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Regional analysis of the pharmacological effects of acute ethanol on extracellular striatal dopamine activity

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Abstract

BACKGROUND—The objective of the present study was to characterize the acute pharmacological effects of ethanol on extracellular dopamine in the dorsomedial and dorsolateral striata. This is the first study to quantify and directly compare the effects of acute ethanol on dopamine in these subregions. Therefore, we also tested the nucleus accumbens as a positive control. We hypothesized that while ethanol may increase extracellular dopamine in the dorsomedial striatum and dorsolateral striatum, the magnitude of this increase and the temporal profiles of extracellular dopamine concentrations would differ among the dorsomedial striatum, dorsolateral striatum, and nucleus accumbens.

METHODS—We performed in vivo microdialysis in adult, male Long Evans rats as they received a single (experiment 1) or repeated (experiment 2) doses of ethanol.

RESULTS—The results of our positive control study validate earlier work by our lab demonstrating that acute intravenous ethanol immediately and robustly increases extracellular dopamine in the nucleus accumbens (Howard et al. 2008). In contrast, a single 1 g/kg dose of intravenous ethanol did not significantly affect extracellular dopamine in the dorsomedial striatum or the dorsolateral striatum. However, following a cumulative ethanol dosing protocol we observed a ramping up of tonic dopamine activity in both the dorsomedial striatum and dorsolateral striatum over the course of the experiment, but this effect was more robust in the dorsomedial striatum.

CONCLUSIONS—These results suggest that distinct mechanisms underlie the stimulating effects of acute ethanol on extracellular dopamine in striatal subregions. Additionally, our findings suggest a role for the dorsomedial striatum and minimal to no role for the dorsolateral striatum in mediating the intoxicating effects of acute moderate to high doses of ethanol.

Keywords

striatum; ethanol; dopamine; ventral tegmental area; substantia nigra

Several studies have demonstrated that the mesolimbic and nigrostriatal dopaminergic systems are sensitive to the pharmacological effects of acute ethanol. In vitro electrophysiological studies by Brodie et al. (1999,1990) demonstrated that ethanol dose-

dependently increases the firing rate of dopamine cell bodies in the ventral tegmental area. Mereu et al. (1984) reported that in paralyzed rats, intravenous administration of low to moderate doses of ethanol (0.5–2.5 g/kg) increased the firing rate of dopamine cell bodies in the substantia nigra, while high doses (4.0 g/kg and higher) suppressed firing rates.

In vivo neurochemical studies sampling from striatal regions innervated by ventral tegmental area dopamine neurons confirm an effect of acute ethanol on mesolimbic dopamine activity, suggesting a dose-dependent, biphasic effect of ethanol on extracellular dopamine activity. Microdialysis and voltammetry studies indicate that low to moderate doses of systemic ethanol (0.5–2.5 g/kg) increase extracellular dopamine in the nucleus accumbens (Robinson et al. 2009; Howard et al. 2008; Yim et al. 2000a; Imperato & Di Chiara 1986). Higher doses of ethanol (5 g/kg), however, appear to depress extracellular dopamine activity in the nucleus accumbens (Imperato & Di Chiara 1986).

Relative to the mesolimbic dopamine system, in vivo microdialysis studies exploring the effects of acute ethanol on nigrostriatal dopamine activity are less consistent. Imperato & Di Chiara (1986) demonstrated that low doses of systemic ethanol (0.25–0.5 g/kg; i.p.) had no significant effect on dorsal striatal dopamine activity, while moderate to high doses (1.0–5.0 g/kg; i.p.) increased extracellular dopamine relative to baseline. In contrast, Blanchard et al. (1993) reported enhanced extracellular dopamine following low doses of ethanol (i.p.) and no effect or decreases following moderate to high doses of ethanol (i.p.). Despite these inconsistent findings, it appears that substantia nigra dopamine neurons are generally less sensitive to acute ethanol as any increases from baseline are less robust in the dorsal striatum relative to the nucleus accumbens.

