Alterations in Brain Serotonin Synthesis in Male Alcoholics Measured Using Positron Emission Tomography

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Background: A consistent association between low endogenous 5HT function and high alcohol preference has been observed, and a number of serotonergic manipulations (uptake blockers, agonists) alter alcohol consumption in animals and humans. Studies have also shown an inverse relationship between alcohol use and cerebrospinal fluid levels of serotonin metabolites, suggesting that chronic alcohol consumption produces alterations in serotonin synthesis or release.

Methods: The objective of the study was to characterize regional brain serotonin synthesis in nondepressed chronic alcoholics at treatment entry in comparison to normal nonalcoholic controls using PET and the tracer α-[^{11}C]-methyl-L-tryptophan.

Results: Comparisons of the alcoholics and controls by SPM found that there were significant differences in the rate of serotonin synthesis between groups. Serotonin synthesis was significantly lower among alcoholics in Brodmann Area (BA) 9, 10, and 32. However, serotonin synthesis among the alcoholics group was significantly higher than controls at BA19 in the occipital lobe and around the transverse temporal convolution in the left superior temporal gyrus (BA41). In addition, there were correlations between regional serotonin synthesis and a quantity-frequency measure of alcohol consumption. Regions showing a significant negative correlation with QF included the bilateral rectus gyri (BA11) in the orbitofrontal area, the bilateral medial frontal area (BA6), and the right amygdala.

Conclusions: Current alcoholism is associated with serotonergic abnormalities in brain regions that are known to be involved in planning, judgment, self-control, and emotional regulation.

Key Words: Serotonin, 5HT, Alcoholism, Positron Emission Tomography, PET, Serotonin Synthesis, α-Methyl-L-tryptophan.

THE ROLE OF serotonin (5HT) in regulating levels of alcohol consumption is an active area of research (Carlson and Drew Stevens, 2006; Sommer et al., 2006). A consistent association between low endogenous 5HT function and high alcohol preference has been observed, and a number of serotonergic manipulations (uptake blockers, agonists, neurotoxins) alter alcohol consumption in animals (Gill and Amit, 1989; Higley et al., 1998; McKenzie-Quirk and Miczek, 2003). In general, manipulations that increase synaptic levels of serotonin reduce alcohol intake. Conversely, lower levels of serotonin have been found in the brains of genetically selected high alcohol-preferring P rats (McBride et al., 1991 and a $5HT_{1B}$ receptor knockout mouse has been shown to consume greater quantities of alcohol compared with wild-type controls (Crabbe et al., 1996). Overall, a number of convergent areas of research have implicated serotonin in the regulation of alcohol consumption in animals.

There may also be an association between alcohol intake and hyposerotonergic activity in humans (LeMarquand et al., 1994a, 1994b; Naranjo and Knoke, 2001). Administration of tryptophan or selective serotonin reuptake inhibitors (SSRIs) have been shown to produce modest decreases in alcohol consumption in humans (Naranjo et al., 1994), although results have been variable (Kranzler et al., 1996). Some studies have focused on identifying patient characteristics such as depression comorbidity that predict the effects of SSRIs on alcohol consumption (Pettinati, 2004). A double-blind, placebo-controlled trial demonstrated that there was no effect of the SSRI sertraline on drinking outcomes for depressed or nondepressed alcoholics (Kranzler et al., 2006). Other studies have demonstrated a positive relationship between the effects of the SSRI citalopram on alcohol intake and the status of central serotonin neurotransmission as assessed by the prolactin response to fenfluramine (Berggren et al., 2001).

