Published in final edited form as:

Alcohol. 2011 September ; 45(6): 549–557. doi:10.1016/j.alcohol.2011.02.304.

ETHANOL DRINKING REDUCES EXTRACELLULAR DOPAMINE LEVELS IN THE POSTERIOR VENTRAL TEGMENTAL AREA OF NONDEPENDENT ALCOHOL-PREFERRING P RATS

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Abstract

Moderate ethanol exposure produces neuroadaptive changes in the mesocorticolimbic dopamine system in nondependent rats, and increases measures of dopamine neuronal activity in vitro and in vivo. Moreover, moderate ethanol drinking and moderate systemic exposure elevates extracellular dopamine levels in mesocorticolimbic projection regions. However, the neuroadaptive changes subsequent to moderate ethanol drinking on basal dopamine levels have not been investigated in the ventral tegmental area (VTA). In the current study, adult female alcohol-preferring (P) rats were divided into alcohol-naïve, alcohol drinking, and alcohol deprived groups. The alcohol drinking group had continuous access to water and ethanol (15%, v/v) for 8 weeks. The alcohol deprived group had 6 weeks of access followed by 2 weeks of ethanol deprivation, 2 weeks of ethanol re-exposure, followed again by 2 weeks of deprivation. The deprived rats demonstrated a robust alcohol deprivation effect upon ethanol reinstatement. The alcohol-naïve group had continuous access to water only. In the last week of the drinking protocol, all rats were implanted with unilateral microdialysis probes aimed at the posterior VTA and no-net-flux microdialysis was conducted to quantify extracellular dopamine levels and dopamine clearance. Results yielded significantly lower basal extracellular dopamine concentrations in the posterior VTA of the alcohol drinking group compared to the alcohol naïve and alcohol deprived groups (3.8 ± 0.3 vs. 5.0 ± 0.5 [$p < 0.02$] and 4.8 ± 0.4 nM, [$p < 0.05$], respectively). Extraction fractions were significantly ($p < 0.0002$) different between the alcohol drinking and alcohol naïve groups (72 ± 2 vs. $46 \pm 4\%$, respectively) and not significantly different ($p = 0.051$) between alcohol deprived and alcohol naïve groups ($61 \pm 6\%$ for the alcohol deprived group). The data indicate that reductions in basal DA levels within the posterior VTA occur after moderate chronic ethanol intake in nondependent P rats. This reduction may result, in part, from increased DA uptake and may be important for the maintenance of ethanol drinking. These adaptations normalize with ethanol deprivation and may not contribute to the alcohol deprivation effect.

Keywords

Quantitative microdialysis; Ventral tegmental area; Dopamine; Alcohol-preferring (P) rats; Ethanol

Introduction

The mesocorticolimbic dopamine (DA) system has been implicated in mediating the rewarding properties of many drugs of abuse, including alcohol (Koob and Bloom, 1988; Wise, 1987). This neuronal pathway consists of dopaminergic neurons in the ventral tegmental area (VTA) that project to limbic and cortical forebrain structures including the nucleus accumbens (NAc) and prefrontal cortex (Swanson, 1982). In addition to DA neurons, the VTA is composed of non-dopaminergic, presumably GABAergic, neurons whose axons synapse onto DA neurons as well as project to corticolimbic sites (Bayer and Pickel, 1991; Nagai et al., 1983; Steffensen et al., 1998).

Through bidirectional 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 ethanol for its pharmacological effects, work to obtain ethanol, self-infuse ethanol directly into the VTA and develop tolerance and dependence after excessive ethanol drinking for a prolonged time (Gatto et al., 1994; Murphy et al., 1986, 1989; Rodd et al., 2005b; Waller et al., 1982).

