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# Microstructural changes to the brain of mice after methamphetamine exposure as identified with diffusion tensor imaging

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ABSTRACT

Methamphetamine (METH) is an addictive psychostimulant inducing neurotoxicity. Human magnetic resonance imaging and diffusion tensor imaging (DTI) of METH-dependent participants find various structural abnormities. Animal studies demonstrate immunohistochemical changes in multiple cellular pathways after METH exposure. Here, we characterized the long-term effects of METH on brain microstructure in mice exposed to an escalating METH binge regimen using *in vivo* DTI, a methodology directly translatable across species. Results revealed four patterns of differential fractional anisotropy (FA) and mean diffusivity (MD) response when comparing METH-exposed ($n=14$) to saline-treated mice ($n=13$). Compared to the saline group, METH-exposed mice demonstrated: 1) decreased FA with no change in MD [corpus callosum (posterior forceps), internal capsule (left), thalamus (medial aspects), midbrain], 2) increased MD with no change in FA [posterior isocortical regions, caudate-putamen, hypothalamus, cerebral peduncle, internal capsule (right)], 3) increased FA with decreased MD [frontal isocortex, corpus callosum (genu)], and 4) increased FA with no change or increased MD [hippocampi, amygdala, lateral thalamus]. MD was negatively associated with calbindin-1 in hippocampi and positively with dopamine transporter in caudate-putamen. These findings highlight distributed and differential METH effects within the brain suggesting several distinct mechanisms. Such mechanisms likely change brain tissue differentially dependent upon neural location.



## 1. Introduction

Methamphetamine (METH) is an addictive psychostimulant that induces central nervous system toxicity and associated neurocognitive impairment, such as impaired behavioral inhibition and attentional control (Nordahl et al., 2003; Scott et al., 2007). Cognitive deficits are thought to be due to a wide variety of neurotoxic effects from METH exposure that induce long lasting changes to the structure and function of the brain (Büttner, 2011). In humans, much of what is known about the impact of METH on the brain comes from magnetic resonance imaging (MRI) studies of adult METH users. These cross-sectional studies have demonstrated structural abnormalities in the frontal lobe, including lower gray matter density or volumes (Kim et al., 2006; Schwartz et al., 2010), larger white matter volumes (Bartzokis et al., 2001), and increased white matter hyperintensities (Berman et al., 2008). Moreover, smaller volumes have been observed in the temporal lobe (Bartzokis et al., 2000), including the hippocampus (Thompson et al., 2004); whereas larger volumes have been observed in the parietal cortex (Jernigan et al., 2005) and striatal regions (Chang et al., 2005) of adult METH users.

It has been speculated that enlarged brain regions reflect inflammatory changes whereas reductions in size reflect neuronal cell loss (Thompson et al., 2004), but the mechanisms remain unclear. Recently, diffusion tensor imaging (DTI) has been used to examine microstructural brain changes associated with METH. By linking macroscopic brain changes to microscopic cellular events, DTI promises to integrate large scale brain changes to cellular changes, providing a more integrated view of the effects of a pathological agent on neural systems than does conventional MRI (Bammer et al., 2006). DTI provides quantitative information about the geometric distribution of water diffusion within imaging voxels (Basser, 2006). An aim of DTI is to infer information about

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the integrity of tissue microstructure from this geometric information. Inferences from diffusion data are typically based on the biological boundary model, which states that at sufficiently long diffusion times, water molecules in biological tissues will diffuse in an isotropic manner until they reach a membrane or large protein boundary (Chanraud et al., 2010; Le Bihan and Johansen-Berg, 2012). Such cell boundaries are believed to reduce overall diffusion magnitude and shape the direction of water movement. Based on this geometric information several measures can be computed including mean diffusivity (MD) and fractional anisotropy (FA), the later a measure of the degree to which water movement is dominated by a single direction (Basser, 2006). Based on the biological boundary model, reduced FA and increased MD implies reduced density of membranes possibly due to tissue loss (Alicata et al., 2009). DTI changes associated with METH abuse or dependence have commonly revealed lower FA in frontal white matter and increased MD in basal ganglia structures, such as the putamen and caudate (Alicata et al., 2009; Chung et al., 2007; Tobias et al., 2010). Differences in the integrity of the corpus callosum are less often found, with some investigators reporting lower FA values in the genu (Kim et al., 2009; Tobias et al., 2010), whereas others report only trends or no callosal effect (Alicata et al., 2009; Salo et al., 2009). When cognition is investigated, lower FA in regions within the frontal cortex or genu has been associated with worse cognitive performance (Chung et al., 2007; Salo et al., 2009).