A potential limitation of these earlier studies is that the dorsal striatal subregions were not tested separately. The dorsomedial and dorsolateral striata have been shown to be distinct functionally and in the origins of their dopamine afferents (Joel & Weiner 2000; Yin et al. 2008; Voorn et al. 2004). The dorsomedial striatum is critical for learning the action-outcome associations that underlie goal-directed behaviors, while the dorsolateral striatum is critical for learning the stimulus-response associations that underlie habit formation (Yin et al. 2008). Dopamine afferents to the dorsomedial striatum originate in the retrorubral area, substantia nigra pars compacta and the ventral tegmental area (Voorn et al. 2004; Joel & Weiner 2000). In contrast, the dorsolateral striatum only receives dopaminergic projections from the substantia nigra and retrorubral area (Voorn et al. 2004; Joel & Weiner 2000). Recent work has demonstrated heterogeneity in the sensitivity of midbrain dopamine neurons to drugs of abuse, including ethanol (Mrejeru et al. 2015; Lammel et al. 2011). Therefore, it is possible that midbrain dopamine projections to the dorsomedial striatum and dorsolateral striatum may differ in their sensitivity to ethanol, which may be assessed by measuring extracellular dopamine concentrations in these target subregions.

In this study we sought to characterize and directly compare the acute pharmacological effects of ethanol on extracellular dopamine in both the dorsomedial striatum and dorsolateral striatum using microdialysis in awake, freely moving animals. Intravenous administration of either a single dose (1 g/kg) or a cumulative dosing procedure was used to minimize the effects of stress and handling on extracellular dopamine activity, as well as to

avoid the potentially confounding effects of behavior, expectation, and motivation on dopamine that may be evident in a self-administration design. Our results indicate that dopamine in these subregions is differentially affected by ethanol.

Methods

Animals

A total of fifty-three adult Long Evans rats were used in these experiments. Twenty-six rats (from Charles River, Raleigh, NC), weighing 300–430 grams on dialysis day, were used for the acute ethanol experiments. Twenty-seven rats (from Harlan, Indianapolis, IN), weighing 310–450 grams on dialysis day, were used for the cumulative dose-response experiments. Rats were housed in a temperature (25° C) and light (12 hours on/12 hours off) controlled room and had access to chow and tap water ad libitum. Upon arrival, the rats were dually-housed for the first week, during which they were handled and weighed daily. All procedures were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin.

Surgery

Cannulation and jugular catheterization surgeries were carried out according to the procedures described in Duvauchelle (1998) and Howard et al. (2008). Intravenous catheters were constructed from silastic tubing (0.30 mm ID, 0.64 mm OD, Fisher Scientific, Hampton, NH), a metal cannula (22 gauge, Plastics One, Roanoke, VA), and silicone adhesive. Rats were anesthetized with isoflurane and an incision was made above the skull. Upon securing the catheter in the jugular vein, it was pulled subcutaneously to the top of the skull. A microdialysis guide cannula (21 gauge, Plastics One, Roanoke, VA) was implanted into the skull directly above the nucleus accumbens (AP +2.0, ML +1.1, DV –3.4), dorsomedial striatum (AP +1.2, ML +1.8, DV –1.0), or the dorsolateral striatum (AP 0.0, ML +3.7, DV –1.0) while the animal was in a stereotaxic frame. The guide cannula, catheter cannula, and a tether bolt were held in place on the skull with dental cement. A Timentin (13.4 mg/kg, Animal Health International) and heparinized saline solution was used as a catheter lock solution to maintain patency. Catheters were flushed with 0.2 mL of heparinized saline at least once a week before dialysis experiments commenced. Following surgeries, rats were individually housed and given at least 6 days of recovery prior to experiments.

Drugs

A 10 % (w/v) solution of ethanol (1 g/kg, 10 ml/kg) in saline was made from 95 % ethanol (Aaper Alcohol and Chemical Co., Shelbyville, KY).

Microdialysis

Approximately 12–18 hours prior to the microdialysis experiment, rats were lightly anesthetized with isoflurane to implant the microdialysis probe through the guide cannula and to secure the animal to the tethering apparatus. The probes (1.5 mm active membrane length, 270 µm OD, 13,000 MWCO) were constructed in the laboratory according to the

procedures described by Pettit & Justice (1991). Probes were continuously perfused with artificial cerebrospinal fluid (ACSF; 149 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂, 0.2 mM ascorbic acid, and 5.4 mM D-glucose) overnight at 0.2 µL/min. The flow rates were increased to 2.0 µL/min at least 2 hours prior to dialysate sample collection and remained at 2.0 µL/min for the duration of the experiment.

