Published in final edited form as:
*Am J Psychiatry*. 2014 August 1; 171(8): 881–888. doi:10.1176/appi.ajp.2014.13121581.

# Prefrontal cortical dopamine transmission is decreased in alcoholism

**Rajesh Narendran, M.D.**[1,2], **Neale Scott Mason, Ph.D.**[1], **Jennifer Paris, MEd, MSL.**[2], **Michael L. Himes, B.S.**[2], **Antoine B. Douaihy, M.D.**[2], and **W. Gordon Frankle, M.D.**[1,2]

[1]Department of Radiology, University of Pittsburgh, Pittsburgh, PA

[2]Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA

## Abstract

**Objective—**Basic studies have demonstrated that optimal levels of prefrontal cortical dopamine are critical to various executive functions such working memory, attention, inhibitory control and risk/reward decisions--all of which are impaired in addictive disorders such as alcoholism. Based on this and imaging studies in alcoholics that have demonstrated less dopamine in the striatum, we hypothesized decreased dopamine transmission in the prefrontal cortex in alcoholism. To test this hypothesis, we used amphetamine and [$^{11}$C]FLB 457 positron emission tomography (PET) to measure cortical dopamine transmission in a group of 21 recently abstinent alcoholics and matched healthy controls.

**Methods—**[$^{11}$C]FLB 457 binding potential ($BP_{ND}$) was measured in subjects with kinetic analysis using the arterial input function both before and after 0.5 mg $kg^{-1}$ of d-amphetamine.

**Results—**Amphetamine-induced displacement of [$^{11}$C]FLB 457 binding potential ($\Delta BP_{ND}$) was significantly smaller in the cortical regions in alcoholics compared to healthy controls. Cortical regions that demonstrated lower dopamine transmission in alcoholics included the dorsolateral prefrontal cortex, medial prefrontal cortex, orbital frontal cortex, temporal cortex and medial temporal lobe.

**Conclusions—**The results of this study for the first time unambiguously demonstrate decreased dopamine transmission in the cortex in alcoholism. Further research is necessary to understand the clinical relevance of decreased cortical dopamine as to whether it is related to impaired executive function, relapse, and outcome in alcoholism.

### Keywords

[$^{11}$C]FLB 457; amphetamine; alcoholism; $D_{2/3}$; dopamine; PET

Correspondence R. Narendran, MD Department of Radiology and Psychiatry University of Pittsburgh University of Pittsburgh PET Facility UPMC Presbyterian, B-938 Pittsburgh, PA 15213 Telephone: (412)-647-5176 Fax: (412)-647-0700 narendranr@upmc.edu.

DISCLOSURES

Drs. Narendran, Mason and Frankle have done contractual research work at the University of Pittsburgh for ONO Pharmaceuticals Co., LTD

## INTRODUCTION

Prefrontal cortical dopamine modulates executive functions such as attention, working memory, and risk/reward decision making (1, 2)--all of which are impaired in alcoholism (3-6). Based on this, it is tempting to postulate decreased dopamine transmission in the prefrontal cortex in alcoholism. Unfortunately, the preclinical literature on this topic is mixed and inconclusive with some studies suggesting increased (7), decreased (8, 9) and no change (10-12) in prefrontal cortical dopamine transmission in alcoholism. Nevertheless, the ability of prefrontal cortical dopamine to modulate alcohol consumption has been demonstrated in microinjection studies using dopamine $D_{2/3}$ antagonist and agonist drugs (13, 14). In humans, the displacement of the $D_{2/3}$ specific PET radiotracer [$^{11}$C]raclopride following an acute amphetamine (or methylphenidate) challenge has been validated as a noninvasive measure of the change in extracellular dopamine concentration induced by the challenge (15). Using this approach, two groups have reported decreased striatal dopamine transmission in alcohol dependent subjects compared to healthy controls (16, 17). A limitation of these studies was that measurements of dopamine transmission were restricted to the striatum and its subdivisions, i.e., caudate, putamen and ventral striatum. Studies were limited to the striatum, because [$^{11}$C]raclopride does not provide sufficient signal-to-noise ratio to quantify $D_{2/3}$ receptors in extrastriatal areas, such as the cortex, where the concentration of $D_{2/3}$ receptors is much lower than in the striatum. Thus, no previous studies have reported on the *in vivo* status of dopamine in the prefrontal cortex in alcoholism.

We recently validated the high affinity $D_{2/3}$ PET radioligand [$^{11}$C]FLB 457 as a tool to image amphetamine-induced dopamine transmission in the human cortex (18). The results of these validation studies demonstrate: low test-retest variability (≤ 15%) for [$^{11}$C]FLB 457 binding potential ($BP_{ND}$) under both baseline and post-amphetamine conditions (19, 20); no carryover mass-induced decrease in $BP_{ND}$ in the imaging paradigm used to measure dopamine (19); a relatively small fraction of $D_{2/3}$ receptor specific binding for [$^{11}$C]FLB 457 in the cerebellar reference region compared to cortical regions of interest (21); and a linear relationship between the amphetamine-induced decreases in [$^{11}$C]FLB 457 $BP_{ND}$ and increases in extracellular dopamine as measured with microdialysis (22). Here, we used amphetamine and [$^{11}$C]FLB 457 PET to contrast cortical dopamine transmission in 21 recently abstinent subjects with alcohol dependence and 21 healthy comparison subjects matched for age, gender, race, and nicotine smoking status.

