Published in final edited form as:

Alcohol. 2010 May ; 44(3): 257–264. doi:10.1016/j.alcohol.2010.02.009.

LIMITED ACCESS TO ETHANOL INCREASES THE NUMBER OF SPONTANEOUSLY ACTIVE DOPAMINE NEURONS IN THE POSTERIOR VTA OF NONDEPENDENT P RATS

Sandra L. Morzorati, Rita L. Marunde, and David Downey

Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA

Abstract

Microdialysis experiments in alcohol-preferring (P) rats have shown that chronic ethanol exposure increases extracellular levels of dopamine in the nucleus accumbens. Since dopamine neuronal activity contributes to the regulation of dopamine overflow in terminal regions, we hypothesized that posterior VTA DA neuron activity (firing frequency, burst activity and/or the number of spontaneously active DA neurons) would be increased in P rats consuming ethanol compared with P rats consuming only water. In vivo electrophysiological techniques were used to evaluate the activity of single dopamine neurons in the posterior VTA. Our findings show that voluntary ethanol intake by nondependent P rats significantly increased the number of spontaneously active dopamine neurons in the posterior VTA compared with P rats that consumed only water. Firing frequency and burst activity did not differ between the two groups. These results suggest that adaptive changes occur in the mesolimbic DA system of nondependent P rats to increase the excitability of posterior VTA DA neurons and enhance DA release from nerve terminals in the nucleus accumbens.

Keywords

dopamine neurons; ventral tegmental area; alcohol-preferring (P) rats; chronic ethanol consumption; spontaneously active dopamine neurons

Introduction

The mesolimbic dopamine (DA) system has been implicated in mediating the rewarding properties of various drugs of abuse, including ethanol (Koob and Bloom, 1988; Wise, 1987). This pathway consists of DAergic neurons in the ventral tegmental area (VTA) that project to limbic and cortical forebrain structures, most notably the nucleus accumbens, prefrontal cortex and extended amygdala (Alheid et al., 1995; Swanson, 1982). Drugs misused by humans, including ethanol, excite VTA DA neurons Appel et al., 2003; Brodie et al., 1990, 1999; Gessa et al., 1985) and elevate DA levels in the nucleus accumbens (DiChiara and Imperato, 1985; Imperato and DiChiara, 1986; Melendez et al., 2002; Weiss

Corresponding author: Sandra L. Morzorati, Ph.D., Indiana University School of Medicine, Institute of Psychiatric Research, 791 Union Drive, Indianapolis, IN 46202. Phone: (317) 274-7815, FAX: (317) 274-1365, smorzora@iupui.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al., 1993; Yoshimoto et al., 1991). These actions are thought to mediate ethanol's rewarding effects (Koob and Weiss, 1992).

Approximately half of midbrain DA neurons are spontaneously active and fire either in a slow irregular pattern of single action potentials or burst fire, with single spike activity interspersed with bursts consisting of 2 or more action potentials (Chiodo, 1988). The remaining DA neurons are inactive because their membrane potentials are hyperpolarized (Chiodo, 1988). DA release in terminal areas is regulated, in part, by firing frequency and burst activity (Grace, 1991). It was shown that burst firing increased DA release to a greater degree than action potentials delivered at the same frequency but with a constant inter-stimulus interval (Gonon, 1988). DA release is also affected by changes in the number of DA neurons that are spontaneously active (Floresco et al., 2003; Moore et al., 1998).

Through selective breeding, investigators at Indiana University have established lines of rats with high and low alcohol preference: the alcohol-preferring P and alcohol-nonpreferring NP lines of rats (Lumeng et al., 1977). The P line has been well characterized and shown to substantially meet the criteria for an animal model of alcoholism (Cicero, 1980). Specifically, P rats voluntarily consume intoxicating amounts of ethanol for its pharmacological effects, work to obtain ethanol, self-infuse ethanol directly into the VTA and develop tolerance and dependence following excessive ethanol drinking for a prolonged time (Gatto et al., 1994; Murphy et al., 1986, 1989; Rodd et al., 2005; Waller et al., 1982).

Repeated ethanol administration (Smith and Weiss, 1999) and chronic ethanol consumption in limited-access (Engleman et al., 2000, 2003) and free-choice (Thielen et al., 2004) situations increased extracellular levels of DA in the nucleus accumbens of P rats approximately 12–24 hrs after the last ethanol exposure. In the study by Thielen et al. (2004), microdialysis parameters indicative of DA transporter function did not differ between alcohol-drinking and alcohol-naïve P rats, indicating that increased basal extracellular DA concentrations in the nucleus accumbens of alcohol-drinking rats were due to increased DA neuronal activity rather than decreased DA reuptake. The present study was undertaken to determine if DA neuron activity in the posterior VTA (pVTA) is altered in P rats chronically consuming ethanol. We hypothesized that pVTA DA neuron activity (firing frequency, burst activity and/or the number of spontaneously active DA neurons) would be increased in P rats consuming ethanol for 6 weeks compared with P rats consuming only water. The pVTA was targeted because intracranial self-administration studies showed that rats self-administer ethanol into the posterior, but not the anterior VTA, suggesting that this region is more sensitive to the reinforcing effects of ethanol (Rodd et al., 2004, 2005; Rodd-Hendricks et al., 2000).