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More direct examination of serotonin functioning in alcoholics has been conducted using single photon emission computed tomography (SPECT) and positron emission tomography (PET). Serotonin transporter (SERT) binding measured using SPECT and the ligand [^{123}I]-β-CIT was shown to be lower in the raphe nuclei of male alcoholics (Heinz et al., 1998). The decrease in SERT binding was significantly correlated with lifetime alcohol consumption, and with ratings of anxiety and depression during withdrawal. However, quantitative autoradiography in postmortem samples using [^{123}I]-β-CIT found that SERT binding was higher in the raphe nuclei of both cocaine and alcohol users (Little et al., 1998). The discrepancy between the SPECT and autoradiography results using the same ligand might be due to *in vitro* labeling of additional pools of transporters that may or may not be functional. Using SPECT and [^{123}I]-β-CIT, Heinz and colleagues (2002) demonstrated that decreased SERT binding in recently abstinent alcoholics was only observed among males. SERT levels were significantly correlated with cerebrospinal fluid (CSF) 5-HIAA levels ($r = -0.55$) as well as the severity of depression ($R = -0.46$). Most recently however, no differences in SERT binding were detected using the ligand [^{11}C]-DASB in aggressive and nonaggressive alcoholics compared with controls (Brown et al., 2007).

The role of serotonin in alcoholism has also been examined by behaviors associated with alcohol use such as impulsivity (Fulwiler et al., 2005). Brain concentrations of the serotonin metabolite 5-HIAA were lower in impulsive/anxious individuals who met the criteria for early-onset alcoholism (Virkkunen and Linnoila, 1993). A number of studies have suggested that there is an inverse relationship between alcohol use and CSF levels of the serotonin metabolite 5-HIAA (LeMarquand et al., 1994a,b). Observed decreases in CSF 5-HIAA following chronic alcohol consumption may reflect a decreased availability of serotonin precursors such as tryptophan, a decreased release of serotonin, or a decrease in the rate of serotonin synthesis (Borg et al., 1985). Alterations in serotonin synthesis or release observed in alcoholics may be a vulnerability trait, or a state induced by the chronic effects of alcohol.

To date, there have been no PET studies examining the rate of serotonin synthesis in alcoholics. The present investigation characterized regional brain serotonin synthesis, in nondepressed chronic alcoholics at treatment entry using PET and the tracer α-[^{11}C]-methyl-L-tryptophan (αMTrp) (Diksic and Young, 2001). αMTrp is a synthetic analog of the serotonin precursor L-tryptophan that is taken up into brain serotonergic neurons and it is a substrate for tryptophan hydroxylase. During a PET scan only a small fraction of the tracer is converted to α-[^{11}C]-methyl-serotonin (αM-5HT), but the trapping of the tracer correlates with conversion of tryptophan to 5-HT. The trapping constant K^* (ml/g/min) represents the irreversible uptake of the tracer and metabolite(s) and it has been used to selectively estimate regional rates of brain 5HT synthesis (Diksic and Young, 2001; Nishizawa et al., 1997; Okazawa and Diksic, 1998; Sakai et al., 2006). This is the first report of brain serotonin synthesis in the living human brain of chronic alcoholics.

MATERIAL AND METHODS

Assessment Procedure

Assessment and recruitment were conducted at the Addictions Unit of the McGill University Health Centre (MUHC). The study was approved by the MUHC and Montreal Neurological Institute and Hospital Research Ethics Boards. All subjects signed an informed consent form before being included in the study. Potential study participants (males aged 18 to 50) were identified by the co-investigators in the course of routine clinical assessment at the Addictions Unit. Initial clinical assessment collected detailed information on the pattern of alcohol use and signs of physical dependence, as well as information on other drug use, family/social functioning, medical status, employment/support, legal status and psychological status using the Addiction Severity Index (ASI) (McLellan et al., 1990). The psychometric properties of the ASI have been found to be excellent with high interrater reliabilities ranging from 0.86 to 0.96 and test-retest reliabilities of 0.92. Patients were also routinely asked to fill out questionnaires measuring psychological distress including the Symptom Checklist-90-R (SCL-90-R) and Beck Depression Inventory (BDI). The SCL-90-R is a standardized self-report inventory covering 9 specific areas of psychological distress (e.g., hostility, somatization, depression, anxiety) experienced in the past week. The instrument has been shown to have sound psychometric properties (internal consistency for various subscales range from 0.77 to 0.90; test–retest reliability from 0.78 to 0.90) (Derogatis, 1983). The BDI is a 21 item self-report that rates cognitive, affective, somatic and vegetative symptoms of depression on a 4-point scale, with the total score reflecting overall level of depression experienced in the week prior to the test (Beck and Steer, 1987).

