

## Effect of Acute Ethanol on Striatal Dopamine Neurotransmission in Ambulatory Rats

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### ABSTRACT

The effect of ethanol on evoked dopamine release in the caudate putamen has been measured in behaving animals with *in vivo* electrochemistry. Dopamine was measured with fast-scan cyclic voltammetry in adult male rats to resolve the competing processes of dopamine uptake and release. Ethanol dose dependently decreased dopamine efflux compared with saline-treated animals: to 89% of controls with 0.5 g/kg, 70% with 1 g/kg, 34% with 2.5 g/kg, and 18% with 5 g/kg. This decrease was not due to a change in uptake, as measured by the rate of dopamine disappearance after stimulation, and therefore can be attributed to decreased dopamine release. Additionally, it was not mediated by a decrease in biosynthesis, as measured by L-DOPA accumulation after NSD 1015 administration. The selective dopamine uptake inhibitor GBR 12909 compensated

for the effects of high doses of ethanol on dopamine release. Moreover, GBR 12909 induced faster restoration of the righting reflex in rats sedated with 2.5 g/kg, but not 5 g/kg, ethanol. In brain slices containing the caudate putamen, ethanol suppressed dopamine release only at the highest dose tested (200 mM). The difference in responses between the slice and the intact animal indicates that ethanol exerts its effects in the cell body regions of dopamine neurons as well as in terminals. These neurochemical results, combined with published accounts of microdialysis measures of extracellular dopamine and electrophysiological recordings of dopamine neurons, demonstrate that ethanol has a profound effect on dopamine neurons whose net result is a suppression of dopamine neurotransmission at high doses.

The nigrostriatal and mesolimbic dopamine pathways are, respectively, implicated in the stimulating and reinforcing aspects of addictive drug pharmacology (McBride et al., 1999; Souza-Formigoni et al., 1999). In general, electrophysiological data provide evidence that acute ethanol stimulates dopamine transmission. Brodie et al. (1999) reported dose-dependent ethanol-induced increases in cell firing of ventral tegmental dopamine neurons *in vitro* over a wide range of ethanol concentrations. Mereu et al. (1984), recording *in vivo* from paralyzed rats, found increased dopamine cell firing in substantia nigra pars compacta (SNc) after low and moderate doses of ethanol, but a transient increase followed by profound inhibition of cell firing after higher doses.

In contrast, neurochemical data give a more complicated view of acute ethanol on dopamine activity. In synaptosomes, ethanol causes a decrease in K<sup>+</sup>-evoked dopamine release that is accompanied by a decrease in Ca<sup>2+</sup> efflux (Woodward et al., 1990). However, the body of microdialysis data suggests a biphasic dopaminergic response to ethanol, with increases in extracellular dopamine in the nucleus accumbens

after ethanol administration of low-to-moderate doses (Yoshimoto et al., 1991), and no effect (Blanchard et al., 1993) or decreases (Imperato and Di Chiara, 1986; Blanchard et al., 1993) in extracellular dopamine at higher doses. Few studies have measured changes in extracellular dopamine in the caudate putamen (CP) after systemic ethanol administration, and those studies are inconsistent. Imperato and Di Chiara (1986) reported no effect of low doses of ethanol on extracellular dopamine, whereas moderate and high doses increased dopamine. Blanchard et al. (1993) found increases in extracellular dopamine after low doses of ethanol, but decreases after higher doses.

Microdialysis recovery of dopamine from the extracellular space can be affected by changes in dopamine uptake (Justice, 1993). If, for example, ethanol administration changed the efficiency of the dopamine transporter, extracellular concentrations measured by traditional microdialysis experiments would be affected accordingly. In fact, there is evidence that ethanol changes the rate of dopamine uptake, although these data are apparently conflicting. Lin and Chai (1995), using chronoamperometry in anesthetized rats, reported that local application of ethanol in the striatum decreased the amplitude and clearance of *N*-methyl-D-aspar-

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tate-induced dopamine release as well as clearance of exogenously applied dopamine. In contrast, Wang et al. (1997), also using chronoamperometry in anesthetized rats, found that both systemic and local ethanol administration decreased  $K^+$ -induced dopamine release, an effect attributed to an increase in dopamine uptake.

Microdialysis and voltammetry are complementary methods measuring different aspects of dopamine neurotransmission. There are temporal differences between the methods, with microdialysis providing samples that are integrated over 5 to 20 min, and fast scan cyclic voltammetry (FSCV) taking real-time measurements every 100 ms. In addition, the size of the carbon fiber electrode used for FSCV is less than one-tenth the size of a typical microdialysis probe, allowing more precise spatial resolution. The most important difference, however, is the nature of the information obtained by each method. Microdialysis provides information on changes in extracellular levels of dopamine that are regulated by multiple mechanisms, including release, uptake, synthesis, and metabolism. In contrast, FSCV measures extracellular dopamine changes after electrical stimulation of cell bodies in the SNc, rather than spontaneous or gradual changes in extracellular dopamine. Thus, FSCV yields the separable aspects of evoked dopamine release and subsequent uptake.

The present experiments were designed to reexamine the effects of ethanol on striatal dopamine transmission using FSCV, particularly at higher sedative doses. We first measured evoked dopamine release after a range of ethanol doses in freely moving rats. The dopamine signal was pharmacologically verified on some rats using the dopamine uptake blocker GBR 12909. In these rats, we observed that GBR 12909 restored the evoked dopamine signal as well as shortened the ethanol-induced sedation in rats. Thus, we further examined this behavioral effect by administering GBR 12909 subsequent to sedative doses of ethanol in a separate group of rats. The neurochemical effects of ethanol on dopamine were further characterized *in vitro* using CP slices from adult rats. Finally, to determine whether ethanol's effect on evoked dopamine release was due to changes in dopamine biosynthesis, we measured L-DOPA accumulation after administration of NSD 1015, an *l*-aromatic acid decarboxylase blocker, in ethanol-treated rats.

