# Decreased frontal white-matter integrity in abstinent methamphetamine abusers

**CINP**

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### Abstract

This study explored differences in frontal white-matter (WM) integrity between methamphetamine (MA) abusers and healthy comparison subjects using diffusion tensor imaging (DTI). Fractional anisotropy (FA) values, which indicate WM integrity, were calculated for regions-of-interest in frontal WM on diffusion tensor images of 32 MA abusers and 30 healthy comparison subjects. Frontal executive functions were also assessed by the Wisconsin Card Sorting test (WCST). MA abusers had significantly lower FA values in bilateral frontal WM at the anterior commissure–posterior commissure (AC–PC) plane and the right frontal WM 5 mm above the AC–PC plane relative to healthy comparison subjects. MA abusers had more total, perseveration and non-perseveration errors in the WCST relative to healthy comparison subjects. FA values of the right frontal WM 5 mm above the AC–PC plane negatively correlated with the number of total and non-perseveration errors in the WCST in MA abusers. In the sub-analysis for gender differences, lower FA values in frontal WM and more errors in the WCST were found only in male MA abusers, not in female MA abusers, relative to comparison subjects of the respective gender. We report that frontal WM integrity of MA abusers is compromised. This finding may also be related to impairment in frontal executive function. In addition, the neurotoxic effect of MA on frontal WM may be less prominent in women than in men, possibly due to oestrogen's neuroprotective effect.

## Introduction

Brain-imaging studies of methamphetamine (MA) abusers have reported various kinds of frontal brain abnormalities (Ernst et al., 2000a; Paulus et al., 2002; Sekine et al., 2003; Volkow et al., 2001). Moreover, the impairment of neuropsychological function has also been reported in MA abusers (Kalechstein et al., 2003; Vorhees et al., 1994). The cognitive impairment in MA abusers may be related to abnormalities of frontal

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lobes of the brain, as shown in prior functional magnetic resonance (MR) studies reporting the failure of normal prefrontal activation during a decision-making task in MA abusers (Paulus et al., 2002, 2003). Recently, we reported that decreased grey-matter densities and glucose metabolism in the frontal region of the brain correlated with the impairment of frontal executive functions in MA abusers (Kim et al., 2005, 2006).

Although most brain-imaging studies in MA abusers have been conducted on the grey matter, frontal white-matter (WM) abnormalities including a decreased level of *N*-acetylaspartate (NAA), a marker of neuronal viability, has also been reported in MA abusers (Ernst et al., 2000a). We have also reported decreased glucose metabolism in frontal WM

(Kim et al., 2005) and increased frontal WM hyperintensities (Bae et al., 2006) in MA abusers.

While studies on WM hyperintensities or WM volumes assess macro-structural changes in WM, diffusion tensor imaging (DTI) enables the measurement of micro-structural changes in WM tracts (Basser, 1995). DTI can provide fractional anisotropy (FA) values, a scalar indicator of WM integrity (Basser, 1995). Although DTI studies in subjects with alcohol and cocaine dependence have been conducted (Lim et al., 2002; Pfefferbaum and Sullivan, 2002), there have been no prior DTI studies in MA abusers.

Effects of MA abuse on the brain may be different between male and female MA abusers. In animal studies, males have been reported to be more susceptible to neurotoxic effects of MA than females (Hirata et al., 1996; Wagner et al., 1993). While frontal lobe abnormalities in MA abusers, including decreased cerebral blood flow, decreased cerebral glucose metabolism and increased WM hyperintensities have been reported to be more prominent in males than females (Bae et al., 2006; Chang et al., 2002; Kim et al., 2005).

Based on previous research suggesting frontal WM abnormalities in MA abusers (Bae et al., 2006; Ernst et al., 2000a; Kim et al., 2005), we hypothesized that MA abusers would have decreased frontal WM integrity, i.e. lower FA values, and impaired frontal executive function relative to healthy comparison subjects. We also hypothesized that, in accord with our prior studies reporting potential gender difference in MA neurotoxicity (Bae et al., 2006; Kim et al., 2005), decreased frontal FA values would be more pronounced in male MA abusers than in female MA abusers.

## Method

### Subjects

Study subjects were recruited through advertisements in local newspapers in Seoul, South Korea. Inclusion criteria for MA abusers were: (1) aged 19–49 years, (2) lifetime diagnosis of DSM-IV MA dependence, as determined by the Structured Clinical Interview for DSM-IV (SCID-IV), (3) abstinence period >4 wk, and (4) cumulative intravenous MA abuse over 50.0 g.

We set abstinence duration as an inclusion criterion in order to avoid the potential confounding effects of acute intoxication, withdrawal and recovery due to recent MA use.

Exclusion criteria for MA abusers and healthy comparison subjects were: (1) lifetime significant medical illness such as hypertension, hepatitis, and diabetes mellitus, (2) comorbid Axis I psychiatric disorders, as determined by SCID-IV, (3) antisocial or borderline personality disorders, as identified by the Personality Disorder Questionnaire-4, (4) lifetime exposure to any other DSM-IV dependence- or abuse-related drugs, except nicotine, caffeine, alcohol drinking and prescribed medications (every subject who drinks >8 g of ethanol per week but does not have a lifetime diagnosis of alcohol-related disorder, was defined as a social drinker), (5) Contraindications to MR scanning, and (6) subjects with grade 1–2 or more WM hyperintensities in deep frontal WM by the modified version of the Coffey classification were also excluded (Bae et al., 2006).