Materials and Methods

Animals

Adult female P rats were obtained from the Indiana University Alcohol Research Center and divided into two groups: alcohol-drinking and alcohol-naïve. At the time of the experiments, the rats weighed 250 – 300 g and had been selectively bred for 54–56 generations. Female P rats were used because they served as subjects in the studies reporting increased accumbal DA levels with chronic ethanol intake (Engleman et al., 2000, 2003; Thielen et al., 2004). Rats were housed singly in clear Plexiglas cages (26 × 46 × 38 cm, W × L × H) and allowed to acclimate for 7 days to a temperature- and humidity-controlled environment with a 12-hr reversed light cycle (lights off at 0900). The animal facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were pre-approved by the Institutional Animal Care and Use Committee

in strict compliance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996).

Alcohol-Drinking Procedure

The alcohol-drinking rats were initially given free-choice access to water and 15% ethanol (v/v) for 7 days before limiting ethanol availability to a daily (including weekends) 1-hr access period at the beginning of the dark cycle. Ethanol intake was recorded following each access period by weighing the ethanol bottle to the nearest 0.1 g and subtracting this value from the pre-access weight. Body weights were recorded at the same time and ethanol intake was converted to average grams of ethanol per kilogram of body weight for that hour. The alcohol-drinking rats were maintained on limited access for 6 weeks; the alcohol-naïve rats drank only water. Standard laboratory chow was available ad libitum to all rats. Experiments were performed in alcohol-drinking rats 24 hrs after the last access session. During the last week of drinking and prior to experimentation, the alcohol-drinking rats were observed for signs of physical dependence such as stiff tail, hyper-reactivity, tremors, aimless locomotion or stereotypical body movements (Waller et al., 1982). In addition, daily vaginal smears were obtained to determine the stage of estrus. Rats were used only when circulating estrogen levels were low because estrogen has been shown to modulate DA neuron function, perhaps through interaction with β estrogen receptors (Chiodo and Caggiula, 1983; Creutz and Kritzer, 2002; Thompson and Moss, 1994). All experiments were conducted at approximately the same time each day. Alcohol-drinking and alcohol-naïve rats were alternately selected for experimentation.

Surgery

General anesthesia was induced and maintained during the surgical procedures with an isoflurane/air mixture. All incision sites and pressure points were infiltrated with a long-acting local anesthetic (2% mepivacaine hydrochloride). A femoral vein was cannulated for the intravenous (iv) administration of fluids. The trachea was intubated with PE260 tubing via an incision on the ventral neck and the rat was positioned in a stereotaxic frame with the skull level. A large craniotomy was made overlying the right VTA and the meninges were carefully retracted. Indifferent and ground screws were threaded into the contralateral skull.

After all surgical procedures were completed, a paralyzing agent, gallamine triethiodide (25 mg/kg, iv), was administered to produce complete paralysis of all skeletal muscles. The isoflurane was discontinued and the tracheal cannula was connected to a volume-controlled respirator. An ophthalmic ointment was placed in the rat's eyes to prevent drying. The rat was allowed to recover from anesthesia for 30 min before recordings were begun. Gallamine was administered at regular intervals throughout the experiment. Body temperature was maintained with a water-circulating heating pad. A paralyzed rather than an anesthetized rat was chosen because general anesthesia was shown to significantly affect DA neuron activity (Kelland et al., 1989, 1990; Windels and Kiyatkin, 2006).

Recording

The exposed skull was marked with a grid delineating the rostrocaudal and mediolateral extents of the pVTA (from bregma, P: 5.3–6.0 mm; from midline, L: 1.3–1.7 mm) (Paxinos and Watson, 1998). Glass micropipettes filled with 2 M NaCl (in vitro impedances 2.0–5.0 M Ω at 135 Hz) were used to record extracellular action potentials (from the brain's surface, V: 7.5–9.5 mm, 6° angle). The number of spontaneously active DA neurons was determined by the cells per track sampling method proposed by Bunney and Grace (1978) and used to compare populations of DA neurons in control and drug-treated animals (Chiodo and Bunney, 1983, Diana et al., 1996, Shen, 2003, Shen and Chiodo, 1993, Shen et al., 2007, White and Wang, 1983). For this study, the recording electrode was systematically lowered

8 times through a stereotaxically defined area covering the pVTA (mm posterior from bregma/mm lateral from midline: 5.3/1.7, 5.5/1.3, 5.5/1.5, 5.5/1.7, 5.7/1.3, 5.7/1.5, 5.7/1.7, 6.0/1.7). The number of DA neurons encountered using 8 tracks is comparable to previous data using 12 tracks (Morzorati and Marunde, 2006). The recording electrode was lowered with the aid of a binocular microscope mounted to the stereotaxic frame. Each electrode descent was made with a uniform, slow speed to ensure that even slowly firing spontaneously active DA neurons were counted. It has been our experience that tissue accumulates on the tip of the electrode as it traverses the brain, making its recording characteristics less than optimal. For this reason, an electrode was exchanged for a newly-prepared one every 2 tracks. DA neurons were identified on the basis of previously established criteria (Freeman and Bunney, 1987; Grace and Bunney, 1983): broad (≥ 2.5 msec), triphasic (+/-/+) action potential waveforms with a notch on the initial rising phase and slow irregular firing pattern. Whenever possible, a neuron was further characterized as DAergic by a decrease in firing frequency after the administration of quinpirole (50 $\mu\text{g/kg}$, iv), a D_2 receptor agonist, followed by an increase in firing frequency after the administration of eticlopride (200 $\mu\text{g/kg}$, iv), a D_2 receptor antagonist.

