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Commentary

Coincident signaling in mesolimbic structures underlying alcohol reinforcement

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ABSTRACT

The medium spiny neurons (MSNs) of the nucleus accumbens function in a critical regard to examine and integrate information in the processing of rewarding behaviors. These neurons are aberrantly affected by drugs of abuse, including alcohol. However, ethanol is unlike any other common drug of abuse, due to its pleiotropic actions on intracellular and intercellular signaling processes. Intracellular biochemical pathways appear to critically contribute to long-term changes in the level of synaptic activation of these neurons, which have been implicated in ethanol dependence. Additionally, these neurons also display a fascinating pattern of up/down activity, which appears to be, at least in part, regulated by convergent activation of dopaminergic and glutamatergic (NMDA) inputs. Thus, dopaminergic and NMDA receptor-mediated synaptic transmission onto these neurons may constitute a critical site of ethanol action in mesolimbic structures. For instance, dopaminergic inputs alter the ability of ethanol to regulate NMDA receptor-mediated synaptic transmission onto accumbal MSNs. Prior activation of D1-signaling cascade through the cAMP-regulated phosphoprotein-32 kD (DARPP-32) and protein phosphatase-1 (PP-1) pathway significantly attenuates ethanol inhibition of NMDA receptor function. Therefore, the interaction of D1-signaling and NMDA receptor signaling may alter NMDA receptor-dependent long-term synaptic plasticity, contributing to the development of ethanol-induced neuroadaptation of the reward pathway.

The development of alcohol dependence is characterized by compulsive intake even in the face of serious social and health problems. Dependence may progress further to outright abuse characterized by significant tolerance and physiological symptoms upon withdrawal [1]. One normal response to ethanol, thought to be a critical component in the development of ethanol dependence, involves its ability to produce euphoria (positive reinforcement). Such mechanisms are largely mediated by the mesocorticolimbic system and a principal neuron in this system is the GABAergic medium spiny neuron of the nucleus accumbens. These neurons process and integrate information from three primary sites including glutamatergic inputs from prefrontal cortex and limbic structures (i.e., the hippocampal formation) and dopaminergic inputs from the ventral tegmental area. These coincident inputs appear to induce complex biochemical interactions with functional effects that very likely encompass critical neuroadaptive alterations involved in ethanol depen-

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dence. Thus, this review is focused on the coincident signaling of these neurotransmitter receptors in mesocorticolimbic structures and their roles in ethanol reinforcement and the development of ethanol dependence.

1. Medium spiny neurons: pharmacology, biochemistry and function

The major components in the brain motivation and reward circuit include the mesolimbic dopamine pathway (Fig. 1) from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and the limbic system including the prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HIP) [2]. A variety of receptors are expressed on the surface of individual MSNs, including ionotropic and metabotropic receptors [3–6]. The co-expression of both glutamatergic and dopaminergic receptors on MSNs further confirms that the interaction of these two neurotransmitter systems in the NAc occurs at the level of individual neuron during cortical information processing.

Evidence indicates that MSNs exhibit a relatively unique bistable membrane potential, which affects the excitability of MSNs. In vivo recordings demonstrate that MSNs in the ventral and dorsal striatum fire irregularly at a very low frequency (<1 Hz) and their membrane potential fluctuates between two steady states, a depolarized up state and a hyperpolarized down state. The up state (about −60 mV) is 100–1000 ms in duration and 10–25 mV in amplitude from the down state (−85mV) [7]. The up state in the NAc is driven by convergent synaptic inputs from the PFC, AMY and HIP, whereas the down state is controlled by inward rectifier potassium channels. The glutamatergic afferents from both the PFC and the HIP are often damaged during the process of slice preparation. The lack of temporally convergent synaptic inputs in slice preparation may explain why the bistable membrane fluctuation is rarely observed in vitro, when MSNs often stay at a very negative membrane potential in the range corresponding to the down state in vivo.

