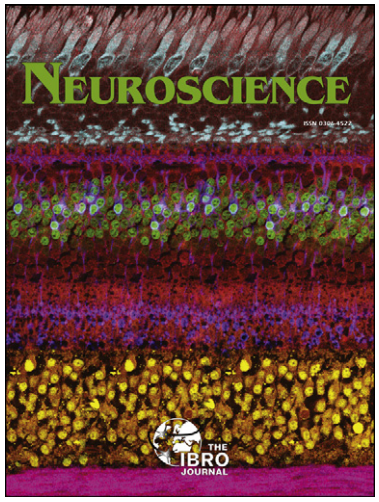


Cortical Thinning in Amphetamine-Type Stimulant Users

Philip Koester, Marc Tittgemeyer, Daniel Wagner, Benjamin Becker, Euphrosyne Gouzoulis-Mayfrank, Jörg Daumann

PII: S0306-4522(12)00667-7
DOI: http://dx.doi.org/10.1016/j.neuroscience.2012.06.049
Reference: NSC 13873

To appear in: *Neuroscience*

Accepted Date: 20 June 2012

Please cite this article as: P. Koester, M. Tittgemeyer, D. Wagner, B. Becker, E. Gouzoulis-Mayfrank, J. Daumann, Cortical Thinning in Amphetamine-Type Stimulant Users, *Neuroscience* (2012), doi: http://dx.doi.org/10.1016/j.neuroscience.2012.06.049

Cortical Thinning in Amphetamine-Type Stimulant Users

Philip Koester[a], Marc Tittgemeyer[b], Daniel Wagner[a], Benjamin Becker[a], Euphrosyne Gouzoulis-Mayfrank[a], Jörg Daumann[a]

[a]Department of Psychiatry and Psychotherapy, University Hospital of Cologne, Kerpener Str. 62, 50937 Cologne, Germany

[b]Max-Planck-Institut für neurologische Forschung, Gleuler Str. 50, 50931 Cologne, Germany

Corresponding Author:

J. Daumann, PhD; Department of Psychiatry and Psychotherapy

University of Cologne

Kerpener Strasse 62, 50924 Cologne, Germany

Tel: +49 221 478 87113, Fax: +49 221 478 97543

E-mail: joerg.daumann@uk-koeln.de

Abstract

Accumulating evidence supports the hypothesis of ecstasy and amphetamine exhibiting neurotoxic properties in human recreational users. The extent and exact location of neuronal degeneration might also be associated with a specific profile of cognitive deterioration described in polydrug users. Voxel-based morphometry and cortical thickness analyses constantly gain attention for answering the question of associated neurological sequelae. We aimed to evaluate the integrity of cortical and subcortical structures in three groups that differ in the consumption of amphetamine-type stimulants. Cortical thickness, cortical grey matter volume and the shape of supposedly vulnerable subcortical structures were compared between 20 experienced users, 42 users with little exposure to these substances and 16 drug- naïve controls. Cortical thinning in experienced users compared to drug-naïve controls and low-exposure users was observed in medio-frontal regions. Effects of ecstasy and amphetamine on cortical volume were similar to those of cortical thickness, with volume reductions primarily in frontal, but also in occipital and parietal regions of low exposure and experienced users. These effects were differently lateralized for the different comparisons. The investigation of subcortical structures revealed non-significant bilateral shape differences in the hippocampi. Our data support the hypothesis that massive recreational amphetamine-type stimulant polydrug use is associated with a thinning of cortical grey matter. Disrupted neuronal integrity in frontal regions does fit well into models of addiction and the cognitive deterioration in amphetamine-type stimulant polydrug users. The exact neurotoxic mechanisms of polydrug ecstasy and amphetamine use, however, remain speculative.

Key Words MDMA, Amphetamine, ATS, Neurotoxicity, Cortical Thickness, Cortical Volume

3,4-Methylenedioxy-N-methylamphetamin (MDMA, "ecstasy") and amphetamine ("speed") belong to the most used illicit drugs worldwide as per the United Nations Office on Drugs and Crime (UNODC, 2011). These synthetic substances are categorized as amphetamine-type stimulants (ATS) (UNODC, 2011). Due to their stimulating and euphorigenic effects, ATS are often used excessively with high cumulative doses on dance-parties. In animals there is a large body of evidence indicating long-lasting neurotoxic effects after administration of MDMA and amphetamine (Kolb, Gorny, Li, Samaha, & Robinson, 2003; Ricaurte, Martello, Katz, & Martello, 1992). Alterations of the dopaminergic and serotonergic systems have been described in laboratory rats and non-human primates (Hatzidimitriou, McCann, & Ricaurte, 1999; Melega et al., 1997; Ricaurte, et al., 1992). However, it is still a matter of debate how to translate animal data to humans (Easton & Marsden, 2006; Gouzoulis-Mayfrank & Daumann, 2009).

It remains unclear whether these neurotoxic effects are expressed in grey matter abnormalities in humans as measured by means of structural magnetic resonance imaging (MRI). A few studies have consistently reported disturbed neuronal integrity which is most probably caused by ATS use (Cowan et al., 2003; J. Daumann et al., 2011; Kish et al., 2010). However, studies differ substantially with respect to the exact locations of grey matter alterations and thus suffer from a clear hypothesis regarding the neuronal substrate underlying structural modification. Differing results might be attributable to differences in sample characteristics (drug use patterns etc.), statistical approaches (region of interest vs. whole brain analyses) and methodological shortcomings associated with the naturalistic study design. The most consistent findings of grey matter integrity alterations have been reported in medial (orbito-) frontal and anterior cingulate (J. Daumann, et al., 2011; Kish, et al., 2010) and, to a lesser extent, in parietal (Kish, et al., 2010) and occipital regions (Cowan, et al., 2003; J. Daumann, et al., 2011). Preceding this study we analyzed structural MRI images regarding brain

morphology in three groups, differing in the amount of substances they have used (J. Daumann, et al., 2011). We aimed to improve the standard voxel based morphometry analysis by relying on image transformations from the tract-based-spatial statistics (TBSS) analysis, thereby reducing the impact of non-linear transformations. Experienced users displayed widespread cortical grey matter density reductions along the medial frontal surface and the anterior cingulate compared to low-exposure users and drug-naïve controls. The absence of differences between the last two groups substantiated the hypothesis of measurable neurotoxicity after low exposure to these substances. It also debilitated the assumption that the co-use of cannabis might have a major impact. In contrast, Cowan et al demonstrated cortical grey matter density reductions which for the most part were restricted to posterior, especially cerebellar and occipital regions (Cowan, et al., 2003).

Due to methodological shortcomings of voxel-based morphometry approaches to investigate white matter architecture, the integrity of white matter tracts is mostly assessed by means of diffusion tensor imaging (DTI). Results of studies using this technique on ATS polydrug users are, however, inconsistent. Whereas some studies did not find any significant group differences on DTI derived measures (J. Daumann, et al., 2011; de Win et al., 2007), other studies reported drug-use induced changes in fractional anisotropy (FA) and apparent diffusion coefficient (ADC) in thalamic (de Win et al., 2008; Moeller, Hasan et al., 2007), callosal (Moeller, Hasan, et al., 2007) and striatal (Alicata, Chang, Cloak, Abe, & Ernst, 2009) regions. The exact mechanisms of conspicuous structural findings are not yet fully understood. Besides drug-induced cell death, microanatomical changes in synaptic strength and structure, altered plasticity, changes in soma size and capillary changes might lead to thinning of the cerebral cortex and changes in shape and size of subcortical structures (Hutton, Draganski, Ashburner, & Weiskopf, 2009).

A recent study was the first to demonstrate cortical thinning is ATS users (Kish, et al., 2010). As this measure has been proven to be more sensitive to micro-anatomical changes in neurological architecture than common voxel-based morphometry analysis (Hutton, et al., 2009), the present study aimed to re-analyze the data which already have been analyzed (J. Daumann, et al., 2011) regarding parameters that in recent studies have proven to be sensitive to the effects of massive ATS use, especially cortical grey matter structure (Cowan, et al., 2003; J. Daumann, et al., 2011) and structural (Alicata, et al., 2009; de Win, et al., 2008) changes in subcortical structures.

Acquiring additional structural parameters in a large sample that previously has been investigated with other methodologies significantly contributes to the knowledge regarding potential vulnerabilities in polydrug ATS users and might thereby add valuable information to the neurobiological model of the neurotoxic profile of ATS use in humans.