Signals from the recording electrode and indifferent screw were differentially amplified and filtered with a bandpass of 300–10,000 Hz, displayed on an oscilloscope and monitored on an audio amplifier. The signals were also routed through an analog-to-digital converter (Cambridge Electronic Design, CED, Cambridge, England) interfaced to a PC running Spike2 (CED) software, displayed on the computer monitor and selected for analysis using a template matching routine. The numbers of spontaneously active DA neurons encountered per electrode descent (7.5 – 9.5 mm) were counted. In addition, the activity of 4–5 DA neurons per rat was recorded for 5–15 continuous minutes for analyses of firing frequency and burst activity (number of bursts and percentage of action potentials in bursts). These analyses were performed off-line on 500 consecutive action potentials (Grace and Bunney, 1984). A DA neuron was considered bursting if $\geq 5\%$ of its action potentials occurred in bursts. Burst onset was defined as the occurrence of two spikes with an interspike interval of ≤ 80 msec and burst termination as the occurrence of an interspike interval of ≥ 160 msec (Grace and Bunney, 1984).

Histology

At the end of the experiment, the rat was deeply anesthetized. A glass micropipette containing 0.5% Fast Green dye in 2 M NaCl was lowered into the rostro-most and caudal-most extents of the recording area and the dye was ejected with positive current (35 μA for 14 min). The rat was decapitated and the brain removed. Serial frozen sections (40 μm) were stained with cresyl violet. The location of the dye deposits and any marks made by the electrode penetrations were determined using light microscopy. Only data from rats in which the pVTA was sampled were used in the analyses.

Statistical Analyses

Intergroup comparisons of firing frequencies, burst parameters and the number of spontaneously active DA neurons encountered per electrode track were performed using separate analyses of variance (ANOVA). Statistical significance was designated as $\alpha = 0.05$.

Results

Ethanol intakes for the alcohol-drinking rats were stable over the 6-week exposure and averaged 1.3 g/kg during the 1-hr access period. Under these conditions, alcohol-drinking P rats did not show overt signs of alcohol dependence or withdrawal. Since estrogen was shown to modulate DA neuron function (Chiodo and Caggiula, 1983; Creutz and Kritzer,

2002; Thompson and Moss, 1994), all electrophysiological recordings were scheduled to avoid proestrus (the stage of estrus in which circulating levels of estrogen are highest). Data are presented for 9 alcohol-drinking rats and 8 alcohol-naïve rats in which the recording area was histologically verified as being in the pVTA. DAergic neurons, recorded from 7.9 mm to 9.3 mm below the surface of the brain, had long-duration (3.7 ± 0.1 ms, mean $\pm$ S.E.M.) triphasic action potentials and slow firing frequencies (3.5 ± 0.2 spikes/s, mean $\pm$ S.E.M.). A typical waveform of a pVTA DA neuron is shown in Figure 1A. The DAergic nature of that neuron was further evidenced by a decrease and increase in firing frequency following infusions of quinpirole and eticlopride, a D_2 receptor agonist and antagonist, respectively (Figure 1B).

Data acquired from the alcohol-drinking and alcohol-naïve P rats is summarized in Table 1. P rats consuming ethanol for 6 weeks in a limited access paradigm had a significantly greater number of spontaneously active DA neurons per electrode track through the pVTA compared with alcohol-naïve P rats ($F(1, 15) = 19.09$, $p = 0.0001$). In both groups of rats, the maximum number of DA neurons encountered in a single electrode track was 5; the minimum number was 1. In each rat, the activity of 4–5 DA neurons was acquired for analysis of firing frequency and burst activity (the number of bursts and the percent of action potentials in bursts). Recordings from 37 DA neurons in the alcohol-drinking rats and 33 DA neurons in the alcohol-naïve rats showed no difference in firing frequencies. DA neurons with 5% or more of their action potentials in bursts were considered bursting. Using this criterion, approximately 93% of the DAergic neurons displayed a burst pattern of activity (34 of 37 neurons in alcohol-drinking rats and 31 of 33 neurons in alcohol-naïve rats). A traditional burst analysis on 500 consecutive action potentials (Grace and Bunney, 1984) showed the number of bursts and the percentage of action potentials in bursts did not differ between alcohol-drinking and alcohol-naïve P rats (Table 1).

