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Beer self-administration provokes lateralized nucleus accumbens dopamine release in male heavy drinkers

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Abstract

Rationale—Although striatal dopamine (DA) is important in alcohol abuse, the nature of DA release during actual alcohol drinking is unclear, since drinking includes self-administration of both conditioned flavor stimuli (CS) of the alcoholic beverage and subsequent intoxication, the unconditioned stimulus (US).

Objectives—Here we used a novel self-administration analog to distinguish nucleus accumbens (NAcc) DA responses specific to the CS and US.

Methods—Right-handed male heavy drinkers ($n=26$) received 3 positron emission tomography (PET) scans with the D₂/D₃ radioligand [¹¹C]raclopride (RAC), and performed a pseudo self-administration task that separately administered a flavor CS of either a habitually consumed beer or the appetitive control Gatorade[®], concomitant with the US of ethanol intoxication (0.06 g/dL IV administration) or IV saline. Scan conditions were Gatorade flavor + saline (Gat&Sal); Gatorade flavor + ethanol (Gat&Eth); and beer flavor + ethanol (Beer&Eth).

Results—Ethanol (US) reduced RAC binding (inferring DA release) in the left (L) NAcc [Gat&Sal > Gat&Eth]. Beer flavor (CS) increased DA in the right (R) NAcc [Gat&Eth > Beer&Eth]. The combination of beer flavor and ethanol (CS + US), [Gat&Sal > Beer&Eth], induced DA release in bilateral NAcc. Self-reported intoxication during scanning correlated with L NAcc DA release. Relative to saline, infusion of ethanol increased alcoholic drink wanting.

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Conclusions—Our findings suggest lateralized DA function in the NAcc, with L NAcc DA release most reflecting intoxication, R NAcc DA release most reflecting the flavor CS, and the conjoint CS+US producing a bilateral NAcc response.

Keywords

ventral striatum; mesolimbic; alcoholism; conditioned response; cue reactivity; reward; operant; alcohol abuse; instrumental conditioning; alcohol seeking

Introduction

Mesolimbic dopamine (DA) in the NAcc (within the ventral striatum; VST) has long been postulated to be central to reward (Robinson and Berridge, 1993, 2008; Schultz *et al*, 1997; Weiss *et al*, 1993). In animals, ethanol provokes NAcc DA release, (e.g. Di Chiara and Imperato, 1988), although NAcc DA transmission is also provoked by alcohol's conditioned stimuli (CS), such as its olfactory and/or gustatory properties (Doyon *et al*, 2005). Drug-paired CS can exert powerful control over behavior, such as eliciting drug-seeking (for review see Crombag *et al*, 2008) or craving (e.g. Grant *et al*, 1996; Tiffany, 1999). We recently reported the first study on the effects of alcohol-related flavor CS on DA in humans. Using positron emission tomography (PET) with the DA D₂/D₃ ligand [¹¹C]raclopride (RAC) we demonstrated that the flavor of a preferred beer (absent a measurable breath alcohol concentration) is sufficient to induce DA release in the right NAcc (Oberlin *et al*, 2013). Alcohol intoxication itself (the unconditioned stimulus; US) has been more widely studied with RAC-PET. In one study, intravenous (IV) alcohol infusion (devoid of alcohol's usual aroma, flavor, and intra-oral sensations) induced bilateral (Ramchandani *et al*, 2011) DA release, although only in subjects carrying the minor allele of the OPRM1 mu-opioid receptor. Other studies found no effect with IV alcohol (Yoder *et al*, 2007; Yoder *et al*, 2005), or found it only when alcohol was unexpected (Yoder *et al*, 2009). However, two RAC-PET studies reported bilateral VST DA release when social drinkers consumed alcoholic beverages orally, thus combining alcohol's intraoral somatosensory CS effects with the US of intoxication (Boileau *et al*, 2003; Urban *et al*, 2010); a similar study of oral consumption reported bilateral VST DA release, but only in high-risk subjects (Setiawan *et al*, 2014).

To more clearly ascribe DA responses to the elements of flavor and other intra-oral sensory properties (the CS) versus intoxication effects (the US), we separated alcohol's pharmacologic actions (administered intravenously) from its normally conditioned sensory properties (delivered orally). We did so using a paradigm that preserves some instrumental aspect of normal human drinking (Sanchis-Segura and Spanagel, 2006 for review). Operant behavior may be crucial to VST's DA signaling (Bjork and Hommer, 2007), as self-administration and approach behavior in animals increases NAcc DA (Phillips *et al*, 2003; Weiss *et al*, 1993). To that end, subjects in this study had PET scans using a DA D₂/D₃ ligand while self-administering flavors of beer or an appetitive flavor control, Gatorade[®], during intravenous infusion of either alcohol or saline (which subjects believed was also self-administered). By manipulating flavor and intoxication independently, we assessed: 1)

the US of alcohol intoxication; 2) the CS of beer flavor; and 3) the combined effect of beer flavor and intoxication (CS plus US) as in naturalistic drinking.

Compared to our previous findings (Oberlin *et al*, 2013) in which the CS (beer flavor) was administered during “extinction” (i.e., without ensuing intoxication), we hypothesized that the added context of ethanol self-administration would increase the NAcc DA response.