Animals were awake and freely moving during the microdialysis experiments. In all experiments, the sample collection interval was 5 minutes and four baseline samples were collected per animal prior to any infusions. Baseline dopamine concentrations were required to have a relative standard deviation <0.16 for data inclusion. To confirm that the dopamine in the dialysate samples was due to calcium-dependent exocytotic release, probes were perfused with calcium-free ACSF for approximately 2 hours at the conclusion of all experiments and additional samples were collected. All dialysate samples were immediately frozen on dry ice upon collection.

Experiment 1: Acute intravenous 1 g/kg ethanol

Following collection of the baseline samples, animals received a manual bolus of saline. Samples were collected for 45 minutes following the infusion. Animals then received a manual bolus of a 1 g/kg dose of ethanol (10% w/v, in saline), and post-infusion samples were collected for 25 minutes (Table 1).

Experiment 2: Cumulative intravenous ethanol

For the cumulative dosing study, animals received 4 infusions of ethanol (10% w/v, in saline) or saline. Infusions were administered 20 minutes apart via a syringe pump (CMA 400, Japan) at a flow rate of 1 mL/min. All animals received a saline infusion at least 1 hour prior to sample collection to habituate them to the sound of the pump. Ethanol infusions were administered in the following order: 0.5, 0.5, 0.75, and 0.75 g/kg (Table 1). This produced cumulative doses of 0.5, 1.0, 1.75, and 2.25 g/kg.

Histology

After the dialysis experiments, rats were overdosed with sodium pentobarbital (150 mg/kg, i.v.) and probes were carefully unimplanted. Their brains were harvested and stored in vials containing 10% formalin for at least 24 hours. The brain tissue was coronally sectioned (120 µm thick), stained with cresyl violet, and examined under a microscope to confirm probe placement. The probe tracks were mapped using the Paxinos & Watson (2007) atlas.

HPLC Analysis

Dialysate dopamine concentrations were quantified via reversed-phase high performance liquid chromatography with electrochemical detection. The HPLC systems consisted of a Luna 50 × 1.0 mm column (C18, 3 µm particle size; Phenomenex, Torrance, CA), 2 mm glassy carbon working electrode electrochemical detector (SenCell; Antec Leyden) at potential +450 mV, an 8125 manual injector (Rheodyne, Cotati, CA), and an INTRO controller (Antec Leyden). Mobile phase was continuously pumped through the systems via either a syringe pump (ISCO 65D, Telodyne) or an LC110S pump (Antec Leyden). The mobile phase consisted of 0.500 g octanesulfonic acid, 0.050 g decanesulfonic acid, 0.128 g

ethylenediaminetetraacetic acid, and 11.08 g NaH_2PO_4 dissolved in 1 liter of deionized water, and methanol as the organic solvent (8–10% v/v). The mobile phase was adjusted to pH 5.6 prior to adding the methanol. The sample injection volume was 5 microliters. External standards (0.3125 to 7.5 nM) were used to quantify the dopamine concentrations. EZChrom Elite software (Agilent, Wilmington, DE) was used to record and analyze all chromatograms. Only dopamine peaks with a signal to noise ratio >6 were included in the analyses.

GC Analysis

For animals that received ethanol infusions, dialysate ethanol concentrations were quantified via gas chromatography (GC) with flame ionization detection. Prior to freezing the dialysate samples, 2 μL aliquots were transferred to 2 mL glass chromatography vials and sealed with a septum. A Varian CP 3800 (Agilent Technologies) or Scion 436 gas chromatograph (Bruker, Netherlands) and a Varian 8200 headspace autosampler was used to analyze the concentrations of ethanol in the samples. The stationary phase was an HP Innowax capillary column (30 m $\times$ 0.53 mm $\times$ 1.0 μm film thickness). For the acute, single-dose studies, helium was used as the carrier gas, but prior to the cumulative dosing studies a hydrogen generator (Model 20H-MD, Parker Hannifin, England) was installed to use hydrogen as the carrier. Resulting ethanol peaks were recorded using either Varian Star Chromatography Workstation or CompassCDS (Bruker, Netherlands) software, and calibration was achieved using external standards (0.3125 to 40 mM).

Statistical analyses

Repeated measures analyses of variance (ANOVA) were performed on both the dialysate dopamine concentrations and the dialysate dopamine values normalized to baseline. Repeated measures ANOVAs were also performed on the dialysate ethanol concentrations. Two animals from experiment 1 (1 each from the nucleus accumbens and dorsolateral striatum groups) were missing 1–2 dialysate ethanol values due to technical issues with the GC. In order to have the animals included in statistical analyses, the mean of the surrounding values was used in place of the missing value. For the overall repeated measures ANOVA on the dialysate ethanol data, the degrees of freedom were appropriately adjusted to account for these missing values.