## Materials and Methods

**Animals.** Ethanol-naïve male Sprague-Dawley rats (Charles River, Raleigh, NC) were housed on a 12:12-h light/dark cycle with food and water *ad libitum*. Rats were group housed before surgery and singly housed after surgery. All protocols were approved by the Institutional Animal Care and Use Committee at the University of North Carolina.

**Drugs.** GBR 12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenylpropyl]piperazine dihydrochloride) and NSD 1015 (3-hydroxybenzylhydrazine dihydrochloride) were purchased from Research Biochemicals International (Natick, MA), and were dissolved in 0.9% saline before injection. Ketamine hydrochloride/xylazine hydrochloride solution and chloral hydrate were purchased from Sigma (St. Louis, MO). Ethanol (95%) was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY), and was diluted to a 20% w/v solution with 0.9% saline. All drugs were administered by *i.p.* injection.

**Surgery for Voltammetric Experiments.** The surgical procedures were as previously described (Garris et al., 1997). In brief, rats

were anesthetized by *i.p.* injection of chloral hydrate (400 mg/kg) or ketamine (80 mg/kg) with xylazine (12 mg/kg) and placed in a stereotaxic frame. A guide cannula (Plastics One, Roanoke, VA) was positioned above the CP (1.2 mm anterior and 2.0 mm medial to bregma, 2.5 mm from skull surface). Reference and auxiliary electrodes were placed in the left forebrain, contralateral to the guide cannula, and all items were secured with skull screws and cranioplastic cement. A detachable micromanipulator containing a carbon fiber electrode was inserted into the guide cannula and lowered into the CP. A bipolar stimulating electrode was then placed directly above the substantia nigra/ventral tegmental area (5.6 mm posterior and 1.0 mm medial to bregma, 7.5 mm from dural surface). Electrical stimulations (60 rectangular pulses, 60 Hz, 120  $\mu$ A, 2 ms/phase, biphasic) were applied via the stimulating electrode, which was lowered at 0.1- to 0.2-mm increments until evoked dopamine release was detected at the carbon fiber electrode. The stimulating electrode was then fixed with cranioplastic cement, and the carbon fiber electrode was removed.

**Voltammetric Experiments.** At least 2 days after surgery, rats (260–380 g) were placed in the test chamber and a new carbon fiber electrode was inserted into the CP. The reference, auxiliary, and carbon fiber electrodes were connected to a head-mounted voltammetric amplifier attached to a swivel at the top of the test chamber. Voltammetric recordings were made at the carbon fiber electrode every 100 ms by applying a triangular waveform (0.4 to +1.0 V, 300 V/s) using a biopotentiostat (EI400; Cypress Systems, Lawrence, KS). Data were digitized (National Instruments, Austin, TX) and stored to a computer. Dopamine release was evoked every 10 min with electrical stimulations (60 rectangular pulses, 60 Hz, 120  $\mu$ A, 2 ms/phase, biphasic) and detected at the carbon fiber electrode. After at least three stimulations a single dose of ethanol (0, 0.5, 1, 2.5, or 5 g/kg *i.p.*) was injected. Stimulations and recordings continued at 10-min intervals for 60 min postinjection. The carbon fiber electrodes were calibrated *in vitro* after each experiment.

To pharmacologically confirm that the signal detected was dopamine, some rats received GBR 12909 after ethanol administration. Single or multiple doses of 10 or 20 mg/kg GBR 12909 were delivered *i.p.* 20 to 120 min after ethanol administration, and subsequent behavior was observed.

**Uptake.** Dopamine uptake follows Michaelis-Menten kinetics and is thus concentration-dependent. Thus, comparison of uptake following evoked release requires analysis of clearance rates at the same absolute dopamine concentration. To determine qualitatively the effect of ethanol on dopamine uptake, three pre-ethanol evoked responses were averaged, truncated to the range of concentrations observed 40, 50, and 60 min after ethanol, and compared with the postethanol responses. To obtain a more quantitative measure of uptake, the slope of the descending phase of the evoked dopamine signal in the absence and presence of ethanol in each rat was measured at an amplitude corresponding to 15% of the maximal concentration of dopamine observed before ethanol administration. The slope was measured as the tangent taken from six points (500 ms). For data from each animal, three slopes obtained before ethanol were averaged and compared with the average slopes determined 40, 50, and 60 min after ethanol. These slopes were compared with a paired *t* test at each dose.

**Biosynthesis Experiments.** To measure dopamine synthesis rates, rats were injected with the *l*-aromatic acid decarboxylase inhibitor NSD 1015 (Carlsson and Lindqvist, 1973; Budygin et al., 1999). Ethanol (0.5, 1, 2.5, or 5 g/kg *i.p.*) or saline was administered 10 min before NSD 1015 (50 mg/kg *i.p.*). The animals were decapitated 30 min later. The whole brain was quickly removed and placed on a glass plate over ice. Striata were dissected and homogenized in 0.1 M HClO<sub>4</sub> containing 100 ng/ml 3,4-dihydroxybenzylamine as an internal standard. Homogenates were centrifuged for 10 min at 10,000g. Supernatants were filtered through 0.22-mm filter and analyzed for levels of L-DOPA using high performance liquid chromatography with electrochemical detection. The volume of injection

was 20  $\mu\text{l}$ . L-DOPA was separated on a reverse phase column (Ultremex C18, 100  $\times$  4.60 mm; Phenomenex, Torrance, CA) with a mobile phase consisting of 50 mM monobasic sodium phosphate, 0.2 mM octyl sodium sulfate, 0.1 mM EDTA, 10 mM NaCl, and 10% methanol (pH 2.6) at a flow rate of 1 ml/min and detected by a glass carbon electrode (BioAnalytical Systems, West Lafayette, IN). The potential applied was +0.65 V.

