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Review

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Temporal Profiles Dissociate Regional Extracellular Ethanol versus Dopamine Concentrations

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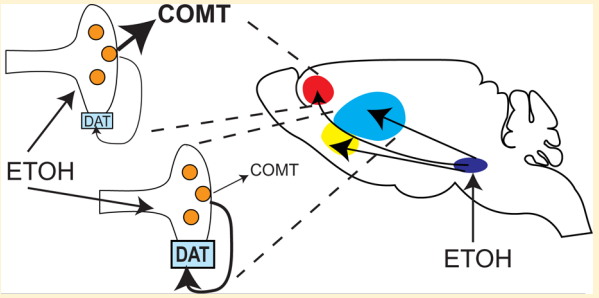
ABSTRACT: In vivo monitoring of dopamine via microdialysis has demonstrated that acute, systemic ethanol increases extracellular dopamine in regions innervated by dopaminergic neurons originating in the ventral tegmental area and substantia nigra. Simultaneous measurement of dialysate dopamine and ethanol allows comparison of the time courses of their extracellular concentrations. Early studies demonstrated dissociations between the time courses of brain ethanol concentrations and dopaminergic responses in the nucleus accumbens (NAc) elicited by acute ethanol administration. Both brain ethanol and extracellular dopamine levels peak during the first 5 min following systemic ethanol administration, but the dopamine response returns to baseline while brain ethanol concentrations remain elevated. Post hoc analyses examined ratios of the dopamine response (represented as a percent above baseline) to tissue concentrations of ethanol at different time points within the first 25−30 min in the prefrontal cortex, NAc core and shell, and dorsomedial striatum following a single intravenous infusion of ethanol (1 g/kg). The temporal patterns of these "response ratios" differed across brain regions, possibly due to regional differences in the mechanisms underlying the decline of the dopamine signal associated with acute intravenous ethanol administration and/or to the differential effects of acute ethanol on the properties of subpopulations of midbrain dopamine neurons. This Review draws on neurochemical, physiological, and molecular studies to summarize the effects of acute ethanol administration on dopamine activity in the prefrontal cortex and striatal regions, to explore the potential reasons for the regional differences observed in the decline of ethanol-induced dopamine signals, and to suggest directions for future research.



KEYWORDS: *Dopamine, ethanol, striatum, prefrontal cortex, microdialysis*

Alcoholism represents the end stage in the transition from voluntary to uncontrolled alcohol consumption. These behavioral transitions are the result of ethanol-induced alterations in the fundamental molecular and cellular processes that regulate cognition, motivation, and reward seeking behaviors. Therefore, characterizing the acute neurochemical effects of ethanol is critical to understanding the development and progression of alcohol use disorders.

Ethanol is believed to exert its reinforcing effects on behavior, at least in part, via activation of the mesolimbic dopamine circuit. This circuit consists of dopamine neurons originating in the ventral tegmental area (VTA) and terminating in the nucleus accumbens (NAc), and is implicated in motivated and goal-directed behaviors.[1] Ethanol has been shown to acutely enhance the firing rate of VTA dopamine neurons in vitro and increase extracellular dopamine in the NAc of awake, freely moving animals[2,3] (for reviews see refs 1 and 4). Additional pharmacological, lesion, and genetic studies have further implicated the mesolimbic dopamine circuit as a target for ethanol.[1,4−6]

Additionally, ethanol has been shown to affect mesocortical and nigrostriatal dopamine activity. Mesocortical dopamine neurons originate in the VTA and terminate in the prefrontal cortex (PFC), and contribute to the regulation of cognition and executive control of goal-directed behaviors.[5] Nigrostriatal dopamine neurons originate in the substantia nigra and innervate the dorsal striatum. These neurons coordinate motor responses relevant to goal-directed and habitual behaviors.[6−8] Neurochemical studies demonstrate that acute ethanol administration results in increased extracellular dopamine levels in the prefrontal cortex.[9,10] In contrast, the nigrostriatal dopamine circuit may be less sensitive to acute ethanol administration,[11,12] but may be gradually recruited with chronic ethanol self-administration.[6,13−15]

This Review summarizes recent in vivo microdialysis studies exploring the effects of acute, passive ethanol administration on dopamine activity in the medial PFC and striatal subregions. Additionally, we conducted post hoc analyses on these published and unpublished data to explore the decline of the ethanol-induced dopamine signal during the descending limb of the ethanol concentration time course in the medial PFC, NAc core and shell, and dorsomedial striatum (DMS). The results of our analyses revealed unexpected differences across these regions. In this review, we discuss the rationale and

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methodology for the post hoc analyses, propose explanations for the observed regional differences, and suggest directions for further research.