In experiment 1, we analyzed the effects of the saline and ethanol infusions separately, using 2-way mixed-model, repeated measures ANOVAs. Separate ANOVAs were performed on the first 9 time points (4 baseline and 5 post-saline infusion) and the second 9 time points (4 baseline and 5 post-ethanol infusion). The four samples prior to each infusion were used to determine basal dopamine concentrations for each animal. In each ANOVA, the between subjects factor was brain region (3 levels: nucleus accumbens, dorsomedial striatum, dorsolateral striatum), and the within subjects factor was time.

In experiment 2, we performed a three-way, mixed model, repeated measures ANOVA. The 2 between-subjects factors were brain region (2 levels: dorsomedial striatum, dorsolateral striatum) and drug (2 levels: saline, ethanol) and the within-subjects factor was time (20 time points). Additionally, because our a priori hypothesis was that cumulative infusions of

ethanol would differentially affect extracellular dopamine within the dorsomedial and dorsolateral striata relative to cumulative saline infusions, we also explored the time by drug interaction within each brain region. Samples 1–4 were used to determine basal dopamine concentrations for each animal. Sample 10 from an animal in the dorsomedial striatum-saline group was contaminated and thus removed from the dataset. The missing value was replaced with the mean of the surrounding samples and the degrees of freedom were appropriately adjusted.

We performed simple effects analyses (with Bonferroni corrections) when appropriate to follow up on any significant interaction effects identified in the overall ANOVAs (Kirk, 1982). Data were analyzed using SPSS software (IBM). Significance was assigned if $p < 0.05$; NS = not significant.

RESULTS

Basal dopamine concentrations

Basal dopamine concentrations for each brain region are reported in Table 1. In experiment 1, the saline and ethanol baseline values for each animal were analyzed separately. Therefore, it was possible for an animal's saline data to be excluded (due to instability of the baseline values) despite the ethanol data meeting all inclusion criteria, and vice versa. Due to significant differences in baseline dopamine concentrations among the 3 brain regions prior to the ethanol infusion ($F_{2,18}=12.05$, $p<0.01$), we did the statistical analyses on the percent basal data for experiment 1. For experiment 2, because there were no significant differences in basal dopamine values, we analyzed the nanomolar dialysate dopamine concentrations.

Single dose 1 g/kg

Control infusions of saline had no significant effect on dopamine activity in any subregion (Fig 1a; $F_{8,144}=1.89$, NS). In contrast, the ethanol infusion stimulated dopamine release in some but not all striatal subregions (subregion x time interaction: $F_{16,144}=2.06$, $p<0.05$). In the nucleus accumbens, extracellular dopamine was significantly elevated above baseline following the ethanol infusion ($n=8$; Fig 1b; simple effect of time: $F_{8,144}=7.98$, $p<0.01$). Post hoc analyses showed that ethanol-induced stimulation of dopamine peaked at 40% above baseline within the first 5 minutes following the infusion ($F_{4,144}=11.16$, $p<0.01$). Furthermore, dopamine remained elevated in the nucleus accumbens 15–25 minutes following the infusion ($F_{4,144}=3.54$ – 5.14 , $p<0.01$ for each time point). In the dorsomedial striatum, the same dose of ethanol stimulated dopamine to 16% above baseline, but this effect was not statistically significant ($n=7$; Fig 1b; simple effect of time: $F_{8,144}=1.49$, NS). In the dorsolateral striatum, there was clearly no effect of ethanol on extracellular dopamine ($n=6$; Fig 1b; simple effect of time: $F_{8,144}=0.22$, NS).

Simple effect analysis indicated that the dopamine response in the sample immediately following the ethanol infusion differed among the three brain regions ($F_{2,138}=11.5$, $p<0.001$). Post hoc tests found that the response in the nucleus accumbens was significantly greater than that in the dorsomedial striatum ($F_{1,138}=8.84$, $p<0.01$) and dorsolateral striatum ($F_{1,138}=22.04$, $p<0.01$). However, extracellular dopamine concentrations in the sample

immediately following the ethanol infusion were not significantly different between the dorsomedial and dorsolateral striatum ($F_{1,138}=3.21$, NS). Additional simple effects and post hoc analyses revealed only significant differences between the nucleus accumbens and the dorsolateral striatum ($F_{1,138}=9.44$, $p<0.01$) at the 80-minute time point (15 minutes following the ethanol infusion). Lastly, the dialysate ethanol time courses were not significantly different among the 3 subregions (Fig 1c; $F_{2,18}=1.76$, NS).

