

Alcohol Stimulates the Release of Dopamine in the Ventral Pallidum but not in the Globus Pallidus: A Dual-Probe Microdialysis Study

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The mesoaccumbens dopamine system has been hypothesized to be a common neural substrate mediating the actions of various drugs of abuse, including ethanol. However, the involvement of the mesopallidal dopamine system has received very little attention. The present study examined the effects of intraperitoneal (IP) ethanol administration on the extracellular levels of dopamine in the ventral pallidum (VP) and globus pallidus (GP) of Wistar rats. Rats were bilaterally implanted with microdialysis probes aimed at the VP and GP or nucleus accumbens (NAc) and dorsal striatum (dSTR). During microdialysis testing, rats with probes located in the VP and GP were injected IP with sterile saline or 15% (v/v) ethanol in saline at doses of 0.75, 1.5, or 2.25 g/kg. Rats with NAc and dSTR probes were injected with saline or 2.25 g/kg ethanol. The IP administration of 1.5 and 2.25 g/kg ethanol significantly ($p < 0.05$) elevated the extracellular levels of dopamine in the VP (maximal increase: 136 and 182% of baseline, respectively) but not in the GP. No effects on extracellular dopamine levels were observed following the IP injections of 0.75 g/kg ethanol or saline. The IP administration of 2.25 g/kg ethanol significantly ($p < 0.05$) elevated the extracellular levels of dopamine in the NAc (maximal increase: 198% of baseline) and dSTR (maximal increase: 155% of baseline). Analysis of the effects of 2.25 g/kg ethanol on dopamine release revealed greater increases in the VP, NAc, and dSTR compared to the GP. The data suggest that the mesopallidal, mesoaccumbens, and nigrostriatal dopamine systems are more sensitive to the effects of ethanol than the nigropallidal dopamine system.

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INTRODUCTION

For the past few decades, the mesoaccumbens dopamine system, which originates from the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc), has been hypothesized to be a common neural substrate involved in the neurochemical and reinforcing effects of various drugs of abuse. It is generally recognized that most drugs of abuse, including ethanol, increase the activity of the mesoaccumbens dopamine system. Consistent with this possibility, *in vivo* electrophysiological data indicate that systemic administration of ethanol stimulates dopaminergic cell firing in the VTA (Gessa *et al*, 1985). Neurochemical

data demonstrate increased metabolism of dopamine in the NAc following intraperitoneal (IP) ethanol administration (Khatib *et al*, 1988). Findings from microdialysis studies have consistently shown that IP administration of ethanol (eg 0.25–3 g/kg) significantly increases the extracellular levels of dopamine in the VTA (Campbell *et al*, 1996; Kohl *et al*, 1998) and NAc (Imperato and Di Chiara, 1986; Yoshimoto *et al*, 1992; Kohl *et al*, 1998; Yan, 1999). Moreover, as determined by quantitative microdialysis methods (ie no-net flux), the ethanol-induced elevations of extracellular dopamine levels in the NAc have been accounted for by increased release and not uptake inhibition (Yim and Gonzales, 2000).

Few *in vivo* microdialysis studies have demonstrated the effects of acute IP ethanol administration on the release of dopamine in regions 'beyond' the NAc and VTA. In the first study of this kind, Imperato and Di Chiara (1986) demonstrated that moderate doses of IP injection of ethanol stimulated the activity of the nigrostriatal dopamine system. Thus, the IP administration of ethanol significantly elevated the extracellular levels of dopamine in the dorsal striatum

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(dSTR). Nevertheless, the authors also demonstrated that the NAc appeared to be more sensitive than the dSTR to the actions of ethanol (Imperato and Di Chiara, 1986). Recently, the effects of systemic ethanol on nonstriatal dopaminergic regions have been examined. Yoshimoto *et al* (1992) demonstrated that IP administration of ethanol significantly elevated, in a dose-dependent fashion, the extracellular levels of dopamine in the central nucleus of the amygdala. The amygdala complex is suggested to receive dopaminergic inputs from the VTA and substantia nigra (SN; Fallow and Ciofi, 1992).

The advent of neuroanatomical techniques has greatly aided in delineating pallidum brain regions (Groenewegen and Russchen, 1984; Alheid and Heimer, 1988; Zahm and Heimer, 1990). Pallidal brain structures include two dopaminoceptive regions: the ventral pallidum (VP) and the globus pallidus (GP; ie dorsal pallidum), which receive their dopaminergic input from two different mesencephalic subdivisions. Dopamine innervation of the VP comes predominantly from neurons within the VTA (Deutch *et al*, 1988; Klitenick *et al*, 1992), whereas dopamine innervation of the GP comes predominantly from neurons in the SN (Hattori *et al*, 1975; Prensa and Parent, 2001).

