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Voluntary Ethanol Intake Enhances Excitatory Synaptic Strength in the Ventral Tegmental Area

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Abstract

Background—Addiction has been considered a disorder of motivational control over behavior, and the ventral tegmental area (VTA), in conjunction with other limbic brain structures, is thought to play a critical role in the regulation of a number of motivated behaviors including seeking of addictive drugs such as alcohol. Of particular interest is the ability of prolonged exposure of addictive drugs to enhance the function of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamatergic receptors (AMPA) in the VTA, as glutamate receptor activation can significantly regulate VTA neuron activity. Here, we examined whether voluntary ethanol intake altered VTA AMPAR function.

Methods—We utilized in vitro electrophysiology to examine glutamatergic function in the VTA neurons 12 to 24 hours after the last self-administration bout, which occurred 35 to 50 days after the initiation of ethanol self-administration under a 2-bottle intermittent access model.

Results—Voluntary intermittent ethanol intake in a 2-bottle paradigm enhanced postsynaptic AMPAR function, indicated by an increased ratio of evoked AMPAR to N-methyl-D-aspartic acid receptor currents, and by an increase in the amplitude of spontaneous miniature excitatory postsynaptic currents (mEPSCs) measured in the presence of tetrodotoxin to prevent action potential-dependent release. In contrast, ethanol self-administration did not alter evoked presynaptic glutamate release, indicated by no change in the paired-pulse ratio of 2 AMPAR EPSCs evoked 50 ms apart, although spontaneous glutamate release was significantly enhanced, indicated by enhanced mEPSC frequency.

Conclusions—Our results suggest that postsynaptic AMPAR function in VTA neurons was significantly enhanced after ethanol self-administration. As increased VTA AMPAR function can significantly regulate firing and enhance the reinforcing and activating effects of drugs of abuse, the increased AMPAR activity observed here may facilitate the drive to consume ethanol.

Keywords

Electrophysiology; Ventral Tegmental Area; Alcohol; α -Amino-3-Hydroxy-5-Methyl-4-Isioxazolepropionic Acid Receptors

THE DOPAMINE NEURONS of the ventral tegmental area (VTA), which project to regions such as the nucleus accumbens (NAc), basolateral amygdala, and prefrontal cortex, are potent modulators of addictive and goal-directed behaviors (Cardinal et al., 2002; Fields

et al., 2007; Paxinos, 1995; Spanagel and Weiss, 1999; Wise, 2004). Several studies have shown that dopamine signaling in the NAc can facilitate self-administration of ethanol (Hodge et al., 1997; Samson et al., 1999; Weiss et al., 1996; but see Rassnick et al., 1993), and direct inhibition of the VTA reduces ethanol self-administration (Hodge et al., 1993, 1996). In addition, animals self-administer ethanol directly into the VTA (Gatto et al., 1994; Rodd-Henricks et al., 2000), suggesting that the VTA may be important for the reinforcing effects of ethanol.

Glutamatergic afferents to the VTA are key modulators of dopamine cell firing and drug-seeking behavior (Canavier and Landry, 2006; Overton and Clark, 1997; You et al., 2007; Zhang et al., 1997) and therefore may play an important role in the manifestation of voluntary alcohol consumption. Of particular interest are the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) on VTA dopamine neurons, which show increased expression (Churchill et al., 1999; Fitzgerald et al., 1996; Ortiz et al., 1995) and thus enhance excitatory synaptic strength (Liu et al., 2005; Saal et al., 2003; Ungless et al., 2001; Zhang et al., 1997) in response to many drugs of abuse, including ethanol. While previous studies have demonstrated that passive administration of drugs of abuse alters excitatory signaling onto VTA neurons, it is not known whether voluntary alcohol consumption can alter synaptic strength. We therefore trained rats to self-administer alcohol in an intermittent access, 2-bottle choice paradigm for ~6 weeks and assayed synaptic changes in the VTA following 12 to 24 hours withdrawal from ethanol.

MATERIALS AND METHODS

All experiments were performed in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the Ernest Gallo Clinic and Research Center and the University of California, San Francisco.

