

SEX DIFFERENCES IN IMPULSIVITY AND BRAIN VOLUMES IN METHAMPHETAMINE USERS

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## ABSTRACT

Methamphetamine (METH) is an addictive stimulant adversely affecting brain structure and function. The aim of this study was to investigate impulsivity and brain structures, and sex differences on these variables, between METH users and non-drug user controls (CON). Structural MRI was performed in 124 subjects: 62 METH (ages  $41.2 \pm 1.4$  years, 34 males, 28 females) and 62 CON (ages  $43.3 \pm 2.3$  years, 36 males, 26 females). FreeSurfer 5.1 automated morphometry was used to measure brain morphometry. Subjects completed the Barratt Impulsiveness Scale (BIS) questionnaire, which measures six factors. 2-way and repeated measures ANCOVA, co-varying for age, education, depressive symptoms, and intracranial volume, evaluated for independent and interactive effects of group status and sex. Relationships between METH usage characteristics, brain volumes, and impulsivity scores with significant group differences were examined with Pearson correlations. METH users had higher impulsivity scores in a majority of the factors ( $p < 0.001-0.05$ ). Male METH users had larger right superior frontal volumes compared to CON, while female METH users had smaller volumes compared to CON ( $p = 0.02$ ). Group-by-volume interactions were found in the right superior frontal ( $p = 0.02$ ) and right insula ( $p = 0.02$ ). In the male METH users, greater volumes in these regions were associated with less self-control. Female METH users had larger accumbens compared to female CON ( $p = 0.03$ ). METH users had consistently thinner left frontal cortical regions compared to CON ( $p = 0.003$ ). Consistent with prior reports, METH users were more impulsive and had thinner left frontal cortices than CON. Male METH users had larger superior frontal and insula volumes than CON, which was associated with less self-control ( $p = 0.009-0.05$ ). This was not seen in the female METH users, possibly due to a protective effect of estrogen from neuroinflammation in these regions. This suggests that men and women may be affected differently, and sex should be accounted for in brain morphometry studies.

## 1. Introduction

Methamphetamine (METH) is an addictive stimulant known to adversely affect behavior and the brain. In 2012, there were an estimated 34 million users of amphetamine-type-stimulants (ATS) worldwide, less educated, used larger quantities of METH, greater impulsivity were found to be younger, less educated, used larger quantities of METH, greater impulsivity were found to be younger, less educated, used larger quantities of METH (Crime, 2014). METH users accounted for an estimated 7% of total admissions to treatment services in the USA, but more significantly, 30% of total admissions in Hawaii (SAMHSA, 2014). This drug is often referred to as 'ice' or 'crystal meth' and is typically smoked, with a subsequent euphoria that can last for hours (Newton et al., 2005).

Structural gray matter differences between METH users and CON have previously been reported. Specifically, METH users have enlarged striatal structures, such as the putamen (Chang et al., 2005a; Ersche et al., 2012), globus pallidus (Chang et al., 2005a), and nucleus accumbens (Jernigan et al., 2005). Chang *et al.* found that METH users with enlarged striatum had relatively normal cognitive performance, suggesting a compensatory response to maintain cognitive function. However, METH-dependent tobacco smokers had smaller gray matter volume in the orbitofrontal cortex and caudate nucleus compared to CON and smaller gray matter volume in the insula compared to control nonsmokers (Morales et al., 2012). METH users also had smaller gray matter volumes (Nakama et al., 2011) in dorsolateral prefrontal, orbitofrontal, and thinner cortices (Thompson et al., 2004) compared to CON.

Impulsive behavior in METH users compared to CON has previously been studied. METH users with greater impulsivity were found to be younger and less educated. They also used larger quantities of METH, were more likely to be binge users, had a larger number of sexual partners, and scored higher on the Beck Depression Inventory compared to METH users with lower impulsivity (Semple et al., 2005). METH users had higher impulsivity scores on the three subscales of the Barratt Impulsiveness scale (BIS) compared to non-METH users (Lee et al., 2009). Furthermore, compared to cocaine-dependent participants, METH-dependent participants had significantly greater BIS nonplanning and total scores (Winhusen et al., 2013). While most studies cannot conclude whether METH use caused abnormal brain structures or impulsive behavior, one study suggested that gray matter dorsal striatum changes may predispose individuals for an increased risk for developing stimulant dependence (Ersche et al., 2012). Ersche et al. evaluated stimulant dependent users, their non-drug using siblings, and unrelated CON and found gray matter changes in the dorsal striatum and higher impulsivity in both the stimulant users and their siblings compared to the CON.

Impulsivity has also been measured through behavioral tasks and correlated with brain volumes. In a study measuring inhibitory control, METH users had lower gray matter intensity in the right pars opercularis and worse inhibitory control than the non-METH users (Tabibnia et al., 2011). Impulsivity, measured with a delay-discounting task, correlated positively with gray matter density in the posterior cingulate cortex and ventral striatum and negatively with that in the left superior frontal gyrus (Schwartz et al., 2010). In non-drug using CON, gray matter volumes of the left OFC, right OFC, and left anterior cingulate cortex (ACC) correlated inversely with BIS total scores, and the left OFC volume also correlated inversely with motor impulsivity (Matsuo et al., 2009). These results suggest that smaller brain structures, perhaps due to effects of drugs, may account for more impulsive behavior observed.

