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Distinct Synaptic Strengthening of the Striatal Direct and Indirect Pathways Drives Alcohol Consumption

Yifeng Cheng[#], Cathy C.Y. Huang[#], Tengfei Ma[#], Xiaoyan Wei, Xuehua Wang, Jiayi Lu, and Jun Wang^{*}

Department of Neuroscience and Experimental Therapeutics, College of Medicine, Texas A&M Health Science Center, Bryan, Texas 77807

Abstract

BACKGROUND—Repeated exposure to addictive drugs and alcohol triggers glutamatergic and GABAergic plasticity in many neuronal populations. The dorsomedial striatum (DMS), a brain region critically involved in addiction, contains medium spiny neurons (MSNs) expressing dopamine D1 or D2 receptors, which form direct and indirect pathways, respectively. It is unclear how alcohol-evoked plasticity in the DMS contributes to alcohol consumption in a cell type-specific manner.

METHODS—Mice were trained to consume alcohol using an intermittent-access two-bottle-choice drinking procedure. Slice electrophysiology was used to measure glutamatergic and GABAergic strength in DMS D1- and D2-MSNs of alcohol-drinking mice and their controls. *In vivo* chemogenetic and pharmacological approaches were employed to manipulate MSN activity and their consequences on alcohol consumption were measured.

RESULTS—Repeated cycles of alcohol consumption and withdrawal in mice strengthened glutamatergic transmission in D1-MSNs and GABAergic transmission in D2-MSNs. *In vivo* chemogenetic excitation of D1-MSNs, mimicking glutamatergic strengthening, promoted alcohol consumption; the same effect was induced by D2-MSN inhibition, mimicking GABAergic strengthening. Importantly, suppression of GABAergic transmission *via* D2 receptor-glycogen synthase kinase-3 β (GSK3 β) signaling dramatically reduced excessive alcohol consumption, as did selective inhibition of D1-MSNs or excitation of D2-MSNs.

CONCLUSIONS—Our results suggest that repeated cycles of excessive alcohol intake and withdrawal potentiates glutamatergic strength exclusively in D1-MSNs and GABAergic strength specifically in D2-MSNs of the DMS, which concurrently contribute to alcohol consumption. These results provide insight into the synaptic and cell type-specific mechanisms underlying

^{*}To whom correspondence should be addressed: Jun Wang, M.D., Ph.D., 8447 State Highway 47, Suite 2106, Bryan, TX 77807, Tel: 979-436-0389, Fax: 979-436-0086, jwang@medicine.tamhsc.edu.

[#]These authors contributed equally to this work.

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alcohol addiction and identify targets for the development of new therapeutic approaches to alcohol abuse.

Keywords

Alcoholism; dorsomedial striatum; GABAergic plasticity; dopamine D2 receptor; GSK3 β ; DREADDs

INTRODUCTION

Addiction is considered to arise from maladaptive learning and memory processes, involving various forms of aberrant synaptic plasticity in different neuronal populations within unique neural circuits (1–3). The striatum, a major area of the basal ganglia, is essential for drug and alcohol addiction (1–3). For instance, human imaging studies have indicated that the striatum is linked to cocaine and alcohol addiction (4, 5). Moreover, rodent studies revealed that striatal glutamatergic inhibition attenuated cocaine sensitization and alcohol intake (6, 7). Similarly, striatal knockdown of GABA receptors or inhibition of GABAergic transmission also reduces alcohol consumption (8, 9). These studies indicate that both excitatory glutamatergic and inhibitory GABAergic activities in the striatum positively control alcohol consumption, although the underlying mechanisms are poorly characterized.

Increasing evidence suggests that the dorsal part of the striatum is essential for drug and alcohol addiction (5, 10, 11). The dorsal striatum can be subdivided into the dorsolateral striatum, which is involved in habit formation (10, 12), and the dorsomedial striatum (DMS), which mediates goal-directed behaviors (10, 12). The DMS has been strongly implicated in drug and alcohol abuse (6, 13–16). The principal cells of the striatum are medium spiny neurons (MSNs). MSNs expressing dopamine D1 receptors (D1-MSNs) project directly to the substantia nigra pars reticulata (SNr); this constitutes the direct pathway, which mediates “Go” actions in rewarding behaviors (17–19). In contrast, D2-MSNs express dopamine D2 receptors (D2Rs) and connect indirectly to the SNr; this indirect pathway regulates “NoGo” behaviors (17, 18). In MSNs, there are two major neurotransmissions: glutamatergic and GABAergic (20). They are known to be regulated by alcohol in the DMS and other brain regions (6, 21, 22). However, it is unclear whether these two types of neurotransmissions are modulated by alcohol in a cell type-specific manner and it is not known how D1- and D2-MSNs distinctly influence alcohol consumption.

In this study, we measured both glutamatergic and GABAergic activity in D1-MSNs and D2-MSNs and found that NMDA receptor (NMDAR) activity in D1-MSNs and GABAergic activity in D2-MSNs were selectively potentiated following cycles of alcohol consumption and withdrawal. Using a chemogenetic approach employing designer receptors exclusively activated by designer drugs (DREADDs), which allowed selective manipulation of D1- or D2-MSN activity (23), we discovered that both of these cell types were not only necessary, but also sufficient, to drive alcohol consumption. Furthermore, we observed that D2R-glycogen synthase kinase-3 β (GSK3 β) signaling regulated GABAergic activity and thus, alcohol consumption. The findings of this study provide detailed mechanistic information

indicating how different forms of neuroplasticity in distinct neuronal populations of the striatal direct and indirect pathways drive alcohol consumption.

METHODS AND MATERIALS

A detailed description of all materials and methods can be found in Supplement 1. The intermittent-access two-bottle-choice drinking procedure was used to establish high levels of alcohol consumption in mice (15, 24–26). Twenty-four hr after the last alcohol-drinking session, animals were sacrificed and 200- μ m coronal sections containing the DMS or sagittal sections containing the GPe in the same thickness were prepared. The external solution contained (in mM): 125 NaCl, 4.5 KCl, 2.5 CaCl₂, 1.3 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 15 sucrose and 15 glucose. The pipette solution contained (in mM): 119 CsMeSO₄, 8 TEA.Cl, 15 HEPES, 0.6 EGTA, 0.3 Na₃GTP, 4 MgATP, 5 QX-314.Cl, 7 Na₂CrPO₄ for measuring NMDAR activity and 125 CsCl, 6 NaCl, 10 HEPES, 1 EGTA, 0.6 Na₃GTP, 2 MgATP, 10 QX-314.Cl, 2 Na₂CrPO₄ for recording GABAergic activity. Neurons were clamped at -70 mV in all recordings. The *hM4Di* or *hM3Dq* viruses were infused into the DMS (Site 1: anterior-posterior, $+1.18$; medial-lateral, ± 1.3 ; dorsal-ventral, -2.9 from Bregma. Site 2: anterior-posterior, $+0.38$; medial-lateral, ± 1.55 ; dorsal-ventral, -2.88). Six weeks after viral infusion, animals were intraperitoneally injected with 3 or 5 mg/kg of CNO 30 min before the start of the drinking sessions, and alcohol or saccharine intake was measured at 1, 4, and 24 hr. Western blot analysis was used to detect alterations of GSK3 β and GABA_A receptor (GABA_AR) levels in dorsostriatal tissues.

