Credit: Brain light/Alamy Stock Photo

Gutted about opioids

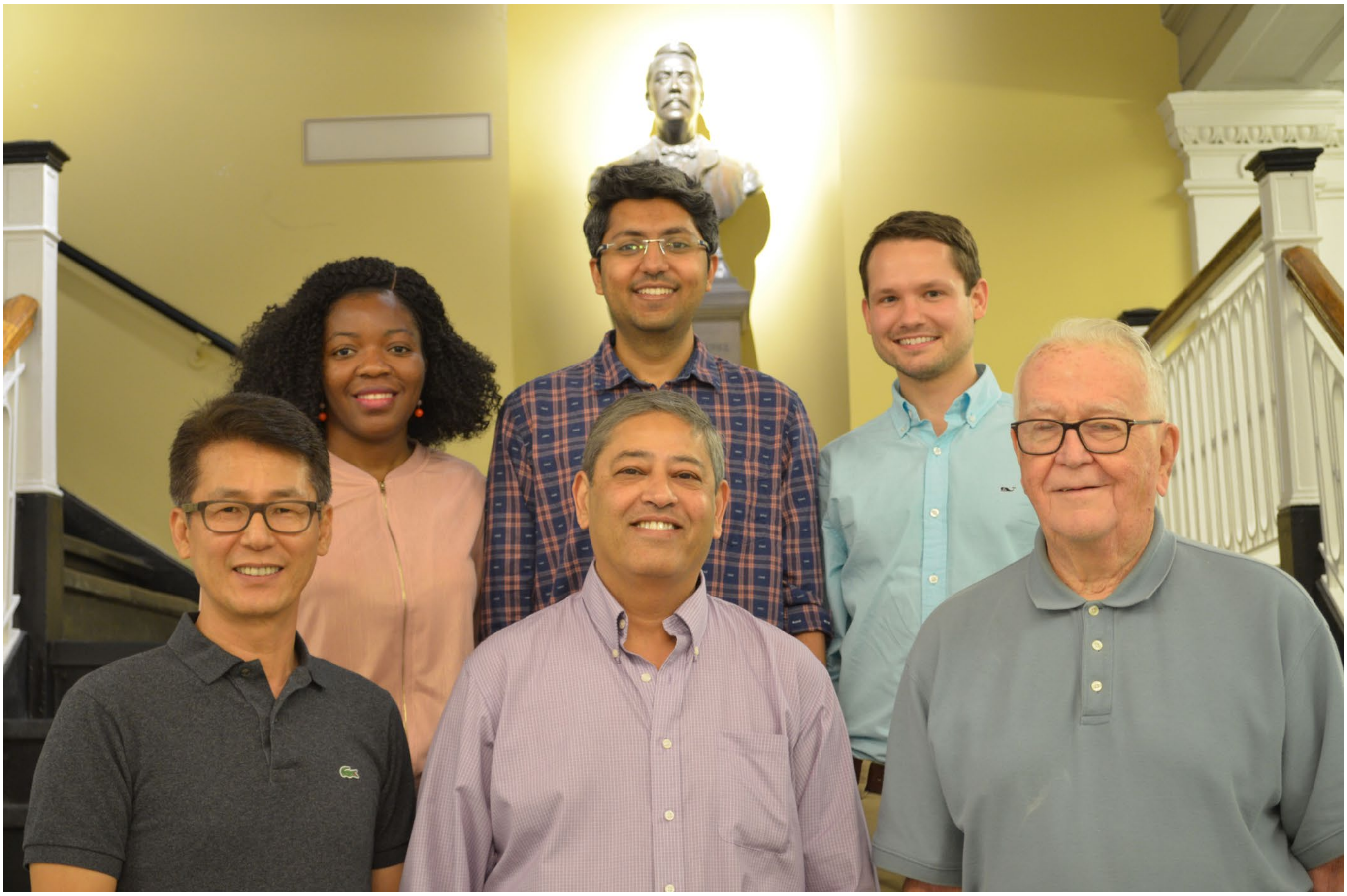
The microbiome may have an influence on addiction

Wudan Yan

On a chilly winter morning this past January, pharmacologist James Kang warmed up a tank of water in a quiet lab room located on the campus of Virginia Commonwealth University in Richmond in preparation for a rodent experiment. After a few minutes, the water became hot enough that any mouse whose tail was dipped into the tank would normally rapidly flick it away within about three seconds.