Recent evidence supports the hypothesis that the mesopallidal dopamine system (VTA to VP dopamine projection neurons) is critically involved in the neurochemical and rewarding actions of cocaine. Intravenous operant self-administration of cocaine significantly elevates the extracellular levels of dopamine in the VP (Sizemore *et al*, 2000). Moreover, intra-VP administration of cocaine increases the extracellular levels of VP-dopamine (Gong *et al*, 1997), induces conditioned place preference (Gong *et al*, 1996), and produces behavioral sensitization to locomotor activation (Napier *et al*, 2001). However, the investigation of the mesopallidal dopamine system in the actions of alcohol has received little attention. An understanding of the mesopallidal dopamine system may provide new insights into the neurobiological mechanisms underlying alcoholism. The present study determined the effects of IP ethanol administration on the extracellular levels of dopamine in the VP and GP of Wistar rats. It is hypothesized that the mesopallidal system will be more sensitive than the nigropallidal system to the ethanol-induced increase in the extracellular levels of dopamine.

METHODS

Animals

Adult male Wistar rats (Harlan Industries, Indianapolis, IN, USA), weighing 275–325 g at the time of surgery, were housed individually in temperature- and humidity-controlled rooms on a normal 12-h light:dark cycle (lights on at 0700 h). Food and water were available *ad libitum*. The animals used in these experiments were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee and are in accordance with the 'Guide for the Care and Use of Laboratory Animals' (Institute of

Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

Surgery and Microdialysis

Rats were stereotactically implanted under 2% isoflurane anesthesia with two 18-gauge guide cannulae (Plastic One, Roanoke, VA) aimed 3 mm above the VP and GP. Similarly, a separate group of animals were stereotactically implanted with guide cannulae aimed 3 mm above the NAc and dSTR. These regions served as comparison positive control sites, because prior studies demonstrated that IP ethanol administration enhances the release of dopamine within these areas (Imperato and Di Chiara, 1986). The cannulae were implanted from the midline (0° angle) using the following coordinates, relative to bregma, with the incisor bar set at –3.3 mm: (a) AP 0 (zero) mm, L +2.6 mm, and D/V –6.5 mm for the VP; (b) AP –0.5 mm, L +3.0 mm, and D/V –5.0 mm for the GP; (c) AP +1.7 mm, L +1.2 mm, and DV –5.5 mm for the NAc (d) AP +0.8 mm, L +3.0 mm, and DV –4.0 mm for the dSTR. Each guide cannula was slowly inserted into position; three stainless-steel screws were placed in the skull to secure the guides, and fixed in place with cranioplastic cement. An 18-gauge stylet was placed into each guide cannula to avoid blockage and minimize risk of infections. Following surgery, rats were individually housed and allowed 4–5 days to recover.

After recovery from surgery, animals were habituated for 2 consecutive days to the handling procedures necessary for IP injections. On the day before microdialysis, animals were briefly anesthetized and microdialysis loop-style probes were inserted through the guides and cemented into place. The loop-style probes were made as previously described (Perry and Fuller, 1992). Briefly, dialysis probes consisted of two lengths of dialysis tubing heat shrunk on polyethylene (PE)-10 tubing fused to PE-20 tubing. The inner diameter of the dialysis membrane (Spectra Por-HC, Spectrum Laboratories, Rancho Dominguez, CA) was 200 μ m (molecular weight cutoff: 13,000). The probe tip for the VP and NAc extended 3.0 mm beyond the guide cannula with the upper 1.5 mm covered with PE tubing and the lower 1.5 mm exposed dialysis membrane. The probe for the GP and dSTR extended 3.0 mm beyond the guide cannula with the upper 2.0 mm covered with PE tubing and the lower 1.0 mm exposed dialysis membrane.

On the day of microdialysis, animals were placed individually in Plexiglas holding cages (40 \times 28 \times 40 cm³) and connected to a perfusion system delivering artificial cerebrospinal fluid (aCSF; composition in mM: NaCl, 145; KCl, 2.7; MgCl₂, 1.0; CaCl₂, 2.5; Na₂HPO₄, 2.0, pH 7.4 adjusted with 0.1 N acetic acid). The 2.5 mM CaCl₂ concentration is slightly higher than the 1.2 mM concentration reported for CSF, but the 2.5 mM CaCl₂ concentration is commonly used in microdialysis and does not appear to produce anomalous results. The aCSF was perfused through both probes at a flow rate of 0.75 μ l/min with a syringe pump (Harvard Instruments, MA, USA) for 90–120 min before baseline samples were collected. Baseline samples were collected every 15 min for an additional 90 min before injections were made. Animals with probes implanted in the VP and GP were injected IP with an equal volume of sterile saline or 15% (v/v) ethanol in saline at doses of 0.75, 1.5, or

2.25 g/kg. Animals with probes implanted in the NAc and dSTR were injected IP with an equal volume of sterile saline or 15% (v/v) ethanol in saline at a dose of 2.25 g/kg. Each rat received only one injection of either saline or ethanol. In rodents, the IP injection of ethanol at the doses presently chosen have been observed to produce blood alcohol concentrations in the range of approximately 50–200 mg/dl (eg Lê *et al*, 1992).