DISSOCIATION OF THE TEMPORAL PROFILES OF DIALYSATE ETHANOL AND DOPAMINE

In vivo microdialysis is frequently employed to monitor and quantify extracellular neurochemical changes in select brain regions induced by pharmacological, behavioral, or environmental manipulations in freely moving animals.[16] Over the past few decades, changes in extracellular dopamine activity in response to acute ethanol have been extensively investigated using in vivo molecular monitoring techniques, including microdialysis. While the temporal resolution of microdialysis is limited, this technique can detect relatively fast changes in extracellular concentrations of various analytes with sampling times as low as 1 min.[17]

Our lab and others have extended its application to monitor the quantity and time course of brain concentrations of ethanol following systemic administration.[9,18−21] Concurrent analyses of both analytes from the same microdialysis sample enables characterization of dopaminergic activity relative to ascending and descending tissue concentrations of ethanol. Using this approach, it was discovered that the time course of the dopamine response in the NAc to acute ethanol did not overlap with the temporal profile of brain ethanol concentrations (Figure 1).[22] Yim et al. reported that, following an intraperitoneal (i.p.) injection of 1 or 2 g/kg ethanol in naïve rats, extracellular dopamine reaches peak concentrations to 140% of baseline levels within the first 15 min sample while ethanol also attains peak brain concentrations 15−30 min following the injection, depending on the dose administered. The accumbal dopamine response returns to baseline 60−90 min post injection, while ethanol remains elevated in the dialysate. Dialysate ethanol concentrations did not return to baseline during the 2 h sampling period postinjection.[22]

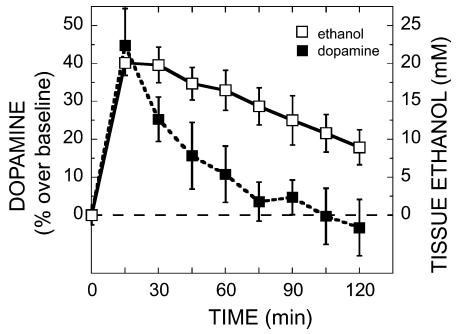

Figure 1. Dialysate concentrations of dopamine in the nucleus accumbens and tissue concentrations of ethanol following acute ethanol administration (1 g/kg, i.p.). There is a dissociation in the time courses of dopamine and ethanol concentrations in which dopamine returns to baseline levels while ethanol remains elevated in the tissue. The ethanol injection occurred at the 0 min time point. Symbols represent mean ± SEM (n = 5). Data from Yim et al.[22]

Interpreting the Dissociation of the Temporal Profiles of Dialysate Ethanol and Dopamine: Relevance to Acute Tolerance. Yim et al. hypothesized that the observed dissociation in the time courses of the dopamine response and dialysate ethanol concentrations following acute ethanol administration may be due to the development of acute tolerance.[22] The dissociation between ethanol and dopamine occurs during the descending phase of the brain ethanol concentration curve, and this temporal pattern aligns with that observed in behavioral studies of acute tolerance in humans and rodents.[23,24] Following a single dose of ethanol in humans, behavioral stimulation is reported during the ascending limb of the blood ethanol curve, while sedation and reduced impairments in the activation of motor responses are reported during the descending limb of the blood ethanol curve.[25,26] Acute tolerance to the stimulating and motor impairing effects of ethanol represents a physiological adaption occurring during a single ethanol exposure,[23,24] and may be relevant in predicting individual vulnerability to alcohol use disorders.[25−27] For example, selectively bred alcohol-preferring rats develop acute tolerance to a single dose of ethanol more rapidly than nonpreferring rats.[28,29] Consistent with this observation, rats displaying high acute tolerance tend to consume larger quantities of ethanol.[27] Together these findings suggest a relationship between the propensity to consume large quantities of ethanol (possibly due to a genetic vulnerability) and the tendency to exhibit rapid acute behavioral tolerance.

In alcohol nonpreferring rats, acute tolerance to the motor impairing effects of ethanol develops within 60−90 min following an i.p. injection of 2 or 2.3 g/kg ethanol.[28,29] This time course overlaps with that of the dissociation between ethanol and dopamine following an i.p. injection of a 1 g/kg dose of ethanol.[22] While dopamine in the NAc likely is not responsible for the specific motor behaviors assessed in the studies by Tampier et al.[27,29] and Waller et al.,[28] dopaminergic mechanisms are hypothesized to contribute to the acute stimulating effects of low to moderate doses of ethanol during the ascending limb of the blood ethanol concentration curve.[30,31] Early work showed that following i.p. administration of 0.25 and 0.5 g/kg ethanol, peak behavioral stimulation (defined as rearing, ambulation, and grooming) correlated with peak extracellular dopamine activity in the NAc at 20 min postinjection, and behavioral activity declined as dopamine levels returned to baseline.[30] Additionally, dopamine antagonists have been shown to dose-dependently reduce the locomotor-stimulating effects of ethanol in FAST mice, a strain of mice that is highly sensitive to the stimulating effects of acute ethanol.[32] However, while dopaminergic mechanisms may contribute to the expression of acute tolerance, the exact cellular and molecular mechanisms underlying this phenomenon are unknown, and therefore one cannot rule out the possibility of additional contributory mechanisms outside of the mesolimbic dopamine system.[33]

Interpreting the Dissociation of the Temporal Profiles of Dialysate Ethanol and Dopamine: Relevance to Ethanol's Mechanism of Action. A temporal dissociation between extracellular dopamine and drug concentrations is not observed with psychostimulants but has been observed with morphine. These effects may be related to differences in the mechanisms of actions of ethanol, psychostimulants, and morphine. Following acute drug administration, psychostimulants demonstrate a direct relationship between brain concentrations of the drug and the dopamine response in the striatum. Using in vivo microdialysis, Kuczenski et al.[34] demonstrated that extracellular concentrations of striatal dopamine and amphetamine showed nearly identical temporal profiles following a single subcutaneous dose of amphetamine (Figure 2A).[34] A similar concentration−response relationship has been observed with cocaine. Following an i.p. injection of