Methods

Subjects

All procedures were approved by the Indiana University Institutional Review Board, and all subjects signed informed consents prior to study procedures. The 28 subjects recruited from the community were right-handed, male beer drinkers in good self-reported physical and mental health who reported beer as being one of their two most-often consumed alcoholic beverages. Two subjects were excluded from analyses; one for equipment failure during the study, and the other for excessive motion. Exclusionary criteria included: positive urine screen for illicit drugs, reported current illicit drug use or past treatment for substance dependence, current treatment-seeking for substance use, self-reported dislike of Gatorade, or self-report of any symptoms, diagnoses, or treatment consistent with a current or past major DSM-IV Axis I psychiatric disorder (aside from alcohol dependence), any history of neurological disease of cerebral origin, head injury resulting in loss of consciousness for > 20 minutes, current use of psychotropic medications, Beck Depression Inventory scores of > 9, or past/current problems in circulatory, endocrine, gastrointestinal, urinary, musculoskeletal, or blood-related disorders. Subjects underwent in-person interviews that included the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA: Bucholz *et al*, 1994), the Timeline Followback (90 day TLFB: Sobell *et al*, 1986) to quantify recent drinking, and the Alcohol Use Disorder Identification Test (AUDIT: Saunders *et al*, 1993). The family history module of the SSAGA was also used to determine familial alcoholism, and to avoid possible fetal alcohol effects by excluding subjects whose mothers had probable alcoholism. Recent drinking ranged from social to heavy (Table 1). Two of the 26 subjects met DSM-IV criteria for alcohol dependence as assessed by the SSAGA; however, these subjects did not differ from the rest of the group in drinks per drinking day (7.2 ± 2.8 and 7.2 ± 2.5 , respectively, mean \pm SD). Four subjects reported weekly marijuana use within the past year, although no use within the past two months (recent abstinence was verified by urine tests); all other subjects' past marijuana use was lower. Regular habitual cigarette smoking was exclusionary, although two subjects reported smoking up to 1 cigarette or cigar per week. None of these subjects participated in our previous RAC-PET gustatory study (Oberlin *et al*, 2013).

Procedure

Subjects were told that they would be responding for flavor (beer or Gatorade) sprays and IV infusion (ethanol or saline) by pressing a mouse button, and that the flavor/infusion combination might vary across scans. Subjects underwent three RAC PET scans on two consecutive days (with an overnight stay at the Clinical Research Center): one scan on the first day and two scans at least two hours (6 half-lives) apart on the second day, with all

three scans occurring at fixed times to minimize circadian variability (day 1/scan 1, 1:00pm; day 2/scan 2, 1:00pm; day 2/scan 3, 3:00pm). Scans involving ethanol infusion were always first and last, and were counterbalanced in order for presentation of beer and Gatorade flavor (beer first, $n=12$). Two flavors of Gatorade tastant (blue Glacier Freeze™ and Orange) were randomized across subjects. Subjects completed three scans while presented with Gatorade and saline (Gat&Sal), Gatorade and ethanol (Gat&Eth), and beer and ethanol (Beer&Eth), as illustrated in Figure 1B, with the two flavors of Gatorade employed so as to avoid one flavor becoming associated with ethanol intoxication. The beer used was each individual subject's most frequently consumed brand.

The study day timeline is outlined in Figure 1A. On the morning of the study day 1, subjects received breakfast, were re-screened for illicit drugs with a urine test, and were tested for ethanol intoxication with a breath alcohol concentration (BrAC) meter. An IV catheter was placed in an antecubital fossa vein for RAC injection and IV infusions. Approximately 30 min prior to imaging, subjects tasted 15 ml of each of the three tastants to ensure flavor recognition. Immediately preceding PET, subjects were instructed to, "Imagine you are drinking in the normal way, by taking sips of your favorite drink. Each mouse button press is the equivalent of taking a sip." Subjects were instructed that both flavor sprays and infusion would be contingent on their response, and that their drinking progress would be shown by the three emptying glasses on the screen (Figure 1A). They were also told that ethanol infusions would be roughly equivalent to three standard drinks. Only the flavor sprays were truly contingent on subject responding, as the ethanol infusion was fixed as described below. The discrete stimulus of self-administered flavor delivery accompanied the diffuse stimulus of ascending BrAC to simulate real-world drinking stimuli. Importantly, subjects were led to believe that the alcohol was being delivered in a manner that reflected the emptying of the glasses.

While supine in the PET scanner, but before imaging, subjects responded to a "press to sip" prompt on the screen for two separate water sprays. This habituated subjects to the procedure and stimulus delivery. Subjects then rated the water to assure that they understood the subjective rating scales, and to provide a behavioral baseline. Self-administration of beer or Gatorade (delivered through a water-chilled jacket) and simultaneous infusion of ethanol or saline began precisely two minutes after RAC administration. Cartoon images of drink glasses preceded the "press to sip" and the button-press prompt. The button-press resulted in flavor delivery and a concomitant drop in the image of the fluid level of the cartoon drinking glass (which remained on-screen for 10s after response). Immediately before tracer injection, subjects were told which flavor/infusion combination they would be getting, both to minimize time spent in expectation and to avoid prediction error (Yoder *et al*, 2011). "Press to sip" prompts were presented in three 5-minute blocks of five prompts each; each prompt resulted in subjects responding for flavor sprays that were 750ms long, for a total of 15 sprays. Subjective ratings were acquired between blocks (i.e. after the 5th, 10th, and 15th flavor sprays; upward arrows in Figure 1A).

Gustatory stimulus delivery

Subjects self-administered flavor sprays using a mouse button, which signaled the computer-controlled “gustometer” (modeled after Marciani *et al*, 2006) to deliver a 0.75 ml spray. Beer, Gatorade, and water were delivered through PTFE (Teflon[®]) tubing via a spray nozzle, which was positioned between subject’s incisors and aimed onto the tongue. Gustometer pump motors were controlled by a PC running DASYS Lab software (ver. 11.0; IOtech, Inc., Cleveland, OH) with a Personal DAQ/3000 module (IOtech). On a separate laptop, E-Prime (ver. 2.0; Psychology Software Tools, Inc., Sharpsburg, PA) was configured to interface with the DASYS Lab software that controlled the gustometer and visual stimuli seen by subjects on a computer monitor.