Discussion

The results of the present study reveal that voluntary ethanol intake by nondependent P rats over a 6-week period significantly increased the number of spontaneously active DA neurons in the pVTA compared with P rats that consumed only water. The number of spontaneously active pVTA DA neurons per track reported here in alcohol-naïve P rats is similar to that reported previously (Morzorati and Marunde, 2006) (2.7 ± 0.1 DA neurons per track reported here; 2.6 ± 0.1 reported previously, mean $\pm$ S.E.M.). DA neuronal activity contributes to the regulation of DA overflow in terminal areas. Thus, these results suggest that the increased levels of accumbal DA observed in microdialysis studies following chronic ethanol exposure (Engleman et al., 2000, 2003; Smith and Weiss, 1999; Thielen et al., 2004) are due, at least in part, to an increase in the number of DA neurons that are spontaneously active. Firing frequency and burst activity did not differ between alcohol-drinking and alcohol-naïve P rats. These data are consistent with reports indicating that extracellular DA levels in terminal regions evaluated by neurochemical methods correlates strongly with the number of spontaneously active DA neurons but not with the average firing frequency or burst activity of individual DA neurons (Floresco et al., 2003, Moore et al., 1998). Those authors further posit that increased firing frequency or burst activity produces a fast phasic release of DA that is limited to the synapse and is rapidly removed (in part by reuptake processes) while increasing the overall number of DA neurons that are firing produces a slow tonic release of DA that is more spatially diffuse and can be readily detected by microdialysis (Floresco et al., 2003).

In general, activity of VTA DA neurons is orchestrated by a balance of excitatory and inhibitory mechanisms. In inactive DA neurons, the balance swings towards more inhibitory influences, bringing the membrane potential further from threshold for firing. On the other

hand, the balance favors excitatory influences in spontaneously active DA neurons. It has been shown that VTA DA neurons receive inhibitory GABAergic as well as excitatory glutaminergic afferents (Christie et al., 1985; Clement and Grant, 1990; Grace, 1987) and a portion of these afferents synapse on GABA_A and NMDA receptors, respectively (Gariano and Groves, 1988; Johnson and North, 1992; Kitai et al., 1999). GABA_A and NMDA receptors belong to the ligand-gated ion channel superfamily and are an important site of ethanol action in the CNS. In the alcohol-drinking P rats, chronic exposure to ethanol may have produced adaptive changes that decreased the sensitivities of GABA_A receptors and/or increased the sensitivities of NMDA receptors. Consistent with this notion are reports indicating that chronic exposure to high doses of ethanol produced region-specific adaptive changes in GABA_A and NMDA receptor function (Allan and Harris, 1987; Kumari and Ticku, 2000; Kang et al., 1996, 1998) that may have resulted from alterations in receptor subunit composition, posttranslational modification, subcellular localization (internalization/externalization), synaptic localization (synaptic/extrasynaptic) and/or responses to neurosteroid secretion (Carpenter-Hyland et al., 2004; Follesa and Ticku, 1995; Holter et al., 2000; Kumar et al., 2004; Tsai and Coyle, 1998). More specifically, GABA and NMDA receptors located on VTA DA neurons were evaluated in mice injected with ethanol and devoid of overt signs of withdrawal hyperactivity at the time of testing (Brodie, 2002). GABA-mediated inhibitions of DA neuronal activity were reduced in ethanol-treated mice compared with ethanol-naïve mice. There were no differences in NMDA-mediated excitations of VTA DA neurons.

VTA DA neurons express receptors for their own neurotransmitter in somatodendritic and axon terminal regions. Stimulation of somatodendritic autoreceptors inhibits the spontaneous activity of DA neurons (Bunney et al., 1973; Groves et al., 1975), whereas stimulation of axonal autoreceptors reduces the excitability of DAergic terminals and inhibits DA synthesis and release (Starke et al., 1989; Tepper et al., 1987; Wolf and Roth, 1990). Microdialysis experiments have suggested that terminal autoreceptor sensitivity was decreased in P rats chronically consuming ethanol in limited and continuous access paradigms (Thielen et al., 2004; Engleman et al., 2003). Hypofunctioning terminal D₂ autoreceptors would lead to increased activity of VTA DA neurons, consistent with our current findings. Likewise, if the sensitivity of somatodendritic D₂ receptors is reduced with chronic ethanol intake, like it is for terminal D₂ receptors, then the activity of pVTA DA neurons would increase (again consistent with our current findings) and result in elevated accumbal DA.

Alternatively, or in addition, the excitability of pVTA DA neurons may reflect alterations in neurotransmitter release from afferents. Activity of neurons in the prefrontal cortex, ventral pallidum and/or nucleus accumbens that project to the pVTA may have been altered with chronic ethanol exposure. This, along with possible changes in transmitter release and/or reuptake, would produce sustained alterations in basal extracellular transmitter levels, affecting receptor sensitivities or simply changing the amount of transmitter to which the receptors are exposed. In either situation (alteration of receptor sensitivities or of afferent tone), pVTA DA neurons would be more excitable with chronic ethanol intake and DA neurons that were once inactive may now fire spontaneously.

