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A Glitch in the Matrix: Aberrant Extracellular Matrix Proteolysis Contributes to Alcohol Seeking

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Drug addiction has been characterized as a disorder of maladaptive synaptic plasticity. Irrespective of the mechanism of action of the drug being abused, they all produce similar behavioral endpoints: craving and vulnerability to relapse during periods of abstinence, loss of control over intake, and continued use despite negative consequences. As such, identifying shared neurobiological features across different classes of drugs may identify points of attack that can be used to develop efficacious therapeutics for the treatment of addiction. One such shared neurobiological trait is aberrant extracellular matrix remodeling, in particular by matrix metalloproteinase-9 (MMP-9). MMPs are Zn^{2+} -dependent proteolytic enzymes that are primary regulators of the composition and integrity of the extracellular matrix. Dysregulation of MMP-9 activity has been reported following chronic use of alcohol, nicotine, opiates, cocaine, and methamphetamine (1). A family of 23 extracellular matrix-remodeling MMPs were originally described for their roles in angiogenesis, tumor cell invasion, and wound healing, and were thought to function primarily in regulating structural support surrounding cells (2). In the brain, however, they have more recently gained recognition for being important signaling molecules that regulate both structural and functional plasticity, primarily at excitatory synapses (1).

The study by Stefaniuk *et al.* (3) in this issue of *Biological Psychiatry* elucidates a novel mechanism by which MMP-9 functions within the central nucleus of the amygdala (CeA) to regulate synaptic plasticity underlying increased motivation to consume alcohol. The major finding of this paper is that mice lacking the *Mmp9* gene (MMP-9 knockout [KO]) have decreased motivation to consume 10% alcohol when forced to work for the drug, despite consuming the same amount of alcohol as their wild-type counterparts when given free access. Additionally, MMP-9 KO mice showed decreased alcohol seeking during withdrawal when they were not able to obtain any intoxicant. This suggests that the pharmacological actions of alcohol are largely conserved in the KO mice, but these mice are less motivated to seek and consume the drug during periods of abstinence. The CeA was identified as the site of action for MMP-9 activity by virally rescuing its expression in KO mice, which rescued motivation to consume alcohol. In an elegant series of morphological and electrophysiological experiments, the authors then investigated the effects of MMP-9 on structural and functional plasticity following alcohol withdrawal: DiOlistic labeling of CeA dendrites

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and spine quantification showed that MMP-9 KO mice had decreased spine density, and patch-clamp experiments examining the basolateral amygdala to CeA projection showed that MMP-9 KO mice did not generate silent synapses evident in wild-type littermates. The authors went on to examine a single nucleotide polymorphism within the human *MMP9* promoter that predisposes carriers to alcoholism. In a population of alcoholics, those who express the T single nucleotide polymorphism at the -1562 position in the *MMP9* gene have a higher motivation to drink alcohol despite negative consequences. This laboratory previously showed that this T/C polymorphism enhances MMP expression in humans (4). These intriguing findings add to a small but growing literature on MMPs in addicted human subjects (Figure 1).

MMP-9 is the most studied metalloproteinase in the brain. Its substrates include matrix molecules that bind and signal through integrins when cleaved (e.g., fibronectin, laminin), cell adhesion molecules (e.g., intercellular adhesion molecule 5, synaptic cell adhesion molecule 2), and pro-forms of growth factors (e.g., pro-transforming growth factor β and pro-brain-derived neurotrophic factor), both of which are activated upon cleavage (1). Thus, by virtue of its remodeling activity in the extracellular matrix, MMP-9 is uniquely situated to affect many diverse signaling pathways relevant to drug addiction. While MMP-9 is well established as both necessary and sufficient for long-term potentiation at hippocampal synapses, very little is known about its role in other brain sites, including the CeA (5). Previously, Michaluk *et al.* (6) showed that rats that over-express an autoactive form of MMP-9 have greater numbers of long, thin spines, with no change in mushroom- or stubby-type spines in the hippocampus. Consistent with this finding, Stefaniuk *et al.* (3) report a decrease in long, thin spines in MMP-9 KO mice, particularly in those that consumed the least amounts of ethanol, with no detected change in other spine subtypes. Wild-type members of the high-alcohol-consuming group had enlargement of both long and mushroom-type spines compared with their low-consuming counterparts, but these effects were blocked in MMP-9 KO mice, implicating the enzyme in spine enlargement. MMP-9 can directly affect dendritic spine morphology by signaling through integrin-linked kinase, an intracellular enzyme that phosphorylates cofilin, leading to increased actin polymerization (1). Consistent with a role for MMP9 in plasticity, MMP-9 gene transcription is upregulated in three key regions in response to fear conditioning: the amygdala, the prefrontal cortex, and the hippocampus. Interestingly, however, a second report from the same group suggests that MMP-9 activity in the CeA is necessary for appetitive but not aversive learning (7).

