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Can Mice Help Us Understand the Role of the Microbiome in Risk of Opioid Abuse?

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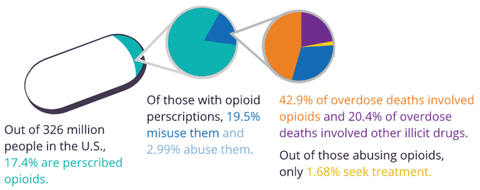
Introduction

Opioid Crisis:

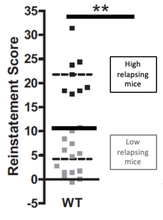
- Out of the 56 million people that were prescribed opioids, only a small percentage of users became abusers[1,2].



The Importance of the Gut Microbiome:

- Plays a modulating role in metabolic diseases and neurological disorders[3,4,5,6]
- Disease states correlate with variation in temporal or individual composition[7]

Research Premise:



- Following a regime of opioid self-administration and extinction, not all wild-type mice relapsed post-abstinence.
- The amount of morphine consumed did not explain differences in relapse[8].

Knowledge Gap:

- Studies linking variation in the gut microbiome to drug abuse liability are lacking.

Study Objectives:

- To establish how individual microbiomes vary in response to morphine
- To establish whether composition or responses to morphine vary between individuals

Results

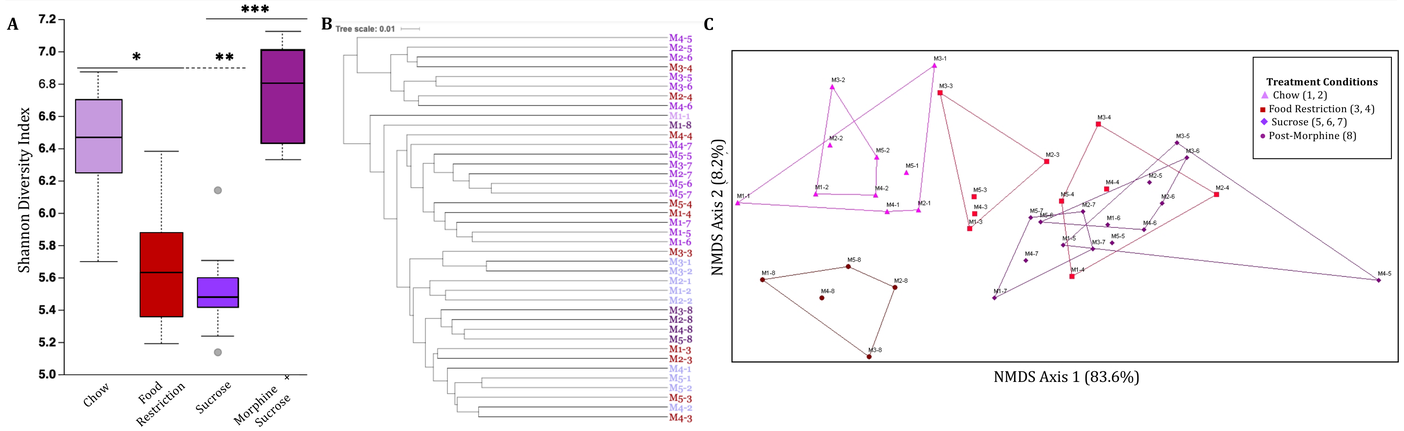


Figure 1: Opioid self-administration increased microbiome diversity but individual microbiomes an their responses were distinct. [1A] Gut microbiome alpha diversity by treatment where significance between treatments was determined using pairwise Kruskal-Wallis test. p-values are (*) = 0.001940, (**) = 0.000065, and (***) = 0.001063. **[1B]** Unweighted Pair-Group Method with Arithmetic Mean (UPGMA) tree built using Unifrac distance matrix, of each mouse (M1 through M5) microbiome at 8 sample dates, representing the four treatment conditions (color coded). **[1C]** Nonmetric multidimensional scaling ordination recommended a two-dimensional solution after 250 runs with the real data and randomized data. Axis one and two of the real data was significant (p-value = 0.040) compared to the randomized data. Final stress for a two-dimensional solution was determined to be 12.70486. Percent variation explained by each axis is listed, with cumulative variation being 91.8%. A pairwise Multi Response Permutation Procedure (MRPP) demonstrated significant differences in between every treatment group (p-value < 0.001).