**Brain Slice Experiments.** To assess the direct effect of ethanol on striatal dopamine terminals, evoked dopamine release was measured in brain slices using FSCV during bath application of ethanol. Slices were prepared and maintained as previously described (Kennedy et al., 1992). Briefly, male Sprague-Dawley rats were sacrificed by decapitation and the brains rapidly removed and cooled in ice-cold, preoxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>), modified Krebs' buffer. The tissue was then sectioned into 400- $\mu\text{m}$ -thick coronal slices containing the CP using a vibrotome (Vibroslice HA752; Campden Instruments, Loughborough, UK). Slices were kept in a reservoir of oxygenated Krebs' at room temperature until required. Thirty minutes before each experiment, a brain slice was transferred to a "Scottish-type" submersion recording chamber, perfused at 1 ml/min with 34°C oxygenated Krebs', and allowed to equilibrate. The Krebs' buffer consisted of 126 mM NaCl, 2.5 mM KCl, 1.2 mM NaH<sub>2</sub>PO<sub>4</sub>, 2.4 mM CaCl<sub>2</sub>, 1.2 mM MgCl<sub>2</sub>, 25 mM NaHCO<sub>3</sub>, 11 mM glucose, 20 mM HEPES, 0.4 mM *l*-ascorbic acid, and was pH adjusted to 7.4.

A bipolar stimulating electrode (Plastics One) was placed on the surface of the brain slice above the CP (tip spacing, 250  $\mu\text{m}$ ), and the carbon fiber working electrode placed about 100 to 200  $\mu\text{m}$  away from the midpoint between the poles, penetrating the slice by at least 75  $\mu\text{m}$ . Dopamine was evoked by a single, rectangular, electrical pulse (300  $\mu\text{A}$ , 2 ms/phase, biphasic) applied every 5 min. Dopamine was detected using FSCV as described above. Once the extracellular dopamine response to electrical stimulation was stable for three successive stimulations, ethanol (100 or 200 mM) was applied to the CP via the superfusate. The concentrations of ethanol chosen were to span the estimated peak brain concentrations following the highest doses given in vivo in this study (Matticci-Schiavone and Ferko, 1984).

In some brain slice experiments, constant-potential amperometry (300 mV versus Ag/AgCl) was used for dopamine detection to reconfirm the assessment of ethanol's effect on dopamine uptake. In general, FSCV is preferred because it provides a determination of the compounds detected as well as their time course. However, amperometry more accurately measures the time course of extracellular dopamine than does FSCV. Adsorption of dopamine to the carbon fiber microelectrode surface occurs between FSCV scans, which can distort the time course of the dopamine signal, potentially masking subtle changes in dopamine uptake. This problem is avoided in amperometry because dopamine is immediately oxidized on contact with the electrode surface, providing no opportunity for adsorption. *l*-Ascorbic acid and any endogenous antioxidants in the slice act to replenish the consumed dopamine by reducing dopamine-*ortho*-quinone (Kawagoe and Wightman, 1994), requiring the use of ascorbate in the in vitro calibration. Data were initially collected with FSCV to identify dopamine release sites, and amperometry was used to establish a baseline of stimulated release before ethanol was applied to the slice. In these experiments, release was evoked with a single-phase stimulation (2 ms) to reduce the electrical artifact. Amperometry signals were low-pass filtered to 30 Hz by the potentiostat and then digitized at 60 Hz (National Instruments). Dopamine uptake was measured from the descending curve of the amperometry signal by slope analysis, as described above.

**Behavioral Experiments.** The hypnotic-sedative effect of ethanol was assessed as loss of righting reflex in rats (330–480 g) after i.p. administration of a single dose of ethanol (2.5 or 5 g/kg), followed by i.p. administration of 10 mg/kg GBR 12909 20 min later. In some rats, 5 g/kg ethanol was followed by 20 mg/kg GBR 12909. Loss of righting reflex was determined when all four paws of a rat did not return to the floor within 15 s after being placed on its side. After

ethanol-induced loss of righting reflex, rats were left on their sides until they spontaneously righted. Then the experimenter tested for the righting reflex 1 to 2 min after spontaneous righting; recovery of righting reflex was determined when the reflex was retained. Criteria for inclusion in the experiment were that rats lose the righting reflex within 10 min of ethanol administration and do not regain it for 20 min. (Preliminary observations revealed that after 2.5 mg/kg ethanol only 60% of rats weighing less than 300 g lost the righting reflex, and these rats had regained the reflex by 20 min.) At 20 min, those rats meeting the criteria were randomly assigned to receive either GBR 12909 or saline.