Ethanol Self-Administration

Male, Wistar rats began ethanol self-administration at PN25. Self-administration was performed using a 2-bottle intermittent ethanol access model (Steensland et al., 2007; Wise, 1973), where animals had 24-hour concurrent access to 2 bottles, 1 with 20% ethanol and 1 with water, starting on the afternoon of Monday, Wednesday, and Friday. Thus, there were 24 or 48 ethanol-free hours between each 24-hour period of ethanol access. The amount of ethanol or water consumed was determined by weighing the bottles before access, after 30 minutes of access, and after 24 hours of access. Animal body weight was determined once per week on Wednesdays, and the daily volume of ethanol consumed in a given week was averaged before the gram-per-kilogram intake was determined.

In Vitro Electrophysiology

In vitro brain slice experiments were performed 35 to 50 days after the initiation of self-administration. Rats were deeply anesthetized with 40 mg/kg pentobarbital (i.p.) and perfused transcardially with ~30 ml of chilled modified artificial cerebrospinal fluid (aCSF) at a rate of ~10 ml/min. The modified aCSF for perfusion contained (in millimolar): 225 sucrose; 119 NaCl, 2.5 KCl, 1.0 NaH₂PO₄, 4.9 MgCl₂, 0.1 CaCl₂, 26.2 NaHCO₃, 1.25 glucose; 1 ascorbic acid; and 3 kynurenic acid. The brain was removed rapidly, and horizontal slices (230 μ m) containing the VTA were cut in this same modified aCSF. Slices recovered at 31°C in carbogen-bubbled aCSF (126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH₂PO₄, 1.2 mM MgCl₂, 2.4 mM CaCl₂, 18 mM NaHCO₃, 11 mM glucose, with pH 7.2 to 7.4 and 301 to 305 mOsm), with 1 mM ascorbic acid added just before the first slice. During experiments, slices were submerged and continuously perfused (~2 ml/min) with

carbogen-bubbled aCSF warmed to 31 to 32°C and supplemented with picrotoxin (50 μ M, to block GABA_A receptors).

All experiments were performed using whole-cell recording and visualized using infrared-DIC with 3.0 to 4.5 M Ω electrodes. The internal solution contained (in millimolar): 120 cesium methanesulfonate, 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA-Cl, 2.5 MgATP, and 0.25 NaGTP, pH 7.2 to 7.3, 270 to 285 mOsm. Electrical signals were recorded using Clampex 9.2 or 10.1 and an Axon 700A or 700B patch amplifier (Axon Instruments, Foster City, CA). Excitatory postsynaptic currents (EPSCs) were evoked using a bipolar stimulating electrode placed rostral to the VTA, and were filtered at 2 kHz, digitized at 10 kHz, and collected on-line using Igor Pro software (Wavemetrics, Lake Oswego, OR). Series resistance (10 to 30 M Ω) and input resistance were monitored on-line with a 4-mV depolarizing step (50 ms) given after every EPSC. To calculate the AMPAR/NMDAR ratio, an average of 12 EPSCs at +40 mV was computed before and after application of the NMDAR blocker 2-amino-5-phosphonopentanoate (AP5, 50 μ M). NMDAR responses were calculated by subtracting the average response in the presence of AP-5 (AMPA only) from the total current before addition of AP5; the peak of the AMPAR EPSC was then divided by the peak of the NMDAR EPSC to yield an AMPAR/NMDAR ratio. Paired-pulse ratio (PPR) experiments, using an interstimulus interval of 50 ms, were calculated as the ratio between the second and the first EPSCs averaged over 10 min. Cell capacitance was determined as described (Hopf et al., 2007). Spontaneous miniature EPSCs (mEPSCs) were recorded in cells voltage-clamped at -70 mV in the presence of tetrodotoxin (TTX, 500 nM) to suppress spontaneous action potential-driven release. mEPSCs were collected, with an acquisition rate of 100 kHz and filtering at 1 kHz, using Clampex (Axon Instruments, Cupertino, CA) and analyzed using Mini Analysis Program (Synaptosoft, Decatur, GA). Detection criteria were set at >7 pA, <1 ms rise time, and <3 ms decay time, with filtering during analysis at 1 kHz. For a given cell, mEPSCs were collected for 5 min or until 300 mEPSCs were collected, and data were only included if the holding potential at -70 mV was <200 pA and the root mean square noise was <2 pA to assure high signal-to-noise ratio. All data were also visually inspected to prevent noise disturbance of the analysis.

