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Recovery of Dopamine Transporters with Methamphetamine Detoxification is not Linked to Changes in Dopamine Release

Nora D. Volkow MD.[1,4], Gene-Jack Wang MD.[1], Lisa Smith MD[2]
Joanna S. Fowler Ph.D.[3], Frank Telang MD., Jean Logan Ph.D.[1]
Dardo Tomasi Ph.D.[1]

1 Laboratory of Neuroimaging, Intramural Program, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA
2 Department of Psychiatry, University of California, Los Angeles, CA, USA
3 Medical Department, Brookhaven National Laboratory, Upton, NY, USA
4 Office of Director, National Institute on Drug Abuse, Bethesda, MD, USA.

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Nora D. Volkow MD.[1,4], Gene-Jack Wang MD.[1], Lisa Smith MD[2]
Joanna S. Fowler Ph.D.3, Frank Telang MD., Jean Logan Ph.D.[1]
Dardo Tomasi Ph.D.[1]

[1]Laboratory of Neuroimaging, Intramural Program, National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA; [2]Department of Psychiatry, University of California, Los Angeles, CA, USA; [3]Medical Department, Brookhaven National Laboratory, Upton, NY, USA; [4]Office of Director, National Institute on Drug Abuse, Bethesda, MD, USA.

Corresponding author:
Nora D. Volkow, M.D.
National Institute on Drug Abuse, NIH
6001 Executive Boulevard, Room 5274,
Bethesda, MD (20892)
Tel. (301) 443-6480
Fax (301) 443-9127
E-mail: nvolkow@nida.nih.gov

Running Title: Recovery of DAT in Methamphetamine Abusers

Abstract

Metamphetamine's widepread abuse and concerns that it might increase Parkinson's disease led us to assess if the reported loss of dopamine transporters (DAT) in methamphetamine abusers (MA) reflected damage to dopamine neurons. Using PET with [^{11}C]cocaine to measure DAT, and with [^{11}C]raclopride to measure dopamine release (assessed as changes in specific bidning of [^{11}C]raclopride between placebo and methylphenidate), which was used as marker of dopamine neuronal function, we show that MA (n=16), tested during early detoxification, had lower DAT (20-30%) but overall normal DA release in striatum (except for a small decrease in left putamen), when compared to controls (n=15). In controls, DAT were positively correlated with DA release (higher DAT associated with larger DA increases), consistent with DAT serving as markers of DA terminals. In contrast, MA showed a trend for a negative correlation (p=0.07) (higher DAT associated with lower DA increases), consistent with reduced DA re-uptake following DAT downregulation. MA who remained abstinent nine-months later (n=9) showed significant increases in DAT (20%) but methylphenidate-induced dopamine increases did not change. In contrast, in controls, DAT did not change when retested 9 months later but methylphenidate-induced dopamine increases in ventral striatum were reduced (p=0.05). Baseline D2/D3 receptors in caudate were lower in MA than in controls and did not change with detoxification, nor did they change in the controls upon retest. The loss of DAT in the MA, which was not associated with a concomitant reduction in dopamine release as would have been expected if DAT loss reflected DA terminal degneration; as well as the recovery of DAT after protracted detoxification, which was not associated with increased dopamine release as would have been expected if DAT increases reflected terminal regeneration, indicate that the loss of DAT in these MA does not reflect degeneration of dopamine terminals.

Key words: addiction, neurotoxicity, dopamine terminal, Parkinson's disease.

Introduction

Methamphetamine (METH) has been shown to be neurotoxic to brain dopamine (DA) neurons in laboratory animals (Seiden LS and KE, 1996), and there is preliminary evidence from two recent epidemiological studies that its abuse might increase the risk for Parkinson's disease (Callaghan et al., 2012; Curtin et al., 2015). Postmortem and brain imaging studies have documented a significant loss of DA transporters (DAT), and of vesicular monoamine transporters (VMAT), which have been used as markers of DA terminals in the brains of methamphetamine abusers (MA)(McCann et al., 1998; Sekine et al., 2001; Volkow ND et al., 2001; Wilson et al., 1996). However, although the initial preclinical studies interpreted the METH-induced decreases of DAT as evidence of irreversible terminal degeneration (Ricaurte and McCann, 1992), studies in rodents (Cass and Manning, 1999; Friedman et al., 1998) and non-human primates (Harvey et al., 2000; Melega et al., 2008) have given evidence of significant recovery with abstinence. Similarly, in humans, two imaging studies, one in 7 MA and another in 8 MA reported DAT recovery within one month of detoxification (Chou et al., 2007; Yuan et al., 2014) and one in 5 MA reported significant recovery following 9 months of detoxification (Volkow et al., 2001a). However, it is still unclear if the loss of DAT in MA reflects damage to DA neurons or downregulation of DAT. Here, we tested the hypothesis that the loss of DAT in the striatum of MA does not reflect degeneration of DA terminals but rather downregulation of DAT and thus would not be associated with decreased DA release in striatum as would be expected if there was degeneration of DA terminals. We also hypothesized that DAT would recover with protracted METH detoxification secondary to DAT upregulation rather than from terminal regeneration and hence DAT increases would not be associated with a parallel increase in DA release. The rationale for this prediction is that if the loss of DAT reflected DA terminal degeneration then DAT increases with protracted detoxification would reflect DA terminal regeneration in which case one would expect increases in DA release. However, if as we hypothesize, the DAT loss in MA reflects downregulation (rather than terminal degeneration) and the DAT increases with detoxification reflect DAT upregulation (rather than regeneration) then this would result in increased DA reuptake into the terminals and with reduced extracellular DA.

