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ARTICLE

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Microbial short-chain fatty acids regulate drug seeking and transcriptional control in a model of cocaine seeking

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Cocaine use disorder represents a public health crisis with no FDA-approved medications for its treatment. A growing body of research has detailed the important connections between the brain and the resident population of bacteria in the gut, the gut microbiome, in psychiatric disease models. Acute depletion of gut bacteria results in enhanced reward in a mouse cocaine place preference model, and repletion of bacterially-derived short-chain fatty acid (SCFA) metabolites reverses this effect. However, the role of the gut microbiome and its metabolites in modulating cocaine-seeking behavior after prolonged abstinence is unknown. Given that relapse prevention is the most clinically challenging issue in treating substance use disorders, studies examining the effects of microbiome manipulations in relapse-relevant models are critical. Here, male Sprague-Dawley rats received either untreated water or antibiotics to deplete the gut microbiome and its metabolites. Rats were trained to self-administer cocaine and subjected to either within-session threshold testing to evaluate motivation for cocaine or 21 days of abstinence followed by a cue-induced cocaine-seeking task to model relapse behavior. Microbiome depletion did not affect cocaine acquisition on an fixed-ratio 1 schedule. However, microbiome-depleted rats exhibited significantly enhanced motivation for low dose cocaine on a within-session threshold task. Similarly, microbiome depletion increased cue-induced cocaine-seeking following prolonged abstinence and altered transcriptional regulation in the nucleus accumbens. In the absence of a normal microbiome, repletion of bacterially-derived SCFA metabolites reversed the behavioral and transcriptional changes associated with microbiome depletion. These findings suggest that gut bacteria, via their metabolites, are key regulators of drug-seeking behaviors, positioning the microbiome as a potential translational research target.

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INTRODUCTION

Psychostimulant use disorder is a recalcitrant neuropsychiatric condition with a relapsing-remitting course leading to profound morbidity and mortality [1]. Although understanding of the cellular and circuit-level adaptations in models of psychostimulant use disorders has advanced tremendously, translating these findings to the clinical arena has proven difficult [2, 3]. As such, there are currently no FDA-approved medications for psychostimulant use disorders. This urgent need has led to increased interest in examining peripheral factors beyond the blood-brain barrier, which may serve as useful therapeutic targets or biomarkers [4, 5].

It has become increasingly clear that the resident population of bacteria within the gastrointestinal tract, collectively termed the gut microbiome, modulates brain function [6, 7]. Robust literature indicates that connections between the brain and the gut microbiome modulate neuropsychiatric disease pathogenesis in animal models and clinical studies of autism, depression, anxiety, and substance use disorders [5, 8–15]. Indeed, previously published work demonstrates gut microbiome depletion results in significantly altered behavioral and transcriptional responses to cocaine and opioids [11, 16–21].

Although the exact mechanisms underlying gut-brain signaling are unclear, evidence suggests that neuroactive metabolites produced by the microbiome play a signaling role in neuropsychiatric diseases [10, 12, 22]. The microbiome is a highly metabolically active ecosystem that produces hundreds of metabolites that are absorbed into the circulation [23–25]. Short-chain fatty acids (SCFA), metabolites produced by the bacterial fermentation of dietary fiber, are key gut-brain signaling mediators [26, 27]. The three primary SCFA (butyrate, acetate, and propionate) have widespread effects on brain function, rescuing

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effects of microbiome depletion at the behavioral and transcriptional level across neuropsychiatric disease models [27–31]. Repletion of SCFA reverses the behavioral effects of microbiome depletion in mouse models of cocaine and morphine conditioned place preference [11, 16].

Microbiome manipulations also affect animal models of opioid tolerance and hyperalgesia [20, 32, 33], and more complex effects of the microbiome on cocaine place preference and sensitization [18, 19]. Importantly, these early studies have focused on shorter-term experimenter-administered drug treatments. Although these studies have advanced understanding of the effects of microbiome manipulations on animal models of substance use disorders, there is a need to examine microbiome effects on models of drug self-administration, which are the most translationally-relevant animal models of substance use disorders [34]. These models provide critical information on both drug-taking and drug-seeking after abstinence, a model for drug relapse [35, 36]. Relapse-type behavior arises in part due to incubation of drug craving, a phenomenon seen in both human rats and animal models in which the desire to administer drugs increases with longer periods of abstinence [34, 37, 38]. Given that relapse prevention is the most difficult challenge in treating patients with psychostimulant use disorder, understanding microbiome effects in these models is critical for future translational insight.

Here, we leveraged our established antibiotic microbiome depletion model to examine the effects of microbiome manipulations on drug-seeking behavior in a translationally-relevant self-administration model for cocaine intake and relapse-like behavior. Antibiotic-induced depletion of the gut microbiome in male rats markedly alters the rewarding effects of cocaine and motivation to seek cocaine after withdrawal. Furthermore, rats with altered microbiomes have altered expression of synaptic plasticity genes following cocaine self-administration. To assess the mechanistic contribution of microbial metabolites, repletion experiments utilizing SCFA were also performed. Crucially, repletion of bacterially-derived SCFA metabolites reversed the behavioral and molecular changes associated with microbiome depletion. This study provides mechanistic insight into the role of the microbiome and its metabolites on striatal gene expression in a translationally-relevant model of cocaine use disorder and relapse.

MATERIALS AND METHODS

Rats

Adult male Sprague-Dawley rats (ENVIGO-Harlan, 300–320 g) were pair-housed under specific-pathogen free conditions in a room with constant 22–25 °C and humidity of 55%. Rats were maintained on a reverse 12-hr light-dark cycle (lights on at 1900 h) with food and water available ad libitum. All animal protocols were approved and conducted in accordance with the policies of the Institutional Animal Care and Use Committee at Mount Sinai.

For the present study only male animals were used as we have found differential effects of microbiome depletion in female animals, and comprehensive studies examining exclusively female animals are underway.

Antibiotic and short-chain fatty acid treatments

Rats were randomly assigned and given ad libitum access to non-absorbable antibiotics administered in the drinking water at concentrations of neomycin 2 mg/ml, vancomycin 0.5 mg/ml, bacitracin 0.5 mg/ml, and pimaricin 1.2 µg/ml (all from Fisher Scientific), in an adaption of prior studies [11, 39]. For short-chain fatty acids (SCFA) experiments, the three principle bacterially-derived SCFA were dissolved in the drinking water at physiological levels (67.5 mM acetate, 40 mM butyrate, 25.9 mM propionate, all from Sigma Aldrich) as described previously [11, 40, 41]. Rats were treated with antibiotics and/or SCFA for two weeks prior to any testing and were maintained on this treatment for the duration of the study.

Cocaine self-administration: acquisition

Cocaine self-administration was performed in standard operant conditioning chambers (MED-Associates) as described previously [42]. Rats were food restricted throughout the duration of training with 18 g food/subject delivered once daily following session completion. Sessions were daily for 3 h. Rats were trained to respond for cocaine on the "active" lever on an FR1 schedule of reinforcement where one lever press resulted in the delivery of a 5.9 s infusion of 0.8 mg/kg/infusion (0.1 ml) cocaine paired with concurrent illumination of the cue light located above the active lever. Full details are included in Supplementary Materials and Methods.