Putative VTA dopamine neurons were identified by the presence of an I_h current (Johnson and North, 1992), using a series of 500-ms hyperpolarizing steps (in 10-mV increments) from a holding potential of -70 mV. Although I_h is present in both dopamine and non-dopamine VTA neurons (Margolis et al., 2006), and we recognize that its presence does not unequivocally identify dopamine neurons in VTA slices, previous work from our lab (Borgland et al., 2006; Sarti et al., 2007) has suggested a high (~80%) correlation between the presence of I_h and tyrosine hydroxylase, indicative of dopamine neurons, in neurons just medial to the medial terminal nucleus of the accessory optic tract (MT), where nearly all the recordings in the present study were performed.

Reagents

AP5 and TTX were purchased from Tocris (Ellisville, MO), and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical Analysis

All data are expressed as mean $\pm$ standard error of the mean. Electrophysiological results were analyzed with a 2-tailed, unpaired *t*-test unless otherwise indicated. Mann-Whitney tests were used in cases where variance was significantly different between groups. Behavioral results were analyzed using a 1-way ANOVA with Bonferroni's Multiple Comparison Test post hoc. Significance was determined using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA) with confidence intervals of at least 95%.

RESULTS

Ethanol self-administration in Wistar rats was performed using a 2-bottle intermittent ethanol access model, where animals had 24-hour concurrent access to 2 bottles, 1 with 20% ethanol and 1 with water, starting on the afternoon of Monday, Wednesday, and Friday (Fig. 1A). Thus, there were 24 or 48 ethanol-free hours between each 24-hour period of ethanol access. As previously described (Steensland et al., 2007; Wise, 1973), animals showed a significant escalation of ethanol intake across weeks of ethanol access (Fig. 1B,C; $F_{(6,133)} = 7.45$, $p < 0.0001$, 1-way ANOVA of g/kg/24 h across weeks, $n = 22$), with an increase from ~4.5 g/kg/24 h on week 1 to ~8 g/kg/24 h on week 5 when drinking levels reached plateau.

We also examined drinking levels during the first 30 min of ethanol access, as it has been widely observed that animals exhibit a period of significant drinking or “loading up” during the initial period of ethanol access within a given self-administration session (Samson and Hodge, 1996). The preference for ethanol [expressed as (ml ethanol)/(ml ethanol + ml water)] during the first 30 min of access was also significantly increased across weeks of ethanol access (Fig. 1D; $p < 0.0001$, Kruskal–Wallis test), although the gram-per-kilogram ethanol consumed during the initial 30 min of ethanol access was not significantly different across weeks of ethanol access (Fig. 1F; $F_{(6,133)} = 1.11$, 1-way ANOVA of g/kg/30 min across weeks, $p = 0.36$). Taken together, these data suggest that animals showed a significant increase in ethanol consumption and preference, which reached a plateau by about the fifth week of access to ethanol.

Studies have suggested an important role for VTA AMPARs in regulation of several reward-related behaviors (see Carlezon and Nestler, 2002). The purpose of the experiments in this study was to examine potential changes in glutamatergic synaptic function in VTA dopamine neurons that occurred as an immediate consequence of ethanol self-administration. Following withdrawal from chronic ethanol, dopaminergic function is decreased (see Weiss and Porrino, 2002 for review), which thus may enhance motivation for future ethanol intake in an effort to compensate for dopamine deficits. Thus, ~12 to 24 hours after the last drinking bout (which was 35 to 50 days after initiation of ethanol self-administration), rats were deeply anesthetized and brain slices containing the VTA were prepared to examine excitatory transmission onto VTA dopamine neurons using in vitro electrophysiology.

We first examined evoked excitatory currents in VTA dopamine neurons by examining evoked EPSCs. Results from ethanol self-administering animals were compared with age-matched naïve rats. Evoked currents were determined at a holding potential of +40 mV in the presence and absence of the NMDA receptor (NMDAR) antagonist AP5 (50 μ M). In this way, we were able to determine the relative contribution of NMDAR and AMPAR currents, and calculate the ratio of AMPAR/NMDAR, which has been used previously as an index of synaptic strength (Bellone and Lüscher, 2006; Malenka and Nicoll, 1999; Perkel and Nicoll, 1993; Ungless et al., 2001). The AMPAR/NMDAR ratio was significantly increased in ethanol self-administering rats (Fig. 2; EtOH: 0.80 ± 0.14 , $n = 9$; naïve: 0.34 ± 0.06 , $n = 7$; Mann–Whitney test, $p = 0.0079$). Furthermore, current densities were calculated for both AMPAR- and NMDAR-mediated currents for both groups (AMPAR current densities: naïve: 0.0090 ± 0.002 pC/pF; EtOH SA: 0.007 ± 0.001 pC/pF; NMDAR current densities: naïve: 0.14 ± 0.033 pC/pF; EtOH SA: 0.070 ± 0.013 pC/pF). Consistent with the increased AMPAR/NMDAR ratio, the ratio of AMPAR/NMDAR-mediated current density was significantly greater in EtOH SA rats (Naïve: 0.063 ± 0.012 ; EtOH SA: 0.14 ± 0.034 ; Mann–Whitney test, $p = 0.049$). In addition, there was no difference in whole-cell capacitance levels between the groups (naïve: 95.3 ± 11.6 pF; EtOH SA: 121.9 ± 12.1 pF). Taken