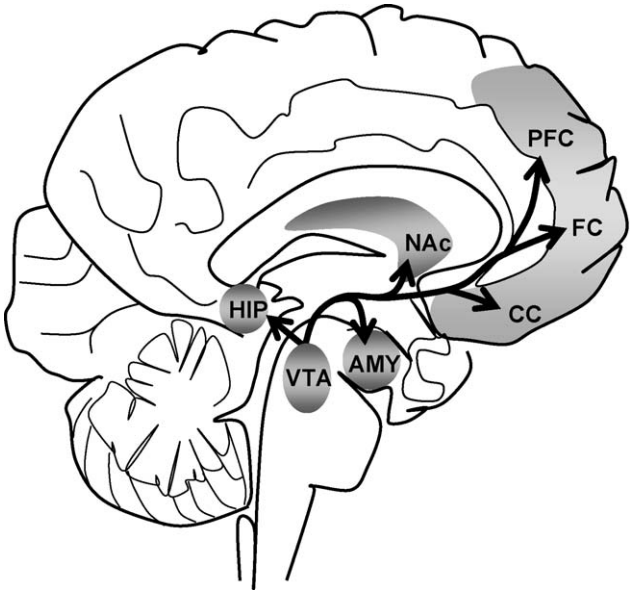

Fig. 1 – The mesocorticolimbic pathway activated in alcohol dependence and reward. The dopaminergic projection from the midbrain ventral tegmental area (VTA) innervates numerous limbic structures including the hippocampal formation (HIP) and amygdala (AMY) as well as cortical regions—the frontal cortex (FC), prefrontal cortex (PFC) and cingulate cortex (CC).

O'Donnell and Grace demonstrated that MSNs display greater excitability at the up state and that the transition to the up state is gated by hippocampal inputs [7]. Spontaneous action potentials are only observed during the up state in response to cortical and amygdaloid inputs. The excitatory hippocampal inputs have a gating effect on bursting of MSNs in response to inputs from PFC and AMY. Finally, lesions of the fornix abolished the bistable membrane potentials and microinjection of lidocaine into the fornix reversibly eliminated the up state [7].

These findings are further supported by anatomical studies. Synapses on the distal dendrites are expected to exert less influence on the neuronal excitability than those on the proximal dendrites and the soma. Glutamatergic afferents from the PFC form synapses on the distal dendrites of MSNs in the NAc, whereas hippocampal afferents form synapses on the proximal dendrites and the cell body thereby exerting a greater control (gating effect) on the transition from the down state to the up state [8]. Furthermore, populations of MSNs in the NAc can shift to the up state synchronously possibly driven by synaptic inputs from the ventral hippocampus. The effects of drugs of abuse on the transition from the up state to the down state and the hippocampus-dependent gating of this transition have not been addressed and this seems to be an important topic for study.

The expression of dopamine receptor subtypes, the correlation between receptor subtypes and projection patterns of MSNs and the intracellular signaling cascades upon activation of postsynaptic dopamine receptors are particularly relevant to accumbal neurons. These neurons express a variety of dopamine receptors normally divided into D1-like subfamily (D1DRs including D1 and D5, also termed as D1a and D1b) and D2-like subfamily (D2DRs including D2, D3 and D4) and are the products of five structurally homologous genes [9]. D1DRs display similar pharmacological profiles such as high affinity to the selective antagonist SCH 23390, but there are no selective ligands to discriminate D1 receptors from D5 receptors [9]. The term "D1DRs" refers to the D1-like subfamily including both D1 and D5 receptors and the term "D2DRs" refers to the D2-like subfamily including D2, D3 and D4 receptors. The term "D1" alone is an abbreviation of "D1-like" unless it is described as "D1 receptor". D1DRs are co-expressed with D2DRs on a small portion of accumbal MSNs and located on both presynaptic and postsynaptic sites. In contrast to the dorsal striatum where at least 50% of MSNs co-express D1DRs and D2DRs [10], D1DRs and D2DRs are co-expressed to a much lesser extent in the NAc (about 22–38%) [11]. The co-expression of both D1DRs and D2DRs suggests that interactions of these two subfamilies of dopamine receptors are present at the level of individual MSNs. Ultrastructural studies demonstrate that D1DRs are distributed on the dendrites and soma of about 50% of accumbal MSNs and also on presynaptic terminals suggesting the presence of both pre-synaptic and post-synaptic sites of

dopaminergic modulation. However, dopaminergic terminals do not form synaptic connections with glutamatergic afferents from the PFC in the NAc suggesting that dopaminergic modulation of Glu transmission is most likely non-synaptic [4].