Experiment 1: within session threshold testing

Following acquisition of FR1 administration, a within session threshold test was performed to assess differences in cocaine motivation and consumption between groups. In this behavioral economics task, rats are given continuous access to cocaine while increasing the effort requirement to obtain the same amount of drug [42, 43]. Importantly, this measure allows for assessment of both motivation and dose-response within a single session. Full details are included in Supplementary Materials and Methods.

Experiments 2 and 3: cue-seeking after abstinence

These experiments were performed in separate groups of rats from the threshold tasks. Following acquisition, rats were returned to their home cages for 21 days of abstinence. Food restriction was lifted during this abstinence period and resumed 1 day before behavioral testing. For the cue-seeking task, rats were returned to the operant chamber for 30 min where active lever pressing resulted in the illumination of the previously drug-paired cue light without a cocaine infusion.

16S sequencing and SCFA metabolomics

Thirty minutes following completion of the cue-induced cocaine-seeking task, rats were euthanized by rapid decapitation, and cecal contents were removed and flash frozen on dry ice. Samples were processed for 16S sequencing and SCFA metabolomics as described previously with full details in Supplementary Materials and Methods.

RNA-sequencing analyses

Rats were euthanized as above, and the nucleus accumbens core was rapidly dissected on ice using the anterior commissure as an anatomical guide. RNA was isolated and cDNA libraries were prepared according to standard protocols. Libraries were sequenced with 150 nucleotide paired-end reads. Filtered reads were mapped to the mouse genome using STAR, and differential gene expression was performed using the DeSeq2 package with significantly regulated genes identified with the predetermined criteria of an FDR adjusted p value < 0.2. Full methodological details in Supplementary Materials and Methods.

Statistical analysis/figures

For analysis of self-administration acquisition and within-subject dose-response, repeated measures ANOVA or mixed effects analyses were utilized as appropriate with Fisher's post-hoc test used in Experiment 1, and Tukey post-hoc tests utilized for reinstatement analyses. For PICRUSt, abundances were z-scored and analyzed using an ANOVA with a Tukey post-hoc. For pairwise behavioral comparisons, two-tailed Student's t-tests were used. Analysis of RNA-sequencing and pathway data are as above. Figures were created using BioRender.com with permission to publish.

RESULTS

Experiment 1: microbiome depletion increases responding for low dose cocaine

To assess the effects of microbiome manipulation on drug self-administration, our previously established protocol of control (H_2O) or non-absorbable antibiotic-treated (Abx) rats (Fig. 1A) was used. Following the initial treatment period, rats were trained to self-administer cocaine on a fixed ratio 1 (FR1) reinforcement schedule at a dose of 0.8 mg/kg/infusion. Initial studies found that microbiome manipulation affected the development of conditioned place preference and locomotor sensitization to low but not high dose cocaine [11], so this higher training dose was

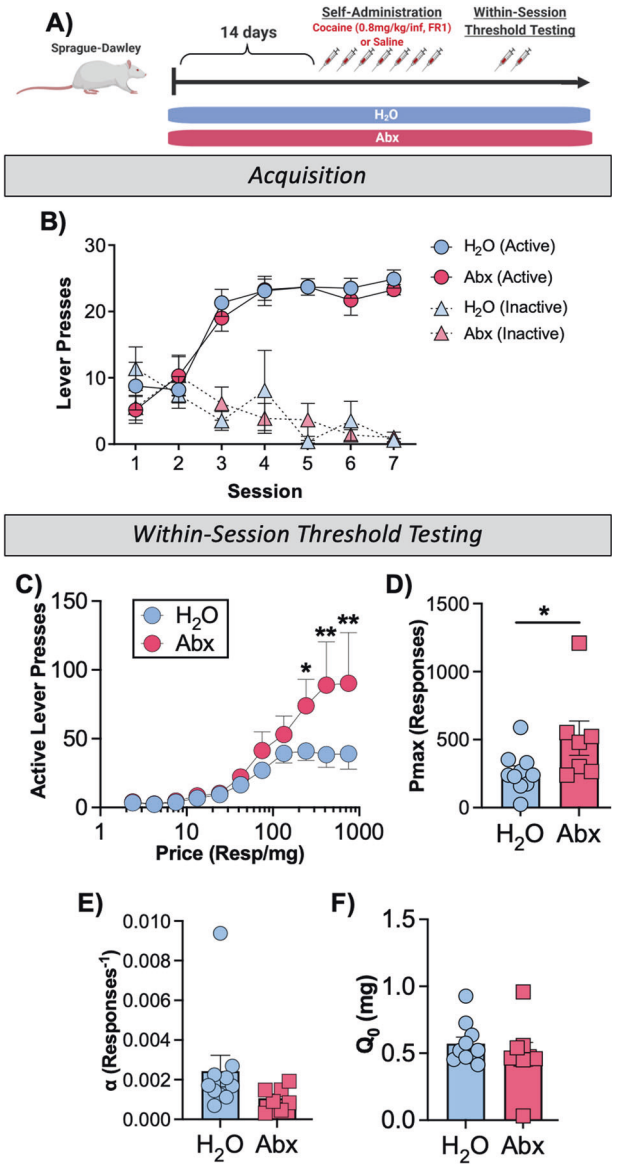

Fig. 1 Abx effects on cocaine taking behaviors. A Schematic diagram of experimental procedures. **B** Acquisition of FR1 cocaine self-administration. Both H_2O and Abx groups demonstrated robust and equal acquisition of active lever pressing (Main effect of day: $p < 0.0001$). **C** Dose-response curve for within-session threshold task demonstrated significant price x treatment interaction ($p = 0.006$) with Abx rats pressing more for lower doses. **D** Calculation of maximal effort rats will exert for cocaine, as measured by the P_{max} calculation, was increased in Abx rats ($p = 0.05$). **E** The alpha measure, which calculates demand elasticity ws not significantly different between groups. **F** Drug intake at minimally constraining price (Q_0) was not significantly different between groups. All data presented as mean ± SEM; *$p \leq 0.05$; **$p < 0.01$. $N = 10$ H_2O; 7 Abx.

selected to allow for equal acquisition of the response and to allow for subsequent testing of drug-seeking at different doses.

Both the H_2O and Abx groups acquired self-administration with a significant main effect of day (Fig. 1B; $F_{(6,102)} = 24.23$, $P < 0.0001$), but no effect of treatment ($F_{(1,102)} = 0.84$, $p = 0.36$) or any interaction ($F_{(6,102)} = 0.41$, $p = 0.87$). Similarly, there were no treatment effects for inactive lever pressing ($F_{(1,17)} = 0.009$, $p = 0.93$). Building from this foundation of equal administration between groups, rats were then tested on the within-session threshold task [42] to assess motivation for cocaine [43, 44]. Figure 1C shows the dose-response curve in which the cocaine dose delivered per lever press decreases over the session—on the x-axis increases in "price" are the number of presses required per mg of cocaine. There was a significant effect of price within these sessions, as was expected ($F_{(10,150)} = 16.91$; $p = 0.0001$). There was no significant main effect of antibiotic treatment ($F_{(1,15)} = 2.61$; $p = 0.13$), but there was a significant price_x_treatment interaction ($F_{(10,150)} = 2.596$; $p = 0.006$) owing to the fact that Abx-treated rats showed more robust responding for higher prices (i.e. lower dose/infusion) of cocaine.

