Journal of Neurochemistry, 2005, **93**, 1469–1481 doi:10.1111/j.1471-4159.2005.03137.x

Effect of operant self-administration of 10% ethanol plus 10% sucrose on dopamine and ethanol concentrations in the nucleus accumbens

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Abstract

Although operant ethanol self-administration can increase accumbal dopamine activity, the relationship between dopamine and ethanol levels during consumption remains unclear. We trained Long-Evans rats to self-administer escalating concentrations of ethanol (with 10% sucrose) over 7 days, during which two to four lever presses resulted in 20 min of access to the solution with no further response requirements. Accumbal microdialysis was performed in rats self-administering 10% ethanol (plus 10% sucrose) or 10% sucrose alone. Most ethanol (1.6 ± 0.2 g/kg) and sucrose intake occurred during the first 10 min of access. Sucrose ingestion did not induce significant changes in dopamine concentrations. Dopamine levels increased within the first 5 min of ethanol availability followed by a return to baseline, whereas brain ethanol levels reached peak concentration more than 40 min later. We found significant correlations between intake and dopamine concentration during the initial 10 min of consumption. Furthermore, ethanol-conditioned rats consuming 10% sucrose showed no effect of ethanol expectation on dopamine activity. The transient rise in dopamine during ethanol ingestion suggests that the dopamine response was not solely due to the pharmacological properties of ethanol. The dopamine response may be related to the stimulus properties of ethanol presentation, which were strongest during consumption.

Keywords: dopamine, nucleus accumbens, operant ethanol self-administration.

J. Neurochem. (2005) **93,** 1469–1481.

The role of accumbal dopamine in ethanol reinforcement remains a complex issue. Blockade of dopamine transmission interferes with responding for ethanol reinforcement (Rassnick *et al*. 1992; Samson *et al*. 1993; Hodge *et al*. 1997; Czachowski *et al*. 2001), whereas ethanol consumption is not as sensitive to this manipulation (Samson *et al*. 1993; Czachowski *et al*. 2001). Several studies have shown, however, that extracellular dopamine concentrations increase during operant ethanol self-administration (Weiss *et al*. 1993; Gonzales and Weiss 1998; Melendez *et al*. 2002; Doyon *et al*. 2003). Furthermore, rats self-administer ethanol directly into the ventral tegmental area (VTA) (Gatto *et al*. 1994; Rodd-Henricks *et al*. 2000), the region from which the neurons of the mesoaccumbens system originate, suggesting a direct link between ethanol reinforcement and dopamine activation.

We recently examined the consummatory component of ethanol reinforcement in more detail by (i) using an operant procedure that distinguished lever responding from ethanol consumption and (ii) measuring intra-accumbal ethanol and dopamine concentrations concurrently during limited-access drinking (Doyon *et al*. 2003). A small, transient increase in accumbal dopamine concentration was observed within 5 min of ethanol access, after which point accumbal ethanol levels continued to rise with no apparent stimulatory effect on dopamine activity. We hypothesized that this rapid dissociation between the dopamine and ethanol time courses could be related to the stimulus properties of ethanol (taste,

Received November 19, 2004; revised manuscript received February 7, 2004; accepted February 11, 2004.

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Abbreviations used: GLM, general linear model; VTA, ventral tegmental area.

smell) rather than its pharmacological action. However, two possible alternative explanations for this transient dopaminergic response are that the mean ethanol intake among the rats was not high enough to induce further neurochemical activity or that the response desensitized over time due to the long-term ethanol exposure that the animals underwent (over 40 days). The latter phenomenon can occur when a reward becomes predictable (Bassareo and Di Chiara 1997; Schultz *et al*. 1997).

Therefore, a more definitive examination of these issues is required to clarify the relationship between intra-accumbens ethanol and dopamine in response to limited-access consumption of ethanol. The present experiments were designed to determine the effect of ethanol intake on dopamine and ethanol concentrations within the accumbens during an early period in the development of reinforced responding. To accomplish these goals, we trained male Long-Evans rats to press a lever for limited access to ethanol (plus sucrose) over 7 days, using an operant procedure that segregated lever pressing from drinking behavior. Microdialysis was performed during self-administration of 10% ethanol (plus 10% sucrose). We followed the time course of intra-accumbal dopamine and ethanol concentrations during the limited-access drinking period to determine the relationship, if any, between intra-accumbal ethanol levels and the accompanying dopamine response. Consumption patterns for both treatment groups were also quantified for comparison with the ethanol and dopamine concentrations over time.

Materials and methods

Subjects

The present study used 41 male Long-Evans rats (Charles River Laboratories, Wilmington, MA, USA) that weighed between 327 and 496 g at the time of testing. Rats were handled and weighed for at least 5 days upon arrival prior to surgery or training. Each rat lived individually in a humidity and temperature-controlled (22°C) environment under a 12-h light/dark cycle (on at 7:00 AM; off at 7:00 PM). Each rat had food and water available *ad libitum* in the home cage except during the procedures indicated below. All procedures complied with guidelines specified by the Institutional Animal Care and Use Committee of the University of Texas at Austin and the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Behavioral apparatus

Standard operant chambers (Medical Associates Inc., St. Albans, VT, USA) modified for microdialysis perfusion were used for self-administration training and microdialysis testing. One wall of each chamber contained a retractable lever on the left side (2 cm above grid floor), which upon activation triggered the entry of a retractable drinking spout on the right side (5 cm above grid floor). The floor was a grid of metal bars in connection with the spout of the drinking bottle, which formed a lickometer circuit (Medical Associates Inc.). A cubicle with the front doors left open during training and testing housed each operant chamber. PC software provided by Medical Associates controlled operant chamber components and acquisition of lickometer data. Activation of an interior chamber light and a sound-attenuating fan accompanied the start of each operant session.

Surgery

Prior to operant training and testing, we surgically prepared the rats for microdialysis by inserting a stainless steel guide cannula (21 gauge; Plastics One Inc., Roanoke, VA, USA) above the left nucleus accumbens. The surgery occurred while the rats were under isoflurane anesthesia (1.5–2.5% in 95%/5% O_2/CO_2, 1–2 L/min), using standard stereotaxic equipment. The following coordinates were used (in mm relative to bregma): +1.7 antero-posterior, +1.0 lateral, −4.0 ventral to the skull surface (Paxinos and Watson 1998). The guide cannula was cemented to the skull by embedding three stainless steel screws into the skull and covering the entire unit, around the base of the cannula, with dental cement (Plastics One Inc.). We also placed a single steel bolt vertically into the hardening cement as an anchor for the microdialysis tether. An obturator was placed inside the guide cannula to prevent blockage prior to the microdialysis session. After 1 week of recovery, the rats began the training procedure.

Self-administration training

Operant sessions occurred once a day for 5 days/week. Subjects were initially divided into two groups (10% ethanol plus 10% sucrose or 10% sucrose) and all were trained to lever press for access to 15% sucrose (w/v). Animals were water deprived (10–22 h) prior to each session (30 min) to facilitate acquisition of the operant response. A reliable bar-pressing response for sucrose occurred in approximately 2–6 days. Rats were not water restricted at any time during the subsequent training periods.