Psychiatric status was determined in a second clinical interview conducted by co-investigators DC, JPB, or JCN, immediately following the assessment interview. Blood tests for standard medical screening to monitor blood (CBC, glucose), liver (ALT, AST, GGT), and thyroid (T4, TSH) function were conducted and a urine sample was collected for toxicology screening.

Application of Study Inclusion/Exclusion Criteria

Following the assessment and psychiatric interviews, the co-investigators reviewed all assessment information in order to apply inclusion criteria. All patients who met criteria for outpatient treatment as well as DSM-IV criteria for alcohol abuse or dependence, were eligible for the study. Patients who were likely to experience withdrawal syndromes, medical complications and/or severe emotional problems (e.g., psychosis, suicidal attempts) necessitating inpatient treatment were excluded. Patients who meet inclusion criteria were asked if they were interested in learning about the PET study, and whether they were willing to speak to the Clinical Research Coordinator (CRC) about participation and informed consent.

Once informed consent was obtained, additional information was collected by the CRC in order to apply all exclusion criteria. (Note that the informed consent explicitly requested the use of all information collected during the Addictions Unit initial assessment, including the results of all routine laboratory tests, for research purposes.) Additional laboratory tests were ordered including albumin/Ca^{++}, plasma vitamin B6, prothrombin, as well as an EKG. The following exclusion criteria were applied once the results of all diagnostic and laboratory tests were obtained:

1. Individuals reporting abuse or dependence (DSM-IV criteria) of any substance other than alcohol or nicotine were excluded.
2. Individuals taking lithium, neuroleptics, antidepressants, anticonvulsants or antianxiety agents (e.g., benzodiazepines) at any time in the past 6 months were excluded.

3. Patients with any history of a neurological condition affecting the CNS, or any current Axis I psychiatric disorder (DSM-IV criteria) were excluded.
4. Subjects with a history of any severe physical illness or abnormalities in the EKG, or abnormalities in laboratory tests for renal, hepatic, hematology, and thyroid function were excluded.
5. Patients who had received previous radiation doses within the past year (over 5 mSv) were excluded.

Subjects

Eight male alcoholic patients (age 38.0 ± 7.3 yr) meeting DSM-IV criteria for alcohol dependence were recruited. The control group consisted of twelve normal male nonalcoholic volunteers (aged 35.0 ± 10.2 years) that were recruited via advertisements. These individuals were extensively screened, and included individuals without any illnesses, including affective disorder. All exclusion criteria listed above were followed for the recruitment of the control group.

PET and MRI

The PET and MRI scans were conducted at the Montreal Neurological Institute. The PET scan was administered within 7 days of assessment and treatment entry at the Addictions Unit. All subjects were asked to refrain from consuming alcohol on the morning of the PET scan. In order to confirm abstinence a detailed account of alcohol/drug intake for the week prior to the scan, as well as a urine sample for toxicology analysis were requested immediately prior to the PET scan. Subjects were scanned using dynamic PET scans on an ECAT HR+ scanner for 60 minutes following the injection of the tracer. A 10-mCi dose of the radiotracer α-[^{11}C]methyl-L-tryptophan (a-MTrp) was administered (total exposure < 5 mSv) intravenously over a 2-minute period in the arm contralateral to one used for blood sampling. All dynamic scans were preceded by a transmission scan for attenuation correction using ^{68}Ga. Thirteen venous blood samples were drawn during the scan, at progressively longer intervals, to obtain a time-radioactivity course in the plasma (input function). Five additional blood samples were drawn to determine the free and total tryptophan levels in the plasma (Nishizawa et al., 1997, 1998).

All of the subjects underwent an MRI (Siemens Vision 1.5; T1-weighted images with 1 mm slice thickness; 160 slices) that was co-registered on the PET images. Co-registration of individual PET and MRI images was performed using an automatic procedure which used averaged tissue activity images obtained from a time period of 5 to 60 minutes of the dynamic PET data (Okazawa and Diksic, 1998; Woods et al., 1993). The MRI images from each subject were transferred into Talairach space automatically (Collins et al., 1994; Talairach & Tourroux, 1988), and co-registration was assessed visually. Using parameters obtained by the automatic co-registration and transformation, the functional PET images were resampled linearly into the stereotaxic coordinate space of Talairach. The PET images were reconstructed with a 5.0 × 5.0 × 5.0 mm resolution, and then blurred to a final resolution of 10 mm FWHM in the transaxial direction using a Gaussian filter. These images were then used for the generation of the regional radioactivity time activity curves or SPM analysis, as described below.