In the present study, all recordings were obtained from dopamine neurons in the pVTA (from bregma, posterior 5.3–6.0 mm). Considerable evidence has accumulated to indicate that the posterior and anterior VTA and their accumbal projections are functionally heterogeneous. A large number of dopaminergic axons from the posterior VTA project to the medial nucleus accumbens shell, whereas dopaminergic axons from the anterior VTA project to the nucleus accumbens core and lateral nucleus accumbens shell (Ikemoto, 2007). Activation of the pVTA - medial shell pathway mediates the reinforcing effects of abused

drugs. Rats readily self-administer ethanol and its metabolite acetaldehyde (Rodd et al., 2004; Rodd-Hendricks et al., 2000; Rodd-Hendricks et al., 2002), nicotine (Ikemoto et al., 2006), carbachol (Ikemoto and Wise, 2002), opiates (Zangen et al., 2002), cannabinoids (Zangen et al., 2006) and cocaine (Rodd et al., 2005) into the posterior VTA, but not the anterior VTA or sites adjacent to the pVTA.

The results of the present study are in contrast to those of others who reported a reduction in the number of spontaneously active VTA DA neurons with chronic ethanol exposure in Sprague-Dawley rats (Diana et al., 1996, Shen, 2003, Shen and Chiodo, 1993, Shen et al., 2007). In addition, Diana et al. (1993) reported that extracellular DA levels in the nucleus accumbens were reduced in Sprague-Dawley rats chronically administered ethanol. This, again, is in contrast to the findings reported by Engleman et al. (2000, 2003), Thielen et al. (2004) and Smith and Weiss (1999) in P rats. Differences in the magnitude (amount and duration) of ethanol exposure appear to underlie the discrepancies. In the previous studies, ethanol was administered in high doses to produce dependence and withdrawal. In the present study, ethanol was self-administered in moderate amounts and no overt signs of dependence and withdrawal were observed. Taken together the data suggest that the activity of the mesolimbic DA system is increased with exposure to moderate amounts of ethanol and decreased when ethanol exposure is high enough to produce dependence. This notion is supported by Weiss et al. (1996) who reported that extracellular DA levels in the nucleus accumbens were elevated in ethanol-nondependent Wistar rats but reduced in ethanol-dependent rats undergoing ethanol withdrawal as compared with rats that were ethanol-naïve.

Findings of the present study may have important implications for the development of alcoholism. Moderate ethanol intake produces adaptive changes in the pVTA that may maintain ethanol consumption. Changes are manifested by an increase in the number of pVTA DA neurons that fire spontaneously and increased extracellular DA levels in the nucleus accumbens. At this point, the organism is not physically ethanol-dependent. Prolonged consumption, possibly associated with cycles of ethanol deprivation and re-exposure, may eventually lead to excessive ethanol intake and further changes in the pVTA to compensate for the continual ethanol-mediated effects on DA neurons. These changes may be manifested by a decrease in the number of VTA DA neurons that fire spontaneously and decreased DA levels in the nucleus accumbens when ethanol is withdrawn. Drinking during withdrawal restores accumbal DA levels. At this point, the organism is addicted to and dependent on ethanol.

Acknowledgments

The authors wish to thank Dr. Larry Lumeng for supplying the selectively bred P rats and Ravi Rayala for developing custom scripts for data acquisition and analysis. This work was supported in part by grants from the National Institute on Alcohol Abuse and Alcoholism: AA12832 and AA07611.