RESULTS

Selective Potentiation of Excitatory Transmission in DMS D1-MSNs Following Repeated Cycles of Excessive Alcohol Consumption and Withdrawal

The NMDAR is one of the major targets of alcohol (21, 27). However, it was unclear whether NMDAR-mediated excitatory transmission in D1- or D2-MSNs was altered by alcohol consumption and withdrawal. To measure NMDAR activity in these two subpopulations of striatal neurons, we generated new lines of mice to visualize fluorescently labeled D1- and D2-MSNs. These new mice were crossed by *drd1a*-Cre (D1-Cre) and *drd2*-Cre (D2-Cre) mice with Cre reporter lines (28). We confirmed that D1-MSNs projected to the internal part of the globus pallidus (GPi) and the SNr in the D1-Cre;Ai14 mice (Figure 1A) (29), whereas D2-MSNs mainly projected to the external part of the globus pallidus (GPe) in the new D2-Cre;Ai14 mice (Figure 1B) (17). Importantly, our transgenic mouse model suggests less than 5% overlap of DMS D1- and D2-MSNs (Figure S1), which is consistent with previous reports (30, 31). The animals were trained for 8 weeks to consume 20% alcohol using the widely used intermittent-access two-bottle-choice drinking procedure (15, 24, 32). They consumed ~ 18 – 20 g/kg/24 hr of alcohol (Figures S2A, S3A), which was considered as excessive alcohol intake in C57BL/6 mice (24, 26, 33). Twenty-four hr after the last alcohol-drinking session, whole-cell recording was conducted to measure NMDAR activity in D1- and D2-MSNs of the DMS. We found that the amplitude of NMDA-induced currents in D1-MSNs was dramatically greater in alcohol-drinking mice than their water controls (Figure 1C; Figure S2B). Surprisingly, the NMDA current in D2-MSNs was

significantly smaller in the alcohol-drinking group than in the water-drinking group (Figure 1D). Since the NMDA-elicited current reflects both synaptic and extrasynaptic NMDAR activity (34), these alcohol-related effects may result from changes in the synaptic and/or extrasynaptic NMDARs. To examine the synaptic NMDARs, we measured the input-output relationship for NMDAR-mediated excitatory postsynaptic currents (EPSCs) in D1- and D2-MSNs from alcohol- and water-treated mice. As shown in Figure 1E and 1F, the EPSC amplitudes were significantly higher in D1-MSNs from the alcohol group than in those from the water group, whereas the EPSC amplitudes in D2-MSNs were identical in these two groups. Furthermore, we observed that the NMDA/AMPA ratio was also increased selectively in D1- but not in D2-MSNs following alcohol consumption (Figure S2C–F). These results suggest that repeated cycles of alcohol consumption and withdrawal selectively potentiated synaptic NMDAR activity in D1-MSNs, but not in D2-MSNs, within the DMS.

The NMDAR is composed of GluN1 and GluN2 (A–D) subunits (35). The activity of GluN2B-containing NMDARs was reported to increase after cycles of excessive alcohol consumption and withdrawal (6, 36). Therefore, we examined whether GluN2B activity was selectively altered in D1- or D2-MSNs of the DMS following excessive alcohol consumption and withdrawal. We observed that the GluN2B/NMDA ratio in D1-MSNs was significantly higher in the alcohol group than in the water group (Figure 1G). However, this effect was not observed in D2-MSNs (Figure 1H). Collectively, these results suggest that alcohol-induced strengthening of the synaptic NMDAR-mediated glutamatergic input onto D1-MSNs of the DMS resulted, at least in part, from facilitation of GluN2B-NMDAR activity.

Selective Potentiation of Inhibitory Transmission in DMS D2-MSNs Following Repeated Cycles of Excessive Alcohol Consumption and Withdrawal

GABAergic changes have been found in many brain regions, including the striatum, following alcohol exposure (9, 37). However, it was unclear whether prolonged excessive alcohol consumption and withdrawal changed GABAergic transmission selectively in D1- or D2-MSNs. Mice were trained and consumed comparable levels of alcohol as above (~20 g/kg/24 hr, Figure S3A); GABAergic activity was measured in D1- and D2-MSNs of the DMS 24 hr after the last drinking session. We found that the amplitude of GABA-induced currents was moderately higher in D1-MSNs and dramatically elevated in D2-MSNs of alcohol-drinking mice, as compared with those of their water controls (Figure 2A). Since GABA_ARs are located at synaptic and extrasynaptic sites in MSNs (38), we investigated whether synaptic GABAergic transmission was affected after prolonged excessive alcohol consumption and withdrawal. Miniature inhibitory postsynaptic currents (mIPSCs) were measured in both populations of MSNs. We found that neither the amplitude nor the frequency of mIPSCs differed in D1-MSNs from the alcohol and water groups (Figure 2B–D). The cumulative probability distributions of mIPSC amplitudes and inter-event intervals were not affected by alcohol exposure (Figure 2C,D). Hence, the increased GABA-induced currents in D1-MSNs of the alcohol group (Figure 2A) may result from enhanced extrasynaptic GABA_AR activity. Indeed, we found that tonic extrasynaptic GABA currents were enhanced in D1-MSNs following excessive alcohol intake (Figure S3B). In contrast,

both the amplitude and frequency of mIPSCs were significantly higher in D2-MSNs in alcohol-drinking mice than in their water controls (Figure 2E–G; Figure S3C–E); this was confirmed by analysis of cumulative probability distributions of mIPSCs. The distribution of mIPSC amplitudes showed a right shift (Figure 2F), indicating an increase in amplitude. The distribution of mIPSC inter-event intervals shifted to the left in alcohol-drinking mice (Figure 2G), demonstrating a reduced duration of mIPSC events, and thus an increased mIPSC frequency. Collectively, these results indicate that inhibitory GABAergic transmission is potentiated selectively in DMS D2-MSNs, but not in D1-MSNs, following repeated cycles of excessive alcohol consumption and withdrawal.

***In vivo* Chemogenetic D1-MSN Excitation or D2-MSN Inhibition Promotes Alcohol Consumption**

The identified potentiation of excitatory glutamatergic transmission in D1-MSNs and of inhibitory GABAergic transmission in D2-MSNs of the DMS following excessive alcohol intake would be predicted to excite D1-MSNs and inhibit D2-MSNs. Since D1-MSNs control “Go” and D2-MSNs control “NoGo” actions in rewarding behaviors (17, 18), we reasoned that the resulting increase in “Go” and reduction of “NoGo” could both promote alcohol consumption. To test these hypotheses, we selectively manipulated D1- or D2-MSN activity using DREADDs (23). To confirm selective expression of a DREADD in the targeted neuronal population, e.g., D1-MSNs, a Cre-inducible adeno-associated virus (AAV) expressing the *hM3Dq* gene (Figure 3A) was bilaterally infused into the DMS region of D1-Cre mice (Figure 3B, left). Expression of *hM3Dq* in the DMS was indicated by a red fluorescent reporter, mCherry (Figure 3B, right), and was restricted selectively to D1-MSNs, as confirmed by their GPi and SNr projections in the direct pathway (Figure 3C). We also confirmed selective expression of *hM4Di* in the DMS D2-MSNs as indicated by mCherry in the GPe of the indirect pathway (Figure S4A).

The effects of DREADDs on MSN activity were confirmed by recording from GPe neurons in the indirect pathway (Figure 3D). We infused two viruses encoding a DREADD and channelrhodopsin-2 (ChR2) into the DMS of D2-Cre mice and confirmed their co-expression in D2-MSN fibers within the GPe (Figure 3D,E). Optical stimulation of ChR2-containing fibers elicited optogenetically-induced inhibitory postsynaptic currents (oIPSCs) in GPe neurons (Figure 3F, left). In DMS slices expressing *hM3Dq*, bath application of the DREADD agonist, clozapine-N-oxide (CNO), significantly increased the amplitude of oIPSCs by 42.4% (Figure 3F, right). In contrast, in DMS slices expressing *hM4Di* (Figure 3G; Figure S4B), CNO significantly reduced the oIPSC amplitude by 51.9% (Figure 3H). We also confirmed the effect of *hM4Di* and *hM3Dq* activation in D1-MSNs (Figure S5). These results confirmed that *hM3Dq* activation excited, or *hM4Di* activation suppressed the MSN output, respectively.