Although sex differences have been reported in other populations, few studies have analyzed sex differences between METH users and CON in terms of brain volumes and impulsivity. In a sample of 125 healthy undergraduate students, men exhibited higher motor impulsivity scores compared to women on two computerized behavioral tests (Lage et al., 2013). In contrast, a study of treatment-seeking drug users found women to be more impulsive than the men (Lejuez et al., 2007). However, this study of drug users did not include a non-drug user control group and therefore could not evaluate group by sex interactions. One study found sex differences in the insula volume in substance-dependent users. The female stimulant-dependent group had smaller insula compared with same-sex CON, whereas the male substance dependent group had larger insula (Tanabe et al., 2013). Although the results may differ, these studies indicate that there are differences between men and women, which need to be accounted for in morphometric studies.

Therefore, the main goal of the current study was to investigate the association between brain structure and impulsivity in METH users and CON, separately for men and women. To address this goal, the following specific Aims and Hypotheses were developed:

Aim 1. To evaluate independent and combined effects of METH use and sex on structural brain morphometry and impulsivity.

Hypothesis 1A: METH users will have larger striatal volumes, based on previous findings regarding a compensatory response due to normal cognitive function found in the METH users (Chang et al., 2005a; Jernigan et al., 2005).

Hypothesis 1B: We also hypothesize *a priori* (Morales et al., 2012; Nakama et al., 2011; Schwartz et al., 2010) that METH users would have smaller and thinner cortices than CON in eight frontal cortical regions

Aim 2. To assess if impulsivity is associated with brain morphometry in METH users and CON

Hypothesis 2: We expect to see higher impulsivity scores in the METH users and an association between higher impulsivity scores and larger cortical brain measures across all participants based on previous studies (Lee et al., 2009; Schwartz et al., 2010).

## 2. Materials and Methods

### 2.1 Participants

Individuals were recruited from the community through flyers and word-of-mouth. They were compensated for their time and participation, with CON receiving compensation in cash and METH users receiving compensation in gift cards. A group of 124 subjects (62 METH users: ages  $41.2 \pm 1.4$  years, 34 men; and 62 CON; ages  $43.3 \pm 1.9$  years, 36 men) were included in this study and enrolled between March 2010 and February 2014. Each participant signed a consent form approved by the institutional review boards at the University of Hawaii and at the Queen's Medical Center. All participants completed detailed clinical evaluations, as detailed below.

All CON had to meet the following criteria:

- 1)  $\geq 18$  years of age;
- 2) able to provide informed consent;
- 3) urine negative for substances of abuse, including METH, amphetamines, cocaine, THC, benzodiazepine, and opiates.

METH users had to meet criteria 1 and 2 above; in addition:

- 3) urine negative for substances of abuse, including cocaine, THC, benzodiazepine, and opiates;
- 4) have a history of METH-dependence according to DSM-IV criteria for greater than 3 years (Past DSM-IV dependence for only marijuana and cocaine was allowed, but METH users could not have any current DSM-IV dependence for other drugs, except tobacco); and
- 5) METH described as the most used substance (in length of time or frequency) and "drug of choice".

Exclusion criteria for both groups included: 1) confounding neurological or psychiatric disorder because they could affect brain morphometry; 2) chronic severe medical condition that could confound outcome variables 3) on medications that could confound outcome measures; 4) pregnancy; and 5) not able to perform MRI brain scans due to metallic objects inside the body (ex: pacemakers, surgical clips) or claustrophobia.

Detailed drug use histories and medical histories were obtained from all subjects by trained research staff. Drug use patterns included frequency, duration of use, route of use, and last use for illicit drugs, tobacco, and alcohol. Structured assessments included the Center for Epidemiologic Study –Depression (CES-D) questionnaire. A psychiatrist interviewed any subject who had a self-reported score of 16 or

greater further to ensure they met the inclusion and exclusion criteria and further evaluated all participants on drug use and medical histories. Urine toxicology tests were done to ensure all subjects met the necessary criteria for participation, and pregnancy tests were done in all women before the MRI scan.

## 2.2 Barratt Impulsiveness Scale

The Barratt Impulsiveness Scale (BIS)(Patton et al., 1995) was administered to all subjects. This self-administered 30-item questionnaire assesses different personality constructs of impulsiveness, where each item was rated from 1 (rarely/never) to 4 (almost always/always). There are six first order factors: attention, cognitive instability, motor, perseverance, self-control, and cognitive complexity, with two first order factors making up one second-order factor for a total of three second order factors: attentional, motor, and nonplanning impulsiveness.

## 2.3 MRI acquisition

All subjects were scanned on a Siemens 3T MR scanner (Tim Trio; Siemens Medical Solutions, Erlangen, Germany) with an 8- or 12-channel head coil. For the structural imaging sequences, a 3-plane localizer (TR/TE = 20/5; 3 by 3 slices, 0.5x0.5x10 mm), a high-resolution 3D magnetization-preparation rapid gradient echo (MP-RAGE, TR/TE/TI = 2200/4.11/1000 ms; 160 slices, 1x1x1 mm), and a fluid-attenuated inversion recovery (FLAIR) sequence (9100/84/2500 ms; 44 slices, 0.9x0.9x3 mm) were acquired. Structural MP-RAGE and FLAIR images were read by a trained physician to ensure subjects did not show any brain lesions or significant abnormalities.

## 2.4 Image analyses

Morphometric measures of brain structures were obtained from the MP-RAGE scan using the Freesurfer 5.0 software (<http://surfer.nmr.mgh.harvard.edu/>). The technical details are described in previous publications (Fischl and Dale, 2000; Fischl et al., 2004; Han et al., 2006; Jovicich et al., 2006). After removing the skull from the MRI scan images, a Talairach transformation was conducted. Automated segmentation was performed for the subcortical white and deep gray matter structures and cortical areas using a probabilistic atlas. The process produced cortical and subcortical reconstruction and volumetric segmentation of the original MRI scans. All regions of interest (ROIs) were visually inspected to ensure accuracy.

