Scientists discover surprising details about xylazine in combination with fentanyl

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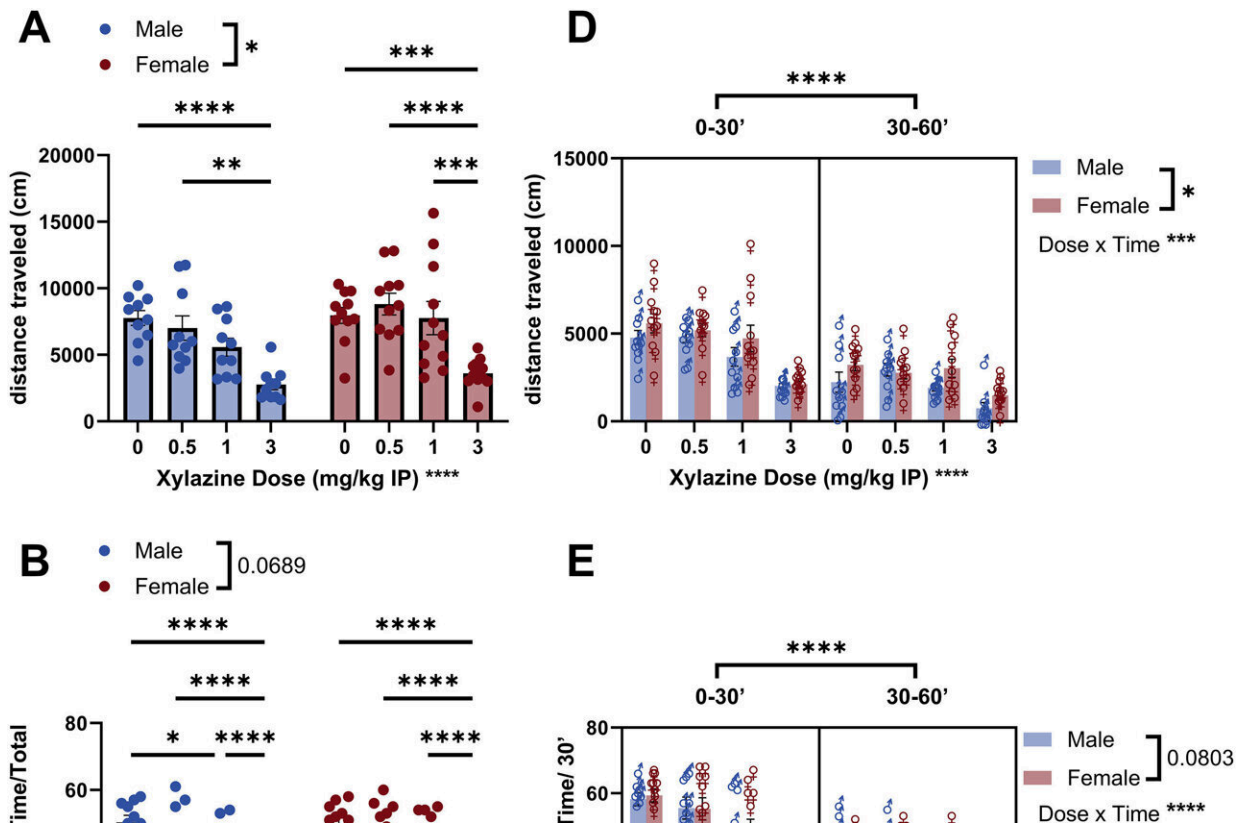

Effect of acute IP xylazine administration on locomotor activity. (A) Cumulative distance traveled, (B) % ambulatory time, and (C) average velocity of male and female mice administered saline or xylazine (D) Distance traveled, (E) % ambulatory time, and (F) velocity split into 30 min bins. Credit: *Addiction Neuroscience* (2024). DOI: 10.1016/j.addicn.2024.100155

Unregulated use of fentanyl and overdose deaths have increased dramatically in recent years, and this trend was made more alarming when authorities found fentanyl laced with the animal tranquilizer xylazine.

Some addiction specialists and public health officials feared the added xylazine would impede the fast-acting effects of the drug naloxone, which can effectively treat patients experiencing respiratory depression—a serious side effect of opioid use that can lead to death.

In a recent research discovery published in the journal *Addiction Neuroscience*, scientists at the University of North Carolina at Chapel Hill found that xylazine is a kappa opioid receptor agonist, meaning it activates kappa opioid receptors in the same way fentanyl activates opioid receptors.

This result was surprising because previously xylazine was thought to only bind to the α_2-adrenergic receptor, and this finding could hint as to why withdrawal from fentanyl in combination with xylazine is so severe.

This research, led by the lab of Zoe McElligott, Ph.D., associate professor of psychiatry and pharmacology at the UNC School of Medicine, provides important insights into the subtle cellular mechanisms underlying opioid use—especially in light of the added anesthetic xylazine to fentanyl—and naloxone, the leading treatment used to prevent death from fentanyl overdose. She is senior author of the *Addiction Neuroscience* paper.

"Many people thought xylazine operated exclusively through a different mechanism in the nervous system," said McElligott, who is also a member of the UNC Bowles Center for Alcohol Studies.