The methods to measure the brain trapping constants K^* [ml/g/min] and its conversion to the 5HT synthesis rate **R** (pmol/g/min) have been described previously (Diksic and Young, 2001; Okazawa and Diksic, 1998). Using the venous sinus-venous plasma normalized input function the K^* values were calculated using the Patlak method. The input function utilized regions of interest (ROIs) of the sinus in the brain images between 0 and 20 minutes and the radioactivity of the venous samples after 20 minutes. The ROIs from different scans were used for between group comparisons (Nishizawa et al., 1997, 1998; Okazawa et al., 2000). It has been shown that this normalization procedure does not introduce any significant bias to the functional images of K^* (Nishizawa et al., 1998).

Because of possible alcohol-induced brain atrophy, the volumes of several brain regions were extracted and statistically compared between controls and patients. A tissue classification algorithm (INSECT) was used to obtain grey and white matter, as well as CSF volumes (Zijdenbos et al., 1998). Subsequently these images were submitted to Automatic Nonlinear Imaging Matching and Anatomical Labeling (ANIMAL) and segmented into 11 anatomical regions (frontal, cingulate, parietal, temporal, occipital and insular cortices, hippocampus, thalamus, globus pallidus, putamen, caudate nucleus). In addition, the K^* values in several brain regions were compared before and after partial volume correction (PVC). The regional brain volumes, as well as the CSF and scalp volumes were used in the K^* PVC. Statistical comparisons of the K^* values before and after PVC (Aston et al., 2002) were conducted using Multivariate Analysis of Variance (MANOVA).

Statistical Parametric Mapping (SPM 2) Analysis

The functional PET images were analyzed with SPM 2. Functional image files were converted to a file format that could be applied to the SPM program, after standardization into Talairach space (Okazawa and Diksic, 1998; Okazawa et al., 2000). To remove the effect of global differences in regional values among the subjects, proportional scaling was used to normalize the images using mean global values (Okazawa et al., 2000). The proportional scaling reduces variance and increases statistical power for subsequent comparisons (Friston et al., 1991). *t*-tests were applied pixel by pixel to compare the regional differences in functional images between the patient and control groups. The *t*-value for each pixel was then converted to a normal standard distribution (z-values), independent of the error degree of freedom as based on the Gaussian random field theory (Worsley et al., 1998). To identify the regions considered to show a significant difference, 2 thresholds were used. First, the height threshold (u) used to interpret the *t*-test in terms of probability levels was set at $p < 0.005$. Secondly, the extent threshold (*k*-number of voxels in a cluster) was set to 80 voxels to remove small noisy clusters, which may reach significance by chance. For analysis of the patient group, correlations with the quantity/frequency (QF) of alcohol consumption in the month prior to the scan were examined with SPM using the height threshold at the cluster level of $p < 0.05$ and the cluster size greater than 80 voxels.

RESULTS

Demographic information and the range of alcohol consumption displayed by the male alcoholic patient group are shown in Table 1. Alcohol consumption was expressed as typical daily intake in grams of absolute ethanol, as well as in standard drink equivalents (e.g., 12 oz of 5%/vol beer, 5 oz of 12%/vol wine, 6 oz wine of 10%/vol wine, or 1.5 oz of 40%/vol spirits). As calculated, the drink equivalents each contain 13.44 grams of absolute ethanol. In addition, a QF value (quantity × frequency) is listed, providing an estimate of the total number of drink equivalents consumed in the month prior to the PET scan.

There was no significant relationship between K^* and age in either the patient or control groups. This is consistent with previous research which showed that K^*, before and after partial volume correction, did not correlate with age (Rosa-Neto et al., 2007).