Subcortical regional volumes were determined in each hemisphere for the amygdala, caudate, hippocampus, globus pallidus, putamen, and nucleus accumbens. The cortical volume, thickness, and area were determined for each of eight *a priori* ROIs: insula, superior frontal, caudal anterior cingulate, rostral anterior cingulate, lateral orbitofrontal, medial orbitofrontal, caudal middle frontal, and rostral middle frontal determined by the Deiskan-Killiany atlas in Freesurfer (Desikan et al., 2006). In addition, a vertex-based analysis was conducted over the whole brain for the same cortical measures.

## 2.5 Statistical analyses

Statistical analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). One-way analysis of variance (ANOVA) was used to compare demographic and clinical characteristics between male METH users, female METH users, male CON, and female CON. Student's t-tests (unpaired, two-tailed) were performed to compare METH usage characteristics between the male and female METH users. Two-way analyses of covariance (ANCOVAs) were used to test the independent and interactive effects of METH status and sex on brain morphometry and impulsivity scores. When necessary, intracranial volume (ICV), education, and CES-D scores were included in the models as covariates. Simes correction(Simes, 1986) for multiple comparisons was performed on the cortical measures, dividing significance values for each hemisphere by eight (for the number of *a priori* frontal cortical regions). Repeated-measures ANCOVA was used to evaluate the effects of METH across the frontal cortical brain consisting of the eight Freesurfer regions in each measure (cortical volume, cortical thickness, and surface area), separately for

each hemisphere. These models included two between-subject variables (drug status and age) and one within-subject factor (frontal cortical brain region).

For the vertex-based analysis on cortical brain measurements in QDEC, Freesurfer's GUI-based statistical engine was used. Data were smoothed using 10-mm full width half maximum Gaussian kernel, and the cluster-wise threshold was set at  $P < 0.05$ . Correction for multiple comparisons was done using Monte Carlo Z simulation. Relationships between METH usage characteristics, brain volumes, and impulsivity scores with significant group differences were examined with Pearson correlations. Some METH use characteristics (days of abstinence, total amount use) were log-transformed prior to analysis to ensure normality. For all statistical analysis,  $p$ -values  $< 0.05$  were considered significant, and trends were noted for  $p$ -values  $< 0.1$ . However, for cortical brain morphometry,  $p < 0.1$  was considered significant since there was an *a priori* directional hypothesis for the eight ROIs.

### 3. Results

#### 3.1 Clinical Characteristics

The METH users and CON were similar in age, ethnic, and racial distributions. The METH users had slightly fewer years of education than the CON ( $p=0.02$ ), but the two groups did not differ in their verbal WTAR IQ scores ( $p=0.11$ ). The METH users had higher CES-D scores than the CON ( $p=0.0002$ ), but all groups were similar in their proportion of tobacco smokers ( $p=0.75$ ), smoking pack years ( $p=0.39$ ), lifetime alcohol used ( $p=0.43$ ), and lifetime marijuana used ( $p=0.53$ ) (Table 1). Male and female METH users did not differ in the amounts and patterns of their METH usage, shown in Table 1.

#### 3.2 BIS Scores

The METH users had significantly higher impulsivity scores in four of the 1<sup>st</sup> order factors: attention, motor, self-control, and cognitive complexity. All three second order factors (attentional, motor, and nonplanning) and the total BIS score were significantly higher in the METH users compared to CON (Fig. 1). There was a METH-by-sex interaction ( $p=0.02$ ) for perseverance, with the male METH users having less perseverance (higher BIS perseverance score) than the female METH users (13%, posthoc  $p=0.04$ ). Male and female control groups had similar perseverance scores.

#### 3.3 Subcortical Brain Volumes (Fig.3)

In the left accumbens, there was a METH main effect ( $p=0.02$ ). Specifically, the METH users had 8% larger left accumbens volume compared to the CON, driven mostly by the 13% larger volume in the female METH users compared to the female CON. However, the METH by sex interaction did not reach significance in this region ( $p=0.18$ ). The right accumbens volume was 10% larger in the female METH users compared to female CON, whereas the difference amongst males was only 1%, resulting in a significant METH-by-sex interaction ( $p=0.03$ ). Amongst the METH users, the men and women had similar right accumbens volume, but amongst the CON, the females had 12% larger volumes compared to the males. All ANCOVA tests were co-varied for age and ICV.

#### 3.4 ROI Cortical Brain Measurements

In both hemispheres, cortical volume, thickness, and area were measured in the eight *a priori* Freesurfer Regions. Two-way ANCOVAs were performed, co-varying for age in all three measurements and ICV for volumes. In the left hemisphere, METH users had thinner cortices in the rostral anterior cingulate (-4%,  $p=0.0008$ ), medial orbitofrontal (-3%,  $p=0.003$ ), and superior frontal (-1%,  $p=0.02$ ) regions compared to CON;  $p$ -values remained significant after Simes correction. METH users had also smaller caudal middle frontal volumes (-4%,  $p=0.01$ ) compared to CON. In the right hemisphere, several regions were smaller in the METH users compared to CON, but only the rostral middle frontal volume (-2%,  $p=0.01$ ) and medial orbitofrontal cortex volumes (-3%,  $p=0.006$ ) remained significant after Simes correction compared to CON. METH users had 4-5% larger right insula volumes compared to CON, independent of sex ( $p=0.06$ ) (Table 2). In the right superior frontal region, the volume showed a METH-by-sex interaction ( $p=0.02$ )

(Fig. 3). Specifically, the female METH users had 6% smaller right superior frontal volumes compared to female CON ( $p=0.06$ ), whereas the male METH users had about 3% larger volumes (not significant). This region was also statistically significant in the vertex-based analysis (Cluster-Wise P-value =0.00005).