Kang, a postdoctoral fellow in Hamid Akbarali's pharmacology lab, had brought several sets of mice to the room with the water tank. The idea was to see whether the gut bacteria transplanted into the animals would affect how they responded to a powerful painkiller. Among the mice were two sets that had received the opioid morphine twice a day for five days—enough time for the mice to become desensitized to the drug and need a higher dose to achieve the same pain mitigation as before. Both these sets of mice had also received fecal transplants from other mice, a process meant to completely overwrite their preexisting, healthy gut microbiomes. One group received their microbiome from mice that were given drug pellets containing morphine, whereas the other group had their microbiome transplants from mice given placebo pellets of cellulose. With the tank of water now hot enough in the lab, Kang treated all the transplanted rodents with morphine and waited 20 minutes for the drug to take effect.

After enough time passed, Kang took each mouse and exposed its tail to the water. Because morphine works by reducing pain, mice treated with the drug are able to withstand the hot water for as long as ten seconds, on average, before flicking their tails away. In contrast, the mice that had received a microbiome transplant from other mice treated with morphine showed signs of being desensitized to the drug: despite receiving injection of the opioid, they moved their tails away within a few seconds of contact with the water. (This was not unexpected: usually, mice desensitized to morphine will flick their tails in about three to five seconds.) But—crucially—when Kang gave a dose of morphine to the mice



Gut sleuths: The Akbarali lab, top row (left to right): Essie Komla, Karan Muchhala, Ryan Mischel; bottom row (left to right): James Kang, Hamid Akbarali, William Dewey. Credit: Hamid Akbarali

that had been treated earlier with five days of the opioid and also received a gut transplant from mice never exposed to the drug, they moved their tails away from the hot water just as languidly as mice that had never been desensitized to morphine.

Kang, at first, thought he made a mistake. But when he and his colleagues repeated the experiment, they kept producing the same data. The findings seemed to suggest that providing healthy bacteria by fecal transplants had overridden any desensitization to morphine. The results fueled an idea that perhaps modifying the microbiome might be one way to prevent people from becoming desensitized—or even addicted—to opioids.

Today, the opioid epidemic is rampant in the US. More than 11% of American adults suffer from chronic pain, and nearly 18% more experience severe pain—defined as very bothersome discomfort that persists most days. However, options for treating chronic pain are limited, and many doctors prescribe patients opioids such as oxycodone, one of the strongest and most effective medicines available. (Oxycodone is synthetically derived from another related compound in opium poppies but is similar in structure to morphine.) Unfortunately, the use and overprescription of oxycodone, some say, have contributed to the expansive opioid epidemic in the US, which is taking more than 130 lives every day from opioid-related overdoses. In light of the scope of the crisis, US National Institutes of Health Director Francis Collins and US National Institute on Drug Abuse Director Nora Voldow called for "all scientific hands on deck" to end the opioid crisis last May, citing the need to develop new, non-addictive treatments for those suffering from chronic pain and technologies to treat opioid addiction.

While there are many variables—both intrinsic to the individual and environmental—that can lead to addiction, researchers are setting out to understand the involvement of the gut microbiome in this process. "If one accepts that the microbiome is shaping brain development, and key circuits underpinning pathology and behavior, then it's not surprising that it will influence the circuits that are underpinning opioid addiction," says John Cryan, a microbiome researcher at University College Cork in Ireland. "It's just that we haven't proven it yet."

The second brain

Although the gut and the brain are separate organs, researchers have speculated since the 1980s that the health of our gut influences higher cognitive functions of the brain via a series of nerves wrapped around the gastrointestinal tract and the trillions of bacteria—collectively known as the microbiome—that live inside it. This is why the gut is sometimes referred to as the 'second brain'. With the growing appreciation of the microbiome in medicine, researchers are finding evidence that it can modulate psychological conditions like depression and anxiety, and are increasingly interested in learning how the microbiome could be linked to drug abuse.

Bacterial detective: Anna Taylor.
Credit: Anna Taylor

Although research into the connection between the microbiome and opioid addiction is still in its infancy, studies are showing that opioids modify the gut microbiome[1,2]. And, at least in mouse experiments, changes in the microbiome from prolonged usage of opioids could lead to animals becoming desensitized to these drugs. When this happens, the same dose of drug loses its ability to allay pain over time and the animal will need higher doses of the drug to manage pain. While no one knows how this desensitization is related to opioid addiction, it may be a contributing factor, Akbarali says.

