Alcohol and other drugs of abuse hijack the mechanisms responsible for neural plasticity, producing changes in brain function that lead to addiction. Plasticity is the strengthening or weakening of connections that occur during periods of heightened or diminished neural transmission, which is thought to underlie the neural basis for learning and memory. A key molecular regulator of neural plasticity is the NMDA receptor, which detects these changes in synaptic transmission and converts them into long-term modifications. Ethanol directly affects NMDA receptors in the nervous system by acutely inhibiting their function (1). However, chronic ethanol intake increases NMDA receptor function. Elucidating the mechanisms behind these effects could provide insight into the disordered physiological processes associated with alcohol use disorders and suggest novel treatments. Using a unique combination of experimental methods, we discovered the central role of a specific NMDA receptor subunit in these effects.

The NMDA receptor consists of two obligatory GluN1 subunits paired with two GluN2 and/or GluN3 subunits. The two variations of the GluN2 subunit most heavily expressed in the adult forebrain are GluN2A and GluN2B, which play key roles in defining many NMDA receptor properties. Unfortunately, efforts to investigate the selective contributions of these NMDA receptor subunits in ethanol’s actions in vivo have been hampered by insufficiently selective drugs. Additionally, deleting or knocking out GluN2B, a standard method for investigating the effects of a gene or protein, results in lethality, thus precluding direct investigation on GluN2B’s specific effects (2). Here, we have taken advantage of a newly generated conditional KO of GluN2B in combination with the use of NMDA receptor-targeted drugs to assess the roles of this subunit in both acute and chronic actions of ethanol on the NMDA receptor. Because glutamate is the neurotransmitter that activates NMDA receptors, we have focused on measuring ethanol’s effects on glutamate signaling at the synaptic level (i.e., the site where transmission occurs between cells) within the bed nucleus of the stria terminalis (BNST). This brain region plays a critical role in the expression of negative affect (e.g., anxiety, depression) that occurs during alcohol abstinence and is a primary contributor to relapse of alcohol intake.

Electrophysiological and biochemical studies confirmed the deletion of GluN2B from the BNST in GluN2B KO mice and revealed that its absence enhanced glutamatergic transmission in this brain region. Moreover, long-term potentiation, a major form of NMDA receptor-mediated synaptic plasticity at glutamatergic synapses, is absent in brain slices prepared from GluN2B KO mice. Strikingly, we found that ethanol inhibits ~50% of NMDA receptor-mediated excitatory transmission in control or normal mice but has no effect in GluN2B KO mice. In contrast, such ethanol sensitivity was maintained in GluN2A KO mice. These data indicate that the GluN2B subunit of the NMDA receptor is critical for alcohol actions in the brain (Fig. P1).

Chronic intermittent ethanol exposure and withdrawal disrupt both excitatory and inhibitory transmission within key brain nuclei like the BNST, an effect that likely plays a major role in alcohol dependence and relapse. We found that the withdrawal from chronic intermittent ethanol enhances NMDA receptor-mediated long-term potentiation in the BNST. This enhancement was absent in GluN2B KO mice, suggesting a key role for GluN2B in this enhanced plasticity. To gain insight into the mechanisms by which GluN2B accomplishes such effects after...
chronic ethanol exposure, we took advantage of the divergent actions of the GluN2B antagonist Ro25-6981. A unique feature of this compound is that it inhibits GluN2B-containing NMDA receptors in the presence of high agonist concentrations. An agonist, such as glutamate, is a molecule that binds to a receptor and leads to its activation. This would occur at NMDA receptors found at the synapses because they are exposed to high glutamate levels. Importantly, this antagonist also increases the affinity of glutamate for NMDA receptors at low agonist concentrations, such as those found at NMDA receptors located outside synapses (extrasynaptic NMDA receptors) (3), leading to enhancement of NMDA receptor activity. Intriguingly, we found that a moderate concentration of Ro25-6981 augments long-term potentiation in the BNST after chronic intermittent ethanol. This effect is blocked by pretreatment with memantine, a drug that preferentially inhibits extrasynaptic NMDA receptors (4). In combination, these data suggest that increased activity of extrasynaptic GluN2B-containing NMDA receptors contributes to the chronic intermittent ethanol-induced enhancement of long-term potentiation in the BNST (Fig. P1, Right).

In conclusion, these studies demonstrate a clear role for GluN2B in plasticity and alcohol-related effects in the BNST, a region critical in modulating behaviors involved in withdrawal and relapse to alcohol use. These data emphasize the importance of both the NMDA receptor subunit’s specificity and localization in the actions of ethanol. Indeed, memantine, which seems to display some localization specificity in its actions, is currently under active investigation as a treatment for alcoholism. Interestingly, it has been shown to reduce withdrawal severity (5). Our data suggest that the effectiveness of this compound may be greatest in withdrawn, dependent alcoholics, in whom an extrasynaptic, memantine-sensitive population of receptors exists.