To detect the current abuse of MA, cocaine, opiate, phencyclidine, and marijuana, urine screening was conducted with the Redwood Biotech® (Santa Rosa, CA, USA) urine strip. Information regarding lifetime exposure to dependence- or abuse-related drugs was obtained from structured interviews. Severity and complications of MA abuse was assessed by the Addiction Severity Index (ASI). The screen for the HIV-positive subjects was not conducted for ethical and legal issues. However, the prevalence of HIV infection in Korea is substantially lower than that in other countries (Kim et al., 2003). Furthermore, only 1.1% of HIV transmissions in South Korea have been reported to be attributable to intravenous street drug injections, as disposable syringes are readily available in pharmacies in South Korea (Kim et al., 2003). HCV Ab and HBs Ag tests were performed to exclude the presence of hepatitis C and hepatitis B.

Screening procedures were as follows. In total, 197 subjects who potentially met inclusion criteria were referred. Out of these 197 subjects, those with a prior exposure history of inhalant, marijuana, MDMA or cocaine ($n=60$, $n=41$, $n=6$, $n=1$, respectively and not mutually exclusive), subjects with a current or past history of alcohol abuse or dependence ($n=49$), subjects with current or lifetime psychiatric disorders (major depressive disorder, $n=35$; schizophrenia and delusional disorder, $n=6$; bipolar I and II, $n=8$; panic disorder, $n=5$; generalized anxiety disorder, $n=4$, and antisocial personality disorders, $n=8$), and subjects with hypertension, hepatitis, and diabetes mellitus ($n=26$, $n=7$, $n=34$, respectively and not mutually exclusive) were excluded from the brain-imaging portion of the study. No study subjects had a current or past history of attention deficit hyperactivity disorder (ADHD) as assessed by interviews and school reports. In addition, all female study subjects were all pre-menopausal and had no current or past history of endocrinal diseases.

The study protocol was approved by the Institutional Review Boards at Seoul National University
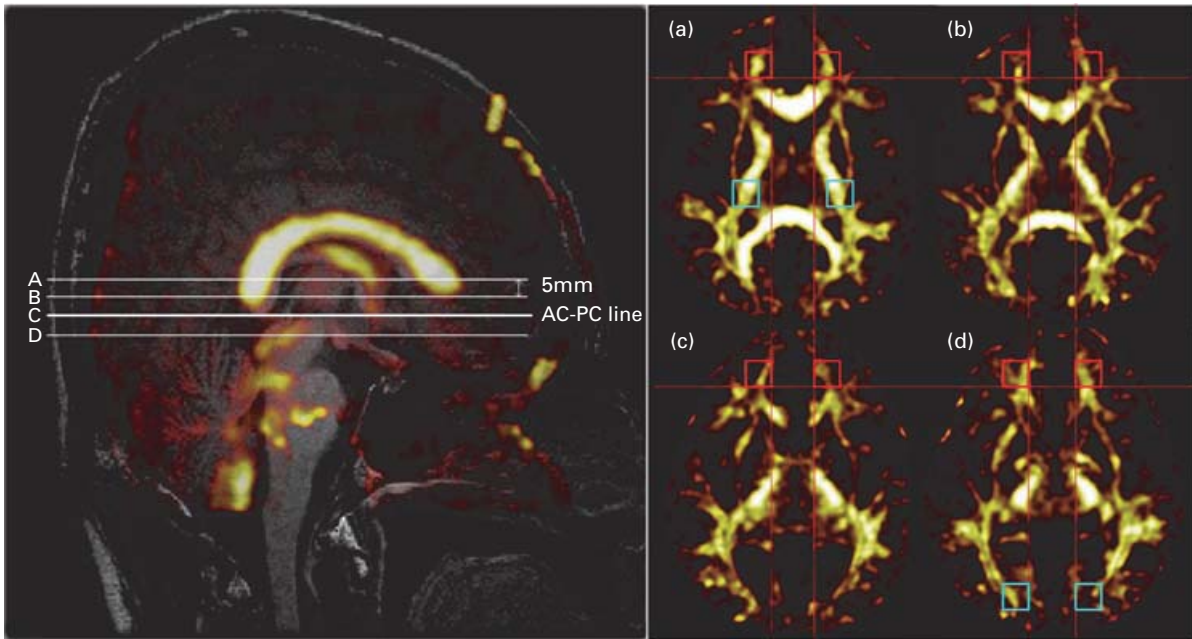
**Figure 1.** Regions of interest (ROIs) for fractional anisotropy (FA) value measurement. Axial view of four frontal white matter (WM) ROIs (red square) and two reference WM (blue square) ROIs locations in co-registered FA maps. (a) Axial FA image, 10 mm above the anterior commissure–posterior commissure (AC–PC) plane. (b) Axial FA image, 5 mm above the AC–PC plane. (c) Axial FA image, the AC–PC plane. (d) Axial FA image, 5 mm below the AC–PC plane.

Hospital, Seoul, South Korea, and McLean Hospital, Massachusetts, USA. After a complete description of the study to the subjects, written informed consent was obtained. All study procedures including MR scans were conducted in South Korea.