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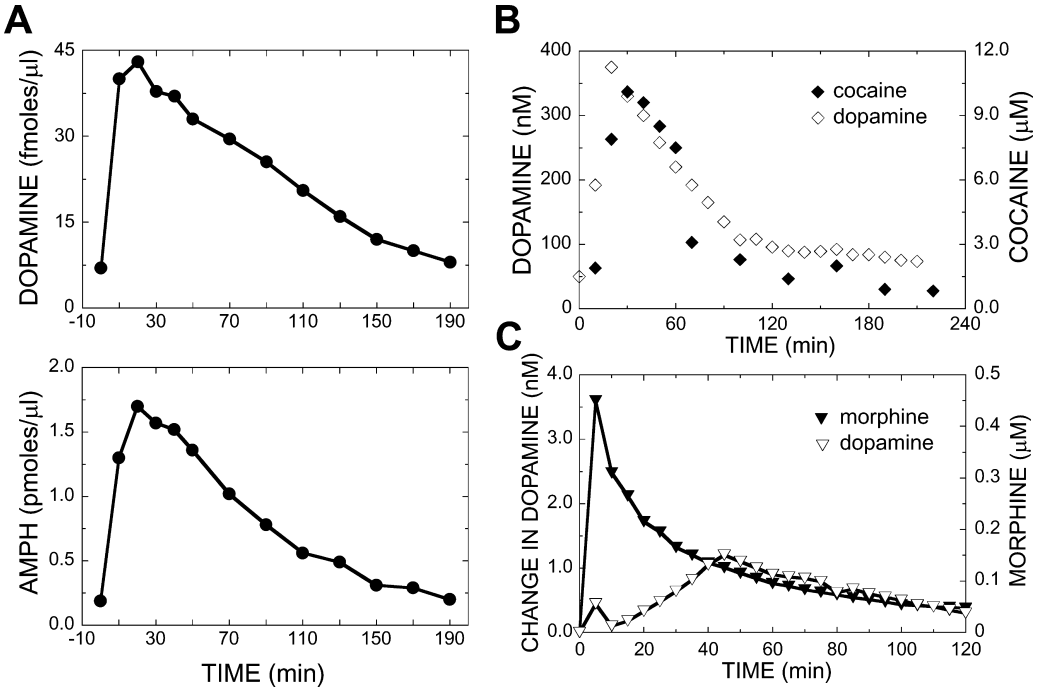

Figure 2. Temporal profiles of extracellular concentrations of amphetamine, cocaine, or morphine and dopamine following acute systemic administration. (A) Extracellular concentrations of dopamine (top left panel) and amphetamine (bottom left panel) in the dorsal striatum demonstrate nearly identical temporal profiles following acute administration of amphetamine (8 mg/kg, s.c.). Symbols represent mean. Reproduced with permission from Kuczenski et al.[34] (B) Extracellular concentrations of cocaine (◆) and dopamine (◇) in the striatum demonstrate similar temporal profiles following cocaine administration (30 mg/kg, i.p.). Symbols represent mean. Reproduced with permission from Nicolaysen et al.[35] (C) Temporal dissociation in dialysate concentrations of morphine (▼) and extracellular dopamine (▽) in the striatum occurs within the first 40 min following acute administration of morphine (1 mg, i.v.). After 40 min, a temporal dissociation is no longer apparent between extracellular levels of morphine and dopamine. Symbols represent mean. Reproduced with permission from Gottås et al.[38]

30 mg/kg, cocaine attains a maximum concentration of 10 μM within 20−30 min post injection (Figure 2B).[35] Extracellular dopamine concentrations in the striatum also peaked at 30 min post cocaine administration. As extracellular concentrations of cocaine and dopamine declined, there was a linear relationship between dialysate dopamine and drug concentrations.[35] The effect of cocaine on extracellular dopamine has also been shown to occur within seconds of an intravenous infusion using fast scan cyclic voltammetry,[36,37] but this method does not allow concurrent analysis of extracellular cocaine concentrations; therefore, the relationship between the drug response and the drug under these conditions is not completely clear.

Interestingly, in contrast to psychostimulants, a dissociation in dialysate concentrations of morphine and extracellular dopamine in the striatum occurs following acute administration of morphine. However, the time course of this dissociation contrasts with that of ethanol in that the dissociation between extracellular concentrations of morphine and dopamine appears to occur primarily during ramping up of the dopamine response, rather than during the decline of the dopamine response. Gottås et al. recently demonstrated that, following intravenous (i.v.) morphine administration, drug concentrations in the brain reached peak levels within 5−7 min (Figure 2C).[38] In contrast, extracellular dopamine in the striatum gradually increased, reaching peak levels approximately 46 min following the i.v. morphine infusion. Thereafter, extracellular morphine and dopamine levels slowly declined toward baseline, but neither reached baseline during the 2 h following the infusion. During the decline of the dopamine signal, the dissociation with extracellular concentrations of morphine was less apparent.[38]

The mechanisms by which psychostimulants and morphine enhance extracellular dopamine are well understood. Cocaine and amphetamine exert their primary effects on dopamine activity at the terminals of dopamine neurons. Cocaine inhibits the dopamine transporter, blocking a major mechanism of dopamine clearance from the synapse and, thus, resulting in increased levels of extracellular dopamine.[35,39] Amphetamine also alters the function of the dopamine transporter in addition to interfering with the storage of dopamine into synaptic vesicles.[40] In contrast, the molecular and cellular mechanisms by which ethanol enhances dopaminergic activity are not clearly understood. The lack of a direct relationship between extracellular ethanol and dopamine is consistent with experimental evidence that ethanol does not directly impair dopamine reuptake.[41,42] Using no net flux in vivo microdialysis, it was demonstrated that a 1 g/kg (i.p.) dose of ethanol increases the equilibrium point where no net flux is observed for dopamine in the NAc, but it does not alter the slope of the no net flux plot.[41]

