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Yin and Yang: Unsilencing Synapses to Control Cocaine Seeking

Julie A. Kauer[1,*] and Abigail M. Polter[1]
[1]Department of Molecular Pharmacology and Physiology, Brown University, Providence, RI 02912, USA
*Correspondence: julie_kauer@brown.edu
http://dx.doi.org/10.1016/j.neuron.2014.08.057

In this issue of *Neuron*, Ma et al. (2014) show that long-term depression of two independent prefrontal cortical inputs to nucleus accumbens modifies behavioral responses controlling incubation of cocaine craving. Intriguingly, one input increases while the other attenuates behavioral responses, hinting that both "prorelapse" and "antirelapse" pathways are strengthened after cocaine self-administration.

If treating addiction were as simple as separating the user from the drug, we would be able to treat and cure substance use disorders. Instead, as anyone who has ever tried to quit can tell you, the quitting is not the hardest part, despite the acute symptoms of physical dependence—instead, it's the constant nagging cravings that develop during abstinence and that, for many, increase rather than diminishing with time (Gawin and Kleber, 1986). Drug-related environmental cues can exacerbate this, seemingly acting as triggers of craving. How can we understand the escalation of drug craving that occurs during withdrawal?

One attractive rodent model of this escalation process has been called incubation of craving (Pickens et al., 2011). Rats are trained to self-administer cocaine in a specific environment and then withdrawn from the drug entirely for different periods of time. Reintroducing the animal to the same environment and cues even without any cocaine results in drug-seeking behavioral responses, i.e., a lever press or nose poke that previously delivered cocaine. Remarkably, the operant responding (despite no drug delivery) increases markedly, as much as 3-fold, from day 1 to day 90 after withdrawal from the original cocaine (Pickens et al., 2011). These behavioral responses appear to reflect the gradually escalating craving for cocaine developing during withdrawal, measured by the willingness of the rat to work to seek the drug.

Synaptic strength and number are widely viewed as essential building blocks by which the nervous system remodels during development, learning, and myriad environmental conditions and stimuli, including exposure to and self-administration of addictive drugs (Lüscher and Malenka, 2011). The paper by Ma et al. (2014) in this issue has identified novel opposing cocaine-induced synaptic modifications during the incubation of cocaine craving in specific prefrontal cortical (PFC) inputs to the nucleus accumbens (NAc), a brain region essential for the development of addiction in both human patients and animal models. The authors first confirmed earlier anatomical work showing that two prefrontal cortical regions, the infralimbic cortex (IL) and the prelimbic cortex (PrL), provide major glutamatergic inputs to the shell (Sh) and core (Co) of the NAc, respectively. The authors then virally delivered channelrhodopsin into the IL or PrL, allowing them later to use light pulses to stimulate selectively glutamatergic synapses arising from either injected brain region. They allowed rats to self-administer cocaine over a 7 day period and then examined functional properties of each pathway in NAc slices prepared at 1 or 45 days after withdrawal. They first measured silent synapses, synapses at which glutamate is released but postsynaptic sites contain NMDARs, but no AMPARs, and thus are functionally silent at resting membrane potentials (Kerchner and Nicoll, 2008). Using selective optogenetic activation of

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the IL-to-shell or PrL-to-core pathway at 1 day of withdrawal from cocaine, Ma and colleagues found that the percentage of silent synapses had increased greatly in both pathways. By 45 days of withdrawal, however, in both regions the percentage of silent synapses had returned to basal values. By the addition of AMPARs, synapses can become unsilenced, as happens for example during NMDAR-dependent LTP, circuit development, and some forms of homeostatic synaptic scaling (Lee, 2012; Hanse et al., 2013). Previous work had indicated that a key component of incubation of cocaine craving is the insertion of GluA2-lacking, Ca^{2+}-permeable AMPARs (CP-AMPARs; Loweth et al., 2014). Did the unsilencing of NAc synapses result from insertion of CP-AMPARs? Nothing so simple: Ma and colleagues discovered at 45 days of withdrawal that while the IL-to-shell synapses did indeed have increased levels of CP-AMPARs, the PrL-to-core synapses did not, becoming unsilenced by inclusion of the far more common Ca^{2+}-impermeable AMPARs (CI-AMPARs). Thus, at the time when animals exhibited 2-fold greater cocaine seeking than on withdrawal day 1, previously silent synapses had become functional via the insertion of AMPARs. Might this synaptic remodeling be responsible for the increased behavioral responding that characterizes incubation of craving? And if so, could this process be reversed?

