Review of: "Perinatal Oxycodone Exposure Causes Long Term Sex-Dependent Changes in Sensory and Reward Processing in Adult Mice"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

This study aims to understand the impact of prolonged vs. short oxycodone on the sensory and reward processing in opioid-exposed offspring. As stated, opioid-exposed neonates exhibit NOWS (neonatal opioid withdrawal syndrome), a constellation of withdrawal signs after birth following an *in utero* exposure of prenatal opioids. Non-pharmacological measures remain the primary method to manage NOWS, however, some neonates continue to manifest severe withdrawal that interferes with basic physiological functions, e.g., feeding, sleeping, growth, such that pharmacological interventions are needed. Using a rodent model, the authors conducted an experiment categorizing mice into 4 groups: Short Oxy, Short Veh, Long Oxy, Long Veh to mimic situations where neonates require postnatal pharmacotherapy compared to those who do not require postnatal pharmacotherapy. The current design extends previous findings, primarily on weight and USVs, to explore long term outcomes including social behavior, anxiety related behaviors, sensorimotor, cognitive, and reward behaviors.

Strengths:

1. Investigators built this study on their published work on the same topic (Minakova, Front Behav Neurosci, 2021).
2. Well-described methodology focusing on several behavioral tasks to understand the impact of oxycodone on neurodevelopmental functions with the elimination of the confounding factors, which are abundant in human studies.
3. The study of sex effects is a crucial component of this study.
4. The use of primiparous females is a clever methodological detail that is important to obtaining robust data.
5. Results showing sex differences on susceptibility to other substance addiction (with females at increased risks) replicate others' studies and are important foundation to understand different pattern of addiction in adults with opioid use disorder (OUD).
6. The authors in their prior published study showed that oxycodone exposure led to decreased weight post weaning, predominantly in males. The current study showed that this trend changes with oxycodone exposure increasing weight gain in adult males. This is a very interesting phenomenon and has important implications for future metabolic diseases, both in animal and human studies. Authors could refer to articles by Toorie AM, Vassoler FM, et al., *Addict Biol*, 2021; Corr TE, et al., *J Dev Orig Health Dis*, 2022; Yen E & Maron JL, *Front Pediatr*, 2022. The utility of indirect calorimetry in addition to the nutritional intake and feeding behavior would help elucidate why oxy-exposed pups experience weight loss.

Weaknesses/comments:

1. The Long Oxy group was intended to examine the postnatal pharmacotherapy effects in NOWS, which is not well studied in neonates. However, in cases where opioid-exposed neonates receive postnatal pharmacotherapy, these neonates usually are given morphine in addition to the breast milk (if moms opt to breastfeed). Since it is unclear how much opioids pass through the breast milk, it would be difficult to extrapolate that Long Oxy group truly resembles opioid-exposed neonates who require pharmacotherapy. In other words, the clinical application of this study would be to understand how provision of opioids through maternal breastmilk affects outcomes, rather than provision of opioids to mitigate severe withdrawal. In order to understand the impact of postnatal pharmacotherapy for severe withdrawal/NOWS, it would make more sense to provide pharmacotherapy to pups in the Long Oxy group to mimic what happens in the clinical realms. Additionally, it would be very helpful to understand the concentration of drug in the neonate by measuring plasma oxycodone, for example.
2. Along those same lines, the early post-natal period in a mouse is equivalent to late gestation in a human. This makes it challenging to interpret with respect to human translational relevance. We recommend including some discussion surrounding this point as this is one component that makes prenatal opioid exposure so challenging to model in rodents.
3. The authors pointed out that based on prior studies where oxy-exposed pups experienced significant dehydration and weight loss post weaning, all pups in the current study were provided supplementation of Hydrogel, Nutro-gel diet, and standard chow, which “appeared to rescue the weight differences between Oxy and Veh groups.” While this may minimize weight loss as a confounding factor, this may be in fact an important variable to understand opioid effects on weight loss, catch-up growth, and feeding reward behavior. Any insight as to why pups experienced significant dehydration and weight loss post weaning? Is it because of the withdrawal symptomatology that prevents them from eating adequately, or is it because they are not interested in feeding? Also, what effects might supplementation and weight rescue do to the hunger signaling and the drive to eat for all groups, especially for the oxy-exposed pups? Discussing this effect would strengthen the paper.
4. While the methods are clearly laid out, it would be helpful to include some more information from past publications, rather than simply referring to the paper. For example, the dose of oxycodone should be available in this manuscript rather than necessitating the reader to look this up in a previous publication.