After reliable lever-pressing behavior was established, subjects in the ethanol plus sucrose group were trained for self-administration of 10% ethanol with 10% sucrose using a modified version of the sucrose fading procedure (Samson 1986), in which we increased the concentration of ethanol (v/v) in the drinking solution across sessions (2–10% over 6 days), but we did not subsequently remove the sucrose (Table 1). Following lever training, the subjects in the

Table 1 Summary of operant self-administration training protocol for ethanol plus sucrose self-administration

Day	Drink solution[a]	Pre-drink wait (min)	Response requirement
1	10S	2	2
2	10S 2E	4	2
3	10S 2E	6	2
4	10S 5E	8	2
5	10S 5E	10	4
6	10S 10E	12	4
7[b]	10S 10E	15	4

The sucrose control group followed the same schedule except that ethanol was not faded into the drink solution.

[a]S equals sucrose and E equals ethanol. Numeral for drink solution represents percentage (w/v for sucrose; v/v for ethanol).

[b]Dialysis session.

sucrose group ($n = 10$) were switched to 10% sucrose (w/v) as reinforcement over the same number of days as the ethanol group. During this period, we gradually habituated the rats to (i) a 15-min 'wait time', which preceded access to the lever and drinking solution and (ii) a response requirement that increased from two to four across sessions. Upon completion of the response requirement, the drinking solution became accessible for 20 min, followed by a 20-min post-drinking period in the absence of the lever and drinking solution. For the dialysis experiment, the response requirement was set at four and the reinforcer was either 10% ethanol (plus 10% sucrose) or 10% sucrose. The sucrose group was never exposed to ethanol. Consumption was monitored during training and during the microdialysis session by a lickometer and by measuring the volume of liquid in the drinking bottle before and after the session, taking care to account for spillage. Body weights were measured each day.

A third group of rats ($n = 7$) was included to control for the non-specific effects of handling on dopamine activity. These animals were placed into the operant chamber for the same periods of time and corresponding number of days as the other groups, except that they did not receive training for self-administration. These rats were never exposed to a lever or a drinking bottle in the chamber. Each rat in the handling group was paired surgically and experimentally with a rat in the ethanol plus sucrose or the sucrose group.

We subsequently included an additional experimental group ($n = 8$) that was trained in exactly the same manner as the ethanol group, except that rather than receiving 10% ethanol (plus 10% sucrose) during the dialysis session, these rats self-administered a solution of 10% sucrose, which did not contain ethanol. Therefore, the only procedural difference between this group (i.e. unexpected sucrose) and the ethanol group occurred on the experiment day, during which time each group consumed a distinct reinforcer.

Microdialysis

The microdialysis probes were constructed according to the methods described by Pettit and Justice (1991). Briefly, fused-silica tubing (inner diameter = 40 μm; Polymicro Technologies, Phoenix, AZ, USA) formed the inlets and outlets of the probes, and hollow cellulose fiber (inner diameter = 200 μm; molecular weight cut-off = 13 000; Spectrum Laboratories Inc., Rancho Dominguez, CA, USA) formed the dialysis membrane. The active dialysis membrane spanned 2.2 mm (the distance between the end of the inlet and the epoxy that sealed the membrane).

Habituation to the microdialysis tethering apparatus occurred within the week preceding testing. This procedure consisted of tethering the rats overnight in the operant testing room, with continued tethering throughout the subsequent day of operant training. Rats were tethered by gently restraining the conscious animal or by sedation with halothane for a few minutes. On the day preceding the dialysis session, we perfused (flow rate = 2 μL/min) the microdialysis probes with artificial cerebral spinal fluid (149 mM NaCl, 2.8 mM KCl, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, and 0.25 mM ascorbic acid, 5.4 mM D-glucose) and slowly inserted them into the brain through the guide cannula while the rat was briefly anesthetized (15–20 min) with 2% halothane in air. This procedure occurred at least 14 h before the start of the experiment. We used a syringe pump (CMA102; CMA, Solna, Sweden) to pump the perfusate through a fused-silica transfer line into a single channel swivel (Instech Solomon, Plymouth Meeting, PA, USA), which hung from a counterbalanced lever arm (Instech Solomon). The swivel formed a connection with the inlet of the probe and a spring tether secured the animal to the swivel. After the rat recovered from the probe implantation procedure (usually within 15 min), the perfusion flow rate was decreased to 0.2 μL/min overnight. The flow rate was returned to 2.0 μL/min 2 h prior to the baseline-sampling period. We manually changed each sample vial, which were immediately frozen on dry ice (excluding the fraction of dialysate that was removed for ethanol analysis, see 'Ethanol analysis') and then stored at −80°C until analyzed.

Experimental design

Dialysis samples were taken every 5 min except as indicated below. Six samples were collected during a baseline period in the home cage (30 min; data not shown). One sample was collected during the period in which the rat was transferred into the operant chamber prior to activation of the operant program (5 min; data not shown). Upon activation of the program, three samples were collected prior to introduction of the drinking spout: two 5-min samples during a waiting period and a third waiting sample (approximately 5.7 min) that included at its end a brief lever-pressing period (0.7 ± 0.2 min; excluding one rat that required 15.3 min). Completion of the response requirement was followed by a 20-min drinking period with unrestricted access (four samples) and then a post-drinking period within the chamber in the absence of the solution (20 min, four samples). At the end of this time, the rat was moved back into the home cage. For the ethanol group, we then collected an additional six samples (at 10-min intervals) to monitor ethanol concentrations in the dialysates, but these sample were not analyzed for their dopamine content. After obtaining all samples, the perfusion solution was switched to one lacking calcium for 45–60 min. A sample (10 min) was then taken to determine the calcium dependency of the dopamine in dialysates.

Histology

After the experiment, the rats were overdosed with chloral hydrate (600 mg/rat) and saline was perfused through the heart, followed by 10% (v/v) formalin. The brains were removed and immersed in 10% formalin/30% sucrose (w/v) for at least 3 days. Brains were cut into coronal sections (48 μm thick) with a cryostat (Bright Instrument Co., Cambridgeshire, England), and the sections stained with cresyl violet. The slides were examined to confirm the placement of the active dialysis membrane (2.2 mm). We determined subregional placement within the core, shell, or core and shell if at least 30% of the dialysis membrane bisected any of these areas.

Dopamine analysis

Two chromatography systems were used to separate and quantify dopamine during these experiments. Both HPLC systems were amperometric and based on reversed phase chromatography using an ion-pairing agent with electrochemical detection. The majority of samples were analyzed with the first system, which included one of three pumps [ISCO 260D (ISCO, Lincoln, NE, USA), LC-10AD (Shimadzu Scientific Instruments Inc., Columbia, MD, USA), and LC-10ADVP (Shimadzu Scientific Instruments Inc.)], a FAMOS autosampler (LC Packings, Sunnyvale, CA, USA), a VT-03 cell (2 mm working electrode diameter, potential: 450 mV against a Ag/AgCl reference; Antec Leyden BV, Zoeterwoude, the Netherlands)