Following the systemic injection, 15-min dialysate samples were collected for an additional 90 min. Samples were immediately frozen on dry ice and stored at -70°C until analysis. At the end of the experiment, rats were killed by CO_2 inhalation. A 1% bromophenol blue solution was perfused through the probes to mark their placements. Brains were removed and stored at -70°C ; frozen $40\text{ }\mu\text{m}$ sections were prepared for histological verification of the location of the probe tips; only rats with proper probe placements were included in data analysis (Figures 1 and 2). However, rats with only one correct probe placement were used in that analysis of data for the area with the correct placement.

Biochemical Assay

Dialysate dopamine concentrations were determined by reverse-phase high-performance liquid chromatography coupled with electrochemical detection. Samples were loaded into a Rheodyne $5.0\text{ }\mu\text{l}$ sample loop and injected onto an analytical column ($100 \times 1.0\text{ mm}^2$; Spherisorb C-18;

BAS, West Lafayette, IN). The mobile phase consisted of 100 mM sodium acetate, 0.5 mM EDTA, 5 mM sodium octyl sulfate, 10 mM NaCl, and 6% acetonitrile (adjusted to pH 4.0 with glacial acetic acid). The flow rate was $70\text{ }\mu\text{l}/\text{min}$, delivered by an ESA 582 (Chelmsford, MA) solvent delivery system. Dopamine was detected using a radial flow detector cell with a 6 mm glassy carbon electrode (BAS, West Lafayette, IN) coupled to a model 400 amperometric detector via an external cell cable (EG&G Princeton Applied Research, Princeton, NJ). The applied potential was set at +450 mV with a sensitivity setting of $1.0\text{ nA}/\text{V}$. The use of the Unijet reference electrode required an applied potential setting that was 100 mV less than the equivalent potential setting for a standard Ag/AgCl reference electrode. The output of the detector was sent to a Chrom Perfect (Justice Innovations, Denville, NJ) chromatography data analysis system. The lower limit of sensitivity for dopamine was approximately $0.5\text{ fmol}/5.0\text{ }\mu\text{l}$ injected onto the column.

Data Analysis

Dopamine concentrations found in dialysates were transformed to percentage of baseline values and operationally defined as 100%. The statistical analysis was performed on the normalized data using an overall mixed factorial ANOVA (Time \times Dose \times Region) with Time as a repeated measure. Results from treatments showing significant overall changes were followed by Dunnett's *post hoc* comparisons. Since the data were collected during an

