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Merger Fever: Can Two Separate Mechanisms Work Together to Explain Why We Drink?

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We often hear how problems associated with alcohol abuse represent a massive economic and public health burden. Yet despite a concerted effort to identify new and effective pharmacotherapeutics, the drug treatment options available to treat alcohol disorders have progressed little over the past few decades. This is not to say there has been no progress in our understanding of how alcohol affects brain chemistry and behavior. On the contrary, there have been major insights into the neuropharmacology of excessive alcohol drinking, revealing key roles and potential targets amongst various neuropeptides and neurotransmitters (1). In a report published in this issue of *Biological Psychiatry*, Nam *et al.* (2) offer some potential new leads.

One exciting and growing body of research points to the importance of the principle excitatory neurotransmitter of the brain, glutamate, both in mediating the behavioral effects of alcohol and in the pathophysiology of alcohol abuse (3). The glutamate *N*-methyl-D-aspartate receptor (NMDAR) subtype seems to be of particular importance. Systemic administration of MK-801 and other NMDAR antagonists potentiate alcohol intoxication and reduce alcohol drinking in rodents (4,5), and human volunteers given an NMDAR blocker such as ketamine report a feeling of intoxication akin to that produced by alcohol (6). These effects likely relate to the pharmacological action of alcohol as a functional NMDAR antagonist (7). This antagonistic action likely explains why chronic alcohol exposure leads to a transient upregulation of NMDARs in forebrain regions regulating reward, cognition, and emotion (8) and the ability of NMDAR blockers to alleviate central nervous system hyperexcitability and anxiety during alcohol withdrawal (5).

Then there is the fact that the NMDAR is also a major player in various forms of synaptic plasticity and learning, the receptor well-placed to regulate the neural adaptations that ultimately drive the progression of alcoholism as a disease. Collectively, these various observations support the idea that NMDAR-mediated glutamate signaling is an important neural mechanism regulating the effects of alcohol on brain and behavior in multiple ways of course, no single mechanism or system can regulate something as complex as alcohol drinking in isolation, and a major challenge for the field is to draw meaningful lines between what ostensibly seem to be disparate mechanisms.

The starting point for the current study by Nam *et al.* is previous work that had shown that gene deletion of the mouse Type 1 equilibrative nucleoside transporter (ENT1), a regulator of extracellular adenosine levels and long-suspected of being an alcohol regulator (9), leads

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to decreased behavioral sensitivity to high (aversive) doses of alcohol and elevated alcohol drinking (10). It was also known that adenosine signaling through the A_1 receptor inhibited glutamate synaptic transmission in the nucleus accumbens (a major node within the reward circuit) and reduced alcohol drinking (10,11). Moreover, ENT1 null mutants had shown evidence of reduced accumbal adenosine tone increased glutamate transmission in the nucleus accumbens (10). On this basis, one could suggest that ENT1 deletion and associated loss of A_1 signaling could lead to glutamatergic disinhibition in the accumbens, and that these changes might underlie the increased alcohol drinking in these mice.

Nam *et al.* employ a range of techniques, including no-net flux microdialysis, proteomics, and drug injections coupled with behavior, to further explore glutamatergic abnormalities in the ENT1-deficient mice and the potential contribution of these aberrations to their drinking phenotype (2). They first verified that the ENT1 mutants had elevated basal extracellular glutamate (and decreased extracellular adenosine) levels in the nucleus accumbens. They also found decreased expression of the glial glutamate transporter EAAT2 (GlyT-1) and increased expression of the glutamate signaling-related molecules calmodulin and neurogranin (Ng). Digging deeper into the intracellular signaling machinery revealed reduced expression of phosphorylated forms of NMDAR GluN1 subunit (Ser890) and Ng (Ser36), both of which are preferentially phosphorylated by protein kinase C γ (PKCγ). Further analysis revealed that the mutants had lesser expression of phosphorylated PKCγ (Thr514) and, possibly because of the upstream changes in Ng and PKCγ, decreased levels of an active form (Thr286) of calmodulin-dependent protein kinase Type II (CaMKII). Further connections were made between these molecular alterations, with the observation that activity of the NMDAR-associated protein phosphatases PP1/PP2A—which are known to dephosphorylate PKCγ (Thr514)—was increased, whereas a phosphorylated form (Ser133) of the CaMKII target cAMP response element binding (CREB) was decreased. Putting all this together provides a detailed picture of the intracellular changes resulting from ENT1 deletion. But to what extent, if any, do they contribute to these increased proclivity of the mutant animals for alcohol?

This was an especially important question to answer because the study's molecular observations were limited to the nucleus accumbens, whereas the ENT1 deletion was brain-wide. Constitutive mutant mice can be valuable tools for studying alcohol-related behaviors, but great care must be exercised when attributing phenotypic abnormalities to molecular changes found in specific brain regions (12,13). Nam *et al.* were able to show that systemically blocking the NMDAR (via 4 days of CGP37849 injections) in the ENT1 mutants normalized both the elevated alcohol consumption (tested in a 24-hour two-bottle free-choice procedure) and many of the molecular abnormalities found in the accumbens. This is good correlative evidence linking these behavioral and molecular changes. Even more compelling will be follow-up studies that go on to show that microinfusion of an NMDAR antagonist into the accumben, and ideally not other region, is sufficient to rescue the mutant drinking phenotype. The authors did employe a site-specific drug administration approach to show that bilateral microinfusion of a peptide inhibitor of pPKCγ into the accumbens of wild-type mice increased CaMKII and CREB activity alcohol drinking. Thus, taken together, these experiments provide initial steps toward establishing the functional contribution of both NMDAR- and PKCγ-related signaling to the excessive drinking associated with ENT1 deletion.

The reward-related effects of alcohol are mediated by a highly interconnected neural circuit. One important question going forward will be to determine whether interactions between ENT1 and glutamate generalize beyond the striatum to other loci, such as the prefrontal cortex and extended amygdala and whether these contribute to drinking or other alcohol-related behaviors. Probably the best way to tackle this and to verify the importance of the

accumbens to the excessive ENT1 mutant drinking phenotype would be to knock down ENT1 within specific regions with next-generation targeted mutation or viral-based tools. Another interesting avenue for future work will be to explore how loss of ENT1 and the resulting functional changes in downstream NMDAR-associated pathways might play a role in the neuroadaptations that result from chronic exposure to alcohol. Nam *et al.'s* observation that ENT1 deletion leads to changes in NMDAR and other molecules critical for plasticity, such as CaMKII and CREB, strongly hints that this might be a fruitful line of inquiry.

A better understanding of how alcohol co-opts the incredible capacity of the brain for plasticity, via these and other mechanisms, to drive changes in circuits and behavior will lay the foundation for developing the next generation of treatments for alcohol dependence and alcoholism.

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