## MATERIALS AND METHODS

### Human Subjects

Seventy-seven alcohol dependent subjects and 36 healthy controls were enrolled in the study to arrive at 21 completers/group. The study was conducted following the approvals of the University of Pittsburgh Institutional Review Board and Radioactive Drug Research Committee. All subjects provided written informed consent. Alcohol dependent subjects and healthy controls were largely recruited through advertisements displayed at local community centers, buses, newspapers and web sites. In addition, addiction medicine clinics and hospital emergency rooms in the community also referred alcohol dependent subjects. Study criteria for alcohol dependence were [1] males or females between 18 and 40 years old of all

ethnic and racial origins; [2] fulfill DSM-IV criteria for alcohol dependence as assessed by SCID; [3] no current or past DSM-IV Axis I disorder other than alcohol abuse or dependence, including abuse or dependence to other recreational drugs (nicotine dependence was allowed); [4] no current (as confirmed by urine drug screen at screening) use of cocaine, opiates, cannabis, sedative-hypnotics, amphetamines, 3,4-methylenedioxy-N-methylamphetamine, and phencyclidine; [5] not currently on any prescription or over the counter medications; [6] no current or past chronic medical or neurological illnesses (including glaucoma, seizure disorders, a focal finding on MRI such as stroke or tumor, chronic obstructive pulmonary disease or respiratory disease, renal problems, and liver problems) as assessed by a complete physical exam and labs; [7] no resting systolic blood pressure > 140 and diastolic blood pressure > 90; [8] no more than one risk factor for coronary artery disease (e.g., smoking, obesity, cholesterol > 240 mg $dl^{-1}$, sedentary life style etc.); [9] no first-degree relative with a psychotic or mood disorder; [10] not currently pregnant; [11] no history of radioactivity exposure from nuclear medicine studies or occupation; [12] no metallic objects in the body that are contraindicated for magnetic resonance imaging (MRI).

Alcohol dependent subjects completed a minimum of 14-days of outpatient abstinence monitored with witnessed urine toxicology. Subjects were monitored for alcohol and recreational drug use with urine alcohol metabolite (ethyl glucuronide and ethyl sulfate) and urine drug screens three times/week for two consecutive weeks. Since alcohol metabolites and common drugs of abuse can be detected for 2 to 3 days after use, subjects were informed that they should not use alcohol or street drugs for the 14 days prior to the PET study. In order to promote abstinence from alcohol during this two-week period, subjects were paid $75 for each urine sample that was negative for ethyl glucuronide and ethyl sulfate. Alcohol dependent subjects were scheduled for the PET scans after successful completion of the abstinence monitoring protocol. Subjects who tested positive for ethyl glucuronide and ethyl sulfate were offered up to three attempts to re-start the abstinence monitoring protocol. This abstinence monitoring protocol ensured that all subjects were abstinent for a minimum of two weeks prior to the PET scan. Alcohol dependent subjects were also monitored for alcohol withdrawal signs and symptoms three times/week during the first week of abstinence using the Clinical Institute Withdrawal Assessment of Alcohol Scale (23). Alcohol dependent subjects who were at risk of severe withdrawal, i.e., scored greater than 19 on the Clinical Institute Withdrawal Assessment of Alcohol Scale and had prior history of alcohol withdrawal seizures or delirium tremens were excluded from the research protocol. The severity of alcohol dependence was assessed in with the Michigan Alcohol Screening Test (24) and Alcohol Dependence Scale (25).

Healthy control subjects matched for age, gender, ethnicity and smoking status had no past or present neurological or psychiatric illnesses including substance abuse (confirmed by urine drug screen both at screening and the day of the PET scan). Healthy controls and alcohol dependent subjects underwent the PET scans as outpatients. Following the completion of the PET scans, alcohol dependent subjects were scheduled for a follow up visit during which they were debriefed of the risks of alcohol abuse and provided a referral for outpatient treatment.

### Image acquisition and analysis

Following a structural MRI, subjects underwent a baseline and a post-amphetamine [$^{11}$C]FLB 457 PET scan in the same experimental session using procedures described in (18).

Briefly, [$^{11}$C]FLB 457 was synthesized using the methodology reported by Halldin, et al. (26). PET imaging sessions were conducted with the ECAT EXACT HR+ camera. Following a transmission scan, subjects received an intravenous bolus injection of [$^{11}$C]FLB 457 and emission data was collected for 90 min. Arterial blood samples were collected to measure the plasma free fraction ($f_P$) for [$^{11}$C]FLB 457 and derive a metabolite corrected arterial input function for modeling using methods described previously (18). The maximum injected mass for [$^{11}$C]FLB 457 was restricted to 0.6 μg (27). The post-amphetamine [$^{11}$C]FLB 457 scan was performed 3 hours after the administration of 0.5 mg kg$^{-1}$ of oral d-amphetamine. During this scan, amphetamine blood levels were measured in three arterial samples drawn at time 0 min, 45 min and 90 min and analyzed using methods described in (28).

PET data were reconstructed and processed with the image analysis software MEDx (Sensor Systems, Inc., Sterling, Virginia) and SPM2 (www.fil.ion.ucl.ac.uk/spm) as described in (18). Frame-to-frame motion correction for head movement and MR-PET image alignment were performed using a mutual information algorithm implemented in SPM2. MRI segmentation was performed using the automated segmentation tool in Functional MRI of the Brain Software Library (29). Cortical (medial temporal lobe, dorsolateral prefrontal cortex, orbital frontal cortex, medial prefrontal cortex, anterior cingulate cortex, temporal cortex, parietal cortex, and occipital cortex) and subcortical (midbrain and cerebellum) regions of interest were defined on the MRI using a segmentation-based and direct identification method described in (19, 30, 31). Regional volumes and time activity curves were then generated in MEDx as outlined in (30, 31). Primary analysis included the eight cortical regions that had been validated in our previous [$^{11}$C]FLB 457 human studies (18-21). Secondary analysis included the midbrain as a region of interest to test if there is convergence between the midbrain dopamine cells and terminal fields. Derivation of [11C]FLB 457 distribution volume ($V_{T)}$ in the regions of interest ($V_{T\ ROI}$) and cerebellum ($V_{T\ CER}$) was performed using a two-tissue compartment kinetic analysis using the arterial input function as described in (18).