Ethanol Infusion

To achieve a uniform course of BrAC in all subjects, IV ethanol (and saline) infusion profiles were individually customized using a physiologically-based pharmacokinetic model that incorporated height, weight, age and sex (O'Connor *et al*, 1998; Ramchandani *et al*, 1999). The IV ethanol infusion began simultaneously with flavor delivery (cartoon drink glasses and “press to sip” prompt), which started two minutes after RAC injection. Six percent alcohol in 0.45% saline was delivered IV by a computer-controlled peristaltic pump (iMed Gemini PC-2 TX). A nonlinear increasing rate during the 15 min “ramp” (the period of increasing BrAC to a target of 0.060 g/dL) was followed by a decreasing infusion rate during the subsequent 28 min “clamp” (the period where BrAC was held constant at 0.060 g/dL for the remainder of the scan). The pharmacokinetically-modeled ascent approximated the BrAC profile resulting from an average height/weight male consuming two to three standard beers in a 15 min period, but avoids the considerable variation associated with oral ingestion. BrACs were assessed once immediately following scan completion with a handheld breath alcohol meter (Dräger Alcotest[®] 6510).

Subjective Ratings

Subjects responded to computerized rating scales at four time points for each scan: once preceding the scan to establish a pre-scan baseline, and three times in the beginning of the scan at 5 min intervals (see Figure 1A). ‘Desire’ to drink alcohol was assessed with 4 items (#11, #18, #21, #32 from the Alcohol Craving Questionnaire: Singleton *et al*, 2000) on a 7-point visual analog scale (VAS; 1 = strongly disagree, 7 = strongly agree). Subjects were asked the number of beers they wanted at the moment, with responses in 0.5 beer increments (assuming a standard 12 oz. beer). Perceived intoxication was rated along a continuous VAS with five labels anchored by “NOT AT ALL” to “MOST EVER”. Flavor pleasantness was measured on a VAS (1 = “Least Pleasant Ever”, 7 = “Most Pleasant Ever”). Perceived flavor intensity utilized Green’s Labeled Magnitude scale (Green *et al*, 1996), which ranged from “barely detectable” to “strongest imaginable”. Rating scales were presented via a computer monitor, and subjects responded with a wireless mouse. Responses during the scan were averaged across time points and compared to baseline.

Image Acquisition and Processing

A 3D magnetization prepared rapid acquisition gradient echo magnetic resonance imaging (MRI) volume was acquired for all subjects using a Siemens 3T Trio-Tim (160 sagittal slices, $1.0 \times 1.0 \times 1.2 \text{ mm}^3$ voxels, FOV = $256 \times 256 \text{ mm}$, TR=2300 ms, TE = 2.91 ms, FA = 9° , duration 9:14). RAC synthesis was as described previously (Fei *et al*, 2004), and RAC PET scans were acquired on a Siemens EXACT HR+ (3-D mode; septa retracted). PET scans were initiated with the IV infusion of $530 \pm 48 \text{ MBq}$ RAC (mass dose $0.105 \pm 0.035 \text{ nmol/kg}$, mean \pm SD) over 1.5 min; dynamic data were acquired for 45 min (per Yoder *et al*, 2009).

Dynamic PET images were generated using Siemens Fourier rebinning (FORE) and filtered backprojection algorithms including corrections for attenuation, random coincidences, scattering, and dead time. Image processing utilized SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>) and was procedurally similar to that described previously (Oberlin *et al*, 2013; Yoder *et al*, 2012). Each subject's anatomic MRI was used as a reference to which an early mean PET image (containing a mixture of blood flow and specific binding) was co-registered. To facilitate motion-correction, all PET frames were then co-registered to the early mean PET image (in native MRI space). After co-registration, rigid body realignment was applied to minimize spatial variance across frames and to evaluate residual motion. Each subject's MRI was spatially transformed into Montreal Neurological Institute (MNI) space; the transformation parameters were applied to all motion-corrected dynamic PET data. A reference region was created from cerebellar gray matter, excluding the vermis (from AAL atlas; Tzourio-Mazoyer *et al*, 2002). Time-activity curves for the cerebellum were generated from dynamic RAC data using scripted commands (AFNI; <http://afni.nimh.nih.gov/afni/>). Spatially normalized D₂/D₃ receptor availability was indexed by binding potential (BP_{ND}), operationally defined as the bound RAC concentration relative to non-displaceable RAC concentration (Innis *et al*, 2007). BP_{ND} was estimated using the multilinear reference tissue model (MRTM2; Ichise *et al*, 2003) for all voxels within striatum, using the cerebellar time-activity curve as the input function. Only voxels with BP_{ND} values greater than 0.75 in all three scans were considered (e.g. Joutsa *et al*, 2012). BP_{ND} images were smoothed with a 4 mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. Group averages and correlations were calculated using BP_{ND} data from each scan extracted from functional clusters defined at a height threshold of $p_{\text{uncorr}} < 0.01$. Mean values from these functional clusters allowed calculation of BP_{ND} for each subject. Changes in BP_{ND} were expressed as a percentage of control condition, defined as:

$$\text{BP}_{\text{ND}} = (\text{BP}_{\text{ND}[\text{Control}]} - \text{BP}_{\text{ND}[\text{Experimental}]}) / \text{BP}_{\text{ND}[\text{Control}]}$$

with 'Control' and 'Experimental' defined as referenced in Figure 1C.

Statistics

Stimuli and Ratings—One-sample *t*-tests tested if BrAC values differed from the target of 0.06%; paired *t*-tests assessed for differences in BrAC between Gat&Eth and Beer&Eth. Delivered volume of tastants and subjective ratings were evaluated using within-subjects ANOVA across four condition levels (Pre-scan Baseline, Gat&Sal, Gat&Eth, Beer&Eth). Significant effects of Condition were followed by paired *t*-tests to identify differences.