Table 1. Demographic Information and Range of Alcohol Consumption

Subject	Age	Years problem use	Daily alcohol intake (grams)[a]	Alcohol intake drink equivalents[b]	QF[c]
1	44	19	242	18	540
2	42	19	141	10.5	315
3	26	4	202	15	300
4	40	25	242	18	234
5	48	5	94	7	182
6	36	3	60.5	4.5	134
7	38	13	202	15	129
8	30	15	134	10	86

[a]Typical daily alcohol intake expressed as grams absolute beverage alcohol (ethanol)/day.
[b]Daily alcohol consumption expressed in standard drink equivalents. A standard drink equivalent is based upon 12 oz of 5%/vol beer, 5 oz of 12%/vol wine, 6 oz of 10%/vol wine, or 1.5 oz of 40%/vol spirits, and contains approximately 13.44 g of ethanol.
[c]QF is a Quantity × Frequency estimate of the total amount of alcohol consumed in the month prior to the PET scan (expressed in standard drink equivalents).

Comparison of Brain Volumes

There were no significant differences between groups in terms of brain volume of grey matter [controls: 961 ± 14 ml (mean ± SEM); patients: 933 ± 30 ml] [$F(1,18) = 0.546$, $p = 0.47$]. Similarly there were no significant differences between groups in terms of the volume of white matter [$F(1,18) = 1.446$, $p = 0.245$] or CSF [$F(1,18) = 0.98$, $p = 0.33$]. Regional brain volumes were estimated separately on the left and the right sides and compared using ANOVA (with repeated measures). No significant left-to-right differences were found [$F(1,16) = 2.15$, $p = 0.16$], and thus left and right volumes were averaged for all group comparisons. There were no significant group differences in the regional brain volumes [$F(1,16) = 0.34$; $p = 0.56$], and no group by region interaction [$F(10,160) = 0.55$, $p = 0.85$]. Statistical comparisons of the K^* values before and after PVC found that there was a marginally significant difference in normal controls [$F(1,22) = 4.21$, $p = 0.0495$], but no significant differences among the patient group [$F(1,14) = 0.462$, $p = 0.51$].

There were no significant differences in plasma tryptophan between controls and patients [controls: 9.7 ± 5.2 nmol/ml (free) and 62 ± 33 nmol/ml (total); patients: 11.9 ± 2.9 nmol/ml (free) and 47 ± 16 nmol/ml (total)] nor in global brain serotonin synthesis between the control (4.44 ± 1.10 μL/g/min) and patient (4.96 ± 0.86 μL/g/min) groups. Individual ANOVAs yielded the following F-values: for free tryptophan $F(1,18) = 1.17$; $p > 0.29$; for total tryptophan $F(1,18) = 1.41$; $p > 0.25$; for global K^* $F(1,18) = 1.44$; $p = 0.25$.

Regional Variations in Serotonin Synthesis

Comparisons of the alcoholics and controls by SPM (uncorrected $p < 0.005$ and $k > 80$), found that there were significant regional differences in the rate of serotonin synthesis between groups. In particular, serotonin synthesis was significantly lower among alcoholics compared with the controls in the medial portions, Brodmann Area (BA) 9, 10, and 32. However, serotonin synthesis among the alcoholics group was significantly higher than controls around the transverse temporal convolution in the superior temporal gyrus (BA41) and at BA19 in the occipital lobe and (see Fig. 1).

There were significant negative correlations between QF in the alcoholic group and α-[^{11}C]MTrp normalized K^* trapping values in the bilateral rectus gyri (BA11) ($r = -0.93$, $p < 0.001$) in the orbitofrontal area and the bilateral medial frontal area (BA6) ($r = -0.98$, $p < 0.0001$) (see Fig. 2, note that only clusters on the right side are shown). In addition, a negative correlation was computed in the subcallosal area

