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Review

Alcohol and the brain: from genes to circuits

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Alcohol use produces wide-ranging and diverse effects on the central nervous system. It influences intracellular signaling mechanisms, leading to changes in gene expression, chromatin remodeling, and translation. As a result of these molecular alterations, alcohol affects the activity of neuronal circuits. Together, these mechanisms produce long-lasting cellular adaptations in the brain that in turn can drive the development and maintenance of alcohol use disorder (AUD). We provide an update on alcohol research, focusing on multiple levels of alcohol-induced adaptations, from intracellular changes to changes in neural circuits. A better understanding of how alcohol affects these diverse and interlinked mechanisms may lead to the identification of novel therapeutic targets and to the development of much-needed novel and efficacious treatment options.

Highlights

Alcohol affects several levels of brain function, from cellular and molecular pathways to circuit-level activity.

The effects of alcohol on the various levels of brain function are closely intertwined and underlie different aspects of AUD.

Understanding this diverse and interlinked molecular, cellular, and circuit landscape will help to guide the development of future therapeutic approaches.

Alcohol-induced neuronal adaptations

AUD affects about 10–15% of the global population, causing significant medical, social, and economic burdens[i]. Although most drinkers consume alcohol for years without escalating to excessive use, a subset of people develop harmful drinking patterns [1]. Unfortunately, efficacious treatment options are limited [2], owing in part to the complex and multifaceted ways in which alcohol intake affects the nervous system. Both acute and chronic alcohol exposure produce molecular and cellular neuroadaptations that influence the activity of discrete brain regions and cell types [3–5]. Consequently, these neuronal adaptations that underlie AUD are influenced by diverse interactions between alcohol and intracellular signaling, the epigenome, neurotransmitters, and modulators, as well as the activity of neuronal circuits, which in turn drive behaviors such as heavy alcohol use, anxiety, craving, and relapse.

We review here recent literature focusing on alcohol-induced neuronal adaptations. We discuss seven distinct but tightly interlinked levels of effects of alcohol on the brain, starting from genetic factors that confer susceptibility to AUD (level 1), through alcohol-induced changes in epigenetic mechanisms (level 2), transcriptional activity (level 3), alternative splicing (level 4), translation (level 5), and post-translational modifications (level 6), to circuit-level activity (level 7). We discuss the molecular mechanisms that contribute to the development of this disorder, and describe evidence outlining potential new avenues for medication development for the treatment of AUD. Finally, we consider recent work examining how alcohol-induced plasticity impacts on the level of neural circuit activity and release of neuromodulators to influence decisions of when and how much to drink.

Level 1: genetic factors in AUD

Common and rare variants in specific genes play a role in both the resilience and susceptibility mechanisms that protect against or promote AUD, respectively [6–9]. Among the most validated functional variants associated with AUD are alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2), both of which participate in alcohol metabolism in the liver [10]. Interestingly, single-nucleotide polymorphisms (SNPs) in both *ALDH1B* and *ALDH2* are protective against the development of AUD [10]. Of note, ALDH2 in cerebellar astrocytes promotes alcohol metabolism, GABA production, and intoxication [11]. A recent genome-wide meta-analysis study in 435 563 individuals of European descent has identified SNPs linked to AUD and problem

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drinking [7]. Among the identified variants are several ADH enzymes as well as in genes such as *PDE4B*, *SYNGAP*, and *BDNF* [7].

A non-synonymous SNP has also been identified within the brain-derived neurotropic factor (*BDNF*) gene (rs6265) that results in the substitution of valine 66 by methionine. Carriers of the SNP display early onset of relapse [12], and transgenic knock-in mice carrying the mutation consume excessive amounts of alcohol despite negative consequences [13], a phenotype which is rescued by viral overexpression of wild-type BDNF in the medial prefrontal cortex (mPFC) or via activation of the BDNF receptor, TrkB [13]. Another example is a SNP in the EE hand-domain containing 2 (*EFHD2*) gene [14]. This SNP is associated with lifelong drinking as well as having a negative association with anxiety phenotypes in healthy adolescence [14], and mice lacking EFhd2 consume more alcohol [14]. These data suggest that both *BDNF* and *EFHD2* are 'resilience' genes, and that malfunction of either BDNF or EFhd2 contributes to the development of AUD.