in connection with an Intro controller (GBC Separations Inc., Hubbardston, MA, USA) and a Polaris 2 × 50 mm column (C18, 3-μm particle size; Varian, Palo Alto, CA, USA). Samples from 39 subjects were analyzed with this configuration. The second system consisted of a LC-10ADVP pump (Shimadzu, Kyoto, Japan), a 5041 analytical cell (potential: 350 mV; ESA Inc., Chelmsford, MA, USA), and a 465 autosampler (ESA Inc.) used in connection with a BDS Hypersil 2.1 × 100 mm column (C18, 3-μm particle size; Thermo Hypersil-Keystone, Bellefonte, PA, USA). Samples from two subjects were analyzed with this configuration. For these systems, the mobile phase composition was altered appropriately, using octanesulfonic acid (0.72–0.77%, w/v) or octanesulfonic acid (0.5%, w/v) plus decanesulfonic acid (0.05%, w/v) in combination with methanol (12–15%, v/v) to resolve dopamine sufficiently. The flow rates were set at 0.3 mL/min for both systems. For the first system, 7 μL of the dialysate was injected using the microliter pickup mode and the transfer fluid was ascorbate oxidase (EC 1.10.3.3; 102.3 U/mg; Sigma, St. Louis, MO, USA). For the second system, 7 μL of the dialysate was incubated with 21 μL of ascorbate oxidase (102.3 U/mg; Sigma) for 1.5 min, and 20 μL was injected. A Shimadzu C-R3A integrator (Shimadzu, Houston, TX, USA) or a computer data acquisition system (EZ Chrome Elite; Scientific Software Inc., Pleasanton, CA, USA) recorded the dopamine peaks. Quantification was carried out by comparing dopamine peak heights or areas from dialysate samples to external standards.

Ethanol analysis

Ethanol was analyzed in all dialysis samples collected after the lever-press period for subjects in the ethanol plus sucrose group. Before freezing the dialysis sample, 2 μL of fluid were transferred into a glass vial and sealed with a septum for analysis of ethanol later that day. A gas chromatograph (Varian CP 3800; Varian, Walnut Creek, CA, USA) with flame ionization detection (220°C) measured the ethanol in the dialysates. Specific details concerning the treatment of dialysate ethanol samples and the components of the gas chromatograph are described by Doyon *et al*. (2003). The limit of detection was 0.03 mM ethanol (signal to noise = 3). Quantification of ethanol in dialysates was done by comparing peak areas obtained with a Star chromatographic analysis system (Varian) to external standards.

Statistical analysis

Dialysate dopamine levels (nM) were analyzed using analysis of variance (ANOVA) with repeated measures. The six home cage samples served as the baseline response to which the transfer and wait samples were compared. The average of the transfer and wait periods (four samples), including the last wait sample that encompassed the lever-press period, defined the baseline response to which the drink and post-drink samples were compared. The lever press period was included as a basal sample because of its short duration. Any potential dopamine activity resulting from this period would be collected in the next sample due to the brief time lag inherent in microdialysis. Technical problems associated with sample collection or HPLC analysis resulted in the loss of some samples (8 of 533). To account for this we estimated these values by averaging adjacent time points and then adjusting the degrees of freedom in the ANOVA. Separate ANOVA tests were conducted to test for group by time interactions during the main phases of the experiment (i.e. basal plus transfer/wait periods; transfer/wait periods plus drink and post-drink periods). *Post hoc* contrasts comparing individual time points to baseline within groups were performed after determining a significant group by time interaction during these periods. Bonferroni corrections were used in the case of *post hoc* contrasts. ANOVA was performed using the Manova routine in SPSS for Windows, and *post hoc* contrasts were carried out using the GLM procedure. Significance for these analyses was determined when $p < 0.05$.

Analysis of the consumption parameters was carried out using multivariate ANOVA (GLM procedure) and *F*-values derived from Wilks' Lambda. Two parameters were log transformed to maintain homogeneity of variance (latency to drink, lever-pressing time). Due to a technical issue, one rat in the ethanol plus sucrose groups and two rats in the sucrose group were excluded from this analysis because we were unable to obtain a value for the duration of bar pressing. The behavioral data shown in Table 2 reflects this change in sample size.

Results

Histological analysis and calcium-dependence of dopamine concentrations

At least 50% of the active dialysis membrane for each probe was within the nucleus accumbens. Examination of the probe positions within subregions of the accumbens showed that, overall, 42% were within the core, 33% in the shell, and 24% bisected both the core and shell (Fig. 1). The placements were random in distribution between each experimental

Table 2 Lickometer parameters for rats self-administering ≥0.8 g/kg ethanol (high ethanol), ≤0.5 g/kg ethanol (low ethanol), and sucrose during dialysis

Parameter	High ethanol $n = 9$	Sucrose $n = 8$	Low ethanol[a] $n = 6$
Lever-pressing time (min)	0.8 ± 0.4	0.6 ± 0.3	3.3 ± 2.7
Latency to begin drinking (min)	0.07 ± 0.02	0.27 ± 0.19	0.47 ± 0.23[c]
Number of bouts	1.7 ± 0.2	1.9 ± 0.2	1.3 ± 0.2
Initial bout duration (min)	6.6 ± 1.2	8.3 ± 0.8	2.0 ± 0.6[b]
Total licks	1855 ± 275	2359 ± 299	349 ± 120[b]
Licks during initial bout	1692 ± 293	2202 ± 297	298 ± 126[b]
Initial bout response rate (licks/min)	266 ± 20	256 ± 27	184 ± 55
Response rate for ½ of initial bout (licks/min)	343 ± 15	328 ± 22	220 ± 63

Bout refers to a period of at least 25 licks, with no more than 2 min between licks. Values shown as mean ± SEM

[a]Significantly different from the high ethanol and sucrose group by multivariate ANOVA ($p < 0.05$).

[b]Significantly different from the high ethanol and sucrose group by univariate ANOVA ($p < 0.05$).

[c]Significantly different from the high ethanol group by univariate ANOVA ($p < 0.05$).

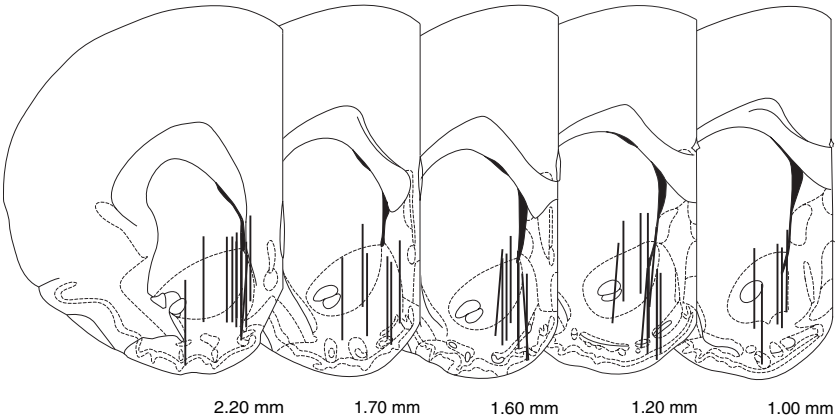

Fig. 1 Coronal sections showing microdialysis probe placement within the nucleus accumbens. Lines indicate the active dialysis regions. Numbers below the figure represent the position of the slice relative to Bregma. The figure was adapted from Paxinos and Watson (1998).

group (sucrose plus ethanol groups: 5 core, 6 shell, 5 core and shell; sucrose group: 6 core, 2 shell, 2 core and shell; handling group: 2 core, 3 shell, 2 core and shell). The core and shell subregions were not examined with regard to differences in dopaminergic activity due to the dispersion of the probes. The dialysate dopamine samples showed very robust calcium dependence (78 ± 3% for the 33 subjects). The calcium dependency of dialysate dopamine exceeded 50% in all subjects except for one, which showed 41%. In addition, approximately 50% of all subjects incurred a certain amount of ventricular damage caused by the insertion of the microdialysis probe. However, these subjects were randomly distributed within each experimental group.