Repeated-Measures ANCOVA revealed that METH users had consistently thinner left frontal cortices compared to CON ( $p=0.003$ ), especially in the medial orbitofrontal and rostral anterior cingulate regions (post-hoc  $p<0.05$ ) (Fig. 4). METH users also had thinner right frontal cortices and consistently smaller frontal volumes in both hemispheres compared to CON ( $p=0.002-0.01$ ).

### 3.5 Vertex-Based Analysis of Cortical Measurements

On vertex-based analyses, METH users had smaller left superior frontal volumes (CWP=0.0003) and thinner lateral orbitofrontal cortices (CWP=0.00006) (Fig. 5) compared to CON. Consistent with the ROI analyses, there was a METH by sex interaction in the right superior frontal volume (CWP=0.00005) (Fig. 3). These three findings remained significant after correction for multiple comparisons by method of Monte Carlo simulation.

### 3.6 Relationship with Clinical Variables

In the left caudal middle frontal region, longer duration of METH use was associated with smaller volume ( $p=0.04$ ,  $r=-0.30$ ) (Fig. 6) independent of sex and co-varying for age and ICV. In the right superior frontal volume, logarithmic scaled days since last METH use negatively correlated with volume, independent of sex and co-varying for age and ICV ( $p=0.01$ ,  $r=-0.32$ ) (Fig.3). Logarithmic number of days since last METH use was used due to the wide range amongst the METH users.

### 3.7 Relationships between Impulsive Measures and Brain Morphometry

Significant one-way ANCOVA group-by-volume interactions were found in several regions: right insula ( $p=0.02$ ), right rostral middle frontal ( $p=0.02$ ), right superior frontal ( $p=0.05$ ) (Fig. 3), and left rostral middle frontal ( $p=0.04$ ) for self-control. Specifically, in male METH users, greater lack of self-control was associated with larger brain volumes in these same regions: right insula ( $p=0.009$ ,  $r=0.44$ ) (Fig. 5), right superior frontal ( $p=0.04$ ,  $r=0.35$ ), right rostral middle frontal ( $p=0.004$ ,  $r=0.48$ ), and left rostral middle frontal ( $p=0.005$ ,  $r=0.47$ ). Similar associations were not observed in the other three groups.

## 4. Discussion

### 4.1 Summary

Our study found several alterations in brain morphometry in METH users. First, METH users, and specifically male users, had larger superior frontal volumes compared to CON. These alterations were associated with a greater lack of self-control and shorter time periods of abstinence from METH. (Fig.3). Similarly, we validated previous findings of larger right insula volumes and thinner frontal cortices in METH users compared to CON.

### 4.2 Superior Frontal Volume in METH Users

Few studies have found differences between METH users and CON in the superior frontal region (Kim et al., 2005). Larger right superior frontal volumes observed in the male METH users could be due to neuroinflammation caused by METH, possibly due to increased water content and cell volumes (Chang et al., 2007) and reactive gliosis and increases in gene and protein expression of factors associated with the innate immune response (Clark et al., 2013). Greater activated microglial density and glial proliferation could be contributing to the neuroinflammatory effects, previously shown in frontal gray matter in METH users (Chang et al., 2005b; Ernst et al., 2000). In our study, larger volumes are associated with more impulsive behavior, specifically a greater lack of self-control. Furthermore, within the male METH users,

greater time periods of METH abstinence was associated with smaller right superior frontal volumes, suggesting that the neural system within this region may recover with abstinence. A previous study found a negative correlation between impulsivity measured by a delay-discounting task and the left superior frontal gyrus gray matter density (Schwartz et al., 2010). However, there was a significant difference in the proportion of smokers between their METH group and their CON, making it hard to discern whether the effects observed were due to tobacco or METH. In our study, we recruited equal proportion of tobacco smokers in all four groups to focus predominantly on the effects of METH.

#### 4.3 Right Insula Volume in METH Users

Although there was only a trend for the right insula being larger in METH users compared to CON ( $p=0.06$ ), this was similar to the result by Tanabe et al. who found substance-dependent men had larger insula compared to sex-matched CON (Tanabe et al., 2013). The insula has been implicated in different modalities of self-control (Dambacher et al., 2014) and decision-making with risk-taking (Paulus et al., 2003; Xue et al., 2010), suggesting it plays a role in drug addiction (Naqvi et al., 2014). However, previous studies have shown mixed results as to whether drug addiction was associated with increased or decreased gray matter volume in the insula. One study found that METH-dependent subjects had smaller left insula compared to CON (Morales et al., 2012), but another study found higher gray matter density in cigarette smokers in the left anterior insula compared to nonsmokers (Zhang et al., 2011). The inconsistencies could be due to differences in methodologies. In our study, automated segmentation of cortical brain regions was performed using FreeSurfer, similar to Tanabe et al. (Tanabe et al., 2013). In one study, smokers with insula lesions had a lower urge to smoke and quit smoking more easily compared to smokers with extra-insular brain injuries (Naqvi et al., 2007). Tanabe et al. suggested that combining Naqvi and Bechara's (Naqvi et al., 2007) results with theirs and another animal study (Contreras et al., 2007) may suggest that a larger or overactive insula may relate to more severe drug-use behavior, while a smaller insula may relate to a decreased drug-related behavior. In our study, there was also an association between larger right insula volumes and greater lack of self-control measured by the BIS in the male METH users, which supports the possibility that larger superior frontal insula volumes could be associated with more drug use behavior. However, more studies need to be done to understand individual differences in insula morphometry.

