RESEARCH HIGHLIGHT

Aversion No MOR: Mu-opioid receptors in habenular $\beta 4$ neurons are key for naloxone aversionMegan E. Fox ¹Neuropsychopharmacology (2020) 45:243–244; <https://doi.org/10.1038/s41386-019-0404-x>

When used as prescribed, opioids are effective for managing severe pain associated with certain surgical procedures or advanced stages of cancer. Recently, the use of opioids has become a public health crisis due to an alarming increase in the number of opioid overdose deaths [1]. Outside of analgesia, opioids can produce a euphoric state that contributes to their abuse liability. Withdrawal from opioids produces an uncomfortable and dysphoric state that is thought to contribute to high rates of relapse after detoxification [2]. Regardless of the type of opioid abused—be it prescription, illicit, or synthetic—the primary target of these compounds is the μ -opioid receptor (MOR) [3].

MORs are inhibitory G-protein coupled receptors with expression distributed widely throughout the brain [3]. The rewarding effects of opioids are attributed to activation of MORs expressed in the nucleus accumbens and other brain areas. In fact, rats will readily self-administer MOR agonist morphine into the ventral tegmental area and nucleus accumbens [4, 5]. Naloxone, the agent commonly used to prevent an opioid overdose, also acts at MORs as a competitive antagonist, blocking the actions of opioids on their receptors. Naloxone is commonly used to precipitate withdrawal in rodents, which produces somatic withdrawal signs and aversive, stress-like states [6]. Naloxone also generates an aversive state in opioid-naïve animals, as measured by the time they spend in a chamber previously paired with naloxone [7]. While the rewarding effect of MOR activation is mainly encoded by mesocorticolimbic circuitry, less is known about the circuits mediating the aversive effects of MOR blockade.

In this issue, Boulos et al. test the hypothesis that medial habenula (MHb) MOR signaling contributes to opioid withdrawal and naloxone aversion [8]. The MHb is associated with aversion and has a high density of MOR expression, making this region a prime target for investigation [7]. Stereotaxic targeting of the MHb can be tricky given the region's small size and proximity to the third ventricle. To manipulate MOR in the MHb, the authors instead use a genetic approach to delete MORs from MHb neurons. Compared with the rest of the brain, the MHb is particularly enriched in nicotinic acetylcholine receptors containing the $\beta 4$ -subunit. The authors target this population of MHb neurons with a mouse line expressing Cre recombinase under the *Chnrb4* promoter (B4-Cre). They cross B4-Cre mice with floxed MOR mice (MOR^{fl/fl}) to delete MOR in the MHb while leaving MOR signaling intact in the rest of the brain. Based on in situ hybridization and qRT-PCR, their approach targets ~50% of MOR expressing neurons in the MHb, affecting both peptidergic and cholinergic cells.

The authors first establish how B4-MOR deletion (B4-MOR^{-/-}) influences well-known behavioral effects of morphine on locomotion and analgesia, and on the rewarding effects of morphine in a conditioned place preference procedure. MOR-intact and B4-MOR^{-/-} mice exhibit similar hyperlocomotion after acute morphine and develop similar analgesic tolerance after repeated morphine. B4-MOR^{-/-} mice also have a similar preference for the morphine-paired chamber as their wild-type littermates. MORs influence motivation to obtain natural rewards, thus the authors next tested how B4-MORs influence processing of palatable food rewards in Pavlovian conditioning tasks. B4-MOR^{-/-} and their wild-type littermates both learn cue-reward associations in an autoshaping task and make similar food-cup approaches during presentation of reward-predictive cues. These findings contrast with the behavior of total MOR knockout mice [9], which exhibit reduced motivation to obtain food rewards and delayed learning about cue-reward associations. Analgesia and reward-related behaviors are normal in B4-MOR^{-/-} mice, indicating MHb MOR signaling must serve another function.

Boulos et al. next tested B4-MOR^{-/-} in a conditioned place aversion procedure. Mice learn to associate a context with a high dose of naloxone, which typically produces conditioned aversion [7]. Both wild-type and B4-MOR^{-/-} mice avoid the naloxone-associated context; however, the magnitude of the place aversion is much greater in wild-type mice. The authors then subject B4-MOR^{-/-} and wild-type mice to a regimen of escalating doses of morphine prior to place conditioning. Several hours after morphine, mice are injected with saline in one context, and naloxone in another. When they test both genotypes, B4-MOR^{-/-} and their wild-type littermates avoid the naloxone-paired context, but mice with intact B4-MORs have a much stronger conditioned avoidance than B4-MOR knockout mice. It is worth noting the dose of naloxone used in this experiment is insufficient to elicit somatic withdrawal signs in morphine-dependent mice. Without any physical symptoms, the authors suggest B4-MORs are involved in an aversive emotional state associated with opioid withdrawal.

