Pavlovian-conditioned opioid tolerance

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Opioid tolerance develops as a learned response to drug-associated cues and is thus a dynamic effect modulated by the interaction between drug and environment.

Opioids are powerful analgesics and are widely used clinically. This class of drug, which includes morphine and heroin, is also highly addictive and misuse is an ongoing health crisis. A major barrier to the safe and effective use of opioids is the rapid development of tolerance, defined as reduced ability of a drug to elicit a particular effect (e.g., analgesia) following repeated administration. Not only does tolerance lead to difficulties in the palliative application of opioids as they quickly lose their analgesic efficacy, tolerance is one of the Diagnostic Statistical Manual-5 criteria for opioid use disorder. Tolerance is, in part, responsible for escalation of opioid intake as higher doses, with reports as high as 500-fold (*1*), are needed to achieve the desired effect. While tolerance is often considered a static property at any given point in time, several decades of research have demonstrated that it is in fact dynamic and, in part, a result of learned associations between the drug and context in which it is administered. In this issue of *Science Advances*, Hou *et al.* (*2*) define a neural pathway implicated in context-dependent tolerance and elucidate a potential receptor target that may mediate the effect.

A LITTLE HISTORY

Medicinal use of opioids predates recorded history. The first known opioid prescriptions, opium prescribed by way of clay tablet, date back more than 8000 years, and opioids have remained highly prevalent for pain management (*3*). The concept of tolerance has also been recognized for many years, with case studies on morphine tolerance at least as early as 1877 (*4*). Interestingly, the development of tolerance does not occur at a steady rate. Rather, different types of opioids induce tolerance with different levels of administration. It is also widely recognized that individuals become tolerant to different effects of opioid action at different rates; for example, most opioids lose the ability to reduce pain faster than their gastrointestinal (e.g., constipation) or respiratory depression effects. This, of course, has implications for the danger of opioid use as tolerance to the analgesic effects of opioids may result in escalation of use. With slower development of tolerance to the respiratory effects, this escalation may bring one closer to, or past, a lethal dose.

In search of a mechanism by which the same dose of drug elicits reduced effects, decades of molecular pharmacology research have focused solely on drug action at the primary target of clinically used opioids: the mu opioid receptor (MOR), named for the compound that led to its discovery: morphine (*5*). Early hypotheses, in accordance with conventional receptor theory, posited that morphine tolerance must be a result of either a decrease in the amount of drug reaching the receptor, through increased drug metabolism, or changes at the receptor level such as receptor internalization (decreased number of available receptors) or desensitization (decreased downstream signaling through the receptor). However, despite many attempts, no consistent evidence was found that repeated morphine leads to receptor internalization or desensitization. Counterintuitively, it has now been proposed that, at least in the case of morphine, tolerance is actually a result of prolonged receptor signaling that leads to cellular adaptations, which counteract the effects of MOR activation (*6*).

Our understanding of analgesic tolerance is complicated further by the discovery that the pharmacodynamic action of opioids can actually be altered in a manner similar to learned behaviors. In a pioneering series of studies by Siegel (*7*) in the 1970s, it was found that, after repeated morphine exposure, the expression of analgesic tolerance depended on the presence of morphine-associated cues at the time of administration and that tolerance was extinguished in the absence of these conditioned cues (Fig. 1A). Indeed, Siegel demonstrated that, even within the same subject, the expression of analgesic tolerance was dynamic depending on whether the morphine challenge, and measurement of pain thresholds, was in the same context where morphine had previously been given. He hypothesized that, akin to Pavlov's dogs salivating at the sound of a bell, opioid-conditioned cues elicit a compensatory physiological response to counteract the anticipated effects of the drug. These findings demonstrate a critical and fundamental property of opioid tolerance. More broadly, the fact that tolerance can be dynamically modulated by environmental context challenges basic receptor theory where receptor-drug interactions are conceptualized as a lock-and-key process.