#### 4.4 Sex Differences in METH Users

The differences between METH users and CON in the superior frontal volume and insula were predominantly in the male, but not female, METH users, suggesting that sex may modulate the effects of METH on brain morphometry. A previous study had found significant decrease in cerebral glucose metabolism in the right superior frontal white matter and impairment in executive function in abstinent male METH users compared to CON (Kim et al., 2005), but this was not seen in the female METH users. Estrogen may be protective in these brain regions against excess neuroinflammatory responses of METH, as has been shown in the nigrostriatal dopaminergic system in female rats (Dluzen and McDermott, 2006).

#### 4.5 Larger N. Accumbens Volumes in METH Users

Our METH users had larger nucleus accumbens compared to the CON, in agreement with a previous study by Jernigan et al (Jernigan et al., 2005). However, in our study, this difference was mostly driven by the female METH users. However, both female and male METH users reported higher BIS scores compared to CON. Therefore, METH may be causing neuroinflammation in the nucleus accumbens through greater activated microglial density, which has been shown previously in the striatum in METH users (Sekine et al., 2008). The effects of neuroinflammation within the nucleus accumbens, which is involved in the rewards pathway, could be negatively impacting impulsive behaviors in METH users.

#### 4.6 Thinner frontal Cortices in METH Users

In the left hemisphere, METH users had thinner cortices in several frontal sub-regions, especially in the medial orbitofrontal cortex and rostral anterior cingulate cortex, similar to findings in amphetamine-type

stimulant users (Koester et al., 2012). The orbitofrontal cortex plays a major role in drug addiction and may be more prone to the neurotoxic effects of METH as shown by lower levels of dopamine D2 receptor availability compared to controls in this region (Volkow et al., 2001). In our study, METH users with greater duration of METH use had smaller left caudal middle frontal volumes, suggesting that longer METH exposure may be more damaging in this region of the cortex. A study of healthy individuals found that smaller gray matter volume in the left and right orbitofrontal cortex and anterior cingulate cortex correlated with higher impulsivity, measured with the BIS (Matsuo et al., 2009). In our study, the caudal middle frontal volume was smaller in the METH users compared to the CON, which is consistent with others who similarly found smaller frontal gray matter volumes in METH users (Morales et al., 2012; Nakama et al., 2011).

#### 4.7 Impulsivity in METH Users

METH users were more impulsive than CON. This is consistent with previous reports that found METH users had higher total and 1<sup>st</sup> order factor scores on the BIS than CON (Lee et al., 2009), as well as other studies in stimulant users (Liu et al., 2011; Perry et al., 2013). Furthermore, the male METH users had less perseverance (more impulsiveness) compared to the female METH users. Greater impulsiveness may make men more susceptible to substance abuse. This may partially account for the increased risk of alcohol problems in men but not in women (Stoltenberg et al., 2008). Perseverance factors into the BIS motor impulsiveness 1<sup>st</sup> order factor. Our results were consistent with studies of non-drug users that found men had higher motor impulsivity scores compared to women on behavioral tasks (Lage et al., 2013) and committed more inhibitory errors than females (Saunders et al., 2008). Similarly, in the laboratory setting, male rats showed more disrupted inhibitory control than female rats (Bayless et al., 2012), and made more premature responses during task acquisition. (Jentsch and Taylor, 2003) Our results differed from those of Perry et al (Perry et al., 2013), who found female substance-dependent individuals (SDI) reported higher impulsive behavior measured by the BIS compared to male SDI. However, their sample may represent a different population since their SDI included those who were also dependent on alcohol, cannabis, and opioids. There could be an unequal proportion of alcohol, cannabis, and opioids dependence amongst the female and male SDI, which may account for the differences observed in impulsivity. Perry et al did not publish the breakdown of these drug dependence between the male and SDI. In our study, although we had six METH users with past histories of DSM-IV dependence for marijuana, lifetime marijuana use did not significantly differ the male and female METH users. Additionally, we did not include any METH users with current or past DSM-IV dependence for alcohol and opioids. It is also possible that our study sample of METH users differed greatly from Perry et al's SDI group. Our METH users were racially diverse, with 24% of Native Hawaiian/Pacific Islander descent. Perry et al, did not include a racial breakdown of their subjects, which makes it difficult to truly compare our METH users to their SDI group.

#### 4.8 Limitations and Future Directions

One limitation of this study is its cross-sectional design. Therefore, we cannot preclude premorbid brain differences in METH users. Additionally, the BIS is a self-report measure of impulsivity and not all subjects may have answered the questions honestly, Therefore, it may not reflect the true impulsivity of the subjects. Furthermore, it would be ideal to recruit METH users without other current or past drug use to solely evaluate the effects of METH; however, there are a very small number of METH users who do not currently use or have used other drugs. We attempted to minimize these confounding effects by recruiting an equal proportion of tobacco smokers in all groups. Furthermore, we excluded anyone who met current dependence according to DSM-IV criteria for other illicit drugs and alcohol.