Finally, 32 MA abusers (23 men and 9 women, $34.0 \pm 7.5$ yr) and 30 healthy comparison subjects (20 men and 10 women, $31.6 \pm 6.7$ yr) were recruited through advertisements in local newspapers and at the Korean Association against Drug Abuse.

*Acquisition and processing of diffusion tensor images*

All MR imaging was performed using a 3.0 T GE whole body imaging system (GE VH/I; General Electric, Milwaukee, WI, USA). A three-dimensional spoiled gradient echo-pulse sequence was used for anatomical localization (TE = 1.4 ms, TR = 5.7 ms, TI = 400 ms, $256 \times 256$ matrix, FOV = 22 cm, Flip angle = 20°, 1 NEX). No brain structural abnormalities were noted for either group of subjects in clinical qualitative readings of axial T2 images and fluid attenuated inversion recovery images.

A dual spin-echo echo-planar imaging (EPI) sequence was used to acquire diffusion tensor images. MR images with 25 non-collinear diffusion gradients and without diffusion gradient were acquired (TE/TR = 90 ms/10 000 ms, B factor = 0, 1000 s/mm², matrix = $256 \times 256$, slice thickness/gap = 3.5 mm/0 mm, FOV = 24 cm, total slice number = 38, scan average = 1).

Twenty-six diffusion weighted images (DWIs) were acquired with time interval. Among them, only one DWI was without diffusion gradient. All other DWIs have their own diffusion gradients. To correct potential motion-related artifacts, all DWIs with diffusion gradients were realigned to the DWI without diffusion gradients. To avoid EPI-induced distortion, the diffusion weighted image of each subject was co-registered to his/her own T1 image. For these re-alignment and co-registration procedures, Statistical Parametric Mapping (SPM2) software was used (Oakes et al., 2005). After the realignment and co-registration procedures, further post-processing such as smoothing or filtering was not performed, as these processes may potentially distort the data considering the small region-of-interest (ROI) size in the current study. FA maps were constructed by calculating FA values on each voxel.

*Placement of ROIs*

Isocubic ROIs ($10 \times 10 \times 10$ mm) were placed on the bilateral frontal WM in the FA map (Figure 1). ROI placements were performed in the following

order: (1) rotation of all FA maps for the anterior commissure–posterior commissure (AC–PC) line to be parallel (Lim et al., 2002), (2) selection of one axial slice of FA map at the level of the AC–PC line for each subjects, (3) placement of ROI for bilateral frontal WM anterior to the corpus callosum in the axial slice at the level of the AC–PC line, (4) selection of three more axial slices at 5-mm intervals (5 mm below, 5 mm above, 10 mm above) parallel to the predetermined axial slice AC–PC line, (5) placement of ROIs in three other axial slices (5 mm below, 5 mm above, 10 mm above the AC–PC plane) according to ROI locations in the axial slice at the level of the AC–PC plane. Since even a minimal overlap of ROIs with adjacent corpus callosum is likely to 'contaminate' FA values due to high mean FA values in the corpus callosum, a potential overlap with corpus callosum was carefully detected semi-automatically in procedures for the ROI placement, and consequently avoided. In each slice, ROIs were fixed at the same vertical locations. ROI placement and FA value calculations were conducted by an experienced research associate (A.C.), blind to the diagnosis and clinical information of study subjects, using Interactive Data Language-based in-house application (IDL, Research Systems Inc., Boulder, CO, USA).

Our ROIs may include grey matter as well as WM. As grey matter has much lower FA values than does WM, the inclusion of grey matter within ROIs may have confounded the findings. For the exclusion of the grey-matter portion, ROI size or location should not be pre-determined but be modifiable according to the size and location of WM in each ROI of each individual. However, this user-dependent selection of ROI locations will potentially decrease test–retest and inter-operator reliabilities. Therefore, we decided to use fixed size and location of ROIs for higher reliability, although ROIs can include the grey-matter volumes as in prior DTI studies of similar methods (Lim et al., 2002). Future measurement of FA values using the tractography-defined ROI would be helpful, as the tractography can both define the WM tracts and have high reliability at the same time.

Intra-operator and inter-operator reliabilities were calculated by FA values of the frontal ROIs in the AC–PC plane. Each FA value was acquired after operator-guided ROI selection and computerized calculation. Intra-operator reliabilities were tested by the same operator who unknowingly measured the same MR imaging sets ($n=30$) over a 1-wk interval. Intra-class correlation coefficients (ICC) for the right and left deep frontal WM in the AC–PC plane were 0.89 and 0.91 respectively. Inter-operator reliability between two independent operators (number of image sets $=30$) were 0.84 and 0.85 for the right and left deep frontal WM in the AC–PC plane respectively.

## Wisconsin Card Sorting test (WCST)

The WCST was conducted to examine the frontal executive function (Robinson et al., 1980). The number of perseveration errors, non-perseveration errors, and total errors (perseveration errors + non-perseveration errors) was used for the statistical analysis.

## Statistical analysis

Group differences in continuous and categorical variables were computed using independent $t$ test, ANCOVA and Fisher's exact test respectively. Associations between continuous variables were calculated using the Pearson correlation analysis. Statistical significance was defined at an $\alpha$-level of $<0.05$ using two-tailed tests. STATA 6.0 for Windows (StataCorp., College Station, TX, USA) was used for computations.