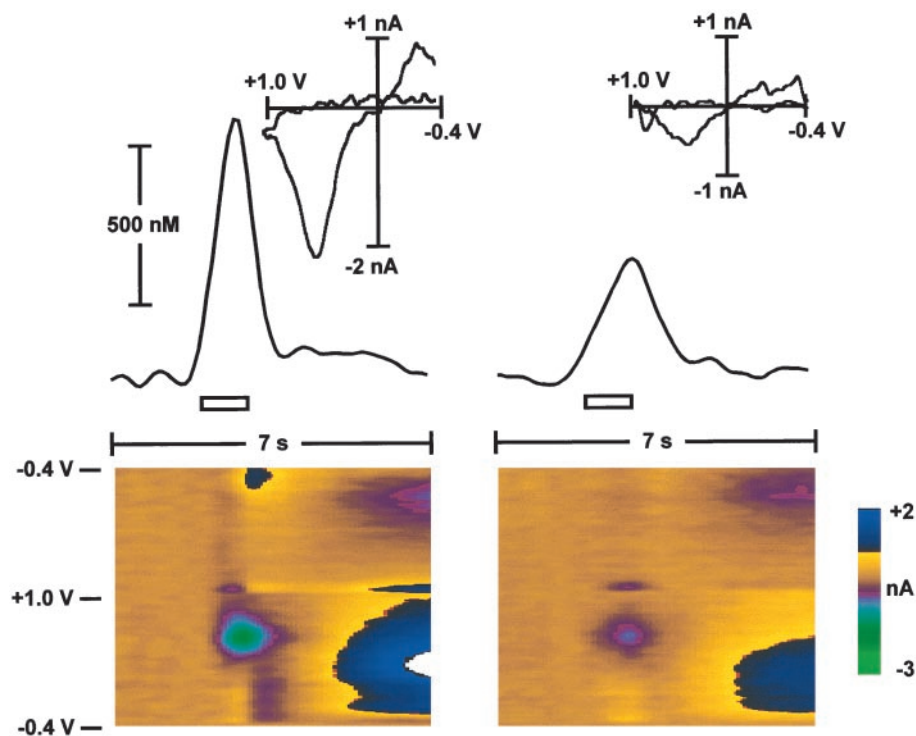
**Statistical Analysis.** All statistical analyses were carried out using Prism (GraphPad Software, Inc., San Diego, CA).

## Results

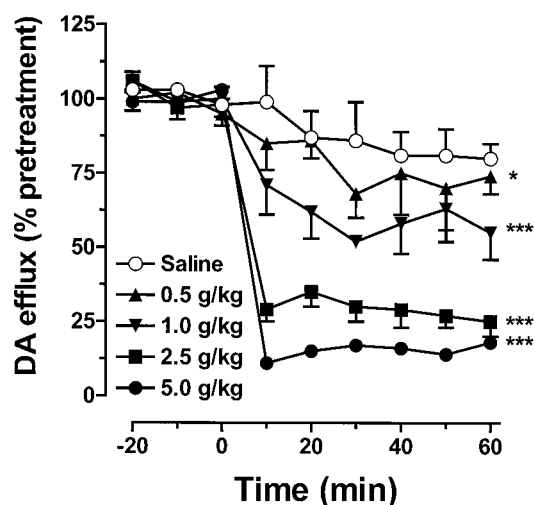
**Ethanol Dose Dependently Decreases Evoked Dopamine Release in the CP of Freely Moving Rats.** Figure 1 represents the neurochemical data obtained in a representative rat. In this study, as in previous reports (Garris et al., 1997), electrical stimulation (60 Hz, 1 s, 120  $\mu\text{A}$ , 2 ms/phase, biphasic rectangular pulses) of mesencephalic dopamine neuronal cell bodies produced a fast rise in extracellular striatal dopamine during the stimulation, followed by a return to the basal level. The behavioral response to this stimulation was typically an ipsilateral turn of the head with no audible vocalization.

Electrically evoked dopamine concentrations in the CP were stable when measured at 10-min intervals before drug treatment (Fig. 2). Following saline administration, the evoked dopamine response did not significantly change over the time course of the experiment. Ethanol, however, dose dependently (Figs. 2 and 3) decreased evoked dopamine concentrations within 10 min after administration, and this effect persisted for at least 60 min. This fast onset is similar in time to electrophysiological data (Mereu et al., 1984), and consistent with the pharmacokinetics of ethanol following i.p. administration (Nurmi et al., 1994). The effect of ethanol on dopamine efflux was assessed by one-way ANOVA on the average of the six postinjection samples ( $F_{4,25} = 161$ ,  $p < 0.001$ ), followed post hoc by Newman-Keuls multiple comparison test. Evoked dopamine was decreased to 89% by 0.5 g/kg ethanol ( $p < 0.05$ ,  $n = 4$ ), 70% by 1.0 g/kg ( $p < 0.001$ ,  $n = 4$ ), 34% by 2.5 g/kg ( $p < 0.001$ ,  $n = 4$ ), and 18% by 5 g/kg ( $p < 0.001$ ,  $n = 3$ ) compared with saline ( $n = 4$ , Fig. 2). Behaviorally, there was a slight increase in locomotor activity after 0.5 g/kg and slight sedation after 1 g/kg ethanol. The higher doses, 2.5 and 5 g/kg ethanol, generally induced profound sedation within the first 10 min.

**Ethanol-Induced Decrease in Dopamine Release Is Not Caused by an Increase in Uptake in Vivo.** On the time scale of these measurements, uptake is the predominant clearance mechanism. This was dramatically shown in mutant mice lacking the dopamine transporter where clearance rates were diminished 300-fold (Giros et al., 1996). In a within-subject comparison of the slope of dopamine disappearance before and after ethanol administration, we found no significant difference observed in the rate of uptake of evoked dopamine (for each group,  $p > 0.05$ , paired *t* test). Thus, the dose-dependent decrease in dopamine release was not due to faster dopamine uptake. This is clearly seen (Fig. 4) by comparison of clearance curves obtained before and after ethanol at the two highest doses.