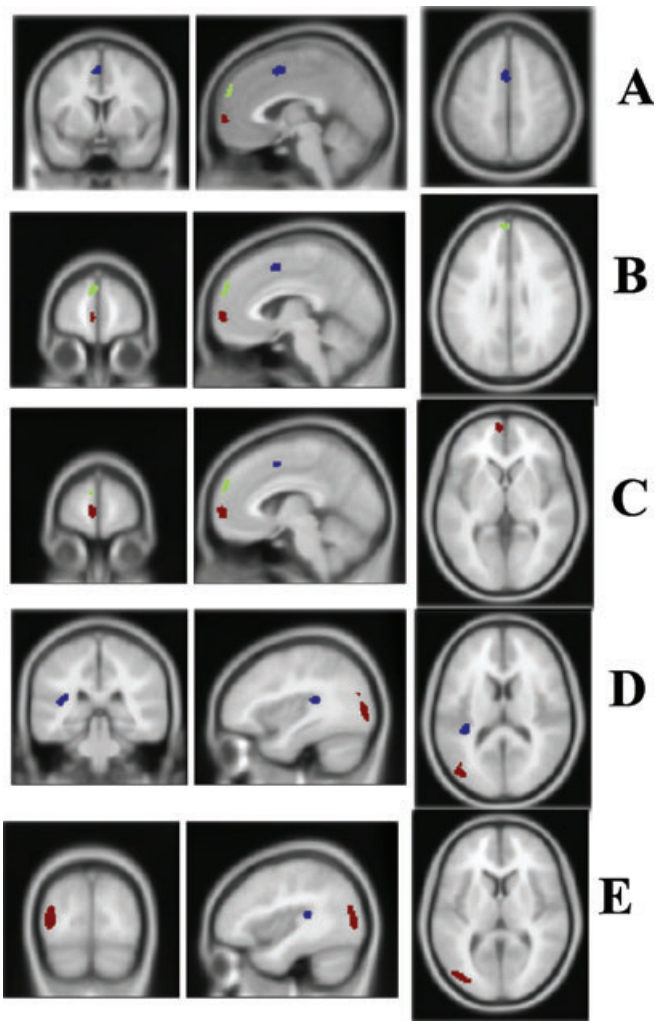

Fig. 1. Serotonin Synthesis: Statistical parametic maps [SPM(t)] showing clusters in Brodmann Area (BA) 32, 9, and 10 (panels **A, B, C,** respectively) where rates of serotonin synthesis were significantly lower among alcoholics compared with normal controls. Significant clusters are superimposed on the rendered MRI images. Note that rates of serotonin synthesis were significantly higher in alcoholics compared with normal controls around the transverse temporal convolution in BA41, the superior temporal gyrus, and in the vicinity of BA19 in the occipital lobe (panels **D** and **E,** respectively).