First, MA abusers ($n=32$) and healthy comparison subjects ($n=30$) were compared. In the sub-analysis, comparisons between male MA abusers ($n=23$) and male comparison subjects ($n=20$) and between female MA abusers ($n=9$) and female comparison subjects ($n=10$) were conducted to explore potential gender effects.

## Results

### Demographic and clinical data

There were no significant differences in age, gender composition, prevalence of social alcohol drinking, handedness or parents' socioeconomic status between MA abusers and healthy comparison subjects. MA abusers had a lower educational level than healthy comparison subjects (independent $t$ test: $t=8.33$, d.f. $=60$, $p=0.01$). It was practically impossible to recruit healthy comparison subjects with educational levels comparable to those in MA abusers. Instead, we matched the socioeconomic status of parents between groups. Prevalence of current cigarette smoking tended to be higher in MA abusers relative to healthy comparison subjects (Fisher's exact test, $p=0.004$). All MA abusers were intravenous abusers. Detailed clinical information, demographic information and detailed drug-related variables of subjects are presented in Table 1.

There were no significant differences in total cumulative dose, average daily dose, mean abstinence

**Table 1.** Demographic characteristics of MA abusers and healthy comparison subjects

| | MA abusers (n=32) | | | | Healthy comparison subjects (n=30) | | | |
|---|---|---|---|---|---|---|---|---|
| | Men (n=23) | | Women (n=9) | | Men (n=20) | | Women (n=10) | |
| Demographic variables | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| Age (yr) | 36.0 | 6.7 | 29.0 | 7.2 | 33.3 | 6.6 | 28.7 | 6.0 |
| Handedness (right) | 22 | 95.7% | 7 | 77.8% | 18 | 90.0% | 9 | 90.0% |
| Parent's SES | | | | | | | | |
|   High | 2 | 8.7% | 2 | 22.2% | 4 | 20.0% | 3 | 30.0% |
|   Middle | 12 | 52.2% | 4 | 44.4% | 11 | 55.0% | 5 | 50.0% |
|   Low | 9 | 39.1% | 3 | 33.3% | 5 | 25.0% | 2 | 20.0% |
| Social alcohol drinking | 17 | 73.9% | 5 | 55.6% | 14 | 70.0% | 5 | 50.0% |
| Current smoking* | 18 | 78.3% | 7 | 77.8% | 9 | 45.0% | 3 | 30.0% |
| MA abuse characteristics | | | | | | | | |
|   Intravenous use | 23 | 100.0% | 9 | 100.0% | – | – | – | – |
|   Total cumulative dose (g) | 411.7 | 542.8 | 133.3 | 149.8 | – | – | – | – |
|   Average daily dose (g) | 0.58 | 0.47 | 0.29 | 0.25 | – | – | – | – |
|   Age of initial use (yr) | 24.4 | 5.2 | 21.9 | 6.8 | – | – | – | – |
|   Duration of abuse (month) | 74.9 | 50.5 | 46.7 | 50.2 | – | – | – | – |
|   Abstinence duration (month) | 24.3 | 37.5 | 43.1 | 65.9 | – | – | – | – |

SES, Socioeconomic status; MA, methamphetamine.

* Fisher's exact test, $p = 0.002$.

period, handedness, or prevalence of social alcohol drinking and current cigarette smoking between male and female MA abusers. Male MA abusers were significantly older than female MA abusers (independent $t$ test: $t = 2.59$, d.f. $= 30$, $p = 0.014$).

There were no significant differences in age, handedness, or prevalence of social alcohol drinking and current cigarette smoking between 23 male MA abusers (age $36.0 \pm 6.7$ yr) and 20 male comparison subjects (age $33.3 \pm 6.6$ yr). There were no significant differences in age, handedness, or prevalence of social alcohol drinking between nine female MA abusers (age $29.0 \pm 7.2$ yr) and 10 female comparison subjects (age $28.7 \pm 6.0$ yr). Prevalence of current cigarette smoking tended to be higher in female MA abusers relative to female comparison subjects (Fisher's exact test, $p = 0.02$).

***Comparison between MA abusers and healthy comparison subjects***

Relative to healthy comparison subjects, MA abusers had significantly lower FA values in three ROIs: the right and left frontal WM at the AC–PC plane and the right frontal WM 5 mm above the AC–PC plane (independent $t$ tests: 11.6% decrease, $0.302 \pm 0.046$ vs.

$0.337 \pm 0.041$, $t = 3.16$, d.f. $= 60$, $p < 0.01$; 7.5% decrease, $0.294 \pm 0.046$ vs. $0.316 \pm 0.041$, $t = 2.01$, d.f. $= 60$, $p < 0.05$; 8.3% decrease, $0.330 \pm 0.036$ vs. $0.360 \pm 0.036$, $t = 3.35$, d.f. $= 60$, $p < 0.01$, respectively) (Figure 2). There were no significant differences between MA abusers and comparisons at other ROIs (Figure 2) including parietal and occipital regions (Figure 3).

MA abusers had significantly more total, non-perseveration, and perseveration errors in the WCST relative to healthy comparison subjects (independent $t$ tests: $t = 3.02$, d.f. $= 60$, $p < 0.01$; $t = 2.73$, d.f. $= 60$, $p < 0.01$; $t = 2.62$, d.f. $= 60$, $p = 0.01$, respectively). Within the healthy comparison group, there were no significant correlations between the number of errors (total, perseveration and non-perseveration errors) of the WCST and FA values in all ROIs.

