

# Heterogeneous Effects of Alcohol on Dopamine Release in the Striatum: A PET Study

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**Background:** A dopaminergic response to alcohol in humans has not been demonstrated consistently with positron emission tomography (PET). We hypothesized that the effect of alcohol on striatal dopamine (DA) release may be anatomically heterogeneous between subjects. Our approach was to identify voxels that exhibited alcohol-induced DA responses within the striatum, and to determine the relationships between DA responses and alcohol-related behavior.

**Methods:** A novel method was developed to examine the anatomic extent and magnitude of striatal DA responses to alcohol across subjects. Thirteen healthy control subjects underwent 2 PET scans with [<sup>11</sup>C]raclopride (1 at baseline, 1 with an IV alcohol infusion to a target breath alcohol concentration of either 60 or 80 mg%). Parametric images of striatal binding potential (BP) were used to create maps of change in BP ( $\Delta$ BP, an index of changes in DA levels). The anatomic extent and magnitude of DA responses were determined with voxel extraction methods. Subjective responses ("High," "Intoxication") to the alcohol infusion and behavioral data from the 90-day time-line follow back were assessed for relationships with DA responses to alcohol.

**Results:** A voxel-wise *t*-test between baseline and alcohol BP images did not show any differences in D<sub>2</sub>/D<sub>3</sub> receptor availability between the conditions. Data from the striatal  $\Delta$ BP maps nevertheless showed that the anatomic extent and magnitude of alcohol-induced DA release in the striatum are correlated with subjective responses to alcohol.

**Conclusions:** The heterogeneity of dopaminergic responses to alcohol across subjects may be a reason for the lack of reports demonstrating DA involvement in alcohol-related behaviors. By allowing for different spatial patterns of DA release within each subject's striata, we showed correlations between alcohol-induced DA release in the striata and behavioral outcomes related to alcohol.

**Key Words:** Dopamine, Alcohol, PET, Raclopride, Behavior.

**D**OPAMINE (DA) IS involved in determining reward salience, valence, expectation, and the acquisition of addictive behaviors (Di Chiara et al., 2004; Grace, 2000; Ikemoto et al., 1997; Salamone et al., 2005; Schultz, 2002). Animal studies have repeatedly demonstrated that alcohol administration increases DA in the nucleus accumbens,

the major efferent target of mesolimbic DA neurons, which constitute an important component of the brain's reward systems (e.g., Gonzales and Weiss, 1998; Imperato and Di Chiara, 1986; Melendez et al., 2002; Yoshimoto et al., 1992).

Recent developments in positron emission tomography (PET) now permit testing such findings in humans. In brief, the radiotracer [<sup>11</sup>C]raclopride ([<sup>11</sup>C]RAC) binds competitively to DA D<sub>2</sub>/D<sub>3</sub> receptors, is sensitive to changes in endogenous DA concentration ([DA]) (Seeman et al., 1989; Young et al., 1991), and can be displaced by endogenous DA release induced by pharmacological manipulation or cognitive stimuli. As a result, there is a consequent decrease in the measured [<sup>11</sup>C]RAC signal relative to the baseline condition. This method has been used successfully to document increases in human striatal DA levels in response to drugs of abuse such as amphetamine (Breier et al., 1997; Drevets et al., 2001; Leyton et al., 2002; Martinez et al., 2003, 2005; Munro et al., 2006a, 2006b; Oswald et al., 2005), methylphenidate (Volkow et al., 1994, 1997, 1999; Wang et al., 1999), and nicotine (Barrett et al., 2004; Brody et al., 2004, 2006). However, studies designed to measure dopaminergic responses to alcohol have been equivocal.

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Early studies of the effect of alcohol on human striatal DA release looked only in the caudate and putamen, and found either no change in DA levels in response to oral alcohol (Salonen et al., 1997;  $n = 7$ ) or a decrease in [DA] after an IV infusion of alcohol (Wong et al., 1993;  $n = 4$ ). However, animal studies suggest that alcohol-induced DA release is primarily localized to the nucleus accumbens (e.g., Imperato and Di Chiara, 1986; Yoshimoto et al., 1992). These initial human PET studies did not specifically target the ventral striatum (which contains the nucleus accumbens) as a region of interest, so it is possible that they may have missed an effect of alcohol on DA release. To our knowledge, there is only one report of DA release in the ventral striatum after ingestion of oral alcohol (Boileau et al., 2003;  $n = 6$ ). While the results of the Boileau study are promising, there was considerable variability in the effect across subjects. The design also included a possible confounding placebo condition instead of a true resting baseline. Placebo conditions themselves have been reported to cause DA release (de la Fuente-Fernandez et al., 2001, 2002; Kaasinen et al., 2004). Finally, a previous study by our group, which used the alcohol clamp technique to control brain exposure to alcohol (O'Connor et al., 2000; Ramchandani et al., 1999), did not find an effect of IV alcohol infusion [to 60 mg% target breath alcohol concentration (BrAC)] on DA release (Yoder et al., 2005;  $n = 8$ ).