Furthermore, an SNP in the gene encoding β-Klotho, a component of the hormone receptors for fibroblast growth factors FGF19 and FGF21, was highly correlated with alcohol consumption in a large genome wide association (GWAS) study of individuals without AUD [15]. Brain-specific knockout of β-Klotho increased alcohol consumption and preference in mice by a so far unknown mechanism [15]. Interestingly, high expression of β-Klotho has previously been observed in mesolimbic regions in mice, such as the nucleus accumbens (NAc) and ventral tegmental area (VTA), which are central to reward-seeking behavior [16]. In addition, a polymorphism in Ras suppressor 1 (*RSU1*), that is involved in the regulation of neuronal actin dynamics through RAC1, has been shown to influence reward anticipation and alcohol consumption in humans [17]. By contrast, an SNP in TRAF family member-associated NF-κB activator (*TANK*) is associated with reduced drinking in humans [15]. A study in *Tank* knockout mice showed that these animals exhibit reduced alcohol drinking and preference, and further studies revealed that TANK is involved in the regulation of alcohol-dependent insular cortical activation of nuclear factor κB (NF-κB) in mice [18].

Level 2: alcohol-induced alterations in epigenetic regulation

An emerging body of evidence suggests that epigenetic regulation represents an important level of alcohol-induced molecular adaptations in the brain (Figure 1). This umbrella term includes covalent modifications of DNA and histones (e.g., methylation, acetylation) resulting in the condensation or relaxation of chromatin (the complex of DNA and associated proteins) leading to repression or enhancement of gene expression, respectively. Importantly, these mechanisms change the landscape of genome accessibility and the proteome, and these in turn contribute to alterations in neuronal cytoarchitecture and activity, and play a crucial role in the development of substance use disorders including AUD. For example, alcohol self-administration in macaques was shown to decrease DNA methylation at specific genomic loci in a dose-dependent manner [19]. Interestingly, several of the affected genes were related to synaptic function, including genes encoding proteins that control neurotransmitter release or receptor trafficking [19], providing further evidence that epigenetic regulation plays a role in alcohol-induced synaptic plasticity.

Acute and chronic exposure to alcohol can have opposite effects on epigenetic regulation. For instance, whereas acute alcohol exposure increased histone acetylation and decreased histone methylation in the central amygdala (CeA), chronic intermittent exposure had opposite effects [20,21]. These findings suggest that the epigenetic landscape undergoes adaptations that might play an important role in the development of AUD.