Cortical thickness and cortical volume were compared between 20 experienced users, 42 users with limited ATS experience and 16 drug-naïve controls. Based on the literature above, we additionally selected six structures that have been found to display noticeable alterations in structural and functional analyses. Although we did not find any differences between the groups on DTI-derived measures in our previous analysis, we were interested whether this analysis might reveal hints towards alterations reported by other groups.

All drug-using participants have been directly recruited from the local dance scene throughout 2006-2009. Control subjects were recruited by word of mouth and newspaper advertisements. As the concomitant use of several ATS is common in the investigated sample, MDMA and amphetamine users have been grouped together. It has shown to be impractical to recruit a reasonable number of participants who use only one substance for recreational purpose. Methamphetamine use, in contrast, was absent in the investigated sample. Based on previous results it is hypothesized that subjects with little exposure to MDMA and amphetamine do not display any alterations of the cortical structure, whereas experienced

users are assumed to exhibit cortical thinning and cortical volume reductions compared to drug-naïve controls and low exposure users. We hypothesized that these effects are most prominent in medial (orbito-) frontal regions. Furthermore, we investigated whether the use of ATS has an effect on the volume and shape of the hippocampus, thalamus, nucleus accumbens, putamen, globus pallidus and nucleus caudatus, all of which have been reported to be functionally or metabolically affected by ATS use (J. Daumann et al., 2005; de Win, et al., 2008; Kish, et al., 2010).

Experimental Procedure

Participants

The most important methodological and procedural characteristics of the study are summarized. Demographic characteristics and drug use patterns of the sample are equivalent to Daumann et al., 2011. Drug-naïve controls must not have had any experience with illicit drugs. Low exposure users were not allowed to have exceeded a lifetime consumption of more than five pills MDMA and/or 5 grams of amphetamine. Experienced users were included if they at least consumed 100 doses of MDMA and/or 50 grams of amphetamine in their life. Due to the frequent concomitant use of Marijuana in the sample, the use of cannabis was allowed for the 2 ATS-experienced groups until one day prior to imaging. All participants were at least 18 years old, had no positive drug screen on the day of the study with the exception of cannabis, had no current or previous severe alcohol abuse or dependence (according to DSM IV criteria) and no psychiatric or neurological disorder. The regular use of other illicit drugs (regular was defined as use once a month or more often over 6 months or longer over the past 2 years), contraindications to MRI and brain morphological irregularities in the MRI scan led to the exclusion of subjects. Eventually, 16 drug-naïve controls (9 men, mean age: 26.31+-4.11), 42 low exposure users (31 men, mean age: 23.6+-5.27) and 20 experienced users (13 men, mean age: 26.6+-7.17) met inclusion criteria and participated in

the study. In the present study the same data were investigated that previously have been analyzed by means of a voxel-based morphometry and tract-based spatial statistics analysis (J. Daumann, et al., 2011). All study specific information is identical to this publication.

Procedure

Urine samples, which were screened for the most important recreationally used substances, have been collected and analyzed on the study day (enzyme-multiplied immunoassay, von Minden GmbH). A positive screening for amphetamines, methamphetamines, cocaine, benzodiazepine, barbiturates and opiates was an exclusion criterion. Self-reported drug histories, assessed by means of a semi-structured interview, have been validated by randomly collected hair samples, analyzed by the Institute of Legal Medicine of the University of Cologne. This way, the quantity of used ATS in the past six months has been validated for experienced and low-exposure users. As on the study day also neuropsychological measures were assessed, all participants were required to stay abstinent from any legal or illegal psychotropic substance except for cannabis seven days prior to imaging. The study was carried out in accordance with the declaration of Helsinki from 1975 and was approved by the local ethics committee. Following a detailed description of the study, written informed consent was obtained from all participants. Subjects had the assurance that they could withdraw from the study at any time without having to explain the reasons and received a payment for their participation. No screened participant was excluded, as all met the inclusion and exclusion criteria.

Image acquisition and processing

A Siemens Magnetom Trio Tim whole body MRI system operating at 3.0 Tesla, and a standard quadrature head coil was used for T1 image acquisition (flip angle=18°, TR=1930ms, TE=5.8ms, slice thickness=1.25mm, voxel size=1.0x1.0x1.25mm). Cortical

thickness and cortical volume for each participants T1 weighted image were reconstructed using the freely available imaging software package Freesurfer v 5.0.0 (http://surfer.nmr.mgh.harvard.edu). The fully automated standard protocol, which has previously been described in detail, was followed (Dale, Fischl, & Sereno, 1999; Fischl & Dale, 2000; Fischl, Sereno, & Dale, 1999). In short, images were normalized, brain extracted and segmented, followed by a tessellation of the grey/white matter junction. The folded surface was inflated and topological defects were corrected. Next, the surfaces of white-, grey matter and pial surfaces were reconstructed. The cortical thickness was computed as the shortest distance between the grey-white matter junction and the pial surface. Cortical volume was computed on the same pre-processed images by multiplying cortical thickness and pial surface. All images were aligned to standard space (MNI 305) and the cortical images were smoothed with a Gaussian kernel of 10 mm full width at half maximum (FWHM) to further reduce local variations in image quality. Despite existing hypotheses regarding the probable location of cortical grey matter abnormalities we decided to perform a fully automated whole brain analysis to validate the results of our previous analysis.

The same raw T1 weighted images were fed into a shape and appearance analysis, performed with FIRST v1.2 (FMRIB's Integrated Registration and Segmentation Tool) (Patenaude, 2007; Patenaude, Smith, Kennedy, & Jenkinson, 2007, 2008), part of FSL 4.1.5 (FMRIB Software Library) (Smith et al., 2004; Woolrich et al., 2009). Six subcortical regions (hippocampus, thalamus, nucleus accumbens, putamen, nucleus caudatus and globus pallidus) have been modeled separately for both hemispheres. The analysis was performed following the automated standard protocol of FIRST. In brief, images were transformed linearly into MNI 152 (Montreal Neurological Institute) space, followed by an automated segmentation of designated structures based on shape models and voxel intensities. In a following step, boundaries between the structures were corrected using FAST (FMRIB's Automated Segmentation Tool) to ensure that no overlap between structures occurred. All (pre-)

processing steps for both analyses were checked for accuracy by visually inspecting skull stripping, segmentation, registration and surface-reconstruction results.

Statistics

Demographic data and drug use parameters were analyzed by means of ANOVA, $\chi 2$ Fischer´s Exact Test and unpaired t-tests. SPSS 17 (SPSS inc., Chicago) was used to compute all reported values.

For the group comparisons of cortical thickness and volume, QDEC (Query, Design, Estimate, Contrast) was used. QDEC is a statistical tool implemented in Freesurfer, fitting the general linear model (GLM) to the group averaged data on a vertex by vertex analysis of variance (ANOVA). All groups were compared to each other, resulting in three contrasts: drug-naïve controls vs. low exposure users, drug-naïve controls vs. experienced users and low exposure users vs. experienced users. Images were smoothed with an isotropic Gaussian kernel of 10mm full width at half maximum (FWHM). Correction for multiple comparisons was performed by means of cluster based (thresholded at $p<.05$) Monte Carlo simulation.

The group comparison regarding the shape of subcortical structures was performed by means of a multivariate general linear model on a per vertex basis, leading to a multivariate F-statistic (Pillai's Trace). Correction for multiple comparisons was performed by means of the false discovery rate (FDR). Both statistical analyses were performed with tools implemented in FSL 4.1.5. Additionally, the volume of these structures were computed by measuring the number voxels for each participant boundary corrected structures and performing three unpaired t-tests on these data, one for each contrast. After correction for multiple comparisons no group effect of volume and shape remained significant.

Results

Demographics and drug use parameters

Demographic characteristics of the sample and drug use patterns of the two ATS using groups are published elsewhere (J. Daumann, et al., 2011). For completeness, the most important results are summarized below and in table 1 and 2.

All groups were comparable in terms of gender distribution ($\chi 2$= 1.24, p=.539) and age (F=2.64, p=.078). ATS using groups did not differ significantly in terms of years of education (F=6.58, p=.74). Drug-naïve controls, however, exhibited a significantly longer education than low exposure users (F=6.58, p=.009) and experienced users (F=6.58, p=.004). There was a clear concomitant use of MDMA and amphetamine in the investigated sample. Low-exposure and experienced users did not differ from each other with respect to the substances they had experience with (χ^2=.984, p=.321). All experienced users consumed MDMA and amphetamine. Only six low-exposure users had no experience with MDMA and 2 have not used amphetamines in their lives.