However, if subjects do not exhibit changes in [DA] in the same striatal regions, traditional methods that average effects over particular regions or voxels across subjects could mask more spatially variable effects across individuals. In this paper, we reanalyzed the Yoder et al. (2005) study data (8 subjects who received an IV infusion with a target BrAC of 60 mg%) using an approach designed to overcome potential anatomical variability in stimulus-induced striatal DA release. This new approach characterizes the overall anatomic extent and magnitude of DA responses in each subject, without requiring that all subjects respond in anatomically identical areas. In addition to the change in method, we added 5 subjects who received a higher dose of IV alcohol (targeting a BrAC of 80 mg%) to explore the possibility of dose-related effects. We hypothesized that larger dopaminergic responses to alcohol would be correlated with a larger subjective effect as well as with greater habitual drinking (Katner and Weiss, 2001; Weiss et al., 1993).

## MATERIALS AND METHODS

### Subjects

All procedures were explained to the subjects and written consent obtained, in accordance with the requirements of the Indiana University Institutional Review Board. Subjects were 13 healthy, nonsmoking, social-drinking volunteers without histories of significant neurological disturbances or psychiatric diagnoses. The existence of alcohol, marijuana, and other drug use problems were assessed with sections of the Semi-Structured Assessment for the

**Table 1.** Subject Demographics

Target BrAc	Experimental protocols	
	60 mg%	80 mg%
<i>n</i>	8	5
Gender	1F	1F
Handedness	7R	4R
Age (mean $\pm$ SD)	25 $\pm$ 2.0	25 $\pm$ 1.4
Race	1B, 7C	1A, 4C
Ethnicity	8 N-H/L	5 N-H/L
Family history positive	2	2
Family history ambiguous	2	0
Family history negative	4	3
TLFB (90-day): mean (range)		
Avg drinks/wk	8.13 (1.09–20.8)	6.75 (2.26–9.57)
Avg drinks/drinking day	3.69 (1.4–6.23)	2.95(1.04–4.83)
# High drinking days	10.9 (0.0–31)	4.6 (0.0–8.0)
Informed of ETOH	Via cues	Verbally
ETOH infusion start (min)	2–3 <sup>a</sup>	5 <sup>b</sup>
Ramp (min)	10	15 <sup>c</sup>
Clamp (min)	30	10

TFLB, time-line follow back (90-day); ETOH, alcohol; F, female; R, right; A, asian; B, black; C, Caucasian; N-H/L, non-Hispanic/Latino; avg, average. Family history positive status was defined as subjects who have at least one first-degree relative with alcoholism plus another first-degree or second-degree relative with alcoholism. Family history ambiguous was defined as subjects with only one first-degree or second-degree relative with alcoholism. Alcohol infusion start time (min) is relative to the start of the [<sup>11</sup>C]RAC scan. "Ramp" refers to the length of the ascension to target BrAc. "Clamp" refers to how long the target BrAc was maintained. Super-script notations indicate exceptions to protocols.

<sup>a</sup>Infusion started at 7.5 min ( $n = 1$ ) and at 17 min ( $n = 1$ ).

<sup>b</sup>Infusion started at 15 min ( $n = 1$ ).

<sup>c</sup>Ascension ("ramp") to 80 mg% occurred over 35 min ( $n = 1$ ).

Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994; Hesselbrock et al., 1999; NIAAA, 2003). Subjects also completed the time-line follow back interview (TLFB; Sobell et al., 1986) to quantify habitual drinking. None of the subjects were taking medications with central nervous system effects. Subjects received a urine drug screen on the day of scanning and all tested negative for amphetamines, barbiturates, benzodiazepines, cannabis, cocaine, and opiates. Subject demographics, family history of alcoholism, and social drinking patterns are listed in Table 1.