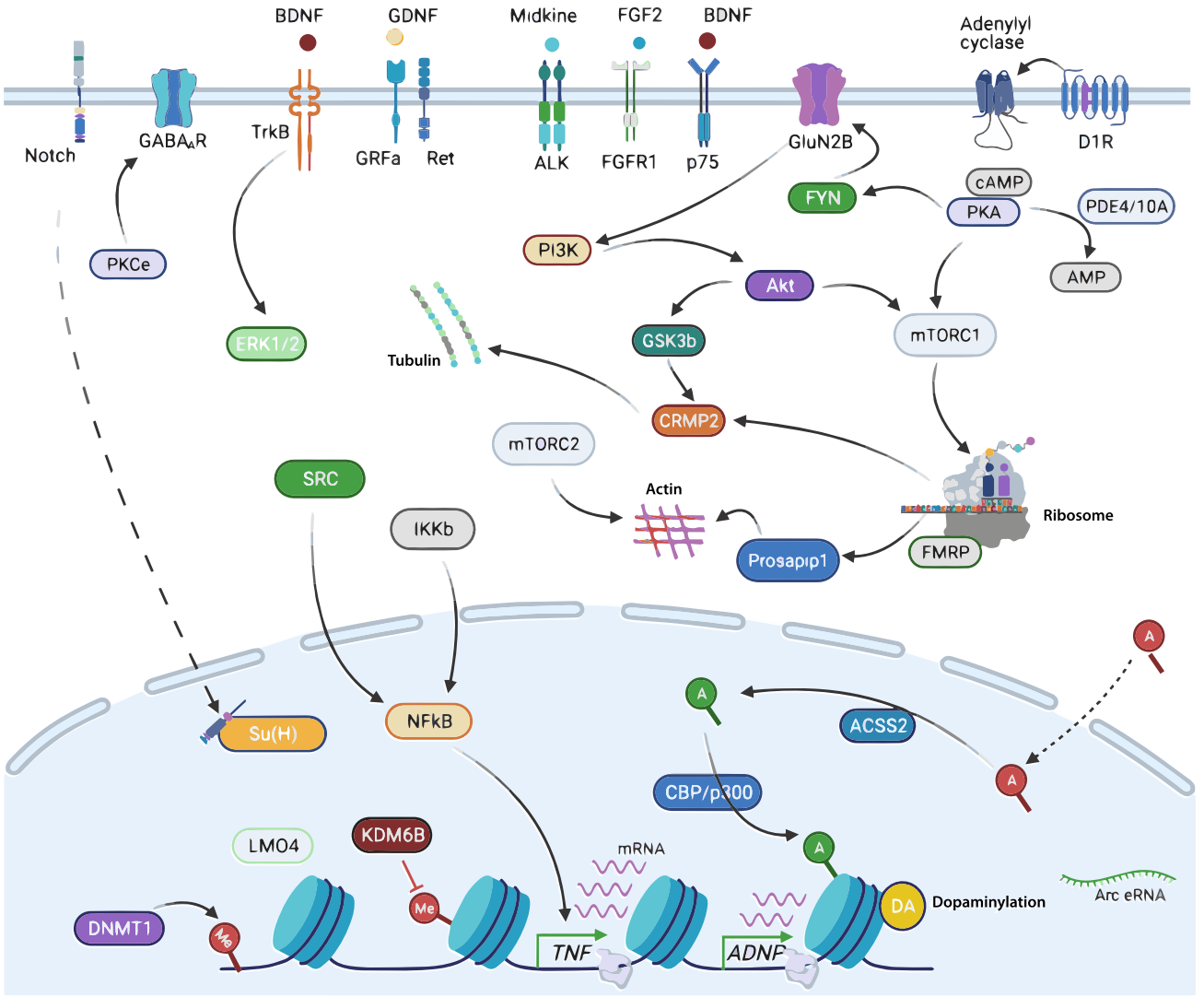

Figure 1. Molecular pathways in alcohol use disorder (AUD). Alcohol binds to several transmembrane receptors including glutamate, GABA, and dopamine receptors, as well as to receptors for different neuropeptides and neurotrophic factors. These in turn affect the activity of several second messenger cascades and intracellular signaling pathways. These pathways mediate long-lasting cellular adaptations that affect, among others, translation and synaptic plasticity, and which contribute to neuronal adaptations underlying AUD. In the nucleus of neurons, alcohol has complex effects on the epigenetic regulation of gene expression. These complex and highly interlinked pathways activate specific gene expression programs which underlie neuronal maladaptations and contribute to the development of AUD. Abbreviations: ACSS2, acetyl-CoA synthetase 2; ADNP, activity-dependent neuroprotector homeobox; ALK, anaplastic lymphoma kinase; BDNF, brain-derived neurotrophic factor; CBP, CREB (cAMP response element-binding protein)-binding protein; CRMP2, collapsing response mediator protein family 2; D1R, dopamine receptor D1; DNTM1, DNA methyltransferase 1; ERK1/2, extracellular signal-regulated kinase 1/2; FGF2, fibroblast growth factor 2; FGFR1, FGF receptor 1; FMRP, fragile X mental retardation protein; GABA$_A$R, γ-aminobutyric acid receptor A; GDNF, glial-derived neurotrophic factor; GFRa1, GDNF family receptor α1; GluN2B, glutamate *N*-methyl-D-aspartate receptor subunit 2B; GSK3β, glycogen synthase kinase 3b; IKKb, inhibitor of nuclear factor κB kinase subunit β; KDM6B, lysine demethylase 6B; LMO4, LIM domain only 4; mTORC1/2, mammalian target of rapamycin complex 1/2; PDE, phosphodiesterase; NF-κB, nuclear factor κB; PKA, protein kinase A; PKCε, protein kinase Cε; PI3K, phosphoinositide-3-kinase; Prosapip1, Prosap2 (proline-rich synapse-associated protein 2)-interacting protein 1; Ret, rearranged during transfection; Su(H), suppressor of hairless; TNF, tumor necrosis factor; TrkB, tropomyosin receptor kinase B. Figure created with BioRender.com.

Epigenetic pathways are tightly interlinked, resulting in increased complexity of alcohol-induced epigenetic dysregulation. For example, chronic exposure to alcohol led to long-lasting reduction of histone H3 acetylated on lysine 27 (H3K27ac) and parallel induction of trimethylation

(H3K27me3) at the immediate early gene *Arc* in the CeA of rats [22]. These acetylation/methylation changes resulted in decreased expression of the non-coding *Arc* eRNA (enhancer RNA; short non-coding RNAs transcribed from enhancers) and affected *Arc* transcription [22]. These findings emphasize that alcohol does not affect specific epigenetic mechanisms in a vacuum, and it is essential to consider potential interactions between these regulatory pathways.

