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An Unusual Suspect in Cocaine Addiction

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Development of drug addiction is extremely complex, but its initiation can be as simple as the flip-flop of glutamatergic receptor subtypes triggered by an "unusual" type of NMDA receptors, as suggested by Yuan et al. (2013) in this issue of *Neuron*.

The development of drug addiction involves complex neural circuits and multidimensional molecular and cellular adaptations. A common initial consequence of exposure to almost all drugs of abuse is activation of the mesolimbic dopamine (DA) system, which includes the ventral tegmental area (VTA) and its target the nucleus accumbens (NAc) (Lüscher and Malenka, 2011). Early insight from studying the development and maintenance of cocaine-induced behavioral sensitization indicates that the VTA, particularly glutamatergic transmission in the VTA, is critical for the initiation phase of addiction-related behaviors (Vanderschuren and Kalivas, 2000; Wolf and Tseng, 2012). Significant effort has been since devoted to understand the adaptive changes induced at excitatory synapses on VTA DA neurons as a starting point for uncovering how drugs of abuse reshape the mesolimbic DA system and other brain regions to eventually lead to addiction.

About a decade ago, a first wave of findings established that a single exposure to cocaine or other drugs of abuse increases the ratio of AMPA receptor (AMPAR)-mediated to NMDA receptor (NMDAR)-mediated responses at excitatory synapses on VTA DA neurons (Ungless et al., 2001). This synaptic adaptation shares core features of classic NMDAR-dependent long-term potentiation (LTP): increase in whole-cell AMPAR current, requirement for GluA1-containing AMPARs, and sensitivity to NMDAR-selective antagonists (reviewed by Lüscher and Malenka, 2011). The second wave of research cast its sites on the underlying molecular mechanisms to reveal two critical features of this cocaine-induced LTP-like phenomenon: the "flip" of the regular calcium-impermeable AMPARs (CI-AMPARs) to GluA2-lacking, calcium-permeable AMPARs (CP-AMPARs) (Bellone and Lüscher, 2006) and the decrease in NMDAR-mediated response (Mameli et al., 2011). The flip to CP-AMPARs leads an increase in AMPAR transmission due to their higher single-channel conductance, and the higher calcium permeability redefines the LTP rules in VTA DA neurons after cocaine exposure (Mameli et al., 2011). These discoveries triggered several critical questions: what governs the reduction of NMDAR response, how is it coordinated with AMPAR regulation, and what are the behavioral consequences of these initial cocaine-induced adaptations?

In this issue of *Neuron*, Yuan et al. (2013) hit a homerun for this line of study by identifying an unexpected player, GluN3A, insertion of which not only mediates the reduced synaptic NMDAR responses but also gates the insertion of CP-AMPARs in VTA DA neurons after cocaine exposure.

Yuan et al. (2013) first examined whether the source of Ca^{2+} was impacted after cocaine exposure by imaging synaptic Ca^{2+} signals in VTA DA neurons in acute slices while recording evoked excitatory postsynaptic currents (EPSCs) in Mg^{2+}-free solutions. They found that 24 hr after a single cocaine injection, the synaptic Ca^{2+} transients showed little sensitivity to NMDAR-selective antagonists, even though NMDAR currents were easily detectable. Instead, the evoked dendritic Ca^{2+} transients were almost exclusively contributed by CP-AMPARs. This raised the possibility that synaptic NMDARs in VTA DA neurons were unexpectedly replaced by other NMDARs with much less Ca^{2+} permeability after cocaine exposure.

Subsequent examination of NMDAR EPSCs revealed increased decay kinetics, enhanced sensitivity to ifenprodil, and decreased sensitivity to Zn^{2+}, which collectively suggest an increased content of GluN2B-containing NMDARs. Importantly, the current-voltage relationship of NMDAR EPSCs showed greatly reduced sensitivity to Mg^{2+}, further suggesting the presence of GluN2C/D or GluN3 subunits. Follow-up pharmacological assays and the use of GluN3A knockout (KO) mice allowed for Yuan et al. (2013) to conclude that GluN3A, the noncanonical NMDAR subunit, was responsible for the reduced Ca^{2+} permeability as well as the reduced Mg^{2+} sensitivity. Given the enhanced content of GluN2B, and the fact that GluN1/GluN3A alone does not bind glutamate (and thus should have little sensitivity to APV), it is most likely that GluN1/GluN2B/GluN3A triheteromers are inserted in VTA DA neuron synapses after a single cocaine injection. Additional results also indicate that insertion of these GluN3A triheteromers was a prerequisite for cocaine-induced upregulation of synaptic CP-AMPARs in VTA DA neurons.