### IV Alcohol Administration

Subjects underwent 2 [<sup>11</sup>C]RAC PET scans, the second of which was coincident with an IV infusion of alcohol, as described previously (O'Connor et al., 2000; Ramchandani et al., 1999). In the 8 subjects of the first protocol, transformations of height, weight, and gender were used to model a linear rise of breath alcohol (BrAC) over 10 minutes to a target concentration of 60 mg% that was then "clamped" to remain constant ( $\pm 5$  mg%, O'Connor et al., 2000) for 30 minutes. The 60 mg% group received visual and olfactory cues indicating that alcohol was about to be administered, as described previously (Yoder et al., 2005).

The 5 subjects in the second group were linearly ramped to a target BrAC of 80 mg% over 15 minutes, and then clamped at this target for 10 minutes. The 80 mg% subjects received a verbal instruction that alcohol was about to be administered at the moment the infusion started ("This is an alcohol scan"). Five minutes after the start of the rest scan, subjects were instructed, "This is a no-alcohol scan." The actual sequences of the infusion protocols are outlined in Table 1. A modification of the Subjective High Assessment Scale (SHAS;

Schuckit, 1980) was used during the scans. Subjects were queried about how *high* (“stimulated, up, feeling good”) and how *intoxicated* (“feeling drunk, affected by alcohol”) (Judd et al., 1977; Morzorati et al., 2002; Schuckit, 1980) they felt; subjects verbally reported a whole number from 0 (baseline) to 100 (the most ever experienced). Scores were recorded periodically during the alcohol scan, and the area under the curve (AUC) for both high and intoxication was calculated for each subject using the trapezoidal rule. The AUC values for high and intoxication were tested for correlations with the extent and magnitude of DA responses.

#### Image Acquisition

Two [<sup>11</sup>C]RAC scans were performed on the same day with the EXACT HR+scanner (CTI, Knoxville, TN). Data were acquired with septa retracted (3D mode). Full-width at half-maximum (FWHM) was 9 mm when images were reconstructed with a 5 mm Hanning filter. [<sup>11</sup>C]Raclopride was synthesized as reported previously (Fei et al., 2004). Radiochemical purity was >99%. Scans were initiated with the IV injection of (mean ± SD) 14.1 ± 2.78 mCi of [<sup>11</sup>C]RAC. The specific activity at the time of injection was 0.80 ± 0.49 Ci/μmol. Total mass injected was 23.8 ± 15.5 nmol per subject per scan. The first scan was conducted in the morning while subjects rested quietly. The second scan, with IV alcohol infusion, was conducted in the afternoon. Scan order was not randomized across subjects because of the potential for persisting and confounding effects of alcohol from a morning scan. Subjects also received a heavily T1-weighted magnetic resonance imaging (MRI; 3D spoiled gradient echo recalled) on a 1.5T GE Echospeed LX scanner (GE Medical Systems, Waukesha, WI).

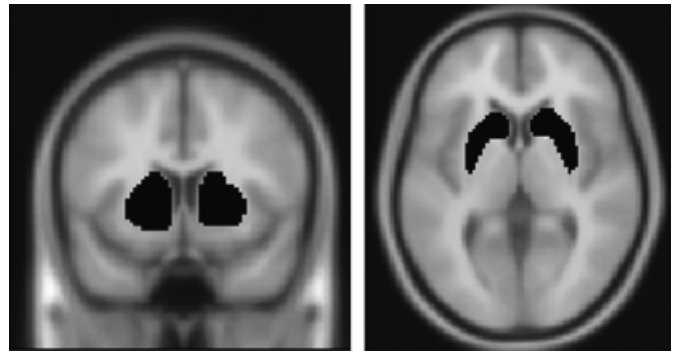
#### Image Processing

MRI and PET images were converted to Analyze format (a widely used image format developed by the Biomedical Imaging Resource at the Mayo Foundation), using MRICro software (<http://www.sph.sc.edu/comd/rorden/mricro.html>). All subsequent data processing steps were performed with Statistical Parametric Mapping 2 (SPM2) software (<http://www.fil.ion.ucl.ac.uk/spm/>). For each scan, a summed image was created from the first 10 minutes of dynamic [<sup>11</sup>C]RAC data using the Realign function in SPM2. These summed images contained a mixture of blood flow and specific striatal D<sub>2</sub>/D<sub>3</sub> binding, permitting accurate registration of all time frames to a single image. The summed image was coregistered to the individual subject’s MRI scan using SPM2. Motion correction was achieved by coregistering individual PET frames to the coregistered, summed PET image. Each subject’s MRI was normalized into Montreal Neurological Institute (MNI) stereotactic space using SPM2’s default normalization parameters. The transformation matrix obtained from this normalization step was applied to the motion-corrected, coregistered PET data from each subject, placing all dynamic PET data in MNI stereotactic space.