Alternative possibilities include an indirect effect of ethanol on the stimulation of dopamine release or a rapid desensitization of the mechanism(s) by which ethanol acts to facilitate increased dopaminergic activity. A mechanism by which morphine increases mesocorticolimbic dopamine activity is through binding to mu opioid receptors (MORs) on specific GABAergic terminals that synapse onto VTA dopamine neurons. Activation of these MORs hyperpolarizes the GABA neuron, removing the tonic inhibition of VTA dopamine

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neurons.[43−45] The possibility of a disinhibitory mechanism of ethanol action on VTA dopamine neurons has been suggested based on evidence demonstrating a reduction in the activity of VTA GABAergic neurons following ethanol administration.[46−48] However, it is not entirely clear if this effect underlies the stimulation of mesocorticolimbic dopamine activity observed in vivo following acute ethanol administration (for review, see ref 4). Furthermore, other groups have reported conflicting results regarding the effect of ethanol on GABAergic transmission in the VTA. For example, ethanol has been shown to potentiate GABA release onto VTA dopamine neurons in vitro.[49,50] Additionally, a recent microdialysis study showed no significant effect of systemic ethanol administration on GABA concentrations in the VTA of alcohol-preferring and alcohol nonpreferring rat lines.[51]

"RESPONSE RATIOS"

The original study by Yim et al. directly compared the time courses of the dopamine response and dialysate ethanol concentrations, focusing on an extended time period encompassing the 15−120 min following the intraperitoneal (i.p.) injection.[22] This allowed comparison with ethanol-induced behaviors that had similar time courses, as discussed above. More recent work has now allowed higher resolution sampling during the microdialysis experiment so that times within the first 30 min of ethanol administration can be analyzed.

A potential confound in the study by Yim et al. is that ethanol administration via i.p. injections may be aversive to naïve rats and, as a result, such studies may include effects of stress on dopamine activity.[52−56] Intravenous ethanol administration minimizes stress in naïve animals because no animal handling is required. Using this route of administration, Howard et al. found a similar dissociation in the decline of the dopamine response relative to descending concentrations of ethanol.[20]

To explore the dissociation in the temporal profiles of extracellular dopamine and brain ethanol concentrations across brain regions, we performed post hoc analyses on our existing body of data. Similar to Yim et al., we computed ratios (referred to as "response ratios") of the dopamine response (represented as a percent over baseline) to tissue concentrations of ethanol.[22] We hypothesized that, within the first 25−30 min following acute ethanol administration, the "response ratios" within each brain region would decline in a similar manner. Contrary to our expectations, we observed regional differences in the temporal profiles of the "response ratios", suggesting distinct mechanisms may underlie the decline of the dopamine signal during the descending limb of the ethanol concentration curve. Here we describe the methods by which we determined the "response ratios" for each brain region, our results, and a limited interpretation of our results.

We analyzed data collected from in vivo microdialysis experiments in the nucleus accumbens core (NAc core) and shell (NAc shell) regions, medial prefrontal cortex (mPFC), and dorsomedial striatum (DMS) following acute i.v. ethanol administration (1 g/kg). In the subsequent sections, we first review the methodological details of our microdialysis experiments and discuss the adjustments made to our calculations to correct for procedural differences across experiments. Due to the lower concentration of endogenous dopamine in the mPFC, methodological modifications, such as an increase in probe lengths and a decrease in the perfusate flow rate, were made to enhance dopamine recovery in this region.

The probes used in our studies are constructed in our laboratory according to the procedures described by Pettit and Justice.[9,20,21,57,58] The probe active area is 1.5 mm for striatal regions and 2.75−3.25 mm for the mPFC.[9,20,21] Probes are continuously perfused with artificial cerebrospinal fluid (ACSF) at a flow rate of 2 μL/min for striatal samples and 1 μL/min for prefrontal cortical samples.[9,20,21] In every experiment, 2−4 samples are collected prior to any infusions to determine basal dopamine levels for each animal. Relative standard deviations are calculated to assess the stability of basal dopamine activity for each animal. Only those animals demonstrating relative standard deviation values < 0.25 were included in the microdialysis experiments. In striatal experiments, samples are collected in 5 min intervals, but the collection time is increased to 10 min for mPFC samples to account for the decreased flow rate. To control for any effects of an i.v. infusion on extracellular dopamine activity, a saline infusion is given either to the same animal prior to the ethanol infusion (for within-subjects study designs) or to a separate group of animals (for between-subjects study designs) and dialysate samples are subsequently collected. The control saline infusions had no significant effects on extracellular dopamine in all of the experiments included in our analyses.[9,20,21] At the conclusion of experiments, the ACSF is replaced with calcium-free ACSF and perfused through the probe for 1−2 h and a final 2 samples are collected. These samples are necessary to confirm calcium-dependent exocytotic dopamine release from neurons surrounding the probe membrane.

In Vivo Extraction Fraction for Ethanol. Dialysate ethanol concentrations are quantified via gas chromatography, but these concentrations are only a fraction of the tissue concentration of ethanol. To determine the in vivo recovery of ethanol for our probes in Long-Evans rats, Howard et al. inhibited ethanol metabolism via intravenous administration of the alcohol dehydrogenase inhibitor 4-methylpyrazole (2 mg/kg) to produce a "pseudo-steady state", and then systemically administered ethanol. A ratio of dialysate ethanol concentrations to blood ethanol concentrations was calculated, and the in vivo extraction fraction for ethanol was determined to be 0.14.[20] This value was used to determine the tissue concentrations of ethanol for each animal included in our analyses.