For this purpose we measured DAT and DA release in MA (n=16) during the early phases of withdrawal (< 6 months from abstinence) and 9 months later after detoxification. In parallel,

we assessed healthy controls (n=15) and re-tested a sub-group 9 months later to assess test-retest reproducibility over a 9 month interval period. Positron Emission Tomography (PET) was used to measure DAT with [^{11}C]cocaine (DAT radioligand)(Volkow et al., 1998a; Volkow et al., 1998b), and to measure DA release (used as marker of DA neuronal function) with [^{11}C]raclopride (D2/D3 receptor radioligand sensitive to endogenous DA)(Volkow et al., 1994). DA release was measured by comparing [^{11}C]raclopride's specific binding in striatum after placebo to that after methylphenidate (60 mg, oral), which is a stimulant drug that increases DA by blocking DAT (Volkow et al., 1998b). Ten MA were able to stay drug-free at least 9 months later (protracted abstinence) and nine of them agreed to have repeated PET scans. Ten of the 15 healthy controls agreed to return for rescanning 9 months after completion of the first set of studies. The differences in the DA increases triggered by methylphenidate (MP) for the measures taken during early detoxification, between the MA who relapsed and the MA who remained abstinent after 9 months of treatment, along with their comparison with healthy controls have been published (Wang et al., 2012).

Materials and Methods

Subjects: Sixteen methamphetamine abusers (MA: 3 female and 13 male, 39.2±4.9 year old) were recruited from Harbor-University of California – Los Angles (HUCLA) Medical Center and the Portland Veterans Administration Medical Center (PVAMC). Fifteeen healthy participants were recruited to serve as the control group (2 female and 13 male, 37.2±4.3 year old) from the local community. Inclusion criteria for MA was a DSM IV diagnosis of METH abuse or dependence with the use of METH via the smoked or intravenous routes, with a frequency of at least 5 times/week and 0.5 g/day; with a minimum 12-month history of METH abuse, a minimum 2-week period of detoxification prior to scanning and the desire to stop using METH. Exclusion criteria for both groups were: present or past history of neurological or DSM IV Axis I psychiatric disease including a past or present history of drug abuse or dependence (other than METH for the MA; and nicotine for either group), medical illness that can affect brain function, past or present history of cardiovascular disease or high blood pressure. Urine screening tests for psychoactive drugs were performed to corroborate absence of drug use. Details for screening have been published (Volkow ND et al., 2001; Volkow et al., 2001a).

Following the first PET scanning session the MA were referred to an outpatient treatment facility where they were followed for 9 months and underwent periodic random urine-screening tests to ensure sobriety. The outpatient treatment program consisted of an intensive outpatient drug rehabilitation protocol that included group and individual therapy and educational group sessions that provided training on early recovery skills and relapse-prevention techniques. MA did not receive any pharmacological treatments during their outpatient treatment. The controls were contacted 9 months after the first PET scanning session and asked to return for the second scanning session. The MA and controls that agreed to return for a second PET scanning session underwent a second medical exam and screening session to ensure that they still met criteria for participation. For the MA this required that they had remained abstinent during the 9 months detoxification period.

Nine of the ten MA who remained abstinent for the 9 months follow-up period after being scanned during early detoxification, agreed to return for the second PET scanning session (1 female, 8 males; 41 ±4 years of age). Ten of the controls (1 females, 9 males; 36 ±4 years of age) agreed to return for the second PET scanning session. All participants (controls and MA) underwent urine testing on the day of the scans to ensure no drug use. The protocol was approved by the Institutional Review Boards at Stony Brook University/Brookhaven National Laboratory, at HUCLA, and PVAMC. Written informed consent was obtained after the experimental procedure was explained to the participants.

Scanning: The PET scans were carried out on an HR+ high-resolution, whole-body PET scanner (4.5 × 4.5 × 4.8 mm full width at half maximum at center of field of view) in three-dimension acquisition mode with 63 planes (Siemens Medical Solutions Inc, Knoxville, Tennessee). An external chinstrap device was used in addition to the individual head holder to minimize head motion during the scan. For all scans, a transmission scan was obtained with a germanium 68 rotating rod source prior to the emission scans to correct for attenuation. For [^{11}C]cocaine, dynamic scans were started immediately after injection of 5–10 mCi of [^{11}C]cocaine (specific activity 0.5–1.5 Ci/μM at end of bombardment) and carried out for a total of 54 minutes as previously described (Volkow et al., 2009). For [^{11}C]raclopride, dynamic scans were started immediately after injection of 4-10 mCi of [^{11}C]raclopride (specific activity 0.5–1.5 Ci/μM at end

of bombardment) and carried out for a total of 60 minutes as described (Volkow et al., 1994). Participants were scanned with [^{11}C]raclopride twice, 2 hours apart from each other; the first scan was done 60 minutes after oral placebo and the second 60 minutes after oral MP (60 mg). The study used a single blind design (participants were blind to the drugs received). Each PET study included repeated arterial measurements to quantify plasma concentration of total radioactivity and to quantify the fraction of labeled radiotracer in plasma.

Image and data analysis. The images were analyzed both using manual-drawn preselected regions of interest (ROI) and statistical parametric mapping (SPM) analyses, which enabled us to make comparisons on a voxel by voxel basis (Friston KJ et al., 1995).

For the ROI analysis we extracted regions in caudate, putamen, ventral striatum and cerebellum by superimposing boundaries from a neuroanatomical atlas as previously described (Volkow et al., 2001b). Briefly, the ROI were initially outlined on the individual's summed [^{11}C]cocaine and the [^{11}C]raclopride images (images obtained between 15 and 54-60 min), and projected into the dynamic images to generate time–activity curves for the striatal regions (caudate, putamen and ventral striatum) and the cerebellum. The time–activity curves for the tissue concentration, along with the time–activity curves for unchanged tracer in plasma were used to calculate the total tissue distribution volume (V_T). V_T corresponds to the equilibrium measurement of the ratio of tissue concentration to plasma concentration and was obtained using a graphical analysis technique for reversible systems (Logan et al., 1990). The ratio of V_T in striatum to that of V_T in cerebellum corresponds to the non-displaceable binding potential BP_{ND} +1. BP_{ND} was used as an estimate of DAT availability from the [^{11}C]cocaine images and of D2/D3 receptor availability from the [^{11}C]raclopride images obtained after placebo and after MP. MP-induced DA increases were quantified as percent change in BP_{ND} between the placebo and the MP scans.