The power of the threshold task is that it assesses motivation and dose-response intake within a single session. Rats will maintain a preferred level of drug when the cost of drug intake is low. As price increases, rats are unable to maintain this dose and reduce their intake [42, 43]. The inflection point at which this happens is P_{max}, the maximal price that the animal is willing to pay to maintain a chosen concentration of drug. Abx-treated rats exhibited a significant increase in P_{max} (Fig. 1D; $t = 2.11$, $p = 0.05$). We also measured alpha (α) which estimates the *rate* at which drug consumption declines in the face of increasing price. There was no significant difference between groups on alpha (Fig. 1E; $t = 1.41$, $p = 0.18$) We also measured drug intake at a minimally constraining price, Q_0 (Fig. 1F; $p = 0.42$). There was no significant change, likely owing to similar drug intake between groups at higher doses when low effort was required to maintain intake.

Experiment 2: Microbiome depletion increases cue-induced drug-seeking after abstinence

We examined whether antibiotic treatment would affect cocaine-seeking behavior in a seeking model of cocaine use disorder. A new cohort of rats was split into two groups, receiving either control (H_2O) or antibiotics (Abx) for the duration of the study (Fig. 2A). For these experiments rats were trained to self-administer cocaine for 2 weeks. They next underwent 21 days of home cage abstinence followed by a cue-induced cocaine-seeking task. Consistent with findings from Experiment 1, no group differences were observed in active (Fig. 2B; $F_{(1,11)} = 3.39$, $p = 0.09$) or inactive (Fig. 2B; $F_{(1,11)} = 0.28$, $p = 0.61$) lever pressing between rats that received either antibiotics or control. A main effect of time was observed, indicating that both groups acquired cocaine self-administration (Fig. 2B active lever $F_{(3,33)} = 6.98$, $p < 0.001$). No significant time by treatment interaction was observed (Fig. 2B active lever $F_{(13,138)} = 0.42$, $p = 0.96$; Fig. 2B inactive lever; $F_{(13,137)} = 0.92$, $p = 0.53$).

On the cue induced seeking task, while both groups showed robust preference for the previously active lever (Fig. 2C; $F_{(1,22)} = 69.60$; $p < 0.0001$), there was a main effect of antibiotics ($F_{(1,22)} = 8.72$; $p = 0.007$), and a treatment x lever interaction ($F_{(1,22)} = 10.42$; $p = 0.004$). Post-hoc analysis demonstrated that this interaction was due to Abx-treated rats pressing at much higher levels for the previously active lever (Fig. 2C Red asterisks; $p \leq 0.001$) compared to H_2O-treated controls, with no treatment effect on the inactive lever (Fig. 2C; $p = 0.99$). Post-hoc analysis further demonstrated that both Abx-treated (Fig. 2C; $p < 0.0001$) and control rats (Fig. 2C; $p = 0.012$) exhibited increased responding for the previously active lever compared to the inactive lever, as expected.

As an additional measure of drug-seeking, we analyzed the latency to first lever press in response to a return to the chamber. Abx-treated rats showed decreased latency to their first active lever press relative to H_2O-treated controls (Fig. 2D; $t_{(10)} = 2.24$, $p = 0.049$). Note that one H_2O sample was excluded from this analysis only for being a high outlier on this measure only. Removal does not affect statistics with appropriate tests, but affects ability to visualize data. Examination of responding over the duration of the session was performed by analysis of cumulative responding for the previously active lever in five-minute bins for the duration of the 30 minute session. Abx-treated

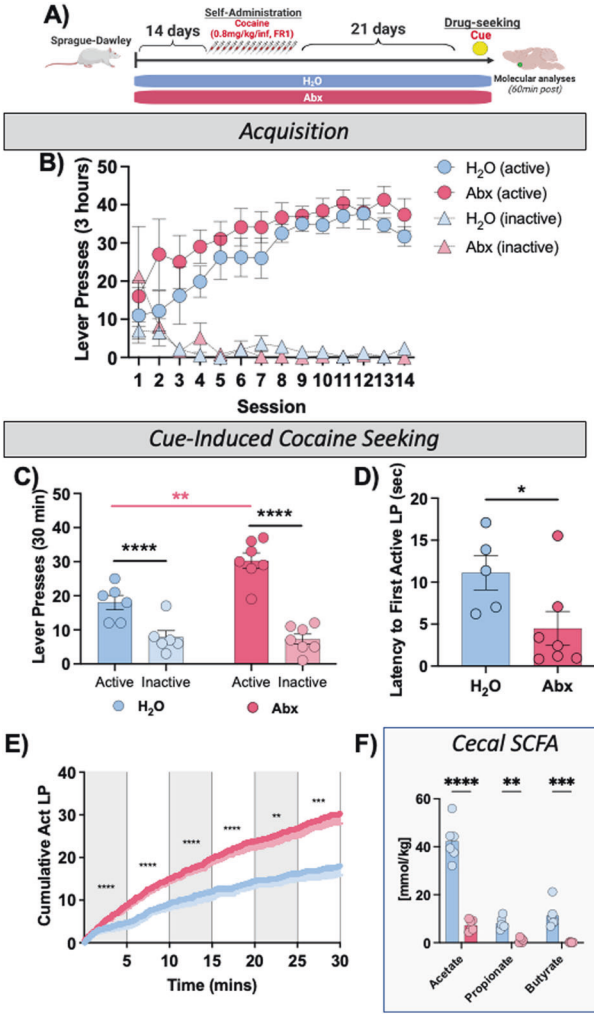

Fig. 2 Abx increases on cocaine-seeking after abstinence. **A** Schematic diagram of experimental procedure for this experiment. **B** Acquisition of FR1 self-administration. Both H_2O and Abx groups showed robust acquisition of active lever pressing (Main effect of day: $p < 0.0001$). **C** On a cue-induced cocaine seeking task, both treatment groups showed robust lever pressing on the previously active lever—but pressing on the previously active lever was markedly increased in Abx-treated rats (red asterisks, $p = 0.001$). **D** The latency to first previously active lever press was significantly lower in Abx-treated rats ($p = 0.049$). **E** Analysis of cumulative active lever pressing across the session in five-minute bins shows Abx-treated rats had higher lever pressing in each bin measured. **F** Antibiotic treatment led to robust depletion of all three major SCFA in cecum. All data presented as mean ± SEM; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. $N = 6$–7/group.