Table 1 Demographic sample characteristics

Characteristics	**C**ontrols (n=16)	**L**ow **E**xposure (n=42)	**E**xperienced (n=20)	$F^1/t^2/\chi^{2^3}/F^4$	p
Present age	26.31 (±4.11)	23.55 (±5.27)	26.6 (±7.17)	2.637[1]	.078
Gender (m:f)	9:7	30:12	13:7	1.235[3]	.539
Education	17.46 (± 2.68)	14.92 (±2.74)	14.38 (±2.18)	6.583[1]	*.009*(C/LE) *.004*(C/E) .740(LE/E)
Experience with…					
MDMA	0	36	20	3.163[3]	.075
amphetamine	0	40	20	.984[3]	.321
cannabis	0	41	19	.298[3]	.585
Last use of…					
MDMA (days)	-	670.03 (938.93)	43.6 (54.7)	2.969[2]	<*.001*
amphetamine (days)	-	393.15 (808.425)	141.8 (484.489)	1.511[2]	.136
cannabis (days)	-	90.17 (225.979)	157.06 (225.881)	-1.005[2]	.324

[1] =F- values with post-hoc test (Scheffé); [2]=t-values were calculated using unpaired t-test; 2-tailed (df = 60); [3]=Comparison tested with χ^2; [4]= post hoc values (Scheffé)

ATS groups were comparable in substances used and all relevant cannabis parameters. Experienced users consumed significantly more MDMA and amphetamine than low exposure users.

Table 2 Specific Drug use characteristics of the sample

Specific Drug Use Patterns	**C**ontrols (n=16)	**L**ow **E**xposure (n=42)	**E**xperienced (n=20)	F^1/t^2	p
Lifetime dose MDMA (pills)	-	2.89 (±2.47)	398 (±342.69)	7.55^2	*<.001*
Lifetime dose Amph (grams)	-	2.81 (±1.5)	258.34 (±320.39)	5.22^2	*<.001*
Lifetime dose Cannabis (grams)	-	531.2 (±825.04)	898.55 (±1289.83)	1.36^2	.180
Daily dose MDMA (pills/occasion)	-	1.02 (±0.69)	3.24 (±2.35)	5.68^2	*<.001*
Daily dose Amph (milligrams/occasion)	-	451.19 (±288.71)	845 (±517.56)	3.85^2	*<.001*
Daily dose Cannabis (joints/occasion)	-	1.85 (±1.48)	1.92 (±1.48)	0.05^2	.853

[1] =F- values with post-hoc test (Scheffé); [2]=t-values were calculated using unpaired t-test; 2-tailed (df = 60)

Cortical Thickness

Results of the analysis are visually summarized in Figure 1. Group differences in cortical thickness were most pronounced in experienced users compared to drug-naïve controls. In the left hemisphere, the middle frontal cortex and the frontopolar region in particular was significantly thinner in experienced users. In contrast, the right hemisphere of experienced users exhibited a cortical thinning near the central sulcus, compared to drug-naïve controls. Comparing experienced users to low-exposure users revealed no differences between the groups in the left hemisphere. In the right hemisphere, however, experienced users exhibited a decreased cortical thickness in the entire middle frontal cortex, extending from orbitofrontal to superior regions of the frontal cortex. Low exposure users did not differ from drug-naïve controls on cortical thickness. Additionally the years of education of all participants were

correlated with cortical thickness, but no significant results survived the correction for multiple comparisons.

Figure 1

Cortical Grey Matter Volume

Results are visually summarized in Figure 1. The group differences in cortical grey matter volume were, for the most part, similar to those obtained in the cortical thickness analyses. Experienced users exhibited decreased volume in frontal regions of the left hemisphere. Again, this effect stretched from the orbitofrontal to the superior frontal cortex. Additional to volume reductions nearby the central sulcus, the cortical volume analysis revealed supramarginal and occipital regions with a decreased volume of experienced users. Similar, yet less widespread results were obtained comparing low exposure users to experienced users for the left hemisphere. Primarily the volume of the middle frontal regions in the left hemisphere exhibited a decreased volume. The same areas were affected in the right hemisphere and additionally the precuneus revealed a decreased volume in experienced users. Small regions in the orbitofrontal (left hemisphere) and occipital (right hemisphere) displayed volume reductions in low-exposure users compared to drug-naïve controls.

Figure 2

Table 1: Summary of imaging statistics for the three contrasts on cortical thickness and cortical volume

Contrast	Structure	Vertices	p
Cortical Thickness			
C vs. EU	LH superior frontal	1201.54	0.001
	RH postcentral	1128.18	0.003
LEU vs. EU	LH	-	-
	RH superior frontal	1202.45	0.001
C vs. LEU	LH	-	-
	RH	-	-
Cortical Volume			
C vs. EU	LH superior frontal	4863.52	<0.001
	LH pericalcarine	2012.85	<0.001
	RH postcentral	1309.50	<0.001
	RH lateral occipital	1108.99	<0.001
	RH lateral occipital	865.16	0.004
	RH inferior parietal	813.80	0.007
LEU vs. EU	LH superior frontal	1828.51	<0.001
	RH superior frontal	2465.86	<0.001
	RH precuneus	783.72	0.009
C vs. LEU	LH rostral middle frontal	950.35	0.001
	RH lateral occipital	1025.93	<0.001

Abbreviations: C= controls; LEU= low-exposure users; EU= experienced users; LH= left hemisphere; RH=right hemisphere

Volumetric Measures of Subcortical Structures

An example of the results is given in Figure 3. Comparing low exposure to drug-naïve controls revealed extensions in the right nucleus accumbens, the left hippocampus and the putamen bilaterally of the ATS group. Experienced users displayed extensions in the right hippocampus compared to low exposure users. Drug-naïve controls exhibited extensions in both hippocampi compared to experienced users.

Volume of these structures, analyzed in a post-hoc manner, revealed no significant main effects between the three groups. The strongest, yet insignificant trend for volumetric group

differences was observed for the left hippocampus.

Figure 3

Discussion

The present study was performed to investigate multiple brain-morphological measures which in recent studies have been shown to be able to detect adverse effects associated with recreational MDMA and amphetamine use. Three groups, which in a former study have been analyzed by means of a tract-based spatial statistics and voxel-based morphometry analysis, were re-analyzed regarding cortical thickness, cortical volume and the shape of subcortical structures. One major strength of the present study is the inclusion of three groups which both ends of typical recreational ATS users, encompassing even a group with massive substance use compared to other studies (Cowan, et al., 2003; Kish, et al., 2010). By including a group of low-exposure users we intended to verify the assumption that low exposure to ATS does not lead to measurable neurotoxic effects and to minimize the influence of confounding factors such as concomitant cannabis use and other pre-existing group differences associated with substance use. Except for years of education, the three groups were comparable on the most important demographic measures. Low-exposure users and experienced users were also comparable in terms of cannabis use patterns, making it implausible that the described effects are attributable to the co use of cannabis.

After correction for multiple comparisons, the analyses on shape and volume of subcortical structures did not reveal any significant group differences. In the literature, reports on neuro-anatomically altered subcortical structures are inconsistent (J. Daumann, et al., 2011; de Win, et al., 2007; Moeller, Hasan, et al., 2007). Several studies have demonstrated differences in fractional anisotropy (FA) of ATS users compared to controls (Alicata, et al., 2009; de Win, et

al., 2008; Moeller, Hasan, et al., 2007), whereas other studies failed to demonstrate differences in DTI-derived measures (J. Daumann, et al., 2011; de Win, et al., 2007). These varying results might be attributable to differences in drug use patterns (e.g. presence of methamphetamine use) and applied statistics (e.g. region of interest vs. whole brain analysis). If group differences in FA have been reported, they were mostly associated with the nucleus caudatus, putamen and the thalamus. Additionally, volumetric differences in subcortical structures between MDMA users and drug-naïve controls have been demonstrated in the globus pallidus, nucleus caudatus, putamen and thalamus in a sub-group of ATS users with the co-use of methamphetamine (Kish, et al., 2010). In our sample, however, we did not find major differences in shape or volume of subcortical structures between the three investigated groups. One reason for the lack of significant group differences in the present study might be that methamphetamine use was absent in our sample. This is particularly important as research has shown that methamphetamine has stronger neurotoxic properties than amphetamine (Hall, Stanis, Marquez Avila, & Gulley, 2008; Yamamoto, Moszczynska, & Gudelsky, 2010; Zolkowska, Rothman, & Baumann, 2006).