FA values in the right frontal WM at 5 mm above the AC–PC plane in MA abusers negatively correlated with the number of total and non-perseveration errors in the WCST (Pearson's correlations: $r = -0.46$, $n = 32$, $p = 0.01$; $r = -0.51$, $n = 32$, $p < 0.01$, respectively), but not with the number of perseveration errors in the WCST (Pearson's correlations: $r = -0.26$, $n = 32$, $p = 0.15$, respectively). FA values in the right frontal WM at 10 mm above the AC–PC plane in MA abusers negatively correlated with the number of
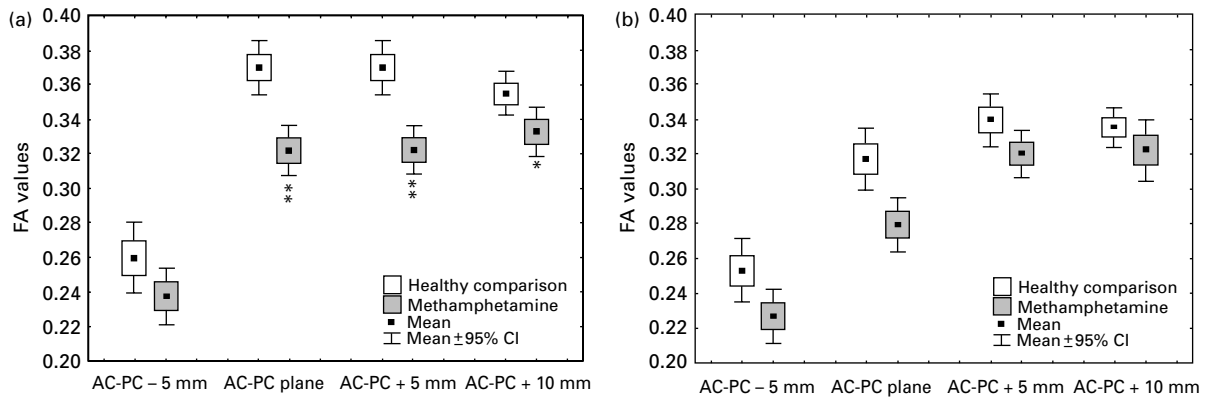
**Figure 2.** (a) Fractional anisotropy (FA) values in right frontal white matter (WM) of methamphetamine abusers ($n = 32$) and healthy comparison subjects ($n = 30$). (b) FA values in left frontal WM of methamphetamine abusers ($n = 32$) and healthy comparison subjects ($n = 30$) (* $p < 0.05$, ** $p < 0.01$). AC–PC, Anterior commissure–posterior commissure plane.
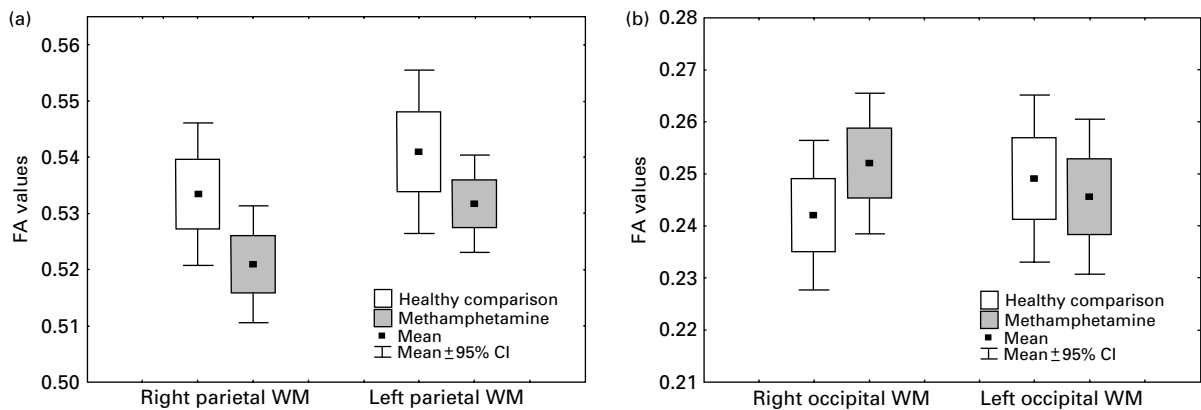


**Figure 3.** (a) Fractional anisotropy (FA) values in parietal white matter (WM) of methamphetamine abusers ($n = 32$) and healthy comparison subjects ($n = 30$). (b) FA values in occipital WM of methamphetamine abusers ($n = 32$) and healthy comparison subjects ($n = 30$). AC–PC, Anterior commissure–posterior commissure plane.

non-perseveration errors in the WCST (Pearson's correlations: $r = -0.42$, $n = 32$, $p = 0.02$, respectively), but not with the number of total and perseveration errors in the WCST (Pearson's correlations: $r = -0.34$, $n = 32$, $p = 0.06$; $r = -0.15$, $n = 32$, $p = 0.42$, respectively).

Age did not correlate with FA values in any ROIs in MA abusers or healthy comparison subjects. There was no correlation either when each gender was tested separately. The influence by potential confounders including educational level and smoking was tested in additional analyses. Additional analyses showed that between-group differences in FA values or the number of errors in the WCST remained significant after controlling for educational levels. There were no significant differences in FA values or the number of errors in the WCST between MA abusers who were smokers

($n = 25$) and those who were not ($n = 7$), or between smokers ($n = 12$) and non-smokers ($n = 18$) in the healthy comparison group.