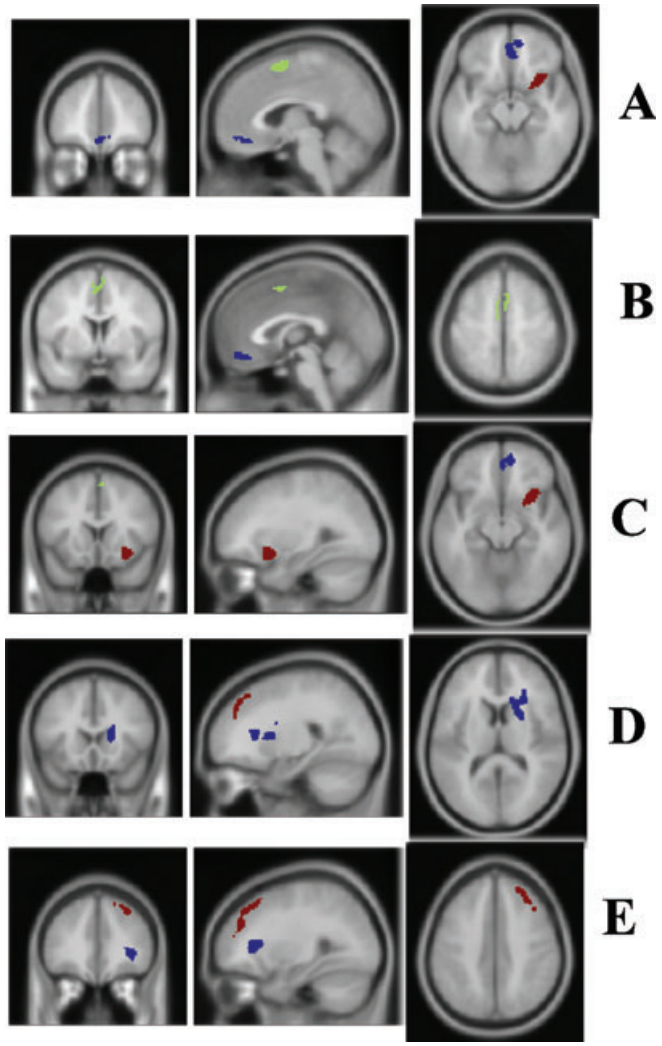

Fig. 2. Correlations between QF and the normalized K^* trapping values ($p < 0.001$). Negative correlations (corrected $p < 0.05$ at the cluster-level) were significant in the left middle frontal gyrus (BA11), the superior frontal gyrus (BA6), and the right inferior frontal gyrus (panels **A, B, C,** respectively). Positive correlations between serotonin synthesis and QF (corrected $p < 0.05$ at the cluster-level) were significant in the right caudate/striatum (panel **D**) and the right lateral prefrontal area (BA8, 9, 10) (panel **E**).

(BA34; Tailarch coordinates 22, 5, −14) around the right amygdala ($r = -0.95$, $p < 0.001$). Significant positive correlations with QF were observed in the right striatum/caudate ($r = 0.96$, $p < 0.001$) and the right lateral prefrontal area (BA8, 9, 10) ($r = 0.95$, $p < 0.001$) (Fig. 2).

DISCUSSION

A number of behavioral and biochemical studies have suggested that there is an effect of alcohol on the brain serotonergic system. The male alcoholic subjects included in this study displayed a wide range of alcohol intake in the month prior to the PET scans, and all subjects had been actively drinking in the week prior to the scan. When comparing brain serotonin synthesis to normal controls, the alcoholic group showed significantly lower rates in BA9 and 10. These Brodmann areas are part of the medial prefrontal cortex which is implicated in planning, self-control, and moderating social behaviors (Knoch and Fehr, 2007). Additionally, serotonin synthesis was lower in the alcoholic group in BA32. This area is a part of the dorsal anterior cingulate gyrus through which the connections between the limbic system and the frontal lobes pass. Comparisons of alcoholics and controls using postmortem whole-hemisphere autoradiography have shown that there is a significant decrease in SERT binding in the dorsal amygdala, the anterior cingulate cortex and striatum (Storvik et al., 2006, 2007). The results from SERT binding studies suggest that there are serotonergic abnormalities in the cortical-striatal-thalamic axis among alcoholics (Storvik et al., 2006). Indirect measures of serotonergic functioning in alcoholics also support this interpretation. In studies utilizing m-Chlorophenylpiperazine (mCPP), a partial $5HT_{2C}$ agonist, regional glucose utilization measured using FDG-PET was demonstrated to be lower among alcoholics compared with controls. Alcoholics showed a blunted neuroendocrine response and less regional activation in the orbital and prefrontal cortices following m-CPP challenge (Hommer et al., 1997). The authors suggested that hyporesponsivity to m-CPP

Table 2. Identification of Significant Clusters Shown in Figs 1 and 2

Row	Talairach coordinates (mm)	Brodmann anatomical area	*t*-value	*z*-value
Figure 1—Regional group differences by SPM ($p < 0.005$)				
A	−4 8 46	BA 32; left medial frontal gyrus	3.78	3.20
B	−4 58 25	BA9; left superior frontal gyrus	4.70	3.75
C	−6 58 −1	BA10; left superior frontal gyrus	5.29	4.06
D	−36 −32 16	BA41; left superior temporal gyrus	3.81	3.22
E	−40 −79 8	Close to BA19; left middle occipital gyrus	5.62	4.22
Figure 2—Correlations between QF and the normalized K^* trapping values ($p < 0.001$)				
A	−2 36 −15	BA11; left middle frontal gyrus	9.59	3.96
B	4 9 55	BA6; right superior frontal gyrus	20.75	4.93
C	28 13 −9	BA13; right inferior frontal gyrus	10.70	4.11
D	18 −1 26	Right caudate/striatum	13.27	4.39
E	32 33 39	BA8, 9, and 10; right lateral prefrontal area	9.63	3.96

may reflect reductions in regional brain serotonergic activity among alcoholics, as confirmed in the present study.

