Journal Club

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A Piriform-Orbitofrontal Cortex Pathway Drives Relapse to Fentanyl-Seeking after Voluntary Abstinence

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Review of Reiner et al.

Fentanyl is a synthetic opioid commonly prescribed to treat severe pain, but its illicit use has contributed to the global opioid epidemic, largely because clandestinely manufactured fentanyl is sold as an alternative to heroin. Furthermore, fentanyl has a high abuse potential and risk of overdose because it is 30–50 times more potent at opioid receptors than heroin, making accurate dosing difficult; it has a short duration of action, encouraging frequent dosing (Han et al., 2019); and it has higher lipophilicity, so it penetrates the brain more rapidly (Moss and Carlo, 2019). These pharmacokinetic differences between fentanyl and heroin suggest that their addictive properties could emerge from non-identical neurobiological mechanisms. It is therefore important to investigate the neural systems underpinning relapse to fentanyl-seeking.

Different patterns of brain activity emerge during relapse depending on how abstinence was induced (Fuchs et al., 2006; Venniro et al., 2017a). Therefore, it is important to implement animal models of addiction that more accurately mimic abstinence in humans. The few rodent self-administration procedures that investigated relapse to fentanyl-seeking behavior incorporated

experimenter-imposed extinction or forced abstinence before relapse. However, humans do not typically undergo extinction of responses to the cue-drug association, and people often cease drug use voluntarily. One procedure that enables the investigation of relapse after voluntary abstinence (Caprioli et al., 2015) gives rats a mutually exclusive choice between highly palatable food and a drug. In a recent study, Reiner et al. (2020) used this protocol to identify the neural circuits, which drive relapse to fentanyl-seeking after voluntary abstinence.

The authors asked whether rats would voluntarily abstain from fentanyl self-administration in favor of palatable food and subsequently relapse to fentanyl-seeking. Consistent with findings that rats voluntarily abstain from heroin in favor of food (Venniro et al., 2017b), when presented with a choice between palatable food and fentanyl, rats with a history of fentanyl self-administration preferred food to the extent of almost entirely abstaining from fentanyl consumption. Despite this period of voluntary abstinence, when rats were later reexposed only to the cues that signaled fentanyl availability, rats robustly relapsed to fentanyl-seeking behavior, despite the drug not being available. Thus, the authors demonstrated a model of relapse to fentanyl-seeking after voluntary abstinence.

To investigate possible brain regions that are driving relapse to fentanyl-seeking, the authors drew on functional imaging studies of opioid-dependent humans.

Such studies indicate that opioid-related cues elicit activity in the orbitofrontal cortex (OFC). Consistent with this, Reiner et al. (2020) found that cue-induced relapse to fentanyl seeking was associated with greatly increased expression of cFos, a marker of neural activity, in the lateral OFC (lOFC) and ventral OFC (vOFC), as well as with a modest increase in the neighboring anterior insular cortex (AIV).

To investigate the necessity of these regions in controlling fentanyl-seeking, the authors temporarily inactivated lOFC, vOFC, or AIV bilaterally by microinjecting a mixture of GABA_{A/B} receptor agonists into each respective region before fentanyl-associated cue exposure (i.e., relapse) as well as when rats were reexposed to fentanyl self-administration conditions (i.e., reacquisition). Inactivation of the lOFC or AIV, but not the vOFC, produced a moderate (30%–40%) reduction in relapse to cue-induced fentanyl-seeking, while reacquisition to fentanyl self-administration was unaffected, indicating that distinct facets of fentanyl abuse are controlled by distinct brain systems.

The authors then asked which inputs to the OFC contribute to relapse. Examining cFos immunoreactivity after the retrograde tracer CTb was infused into the ventrolateral OFC (vlOFC) revealed that projections from the piriform cortex (Pir) to the vlOFC were activated during relapse to fentanyl-seeking. The Pir is a key olfactory structure that has rarely been investigated within the context of

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addiction. Reiner et al. (2020) showed that pharmacologically inactivating the Pir reduced fentanyl relapse by ~30–40%, without affecting reacquisition of fentanyl self-administration. Because this effect closely mirrored that of IOFC inactivation, the authors hypothesized that the Pir-OFC pathway is critical for relapse to fentanyl-seeking. To test this hypothesis, the authors inactivated the connection between the Pir and vOFC, before testing relapse to fentanyl-seeking and reacquisition of fentanyl self-administration. As this connection is predominantly ipsilateral, the authors infused GABA_{A/B} receptor agonists unilaterally into the Pir, and either the ipsilateral or contralateral vOFC. Ipsilateral inactivation leaves the Pir-OFC circuit intact in one hemisphere, which had no impact on relapse or reacquisition behavior. In contrast, contralateral Pir and vOFC inactivation resulted in disconnection of the Pir-OFC circuit in both hemispheres, which significantly reduced relapse to fentanyl-seeking by ~30–40%, while leaving reacquisition of fentanyl self-administration intact. This recapitulated the effects of inhibiting the Pir or the OFC individually, together indicating that the Pir-OFC connection controls relapse to fentanyl-seeking.