D1 and D2 receptors are highly expressed in the NAc and are often co-expressed with different peptides in different populations of MSNs in the NAc as well as the dorsal striatum. Several studies using double in situ mRNA hybridization suggest that D1 receptors and substance P (SP) are co-expressed in about 50% of MSNs, which are distinguished from MSNs co-expressing D2 receptors and enkephalin (ENK) in the ventral and dorsal striatum in rodents as well as in primates [10,12,13]. Only a small proportion (2–5%) of MSNs co-express D1 and D2 receptors [12,13]. MSNs co-expressing D1 receptors and SP are mostly restricted in the shell, and those co-expressing D2 receptors and ENK tend to be located in the core of the NAc [14]. In contrast to the striatum, D3 receptors are highly expressed in the NAc and co-expressed with D1 receptors in a significant proportion of SP-positive neurons in both the shell and core regions of the NAc [15]. D4 and D5 receptors are expressed at very low levels and therefore have been less studied.

MSNs selectively expressing D1 or D2 receptors tend to project to different targets. For example, in the striatum, the vast majority of MSNs containing D1/SP tend to project to the substantia nigra, whereas neurons containing D2/ENK primarily form the striatopallidal connection [12]. In contrast to the selective mesencephalic projection pattern of D1/SP neurons in the dorsal striatum, neurons co-expressing D1/SP in the NAc (arising mostly from the shell) project to the VTA as well as the ventral pallidum. D2/ENK containing neurons in the NAc (mostly in the core) selectively project to the ventral pallidum [16].

The immediate early gene, Fos is expressed in both dorsal and ventral striatum, and is a putative marker for neuronal activity that is enhanced by activating D1DRs or blocking D2DRs. Robertson and Jian demonstrated that in the NAc, a D1DR agonist induced Fos immunoreactivity in MSNs projecting primarily to the VTA and about half of these neurons also projected to the ventral pallidum, whereas D2DR antagonist-induced Fos immunoreactivity was primarily located in neurons projecting to the ventral pallidum [17]. Taken together, these data provide anatomical and functional support for the topographic projection patterns of MSNs likewise with differential dopaminergic receptor expression patterns in the NAc and suggest a role of D1DRs in the reciprocal innervation between the NAc and VTA.

Dopamine and cAMP-regulated phosphoprotein-32 kDa (DARPP-32) is an integrator of neurotransmission in dopaminoceptive neurons in multiple brain regions. DARPP-32 was identified initially as a target of D1DR-activated adenylyl cyclase in the striatum by Greengard and colleagues [18]. Immunohistochemical studies demonstrate that DARPP-32 is highly expressed in the NAc. DARPP-32 is encoded by a single gene [19], and the distribution of DARPP-32 mRNA is consistent with immunohistochemical studies. Specifically in the ventral and dorsal striatum, DARPP-32 is restricted to MSNs [20]. At the ultrastructural level, immunoactivity of DARPP-32 has been observed in most subcellular compartments including cytoplasm, dendrites, some nuclei, axons and axon terminals [21]. It is well accepted that DARPP-32 functions as a crucial intracellular mediator of electrophysiological, biochemical, transcriptional and behavioral effects of dopamine [22].

The function of DARPP-32 is regulated by phosphorylation at four sites including Thr34, Thr75, S102 and S137. DARPP-32 is phosphorylated at Thr34 through actions of PKA and cGMP-dependent protein kinase (PKG). Phosphorylated DARPP-32 at Thr34 binds to the active site of protein phosphatase-1 (PP-1) and prevents its access to phosphorylated targets such as NMDA receptors [22]. Cyclin-dependent kinase 5 (Cdk5)-mediated phosphorylation of DARPP-32 at Thr75 converts DARPP-32 into an inhibitor of PKA, reducing the efficacy of dopaminergic signaling [23]. S102 and S137 are phosphorylated by CK2 and CK1, respectively. Phospho-S102-DARPP-32 increases the rate of phosphorylation of Thr34 by PKA and phospho-S137-DARPP-32 decreases the rate of PP2B-mediated dephosphorylation of phospho-Thr34-DARPP-32, thereby phosphorylation of both sites potentiates D1 signaling through DARPP-32/PP-1 cascade [24]. Thus, depending on which particular amino acid residue is phosphorylated, DARPP-32 functions as either a kinase (PKA) or phosphatase (PP-1) inhibitor. PP-2B and PP-2A are the most effective phosphatases in dephosphorylating DARPP-32 at Thr34 and Thr75, respectively.