Effect of Methodological Differences on in Vivo Ethanol Recovery. To account for the differences in microdialysis parameters across experiments, we made adjustments to our calculations of ethanol tissue concentrations. A linear relationship approximates the increase in ethanol recovery across a probe as a function of probe length in the range of 1−3 mm.[59] Therefore, the in vivo recovery constant for ethanol was adjusted accordingly for the mPFC data. For example, the extraction fraction for ethanol for a probe with a length of 3 mm would be doubled to 0.28. Additionally, the microdialysis experiments sampling from the mPFC used a lower perfusate flow rate than the striatal experiments. An inverse relationship exists between perfusate flow rate and analyte extraction fraction, where the percent of relative in vivo recovery declines exponentially as the flow rate is increased.[60−63] As a result, the in vivo extraction fraction for ethanol was also increased by a factor of 1.56 for animals in the mPFC experiments. Therefore, with both adjustments accounting for the increased probe length and decreased flow rate, the

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final extraction fraction for ethanol for the mPFC dialysate samples was 0.364−0.437.

Methods. Using a similar method to that described by Yim et al.,[22] we calculated tissue concentrations of ethanol and "response ratios" for each animal within the first 25−30 min following the ethanol infusion (1 g/kg). It should be noted that because the studies were not conducted simultaneously, we are unable to directly compare the "response ratios" across the four brain regions. Given the variability in basal dopamine levels across the NAc core and shell, mPFC, and DMS, we focused on the percent change in dopamine levels relative to baseline. However, we also conducted the same analyses on the raw dopamine values and obtained similar temporal patterns in the "response ratios" for each brain region (data not shown). The equations used to determine tissue concentrations of ethanol and "response ratios" are listed below:

$$\text{tissue [EtOH]} = \frac{\text{dialysate [EtOH]}}{\text{extraction fraction}}$$

$$\text{response ratio} = \frac{\text{dopamine response}}{\text{tissue [EtOH]}}$$

"Response Ratios" for NAc Core and Shell. Following i.v. ethanol administration, the dopamine response in the NAc shell peaked to 40% over baseline within the first 5 min sample and then declined faster than dialysate ethanol concentrations. For the "response ratio" analyses, 23 animals from 3 studies[20,21] (the third study is unpublished) were included. It should be noted that a subset of these animals (n = 5) received a hypotonic ethanol solution, though it is unlikely that this had any significant effects on extracellular dopamine in the NAc, as hypotonic and isotonic ethanol solutions produced no differential effects on extracellular dopamine in the mPFC.[9] There were no statistically significant changes in the "response ratios" during the initial 25 min following the ethanol infusion for the NAc shell (Figure 3; F(4, 88) = 1.82, n.s.). Of the

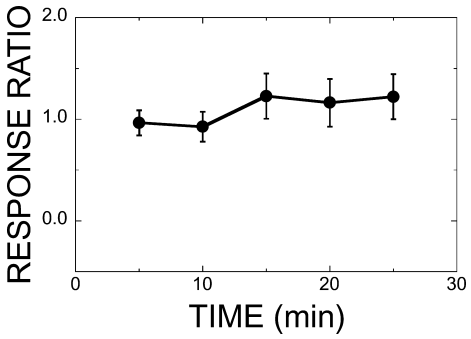

Figure 3. "Response ratios" in the nucleus accumbens shell (NAc shell) region for the first 25 min following intravenous ethanol administration (1 g/kg). The ratios are the dopamine response (represented as a percent over baseline) relative to tissue concentrations of ethanol. Symbols represent mean ± SEM (n = 23).

animals included in the analyses, there were four animals whose dopamine response returned to or dropped below baseline within the 25 min following the ethanol infusion.

For the NAc core, six animals from one study[20] were included in the "response ratio" analyses, and these animals also received a hypotonic ethanol solution. Within the first 25 min following the ethanol infusion, there were no significant changes in the "response ratios" in the core (Figure 4; F(4,

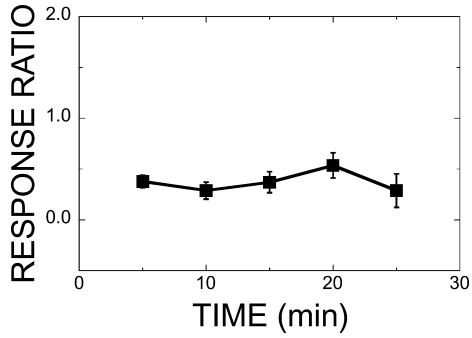

Figure 4. "Response ratios" in the nucleus accumbens core (NAc core) region for the first 25 min following intravenous ethanol administration (1 g/kg). The ratios are the dopamine response (represented as a percent over baseline) relative to tissue concentrations of ethanol. Symbols represent mean ± SEM (n = 6).

20) = 1.05, n.s.). The ethanol-induced dopamine response returned to or dropped below baseline in 2 of the 6 animals within 25 min following the ethanol infusion. Three additional animals had extracellular dopamine levels return to near baseline levels within the last 5 min sample.