To test the hypothesis that excitation of D1-MSNs is sufficient to increase alcohol consumption, we infused a viral vector encoding the excitatory *hM3Dq* into the DMS of D1-Cre mice. The animals were trained to consume alcohol as described above (15, 24). CNO was administered systemically 30 min before the drinking session and alcohol consumption was measured at 1, 4, and 24 hr. We found that 1-hr alcohol consumption was significantly

increased following CNO administration (Figure 3I). In addition, 1-hr alcohol preference was also enhanced (Figure 3J). The CNO effects on alcohol consumption and preference were time-dependent (Figure 3K; Figure S6A,B). Interestingly, systemic administration of CNO also significantly decreased 1-hr water intake (Figure 3L), but not in 4- or 24-hr intake (Figure S6C).

To test the hypothesis that inhibition of D2-MSNs promotes alcohol consumption, we expressed the inhibitory *hM4Di* in DMS D2-MSNs of D2-Cre mice. The animals underwent the drinking procedure and CNO treatment described above. We observed that CNO administration caused a significant increase in 1-hr alcohol drinking (Figure 3M) and preference (Figure 3N) in D2-Cre mice. The CNO effects on alcohol drinking and preference were diminished over time (Figure 3O; Figure S6D,E). Water intake was not altered by systemic administration of CNO (Figure 3P; Figure S6F).

Taken together, these results suggest that alterations of D1- and D2-MSN activity in the DMS are sufficient to drive alcohol consumption, and that these exert opposite influences on this behavior.

Direct *In Vivo* Chemogenetic Inhibition of D1-MSNs or Excitation of D2-MSNs Attenuates Excessive Alcohol Consumption

Next, we examined whether direct manipulations of these two neuronal populations reduced excessive alcohol consumption. First, we asked whether *in vivo* chemogenetic inhibition of D1-MSNs, which presumably suppresses “Go” actions, decreased excessive alcohol consumption in mice. These mice were trained to consume alcohol using the same procedure as above. We discovered that high levels of 1-hr alcohol consumption (Figure 4A) and preference (Figure 4B) were dramatically attenuated by administration of CNO. The CNO effects on alcohol intake and preference exhibited a time-dependent manner (Figure 4C; Figure S7A,B). However, neither water intake (Figure 4D; Figure S7C) nor saccharin intake (Figure 4E; Figure S7D) was altered by administration of CNO. These findings suggest that inhibition of the output of DMS D1-MSNs in the direct pathway selectively decreases alcohol consumption by suppressing the preference for alcohol intake.

Conversely, we assessed whether excitation of D2-MSNs, which presumably enhances “NoGo” actions, reduced excessive alcohol consumption. An *hM3Dq* vector was bilaterally infused into the DMS of D2-Cre mice. We found that both 1-hr alcohol consumption (Figure 4F) and preference (Figure 4G) were significantly suppressed by CNO administration. The inhibitory effect of CNO on alcohol-drinking behavior was time-dependent (Figure 4H; Figure S7E,F). Intake of water (Figure 4I; Figure S7G) or saccharin (Figure 4J; Figure S7H) was not affected by CNO injection. These results indicate that excitation of DMS D2-MSNs in the indirect pathway specifically decreases alcohol consumption by suppressing the preference for alcohol intake. In addition, we found that D2-MSN excitation prevented expression of alcohol-induced conditioned place preference (Figure S8), which provides further evidence that D2-MSNs play a negative role in alcohol-related behavior.

Collectively, these results suggest that DMS D1- and D2-MSN activities are necessary to drive alcohol consumption and again in an opposite way.

D2R Signaling via GSK3 β Suppresses GABAergic Transmission in D2-MSNs and Inhibits Excessive Alcohol Consumption

Lastly, we asked whether and how D1- and D2-signaling contribute to alcohol consumption. Previously, we found that pharmacological inhibition of D1 receptors attenuated excessive alcohol consumption (15). Here, we investigated whether pharmacological modulation of D2R signaling suppressed alcohol consumption. Since the D2R agonist, quinpirole, was shown to suppress cortical GABAergic transmission (39, 40) and GABAergic activity is increased preferentially in D2-MSNs by alcohol (Figure 2), this compound may inhibit GABAergic transmission in D2-MSNs and thus decrease alcohol consumption. To assess this possibility, electronically-induced IPSCs (eIPSCs) were recorded in D2-MSNs from alcohol-drinking D2-Cre;Ai14 mice. We found that bath application of quinpirole significantly reduced eIPSC amplitudes (Figure 5A,C). We next explored the potential downstream target of D2R activation that may mediate quinpirole-induced inhibition of GABAergic transmission. Thus, we treated DMS slices with a GSK3 β inhibitor (SB216763) and observed that this completely abolished the inhibitory effect of quinpirole on eIPSCs (Figure 5B,C). These results suggest that activation of D2Rs in D2-MSNs of the DMS suppresses GABAergic transmission in a GSK3 β -dependent manner.

Additionally, we observed that GSK3 β phosphorylation on the serine 9 residue was increased without alternation of its protein levels following cycles of excessive alcohol intake and withdrawal (Figure 5D–G). Furthermore, protein levels of GABA_AR β 3 subunit, which predominantly contributes to GABA_AR activity in D2- rather than D1-MSNs (41, 42), was also significantly augmented in the same animal (Figure 5D,H). Given that GSK3 β phosphorylation decreases the kinase activity (43, 44), these data indicate that prolonged alcohol consumption and withdrawal suppresses GSK3 β activity, which, in turn, enhances GABA_AR expression.

To test whether activation of D2Rs reduced alcohol intake, we bilaterally infused quinpirole or vehicle into the DMS of alcohol-drinking C57BL/6 mice and measured alcohol consumption at 2, 4, and 24 hr. We measured alcohol intake starting at 2 hr post-infusion, but not at 1 hr as in chemogenetic experiments, because local agent infusion took a longer time than the i.p. injection used in chemogenetic experiments, and because 2-hr alcohol intake post-infusion was stable (15). We found that 2-hr alcohol consumption was significantly reduced in the quinpirole-treated group, as compared with the vehicle group, without alternation of water consumption (Figure 5I; Figure S9A). Moreover, infusion of both quinpirole and SB216763 into the DMS did not significantly affect 2-hr alcohol or water intake, as compared with vehicle infusion (Figure 5I; Figure S9B), suggesting that GSK3 β inhibition abolished the quinpirole effect on alcohol consumption. Infusion of SB216763 alone did not affect alcohol intake (Figure 5J). The inhibitory effect of quinpirole on alcohol consumption was also observed at 4-hr, but not at 24-hr (Figure 5K). These results suggest that activation of D2Rs in the DMS suppresses alcohol consumption in a GSK3 β -dependent manner.

Furthermore, we assessed whether direct inhibition of GABAergic activity suppressed alcohol consumption. A GABA_AR inhibitor, picrotoxin, was bilaterally infused into the DMS of alcohol-drinking mice. We found that picrotoxin infusion produced a significant

reduction in 2-hr alcohol consumption, but not water intake (Figure 5L; Figure S9C). This inhibitory effect of picrotoxin on alcohol consumption gradually diminished over time (Figure 5L).

Collectively, these findings indicate that D2R signaling *via* GSK3 β suppresses GABAergic transmission in D2-MSNs of the DMS and inhibits excessive alcohol consumption.

DISCUSSION

The present study found that repeated cycles of excessive alcohol intake and withdrawal selectively potentiated GluN2B-NMDAR activity in direct-pathway D1-MSNs and GABAergic transmission in indirect-pathway D2-MSNs of the DMS. These changes in synaptic strength serve to excite D1-MSNs and inhibit D2-MSNs and we discovered that D1-MSN excitation or D2-MSN inhibition using an *in vivo* chemogenetic approach promotes alcohol consumption. Conversely, D1-MSN inhibition or D2-MSN excitation suppress alcohol consumption. These findings suggest that changing the activity of either type of MSNs is sufficient and necessary to regulate alcohol-drinking behavior. Importantly, we demonstrated that the potentiation of D2-MSN GABAergic transmission from alcohol-drinking mice was reduced by pharmacological activation of D2Rs and subsequent GSK3 β signaling. D2R activation or local GABAergic inhibition in the DMS reduced alcohol consumption. This study elucidated the detailed mechanism involved in the modulation of alcohol-drinking behavior by alcohol-mediated changes in neurotransmission onto distinct neuronal populations in the same brain region, i.e., the DMS (Figure 6).