Generation of silent synapses (i.e., synapses with *N*-methyl-D-aspartate but no α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors) is a second adaptation shared across classes of drugs, and is believed to be crucial for circuit remodeling leading to behavioral phenotypes of addiction (8). While these have not been examined previously specifically within the CeA, withdrawal from cocaine or morphine has been shown to generate silent synapses in the nucleus accumbens (8). However, these two drugs seem to have opposite effects on overall spine density and differing mechanisms of generating silent synapses. Withdrawal from cocaine results in increased spine density and an increased percentage of silent synapses from <10% to up to 30%. Corresponding imaging suggests that these silent synapses are formed de novo as a result of cocaine experience. Contrarily,

withdrawal from morphine causes a decrease in spine density in the nucleus accumbens, and the increase in silent synapses is believed to result from complete internalization of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors within previously mature spines (8). Here, Stefaniuk *et al.* (3) go on to show, for the first time, that alcohol withdrawal increases the number of silent synapses within the CeA, and this effect is absent in MMP-9 KO mice. It has been previously shown that MMP-9 signaling through beta-1 integrin subunits controls *N*-methyl-D-aspartate receptor surface diffusion into synapses, providing a potential mechanism by which MMP-9 deletion reduces *N*-methyl-D-aspartate currents and silent synapse formation (9). In this previous report, MMP-9 mutation did not affect GluA2 alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor subunit motility under baseline conditions, suggesting that there is a parallel mechanism by which alcohol prevents synapses from maturing. However, in conditions of chemically induced long-term potentiation MMP-9 is activated locally at synapses and its proteolytic activity is responsible for initiating signaling events to drive calcium-permeable GluA1 receptors into the synaptic cleft (10). The effect of withdrawal from chronic alcohol exposure on dendritic spine density within the CeA has not been previously reported, and thus a major question raised by this manuscript is which of these two mechanisms may be responsible. Evidence reported here show that MMP-9 KO mice have lower total spine density and a higher baseline fraction of silent synapses, but no alcohol-induced increase in silent synapse formation. Coupled with previous evidence that MMP-9 activity drives new spine formation, this makes it tempting to speculate that these synapses are indeed synthesized *de novo*.

The translational potential for therapeutic manipulation of MMP-9 has been examined in a large number of neuropsychiatric disorders [for a review, see Vafadari *et al.* (5)], although as of yet no successful MMP-9-directed drug candidates have come to fruition (5). Minocycline is a commonly used tetracycline antibiotic that has been shown to reduce MMP-9 activity and expression, raising the question of whether this drug could be effectively used for treatment of neuropsychiatric disorders. The interesting clinical data in this report demonstrating that a known gain-of-function single nucleotide polymorphism in the *MMP9* gene is associated with increased motivation to consume alcohol despite negative consequences adds to literature encouraging examination of MMP-9 as a pharmacotherapeutic target for addiction. Previously, increased serum MMP-9 expression has been demonstrated in both alcohol-dependent and heroin-dependent patients (5). Outside of drug addiction, either serum MMP-9 levels or the C-1562T polymorphism have been correlated with a number of disorders, most notably mild cognitive impairment, Alzheimer's disease, major depressive disorder, and fragile X syndrome. Chemically modified tetracyclines that retain MMP-9 inhibition properties, but have no danger of conferring antibiotic resistance, have been developed and been confirmed to be safe in phase 1 clinical trials. I propose that these compounds should be further developed and tested in phase 2 trials for addiction and other neuropsychiatric disorders.

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Figure 1.

Proposed model of synaptic plasticity underlying alcohol seeking in wild-type (top) and matrix metalloproteinase-9 (MMP-9) knockout (KO) (bottom) mice. In wild-type animals, repeated alcohol consumption and withdrawal causes increased silent synapse formation and number of long spines was positively correlated with motivation to consume alcohol. In MMP-9 KO mice, there is a higher baseline fraction of silent synapses, but alcohol intake does not cause an increase in silent synapse formation, and these mice show less motivation to consume alcohol during progressive ratio testing and during withdrawal. Alc., alcohol; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; NMDA, *N*-methyl-D-aspartate.