Biological Psychiatry: Celebrating 50 Years

Exploring the Bridge From Extracellular Signals to Intracellular Plasticity

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Biological and environmental factors contribute to all stages of addiction, from initial drug use and abuse to relapse. The learned associations between drugs, cues, and the environment create enduring changes in underlying brain circuitry. During periods of abstinence, exposure to drugs, stressors, or cues trigger transient changes in the brain leading to increased drug-seeking behavior and relapse. These external stimuli have a profound impact on the internal structure and function of the brain, making it difficult to break the cycle of abstinence and relapse. Understanding the enduring and transient changes that occur after drug use and relapse is critical to designing and providing effective therapies for addiction.

Attention has primarily focused on the changes inside neurons when examining drug-induced changes in the brain. However, the environment outside neurons also significantly changes after exposure to and abstinence from drugs of abuse. The environment surrounding neurons is diverse, forming an extracellular matrix (ECM) including proteoglycans, link proteins, extracellular receptors, and enzymes. Extracellular receptors produce and anchor ECM molecules to the cell membrane, while extracellular enzymes like matrix metalloproteases (MMPs) degrade and remodel the ECM. Extracellular molecules have many functions, including structural support, cell migration, and neural plasticity (1); some of these functions may be facilitated by signaling components or sequences exposed after MMP activity (1–3).

The ECM contributes to various forms of plasticity, including drug-induced plasticity [for review, see (1,2)]. Transient synaptic plasticity (t-SP) is a phenomenon observed after cocaine experience and is assessed by elevated glutamatergic signaling and larger dendritic spine heads (4,5). Extracellular regulation of t-SP may seem unlikely because glutamatergic signaling and dendritic spines are features of the neurons themselves; however, a plethora of studies connect drug-induced plasticity to active MMP-9 [for review, see Mulholland *et al.* (2)], although the pathway remains unclear. In the current issue of *Biological Psychiatry,* Garcia-Keller *et al.* (6) explored the pathway from extracellular degradation via MMP-9 to t-SP in the nucleus accumbens core and cue-induced reinstatement of cocaine self-administration behavior.

A bridge from the extracellular environment to intracellular signaling is integrin, the cell to ECM adhesion receptor. The integrin receptor is composed of α and β subunits, with the α subunit determining ligand binding and the β subunit determining the intracellular signaling pathway(s) (3). Earlier work by Wiggins *et al.* (5,7) demonstrated that cocaine experience, both in noncontingent behavioral sensitization and in intravenous self-administration models, alters the expression of two integrin β subunit isoforms, β1 and β3. Based on these studies implicating cocaine-induced changes to the β1 and β3 subunits, the current study assessed a more causal relationship between cue-induced reinstatement, t-SP, and both subunits using a morpholino knockdown method. Mirroring earlier work identifying increased β3 protein levels after cocaine-induced reinstatement (5), knockdown of β3 decreased responding on the active, previously cocaine-reinforced lever and decreased both measures of t-SP (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/*N*-methyl-D-aspartate (NMDA) ratio and diameter of dendritic spine heads). Knockdown of β3 had no impact on responding on the active, previously reinforced lever when sucrose was the earned reward. In addition, the active lever presses during reinstatement were positively correlated with the AMPA/NMDA ratio and the dendritic spine head diameter. These findings suggest a relationship between the functionality of β3 and the manifestation of reinstatement behavior.

In contrast to β3, the role of β1 appears to be more complex. Morpholino knockdown of β1 or β3 was sufficient to observe changes in behavior after administration; however, it was not sufficient to completely knock out all β1 or β3 protein levels. Notably, after administration of the β1 morpholino, β1 protein levels were lower, but β3 and integrin-linked kinase (ILK) protein levels were elevated, suggesting a unidirectional compensatory mechanism for the loss of β1 because a similar pattern was not observed after the loss of β3. This pattern of lower β1 and higher β3 levels echoes the changes to these subunits after acute, noncontingent cocaine exposure (7). After knockdown of β1, cue-induced self-administration increased, possibly because of the elevated levels of β3 and ILK. Given these findings, one may hypothesize that a corresponding inversion of the relationship between cue-induced reinstatement and t-SP would be observed after administration of the β1 morpholino. These studies provide compelling evidence that cocaine and related cues modify the β subunit of the integrin receptor and that these modifications contribute to the transient plasticity underlying the manifestation of cocaine-seeking behavior.