Ethanol intake patterns during the training procedure

Due to clear differences in ethanol preference during training and dialysis (Fig. 2), we divided the rats in the ethanol plus sucrose group into two subgroups: a high ethanol group (with intakes ≥ 0.8 g/kg; $n = 10$) and a low ethanol group (with intakes ≤ 0.5 g/kg; $n = 6$), based on intake during the dialysis session. The ethanol intake levels between the high and low ethanol groups differed significantly across the training period (group: $F_{1,14} = 6.38$, $p < 0.05$; group × time: $F_{5,70} = 8.59$, $p < 0.05$). The separation into subgroups was also justified by a histogram analysis of the ethanol intakes during dialysis, which showed that the population as a whole did not follow a normal distribution, with the low ethanol-drinking rats skewed to the left of the distribution.

Dopamine concentrations and operant activity prior to consumption

Mean dialysate dopamine concentrations during the home-cage baseline period were 1.5 ± 0.1 nM for the high ethanol plus sucrose group, 1.7 ± 0.6 nM for the low ethanol plus sucrose group, 1.9 ± 0.4 nM for the sucrose group, and 1.4 ± 0.1 nM for the handling group. Home-cage baseline dopamine concentrations were not significantly different among the groups (group: $F_{3,29} = 0.04$, $p > 0.05$; group by time interaction: $F_{15,145} = 1.05$, $p > 0.05$). Examination of

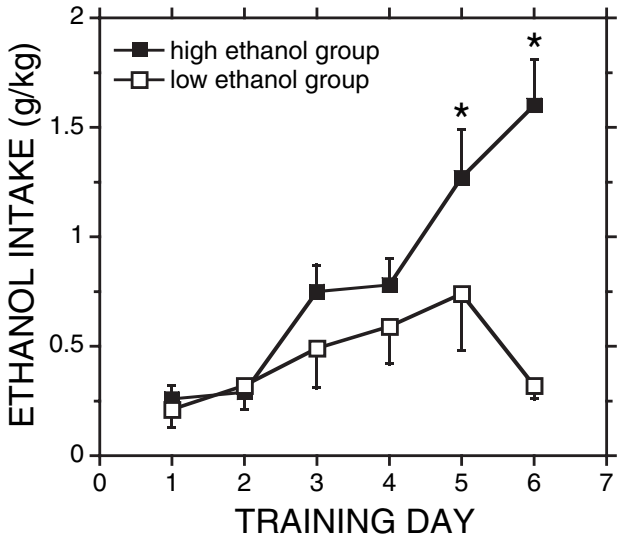

Fig. 2 Ethanol intake levels (g/kg) for the high and low ethanol groups during the operant training procedure. Intakes differed significantly across the training period between the groups, most noticeably on days 5–6 when the ethanol concentration in the drinking solutions increased to 10%. Asterisks denote significance compared with the low ethanol group by ANOVA.

the home cage baseline, transfer, and wait periods also showed that none of the groups differed significantly from one another across this time frame (group: $F_{3,25} = 0.03$, $p > 0.05$; group by time interaction: $F_{12,112} = 0.77$, $p > 0.05$). An effect of time, however, was observed across all groups ($F_{4,112} = 12.68$, $p < 0.05$), including the handling group, which was exposed to the operant chamber but did not self-administer a solution. *Post hoc* contrasts indicated that dopamine increased significantly during the period in which the rats were transferred from the homecage ($F_{1,32} = 54.68$, $p < 0.05$; data not shown) into the operant chamber and during each of the samples that followed (i.e. the wait period). In the subsequent analysis of the drink and post-drink periods we used the dopamine samples from the

transfer and wait periods as a baseline rather than the home-cage samples. All groups showed stable and similar dopamine responses during the transfer and wait periods (time: $F_{3,83} = 1.85$, $p > 0.05$; group by time: $F_{9,83} = 0.79$, $p > 0.05$; Figs 3a–d). Mean dialysate dopamine concentrations during these periods were 1.9 ± 0.2 nM for the high ethanol plus sucrose group, 2.0 ± 0.5 nM for the low ethanol plus sucrose group, 2.2 ± 0.4 nM for the sucrose group, and 1.7 ± 0.2 nM for the handling group.

ANOVA indicated that the high ethanol, low ethanol, and sucrose groups did not differ significantly in terms of the duration of the operant response during the dialysis experiment. The time required to complete the response requirement was 0.8 ± 0.4 min for the high ethanol group, 0.6 ± 0.3 min for the sucrose group, and 3.3 ± 2.7 min for the low ethanol group ($F_{2,23} = 1.42$, $p > 0.05$; Table 2). The variability seen in the low ethanol group was due to a single rat that stalled during the lever-pressing period (15.3 min) but eventually finished the response requirement.

Drinking behavior and dopamine concentrations during consumption

The low ethanol group ($n = 6$), consumed 0.32 ± 0.06 g/kg, ranging from 0.2 to 0.5 g/kg. In contrast, the high ethanol group ($n = 10$) drank 1.6 ± 0.2 g/kg with intakes ranging from 0.8 to 2.8 g/kg. The sucrose and high ethanol groups ingested similar amounts of fluid within the 20-min drink period (sucrose group: 11.4 ± 1.6 mL; high ethanol group: 8.3 ± 1.1 mL). The low ethanol group ingested 1.7 ± 0.3 mL, which was significantly lower than that consumed by the sucrose and high ethanol groups ($F_{1,25} = 27.77$, $p < 0.05$).

Upon completion of the response requirement, mean dopamine levels increased (20 ± 6% above baseline) within 5 min of access to 10% ethanol plus 10% sucrose for the high ethanol group (Fig. 3a). This elevated state was brief, and values returned to baseline during the subsequent seven samples. Eight of 10 rats in the high ethanol group showed peak dopamine responses within the first 10 min (two