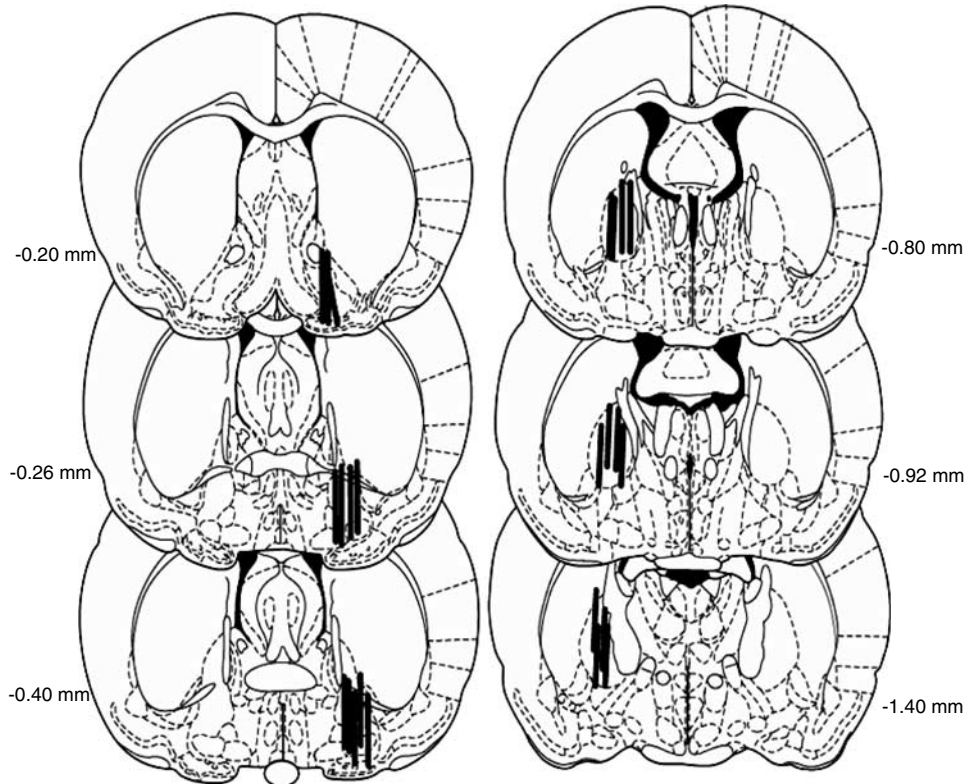


Figure 1 Representative locations of microdialysis probes in the ventral pallidum (VP; left panel) and globus pallidus (GP; right panel). Lines indicate the active regions of the probes used in this study verified by perfusion with bromophenol blue dye. Overlapping placements are not shown; therefore the complete quantitative distribution of the placements is not indicated here. The line represents the 1.5 and 1 mm active dialysis membrane of the probe located in the VP and GP, respectively. Probe coordinates are relative to bregma, according to the stereotaxic atlas of Paxinos and Watson (1998).

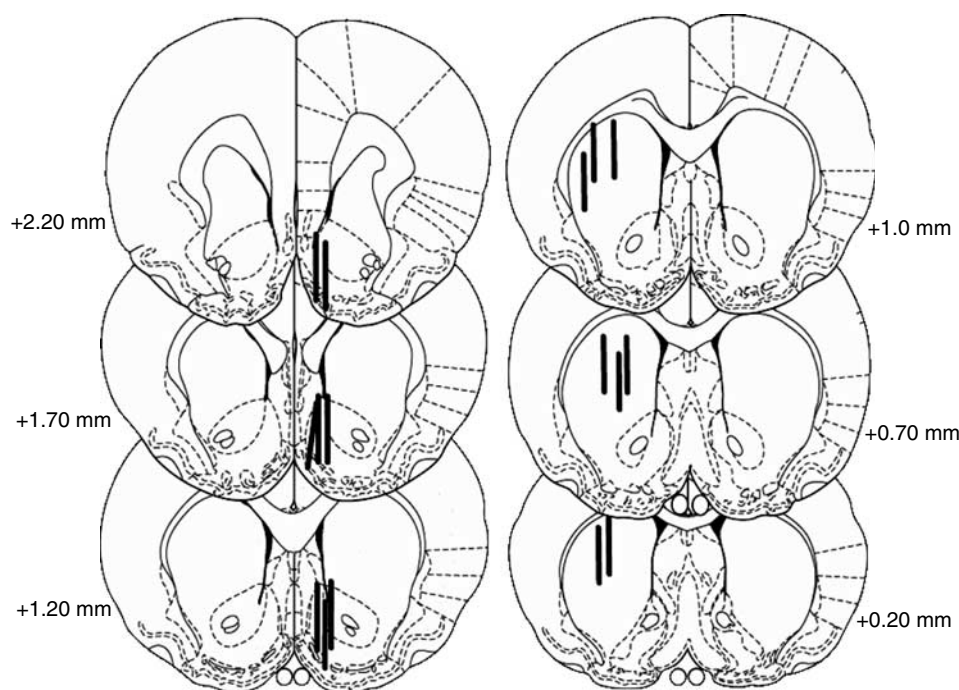


Figure 2 Locations of microdialysis probes in the nucleus accumbens (NAc; left panel) and dorsal striatum (dSTR; right panel). Lines indicate the active regions of the probes used in this study verified by perfusion with bromophenol blue dye. The line represents the 1.5 and 1 mm active dialysis membrane of the probe located in the NAc and dSTR, respectively. Probe coordinates are relative to bregma, according to the stereotaxic atlas of Paxinos and Watson (1998).

overlapping time period, a direct comparison of the effects of IP administration of 2.25 g/kg ethanol on dopamine release (ie average of the 15, 30, and 45 min collection periods) across all four brain regions was analyzed using a one-way ANOVA. Significant effects were followed by *post hoc* comparisons using Tukey's *b*-test. A significance level of 0.05 was used for all tests.

RESULTS

Only animals with proper probe placements in either the VP or GP (Figure 1), or NAc or dSTR (Figure 2) were included in the data analysis. Approximately 75–80% of the animals had both probes correctly implanted in the VP and GP or NAc and dSTR. As shown in Figure 1 (left panel), most of the length of the probe was in the VP with some extension into the substantia innominata, nucleus of the horizontal limb of the diagonal band, and/or the magnocellular preoptic nucleus. Probes in the GP were located along the anterior–posterior region (Figure 1, right panel). As shown in Figure 2 (left panel), most probes in the NAc were located in the 'shell' region with some placements in the shell plus the 'core'. For the dSTR, all probes were located in the dorsal anterior region of the striatum (right panel). The mean (\pm SEM) basal dialysate concentration of dopamine was as follows (in nanomolar): 0.43 ± 0.04 for the VP ($n=22$); 1.1 ± 0.1 for the GP ($n=22$); 0.71 ± 0.16 for the NAc ($n=8$); and 4.8 ± 0.3 for the dSTR ($n=8$).