#### Parametric Binding Potential (BP) Images

Binding potential ( $BP \equiv B'_{\max}/K_D$ ) indexes DA D<sub>2</sub>/D<sub>3</sub> receptor availability, and changes in BP can be used as an index of change in [DA]. If [<sup>11</sup>C]RAC BP values from an experimental scan condition are different from baseline BP values, the changes in BP are presumed to be caused by changes in endogenous [DA] (Dewey et al., 1992, 1993; Seeman et al., 1989; Young et al., 1991). Increases in BP relative to baseline indicate decreases in [DA], and decreases in BP relative to the baseline BP indicate increases in [DA].

Parametric BP images were generated using an in-house script written in MATLAB (The Mathworks, Inc., Natick, MA) that implemented a multilinear reformulation of the Logan plot (Ichise et al., 2002; Logan et al., 1996). This graphical analysis method



**Fig. 1.** Coronal (left) and axial (right) views of the mask used to create striatal binding potential images, shown at the level of the ventral striatum. See text for details.

requires an input function from a “reference region” (an area devoid of D<sub>2</sub>/D<sub>3</sub> receptors, e.g., the cerebellum) in lieu of an arterial plasma input function. For this purpose, a region of interest was created for each posterior cerebellar hemisphere using the MarsBaR Toolbox for SPM2 (<http://marsbar.sourceforge.net/>). Time-activity curves (TACs) for the right and left cerebellum were extracted using MarsBaR, and written to text files using an in-house Toolbox for SPM2. The right and left cerebellar TACs were averaged, and the averaged cerebellar TAC was used as the input function for the voxel-wise Logan graphical analysis. The resultant parametric whole brain BP images were smoothed with an 8 mm kernel (Costes et al., 2005; Picard et al., 2006; Ziolkowski et al., 2006).

We restricted the search area during the voxel-wise paired *t*-test analysis, as (1) our sole focus was the striatum, and (2) high-affinity [<sup>11</sup>C]RAC binding is confined to the striatum; other ligands are required to examine DA receptor availability in extrastriatal areas (Christian et al., 2004; Mukherjee et al., 1997, 2005). A bilateral striatal binary mask (Fig. 1) was created from the left and right caudate and left and right putamen regions of interest found in the MarsBaR Automated Anatomic Labeling Region of Interest library (<http://marsbar.sourceforge.net/>). The binary mask was smoothed with a 10 mm kernel. The anatomic descriptions of the regions used for the striatal mask in the present work are described in Tzourio-Mazoyer et al. (2002). The mask was applied to the whole brain BP images to create striatal BP images that were used for all analyses reported herein.

To test for effects of alcohol infusion on [DA] via changes in BP, voxel-wise paired *t*-tests of the baseline and alcohol striatal BP images were conducted in SPM2. Separate analyses were done for the 60 and 80 mg% groups. SPM2 conducts unidirectional analyses; as such, 2 contrasts were run for each alcohol group: baseline BP > alcohol BP (which tested for DA release) and alcohol BP > baseline BP (which tested for decreases in [DA]). The statistical threshold for the SPM results was  $p < 0.001$  (uncorrected), which is a standard and conservative threshold for comparisons across multiple voxels.

#### ΔBP Images and Voxel-Wise Extraction of DA Responses

Maps of striatal DA responses were created to visualize individual responses to alcohol, and, more importantly, to extract potentially useful information about a subjects’ dopaminergic responses to alcohol across the striatum in an objective manner. Dopamine responses, which include increases and decreases in [DA], can be indexed by change in BP (ΔBP), defined here as  $(BP_{\text{baseline}} - BP_{\text{alcohol}})/BP_{\text{baseline}}$ . Striatal ΔBP maps for each subject were created using this formula and the ImCalc function in SPM2. These maps were used to visually compare each subject’s dopaminergic response to alcohol. The MarsBaR toolbox for SPM2 was used to facilitate

the voxel-based extraction of  $\Delta BP$  values. First, a region of interest was made from the same binary mask that was used to create the striatal search area. Second, this region of interest was used to extract the  $\Delta BP$  values of all voxels contained in the striatal  $\Delta BP$  images which were then written to a MATLAB file. Third, MATLAB was used to extract information about the DA responses within each subject's striatal  $\Delta BP$  image. Dopamine responses consisted of either voxels with positive  $\Delta BP$  values ( $\Delta BP > 0$ ), which indicated increases in [DA], or voxels with negative  $\Delta BP$  values ( $\Delta BP < 0$ ), which indicated decreases in [DA]. The spatial extent of a DA response was defined as the number of striatal voxels with  $\Delta BP$  values either  $> 0$  or  $< 0$ . The magnitude of a DA response was defined as the sum of the  $\Delta BP$  values from the extent voxels of the respective response (increased or decreased [DA]).