PET outcome variables are defined in accordance to the consensus nomenclature for *in vivo* imaging of reversibly binding radioligands (32). $D_{2/3}$ receptor availability at baseline and post-amphetamine was estimated using $BP_{ND}$, i.e., binding potential relative to non-displaceable uptake, which was derived as

$$BP_{ND} = \frac{V_{T\ \ ROI} - V_{T\ \ CER}}{V_{T\ \ CER}} = f_{ND} * \frac{B_{avail}}{K_D} \quad \text{Eq 1}$$

where, $f_{ND}$ ($=f_P/V_{T\ CER}$) is the free fraction of [$^{11}$C]FLB 457 in the non-displaceable compartment, $B_{avail}$ is the density of $D_{2/3}$ receptors (nmol L$^{-1}$) available to bind to

[$^{11}$C]FLB 457 *in vivo*, $K_D$ is the *in vivo* equilibrium dissociation constant of [$^{11}$C]FLB 457 (nmol $L^{-1}$)

The amphetamine-induced change in $BP_{ND}$ ($\Delta BP_{ND}$) was calculated as the difference between $BP_{ND}$ measured in the post-amphetamine condition ($BP_{ND\ AMPH}$) and $BP_{ND}$ measured in the baseline condition ($BP_{ND\ BASE}$), and expressed as a percentage of $BP_{ND\ BASE}$:

$$\Delta BP_{ND} = 100 * \frac{BP_{ND_{AMPH}} - BP_{ND_{BASE}}}{BP_{ND_{BASE}}} \quad \text{Eq 2}$$

Finally, as our validation work with [$^{11}$C]FLB 457 demonstrated $D_{2/3}$ specific binding in the cerebellum there was concern that any amphetamine-induced change in $V_{T\ CER}$ could bias the dopamine-release outcome measure, $\Delta BP_{ND}$ ($BP_{ND}$ is dependent on $VT_{\ CER,}$ see Eq 1). Therefore, to arrive at a dopamine release measurement in the cortex (i.e., $D_{2/3}$ receptor occupancy following amphetamine) that is independent of $V_{T\ CER}$, we analyzed the baseline and post-amphetamine $V_T$ values from the eight cortical regions of interest with Lassen plots as described in (33). Briefly, the equation for the line [y=mx+b], where $y=[V_{T\ BASELINE} - V_{T\ AMPHETAMINE}]$, and $x=V_{T\ BASELINE}$, produced a linear relationship with slope of line equal to receptor occupancy (m). This approach assumed that there is uniform receptor occupancy across the cortical regions.

### Statistical analysis

Comparison between scan conditions (baseline vs. post-amphetamine) was performed with paired t-tests. Comparisons between groups (alcohol dependence vs. healthy controls) were performed with unpaired t-tests (regions of interest level) and repeated measures of ANOVA (amphetamine blood levels). Furthermore, to test for a global effect of diagnosis (alcohol dependence vs. healthy controls) on baseline cortical $BP_{ND}$ and $\Delta BP_{ND}$, a linear mixed model analysis was performed with cortical regions of interest as a repeated measure and diagnostic group as the fixed factor (IBM SPSS Statistics). Relationship between PET data and clinical characteristics (years of drinking, amount of drinks/day, Alcohol Dependence Scale and Michigan Alcohol Screening Test) were assessed by Pearson product moment correlation coefficient. A two-tailed probability value of $p < 0.05$ was selected as the significance level for all analyses. A false discovery rate correction with $\alpha = 0.05$ was applied to correct for multiple comparisons in the regions of interest (34).

## RESULTS

Twenty-one alcohol dependent subjects were matched with 21 healthy controls on age, gender, ethnicity and smoking status (including the number of cigarettes smoked per day rounded to the nearest one-half pack, i.e., 10 cigarettes). **Table 1** lists demographics variables and measures of alcohol use in alcohol dependent subjects.

## Scan parameters

**Table 2** shows the [$^{11}$C]FLB 457 scan parameters for healthy controls and alcohol dependent subjects under baseline and post-amphetamine conditions. No significant differences between the baseline and post-amphetamine condition were observed in any of these scan parameters in healthy controls and alcohol dependent subjects. Notably, there was no significant change in $V_{T\ CER}$ following amphetamine in both groups ($\Delta V_{T\ CER}$, healthy controls = −2.5 ± 15.1%; alcohol dependence = −0.9 ± 10.8%, t=0.41, df= 40, p=0.68). This justified the use of $\Delta\ BP_{ND}$ to contrast differences in amphetamine-induced dopamine transmission between the healthy controls and alcohol dependence group (see Eq 1, refer to 35).

The amphetamine blood levels measured at time, t =0, 45 and 90 min relative to post-amphetamine [$^{11}$C]FLB 457 scan in healthy controls (80 ± 10, 73 ± 9 and 70 ± 10 ng $mL^{-1}$) was not significantly different from that measured in alcohol dependent subjects (75 ± 8, 69 ± 7 and 66 ± 7 ng $mL^{-1}$; repeated measures of ANOVA, effect of diagnosis: F=3.08, df=1, p =0.09; effect of time: F = 57.20, df=2 , p <0.001; diagnosis x time interaction: F=0.07, df=2, p =0.93).

## Regional volumes

No between-group differences were found in the cortical regions, midbrain and cerebellum volumes determined from the MRI scans (**data not shown**, all p-values ≥ 0.2), suggesting lack of measurable volumetric changes in alcohol dependence.

## Cortex

**$D_{2/3}$ receptor availability ($BP_{ND}$) under baseline conditions—**As shown in **Figure 1**, no differences in baseline [$^{11}$C]FLB 457 $BP_{ND}$ were observed in alcohol dependence compared to healthy controls (linear mixed model, effect of diagnosis, F (1, 40) = 0.89, p =0.35; effect of region, F (7, 280) = 332.65, p < 0.001; region x diagnosis interaction, F (7, 280) = 1.71, p = 0.11). In addition, unpaired t-tests conducted at the level of the individual regions of interest failed to show any significant differences between the two groups.

**Amphetamine-induced reduction in $D_{2/3}$ receptor availability ($\Delta\ BP_{ND}$)—** Amphetamine led to a significant reduction in [$^{11}$C]FLB 457 $BP_{ND}$ in healthy controls (**Figure 2, left panel**), but not in alcohol dependence (**Figure 2, right panel**). The amphetamine-induced **Δ** [$^{11}$C]FLB 457 $BP_{ND}$ was significantly lower in alcohol dependence compared to healthy controls (linear mixed model, effect of diagnosis, F (1, 40) = 11.03, p =0.002; effect of region, F (7, 280) = 1.99, p < 0.056; region x diagnosis interaction, F (7, 280) = 0.65, p = 0.71). The inclusion of mean amphetamine blood levels as a co-variate in the model did not change the significance of the results (linear mixed model, effect of diagnosis (F (1, 39) = 9.44, p=0.004). Unpaired t-tests conducted at the level of the individual regions of interest were significant in all of the cortical regions except the anterior cingulate cortex (see **Table 3**). All significant comparisons, except in the parietal and occipital cortex survived the false discovery rate correction. Also, consistent with the Δ $BP_{ND}$ results, the amphetamine-induced dopamine release's occupancy of $D_{2/3}$ receptors (derived using Lassen plots that is independent of $V_{T\ CER}$) was significantly lower in

alcohol dependent subjects compared to healthy controls (healthy controls = 16.0 ± 15.6%; alcohol dependence = −1.2 ± 19.5%, t= −3.16, df=40, p=0.003).