Differences in DAT between groups were tested using a two group factor ANOVA (Controls vs MA) and differences in MP-induced DA increases were tested using a repeated measure ANOVA model with two conditions (Placebo and MP) and two groups (Controls vs MA) including all the participants that underwent the first scanning session. *Post-hoc t*-tests were used to assess the direction of the differences. Differences in DAT, in MP-induced DA increases

(% change in [^{11}C]raclopride's BP_{ND} from placebo) and in D2/D3 receptor availability (D2/D3R) (placebo measures) between the first and second evaluations were tested using a two group (Controls vs MA) repeated measures ANOVA (first evaluation vs second evaluation). Post hoc t tests were done to assess the direction of the differences.

The ROI measures were used to assess the correlations between DAT and MP-induced DA increases, between DAT and D2/D3R, and between D2/D3R and MP-induced DA increases. The ROI measures were also used to assess the correlations between DAT and the clinical characteristics of the MA and for the correlations between plasma MP concentrations and MP-induced DA increases.

For the voxelwise analyses we used SPM8 (Wellcome Trust Centre for Neuroimaging). For this purpose, we first estimated the V_T for each voxel and then normalized each voxel to the activity in cerebellum (left and right ROIs) in order to obtain BP_{ND} images. Custom Montreal Neurological Institute (MNI) templates, which were previously developed using the DV images from 34 healthy subjects acquired with [^{11}C]raclopride (Wang et al., 2012) were used for the spatial normalization of the BP_{ND} images. The BP_{ND} images were spatially smoothed using an 8-mm Gaussian kernel to minimize the variability of the brain anatomy across subjects.

The V_T ratio images (BP_{ND}) from [^{11}C]cocaine scans were included into a repeated measures Statistical group analyses based on an SPM8 full factorial model with two groups (Controls and MA) and two repeated conditions (First evaluation and Second evaluation), covariates (parts per million CO levels and the age of participants) were used to assess group differences at baseline. A full factorial model with two groups (Controls and MA), two repeated conditions (First evaluation and Second evaluation) and two covariates were used to assess the effect of treatment and treatment × group interaction effects. For the comparisons of MP-induced DA increases we used a full factorial model with two groups (Controls and MA) and two repeated measures (placebo and MP) and conditions (First evaluation and Second evaluation). A mask of regions ('V_T-mask') with high radiotracer binding was developed using an intensity threshold max (V_T)/3. The statistical significance was set by a cluster-level threshold $P_{FWE} < 0.05$, corrected for

multiple comparisons with the family-wise error (FWE) within regions of high radiotracer binding using the V_T-mask.

For all the analyses we used carbon monoxide (CO), a marker for tobacco smoking (Middleton and Morice, 2000) and age as covariates because the groups differed in smoking prevalence and age, and aging is associated with a decline in DAT (around 6.6% decline per decade)(Volkow et al., 1996; Volkow et al., 1994).

RESULTS

Participant Characteristics

The initial groups of MA (n=16) differed from the initial group of controls (n=15) in that they had a greater prevalence of active cigarette smokers (11 MA vs 4 controls, P<0.001) and consequently higher CO levels (16±11 vs 6±7, p=0.009) and, although not significant, they tended to be older than controls (39.2±5 vs 35.9±6 years, p=0.11). The MA had a history of 13±7 years of METH use and an average consumption of 1.2±1.0 g per day. They reported having had the least METH binge episode, on average, 102±49 days prior to recruitment.

The MA who were able to stay abstinent for nine months following their initial PET studies and who completed the second evaluation (n=9) were significantly older than the controls (n=10) who completed the second evaluation (41.3±4 vs 36.0±4 years, p=0.02). Prevalence of active cigarrete smoking (5 MA versus 2 controls), or CO levels did not differ significantly between the groups (MA 10±7 vs Controls 6±8). The subgroup of MA who underwent repeated evaluations had a history of 13±9 years of METH use and an average consumption of 1.6±1 g per day and reported 262±50 days of abstinence at the time of the second evaluation.

Baseline Differences in DAT between Controls and MA.

SPM analysis revealed that DAT were significantly lower in MA (n=16) than in controls (n=15) (Figure 1). The ROI analysis corroborated lower DAT in MA than controls in caudate (CD: 0.55±0.09 vs 0.81±0.07; p=0.0001; 32% lower), putamen (PUT: 0.68±0.01 vs 0.97±0.13; p=0.0001; 29% lower) and ventral striatum (VS: 0.57±0.11 vs 0.72±0.21, p=0.02; 20% lower).

Baseline Differences in MP-induced DA increases between Controls and MA

There were no group differences in the concentration of MP in plasma at any of the time points between controls and MA. MP plasma concentrations were not correlated with MP-induced DA increases in controls nor in MA.

SPM analysis did not reveal differences in MP-induced DA increases in striatum (decreases in BP_{ND}) between the groups (Figure 1). Using less stringent statistics (small volume corrections using a 10 mm diameter sphere) we had identified a small region in the left putamen where the values were lower in MA than in controls ($p<0.05$) (Wang et al., 2012).

Correlations between Baseline Measures of DAT and D2/D3R availability

We had previoulsy reported lower baseline D2/D3R availability in CD in the MA compared with the controls (Wang et al., 2012). Here, we assessed if the baseline D2/D3R measures correlated with DAT. In controls, DAT availability showed a significant positive correlation with D2/D3R in CD (r=0.63, p=0.01), PUT (r=0.81, p=0.0003) and VS (r=0.84, p=0.0001). In contrast, in MA, the correlations were not significant in any of the striatal regions: CD (r=0.12), PUT (r=0.32) or VS (r=0.29).