Activation of D1DRs and PKA stimulates the phosphorylation of DARPP-32 at Thr34. Activation of D2DRs has antagonistic effects by inhibiting adenylyl cyclase (direct mechanism) and mobilizing intracellular calcium stores via PLC activation thereby increasing dephosphorylation of phospho-Thr34-DARPP-32 mediated by Ca^{2+}-dependent PP-2B (indirect mechanism) [25]. Furthermore, by activating PP-2A, activation of D1DRs decreases the state of DARPP-32 phosphorylation at Thr75 thereby removing the inhibitory effects of phospho-Thr75-DARPP-32 on PKA and amplifying D1-dopaminergic signaling through a positive feedback loop.

Glutamate produces complex regulation of the level of DARPP-32 phosphorylation [26]. For instance, elevation of intracellular calcium following activation of NMDA receptors, with subsequent activation of calcium-dependent phosphatases such as PP-2B, can result in dephosphorylation of phospho-Thr34-DARPP-32. This increase of intracellular Ca^{2+} may also be mediated by influx via voltage-gated calcium channels (VGCCs) induced by depolarization following by strong activation of AMPA receptors. Conversely, activation of NMDA and AMPA receptors also decreases DARPP-32 phosphorylation at Thr75 via Ca^{2+}-dependent PP-2A activation, although the mechanism is less well characterized. Since phospho-Thr75-DARPP-32 is a PKA inhibitor, activation of ionotropic glutamate receptors may reduce the inhibitory effects of phospho-Thr75-DARPP-32 on PKA. Thus, glutamate signaling may potentiate or depress D1DR signaling through the PKA/DARPP-32/PP-1 pathway. Taken together, the state of DARPP-32 phosphorylation may be tonically regulated by excitatory transmission onto MSNs (Fig. 2).

2. Accumbal excitatory synaptic transmission and plasticity

Alterations in excitatory synaptic transmission impinging upon medium spiny neurons are increasingly thought to be a

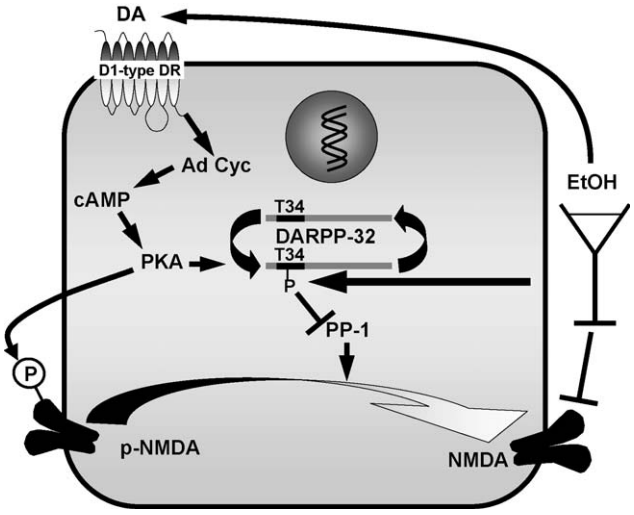

Fig. 2 – Dopaminergic coincident signaling in medium spiny neurons. Activation of D1-like dopaminergic receptors initiates a complex cascade whereby adenylyl cyclase (Ad Cyc), cAMP and protein kinase A (PKA) culminate in phosphorylation of DARPP-32 at the Thr-34 residue. This phospho-isoform of DARPP-32 potently inhibits protein phosphatase 1 (PP1) and thereby the phosphorylation state and ethanol sensitivity of NMDA receptors. Thus, D1-like dopamine receptors on MSNs may regulate the ability of NMDA receptors to modulate synaptic plasticity during ethanol experience.

critical aspect of neuroadaptation induced by drug exposure and the NMDA sub–type of glutamate receptors are an important component of the machinery underlying accumbal plasticity. Glutamatergic synapses on MSNs display both LTP (long-term potentiation) and LTD (long-term depression): two opposing forms of long-term synaptic plasticity. Most of the work has been conducted in the corticostriatal pathway, where high frequency stimulation induces either LTP or LTD under different conditions [27]. Corticostriatal LTP has been shown to be NMDA receptor-dependent and requires the activation of D1DR signaling through the DARPP-32/PP-1 pathway [27,28]. Consistent with these findings, LTP is inhibited by an NMDA receptor antagonist, facilitated by removal of extracellular Mg^{2+} and is absent in DARPP-32 knockout mice [27]. Accordingly, Wickens and colleagues showed that either dopamine depletion or application of a D1DR antagonist (SCH 23390) blocked corticostriatal NMDA receptor-dependent LTP in rats [28]. In contrast, D2DRs seem to have no effects or opposing effects to the actions of D1DRs during the induction phase of striatal LTP [28,29]. Furthermore, a recent study discriminated the roles of D1 and D5 receptors in striatal LTP: ablation of D1 receptors located on MSNs disrupted corticostriatal LTP, whereas blockade of D5 receptors located on nitric oxide-producing interneurons prevented LTD [30].

