



The role of dopamine in alcohol self-administration in humans: Individual differences

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KEYWORDS

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Abstract

Objective: To clarify dopamine's role in alcohol self-administration in a heterogeneous sample of drinkers using acute phenylalanine/tyrosine depletion (APTD). **Methods:** Sixteen men with variable drinking histories were characterized on their ethanol-induced cardiac response, a marker previously proposed to index dopamine system reactivity and vulnerability to alcohol abuse. During separate sessions participants were administered (i) a nutritionally balanced (BAL) amino acid (AA) mixture, (ii) a mixture lacking the dopamine precursors, phenylalanine and tyrosine, and (iii) APTD followed by the dopamine precursor, L-DOPA. Five hours after AA administration, participants could earn units of alcohol using a progressive ratio breakpoint task. **Results:** Alcohol self-administration was reduced in the APTD and APTD+L-DOPA conditions relative to the BAL condition. In both cases the changes were predicted by ethanol-induced cardiac change. **Conclusions:** The motivation to drink is likely regulated by more than one neurobiological mechanism. Individual differences in cardiac responsivity to ethanol might provide a peripheral marker of responsiveness to pharmacological manipulations of dopamine. © 2008 Elsevier B.V. and ECNP. All rights reserved.

1. Introduction

Alcohol administration increases mesolimbic dopamine (DA) neurotransmission (e.g., Samson et al., 1992), an action believed to affect the ability of a wide range of abused substances to be reinforcing and sustain interest (Di Chiara and Imperato, 1988; Berridge and Robinson, 1998). In rodents, the initiation of alcohol ingestion is time-locked to increases in DA overflow (Weiss et al., 1992), and DA antagonists attenuate intake (Rassnick et al., 1992; Files et al., 1998). In humans, functional neuroimaging studies suggest that alcohol ingestion

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increases striatal DA release (Boileau et al., 2003) while decreasing DA neurotransmission reduces alcohol self-administration in social drinkers (Leyton et al., 2000a; Enggasser and de Wit, 2001) and patients meeting criteria for alcohol dependence (Modell et al., 1993). Despite the consistency of these findings, DAergic reductions do not appear to decrease alcohol consumption in all subjects (Modell et al., 1993; Enggasser and de Wit, 2001). These and other observations have led to the proposition that multiple pathways to alcohol abuse likely exist, and that for each the relevant neurobiology may differ (e.g., Pihl and Peterson, 1995).

Specific subtypes of alcoholism have been proposed (Windle and Scheidt, 2004; Babor et al., 1992; Cloninger, 1987; Conrod et al., 2000). Genetic markers for DAergic, GABAergic and serotonergic pathways for alcoholism have been described (Noble et al., 1998; Young et al., 2004; Hinckers et al., 2006), and recent neuroanatomical and pharmacological challenge studies in alcohol dependent subjects suggest that DAergic abnormalities may be restricted to a sub-group (e.g., Tupala and Tiihonen, 2004, 2005). Clinically the efficacy of pharmacotherapies that directly or indirectly target DA neurotransmission also varies, and it has been proposed that this reflects, at least in part, the degree to which DA is involved in a given individual's symptom profile and vulnerability for relapse (Walter et al., 2001).

Individual differences in the DAergic response to alcohol have been proposed to be indexed by differences in the heart rate (HR) response during the ascending limb of the blood alcohol curve, and a high HR response has been suggested to represent a peripheral marker that identifies individuals with DA-specific alcohol reinforcement (Brunelle et al., 2004; Conrod et al., 2001). This hypothesis is supported by preliminary neuroimaging results suggesting that alcohol-induced cardiac effects are proportional to limbic DA release (Boileau et al., 2003); however, direct evidence linking alcohol's HR effects with DA-mediated motivational effects is currently lacking. In an effort to better clarify DA's role in human alcohol self-administration, the present study investigated the effect of decreasing DA neurotransmission on alcohol self-administration in a sample of non-dependent social drinkers who varied across a number of domains including current drinking patterns, individual and family drinking histories, and alcohol-induced cardiac responsivity.