While the finding of larger insula volumes in the METH users is consistent with previous work (Tanabe et al., 2013), larger superior frontal volumes in the male METH users have not been reported previously. Our findings suggest that men and women may be affected differently by METH, and sex should be accounted for in brain morphometry studies. Brain morphometry may be a useful biomarker for understanding brain volumes involved in impulsivity and METH use and for assessing whether neurotransmitter pathways involved in these regions could provide potential treatment targets for drug addiction. Future studies could evaluate some genotype differences that could account for the variability

and possible group differences in age-dependent changes. Diffusion-tensor imaging and MRS studies, specifically measuring myo-inositol in these frontal regions, could be done to further evaluate neuroinflammation in METH users.

Table 1. Clinical Characteristics of Research Participants (mean  $\pm$  S.E.)

	Meth (n=62)		Control (n=62)		p-value
	M (n=34)	F (n=28)	M (n=36)	F (n=26)	
Age (years)	39.6 $\pm$ 1.9	43.2 $\pm$ 2.0	41.3 $\pm$ 2.0	46.2 $\pm$ 3.7	0.27
Age range	22-57	22-60	18-62	18-78	
Ethnicity (Hispanic/NonHispanic)	1/33	4/24	4/32	2/24	0.43
Race					0.50
Asian	9	6	7	8	
Black/African American	0	0	1	0	
Native Hawaiian/Other Pacific Islander	7	8	5	2	
White	12	7	14	13	
More than One Race	6	7	9	3	
Education (years)	12.8 $\pm$ 1.7	12.8 $\pm$ 0.3	13.8 $\pm$ 0.3	14.0 $\pm$ 0.5	<b>0.02</b>
Education range	9-16	10-16	12-18	11-20	
Estimated verbal IQ (WTAR)	99.4 $\pm$ 2.0	99.0 $\pm$ 1.8	102.0 $\pm$ 1.6	105.0 $\pm$ 1.7	0.11
Depression scores (CES-D)	18.2 $\pm$ 2.0	18.6 $\pm$ 2.6	8.7 $\pm$ 0.9	9.8 $\pm$ 2.1	<b>0.0002</b>
Scanner upgrade (before/after)	6/28	3/25	2/34	2/24	0.39
Smokers (past and current)	21 (62%)	15 (54%)	22 (61%)	13 (50%)	0.75
Smoking pack years	18.0 $\pm$ 6.5	8.6 $\pm$ 1.9	14.2 $\pm$ 3.0	10.1 $\pm$ 2.6	0.39
Lifetime Alcohol Use (mL)	139499.2 $\pm$ 37181.5	83545.9 $\pm$ 33661.6	73270.5 $\pm$ 28374.7	80124.6 $\pm$ 39408.2	0.43
Lifetime Marijuana Use (g)	2977.0 $\pm$ 1486.6	2993.1 $\pm$ 2319.7	492.8 $\pm$ 223.8	1548.3 $\pm$ 1429.2	0.53
Lifetime Meth Use (g)	3429.0 $\pm$ 853.0	4956.2 $\pm$ 1698.7			0.40
Range	2.11- 18806.0	55.4- 46689.7			
Duration of Use (months)	160.6 $\pm$ 17.9	199.1 $\pm$ 19.3			0.15
Range	24-349	36-427			
Age at First Use (years old)	24.9 $\pm$ 1.7	24.5 $\pm$ 1.7			0.87
Range	9-50	11-42			
Route of Use					0.11
Inhaled	29	28			
IV	2	0			
Nasal	3	0			
Current/Past (over 1 year last use)	29/5	19/9			0.1
Days Since Last Use (abstinence)	491.0 $\pm$ 249.4	753.6 $\pm$ 330.6			0.52
Range	0-7445	0-8222			

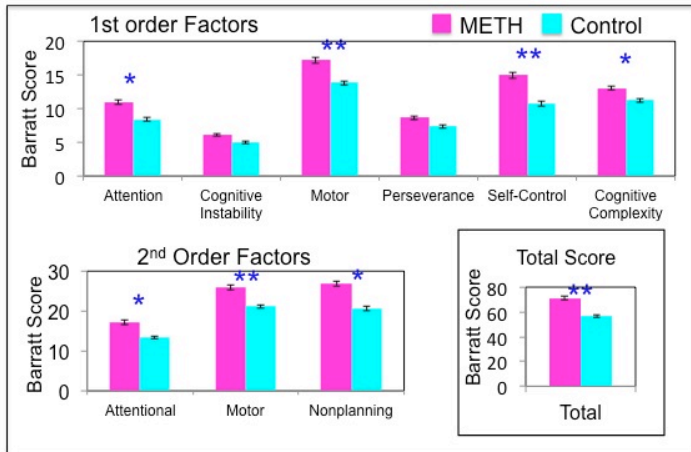


Fig. 1. 1<sup>st</sup> order, 2<sup>nd</sup> order, and Total BIS Scores in METH users and CON. Two-way ANCOVA showing main METH effect. \*p=0.0001- 0.005. \*\*p<0.0001

Table 2. Percent Differences and 2-way ANCOVA METH Effect P-values for Cortical Volume, Thickness, and Area in Right and Left Cortical ROIs Between METH Users and CON