Correlations between Baseline Measures of DAT and MP-induced DA increases

In controls, DAT availability showed a significant positive correlation with MP-induced DA increases in CD (r=0.54, p=0.04) and in PUT (r=0.68, p=0.006), such that the higher the DAT the larger the DA increases (Figure 2). In contrast, in MA the correlation for PUT showed a trend in the *opposite* direction (r=-0.46, p=0.07), such that the lower the DAT the larger the DA increases (r=0.47, p=0.07) (Figure 2). The comparsions between the correlations (DAT and DA release) in MA and controls differed significantly in CD (p=0.001) and in PUT (p=0.001).

Correlations between Baseline Measures of D2/D3R and MP-induced DA increases

In controls, D2/D3R showed a significant positive correlation with MP-induced DA increases in PUT (r=0.67, p=0.006) and showed a trend in CD (r=0.47, p=0.08), such that the higher the

D2/D3R the larger the DA increases; the correlations in VS were not significant (r=0.09). In MA, the correlations were also positive and significant in CD (r=0.57, p=0.02) and showed a trend in VS (r=0.45, p=0.08); the correlations in PUT were not significant (r=0.29).

Effects of METH detoxification on DAT in MA and test-retest measures in controls.

SPM analysis in the 9 MA and in the 10 controls who underwent repeated scans after the first visit (baseline measures) showed significant increases in DAT between the first and second evaluation in MA but showed no changes in the controls (Figure 3). The SPM results revealed that the group by evaluation interaction effect was significant (Figure 4).

The ROI analysis corroborated the SPM findings. The group by evaluation interaction was significant in CD (F=9, p=0.008) and PUT (F=12, p=0.003). Post hoc t tests showed significant increases in DAT in MA between the first and second evaluation in CD (0.533±0.08 vs 0.664±0.09, p= 0.0023; 20% increase) and PUT (0.67±0.08 vs 0.828±0.08 p=0.0014; 22% increase) but no differences between test-retest in controls. The DAT increases with detoxification were negatively associated with the age of initiation of METH abuse in CD (r=0.78, p=0.01) and VS (r=0.74, p=0.02); such that the younger the age of initation the less the DAT recovery with detoxification. Neither the doses of METH used nor the years of METH abuse show a correlation with recovery.

Effects of METH detoxification on D2/D3R in MA and test-retest measures in controls.

SPM comparison of D2/D3R between the MA and the controls that underwent repeated scans showed a significant group effect in CD but neither the repeated measures (first vs second evaluation) nor the interaction effects were significant. Similarly, the ROI analysis showed a significant group effect in CD D2/D3R (F=4.4, p=0.05) but no effect of evaluation or of interaction. Post hoc analysis showed that D2/D3R in CD were lower in MA than in controls (p=0.05). Neither the group not the evaluation effects were significant in PUT or VS.

Effects of METH detoxification on MP-induced DA increases and test-retest measures in controls.

SPM comparison for MP-induced DA increases between the first and second evaluation showed

no differences between the evaluations in MA (9 participants who completed detoxification) nor in controls (10 participants who completed test-retest) (Figure 5). The ROI analysis corroborated a lack of an effect of detoxification in MP-induced DA increases (quantified as percent change from placebo) in MA between the first and second evaluation in CD (6.4 ±5% vs 3.5 ±9%), PUT (7.4 ±5% vs 5.1 ±9%) or VS (5.3 ±12% vs 5.2 ±27%). However, in controls the ROI showed that MP-induced DA increases were significantly attenuated between the first and the second evaluation in VS (12 ±15% vs 3 ±12%, p=0.02) but did not differ in CD (4.4 ±10% vs 4.4 ±8%) or PUT (10.2 ±12% vs 6 ±10%). There were no significant correlations between the history of METH abuse and MP-induced DA increases either for the first or second evaluations.

Comparisons of DAT in MA after protracted detoxification with controls.

SPM analysis for the group comparison between DAT measures taken from the MA during protracted detoxification and from the controls during their second visit showed no significant group differences. In contrast, the ROI analysis, showed that, in MA, the DAT measures during protracted detoxification were still significantly lower than in the controls (CD p=0.02; PUT p=0.005; and VS p=0.03). The significance observed for the ROI analysis but not for the SPM analysis most likely reflects the more stringent criteria for significance from SPM (p_{FWE}) than for ROI.

Comparisons of baseline DAT in MA who relapsed and those who did not.

Comparison of the baseline DAT measures between MA who would go on to relapse (N=6) and those who did not (n=10), did not differ significantly for any of the striatal regions: CD (0.58 ±0.11 vs 0.53 ±0.06), PUT (0.69 ±0.13 vs 0.67 ±0.08) or VS (0.58 ±0.08 vs 0.56 ±0.12) respectively.

DISCUSSION

Here we show significantly lower DAT in the striatum of MA than in controls and document significant increases in DAT between early and protracted METH detoxification. We also report that whereas in controls DAT availability was positively correlated with DA release (MP-induced DA increases), such that the higher the DAT the larger the DA increases, in MA this

correlation, while not significant (p=0.07), was in the opposite direction, such that the lower the DAT the larger the DA increases. Finally, although DAT availability recovered with protracted detoxification in MA, DA release (MP-induced DA increases) did not change. The significant DAT decreases but mostly normal DA release indicates that the striatal DAT losses per se do not reflect degeneration of DA terminals. Similarly, the increases in DAT but the lack of changes in DA release with protracted detoxification are consistent with DAT recovery reflecting upregulation rather than the regeneration of DA terminals in MA. However, our results cannot rule out the possibility of DA terminal degeneration in the MA who were unable to remain abstinent and in whom we had previoulsy shown had reduced DA release (Wang et al., 2012).