"Response Ratios" for mPFC. Nineteen animals from one study[9] were included in the "response ratio" analyses for the mPFC. The "response ratios" significantly declined at a relatively linear rate over the first 30 min following the ethanol infusion (Figure 5; F(2, 36) = 5.66, p = 0.007). In 3 of the 19

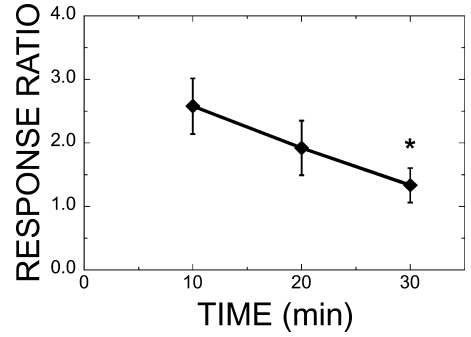

Figure 5. "Response ratios" in the medial prefrontal cortex (mPFC) region for the first 30 min following intravenous ethanol administration (1 g/kg). The ratios are the dopamine response (represented as a percent over baseline) relative to tissue concentrations of ethanol. Symbols represent mean ± SEM (n = 19). *Post hoc t tests indicate significance when compared to the 10 min time point following overall significance in the ANOVA; $p < 0.05$.

animals included in the analyses, the dopamine response returned or dropped below baseline within the 30 min following the ethanol infusion.

"Response Ratios" for DMS. The DMS "response ratio" analyses included nine animals from one study (unpublished data). There was no main effect of time in the overall ANOVA for the "response ratios" in this region (Figure 6; F(4, 32) = 0.553, n.s.). There were five animals whose dopamine responses returned to or dropped below baseline within the first 25 min following the ethanol infusion.

INTERPRETATION

"Response ratios" were calculated for the first 25−30 min following acute i.v. ethanol and thus are likely not relevant to acute behavioral tolerance, as behavioral tolerance occurs on

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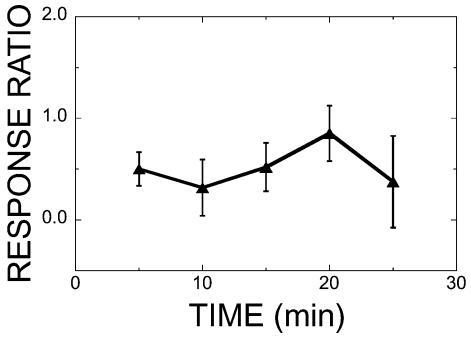

Figure 6. "Response ratios" in the dorsomedial striatum (DMS) region for the first 30 min following intravenous ethanol administration. The ratios are the dopamine response (represented as a percent over baseline) relative to tissue concentrations of ethanol. Symbols represent mean ± SEM (n = 9).

the time course of hours, as discussed above. However, within this short time frame, acute tolerance may be developing to the pharmacological mechanisms by which ethanol stimulates mesocorticolimbic dopamine activity. Our analyses of "response ratios" do not directly assess the mechanism by which ethanol stimulates extracellular dopamine concentrations. However, these analyses did reveal interesting regional differences in the decline of the dopamine signal during the descending limb of the blood ethanol concentration curve. Below, we speculate about possible reasons for the faster decline in the "response ratios" in the PFC versus striatal regions.

Projection-Specific Subpopulations of Midbrain Dopamine Neurons May Be Differentially Affected by Ethanol. Recent work has suggested that midbrain dopamine neurons are physiologically, molecularly and functionally distinct, and therefore may be differentially affected by commonly abused drugs. While there is not yet a consensus in the field regarding the specific differences among midbrain dopamine neurons, and species-specific differences are apparent, recent work has demonstrated that specific characteristics of midbrain dopamine neurons vary depending on neuronal projection targets. Some of this recent work as well as the general physiological and molecular characteristics of midbrain dopamine neurons have been reviewed previously[4,45,64−67] and thus will be only briefly summarized here.

Specifically, VTA dopamine neurons projecting to the PFC, NAc core, NAc medial shell, and basolateral amygdala (BLA) do not universally display the characteristics historically used to identify dopamine neurons. For example, recordings from adult mouse brain slices demonstrate that in response to low current levels, these dopamine neurons fire action potentials at frequencies in the range of 10−15 Hz, which are significantly higher than the firing frequencies of those projecting to the NAc lateral shell and nigrostriatal dopamine neurons (3−6 Hz) in vitro.[64,65,68,69] Furthermore, these fast-firing dopamine neurons are able to sustain these higher firing frequencies for several seconds.[68] Another key physiological difference is the lack of an I_h current in the fast-firing dopamine neurons in vitro, which contrasts the large I_h current observed in dopamine neurons projecting to the NAc lateral shell.[64,65]

Additionally, molecular differences exist among these distinct subpopulations of midbrain dopamine neurons, including the expression of somatodendritic D2-like autoreceptors, which has historically been used as a criterion for identifying dopamine neurons. Using transgenic mice that lacked specifically D2-subtype autoreceptors on dopamine neurons, but expressed postsynaptic D2 receptors on nondopaminergic neurons, Bello et al. recorded the activity of presumed midbrain dopamine neurons in horizontal brain slices.[70] These neurons did not respond to bath application of quinpirole, while those from control mice demonstrated hyperpolarization. This work provides strong evidence that within the D2-like receptor family, D2-subtype receptors are the primary mediators of autoinhibition at the level of the cell body in midbrain dopamine neurons.[70] However, the projection targets of the recorded neurons were not identified, which is critical given the profound heterogeneity observed among midbrain dopamine neurons. Furthermore, the identification criteria for dopamine neurons used by Bello and colleagues may have prevented sampling from mesocortical neurons, which appear to lack somatodendritic autoreceptors altogether.[65,68,71,72] Lammel and colleagues reported that, in coronal midbrain slices of adult mice, bath application of 100 μM dopamine did not alter the firing frequencies of mesocortical dopamine neurons while hyperpolarizing all other VTA dopamine neurons.[68] It should be stated, however, that species-specific variation may exist with regard to the expression of somatodendritic autoreceptors on mesocortical dopamine neurons. Margolis and colleagues identified PFC-projecting tyrosine hydroxylase-positive neurons that were hyperpolarized by bath application of quinpirole in horizontal brain slices from adolescent rats.[73]