In addition to decreases in the brain regions involved in behavior mentioned above, 5-HT synthesis was significantly higher among the alcoholic group in the occipital region and the superior temporal gyrus. Alcoholic patients can experience delirium, including visual and auditory hallucinations during alcohol withdrawal (First et al., 1995). BA19 is a visual association area with multimodal integrating functions. The region around the transverse temporal convolution is close to the primary auditory receptive area. The alcoholic group had abstained from alcohol on the morning of the PET scan, however, none reported discomfort and it is unlikely that the alcoholic group were in withdrawal at the time of the PET scan. Statistical comparisons of the K^* values before and after PVC found that there were no significant differences among the patient group. In addition, there were no significant differences in the overall or regional brain volumes between controls and patients. These findings suggest that there was no significant brain atrophy in the patient group. This may be related to the relatively young age of the patient group, the small number of patients studied and/or the wide range of years of alcohol abuse (Table 1).

There were no alterations in serotonin synthesis detected in the raphe nucleus. Most recently, a morphometric analysis of dorsal raphe serotonin neurons in postmortum samples of alcoholics showed that there was no variation in serotonin cell counts (Underwood et al., 2007), however, there was a significant increase in tryptophan hydroxylase immunoreactivity. This suggested that there may be a compensatory response to impaired serotonergic transmission within the dorsal raphe among alcoholics. This was not confirmed in the present study, however, the dorsal raphe is a rather small structure in the relation to the resolution of the PET scanner used (e.g., around 6 mm) and given the stringent statistical criteria used in the comparisons (e.g., image resolution, cluster size) variations in the dorsal raphe may have been missed.

Correlational analysis between regional serotonin synthesis and QF yielded both positive and negative associations. QF is an estimate of the total alcohol consumed during the month prior to the scan. Significant negative correlations were observed in the amygdala and bilateral orbitofrontal areas, indicating that higher QF alcohol consumption was associated with lower serotonin synthesis. Severe alcoholism often leads to a number of additional symptoms including depression and anxiety as well as a higher rate of suicidality (Roy and Janal, 2007). Leyton and colleagues (2006) found lower α-[^{11}C]-methyl-L-tryptophan trapping (an index of 5-HT synthesis) in the orbital and ventromedial prefrontal cortex (BA11) in a group of patients who had attempted suicide. The suicidal group included a number of individuals with a history of drug or alcohol abuse, and it appears possible that low 5HT synthesis in these regions may be common to more than one psychiatric syndrome. This is not unexpected given the large degree of comorbidity and symptom overlap among disorders, as well as the potential for other overlapping etiological and genetic factors. The comorbidity between alcoholism and depression is significant from a clinical viewpoint (Charney et al., 2005), and it has not been possible to determine whether alcoholism is distinct from depression in terms of state and/or trait-dependent effects on the serotonin system. Note, however, that the alcoholic group in the current study were not depressed at the time of the PET scan, as evidenced by clinical interview and self-report measures.

On the other hand, a positive correlation between serotonin synthesis and QF was observed in the right striatum, suggesting that serotonin synthesis was increased by alcohol consumption. Chronic alcohol consumption by alcohol-preferring P rats has been shown to produce alterations in $5HT_3$ receptor function and activity of the mesolimbic DA system (McBride et al., 2004). Nigrostriatal dopaminergic (DA) neurons project to the striatum and interactions between serotonin and dopamine have been reported (Liu et al., 2006; McBride et al., 2004), although they are complex involving both inhibitory and excitatory actions (Esposito, 2006).

In summary, alcoholics have altered rates of serotonin synthesis in several brain regions including the prefrontal cortex, and negative correlations between regional serotonin synthesis and a quantity-frequency measure of alcohol consumption were observed in the amygdala and bilateral orbitofrontal region. The results suggest alcoholism is associated with serotonergic abnormalities in brain regions that are known to be involved in planning, judgment, self-control, and emotional regulation.

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