Potentiation of Excitatory and Inhibitory Transmission Separately in D1- and D2-MSNs by Excessive Alcohol Intake

One major finding of this research is that NMDAR-mediated transmission is potentiated selectively in D1-MSNs, while GABAergic transmission is potentiated exclusively in D2-MSNs, following cycles of excessive alcohol consumption and withdrawal. We further observed that the NMDA/AMPA ratio was increased in D1- but not D2-MSNs. Given that both the AMPAR (15) and NMDAR activities are potentiated in D1-neurons following alcohol consumption, the increased NMDA/AMPA ratio suggests that cycles of alcohol consumption and withdrawal causes greater potentiation of NMDAR activity than of AMPAR activity. This conclusion is line with previous findings that the NMDAR, rather than the AMPAR, is the primary and direct target of alcohol (16, 45) and that a similar change at the corticostriatal synapses in D1-MSNs was induced following chronic cocaine exposure (46). Since NMDAR activity is known to be facilitated by dopamine activation of D1 receptors (17, 47), this selective change of NMDARs in D1-MSNs may result from alcohol-induced elevation of striatal dopamine levels (48). The increased NMDAR activity is consistent with drug-induced “silent” synapses (49). In contrast, a decrease in the NMDA-induced current was observed in D2-MSNs, which may reflect NMDAR inhibition by dopamine activation of D2Rs (50). One mediator of the downstream signaling of D2R activation is GSK3 β . This kinase, however, seems less likely involved in the reduction of NMDA currents in D2-MSNs since cycles of alcohol intake and withdrawal inhibit the kinase activity (as shown herein) (51, 52) and GSK3 β inhibition has been reported to

enhance NMDA currents (53). Additionally, we measured GABAergic activity in these two types of striatal neurons. In D1 neurons, an increased GABA-induced current without mIPSC alteration suggests that extrasynaptic GABA_AR activity is enhanced following repeated cycles of alcohol exposure and withdrawal and confirmed by the increased GABAergic tonic current. Importantly, we observed enhancement of the mIPSC frequency in D2-MSNs, indicating a potential increase in GABAergic inputs onto this neuronal population. This concept is in agreement with a previous study indicating that withdrawal from repeated exposure to alcohol caused an allostatic state including dopaminergic deficiency in the striatum (54, 55); this deficiency was reported to increase GABAergic interneuron connectivity onto D2-MSNs (56).

D2Rs Regulate GABAergic Transmission *via* GSK3 β Contributing to Excessive Alcohol Consumption

The second major finding is that D2R activation suppresses D2-MSN GABAergic transmission and alcohol intake in a GSK3 β -dependent manner, indicating that D2R-GSK3 β signaling regulates alcohol intake (Figure 6). Chronic alcohol consumption has been reported to decrease protein levels of D2Rs and GSK3 β activity in the striatum (5, 51). Since GSK3 β negatively regulates GABA_AR trafficking and thereby GABAergic transmission (40, 57, 58), excessive alcohol consumption leads to downregulation of D2R-GSK3 β signaling and thereby increases GABAergic activity. Consistent with this notion, we observed an enhanced GSK3 β phosphorylation and β 3-containing GABA_AR expression following excessive alcohol intake, suggesting that alcohol exposure and withdrawal downregulates GSK3 β activity and consequently enhances GABA_AR expression. The β 3-containing GABA_AR, like the δ -containing one (9), mediates tonic inhibition (41, 42) and are activated by ambient GABA that are likely released from tonically active GABAergic interneurons (59). This indicates an important role of GABAergic interneurons in alcohol-drinking behavior and it would be of interest to examine the exact role in the future. These results also explain, in part, our observation that the GABA-induced currents and the mIPSC amplitudes in D2-MSNs were potentiated following excessive alcohol intake. Conversely, we found that D2R activation and signaling *via* GSK3 β reduced GABAergic activity in D2-MSNs in alcohol-drinking animals. The D2R-GSK3 β -mediated reduction of GABAergic transmission in the DMS inhibited alcohol consumption but not water intake, a finding that is supported by other reports indicating that GABAergic inhibition or GABA receptor knockdown in the striatum reduced alcohol intake (8, 9).

Contrasting Roles of D1- and D2-MSNs in Alcohol Consumption

The third major finding of this research is that bidirectional chemogenetic manipulations of two different subtypes of MSNs produced distinct changes in alcohol-drinking behavior, revealing the opposing roles of D1- and D2-MSNs in alcohol consumption and preference in mice.

Specifically, chemogenetic excitation of D1-MSNs promoted alcohol consumption, whereas inhibition of their activity attenuated alcohol consumption. This indicated a positive role of D1-MSNs in regulation of alcohol consumption. Our results are supported by pharmacological and genetic studies (15, 60–62). Importantly, our study on D2-MSNs

advances the understanding of their negative role in alcohol consumption, which has not been seen in previous pharmacological studies using D2R agonists/antagonists (15, 62–65), due to complex expression pattern of striatal D2Rs (47).

In summary, we found that repeated cycles of alcohol consumption and withdrawal potentiate excitatory glutamatergic strength in D1-MSNs and inhibitory GABAergic strength in D2-MSNs of the DMS. The excitatory potentiation may occur at the corticostriatal input, which has been reported in other drugs of abuse (46), and the inhibitory strengthening may result from GABAergic interneurons. Since the DMS is part of the cortico-striato-thalamo-cortex circuit, which is known to control action-outcome learning and goal-directed behavior (66), these glutamatergic and GABAergic changes presumably enhance process of goal-direct information in this circuit. Consequently, these changes increase D1-MSN-mediated “Go” and decrease D2-MSN-mediated “NoGo” actions controlling alcohol-associated behaviors (Figure 6). Both of these effects serve to reinforce alcohol consumption, leading to pathological excessive use of alcohol. This study provides an insight into the detailed mechanisms underlying the control of alcohol consumption, and perhaps the intake of other drugs, by excitatory and inhibitory neurotransmission onto striatal neuronal subpopulations, identifying both synaptic and neuronal therapeutic targets for the development of new approaches to the treatment of alcohol abuse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Luscher C, Malenka RC. Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. *Neuron*. 2011; 69:650–663. [PubMed: 21338877]
2. Koob GF, Volkow ND. Neurocircuitry of addiction. *Neuropsychopharmacology*. 2010; 35:217–238. [PubMed: 19710631]
3. Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci*. 2006; 29:565–598. [PubMed: 16776597]
4. Filbey FM, Claus E, Audette AR, Niculescu M, Banich MT, Tanabe J, et al. Exposure to the taste of alcohol elicits activation of the mesocorticolimbic neurocircuitry. *Neuropsychopharmacology*. 2008; 33:1391–1401. [PubMed: 17653109]
5. Volkow ND, Morales M. The Brain on Drugs: From Reward to Addiction. *Cell*. 2015; 162:712–725. [PubMed: 26276628]
6. Wang J, Lanfranco MF, Gibb SL, Yowell QV, Carnicella S, Ron D. Long-lasting adaptations of the NR2B-containing NMDA receptors in the dorsomedial striatum play a crucial role in alcohol consumption and relapse. *J Neurosci*. 2010; 30:10187–10198. [PubMed: 20668202]
7. Brown TE, Lee BR, Mu P, Ferguson D, Dietz D, Ohnishi YN, et al. A silent synapse-based mechanism for cocaine-induced locomotor sensitization. *J Neurosci*. 2011; 31:8163–8174. [PubMed: 21632938]