Imaging—BP_{ND} images were analyzed voxelwise using a factorial model in SPM8 with three levels (Gat&Sal, Gat&Eth, Beer&Eth); peak significance was set to $p_{FWE} < 0.05$, corrected for family wise error (FWE) within a search volume approximating the left or right NAcc (as defined by Neto *et al*, 2008). *Post hoc* analyses tested other factors of interest that may have influenced DA release. Mean extracted BP_{ND} in responding regions ($p_{uncorr} < 0.01$) was calculated to assess the effects of family history (FH) and scan order among significant contrasts that emerged from the voxelwise analyses. Spearman's rank-order correlation assessed putative relationships between BP_{ND} and self-reported drinking, subjective effects, and BrAC ($\alpha = 0.05$, uncorrected). To further assess the potential role of recent drinking, subjects were classified on drinks/week to Low or High groups (less or greater than the interquartile range, respectively; $ns=6$), and *t*-tests assessed differences in BP_{ND} in responding regions. Laterality effects were tested with paired *t*-tests of mean extracted values from both the responding region and its contralateral homologous area (e.g. Wong *et al*, 2006). Data values in-text are presented as mean \pm standard error of the mean (SEM), where reported mean BP_{ND} values illustrate effect size. Exploratory analyses were performed in caudate and putamen (defined by the AAL atlas) using the anterior commissure to delineate anterior and posterior subregions.

Results

Stimuli

Measured BrAC differed neither from target ($0.059\% \pm 0.001$ SEM and $0.060\% \pm 0.002$ in the Gat&Eth and Beer&Eth alcohol infusion conditions, respectively; $ps > 0.7$) nor between conditions ($p > 0.6$). Tastant volume (12.2 ml \pm 0.2; 12.4 ml \pm 0.2; 12.1 ml \pm 0.2 for Gat&Sal, Gat&Eth, and Beer&Eth scans, respectively) did not differ between conditions ($p > 0.6$).

Subjective Ratings

Stimulus qualities—Flavors (beer and Gatorade) were rated as more pleasant and intense than water ($ts(25) > 5.2$, $ps < 0.001$, but the flavors did not differ from each other ($ps > 0.2$).

Desire to drink—The ethanol conditions increased both Desire and Wanting relative to baseline and the Gat&Sal condition, [ANOVA; $F(3,75) = 18.8$, $p < 0.001$, paired-*t*; $ts(25) > 3.4$, $ps = 0.002$]. The ethanol conditions did not differ in Desire ($p = 0.09$), but Beer&Eth showed a trend to increase number of beers wanted when compared to Gat&Eth ($p = 0.052$; Figure 2A).

Subjective intoxication—Ethanol infusions increased perceived intoxication relative to baseline and Gat&Sal, [ANOVA; $F(3,75) = 34.7$, $p < 0.001$, paired-*t*; $ts(25) > 5.6$, $ps < 0.001$], but ethanol infusions did not differ from each other ($p > 0.9$; Figure 2B).

Imaging: stimulus effects

Effects of ethanol—Compared to Gat&Sal, Gat&Eth significantly reduced left NAcc RAC BP_{ND}, indicating higher DA in the alcohol condition ($n = 26$, peak voxel $[-10, 6,$

-10], $Z = 3.91$, $p_{\text{FWE}} = 0.001$; Figure 3A). BP_{ND} (percent change) within the responding cluster was $5.9\% \pm 0.8\%$. There was no effect in R NAcc.

Effects of beer flavor—Compared to Gat&Eth, Beer&Eth significantly reduced RAC BP_{ND} (increased DA in response to beer flavor) in the right NAcc (peak voxel [12, 10, -10], $Z = 2.71$, $p_{\text{FWE}} = 0.043$, Figure 3B). BP_{ND} within the responding cluster was $6.8\% \pm 2.6\%$. There was no effect in L NAcc.

Effects of beer flavor plus alcohol—Compared to the baseline of Gat&Sal, the combined exposure to both beer flavor and ethanol intoxication (i.e., Beer&Eth) significantly reduced RAC BP_{ND} (increased DA) in both left ([-14, 6, -12], $Z = 3.19$, $p_{\text{FWE}} = 0.013$) and right [12, 8, -10], $Z = 3.75$, $p_{\text{FWE}} = 0.002$; Figure 3C) NAcc. Average BP_{ND} was $5.8\% \pm 1.5\%$ and $6.8\% \pm 2.2\%$ for the responding L and R clusters, respectively.

Imaging: factors modulating DA response

Perceived intoxication—Ethanol plus flavor-induced displacement of RAC in L NAcc (Beer&Eth vs. Gat&Sal) correlated positively with perceived intoxication ($\rho = 0.399$, $p = 0.044$; Figure 4A), with higher perceived intoxication corresponding to greater DA response to alcohol plus beer flavor. No correlation was observed on the right. Self-reported intoxication did not correlate with L NAcc DA response in the Gat&Eth vs. Gat&Sal comparison.

BrAC—Measured BrAC in the Gat&Eth scan showed a trend-level correlation with BP_{ND} in L NAcc in the Gat&Eth vs. Gat&Sal comparison ($\rho = 0.361$, $p = 0.070$). No other correlations with BrAC were detected.

Family history—As the FH groups differed unexpectedly in BrAC, $t_s(24) > 3.4$, $p_s < 0.003$, FH tests of BP_{ND} in each responding region included a BrAC covariate. There were no differences in BP_{ND} by FH group in any outcome ($p_s > 0.19$).

Order effects— BP_{ND} did not differ based on which condition was presented first, i.e. Beer&Eth or Gat&Eth, in any of the responding regions ($p_s > 0.10$).

Other factors—Subjective ratings of flavor pleasantness, intensity, desire, or wanting for alcohol did not correlate with BP_{ND} in any of the responding regions in their respective contrasts, i.e. L NAcc response to the Gat&Eth vs. Gat&Sal comparison, the R NAcc response to the Beer&Eth vs. Gat&Eth comparison, or in either the L or R NAcc response to the Beer&Eth vs. Gat&Sal comparison. No correlations of BP_{ND} with age, age of first or regular drinking, or recent drinking frequency were observed. Low and High drinker groups did not differ in responding regions ($p_s > 0.27$).

Imaging: other analyses

Laterality—The R NAcc region responding to flavor, i.e. [Gat&Eth > Beer&Eth], showed a larger DA response than the corresponding region on the left, $t(25) = 2.2$, $p = 0.037$. The L

NAcc region responding to intoxication, i.e. [Gat&Sal > Gat&Eth], showed a strong trend of difference ($p = 0.057$) compared to the homologous area on the right; Figure 4B.