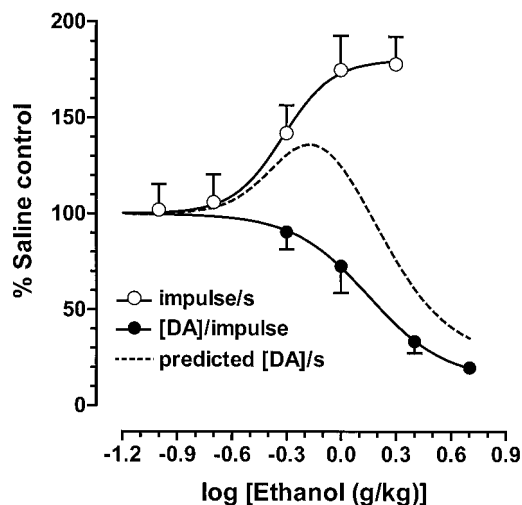


**Fig. 1.** Representative topographical plots, concentration-time plots, and cyclic voltammograms of in vivo dopamine in the rat striatum. In vivo dopamine release evoked by a single experimenter-delivered stimulus train in the SNc (1 s, 60 Hz, 120  $\mu$ A, 2 ms/phase, biphasic) before (left) and after (right) 2.5 g/kg ethanol in the same animal. The time of stimulus train delivery is shown by the rectangle above the time axis. The color plot topographically depicts the voltammetric data, with time on the x-axis, applied scan potential on the y-axis (scan direction from bottom to top), and background-subtracted faradaic current measured on the z-axis in pseudocolor. Directly above the color plot is the faradaic current (solid line) at the oxidation potential for dopamine extracted from the color plot, depicted so that an increase in extracellular dopamine would produce an upward deflection. The change in pH seen in the last 3 s of the color plot was subtracted from the dopamine signal. At the top is a background-subtracted cyclic voltammogram taken at the peak response. This has an oxidation peak at around 600 mV and a reduction peak at  $-200$  mV versus Ag/AgCl, identifying the released species as dopamine. For further explanation of this type of data representation, see Michael et al. (1998).



**Fig. 2.** In vivo striatal dopamine overflow following a single i.p. injection of ethanol. Dopamine was measured following an experimenter-delivered stimulus train (1 s, 60 Hz, 120  $\mu$ A) every 10 min, with ethanol (0.5–5 g/kg) or saline administration at 0 min. Dopamine overflow was decreased after all doses of ethanol relative to saline. Data are plotted as mean  $\pm$  S.E.M. \* $p < 0.05$ , \*\*\* $p < 0.001$  versus saline controls after ethanol administration (Newman-Keuls post hoc test).

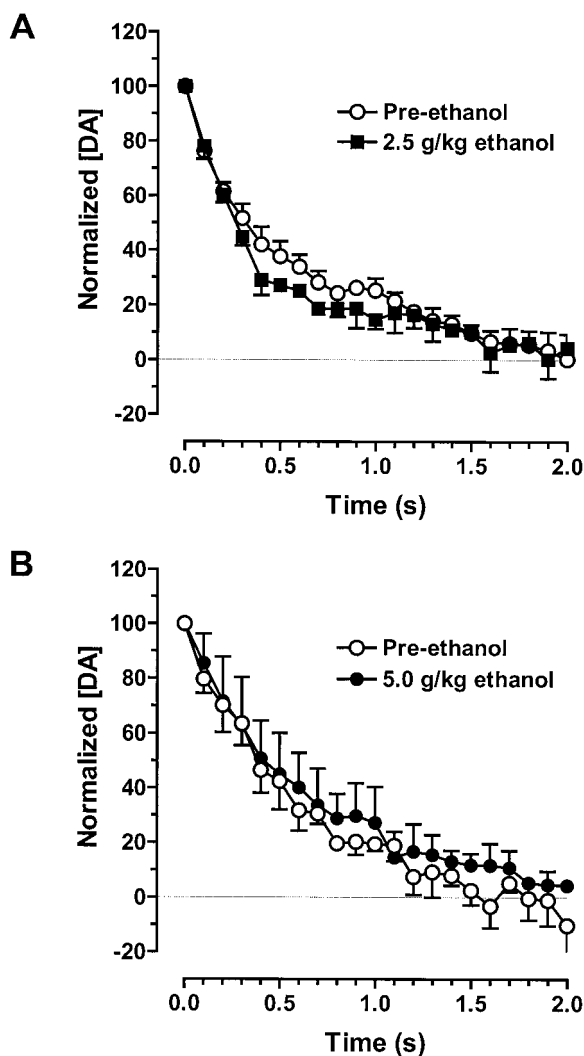
**Ethanol-Induced Decrease in Dopamine Release Is Not Caused by an Increase in Biosynthesis in Vivo.** To determine whether a decrease in dopamine biosynthesis could explain the ethanol-induced decrease in evoked dopamine, we measured L-DOPA accumulation in the CP. A one-way ANOVA showed a significant effect of group on striatal levels of L-DOPA ( $F_{4,18} = 5.48$ ,  $p < 0.01$ ). Lower doses of ethanol, 0.5 to 2.5 g/kg, did not alter L-DOPA accumulation following NSD 1015 compared with controls ( $n = 4$ –5/group, Fig. 5). The highest dose (5 g/kg) of ethanol significantly increased tissue levels of L-DOPA to 61% above the control value ( $p < 0.01$ ; Newman-Keuls post hoc test). Therefore, the



**Fig. 3.** Measures of dopamine neurotransmission as a function of ethanol dose. The voltammetric data (●) are the mean evoked dopamine concentration after ethanol normalized by the time-matched controls. The electrophysiological data (○) are adapted from Mereu et al. (1984). Data are expressed as mean percentages of the saline controls  $\pm$  S.E.M. The solid lines are sigmoidal curves obtained by nonlinear regression of the data points. The predicted rate of dopamine outflow (dashed line), and thus extracellular dopamine concentration, is the product of the two data sets. Note that doses up to 2.5 g/kg from Mereu et al. (1984) are presented; at 4 g/kg, they reported a greater initial increase followed by a profound decrease in cell firing, which may further reduce the predicted extracellular dopamine concentrations at higher doses.

dose-dependent decrease in dopamine release was not due to a decrease in dopamine biosynthesis and subsequent reduced vesicular dopamine content.