Results of studies investigating the grey matter are also inconsistent, although most evidence points towards neuronal density and cortical thickness reductions in frontal regions (Cowan, et al., 2003; J. Daumann, et al., 2011; Kish, et al., 2010). Despite this evidence we deliberately decided to perform a fully automated whole-brain analysis to validate previous results and to exclude the possibility of any operator bias. Cortical thinning and cortical grey matter volume reductions have been observed and are probably the result of regular and massive ATS polydrug use. These effects were most pronounced in medial frontal regions. The strongest effects on cortical thickness and volume were observed by comparing experienced users to either drug-naïve controls or low-exposure users. Expectedly, low exposure users did not differ from drug-naïve controls on cortical thickness. However, cortical grey matter volume

reductions in low exposure users have been found for this comparison.

These results add to findings regarding the effects of ATS use on neuronal integrity. Recent reports suggest that the cortex might be especially prone to the neurotoxic effects of ATS (Cowan, et al., 2003; J. Daumann, et al., 2011; Gouzoulis-Mayfrank & Daumann, 2009; Kish, et al., 2010). Grey matter concentration reductions (Cowan, et al., 2003; J. Daumann, et al., 2011) and cortical thinning (Kish, et al., 2010) have been demonstrated in medial frontal and cingulate but also in parietal and occipital (Cowan, et al., 2003) regions.

Studies on functional MRI, psychopathology and cognitive impairment of ATS user further validate the finding of primarily frontal structures being vulnerable to the neurotoxic effects of ATS polydrug use. The (pre-) frontal cortex has been associated with higher order cognitive abilities and top-down information processing (Koechlin & Hyafil, 2007; Koenigs et al., 2007). Studies investigating cognitive functions in recreational ATS users described impairments in cognitive functions associated to the orbitofrontal and the dorsolateral prefrontal cortex, such as working memory, decision making and cognitive as well as inhibitory control (Gouzoulis-Mayfrank & Daumann, 2006; Gouzoulis-Mayfrank et al., 2000; Hanson, Luciana, & Sullwold, 2008; Moeller, Steinberg et al., 2007; Morgan, Impallomeni, Pirona, & Rogers, 2006; Quednow et al., 2007; Rogers et al., 1999). Response patterns of ATS users on a decision making task have been reported to be comparable to patients with damage to the orbitofrontal cortex, displaying disinhibited and impulsive decisions (Rogers, et al., 1999). Similar cognitive impairments on inhibitory control and decision making have been described in amphetamine and methamphetamine users (Aron & Paulus, 2007; Ornstein et al., 2000). It is assumed that primarily the disinhibited and compulsive behavior is responsible for the poor performance of polydrug users on decision making and executive functioning tasks. Accompanied by these noticeable cognitive functions, recreational ATS users have shown

abnormal activity in the underlying frontal networks (Cowan, et al., 2003; J. Daumann, Jr., Fischermann, Heekeren, Thron, & Gouzoulis-Mayfrank, 2004; Valdes et al., 2006).

During the last years, neural alterations, particularly in the OFC, have been suggested to play a major role in animal and human models of addiction (Koob & Simon, 2009; Schoenbaum, Roesch, & Stalnaker, 2006; Schoenbaum & Shaham, 2008). Most authors suggest that drug use is, in part, mediated by drug-induced changes in orbitofrontal functions and structures (Koob & Simon, 2009; Schoenbaum & Shaham, 2008). Cellular adaptations in dopaminergic and/or glutamatergic projections as a result of chronic drug use have been proposed to underlie alterations in OFC (Schoenbaum & Shaham, 2008; Volkow, Fowler, Wang, Baler, & Telang, 2009). Results of the present study, together with previous findings indicating thinning of the frontal cortex in ATS cocaine, methamphetamine and alcohol dependent users, are in line with this hypothesis (Durazzo et al., 2011; Kim et al., 2006; Makris et al., 2008; Tanabe et al., 2009; Thompson et al., 2004).

Although not consistent, neuropsychological, neurophysiological and functional fMRI data also support the hypothesis of neurological alterations in parietal and occipital regions. The parietal cortex, with a dense interconnection to the dorsolateral prefrontal cortex, has been associated spatial and attentional processes (Coull, Frith, Frackowiak, & Grasby, 1996; Hopfinger, Buonocore, & Mangun, 2000). MDMA polydrug users have been found to perform functionally below control subjects on tasks visual recognition and spatial working memory tasks (Fox et al., 2002). Additionally, a hypoactivation of the parietal cortex has repeatedly been associated with deficits in attentional shifting and rigid stimulus-response patterns in methamphetamine users compared to drug naïve controls (Paulus, Hozack, Frank, Brown, & Schuckit, 2003; Paulus, Tapert, & Schuckit, 2005). An attenuated neural response in the parietal lobe has also been described in event-related potential (ERP) recordings during verbal recognition (Burgess, Venables, Jones, Edwards, & Parrott, 2011). The role of serotonergic functions has also been discussed in conjunction with deficits in visual orienting,

which is mostly associated with the primary visual cortex (Brown, Edwards, McKone, & Ward, 2007). This low-level visual processing has found to be altered in MDMA polydrug users, which was interpreted in terms of serotonergic dysfunction in the occipital cortex (Dickson, Bruno, & Brown, 2009).

The specific mechanisms underlying cortical thinning in ATS users in the present study are not known. Structural MR-imaging cannot provide information about the microstructure of the brain or cytoarchitectonic details. Besides possibly pre-existing group differences in the current sample, drug induced changes in synaptic strength and structure, altered plasticity, changes in soma and nuclei size as well as changes in glia and cappilar changes and cell death might be responsible for cortical thinning in ATS polydrug users (Hutton, et al., 2009). In addition, the direction of the causal relationship between structural alteration and drug use, and how these are related to dysfunctional neuronal activity, psychopathology and cognition, is not yet understood.

A second conclusion emerging from the results of the present study refers to the ambiguity of affected regions in conjunction with the used methodology. Whereas the analysis of cortical thickness exclusively demonstrated group differences in frontal regions, thereby supporting recent findings from our previous analysis in the same sample, the analysis on cortical volume additionally revealed occipital and parietal group differences (J. Daumann, et al., 2011). Although other studies also reported posterior cortical grey matter volume reductions (Cowan, et al., 2003; Kish, et al., 2010), these results are surprising as neither the previously applied VBM analysis on the same sample (J. Daumann, et al., 2011) nor the cortical thickness analysis of the present study revealed any group differences in occipital regions. Furthermore, the analysis unexpectedly displayed minor cortical volume reductions in low exposure users compared to drug-naïve controls. It is unlikely that this result is caused by pre-existing group differences or low exposure to ATS alone, but rather is caused by the differing methodological

approach. Cortical thickness is a specific structural parameter, which is even able to detect minor changes in thickness induced by cognitive training (Engvig et al., 2010). However, it does not reveal any information on exact neuronal mechanisms. The analysis on cortical volume might be hyper-sensitive to micro-anatomical changes. Cortical thickness specifically measures the distance of pial and grey matter surface at each voxel, whereas volumetric voxel based measures are additionally dependent on the surface area and hence cortical folding patterns. Due to this, common volumetric VBM studies have been shown to be more sensitive to noise (Hutton, et al., 2009). In our previous study we aimed to minimize these effects by using DTI space, hypothesizing that this would alleviate a bias due to non-linear transformations. The results of the previous study have been validated, as in the cortical thickness analysis primarily frontal areas have found to be affected. Additional alterations in parietal and occipital areas might be associated with differing methodologies of the analyses. As these posterior regions have also been found to be affected in other studies (Cowan, et al., 2003; Kish, et al., 2010), these results are also assumed to give a valid impression of possible neurotoxic damages associated with ATS use.