ROI	2-way ANCOVA METH effect P-value		
	Volume**	Thickness*	Area*
L Rostral Anterior Cingulate	(-2%) 0.18	<b>(-4%) 0.0008</b>	(+2%) 0.57
L Caudal Anterior Cingulate	(-0.5%) 0.68	(-2%) 0.24	(+0.3%) 0.99
L Medial OrbitoFrontal	(-0.8%) 0.45	<b>(-3%) 0.003</b>	(+1%) 0.52
L Lateral OrbitoFrontal	(-0.05%) 0.46	<b>(-2%) 0.04♣</b>	(+1%) 0.49
L Rostral Middle Frontal	(-2%) 0.03	(-1%) 0.07	(-0.8%) 0.70
L Caudal Middle Frontal	<b>(-4%) 0.01</b>	(-0.4%) 0.36	(-3%) 0.07
L Superior Frontal	<b>(-1%) 0.02♣</b>	<b>(-1%) 0.02</b>	(+0.5%) 0.86
L Insula	(+2%) 0.52	(+0.5%) 0.94	(+1%) 0.48
R Rostral Anterior Cingulate	(-2%) 0.43	(-4%) 0.04	(+2%) 0.47
R Caudal Anterior Cingulate	(-0.5%) 0.32	(-2%) 0.23	(+0.3%) 0.87
R Medial OrbitoFrontal	(-0.8%) 0.04	<b>(-3%) 0.006</b>	(+1%) 0.87
R Lateral OrbitoFrontal	(-0.05%) 0.10	(-2%) 0.20	(+1%) 0.64
R Rostral Middle Frontal	<b>(-2%) 0.01</b>	(-1%) 0.21	(-0.8%) 0.25
R Caudal Middle Frontal	(-4%) 0.32	(-0.4%) 0.19	(-3%) 0.93
R Superior Frontal	(-1%) 0.02	(-1%) 0.24	(+0.5%) 0.63
R Insula	(+2%) 0.06	(+0.5%) 0.46	(+1%) 0.09

\*Co-varied for age, \*\*Co-varied for age and ICV. P-values in red were significant after Simes correction.

♣Significant in vertex-based analysis after correction for multiple comparisons. .

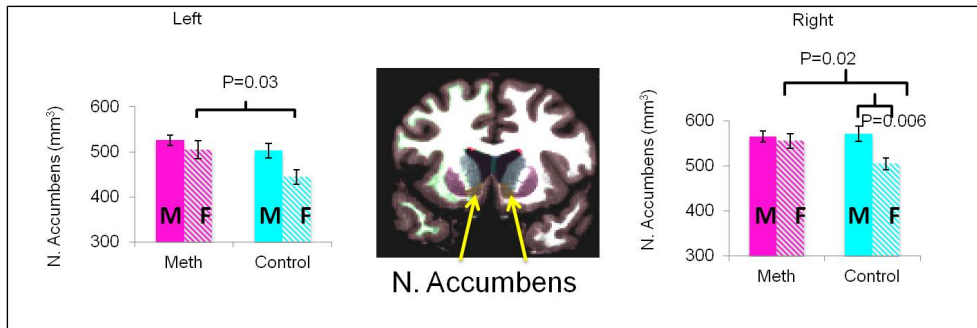


Fig. 2. Center: 2-D Coronal Slice of the Brain. In the left accumbens, the METH users had larger N. accumbens compared to the CON. In the right accumbens, the female METH users had larger N. accumbens compared to female CON. Left: METH main effect ( $p=0.02$ ). METH users had 8% larger left accumbens volume compared to the CON, driven mostly by the 13% larger volume in the female METH users compared to the female CON (post-hoc  $p=0.03$ ). Right: METH-by-sex interaction ( $p=0.03$ ). Right accumbens volume was 10% larger in the female METH users than female CON (post-hoc  $p=0.02$ ), compared to a 1% difference in the two male groups. METH users: the men and women had similar right accumbens volume, CON: the female CON had 12% larger volumes compared to the male CON (post-hoc  $p=0.006$ ).

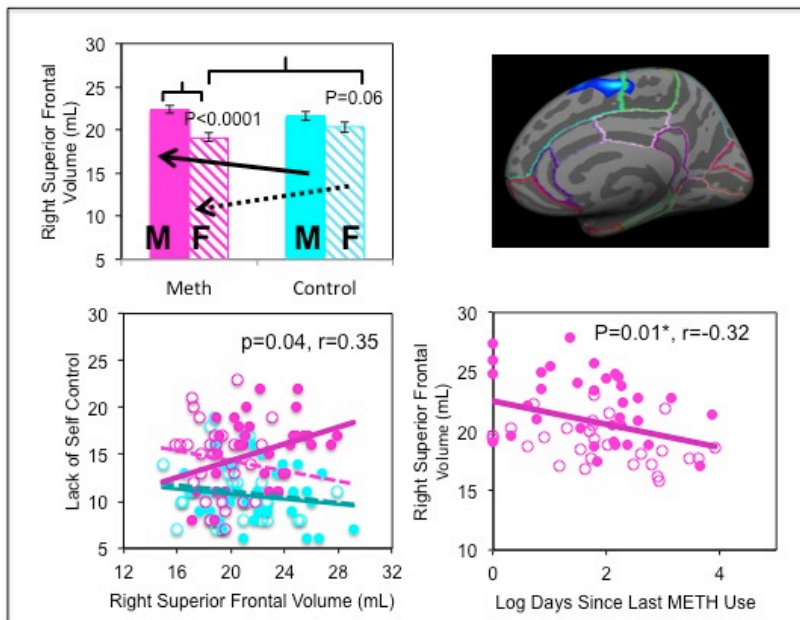


Fig. 3. Male METH users had larger superior frontal volumes compared to CON, which was associated with greater lack of self-control and shorter time periods of abstinence. Top Left: Two-Way ANCOVA METH-by-sex interaction significant in ROI ( $p=0.02$ ) and vertex-based (cluster-wise  $p=0.00005$ ) analysis. Right: Location of significant cluster within superior frontal region in vertex based analysis. Bottom Left: One-way ANCOVA group-by-volume interaction:  $p=0.02$  (co-varied for age and ICV). Male METH users: Male METH users: larger superior frontal volumes associated with greater lack of self-control (Pearson correlation  $p=0.04$ ,  $r=0.35$ .) Bottom Left: METH users: Longer periods of abstinence associated with smaller right superior frontal volumes (\*Pearson correlation  $p=0.01$ ,  $r=-0.32$ , co-varied for age and ICV).