### Midbrain

**$D_{2/3}$ receptor availability ($BP_{ND}$) under baseline conditions—**No significant differences were observed in midbrain [$^{11}$C]FLB 457 $BP_{ND}$ in alcohol dependence compared to healthy controls ($BP_{ND}$: healthy controls = 2.50 ± 0.62; alcohol dependence = 2.23 ± 0.38, t= −1.67, df= 40, p=0.10).

**Amphetamine-induced reduction in $D_{2/3}$ receptor availability ($\Delta BP_{ND}$)—** Amphetamine led to a significant reduction in [$^{11}$C]FLB 457 $BP_{ND}$ in the midbrain in healthy controls (baseline = 2.50 ± 0.62; post-amphetamine = 2.09 ± 0.37, t=3.16, df =20, p=0.005), but not in alcohol dependence (baseline = 2.23 ± 0.38; post-amphetamine = 2.35 ± 0.63, t= −1.49, df =20, p=0.15). The amphetamine-induced **Δ** [$^{11}$C]FLB 457 $BP_{ND}$ in the midbrain was significantly lower in alcohol dependence compared to healthy controls ($\Delta BP_{ND,}$ healthy controls = −13.2 ± 15.2%; alcohol dependence = 5.0 ± 14.6%, t = 3.91, df= 40, p=0.0003). This comparison survived the false discovery rate correction.

### Clinical correlations

Correlation analyses revealed no significant associations between **Δ** $BP_{ND}$ in the regions of interest (cortex and midbrain) and mean amphetamine blood levels in healthy controls and alcohol dependence. In addition, there was no significant associations between **Δ** $BP_{ND}$ and any of the clinical measures (alcohol frequency, amount, duration of abuse, Michigan Alcohol Screening Test, or Alcohol Dependence Scale scores) in alcohol dependence.

## DISCUSSION

In this PET study, we found less displacement of [$^{11}$C]FLB 457 $BP_{ND}$ in the cortex and midbrain after amphetamine in recently abstinent alcoholics compared to healthy controls. In a previous study using PET and microdialysis, it was shown that 1% displacement of [$^{11}$C]FLB 457 $BP_{ND}$ in the cortex corresponds to a 57% increase in extracellular dopamine concentration (22). Extending this relationship to the current dataset (mean $\Delta BP_{ND}$ in healthy controls = −9 to −14%; alcohol dependence = +9 to −4% in **Table 4**) suggests that cortical dopamine in healthy controls and alcohol dependent subjects increases by ~513-798% and 0-228% respectively following the same dose of amphetamine. This result for the first time unequivocally demonstrates that there is decreased dopamine transmission in the cortex in alcoholism. These data also for the first time show convergence between the midbrain dopamine cells and terminal fields with respect to decreased dopamine transmission in alcoholism. Such a blunting in mesocortical dopamine transmission in alcoholics is consistent with what has been previously reported in the nigrostriatal system that includes the limbic-related nucleus accumbens (16, 36). Decreased dopamine transmission in the mesolimbic regions, such as the ventral striatum and medial temporal lobe, likely contributes to anhedonia, amotivation, and decreased reward sensitivity in alcohol dependence. This has led to the conceptualization of alcohol dependence as a reward-deficit disorder with a higher reward threshold for both natural and drug/alcohol

reinforcers (37, 38). The fact that there is also less dopamine in the prefrontal cortex, which governs executive functions, is important because it could impair the addict's ability to learn and utilize informational/behavioral strategies critical to relapse prevention. This is supported by literature that links prefrontal cortical dopamine with executive functions, such as attention, working-memory, behavioral flexibility and risk/reward decision-making --all of which are impaired in addictive disorders such as alcoholism (3, 39). Floresco and Magyar, in a study using a rodent version of the Iowa Gambling Task (a task that measures risk preference decision-making), demonstrated that blocking dopamine transmission in the prefrontal cortex leads to a response decision that fails to integrate the consequences of conditioned punishment (39). Based on this study, it is tempting to speculate that the failure to incorporate past negative consequences in a decision to drink alcohol during abstinence is related to decreased prefrontal cortical dopamine in alcoholism. If this hypothesis were confirmed, it would support a role for medications that increase prefrontal cortical dopamine to prevent relapse in alcoholism.

Alcohol-induced potentiation of GABA, the major inhibitory transmitter in the brain, inhibits GABA-ergic interneurons in the ventral tegmental area and substantia nigra, and leads to increased phasic (or synaptic) dopamine transmission (40, 41). However, chronic and repeated use of alcohol leads to decreased phasic dopamine via adaptations in the tonic (or extracelluar) dopamine and glutamatergic systems in the cortico-limbic pathways (for detailed review, refer to 40). If decreased dopamine transmission is the result of an adaptation in the cortico-limbic circuits, it might be possible to reverse this deficit in alcoholics with prolonged abstinence. On the other hand, alcoholics also demonstrate signs of inflammation (i.e., greater activated microglia and pro-inflammatory cytokines) and a reduction of dopamine neuronal markers in the brain (42, 43). Therefore, the possibility of a toxic irreversible loss of dopamine neurons in alcoholism cannot be ruled out. This may explain the persistent and enduring cognitive impairments that have been reported in abstinent alcoholics (44). Also unclear is whether decreased dopamine transmission in alcoholism represents a premorbid trait or alcohol-induced state. Future dopamine imaging studies in recovering alcoholics with prolonged periods of abstinence and non-human primates that can be imaged both pre- and post- alcohol exposure are necessary to evaluate these issues.