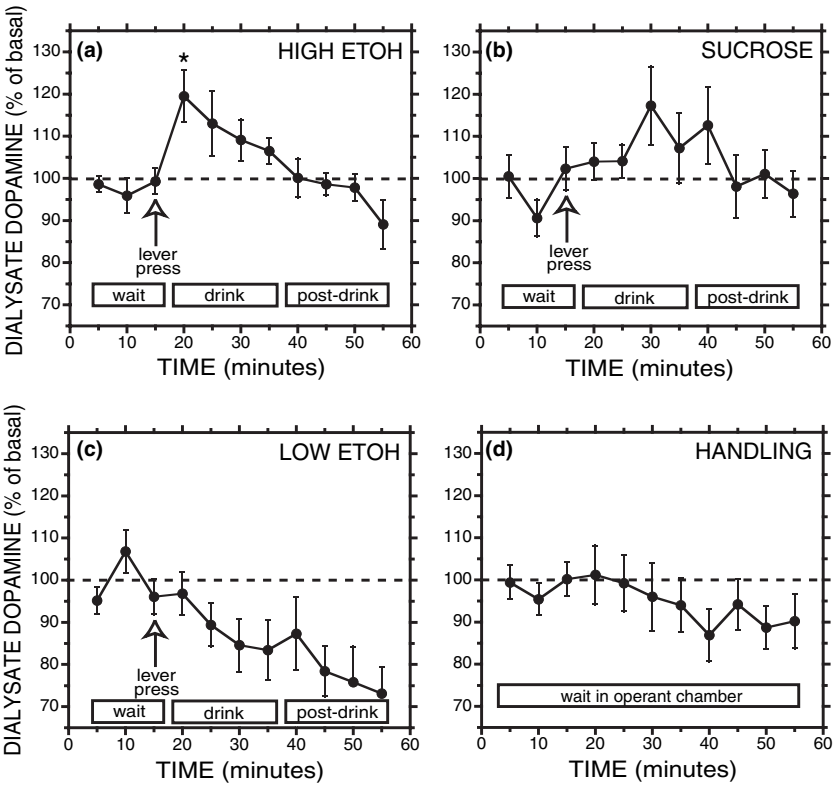

Fig. 3 Effect of operant self-administration of 10% ethanol plus 10% sucrose and 10% sucrose (b) on extracellular accumbal dopamine levels. The rats in the ethanol plus sucrose group were subdivided into high (≥ 0.8 g/kg; panel a) and low (≤ 0.5 g/kg; panel c) drinking groups based on intake. A significant increase in mean dopamine levels occurred briefly during the first 5 min of the drink period for the high ethanol group, but not at any point for the sucrose or low ethanol group. Wait refers to the time in which the rat was in the operant chamber prior to access of the drinking solution. Lever press (open arrow) is the time at which lever pressing occurred. Drink refers to the 20-min free-access drinking period in the absence of lever pressing. Post-drink refers to dopamine while the rat was in the operant chamber in the absence of the drinking solution. Each point represents the mean ± SEM ($n = 10$ for the high ethanol group, $n = 10$ for the sucrose group; $n = 6$ for the low ethanol group). (d) The effect of the operant environment on extracellular accumbal dopamine in rats not trained for operant self-administration ($n = 7$). Asterisk denotes significance compared with the wait period by *post hoc* simple contrasts ($p < 0.05$).

samples) of the 20 min drink period. In contrast, dopamine concentrations remained at baseline levels during this period for the sucrose and low ethanol groups (Figs 3b and c). ANOVA showed a significant overall group by time interaction ($F_{8,90} = 2.70$, $p < 0.05$) during the drink period between the high ethanol, low ethanol, and sucrose groups. Further analysis revealed that the groups differed during the first dopamine sample of the drink period. At this point, a group by time interaction existed between the high ethanol group and (i) the sucrose group ($F_{1,18} = 5.04$, $p < 0.05$) and (ii) the low ethanol group ($F_{1,14} = 7.90$, $p < 0.05$), but not between the sucrose and low ethanol groups ($F_{1,14} = 1.01$, $p > 0.05$). Within-group *post hoc* contrasts indicated a significant increase in dopamine levels during the first drink sample compared with baseline for the high ethanol group ($F_{1,9} = 12.13$, $p < 0.05$; Fig. 3a). In contrast, the sucrose and the low ethanol groups did not show a significant dopamine response at any point during the drink period. Furthermore, because of the apparent trend towards a decrease in dopamine levels following low ethanol consumption, we also analyzed the dopamine response between the low ethanol and handling groups. However, there was no significant difference between these groups (group × time: $F_{8,88} = 1.70$, $p > 0.05$).

Licking behavior during consumption of ethanol and sucrose

Multivariate analysis revealed an overall group effect between the high ethanol, low ethanol, and sucrose groups during consumption with respect to several parameters ($F_{16,26} = 2.15$, $p < 0.05$; Table 2). The high ethanol and sucrose groups were not statistically different ($F_{4,12} = 0.64$, $p > 0.05$), whereas the low ethanol group differed from both the high ethanol ($F_{4,10} = 7.96$, $p < 0.05$) and sucrose groups ($F_{4,9} = 10.57$, $p < 0.05$). Univariate ANOVA showed that high ethanol and sucrose groups differed from the low ethanol group in three parameters (Table 2): (i) duration of the first bout, (ii) total number of licks during the first drinking bout, and (iii) total number of licks during the drink period. This analysis also showed that the latency to begin drinking after completion of the response requirement was only different between the high ethanol and low ethanol groups (Table 2). Consumption (licking) in all groups began almost immediately after completion of the operant response (i.e. latency to begin drinking), with 89 ± 4% of spout licks occurring during the first bout. Consumption during the first bout for the sucrose and low ethanol groups was comparable (93 ± 2% and 83 ± 12%, respectively). Figure 4 (inset) shows the average number of licks within each 5-min epoch of the drink period for the high ethanol group.

Accumbal ethanol concentrations during consumption

In addition to dopamine in dialysates, we also quantified the ethanol concentration in each sample collected after completion of the response requirement for the ethanol plus sucrose groups. Ethanol appeared in dialysates within 5 min of ethanol availability in all rats. For the high ethanol group, mean dialysate ethanol concentrations increased progressively (Fig. 4), reaching peak concentration (2.8 ± 0.5 mM) approximately 40 min after drinking began, before declining. The low ethanol group showed low mean dialysate ethanol levels (data not shown), which reflected the amount of ethanol this group consumed (1.7 ± 0.3 mL). The peak ethanol concentration for the low ethanol group was 0.4 ± 0.3 mM. Examination of the time course data indicates that ethanol levels remained very close to this value throughout the drink and post-drink periods. Overall, individual ethanol time courses varied substantially between the animals, including parameters such as peak ethanol concentration and clearance from the dialysates. Pooling the data from the high and low ethanol groups, regression analysis indicated that a significant, positive correlation existed between (i) intake (g/kg) and the area under curve ($F_{1,13} = 27.23$, $p < 0.05$; Fig. 5a) and (ii) intake and peak dialysate ethanol concentration ($F_{1,13} = 33.77$, $p < 0.05$).

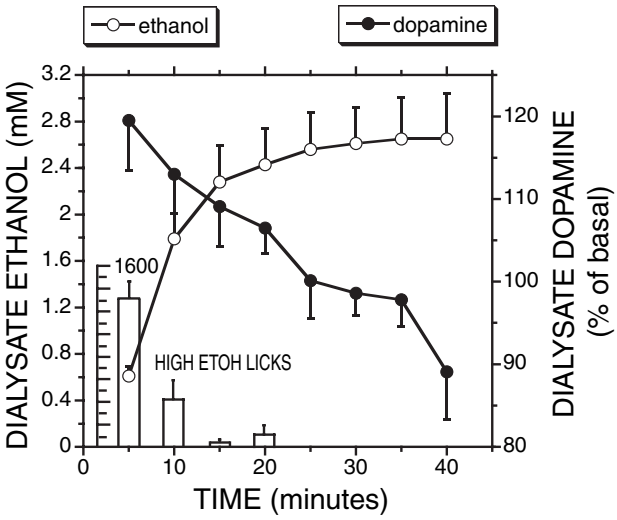

Fig. 4 Mean dialysate dopamine and ethanol levels from the nucleus accumbens during drinking and post-drinking periods for the high ethanol group. Dopamine data are from the same rats shown in Fig. 2(a). Ethanol was analyzed in the same samples from which the dopamine analysis was done. Inset shows periods of ethanol ingestion during the drink period for the high ethanol group. Although there was no direct relationship between the dialysate dopamine and ethanol time courses during any phase of the experiment, the peak dopamine response coincided with the period in which most ethanol intake occurred. Each point is the mean ± SEM ($n = 10$).