DA neurotransmission was decreased using the acute phenylalanine/tyrosine depletion (APTD) method (Moja et al., 1996; Sheehan et al., 1996; McTavish et al., 1999a,b; Leyton et al., 2000b). In laboratory animals, APTD decreases stimulated DA release (McTavish et al., 1999a,b; Le Masurier et al., 2004a; Jaskiw and Bongiovanni, 2004) and cFos activation (Le Masurier et al., 2004b), as well as striatal tissue concentrations of DA (Biggio et al., 1976) and cerebrospinal fluid levels of the DA metabolite, homovanillic acid (Palmour et al., 1998). In humans, APTD decreases extracellular DA levels both at rest (Montgomery et al., 2003) and in response to drug administration (Leyton et al., 2004). APTD offers several advantages as a research tool. Its behavioural effects develop within 3 to 4 h (McTavish et al., 2001; Leyton et al., 2005), it appears to be devoid of many side effects often associated with other treatments that reduce DA transmission (Brogdén et al., 1981; McCann et al., 1990), and, with the possible exception of some trace amines, its neurochemical effects appear to be specific to the catecholamines (Palmour et al., 1998; McTavish et al.,

1999a,b). By acting pre-synaptically, APTD should decrease neurotransmission at all DA receptor subtypes. Recent studies suggest that APTD decreases cocaine- and cocaine cue-induced craving (Leyton et al., 2005), and the salience of reward-related cues and the ability to respond to them preferentially (McLean et al., 2004; Rosier et al., 2005; Scarna et al., 2005; Leyton et al., 2007). In the present study, we predicted that APTD would decrease alcohol self-administration behaviour in at least some drinkers. In addition, we sought to determine whether any APTD-related effects could be prevented through use of the immediate DA precursor, L-DOPA.

2. Experimental procedures

2.1. Participants

Male participants between the ages of 19 and 30 were recruited from the community through advertisements in newspapers and university websites. An initial telephone interview excluded those reporting medical or psychiatric illness, a history of adverse consequences from alcohol consumption, current substance abuse or dependence, a lack of familiarity with the alcohol doses to be administered, or insufficient knowledge about the history of alcohol-related problems in their biological relatives. Potential participants were told that the study would involve three full days of testing, plus a half-day for screening and an alcohol challenge day for cardiac responsivity testing, and that they would be required to remain abstinent from all prescription and illicit drugs for the duration of the study.

Twenty-nine individuals meeting these criteria were invited to complete the full screening. All were assessed by a routine medical exam with standard blood work and all were evaluated during a semi-structured clinical interview using DSM-IV criteria (First et al., 1995). Participants also completed the individual and parental form of the Michigan Alcoholism Screening Test (MAST, Pokorny et al., 1972 and FMAST, MMAST, Crews and Sher, 1992), and provided further details about the alcohol use histories of all first- and second-degree relatives using the Family History Research Diagnostic Criteria (Andreassen et al., 1977). Following this screening 18 individuals were deemed eligible to complete the study. Those invited to participate were medically healthy, free of current and past mood, anxiety and psychotic disorders as well as current substance use disorders and were able to provide information about the presence or absence of current or past alcohol abuse or dependence in each of their first- and second-degree relatives. The study was carried out in accordance with the Declaration of Helsinki, and was approved by the McGill University Health Centre's Research Ethics Board. All subjects provided written, informed consent.

2.2. Amino acid administration

Participants ingested AA mixtures on three test sessions, each conducted a minimum of three days apart, double-blind and in counterbalanced randomized order. On these sessions, subjects arrived at 8:30 am and, following baseline assessments, received the APTD mixture, APTD followed by L-DOPA/Carbidopa (Sinemet, 100 mg/25 mg, administered p.o. at 1 and 3 h after AA ingestion), or a nutritionally balanced mixture (BAL). On the APTD and BAL test days participants ingested identical looking placebo pills in lieu of L-DOPA/Carbidopa. The APTD mixture's composition, preparation and administration were based on a 102.3 gm balanced mixture with phenylalanine and tyrosine withheld (Young et al., 1985; Leyton et al., 2000b).

On the day prior to testing, participants were fed a low protein diet supplied by the investigators. They were also asked to refrain from drinking any alcohol on this day, to restrict themselves to three cups of caffeinated beverages, and to fast from midnight. On the morning of each test day participants provided a urine sample to ensure that they were drug-free (Triage™ Panel for Drugs of Abuse,

sensitive to cocaine, amphetamines, barbiturates, benzodiazepines, Δ^9 -tetrahydrocannabinol, opiates, and phencyclidine. Biosite Diagnostics ©, San Diego, CA, USA) and a breath alcohol sample using an alco-sensor III intoximeter (Thomas Security, Montreal, Canada) to ensure alcohol abstinence.