Acute administration of either forced (experimenter administered) or consumed ethanol increases extracellular DA levels in the NAc in various rodent strains and under varied conditions (Gonzales et al., 2004). In addition, moderate ethanol exposure (exposure that does not produce overt signs of dependence) has been shown to produce adaptive and enduring elevations in extracellular DA levels. Quantitative microdialysis experiments have revealed that repeated (Smith and Weiss, 1999) or single (Franklin et al., 2009) systemic administration of a moderate ethanol dose(s) resulted in elevated extracellular DA levels in the NAc of adult P and Wistar rats, and adolescent (Badanich et al., 2007) Sprague-Dawley rats, the day following the final administration. Elevations in accumbal DA levels were also observed in P rats moderately consuming ethanol in a 24 hour free-choice paradigm with access to ethanol and water as well as in P rats deprived of ethanol for 2 weeks (Thielen et al., 2004). In addition to increasing DA levels in the NAc, preliminary studies found that moderate ethanol intake by P rats increased DA levels in other VTA DA neuron projection areas, such as the ventral pallidum and the medial prefrontal cortex (unpublished observation). On the other hand, extraction fraction, a parameter indicative of DA clearance, did not change (Franklin et al., 2009; Smith and Weiss, 1999; Thielen et al., 2004), indicating that increased accumbal DA levels were likely due to increased DA neuronal activity and/or local release, rather than decreased reuptake. Consistent with this notion, moderate ethanol consumption was found to increase the number of spontaneously active DA neurons in the posterior VTA of P rats (Morzorati et al., 2010) which are known to innervate the NAc (Ikemoto, 2007).

Although many studies have shown that moderate ethanol intake elevates extracellular DA levels in VTA projection regions, to date no studies have examined the effects of ethanol drinking and deprivation on extracellular levels in the VTA of P rats. Thus, the objective of the current study was to use quantitative (no-net-flux) microdialysis to examine the effects of moderate ethanol drinking on extracellular DA levels and DA clearance in the posterior VTA of nondependent P rats. The posterior VTA was targeted because intracranial self-administration studies suggest that the posterior rather than the anterior VTA is more sensitive to the reinforcing effects of ethanol (Rodd et al., 2004, 2005a; Rodd-Hendricks et al., 2000). In addition, DA transmission was assessed in rats that have the same overall period of ethanol access only with ethanol deprivation and re-exposure events. As a mechanism to increase the numbers of active DA neurons in the posterior VTA (Morzorati

et al., 2010) and elevate the extracellular levels of DA in projection regions (Thielen et al., 2004), we hypothesized that ethanol intake would produce an adaptive reduction in extracellular DA levels in the posterior VTA of P rats consuming moderate amounts of ethanol.

Materials and Methods

Animals

Adult female P rats were obtained from the Indiana University Alcohol Research Center. Female P rats were used because they served as subjects in prior microdialysis and electrophysiological experiments examining the effects of moderate ethanol intake on the mesolimbic DA system (Engleman et al., 2000, 2003, Thielen et al., 2004, Morzorati et al., 2010). Because the squads of rats were processed at different times, any effects of the estrus cycle should have been distributed over the different experimental conditions. Initially, rats were double housed in plastic tubs and allowed at least 14 days to acclimate to a temperature- and humidity-controlled environment with a normal 12-hr light-dark cycle (lights on at 7:00 AM). Laboratory chow and water were available ad libitum throughout the experiment. 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

Following acclimation, rats, weighing 200-300 g, were housed individually in hanging metal cages (18 × 25 × 18 cm, W × L × H) and assigned to one of three groups: alcohol naïve, alcohol drinking or alcohol deprived (Thielen et al., 2004). The alcohol drinking schedules used for the two alcohol exposed groups were designed to coincide with previous microdialysis work (Thielen et al., 2004), and behavioral data that demonstrate a significant elevation in ethanol drinking known as the alcohol deprivation effect (ADE) that occurs after a period of ethanol deprivation (McKinzie et al., 1998; Rodd-Henricks, et al., 2000). The design allows for the demonstration of an ADE in deprived rats as compared to the non-deprived alcohol drinking rats after an initial deprivation period. This is followed by assessment of neuroadaptive changes using in vivo microdialysis after additional periods of ethanol re-exposure and deprivation which have been shown to produce a more persistent ADE than that observed after a single deprivation (Rodd-Henricks, et al., 2000). The alcohol drinking rats had access to only water for four weeks, after which they were given continuous access to 15% ethanol (v/v) and water for 8 weeks. The alcohol deprived rats had 6 weeks of access to ethanol and water, deprived of ethanol for 2 weeks, re-exposed to ethanol for 2 weeks and deprived for a second 2-week period. The alcohol naïve rats had access to water only throughout the same period of time. Following this schedule, rats in all three groups in a given squad were ready for experimentation on the same day. Fluid intakes were recorded to the nearest 0.1 g by weighing the ethanol and water bottles every 24-48 hours. Body weights were recorded at the same time and ethanol intake was converted to average grams of ethanol per kilogram of body weight per day. Using a 24-hr free choice access paradigm, adult female P rats drink in bouts, primarily in the dark cycle. Drinking often exceeds 1 g/kg of ethanol per bout which can result in peak blood alcohol concentrations of approximately 90 mg% (unpublished observation; Murphy et al., 2002). To insure that plasma and brain concentrations of ethanol were negligible, the drinking bottles containing ethanol were removed from the alcohol drinking rats 3 hours prior to beginning the experiment and alcohol concentration was determined in the first dialysate samples.