Dose–effect relationships between ethanol intake and dopamine response

Regression analysis showed significant, positive correlations between intake and ethanol-induced dopamine activity

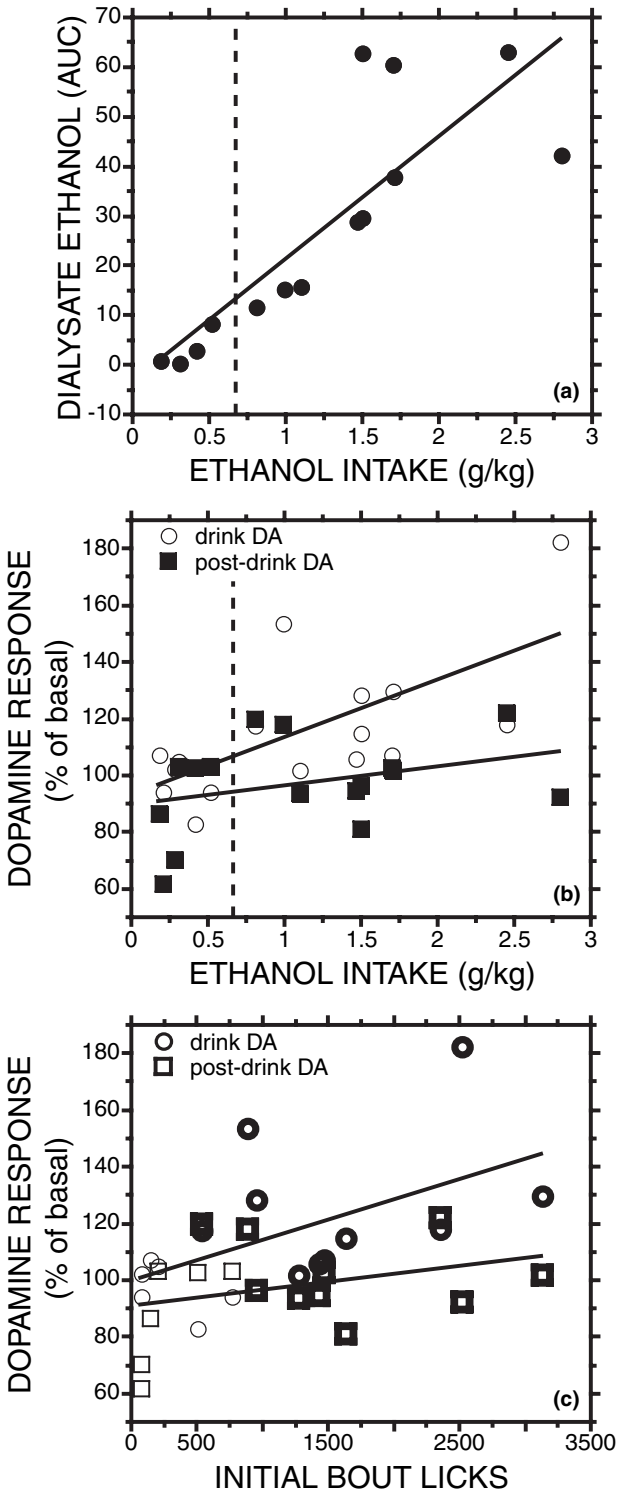

Fig. 5 Dose–effect relationships between (a) ethanol intake (g/kg) and ethanol area under curve, (b) ethanol intake (g/kg) and peak dopamine response (DA) during the initial 10 min of the drink period and first 5 min of the post-drink period, and (c) consummatory behavior (licks during first drinking bout) and peak dopamine response (DA) during the first 10 min of the drink period and first 5 min of the post-drink period. Regression curves represent data pooled from the high and low ethanol-drinking groups ($n = 16$). Vertical dashed lines distinguish the low ethanol from the high ethanol group (a and b). Data points in bold on (c) identify the high ethanol group, whereas the points not in bold are of the low ethanol group. Ethanol intake (g/kg) correlated positively with accumbal ethanol levels (area under curve; $r = 0.83$, $p < 0.05$) and peak dopamine response during the initial 10 min of the drink period ($r = 0.69$, $p < 0.05$), but not with the dopamine response during the first 5 min of the post-drink period ($r = 0.33$, $p > 0.05$). Similarly, initial bout licks correlated positively with peak dopamine levels during the initial 10 min of the drink period ($r = 0.55$, $p < 0.05$), but not during the post-drink period ($r = 0.31$, $p > 0.05$).

during the drink period for the high and low ethanol groups combined. For example, ethanol intake (g/kg) correlated positively with dopamine levels during the first 5 min of the drink period ($F_{1,15} = 8.42$, $p < 0.05$) and with the peak response within the first 10 min of the drink period ($F_{1,15} = 11.36$, $p < 0.05$; Fig. 5b), when most animals showed their maximal dopamine response. However, intake did not correlate with dopamine levels during the post-drink period ($F_{1,15} = 1.73$, $p > 0.05$; first 5 min sample), in which the drinking spout was absent and consumption could not occur. As the majority of ethanol consumption occurred during the first drinking bout (i.e. 85 ± 5% of all licks occurred within 6.6 ± 1.2 min), we also analyzed initial bout licks with the dopamine response at various time points. Initial bout licks correlated positively with dopamine levels during the first 5 min ($F_{1,15} = 5.06$, $p < 0.05$) and the peak response during the first 10 min ($F_{1,15} = 5.97$, $p < 0.05$) of the drink period, but not during the post-drink period ($F_{1,15} = 1.48$, $p > 0.05$; first 5-min sample, Fig. 5c). In contrast, for the sucrose-drinking rats we found no significant correlations between licking and dopamine levels [e.g. initial bout licks vs. peak dopamine response during the first 10 min of the drink period ($F_{1,8} = 0.07$, $p > 0.05$)]. Lastly, there was no significant relationship between peak ethanol and peak dopamine levels in dialysates ($F_{1,13} = 2.01$, $p > 0.05$).

Dopamine concentrations during unexpected sucrose self-administration

We next examined the potential effect of expectation of ethanol reinforcement on the transient dopamine response observed in the high ethanol group. For this experiment, we trained a group of rats in exactly the same manner as the high and low ethanol groups. However, on the experiment day, these animals self-administered a solution of 10% sucrose, which did not contain ethanol. As with the other treatment groups, at least 50% of the active dialysis membrane for each probe was located within the nucleus accumbens, with the probe positions distributed randomly within the core and shell subregions. The dopamine samples showed excellent calcium dependence (81 ± 4%). Daily ethanol intake levels

across the training procedure mirrored those of the high ethanol group. For example, these rats ingested 1.4 ± 0.3 g/kg ethanol on the day prior to dialysis. The unexpected sucrose group also displayed comparable mean dialysate dopamine concentrations during the baseline periods (home-cage: 1.9 ± 0.4 nM; operant wait: 2.2 ± 0.4 nM) with respect to the other treatment groups. Dopamine concentrations remained at baseline levels during consumption (Fig. 6). The high ethanol group differed significantly from the unexpected sucrose group across the first drink sample (group × time: $F_{1,16} = 4.98$, $p < 0.05$; $n = 8$). The unexpected sucrose group showed very similar lickometer-parameter values compared with the sucrose and high ethanol groups (i.e. total licks: 2209 ± 203; initial bout licks: 2182 ± 208; initial bout duration: 9.1 ± 1.0 min; latency to begin drinking: 0.11 ± 0.07 min). Figure 6 (inset) shows the mean number of licks within each 5-min epoch of the drink period. The lever press time for this group (0.50 ± 0.11 min) was also comparable to those of the other groups.