### Sex differences of the frontal WM integrities

#### Findings in male MA abusers

Relative to male comparison subjects, male MA abusers had significantly lower FA values in five ROIs: the left frontal WM at 5 mm below the AC–PC plane, the right and the left frontal WM at the AC–PC plane, the right frontal WM at 5 mm above the AC–PC plane, and the right frontal WM at 10 mm above the AC–PC plane (independent $t$ tests: $t = 2.31$, d.f. $= 41$, $p = 0.02$; $t = 4.15$, d.f. $= 41$, $p < 0.001$; $t = 3.26$, d.f. $= 41$, $p < 0.01$; $t = 4.70$, d.f. $= 41$, $p < 0.001$; $t = 2.44$, d.f. $= 41$, $p = 0.02$, respectively) (Figure 4). There were no significant
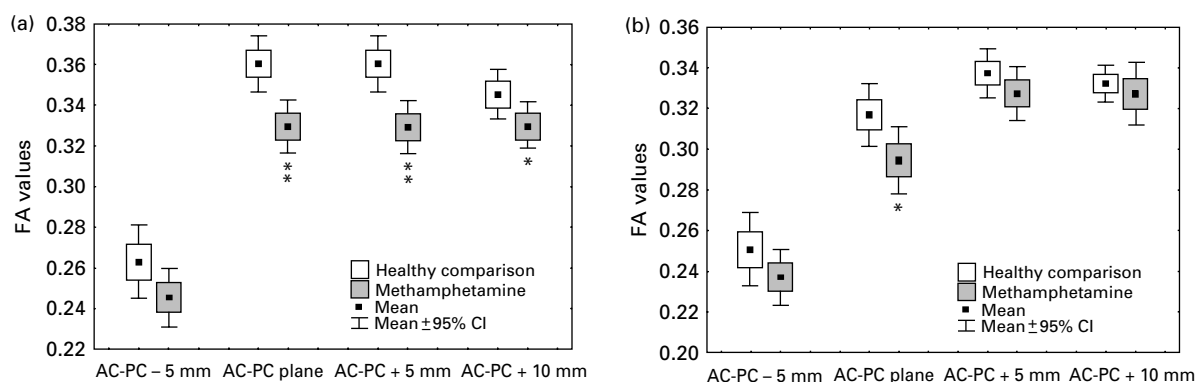
**Figure 4.** (a) Fractional anisotropy (FA) values in right frontal white matter (WM) of male methamphetamine abusers ($n=23$) and male comparison subjects ($n=20$). (b) FA values in left frontal WM of male methamphetamine abusers ($n=23$) and male comparison subjects ($n=20$) (* $p<0.05$, ** $p<0.01$). AC–PC, Anterior commissure–posterior commissure plane.
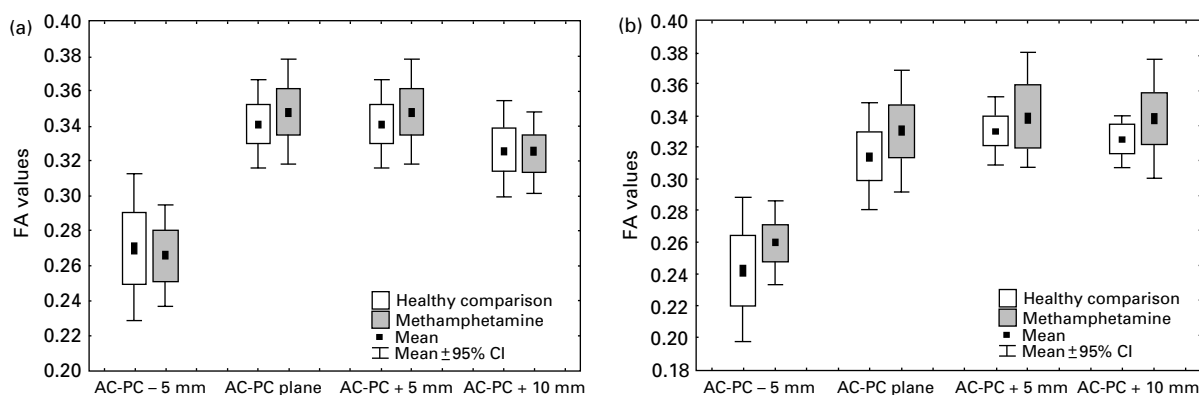


**Figure 5.** (a) Fractional anisotropy (FA) values in right frontal white matter (WM) of female methamphetamine abusers ($n=9$) and female comparison subjects ($n=10$). (b) FA values in left frontal WM of female methamphetamine abusers ($n=9$) and female comparison subjects ($n=10$). AC–PC, Anterior commissure–posterior commissure plane.

differences in FA values in the other three frontal WM ROIs (Figure 4). Male MA abusers had significantly more total errors, perseveration errors and non-perseveration errors in the WCST relative to male comparison subjects (independent $t$ test: $t=3.10$, d.f. $=21$, $p<0.01$; $t=2.78$, d.f. $=21$, $p<0.01$; $t=2.63$, d.f. $=21$, $p=0.01$, respectively).