Cumulative dose-response

The objective of Experiment 2 was to use a cumulative-dosing design to test whether a large ethanol dose range had differential effects on extracellular dopamine in the dorsomedial striatum and dorsolateral striatum. There were 4 groups: dorsomedial striatum-ethanol, dorsomedial striatum-saline, dorsolateral striatum-ethanol, and dorsolateral striatum-saline. Within each group, the within-subjects factor was time. Infusions occurred at the 20, 40, 60, and 80-minute time points.

The overall ANOVA conducted on the raw (untransformed; Figs 2a and 2c) nanomolar dopamine concentrations indicated significant main effects of time (within-subjects; $F_{19,436}=3.02$, $p<0.001$) and drug (between-subjects; $F_{1,23}=8.28$, $p<0.01$) on extracellular dopamine. While the three-way interaction between time, drug, and brain region was not significant ($F_{19,436}=0.94$, NS), there were significant interactions of time by drug ($F_{19,436}=5.08$, $p<0.001$) and of time by brain region ($F_{19,436}=2.08$, $p<0.01$). Post-hoc analyses following up on the interaction of time and drug indicated a significant simple effect of time only for the ethanol groups ($F_{19,436}=8.43$, $p<0.001$; collapsed across dorsomedial striatum and dorsolateral striatum). Cumulative saline infusions had no significant effect on extracellular dopamine concentrations ($F_{19,436}=1.04$, NS; collapsed across dorsolateral striatum and dorsomedial striatum). Additional post hocs examining drug effects indicated significant differences between the saline and ethanol groups at time points 55–90 minutes ($F_{1,28}=5.40$, $p<0.01$; collapsed across dorsomedial striatum and dorsolateral striatum).

Following up on the time by brain region interaction, post hoc analyses indicated a significant simple effect of time on extracellular dopamine only in the dorsomedial striatum ($F_{19,436}=5.40$, $p<0.001$; collapsed across saline and ethanol groups). However, subsequent post hoc analyses comparing baseline vs individual time points were not statistically significant (Fig 2a). The effect of time was not significant for the dorsolateral striatum ($F_{19,436}=1.38$, NS; collapsed across saline and ethanol groups). Additionally, because our a priori hypothesis was that ethanol would differentially affect dopamine concentrations in the dorsomedial and dorsolateral striatum, we also analyzed drug effects within each subregion. A significant time by drug interaction was observed in both the dorsomedial ($F_{19,436}=3.26$, $p<0.001$) and dorsolateral ($F_{19,436}=2.71$, $p<0.001$) striatum. However, subsequent post hoc analyses comparing ethanol time points vs. baseline or ethanol vs. saline at individual time points were not statistically significant for either subregion.

Separate analyses were performed on the normalized data (Figure 3). Similar to the results from the analyses on the raw data, the overall ANOVA indicated significant main effects of time (within-subjects; $F_{19,436}=2.93$, $p<0.001$) and drug (between-subjects; $F_{1,23}=10.84$,

$p < 0.01$), as well as a significant interaction of time by drug ($F_{19,436} = 10.58$, $p < 0.001$). The three-way interaction between time, drug, and brain region was not significant ($F_{19,436} = 0.90$, NS), but the two way interaction between time and brain region trended towards significance ($F_{19,436} = 1.46$, $p = 0.097$). Post hoc analyses following up on the overall time by drug interaction indicated a significant simple effect for the ethanol groups only ($F_{19,436} = 8.57$, $p < 0.001$; collapsed across dorsomedial and dorsolateral striatum). Cumulative saline infusions had no significant effect on extracellular dopamine ($F_{19,436} = 1.09$, NS; collapsed across dorsomedial and dorsolateral striatum).

Although the interaction between time and brain region did not meet the criteria for statistical significance in our analyses of the normalized dopamine data, our a priori hypothesis was that ethanol would exert differential effects on dopamine based on subregion. Therefore, we analyzed drug and time effects within each brain region. A significant time by drug interaction was observed in both the dorsomedial striatum (Fig 3a; $F_{19,436} = 3.77$, $p < 0.001$) and dorsolateral striatum (Fig 3b; $F_{19,436} = 2.39$, $p < 0.001$). Additionally, the simple effect of time was significant in both the dorsomedial striatum ($F_{19,436} = 8.17$, $p < 0.001$) and dorsolateral striatum ($F_{19,436} = 2.38$, $p < 0.001$), only for the ethanol groups and not the saline groups. However, subsequent post hoc analyses comparing ethanol time points vs. baseline or ethanol vs. saline at individual time points were statistically significant only for the dorsomedial striatum (Fig 3a).