Discussion

This is the second study to examine the relationship between intra-accumbal dopamine and ethanol concentrations using an operant procedure that specifically distinguished ethanol consumption from lever-pressing behavior (Doyon *et al*. 2003). This study extends previous findings (Weiss *et al*. 1993; Melendez *et al*. 2002; Doyon *et al*. 2003) by demonstrating that accumbal dopamine levels can clearly undergo transient elevations in response to ethanol (plus sucrose) intake. The magnitude of the dopamine response was dependent upon the amount of ethanol consumed, but not on the concentration of ethanol reaching the accumbens. Examination of the time courses of individual rats showed that the rise in dopamine levels occurred predominantly within 5–10 min of ethanol access before declining to baseline. At this time, accumbal ethanol concentrations were still in the rising phase of their time course, reaching peak levels over 40 min later. Although quantitative microdialysis was not performed, we estimated the mean tissue concentration of ethanol to be 13.8 ± 1.7 mM 10 min into the drink period and 21.7 ± 3.5 mM at its peak. These estimations were based on dialysate concentrations and an *in vivo* extraction fraction for ethanol of 0.13 (Robinson *et al*. 2000) and are comparable to previous reports of brain ethanol levels following oral self-administration (Nurmi *et al*. 1999).

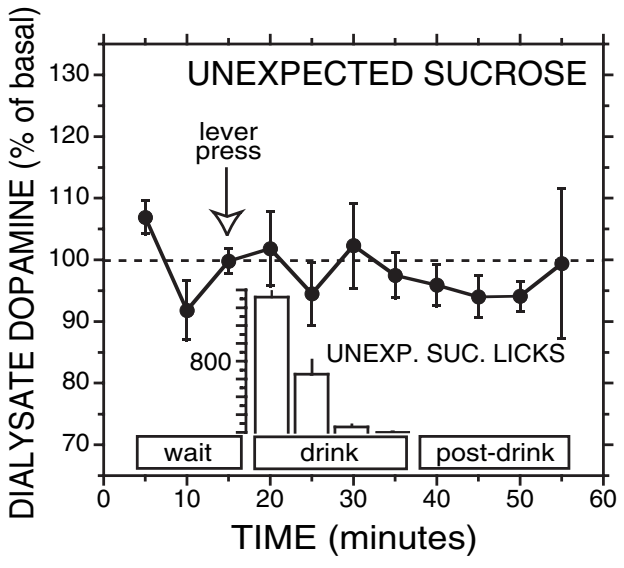

Fig. 6 Effect of operant self-administration of 10% sucrose on extracellular accumbal dopamine levels in rats previously reinforced with 2–10% ethanol (plus 10% sucrose). In contrast to the high ethanol group, no significant changes in dopamine occurred during the consumption period. Each point represents the mean ± SEM ($n = 8$). The dialysis sampling periods shown here are identical to those in Fig. 3. Inset shows the periods of sucrose ingestion during each 5-min epoch within the drink period.

Our previous results also demonstrated a transient dopamine response to limited-access ethanol consumption (Doyon *et al*. 2003), which was smaller in magnitude but with a remarkably similar time course to the one observed here. We originally suggested that the discrepancy between the ethanol and dopamine time courses could be due to (i) intakes (0.45 ± 0.04 g/kg) that were not sufficiently high enough to produce a sustained ethanol-induced dopamine response or (ii) desensitization with a long-term reinforcement schedule, thereby causing a blunted ethanol-induced dopamine response over time. The present study indicates that ethanol intakes of 1.6 ± 0.2 g/kg (over three times higher than the previous report) were not sufficient for stimulation of mean dopamine activity beyond the first 5 min of consumption, suggesting that low levels of ethanol intake do not adequately explain our previous data. Moreover, the results reported here suggest that the dopaminergic response does not desensitize, as relatively limited ethanol exposure (6 days of ethanol reinforcement) failed to produce sustained increases in extracellular dopamine levels.

The transience of the ethanol-induced dopamine response observed here is inconsistent with prior studies of operant ethanol self-administration (Weiss *et al*. 1993; Gonzales and Weiss 1998; Melendez *et al*. 2002), in which dopamine levels remained elevated above basal throughout a limited-access drinking period. This discrepancy cannot be attributed to differences in consumption, because mean intakes (g/kg) were comparable to, if not greater than, those reported previously. It is possible that intrinsic neurochemical differences between the rat strains used in these studies could account for some of these data. The present study employed male Long-Evans rats, whereas others have used alcohol-preferring male (Weiss *et al*. 1993) and female P rats (Melendez *et al*. 2002) and male Wistar rats (Weiss *et al*.

1993; Gonzales and Weiss 1998). Alcohol-preferring rat lines display lower accumbal dopamine levels (Murphy *et al*. 1987; Gongwer *et al*. 1989; Katner and Weiss 2001) and exhibit greater dopaminergic responsiveness to oral ethanol (Weiss *et al*. 1993; Katner and Weiss 2001) compared with non-preferring lines. Although critical neurochemical differences could exist between Wistar and Long Evans rats, it does not seem likely considering that both lines are genetically heterogeneous and are closely related in background. A notable aspect of this study that distinguishes it from others is the operational distinction between consumption and appetitive responding, which resulted in a clearly defined dopamine response during the initial phases of ethanol self-administration, providing a possible explanation for the inconsistencies between these studies. However, the motivational and neurochemical consequences of dispensing reinforcement in 'lump sum', compared with small amounts that are contingent upon further behavior (Weiss *et al*. 1993; Gonzales and Weiss 1998; Melendez *et al*. 2002), are not understood. Further work is necessary to determine whether differences exist between these types of response–outcome procedures and which approach best models human alcohol drinking.

The present study strongly supports the suggestion by Doyon *et al*. (2003) that the ethanol-induced dopamine response produced during operant self-administration may not be solely pharmacological in nature, as others have put forward (Weiss *et al*. 1993; Gonzales and Weiss 1998), but may instead be related to the stimulus properties of ethanol presentation. The occurrence of prominent elevations in accumbal ethanol concentrations well after the dopamine response had subsided indicates that any pharmacological effect of ethanol was transient at best. Regression analysis showed significant correlations between intake and dopamine response within the first 10 min of the drink period but not 15 min later during the post-drink period. The fact that dialysate ethanol levels were not related to the peak dopamine response further suggests that the dopaminergic activity was not entirely due to the pharmacological actions of ethanol. Importantly, the absence of a dopamine response in ethanol-conditioned rats self-administering 10% sucrose indicates that the observed dopamine response is dependent on the presence of ethanol, and is not merely an artifact related to operant responding or the expectation of ethanol reinforcement, for example. These results are consistent with a previous report by Katner *et al*. (1996), which showed no effect of ethanol expectation in heterogeneous Wistar rats. On the contrary, our hypothesis concerning a cue-induced dopamine increase is not fully supported by recent data from rats performing a second-order schedule of reinforcement for cocaine, in which a conditioned stimulus preceding cocaine presentation failed to evoke an increase in accumbal dopamine activity (Ito *et al*. 2000). However, several methodological differences could contribute to this apparent discrepancy. Ito *et al*. (2000) utilized a delay (up to 20 min) between the conditioned stimulus and the onset of reinforcement, whereas in the present study the stimulus cues of ethanol (i.e. taste and odor) coincided with the acquisition of the reinforcer. Therefore, the temporal contiguity between a conditioned stimulus and the onset of reinforcement may be an important factor for predicting cue-related dopamine responses measured with microdialysis. Alternatively, the stimulus strength of the visual stimulus used in the Ito *et al*. (2000) study may not be as strong as the sensory stimuli in the present study for eliciting an accumbal dopamine response.