**Ethanol Alters Evoked Dopamine Release but Not Uptake in Vitro at the Highest Dose Tested.** In brain slices using FSCV, the dopamine response to a single stimulation pulse was significantly decreased to 63% of control ( $p < 0.05$ , one-way ANOVA with Dunnett's post hoc test,  $n =$

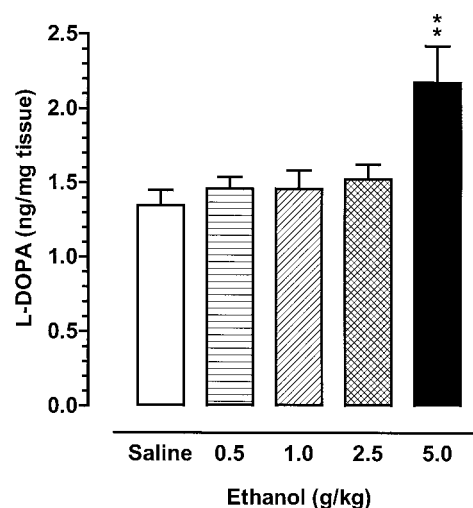


**Fig. 4.** Effect of ethanol on dopamine uptake in vivo. The descending portion of the evoked dopamine signals before and after ethanol administration is compared for the 2.5-g/kg (A) and 5.0-g/kg (B) doses. The three pre-ethanol evoked responses were averaged ( $\pm$ S.E.M.), truncated to the range of concentrations observed 40, 50, and 60 min after ethanol, and compared with the postethanol responses. Values are set to 100% as the maximum of the concentration range.

4) after 15 min and 71% ( $p < 0.05$ ) after 20 min of 200 mM ethanol application, and the effect was reversed on ethanol washout (Fig. 6A). However, 100 mM ethanol did not significantly affect evoked dopamine ( $p > 0.05$ ,  $n = 5$ ).

In vitro data collected with amperometry confirmed that 200 mM ethanol decreased the dopamine response. These data showed no significant difference in the rate of uptake of evoked dopamine, as determined by the slope of dopamine disappearance, measured before and during ethanol application ( $p > 0.05$ , paired  $t$  test,  $n = 4$  animals). The average clearance curves obtained before and after ethanol are shown in Fig. 6B.

**GBR 12909 Antagonizes the Neurochemical and Behavioral Effects of Ethanol in Vivo.** GBR 12909 (10 mg/kg,  $n = 3$ , or 20 mg/kg,  $n = 3$ ), administered postethanol, increased the evoked dopamine concentration 2- to 4-fold, providing additional pharmacological evidence that the signal measured was indeed dopamine. A representative response is shown in Fig. 7. As expected (Budygin et al., 1999),



**Fig. 5.** L-DOPA accumulation in striatal tissue 40 min after ethanol or saline administration (30 min after NSD1015). L-DOPA accumulation was significantly greater after 5 g/kg ethanol compared with saline. Data plotted as mean  $\pm$  S.E.M. ( $n = 4-5$ ). \*\* $p < 0.01$  versus saline controls (Newman-Keuls post hoc test).

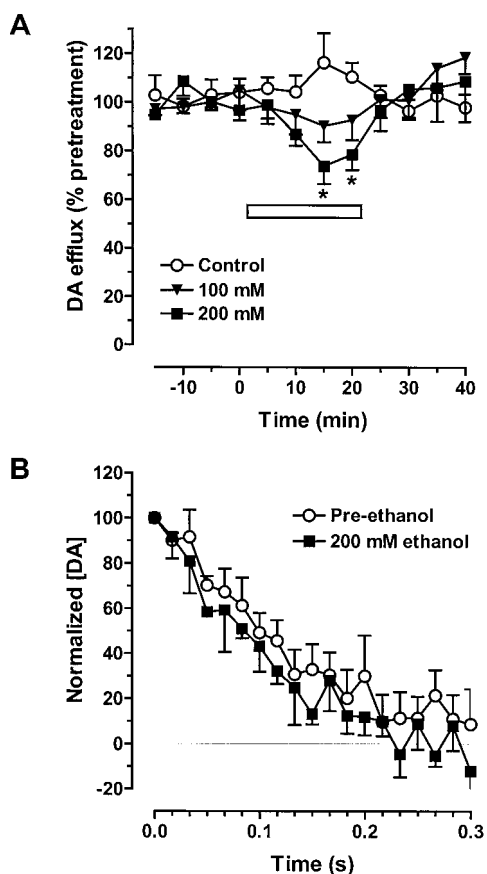
GBR 12909 decreased the rate of dopamine uptake. In addition, we observed behavioral activation after GBR 12909 administration, waking the rats from ethanol-induced sedation/hypnosis.

To quantify the behavioral effects of GBR 12909, we measured the righting reflex in rats after 2.5 or 5 g/kg ethanol (Table 1). GBR 12909 (10 mg/kg) induced faster recovery of righting reflex after 2.5 g/kg ethanol than saline. Rats receiving GBR 12909 ( $n = 6$ ) regained the righting reflex at  $17 \pm 3$  min postinjection, whereas those receiving saline ( $n = 7$ ) did not recover until  $62 \pm 15$  min (unpaired  $t$  test,  $p < 0.05$ ). Interestingly, an effect of GBR 12909 (10 or 20 mg/kg) was not apparent after 5 g/kg ethanol (unpaired  $t$  test,  $p > 0.05$ ), because all rats regained the righting reflex after approximately 9 h.