Surgery

Rats were anesthetized with an isoflurane/air mixture and positioned in a stereotaxic frame with the skull level. An 18 gauge stainless steel guide cannula (Plastics One, Roanoke, VA) was implanted unilaterally 3 mm above the base of the posterior VTA (from bregma: P −5.8 mm, L +2.1 mm; from the skull surface: V −6.0 mm, 10° angle from vertical) (Paxinos and Watson, 1998). The guide cannula was secured to the skull with cranioplastic cement and anchored with stainless steel screws. To avoid blockage and minimize the risk of infection, a dummy cannula was inserted into the guide cannula. Rats were allowed to recover for 4 days before being habituated to the clear Plexiglas microdialysis chambers (22 × 44 × 38 cm, W × L × H) for 2 days, 2 hours each day (11:00 AM – 1:00 PM). The day before microdialysis, the rats were briefly anesthetized, the dummy cannula was removed from the guide cannula and a microdialysis probe was inserted and glued into place. The probes were loop style, constructed as previously described (Kohl et al., 1998), with a 3.0 mm loop of active dialysis surface (molecular weight cutoff 13.0 kD, Spectrum Laboratories, Rancho Dominguez, CA). The probe extended 3 mm below the guide cannula.

Dopamine No-Net-Flux

The no-net-flux experiments were conducted in freely moving rats as described previously (Cosford et al., 1996; Justice, 1993; Thielen et al., 2004). The rats were placed in the microdialysis chambers and microdialysis probes were perfused at a flow rate of 0.5 µl/min with artificial cerebral spinal fluid (aCSF) (140 mM NaCl, 3 mM KCl, 1.2 mM CaCl₂, 2 mM Na₂PO₄, 1.0 mM MgCl₂, 0.2 mM ascorbate; pH 7.4) using a microliter syringe pump (Harvard Apparatus, Holliston, MA). After a 2-h equilibration period, 5 baseline samples were collected every 20 min in Microfuge tubes containing 2 µl of a collection cocktail consisting of 0.2 mM EDTA, 0.33 mM L-cysteine and 0.05 mM L-ascorbic acid diluted in 0.1N acetic acid. After collection of baseline samples, the posterior VTA was perfused, in random order, with aCSF containing 5, 10 or 20 nM dopamine for 100 min each. For each switch in DA concentration, the flow to the probe was briefly interrupted and lines containing the new DA concentration in aCSF were attached to the inlet assembly. The new concentration of DA was perfused for an initial 20-min period to allow for equilibration; these samples were discarded. An additional four 20-min samples were then collected, after which the rats were switched to the next concentration of DA as described above. After perfusion with all three concentrations of DA, aCSF alone was perfused for an additional 60 min (three samples). All dialysate samples were immediately frozen on dry ice and stored at −70° C until assayed.

Determination of Dopamine and Ethanol Levels in Dialysates

Dialysate samples were analyzed for DA levels using microbore high-performance liquid chromatography with electrochemical detection (HPLC/EC). Dialysates were injected (5-µl loop; Rheodyne, Cotati, CA) onto a reverse-phase microbore column (1.0 × 100 mm SepStik Spherisorb C18 column, 3 µm particle size; Bioanalytical Systems, West Lafayette, IN) and separated using a mobile phase composed of 100 mM sodium acetate, 0.5 mM EDTA, 5.0 mM sodium octylsulfonic acid, 10 mM NaCl, and 6% acetonitrile, (pH 5.0 adjusted with glacial acetic acid), at a flow rate of 0.07 ml/min. Levels of DA in the dialysates were determined by electrochemical detection (+550 mV potential, 2-nA sensitivity; PerkinElmer Life Sciences, Princeton, NJ) using a 6 mm radial-flow, glassy-carbon electrode (Bioanalytical Systems, West Lafayette, IN). The injector, column, and electrode were contained in a UniJet CC-6 cabinet (Bioanalytical Systems), which allows for very low dead volume and greater sensitivity when using smaller sample volumes. Output from the detector was sent to a microprocessor and integrated by using ChromPerfect Spirit (version 4.4.21; Justice Innovations, Palo Alto, CA). The DA detection limit was 100 pM.