References

- Alheid, GF.; De Olmos, JS.; Beltramino, CA. Amygdala and extended amygdala. In: Paxinos, G., editor. *The Rat Nervous System*. 2. San Diego: Academic Press; 1995. p. 495-578.
- Allan AM, Harris RA. Involvement of neuronal chloride channel in ethanol intoxication, tolerance, and dependence. *Recent Dev Alcohol* 1987;5:313–325. [PubMed: 2436258]
- Appel SB, Liu Z, Mcelvain A, Brodie MS. Ethanol excitation of dopaminergic ventral tegmental area neurons is blocked by quinidine. *J Pharmacol Exp Ther* 2003;306:437–446. [PubMed: 12721326]
- 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, Pesold C, Appel SB. Ethanol directly excites dopaminergic ventral tegmental area reward neurons. *Alcohol Clin Exp Res* 1999;23:1848–1852. [PubMed: 10591603]
- Brodie MS, Shefner SA, Dunwiddie TV. Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Res* 1990;508:65–69. [PubMed: 2337793]
- Bunney BS, Aghajanian GK, Roth RH. Comparison of effects of L-dopa, amphetamine and apomorphine on firing rate of rat dopaminergic neurons. *Nature New Biology* 1973;245:123–125.
- Bunney BS, Grace AA. Acute and chronic haloperidol treatment: comparisons of effect on nigral dopaminergic activity. *Life Sci* 1978;23:1715–1728. [PubMed: 31529]
- 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]
- Chiodo LA. Dopamine-containing neurons in the mammalian central nervous system: electrophysiology and pharmacology. *Neurosci Biobehav Rev* 1988;12:49–91. [PubMed: 3287242]
- Chiodo LA, Bunney BS. Typical and atypical neuroleptics; Differential effects of chronic administration on the activity of A9 and A10 midbrain dopaminergic neurons. *J Neurosci* 1983;3:1607–1619. [PubMed: 6135762]
- Chiodo LA, Caggiula AR. Substantia nigra dopamine neurons: alterations in basal discharge rates and autoreceptor sensitivity induced by estrogen. *Neuropharmacology* 1983;22:593–599. [PubMed: 6877536]
- Christie MJ, Bridge S, James LB, Beart PM. Excitotoxic lesions suggest an aspartatergic projection from the medial prefrontal cortex to the ventral tegmental area. *Brain Res* 1985;333:169–172. [PubMed: 2859910]
- Cicero TJ. Animal models of alcoholism. In: Eriksson, K.; Sinclair, JD.; Kiianmaa, K., editors. *Animal Models in Alcohol Research*. New York: Academic Press; 1980. p. 99–117.
- Clement JR, Grant S. Glutamate-like immunoreactivity in neurons of the rat laterodorsal and pedunculopontine nucleus in the rat. *Neurosci Lett* 1990;120:70–73. [PubMed: 2293096]
- Creutz LM, Kritzer MF. Estrogen receptor- β immunoreactivity in the midbrain of adult rats: regional, subregional, and cellular localization in the A10, A9, and A8 dopamine cell groups. *J Comp Neurol* 2002;446:288–300. [PubMed: 11932944]
- 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 USA* 1993;90:7966–7969. [PubMed: 8367449]
- Diana M, Pistis M, Muntoni A, Gessa G. Mesolimbic dopaminergic reduction outlasts ethanol withdrawal syndrome: Evidence of protracted abstinence. *Neuroscience* 1996;71:411–415. [PubMed: 9053796]
- DiChiara G, Imperato A. Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. *Eur J Pharmacol* 1985;115:131–132. [PubMed: 4043232]
- Engleman EA, McBride WJ, Li TK, Lumeng L, Murphy JM. Ethanol drinking experience attenuates (–)sulpiride-induced increases in extracellular dopamine levels in the nucleus accumbens of alcohol-preferring (P) rats. *Alcohol Clin Exp Res* 2003;27:424–431. [PubMed: 12658107]
- Engleman EA, McBride WJ, Wilber AA, Shaikh SR, Eha RD, Lumeng L, Li T-K, Murphy JM. Reverse microdialysis of a dopamine uptake inhibitor in the nucleus accumbens of alcohol-preferring rats; effects on dialysate dopamine levels and ethanol intake. *Alcohol Clin Exp Res* 2000;24:795–801. [PubMed: 10888067]
- Floresco SB, West AR, Ash B, Moore H, Grace AA. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nature Neurosci* 2003;6:968–973. [PubMed: 12897785]
- Follesa P, Ticku MK. Chronic ethanol treatment differentially regulates NMDA receptor subunit mRNA expression in rat brain. *Mol Brain Res* 1995;29:99–106. [PubMed: 7770006]
- Freeman AS, Bunney BS. Activity of A9 and A10 dopaminergic neurons in unrestrained rats: Further characterization and effects of apomorphine and cholecystokinin. *Brain Res* 1987;405:46–55. [PubMed: 3032350]

- Gariano RF, Groves PM. Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. *Brain Res* 1988;462:194–198. [PubMed: 3179734]
- 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]
- Gessa GL, Muntoni F, Collu M, Vargiu L, Mereu G. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Res* 1985;348:201–203. [PubMed: 2998561]
- Gonon F. Nonlinear relationship between impulse flow and dopamine released by rat midbrain neurons as studied by in vivo electrochemistry. *Neuroscience* 1988;24:19–28. [PubMed: 3368048]
- Grace, AA. The regulation of dopamine neuron activity as determined by in vivo and in vitro intracellular recordings. In: Chiodo, LA.; Freeman, AS., editors. *Neurophysiology of Dopaminergic Systems – Current Status and Clinical Perspectives*. Detroit, MI: Lakeshore Publishing; 1987. p. 1-66.
- Grace AA. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience* 1991;41:1–24. [PubMed: 1676137]
- Grace AA, Bunney BS. Intracellular and extracellular electrophysiology of nigral dopaminergic neurons. I. Identification and characterization. *Neuroscience* 1983;10:301–315. [PubMed: 6633863]
- Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci* 1984;4:2877–2890. [PubMed: 6150071]
- Groves PM, Wilson CJ, Young SJ, Rebec GV. Self-inhibition by dopaminergic neurons. *Science* 1975;190:522–529. [PubMed: 242074]
- Holter SM, Danysz W, Spanagel R. Novel uncompetitive N-methyl-D-aspartate (NMDA)-receptor antagonist MRZ 2/579 suppresses ethanol intake in long-term ethanol-experienced rats and generalizes to ethanol cue in drug discrimination procedure. *J Pharmacol Exp Ther* 2000;292:545–552. [PubMed: 10640291]
- Ikemoto S. Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res Rev* 2007;56:27–78. [PubMed: 17574681]
- Ikemoto S, Qin M, Liu ZH. Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. *J Neurosci* 2006;26:723–730. [PubMed: 16421292]
- Ikemoto S, Wise RA. Rewarding effects of the cholinergic agents carbachol and neostigmine in the posterior ventral area. *J Neurosci* 2002;22:9895–9904. [PubMed: 12427846]
- Imperato A, DiChiara G. Preferential stimulation of dopamine release in the n. accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther* 1986;238:219–228. [PubMed: 3761194]
- Johnson SW, North RA. Two types of neurons in the rat ventral tegmental area and their synaptic inputs. *J Physiol (Lond)* 1992;450:455–468. [PubMed: 1331427]
- Kang MH, Spigelman I, Olsen RW. Alteration in the sensitivity of GABA(A) receptors to allosteric modulatory drugs in rat hippocampus after chronic intermittent ethanol treatment. *Alcohol Clin Exp Res* 1998;22:2165–2173. [PubMed: 9884165]
- Kang MH, Spigelman I, Sapp DD, Olsen RW. Persistent reduction of GABA(A) receptor-mediated inhibition in rat hippocampus after chronic intermittent ethanol treatment. *Brain Res* 1996;709:221–228. [PubMed: 8833758]
- Kelland MD, Chiodo LA, Freeman AS. Anesthetic influences on the basal activity and pharmacological responsiveness of nigrostriatal dopamine neurons. *Synapse* 1990;3:207–209. [PubMed: 1978421]
- Kelland MD, Freeman AS, Chiodo LA. Chloral hydrate anesthesia alters the responsiveness of identified midbrain dopamine neurons to dopamine agonist administration. *Synapse* 1989;3:30–37.
- Kitai ST, Shepard PD, Callaway JC, Scroggs R. Afferent modulation of dopamine neuron firing patterns. *Curr Opin Neurobiol* 1999;9:690–697. [PubMed: 10607649]
- Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. *Science* 1988;242:715–723. [PubMed: 2903550]

