The Effects of Oral Self-Administration of Fentanyl on Sleep Patterns, Precipitated Withdrawal, and Affective Behaviors in Female and Male Mice

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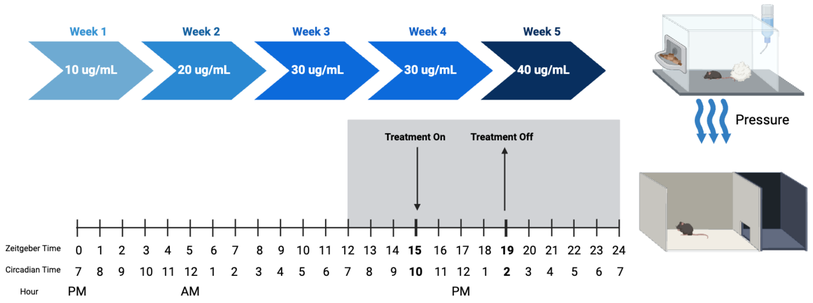
Introduction

- Opioid Use Disorder (OUD) is a chronic, relapsing disorder characterized by craving, bingeing, and withdrawal.[1]
- There have been more than 500,000 deaths from opioid overdoses in the past 15 years. The Director of the Office of National Drug Control Policy estimates that the number of annual opioid-induced deaths could reach 165,000 by 2025.[2]
- Most of these opioid overdose deaths are due to the increased availability of synthetic opioids, such as fentanyl.[3]
- Many animal models used to study OUD involve intravenous administration, but oral administration in the form of pills and patches is also common.[4]
- Withdrawal symptoms like sleep dysregulation and anxiety are implicated in relapse and continued drug consumption. However, few, studies have explored sleep dysregulation from fentanyl consumption.
- We explored how oral self-administration of fentanyl alters sleep, promotes precipitated withdrawal behaviors, and induces long-term affective changes in mice.

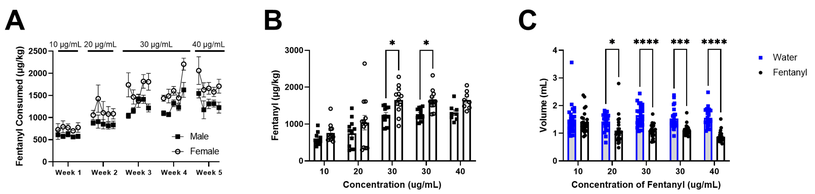
Methodology



- 3 cohorts, each of 16 C57BL/6J female (8) and male (8) mice that were at least eight weeks in age.
- Animals were single-housed in a reverse-light cycle.
- Half of the cohort received solid fentanyl mixed in normal water, other half received normal water.
- First two cohorts underwent the paradigm in the PiezoSleep 2.0 chambers.
- On the final day of the 40 ug/mL dose, all mice underwent precipitated withdrawal, receiving an intraperitoneal injection of 1 mg/kg naloxone.
- About one and a half weeks into withdrawal, we performed the Light-Dark Box assessment for approach/avoidance behaviors.