Samples of microdialysate collected during the 2 hr equilibration period prior to collection of samples for DA no-net-flux were analyzed for ethanol content using an Analox Analyzer (model GL5; Analox Instruments USA, Lunenburg, MA). The ethanol concentration in 5 μ l samples was determined by measuring oxygen uptake generated by the oxidation of ethanol to acetaldehyde and hydrogen peroxide by ethanol oxidase.

Probe Placements

At the end of the experiment, the rats were killed with CO₂ inhalation. Probes were perfused with 1% methylene blue dye (Sigma, St. Louis, MO) and the brains were extracted and frozen at -10° C. The brains were later sectioned on a cryostat into 40 μ m slices; the slices were mounted on slides and alternate slides were stained with cresyl violet. Only rats with at least 75% of the active probe membrane in the posterior VTA were included in the data analysis.

Data analysis

Daily averages of ethanol intake were compared between alcohol drinking and alcohol deprived rats using an independent Student *t* test. To examine the ethanol deprivation effect, an ANOVA was used to compare daily alcohol intakes in alcohol drinking and alcohol deprived rats for 3 days prior to removal of ethanol and for 5 days after alcohol re-exposure (Thielen et al., 2004).

The extracellular DA concentrations and extraction fractions (a measure of DA clearance) were obtained from multiple linear regression modeling. A difference score of the concentration of DA perfused through the probe minus the concentration of DA recovered out of the probe ($DA_{in} - DA_{out}$, nM) was calculated for each sample and plotted against the concentrations of DA perfused through the probe (5, 10, 20 nM). The x-intercept and slope (E_d) were determined using the data from all rats within a group. The E_d represents the extraction fraction or probe recovery of DA. Changes in the extraction fraction are altered by changes in DA clearance around the probe, which, in turn, alters in vivo probe recovery. Thus, E_d is an indirect measure of DA reuptake. The x-intercept represents the point at which there is no net flux of transmitter across the dialysis membrane and is the true extracellular concentration of DA. As previously applied (Thielen et al., 2004), multiple linear regression analysis was used to compare the effect of drinking history on the x-intercepts and slopes. Probabilities of $p < 0.05$ were considered significant.

Results

Representative probe placements are shown in Figure 1 for the 9 alcohol naïve, 10 alcohol drinking and 9 alcohol deprived P rats. Probes were placed between -5.3 and -6.0 posterior to bregma according to the atlas of Paxinos and Watson (1998). At least 75% of the active membrane of the probes was located in the posterior VTA.

Ethanol consumption did not differ between alcohol drinking and alcohol deprived P rats. Ethanol intakes for the alcohol drinking rats averaged (mean $\pm$ SEM) 4.8 ± 0.3 g/kg/day for the 8 weeks of ethanol access, while ethanol intakes for the alcohol deprived rats averaged 5.3 ± 0.4 g/kg/day for the 6 weeks of ethanol access prior to deprivation. Re-exposure to ethanol produced a significant ADE in the alcohol deprived P rats. Figure 2 shows ethanol intakes for the alcohol deprived rats 3 days prior to ethanol removal and the first 5 days after ethanol re-exposure; and for the alcohol drinking rats over the same days. Both the alcohol deprived and alcohol drinking rats had an average daily intake (mean $\pm$ SEM) of 4.7 ± 0.4 g/kg/day for the 3 days before ethanol removal in the alcohol deprived rats. A comparison of intakes between alcohol deprived and alcohol drinking P rats prior to and after ethanol re-

exposure in the deprived rats revealed a significant increase in ethanol intake in the alcohol deprived rats on the first 2 days of ethanol re-exposure ($F(1,17) = 5.764$, $p < 0.03$).

Alcohol concentrations in the dialysate samples collected at the beginning of the microdialysis experiments were 0 mg%, indicating that the experimental results are not confounded by elevated brain ethanol levels.