2.3. Plasma amino acid measurements

Plasma samples were collected at morning baseline as well as 4.5 h post-AA ingestion. Plasma phenylalanine and tyrosine concentrations were determined by HPLC with pre-column derivatization and fluorometric detection. Plasma samples were missing from 8 of the 48 test days.

2.4. Alcoholic beverages

Prior to the study, each participant selected an alcoholic beverage to consume on each AA test day. The beverage could consist of any 80-proof liquor (including preferred brand) and a non-alcoholic mixer, and the same beverage was to be consumed on all three test days. Choice of alcoholic beverage was restricted to 80-proof liquors due to the high variability in the alcohol contents of commercially available brands of beer, wines and coolers. Non-alcoholic mixers were restricted to those without caffeine or aspartame (which contains phenylalanine). Participants were informed that on each test day they would be required to consume a minimum of the equivalent of one standard drink containing 12 g of alcohol, and that the maximum dose of alcohol that could be consumed on any day was 72 g, the equivalent of six standard drinks.

2.5. Alcohol exposure and administration

The alcohol administration phase of the study was scheduled to begin 5 h after the AA ingestion to coincide with the time frame of pharmacological and behavioural effects observed in previous APTD studies (e.g., Leyton et al., 2000a, 2004, 2005). Fifteen minutes prior to each alcohol administration session participants were comfortably seated in a chair in front of a computer on a large table and were presented with a glass containing 100 ml of water. Participants were instructed to handle the glass, look at it, and smell the drink but not to consume any of it. After 3 min the subject completed a subjective assessment. Approximately 7.5 min prior to alcohol administration participants were presented with their preferred alcoholic beverage (12 g of alcohol plus 100 ml of mix). Again they were instructed to handle the glass and both look at and smell the beverage but not to consume any of it. After 3 min had elapsed they completed another subjective state assessment.

Following the cue exposure, participants were instructed to consume the previously presented alcoholic beverage within 10 min. This initial drink was included to measure responses to a fixed dose of alcohol, to normalize drinking in the laboratory, to model the influence of an initial drink on subsequent drinking behaviour in the self-administration paradigm, and to enable comparisons with other studies using a similar self-administration paradigm (Petrakis et al., 2002; Barrett et al., 2006).

2.6. Alcohol and water self-administration

Following the consumption of the first alcoholic drink, participants had the opportunity to earn up to 10 mixed alcoholic drinks, each containing 6 g of alcohol and 50 ml of mix and/or 10 100 ml drinks of water using a progressive ratio (PR) task. To earn each alcoholic beverage participants were required to repeatedly press the letters 'd' and 'r' a predetermined number of times, while water was similarly earned using the letters 'w' and 'a'. For each type of drink, the first earned beverage required 40 button presses and the number of button presses required to earn each subsequent drink of the kind increased

one-and-one-half times (i.e., 60, 90, 135, 203, 304, 456, 684, 1,026, and 1,538 clicks). Each type of drink required a total of 4536 button presses to reach the maximum amount allowed. Each session lasted until the maximum number of alcohol or water drinks were earned or to a maximum of 2 h, whichever came first. Participants were not required to earn any drinks during the sessions, but were required to remain seated in the testing room until each session was completed. Upon completion of the self-administration task, participants were brought a meal and remained in the laboratory until their blood alcohol concentration reached 0.04. They were then safely escorted home by one of the researchers or by taxi.

2.7. Subjective state

Participants were administered visual analogue scales (VAS) immediately prior to ingesting the AA mixture, following water exposure, following alcohol exposure and then following every 12 g of alcohol. Items were rated on a 10 cm line labelled with the integers 1–10 and anchored with the words "least" and "most". Items included in the VAS were 'high', 'euphoric', 'sedated', 'intoxicated', 'rush', 'excited', 'anxious', 'energetic', 'mind racing', 'alert', 'like drink', 'want drink', 'urge for drink', 'desire drink', and 'crave drink'. In addition, the final 13 participants who completed the protocol verbally rated their level of nausea immediately prior to receiving their priming dose of alcohol on each test day using a 10-point scale (1 = not at all, to 10 = extreme).