Loss of DAT in the striatum of METH abusers

Our current findings of loss of DAT in the striatum of MA is consistent with prior brain imaging studies reporting lower DAT in MA (McCann et al., 1998; Volkow et al., 2001a; Wilson et al., 1996). Studies in laboratory animals have also consistently found decreases in DAT in striatum with METH exposure, with some additionally reporting evidence of neurotoxicity to DA neurons (Friedman et al., 1998; Krasnova et al., 2010; Volz et al., 2007; Wallace et al., 1999). However, preclinical studies that reported neurotoxicity used much larger doses of METH (binge models) than those typically used by humans, who initiate their trajectory using low METH doses and then progresssively escalate them. This is relevant since preclinical studies have also shown that priming rodents with a low dose of METH prevents the neurotoxicity resulting from subsequent larger METH doses (Cadet et al., 2009; Hodges et al., 2011). The magnitude of the DAT losses reported by preclinical studies that used regimens of METH administration relevant to humans are smaller than those reported using binge models of METH administration (Groman et al., 2012; McFadden et al., 2012; Schwendt et al., 2009) and have not shown changes associated with neurotoxicity (Brennan et al., 2010; Schwendt et al., 2009). This indicates that the typically escalating pattern of METH abuse by humans might protect MA against the DA neuronal toxicity observed with binge models of METH administration (Krasnova and Cadet, 2009).

Association between DAT and DA release: disruption in MA

In healthy controls we document a positive association between DAT and MP-induced DA release in dorsal striatum, such that the greater the DAT the larger the DA increases, which would be consistent with the DAT measures reflecting DA terminal density. Interestingly, in the MA, the relationship between DAT and DA increases, albeit not significant (p=0.07), was in the opposite direction to what we observed in controls. That is, in MA, the lower the DAT the larger the DA increases, which would be consistent with the loss of DAT reflecting downregulation (Melikian and Buckley, 1999), since DAT downregulation would result in reduced DA reuptake into the terminal and hence higher levels of extracellular DA. On the other hand if DAT loss in the MA reflected DA terminal degeneration (less DAT because there are lesss DA terminals) then one would have expected that the larger the loss of DAT the greater the attenuation of MP-induced DA increases (Palner et al., 2011).

In MA, MP-induced DA increases in striatum did not differ significantly from those in controls, except for a small region in left putamen, which was observed when using a small volume correction (Wang et al., 2012). This contrasts with findings in cocaine abusers and in alcoholics in whom MP-induced DA increases throughout the striatum were dramatically reduced (50-80% lower than in controls) (Volkow et al., 2014; Volkow et al., 1997; Volkow et al., 2007). Since degeneration of DA terminals in rodents is associated with attenuated stimulant-induced DA increases (Palner et al., 2011), the mostly normal DA responses to MP in the MA observed in this study are not consistent with then notion that DAT losses reflect DA terminal degeneration.

In a prior report on these participants we showed that the MA who relapsed had lower DA release in striatum than controls whereas the MA who were able to complete the detoxification did not differ from controls as assessed with their baseline measures (Wang et al., 2012). On the other hand, in the current study, we did not see differences in baseline measures of DAT between MA who relapsed and those who did not, and both groups showed lower DAT than controls. This indicates that measures of DA release are better predictors of clinical outcomes in MA than DAT.

DAT increased with detoxification but neither DA release nor D2/D3R changed

This study documents significant recovery of DAT with protracted abstinence in MA after 9 months following their initial evaluation. These results replicate our prior findings of significant DAT recovery in 5 MA who remained abstinent also for a 9 month period (Volkow et al., 2001a). DAT recovery has also been reported in MA after short abstinence (1 month or less) (Chou et al., 2007; Yuan et al., 2014). However, as was the case in our prior study, despite significant DAT recovery with METH detoxification, DAT availability, as assessed with ROI (though not SPM), was still lower than in healthy controls (Volkow et al., 2001a). Similarly, in non-human primates chronically exposed to METH, the reduction in DAT, while increasing between 2 and 7 weeks post METH discontinuation, remained lower than their baseline measures (Groman et al., 2012). Thus, our current results, together with findings from prior studies, indicate that DAT downregulation from chronic METH exposure is long lasting.

Despite the DAT increases with protracted detoxification there were no changes in DA release with METH detoxification. This lack of a concomitant increase in DA release is consistent with an upregulation of DAT with METH detoxification rather than with a regeneration of DA terminals.

The mechanisms underlying loss of DAT with chronic exposure to METH and their recovery with protracted detoxification are unclear. The loss could reflect adaptive compensatory downregulation to minimize entry of METH to DA terminals (since METH is a substrate for the DAT). Indeed, DAT downregulation has been shown to reduce MDMA-induced toxicity to DA neurons (Peraile et al., 2010). Loss of DAT could also reflect disruption of trafficking and internalization of DAT (Melikian and Buckley, 1999). METH transiently downregulates the surface expression of DAT through a protein kinase C-dependent mechanism (Hong and Amara, 2013; Richards and Zahniser, 2009). However, this mechanism cannot explain the long lasting loss of DAT in MA. Epigenetic modifications to the DAT gene could provide a mechanism for long lasting decreases in DAT expression (Shumay et al., 2010). However, to our knowledge, such studies have not been reported.

In MA the baseline measures of D2/D3R were lower in caudate than in controls and these measures did not recover with detoxification; in contrast to the DAT, which while also lower in

MA showed significant recovery with detoxification. Moreover, the correlations between DAT and D2/D3R were not significant. This suggests distinct neurodaptation responses to chronic METH exposure between presynaptic and postynapic markers. DAT reflecting responses in the DA terminal and hence in DA neurons. Though D2/D3R are expressed both in DA terminals (presynaptic receptors) and in striatal middle spiny neurons (postsynaptic receptors), the lack of a correlation between D2/D3R and DAT suggests that their downregulation predominantly reflects adaptations in postsynaptic receptors.