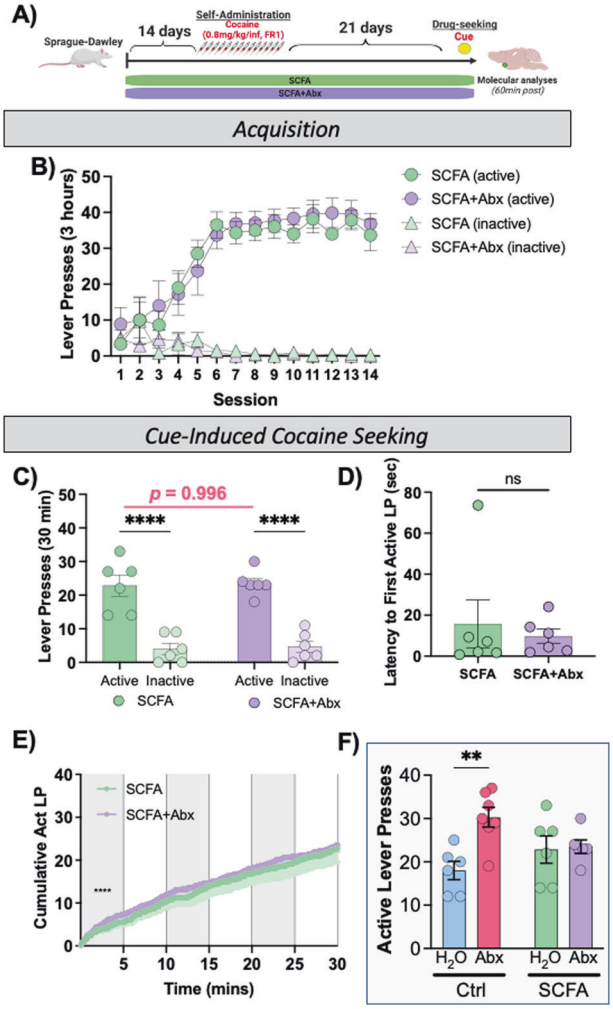

Fig. 3 SCFA repletion reverses effects of microbiome depletion on drug-seeking. **A** Schematic diagram of experimental procedure. **B** SCFA and SCFA+Abx rats both acquires FR1 cocaine self-administration (Main effect of time: $p < 0.0001$). **C** On a cue-induced seeking task both groups showed robust responding on the previously active lever. However, in this case, rats treated with SCFA+Abx show equal total active responding to SCFA controls. **D** SCFA+Abx rats showed a trend toward increased latency to first response, but the effect was not significant. **E** Cumulative lever pressing over time shows parallel trajectories of responding between the two groups aside from the first five minutes. **F** Comparison of active lever presses on the cue seeking task for all four groups. All data presented as mean ± SEM; $^{****}p < 0.0001$. $N = 6$–7/group.

rats exhibited significantly increased sampling of the active lever at all time bins throughout the duration of the session (Fig. 2E; $p < 0.01$ for all bins).

To assess for underlying molecular effects of microbiome depletion, we also quantified the three most abundant short-chain fatty acids (SCFA). We found that as expected antibiotic treatment robustly reduced levels of all three SCFA tested (Fig. 2F; Effect of treatment: $F_{(1,30)} = 161.3$, $p < 0.0001$; Effect of analyte: $F_{(2,30)} = 88.87$, $p < 0.0001$; Interaction: $F_{(2,30)} = 38.87$, $p < 0.0001$).

Experiment 3: SCFA repletion reverses the effects of antibiotics on cocaine-seeking after abstinence

Given that SCFA were decreased following antibiotic treatment and that prior studies demonstrate SCFA repletion reverses effects of antibiotic treatment on mouse conditioned place preference for cocaine and morphine [11, 16], we hypothesized that SCFA repletion would likewise reverse the effects of antibiotic treatment on cocaine-seeking. The experimental design was similar to Experiment 2 except rats received either SCFA alone or a combination of SCFA+Abx in their drinking water (Fig. 3A).

Again, during acquisition no group differences were observed in either active (Fig. 3B; $F_{(1,11)} = 0.29$, $p = 0.60$) or inactive (Fig. 3B; $F_{(1,11)} = 0.02$, $p = 0.89$) lever pressing between rats that received either SCFA or SCFA+Abx. Both groups acquired cocaine self-administration, as indicated by a main effect of time (Fig. 3B active $F_{(2.6,27.6)} = 21.03$, $p < 0.0001$; inactive $F_{(2.7,28.8)} = 3.09$, $p = 0.048$). Time by treatment interaction was not observed (Fig. 3B active $F_{(13,139)} = 0.32$, $p = 0.98$; inactive $F_{(13,139)} = 0.83$, $p = 0.63$).