- Koob GF, Weiss F. Neuropharmacology of cocaine and ethanol dependence. *Recent Dev Alcohol* 1992;10:201–233. [PubMed: 1350359]
- Kumar S, Fleming RL, Morrow AL. Ethanol regulation of γ -aminobutyric acid_A receptors: genomic and nongenomic mechanisms. *Pharmacol Ther* 2004;101:211–226.
- Kumari M, Ticku MK. Regulation of NMDA receptors by ethanol. *Prog Drug Res* 2000;54:152–189. [PubMed: 10857388]
- Lumeng, L.; Hawkins, DT.; Li, T-K. New strains of rats with alcohol preference and nonpreference. In: Thurman, RG.; Williamson, JR.; Drott, H.; Chance, B., editors. *Alcohol and Metabolizing Systems*. Vol. 3. New York: Academic Press; 1977. p. 537-544.
- Melendez RI, Rodd-Hendricks ZA, Engleman EA, Li T-K, McBride WJ, Murphy JM. Microdialysis of dopamine in the nucleus accumbens of alcohol preferring (P) rats during anticipation and operant self-administration of ethanol. *Alcohol Clin Exp Res* 2002;26:318–325. [PubMed: 11923583]
- Moore H, Todd CL, Grace AA. Striatal extracellular dopamine levels in rats with haloperidol-induced depolarization block of substantia nigra dopamine neurons. *J Neurosci* 1998;18:5068–5077. [PubMed: 9634572]
- Morzorati SL, Marunde RL. Comparison of VTA dopamine neuron activity in lines of rats selectively bred to prefer or avoid alcohol. *Alcohol Clin Exp Ther* 2006;30:991–997.
- Murphy JM, Gatto GJ, McBride WJ, Lumeng L, Li TK. Operant responding for oral ethanol in the alcohol-preferring P and alcohol-nonpreferring NP lines of rats. *Alcohol* 1989;6:127–131. [PubMed: 2713085]
- Murphy JM, Gatto GJ, Waller MB, McBride WJ, Lumeng L, Li TK. Effects of scheduled access on ethanol intake by the alcohol-preferring (P) line of rats. *Alcohol* 1986;3:331–336. [PubMed: 3778650]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 4. New York: Academic Press; 1998.
- Rodd ZA, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride JM. Intracranial self-administration of cocaine within the posterior ventral tegmental area of Wistar rats: evidence for involvement of serotonin-3 receptors and dopamine neurons. *J Pharmacol Exp Ther* 2005;313:134–145. [PubMed: 15650115]
- Rodd ZA, Bell RL, Zhang Y, Murphy JM, Goldstein A, Zaffaroni A, Li TK, McBride WJ. Regional heterogeneity for intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. *Neuropsychopharmacology* 2005;30:330–338. [PubMed: 15383830]
- Rodd ZA, Melendez RI, Bell RL, Kuc KA, Zhang Y, Murphy JM, McBride WJ. Intracranial self-administration of ethanol within the ventral tegmental area of male Wistar rats: evidence for regional heterogeneity and involvement of dopamine neurons. *J Neurosci* 2004;24:1050–1057. [PubMed: 14762123]
- Rodd-Hendricks 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* 2000;149:217–224. [PubMed: 10823401]
- Rodd-Hendricks ZA, Melendez RI, Zaffaroni A, Goldstein A, McBride WJ, Li TK. The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacol Biochem, Behav* 2002;72:55–64. [PubMed: 11900769]
- Shen RY. Ethanol withdrawal reduces the number of spontaneously active VTA dopamine neurons in conscious animals. *J Pharmacol Exp Ther* 2003;307:566–572. [PubMed: 12966156]
- Shen RY, Chiodo LA. Acute withdrawal after repeated ethanol treatment reduces the number of spontaneously active dopaminergic neurons in the ventral tegmental area. *Brain Res* 1993;622:289–293. [PubMed: 8242369]
- Shen RY, Choong KC, Thompson AC. Long-term reduction in ventral tegmental area dopamine neuron population activity following repeated stimulant or ethanol treatment. *Biol Psychiatry* 2007;61:93–100. [PubMed: 16697354]
- Smith AD, Weiss F. Ethanol exposure differentially alters central monoamine neurotransmission in alcohol-preferring versus –nonpreferring rats. *J Pharmacol Exp Ther* 1999;288:1223–1228. [PubMed: 10027862]