When considering these molecular and physiological distinctions, it is not surprising that midbrain dopamine neurons also demonstrate significant pharmacological and functional heterogeneity that is also associated with their projection targets. In a series of studies, Westerink and colleagues[5,7,74] demonstrated significant differential responsiveness of mesocortical, mesolimbic, and nigrostriatal dopamine neurons to various pharmacological manipulations. For example, infusion of the $GABA_a$ receptor agonist muscimol into the VTA through a microdialysis probe significantly decreased extracellular dopamine in the PFC and NAc, but in contrast muscimol infused into the SNc significantly elevated extracellular dopamine levels in the dorsal striatum.[5,74] Administration of NMDA and the $GABA_b$ receptor agonist baclofen into the VTA or SNc via a microdialysis probe also produced differential effects on the percent change in and the temporal pattern of extracellular dopamine in the PFC, NAc, and dorsal striatum.[5]

Rewarding and aversive stimuli also have been shown to produce differential effects on extracellular dopamine in cortical and striatal regions. Acute exposure to rewarding or appetitive stimuli such as drugs of abuse significantly increases extracellular dopamine in the NAc and PFC, but the dorsal striatum appears to be acutely less sensitive to such stimuli.[9,12,20,21,75−79] Aversive and stressful stimuli have been shown to increase extracellular dopamine in the PFC to a much greater extent than in the NAc or dorsal striatum.[53−55,75−77] Additionally, aversive stimuli increase the AMPAR/NMDAR ratio only in dopaminergic cells projecting to the PFC and lateral NAc shell, indicating modulation of excitatory synapses on these subpopulations of dopamine neurons.[80] In contrast, AMPAR/NMDAR ratios increased only in those dopamine neurons projecting to medial and lateral NAc in response to acute cocaine reward.[64,80] Similarly, rats exposed to a single high dose of toluene vapor demonstrated significant increases in AMPA/NMDA ratios in VTA dopamine neurons projecting to the NAc core and medial shell, but not in mesocortical

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dopamine neurons.[81] Therefore, subpopulations of midbrain dopamine neurons appear serve distinct roles in the response to salient events depending on the motivational valence of the event (for reviews, see refs 45 and 66), and this may have functional relevance to the stimulation of dopamine activity observed in specific target regions following acute ethanol administration

While the cellular and molecular mechanisms by which ethanol stimulates mesocorticolimbic dopamine activity are not entirely understood, ethanol may exert differential effects on midbrain dopamine neuron subpopulations. Therefore, the anatomical distribution and physiological, molecular, and functional heterogeneity of midbrain dopamine neurons may contribute to the regional differences observed in the "response ratio" analyses. Ethanol has been shown to directly stimulate VTA dopamine neurons,[2,3] but the projection targets of the recorded neurons were not identified. Differential effects of ethanol have been observed in the VTA with respect to the anterior and posterior regions. Rats will self-administer various doses of ethanol directly into the posterior VTA but not the anterior VTA.[82] Recently, ethanol has been shown to increase the firing rate of dopamine neurons located in the posterior VTA, but it suppresses the firing rate of dopamine neurons originating in the anterior VTA.[83] These differential effects of ethanol on the anatomical divisions of the VTA may contribute to the differences seen in the "response ratios" in target regions. The dopamine neurons projecting to the PFC, NAc core and medial shell, and BLA form distinct populations within the medial posterior VTA.[64,68] In contrast, dopamine neurons projecting to the lateral NAc shell are found in the lateral posterior and anterior VTA, with a significant number of these neurons also located in the SNc.[68]

Additionally, acute ethanol may selectively modulate excitatory (and/or inhibitory) synapses on VTA dopamine neurons, similar to the effect observed following acute cocaine or toluene administration.[80,81] Acute systemic administration of ethanol has been shown to strengthen excitatory synapses on VTA dopamine neurons, as indicated by increased AMPAR/NMDAR ratios,[84] but because the projection targets of these neurons were not identified, it is unclear if this effect is uniform across dopamine neurons. Ethanol may exert differential effects on midbrain dopamine neurons, such as selectively enhancing firing rates or excitatory/inhibitory synapses, which could alter dopamine activity in target regions and thus potentially contribute to the regional differences in "response ratios".