together, these data suggest that ethanol self-administration caused an increase in AMPA receptor function in VTA dopamine neurons.

Next, to determine whether ethanol self-administration might alter presynaptic glutamate release dynamics, we examined the response to a pair of evoked EPSCs with 50 ms between the pulses; changes in the PPR (amplitude of the second EPSC divided by the first EPSC) is a measure that changes in a predictable relationship with release probability (Dobrunz and Stevens, 1997; Perkel and Nicoll, 1993). However, in contrast to the AMPAR/NMDAR ratio, PPRs from self-administering rats were not significantly different from naïve controls (Fig. 3; EtOH: 0.89 ± 0.11 , $n = 8$; naïve: 0.92 ± 0.05 , $n = 5$; $t(11) = 0.21$, $p = 0.84$), suggesting that presynaptic release dynamics in the VTA during evoked release were not altered following ethanol self-administration.

To further characterize how ethanol self-administration altered glutamatergic function in the VTA, we examined spontaneous miniature EPSCs (mEPSCs) at a -70 mV holding potential, which are mediated primarily by AMPARs (Borgland et al., 2006). Analysis of mEPSCs is a standard method for determining the locus of synaptic change, as an increase in the amplitude of mEPSCs is routinely attributed to a postsynaptic change in receptor function, while an increase in the frequency of mEPSCs can indicate an increase in presynaptic glutamate release or the number of synaptic contacts (Malenka and Nicoll, 1999). The frequency of mEPSCs was significantly enhanced following ethanol self-administration (Fig. 4A,B,E,F; EtOH: 2.43 ± 0.51 Hz, $n = 9$; naïve: 0.86 ± 0.12 Hz, $n = 8$; Mann–Whitney test, $p = 0.021$). Importantly, mEPSC amplitude was also significantly increased by ethanol self-administration (Fig. 4A–D; EtOH: 14.0 ± 1.9 pA, $n = 9$; naïve: 10.3 ± 0.7 pA, $n = 8$; Mann–Whitney test, $p = 0.046$), consistent with the increase in AMPAR/NMDAR ratio and further suggesting that postsynaptic AMPA receptor function was significantly enhanced by ethanol self-administration.

DISCUSSION

The central finding of this study is that voluntary ethanol intake significantly enhanced excitatory synaptic strength onto VTA neurons. In particular, the increased amplitude of mEPSCs observed here is often suggested to represent an increase in postsynaptic function of AMPARs (Malenka and Nicoll, 1999). Additionally, the increase in mEPSC amplitude strongly suggests that the increased AMPAR/NMDAR ratio after ethanol self-administration reflected an enhancement of postsynaptic AMPAR currents rather than a reduction in NMDAR currents, although we cannot completely rule out the latter possibility. The present results also agree with a previous study that found increased protein levels of the AMPAR subunit GluR1 in the VTA after chronic ethanol (Ortiz et al., 1995). Previous studies of VTA NMDAR function after chronic ethanol have produced mixed results, with evidence for increased NMDAR subunit expression (Ortiz et al., 1995) as well as decreased NMDA modulation of firing, although the latter result may reflect decreased basal firing after chronic ethanol rather than altered NMDA receptor function (Bailey et al., 1998). Thus, taken together, these results strongly suggest that AMPAR function was significantly enhanced by ethanol self-administration.