8. Hyttia P, Koob GF. GABAA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *Eur J Pharmacol.* 1995; 283:151–159. [PubMed: 7498304]
9. Nie H, Rewal M, Gill TM, Ron D, Janak PH. Extrasynaptic delta-containing GABAA receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. *Proc Natl Acad Sci U S A.* 2011; 108:4459–4464. [PubMed: 21368141]
10. Everitt BJ, Robbins TW. From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neurosci Biobehav Rev.* 2013; 37:1946–1954. [PubMed: 23438892]
11. Wang J, Carnicella S, Phamluong K, Jeanblanc J, Ronesi JA, Chaudhri N, et al. Ethanol induces long-term facilitation of NR2B-NMDA receptor activity in the dorsal striatum: implications for alcohol drinking behavior. *J Neurosci.* 2007; 27:3593–3602. [PubMed: 17392475]
12. Balleine BW, O'Doherty JP. Human and rodent homologues in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology.* 2010; 35:48–69. [PubMed: 19776734]
13. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Childress AR, et al. Cocaine cues and dopamine in dorsal striatum: mechanism of craving in cocaine addiction. *J Neurosci.* 2006; 26:6583–6588. [PubMed: 16775146]
14. Nam HW, Hinton DJ, Kang NY, Kim T, Lee MR, Oliveros A, et al. Adenosine transporter ENT1 regulates the acquisition of goal-directed behavior and ethanol drinking through A2A receptor in the dorsomedial striatum. *J Neurosci.* 2013; 33:4329–4338. [PubMed: 23467349]
15. Wang J, Cheng Y, Wang X, Roltsch Hellard E, Ma T, Gil H, et al. Alcohol Elicits Functional and Structural Plasticity Selectively in Dopamine D1 Receptor-Expressing Neurons of the Dorsomedial Striatum. *J Neurosci.* 2015; 35:11634–11643. [PubMed: 26290240]
16. Wang J, Ben Hamida S, Darcq E, Zhu W, Gibb SL, Lanfranco MF, et al. Ethanol-mediated facilitation of AMPA receptor function in the dorsomedial striatum: implications for alcohol drinking behavior. *J Neurosci.* 2012; 32:15124–15132. [PubMed: 23100433]
17. Gerfen CR, Surmeier DJ. Modulation of striatal projection systems by dopamine. *Annu Rev Neurosci.* 2011; 34:441–466. [PubMed: 21469956]
18. Maia TV, Frank MJ. From reinforcement learning models to psychiatric and neurological disorders. *Nat Neurosci.* 2011; 14:154–162. [PubMed: 21270784]
19. Sippy T, Lapray D, Crochet S, Petersen CC. Cell-Type-Specific Sensorimotor Processing in Striatal Projection Neurons during Goal-Directed Behavior. *Neuron.* 2015
20. Kreitzer AC. Physiology and pharmacology of striatal neurons. *Annu Rev Neurosci.* 2009; 32:127–147. [PubMed: 19400717]
21. Lovinger DM, White G, Weight FF. NMDA receptor-mediated synaptic excitation selectively inhibited by ethanol in hippocampal slice from adult rat. *J Neurosci.* 1990; 10:1372–1379. [PubMed: 2158533]
22. Roberto M, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Increased GABA release in the central amygdala of ethanol-dependent rats. *J Neurosci.* 2004; 24:10159–10166. [PubMed: 15537886]
23. Urban DJ, Roth BL. DREADDs (Designer Receptors Exclusively Activated by Designer Drugs): Chemogenetic Tools with Therapeutic Utility. *Annu Rev Pharmacol Toxicol.* 2015; 55:399–417. [PubMed: 25292433]
24. Hwa LS, Chu A, Levinson SA, Kayyali TM, DeBold JF, Miczek KA. Persistent escalation of alcohol drinking in C57BL/6J mice with intermittent access to 20% ethanol. *Alcohol Clin Exp Res.* 2011; 35:1938–1947. [PubMed: 21631540]
25. Warnault V, Darcq E, Levine A, Barak S, Ron D. Chromatin remodeling--a novel strategy to control excessive alcohol drinking. *Transl Psychiatry.* 2013; 3:e231. [PubMed: 23423140]
26. Darcq E, Warnault V, Phamluong K, Besserer GM, Liu F, Ron D. MicroRNA-30a-5p in the prefrontal cortex controls the transition from moderate to excessive alcohol consumption. *Mol Psychiatry.* 2015; 20:1219–1231.
27. Holmes A, Spanagel R, Krystal JH. Glutamatergic targets for new alcohol medications. *Psychopharmacology (Berl).* 2013; 229:539–554. [PubMed: 23995381]

28. Gong S, Doughty M, Harbaugh CR, Cummins A, Hatten ME, Heintz N, et al. Targeting Cre recombinase to specific neuron populations with bacterial artificial chromosome constructs. *J Neurosci.* 2007; 27:9817–9823. [PubMed: 17855595]
29. Madisen L, Zwingman TA, Sunkin SM, Oh SW, Zariwala HA, Gu H, et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. *Nat Neurosci.* 2010; 13:133–140. [PubMed: 20023653]
30. Bertran-Gonzalez J, Bosch C, Maroteaux M, Matamalas M, Herve D, Valjent E, et al. Opposing patterns of signaling activation in dopamine D1 and D2 receptor-expressing striatal neurons in response to cocaine and haloperidol. *J Neurosci.* 2008; 28:5671–5685. [PubMed: 18509028]
31. Valjent E, Bertran-Gonzalez J, Herve D, Fisone G, Girault JA. Looking BAC at striatal signaling: cell-specific analysis in new transgenic mice. *Trends Neurosci.* 2009; 32:538–547. [PubMed: 19765834]
32. Becker HC, Ron D. Animal models of excessive alcohol consumption: recent advances and future challenges. *Alcohol.* 2014; 48:205–208. [PubMed: 24811154]
33. Anacker AM, Loftis JM, Kaur S, Ryabinin AE. Prairie voles as a novel model of socially facilitated excessive drinking. *Addict Biol.* 2011; 16:92–107. [PubMed: 20579002]
34. Carpenter-Hyland EP, Woodward JJ, Chandler LJ. Chronic ethanol induces synaptic but not extrasynaptic targeting of NMDA receptors. *J Neurosci.* 2004; 24:7859–7868. [PubMed: 15356198]
35. Paoletti P, Bellone C, Zhou Q. NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nat Rev Neurosci.* 2013; 14:383–400. [PubMed: 23686171]
36. Kash TL, Baucum AJ 2nd, Conrad KL, Colbran RJ, Winder DG. Alcohol exposure alters NMDAR function in the bed nucleus of the stria terminalis. *Neuropsychopharmacology.* 2009; 34:2420–2429. [PubMed: 19553918]
37. Weiner JL, Valenzuela CF. Ethanol modulation of GABAergic transmission: the view from the slice. *Pharmacol Ther.* 2006; 111:533–554. [PubMed: 16427127]
38. Luo R, Partridge JG, Vicini S. Distinct roles of synaptic and extrasynaptic GABA receptors in striatal inhibition dynamics. *Front Neural Circuits.* 2013; 7:186. [PubMed: 24324406]
39. Li YC, Kellendonk C, Simpson EH, Kandel ER, Gao WJ. D2 receptor overexpression in the striatum leads to a deficit in inhibitory transmission and dopamine sensitivity in mouse prefrontal cortex. *Proc Natl Acad Sci U S A.* 2011; 108:12107–12112. [PubMed: 21730148]
40. Li YC, Wang MJ, Gao WJ. Hyperdopaminergic modulation of inhibitory transmission is dependent on GSK-3beta signaling-mediated trafficking of GABAA receptors. *J Neurochem.* 2012; 122:308–320. [PubMed: 22676038]
41. Janssen MJ, Ade KK, Fu Z, Vicini S. Dopamine modulation of GABA tonic conductance in striatal output neurons. *J Neurosci.* 2009; 29:5116–5126. [PubMed: 19386907]
42. Janssen MJ, Yasuda RP, Vicini S. GABA(A) Receptor beta3 Subunit Expression Regulates Tonic Current in Developing Striatopallidal Medium Spiny Neurons. *Front Cell Neurosci.* 2011; 5:15. [PubMed: 21847370]
43. Beaulieu JM, Gainetdinov RR, Caron MG. Akt/GSK3 signaling in the action of psychotropic drugs. *Annu Rev Pharmacol Toxicol.* 2009; 49:327–347. [PubMed: 18928402]
44. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther.* 2015; 148:114–131. [PubMed: 25435019]
45. Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. *Science.* 1989; 243:1721–1724. [PubMed: 2467382]
46. Pascoli V, Terrier J, Espallergues J, Valjent E, O'Connor EC, Luscher C. Contrasting forms of cocaine-evoked plasticity control components of relapse. *Nature.* 2014; 509:459–464. [PubMed: 24848058]
47. Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev.* 2011; 63:182–217. [PubMed: 21303898]
48. Sulzer D. How addictive drugs disrupt presynaptic dopamine neurotransmission. *Neuron.* 2011; 69:628–649. [PubMed: 21338876]
49. Huang YH, Lin Y, Mu P, Lee BR, Brown TE, Wayman G, et al. In vivo cocaine experience generates silent synapses. *Neuron.* 2009; 63:40–47. [PubMed: 19607791]