2.8. Alcohol-induced cardiac reactivity

Participants' cardiac reactivity to alcohol ingestion was measured on a 4th day in a separate testing facility. Participants arrived at the laboratory in the morning, having fasted a minimum of 4 h and abstaining from alcohol for a minimum of 24 h. A ten-minute sober baseline HR measurement was taken using the Polar S810 monitor (Polar electro, Finland). Participants then consumed 0.75 g of pure ethanol per kg of body weight mixed with 5 parts orange juice separated into two glasses. The time interval for the ingestion of each glass was 7.5 min. Following a 15-minute wait, HR was again measured for 10 min (between 30 and 40 min post-onset of alcohol ingestion). During each HR measurement, the first 5 min were not used in the calculation of the average HR as they served to control for adjustment to the procedure. Participants were explicitly told to remain still, and compliance to this instruction was verified through observation via a camera placed in the test room. Ethanol-induced cardiac change was calculated by subtracting the baseline HR value from the intoxicated HR value and then dividing the difference by baseline HR, thereby producing a percent change value. This HR measurement procedure has been demonstrated to provide a reliable and valid index of ethanol-induced cardiac change in male social drinkers (Conrod et al., 2001; Brunelle et al., 2004). In 11 cases the HR assessment was scheduled prior to commencement of the amino acid test sessions, while in the remaining 5 cases it was scheduled following the completion of the amino acid test sessions. Timing of HR assessment was not systematically associated with alcohol-induced cardiac response or any other participant characteristic.

2.9. Statistical analyses

All data were analyzed using SPSS, version 12.0. The primary outcome variables in this study were the number of button presses during the PR task to earn alcohol and water in each AA session. Because the behavioural PR data increase geometrically, they were screened for normality using the Kolmogorov–Smirnov method, and it was determined that logarithmic transformations were necessary for each variable to satisfy the normality assumption. The data were then analyzed using a 3×2 repeated-measures ANOVAs using AA condition (BAL, APTD, & APTD+L-DOPA) and beverage type (water & alcohol) as within subjects factors. Stepwise linear regressions were

used to examine how changes in alcohol self-administration in the APTD and APTD+L-DOPA conditions relative to the BAL condition were related to various alcohol-related variables. Correlations among the alcohol-related variables were evaluated with Pearson's correlation coefficient. Family-wise Bonferroni corrections were applied when related analyses were conducted on several variables. Analyses of the VAS data were carried out using mixed modeling. Using a first order autoregressive covariance structure, AA Mixture (BAL, APTD, APTD+L-DOPA), and Time (post-water cue, post-alcohol cue, post-alcohol consumption) were entered as fixed and repeated factors, while morning baseline scores were entered as a time-varying covariate.

3. Results

3.1. Participants

Sixteen participants (age: 21.8±3.3 years old) completed the protocol, while two others discontinued during their first AA test day. One withdrew due to experiencing nausea following the ingestion of the AA mixture (APTD+L-DOPA) while the second participant withdrew after completing the BAL session citing concerns about the study's dietary restrictions. Table 1 presents the alcohol-related data for participants who completed the study. Although none met DSM-IV criteria for current alcohol abuse or dependence, individual and family drinking histories varied considerably.

3.2. Amino acid depletion

APTD lowered plasma concentrations of phenylalanine and tyrosine, as reflected by significant AA Mixture×Time interac-

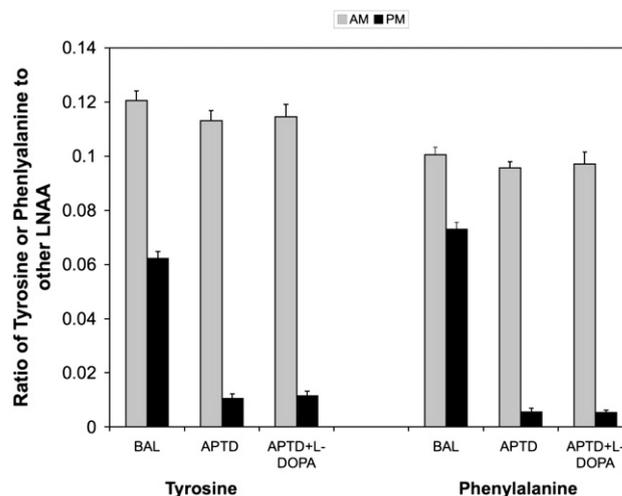


Figure 1 Mean (+SEM) ratios of plasma tyrosine and phenylalanine to other large neutral amino acids (LNAA) at morning baseline and 4.5 h following AA ingestion each AA test condition. All three AA mixtures significantly decreased tyrosine/LNAA and phenylalanine/LNAA ratios ($p < 0.001$). However, the magnitude of these effects were significantly more pronounced in the APTD and APTD+L-DOPA conditions relative to the BAL condition ($p < 0.001$).