Regional Differences in Dopamine Clearance. The observed regional differences in the temporal profiles of the "response ratios" may be due, at least in part, to regional differences in the mechanisms of dopamine clearance or variations in the sensitivity of clearance mechanisms to ethanol. Early on it was demonstrated that regional differences exist in the dynamic regulation of extracellular dopamine. Garris and Wightman determined ratios of dopamine release to uptake to quantify and compare the regulation of extracellular dopamine across the PFC and striatal regions.[85] In striatal regions, this ratio is low, indicating "uptake-dominant" regulation of extracellular dopamine concentrations. In contrast, this ratio is 5−10 times larger in the PFC, indicating "release-dominant" dynamics of interstitial dopamine. Furthermore, dopamine terminals in the PFC show a reduced density of dopamine transporters relative to striatal regions.[86,87]

Clearance of evoked dopamine in the PFC appears slower than that in the striatum and uptake by high affinity dopamine transporters (DAT) is not the primary mechanism of clearance.[87,88] Studies comparing the effect of DAT blockade across brain regions consistently demonstrate reduced efficacy of DAT inhibition on extracellular dopamine in the PFC relative to striatal regions.[87,89−92] For example, the dopamine uptake inhibitor GBR-12909 increases the amplitude and time course of dopamine signals by 200% in the striatum, which contrasts with the 30−40% increase in these parameters observed in the PFC.[87,93]

Other work has focused on the predominant role of metabolism relative to catecholamine uptake mechanisms on dopamine clearance in the PFC. Using in vitro voltammetry, Wayment et al. demonstrated a linear rate of clearance in the PFC, but pharmacological blockade of DAT/NET (norepinephrine transporter) and inhibition of monoamine oxidase (MAO) produced a biphasic dopamine clearance profile due to an additive effect of the drugs. Based on these findings, Wayment and colleagues concluded that dopamine clearance velocity in the PFC is 50−70% dependent on uptake mechanisms (DAT/NET) and 30−50% dependent on MAO.[90] However, this study did not address the role of metabolism by catechol-O-methyltransferase (COMT), which is particularly important for dopamine clearance in regions where DAT density is low and has been demonstrated to play a significant role in dopamine clearance in the PFC.[88,92,94,95] COMT mRNA expression is significantly higher in the PFC than the striatum in human and rat brains.[96] COMT metabolizes dopamine to 3-methoxytyramine (3-MT), which accounts for approximately 60% of the total dopamine turnover in the frontal cortex but only 15% in the striatum.[97] Additionally, pharmacological inhibition of COMT by tolcapone in the PFC significantly increases evoked extracellular dopamine.[94,98] In contrast, systemic administration of tolcapone does not alter extracellular dopamine in the striatum except under the conditions of dopamine uptake inhibition.[89] In summary, dopamine clearance in the PFC relies heavily on metabolism, while in striatal regions dopamine clearance is driven by reuptake mechanisms. If the rate of decline of the dopamine signal is differentially regulated across brain regions, then this could be a potential explanation for the observed regional differences in the temporal profiles of the "response ratios".

Examination of the interaction between ethanol and dopamine clearance mechanisms has predominantly focused on DAT. Acute ethanol administration has been shown to enhance,[99,100] decrease,[101] or not affect[41,42] DAT uptake velocity in the striatum. Of note, however, is that, despite the discrepant observations of ethanol's effects on DAT activity, there appears to be agreement that ethanol does not alter the transporter's affinity for dopamine.[100,101] Genetic manipulations may provide a means of resolving the discrepant results. DAT-knockout (DAT-KO) mice show similar increases in extracellular dopamine in the dorsal striatum as wild type (WT) mice following acute systemic administration of ethanol, which is consistent with previous work demonstrating that direct inhibition or reduction in DAT activity by ethanol is not a primary mechanism underlying stimulation of striatal dopamine activity.[102] Furthermore, fast-scan cyclic voltammetry in brain slices from DAT-KO and WT mice demonstrated no effect of 20 or 200 mM ethanol on the rate of dopamine clearance in the dorsal striatum.[102] However, to date, there are no published studies exploring the effect of acute ethanol on DAT in the PFC.

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At this time, limited work has explored the interaction between acute ethanol and dopaminergic metabolic mechanisms in the PFC. While early studies demonstrated increased tissue concentrations of dopamine metabolites in the striatum and PFC of animals that received acute systemic ethanol administration, it is unclear if these elevations are a direct result of ethanol-induced increases in extracellular dopamine or if these effects vary depending on the ethanol dose.[103−105] Further research is necessary to determine if ethanol directly affects the activity of enzymes involved in dopamine metabolism, specifically within the PFC, as these enzymes may be potential therapeutic targets in alcohol use disorders.

CONCLUSION

In conclusion, a dissociation exists in the temporal profiles of extracellular concentrations of dopamine and tissue concentrations of ethanol, which may be attributable to ethanol's mechanism of action. Within the first 25−30 min following acute i.v. ethanol administration, the time course of this dissociation demonstrates regional variability. Such variability may be due to ethanol's pharmacological interactions with a heterogeneous population of midbrain dopamine neurons, regional differences in dopamine clearance mechanisms, and/or acute modulation of dopamine clearance mechanisms by ethanol. Further investigation is necessary to determine if ethanol exerts such effects on dopamine activity, the precise cellular and molecular mechanisms by which ethanol enhances mesocorticolimbic dopamine activity, and if the ethanol-induced transient rise and decline in extracellular dopamine contributes to the development of acute tolerance to the stimulating effects of ethanol.

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ABBREVIATIONS

3-MT, 3-methoxytyramine; ACSF, artificial cerebrospinal fluid; COMT, catechol-*O*-methyltransferase; DAT, dopamine transporter; DMS, dorsomedial striatum; i.p., intraperitoneal; i.v., intravenous; MAO, monoamine oxidase; MOR, mu opioid receptor; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; NET, norepinephrine transporter; SNc, substantia nigra, pars compacta; VTA, ventral tegmental area; WT, wild type

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