These findings contrast with those reported in Parkinson's Disease (PD) for which the degeneration of DA neurons and their terminals is associated with DAT loss, and during the early stages of a diseases with an upregulation of striatal D2/D3R (Brooks, 2010; Niccolini et al., 2014; Zhu et al., 2014). As PD progresses, DAT loss worsens (reviewed (Brooks, 2010)) and treatment initiation is associated with a downregulation of D2/D3R (reviewed (Niccolini et al., 2014)). The distinct patterns observed in the MA with detoxification relative to those described in the progression of PD also indicates that the changes we are observing are not consistent with those observed with DA neuronal degeneration.

Clinical Implications

Our findings are not consistent with degeneration of DA neurons in the brain of MA. This has clinical implications regarding the concern for a higher risk for PD that could result from METH abuse in humans. However, although our results do not support evidence of DA neuron degeneration as would be expected if METH increased the risk of PD, we cannot rule out the possibility of an interaction of METH abuse with aging such as an acceleration of the age-related loss of DA neuronal function. If such interaction exists, it could explain the preliminary reports of an increased risk of PD in METH abusers (Callaghan et al., 2012; Curtin et al., 2015). It is also possible that, while we did not observe evidence of DA neuronal degeneration in MA, individuals with genetic variants that make DA neurons more sensitive to neurotoxicity might have shown evidence of DA neuronal degeneration. Moreover, the clinical outcome for our MA participants was good (10 out of 16 were able to sustain abstinence for 9 months) so it is possible that MA with more severe phenotypes might have shown evidence of DA neuronal degeneration. In this respect, it is relevant that the 6 MA who relapsed had reduced DA release (Wang et al.,

2012).

Significant DAT recovery with detoxification indicates that the brain recovers even after long lasting METH exposures. Interestingly, we show that the ability to recover decreases when METH abuse was initaited at an early age, which is consistent with prior findings in substance abusers showing worse outcomes with earlier age of drug initiation (Hser et al., 2008).

The lack of changes in DA release in the MA is therapeutically relevant for it indicates that MA will be sensitive to medications that rely on DA release. Indeed, stimulant substitution treatments (amphetamine or methylphenidate), akin to the strategies used to treat heroin addiction, have been proposed for the treament of MA (Moeller et al., 2008). Interestingly, preliminary positive results have been reported by clinical trials that used MP in the treatment of MA (Malmberg et al., 1994; Rezaei et al., 2015) although others have reported positive effects only on retention (Miles et al., 2013).

Finally our current findings when contrasted to those of our prior report showing that MA who would go on to relapse had impaired DA release (Wang et al., 2012) highlights the heterogeneity in the responses of the human brain to METH. These findings also indicate worse outcome among MA with disrupted DA function, which is clinically relevant for it emphasizes the need to tailor therapeutic interventions to the severity of the neurobiological presentation.

Limitations

[^{11}C]Raclopride cannot distinguish between D2 and D3 receptors (Malmberg et al., 1994) and since D3 receptors have been shown to be higher than normal in MA (Boileau I et al., 2012), this confounds our measurement of DA changes in ventral striatum where the expression of D3 receptors is relatively high (Tziortzi et al., 2011). Another limitation of [^{11}C]raclopride is that it cannot distinguish between pre and postynaptic receptors. Although we are interpreting our findings of DAT loss but normal DA release in the MA as evidence that DAT losses do not reflect DA terminal degeneration we cannot rule out the possibility that non-damaged DA terminals compensate by releasing more DA. Our study included very few females and thus further studies are needed to assess whether there are differences between male and female MA.

Summary

The results from this study provide evidence that the DAT losses in MA are not associated with a concomitant decrease in DA release and that they recover with protracted detoxification This indicates that DAT lossess in MA are unlikely to reflect DA terminal degeneration. However, our results cannot rule out the possibility of DA terminal degeneration in MA who were unable to sustain abstinence, who differed from those that succeeded, in that they had reduced DA release (Wang et al., 2012).

Figure Legends

Figure 1. Statistical SPM maps for the group comparisons for DAT and for DA release (Delta BP_{ND}) between normals and METH abusers for the scans taken during the first evaluation (baseline measures). Note the significanlty lower DAT availability in the METH abusers than in the controls and the lack of significant differences in DA release between controls and METH abusers (except for a small region in left putamen).

Figure 2. Regression plots for the correlations between DAT and MP-induced DA increases (observed as reductions in BP_{ND}) in the controls (black symbols) and in the METH abusers (gray symbols) in caudate (CD) and in putamen (PUT). For controls, the correlations were significant in CD (r=0.54, p=0.04) and PUT (r=0.68 p=0.006) such that higher DAT were associated with larger DA increases; for METH the correlations though not significant were in the opposite direction (CD r=-0.36, p=0.17; PUT r=-0.49, p=0.07) such that higher DAT were associated with lower DA increases.

Figure 3. A. Voxelwise measures of DAT availability for the first (visit 1) and second (visit 2) evaluation in the controls and statistical SPM maps for the comparisons between them. B. Voxelwise measures of DAT availability for the first (visit 1) and second (visit 2) evaluation in the METH abusers and statistical SPM maps for the comparisons between them. Note the significant increases in DAT in METH abusers between the first and second evaluations and the lack of differences in the controls.

Figure 4. Statistical SPM maps showing the Group by Visit interaction in DAT availability. The differences between Visit 1 and Visit 2 differed between groups in that DAT changed significantly in METH abusers between the first and the second visit whereas in controls they did not.

Figure 5. Statistical SPM maps for the measures of DA release (Delta BP_{ND}) for the first (visit 1) and second evaluation (visit 2) in the controls and in the METH abusers. The contrast corresponds to PL > MP and denotes the regions where MP increased DA reducing BP_{ND}. Note the reproducibility of the effects between visit 1 and visit 2 and the lack of differences between controls and METH abusers.