tions: tyrosine, $F(2, 18) = 331.66, p \leq 0.0001$; phenylalanine, $F(2, 18) = 556.14, p \leq 0.0001$. Relative to the morning baseline the BAL mixture increased plasma phenylalanine by 237% and tyrosine by 166%. APTD decreased phenylalanine to 16% and tyrosine to 27% of morning levels. The administration of L-DOPA did not affect the degree of AA depletion produced by APTD.

Ingestion of all three mixtures also significantly decreased the ratios of plasma tyrosine and phenylalanine to other large neutral amino acids (LNAA; tryptophan, tyrosine, phenylalanine, leucine, isoleucine, valine) ($p < 0.001$). However, Mixture×Time interactions revealed that these reductions were significantly more pronounced in the APTD and APTD+L-DOPA conditions relative to the BAL condition: tyrosine, $F(2, 18) = 62.62, p \leq 0.0001$; pheny-

Table 1 Individual and family drinking characteristics

Subject	Weekly alcohol drinks – current ^a	Weekly alcohol drinks – peak ^b	MAST	Alcohol dependent relatives ^c	Alcohol-induced cardiac change (%)
1	6	18	0	0	+2.3
2	7	7	0	0	+16.67
3	7	7	0	0	+15.80
4	2	8	0	0	+26.92
5	20	20	0	3	+22.92
6	3	24	0	3.5	-6.00
7	13	30	0	0	+11.48
8	8	18	0	0	+1.61
9	13	30	0	3	+4.48
10	14	28	0	2.5	+6.90
11	18	48	5	3	+8.16
12	16	30	4	2	+5.36
13	2	24	2	0	+14.00
14	17	18	0	0	+18.03
15	9	24	2	3	+11.86
16	9	26	0	0	+25.45

^a Reflects average weekly number of alcoholic beverages consumed over the preceding 30 days.

^b Reflects average weekly number of alcoholic beverages consumed during the month of heaviest use.

^c Number of relatives with a lifetime diagnosis of alcohol dependence. First-degree relatives received a weight of 1 and second-degree relatives were weighted 0.5.

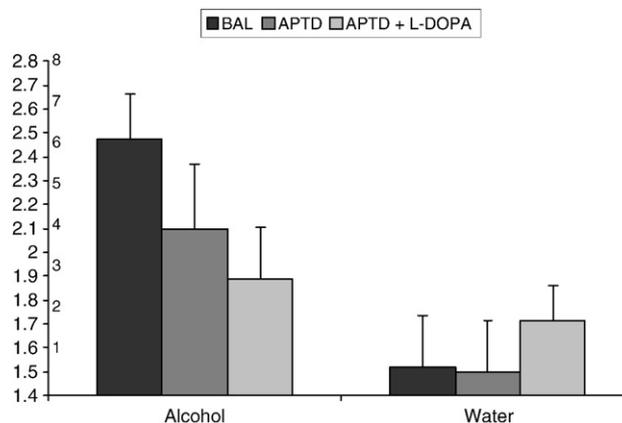


Figure 2 Log transformed progressive ratio (PR) values (outer value on y-axis) and number of alcohol and water units (inner value on y-axis) earned across AA conditions. Alcohol self-administration was significantly reduced in both the APTD and the APTD+L-DOPA conditions relative to BAL. Water self-administration did not significantly differ among the conditions.