Gatorade flavor—The inverse contrasts to those reported above (i.e. Gat&Sal BP_{ND} vs. Gat&Eth BP_{ND}, DA release to Gatorade), showed no significant effects in either L or R NAcc.

Extra-NAcc effects—Exploratory analyses of dorsal anterior and posterior caudate and putamen did not show significant effects in any contrast at the threshold $p_{FWE} < 0.05$, corrected by region.

Discussion

Utilizing a novel analog of self-administration paradigm in heavy drinkers that separates ethanol's conditioned sensory properties from intoxication, we found that relative increases in NAcc DA transmission were localized to the right side for an alcohol-related CS (flavor) and to the left for the US (intoxication). This accords with our previous finding that beer flavor alone increased right NAcc DA in a similar population of heavy drinkers (Oberlin *et al*, 2013), and that (unexpected) intravenous ethanol intoxication increased left VST DA (Yoder *et al*, 2009). Previous reports showed bilateral VST DA increases resulting from ethanol CS and US presented together (Boileau *et al*, 2003; Setiawan *et al*, 2014; Urban *et al*, 2010), although in these studies, fruit juice was the tastant (not necessarily the preferred mixer), meaning that ethanol's orosensory properties could have acted as a CS. If we regard these previous studies as concomitant alcohol CS and US presentation, these data comport with our current findings of bilateral NAcc DA release to combined alcohol CS and US presentation. We can thus infer that the DA response to naturalistic alcohol drinking, which includes CS and US presentation, is evident in bilateral NAcc responses, but with lateralized processing of stimulus types.

Although interpreting lateralized DA responses to alcohol CS and US as separate information processing streams has an attractive simplicity, an alternate conceptualization is that *both* cue-reactivity and DA responses to intoxication are *conditioned responses*. For example, there is considerable evidence in animals showing that the US of a drug can reinstate (conditioned) drug-seeking behaviors (for review, see Epstein *et al*, 2006), even when the US is an entirely different drug; that is, multiple drugs are capable of reinstating cocaine or heroin responding (de Wit and Stewart, 1981, 1983). This implies that internal state changes induced by a drug of abuse acquire properties that act as discriminative stimuli which motivate behavior (Gerber and Stretch, 1975). Studies reveal that reinstatement depends, at least in part, on intact DA functioning (Alleweireldt *et al*, 2002; Xi *et al*, 2006). Similarly, conditioned (discriminative) stimuli that are paired with drug availability and seeking also motivate drug-taking behavior, and appear to depend on DA function (Bossert *et al*, 2007). As both internal and external stimuli can become conditioned to particular effects of a drug, a wide array of conditioning possibilities exist such that the line between a conditioned response and the effects from a US becomes blurred, that is, both can act as discriminative stimuli. Given that drug-paired CS and drug US both activate appetitive motivational mechanisms, which increase motivated behaviors, they also both fit the

classical definition of a reinforcer. In this light, lateralized conditioned responses may simply reflect specialized sensitivity to the nature of the drug stimuli, such that the R NAcc is more sensitive to conditioned cues (flavor) signaling impending intoxication, while L NAcc may be more differentially sensitive to the perceived salient changes in internal states brought on by intoxication, and learned through experience (e.g., reward signaling, dizziness).

This interpretation is consistent with a previous study (Besson and Louilot, 1995) that utilized *in vivo* voltammetry and a conditioned taste aversion paradigm in which a flavor was conditioned to a nausea-inducing lithium salt. The CS that predicted nausea reduced L NAcc DA transmission (suggesting that L NAcc DA responds to internal state changes, and perhaps valence), while the R NAcc showed a stronger response to an appetitive (external) stimulus. Importantly, both responses had been learned (conditioned) (Besson *et al*, 1995). The synthesis of these prior data and the current findings suggests that NAcc DA may instantiate learned responses in a lateralized fashion, such that L NAcc is more linked to signaling interoceptive changes, while R NAcc signals salient exteroceptive stimuli.

The R NAcc response, which was relatively weak when both beer and control flavors were compared under ethanol (Beer&Eth vs. Gat&Eth), responded robustly in conjunction with the left NAcc when beer flavor under ethanol intoxication was compared to Gatorade with saline (i.e., the combination of stimuli closest to naturalistic alcohol ‘drinking’). These DA responses support the notion that intoxication is additive to DA effects induced by the CS of an alcoholic drink’s characteristic flavor, perhaps due to the presence of the additional CS of internal state changes. Further evidence for this type of stimulus synergy is observed in the correlation of subjective intoxication with BP_{ND} in L NAcc, which was only detectable when beer flavor was present. This finding was not replicated on the right, implicating a greater relative contribution of sensitivity to interoceptive effects on the left.

Viewing the effect of intoxication as a learned response comports with previous data suggesting that a history of heavy drinking may be needed for ethanol’s ability to induce VST DA release, as DA response was not observed in social drinkers (Yoder *et al*, 2007; Yoder *et al*, 2005), or in social drinkers with the major (common) allele of the functional μ -opioid receptor gene, *OPRM1* (Ramchandani *et al*, 2011). Notably, the subjects of Urban *et al*. (2010) study drank more heavily than typical social drinkers, and showed DA release to orally consumed ethanol. Given the lack of correlation in drinking history with DA response, or even a difference between extremes of drinking in this sample (which presumably differ in conditioning history), the effect of conditioning may not be strictly linear, but instead reflects a threshold of conditioning. If true, this conditioning might be formed after a very few intoxication sessions; alternatively, a heightened capacity for a DA conditioned response to intoxication might be mediated by other factors—additional studies will be needed to inform these hypotheses.

Other studies have shown lateralized striatal effects that suggest specialization by hemisphere. Congruent with the idea that R striatal DA signaling may be more sensitive to CS, Wong *et al*. (2006) reported that cocaine addicts (‘cravers’) showed greater R dorsal putamen DA release to audiovisual cues than non-cravers. A study in healthy subjects

revealed that R VST DA preferentially responded to unexpected monetary rewards relative to L VST (Martin-Soelch *et al*, 2011). Another study in healthy controls showed that incentive motivation correlated with resting D2 receptor availability in the left putamen; an interpretation of these data is that greater endogenous right putamen DA (relative to left putamen) predicts higher drive (Tomer *et al*, 2008). In Parkinson's patients, right hemisphere DA deficits corresponded with reduced novelty-seeking, relative to controls (Tomer and Aharon-Peretz, 2004), also suggesting lateralized striatal DA function.