Extent and magnitude of DA responses were also assessed for  $\Delta BP$  values  $\geq 0.1$  and  $\leq -0.1$  (see Appendix A for explicit mathematical definitions). These conservative thresholds are based on the 10% intrasubject test-retest variability for single bolus [ $^{11}\text{C}$ ]RAC studies, which was established by Volkow et al. (1993). This work by Volkow and that of others (single bolus, Hietala et al., 1999; bolus-infusion, Mawlawi et al., 2001) suggest that changes in  $BP \pm 10\%$  from baseline (e.g.,  $\Delta BP$  values between  $-0.1$  and  $0.1$ ) are not reliable indicators of true changes in [DA].

#### Other Statistical Analyses

Two-sample *t*-tests were used to test for differences in the anatomical extent and magnitude of DA responses between the 60 and the 80 mg% groups. Pearson's correlation coefficient was used to test for relationships between DA responses and subjective responses and TLFB (90-day) variables of interest. Similar methods have been reported previously (Christian et al., 2006). Statistical significance was set at  $p < 0.05$ . Trend-level significance was defined as  $0.05 < p < 0.1$ .

## RESULTS

#### Voxel-Wise Paired *t*-Test Results

The voxel-wise paired *t*-test analysis of baseline versus alcohol striatal BP images (statistical threshold,  $p < 0.001$ , uncorrected) did not reveal any DA responses (either increases or decreases in [DA]) as a result of either 60 or 80 mg% alcohol infusion.

#### Striatal DA Responses

The striatal  $\Delta BP$  maps for each subject are shown in Fig. 2. Note the degree of individual variability in DA responses to either 60 or 80 mg% alcohol.

Table 2 contains the extent and magnitude data for striatal  $\Delta BP > 0$  (increased [DA]) and  $\Delta BP < 0$  (decreased [DA]). Extent and magnitude for either increases or decreases in [DA] were not statistically different between the 60 and 80 mg% groups (*t*-test for independent samples). Similarly, the extent and magnitude data for the 10% threshold data ( $\Delta BP \geq 0.1$  and  $\leq -0.1$ ; Table 3) also show no group differences in these indices.

As there were no statistical differences between groups with respect to the extent and magnitude of DA responses, data from both groups were combined for correlational analyses with SHAS and TLFB variables.

#### Correlations Between DA Responses and Subjective Responses to Alcohol Infusion

The magnitude of [DA] increase, defined by voxels with  $\Delta BP > 0$ , was significantly correlated with intoxication ( $p = 0.007$ ,  $r = 0.70$ ). The spatial extent of this increase in [DA] had a trend-level correlation with intoxication ( $p = 0.08$ ,  $r = 0.50$ ). Neither the extent nor the magnitude of DA response in voxels with  $\Delta BP > 0$  correlated with high.

When increases in [DA] were assessed using the conservative threshold of  $\Delta BP \geq 0.1$ , the correlations were strengthened. Both spatial extent and magnitude of increased [DA] correlated significantly with intoxication scores ( $p = 0.004$ ,  $r = 0.73$  and  $p = 0.007$ ,  $r = 0.71$ , respectively). A trend-level correlation between spatial extent of increased [DA] and high was detected ( $p = 0.08$ ,  $r = 0.51$ ).

There were no correlations between the anatomical extent or magnitude of decreases in [DA] and high or intoxication (either for  $\Delta BP < 0$  or for  $\Delta BP \leq -0.1$ ).