Alcohol-induced epigenetic alterations are often mediated by altered expression or activity of epigenetic enzymes, which thus represent a promising new avenue for targeted therapeutic interventions. For example, increased enrichment of DNA methylation in the mPFC was linked to enhanced DNA methyltransferase (DNMT) activity [23]. Inhibition of DNMT rescued the methylation and transcriptional changes, and prevented the escalation of alcohol intake [23]. Other examples include CBP and p300 [20], as well as lysine demethylase LSD1 [21]. Decreased binding of CBP and lysine demethylase KDM6B was also shown at specific target genes upon adolescent intermittent alcohol exposure, resulting in anxiety-like behaviors in adult rats [22].

Recently, a previously unanticipated mechanism was identified that links alcohol metabolism to alcohol-induced epigenetic impairments by way of direct incorporation of alcohol-derived acetate into brain histone acetylation [24]. This was driven by nuclear translocation of the metabolic enzyme acetyl-CoA synthetase 2 (ACSS2), inhibition of which prevented alcohol-induced changes of histone acetylation and gene expression, and blocked conditioned place preference to alcohol [24]. This and related epigenetic-metabolic pathways [25] represent a radically novel mechanism of alcohol-induced transcriptional changes.

Level 3: the effects of alcohol on transcriptional activity

Transcription factors often form large multimeric protein complexes that bind to target gene promoters or enhancers to regulate the expression of mRNA. Chronic alcohol exposure in rodents upregulates gene expression in neurons, astrocytes, and microglia [26–28], which raises the possibility that transcription factors serve as one of the master regulators of the neuroadaptations induced by alcohol. The mechanisms that drive alcohol-dependent transcriptional alterations are still being unraveled (Figure 1). For example, the transcriptional activity of NF-κB is controlled through the activity of inhibitor κβ kinase (IKKβ). Using pharmacologic and genetic approaches, IKKβ was shown to contribute to excessive alcohol intake in mice [29], and its action is localized to neurons at least in the NAc and CeA [29]. Another example is the transcriptional regulator, LIM domain only 4 (LMO4), which was shown to drive vast changes in gene expression in the basolateral amygdala (BLA) of mice in response to repeated exposure to alcohol and to the regulation of alcohol intake [30]. In addition to contributing to the mechanisms that drive excessive drinking (GO signaling), transcription factors are likely to contribute to the gating of alcohol intake (STOP signaling). For example, the activity-dependent neuroprotective protein (ADNP) is a transcription factor that protects against excessive alcohol intake and relapse in female rodents [31].

Level 4: alternative splicing

An important mechanism underlying phenotypic variability is alternative splicing, which allows the expression of different transcripts from a single gene. Intriguingly, environmental insults including repeated exposure to alcohol have been shown to impair this mechanism across many species. In postmortem analyses of the striatum and amygdala of individuals with AUD, thousands of transcripts in these brain regions were differentially spliced [32]. Relatedly, hundreds of splice variants were affected by *in utero* alcohol exposure in fetal cortical tissue [33]. Alcohol-induced impairments of alternative splicing were observed across several species, thus facilitating the study of these mechanisms in preclinical models of alcohol use. For example, changes in RNA splicing have been identified in the cortex of rodents and monkeys following chronic alcohol

exposure [34]. In mice, acute exposure to a single injection of alcohol induced differential expression of >10 000 exons. Strikingly, these changes showed a large overlap with those observed in mice treated with antidepressants, potentially outlining a novel mechanism for the rapid antidepressant effects of alcohol [35]. In the brain of fruit flies, lasting changes in the expression of distinct splice variants were linked to alcohol-related associative learning, and knock-down of spliceosome-associated proteins prevented the formation of alcohol memories [36]. Further, alcohol was shown to alter dopamine D2R splicing in the reward circuitry of flies via activation of Notch signaling, leading to its interaction with the transcription factor, suppressor of hairless Su(H) [37]. This pathway underlies memory formation for alcohol-associated cues.

Splicing of mRNA molecules can also occur at distant cellular compartments including the synapse, and thus have a direct effect on the activity of neuronal circuits. Intriguingly, alcohol markedly perturbs the synaptic spliceosome in the cortex of mice, thereby affecting the local translation of proteins involved in synaptic function [38]. These changes are particularly pronounced following repeated exposure to alcohol and were proposed to regulate sensitization [38].