However, human MRI and DTI studies of METH use have several acknowledged limitations. DTI studies only examined only a few regions of interest within the brain, and all studies used cross-sectional designs that do not disentangle markers that predate drug use, effects of abstinence duration, and different use patterns. Further, differences in the maximal dose, days of usage per week/month, and total quantity of METH intake along with poly-substance abuse among participants could result in different neuropathological and cognitive outcomes (Berman et al., 2008; Salo and Fassbender, 2012). Given the challenges of establishing the direct effects of METH in humans, investigators have performed animal experiments to obtain basic neuroscience data on METH exposure. In mice, exposure to neurotoxic METH doses led to decreased dopamine function in the cortex and striatum. For example, decreases in dopamine (Achat-Mendes et al., 2005; Fantegrossi et al., 2008) and tyrosine hydroxylase levels (Achat-Mendes et al., 2005; Bowyer et al., 2008; Deng et al., 1999; Fantegrossi et al., 2008) in the striatum and cortex suggest decreased dopaminergic innervation after METH exposure. METH also induces neuronal death in the striatum, frontal and parietal cortices, hippocampus, and olfactory bulb in a process akin to neuronal apoptosis (Cadet et al., 2005; Cunha-Oliveira et al., 2008); as well as leading to reactive astrocytosis (Deng et al., 1999; Zhu et al., 2005) and microgliosis (Bowyer et al., 2008; Fantegrossi et al., 2008; Thomas and Kuhn, 2005). Within the caudate-putamen METH-induced dopaminergic neurotoxicity has been found to be mediated by the dopamine transporter [DAT; (Fumagalli et al., 1998)]. Given the evidence that METH has a variety of effects on neuronal structure and function at the cellular level in animals and at the brain systems level in humans, it is unfortunate that there has been little work using neuroimaging to directly link these two levels of neural organization. New knowledge from tightly controlled animal experiments employing the same *in vivo* neuroimaging techniques as those used in human studies offers a powerful translational context to better understand the impact of METH on the brain.

The aim of the present study was to utilize DTI in mice previously exposed to a METH binge regimen in order to characterize the long-term effects of METH on the microstructure of the brain using neuroimaging techniques that are translatable across species. Consistent with evidence for METH-induced tissue damage, we hypothesize that decreased FA will be associated with increased MD in METH-exposed mice compared to saline control mice within the frontal and striatal gray matter and their connecting pathways, hippocampus, and white matter tracts such as the corpus callosum and internal capsule. In addition, the whole brain was examined to explore additional microstructural DTI changes given the heterogeneity of METH effects including the potentially differential impact on neuronal cell bodies and their processes versus surrounding glial and astrocyte cells. In exploratory analyses we also investigated potential associations between DTI markers of tissue damage and cellular changes in the caudate-putamen and hippocampus, two brain regions known to be adversely impacted by METH. Specifically, we examined links between MD and immunohistochemical assays of DAT in the caudate-putamen and calibindin-1 in the dorsal hippocampi of a subgroup of METH-exposed mice. Calibindin-1, a marker of hippocampal function associated with calcium buffering, has been implicated in the effects of METH (Kuczenski et al., 2007).

## 2. Methods

### 2.1. Animals

This study was part of a larger examination of the individual and combined effects of human immunodeficiency virus and METH conducted by The Translational Methamphetamine AIDS Research Center (TMARC). Male mice from a C57BL/6 × DBA (BDF1) genetic background ($N=27$) were tested in the current study. Mice were grouped-housed with 2–4 mice per group in a climate-controlled environment with a reversed day/night cycle (lights on at 19:00 h, off at 07:00 h). Since mice are nocturnal animals and active during night, the day/night cycle was reversed so that behavioral assessments could occurred during the day. For MRI mice were housed under the same conditions for consistency. The animals were given free access to food (diet #8626, Haran Teklad, Madison, Wisconsin, USA) and water for the duration of the testing. All procedures were approved by the UCSD Institutional Animal Care and Use Committee and conformed to NIH guidelines. Mice were 4–5 months old at the beginning of study procedures and 9–10 months old at time of MRI, (see below). These mice were previously tested in a battery of cognitive tasks (Kesby et al., 2015a; Kesby et al., 2015b).

### 2.2. Methamphetamine regimen

METH (Sigma, St. Louis, Missouri, USA) was dissolved in saline and administered subcutaneously with a 5 ml/kg injection volume (doses are expressed as salt). Stock solutions of the drug were prepared every three to four days and diluted as needed during the drug regimen. Mice were exposed to an escalating dose-multiple binge METH regimen that was originally established in rats (Kuczenski et al., 2007). While the neurotoxic effects of METH have been typically induced by an acute “binge” procedure (4 injections of high doses in drug-naïve rodents; Davidson et al., 2001), it has been postulated that the inclusion of an escalating dose pretreatment regimen represents a more accurate simulation of the gradual dose progression in human abusers (Segal and Kuczenski, 1997). Prior work indicated that inclusion of this escalation paradigm attenuates the hyperthermic effects of higher METH doses in rats, while still inducing neuropathological and behavioral effects in both rats and mice (Henry et al., 2013; Kesby et al., 2015a, 2015b; Kuczenski et al., 2007). Mice were treated three times per day (10:00; 13:15; 17:30 h) for 14 days with vehicle (saline; $n=13$) or escalating doses of METH ($n=14$), starting