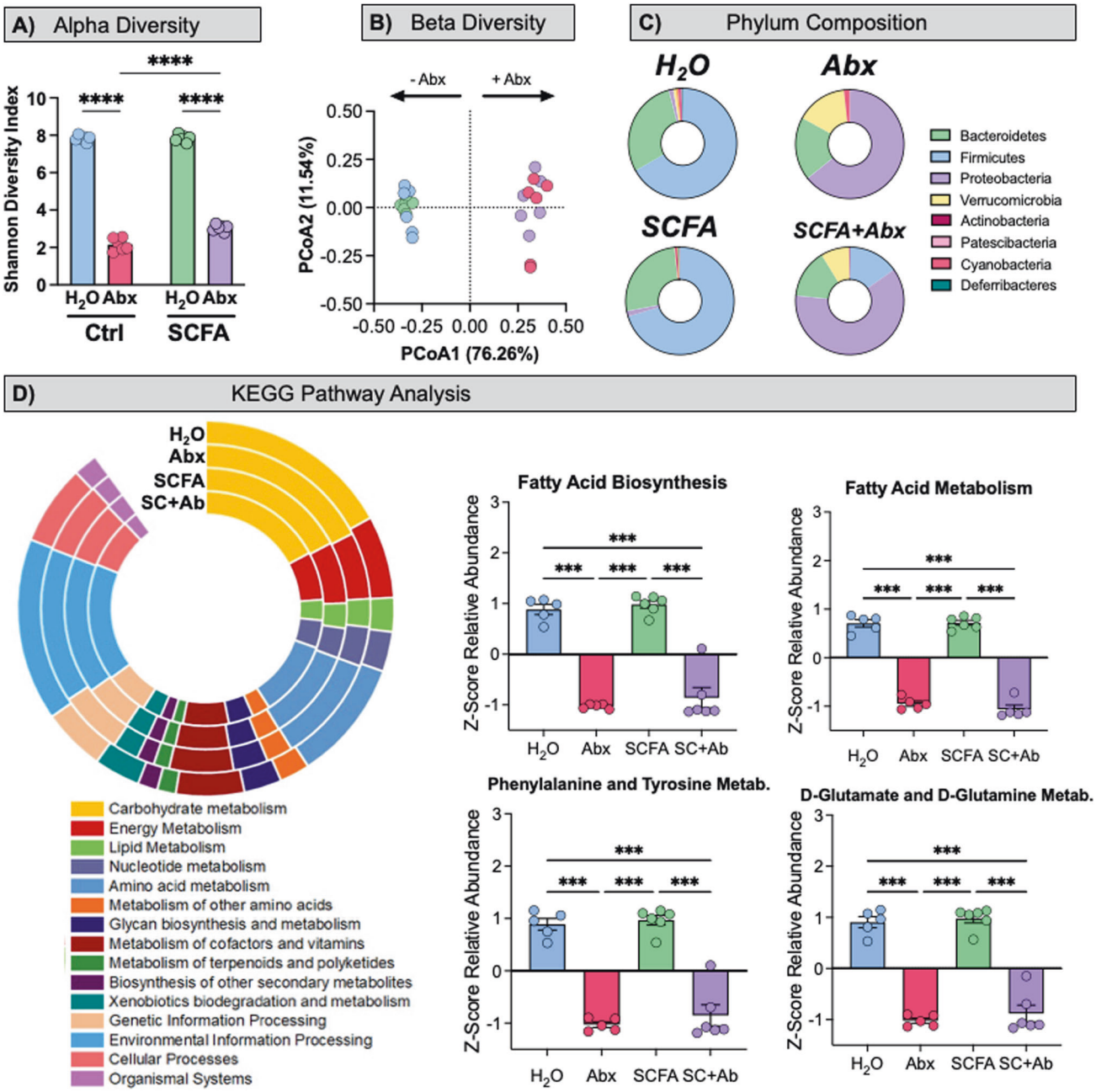

Fig. 4 Effects of Abx and SCFA on microbiome composition and function. A Using the Shannon measure of alpha diversity, a measure of within sample diversity, there are marked reductions in diversity with antibiotictreatment in both groups (Main effect of Abx $p < 0.0001$), and also a less robust but statistically significant main effect of SCFA treatment ($p = 0.0001$). **B** Weighted UniFrac dissimilarity analysis of beta diversity demonstrates antibiotic treatment is the main driver of between rats diversity changes. **C** Donut plots of relative phylum composition show unique changes in bacterial composition between groups. Importantly, these plots show percentage of total, not absolute quantification. **D** High-level overview of PICRUSt data highlights KEGG abundances between treatment groups. KEGG pathway relative abundance is represented in a chipped-donut plot with group labels at the top of the plot. Highly differential pathways involved in fatty acid biosynthesis, fatty acid metabolism, phenylalanine and tyrosine metabolism, and glutamate metabolism are shown as z-scores ± SEM to the right. All plots demonstrate a down regulation in the Abx and Abx+SCFA groups compared to H_2O and SCFA groups. $N = 5–7$/group.

After 21 days of abstinence, both groups exhibited cocaine-seeking with a main effect of active lever pressing (Fig. 3C; $F_{(1,22)} = 72.63$, $p < 0.0001$). As hypothesized, SCFA repletion attenuated the effects of antibiotics on cue-induced cocaine-seeking. There was no main effect of antibiotic treatment (Fig. 3C; $F_{(1,22)} = 0.85$, $p = 0.37$), nor was there a significant interaction (Fig. 3C; $F_{(1,22)} = 0.027$, $p = 0.87$). Tukey's post-hoc analysis demonstrated no differences in responding for either the previously active lever (Fig. 3C; $p = 0.87$) or the inactive lever (Fig. 3C; $p = 0.95$). Post-hoc analysis further demonstrated that both SCFA+Abx-treated (Fig. 3C; $p < 0.0001$) and SCFA-treated rats (Fig. 3C; $p < 0.0001$) exhibited increased responding for the previously active lever compared to the inactive lever, as expected.

No differences were observed in latency to first active lever press (Fig. 3D; $t_{(11)} = 1.73$, $p = 0.11$). Cumulative responding analysis revealed that SCFA+Abx rats exhibited increased responding for the previously active lever only during the first

five minutes of the session (Fig. 3E; $F_{(1,3896)} = 289.0$, $p < 0.0001$). No differences in responding were observed for the other time bins across the 30-minute session.

Active lever presses for all groups on the cue seeking were then compared directly as shown in Fig. 3F. For these analyses there was found a main effect of Abx ($F_{(1,21)} = 7.59$, $p = 0.01$) as well as an Abx x SCFA interaction ($F_{(1,21)} = 6.1$, $p = 0.02$). On comparisons to the H_2O-Ctrl rats, only the Abx group was significantly different ($p = 0.003$, Tukey post-hoc), while both SCFA and SCFA+Abx were statistically equivalent.

Microbiome depletion decreases microbial diversity and predicted SCFA metabolism

To explore the effects of antibiotic-treatment and SCFA repletion microbial diversity, we performed 16S sequencing on cecal contents from rats that underwent behavioral testing for cue-induced cocaine-seeking following abstinence (Figs. 2A and 3A). As expected, there was a robust main effect of antibiotics on alpha diversity (Fig. 4A; $F_{(1,20)} = 3194$, $p < 0.0001$). Interestingly, there were main effects of SCFA treatment ($F_{(1,20)} = 23.38$, $p = 0.0001$) and an antibiotics x SCFA interaction ($F_{(1,20)} = 23.03$, $p = 0.0001$). On post-hoc testing there were between group differences for Abx-treated and non-Abx-treated groups. While there was no difference between control H_2O-treated and SCFA-treated rats ($p = 0.98$), there was a modest but significant difference between Abx-treated and Abx+SCFA-treated rats for alpha diversity (Fig. 4A red/purple comparison; $p < 0.0001$).

Beta diversity analysis using a weighted UniFrac dissimilarity matrix showed that the primary driver of diversity was antibiotic treatment (Fig. 4B x-axis accounts for 76.26% of variability); within these groups the control and SCFA treatment groups largely co-clustered. Antibiotic treatment resulted in significant decreases in the relative abundance of Actinobacteria, Deferribacteres, Firmicutes, Patescibacteria and Tenericutes, and additional unclassified phyla (Fig. 4C). SCFA treatment groups looked similar to their control counterparts, but with SCFA+Abx displaying some shifts in

Fig. 5 RNA sequencing analysis of NAc after drug seeking. A Volcano plots of gene expression in all three treatment groups relative to H_2O controls. Colored points in each panel are statistically significant after FDR correction. **B** UpSet plot of unique and overlapping gene expression patterns between each group. Left bar graphrepresents the number of significant genes in each treatment category. Right graph shows the number of genes in each treatment or treatment combination as indicated by filled circles. **C** Significant GO terms of interest from the unique "Abx Signature" gene list. **D** Predicted protein-protein interactions among genes from synapse and cell junctions gene lists. **E** Heatmap showing z-scored FPKM values of genes from the Abx signature list demonstrating moderation of SCFA+Abx compared to Abx only. **F** Heatmap showing significant module-trait relationships from WGCNA analysis, asterisks indicate significant association with that treatment. **G** Highlighted GO terms from the Pink module, which is the largest module associated with Abx treatment. **H** Key driver analysis from the Pink module using maximal clique centrality analysis. $N = 5$ H_2O, 4 Abx, 6 SCFA, 5 SCFA+Abx; $^*p < 0.05$, $^{**}p < 0.01$.

Proteobacteria, Bacteroidetes, and Firmicutes relative to Abx-treated rats.

To gain more granular insight into the relative changes induced by antibiotics and SCFA treatment, we performed PICRUSt2 analysis. PICRUSt2 predicts the functional potential of bacterial communities based on sequencing profiles. These predictions are supported by integration into the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthologs (KO) and Enzyme Commission numbers (EC) to explore related functional changes. A high-level overview of abundance of KEGGs categorized by metabolic function is shown in Fig. 4D-left as a chipped donut plot of KEGG abundance categorized by KEGG mapper. To further explore relevant pathways, we evaluated KOs related to fatty acid biosynthesis and metabolism as well as essential amino acids required for neurotransmitter production. Significant differences in abundance following antibiotic treatment were observed in the KOs for Fatty Acid Biosynthesis (Fig. 4D-TopMiddle; $F(3,18) = 73.86$; $p < 0.001$), Fatty Acid Metabolism (Fig. 4D-Top-Right; $F(3,17) = 218.8$; $p < 0.001$), Phenylalanine, Tyrosine, and Tryptophan Metabolism (Fig. 4D-BottomMiddle $F_{(3,18)} = 64.18$; $p < 0.001$), and D-Glutamate and D-Glutamine Metabolism (Fig. 4D-BottomRight; $F_{(3,18)} = 91.43$; $p < 0.001$), respectively. Post-hoc analysis for each of the aforementioned KOs revealed significantly decreased biosynthesis and metabolism in the Abx and SCFA+Abx groups compared to the H_2O and SCFA groups. Further analysis of microbiome data is available as Supplementary Figs. 2–4.