Level 5: alcohol and protein translation

The kinase mTOR in complex 1 (mTORC1) plays a crucial role in synaptic plasticity, learning, and memory by orchestrating the translation of several dendritic proteins [39]. mTORC1 is activated by alcohol in discrete brain regions, resulting in the translation of synaptic proteins such as collapsin response-mediated protein 2 (CRMP2) [40] and ProSap-interacting protein 1 (Prosapip1) [41], as well as of Homer1 and PSD-95, GluA2, and Arc [40,42,43]. Through the translation of these transcripts and others, mTORC1 contributes to mechanisms underlying alcohol seeking and drinking as well as the reconsolidation of alcohol reward memories and habit [44–46]. Further, protein translation plays a role in additional alcohol-dependent phenotypes (Figure 1). For example, the activity of the mRNA-binding protein, fragile-X mental retardation protein (FMRP), which plays an important role in translation [47], is enhanced by alcohol in the hippocampus of mice, resulting in an alteration in the expression of synaptic proteins [48]. In addition, FMRP in the hippocampus plays a role in the acute antidepressant actions of alcohol [49]. Interestingly, rapid antidepressants require the coordinated actions of FMRP and mTORC1 [50], raising the possibility that such coordination may also be relevant in the context of alcohol action.

Level 6: the role of post-translational modifications

Post-translational modifications such as phosphorylation are core molecular signaling events. Not surprisingly, protein kinases play a central role in AUD (Figure 1) [3]. For instance, the protein tyrosine kinase (PTK) Fyn, through the phosphorylation of GluN2B in the dorsomedial striatum (DMS) of rodents, contributes to molecular and cellular neuroadaptations that drive goal-directed alcohol consumption [51,52]. Interestingly, Fyn also plays a role in heroin use [53], suggesting a more generalized role of the kinase in addiction. Furthermore, GsDREADD-dependent activation of the serine/threonine kinase protein kinase A (PKA) in the DMS of mice activates Fyn specifically in D1R medium spiny neurons (MSNs) to enhance alcohol consumption, suggesting that PKA is upstream of Fyn [54]. Indeed, a large body of evidence supports the role of PKA signaling in the actions of alcohol [3]. Interestingly, phosphodiesterases 4 and 10a (PDE4 and PDE10A), enzymes that are required for the termination of PKA activity [55], have also been implicated in AUD [56]. Furthermore, a GWAS identified PDE4B as a risk factor in elevated alcohol consumption [6,7]. The intracellular compartmentalization of PKA and PDE is tightly regulated [55], and it is highly likely that this is reflected by the seemingly opposing actions of alcohol on components of the PKA signaling cascade. Repeated alcohol exposure in mice activates another PTK, Src, which in turn stimulates NF-κB/TNF-α signaling in microglia, resulting in microglia engulfment of mPFC synapses, as well as synaptic pruning and increased anxiety-like

behaviors [57]. Another serine/threonine kinase that participates in neuroadaptations underlying AUD is GSK3β [58]. Specifically, GSK3β in the mPFC participates in mechanisms underlying motivation to consume alcohol and alcohol withdrawal-induced anxiety [58]. Furthermore, genetic analysis in humans indicated that GSK3β is an alcohol dependence risk factor, suggesting a central role of GSK3β in AUD [58]. Surprisingly however, GSK3β in the NAc is inhibited by alcohol in rats [40], emphasizing the region-specificity of alcohol action. Like Fyn, the kinase mTORC2 is specifically activated by alcohol in the DMS of mice [59]. Alcohol-dependent activation of mTORC2 in the DMS promotes F-actin assembly, the formation for mature spines, and alcohol intake [59].

In addition, receptor tyrosine kinases (RTKs) which are activated by growth factors and cytokines play a role in alcohol consumption [60]. For example, alcohol-dependent activation of the anaplastic lymphoma kinase (ALK) in the hippocampus and PFC activates STAT signaling leading to changes in gene expression, and systemic administration of ALK or STAT3 inhibitors attenuates alcohol intake in mice [61,62]. Surprisingly, several growth factors/RTKs such as BDNF and the glial-derived neurotrophic factor (GDNF) are endogenous factors that limit alcohol use [60,63]. Interestingly, activation of Midkine/ALK signaling also acts to limit alcohol intake in mice [64,65]. In contrast to BDNF, GDNF, and Midkine, fibroblast growth factor 2 (FGF2)/FGF receptor 1 (FGFR1) signaling promotes excessive drinking in rodents [66,67].

Level 7: impact of chronic drinking on neuromodulators and neural circuits

Recent advances in neurotechnologies have opened new avenues of investigation into how alcohol-induced alterations in neural circuit activity influence ongoing behaviors and decision making (Figure 2) [4,68]. We review these advances in the following, focusing on circuit- and receptor-level studies (brain-wide neuronal networks are reviewed in [69]). Recently, a genome-wide transcriptional assessment of human striatum found that G protein-coupled receptors, the primary targets of many neurotransmitters and neuromodulators, were the top canonical pathway affected in striatum of AUD patients [70]. Reverse translation of these findings into a rodent model demonstrated putative therapeutic potential for a positive allosteric modulator of the muscarinic M4 receptor which, when delivered systemically in rats, reduced a wide range of alcohol self-administration behaviors [70].