Multiple linear regression analyses of the posterior VTA dopamine no-net flux results for alcohol naïve, alcohol drinking and alcohol deprived P rats are illustrated in Figure 3A. X-intercepts indicate the quantitative basal extracellular DA levels. The slopes of the regression lines indicate the extraction fractions, an indirect measure of DA clearance. The basal extracellular DA concentrations and DA extraction fractions in the posterior VTA were, respectively, (mean $\pm$ SEM) 5.0 ± 0.5 nM and $46 \pm 4\%$ for the alcohol naïve rats, 3.8 ± 0.3 nM and $72 \pm 2\%$ for the alcohol drinking rats and 4.8 ± 0.4 nM and $61 \pm 6\%$ for the alcohol deprived rats (Figures 3B and 3C). An ANOVA revealed that the x-intercept, corresponding to the point of no-net-flux and the interpolated DA concentration, was significantly reduced in the posterior VTA of alcohol drinking compared with alcohol naïve P rats ($F(1,212) = 7.9$, $p < 0.02$) and alcohol deprived P rats ($F(1,207) = 4.0$, $p < 0.05$) (Figure 3B). The slope of the regression line, or the extraction fraction (E_d), an indirect measure of DA clearance, was significantly elevated in the posterior VTA of alcohol drinking compared with alcohol naïve P rats ($F(1,211) = 15.2$, $p < 0.0002$) (Figure 3C). Slope differences were not statistically significantly different between alcohol naïve and alcohol deprived P rats ($F(1,181) = 3.9$, $p = 0.051$) (Figure 3C).

Discussion

The current data indicate that eight weeks of moderate ethanol drinking reduced extracellular DA levels in the posterior VTA of female P rats, but extracellular DA levels recovered to control levels after 2 weeks of ethanol deprivation (Figure 3B). The reduction in extracellular DA levels was associated with an increase in DA clearance as measured by in vivo extraction fraction in the alcohol drinking group (Figure 3C).

Evidence indicates that repeated ethanol exposure produces adaptive changes in the mesocorticolimbic dopamine system. For example, repeated microinjection of ethanol directly into the posterior VTA sensitized VTA DA neurons to stimulation by subsequent ethanol administration (Ding et al., 2009b). Likewise, chronic ethanol drinking enhanced the intracranial self-administration of ethanol into the posterior VTA, again suggesting that alcohol exposure increased the sensitivity of the posterior VTA DA neurons (Rodd et al., 2005a). These adaptive effects of ethanol exposure to increase the responsiveness of VTA DA neurons are consistent with data showing that moderate ethanol exposure either through systemic administration (Franklin et al., 2009; Smith and Weiss, 1999) or drinking (Thielen et al., 2004) resulted in elevated basal extracellular DA levels in the NAc. Similar effects were observed in other VTA projection regions including the medial prefrontal cortex and ventral pallidum (unpublished observations) and with peri-adolescent ethanol exposure (Badanich et al., 2007). It should be noted that these elevations were observed in the absence of ethanol, and in some cases were also observed after at least two weeks of ethanol deprivation (Badanich et al., 2007; Thielen et al., 2004). Together these studies suggest that moderate ethanol exposure results in an enduring elevation of basal extracellular DA levels in projection regions that occurs concurrently with an increase in the number of spontaneously active DA neurons in the posterior VTA (Morzorati et al., 2010).

The current data reveal a neuroanatomical divergence in the effect of ethanol drinking on extracellular DA levels in the posterior VTA as compared to projection regions as levels are

increased in projection regions but reduced in the posterior VTA. One mechanism contributing to the reduction in posterior VTA DA levels is likely an increase in DA reuptake, as suggested by the increase in extraction fraction observed in the alcohol drinking rats (Fig 3C). This adaptation dissipates with alcohol deprivation (Fig. 3C) in rats that show an ADE (Fig. 2). One hypothetical result of an adaptive reduction in extracellular DA levels in the posterior VTA would be to reduce feedback inhibition locally at D₂ autoreceptors on the VTA DA neurons which project to forebrain areas. There is ample evidence that VTA DA neurons are under tonic D₂ receptor-mediated auto-inhibition (Kalivas, 1993; White, 1996), and VTA DA neuronal activity (firing frequency, the number of neurons that are spontaneously active) can be regulated by activation/inactivation of D₂ receptors (Jeziorski and White 1989; Kohl et al., 1998; Valenti and Grace, 2010; Wang, 1981; White and Wang, 1984). Importantly, control of D₂ autoreceptors by extracellular DA levels in the VTA was shown to be limited by local DA clearance (Ford et al., 2010), indicating that increased DA clearance in the VTA may reduce feedback inhibition at somatodendritic D₂ autoreceptors. A recent study suggests a more prominent role of D₂ receptors in regulating VTA DA neurons in the posterior compared to the anterior VTA (Ding et al., 2009a). Previous studies examining possible mechanisms involved in the adaptive elevation of DA levels in mesolimbic projection areas resulting from moderate ethanol exposure suggest that D₂ terminal autoreceptors may have a reduced capacity to inhibit DA release (Engleman et al., 2003; Franklin et al., 2009; Thielen et al., 2004).