All conclusions are inherently linked to limitations associated with the design of the present study. Most importantly, no randomization has taken place. Pre-existing group differences and associated alternative hypotheses cannot be ruled out. Drug naïve controls exhibited significantly more years of education than low-exposure and experienced users. However, correlational analyses did not reveal any association between years of education and cortical thickness and cortical volume. Data on alcohol and nicotine consumption have not been collected. Both substances have been associated with reduced grey matter volume and cortical thickness in the prefrontal cortex and the dorsal ACC (Gallinat et al., 2006; Kuhn, Schubert, & Gallinat, 2010; Lawyer et al., 2010; Mechtcheriakov et al., 2007) and might have, if differentially distributed across groups, affected the results. A third limitation inherent to this

design is the inability to disentangle the discrete effects of these substances. As can be seen in table 1, virtually all participants were polydrug ATS users. It is therefore impossible to attribute cortical thickness and volume reductions to either MDMA, amphetamine, cannabis or a combination of these. However, the primary goal of the study was to demonstrate the effects of recreational ATS use on brain-morphological measures in a sample of typical users. A last limitation is based on the fact that drug use patterns were based on self-reported histories which may be inaccurate. To account for this, randomly taken hair samples were collected from some participants and analyzed by the Institute of Legal Medicine of the University of Cologne, which verified the consumed quantity of ATS. Furthermore, studies validating self-reported voluntary substance use found a high reliability of the reported drug quantity (Martin & Newman, 1988; Rothe et al., 1997).

The results of the present study might add important information to the otherwise scarce body of literature on direct neurotoxic effects of MDMA and amphetamine in recreational human users. First, the suspicion substantiates that the (frontal) cortex is prone to the neurotoxic effects of either MDMA, amphetamine or both. The most consistent findings about the neurotoxicity of human polydrug ATS use have been found here (Cowan, et al., 2003; J. Daumann, et al., 2011; Kish, et al., 2010) and were replicated in the present study. These findings are also coherent with knowledge about brain functioning and performance of users on cognitive tasks with a high load on pre-frontal brain regions and psychopathology. It is supposed that serotonergic neurons, originating in the raphe nucleus, are vulnerable to the effects of MDMA. This hypothesis is also supported by the finding that the neurotoxic effects in animals are reversible, and that the time of recovery is dependent on the distance neurons have to re-innervate from the raphe nucleus to the affected region (Gouzoulis-Mayfrank & Daumann, 2009). In contrast, we did not find any significant group differences in shape and volume of subcortical structures, which were supposed to be vulnerable to the effects of ATS. Due to the design of the study at hand, we cannot conclusively answer the questions whether

grey matter thickness and cortical volume differences are a consequence or rather a cause of ATS use.

Acknowledgement

This study was supported in part by a grant to E. Gouzoulis-Mayfrank and J. Daumann from the Deutsche Forschungsgemeinschaft (DFG GO 717/6-1/2).

Financial disclosure

All authors certify that there is no actual or potential financial interest in relation to the subject matter or materials discussed in this manuscript.

Captions:

Fig. 1 Results of cortical thickness analysis overlaid onto standard brain, $p<.05$, corrected for multiple comparisons by means of Monte Carlo Z Simulation. Values indicate –log(10) transformed p-statistics. Abbreviations: RH= right hemisphere, LH= left hemisphere, C=control subjects, LEU= low exposure users, EU=experienced users

Fig. 2 Results of cortical volume analysis overlaid onto standard brain, $p<.05$, corrected for multiple comparisons by means of Monte Carlo Z Simulation. Values indicate –log(10) transformed p-statistics. Abbreviations: RH= right hemisphere, LH= left hemisphere, C=control subjects, LEU= low exposure users, EU=experienced users. Different colors represent varying effect sizes.

Fig. 3 Results of Vertex analysis of the hippocampus for the comparison of controls > experienced users before correcting for multiple comparisons. The arrows indicate the direction of extension/constriction within the structure. After correction these results did not remain significant.

References

Alicata, D., Chang, L., Cloak, C., Abe, K., & Ernst, T. (2009). Higher diffusion in striatum and lower fractional anisotropy in white matter of methamphetamine users. *Psychiatry Res, 174*(1), 1-8. doi: S0925-4927(09)00087-0 [pii]
10.1016/j.pscychresns.2009.03.011

Aron, J. L., & Paulus, M. P. (2007). Location, location: using functional magnetic resonance imaging to pinpoint brain differences relevant to stimulant use. *Addiction, 102 Suppl 1*, 33-43. doi: ADD1778 [pii]
10.1111/j.1360-0443.2006.01778.x

Brown, J., Edwards, M., McKone, E., & Ward, J. (2007). A long-term ecstasy-related change in visual perception. *Psychopharmacology (Berl), 193*(3), 437-446. doi: 10.1007/s00213-007-0785-0

Burgess, A. P., Venables, L., Jones, H., Edwards, R., & Parrott, A. C. (2011). Event related potential (ERP) evidence for selective impairment of verbal recollection in abstinent recreational methylenedioxymethamphetamine ("Ecstasy")/polydrug users. *Psychopharmacology (Berl), 216*(4), 545-556. doi: 10.1007/s00213-011-2249-9

Coull, J. T., Frith, C. D., Frackowiak, R. S., & Grasby, P. M. (1996). A fronto-parietal network for rapid visual information processing: a PET study of sustained attention and working memory. *Neuropsychologia, 34*(11), 1085-1095. doi: 0028-3932(96)00029-2 [pii]

Cowan, R. L., Lyoo, I. K., Sung, S. M., Ahn, K. H., Kim, M. J., Hwang, J. (2003). Reduced cortical gray matter density in human MDMA (Ecstasy) users: a voxel-based morphometry study. *Drug Alcohol Depend, 72*(3), 225-235. doi: S037687160300231X [pii]

Dale, A. M., Fischl, B., & Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage, 9*(2), 179-194. doi: S1053-8119(98)90395-0 [pii]
10.1006/nimg.1998.0395

Daumann, J., Fischermann, T., Heekeren, K., Henke, K., Thron, A., & Gouzoulis-Mayfrank, E. (2005). Memory-related hippocampal dysfunction in poly-drug ecstasy (3,4-methylenedioxymethamphetamine) users. *Psychopharmacology (Berl), 180*(4), 607-611. doi: 10.1007/s00213-004-2002-8

Daumann, J., Jr., Fischermann, T., Heekeren, K., Thron, A., & Gouzoulis-Mayfrank, E. (2004). Neural mechanisms of working memory in ecstasy (MDMA) users who continue or discontinue ecstasy and amphetamine use: evidence from an 18-month longitudinal functional magnetic resonance imaging study. *Biol Psychiatry, 56*(5), 349-355. doi: 10.1016/j.biopsych.2004.06.011
S0006-3223(04)00676-6 [pii]

Daumann, J., Koester, P., Becker, B., Wagner, D., Imperati, D., Gouzoulis-Mayfrank, E. (2011). Medial prefrontal gray matter volume reductions in users of amphetamine-type stimulants revealed by combined tract-based spatial statistics and voxel-based morphometry. *Neuroimage, 54*(2), 794-801. doi: S1053-8119(10)01162-6 [pii]
10.1016/j.neuroimage.2010.08.065

de Win, M. M., Jager, G., Booij, J., Reneman, L., Schilt, T., Lavini, C. (2008). Sustained effects of ecstasy on the human brain: a prospective neuroimaging study in novel users. *Brain, 131*(Pt 11), 2936-2945. doi: awn255 [pii]