Another interesting observation in this study is the lack of differences in baseline $D_{2/3}$ receptor binding potential in both the cortex and midbrain in alcoholics compared to controls (Figure 1). This is in contrast to previous [$^{11}$C]raclopride imaging studies that have reported ~10-20% decrease in $D_{2/3}$ receptor $BP_{ND}$ in the striatal subdivisions in alcoholics (16, 36). The exact reason and physiological relevance for decreased $D_{2/3}$ receptor $BP_{ND}$ in the striatum, but not in the extrastriatal regions in alcoholism is not clear. Reasons that may have contributed to the inability to detect group differences in this study include diminished power due to greater between-subject variability in cortical $D_{2/3}$ receptor binding potential and/or a more pronounced reduction in baseline dopamine levels (i.e., prior to amphetamine stimulation) in the cortex compared to striatum in alcoholics. PET studies in alcoholism with alpha-methyl-para-tyrosine that can deplete baseline dopamine in the striatal and extrastriatal regions are necessary to further understand this issue.

The strengths of this study are: inclusion of relatively young individuals (≤ 40 years) with mild to moderate alcohol dependence; exclusion of individuals with comorbid medical, psychiatric or drug abuse; monitored abstinence prior to imaging; use of a validated imaging paradigm to measure cortical dopamine transmission; use of compartmental modeling with an arterial input function to derive PET outcome measures; ruled out changes in [$^{11}$C]FLB 457 non-specific binding ($V_{ND}$) as a significant contributor to $\Delta BP_{ND}$ in patients and controls; and measurement of amphetamine blood levels. The limitations of the study are: the exclusion of older individuals with severe alcohol dependence; and no relationship between $\Delta BP_{ND}$ and alcohol measures such as frequency, amount, severity and duration of abuse. One possible reason for the failure to demonstrate a relationship between $\Delta BP_{ND}$ and alcohol measures is the limited range of values observed in the alcohol abuse measurements (see Table 1). This is likely an unintended consequence of excluding individuals with more severe alcoholism and comorbid disorders. In conclusion, we found decreased dopamine transmission in abstinent alcoholics in several of the cortical regions that have been implicated in addiction including the prefrontal cortex and medial temporal lobe. The results of these studies suggest that dopamine dysfunction in alcohol dependence is more widespread than previously conceptualized and not restricted to the striatum. Further studies are necessary to understand the mechanisms that contribute to blunted dopamine transmission and its clinical relevance in alcoholism.

## Acknowledgments

The project described was supported by Award Numbers R01AA018330 from the National Institute on Alcohol Abuse and Alcoholism (NIAAA). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIAAA or the National Institutes of Health.

## REFERENCES

1. Brozoski TJ, Brown RM, Rosvold HE, Goldman PS. Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science. 1979; 205(4409):929–32. [PubMed: 112679]
2. Sawaguchi T, Goldman-Rakic PS. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science. 1991; 251(4996):947–50. [PubMed: 1825731]
3. Bickel WK, Jarmolowicz DP, Mueller ET, Gatchalian KM, McClure SM. Are executive function and impulsivity antipodes? A conceptual reconstruction with special reference to addiction. Psychopharmacology (Berl). 2012; 221(3):361–87. [PubMed: 22441659]
4. Longmore BE, Knight RG. The effect of intellectual deterioration on retention deficits in amnesic alcoholics. J Abnorm Psychol. 1988; 97(4):448–54. [PubMed: 3204231]
5. Goldman MS. Cognitive impairment in chronic alcoholics. Some cause for optimism. Am Psychol. 1983; 38(10):1045–54. [PubMed: 6357006]
6. Oscar-Berman M, Shagrin B, Evert DL, Epstein C. Impairments of brain and behavior: the neurological effects of alcohol. Alcohol Health Res World. 1997; 21(1):65–75. [PubMed: 15706764]
7. De Montis MG, Grappi S, Gambarana C, Leggio B, Nanni G, Scheggi S, Tagliamonte A. Sardinian alcohol-preferring rats show low 5-HT extraneuronal levels in the mPFC and no habituation in monoaminergic response to repeated ethanol consumption in the NAcS. Brain Res. 2004; 1006(1): 18–27. [PubMed: 15047020]
8. Engleman EA, Ingraham CM, McBride WJ, Lumeng L, Murphy JM. Extracellular dopamine levels are lower in the medial prefrontal cortex of alcohol-preferring rats compared to Wistar rats. Alcohol. 2006; 38(1):5–12. [PubMed: 16762687]