The effects of IP administration of saline and ethanol (0.75, 1.5, and 2.25 g/kg) on extracellular levels of dopamine in the VP and GP are shown in Figure 3. A mixed factorial

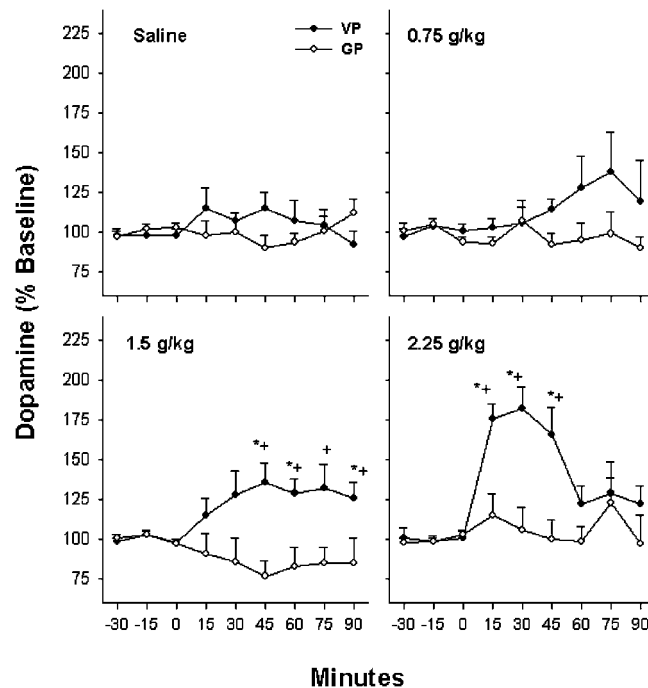


Figure 3 Time course effect of sterile saline ($n=5$) or 15% (v/v) ethanol ($n=5$ –6/dose) in saline at doses of 0.75, 1.5, or 2.25 g/kg on the extracellular levels of dopamine in the VP and GP of Wistar rats. Values represent mean \pm SEM expressed as a percentage of baseline values. The IP administration of saline or 0.75 g/kg failed to significantly alter the extracellular levels of dopamine in either the VP or the GP ($p>0.05$). * $p<0.05$ as compared to saline (Dunnett's *t*); + $p<0.05$ as compared to the GP (one-way ANOVA).

ANOVA (Time \times Dose \times Region) with Time as a repeated measure on the effects of IP administration of saline and ethanol (0.75, 1.5, and 2.25 g/kg) on extracellular dopamine levels in the VP and GP revealed a significant effect of Time ($F_{8,288} = 6.7$; $p < 0.001$), Dose ($F_{3,36} = 0.02$; $p < 0.001$), Region ($F_{1,36} = 21.4$; $p < 0.001$), and Time \times Dose \times Region ($F_{24,288} = 1.6$; $p = 0.04$). Further analysis revealed that ethanol significantly elevated the extracellular levels of dopamine over time in the VP (Time \times Dose interaction, $F_{24,144} = 2.4$; $p = 0.001$) but not in the GP (Time \times Dose interaction, $F_{24,144} = 0.8$; $p = 0.80$). One-way ANOVA across all ethanol doses was performed at each 15-min interval ($F_{3,18} > 3.6$; $p < 0.03$). Comparisons of each ethanol dose with saline revealed that (a) a 1.5 g/kg ethanol dose significantly elevated dopamine levels 45–90 min after injection, and (b) 2.25 g/kg ethanol significantly elevated the extracellular levels of dopamine in the VP during the 15, 30, and 45 min periods (maximal increase 182% of baseline; $p < 0.01$, Dunnett's *post hoc*). The 0.75 g/kg ethanol dose produced a small, statistically nonsignificant effect on extracellular levels of dopamine.

Further analysis revealed that 1.5 and 2.25 g/kg ethanol significantly elevated the extracellular levels of dopamine in the VP compared to the GP (Time \times Region interaction, $F_{8,80} > 3.5$; $p < 0.003$). One-way ANOVA of the effects of 1.5 g/kg ethanol was performed at each 15-min interval. As shown in Figure 3, compared to the extracellular levels of dopamine in the GP, VP-dopamine levels were elevated and sustained throughout the 45–90 min periods (maximal difference: 136 vs 77% of baseline; $F_{1,11} > 7.1$; $p < 0.03$). One-way ANOVA of 2.25 g/kg ethanol revealed that VP-dopamine levels, compared to the GP, were elevated and sustained throughout the 15–45 min periods (maximal difference 182 vs 106% of baseline; $F_{1,11} > 9.8$; $p < 0.01$).