"But because we show xylazine is an agonist at kappa opioid receptors in

the brain and body, in addition to acting at other targets, we may have gleaned insight into why withdrawal from the combination of fentanyl and xylazine is so harsh."

Because severe withdrawal results in extremely aversive symptoms, people often choose to continue using drugs to keep the physical and psychological effects of withdrawal at bay, according to McElligott.

Her lab's discovery, accomplished in collaboration with other researchers at UNC-Chapel Hill, could have big implications for future scheduling recommendations for xylazine and how clinicians might treat patients in the future.

"A big 'take-home' message is that we want to make sure people are administered naloxone as a life-saving treatment," McElligott said. "When xylazine first came on the scene, there was a lot of talk about how it wouldn't respond to naloxone. Our data suggest otherwise, and we don't want people to not administer naloxone because they suspect someone has xylazine in their system."

McElligott's lab began its study when UNC Pharmacology graduate student Madigan Bedard poked her head into McElligott's office on January 4, 2023, when no one else was in the lab. Bedard, who is the co-first author on this paper, asked if McElligott had heard that people had been using xylazine in combination with fentanyl.

"I was blown away because xylazine is what many scientists use to anesthetize animals for lab experiments usually in combination with ketamine, and large and small animal veterinarians use it as a sedative as well," McElligott said.

"We were interested because early on we read case reports that withdrawal from fentanyl/xylazine is particularly bad, and we study the

effects of withdrawal, how withdrawal changes brain circuits and promotes the continued use of drugs and alcohol. So, we were curious what was going on here."

First, they set up a strategic dosing experiment to study withdrawal in mice that received saline as a control, or fentanyl, fentanyl/xylazine. To be extra rigorous, Bedard set up an additional control looking at xylazine alone.

"We thought xylazine alone would be a second control," McElligott said. "We thought those mice would react like the mice given saline." When the saline mice got naloxone, there were very minor withdrawal symptoms, hinting at the role that endogenous opioids play in the brain.

"But when the xylazine mice were treated with naloxone, we saw major withdrawal symptoms, akin to what we observed in mice treated with fentanyl, especially in female mice," she said. "In the female mice, and at these doses, fentanyl and xylazine synergized to make withdrawal worse than either drug alone. Immediately, we knew something strange was going on here."

McElligott and Bedard began thinking through the possibilities. Xylazine is known to target α_2– adrenergic receptors throughout the nervous system, and so they thought maybe naloxone was somehow bumping xylazine off those receptors to promote withdrawal. That seemed unlikely, she said, and they performed experiments with an α_2-adrenergic receptor inhibitor that did not resemble what they saw with naloxone.

They thought xylazine might somehow increase the opioid tone of the brain through naturally occurring endogenous opioid peptides in the brain, such as endorphins. Or, they thought maybe xylazine is just a dirty drug, one that unintentionally binds to different kinds of brain receptors.

"Well, we're in pharmacology at UNC," McElligott said, "so we thought we'd ask a colleague for help."

They turned to Bryan L. Roth, MD, Ph.D., the Michael Hooker Distinguished Professor of Pharmacology who runs the NIH Psychoactive Drug Screening Program from his lab at the UNC School of Medicine. He holds a joint appointment at the UNC Eshelman School of Pharmacy.

His team ran a rapid screen to see what receptors xylazine might target. A week later, Roth showed them the data. The drug latches on to kappa opioid receptors.

"That was not what we expected," McElligott said, "We were very excited, but we didn't want to hang all our data on that one screen."

Roth's lab wound up running a full profile on xylazine, including a slew of assays to be sure xylazine was activating kappa opioid receptors in addition to adrenergic receptors. It was.

McElligott's lab ran further experiments to produce more data in mouse models for the effects of naloxone and another drug called norbinaltorphimine, also known as nor-BNI, with pretreatment of mice surprisingly made withdrawal worse in the xylazine mice that had been later treated with naloxone.

These effects were more profound in female mice. This, too, adds another layer of evidence; kappa opioid receptors behave differently in male and female mice.

"We have lots of tantalizing early data in this paper, and we have so much to follow up on," McElligott said. "But we think our work suggests scientists and clinicians need to investigate different strategies for

mitigating some of the withdrawal responses we are now seeing in individuals, especially those exposed to fentanyl laced with xylazine.

"Additionally, we've begun to collaborate with Nabarun Dasgupta at UNC's Injury Prevention Research Center at the UNC Gillings School of Global Public Health. Nab runs UNC's Street Drug Analysis Lab and is the best source for understanding the current landscape of the unregulated drug supply.

"By collaborating with Nab and his team, we hope to be equipped to rapidly adapt our research for the next unexpected adulterant to the drug supply."

The unapproved use of xylazine in humans not only poses challenges for scientific and clinical understanding of the drug, especially in combination with other drugs, but also for law enforcement, first responders and treatment facilities, who work daily to navigate the adverse effects of the unregulated drug supply.

More information: Madigan L. Bedard et al, Xylazine is an agonist at kappa opioid receptors and exhibits sex-specific responses to opioid antagonism, *Addiction Neuroscience* (2024). DOI: 10.1016/j.addicn.2024.100155

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