9. Murphy JM, Stewart RB, Bell RL, Badia-Elder NE, Carr LG, McBride WJ, Lumeng L, Li TK. Phenotypic and genotypic characterization of the Indiana University rat lines selectively bred for high and low alcohol preference. Behav Genet. 2002; 32(5):363–88. [PubMed: 12405517]
10. Bassareo V, Tanda G, Petromilli P, Giua C, Di Chiara G. Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. Psychopharmacology (Berl). 1996; 124(4):293–9. [PubMed: 8739543]
11. Hegarty AA, Vogel WH. Modulation of the stress response by ethanol in the rat frontal cortex. Pharmacol Biochem Behav. 1993; 45(2):327–34. [PubMed: 8327538]
12. Murphy JM, McBride WJ, Gatto GJ, Lumeng L, Li TK. Effects of acute ethanol administration on monoamine and metabolite content in forebrain regions of ethanol-tolerant and -nontolerant alcohol-preferring (P) rats. Pharmacol Biochem Behav. 1988; 29(1):169–74. [PubMed: 2451255]
13. Hodge CW, Chappelle AM, Samson HH. Dopamine receptors in the medial prefrontal cortex influence ethanol and sucrose-reinforced responding. Alcohol Clin Exp Res. 1996; 20(9):1631–8. [PubMed: 8986215]
14. Samson HH, Chappell A. Dopaminergic involvement in medial prefrontal cortex and core of the nucleus accumbens in the regulation of ethanol self-administration: a dual-site microinjection study in the rat. Physiol Behav. 2003; 79(4-5):581–90. [PubMed: 12954398]
15. Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, deBartolomeis A, Weinberger DR, Weisenfeld N, Malhotra AK, Eckelman WC, Pickar D. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: Evidence from a novel positron emission tomography method. Proc Natl Acad Sci USA. 1997; 94(6):2569–74. [PubMed: 9122236]
16. Volkow ND, Wang GJ, Telang F, Fowler JS, Logan J, Jayne M, Ma Y, Pradhan K, Wong C. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. J Neurosci. 2007; 27(46):12700–6. [PubMed: 18003850]
17. Martinez D, Gil R, Slifstein M, Hwang DR, Huang Y, Perez A, Kegeles L, Talbot P, Evans S, Krystal J, Laruelle M, Abi-Dargham A. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biological psychiatry. 2005; 58(10):779–86. [PubMed: 16018986]
18. Narendran R, Frankle WG, Mason NS, Rabiner EA, Gunn RN, Searle GE, Vora S, Litschge M, Kendro S, Cooper TB, Mathis CA, Laruelle M. Positron emission tomography imaging of amphetamine-induced dopamine release in the human cortex: a comparative evaluation of the high affinity dopamine D2/3 radiotracers [11C]FLB 457 and [11C]fallypride. Synapse. 2009; 63(6): 447–61. [PubMed: 19217025]
19. Narendran R, Mason NS, May MA, Chen CM, Kendro S, Ridler K, Rabiner EA, Laruelle M, Mathis CA, Frankle WG. Positron emission tomography imaging of dopamine D/ receptors in the human cortex with [(1)(1)C]FLB 457: reproducibility studies. Synapse. 2011; 65(1):35–40. [PubMed: 20506186]
20. Narendran R, Himes M, Mason NS. Reproducibility of Post-Amphetamine [(11)C]FLB 457 Binding to Cortical D2/3 Receptors. PLoS One. 2013; 8(9):e76905. [PubMed: 24098812]
21. Narendran R, Mason NS, Chen CM, Himes M, Keating P, May MA, Rabiner EA, Laruelle M, Mathis CA, Frankle WG. Evaluation of dopamine D/ specific binding in the cerebellum for the positron emission tomography radiotracer [(1)(1)C]FLB 457: implications for measuring cortical dopamine release. Synapse. 2011; 65(10):991–7. [PubMed: 21360596]
22. Narendran R, Jedema HP, Lopresti B, Mason NS, Gurnsey K, Ruskiewicz J, Chen C-M, Deuitch L, Frankle G, Bradberry C. Imaging dopamine transmission in the frontal cortex: a simultaneous microdialysis and [11C]FLB 457 PET study. Mol Psychiatry. 2013 In press.
23. Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM. Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). Br J Addict. 1989; 84(11):1353–7. [PubMed: 2597811]
24. Selzer ML. The Michigan Alcoholism Screening Test: the quest for a new diagnostic instrument. Am J Psychiatry. 1976; 127:1653–8. [PubMed: 5565851]

25. Skinner HA, Allen BA. Alcohol dependence syndrome: measurement and validation. J Abnorm Psychol. 1982; 91(3):199–209. [PubMed: 7096790]
26. Halldin C, Farde L, Hogberg T, Mohell N, Hall H, Suhara T, Karlsson P, Nakashima Y, Swahn CG. Carbon-11-FLB 457: a radioligand for extrastriatal D2 dopamine receptors. J Nucl Med. 1995; 36(7):1275–81. [PubMed: 7790956]
27. Sudo Y, Suhara T, Inoue M, Ito H, Suzuki K, Saijo T, Halldin C, Farde L. Reproducibility of [11 C]FLB 457 binding in extrastriatal regions. Nucl Med Commun. 2001; 22(11):1215–21. [PubMed: 11606887]
28. Reimer ML, Mamer OA, Zavitsanos AP, Siddiqui AW, Dadgar D. Determination of amphetamine, methamphetamine and desmethyldeprenyl in human plasma by gas chromatography/negative ion chemical ionization mass spectrometry. Biol Mass Spectrom. 1993; 22(4):235–42. [PubMed: 8481411]
29. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, Matthews PM. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 2004; 23(Suppl 1):S208–19. [PubMed: 15501092]
30. Abi-Dargham A, Martinez D, Mawlawi O, Simpson N, Hwang DR, Slifstein M, Anjilvel S, Pidcock J, Guo NN, Lombardo I, Mann JJ, Van Heertum R, Foged C, Halldin C, Laruelle M. Measurement of striatal and extrastriatal dopamine D1 receptor binding potential with [11C]NNC 112 in humans: validation and reproducibility. J Cereb Blood Flow Metab. 2000; 20(2):225–43. [PubMed: 10698059]
31. Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M. Prefrontal dopamine D1 receptors and working memory in schizophrenia. J Neurosci. 2002; 22(9):3708–19. [PubMed: 11978847]
32. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab. 2007; 27(9):1533–9. [PubMed: 17519979]
33. Cunningham VJ, Rabiner EA, Slifstein M, Laruelle M, Gunn RN. Measuring drug occupancy in the absence of a reference region: the Lassen plot re-visited. J Cereb Blood Flow Metab. 2010; 30(1):46–50. [PubMed: 19738632]
34. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B. 1995; (57):289–300.
35. Narendran R, Mason NS, Laymon C, Lopresti B, Velasquez N, May M, Kendro S, Martinez D, Mathis C, Frankle G. A comparative evaluation of the dopamine D2/3 agonist radiotracer [11C]NPA and antagonist [11C]raclopride to measure amphetamine-induced dopamine release in the human striatum. Journal of Pharmacology and Experimental Therapeutics. 2010; 63(7):574–84.
36. Martinez D, Gil R, Slifstein M, Hwang DR, Huang Y, Perez A, Kegeles L, Talbot P, Evans S, Krystal J, Laruelle M, Abi-Dargham A. Alcohol dependence is associated with blunted dopamine transmission in the ventral striatum. Biol Psychiatry. 2005; 58(10):779–86. [PubMed: 16018986]
37. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010; 35(1):217–38. [PubMed: 19710631]
38. Koob GF. Theoretical frameworks and mechanistic aspects of alcohol addiction: alcohol addiction as a reward deficit disorder. Curr Top Behav Neurosci. 2013; 13:3–30. [PubMed: 21744309]
39. Floresco SB, Magyar O. Mesocortical dopamine modulation of executive functions: beyond working memory. Psychopharmacology (Berl). 2006; 188(4):567–85. [PubMed: 16670842]
40. Grace AA. The tonic/phasic model of dopamine system regulation and its implications for understanding alcohol and psychostimulant craving. Addiction. 2000; 95(Suppl 2):S119–28. [PubMed: 11002907]
41. Urban NB, Kegeles LS, Slifstein M, Xu X, Martinez D, Sakr E, Castillo F, Moadel T, O'Malley SS, Krystal JH, Abi-Dargham A. Sex differences in striatal dopamine release in young adults after

oral alcohol challenge: a positron emission tomography imaging study with [(1)(1)C]raclopride. Biological psychiatry. 2010; 68(8):689–96. [PubMed: 20678752]