As quickly as a day after mice start receiving opioids, pathogenic bacteria, such as *Enterococcus faecalis*, colonize the gut and begin releasing proteins that create an inflammatory milieu[1]. Research from Anna Taylor's lab at the University of Alberta in Canada suggests that 'good' bacteria, such as *Lactobacillus*, declined in relative abundance in mice treated with opioids[3].

Once in the body, morphine first gets converted into a metabolite called morphine-3-glucuronide (M3G) in the liver. M3G then gets shuttled to the gut where it needs to be converted back to morphine by bacterial enzymes in order to return to blood circulation and exert its analgesic effects, according to pharmacologist Sabita Roy at the University of Miami in Florida. But the microbes responsible for this conversion—likely *Bacteroides* and *Bifidobacteria*—are depleted over time in the microbiomes of mice treated with chronic doses of morphine, Roy and her colleagues have found[1]. While it's currently not known why certain types of bacteria disappear from repeated exposure to morphine, Roy speculates that it's the disappearance of these bacteria that may be, in part, leading animals to lose sensitivity to opioids. Roy and her colleagues have administered probiotics that contain the necessary bacteria to morphine-treated mice in ongoing and unpublished studies and found evidence that suggests that these probiotics have been able to prolong the effects of morphine.

The dynamics of the microbiome shift with continued exposure to morphine, and as in other microbiome studies, it's hard to pin down which gut bacteria—or lack thereof—are desensitizing animals to the effects of opioids. It may very well be that many families of bacteria are doing the legwork rather than a single one and that pathogenic bacteria proliferate and helpful bacteria decline. Ongoing and unpublished studies from Akbarali's group suggest that transplanting a healthy microbiome into mice that have already become desensitized to morphine can make the animals responsive to the drug again. What this suggests, Akbarali says, is that the microbiome seems to be very instrumental—at least in mice—in causing animals to lose some sensitivity to painkillers.

Looking at leaks

The effects of opioids on the gut may go beyond just modifying the composition of bacteria inside it. Opioids can also disrupt the tight junctions between cells lining the gut epithelium, causing bacteria to leak out from the large intestiine[4,5].

It's possible that these bacteria that have leaked into the colon are also feeding information back to the brain. Researchers hypothesize that these bacteria secrete factors, including proteins, which then leach into the systemic circulation or act on the afferent nerve fibers in the lining of the colon that are connected to the brain. When researchers in Akbarali's lab took a sample of the colonic milieu—containing all the factors secreted by the bacteria in the bowel—from a mouse treated chronically with morphine and exposed the sample in vitro to neurons isolated from a separate mouse that had never been exposed to opioids, they found that, on a cellular level, the neurons were already desensitized to the painkiller[6]. The sodium channels in the neurons that are typically blocked by morphine had already become inactivated by a factor found in the colonic milieu. "These results are telling us that there's something within the wall of the colon that's being released when mice are treated with chronic morphine," says Akbarali. "It could be anything, but we don't know what it is yet."

And, intriguingly, eliminating the majority of gut bacteria in mouse experiments with broad-spectrum antibiotics—or using mice born in a completely sterile environment and thereby lacking a microbiome—could prevent animals from needing higher doses of opioids for pain relief[7]. Because there are fewer bacteria—or none whatsoever—the animals don't experience side effects, like constipation, associated with opioid use, Roy says.

Even if changes in the microbiome do contribute to desensitization to painkillers, experts note that the pain tolerance pathway is separate from the reward pathway. "What drives someone from taking something for pain management to getting addicted is still a million-dollar question," Roy says.

Researchers like Akbarali, Roy and Taylor hope to one day test their findings from mice in a human clinical trial by performing fecal transplants. If their laboratory findings hold up, the clinical implications for this work could be vast. "I feel there is a window of opportunity to target the microbiome for therapeutic purposes to retain morphine as an analgesic agent without its detrimental side effects," Roy says.

If the results bear out, patients prescribed opioids might one day take probiotics to maintain a healthier microbiome that could prevent bacteria that take over with prolonged opioid treatment from wreaking havoc. "If you can alter the microbiome and keep it healthy, the potential of actually becoming desensitized to opioids and needing a higher dose is reduced. And then the potential of overdosing is reduced, too," Akbarali says. "You're not going to take more than you need." ❒

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