Repeated moderate exposure to ethanol also increases the overall number of active DA neurons (but not their firing rates or patterns) in the posterior VTA of P rats (Morzorati et al., 2010). These data are consistent with in vitro data showing that moderate ethanol administration had no effect on the basal firing rates of DA neurons, but increased the sensitivity of VTA neurons to subsequent ethanol exposure (Brodie, 2002) which occurred in concert with a reduction in the potency of GABA to inhibit VTA DA neurons.

A direct change in D₂ autoreceptor function is another mechanism by which ethanol exposure might affect VTA DA neurons. Ethanol drinking has been reported to reduce the stress-induced elevation in D₂ receptors in the VTA of Kyoto Wistar rats (Yaroslavsky and Tejani-Butt, 2010). Thus, increasing clearance of DA and reducing extracellular DA levels, in addition to a possible reduction in the number of somatodendritic D₂ autoreceptors in the VTA, may reduce inhibitory input (IPSPs) at DA cell bodies. Since somatodendritic D₂ autoreceptors are thought to modulate the activity of dopamine neurons to afferent input (Trent and Tepper, 1991; Tepper et al., 1997), a change in D₂ receptors may favor afferent activity contributing to the spontaneous firing of dopamine neurons. This could translate into an increase in the number of spontaneously active posterior VTA DA neurons (Morzorati et al., 2010) and lead to elevations in extracellular DA levels observed in projection regions (unpublished observation; Franklin et al., 2009; Smith and Weiss, 1999).

Combining the current data from the posterior VTA with the data from projection regions provides a basis to propose a theoretical construct characterizing the adaptive changes in DA levels within the mesocorticolimbic system associated with moderate ethanol exposure (Figure 4). A series of interrelated events might begin with normal functioning of the mesolimbic system being altered by moderate ethanol drinking resulting in increased DA clearance in the posterior VTA. The increased clearance in this region would reduce local feedback regulation at somatodendritic D₂ autoreceptors located on DA cell bodies and dendrites, resulting in reduced inhibitory input and increasing the number of active DA neurons. An increase in the number of spontaneously active posterior VTA DA neurons projecting to forebrain areas would be reflected in elevated extracellular DA levels in projection regions such as the NAc.

The data from the current study also indicate that the adaptive changes that occur in the posterior VTA with moderate ethanol intake appear to be reversible, as both extracellular DA levels and DA clearance normalize after 2 weeks of ethanol deprivation (Figure 3). The timing of this measurement in the deprived rats coincides with a robust increase in ethanol drinking (an ADE) that can persist for up to two days (McKinzie et al., 1998; Rodd-Henricks, et al., 2000). These data suggest that alterations in DA levels in the posterior VTA may not be involved in the ADE.

These findings are inconsistent with effects observed in the NAc where elevations in extracellular DA levels persisted throughout the two week period of ethanol deprivation and may be implicated in the ADE (Thielen et al., 2004). Moreover, moderate ethanol exposure does not typically lead to adaptive changes in DA clearance in projection regions of the mesocorticolimbic DA system (unpublished observations; Franklin et al., 2009; Smith and Weiss, 1999; Thielen et al., 2004). However, consistent with the current data in the posterior VTA, ethanol exposure has been shown to enhance DA transport in cells transfected with the DA transporter (Riherd, et al., 2008) and in mice after ethanol exposure in vapor chambers (Healey et al., 2008), indicating that ethanol exposure can lead to increases in DA clearance. Together these data suggest a fundamental difference in how DA clearance responds to repeated moderate ethanol exposure in the posterior VTA and mesocorticolimbic projection regions and indicate that other mechanisms may be involved in the persistent elevations in DA levels observed in projection regions. Possible mechanisms may include a regionally specific reduction in terminal D₂ autoreceptor function in mesolimbic projection regions which has been shown to endure through periods of ethanol deprivation (Engleman et al., 2003; Thielen et al., 2004).