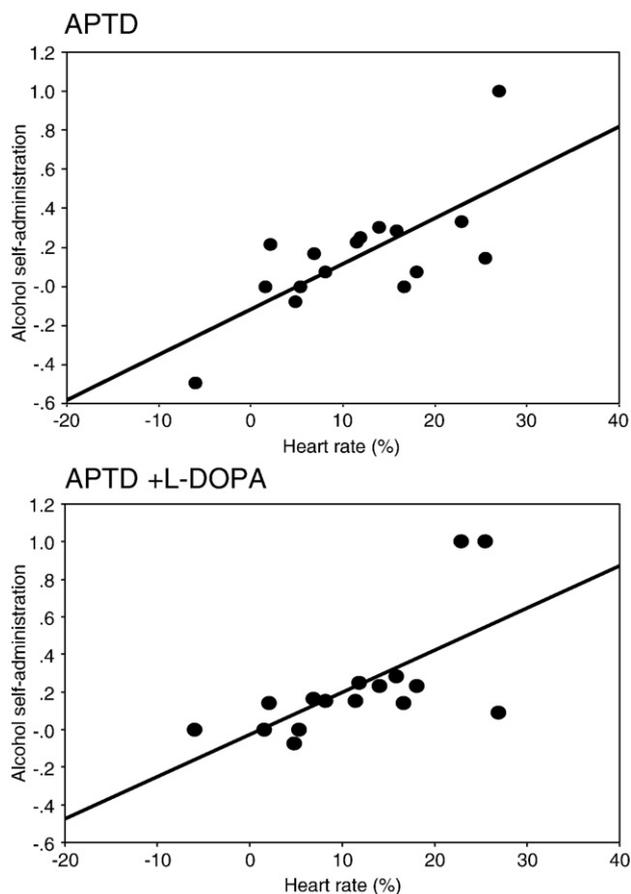


Figure 3 The association between alcohol-induced changes in heart rate and the effect of APTD and APTD+L-DOPA on alcohol self-administration. The graphs plot the percent change in progressive ratio values (log transformed) vs. the percent change in heart rate following acute alcohol challenge. The ethanol-induced increase in heart rate was the sole statistically significant predictor of the effects of APTD and APTD+L-DOPA on alcohol self-administration ($p \leq 0.006$).

alanine, $F(2, 18)=142.81$, $p \leq 0.0001$ (Fig. 1). In contrast no differences were evident among the conditions in the tyroptophan/LNAA ratios; $F(2, 18)=0.15$, $p=0.859$.

3.3. Nausea

Analyses revealed no significant differences among the AA conditions in reported levels of nausea ($F(2, 24)=1.34$, $p=0.281$).

3.4. Alcohol and water self-administration progressive ratio breakpoints

Analyses revealed main effects of Beverage type $F(1, 15)=4.56$, $p \leq 0.05$, as well as a significant AA Condition \times Beverage type interaction $F(2,30)=3.52$, $p \leq 0.04$ (Fig. 2). Because there appeared to be systematic differences in the self-administration of alcohol and water, additional analyses were performed considering each beverage type separately. There were no significant differences for water self-administration among the AA Conditions ($F(2, 30)=0.82$, $p \geq 0.449$). There was however a significant main effect of AA Condition for alcohol self-administration ($F(2, 30)=4.75$, $p \leq 0.016$), reflecting greater alcohol intake on the BAL test session relative to the APTD ($p \leq 0.03$) and APTD+L-DOPA ($p \leq 0.01$) conditions. Beverage administration on the APTD and APTD+L-DOPA sessions did not differ significantly ($p \geq 0.35$).

To determine whether the observed changes in alcohol self-administration were accounted for by various alcohol-related variables, stepwise linear regressions were performed using current and peak number of weekly alcoholic beverages consumed, number of first- and second-degree relatives with alcohol dependence, MAST score, and alcohol-induced HR change scores as potential predictor variables of the PR values (log transformed) from the APTD test sessions vs. the BAL session. For both APTD ($r=0.713$, $p \leq 0.002$) and APTD+L-DOPA ($r=0.651$, $p \leq 0.006$) induced changes in alcohol self-administration, the sole statistically significant predictor was ethanol-induced HR change (Fig. 3). In order to verify specificity of these relationships as well to determine the associations among the alcohol-related variables a series of Pearson's bi-variate correlations were performed.

Results from the correlational analyses are presented in Table 2. The only statistically significant predictor of the APTD and APTD+L-DOPA induced changes in alcohol self-administration was the alcohol-induced HR change, and this association survived family-wise Bonferroni corrections ($p \leq 0.007$). In comparison, no other variables were associated with change in drinking or with alcohol-induced cardiac change ($p \geq 0.09$). A significant positive correlation between peak number of weekly drinks and MAST score was evident, but no other associations exceeded the corrected threshold for statistical significance ($p \geq 0.007$).