We also observed an increase in mEPSC frequency in the VTA following ethanol self-administration. While this could be due in part to an increase in presynaptic release probability, this seems unlikely, as PPRs were not altered by ethanol self-administration. Changes in mEPSC frequency are often observed without changes in release probability during evoked release (Cooke and Woolley, 2005; Ungless et al., 2001) and could possibly reflect an increase in the total number of excitatory synapses in the VTA following weeks of

voluntary ethanol intake, which could serve to further excite dopamine neurons and promote ethanol self-administration.

Enhancements in AMPAR function after ethanol self-administration might occur as a homeostatic neuroadaptation to neuronal inhibition, e.g., by ethanol activating GABA receptors or inhibiting NMDARs (Diamond and Gordon, 1997). However, a number of lines of evidence support the idea that exposure to ethanol can act directly on the VTA dopamine system (see Brodie et al., 2007; Gonzales et al., 2004). Additionally, numerous studies have suggested that excitability of VTA dopamine neurons is significantly reduced during the early stages of withdrawal from ethanol exposure (Bailey et al., 1998; Diana et al., 1993; Shen, 2003; Weiss et al., 1996; but see Brodie, 2002). Thus, increased AMPAR currents could represent a counter-adaptation to such periods of low dopamine cell activity. This increase in number and/or function of AMPAR following acute withdrawal from ethanol could act to increase motivation for ethanol. As AMPA can induce VTA neuron firing (Zhang et al., 1997), increased AMPAR function might produce an enhanced excitatory state that could also increase the motivation or reactivity toward rewarding-related stimuli. Consistent with this idea, previous studies have demonstrated that enhancing levels of the AMPAR subunit GluR1 in the VTA can modulate drug-related locomotor activity and place conditioning (Carlezon and Nestler, 2002). These observations support the idea that increased AMPAR function in the VTA could reflect a state of altered motivational processing to promote further drug-seeking behavior.

In summary, we have shown that voluntary ethanol intake enhanced postsynaptic AMPAR function, indicated by an increased AMPAR/NMDAR ratio and increased mEPSC amplitude. Increased VTA AMPAR function can critically regulate VTA neuron firing (Blythe et al., 2007; Canavier and Landry, 2006; Overton and Clark, 1997), and has been proposed to enhance the reinforcing and activating properties of addictive drugs such as cocaine and morphine (Carlezon and Nestler, 2002). Thus, the increased AMPAR function following weeks of voluntary ethanol consumption may facilitate ethanol seeking and intake, and thus represent an important and novel regulator of ethanol-directed motivation.

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REFERENCES

- Bailey CP, Manley SJ, Watson WP, Wonnacott S, Molleman A, Little HJ. Chronic ethanol administration alters activity in ventral tegmental area neurons after cessation of withdrawal hyperexcitability. *Brain Res* 1998;803:144–152. [PubMed: 9729345]
- Bellone C, Lüscher C. Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nat Neurosci* 2006;9:636–641. [PubMed: 16582902]
- Blythe SN, Atherton JF, Bevan MD. Synaptic activation of dendritic AMPA and NMDA receptors generates transient high-frequency firing in substantia nigra dopamine neurons in vitro. *J Neurophysiol* 2007;97:2837–2850. [PubMed: 17251363]
- Borgland SL, Taha SA, Sarti F, Fields HL, Bonci A. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. *Neuron* 2006;49:589–601. [PubMed: 16476667]
- Brodie MS. Increased ethanol excitation of dopaminergic neurons of the ventral tegmental area after chronic ethanol treatment. *Alcohol Clin Exp Res* 2002;26:1024–1030. [PubMed: 12170113]