Between the dorsomedial striatum and the dorsolateral striatum, there were no significant differences in the temporal profiles of the dialysate ethanol concentrations (Figs 2b and 2d, respectively; $F_{1,13} = 0.92$, NS). Dialysate ethanol concentrations increased with each ethanol infusion, peaking at 8.5 ± 0.3 mM in the dorsomedial striatum and 7.9 ± 0.4 mM in the dorsolateral striatum.

Histologies and calcium-dependency

Histological analyses were performed to confirm probe placements, and for inclusion in the final dataset, probes were required to have at least 50% of the active area in the region of interest (Figure 4). Additionally, all animals had at least 50% calcium dependent dopamine release (range: 55–89% calcium dependency).

DISCUSSION

The current study is the first to compare the effects of acute systemic ethanol on dorsomedial and dorsolateral striatal dopamine activity. In experiment 1, we showed that striatal subregions significantly differ in their extracellular dopamine responses to a single dose of intravenous ethanol (1 g/kg), with the nucleus accumbens demonstrating an immediate response to the drug administration that was more robust than either the dorsomedial or dorsolateral striatum. In experiment 2, our findings indicate an overall dose-dependent effect of non-contingent, systemic ethanol on dorsal striatal dopamine, and this stimulation was driven by a stronger effect in the dorsomedial striatum (Figure 3). While we observed slightly higher basal dopamine concentrations in the ethanol groups relative to the saline groups in experiment 2, this difference was not statistically significant. However, in order to

directly compare drug effects, we reconciled the differences in basal dopamine by transforming the raw data to percent of baseline (Figure 3).

The present work is the first to demonstrate that the direct pharmacological effect of acute ethanol on extracellular dopamine differs across dorsal striatal subregions. Additionally, the present work supports the previously reported difference between the ventral and dorsal striatum in the dopamine response to acute ethanol (Melendez et al. 2003; Imperato & Di Chiara 1986). The use of intravenous ethanol administration minimizes the impact of handling stress, motivation, and behavior on striatal dopamine activity. Therefore, the subregional differences we observed reflect the direct pharmacological effects of ethanol. Here we report that administration of a single dose of ethanol (1 g/kg) produces an immediate and robust increase in extracellular dopamine content to about 140–150% of basal levels in the nucleus accumbens, which is consistent with previous work (Howard et al. 2008; Melendez et al. 2003; Yim & Gonzales 2000b; Imperato & Di Chiara 1986). Furthermore, the temporal profiles of extracellular dopamine in the dorsomedial striatum and dorsolateral striatum starkly contrast that of the nucleus accumbens following a bolus of ethanol. In the nucleus accumbens, a 1 g/kg infusion of ethanol produces an immediate transient increase in dopamine. Conversely, in the dorsomedial striatum we observe a gradual, but non-significant increase in dopaminergic tone, and little or no effect of ethanol in the dorsolateral striatum.

Previous in vivo microdialysis studies in rats have also demonstrated a blunted dopamine response in the dorsal striatum relative to the ventral striatum following acute systemic ethanol administration (Imperato & Di Chiara, 1986; Melendez et al., 2003). Melendez and colleagues (2003) demonstrated that following a single dose of ethanol (2.25 g/kg, i.p.), dopamine peaks at 198% and 156% of baseline levels in the nucleus accumbens and dorsal striatum, respectively. Additionally, Imperato and Di Chiara (1986) reported that low doses of ethanol (0.25 and 0.5 g/kg, i.p.), which stimulated accumbal dopamine activity to 135–180% of basal levels, failed to stimulate dopamine activity in the dorsal striatum. Higher doses of ethanol (2.5 and 5.0 g/kg, i.p.) also had a greater effect on extracellular dopamine content in the nucleus accumbens relative to the dorsal striatum.