50. Li YC, Xi D, Roman J, Huang YQ, Gao WJ. Activation of glycogen synthase kinase-3 beta is required for hyperdopamine and D2 receptor-mediated inhibition of synaptic NMDA receptor function in the rat prefrontal cortex. *J Neurosci*. 2009; 29:15551–15563. [PubMed: 20007479]
51. Neasta J, Ben Hamida S, Yowell QV, Carnicella S, Ron D. AKT signaling pathway in the nucleus accumbens mediates excessive alcohol drinking behaviors. *Biol Psychiatry*. 2011; 70:575–582. [PubMed: 21549353]
52. Liu F, Laguesse S, Legastelois R, Morisot N, Ben Hamida S, Ron D. mTORC1-dependent translation of collapsin response mediator protein-2 drives neuroadaptations underlying excessive alcohol-drinking behaviors. *Mol Psychiatry*. 2016
53. Xi D, Li YC, Snyder MA, Gao RY, Adelman AE, Zhang W, et al. Group II metabotropic glutamate receptor agonist ameliorates MK801-induced dysfunction of NMDA receptors via the Akt/GSK-3beta pathway in adult rat prefrontal cortex. *Neuropsychopharmacology*. 2011; 36:1260–1274. [PubMed: 21326193]
54. Barak S, Carnicella S, Yowell QV, Ron D. Glial cell line-derived neurotrophic factor reverses alcohol-induced allostasis of the mesolimbic dopaminergic system: implications for alcohol reward and seeking. *J Neurosci*. 2011; 31:9885–9894. [PubMed: 21734280]
55. Koob GF, Le Moal M. Addiction and the brain antireward system. *Annu Rev Psychol*. 2008; 59:29–53. [PubMed: 18154498]
56. Gittis AH, Hang GB, LaDow ES, Shoenfeld LR, Atallah BV, Finkbeiner S, et al. Rapid target-specific remodeling of fast-spiking inhibitory circuits after loss of dopamine. *Neuron*. 2011; 71:858–868. [PubMed: 21903079]
57. Tyagarajan SK, Fritschy JM. Gephyrin: a master regulator of neuronal function? *Nature Reviews Neuroscience*. 2014; 15:141–156. [PubMed: 24552784]
58. Rui Y, Myers KR, Yu K, Wise A, De Blas AL, Hartzell HC, et al. Activity-dependent regulation of dendritic growth and maintenance by glycogen synthase kinase 3beta. *Nat Commun*. 2013; 4:2628. [PubMed: 24165455]
59. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron*. 2012; 73:23–34. [PubMed: 22243744]
60. D'Souza M. Chronic D1 agonist and ethanol coadministration facilitate ethanol-mediated behaviors. *Pharmacology Biochemistry and Behavior*. 2003; 76:335–342.
61. El-Ghundi M, George SR, Drago J, Fletcher PJ, Fan T, Nguyen T, et al. Disruption of dopamine D1 receptor gene expression attenuates alcohol-seeking behavior. *Eur J Pharmacol*. 1998; 353:149–158. [PubMed: 9726645]
62. Hodge CW, Samson HH, Chappelle AM. Alcohol self-administration: further examination of the role of dopamine receptors in the nucleus accumbens. *Alcohol Clin Exp Res*. 1997; 21:1083–1091. [PubMed: 9309321]
63. Thanos PK, Volkow ND, Freimuth P, Umegaki H, Ikari H, Roth G, et al. Overexpression of dopamine D2 receptors reduces alcohol self-administration. *J Neurochem*. 2001; 78:1094–1103. [PubMed: 11553683]
64. Phillips TJ, Brown KJ, Burkhart-Kasch S, Wenger CD, Kelly MA, Rubinstein M, et al. Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. *Nat Neurosci*. 1998; 1:610–615. [PubMed: 10196569]
65. Dyr W, McBride WJ, Lumeng L, Li TK, Murphy JM. Effects of D1 and D2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats. *Alcohol*. 1993; 10:207–212. [PubMed: 8507389]
66. Gunaydin LA, Kreitzer AC. Cortico-Basal Ganglia Circuit Function in Psychiatric Disease. *Annu Rev Physiol*. 2016; 78:327–350. [PubMed: 26667072]

Figure 1.

Selective potentiation of NMDAR activity in D1-MSNs following repeated cycles of excessive alcohol consumption and withdrawal. (A) Verification of D1-MSNs from a D1-Cre;Ai14 mouse, based on their GPe and SNr projections in a sagittal section (right), counter-stained with NeuroTrace green (NT-green). Scale bar: 1 mm. Five striatal neurons from an indicated box are shown (left) stained with NT-green (top); three neurons expressed tdTomato (D1-MSNs, middle) and were yellow in the merged image (bottom). Scale bar: 10 μ m. (B) Verification of D2-MSNs from a D2-Cre;Ai14 mouse based on the GPe projections.

Four NT-green-stained striatal neurons are shown (left), two of which expressed tdTomato (D2-MSNs, middle). (C) Cycles of excessive alcohol consumption and withdrawal significantly increased NMDA-induced currents in D1-MSNs. Changes in holding currents were measured after NMDA (10 μ M, 30 s) was applied to the slices (left). The peak amplitudes observed in these study groups (14 neurons, 3 mice per group) were summarized (right). $t_{(26)} = 4.77$, $p < 0.001$, unpaired t test. (D) Cycles of excessive alcohol consumption and withdrawal reduced NMDA-induced currents in D2-MSNs. Changes in holding currents were measured after NMDA was bath-applied (left) and the peak amplitudes in D2-MSNs from the alcohol (15 neurons, 3 mice) and water (14 neurons, 3 mice) groups of mice were compared (right). $t_{(27)} = -2.8$, $p < 0.01$, unpaired t test. (E) Cycles of excessive alcohol consumption and withdrawal significantly increased the NMDAR-EPSC amplitude in D1-MSNs. Representative EPSC traces evoked by a range of stimulation intensities in slices from the alcohol (16 neurons, 8 mice) and water (12 neurons, 6 mice) groups (left), with the corresponding input-output curves (right). $F_{(1,26)} = 7.43$, $^{\#}p < 0.05$, two-way RM-ANOVA. Scale bars: 200 pA, 100 ms. (F) Cycles of excessive alcohol consumption and withdrawal did not alter NMDAR-EPSCs in D2-MSNs. Representative EPSC traces evoked by a range of stimulation intensities in slices from the indicated groups of mice (left), with the corresponding input-output curves from the alcohol (12 neurons, 7 mice) and water (11 neurons, 6 mice) groups (right). $F_{(1,21)} = 0.43$, $p > 0.05$, two-way RM-ANOVA. (G) Cycles of excessive alcohol drinking and withdrawal increased the GluN2B/NMDA ratio in D1-MSNs. Sample GluN2B- and NMDAR-EPSC traces in the indicated groups (left) and the GluN2B/NMDA ratios in D1-MSNs from the alcohol (10 neurons, 6 mice) and water (8 neurons, 4 mice) groups (right). $t_{(16)} = -2.52$, $p < 0.05$, unpaired t test. (H) Cycles of excessive alcohol consumption and withdrawal did not change the GluN2B/NMDA ratio in D2-MSNs. Sample traces in the indicated groups (left), with the GluN2B/NMDA ratios in D2-MSNs from the alcohol (8 neurons, 5 mice) and water (8 neurons, 6 mice) groups (right). $t_{(14)} = -1.22$, $p > 0.05$, unpaired t test. Scale bars (F–H): 100 pA, 100 ms. Statistical comparisons between Alcohol and Water groups at the same levels are indicated by * for $p < 0.05$ and ** for $p < 0.01$ and *** for $p < 0.001$, respectively.