The results of the current study indicate that some mechanism must be functioning to stimulate extracellular dopamine activity transiently during ethanol consumption. We propose that this mechanism could involve (i) an increase in the firing rate of VTA dopamine cells due to sensory-mediated excitatory drive or (ii) a very rapid acute functional tolerance to ethanol within the mesoaccumbens system. According to Grace (2000), the extracellular dopamine response to ethanol could be mediated by phasic increases in the firing rate of VTA dopamine cells. Burst-mediated release of dopamine is significantly higher in conscious animals compared with anesthetized ones (Freeman and Bunney 1987) and a variety of salient environmental stimuli evoke burst activity (Overton and Clark 1997; Horvitz 2000), indicating that these events are linked to sensory stimulation. Excitatory glutamatergic activity within the VTA, possibly conveyed by sensory input, is one source of this phasic dopaminergic activtiy (Murase *et al*. 1993; Zheng and Johnson 2002; Floresco *et al*. 2003). In the present study, ethanol drinking occurred predominantly within the first 10 min of access, corresponding to the period in which all but two rats showed peak dopamine responses. During this period, the stimulus properties of the ethanol solution (i.e. taste, smell) were maximal. Rats consuming sucrose for a comparable amount of time did not show such an enhancement of dopamine, suggesting that this effect was specific to ethanol and not common to all reinforcing stimuli. Therefore, the mismatch between the dopamine and ethanol time courses observed here could be due to transient sensory-mediated stimulation of the dopamine system that occurred with ethanol ingestion. This hypothesis is supported by the positive correlation between ethanol licks and the dopamine response during the initial drinking bout and by the absence of a dopamine response in sucrose-drinking rats previously conditioned for ethanol self-administration. The operational segregation of the consummatory phase of operant ethanol self-administration may have revealed or enhanced this effect by providing the ethanol stimulus in a bolus-like manner.

Alternatively, the brief dopamine response may be partially due to an extremely rapid tolerance to the acute effects of ethanol within the mesoaccumbens system. Therefore, ethanol concentrations reaching the brain during the ascending

phase of the ethanol time course could exert greater regulation over extracellular dopamine accumulation than could peak ethanol concentrations or falling phase concentrations. Functional tolerance has been widely demonstrated in both human (Hiltunen 1997a,b) and animal studies (Waller *et al*. 1983; Lewis and June 1990; Le and Kalant 1992; Erwin and Deitrich 1996; Ponomarev and Crabbe 2002). Doses of ethanol that are self-administered by rats can stimulate locomotor activity during the ascending phase of blood-ethanol concentrations, an effect that is absent during the descending phase (Lewis and June 1990). There is a lack of information, however, regarding neurochemical tolerance to acute administration of ethanol. Previous work demonstrates that the dopamine response to systemically administered ethanol does not desensitize or undergo tolerance in animals given the drug chronically (Rossetti *et al*. 1993). Yim *et al*. (2000) showed that intra-accumbal ethanol concentrations dissociate from the dopamine response about 45 min after i.p. injection. The pharmacokinetics of acute ethanol administration, however, are clearly different from those produced by the oral route. Therefore, a direct comparison of these routes of administration with the dissociation between their respective dopamine and ethanol time courses is not fully valid. Taken together, if acute functional tolerance contributed to the present results, this would be an extremely rapid instance of the phenomenon within the mesoaccumbens system.

All experimental groups displayed significant elevations in extracellular dopamine during the period in which they were transferred into the operant chamber from the home cage and during the 15-min wait period that preceded drinking. This pre-drinking increase in dopamine across the groups, however, does not appear to be related to expectation of ethanol or sucrose reinforcement *per se*, as the handling group that was not trained for operant reinforcement showed a similar response during the same periods. Our previous data demonstrating a non-specific effect of handling on dopamine activity corroborate these results (Doyon *et al*. 2003). We concluded that this phenomenon was due to the physical handling of the rats as they are placed into the operant chamber, a change of environment, or a combination of these factors. These conclusions are supported by studies showing that tactile stimulation can evoke increases in extracellular dopamine in a variety of terminal areas (Inglis and Moghaddam 1999; Adams and Moghaddam 2000). Our observations, however, are not in agreement with certain studies in which a rise in dopamine levels did not occur during the transfer of rats from one environment to another (Weiss *et al*. 1993; Damsma *et al*. 1992; Humby *et al*. 1996). The reason for these inconsistencies is unclear, but could be due to procedural differences between these studies.

For perspective, we should also note that a role for dopamine in ethanol reinforcement is not as widely accepted as dopamine's role in psychostimulant reinforcement, for example. Although there is a large and diverse body of work that supports a dopamine hypothesis of ethanol reinforcement (Weiss and Porrino 2002; Gonzales *et al*. 2004), there is also a substantial amount of negative data. Microinjection studies consistently show that disruption of dopamine transmission within the accumbens reduces responding for ethanol (Rassnick *et al*. 1992; Samson *et al*. 1993; Hodge *et al*. 1997; Czachowski *et al*. 2001). In terms of ethanol consumption, however, most studies show that blockade of dopamine receptors has little or no effect on ethanol intake (Samson *et al*. 1993; Silvestre *et al*. 1996; Czachowski *et al*. 2001). Furthermore, ablation of accumbal neurons does not disrupt ethanol consumption (Ikemoto *et al*. 1997) or operant responding for ethanol (Rassnick *et al*. 1993) in rats previously conditioned to ethanol. In addition, microdialysis studies do not show that ethanol strongly stimulates extracellular dopamine to the degree that psychostimulants do (Mocsary and Bradberry 1996; Nurmi *et al*. 1996; Bradberry 2002; Doyon *et al*. 2003). These studies, along with the results of the present one, indicate that the functional significance of accumbal dopamine activity in ethanol reinforcement remains complex and further work in this area is certainly needed.

In summary, our study clearly demonstrates the occurrence of a rapid dissociation between accumbal dopamine and ethanol time courses during consummatory periods of ethanol self-administration. Although the dopamine response observed during ethanol drinking correlated with the amount of ethanol consumed (g/kg or licks of ethanol), a pharmacological relationship between ethanol and dopamine is not fully supported here due to the transient nature of the effect. These results may be due to the stimulus-mediated properties of ethanol, which may evoke phasic increases in dopamine activity during consummatory phases of self-administration. We do not discount the possibility, however, that other factors contributed, such as a very rapid tolerance to the acute effects of ethanol within the mesoaccumbens system.

Acknowledgements

The authors thank Dr Patrick K. Randall at the Medical University of South Carolina for aid with the statistical analysis of the data and Judith Randall, Traci Fritz, Chi-Chun Wu, and Angela Bird for excellent technical assistance. We are also grateful to Dr Hank Samson for many discussions during the course of the study. This work was funded by grants from NIAAA (AA11852) and the Texas Commission on Alcohol and Drug Abuse (517-9-8444). WMD was supported by training grants from NIMH (MH018882) and NIAAA (AA14849-01), and an award from the Texas Research Society on Alcoholism.

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