While interpretations differ as to the meaning of mesolimbic DA responses, it is clear that alcohol and other drugs of abuse potentiate DA release in the midbrain (e.g., Brodie *et al*, 1990) and NAcc (Di Chiara *et al*, 1988; Imperato and Di Chiara, 1986; Sullivan *et al*, 2011). NAcc DA may also be necessary for CS-induced drug-seeking behavior, as local infusion of a DA receptor antagonist in NAcc reduces both alcohol seeking and consumption (Czachowski *et al*, 2001); similarly, the ability of alcohol's contextual cues to induce alcohol-seeking is blocked by DA receptor antagonists (Hamlin *et al*, 2007). Renewal-induced transcription factor induction (c-Fos) in NAcc requires normal DA D1 receptor function, suggesting that NAcc DA modulates long-term changes in NAcc gene expression specific to alcohol-seeking behavior (Hamlin *et al*, 2007), and providing a possible mechanism for learned responses in NAcc.

Some limitations to this work should be considered. While the paradigm was truly CS self-administration, it was not drug self-administration in the strictest sense. From the standpoint of expectations, the paradigm was self-administration in the fullest sense as subjects were instructed that infusion depended on their responses. Importantly, this method captured the essence of any traditional self-administration paradigm: the presence of an instrumental response for more drug during a period of increasing drug plasma concentration. The most conservative statistical correction for the imaging data would employ bilateral NAcc search regions. Analyzed in this way, all but one of our results remained significant ($p_{S_{FWE}} < 0.05$). Only the effect of beer flavor in the R NAcc (which replicates our earlier finding in a much larger sample; Oberlin *et al*, 2013) became a trend-level result ($p_{FWE} = 0.086$). Family history of alcoholism may also influence how subjects respond to alcohol CS and US. However, we did not find family history differences in DA responses in the current study. Although this is discrepant with our prior data (Oberlin *et al*, 2013), this sample is somewhat smaller, and the family history effects of our earlier report occurred to a flavor CS without concomitant ethanol intoxication or operant requirement (see Kareken *et al*, 2010 for attenuation of conditioned responses by alcohol in FHP). We also note that at least 8 subjects were non-responders in the L NAcc to the Beer&Eth condition. In combination with the lack of relationship between recent drinking history and DA release, this heterogeneity of DA response suggests that other important factors might be mediating DA processing of alcohol stimuli. Larger samples will likely be required to characterize these factors.

In summary, heavy beer drinkers showed increased VST DA release on the right to beer flavor, on the left to intoxication, and bilaterally in a combination that mimicked drinking as it occurs naturalistically (CS plus US). These data show that, within the same individuals, the independent components of an alcoholic drink have additive and lateralized effects on

VST DA release, and that these effects may reflect interoceptive and exteroceptive stimulus processing.

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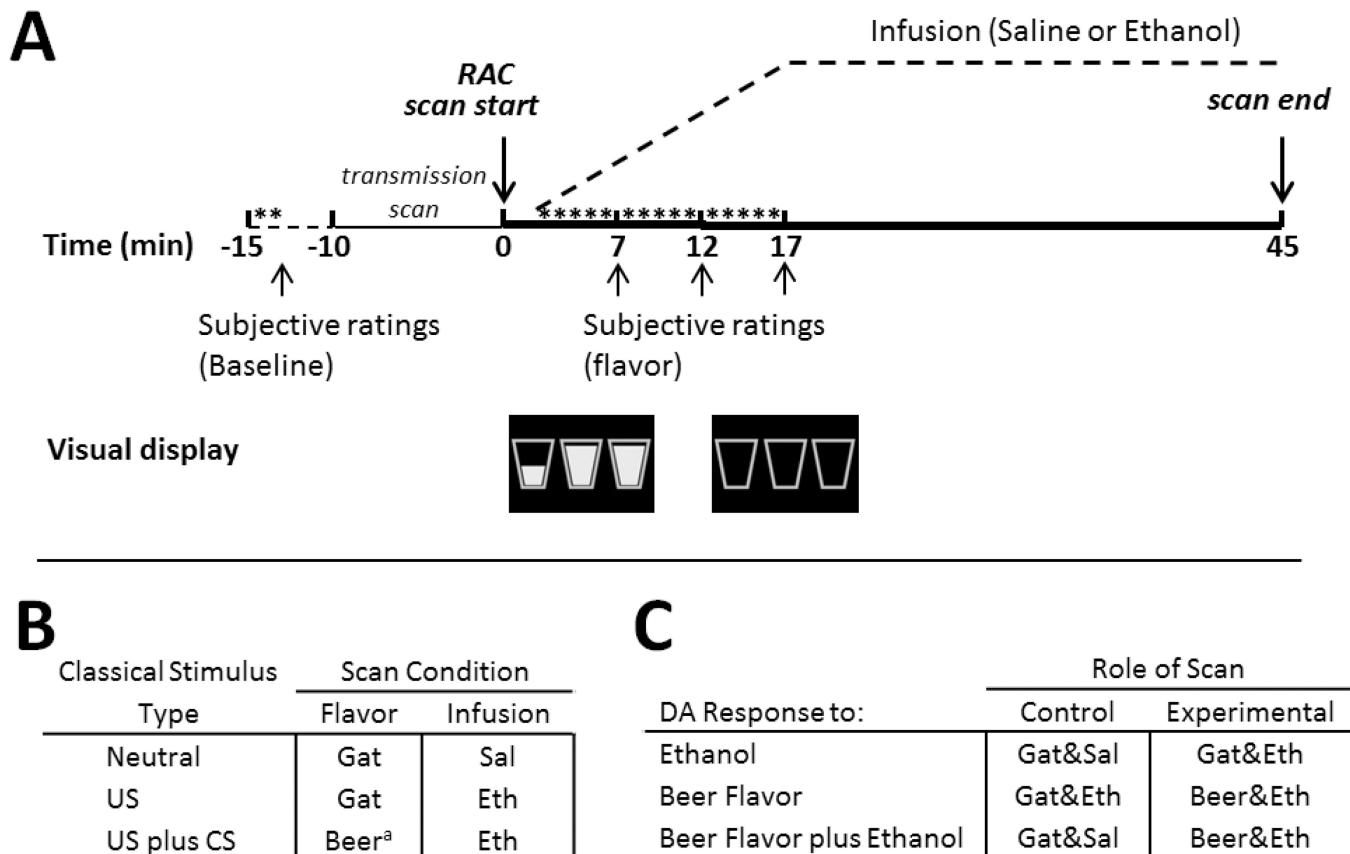