## Discussion

We report that a single i.p. injection of ethanol produces a dose-dependent decrease in evoked dopamine efflux in the CP of ambulatory male rats as measured by FSCV. This effect was apparent within 10 min and lasted for at least 60 min at all doses tested, consistent with the time course of electrophysiological and pharmacokinetic studies. A similar, but attenuated, response to ethanol was seen in striatal slices. The ethanol-induced decrease in evoked dopamine was not due to an increase in dopamine uptake rates or a decrease in biosynthesis, but rather was due to a direct suppression of release. Moreover, the dopamine uptake blocker GBR 12909 reversed the decrease in evoked dopamine efflux, and counteracted the behavioral sedation in a similar time course. Together, these data provide valuable insight to the mechanisms of ethanol's actions.

Two opposing forces maintain the extracellular striatal dopamine concentration: neuronal release of dopamine and its subsequent clearance. Normally, clearance of dopamine is predominantly by uptake via the dopamine transporter (Giros et al., 1996). Electrical stimulations of the SNc as used in this study evoke transient extracellular dopamine overflow

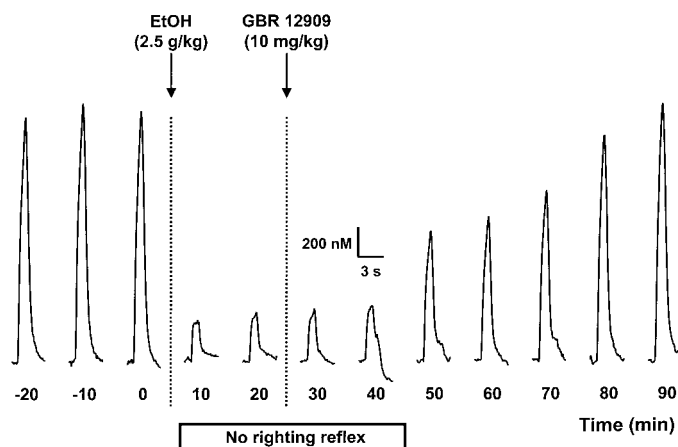


**Fig. 6.** Effect of ethanol on locally evoked dopamine efflux in rat striatal slices. A, locally evoked (single  $300 \mu\text{A}$ , 2 ms/phase, electrical biphasic pulse) dopamine overflow measured by FSCV in slices before, during, and after ethanol (0, 100, or 200 mM,  $n = 4\text{--}5$  animals/group) bath application. Ethanol, as indicated by the bar, was present for 20 min.  $*p < 0.05$  versus time-matched control values. B, dopamine uptake measured by amperometry in slices before and after 200 mM ethanol application. Three pre-ethanol evoked responses were averaged ( $\pm$ S.E.M.), truncated to the range of concentrations observed after 20, 25, and 30 min of ethanol exposure, and compared with the postethanol responses. Values are set to 100% as the maximum of the concentration range.

in the CP that rises above the basal concentration. The rising phase is reciprocally controlled by dopamine release and uptake, and the falling phase by uptake alone. Therefore, the maximal dopamine concentration may be reduced by a decline in release or an increase in uptake. However, the data during the falling phase show that uptake is unchanged, even with high doses of ethanol. Thus, we can attribute the decline in signal following ethanol to decreased dopamine release.

Dopamine release is thought to proceed via two mechanisms (Grace, 1991): phasic (impulse-dependent) release, initiated by the arrival of an action potential at the terminals, and tonic (impulse-independent) release, caused by local depolarization such as by glutamate interactions at *N*-methyl-D-aspartate receptors on presynaptic dopamine terminals. In this study evoked dopamine was measured, providing an index of phasic release. Although elevated tonic release is not measured directly by FSCV, it may decrease evoked dopamine release indirectly through terminal autoreceptors.

Phasic release is influenced by the rate of arrival of impulses at release sites, the number of vesicles released per impulse, and the average amount of dopamine in each vesicle



**Fig. 7.** Neurochemical and behavioral effects of GBR 12909 in ethanol-treated rats. Dopaminergic and behavioral response to 2.5 g/kg ethanol followed by 10 mg/kg GBR 12909 in a representative rat. Evoked dopamine, depicted as the current at the oxidation potential for dopamine across time, was dramatically reduced after ethanol administration, which coincided with a loss of righting reflex. Within 30 min after GBR 12909 administration, dopamine overflow increased and the righting reflex was regained.

TABLE 1

Time for righting reflex recovery (minutes) following GBR 12909 or saline 20 min after ethanol administration

Data shown are means  $\pm$  S.E.M. of time (minutes) to recover righting reflex following administration of GBR 12909 or saline. Voltammetric measurements were not made in these rats.

	2.5 g/kg Ethanol	5 g/kg Ethanol
Saline	$62.4 \pm 15.1$ ( $n = 7$ )	$526 \pm 56$ ( $n = 4$ )
10 mg/kg GBR 12909	$17.3 \pm 2.6^*$ ( $n = 6$ )	$573 \pm 39$ ( $n = 3$ )
20 mg/kg GBR 12909		$556 \pm 12$ ( $n = 3$ )

$^*$  Significantly different from saline group,  $p < 0.05$ .

released. In this study, the rate of impulses was predetermined (although the membrane potential may influence the propagation of the impulses). Therefore, the recordings should be most influenced by alterations in vesicular content or in the number of vesicles released per impulse. A major element controlling vesicular content is dopamine biosynthesis (Pothos et al., 1998). However, post-mortem assays revealed no change in dopamine biosynthesis rate for low-to-moderate doses of ethanol, and an increase at the highest dose. Under slightly different experimental conditions, Carlsson and Lindqvist (1973) found increased biosynthesis at moderate as well as high doses. Thus, the decrease in evoked striatal dopamine release following ethanol is not due to reduced vesicular content following reduced biosynthesis. We propose, therefore, that the number of dopamine vesicles released per impulse is reduced by the action of ethanol. In general, this parameter is controlled by the vesicle availability at release sites (Leenders et al., 1999), vesicle docking (Schafer et al., 1987), calcium concentrations (Mundorf et al., 2000), and the membrane potential (Takeuchi and Takeuchi, 1962).