10.1093/brain/awn255
de Win, M. M., Reneman, L., Jager, G., Vlieger, E. J., Olabarriaga, S. D., Lavini, C. (2007). A prospective cohort study on sustained effects of low-dose ecstasy use on the brain in new ecstasy users. *Neuropsychopharmacology, 32*(2), 458-470. doi: 1301225 [pii]
10.1038/sj.npp.1301225
Dickson, C., Bruno, R., & Brown, J. (2009). Investigating the role of serotonin in visual orientation processing using an 'ecstasy' (MDMA)-based research model. *Neuropsychobiology, 60*(3-4), 204-212. doi: 000253556 [pii]
10.1159/000253556
Durazzo, T. C., Tosun, D., Buckley, S., Gazdzinski, S., Mon, A., Fryer, S. L. (2011). Cortical thickness, surface area, and volume of the brain reward system in alcohol dependence: relationships to relapse and extended abstinence. *Alcohol Clin Exp Res, 35*(6), 1187-1200. doi: 10.1111/j.1530-0277.2011.01452.x
Easton, N., & Marsden, C. A. (2006). Ecstasy: are animal data consistent between species and can they translate to humans? *J Psychopharmacol, 20*(2), 194-210. doi: 20/2/194 [pii]
10.1177/0269881106061153
Engvig, A., Fjell, A. M., Westlye, L. T., Moberget, T., Sundseth, O., Larsen, V. A. (2010). Effects of memory training on cortical thickness in the elderly. *Neuroimage, 52*(4), 1667-1676. doi: S1053-8119(10)00773-1 [pii]
10.1016/j.neuroimage.2010.05.041
Fischl, B., & Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci U S A, 97*(20), 11050-11055. doi: 10.1073/pnas.200033797
200033797 [pii]
Fischl, B., Sereno, M. I., & Dale, A. M. (1999). Cortical surface-based analysis. II: Inflation, flattening, and a surface-based coordinate system. *Neuroimage, 9*(2), 195-207. doi: S1053-8119(98)90396-2 [pii]
10.1006/nimg.1998.0396
Fox, H. C., McLean, A., Turner, J. J., Parrott, A. C., Rogers, R., & Sahakian, B. J. (2002). Neuropsychological evidence of a relatively selective profile of temporal dysfunction in drug-free MDMA ("ecstasy") polydrug users. *Psychopharmacology (Berl), 162*(2), 203-214. doi: 10.1007/s00213-002-1071-9
Gallinat, J., Meisenzahl, E., Jacobsen, L. K., Kalus, P., Bierbrauer, J., Kienast, T. (2006). Smoking and structural brain deficits: a volumetric MR investigation. *Eur J Neurosci, 24*(6), 1744-1750. doi: EJN5050 [pii]
10.1111/j.1460-9568.2006.05050.x
Gouzoulis-Mayfrank, E., & Daumann, J. (2006). Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: how strong is the evidence for persistent brain damage? *Addiction, 101*(3), 348-361. doi: ADD1314 [pii]
10.1111/j.1360-0443.2006.01314.x
Gouzoulis-Mayfrank, E., & Daumann, J. (2009). Neurotoxicity of drugs of abuse--the case of methylenedioxyamphetamines (MDMA, ecstasy), and amphetamines. *Dialogues Clin Neurosci, 11*(3), 305-317.
Gouzoulis-Mayfrank, E., Daumann, J., Tuchtenhagen, F., Pelz, S., Becker, S., Kunert, H. J. (2000). Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). *J Neurol Neurosurg Psychiatry, 68*(6), 719-725.
Hall, D. A., Stanis, J. J., Marquez Avila, H., & Gulley, J. M. (2008). A comparison of amphetamine- and methamphetamine-induced locomotor activity in rats: evidence for qualitative differences in behavior. *Psychopharmacology (Berl), 195*(4), 469-478. doi: 10.1007/s00213-007-0923-8
Hanson, K. L., Luciana, M., & Sullwold, K. (2008). Reward-related decision-making deficits

and elevated impulsivity among MDMA and other drug users. *Drug Alcohol Depend, 96*(1-2), 99-110. doi: S0376-8716(08)00071-9 [pii]
10.1016/j.drugalcdep.2008.02.003
Hatzidimitriou, G., McCann, U. D., & Ricaurte, G. A. (1999). Altered serotonin innervation patterns in the forebrain of monkeys treated with (+/-)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. *J Neurosci, 19*(12), 5096-5107.
Hopfinger, J. B., Buonocore, M. H., & Mangun, G. R. (2000). The neural mechanisms of top-down attentional control. *Nat Neurosci, 3*(3), 284-291. doi: 10.1038/72999
Hutton, C., Draganski, B., Ashburner, J., & Weiskopf, N. (2009). A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. *Neuroimage, 48*(2), 371-380. doi: S1053-8119(09)00679-X [pii]
10.1016/j.neuroimage.2009.06.043
Kim, S. J., Lyoo, I. K., Hwang, J., Chung, A., Hoon Sung, Y., Kim, J. (2006). Prefrontal grey-matter changes in short-term and long-term abstinent methamphetamine abusers. *Int J Neuropsychopharmacol, 9*(2), 221-228. doi: S1461145705005699 [pii]
10.1017/S1461145705005699
Kish, S. J., Lerch, J., Furukawa, Y., Tong, J., McCluskey, T., Wilkins, D. (2010). Decreased cerebral cortical serotonin transporter binding in ecstasy users: a positron emission tomography/[(11)C]DASB and structural brain imaging study. *Brain, 133*(Pt 6), 1779-1797. doi: awq103 [pii]
10.1093/brain/awq103
Koechlin, E., & Hyafil, A. (2007). Anterior prefrontal function and the limits of human decision-making. *Science, 318*(5850), 594-598. doi: 318/5850/594 [pii]
10.1126/science.1142995
Koenigs, M., Young, L., Adolphs, R., Tranel, D., Cushman, F., Hauser, M. (2007). Damage to the prefrontal cortex increases utilitarian moral judgements. *Nature, 446*(7138), 908-911. doi: nature05631 [pii]
10.1038/nature05631
Kolb, B., Gorny, G., Li, Y., Samaha, A. N., & Robinson, T. E. (2003). Amphetamine or cocaine limits the ability of later experience to promote structural plasticity in the neocortex and nucleus accumbens. *Proc Natl Acad Sci U S A, 100*(18), 10523-10528. doi: 10.1073/pnas.1834271100
1834271100 [pii]
Koob, G. F., & Simon, E. J. (2009). The Neurobiology of Addiction: Where We Have Been and Where We Are Going. *J Drug Issues, 39*(1), 115-132.
Kuhn, S., Schubert, F., & Gallinat, J. (2010). Reduced thickness of medial orbitofrontal cortex in smokers. *Biol Psychiatry, 68*(11), 1061-1065. doi: S0006-3223(10)00818-8 [pii]
10.1016/j.biopsych.2010.08.004
Lawyer, G., Bjerkan, P. S., Hammarberg, A., Jayaram-Lindstrom, N., Franck, J., & Agartz, I. (2010). Amphetamine dependence and co-morbid alcohol abuse: associations to brain cortical thickness. *BMC Pharmacol, 10*, 5. doi: 1471-2210-10-5 [pii]
10.1186/1471-2210-10-5
Makris, N., Gasic, G. P., Kennedy, D. N., Hodge, S. M., Kaiser, J. R., Lee, M. J. (2008). Cortical thickness abnormalities in cocaine addiction--a reflection of both drug use and a pre-existing disposition to drug abuse? *Neuron, 60*(1), 174-188. doi: S0896-6273(08)00706-X [pii]
10.1016/j.neuron.2008.08.011
Martin, G. L., & Newman, I. M. (1988). Assessing the validity of self-reported adolescent cigarette smoking. *J Drug Educ, 18*(4), 275-284.
Mechtcheriakov, S., Brenneis, C., Egger, K., Koppelstaetter, F., Schocke, M., & Marksteiner,

J. (2007). A widespread distinct pattern of cerebral atrophy in patients with alcohol addiction revealed by voxel-based morphometry. *J Neurol Neurosurg Psychiatry, 78*(6), 610-614. doi: jnnp.2006.095869 [pii]
10.1136/jnnp.2006.095869
Melega, W. P., Raleigh, M. J., Stout, D. B., Lacan, G., Huang, S. C., & Phelps, M. E. (1997). Recovery of striatal dopamine function after acute amphetamine- and methamphetamine-induced neurotoxicity in the vervet monkey. *Brain Res, 766*(1-2), 113-120. doi: S0006-8993(97)00548-9 [pii]
Moeller, F. G., Hasan, K. M., Steinberg, J. L., Kramer, L. A., Valdes, I., Lai, L. Y. (2007). Diffusion tensor imaging eigenvalues: preliminary evidence for altered myelin in cocaine dependence. *Psychiatry Res, 154*(3), 253-258. doi: S0925-4927(06)00198-3 [pii]
10.1016/j.pscychresns.2006.11.004
Moeller, F. G., Steinberg, J. L., Lane, S. D., Buzby, M., Swann, A. C., Hasan, K. M. (2007). Diffusion tensor imaging in MDMA users and controls: association with decision making. *Am J Drug Alcohol Abuse, 33*(6), 777-789. doi: 783770469 [pii]
10.1080/00952990701651564
Morgan, M. J., Impallomeni, L. C., Pirona, A., & Rogers, R. D. (2006). Elevated impulsivity and impaired decision-making in abstinent Ecstasy (MDMA) users compared to polydrug and drug-naive controls. *Neuropsychopharmacology, 31*(7), 1562-1573. doi: 1300953 [pii]
10.1038/sj.npp.1300953
Ornstein, T. J., Iddon, J. L., Baldacchino, A. M., Sahakian, B. J., London, M., Everitt, B. J. (2000). Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. *Neuropsychopharmacology, 23*(2), 113-126. doi: S0893-133X(00)00097-X [pii]
10.1016/S0893-133X(00)00097-X
Patenaude, B. (2007). *Bayesian Statistical Models of Shape and Appearance for Subcortical Brain Segmentation.* Thesis.
Patenaude, B., Smith, S., Kennedy, D., & Jenkinson, M. (2007). *FIRST - FMRIB's integrated registration and segmentation tool.* Paper presented at the Human Brain Mapping Conference.
Patenaude, B., Smith, S., Kennedy, D., & Jenkinson, M. (2008). *Improved Surface Models for FIRST.* Paper presented at the Human Brian Mapping Conference.
Paulus, M. P., Hozack, N., Frank, L., Brown, G. G., & Schuckit, M. A. (2003). Decision making by methamphetamine-dependent subjects is associated with error-rate-independent decrease in prefrontal and parietal activation. *Biol Psychiatry, 53*(1), 65-74. doi: S0006322302014427 [pii]
Paulus, M. P., Tapert, S. F., & Schuckit, M. A. (2005). Neural activation patterns of methamphetamine-dependent subjects during decision making predict relapse. *Arch Gen Psychiatry, 62*(7), 761-768. doi: 62/7/761 [pii]
10.1001/archpsyc.62.7.761
Quednow, B. B., Kuhn, K. U., Hoppe, C., Westheide, J., Maier, W., Daum, I. (2007). Elevated impulsivity and impaired decision-making cognition in heavy users of MDMA ("Ecstasy"). *Psychopharmacology (Berl), 189*(4), 517-530. doi: 10.1007/s00213-005-0256-4
Ricaurte, G. A., Martello, A. L., Katz, J. L., & Martello, M. B. (1992). Lasting effects of (+-)-3,4-methylenedioxymethamphetamine (MDMA) on central serotonergic neurons in nonhuman primates: neurochemical observations. *J Pharmacol Exp Ther, 261*(2), 616-622.
Rogers, R. D., Everitt, B. J., Baldacchino, A., Blackshaw, A. J., Swainson, R., Wynne, K. (1999). Dissociable deficits in the decision-making cognition of chronic amphetamine

abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. *Neuropsychopharmacology, 20*(4), 322-339. doi: S0893133X98000918 [pii]
10.1016/S0893-133X(98)00091-8

Rothe, M., Pragst, F., Spiegel, K., Harrach, T., Fischer, K., & Kunkel, J. (1997). Hair concentrations and self-reported abuse history of 20 amphetamine and ecstasy users. *Forensic Sci Int, 89*(1-2), 111-128. doi: S0379-0738(97)00123-0 [pii]

Schoenbaum, G., Roesch, M. R., & Stalnaker, T. A. (2006). Orbitofrontal cortex, decision-making and drug addiction. *Trends Neurosci, 29*(2), 116-124. doi: S0166-2236(05)00324-3 [pii]
10.1016/j.tins.2005.12.006

Schoenbaum, G., & Shaham, Y. (2008). The role of orbitofrontal cortex in drug addiction: a review of preclinical studies. *Biol Psychiatry, 63*(3), 256-262. doi: S0006-3223(07)00534-3 [pii]
10.1016/j.biopsych.2007.06.003

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage, 23 Suppl 1*, S208-219. doi: S1053-8119(04)00393-3 [pii]
10.1016/j.neuroimage.2004.07.051

Tanabe, J., Tregellas, J. R., Dalwani, M., Thompson, L., Owens, E., Crowley, T. (2009). Medial orbitofrontal cortex gray matter is reduced in abstinent substance-dependent individuals. *Biol Psychiatry, 65*(2), 160-164. doi: S0006-3223(08)00971-2 [pii]
10.1016/j.biopsych.2008.07.030

Thompson, P. M., Hayashi, K. M., Simon, S. L., Geaga, J. A., Hong, M. S., Sui, Y. (2004). Structural abnormalities in the brains of human subjects who use methamphetamine. *J Neurosci, 24*(26), 6028-6036. doi: 10.1523/JNEUROSCI.0713-04.2004
24/26/6028 [pii]

UNODC. (2011). 2011 Global ATS Assessment. *United Nations Publication*, http://www.unodc.org/documents/ATS/ATS_Global_Assessment_2011.pdf.

Valdes, I. H., Steinberg, J. L., Narayana, P. A., Kramer, L. A., Dougherty, D. M., Swann, A. C. (2006). Impulsivity and BOLD fMRI activation in MDMA users and healthy control subjects. *Psychiatry Res, 147*(2-3), 239-242. doi: S0925-4927(06)00021-7 [pii]
10.1016/j.pscychresns.2006.01.014

Volkow, N. D., Fowler, J. S., Wang, G. J., Baler, R., & Telang, F. (2009). Imaging dopamine's role in drug abuse and addiction. *Neuropharmacology, 56 Suppl 1*, 3-8. doi: S0028-3908(08)00148-2 [pii]
10.1016/j.neuropharm.2008.05.022

Woolrich, M. W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T. (2009). Bayesian analysis of neuroimaging data in FSL. *Neuroimage, 45*(1 Suppl), S173-186. doi: S1053-8119(08)01204-4 [pii]
10.1016/j.neuroimage.2008.10.055

Yamamoto, B. K., Moszczynska, A., & Gudelsky, G. A. (2010). Amphetamine toxicities: classical and emerging mechanisms. *Ann N Y Acad Sci, 1187*, 101-121. doi: NYAS5141 [pii]
10.1111/j.1749-6632.2009.05141.x

Zolkowska, D., Rothman, R. B., & Baumann, M. H. (2006). Amphetamine analogs increase plasma serotonin: implications for cardiac and pulmonary disease. *J Pharmacol Exp Ther, 318*(2), 604-610. doi: jpet.106.101618 [pii]
10.1124/jpet.106.101618