The work presented here is consistent with these earlier studies; however, we further delineate the effect of cumulative ethanol doses on dopamine in dorsal striatal subregions. With higher doses of ethanol (in the range of 1.75–2.5 g/kg), we observe enhanced dopaminergic tone in the dorsomedial striatum that stabilizes at 130% of basal dopamine concentrations and endures as brain ethanol levels decline. In contrast, we observed little to no effect of ethanol on dopamine in the dorsolateral striatum at any dose. The overall conclusion from these experiments is that dopamine neurons projecting to the dorsomedial striatum and dorsolateral striatum show differential sensitivity to a systemic ethanol challenge. However, a caveat is that the difference in the response to acute ethanol between these subregions did not reach statistical significance in all of our analyses, suggesting that the overall difference between these two regions is not very robust.

The current data add to the body of literature suggesting that midbrain dopamine circuits differ in their sensitivity to the stimulant effects of ethanol (Vena & Gonzales 2014;

Melendez et al. 2003; Gessa et al. 1985). Gessa et al. (1985) were the first to report that ethanol stimulated ventral tegmental area dopamine neuron firing rate in vivo with higher potency relative to substantia nigra dopamine neurons in awake, paralyzed rats. These investigators showed that the ethanol dose-response curve was shifted to the right for electrodes placed in the substantia nigra compared to the ventral tegmental area. Microdialysis studies by Melendez et al. (2003) also provide evidence for a higher ethanol sensitivity of ventral tegmental area dopamine neurons compared with those in the substantia nigra in awake, behaving rats. They showed that an injection of ethanol (2.25 g/kg, i.p.) in naïve rats produced a significant dopamine response in the ventral pallidum, which receives dopaminergic input primarily from the ventral tegmental area. In contrast, no dopamine response to ethanol was observed in the globus pallidus, which receives dopaminergic input primarily from the substantia nigra (Fuchs & Hauber 2004; Prensa & Parent 2001; Lindvall & Björklund 1979). The differential sensitivity of ventral tegmental area and substantia nigra dopamine neurons to ethanol likely contributes to the differences we report here in the magnitude and temporal profile of the dopamine response to acute ethanol among the dorsomedial striatum, dorsolateral striatum, and nucleus accumbens. In rats, dopaminergic projections to the dorsomedial striatum and dorsolateral striatum arise primarily from distinct groups of neurons within the substantia nigra, but the dorsomedial striatum also receives some innervation by dopamine neurons with cell bodies located in the ventral tegmental area (Joel & Weiner 2000; Gerfen et al. 1987). This ventral tegmental area innervation of the dorsomedial striatum may explain, in part, our finding of a dose-dependent stimulation of dopamine release by ethanol in the dorsomedial striatum, but not in the dorsolateral striatum. Interestingly, differences in the magnitudes and temporal profiles of the dopamine responses between the dorsomedial and dorsolateral striatum have also been reported following electrically stimulated dopamine activity. Following stimulation of the medial forebrain bundle, the dopamine response in the dorsomedial striatum is larger than that in the dorsolateral striatum (Taylor et al. 2015), which is similar to what we observe following acute ethanol administration.

The mechanisms that underlie the apparent increased sensitivity of the ventral tegmental area dopamine neurons to ethanol relative to the substantia nigra are unknown. Numerous possibilities could contribute to this differential vulnerability to ethanol, and a complete exploration of these possibilities is beyond the scope of the present report. However, the literature provides some clues that would need further experimentation to verify. For example, recent work has demonstrated functional diversity between substantia nigra and ventral tegmental area dopamine neurons in motivated behaviors, which may be due to regulation by contrasting sources of excitatory input (Watabe-Uchida et al. 2012; Matsumoto & Hikosaka 2009). Substantia nigra dopamine neurons primarily receive input from somatosensory and motor cortices in addition to the subthalamic nucleus. On the other hand, ventral tegmental area dopamine neurons receive projections from the lateral hypothalamus. These projections from the lateral hypothalamus are rich in neuropeptides, which induce burst firing in ventral tegmental area neurons (Watabe-Uchida et al. 2012; Korotkova et al. 2004). It is possible that ethanol may be modulating activity at or upstream from these excitatory synapses in the ventral tegmental area while having minimal effects at afferents to the substantia nigra. However, additional distinctions exist between ventral tegmental area

and substantia nigra dopamine neurons including receptor expression patterns and mechanisms of terminal regulation in projection regions (Roeper 2013; Korotkova et al. 2004; Cass & Gerhardt 1995; Cass et al. 1993). Further work is clearly needed to determine if the differences ethanol sensitivity between the dorsomedial striatum and dorsolateral striatum we observed in the present study could be due to ethanol's direct effects on dopamine cell bodies, afferents regulating midbrain dopamine activity, effects at the level of the dopamine terminals in the striatal subregions, or some combination of these factors.