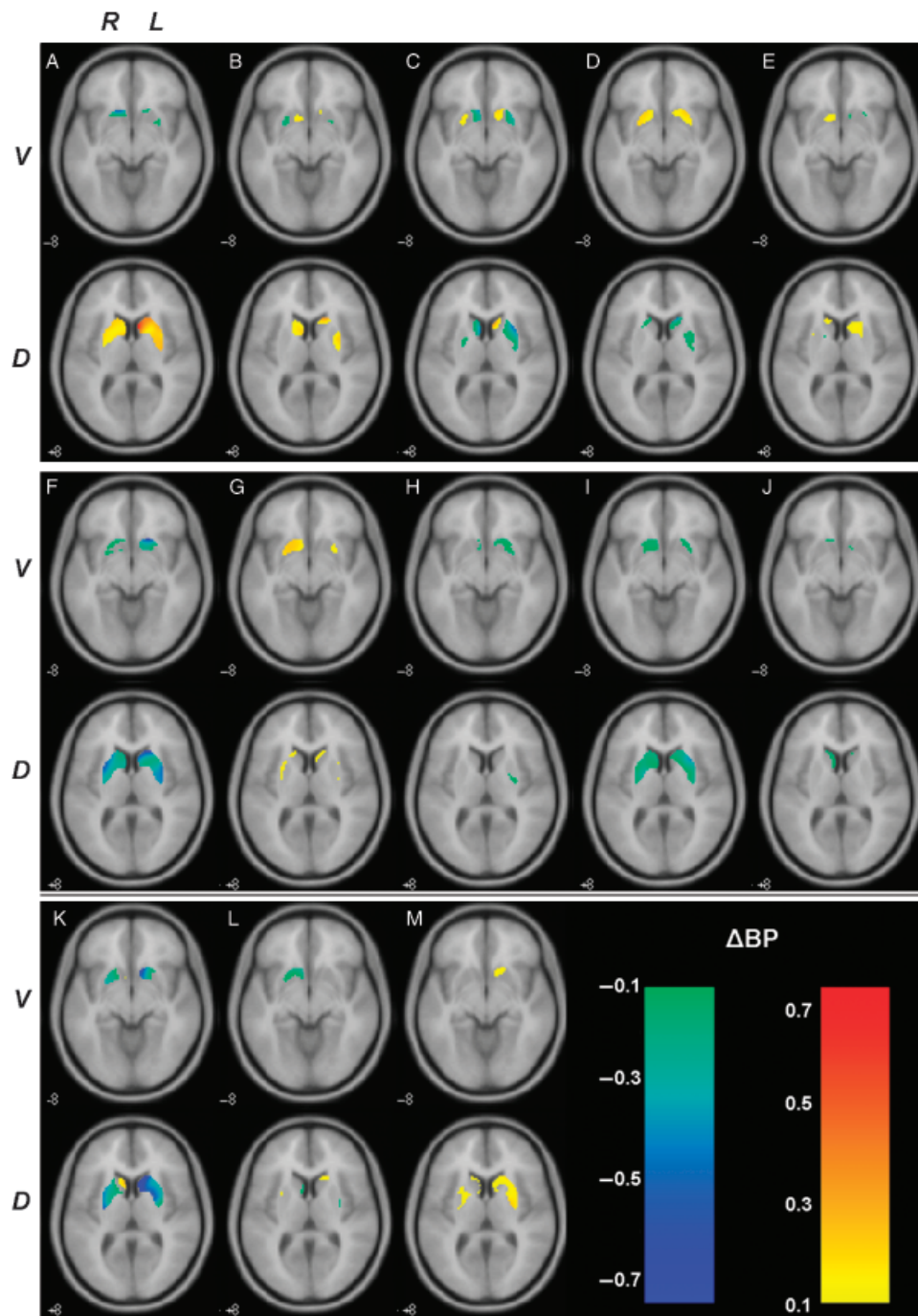
#### Correlations Between DA Responses and Drinking Patterns

There were no significant correlations between the extent or magnitude of the DA responses (unthresholded or thresholded) and drinking habits, which included TLFB (90-day) measurements of total drinks during the 90-day period, drinks per week, total number of drinking days, total number of heavy drinking days, and average number of drinks per drinking day.

## DISCUSSION

Detection of robust dopaminergic system responses to alcohol in humans has not been easy to demonstrate, possibly because of variability in responses across individuals. This appears to be true of our data; there were no consistent effects of alcohol on [ $^{11}\text{C}$ ]RAC BP in the striatum across all subjects. This study describes a method that provides for characterization of the effects of alcohol on [DA] across individuals without requiring that the responses cluster consistently in any one anatomic area. Quantification of  $\Delta BP$  at the voxel level yielded indices of the anatomic extent of DA responses and the magnitude of these responses. Here, we show that the anatomic extent and the magnitude of alcohol-induced DA release in the striatum are related to subjective perceptions of high and intoxication induced by IV alcohol, but were not associated with social drinking patterns. Decreases in [DA] were not correlated with SHAS scores or TLFB variables. As far as we are aware, this study is the first to link alcohol-induced striatal DA release in humans with subjective responses to alcohol.