Figure 1

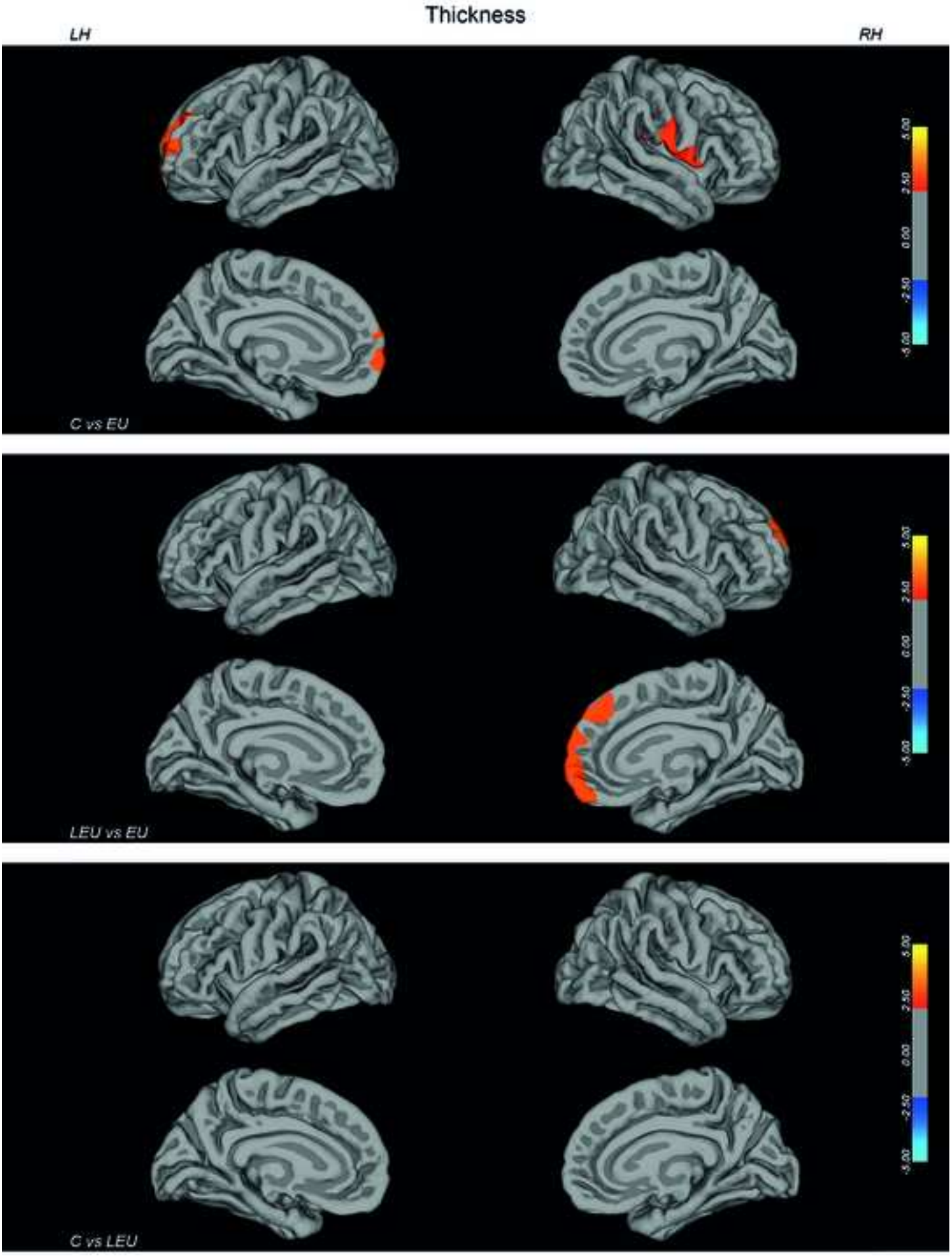


Figure 2

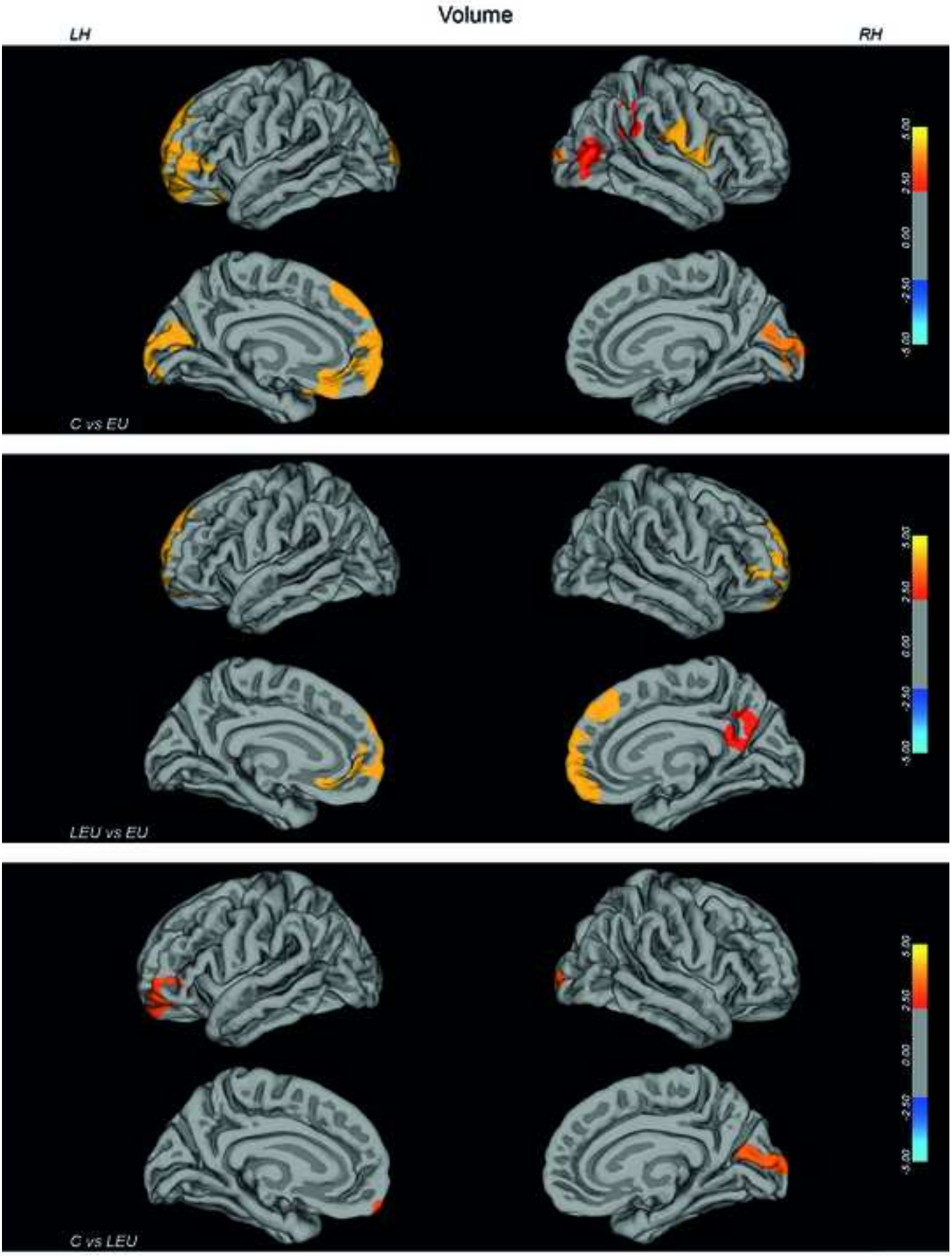
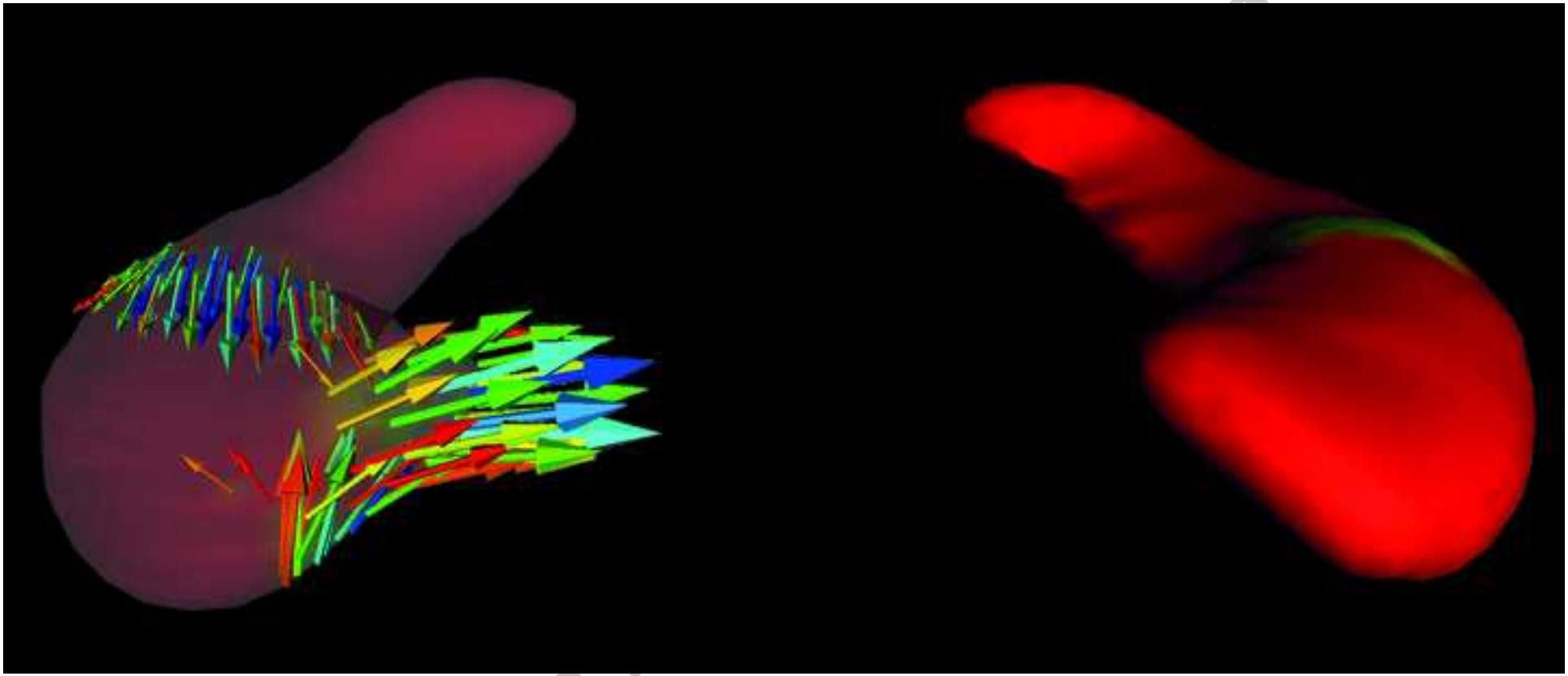


Figure 3



We investigated three groups of amphetamine and MDMA users

Data on cortical thickness, cortical volume and subcortical structures have been collected

Heavy users displayed cortical thinning and volume reduction in frontal and parietal regions

Drug naïve controls did not differ from low exposure users

Neuronal degeneration might be associated with the use of amphetamine derivates