A major form of striatal LTD has been shown to be non-NMDA receptor-dependent and displays a presynaptic mechanism, which requires activation of postsynaptic mGluRs, activation of PKG, formation of retrograde endocannabinoids and activation of presynaptic CB1 receptors [27]. D1DRs and D2DRs interact synergistically to allow LTD formation [31]. Taken together, these findings have significance for understanding the roles of dopamine and the mechanisms underlying learning and memory and addiction to drugs of abuse in the corticostriatal pathway. In the NAc, little information concerning drugs of abuse and LTD has been forthcoming though alterations LTD of excitatory synaptic transmission onto accumbal MSNs in cocaine sensitized mice have been reported [32].

Synaptic potentiation in the ventral striatum (NAc) has been less well characterized and displays similarities to that seen in the dorsal striatum. High frequency stimulation induces NMDA receptor-dependent LTP [33]. Consistent with studies from the dorsal striatum, NMDAR-LTP in the NAc is highly facilitated by reducing extracellular Mg^{2+} [34]. LTP induction has been shown to be developmentally regulated in the dorsal striatum [35]. In contrast to the roles of D1DRs during NMDAR-LTP induction in the dorsal striatum, a few reports fail to show a positive interaction between D1DRs and NMDAR-LTP in the NAc [33]. Since dopamine-dependent potentiation of neuronal firing has been shown in vivo [36], the discrepancy may best be explained by the in vitro method used in these two studies.

The NMDAR- and D1DR-dependent LTP likely involves multiple mechanisms. First, activation of D1DRs selectively increased the NMDA receptor-mediated component of synaptic transmission, which was blocked by the selective D1DR antagonist SCH 23390 [37,38]. Second, activation of D1DRs and PKA leads to AMPAR trafficking onto the surface of cultured accumbal MSNs, a possible cellular mechanism by which dopamine may promote LTP [39], although the effects of D1DRs on AMPA receptor trafficking have not been characterized in preparations other than neuronal culture. Third, a trophic role of dopamine on the morphology of MSNs in the NAc has been demonstrated [40]. Collectively, these studies indicate that the dopamine–glutamate interactions at the postsynaptic level may promote NMDAR-LTP.

Drugs of abuse have been thought to usurp synaptic plasticity and induce enduring cellular alterations in the reward and motivation pathways [41]. A variety of drugs of abuse have been reported to induce LTP in the mesolimbic dopamine pathway. For example, a single in vivo administration of commonly abused drugs with different molecular mechanisms of action (cocaine, amphetamine, morphine, nicotine and ethanol) induced LTP at glutamatergic synapses onto VTA dopamine neurons which was blocked by local injection of an NMDA receptor antagonist [42]. Also, excitatory drive involving NMDA receptor activation from limbic structures targeting the VTA dopamine neurons re-initiated cocaine responding [43]. These data suggest that drugs of abuse could induce NMDAR-LTP in the mesolimbic pathway, which may underlie drug seeking behavior.

The viewpoint that drug exposure induces synaptic potentiation in the mesolimbic pathway is further supported by morphological changes of dendritic structures in the NAc of rats showing behavioral sensitization. Persistent structural changes in the dendrites of accumbal MSNs were observed in amphetamine-sensitized rats and included an increase in the number of dendritic branches and density of dendritic spines

[44]. Although the link between such dendritic changes and behavioral sensitization remains unclear, some evidence suggests that the structural plasticity in the NAc is associated with the induction of psychostimulant-induced behavioral sensitization (i.e., the degree of behavioral sensitization is positively correlated with the spine density) [45]. These kinds of structural changes are similar to NMDAR-dependent synaptic potentiation in the hippocampus and cortex during learning and memory in rodents and postulated to be induced by activating nerve terminals following NMDAR activation [46]. Taken together, these studies suggest that an enhancement of glutamatergic synaptic transmission in the mesolimbic structures may contribute to the development of drug addiction.