42. He J, Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol. 2008; 210(2):349–58. [PubMed: 18190912]
43. Gilman S, Koeppe RA, Adams KM, Junck L, Kluin KJ, Johnson-Greene D, Martorello S, Heumann M, Bandekar R. Decreased striatal monoaminergic terminals in severe chronic alcoholism demonstrated with (+)[11C]dihydrotetrabenazine and positron emission tomography. Ann Neurol. 1998; 44(3):326–33. [PubMed: 9749598]
44. Kopera M, Wojnar M, Brower K, Glass J, Nowosad I, Gmaj B, Szelenberger W. Cognitive functions in abstinent alcohol-dependent patients. Alcohol. 2012; 46(7):665–71. [PubMed: 22703992]

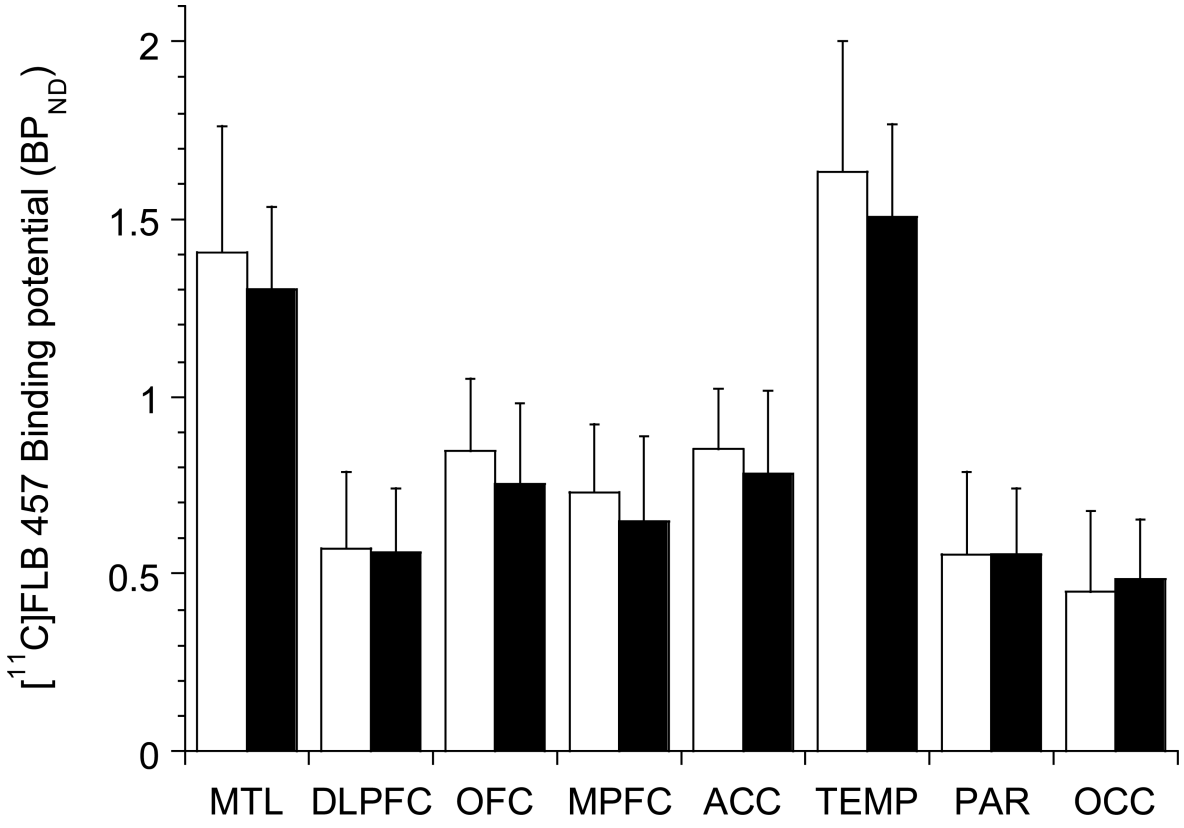


**Figure 1.**

shows the lack of difference in $D_{2/3}$ receptor availability in cortical regions of interest in alcohol dependent subjects (black bars) compared to healthy controls (white bars). MTL: medial temporal lobe, DLPFC: dorsolateral prefrontal cortex, OFC: orbital frontal cortex, MPFC: medial prefrontal cortex, ACC: anterior cingulate cortex, TEMP: temporal cortex, PAR: parietal cortex, and OCC: occipital cortex