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9. Figure1

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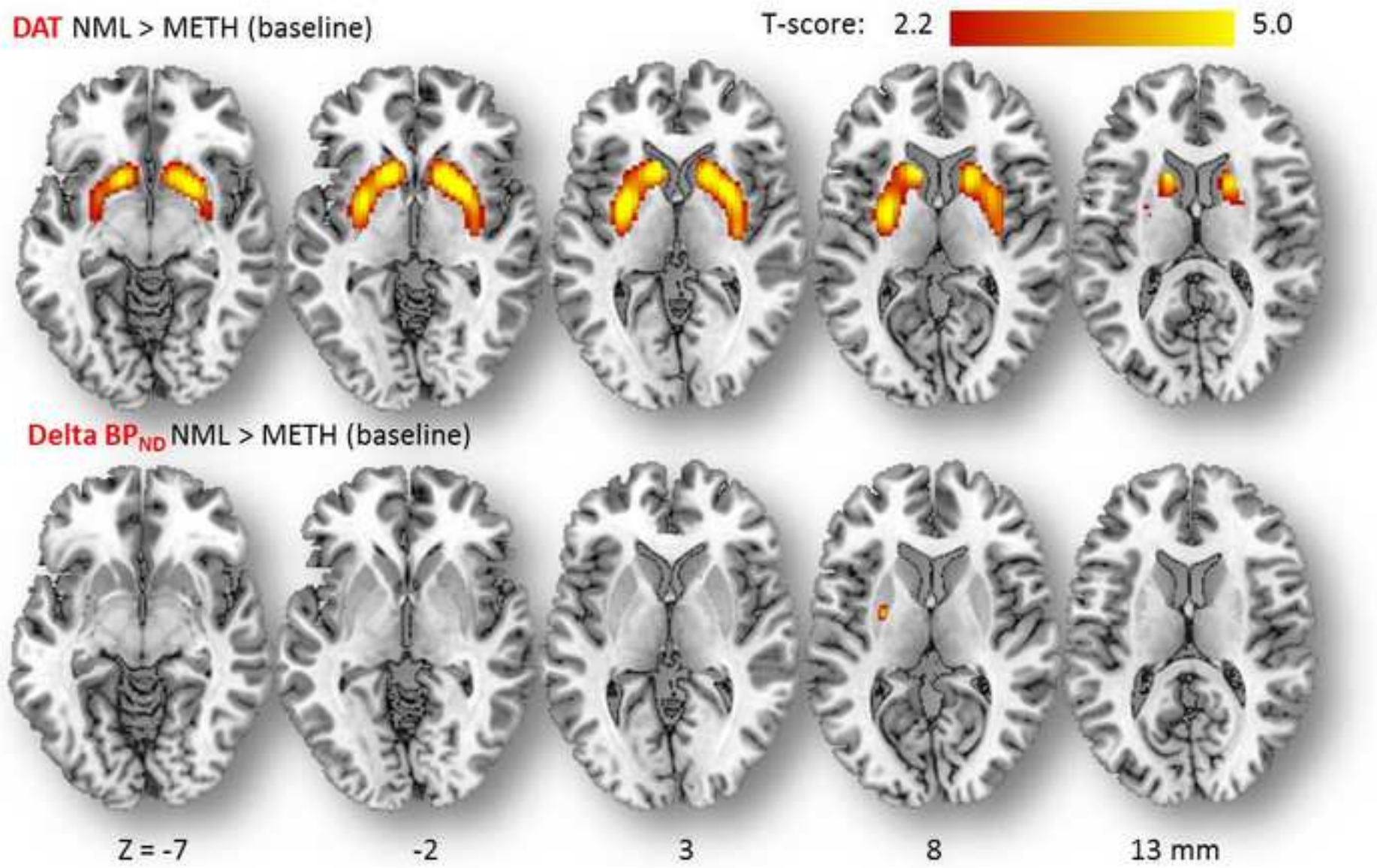

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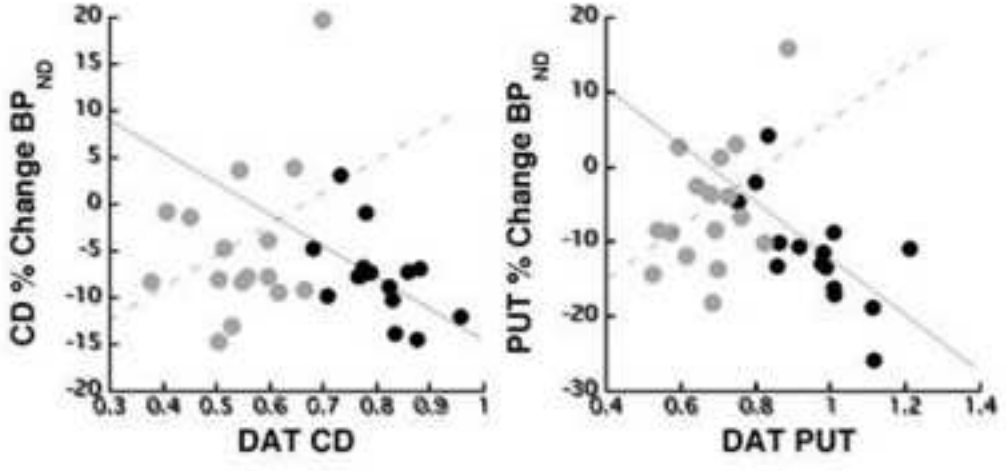