Numerous studies clearly indicate that dopamine enhances NMDA receptor function through activation of D1DRs. Activation of D1DRs enhanced NMDA-evoked postsynaptic inward currents as well as isolated native NMDA receptor-mediated synaptic currents in the NAc [47]. Similar dopaminergic effects on NMDA receptor function were also observed in the dorsal striatum and PFC. Iontophoretic application of dopamine into the neostriatum potentiated NMDA-induced responses [48]. D1DR agonists dose-dependently enhance NMDA-induced responses. The effects of D1DR agonists were mimicked by PP-1 inhibitors and were significantly reduced in DARPP-32 KO mice [37,38]. Furthermore, Seamans and colleagues demonstrated that in the PFC slices, D1DR agonists enhanced depolarization induced by tetanic stimulation by summating NMDA receptor-mediated components of EPSPs [37]. Taken together, these data indicate that activation of D1DRs potentiates NMDA receptor function.

Activation of D1DRs may potentiate NMDA receptor function by regulating NR1 phosphorylation through the DARPP-32/PP-1 signaling pathway. NMDA receptor function was enhanced by activation of PKA [49] or inhibition of PP-1 [50]. PKA-dependent phosphorylation of NR1 subunits at Ser897 enhanced NMDA receptor-mediated currents in spinal cord motor neurons [49]. Additionally, PP-1 decreases the opening probability of single NMDA receptor channels and inhibition of PP-1 activity by phosphatase inhibitors prolongs the duration of single NMDA channel opening and enhanced NMDA-induced currents [50]. Consistent with the previous studies, Blank and colleagues demonstrated the importance of DARPP-32, the endogenous protein phosphatase inhibitor, in regulating NMDA receptor function. In oocytes selectively expressing rat hippocampal poly(A)$^+$ mRNAs, stimulation of PKA had no effect on NMDA responses, but addition of RNAs for DARPP-32 potentiated NMDA responses induced by stimulation of PKA. Correspondingly, in oocytes selectively expressing rat striatal poly(A)$^+$ mRNAs, activation of PKA potentiated NMDA responses and this effect was blocked by PP-1 inhibitor (calyculin A) or antisense oligodeoxynucleotides against DARPP-32 [51]. Finally, Snyder and colleagues demonstrated that activation of D1DRs increased the phosphorylation of NR1 at Ser897 in NAc slices and that this D1DR-dependent phosphorylation of NR1 was absent in DARPP-32 KO mice [52]. Taken together, activation of D1DRs may increase the function of NMDA receptors by enhancing the NR1 phosphorylation through DARPP-32/PP-1 pathway.

3. NMDA receptors: a critical ethanol target

NMDA receptors mediate the excitatory synaptic transmission in the brain and are major targets of ethanol [53]. NMDA receptors with different receptor subunit composition display different ethanol sensitivity and a large literature indicates that NR2B-containing receptors display the greatest ethanol sensitivity. Recombinant studies in Xenopus oocytes indicate that 25 mM ethanol produced nearly maximal inhibition of NMDA-induced currents mediated by NR1/2B recombinant receptors and 50 or 100 mM ethanol produced effects, which were not significantly different from that of 25 mM ethanol [54]. NR1/2A recombinant receptors displayed dose-dependent inhibition by ethanol ranging from 25 to 100 mM [54,55]. NR1/2C-containing receptors were significantly inhibited only by 100 mM ethanol, whereas NR1 homomeric receptors did not display significant sensitivity to ethanol at 100 mM [54]. Finally, the addition of a NR2A subunit into any other combination (NR1/2B or NR1/2C) does not significantly alter receptor sensitivity to ethanol [55].

However, in native systems, it is important to note that the sensitivity of NMDA receptors to acute ethanol varies with brain regions. For example, ethanol potently inhibits the function of NMDA receptors in the hippocampus, cerebellum and nucleus accumbens [56–58]. The regional difference of ethanol sensitivity could be explained by developmentally regulated subunit composition of NMDA receptors in different brain regions [59].