Microbiome depletion alters gene expression in the nucleus accumbens

Although many brain regions contribute to drug reward and the development of substance use disorders, the nucleus accumbens (NAc) is the most heavily implicated as a primary reward center in the brain, playing key roles in drug-seeking after abstinence [45, 46]. Previous work demonstrates that manipulations of the microbiome can markedly affect transcriptional regulation in the brain [11, 16, 31, 47, 48]. To explore the impact of microbiome depletion and SCFA repletion on gene expression in brain reward circuitry, we performed full transcriptomic RNA sequencing on NAc punches from rats that underwent behavioral testing for cue-induced cocaine-seeking following abstinence (Figs. 2A and 3A). When compared to the H_2O controls, we find that all three treatment groups exhibited altered gene expression with 551 DEGs in the Abx group, 262 in the SCFA+Abx group, and 149 in the SCFA group (Fig. 5A). Significant gene ontologies of interest from each group are provided as Supplementary Fig. 5; full list of significant GO terms from each is available as Supplementary Tables 1–3. To dissect the individual differences and overlap between the treatment groups, we created an UpSet plot [49] (Fig. 5B). The number of statistically significant genes in each group is shown on the lower left horizontal bar graph. Intersections between groups are demonstrated in the right side of the graphic with filled in circles in the dot plot representing the genes found in each individual group or combination of groups. This analysis demonstrates that Abx-treated mice have the highest number of differentially expressed genes relative to controls (Fig. 5-right)—and importantly that the majority of these significant genes are unique to the Abx-treatment group (Fig. 5B-right, red bar, 421 for Abx). Rats treated with SCFA+Abx have a significant difference of only 123 unique genes, suggesting that addition of the SCFA to rats with depleted microbiomes led to normalization of transcriptional regulation as well as drug-seeking behaviors.

A detailed analysis of these "Abx Signature" genes that were uniquely altered in Abx-treated rats revealed marked alterations in genes related to synaptic pathways, nervous system development, and membrane function (Fig. 5C and Supplementary Table 4). STRING analysis of predicted protein-protein interactions amongst these gene products in the synapse and cell junction pathways predicted changes in a highly interactive group of pre- and post-synaptic functions (Fig. 5D; High resolution larger image is available as Supplementary Fig. 6 and full network interaction statistics are available as Supplementary Table 5). Finally, heatmap analysis of the genes from this antibiotic signature shows that while these genes were all markedly different from H_2O controls, there was a tempering effect particularly noted in the Abx+SCFA group (Fig. 5E-right column). These data demonstrate that depletion of the microbiome leads to marked transcriptional and behavioral changes—and that SCFA metabolite repletion can attenuate some of the molecular and behavioral phenotypes.

To interrogate the effects of our microbiome and metabolite manipulations in more detail, we also performed weighted gene coexpression network analysis as described previously (WGCNA) [50, 51]. This advanced analysis allows for threshold free detection of clusters of genes that share co-expression patterns allowing them to be grouped into modules without a priori hypotheses about gene function. As a result, genes are grouped into modules based only on coexpression size, and named after arbitrary colors based on descending size of the modules. In Fig. 5F we show all modules that showed significant associated with particular treatment groups as indicated by asterisks in the field (Full module-trait association matrix is Supplementary Table 7; gene membership of all significant modules is provided as Supplementary Table 6). Among the modules associated with Abx treatment, the Pink module was by far the largest and had the most robust functional annotation. Here we performed G:Profiler analysis on the genes from this module and find many predicted functional changes as those from the Abx signature genes (Fig. 5G) with significant pathways related to synapse function, nervous system development, and cell junctions (full Pink module gene ontology analysis available as Supplementary Table 7). By using this additional unbiased clustering analysis we again identify clear patterns of genes affected by microbiome depletion and metabolite replacement. Further, we performed key gene driver analysis on the genes from the Pink module by using maximal clique centrality analysis to identify the most interconnected genes in this module (Fig. 5H). These analyses will provide additional fodder for determining the genes that drive microbiome dependent alterations in central gene expression.

DISCUSSION

We show that antibiotic induced microbiome depletion has marked effects on both active cocaine self-administration and drug-seeking after abstinence. Rats treated with antibiotics show normal acquisition of cocaine self-administration for high dose (0.8 mg/kg/inf) cocaine on an FR1 schedule. However, when using a within-session threshold task, we found that microbiome depletion was associated with enhanced drug intake at the lower bounds of the dose range (Fig. 1). This suggests enhanced motivational effects of cocaine or a shift in the dose-response curve induced by microbiome depletion. While within-session threshold testing is a useful measure of drug-seeking during active use, we wanted to examine the role of gut-brain signaling in drug-seeking following abstinence using a relapse model. We found that rats with a depleted microbiome exhibit increased drug-seeking in response to a drug-paired cue following three weeks of forced abstinence (Fig. 2). This is in line with the threshold task data, suggesting that depletion of the microbiome enhances the motivational effects of cocaine. Importantly, the effects of microbiome depletion could be reversed by repletion of SCFAs, suggesting a possible mechanism for the observed behavioral effects (Fig. 3). Functional analysis found marked changes in numerous bacterial metabolic pathways due to Abx treatment (Fig. 4). When we examined how microbiome depletion and SCFA repletion affected transcriptional regulation following drug-

seeking, we found that alterations in the microbiome and its metabolites played an important role in shifting the transcriptional landscape (Fig. 5). Taken together, these findings demonstrate a role for the microbiome and its metabolites in drug-taking and seeking, laying the foundation for future translational work in this space.

We previously found that depletion of the gut microbiome enhanced cocaine conditioned place preference and locomotor sensitization at low—but not high—doses of cocaine [11]. These behavioral effects were also reversed by repletion of SCFA in antibiotic-treated mice. Lee and colleagues demonstrated that a shorter treatment with a different antibiotic cocktail led to a reduction in cocaine place preference at an intermediate dose [52]. Recent studies demonstrated production of gut microbiome-derived glycine can have marked effects on behavioral responses to cocaine [19]. Interesting recent work has even identified interactions between the microbiome and social stimuli in regulating cocaine place preference [18]. Studies of microbiome effects on non-stimulant drugs of abuse have also shown marked effects of the microbiome on behavior. Recently, we found that depletion of the microbiome using the same antibiotic regimen led to decreased preference for morphine across a wide dose range, and that this microbiome effect was again reversable by SCFA repletion [16]. These results are interesting, as they use identical manipulations of the microbiome and its byproducts—but show opposite effects with opioids. A number of well-crafted studies have demonstrated that a diverse microbiome is important for normal development of tolerance to morphine over time; both germ-free and antibiotic-treated mice show reduced tolerance for morphine [21, 32]. All of these previous studies have utilized shorter term experimenter-administered drugs. The studies in this manuscript are the first to utilize voluntary cocaine self-administration and to employ a method for examining drug-seeking in a model for relapse.