The effects of IP administration of saline and 2.25 g/kg ethanol on extracellular levels of dopamine in the NAc and dSTR are shown in Figure 4. A mixed factorial ANOVA (Time \times Dose \times Region) with time as a repeated measure indicated a significant effect of Time ($F_{8,96} = 7.6$; $p < 0.001$), Dose (saline vs 2.25 g/kg ethanol; $F_{1,12} = 29.7$; $p < 0.001$), Region ($F_{1,12} = 10.3$; $p = 0.007$), and a Time \times Dose interaction ($F_{8,96} = 6.6$; $p < 0.001$). Further analysis revealed that 2.25 g/kg ethanol significantly elevated the extracellular levels of dopamine over time in the NAc (Time \times Dose interaction, $F_{8,48} = 3.5$; $p < 0.002$) and in the dSTR (Time \times Dose interaction, $F_{24,144} = 4.1$; $p < 0.004$). One-way ANOVAs were performed on the effects of 2.25 g/kg ethanol and saline at each 15-min interval for the NAc and dSTR. Compared to saline injection, 2.25 g/kg ethanol significantly elevated the extracellular levels of dopamine in the NAc and dSTR during the 30, 75, and 90 min periods (maximal increase: 198% of baseline; $F_{1,6} > 5.3$; $p < 0.05$, Dunnett's *t*) and 15 and 30 min periods (maximal increase: 156% of baseline; $F_{1,6} > 10.5$; $p < 0.02$, Dunnett's *t*), respectively. Analysis of the ethanol-induced increases in dopamine levels revealed no significant differences between the effects observed in the NAc compared to the dSTR (Time \times Region interaction, $F_{8,48} > 1.0$; $p = 0.45$).

The effects of IP administration of 2.25 g/kg ethanol on dopamine release (ie average response during the 15, 30, and 45 min periods) within the VP, GP, NAc, and dSTR are shown in Figure 5. One-way ANOVA revealed a significant

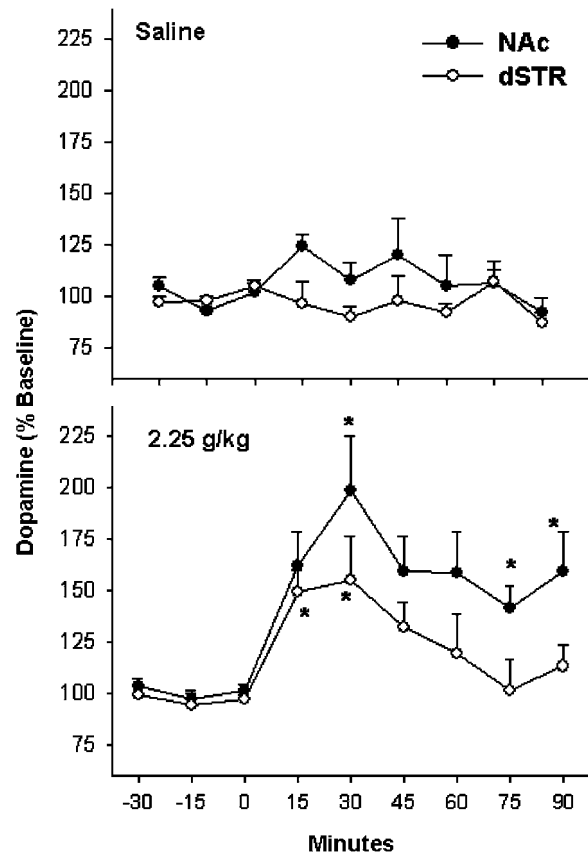


Figure 4 Time course effect of saline ($n=4$) and 2.25 g/kg ethanol ($n=4$) on the extracellular levels of dopamine in the NAc and dSTR of Wistar rats. Values represent mean \pm SEM expressed as a percentage of baseline values. There were no significant differences in extracellular levels of dopamine between the NAc and dSTR after the injection of 2.25 g/kg ethanol. * $p < 0.05$ as compared to saline (Dunnett's *post hoc*).

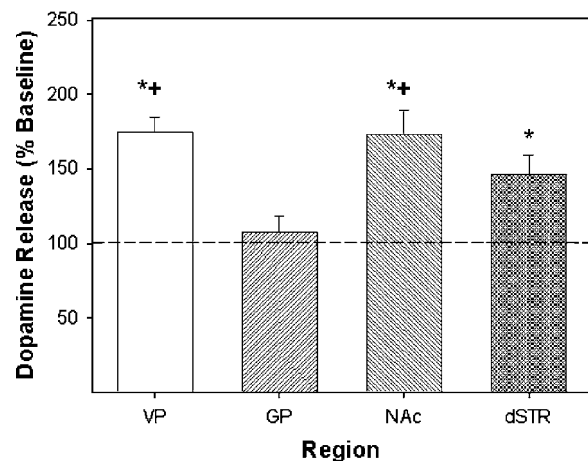


Figure 5 Effects of 2.25 g/kg ethanol on the extracellular levels of dopamine in the VP, GP, NAc, and the dSTR. Dopamine release was defined as the average of the initial three collection periods (ie 15, 30, and 45 min periods). Values represent mean \pm SEM expressed as a percentage of baseline. * $p < 0.05$ compared to the GP (Tukey's *b*), + $p < 0.05$ compared to the GP and dSTR.

effect of Region ($F_{3,16} = 7.72$; $p = 0.002$). *Post hoc* comparisons indicated that mean dopamine release after the injection of 2.25 g/kg ethanol was significantly greater in

the VP, NAc, and dSTR compared to the GP and that the percent mean release was greater in the VP and NAc than in the dSTR and GP ($p < 0.05$; Tukey's *b*).