A fairly strong literature indicates that the state of phosphorylation of NMDA receptors regulates NMDA receptor sensitivity to ethanol. NMDA receptors are phosphorylated by a variety of kinases [60]. Fyn tyrosine kinase has been demonstrated to be particularly important for regulating NMDA receptor sensitivity to ethanol. Moon and colleagues reported that NR2B subunits were the major tyrosine-phosphorylated targets in the postsynaptic density (PSD) [61]. Several investigators demonstrated that ethanol administration enhanced Fyn-mediated phosphorylation of NR2B subunits in hippocampal neurons, which was correlated with an acute tolerance to ethanol inhibition of synaptic NMDA responses [62]. PKA and PKC phosphorylates NR1 at Ser897 and Ser890, respectively [60] and potentiate NMDA receptor function [63,64]. Li and Kendig reported that tyrosine kinase and PKC were involved in NMDAR-mediated hyperexcitability during ethanol withdrawal in spinal cord motor neurons [65]. Little is known about the role of PKA in regulating NMDA receptor sensitivity to ethanol.

The inhibitory effects of ethanol on NMDA receptor function are also regulated by the extracellular concentration of Mg^{2+}. At resting membrane potentials, the NMDA receptor channel is blocked by Mg^{2+} ion. Several labs have reported that ethanol produces a reduced inhibitory effect on NMDA receptor function at reduced Mg^{2+} concentrations, although the mechanisms underlying the interaction between ethanol and Mg^{2+} are unclear. For example, ethanol produces more inhibition of NMDA-induced depolarization, NMDA receptor-mediated population excitatory postsynaptic potentials (pEPSPs) and NMDA EPSCs in the presence of 1 mM Mg^{2+} than 0 Mg^{2+}, evidenced by a shift in the IC50 of ethanol from about 50 mM with Mg^{2+} to above 100 mM without Mg^{2+} [66,67].

Interestingly, the interaction of ethanol and Mg^{2+} on NMDA receptor function coincides with the hypomagnesemia that occurs in chronic alcoholism [68].

Although the exact sites of ethanol action on NMDA receptor remain unclear, most studies indicate that ethanol exerts its actions by directly inhibiting the NMDA receptor channel. Single-channel analysis by using outside-out patches demonstrated that high concentration of ethanol (IC50 174 ± 23 mM with very low concentration of Mg^{2+} $\sim$0.4 μM) decreased the channel open probability (by 31%) and mean open time (by 28%) of NMDA-activated currents without affecting open channel conductance [69]. Furthermore, the degrees of ethanol-induced inhibition of NMDA currents are not significantly different between whole-cell recordings and single-channel recordings using outside-out patches [69] thereby suggesting the sites of ethanol action on NMDA receptors are likely directly on receptor channel protein. In addition, the interaction between ethanol and Mg^{2+} on NMDA receptor function suggests that the action sites of ethanol may be in the vicinity of Mg^{2+}-binding site on NMDA receptor channel [66]. Finally, other investigators demonstrated that ethanol did not inhibit NMDA receptor function in inside-out membrane patches thereby indicating that the site of ethanol action is only accessible from the extracellular environment [70].

Ethanol may inhibit NMDA receptor function by acting on specific amino acid residues on transmembrane domains of this receptor. Woodward and colleagues demonstrated that amino acids on M3 domain of NR1 subunits might be involved in regulating agonist binding therefore affecting ethanol action on NMDA receptor function. The phenylalanine residues on M3 of NR1 subunits seem particularly important and may be related to channel gating. Mutagenesis of phenylalanine to alanine (F639A) on NR1 M3 domain reduced ethanol inhibition of NMDA receptor function in oocytes and HEK 293 cells [71]. However, this does not explain the higher ethanol sensitivity of NR2B-containing NMDA receptors [54,55].

Ethanol significantly and reversibly inhibits several forms of NMDA receptor-dependent LTP in multiple brain regions. The acute effects of ethanol on NMDAR-LTP in the mesolimbic dopamine pathway have not been characterized. However, there is one report related to the chronic effects of ethanol on NMDAR-LTP in the striatum. Yamamoto and colleagues reported that high frequency stimulation induced NMDAR-LTP in the dorsal striatum in ethanol withdrawn rats while LTD was induced in ethanol naïve rats suggesting that glutamatergic synaptic transmission might be enhanced during chronic ethanol treatment in mesolimbic structures [72]. Therefore, acute and chronic ethanol treatment may produce opposing effects on NMDAR-LTP in the mesolimbic pathway.