The proposed method has a particular application when attempting to characterize neurochemical responses whenever intersubject variability may be of concern. Alcoholism is a heterogeneous disorder with many phenotypes, each of which are the culmination of multiple interactions



**Fig. 2.** Striatal change in binding potential ( $\Delta BP$ ) maps demonstrating the wide range of increases and decreases in dopamine concentration ([DA]) in response to IV alcohol. Two axial slices are shown for each subject, one at the level of the ventral striatum ( $-8$  mm ventral to the origin of MNI space) and another 16 mm above the ventral slice ( $+8$  mm dorsal to the origin). Subjects A–I were in the 60 mg% group; subjects J–M received a target alcohol dose of 80 mg%. Positive and negative  $\Delta BP$  values indicate increases and decreases in [DA], respectively. R, right; L, left; V, ventral; D, dorsal.

between genes, environment, personality, brain development, and response to alcohol (Hines et al., 2005; Li, 2000; Matthews et al., 2005). Animal studies, for example, have shown that genetically disparate mouse strains have different functional neuroanatomy and neurochemistry of the basal ganglia, including large variations in markers of dopaminergic function (for review, see Hitzemann et al., 1995). To complicate matters, alcohol itself has a very

large neuropharmacological repertoire, and therefore exerts effects on multiple neurotransmitter systems. It is within this context that the potential for varied striatal DA responses to alcohol exists; we believe our approach may have the requisite sensitivity to detect heterogeneous sets of striatal DA responses. The resulting correlations with the perceived effects of alcohol support the validity of this method.

**Table 2.** Unthresholded Striatal Dopamine Responses

	60 mg%, <i>n</i> = 8	80 mg%, <i>n</i> = 5	<i>p</i>
$\Delta BP > 0$			
DA $\uparrow$ extent	1278 $\pm$ 788	861 $\pm$ 931	0.43
DA $\uparrow$ magnitude	128 $\pm$ 121	56 $\pm$ 82	0.23
$\Delta BP < 0$			
DA $\downarrow$ extent	1237 $\pm$ 788	1654 $\pm$ 931	0.43
DA $\downarrow$ magnitude	-162 $\pm$ 225	-272 $\pm$ 304	0.51

Data are mean  $\pm$  SD of the extent and magnitude of DA responses for the 60 and 80 mg% groups. Dopamine responses are defined either as voxels with  $\Delta BP > 0$  (indicating increases in [DA], DA  $\uparrow$ ) or with  $\Delta BP < 0$  (indicating decreases in [DA], DA  $\downarrow$ ). The *extent* of a DA response is the number of voxels for the respective response; the *magnitude* of a DA response is the sum of  $\Delta BP$  values from the extent voxels of that response. *p*-values are from 2-sample *t*-tests between the groups (equal variances not assumed).

DA, dopamine; [DA], DA concentration.

The ability to characterize neurochemical responses in spite of spatial heterogeneity across people may prove valuable in understanding the effects of alcohol in humans. The correlations between the anatomical extent and/or magnitude of DA release and the subjective effects of alcohol intoxication reported here comport well with other work showing meaningful relationships between drug-induced increases in DA levels and the subjective effects of the drugs, including methylphenidate (Volkow et al., 1999), amphetamine (Abi-Dargham et al., 2003; Drevets et al., 2001; Martinez et al., 2003; Oswald et al., 2005), nicotine (Barrett et al., 2004), and psilocybin (Vollenweider et al., 1999).

There are several limitations to this study. First, the sample sizes for the groups are small. It is possible that a much larger cohort is required to detect anatomically consistent effects of alcohol on [DA]. Second, differences in timing of the alcohol infusion across subjects could confound detection of statistically significant changes in BP (Yoder et al., 2004). However, examination of our data on a case-by-case basis suggests that even similar alcohol infusion protocols do not yield similar results across subjects. Third, the demographics of the subjects were varied, and gender could exert an effect that we do not yet have the power to detect. For example, Munro et al.