Several recent studies have built on classic literature to further detail the mechanisms by which presynaptic dopamine signaling and postsynaptic activity of MSNs orchestrate motivated behavior and its dysregulation by chronic alcohol drinking [71,72]. Although dopamine signaling in the striatum has long been known to be crucial in regulating alcohol-drinking behaviors [73], precise monitoring and manipulation of striatal dopamine release with dopamine biosensors and chemogenetics revealed complex, subregion-specific dopamine release patterns that underlie alcohol drinking and seeking in rodents [71,72]. In addition, alcohol also engages feeding circuits in the hypothalamus which in turn indirectly modulate dopamine neuron activity [74]. Studies in animal models indicate that, following long-term use of alcohol, striatal circuits and receptors undergo a range of adaptations [75,76]. Although the specifics vary between males and females and across brain regions, these adaptations are generally thought to be crucial determinants in dysregulated drinking behaviors.

Multiple classes of neuropeptide releasing neurons and neuropeptide receptors have been implicated as key mediators of drinking behaviors, including neurotensin [77], neuropeptide Y [78], oxytocin [79], opioid peptides [80,81], and corticotrophin-releasing factor (CRF). For instance, in rats and mice, chronic alcohol use alters the activity of the CeA through dysregulation of endocannabinoid, substance P, and CRF signaling [82–84]. The bed nucleus of the stria

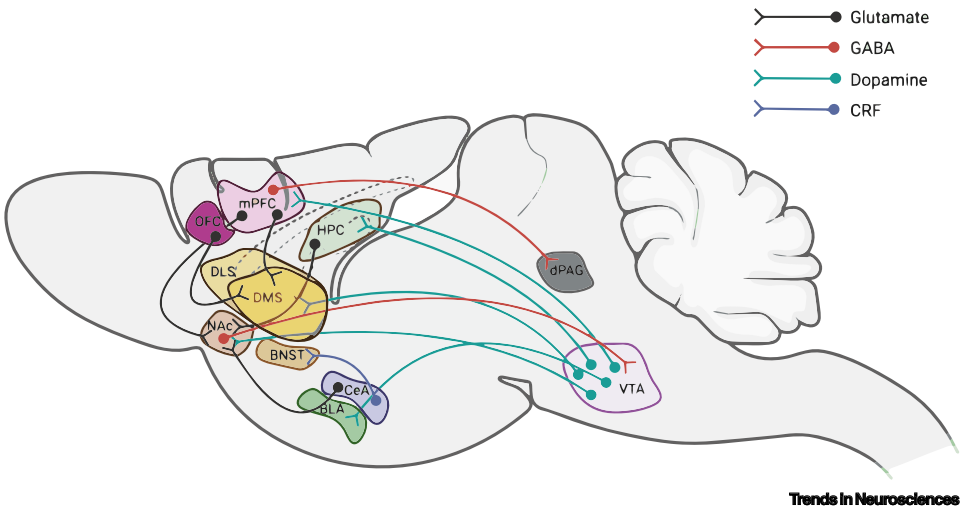

Figure 2. Neuronal circuits affected by alcohol. Acute and chronic use of alcohol affects the activity of multiple neuronal circuits, depicted here schematically in the context of a rodent brain. For example, alcohol activates the mesocorticolimbic brain reward circuit, which encompasses dopaminergic projections from the VTA in the midbrain to several forebrain structures including the striatum and cortex. These circuits underlie the rewarding effects of alcohol. The anxiolytic effects of ethanol have been linked to the amygdala. In addition, CRF neurons projecting from the central amygdala to the BNST were shown to contribute to the escalation of alcohol intake. Prefrontal cortical circuits have been implicated in impaired executive control that underlies excessive drinking, as well as weakened cognitive function in alcohol use disorder (AUD). For example, projections from the mPFC to the dorsal striatum have been linked to habitual alcohol drinking and continued use despite negative consequences. Further, neurons projecting from the mPFC to the dPAG play a central role in compulsive drinking. Strikingly, mice that display inhibitory activity in this circuit during the first alcohol exposure are more likely to develop compulsive drinking behavior. Abbreviations: BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; CRF, corticotropin-releasing factor; DLS, dorsolateral striatum; DMS, dorsomedial striatum; dPAG, dorsal periaqueductal grey; HPC, hippocampus; LA, lateral amygdala; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OFC, orbitofrontal cortex; VTA, ventral tegmental area. Figure created with BioRender.com.

terminalis (BNST) also exhibits plasticity in endocannabinoid- and CRF-expressing neurons as a result of chronic alcohol use, and these alterations modulate drinking, withdrawal-induced negative affect, and stress-induced alcohol seeking in mice [85,86]. Furthermore, the CeA and BNST regions are anatomically connected, and inhibition of CRF neurons projecting from the CeA to the BNST decreases escalation of alcohol intake and somatic withdrawal symptoms in rats [87].