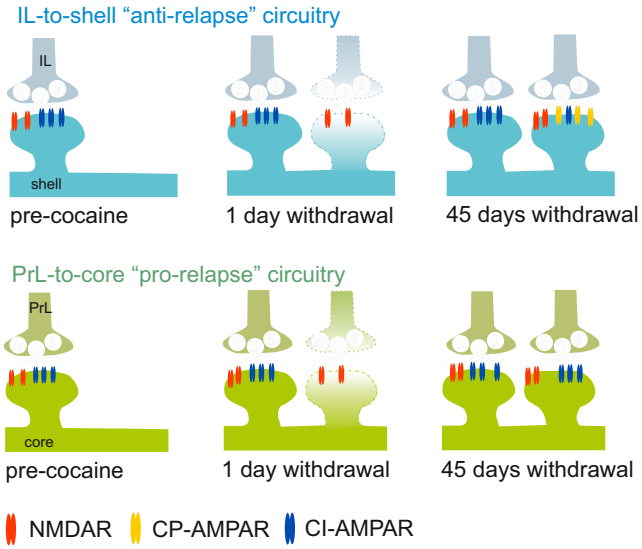

Figure 1. Synaptic Changes during Incubation of Craving
Diagram of two glutamatergic synapses in the nucleus accumbens before cocaine exposure and at two withdrawal time points during the incubation of cocaine craving.

In many brain regions, different forms of long-term synaptic depression (LTD) are mediated by the removal of AMPARs, and synapses in the NAc can exhibit different forms of LTD (Malenka and Bear, 2004; Lüscher and Huber, 2010). Again using light stimulation of each optogenetically labeled pathway, Ma and colleagues next tested whether LTD could be induced in the IL-to-shell or the PrL-to-core pathways in brain slices from rats exhibiting incubation of craving. They found that LTD could be induced in both pathways, successfully removing either the CI-AMPARs (PrL-to-core) or CP-AMPARs (IL-to-shell) present at withdrawal day 45. Presumably as a consequence of this removal, the percentage of silent synapses in each pathway was also increased. Importantly for the in vivo experiment to come, the protocol chosen for LTD induction had little effect on synaptic strength in animals that only received saline during the behavioral experiments, suggesting that fully established synapses remained unaffected by the LTD protocol.

The optogenetic approach provides a powerful way to forge links between in vitro experiments investigating a specific brain circuit and in vivo behavioral experiments in which the same circuit is manipulated. Here, Ma et al. used this approach to test the hypothesis that resilencing the synapses in one or both of the PFC-to-NAc pathways would reverse the incubation of cocaine craving observed behaviorally. When PrL-to-core synapses were resilenced using the optogenetic LTD protocol, animals sharply reduced their nose-poking responses—they appeared to have lost the incubation of craving. Surprisingly, the IL-to-shell synapses may play an opposing role: when these synapses were resilenced after LTD, the rats considerably increased their nose-poking response, as if resilencing these synapses removed a brake on the incubation of craving. Together these experiments show that cocaine self-administration rapidly increases the percentage of silent glutamatergic synapses in the NAc and that synaptic maturation characterized by either CP-AMPAR or CI-AMPAR synapses is apparent after several weeks (Figure 1). LTD protocols used to reverse the maturation process significantly alter behavioral consequences in the incubation of craving model of relapse.

Silent synapses are prominent early in development, but diminish during adulthood. Their reappearance in the more mature nervous system could be considered as the reopening of a plastic period, enabling adaptation to a new set of conditions. What explains the proliferation of synapses after taking cocaine? One likely hypothesis is that a form of homeostatic plasticity is initiated, as occurs in response to functional deprivation. Perhaps during repeated cocaine administration the excitability of NAc medium spiny neurons is reduced sufficiently to drive reactive formation of new synapses. Previous work demonstrated that repeated exposure to cocaine or amphetamine markedly increased dendritic branch number and density of dendritic spines (sites of glutamatergic synapses) on NAc medium spiny neurons (Robinson and Kolb, 2004), suggesting that functionally silent synapses seen on withdrawal day 1 could be nascent synapses, perhaps located on newly formed dendritic spines. During withdrawal, these silent synapses can then be unsilenced, perhaps representing a more mature synaptic condition. The insertion of AMPARs, especially CP-AMPARs, is a recurring adaptation reported in several different brain regions in response to environmental changes. For example, CP-AMPARs increase in the hippocampus after ischemia (Liu and Zukin, 2007), in the lateral amygdala after fear conditioning (Clem and Huganir, 2010), in the visual cortex upon dark rearing (Lee 2012), and