Fig. 1.

(A) *Single scan: stimuli and infusion.* Baseline ratings followed two water sprays. For illustration, the drink images are shown here after 3 sips and 15 sips (empty). Time is relative to RAC injection. *Self-administered sprays of water or flavor. Dashed line illustrates ascending and then clamped breath alcohol concentration. For saline, the same pump rates were used as those needed to achieve the same alcohol time course. (B) *Three-scan study design.* ^aPreferred beer. US = unconditioned stimulus, CS = conditioned stimulus. (C) *Relevant comparisons.* Gat = Gatorade, Sal = saline, Eth = ethanol infusion targeted to breath alcohol concentration of 0.06 g/dL.

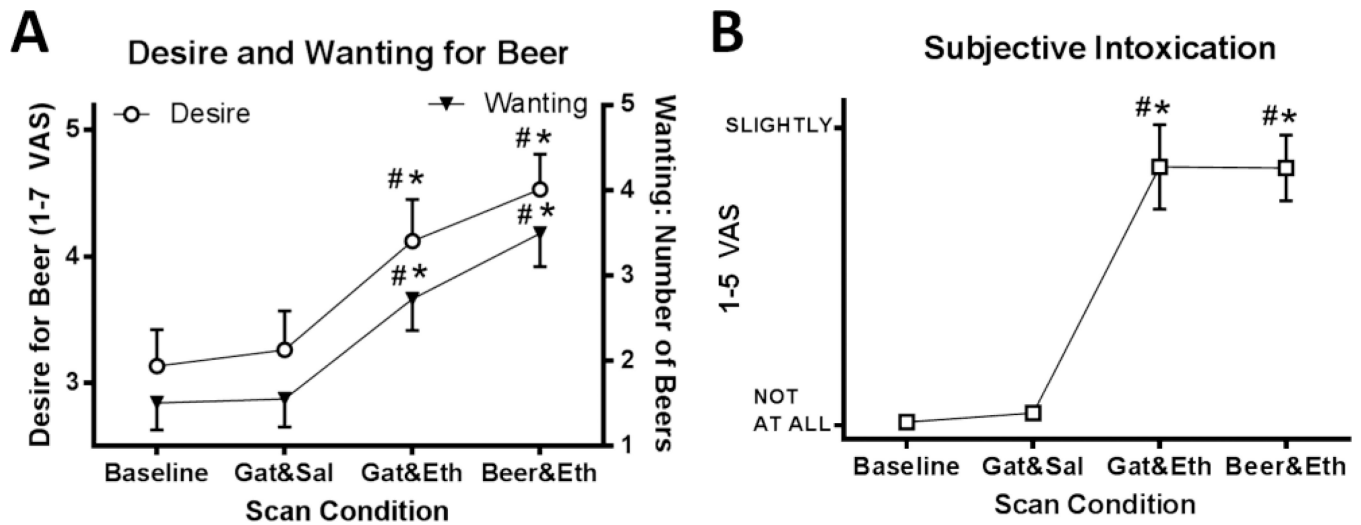


Fig. 2. *Subjective States.* (A) All 26 subjects rated the desire for beer (open circles, left y-axis), and the number of beers wanted (filled triangles, right y-axis) with saline or alcohol infusion. (B) Subjects rated their subjective state of intoxication (open squares). Mean \pm SEM are presented; # = differed from water Baseline, * = differed from Gatorade-Saline; ($p < 0.05$). The Baseline condition was evaluated after two water sprays and prior to each flavor-infusion scan; these are averaged across flavor conditions. VAS = Visual Analog Scale, Gat = Gatorade flavor, Sal = saline infusion; Alc = alcohol infusion.

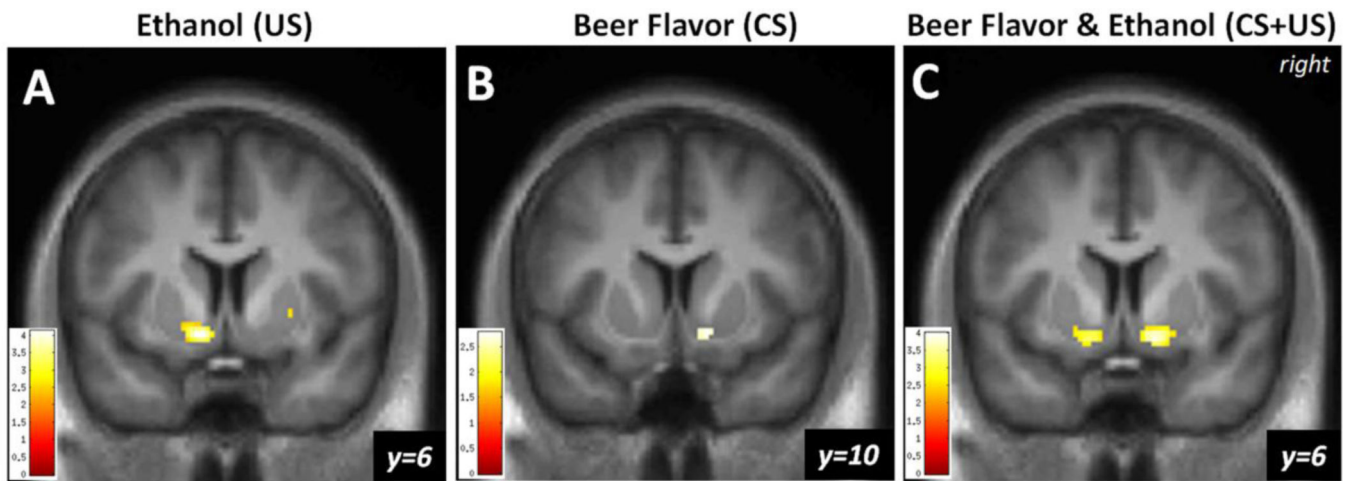


Fig. 3.