Extensive evidence indicates that NMDA receptors play an important role in ethanol reinforcement. Koob and colleagues directly demonstrated that microinjection of NMDA receptor antagonist APV into the NAc suppressed ethanol intake [73]. Studies using systemic administration of NMDA receptor antagonists also indicate the importance of NMDA receptors in ethanol reinforcement. Shelton and Balster showed that selective NMDA receptor antagonists substantially suppressed ethanol responding in ethanol discrimination test [74]. Biala and Kotlinska showed that NMDA receptor antagonists blocked conditioned place preference related to ethanol [75]. Camarini and colleagues reported that the NMDA receptor channel blocker MK-801 blocked ethanol-induced behavioral sensitization in mice [76]. These data suggest that NMDA receptors, particularly those in the NAc, are crucial for ethanol intake.

In contrast, Carlezon and Wise showed that rats will self-administer NMDA receptor antagonists into the NAc in a dopamine-independent manner suggesting that multiple mechanisms may exist in brain reward [77]. In addition, a drug discrimination study indicated that NMDA receptor antagonists could substitute for ethanol suggesting ethanol-induced inhibition of NMDA receptors in the NAc may be reinforcing [78]. However, such findings are very limited and often contradictory. Hunt and colleagues reported that NMDA receptor antagonists might exert inhibitory effects on glutamate release into the NAc and that D2/D4 receptors may be involved [79]. In contrast, Chartoff and colleagues reported that dopamine was not required for locomotor and molecular effects of NMDA receptor antagonists and that glutamatergic transmission into the striatum was enhanced following administration of NMDA receptor antagonists [80]. Most importantly, studies have demonstrated that accumbal NMDA receptors are crucial for ethanol reinforcement as described above [73]. Furthermore, NMDA receptor channel blocker MK-801 has been shown to block the development of behavioral sensitization induced by repeated exposure to other drugs of abuse such as cocaine [81]. Therefore, the preponderance of the data indicates that ethanol intake is dependent, at least in part, on activation of NMDA receptors and dopamine receptors in the NAc.

Studies indicate that D1DRs in the NAc are directly involved in ethanol consumption [73,82]. Ethanol self-administration was disrupted in animals with bilateral lesions of the accumbal dopaminergic nerve terminals [83,84] and by systemic or intra-nucleus accumbens administration of dopamine receptor antagonists [73]. Furthermore, the D1DR signaling through the DARPP-32 pathway has been shown to be crucial for ethanol consumption. El-Ghundi and colleagues demonstrated that either disruption of the D1DR gene expression or blockade of D1DRs in the NAc markedly reduced ethanol-seeking behaviors in mice [82]. Consistent with studies of D1DRs, Risinger and colleagues demonstrated that mice with genetic deletion of DARPP-32 also failed to display ethanol consumption patterns especially at higher ethanol concentration as observed in wild-type mice [85]. These studies strongly indicate that the activation of D1DRs promotes ethanol intake.

4. Commentary: NMDA receptors and the paradox of ethanol reinforcement

One major recent hypothesis concerning the neurobiology of addiction posits that drugs of abuse may usurp the cellular mechanisms similar to those observed in Hebbian conditioning (e.g. LTP) and thereby induce long-lasting neuroadaptations underlying dependence [41]. Likewise, aberrant ethanol intake may be considered a learned behavior. The inhibitory effects of ethanol on NMDA receptor-mediated synaptic transmission would be expected to disrupt such a learning process of aberrant, excessive ethanol intake. Apparently, this

is not the case. We hypothesize that elevated dopamine concentrations in the NAc during ethanol exposure attenuates the inhibitory effects of ethanol on NMDA receptor-mediated synaptic transmission by activating the D1DR signaling cascades and thereby may contribute to ethanol reinforcement. Indeed, attenuation of NMDAR sensitivity to ethanol in accumbal MSNs is apparent and is appears to be is primarily through activation of the D1DR signaling cascades of DARPP-32 pathway [86]. A presynaptic component of D1-regulation of NMDA receptor sensitivity to ethanol has also been observed, although the mechanism is not completely understood [87]. Thus, we suggest that D1-regulation of ethanol inhibition of NMDA receptor-dependent synaptic plasticity in the NAc is of significant interest for understanding mechanisms of ethanol dependence development.

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