DISCUSSION

The results of the present study support the hypothesis that the mesopallidal dopamine system is more sensitive than the nigropallidal dopamine system to the effects of ethanol. The effects of ethanol on VP-dopamine appeared to be dose related, because 1.5 and 2.25 g/kg ethanol increased the extracellular levels of dopamine approximately 1.3 and 1.7 times more than the saline injection, respectively (Figure 3). The effects of 2.25 g/kg ethanol on extracellular VP-dopamine levels were qualitatively in agreement with the results obtained in the NAc and dSTR (Figure 3). In contrast, the IP administration of ethanol at all doses tested (ie 0.75, 1.5, and 2.25 g/kg) failed to significantly enhance the extracellular levels of dopamine in the GP (Figure 3). In the NAc, data indicate that the effect of ethanol on increasing the extracellular levels of dopamine is a result of increased release rather than a result of inhibiting the reuptake of dopamine (Imperato and Di Chiara, 1986; Yoshimoto *et al*, 1992; Yim and Gonzales, 2000). On the basis of these findings, the present data suggest that IP ethanol administration stimulates the release of dopamine in the VP but not the GP, thus supporting the involvement of the mesopallidal dopamine system in the actions of ethanol.

To our knowledge, the present study is the first to demonstrate an effect of ethanol on the extracellular levels of dopamine in the VP. The stimulating effect of ethanol is qualitatively in agreement with a number of previous reports showing ethanol's activating effect on the mesoaccumbens and nigrostriatal dopamine system. *In vivo* microdialysis studies have demonstrated that IP ethanol (at doses ranging from 0.5 to 3 g/kg) dose-dependently increased the extracellular levels of dopamine in the VTA (Campbell *et al*, 1996; Kohl *et al*, 1998), NAc (Imperato and Di Chiara, 1986; Yoshimoto *et al*, 1992; Kohl *et al*, 1998; Yan, 1999), and dSTR (Imperato and Di Chiara, 1986). These data are also consistent with *in vivo* electrophysiological studies indicating that systemic ethanol stimulates the firing rate of VTA and SN dopamine neurons (Gessa *et al*, 1985).

The mechanism(s) responsible for the differences in sensitivity between the mesopallidal and nigropallidal dopamine systems to the activating effects of ethanol are not known. However, because the VP receives its dopaminergic input primarily from the VTA (Deutch *et al*, 1988; Klitenick *et al*, 1992), whereas the GP is primarily innervated by the SN (Hattori *et al*, 1975; Prensa and Parent, 2001), the effects of ethanol may be explained in part by a difference in sensitivity between the VTA and SN. A high sensitivity of VTA-dopamine neurons to ethanol has been demonstrated by Gessa *et al* (1985), which indicated that VTA-dopamine neurons are about five-fold more sensitive (ie increased firing rates) to the systemic effects of ethanol than SN-dopamine neurons. Such sensitivity may be due to differences in receptors modulating the activity of VTA- and SN-dopamine neurons. Sensitivity to the

stimulating effects of ethanol is mediated in part through GABA_A receptors (Harris *et al*, 1995; Korpi, 1994), NMDA receptors (Weight *et al*, 1991), serotonin (5-HT) receptors (Brodie *et al*, 1995), and in particular 5-HT₃ receptors (Campbell *et al*, 1996). Along these lines, ethanol has been shown to inhibit the activity of nondopamine neurons in the SN, which are thought to exert an inhibitory (GABAergic) control on SN-dopamine neurons (Mereu and Gessa, 1985). Thus, the distribution of the GABA receptors within a population of SN-dopamine neurons projecting to the GP may also be different and result in a lack of sensitivity (eg greater inhibition) to the stimulating effects of ethanol.

Alternatively, the differences in sensitivity between the mesopallidal and nigropallidal dopamine systems may reside in the effects of ethanol within the terminal regions. For instance, intra-NAc application of ethanol elevates the extracellular levels of NAc-dopamine (Wozniak *et al*, 1991; Yim *et al*, 1998). Moreover, the ethanol-induced increase in NAc-dopamine is abolished by coapplication of the 5-HT₃ antagonist, ICS 205-930 (Yoshimoto *et al*, 1992). One of the major targets of striatal projection neurons is the pallidum. Particularly, the VP and GP receive their striatal innervation from two different subdivisions of the striatum with the VP receiving afferents from the ventral striatal regions and the GP receiving afferents from the more dorsally located striatal regions (Nauta *et al*, 1978; Haber *et al*, 1985; Zahm, 1989).