One potential mechanism by which manipulations of the gut microbiome can affect neurobiological plasticity and subsequent behavior is via effects on transcriptional regulation in the brain. Previous studies have demonstrated that lack of a diverse gut microbiome modifies transcriptional regulation in the amygdala and prefrontal cortex following fear conditioning [47, 48, 53, 54]. Microbiome depletion significantly alters regulation of neuroplasticity-related genes in the NAc following cocaine exposure [11]. Likewise, in opioid-exposed rats, microbiome depletion led do dysregularities in immediate early gene activation patterns [55] in addition to transcriptome-wide dysregulation [16]. Experiments presented here have the important distinction that transcriptional profiling was performed three weeks after final drug exposure and following a cue-induced drug-seeking task. As a likely result of this difference, transcriptional effects are more modest than those seen immediately after drug intake (Fig. 5). However, these findings are similar to those seen after a fear conditioning task in which there was a significant microbiome x behavior interaction in transcriptional regulation [48].

Importantly, repletion of the microbiome-derived SCFA metabolites leads to reversal of the microbiome effects on drug-seeking and alters transcriptional regulation in the NAc. We examined these metabolites as there is a robust literature demonstrating SCFA as key gut-brain signaling molecules [56]. These molecules, which are produced by gut bacteria in the process of fermentation of dietary fiber and are nearly completely eliminated by antibiotic treatment (Fig. 2F), are implicated in myriad gut-brain signaling pathways from control of blood-brain barrier integrity and microglial function to regulation of food intake [27, 29, 56, 57]. It is noteworthy that all rats in this study were food deprived for the procedure, and while we do not expect an interaction with this and the antibiotic treatment, it does remain a possibility. Previously, SCFA are shown to regulate behavioral responses to both experimenter-administered cocaine and opioids [11, 16]. There is robust evidence that the SCFA modulate transcriptional regulation in the brain. All of these small molecules function as histone deacetylase inhibitors, with butyrate and acetate having the strongest effects [58]. Recently gut-derived acetate was found to disrupt patterns of histone acetylation in the brain, affecting consolidation of memory and behavioral response to alcohol [59, 60]. All SCFA regulate activation of the CREB transcription factor and alter expression of numerous gene products including tyrosine hydroxylase, c-Fos and enkephalin [61–67]. Additionally, in these studies we find that the treatment with antibiotics markedly alters both alpha and beta diversity of the microbiome and has wide-ranging effects on numerous metabolic pathways with potential import for gut-brain signaling—including fatty acid metabolism, phenylalanine metabolism, and glutamate metabolism (Fig. 4). While behavioral and transcriptional effects of microbiome depletion were to some extent reversed by repletion of SCFA compounds, the full contribution of the microbiome and its byproducts to brain and behavior is likely much more complex.

Our understanding of how the gut microbiome and its resultant byproducts can alter brain and behavior is advancing at a rapid rate. These findings provide the first evidence that manipulation of the gut microbiome and its metabolites can are mediators of cocaine self-administration and drug-seeking after relapse. These studies provide important foundational data to move gut-brain signaling towards further translational studies, which should examine how specific microbial composition and metabolite levels work to drive both drug-seeking and other motivated behaviors. Ultimately, there is potential for these microbial signaling pathways to be explored as either biomarkers or treatments for patients with substance use disorders. While much remains to be done, this work suggests strong potential for these pathways to be harnessed from the bench to the bedside.

REFERENCES

1. Substance Abuse and Mental Health Services Administration. Facing Addiction in America: The Surgeon General's Report on Alcohol, Drugs, and Health. in Facing Addiction in America: The Surgeon General's Report on Alcohol, Drugs, and Health (ed. U.S. Department of Health and Human Services (HHS), O. of the S. G.) chap. 6 (HHS, 2016). https://www.ncbi.nlm.nih.gov/books/NBK424857/pdf/Bookshelf_NBK424857.pd.
2. Mews P, Calipari ES. Cross-talk between the epigenome and neural circuits in drug addiction. Prog Brain Res. 2017;235:19–63.
3. Walker DM, Nestler EJ. Neuroepigenetics and addiction. Handb Clin Neurol. 2018;148:747–65.
4. Hofford RS, Russo SJ, Kiraly DD. Neuroimmune mechanisms of psychostimulant and opioid use disorders. Eur J Neurosci. 2018. https://doi.org/10.1111/ejn.14143.
5. Lucerne KE, Kiraly DD. The role of gut-immune-brain signaling in substance use disorders. Int Rev Neurobiol. 2020. https://doi.org/10.1016/bs.irn.2020.09.005.
6. Dinan TG, Cryan JF. Microbes Immunity and Behavior: Psychoneuroimmunology Meets the Microbiome. Neuropsychopharmacology. 2017;42:178–92.
7. Dinan TG, Cryan JF. Brain-Gut-Microbiota Axis and Mental Health. https://doi.org/10.1097/PSY.0000000000000519.
8. Vuong HE, Hsiao EY. Emerging Roles for the Gut Microbiome in Autism Spectrum Disorder. Biol Psychiat. 2017;81:411–23.
9. Hsiao EY, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013;155:1451–63.
10. Meckel KR, Kiraly DD. A potential role for the gut microbiome in substance use disorders. Psychopharmacology. 2019;236:1513–30.
11. Kiraly DD, et al. Alterations of the host microbiome affect behavioral responses to cocaine. Sci Rep. 2016;6:1–12.
12. Lach G, Schellekens H, Dinan TG, Cryan JF. Anxiety, Depression, and the Microbiome: A Role for Gut Peptides. Neurotherapeutics. 2018;15:36–59.
13. De Palma G, et al. Microbiota and host determinants of behavioural phenotype in maternally separated mice. Nat Commun. 2015;6:7735.
14. Savignac HM, Tramullas M, Kiely B, Dinan TG, Cryan JF. Bifidobacteria modulate cognitive processes in an anxious mouse strain. Behav Brain Res. 2015;287:59–72.
15. Lucerne KE, Osman A, Meckel KR, Kiraly DD. Contributions of neuroimmune and gut-brain signaling to vulnerability of developing substance use disorders. Neuropharmacology. 2021;192:108598.