Signaling through integrins can be outside-in, meaning that signals originate outside the cell and signal internally, or inside-out, meaning that signals originate inside the cell and signal externally (3). Two kinases involved in integrin signaling are focal adhesion kinases (FAKs) and ILK (3). Integrin subunit β1 is thought to primarily signal through ILK, while β3 can signal through both ILK and FAK (8). Cocaine sensitization is prevented by silencing ILK in the nucleus accumbens (9), and protein levels of phosphorylated FAK are not different after acute or chronic noncontingent cocaine exposure (7). These

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studies suggest that ILK, potentially mediated through β1, is involved in the enduring synaptic plasticity underlying cocaine sensitization. In contrast, the current study found that FAK but not ILK was involved in mediating cue-induced reinstatement. These apparent conflicting findings may be due to different routes of cocaine administration (noncontingent vs. self-administration), priming method (cocaine vs. cue), and/or method of blocking ILK function (selective small interfering RNA in neurons vs. small molecule inhibitor in the nucleus accumbens). While these findings offer conflicting contributions of ILK and FAK to drug-induced plasticity, they may suggest unique contributions of integrin-mediated signaling to enduring and transient plasticity. In addition, they may target unique downstream molecules, leading to different outcomes—for instance, regulating postsynaptic density protein 95 and synapsin-I (9) or trafficking glutamate receptors (5).

Garcia-Keller *et al.* (6) connected the pathway from active MMP-9 through β3 and FAK to mediate t-SP and cue-induced reinstatement behavior [see Figure 7 in Garcia-Keller *et al.* (6) for a diagram of the pathway]. One cleavage product of MMP-9 explored here was the exposed arginine, glycine, and aspartate (RGD) binding domain, present in several ECM molecules (3). Active MMP-9 and RGD domains increased cue-induced reinstatement in a similar manner (6). Interestingly, reinstatement was completely ablated when MMP-9 was active and FAK was inhibited (6). This could indicate that in conditions with high levels of active MMP-9, some signaling occurs through β1, ILK, or other unexplored signaling molecules, because lower levels of β3 and active MMP-9 resulted in lower reinstatement.

MMP-9 cleaves ECM molecules to expose RGD signaling domains that can signal through β3 and FAK leading to t-SP. While description of this pathway marks significant advances in the addiction and ECM fields, it also raises fascinating questions. For instance, where do the RGD sequences originate? Do some ECM molecules contain more RGD sequences than others? Can other ECM molecules bind to integrins to stimulate these signaling pathways? One potential candidate to answer these questions is the ECM molecule laminin, which contains RGD domains. In the hippocampus, laminin promotes long-term potentiation and may also contribute to other types of plasticity (1). Another potential mechanism through which ECM signals could transform into intracellular signals is the binding of tenascin-C and tenascin-R to integrin receptors. In the hippocampus, tenascin-C and tenascin-R also promote long-term potentiation (1).

Both laminin and tenascins act as bridges between integrins and other ECM molecules, like proteoglycans. Proteoglycans are prominent ECM molecules, and in distinct regions of the brain surrounding a subset of cells they aggregate and form structures called perineuronal nets. These nets are implicated in drug-induced plasticity (10) and may create a microenvironment with higher levels of ECM molecules. This may allow for sustained degradation and remodeling via MMPs to continually generate signaling sequences for integrins. However, this connection has yet to be explored, and the turnover of ECM molecules in the brain remains unknown.

The ECM is not homogeneous throughout the brain or around individual neurons within a region. This leads to intriguing questions about ECM-regulated plasticity across brain regions, neuronal ensembles, and potential contributions to t-SP and other types of plasticity. Does the extracellular microenvironment around a neuron impact intracellular signaling or the neuron's involvement in an ensemble? Are different ECM-mediated signaling pathways engaged in distinct brain regions? Does ECM-mediated signaling in one region contribute to ECM-mediated signaling in another region? How do glia contribute to ECM remodeling and signaling? Are different ECM signaling pathways engaged during different stages or models of addiction?

While different addiction paradigms (cocaine sensitization, cue-induced reinstatement, etc.) engage unique signaling pathways, there appears to be a clear connection between extracellular degradation and synapse remodeling occurring during addiction. In this study, Garcia-Keller *et al.* (6) summarize this connection in a detailed diagram (Figure 7 in their article) that depicts this novel signaling pathway originating from MMP-9 activity in the ECM to t-SP and ultimately a relapse-like behavior. Understanding this pathway leads to the crucial question: are ECM-mediated signaling pathways an effective target for therapies treating addiction and other neuropsychiatric disorders?

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