It is well established that acute ethanol has excitatory activity at low doses and induces sedation at high doses. This is consistent with the biphasic striatal dopamine change obtained with microdialysis (Blanchard et al., 1993), because extracellular dopamine is positively linked to "behavioral alertness" (Schultz, 1994). However, to interpret and compare data obtained with different neurochemical methodol-

ogy, it is important to understand which components controlling extracellular dopamine contribute to the measured response. Although evoked dopamine release provides an index of phasic release and uptake, extracellular dopamine concentrations reported from microdialysis are additionally influenced by cell firing rates and tonic release.

The biphasic change in extracellular dopamine measured with microdialysis with increasing doses of ethanol suggests that two opposing mechanisms, excitatory and inhibitory, are affected by ethanol at different potencies. Consistent with this, electrophysiological studies (Mereu et al., 1984) reveal dose-dependent elevations in dopamine cell firing with an  $ED_{50}$  near 0.5 g/kg in the SNc, whereas the inhibition of dopamine released in the CP with constant number of impulses seen in this study has an  $ED_{50}$  closer to 1.5 g/kg. A simplistic prediction (e.g., ignoring tonic release) is that extracellular dopamine sampled by microdialysis should be the product of cell firing rate and the amount of dopamine available for release. Indeed, the product of our FSCV data and the electrophysiological data is a biphasic dose-response curve, similar in form to much of the microdialysis data (Fig. 3).

Although ethanol can directly increase firing rate as shown in dissociated dopamine neurons (Brodie et al., 1999), many of its effects are mediated by interactions with  $\gamma$ -aminobutyric acid<sub>A</sub> receptors (Grobin et al., 1998). For example, ethanol decreases firing of neurons in the substantia nigra pars reticulata through with  $\gamma$ -aminobutyric acid<sub>A</sub> receptors (Mereu and Gessa, 1985). The decreased firing rate of substantia nigra pars reticulata neurons induced by ethanol has been proposed to disinhibit dopamine cells, leading to their increased firing rate. Furthermore, there is evidence for a parallel mechanism in the ventral tegmental area (Gallegos et al., 1999). The in vivo response is accompanied by a reduction in action potential amplitude and an increased tendency for burst firing (Mereu et al., 1984), properties that accompany membrane depolarization. Membrane depolarization would also decrease evoked dopamine release (Takeuchi and Takeuchi, 1962; Iravani and Kruk, 1996).

In addition to its effect at the cell body, ethanol also can reduce evoked dopamine efflux by its actions at terminals. In brain slices, electrically evoked dopamine release was decreased by ethanol applied at doses that encompass the estimated peak brain concentrations (Mattucci-Schiavone and Ferko, 1984) reached with the higher doses used in the present in vivo studies. The in vitro effects were much less than observed in vivo. At these doses, ethanol directly inhibits calcium influx (Harris and Hood, 1980) and the accompanying dopamine release from synaptosomes (Woodward et al., 1990). Ethanol also increases extracellular adenosine (Nagy et al., 1990), which inhibits dopamine release by suppressing  $Ca^{2+}$  influx (Fredholm and Dunwiddie, 1988), consistent with the antidotal use of caffeine, an adenosine antagonist, for alcohol intoxication. Nevertheless, the terminal effects of ethanol seen in the brain slice are insufficient to explain the full extent of suppression of dopamine release observed in vivo, indicating that the cell body effects of ethanol described above also play an important role.

GBR 12909 counteracted both the suppression of dopamine release and the sedation caused by 2.5 g/kg ethanol in vivo. GBR 12909 increases extracellular dopamine evoked by a stimulus train or by normal impulse flow by selectively de-

creasing the rate of uptake that occurs between the action potentials within a burst. The time course of this neurochemical effect closely correlates with the behavioral activation caused by GBR 12909 alone (Budygin et al., 2000). Following 2.5 g/kg ethanol, the effects of GBR 12909 greatly shortened the time the animals were sedated. The present behavioral finding is consistent with reports that a variety of drugs that enhance striatal extracellular dopamine are capable of reducing the hypnotic effects of ethanol. These include the dopamine releaser amphetamine (Toddy et al., 1978), and the dopamine release enhancers amantadine (Messiha, 1978) and amfonelic acid (Menon et al., 1987). Moreover, the anti-hypnotic effect of amfonelic acid was blocked by the selective postsynaptic dopamine antagonist pimozide (Menon et al., 1987). Together, these findings coupled with the direct measurement of evoked dopamine overflow strongly suggest that pharmacological enhancement of extracellular dopamine can override the hypnotic effects of ethanol. An improvement in the righting reflex with GBR 12909 after 5 g/kg ethanol was not seen, however. At this dose, ethanol causes an initial stimulation and then a profound decrease in firing rate of dopamine neurons (Mereu et al., 1984). The combination of decreased firing rate and decreased number of vesicular release events would lead to minimal release of dopamine so that, even with uptake blockade, extracellular dopamine would not substantially increase.

The results of this study show that ethanol induces a dose-dependent depression in the amount of evoked dopamine release. Since the neurochemical depression is greater than observed in brain slices, the interactions of ethanol with dopamine neurotransmission involve mechanisms at various sites on the neurons and may also involve interplay among multiple neuronal systems. These effects are not always apparent in anesthetized animals (Yavich and Tiihonen, 2000), indicating the importance of measurements in intact, awake animals. The reversal of both the neurochemical attenuation of evoked dopamine release and the accompanying behavioral sedation clearly shows that the some of the behavioral effects of ethanol are mediated directly by its actions on the nigrostriatal dopamine pathway.

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