16. Hofford RS, et al. Alterations in microbiome composition and metabolic byproducts drive behavioral and transcriptional responses to morphine. Neuropsychopharmacology. 2021. https://doi.org/10.1038/s41386-021-01043-0.
17. Hofford RS, et al. Changes in gut microbiome composition drive fentanyl intake and striatal proteomic changes. Preprint at 2022. https://doi.org/10.1101/2022.11.30.518531.
18. García-Cabrerizo R, Barros-Santos T, Campos D, Cryan JF. The gut microbiota alone and in combination with a social stimulus regulates cocaine reward in the mouse. Brain Behav Immun. 2023;107:286–91.
19. Cuesta S, Burdisso P, Segev A, Kourrich S, Sperandio V. Gut colonization by Proteobacteria alters host metabolism and modulates cocaine neurobehavioral responses. Cell Host Microbe. 2022;30:1615–29.e5.
20. Wang F, et al. Morphine induces changes in the gut microbiome and metabolome in a morphine dependence model. Sci Rep. 2018;8:3596.
21. Zhang L, et al. Morphine tolerance is attenuated in germfree mice and reversed by probiotics, implicating the role of gut microbiome. Proc Natl Acad Sci USA. 2019;116:13523–32.
22. O'mahony SM, Clarke G, Borre YE, Dinan TG, Cryan JF. Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. Behavioural Brain Res. 2015;277:32–48.
23. Visconti A, et al. Interplay between the human gut microbiome and host metabolism. Nat Commun. 2019;10:1–10.
24. Vojinovic D, et al. Relationship between gut microbiota and circulating metabolites in population-based cohorts. Nat Commun. 2019;10:1–7.
25. Vuong HE, et al. The maternal microbiome modulates fetal neurodevelopment in mice. Nature. 2020;586:281–6.
26. Sun J, Chang EB. Exploring gut microbes in human health and disease: Pushing the envelope. Genes Dis. 2014;1:132–9.
27. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell. 2016;165:1332–45.
28. Sun J, et al. Antidepressant-like effects of sodium butyrate and its possible mechanisms of action in mice exposed to chronic unpredictable mild stress. Neurosci Lett. 2016;618:159–66.
29. Erny D, et al. Microbiota-derived acetate enables the metabolic fitness of the brain innate immune system during health and disease. Cell Metab. 2021;33:2260–76.e7.
30. Erny D, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci. 2015;18:965–77.
31. Thion MS, et al. Microbiome Influences Prenatal and Adult Microglia in a Sex-Specific Manner. Cell. 2018;172:500–16.e16.
32. Kang M, et al. The effect of gut microbiome on tolerance to morphine mediated antinociception in mice. Sci Rep. 2017;7:1–17.
33. Lee K, et al. The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence. Neuropsychopharmacology. 2018;1. https://doi.org/10.1038/s41386-018-0211-9.
34. Li X, Venniro M, Shaham Y. Translational Research on Incubation of Cocaine Craving. JAMA Psychiat. 2016;73:1115–6.
35. Dong Y, Taylor JR, Wolf ME, Shaham Y. Circuit and Synaptic Plasticity Mechanisms of Drug Relapse. J Neurosci. 2017;37:10867–76.
36. Lu L, Grimm JW, Dempsey J, Shaham Y. Cocaine seeking over extended withdrawal periods in rats: different time courses of responding induced by cocaine cues versus cocaine priming over the first 6 months. Psychopharmacology. 2004;176:101–8.
37. Pickens CL, et al. Neurobiology of the incubation of drug craving. Trends Neurosci. 2011;34:411–20.
38. Parvaz MA, Moeller SJ, Goldstein RZ. Incubation of Cue-Induced Craving in Adults Addicted to Cocaine Measured by Electroencephalography. JAMA Psychiat. 2016;73:1127–34.
39. Bercik P, et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011;141:599–609.
40. Smith PM, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science. 2013;341:569–73.
41. Braniste V, et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med. 2014;6:263ra158.
42. Calipari ES, et al. Granulocyte-colony stimulating factor controls neural and behavioral plasticity in response to cocaine. Nat Commun. 2018;9:9.
43. Siciliano CA, Jones SR. Cocaine potency at the dopamine transporter tracks discrete motivational states during cocaine self-administration. Neuropsychopharmacology. 2017;42:1893–904.
44. Oleson EB, Roberts DCS. Behavioral economic assessment of price and cocaine consumption following self-administration histories that produce escalation of either final ratios or intake. Neuropsychopharmacology. 2009;34:796–804.
45. Zito KA, Vickers G, Roberts DCS. Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. Pharmacol, Biochem Behav. 1985;23:1029–36.
46. Salgado S, Kaplitt MG. The nucleus accumbens: A comprehensive review. Stereotact Funct Neurosurg. 2015;93:75–93.
47. Chu C, et al. The microbiota regulate neuronal function and fear extinction learning. Nature. 2019;574:543–8.
48. Hoban AE, et al. The microbiome regulates amygdala-dependent fear recall. Mol Psychiat. 2018;23:1134–44.
49. Lex A, Gehlenborg N, Strobelt H, Vuillemot R, Pfister H. UpSet: Visualization of Intersecting Sets. IEEE Trans Vis Comput Graph. 2014;20:1983–92.
50. Sens JP, Hofford RS, Kiraly DD. Effect of germ-free status on transcriptional profiles in the nucleus accumbens and transcriptomic response to chronic morphine. Mol Cell Neurosci. 2023;103874. https://doi.org/10.1016/j.mcn.2023.103874.
51. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinforma. 2008;9:559.
52. Lee K, et al. The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence. Neuropsychopharmacology. 2018;43:2606–14.
53. Hoban AE, et al. Regulation of prefrontal cortex myelination by the microbiota. Transl Psychiatry. 2016;6:e774.
54. Stilling RM, et al. Microbes & neurodevelopment-Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. Brain Behav Immun 2015;50:209–20.
55. Simpson S, et al. Depletion of the Microbiome Alters the Recruitment of Neuronal Ensembles of Oxycodone Intoxication and Withdrawal. eNeuro. 2020;7:ENEURO.0312-19.2020.
56. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol. 2019;16:461–78.
57. Frost G, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat Commun. 2014;5:3611.
58. Waldecker M, Kautenburger T, Daumann H, Busch C, Schrenk D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. J Nutr Biochem. 2008;19:587–93.
59. Mews P, et al. Alcohol metabolism contributes to brain histone acetylation. Nature. 2019;574:717–21.
60. Mews P, et al. Acetyl-CoA synthetase regulates histone acetylation and hippocampal memory. Nature. 2017;546:381–6.
61. Macfabe DF. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. Microb Ecol Health Dis. 2012;23.
62. MacFabe DF, et al. Neurobiological effects of intraventricular propionic acid in rats: possible role of short chain fatty acids on the pathogenesis and characteristics of autism spectrum disorders. Behav Brain Res. 2007;176:149–69.
63. Nankova BB, Agarwal R, MacFabe DF, La Gamma EF. Enteric bacterial metabolites propionic and butyric acid modulate gene expression, including CREB-dependent catecholaminergic neurotransmission, in PC12 cells-possible relevance to autism spectrum disorders. PLoS One. 2014;9:e103740.
64. Rabelo FLA, et al. Inhibition of ERK1/2 and CREB phosphorylation by caspase-dependent mechanism enhances apoptosis in a fibrosarcoma cell line treated with butyrate. Biochem Biophys Res Commun. 2003;303:968–72.
65. Mally P, et al. Stereospecific regulation of tyrosine hydroxylase and proenkephalin genes by short-chain fatty acids in rat PC12 cells. Pediatr Res. 2004;55:847–54.
66. Parab S, Nankova BB, La Gamma EF. Differential regulation of the tyrosine hydroxylase and enkephalin neuropeptide transmitter genes in rat PC12 cells by short chain fatty acids: concentration-dependent effects on transcription and RNA stability. Brain Res. 2007;1132:42–50.
67. Shah P, Nankova BB, Parab S, La Gamma EF. Short chain fatty acids induce TH gene expression via ERK-dependent phosphorylation of CREB protein. Brain Res. 2006;1107:13–23.

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AUTHOR CONTRIBUTIONS

DDK, ESC, and KRM designed the experiments. KRM, AG, EGP, ESC, RSH, and DDK performed experiments. KRM, SSS, AG, JSP, MZL, OG, ESC, RSH & DDK analyzed data. KRM & DDK wrote the manuscript. All authors provided critical edits and feedback of the finalized manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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