(A) *DA response to ethanol, with Gatorade flavor.* Statistical map illustrating the nucleus accumbens (NAcc) DA response to ethanol infusion with Gatorade flavor [$\text{Gat\&Sal BP}_{\text{ND}} > \text{Gat\&Eth BP}_{\text{ND}}$] in male drinkers ($n = 26$) as indexed by [^{11}C]raclopride displacement. (B) *DA response to beer flavor, with ethanol.* The NAcc response to beer flavor relative to Gatorade, both under ethanol, [$\text{Gat\&Eth BP}_{\text{ND}} > \text{Beer\&Eth BP}_{\text{ND}}$]. (C) *DA response to beer flavor plus ethanol.* The NAcc response to beer flavor and ethanol relative to Gatorade and saline, [$\text{Gat\&Sal BP}_{\text{ND}} > \text{Beer\&Eth BP}_{\text{ND}}$]. The color bar shows the voxel-wise t statistic. Display threshold at $p < 0.01$, uncorrected, and $k = 20$. All results displayed, $p_{\text{FWE}} < 0.05$, after correction for NAcc search volumes.

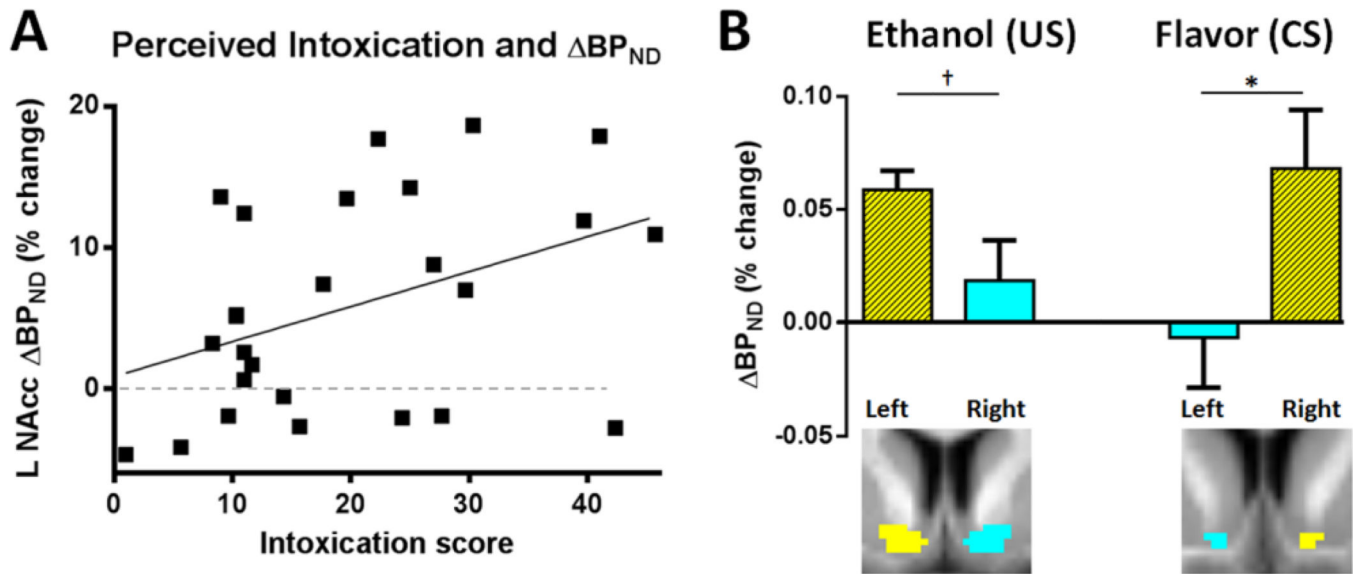


Fig. 4.

(A) *DA Effects of ethanol and subjective response.* In all subjects ($n = 26$), self-reported intoxication correlated with increased displacement of [^{11}C]raclopride (ΔBP_{ND}) to ethanol with beer flavor in L NAcc, as illustrated in Fig 3A; $\rho = 0.399$, $p = 0.044$. The dashed line indicates unchanged ΔBP_{ND} . (B) *Laterality.* Mean reduction in ΔBP_{ND} in response to ethanol and beer flavor (shown in Figs 3A,B) showed a lateralized response, as compared to the homologous region. Columns and spatial extent of responding regions shown in (hatched) yellow, with homologous regions (coronal view, *below*) indicated in cyan. * $p < 0.05$, † $p = 0.057$

Table 1

Subject Characteristics.

	Mean \pm (SD)	Range	n(%)
Age	23.1 (3.3)	21–34	
Caucasian ¹	-	-	25 (96%)
Education	14.8 (1.4)	12–20	
Drinks per week ²	23.0 (12.0)	9–64	
Drinks per drinking day ²	7.2 (2.5)	3–12	
Heavy drinking days per week ^{2,3}	2.2 (1.0)	1–4	
Age of first intoxication	16.4 (2.2)	12–21	
AUDIT ⁴	13.8 (4.5)	9–27	

¹Two subjects self-identified as Hispanic/Latino.

²From the Timeline Followback Interview.

³Greater than 4 drinks per day.

⁴Alcohol Use Disorder Identification Test.