Associative opioid tolerance has major ramifications for the safety of opioid use. A retrospective study of heroin users found that, for many, the occasion of use that resulted in an overdose was in a context not previously associated with the drug (*8*). The causal role of this shift in context was explored in rats, and it was

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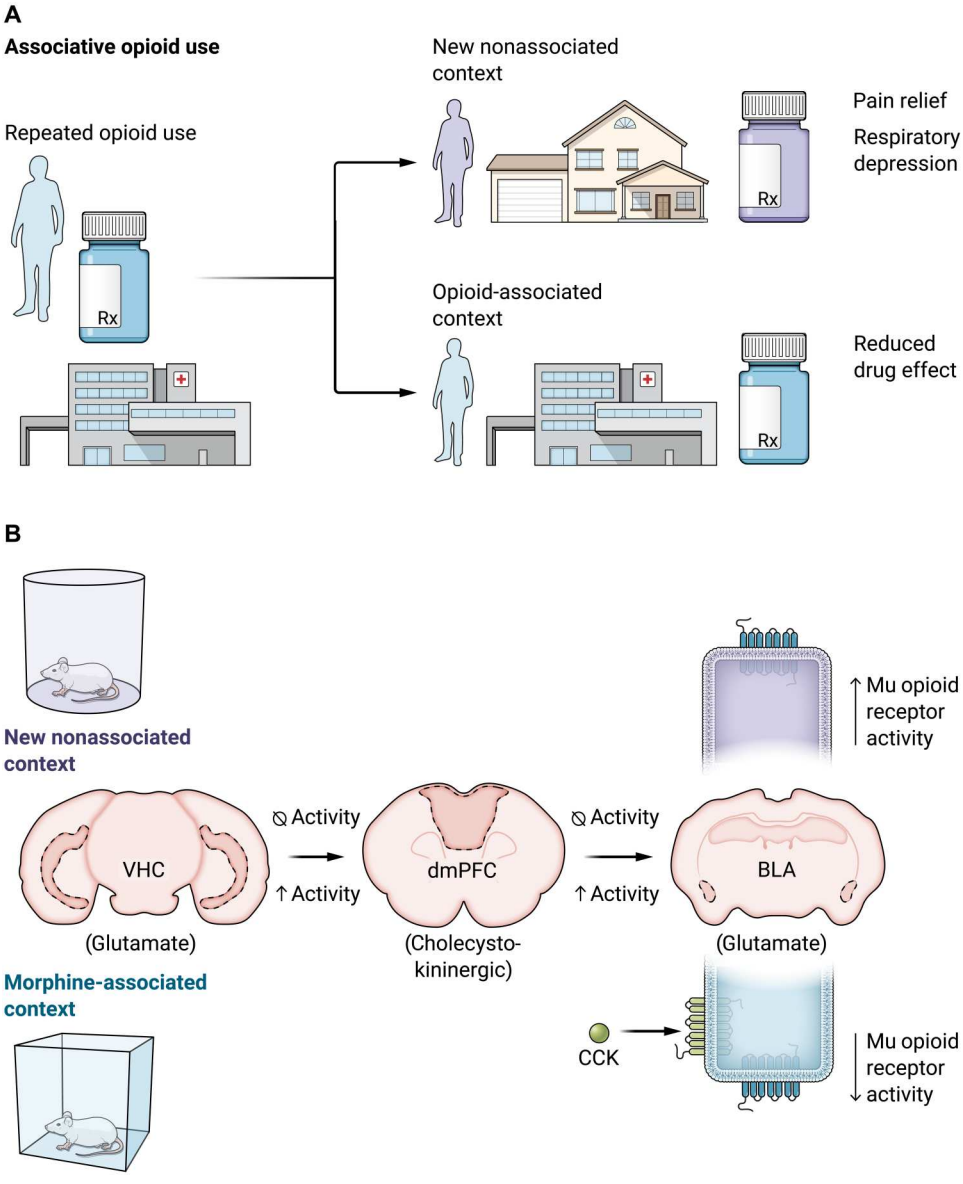

