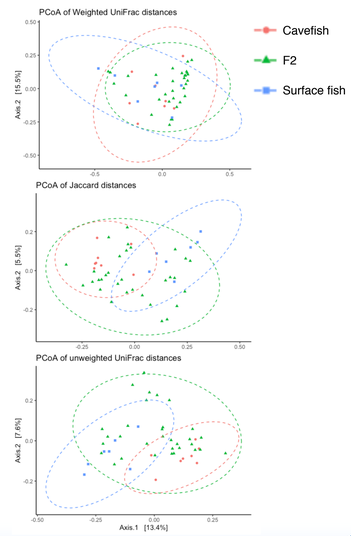


Enrichment of gut OTUs: SF guts had high firmicutes



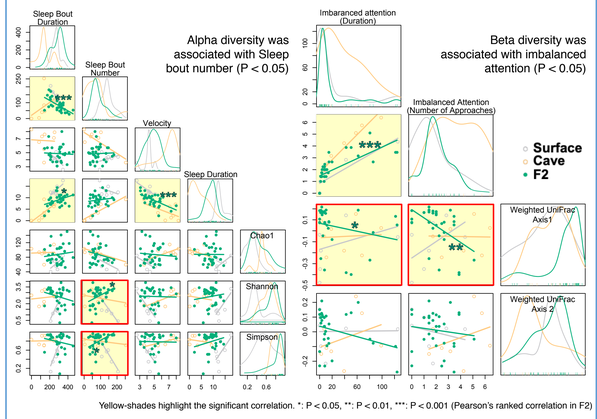


Beta diversity scores: SF and CF were distinguishable



*: P < 0.05, **: P < 0.01, ***: P < 0.001, NS: not significant (Mann-Whitney non-parametric test after Bonferroni correction)

Diversity of gut microbiota was associated with Sleep bout and Imbalanced attention



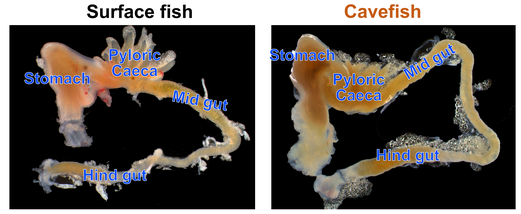
Yellow-shades highlight the significant correlation. *: P < 0.05, **: P < 0.01, ***: P < 0.001 (Pearson's ranked correlation in F2)

CONCLUSION

- Swimming velocity (hyperactivity) was negatively correlated with sleep duration as reported before[11].
- The composition of surface fish's gut microbiota showed less alpha diversity than those of cavefish and F2.
- The gut microbiota composition of surface fish and cavefish was distinguishable; and F2's covered the most of diversities of both surface fish's and cavefish's
- Alpha and beta diversity of the gut microbiota composition were correlated with sleep and attention phenotypes, respectively → suggesting a potential involvement of gut bacteria in the expression of these behaviors

Genotype (gut bacteria) × Genotype (autism risk genes) → Phenotype (autism-like behavior)

Ongoing Research



Using dissected brain samples from 40 of F2 and gut morphometrics (ex. above pic), we will address associations between brain gene expression, gut microbiota, gut phenotype and autism-like traits.

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