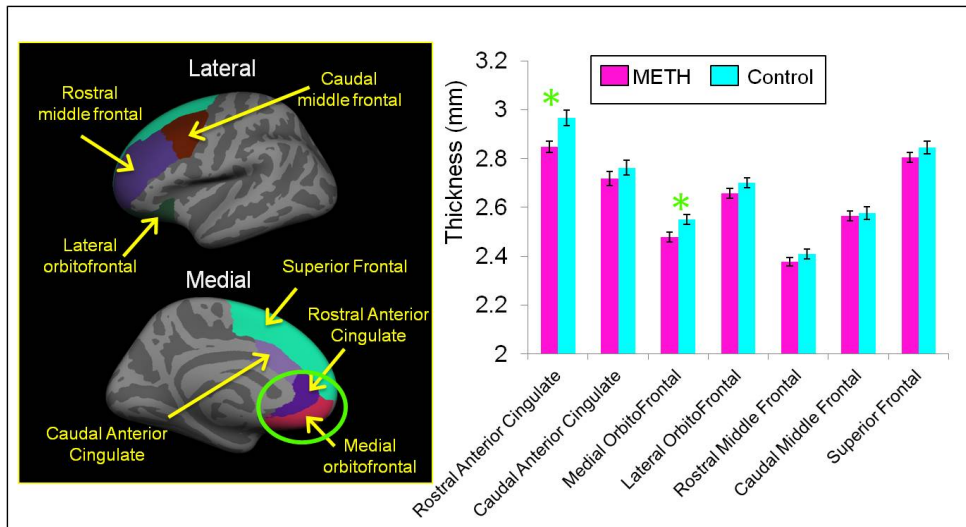


Fig. 4. METH users had consistently thinner left frontal cortices compared to CON. \*indicates the regions that were significant after post-hoc analysis ( $p < 0.05$ ).

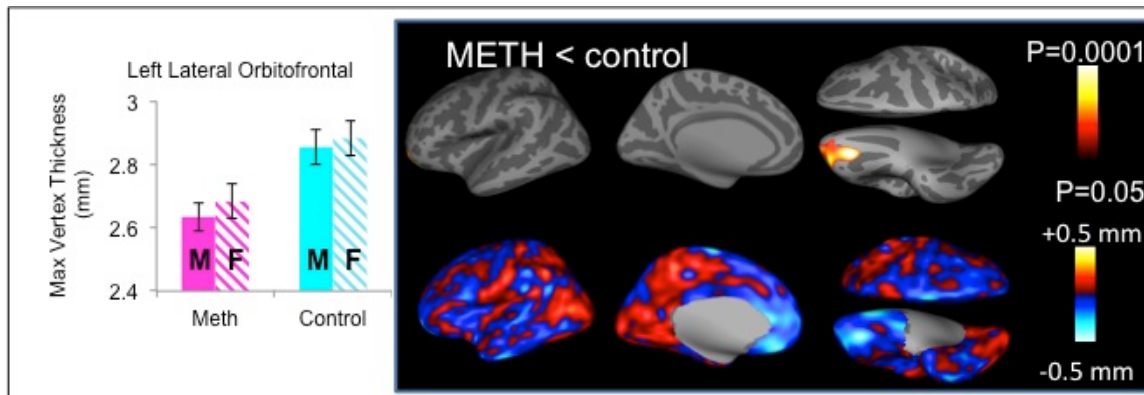


Fig. 5. METH users had thinner cortices in the left lateral orbitofrontal region compared to CON in both ROI and vertex-based analysis (after corrections for multiple comparisons). Left: ROI Two-Way ANCOVA METH effect  $p$ -value = 0.04. Vertex-based analysis cluster-wise  $p$ -value = 0.00006. Both  $p$ -values calculated co-varying for age and ICV. Right: significant  $p$ -value maps (top) and difference maps (bottom) between METH users and CON.

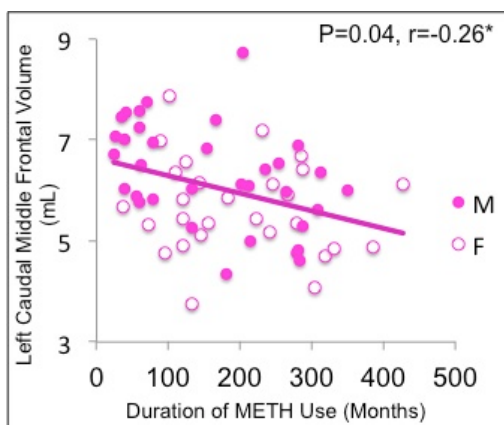


Fig. 6. Smaller caudal middle frontal volumes correlated with longer duration of METH use in the METH users. \*Pearson correlation, co-varied for age and ICV

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