Figure 2.

Selective potentiation of synaptic GABAergic activity in D2-MSNs after repeated cycles of excessive alcohol consumption and withdrawal. GABAergic activity was measured in DMS D1- and D2-MSNs 24 hr after the last alcohol exposure. (A) GABA-induced currents in D1- and D2-MSNs in the indicated groups. Changes in holding currents recorded in D1-MSNs (left; 17 neurons from 5 mice for Water; 18 neurons from 6 mice for Alcohol) and D2-MSNs (middle; 19 neurons from 4 mice for Water; 20 neurons from 5 mice for Alcohol) after bath application of GABA (100 μ M, 30 sec) to DMS slices and their peak amplitudes (right). $t_{(33)} = -2.28$, $p < 0.05$ (D1-MSNs); $t_{(37)} = 2.79$, $p < 0.01$ (D2-MSNs), unpaired t test. (B), Representative mIPSC traces in D1-MSNs from the indicated mice. (C,D) Cycles of excessive alcohol consumption and withdrawal did not alter mIPSCs in D1-MSNs.

Cumulative probability plots for the distributions of mIPSC amplitudes (C) and inter-event intervals (D) in D1-MSNs from alcohol-drinking mice (27 neurons, 5 mice) and their water controls (19 neurons, 4 mice). Inset bar graphs summarize the respective average mIPSC amplitudes (C) and frequencies (D). $t_{(44)} = -0.6$, $p > 0.05$ for amplitude; $t_{(44)} = 0.8$, $p > 0.05$ for frequency, unpaired t test. (E), Representative mIPSC traces in D2-MSNs from the indicated mice. (F,G) Cycles of excessive alcohol drinking and withdrawal increased the amplitude and frequency of mIPSCs in D2-MSNs. Cumulative probability plots showing the distributions of mIPSC amplitudes (F) and inter-event intervals (G) in D2-MSNs from alcohol-drinking mice (17 neurons, 5 mice) and water controls (17 neurons, 4 mice). Inset bar graphs present the respective average mIPSC amplitudes (F) and frequencies (G). $t_{(32)} = -2.23$, $p < 0.05$ for amplitude; $t_{(32)} = -4.20$, $p < 0.001$ for frequency, unpaired t test. Scale bars: 30 pA, 0.5 sec (B, E). Statistical comparisons between Alcohol and Water groups are indicated by * for $p < 0.05$ and ** for $p < 0.01$ and *** for $p < 0.001$, respectively.

Figure 3.

In vivo chemogenetic excitation of D1-MSNs in the direct pathway or inhibition of D2-MSNs in the indirect pathway promotes alcohol consumption in mice. (A) Expression of the *hM3Dq* gene was driven by Cre recombinase. (B) Stereotaxic infusion of an AAV-DIO-*hM3Dq*-mCherry virus into the DMS region of D1-Cre mice (left) led to *hM3Dq*-mCherry expression, as indicated by the red mCherry fluorescence (right) in a section stained with NT-Green. Scale bar: 1 mm. (C) Verification of selective *hM3Dq* expression in D1-MSNs from a D1-Cre mouse infused with the virus (right). Note that *hM3Dq*-expressing D1-MSNs

projected to the GPi and SNr. Scale bar: 1 mm. Inset, micrographs showing 4 striatal neurons from the indicated box (right) stained with NT-green (top); 2 of these expressed mCherry (middle) and were yellow in the merged image (bottom). Scale bar: 10 μ m. **(D)** Schematic diagram illustrating *ex vivo* electrophysiology validation of hM3Dq enhancement of D2-MSN-mediated synaptic transmission in GPe neurons. A cocktail of AAV-DIO-hM3Dq-mCherry and AAV-DIO-ChR2-eYFP vectors was infused into the DMS of D2-Cre mice, and ChR2-mediated striatopallidal oIPSCs were recorded in GPe neurons. **(E)** Representative low-magnification sagittal view of mCherry and eYFP fluorescence in a brain slice from a D2-Cre mouse infused with the viral cocktail. Scale bar: 1 mm. **(F)** Bath application of CNO (10 μ M) increased the amplitude of ChR2-mediated oIPSCs in GPe neurons from D2-Cre mice infused with the *hM3Dq* virus in the DMS. Left, sample traces of oIPSCs at baseline (BL) and during CNO application. Scale bars: 40 ms, 100 pA. Right, Time course of oIPSCs showing CNO significantly increased oIPSC amplitudes (8 neurons, 5 mice). $t_{(7)} = -4.3$, $p < 0.001$, paired *t* test. **(G)** Schematic showing *ex vivo* electrophysiology validation of hM4Di, as described for D. **(H)** CNO application to slices decreased the oIPSC amplitude in GPe neurons from D2-Cre mice infused with *hM4Di* and *ChR2* viruses in the DMS. Left, Sample traces of oIPSCs at baseline and during CNO application. Scale bars: 40 ms, 30 pA. Right, Time course of oIPSC amplitudes before, during, and after CNO application (6 neurons, 3 mice). $t_{(5)} = 4.01$, $p < 0.01$, paired *t* test. **(I)** Excitation of D1-MSNs by systemic administration of CNO (3 mg/kg) reversibly promoted 1-hr alcohol consumption in D1-Cre mice expressing *hM3Dq*. $F_{(2,13)} = 7.81$, $p < 0.01$. $n = 14$ mice. **(J)** CNO excitation of D1-MSNs reversibly increased 1-hr alcohol preference in D1-Cre expressing *hM3Dq*. $F_{(2,13)} = 8.45$, $p < 0.01$. $n = 14$ mice. **(K)** Time-dependency of the CNO-mediated enhancement of alcohol consumption in D1-Cre mice expressing *hM3Dq*. $F_{(2,13)} = 8.05$, $p < 0.01$. $n = 14$ mice. **(L)** CNO excitation of D1-MSNs reduced water intake in D1-Cre mice expressing *hM3Dq*. $t_{(13)} = 3.19$, $p < 0.01$, paired *t* test. $n = 14$ mice. **(M)** CNO inhibition of D2-MSNs reversibly increased 1-hr alcohol consumption in D2-Cre mice infused with the *hM4Di* virus. $F_{(2,6)} = 5.87$, $p < 0.05$. $n = 7$ mice. **(N)** CNO inhibition of D2-MSNs led to a reversible increase in the preference for alcohol in D2-Cre mice with *hM4Di* infusion. $F_{(2,6)} = 5.43$, $p < 0.05$. $n = 7$ mice. **(O)** Time-dependency of the CNO-mediated enhancement of alcohol consumption in D2-Cre mice with *hM4Di*. $F_{(2,6)} = 4.72$, $p < 0.05$. $n = 7$ mice. **(P)** CNO inhibition of D2-MSNs did not alter 1-hr water intake in D2-Cre mice expressing *hM4Di*. $t_{(6)} = 1.63$, $p > 0.05$, paired *t* test. $n = 7$ mice. Statistical comparisons within experimental groups are indicated by * for $p < 0.05$ and ** for $p < 0.01$, respectively.