In male MA abusers, the number of total errors and non-perseveration errors in the WCST negatively correlated with FA values in the right frontal WM at 5 mm above the AC–PC plane (Pearson's correlations: $r=-0.42$, $n=23$, $p<0.05$; $r=-0.50$, $n=23$, $p=0.02$, respectively). In addition, the number of total errors and non-perseveration errors in the WCST negatively correlated with FA values in the right frontal WM at 10 mm above the AC–PC plane ($r=-0.43$, $n=23$, $p<0.05$; $r=-0.51$, $n=23$, $p=0.01$, respectively).

*Findings in female MA abusers*

There were no significant differences in FA values in all ROIs between female MA abusers and female comparison subjects (Figure 5). Moreover, there were no significant differences in total errors, perseveration errors and non-perseveration errors in the WCST between female MA abusers and female comparison subjects. There were no significant correlations of the number of errors (total, perseveration and non-perseveration errors) in the WCST with FA values in all ROIs in female MA abusers.

**Discussion**

We report decreased frontal WM integrities in MA abusers relative to healthy comparison subjects.

In sub-analysis to investigate potential gender differences, our findings of decreased frontal FA values were found only in male MA abusers, not in female MA abusers.

To the best of our knowledge, the current study is the first DTI study in MA abusers. Our strict screening procedure for the selection of study subjects, who were without lifetime exposures to illicit drugs or comorbid psychiatric disorders, suggests that our findings are due to the effects of MA. As MA is much more easily available than other illicit drugs in Korea, MA abuse or dependence comprises 74.2% of all prosecutions for illicit drug abuse (Department of Justice, South Korea). Consequently, subjects with the sole' diagnosis of MA dependence were efficiently recruited for this study.

In accord with our first hypothesis, MA abusers had decreased FA values in bilateral frontal WM. Our findings were consistent with a prior report showing decreased NAA level, decreased glucose metabolism and increased WM hyperintensities in the frontal WM of MA abusers (Bae et al., 2006; Ernst et al., 2000a; Kim et al., 2005). Our findings of low FA values in the frontal WM of MA abusers suggest potential frontal WM deficits. WM deficits in MA abusers may be related to an altered myelination (Albertson et al., 2004; Melo et al., 2006). In animal studies, MA has been reported to induce abnormal myelination process (Melo et al., 2006). Abnormal myelin productions have also been reported in abusers of cocaine, which is also a very addictive psychostimulant (Albertson et al., 2004). Cell body injury by MA exposure may be another plausible mechanism for WM deficits. MA-induced cell body injury, by apoptosis (Deng et al., 2001) or dopaminergic overflow (Sulzer et al., 1995), may induce Wallerian degeneration of axons, which are closely related to WM deficits observed in this study.

The frontal cortex and striatum are two of the most vulnerable regions to neurotoxic effects of MA (Sekine et al., 2003; Volkow et al., 2001). These two regions are functionally connected to each other, as shown in a study reporting correlations between striatal dopamine $D_2$ receptor levels and the metabolism of the orbitofrontal cortex in MA abusers (Volkow et al., 2001). Our findings may suggest that, as well as frontal cortex and striatum per se, WM located between these two areas may also be vulnerable to MA. However, to confirm whether the current finding of compromised frontal WM integrity is related to changes in structural connectivity between the frontal cortex and striatum, a future study defining WM tracts connecting these two regions using tractrography and assessing their FA values is recommended.

In our study, the decrease in FA values of frontal WM correlated with decreased WCST performances in MA abusers. This correlation supports the view that decreased frontal WM integrity may underlie impairment in frontal executive function observed in MA abusers. This is also in line with our recent study reporting the correlation between decreased glucose metabolism in frontal WM and the impaired frontal executive function in MA abusers (Kim et al., 2005).

Besides this dysfunction in decision making, clinical manifestations of drug dependence, such as craving or compulsive drug-seeking, have been suggested to be associated with abnormalities in the prefrontal cortex (Goldstein et al., 2002). Decreased frontal WM integrities, assessed by decreased FA values, have also been reported in subjects with other drug dependencies (Lim et al., 2002; Pfefferbaum and Sullivan, 2002). Therefore, decreased frontal WM integrities may also be related to common clinical manifestations of drug dependence.

In line with our second hypothesis, the decreased FA values in frontal WM and impairment in the frontal executive function were found only in male MA abusers. These finding of gender difference (Dluzen et al., 2003; Garcia-Segura et al., 1999) are in accord with previous studies reporting more pronounced hypoperfusion, hypometabolism and WM hyperintensities in frontal regions of male MA abusers relative to female MA abusers (Bae et al., 2006; Chang et al., 2002; Kim et al., 2005). Similar gender differences in cerebral perfusion and metabolites of frontal WM have also been reported in those who abuse cocaine, another addictive psychostimulant (Chang et al., 1999; Ernst et al., 2000b).

Effects of oestrogen may be the most probable mediating factor which may underline the gender–MA interaction observed in our study. Protective effects of oestrogen against MA had been reported in animal studies (Culmsee et al., 1999; Gao and Dluzen, 2001). Neuroprotective effects of oestrogen can be mediated by a number of factors, including a cerebrovascular protective effect (Paganini-Hill et al., 1988), antioxidant effects (Sawada et al., 1998), inhibiting $Ca^{2+}$ channels in striatal neurons (Mermelstein et al., 1996), inhibiting dopamine transporter function (Wirz-Justice et al., 1974), or reduction of MA-induced hyperthermia (Dluzen et al., 2002). Assessment of the relationship between the oestrogen level and frontal FA values in female MA abusers would be helpful in verifying the neuroprotective

effects of oestrogen against MA. However, the status of oestrogen level was not measured in this study, although our female subjects were all of pre-menopausal status.