An important outcome of this study is the demonstration that different OFC subregions play distinct roles in relapse to fentanyl-seeking. Specifically, despite the increased expression of cFos in the vOFC after relapse to fentanyl seeking, inactivating the vOFC did not significantly reduce relapse. This may be because OFC subregions play a more nuanced role in reward-seeking behavior than what is captured by cue-induced relapse models. For example, the IOFC is activated on exposure to reward-predictive stimuli, with peak activation occurring during the initiation of reward-seeking behavior (Moorman and Aston-Jones, 2014). Conversely, the vOFC encodes expectations about the time until the reward will be available after cue exposure, as a means of stabilizing value expectations and maintaining choice preferences (Stolyarova and Izquierdo, 2017). Collectively, this suggests that IOFC inactivation may prevent the initiation of movement toward the drug-associated cue, resulting in reduced lever pressing. However, because the vOFC is involved in encoding value expectations, and the rat is not faced with a value-based choice within the relapse session, inactivation did not significantly reduce relapse in the current paradigm. Nonetheless, the vOFC may drive fentanyl-seeking or self-administration

when an alternative reward is present. Further insights into the function of the vOFC may be gained by inactivating the vOFC during drug-food choice procedures.

The discovery of the Pir and Pir-OFC connection as a substrate for relapse to fentanyl seeking is highly novel, given that the Pir has primarily been studied for its role in olfaction, and little is known about its role in addiction. Interestingly, relapse to alcohol-seeking in rats after exposure to an alcohol-associated cue did not increase cFos expression in the Pir (Dayas et al., 2007), suggesting that Pir involvement may be unique to opioids or fentanyl. Additionally, there is the possibility that Pir involvement in relapse could be unique to rodents, which use olfactory circuits to learn instrumental tasks (Slotnick, 2001). It will be important to determine whether the human Pir is involved in cue-elicited opioid craving.

It is important to note that pharmacological disconnection techniques do not allow one to infer directionality of the reciprocally connected Pir-OFC pathway. Therefore, although the retrograde tracing data implicate the Pir→OFC projection in fentanyl relapse, the disconnection data equally support the notion that the OFC→Pir projection contributes to fentanyl-seeking. Future studies could use optogenetic or chemogenetic inhibition to isolate these reciprocally connected pathways. However, such interventions typically only indicate whether a particular structure is involved in relapse: they do not necessarily inform us about how the structure is involved. Given that OFC subregions are differentially involved in value-based decision-making (should the rat press the lever?) and integration of reward-associated cues (what does the cue signal to the rat about reward availability?), it would be valuable to understand the temporal patterns of vOFC, IOFC, and Pir→vOFC activity during relapse. For example, using fiber photometry or GRIN-lens calcium imaging, one could determine whether OFC, or Pir→vOFC cells become active: (1) in response to the discriminatory stimulus that signals fentanyl-lever availability; (2) immediately before cue-seeking; and/or (3) in response to presentation of the drug-associated cue.

Last, the identification of the AIV as a region involved in fentanyl relapse is intriguing given that the relapse-induced cFos counts were 3–4 times higher in the OFC than the AIV, and yet, inactivation of the OFC and AIV produced similar reductions in relapse. Therefore, the

relatively modest population of AIV cells that are activated during relapse exerts a substantial influence over fentanyl-seeking. Indeed, the AIV is well positioned to coordinate reward and addiction processes because it receives input from dopaminergic and noradrenergic cells (Lindvall et al., 1978), provides glutamatergic input to the central amygdala (Venniro et al., 2017a), and connects with striatal and limbic structures (Reynolds and Zahm, 2005). Given that the AIV also controls relapse to cocaine (Cosme et al., 2015), nicotine (Pushparaj et al., 2015), alcohol (Campbell et al., 2019), methamphetamine, and heroin (Venniro et al., 2017a), it would be beneficial to discover which AIV circuits and neurochemicals are involved in regulating relapse across all drug classes, as it is an exciting target for the development of addiction therapies.

Overall, the study by Reiner et al. (2020) raises several important questions about the neurobiology of fentanyl relapse after voluntary abstinence. For example, what do the ventral and lateral OFC subdivisions uniquely contribute to addiction? Is the involvement of the Pir unique to rodents, or does it contribute to opioid craving in humans as well? How is the AIV able to control relapse across all major drugs of abuse? Future studies using circuit-defined imaging and manipulation techniques may shed further light on the role of these cortical regions in opioid relapse.

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