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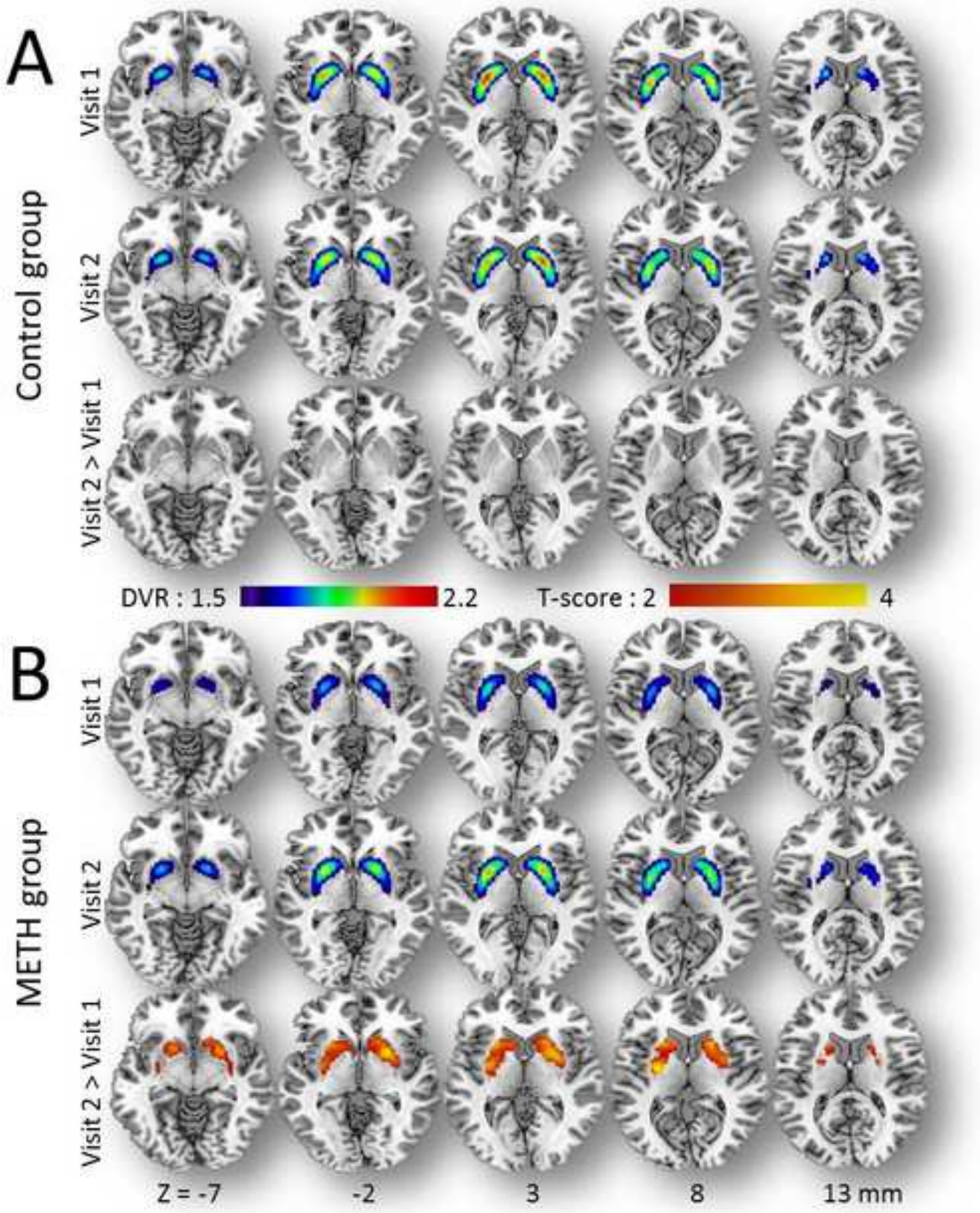

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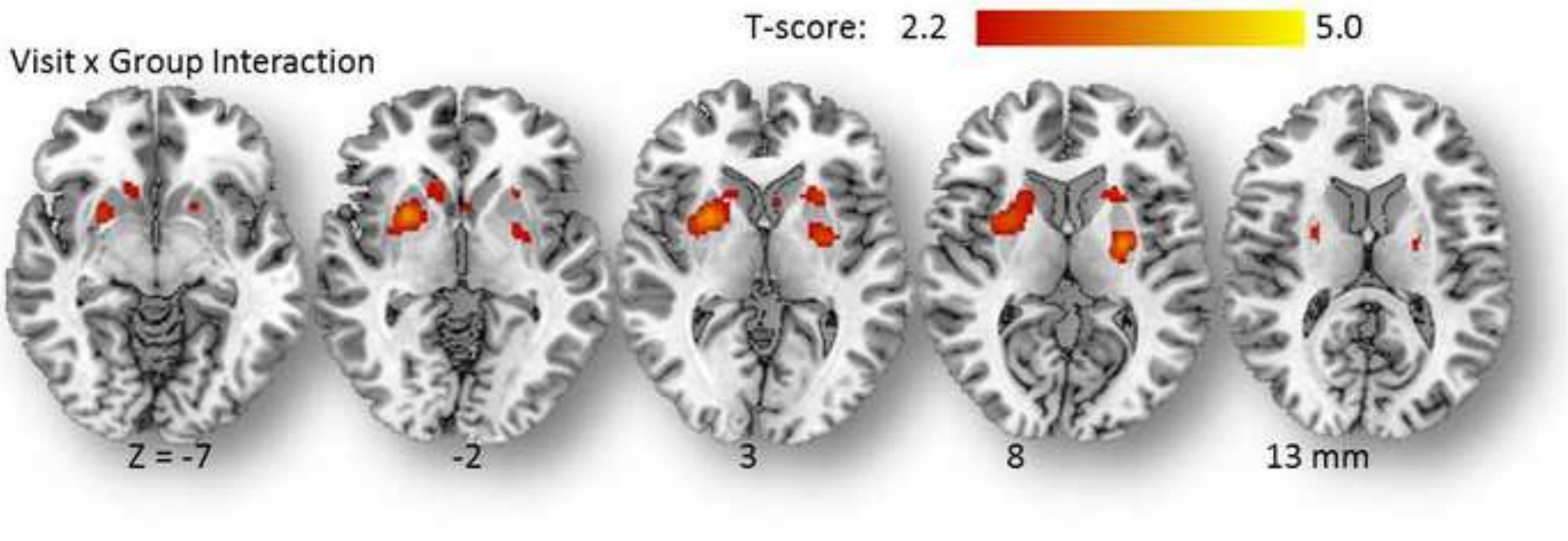

9. Figure5

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