However, there may be other factors playing additional roles in the gender differences of MA effects, as men have been reported to be unable to benefit from the protective effects of oestrogen against MA (Dluzen and McDermott, 2002). Female mice have been reported to express augmented mRNA of glial fibrillary acidic protein to MA exposure, which is associated with glial repair response to brain damage (Dluzen et al., 2003; Garcia-Segura et al., 1999). Therefore, the maintenance of frontal WM integrities in female MA abusers in our study may be related to a more augmented glial repair response in female MA abusers than in male MA abusers.

Study subjects in this study overlapped in part with those in our three previous studies of voxel-based morphometry (VBM) (Kim et al., 2006), positron emission tomography (PET) (Kim et al., 2005) and WM hyperintensities (Bae et al., 2006). Twenty-two MA users (14 males, 8 females) and 25 comparison subjects (18 males, 7 females) participated both in the current study and our previous WM hyperintensities study. Nineteen MA users (17 males, 2 females) and 16 comparison subjects (14 males, 2 females) participated both in the current study and our previous VBM study. Twenty-two MA users (18 males, 4 females) and 14 comparison subjects (12 males, 2 females) participated both in the current study and our previous PET study. Reasons for these partial overlaps include that not all subjects have completed all the protocols of the imaging study of brain MRI, SPECT, and PET. Image quality problems including motion artifacts and individual technical problems for specific scanning parameters of T1 SPGR, T2, and DTI were other reasons.

We have conducted additional analyses for over-lapped subjects between the current study and our two previous studies of VBM and PET, in order to further investigate relationships between imaging modalities. In the VBM study, we reported lower grey-matter density in the right middle frontal cortex in MA abusers. The grey matter density in the right middle frontal cortex in MA abusers did not correlate with FA values in any ROIs. In the PET study, we reported glucose hypometabolism in the right superior frontal WM of MA abusers. The glucose metabolism in the right superior frontal WM in MA abusers positively correlated with FA values of the right frontal WM at the AC–PC plane and 5 mm above the AC–PC plane ($r = 0.39$, $p = 0.02$; $r = 0.37$, $p = 0.03$, respectively).

In summary, low frontal FA values in our study correlated only with glucose hypometabolism in the frontal WM, but not with the frontal grey-matter density decrease. These results suggest that WM integration is more related to WM glucose metabolism than to deficits in grey-matter density. This relationship makes sense, as WM regions of less integrity in DTI may consume a lesser degree of glucose, as assessed by PET. On the contrary, the association between frontal WM integration and frontal grey-matter density might be not strong enough to attain statistical significance. In order to investigate the relationship between grey-matter and WM abnormalities in MA abusers, further studies are recommended.

Limitations of our study include the small sample size of female MA abusers. Considering the small sample size, the gender difference in our study should not be considered as evidence that MA abuse is not harmful for women at reproductive ages. However, there were consistent gender differences in frontal executive functions, which in turn correlated with the decreased frontal WM integrities. Therefore, although our finding may suggest that the degree of MA-induced neurotoxicity on frontal WM might be different according to gender, it does not imply the absence of MA-induced neurotoxicity in women. Future studies with a larger female sample size would be helpful to confirm and extend this finding.

Moreover, although there were no significant differences in age between male/female MA abusers and their respective gender-matched comparison subjects, there was a significant difference in age between female and male MA abusers. Therefore, the gender difference in FA value decrease with MA abuse may be potentially induced by age differences between male and female MA abusers. However, as there were no correlations between age and frontal FA value, it is unlikely that our gender–MA interaction was confounded by the potential age–MA interaction.

Another limitation of our study was the higher prevalence of the current smoking in our MA abusers than in healthy comparison subjects. However, as our findings did not change after controlling current smoking, it seems unlikely that the difference in smoking biased the current findings. In addition, although there were no significant differences of age between male/female MA abusers and their respective gender-matched comparison subjects, there was a significant difference in age between female and male MA abusers. However, as there were no correlations between age and frontal FA values, it is unlikely that

our gender–MA interaction was confounded by the potential age–MA interaction.

Strict screening procedure can be also considered a limitation of our study from one perspective as well as a strength from another perspective. Our sample of MA abusers may not be representative of the 'typical' MA abusers, as a number of MA abusers commonly have comorbid psychiatric disorder or substance abuses. Therefore, our findings may not be generalized to MA abusers with other psychiatric disorders or substance abuse. However, we initially intended to assess the neurobiological effects of MA, which was not confounded by other comorbid conditions. Consequently, we applied strict exclusion criteria for MA abusers, rather than including heterogeneous MA abusers.

In conclusion, we report that abstinent MA abusers had disrupted integrities in frontal WM. This decrease of frontal WM integrity in MA abusers may be, in part, associated with clinical manifestations including the impairment in the frontal executive function. Further, as disrupted frontal WM integrities were found only in male MA abusers, our finding suggests that men, rather than women, are more vulnerable to MA-induced effects on frontal WM.

## Statement of Interest

None.

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