Fig. 1. Associative opioid tolerance. (**A**) Context matters when it comes to opioid tolerance. After repeated use in one context, the same dose of opioid has a reduced effect. In a different context not previously associated with the drug, this tolerance does not occur, and the effects are similar to initial use. (**B**) The neural pathway implicated in associative opioid analgesic tolerance as described by Hou *et al.* Illustration credit: Austin Fisher, *Science Advances*.

demonstrated that mortality was markedly increased with the same dose of morphine in a nonassociated context compared to the context previously paired with opioid administration (*8*, *9*). In fact, it has even been suggested that the term "overdose" is a misnomer and that a more accurate description is a "failure of tolerance" (*10*). Conceptualizing opioid overdose as a failure of tolerance is congruent with that fact that, in many cases, fatal exposures occur at doses no higher than those nonfatally consumed by opioid-experienced users.

A PATHWAY TO FOLLOW

Despite decades of detailed behavioral studies of associative opioid tolerance, little attention has been given to this phenomenon in modern systems neuroscience; Hou and colleagues take a major stride toward understanding the neurobiological basis of associative analgesic tolerance. To induce and quantify associative opioid analgesic tolerance (AOAT) in mice, the authors administered morphine over consecutive days either in a distinct context [contextual conditioned (CC)] or in a home cage (HC). In line with previous reports, CC mice developed analgesic tolerance at a much faster rate than HC mice. With the use of rigorous controls, Hou *et al.* established that this effect generalized across opioids and types of pain, presented similarly across male and female subjects, was not a feature of the distinct context itself but rather the association of drug

and context, and occurred at the supraspinal level. Taking an unbiased approach, they next asked: What brain regions are activated during the development of AOAT, and in what order do these regions interface one another? By assessing activation patterns in response to morphine-context pairing and selective neuronal ablation in each region, they determined that the development of AOAT occurred through a ventral hippocampus (vHPC) to dorsomedial prefrontal cortex (dmPFC) to basolateral amygdala (BLA) pathway. Hou *et al.* then assessed how AOAT altered signaling in each region and whether activation of each projection of this circuit was necessary and/or sufficient in driving the effect.

First, they found that the development of AOAT resulted in the increased excitability of glutamatergic vHPC to dmPFC projections, and activation of these neurons was both necessary and sufficient to this associative tolerance. Next, they used calcium imaging to establish that neurons projecting from the dmPFC to the BLA are activated when the mouse is exposed to the distinct context with which morphine was paired. In search of the neurotransmitter by which activation of these dmPFC neurons was conferring its effect, the authors found that a substantial proportion expressed cholecystokinin (CCK), a peptide considered "anti-opioid" in action and previously implicated in AOAT (*11*), and that these cells were again necessary and sufficient in driving AOAT. A thorough investigation of the final region in this circuit, the BLA, demonstrated that MORs in the region directly mediate an opioid analgesic effect, and AOAT results in disruption of their signaling through CCK receptor activation.

CIRCUITS TO TARGET

Hou *et al.* present a previously unidentified circuit implicated in the development of AOAT and demonstrate the causality of each projection in this pathway by assessing both necessity and sufficiency (Fig. 1B). By establishing an important role of CCK signaling in mediating this effect, the authors present a therapeutic target worthy of further investigation as a potential adjunct treatment in clinical cases of opioid use. Critically, they demonstrate that this circuit is specifically implicated in the associative tolerance of analgesic effects, highlighting that this intervention point may be used to reduce the dose needed for the palliative benefits of opioid use without modulating tolerance to effects such as respiratory depression, thus maximizing safety. However, it will also be important for future work to establish the circuitry implicated in respiratory depression to limit the loss of that tolerance in environments not associated with opioid administration. Together, this work uses systematic circuit tracing and a variety of techniques to uncover the neurobiological processes underlying a phenomenon with major clinical implications for the health and safety of opioid users.

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