**Table 3.** Striatal Dopamine Responses With 10% Threshold

	60 mg%, <i>n</i> = 8	80 mg%, <i>n</i> = 5	<i>p</i>
$\Delta BP \geq 0.1$			
DA $\uparrow$ extent	514 $\pm$ 495	211 $\pm$ 391	0.25
DA $\uparrow$ magnitude	94 $\pm$ 113	28 $\pm$ 50	0.18
$\Delta BP \leq -0.1$			
DA $\downarrow$ extent	553 $\pm$ 823	941 $\pm$ 1011	0.49
DA $\downarrow$ magnitude	-133 $\pm$ 231	-240 $\pm$ 312	0.53

Mean  $\pm$  SD of the extent and magnitude of DA responses for  $\Delta BP$  values  $\geq 0.1$  and  $\leq -0.1$  for the 60 mg% (*n* = 8) and 80 mg% (*n* = 5) groups. DA  $\uparrow$ , increases in [DA]; DA  $\downarrow$ , decreases in [DA]. See text for details. *p*-values are from 2-sample *t*-tests between the groups (equal variances not assumed).

DA, dopamine; [DA], DA concentration.

(2006b) recently demonstrated gender differences in amphetamine-induced increases in [DA]. Fourth, all subjects were made aware that they would receive alcohol immediately before the infusion began. Given the involvement of striatal DA in reward expectation and learning processes, the data could be confounded by reward expectation, prior alcohol experiences, and alcohol-related expectancies. Fifth, the strongest correlational results of this study rest on the assumption that only changes in BP  $> \pm 10\%$  from baseline represent true DA responses. If the actual test-retest variability of the subjects' basal BP were to exceed this threshold, the assumption would be violated; unfortunately, test-retest data were not available for these subjects. Finally, future studies are needed to verify that the anatomical and directional heterogeneity in alcohol-induced DA responses are reliable within individuals.

In summary, we observed heterogeneous striatal DA responses to IV infusion of alcohol that are not easily captured or quantified by traditional methods that require a group of subjects to respond within spatially identical neuroanatomical foci. Our spatially flexible analysis demonstrates that the total number of striatal voxels that exhibit DA release and the sum of  $\Delta BP$  from these voxels appear to be associated with the subjective response to alcohol. With further investigation and validation, our approach could yield important insights into striatal DA function in alcoholism, and into the neurobiology of subjects at risk for alcoholism.

#### Appendix A

This section provides a mathematical explanation of the 10% thresholding. For each voxel, DA responses were placed into 1 of 3 categories, based on the calculated  $\Delta BP$ : increased DA concentration (DA  $\uparrow$ ) and decreased DA concentration (DA  $\downarrow$ ), or no response (neither DA  $\uparrow$  nor DA  $\downarrow$ ). These categories were defined formally as

$$DA \uparrow = \begin{cases} 1, & \Delta BP \geq 0.1 \\ 0, & \Delta BP < 0.1 \end{cases} \quad (1)$$

$$DA \downarrow = \begin{cases} 0, & \Delta BP > -0.1 \\ 1, & \Delta BP \leq -0.1 \end{cases} \quad (2)$$

One dependent variable in this study was the number of striatal voxels that responded to alcohol. Specifically, we used the number of nonzero voxels with either DA  $\uparrow$  or DA  $\downarrow$  to reflect the anatomical extent of the particular DA response. Given the definitions of DA responses (Eqs. 1 and 2), the anatomical extent of DA responses (DA  $\uparrow_E$  and DA  $\downarrow_E$ ) can be expressed as

$$DA \uparrow_E \equiv \sum_{i=1}^{n_s} (DA \uparrow)_i \quad (3)$$

$$DA \downarrow_E \equiv \sum_{i=1}^{n_s} (DA \downarrow)_i \quad (4)$$

where index  $i$  refers to the  $i$ th voxel in an individual, and  $n_s$  is the number of voxels in the striatum.

A second dependent variable was the magnitude of the alcohol effects on  $[DA]$ , which was represented by a summation of the  $\Delta BP$  values for the respective  $DA \uparrow_E$  and  $DA \downarrow_E$ . The magnitude of  $DA$  responses ( $DA \uparrow_M$  and  $DA \downarrow_M$ ) was defined as either

$$DA \uparrow_M \equiv \sum_{i=1}^{n_s} (DA \uparrow)_i (\Delta BP)_i \quad (5)$$

or

$$DA \downarrow_M \equiv \sum_{i=1}^{n_s} (DA \downarrow)_i (\Delta BP)_i. \quad (6)$$

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