Finally, Yuan et al. (2013) heroically identified an mGluR1-Shank/homer-IP3-mTOP signaling pathway whose activation removed GluN2B/GluN3A- and reinserted GluN2A-containing NMDARs and removed CP-AMPARs, thus restoring VTA excitatory synapses in cocaine-exposed animals. Although some cocaine-induced behaviors such as behavioral sensitization and

conditioned place preference remained normal upon prevention of GluN3A-based synaptic alterations in VTA DA neurons, considering this is not the first dissociation between cocaine-induced LTP in the VTA and behavioral sensitization (Wolf and Tseng, 2012), the newly characterized role of GluN3A in cocaine-evoked plasticity in VTA neurons remains exciting. This comprehensive study not only links significant initial adaptive changes in VTA DA neurons in response to cocaine but also provokes several lines of thinking that hold the promise of providing a deeper understanding of addiction-associated cellular and circuitry plasticity.

The first provocative idea centers on GluN3A expression and its relationship to addiction. Reminiscent of other examples of developmental mechanisms that are reinvigorated by addictive drugs to reshape neural circuits, GluN3A is highly expressed early in development but is sharply downregulated during the first few postnatal weeks and remains low until cocaine comes into play in the adult. At the molecular level, critical factors for synaptogenesis and circuitry formation such as CREB and BDNF are activated/upregulated in the VTA and NAc of the developed brain after cocaine exposure (Chao and Nestler, 2004; Grimm et al., 2003). At the cellular level, cocaine exposure generates silent excitatory synapses in the NAc (Huang et al., 2009; Brown et al., 2011; Koya et al., 2012), thought to be like immature excitatory synaptic contacts that are otherwise only abundant in the developing brain. Indeed, recent evidence suggests that maturation of cocaine-generated silent synapses after withdrawal intensifies cocaine seeking (Lee et al., 2013). Together with these drug-reinitiated developmental mechanisms, upregulation of GluN3A may redevelop and redirect the brain toward addiction-related emotional and motivational states. During early development, GluN3A limits synaptic insertion of AMPARs (Roberts et al., 2009), whereas Yuan et al. (2013) reveal that GluN3A could be essential for synaptic insertion of CP-AMPARs after cocaine exposure. This raises interesting new questions such as: (1) does GluN3A differentially gate synaptic insertion of CP-AMPARs versus CI-AMPARs? (2) Alternatively, is the role of GluN3A in regulating AMPARs completely inverted after cocaine exposure, or is this a newly assigned role by cocaine exposure? And (3) what molecular signaling and cellular processes mediate GluN3A-dependent synaptic insertion of CP-AMPARs? Answering these questions would form a stronger understanding of how GluN3A exerts the described synaptic changes and their link to drug addiction.

The second novel idea sheds new light on the functional "flip-flop" of AMPARs and NMDARs. The classic role of synaptic AMPARs is as a "workhorse" in synaptic transmission, whereas NMDARs provide regulatory Ca^{2+} signaling. Yet, with a single exposure to cocaine, synaptic AMPARs become Ca^{2+} permeable and their Ca^{2+} influx then regulates synaptic plasticity (Mameli et al., 2011), while synaptic NMDARs lose their Ca^{2+} permeability. Low Ca^{2+} permeability may compromise traditional NMDAR-dependent plasticity, but these newly inserted GluN3A may endow NMDARs with new functions, such as insertion of CP-AMPARs (Yuan et al., 2013). This functional flip-flop of AMPARs and NMDARs may be among the earliest drug-induced metaplastic events, which redefine plasticity rules to set up the mesolimbic dopamine system for subsequent synaptic alterations after prolonged drug exposure and withdrawal.

Finally, we also gain new insight into the role of mGluR1. Activation of mGluR1 leads to the internalization of cocaine-induced synaptic CP-AMPARs in VTA DA neurons (Bellone and Lüscher, 2006). Discovering that mGluR1 restores NMDAR function by insertion of typical, GluN2-containing NMDARs (Yuan et al., 2013) and knowing that mGluR1 activity also gates the emergence of CP-AMPARs in the NAc (Mameli et al., 2011; Loweth et al., 2013), where secondary adaptations may occur to mediate the maintenance phases of some addiction-related behaviors (Vanderschuren and Kalivas, 2000), suggest a compelling therapeutic potential for mGluR activation in addiction.

With the excitement of discovering a new synaptic component in cocaine response, it is humbling to realize that "the more we know, the less we know." The winding paths that led to the discovery of GluN3A in cocaine-induced adaptations now converge to grand avenues with newly painted road signs clearly in view: does GluN3A respond differently following single versus repeated cocaine exposure? Is GluN3A also expressed in the axons of VTA DA neurons with cocaine exposure to potentially enhance DA release at the terminals? And importantly, what are the behavioral consequences of this cocaine-induced GluN3A upregulation? Answering these questions will help set up the next big hit: understanding how initial cocaine-induced changes trigger subsequent adaptations that occur after chronic drug exposure and drug withdrawal to lead to the long-lasting addictive state.

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