The κ-opioid receptor (KOR) and its endogenous ligand peptide, dynorphin, have been an area of great interest. Reduced dynorphin activity or blockade of KORs in several brain regions, including the CeA [88,89], BNST [90,91], and the striatum, reduces alcohol consumption in mice and rats. KORs have also been shown to modulate the acute actions of alcohol [92] and negative affect during withdrawal [93], and the sensitivity of this receptor is augmented after chronic alcohol use [73]. Fast-acting and selective KOR antagonists have been developed and evaluated in preclinical models using rats, yielding promising results that suggest therapeutic potential for treating AUD [94].

A major theme of recent alcohol research has been to leverage animal models and circuit analysis approaches to link neural circuit activity with specific aspects of AUD [95]. For example, in mice, chronic alcohol exposure decreased the excitability of OFC outputs to the DMS [96], and alcohol-induced synaptic plasticity in the OFC has been linked to excessive alcohol use in both mouse and monkey models [97,98]. In addition, using a combination of activity-dependent genetic tools and chemogenetic manipulations, a small ensemble of mPFC neurons was shown to serve as a memory to cue induced relapse to alcohol use [99]. Interestingly, like the molecular

mechanisms that gate the development of AUD [3], STOP mechanisms also occur at the level of circuitries [100]. Specifically, a subset of infralimbic cortical neurons serve to protect against relapse to alcohol use [100].

Projections from mPFC to the striatum have been implicated in mediating specific aspects of drinking behaviors [101–103]. These projections have been targeted to exert bidirectional, long-lasting control of alcohol drinking [103]. Specifically, using optogenetic stimulation protocols to induce plasticity at cortical synapses onto dopamine D1R MSNs in the DMS, recent studies showed that induction of long-term potentiation (LTP) resulted in lasting increases in drinking, whereas induction of long-term depression (LTD) at these synapses produced decreased drinking [101,103]. Furthermore, dysregulation of striatal function can produce pathological drinking behaviors. For instance, manipulations of striatal D2Rs, adenosine 2A receptors, or the activity of fast-spiking interneurons, among others, alter excessive drinking behaviors [104–106]. Further, disrupted GABAergic transmission in this region is also linked to alcohol-induced cognitive impairments [107]. Together, altered excitability of striatal neurons and upstream cortical regulation of striatal activity influence a diverse range of drinking behaviors, which likely can be attributed to distinct striatal output circuits [108].

Concluding remarks and future perspectives

Advances in neuroscience continue to shed light onto regulatory mechanisms relevant to alcohol use. A striking example is the discovery that particular neurotransmitters, such as serotonin [109] and dopamine [110], can covalently bind to histones and act as epigenetic marks to regulate gene expression. Histone dopaminylation was further shown to influence addiction-like behaviors in the context of cocaine exposure in mice [110]. This novel mechanism could have far-reaching implications for other drugs of abuse, including alcohol, which are known to increase dopamine levels in the mesolimbic system [72]. Another example of a recent discovery facilitated by novel approaches is that ALDH2 in cerebellar astrocytes promotes alcohol metabolism, GABA production, and ethanol-induced intoxication in mice [11]. Importantly, the neurobiological basis of AUD appears in many cases to manifest in a sex-specific manner. Understanding the convergence and divergence between mechanisms in males and females will continue to be crucial in moving forward [111,112].