- Starke K, Gothert M, Kilbinger H. Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol Rev* 1989;69:864–989. [PubMed: 2568648]
- Swanson LW. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull* 1982;9:321–353. [PubMed: 6816390]
- Tepper, JM.; Gariano, RF.; Groves, PM. The neurophysiology of dopamine nerve terminal autoreceptors. In: Chiodo, LA.; Freeman, AS., editors. *Neurophysiology of Dopaminergic Systems: Current Status and Clinical Perspectives*. Detroit, MI: Lakeshore Publishing; 1987. p. 93–127.
- Thielen RJ, Engleman EA, Rodd ZA, Murphy JM, Lumeng L, Li TK, McBride WJ. Ethanol drinking and deprivation alter dopaminergic and serotonergic function in the nucleus accumbens of alcohol-preferring rats. *J Pharmacol Exp Ther* 2004;309:216–225. [PubMed: 14718606]
- Thompson TL, Moss RL. Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. *J Neurochem* 1994;62:1750–1756. [PubMed: 8158125]
- Tsai G, Colye JT. The role of glutaminergic neurotransmission in the pathophysiology of alcoholism. *Annu Rev Med* 1998;49:173–184. [PubMed: 9509257]
- Waller MB, McBride WJ, Lumeng L, Li TK. Induction of dependence on ethanol by free-choice drinking in alcohol preferring rats. *Pharmacol Biochem Behav* 1982;16:501–507. [PubMed: 7200611]
- Weiss F, Lorang MT, Bloom FE, Koob GF. Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: Genetic and motivational determinants. *J Pharmacol Exp Ther* 1993;267:250–258. [PubMed: 8229752]
- 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;16:3473–3485.
- Windels F, Kiyatkin EA. General anesthesia as a factor affecting impulse activity and neuronal responses to putative neurotransmitters. *Brain Res* 2006;1086:104–116. [PubMed: 16600189]
- Wise RA. The role of reward pathways in the development of drug dependence. *Pharmacol Ther* 1987;35:227–263. [PubMed: 3321101]
- White FJ, Wang RY. Comparison of the effects of chronic haloperidol treatment on A9 and A10 dopamine neurons in the rat. *Life Sci* 1983;32:983–993. [PubMed: 6827927]
- Wolf ME, Roth RH. Autoreceptor regulation of dopamine synthesis, *Ann. NY Acad Sci* 1990;604:323–343.
- Yoshimoto K, McBride WJ, Lumeng L, Li TK. Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. *Alcohol* 1991;9:17–22. [PubMed: 1370758]
- Zangen A, Ikemoto S, Zadina JE, Wise RA. Rewarding and psychomotor stimulant effects of endomorphin-1: anteroposterior differences within the ventral tegmental area and lack of effect in nucleus accumbens. *J Neurosci* 2002;22:7225–7233. [PubMed: 12177217]
- Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA. Two brain sites for cannabinoid reward. *J Neurosci* 2006;26:4901–4907. [PubMed: 16672664]

Figure 1.

A. Typical action potential waveform of a dopamine neuron recorded from the posterior ventral tegmental area in a paralyzed rat. **B.** Rate histogram of the ventral tegmental area dopamine neuron shown in A. The intravenous infusion of quinpirole (a D_2 receptor agonist) silenced the neuron while a successive infusion of eticlopride (a D_2 receptor antagonist) reversed the effect and increased neuronal firing frequency above baseline level. Arrows indicate the times of the drug infusions.

Table 1

Characteristics of Posterior VTA Dopamine Neurons in Alcohol-Preferring (P) Rats **Drinking Moderate Amounts of Alcohol^a** and in Alcohol Naive P Rats

All Neurons	Alcohol Drinking	Alcohol Naive
Number of Rats	9	8
Number of Neurons	37	33
Firing Frequency (spikes/s)	3.4±0.6	3.6±0.6
Bursting Neurons ^b	Alcohol Drinking	Alcohol Naive
Number of Neurons	34	31
Firing Frequency (spikes/s)	3.5±0.2	3.7±0.2
Number of Bursts ^c	56±4	55±6
% APs in Bursts ^c	38.0±3.4	36.1±3.2
Number of DA Neurons/Track	3.5±0.1	2.7±0.1 *

Mean±S.E.M.; AP=action potential.

^a Alcohol intake averaged 1.3 g/kg over a 1-hr limited access period for 6 weeks.

^b Dopaminergic neurons with ≥ 5% APs in bursts.

^c Calculated from 500 consecutive APs.

* p=0.0001, alcohol-drinking vs alcohol-naive P rats (averages are based on sampling neurons in 8 tracks per rat).