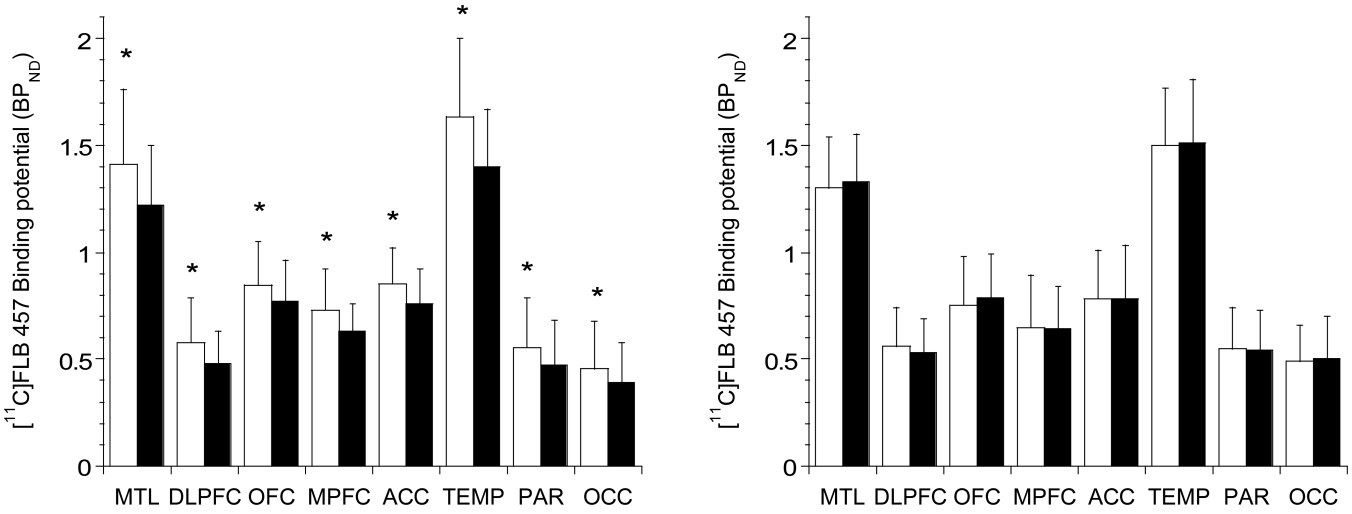


**Figure 2.**

shows [$^{11}$C]FLB 457 $BP_{ND}$ under baseline (white bars) and post-amphetamine (black bars) conditions in healthy controls (left panel) and alcohol dependent subjects (right panel). Amphetamine led to a significant decrease in [$^{11}$C]FLB 457 $BP_{ND}$ in healthy controls, but not in alcohol dependence (* represents $p < 0.05$, following the false discovery rate correction for multiple comparisons). MTL: medial temporal lobe, DLPFC: dorsolateral prefrontal cortex, OFC: orbital frontal cortex, MPFC: medial prefrontal cortex, ACC: anterior cingulate cortex, TEMP: temporal cortex, PAR: parietal cortex, and OCC: occipital cortex

**Table 1**

Demographic and clinical parameters for Healthy controls and Alcohol dependent subjects (n= 21/group)

| | **Healthy Controls** | | **Alcohol dependence** | |
|---|---|---|---|---|
| | **Mean** | **SD** | **Mean** | **SD** |
| Gender | | | | |
| *Male* | 16 | | 16 | |
| *Female* | 5 | | 5 | |
| Ethnicity | | | | |
| *African American* | 2 | | 4 | |
| *Asian* | 2 | | 0 | |
| *Caucasian* | 17 | | 17 | |
| Smoking status | | | | |
| *Yes* | 12 | | 12 | |
| *No* | 9 | | 9 | |
| Positive family history for alcoholism | 0 | | 18 | |
| Age | 28 | 4 | 28 | 5 |
| Weight (Kg) | 74 | 12 | 77 | 14 |
| Duration of abuse (years) | - | - | 11 | 6 |
| Alcohol frequency (days/week) | - | - | 5.7 | 1.5 |
| Alcohol amount (standard drinks/day) | - | - | 13 | 5 |
| Michigan Alcohol Screening Test (scoring range 0 to 22) | - | - | 13 | 4 |
| Alcohol dependence scale (scoring range 0 to 47) | - | - | 21 | 6 |
| Abstinence before scans (days) | - | - | 33 | 18 |

**Table 2**

[$^{11}$C]FLB 457 PET scan parameters

| | Condition | | Injected dose (mCi) | Specific activity (Ci mmol$^{-1}$) | Injected mass (ug) | Free Fraction in plasma (%) | $V_{ND}$ (mL cm$^{-3}$) |
|---|---|---|---|---|---|---|---|
| Healthy Controls | Baseline | Mean | 7.1 | 8891 | 0.39 | 34.7 | 4.27 |
| | | SD | 1.7 | 5689 | 0.17 | 5.9 | 1.23 |
| | Amphetamine | Mean | 7.7 | 8741 | 0.39 | 34.3 | 4.04 |
| | | SD | 1.4 | 4307 | 0.13 | 5.1 | 0.86 |
| Alcohol Dependence | Baseline | Mean | 7.6 | 11369 | 0.36 | 38.9 | 4.43 |
| | | SD | 1.4 | 8871 | 0.17 | 7.6 | 1.01 |
| | Amphetamine | Mean | 6.8 | 6995 | 0.46 | 39.3 | 4.37 |
| | | SD | 1.9 | 4736 | 0.16 | 7.3 | 0.97 |

Values are mean and standard deviation (SD), n = 21 per group

* $p \leq 0.05$, paired t-tests (baseline vs. amphetamine)

*Am J Psychiatry*. Author manuscript; available in PMC 2015 August 01.

**Table 3**

Amphetamine-induced displacement of [$^{11}$C]FLB 457 binding potential ($BP_{ND}$)

| Region | Healthy controls | | Alcohol dependence | | Two-tailed, unpaired t-test | | p |
|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | t | df | |
| Medial temporal lobe | −11.1 | 16.2 | 3.1 | 15.9 | 2.88 | 39.98 | 0.006* |
| Dorsolateral prefrontal cortex | −13.7 | 14.7 | −3.9 | 12.7 | 2.30 | 39.12 | 0.027* |
| Orbital frontal cortex | −8.6 | 12.4 | 8.5 | 18.6 | 3.50 | 34.87 | 0.001* |
| Medial prefrontal cortex | −11.6 | 15.1 | 5.4 | 27.3 | 2.48 | 31.62 | 0.018* |
| Anterior cingulate cortex | −9.0 | 17.7 | 1.1 | 19.6 | 1.75 | 39.61 | 0.089 |
| Temporal cortex | −12.2 | 13.4 | 0.4 | 10.4 | 3.39 | 37.80 | 0.002* |
| Parietal cortex | −11.7 | 18.6 | −1.6 | 12.2 | 2.09 | 34.65 | 0.044 |
| Occipital cortex | −10.6 | 19.2 | 1.3 | 17.2 | 2.11 | 39.52 | 0.041 |

Values are mean and standard deviation (SD), n = 21 per group

*significant following multiple corrections using false discovery rate