in the NAc after cocaine self-administration (Lüscher and Malenka, 2011; Loweth et al., 2014; Ma et al., 2014). CI-AMPARs and CP-AMPARs have different channel conductances, and modulation by endogenous polyamines provides short-term plasticity properties to CP-AMPAR synapses absent in CI-AMPAR synapses. Moreover, allowing intracellular Ca^{2+} through synaptic CP-AMPAR channels without the checks and balances that restrict Ca^{2+} entry through NMDARs or voltage-gated Ca^{2+} channels risks unhealthy levels of intracellular Ca^{2+} during ordinary neurotransmission, or may drive plastic changes at the affected input to the exclusion of other inputs (Liu and Zukin, 2007). Whether these dire predictions occur following insertion of CP-AMPARs at IL-to-shell synapses and whether CP-AMPARs remain at these synapses indefinitely remains to be tested.

Only one of the two pathways examined in this study developed unsilenced synapses via inclusion of CP-AMPARs, while the other utilized more common CI-AMPARs. As demonstrated in the NAc by Ma et al., it is possible to remove new AMPARs of either type using an LTD protocol. Perhaps most important in the context of drug craving is the fate of resilenced synapses after undergoing LTD. Ma et al. induced LTD immediately prior to the final drug-seeking behavior session. For how long do these synapses now remain silent? Is this functional silencing a transient condition? During NMDAR-dependent LTD (such as occurs in the PrL-to-core synapses), NMDARs as well as AMPARs can be removed, and this overall weakening of the synapse may presage synapse elimination (Hanse et al., 2013). The LTD protocol does not necessarily "reset" synapses in the PFC-to-NAc pathways to the cocaine-naive state, but to the state at the beginning of withdrawal, i.e., there is still an increased number of silent synapses relative to those in a drug-naive animal. The duration of in vivo LTD in these accumbens synapses and its behavioral outcomes on cocaine-seeking represent important future directions. Rats trained to self-administer other addictive substances such as heroin, alcohol, nicotine, and even sucrose also exhibit incubation of craving, suggesting that this phenomenon is a general mechanism induced by withdrawal from highly motivating stimuli (Pickens et al., 2011). It will be interesting to see if a similar reversible remodeling of synapses in these PFC-to-NAc pathways also regulates incubation of craving for these other substances.

The NAc is innervated by multiple excitatory afferents beyond the prefrontal cortex, including the basolateral amygdala, hippocampus, and thalamus. Intriguing complementary studies utilizing optogenetics indicate clearly that activation of perhaps all of these inputs contributes to motivated behavior (Britt et al., 2012), and even to the incubation of craving (Lee et al., 2013); however, all of the pieces do not yet fit neatly together. We are still at a stage where our understanding of this fascinating and complex circuit is reminiscent of the blind man and the elephant. While it will be some time before we can fit all the information together to provide a coherent view of how the NAc generates and controls motivated behavior, this piecemeal approach is absolutely essential in these early days to develop a catalog linking region-specific and cell-type-specific drive with behavior.

Cocaine is well known to induce prorelapse or proaddictive neuroadaptations in multiple brain regions. By discovering the existence of cocaine-triggered adaptations in the IL-to-shell that form a novel endogenous "antirelapse" mechanism, the study by Ma et al. suggests important new synaptic and circuit-level targets for intervening to reduce drug craving. In the rodent model of incubation of craving, the magnitude of responding is influenced by a variety of factors, including environmental enrichment, age, exercise, and the estrus cycle, and we speculate that selective interactions with environmental/hormonal factors could preferentially modulate synapses in one of the two prefrontal cortex-to-NAc pathways (Pickens et al., 2011). Genetic or environmentally altered differences in the two PFC-to-NAc circuits or their relative activity levels could contribute to an individual's relative susceptibility to substance abuse disorders, and the mechanistic understanding provided by Ma and colleagues of how these important "antirelapse" synapses can be strengthened might be used to treat cocaine craving.

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