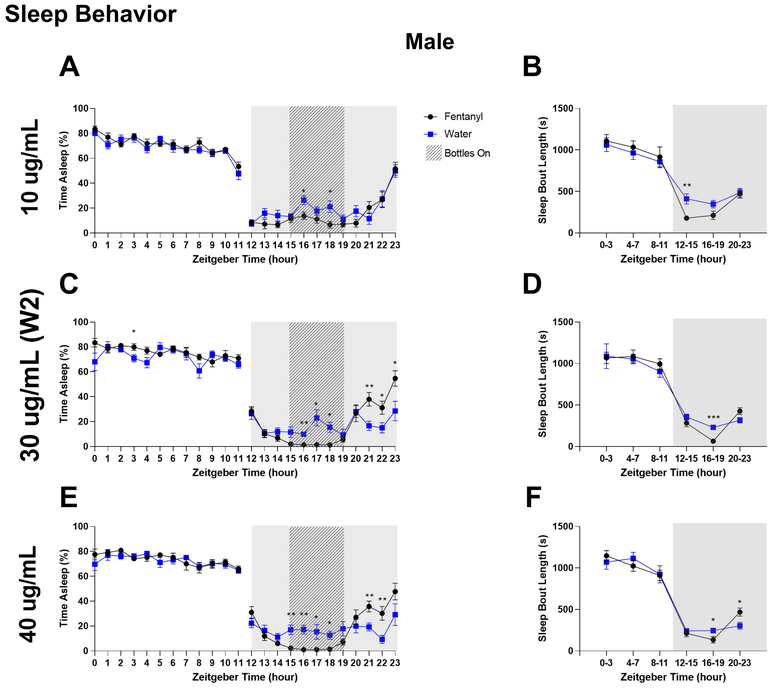
Results

Drinking in the Dark Paradigm

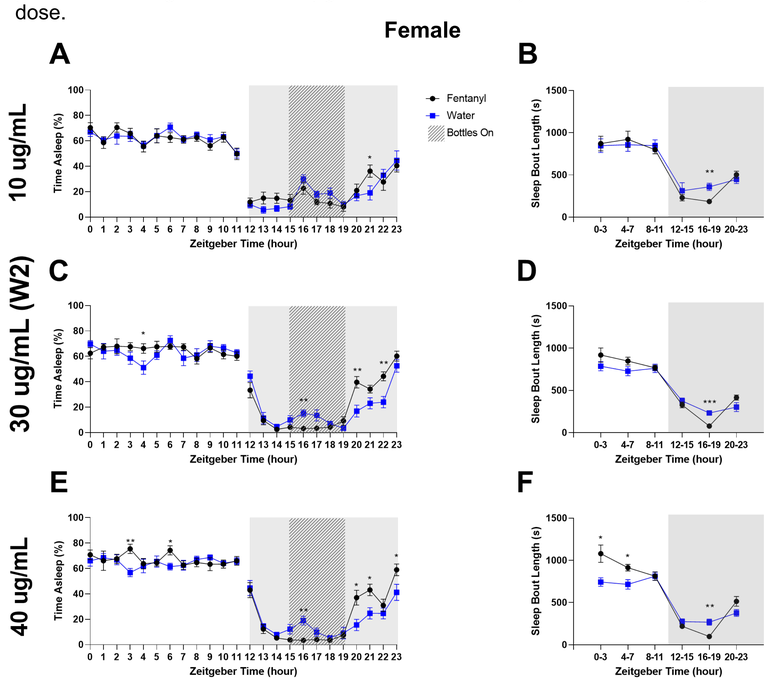


Volume and fentanyl consumed throughout five-week fentanyl drinking paradigm. **A** Both sexes consumed more fentanyl each week, peaking at 30 µg/mL dose. **B** Females consumed more fentanyl than males, on average. **C** Fentanyl groups consumed significantly less fluid than water groups at the 20 µg/mL dose and greater.

Sleep Behavior

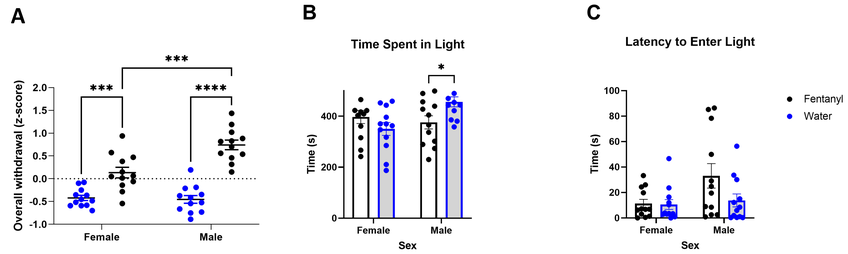


Long-term fentanyl consumption leads to changes in sleep behavior in male mice. **A, C, E** Fentanyl group slept less by hour than the water group at all doses during the drinking period and more once treatment was removed at the 30 and 40 µg/mL doses. **B, D, F** Fentanyl group revealed shorter sleep bouts during the drinking period at doses greater than 10 µg/mL and immediately before at the 10 µg/mL dose.



Long-term fentanyl consumption leads to changes in sleep behavior in female mice. **A, C, E** Fentanyl group slept less by hour than the water group during the drinking period and more immediately after at doses greater than 10 µg/mL. **B, D, F** The fentanyl group revealed shorter sleep bouts during the drinking period at all doses.

Precipitated Withdrawal and Affective Behaviors



Fentanyl consumption produces precipitated withdrawal behaviors and affective changes in adult mice. **A** Fentanyl groups exhibited more withdrawal behaviors than water groups in both sexes. Males exhibited more withdrawal behaviors than females. **B** The male fentanyl group spent less time in the light overall than the male water group, while females did not show significant differences. **C** There were no significant differences in the latency, in seconds, to enter the light side.

Conclusions

- Females and males consumed more fentanyl by weight as we increased the dose, peaking at the 30 µg/mL dose and stabilizing into the 40 µg/mL dose
- Fentanyl groups showed significantly decreased time asleep and shorter sleep bouts when treatment was on and jumped into a “recovery sleep” immediately after. Differences became more significant as dose increased.
- Both fentanyl groups showed more overall precipitated withdrawal behaviors.
- Males spent less total time in light, showing more avoidance behavior.
- Males showed more sleep disruption and withdrawal behaviors while consuming less fentanyl → could males be more affected by lower doses of fentanyl?

Future Directions

- Explore sleep more extensively, such as time spent in different stages of sleep and sleep post-fentanyl consumption or during more long-term. fentanyl consumption.
- We have also been studying acute and protracted withdrawal behaviors, such as approach/avoidance, anhedonia, and fear and extinction learning.
- We are also currently exploring the pathways and brain regions that might be involved in these behaviors.

References

1. Strang, J. *et al.* Opioid use disorder. *Nature Reviews Disease Primers* **6**, 3 (2020). https://doi.org:10.1038/s41572-019-0137-5
2. Lee, YK *et al.* Opioid use disorder. *Front. Public Health* **11**, (2024). https://doi.org/10.3389/fpubh.2023.1274719
3. Jeffery, M. M., Stevens, M., D'Onofrio, G. & Melnick, E. R. Fentanyl-Associated Overdose Deaths Outside the Hospital. *The New England journal of medicine* **389**, 87-88 (2023). https://doi.org:10.1056/NEJMc2304991
4. McCabe, S. E., Cranford, J. A., Boyd, C. J. & Teter, C. J. Motives, diversion and routes of administration associated with nonmedical use of prescription opioids. *Addictive Behaviors* **32**, 562-575 (2007). https://doi.org:https://doi.org/10.1016/j.addbeh.2006.05.022

Acknowledgements

McElligott Lab

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