- Brodie MS, Scholz A, Weiger TM, Dopico AM. Ethanol interactions with calcium-dependent potassium channels. *Alcohol Clin Exp Res* 2007;31:1625–1632. [PubMed: 17850640]
- Canavier CC, Landry RS. An increase in AMPA and a decrease in SK conductance increase burst firing by different mechanisms in a model of a dopamine neuron in vivo. *J Neurophysiol* 2006;96:2549–2563. [PubMed: 16885519]
- Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci Biobehav Rev* 2002;26:321–352. [PubMed: 12034134]
- Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci* 2002;25:610–615. [PubMed: 12446127]
- Churchill L, Swanson CJ, Urbina M, Kalivas PW. Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. *J Neurochem* 1999;72:2397–2403. [PubMed: 10349849]
- Cooke BM, Woolley CS. Sexually dimorphic synaptic organization of the medial amygdala. *J Neurosci* 2005;25:10759–10767. [PubMed: 16291949]
- Diamond I, Gordon AS. Cellular and molecular neuroscience of alcoholism. *Physiol Rev* 1997;77:1–20. [PubMed: 9016298]
- Diana M, Pistis M, Carboni S, Gessa GL, Rossetti ZL. Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. *Proc Natl Acad Sci U S A* 1993;90:7966–7969. [PubMed: 8367449]
- Dobrunz LE, Stevens CF. Heterogeneity of release probability, facilitation, and depletion at central synapses. *Neuron* 1997;18:995–1008. [PubMed: 9208866]
- Fields HL, Hjelmstad GO, Margolis EB, Nicola SM. Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu Rev Neurosci* 2007;30:289–316. [PubMed: 17376009]
- Fitzgerald LW, Ortiz J, Hamedani AG, Nestler EJ. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J Neurosci* 1996;16:274–282. [PubMed: 8613793]
- Gatto GJ, McBride WJ, Murphy JM, Lumeng L, Li TK. Ethanol self-infusion into the ventral tegmental area by alcohol-preferring rats. *Alcohol* 1994;11:557–564. [PubMed: 7865158]
- Gonzales RA, Job MO, Doyon WM. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. *Pharmacol Ther* 2004;103:121–146. [PubMed: 15369680]
- Hodge CW, Haraguchi M, Chappelle AM, Samson HH. Effects of ventral tegmental microinjections of the GABAA agonist muscimol on self-administration of ethanol and sucrose. *Pharmacol Biochem Behav* 1996;53:971–977. [PubMed: 8801605]
- Hodge CW, Haraguchi M, Erickson H, Samson HH. Ventral tegmental microinjections of quinpirole decrease ethanol and sucrose-reinforced responding. *Alcohol Clin Exp Res* 1993;17:370–375. [PubMed: 8098187]
- 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]
- Hopf FW, Martin M, Chen BT, Bowers MS, Mohamedi MM, Bonci A. Withdrawal from intermittent ethanol exposure increases probability of burst firing in VTA neurons in vitro. *J Neurophysiol* 2007;98:2297–2310. [PubMed: 17699688]
- Johnson SW, North RA. Two types of neurons in the rat ventral tegmental area and their synaptic inputs. *J Physiol* 1992;450:455–468. [PubMed: 1331427]
- Liu QS, Pu L, Poo MM. Repeated cocaine exposure *in vivo* facilitates LTP induction in midbrain dopamine neurons. *Nature* 2005;437:1027–1031. [PubMed: 16222299]
- Malenka RC, Nicoll RA. Long-term potentiation—a decade of progress? *Science* 1999;285:1870–1874. [PubMed: 10489359]
- Margolis EB, Lock H, Hjelmstad GO, Fields HL. The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? *J Physiol* 2006;577:907–924. [PubMed: 16959856]