Signaling events that produce transcriptional and translational changes are, in essence, the molecular transducers of the long-lasting cellular adaptations that drive AUD, and targeting some of these signaling molecules could provide an avenue for the development of new therapeutics [12]. An area of focus for drug development efforts has been kinases: over the past 20 years or so this class of signaling molecules has been one of the leading targets for drug development by pharmaceutical and biotechnology companies [113]. Examples of kinase inhibitors that show promise in preclinical rodent AUD models are the ALK inhibitors NVP-TAE684 and alectinib [61], the Fyn kinase inhibitor AZD0530 [52], and the mTORC1 inhibitor, rapamycin [12]. Although rapamycin is already used in the clinic for indications such as preventing organ rejection after kidney transplantation [114], its potential use for the treatment of AUD is hampered by adverse effects as a result of inhibition of mTORC1 in the periphery [115]. To overcome this limitation, a dual drug strategy which enables the selective inhibition of mTORC1 in the brain while preserving its activity in the periphery was recently developed [116,117]. This strategy, which at least in mice eliminates the side effects that result from prolonged mTORC1 inhibition in the periphery, has shown promising results in preclinical alcohol drinking models [117]. Another promising target is protein kinase Cε (PKCε), a kinase long known to be linked to AUD [118,119]. New, selective small-molecule inhibitors of this kinase have been recently developed and have shown promising results in preclinical mouse models of AUD [120]. Other potential

Outstanding questions

Recent studies have outlined several levels of alcohol effects on the brain. The precise mechanistic links between these levels, however, remain in many cases unknown. How do the specific levels of alcohol-induced adaptations interact and influence each other to result in complex behavioral changes?

Which molecular pathways and circuits could serve as the most promising potential therapeutic targets for AUD? Given that AUD is a multifaceted disorder, combinatorial therapeutic approaches that target multiple levels of alcohol-induced maladaptations might be most effective in treating AUD and related conditions.

Which mechanisms determine individual vulnerability to excessive alcohol consumption? Only a small proportion of individuals consuming alcohol go on to develop AUD. A more complete understanding of the molecular pathways, circuits, and behavioral traits that contribute to individual and sex differences in susceptibility could guide targeted approaches in treatment and prevention.

Can alcohol-dependent changes in the molecular landscape be explained, at least in part, by spatial and/or temporal alterations of protein compartmentalization? For instance, lipid rafts consisting of membrane lipids and cholesterol serve as important platforms for signaling pathways. Alcohol is known to alter membrane lipid fluidity – does alcohol therefore affect signaling cascades by altering lipid raft composition?

AUD is a polygenic disorder that has relatively minor contributions from individual genes. How is it possible that manipulating single genes in animal models can result in animals that are resilient to AUD? Does this limit the translational validity of findings from available animal models?

AUD has a significant heritable component, but only a relatively small number of SNPs have been identified as being associated with AUD. Are there additional, currently unknown variants that confer susceptibility to, or resilience against, developing problem drinking and AUD? An integrative approach in combination with large sample size may reveal additional insights.

inhibitors are MY10, which targets receptor protein tyrosine phosphatase βζ (PTPRZ1) [121], lacosimide, which prevents CRMP2-dependent microtubule assembly [40], and PDE inhibitors such as ibudilast [56]. Finally, a potential treatment approach that emerged recently in the context of AUD is ketogenic diet, that is characterized by high fat and low carbohydrate intake. In a study in rats, ketogenic diet has been shown to reduce withdrawal-induced symptoms and craving, as well as alcohol self-administration [122].

Another area requiring further research relates to individual differences in resilience and susceptibility to AUD. Future studies will be necessary to better understand the mechanisms underlying these individual differences. Studies in animal models provide initial hints to possible contributors to these differences. Studies in outbred rats have shown that ~15% of the animals prefer alcohol to a sweetened solution and display AUD-like phenotypes such as alcohol consumption despite negative consequences [123], and much of this variance can be explained by differences in excitability of PKCδ-expressing interneurons in the CeA [124]. Furthermore, rats undergoing intermittent access to 20% alcohol in a two-bottle choice paradigm exhibit distinct profiles of intake ranging from low alcohol consumers to rats that exhibit slow or rapid escalation of excessive drinking [125]. In a recently developed behavioral procedure for quantifying the development of compulsive drinking in mice, the activity of a small population of neurons that connect the mPFC to the dorsal periaqueductal grey were shown to confer vulnerability to compulsive alcohol drinking, and activity in this circuit differentiated subjects that were prone to compulsive alcohol drinking before the expression of the behavior [126].

Together, the studies reviewed here illustrate the complexity of AUD, which results from the interaction of diverse levels of molecular neuroadaptations in different brain regions and neural circuit changes throughout the brain [127]. The specific molecular pathways and circuits that could serve as the most promising therapeutic targets remain to be delineated (see Outstanding questions). Finally, the development of cutting-edge tools for neurotransmitter sensing, circuitry mapping, and manipulation on a more precise spatial and temporal scale will enable further advances in our understanding of how neural activity and communication are altered by chronic alcohol use to produce excessive drinking behaviors.

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Declaration of interests

The authors declare no conflicts of interest.

Resources

[i]https://www.who.int/substance_abuse/publications/global_alcohol_report/en/

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