Since the majority of striatopallidal neurons use GABA as their transmitter, it is possible that ethanol may be acting on GABA receptors within the pallidum, which in effect could modulate the release of dopamine. The involvement of VP-GABA_A receptors in the reinforcing effects of ethanol has recently been observed (Harvey *et al*, 2002). Furthermore, Gong *et al* (1988) show that intra-VP blockade of GABA_A and GABA_B receptors significantly elevates the extracellular levels of dopamine in the VP. Thus, a difference in the distribution and location of GABA innervation within the GP compared to VP (as well as the NAc and dSTR) may account in part for a difference in sensitivity to the activating effects of ethanol.

An issue relevant for the interpretation of the present results relates to the differences in the temporal profile of extracellular dopamine levels in the VP across the ethanol doses tested (Figure 3). Following the injection of 0.75 and 1.5 g/kg ethanol, VP-dopamine levels were only transiently increased and sustained throughout the latter portion of the sampling periods (ie 45–90 min postinjection). In light of the slow onset of the dopaminergic effect in the VP, it is possible that the effects seen at the low and intermediate doses are secondary rather than primary effects of ethanol. On the other hand, following the injection of 2.25 g/kg ethanol, dopamine levels were significantly elevated within the first 15 min, peaked at about 30–45 min, and then declined rather sharply. The onset of dopamine's effect following 2.25 g/kg ethanol is consistent with the rise in brain ethanol concentrations that one would expect under these conditions (eg Ferraro *et al*, 1991; Yim and Gonzales, 2000).

Interestingly, the time profile of VP-dopamine levels following 2.25 g/kg ethanol was different, in part, to NAc-dopamine efflux (Figure 4). Whereas dopamine levels peaked at about 30 min in the VP and then declined rather

sharply, NAc-dopamine levels peaked in a similar fashion as the VP but remained elevated throughout the 90 min sampling period. In a similar fashion, using dual probe microdialysis, Kohl *et al* (1998) demonstrated that IP administration of 3.0 g/kg ethanol significantly elevated and sustained the extracellular levels of dopamine in the NAc but not in the VTA. As indicated, VTA-dopamine levels peaked at about 30 min but declined rather sharply beginning about 40 min postinjection. Together, these findings suggest that ethanol's action on the release of dopamine may be independently determined within each structure by a combination of mechanisms, including activity of midbrain dopamine neurons, and regulation of somatodendritic and terminal release of dopamine.

The findings demonstrating that dopamine release after the injection of 2.25 g/kg ethanol was greater in the VP and NAc compared to the GP and dSTR (Figure 5) suggests that the ventral regions of the striatopallidal complex are more sensitive to the activating effects of ethanol than the dorsal striatopallidal regions. The projections from the SN to the dorsally located GP and dSTR are paralleled by projections from the VTA to the VP and NAc (Groenewegen *et al*, 1991). Also, the projections from the dSTR to the GP are paralleled by projections from the NAc to the VP (Heimer and Alheid, 1991). Such topographical organization has contributed to the popular view of 'functional dichotomy' between extrapyramidal and limbic functions associated with dorsal and ventral systems, respectively (Groenewegen *et al*, 1991). Furthermore, the greater sensitivity of the ventral circuits to ethanol is consistent with the hypothesis that these circuits are critical targets of various drugs of abuse, including ethanol. However, the level to which these circuits are segregated (ie parallel processing) or whether they interact (ie convergent processing) remains inconclusive and beyond the scope of this study.

A potential weakness in the current study is that only relatively high doses of ethanol (in a range that is often sedative to intoxicating) were found to produce increases in dialysate concentrations of dopamine. This effect may be partly due to a highly efficient dopamine reuptake system, in addition to limitations of the microdialysis technique, that is, only dopamine released in close proximity of the probe can be detected. Therefore, the lower ethanol doses may not activate sufficient numbers of dopamine neurons to detect an increase, whereas higher doses of ethanol may cause sufficient activation to detect the nanomolar concentrations of dopamine in the extracellular space.

CONCLUSION

The data from the present study indicate that the mesopallidal, mesoaccumbens, and nigrostriatal dopamine systems are more sensitive to the effects of ethanol than the nigropallidal dopamine system. Furthermore, the greater sensitivity of VP- and NAc-dopamine to ethanol is consistent with the hypothesis that the ventral-striatopallidal circuits are a particular target of drugs of abuse, and may play a critical element in addiction. Additional studies are necessary to establish a role for the mesopallidal dopamine system in the behavioral and reinforcing actions of ethanol. Such approaches should advance the

current understanding on the neurobiological basis of alcoholism.

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