The authors next examine how B4-MORs mediate somatic withdrawal symptoms that were absent in the previous experiment. Using the same schedule of escalating morphine doses, they precipitate withdrawal signs with a higher naloxone dose. Global withdrawal scores are reduced in B4-MOR^{-/-} mice compared with the littermate controls, attributed mainly to decreased incidence of tremor, wet dog shakes, sniffing, and escape jumps. Although MOR deletion in B4-neurons does not abolish all somatic withdrawal symptoms, they clearly play a role in the physical discomforts associated with opioid withdrawal.

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Coupled with the aversion noted even in the absence of somatic withdrawal signs, this work clearly implicates MHb B4-MORs in naloxone aversion.

These findings represent an important step towards understanding how MORs signal in different circuits to balance reward and aversion. However, B4-MORs do not generalize to all aversive responses, since B4-MOR^{-/-} mice develop normal conditioned taste aversion. It is also worth noting the B4-MOR^{-/-} mice are developmental knockouts, which may lead to compensatory adaptations in B4-neurons that contribute to the observed phenotype. While B4-MOR deletion clearly diminishes naloxone aversion and withdrawal signs, it remains unclear how MOR signaling (or lack thereof) alters MHb activity to control these responses. One might predict MOR antagonism increases MHb activity, which, through its projections to the interpeduncular nucleus [10], drives aversion that is absent in MOR knockout mice. It should be relatively straightforward to determine this possibility with B4-cre mice and genetically encoded calcium indicators for monitoring neural activity, or optogenetics/chemogenetics to manipulate activity and oppose/enhance the effects of naloxone.

The data from Boulos et al. add to an important literature on the rewarding and aversive effects of opioids and their withdrawal. The findings are also important from a translational perspective—if we can reduce the aversive state created by opioid withdrawal, we might also reduce relapses driven by negative affect. Indeed, medication-assisted treatments for opioid dependence (e.g., methadone) are based on the premise that removing withdrawal symptoms reduces the likelihood of an individual resuming opioid use. The present data will hopefully inspire future pharmacotherapies geared towards MORs on select neural populations to mitigate the symptoms of acute and protracted opioid withdrawal.

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ADDITIONAL INFORMATION

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REFERENCES

1. Chen Q, Larochelle MR, Weaver DT, Lietz AP, Mueller PP, Mercaldo S, et al. Prevention of prescription opioid misuse and projected overdose deaths in the United States. *JAMA Netw Open*. 2019;2:e187621.
2. Wikler A, Fraser HF, Isbell H. N-Allylnormorphine: effects of single doses and precipitation of acute abstinence syndromes during addiction to morphine; methadone or heroin in man (post addicts). *J Pharmacol Exp Ther*. 1953;109: 8–20.
3. Pasternak GW, Pan Y-X. Mu opioids and their receptors: evolution of a concept. *Pharm Rev*. 2013;65:1257–317.
4. Bozarth MA, Wise RA. Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sci*. 1981;28:551–5.
5. Olds ME. Reinforcing effects of morphine in the nucleus accumbens. *Brain Res*. 1982;237:429–40.
6. Schulteis G, Heyser CJ, Koob GF. Differential expression of response-disruptive and somatic indices of opiate withdrawal during the initiation and development of opiate dependence. *Behav Pharmac*. 1999;10:235–42.
7. Mucha RF, van der Kooy D, O'Shaughnessy M, Bucenieks P. Drug reinforcement studied by the use of place conditioning in rat. *Brain Res*. 1982;243:91–105.
8. Boulos L, Ben Hamida S, Bailly J, Maitra M, Ehrlich A, et al. Mu opioid receptors in the medial habenula contribute to naloxone aversion. *Neuropsychopharm*. 2019. <https://doi.org/10.1038/s41386-019-0395-7>. [Epub ahead of print].
9. Boulos L-J, Nasseef MT, McNicholas M, Mechling A, Harsan LA, Darcq E, et al. TouchScreen-based phenotyping: altered stimulus/reward association and lower perseveration to gain a reward in mu opioid receptor knockout mice. *Sci Rep*. 2019;9:4044.
10. Zhao-Shea R, DeGroot SR, Liu L, Vallaster M, Pang X, Su Q, et al. Increased CRF signalling in a ventral tegmental area-interpeduncular nucleus-medial habenula circuit induces anxiety during nicotine withdrawal. *Nat Commun*. 2015;6:6770.