3.5. Subjective effects

Due to considerable variability in the rate and volume of alcohol ingestion following the administration of the initial mandatory alcoholic drink, only VAS data associated with the cue presenta-

Table 2 Correlations among alcohol-related variables

	Weekly drinks –peak	MAST	Alcoholic relatives	Alcohol cardiac change	Change in alcohol earned (APTD)	Change in alcohol earned (APTD+L-DOPA)
Weekly drinks –current	0.52*	0.30	0.41	0.06	-0.16	0.26
Weekly drinks –peak		0.65**	0.56*	-0.36	-0.35	-0.05
MAST			0.36	-0.17	-0.08	-0.18
Alcoholic Relatives				-0.40	-0.40	-0.04
Alcohol cardiac change					0.71**	0.65**
Change in alcohol (APTD)						0.22

* $p \leq 0.05$, ** $p \leq 0.007$.

tions and priming drinks of each AA condition were analysed. None of the subjective variables were found to be significantly associated with AA mixture and there were no AA Mixture \times Time interactions. In comparison, there were significant main effects of Time for 7 VAS items, euphoria ($F(1, 89.6)=3.16; p \leq 0.05$), intoxicated ($F(1, 89.1)=5.27; p \leq 0.01$), like drink ($F(1, 60.8)=9.78; p \leq 0.001$), want drink ($F(1, 111.5)=15.9; p \leq 0.001$), urge for drink ($F(1, 108.4)=6.87; p \leq 0.01$), desire drink ($F(1, 110.3)=7.86; p \leq 0.001$) and crave drink ($F(1, 106.3)=3.51; p \leq 0.05$). Post hoc analyses revealed that only two of these (increased euphoria, increased intoxication; $p \leq 0.001$) were associated with alcohol ingestion per se, while the remaining increases resulted from alcohol cue presentation ($p \leq 0.01$).

4. Discussion

In previous studies, decreasing DA neurotransmission decreased alcohol intake in male (Enggasser and de Wit, 2001) and female social drinkers (Leyton et al., 2000a) and in those meeting criteria for alcohol dependence (Modell et al., 1993). The present study extends these observations in two ways. First, to our knowledge, this is the first report that decreasing DA transmission also decreases alcohol progressive ratio breakpoints in humans, a change that is thought to provide an objective behavioural measure of the motivation to obtain reward. Second, this is also, to our knowledge, the first study to identify a physiological marker that predicts the ability of a DA manipulation to alter drinking behaviour. The latter finding raises the possibility that there are individual differences in the mechanisms that regulate motivation to drink; cardiac responses to ethanol might identify patients who would benefit from treatments aimed at distinct targets.

APTD did not alter the subjective effects associated with the presentation of alcohol or with the consumption of a single dose. This observation does not support the proposition that DA mediates the hedonic responses associated with alcohol reward (see also Leyton, in press). Although drug-induced increases in DA release have been reported to correlate with both euphoric (Laruelle et al., 1995; Volkow et al., 1994; Drevets et al., 2001; Martinez et al., 2003; Abi-Dargham et al., 2003; Barrett et al., 2004; Oswald et al., 2005) and craving responses to abused substances (Leyton et al., 2002; Oswald et al., 2005), decreasing DA transmission has not consistently diminished the mood-elevating effects of alcohol (Leyton et al., 2000a; Enggasser and de Wit, 2001), tobacco (Casey et al., 2006), amphetamine (Brauer and de Wit, 1996, 1997; Leyton et al., 2007) or cocaine (Gawin, 1986; Sherer, 1988; Evans et al., 2001; Romach et al., 1999; Leyton et al., 2005). In comparison, decreasing DA transmission has been found to decrease cocaine- and cocaine cue-induced craving (Berger et al., 1996; Leyton et al., 2005), and the ability to identify and preferentially respond to reward-paired cues (Leyton et al., 2007, see also McLean et al., 2004; Rosier et al., 2005; Scarna et al., 2005). Though DA may signal the availability of pleasurable events (e.g., Schultz, 1998), increasing the salience of the cues and facilitating their ability to sustain interest (Stewart et al., 1984; Stewart, 1992; Blackburn et al., 1992; Berridge and Robinson, 1998), and perhaps altering the evaluative appraisals that influence mood (Murphy et al., 1971; Fibiger, 1995; Willner, 1995), accumulating evidence suggests that it does not mediate the pleasure, *per se* (Berridge