Of course a number of additional mechanisms have been proposed to produce adaptive changes in the mesocorticolimbic system resulting from moderate ethanol exposure. One possible mechanism involves ethanol-induced adaptations in VTA glutamate transmission. Ethanol exposure is thought to enhance excitatory synaptic transmission on VTA DA neurons by increasing the ratio of AMPA receptor-mediated synaptic currents to NMDA receptor-mediated synaptic currents (Saal et al., 2003; Stuber et al., 2008), which is consistent with enhanced function and/or number of AMPA receptors with ethanol exposure (Ortiz et al., 1995; Stuber et al., 2008). Moreover, ethanol exposure can decrease glutamate clearance and increase extracellular levels of glutamate (Melendez et al., 2005). These adaptations in glutamate transmission may lead to enhanced excitability of DA neurons. Another mechanism may involve neuroadaptations in VTA GABA neurons. Ethanol exposure has been shown to enhance subsequent ethanol inhibition of GABA interneurons, leading to potentially greater disinhibition of DA neurons (Gallegos et al., 1999). In addition, the kappa-opioid system has been shown to regulate ethanol-induced DA release in the NAc (Doyon et al., 2006), and repeated ethanol exposure has been shown to enhance the effect of kappa-receptor ligands in this brain area (Lindholm et al., 2007). Thus, a difference in the neuroadaptive response/recovery time frame for the kappa-opioid system could also play a role in the differential effects observed in the present and previous studies.

Acknowledgments

The authors wish to thank Dr. Larry Lumeng for supplying the selectively bred P rats. This work was supported by a grant from the National Institute on Alcohol Abuse and Alcoholism: AA12832.

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Fig. 1.

Anteroposterior coronal sections illustrating the location of the microdialysis probes in the posterior VTA of P rats. The lines represent the active regions of the probes in alcohol naive (solid lines), alcohol drinking (large dash), and alcohol deprived (small dash) rats. Some overlapping probe placements are not shown. Anteroposterior plates with reference to bregma (Paxinos and Watson, 1998).

Fig. 2.

Ethanol intake and the ethanol deprivation effect. Rats in the alcohol drinking ($n = 10$) and deprived ($n = 9$) groups showed similar ethanol intakes 3 days prior to the two-week period when ethanol was removed from the alcohol deprived group. A significant alcohol deprivation effect (ADE) was observed for the alcohol deprived rats the first two days of ethanol re-exposure ($p < 0.03$ for both days).

Fig. 3.

A. Multiple linear regression analyses of the posterior VTA dopamine no-net flux results for alcohol naïve (square; $n = 9$), alcohol drinking (triangle; $n = 10$) and alcohol deprived (circle; $n = 9$) P rats. X-intercepts indicate the quantitative basal extracellular DA levels. The slopes of the regression lines indicate the extraction fractions, an indirect measure of DA clearance. B. Extracellular levels of DA (nM) in the pVTA were reduced in the alcohol drinking P rats compared to alcohol naïve ($p < 0.02$) and alcohol deprived ($p < 0.05$) P rats. C. DA clearance was significantly elevated in alcohol drinking compared to alcohol naïve P rats ($p < 0.0002$). DA clearance was not statistically different ($p = 0.051$) between alcohol naïve and alcohol deprived P rats.

Fig 4.

Theoretical diagrams illustrating the adaptive changes of moderate ethanol exposure on extracellular DA levels in the mesocorticolimbic system. The top diagram depicts the alcohol-naïve brain, with DA projections from the VTA to forebrain areas (straight arrows), as well as feedback from somatodendritic release (curved arrow). The lower diagram details the effects of moderate ethanol drinking or moderate systemic ethanol exposure to reduce extracellular DA levels in the posterior VTA, likely resulting from increased DA clearance. This would reduce local feedback inhibition on DA neurons (smaller curved arrow), increasing the number of VTA DA neurons that are active, which in turn elevates DA levels in projection regions (larger straight arrows).