In conclusion, our microdialysis experiments indicate differential pharmacological effects of acute ethanol on extracellular dopamine in the dorsomedial striatum, dorsolateral striatum, and nucleus accumbens. Specifically, the temporal profiles and magnitudes of the dopamine response to an intravenous bolus of ethanol significantly differed across these regions. Therefore, our results indicate distinctions in the regulation of extracellular neurochemical activity among the dorsomedial striatum, dorsolateral striatum, and nucleus accumbens, which may contribute to their proposed functional distinctions. Furthermore, the doses used in the present studies induced varying degrees of ataxia and sedation, suggesting that they are acutely intoxicating (Majchrowicz 1975). Therefore, our findings suggest a role for the dorsomedial striatum in mediating the intoxicating effects of acute ethanol.

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Figure 1.

Dialysate dopamine in the DMS, DLS, and NAc represented as a percent of basal levels following intravenous administration of (a) saline (0.9% NaCl) and (b) ethanol (1 g/kg). Figure (c) shows the temporal profiles of dialysate ethanol concentrations in the DMS, DLS, and NAc. Symbols represent the mean. Error bars are SEM, but not all are shown for clarity. Asterisk (*) indicates significant difference from baseline ($p < 0.05$; nucleus accumbens only), and the caret (^) indicates significant differences among the three brain regions at that time point ($p < 0.01$; Bonferroni). The arrow indicates the time of the infusions.

Figure 2.

Temporal profiles of dialysate dopamine (nM) and ethanol concentrations (mM) for DMS (a and b, respectively) and DLS (c and d, respectively) following cumulative intravenous infusions. Separate groups of rats received either ethanol doses (0.5, 0.5, 0.75, and 0.75 g/kg; filled symbols) or control saline infusions (open symbols). Symbols represent the mean. Error bars are SEM, but not all are shown for clarity. The arrow indicates the time of the infusion.

Figure 3.

From Experiment 2, the dose-response effect of cumulative infusions of ethanol and saline on dialysate dopamine in (a) the dorsomedial striatum and (b) the dorsolateral striatum, represented as a percent of baseline. Asterisks (*) indicate statistical significance ($p < 0.05$) relative to baseline and relative to carets (^) indicate statistical significance ($p < 0.05$) between the ethanol and saline groups at specific time points. Symbols represent the mean. Error bars are SEM, but not all are shown for clarity.

Figure 4.

Histologies to show probe placements for (a) experiment 1 and (b) experiment 2. Each line represents the 1.5 mm active area of a single probe.

Table 1

Timeline of microdialysis experiments

Experiment 1	
Sample	Experimental phase
1–4	Baseline samples
5	Saline infusion
6–9	Post-saline infusion
10–13	Baseline samples
14	Ethanol infusion (1 g/kg)
15–18	Post-ethanol infusion

Experiment 2	
Sample	Experimental phase
1–4	Baseline samples
5	Ethanol (0.5 g/kg) or saline infusion
6–8	Post infusion 1
9	Ethanol (0.5 g/kg) or saline infusion
10–12	Post infusion 2
13	Ethanol (0.75 g/kg) or saline infusion
14–16	Post infusion 3
17	Ethanol (0.75 g/kg) or saline infusion
18–20	Post infusion 4

Basal dialysate dopamine concentrations and number of animals included in analyses for each region

Table 2

Experiment 1				Experiment 2			
Saline Infusion		Ethanol Infusion*		Saline Group		Ethanol Group	
Region	<i>n</i>	[DA] (nM)	<i>n</i>	[DA] (nM)	<i>n</i>	[DA] (nM)	<i>n</i>
DMS	9	1.8 ± 0.1	7	1.8 ± 0.1	5	1.5 ± 0.3	8
DLS	7	1.3 ± 0.2	6	1.3 ± 0.2	7	1.7 ± 0.2	7
Nac	5	1.3 ± 0.3	8	0.9 ± 0.1	n/a	n/a	n/a

Basal dopamine concentrations for each brain region (mean ± SEM). In experiment 1, separate baseline samples were collected prior to both the saline and ethanol infusions. In experiment 2, basal dopamine concentrations were determined from the first 4 dialysate samples.

The asterisk (*) indicates significance difference in basal values across brain regions ($p < 0.05$).