Figure 4.

In vivo chemogenetic inhibition of D1-MSNs in the direct pathway or excitation of D2-MSNs in the indirect pathway reduces excessive alcohol consumption. (A) Inhibition of D1-MSNs by administration of CNO (5 mg/kg) significantly and reversibly reduced 1-hr alcohol consumption in D1-Cre mice expressing *hM4Di*. $F_{(2,11)} = 14.07$, $p < 0.001$. $n = 12$ mice. (B) Inhibition of D1-MSNs by CNO administration reduced 1-hr alcohol preference in D1-Cre mice expressing *hM4Di*. $F_{(2,11)} = 12.68$, $p < 0.001$. $n = 12$ mice. (C) Time-dependent inhibition of alcohol consumption by CNO in D1-Cre mice expressing *hM4Di*. $F_{(2,11)} = 9.00$, $p < 0.01$. $n = 12$ mice. (D,E) Administration of CNO did not alter 1-hr water (D) or saccharin (0.033% w/v) (E) intake in D1-Cre mice expressing *hM4Di*. $t_{(11)} = 0.28$, $p > 0.05$ (D); $t_{(9)} = 1.23$, $p > 0.05$ (E), paired t test. $n = 12$ (D) and 10 (E) mice. (F) Excitation of D2-MSNs by administration of CNO significantly and reversibly reduced 1-hr alcohol consumption in D2-Cre mice expressing *hM3Dq*. $F_{(2,11)} = 16.33$, $p < 0.001$. $n = 12$ mice. (G) CNO excitation of D2-MSNs reversibly reduced 1-hr alcohol preference in D2-Cre mice expressing *hM3Dq*. $F_{(2,11)} = 9.42$, $p < 0.01$. $n = 12$ mice. (H) Inhibition of alcohol consumption by CNO excitation of D2-MSNs was time-dependent. $F_{(2,11)} = 27.91$, $p < 0.001$. $n = 12$ mice. (I,J) CNO excitation of D2-MSNs did not alter 1-hr water (I) or saccharin (J) intake in D2-Cre mice expressing *hM3Dq*. $t_{(11)} = 0.19$, $p > 0.05$ (I); $t_{(5)} = 0.82$, $p > 0.05$ (J), paired t test. $n = 12$ (I) and 6 (J) mice. Statistical comparisons within experimental groups are indicated by * for $p < 0.05$ and ** for $p < 0.01$ and *** for $p < 0.001$, respectively.

Figure 5.

D2R signaling *via* GSK3 β suppresses GABAergic transmission in D2-MSNs and attenuates excessive alcohol consumption. (A) D2R activation reduced the eIPSC amplitude in D2-MSNs in alcohol-drinking mice. D2-Cre;Ai14 mice were trained to consume high levels of alcohol for 8 weeks, and eIPSCs were recorded in D2-MSNs 24 hr after the last drinking session. The D2R agonist, quinpirole (Quin; 10 μ M), was bath-applied for 10 min. Left, Representative traces of eIPSCs at baseline (BL) and during quinpirole application. Scale bars: 100 pA, 100 ms. Right, Time course of eIPSC amplitudes before, during, and after quinpirole application (10 neurons, 8 mice). (B) Treatment of DMS slices with a GSK3 β antagonist, SB216763 (SB; 10 μ M), abolished quinpirole-mediated inhibition of eIPSCs in

D2-MSNs. The slices were pre-treated for 25 min with SB216763, which was continuously applied as indicated during the recording period (10 neurons, 5 mice). (C) Bar graphs comparing the effects of quinpirole and quinpirole plus SB216763 on eIPSC amplitudes. The amplitudes were averaged from 0–10 min (baseline) and from 15–20 min (drug applications). $t_{(9)} = 2.59$, $p < 0.05$ for quinpirole vs vehicle, paired t test. $t_{(9)} = -0.68$, $p > 0.05$ for quinpirole plus SB216763 vs vehicle, paired t test. n.s., not significant. (D) Representative western-blots images showing the phosphorylation levels of GSK3 β , protein levels of GSK3 β , GABA $_A$ R, and β -actin. (E–H) Western blot quantification of the phosphorylation levels of GSK3 β (p-GSK3 β) (E), total protein levels of GSK3 β (F), ratio of p-GSK/total GSK3 β (G), and GABA $_A$ R (H). $t_{(16)} = -3.36$, $p < 0.01$ (E); $t_{(16)} = -0.52$ (F), $p > 0.05$; $t_{(16)} = -3.89$, $p < 0.01$ (G); $t_{(16)} = -3.93$, $p < 0.01$ (H), unpaired t test. n = 8 (Water) and 10 (Alcohol) mice. (I) Intra-DMS infusion of quinpirole (6 μ g/0.5 μ l in DMSO), but not of both quinpirole and SB216763 (40 ng/ μ l in DMSO), significantly reduced 2-hr alcohol consumption. $t_{(15)} = 3.33$, $p < 0.01$ for quinpirole vs vehicle; $t_{(16)} = 0.20$, $p > 0.05$ for quinpirole plus SB216763 vs vehicle, unpaired t test. n = 9 (Veh, DMSO), 8 (Quin), and 9 (Quin + SB) mice. (J) Intra-DMS infusion of SB216763 alone did not alter 2-hr alcohol drinking. $t_{(14)} = 0.23$, $p > 0.05$, unpaired t test. n = 8 mice per groups. (K) The inhibitory effect of quinpirole on alcohol consumption was time-dependent. $F_{(2,15)} = 15.19$, $p < 0.001$, two way RM-ANOVA. n = 9 (Veh) and 8 (Quin) mice. (L) Intra-DMS infusion of a GABA $_A$ receptor antagonist, picrotoxin (PTX; 1 μ g/ μ l in DMSO) reduced alcohol drinking at 2 and 4 hr, but not at 24 hr. $F_{(2,11)} = 4.81$, $p < 0.05$, two way RM-ANOVA. n = 8 (Veh) and 5 (PTX) mice. Statistical comparisons between Treatment and Control groups are indicated by * for $p < 0.05$ and ** for $p < 0.01$, or comparisons within experimental groups are indicated by # for $p < 0.05$ and ### for $p < 0.001$, respectively.

Figure 6.

Hypothetical model of alcohol-induced changes in glutamatergic and GABAergic strength in D1- and D2-MSNs of the DMS leading to excessive alcohol consumption. Left, in the normal brain, D1-MSNs express GluN2B-NMDARs and D2-MSNs contain GABA_ARs, as well as D2Rs and GSK3 β . D2R activation stimulates GSK3 β signaling, which negatively regulates GABA_AR activity. D1-MSN or D2-MSN excitation facilitates selection of “Go” or “NoGo” actions in reward behavior, respectively. Right, in the alcoholism brain, repeated cycles of alcohol intake and withdrawal increase GluN2B-NMDAR activity selectively in D1-MSNs, which facilitates selection of “Go” actions and consequently drives excessive alcohol consumption. Meanwhile, cycles of alcohol intake and withdrawal also enhance synaptic GABAergic activity in D2-MSNs by decreasing D2R-GSK3 β signaling. This suppresses selection of “NoGo” actions. Together, the abnormally facilitated “Go” and suppressed “NoGo” actions reinforce excessive alcohol consumption. Note that the changes in activities of receptors, signaling, and behaviors are indicated by alterations of their sizes.