and Robinson, 1998; Wise, 2004; Leyton, in press). Instead, it has been proposed, reward-related hedonic effects might be more closely related to changes in GABAergic (Reynolds and Berridge, 2002) and endogenous opioid transmission (Pecina and Berridge, 2000, see also Berridge and Robinson, 2003; Burgdorf and Panksepp, 2006), both of which are potentially engaged by ethanol (e.g., Dai et al., 2002; Koob, 2004).

The present results should be interpreted in light of the following considerations. First, the inability of L-DOPA to reverse the effect of APTD on progressive ratio breakpoints was unexpected. Since studies in rodents indicate that acute L-DOPA administration enhances tonic DA neurotransmission (Butcher and Engel, 1969; Freed and Murphy, 1978; Eldrup et al., 1995) but potentially disrupts phasic DA cell firing under conditions where DA function has been compromised (Harden and Grace, 1995; Robinson et al., 2004), the present study raises the possibility that L-DOPA disrupts behaviours that require phasic DA cell bursts (Grace, 2000). The sustained focus to obtain reward in a relatively novel task might benefit from both phasic and tonic DA release (O'Donnell, 2003; Volkow et al., 2004). Alcohol's ability to increase DA transmission requires impulse-dependent mechanisms (Brodie et al., 1999). Second, APTD might decrease norepinephrine (NE) synthesis (Palmour et al., 1998), and it is possible that the observed effects on alcohol administration in part reflect changes to NE neurotransmission. However, accumulating evidence suggests that, despite apparent changes in NE metabolism, APTD does not decrease NE release (Sheehan et al., 1996; McTavish et al., 1999a,c; Le Masurier et al., 2004b). Third, the present protocol only tested men and it is possible that the findings may not extend to women. In a previous study, APTD was shown to reduce alcohol self-administration in female social drinkers (Leyton et al., 2000a), though the degree to which this is associated with cardiac response to ethanol remains unknown. Fourth, despite considerable heterogeneity in participants' individual and family drinking histories, the present protocol excluded individuals with current substance (including alcohol) abuse or dependence, and the extent to which the present findings extend to clinical populations needs to be determined. Fifth, because the protocol imposed limits on the amount of alcohol consumed as well as on the length of the drinking session it is possible that ceiling limits may have affected the magnitude of the observed effects. Two of the six participants that did not decrease alcohol self-administration following APTD earned the maximum number of alcohol beverages in each condition and it is possible that differences may have emerged had their sessions been able to continue. Finally, although the sample size in this study was modest ($n=16$), it was within the norms for investigations assessing within subject drug effects in humans; small sample size is typically associated with increased incidents of type II but not type I error. Nevertheless, since we wished to examine APTD effects in a heterogeneous sample of drinkers it is likely that further research will be required to evaluate the role of DA in additional sub-groups.

In conclusion, DA precursor depletion decreased alcohol self-administration in a subset of non-dependent male social drinkers, and this was predicted by cardiac responses to acute alcohol administration. These individual differences might have relevance for determining the most effective treatment for sub-groups of alcohol abusing populations.

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Funding for this study was provided by the Canadian Institutes of Health Research (CIHR); the CIHR had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Sean Barrett was a graduate student who worked on the project. He recruited and tested subjects in the dopamine depletion component of the study, and wrote the first draft of the manuscript. Caroline Brunelle was a graduate student who worked on the project. She recruited subjects, and tested them for ethanol-induced cardiac reactivity. Chawki Benkelfat contributed to the research design, and was the medical investigator for the project. Simon Young contributed to the research design, and oversaw the measurement of plasma amino acid levels. Bob Pihl contributed to the research design, supervised the ethanol-induced cardiac reactivity component, and, with Marco Leyton sought and obtained the project's funding. Marco Leyton was the project's principal investigator. He conceived of the study, designed it, wrote the protocol, oversaw all aspects of the study, and, with Sean Barrett wrote the paper. All authors contributed to and have approved the final manuscript.

Conflict of interest

No conflict declared.

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