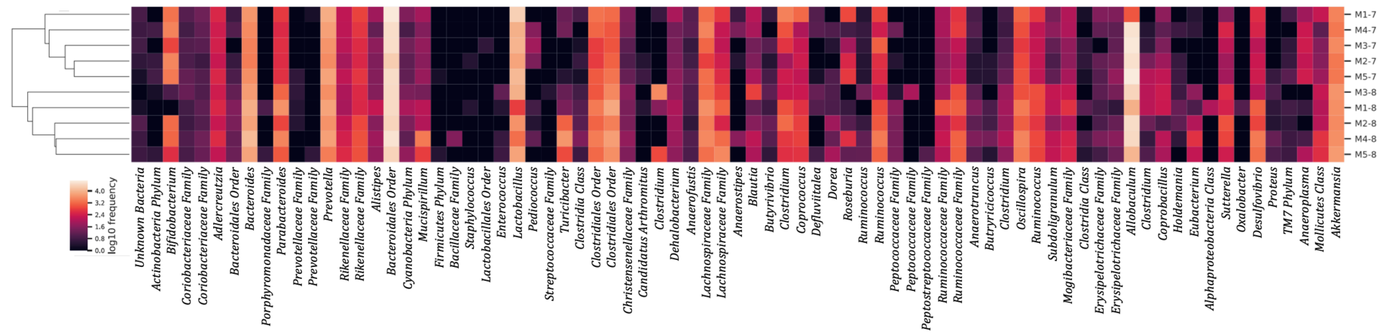


Figure 2: Exposure of gut microbiomes to morphine decreased probiotic bacteria, such as *Bifidobacterium* and *Lactobacillus*, and increased potentially pathogenic bacteria, including *Clostridium*. Dendrogram of species abundance of individual mice pre- and post-morphine was generated using Bray-Curtis distance in QIIME 2. Taxonomy assignment was determined at the genus level and labeled at the lowest classification possible. Color gradient indicates log frequency of species abundance occurring in each individual mouse.

Variable	Df	Sums of Squares	F-Statistic	R^2	P-value
Mouse	4	0.07898	2.629	0.136	0.006
Treatment	3	0.23218	10.305	0.401	0.001
Mouse : Treatment	12	0.11752	1.304	0.203	0.130
Residuals	20	0.15020		0.259	
Total	39	0.57889		1.000	

Table 1: Both treatment and individual mouse impacted microbiome composition. A Permutation Multivariate Analysis of Variance (perMANOVA) test determined whether individual mice, treatments, or the interactions between them explained variation. (Df) Degrees of freedom. (F-Statistic) Tests null hypothesis. (Sum of Squares) Within-group and among-group variance.

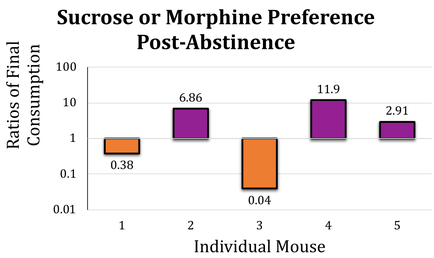


Figure 3: Mice exhibit differences in morphine preference post-abstinence. Ratios less than one indicate mice prefer morphine in a sucrose solution (orange), and ratios greater than one indicate mice prefer sucrose (blue) in a two bottle test of free administration.

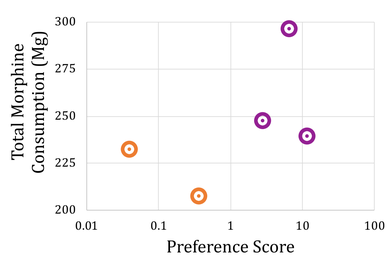


Figure 4: Morphine preference post-abstinence (relapse) did not correlate with higher morphine consumption during the experiment. The preference scores for morphine (from fig. 3) plotted as a function of total amount of morphine in Mg consumed pre-abstinence for each animal. Colors correspond to morphine preference as before.

Conclusions

- Morphine increased gut microbiome diversity, but also increased potentially pathogenic bacteria and decreased probiotic bacteria.
- Composition of the gut microbiome was influenced by treatment and also individual mouse.
- The two mice whose microbiomes were most distinct showed strong preference for morphine post-abstinence.

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Materials and Methods

Mouse model experiments for opioid use:

- Five mice were individually housed for the duration of the study, and fecal samples were collected at eight time points throughout the treatments
- All five mice underwent the same treatments: chow diet, restricted chow diet, sucrose solution (reward), morphine sweetened with sucrose solution, morphine abstinence, and a two bottle choice period (sucrose and sucrose + morphine)
- Initial drug consumption was measured in the home cage in which mice had 30 days ad lib access to 0.5 mg/mL morphine sulfate (MS) in a 20% sucrose solution
- After 10 days abstinence, "relapse" to drug seeking was measured using a two bottle choice between 20% sucrose alone or 20% sucrose with 0.5 mg/mL MS

16S rDNA amplicon generation and sequencing:

- Microbial community DNA was extracted from preserved samples using the Zymo Research Quick-DNA Fecal/Soil Microbe kit and its protocols.
- A mock community of known composition was extracted in parallel as a reference and control for downstream analysis
- Microbiome profiles were determined through amplicon sequencing of the V4-V5 region of the 16S marker gene using protocols of the Earth Microbiome Project[9] with slight modifications made by the Hubbard Center for Genome Studies at UNH
- Sequencing was performed on the Illumina HiSeq platform and reads de-multiplexed prior to processing and analysis

Bioinformatic analyses:

- QIIME 2 pipeline[10] was used for processing and quality control of raw sequences. Alpha diversity boxplots were determined using Shannon's Diversity Index, and significance was calculated using the pairwise Kruskal-Wallis test. Beta diversity was determined using unweighted unifrac distance and was represented by a rarefied phylogenetic tree with samples clustered by UPGMA. Dendrogram of species abundance was generated using Bray-Curtis distance.
- PC-ORD software[11] was used for data transformation, ordination, and statistical tests. Species abundance data was log (x + 1) transformed and subsequently used for a NMDS ordination. MRPP was used to test for significant differences between treatment groups.
- R Software vegan package[12] was used to conduct a perMANOVA to test for significant differences among experimental factors.