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RESEARCH HIGHLIGHT

Morphine, the microbiome, and fatty acids: short chains make a big link in opioid reward

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Influences of the gastrointestinal tract on mood and mental health have been recognized for centuries, and accumulating results over the past decade have supported a key role for gut bacteria (collectively known as the gut microbiome) in many aspects of brain health and disease [1]. While the mechanistic underpinnings of microbiome-brain interactions are complex, emerging evidence suggests that gut microbiota produce multiple brain-penetrant metabolites with gene regulatory potential, such as co-factors for epigenetic modifications [2–4]. This observation has the potential to link differences in gut microbiota to dysregulated gene expression and chromatin patterns that are observed in multiple neuropsychiatric disorders, including major depression, stress-related conditions, and substance abuse [5]. Although bacterial metabolites have recently been implicated in the orchestration of gut-brain interactions [2], evidence supporting a direct role for specific factors in complex motivated behaviors and drug-induced transcriptional reprogramming remains limited.

In this issue of Neuropsychopharmacology, Hofford et al. [6] explored the contribution of gut microbiome diversity and composition in morphine conditioned place preference (CPP) and locomotor sensitization using an oral antibiotic knockdown model (Abx; an antibiotic cocktail delivered via drinking water). The authors first show that oral antibiotic administration reduced bacterial complexity and altered the relative proportion of bacterial phyla, including decreases in *Firmicutes* bacteria. This common gut microbiome phylum is found in healthy individuals and is known to generate short-chain fatty acids (SCFAs) (such as butyrate), which act both as histone deacetylase inhibitors and are further metabolized into co-factors for acetylation of histone proteins. Notably, loss of bacterial diversity was associated with decreased levels of at least two SCFAs produced by gut bacteria—butyrate and propionate. Next, the manuscript reports that microbiome depletion blunts morphine locomotor sensitization and abolishes morphine CPP, commonly used measures of behavioral effects of drugs. Even more strikingly, morphine CPP was completely restored when drinking water was supplemented with SCFAs, demonstrating reversible control of morphine reward as well as necessity for SCFAs in this process.

Given that SCFAs have a known role in gene regulation via epigenetic modulation, Hofford and colleagues next explored whether oral Abx also alters transcriptional changes in the nucleus accumbens (a key brain region linked to drug reward) following repeated morphine administration. Consistent with behavioral observations, microbiome depletion resulted in a dramatically distinct transcriptional responses to morphine, as measured with RNA-seq. While a number of genes were similarly altered by morphine in both control and Abx conditions, microbiome depletion also resulted in additional morphine-regulated changes, largely in functional categories related to histone modification and chromatin dynamics. In addition, SCFA replenishment restored transcriptional changes induced by Abx at several genes, including inducible transcription factors previously identified to be important in drug responses (e.g., the immediate early genes *Egr2* and *Egr4*). Thus, like the behavioral effects of microbiome depletion, at least some effects on morphine-induced gene expression changes are reversible when SCFAs are replaced in the diet.

Together, these findings mark a fundamental first step for continued exploration of gut-brain interactions in the regulation of morphine action. However, many additional questions remain. For example, although this report implicates SCFAs in gut-brain interactions, it is not clear whether (or how) SCFAs might modulate specific gene programs differently in unique cell populations of the NAc, or why some genes are more affected than others. Recent single-cell RNA-seq approaches have identified cell-selective transcriptional patterns in the nucleus accumbens resulting from morphine administration, including surprising effects on microglia [7]. Thus, it will be critical to determine which cell populations are most affected by microbiome depletion, and whether other brain structures are also involved. Likewise, it will be important to define whether SCFA effects are mediated entirely by epigenetic modulation, via other known routes of SCFA function [2], or via unknown mechanisms yet to be discovered. Genome-wide epigenomic profiling may be useful in unravelling the specific epigenetic modifications and genomic locations that are most sensitive to SCFA depletion and replacement. Finally, while this study demonstrated several effects of antibiotics on gut bacteria and SCFAs in male mice, there is ample evidence to suggest that sex differences may contribute to gut-brain interactions [8]. Future studies will be required to identify whether these effects are similar in female mice, and whether gut microbiome contributions to drug responses in humans differ based on sex.

The finding that gut bacteria and their metabolites influence morphine reward and transcriptional changes in a brain area critical to opioid reward add to a growing body of literature supporting a role for the gut microbiome in animal models of substance use. As outlined by Hofford and colleagues, prior

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reports have revealed that microbiome depletion influences analgesic effects of opioids, modulates neuronal encoding of opioid experience, and enhances behavioral responses to cocaine [6, 9]. Given that morphine reward was impaired by microbiome depletion, these findings identify the potential for selectivity and variability of gut microbiome manipulations across drug classes. This contrast highlights another essential aspect of microbiota regulation of nervous system function, which is that an individual's microbiota may be influenced by multiple factors, including early life adversity, experience with psychoactive medications, and even heritable factors transmitted across generations [1, 8]. Together, these factors are likely to shape the contributions of the gut microbiome to vulnerability and resilience for neuropsychiatric disease. Moreover, gut metabolites like SCFAs present under-explored therapeutic avenues for the reversal of brain disease states linked to microbiome dysfunction. While future work will be required to chain together the critical mechanistic contributions of gut microbiome metabolites across the lifespan, this manuscript provides a key link in this chain.

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AUTHOR CONTRIBUTIONS

JJD and JJT wrote and edited the manuscript.

ADDITIONAL INFORMATION

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