- Ortiz J, Fitzgerald LW, Charlton M, Lane S, Trevisan L, Guitart X, Shoemaker W, Duman RS, Nestler EJ. Biochemical actions of chronic ethanol exposure in the mesolimbic dopamine system. *Synapse* 1995;21:289–298. [PubMed: 8869159]
- Overton PG, Clark D. Burst firing in midbrain dopaminergic neurons. *Brain Res Brain Res Rev* 1997;25:312–334. [PubMed: 9495561]
- Paxinos, G. *The Rat Nervous System Atlas*. Academic Press; New York: 1995.
- Perkel DJ, Nicoll RA. Evidence for all-or-none regulation of neurotransmitter release: implications for long-term potentiation. *J Physiol* 1993;471:481–500. [PubMed: 7907145]
- Rassnick S, Stinus L, Koob GF. The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat. *Brain Res* 1993;623:16–24. [PubMed: 8221085]
- Rodd-Henricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ. Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. *Psychopharmacology (Berl)* 2000;149:217–224. [PubMed: 10823401]
- Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* 2003;37:577–582. [PubMed: 12597856]
- Samson HH, Chappell A, Slawecki C, Hodge C. The effects of microinjection of d-amphetamine into the n. accumbens during the late maintenance phase of an ethanol consumption bout. *Pharmacol Biochem Behav* 1999;63:159–165. [PubMed: 10340537]
- Samson, SG.; Hodge, CW. Neurobehavioral regulation of ethanol intake. In: Deitrich, RA.; Erwin, VG., editors. *Pharmacological Effects of Ethanol on the Nervous System*. CRC Press; New York: 1996. p. 203-226.
- Sarti F, Borgland SL, Kharazia VN, Bonci A. Acute cocaine exposure alters spine density and long-term potentiation in the ventral tegmental area. *Eur J Neurosci* 2007;26:749–756. [PubMed: 17686047]
- Shen RY. Ethanol withdrawal reduces the number of spontaneously active ventral tegmental area dopamine neurons in conscious animals. *J Pharmacol Exp Ther* 2003;307:566–572. [PubMed: 12966156]
- Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. *Trends Neurosci* 1999;22:521–527. [PubMed: 10529820]
- Steensland P, Simms JA, Holgate J, Richards JK, Bartlett SE. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, selectively decreases ethanol consumption and seeking. *Proc Natl Acad Sci U S A* 2007;104:12518–12523. [PubMed: 17626178]
- Ungless MA, Whistler JL, Malenka RC, Bonci A. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature* 2001;411:583–587. [PubMed: 11385572]
- Weiss F, Parsons LH, Schulteis G, Hyttia P, Lorang MT, Bloom FE, Koob GF. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neurosci* 1996;16:3474–3485. [PubMed: 8627380]
- Weiss F, Porrino LJ. Behavioral neurobiology of alcohol addiction: recent advances and challenges. *J Neurosci* 2002;22:3332–3337. [PubMed: 11978808]
- Wise RA. Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia* 1973;29:203–210. [PubMed: 4702273]
- Wise RA. Dopamine, learning and motivation. *Nat Rev Neurosci* 2004;5:483–494. [PubMed: 15152198]
- You ZB, Wang B, Zitzman D, Azari S, Wise RA. A role for conditioned ventral tegmental area glutamate release in cocaine seeking. *J Neurosci* 2007;27:10546–10555. [PubMed: 17898226]
- Zhang XF, Hu XT, White FJ, Wolf ME. Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *J Pharmacol Exp Ther* 1997;281:699–706. [PubMed: 9152375]

Fig. 1.

Voluntary ethanol intake under an intermittent access, 2-bottle choice paradigm, 1 bottle with 20% ethanol and the other with water. **(A)** Model of intermittent ethanol access paradigm. **(B)** Animals showed an escalation of ethanol consumed in a 24-hour access period across weeks of access to ethanol; animal body weight was determined once per week, and the daily volume of ethanol consumed in a given week averaged before the gram-per-kilogram intake was determined. **(C)** No change in water intake across weeks. **(D, E)** Preference for ethanol in the first 30 minutes of access increased across weeks of ethanol intake. **(F)** No change in gram-per-kilogram ethanol intake in the first 30 minutes of access across weeks.

Fig. 2.

Ventral tegmental area neuron AMPAR/NMDAR ratios, determined at a holding potential of +40 mV, were significantly enhanced by ethanol self-administration. Example traces of compound currents (left) and AMPAR and NMDAR currents (right) from (A) naïve and (B) ethanol self-administration animals; AMPAR currents were determined in the presence of the NMDAR antagonist AP5 (50 μ M), and NMDAR currents were determined by subtracting the AMPAR current from the total current evoked before AP5 addition. (C) The ratio of peak AMPAR relative to NMDAR currents was significantly enhanced by ethanol intake (* p = 0.0079).

Fig. 3. Ventral tegmental area neuron paired-pulse ratios, the ratio of the second evoked EPSC over the first evoked EPSC (50 ms apart, -70 mV holding potential) was not altered by ethanol self-administration, suggesting no change in evoked glutamate release. Example traces of paired-pulse currents evoked in (A) naïve and (B) ethanol self-administration animals. (C) The paired-pulse ratio was not altered by ethanol intake.

Fig. 4. Ventral tegmental area neuron miniature EPSC (mEPSC) amplitude and frequency were significantly enhanced by ethanol self-administration. Example traces of mEPSCs in (A) naive and (B) ethanol self-administration animals. Cumulative probability distribution from (C) example cells and (D) grouped data showing that the mEPSC amplitude was significantly enhanced by ethanol intake (* $p = 0.046$), suggesting an increase in postsynaptic AMPAR function. Cumulative probability distribution from (E) example cells and (F) grouped data showing that the mEPSC frequency was significantly enhanced by ethanol intake (* $p = 0.021$), suggesting an increase in spontaneous glutamate release.