White matter microstructural alterations induced by chronic cocaine self-administration: a Diffusion Tensor Imaging study in the rat

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Introduction

Chronic cocaine abuse leads to long-lasting neurofunctional and neurometabolic abnormalities, a finding consistently reproduced in different studies using various neuroimaging modalities [1,2,3]. Magnetic resonance imaging studies have recently shown that these changes are often associated to frontal cerebral white matter (WM) alterations [4,5], a result that provides a plausible neuroanatomical substrate for some of the functional deficits observed in cocaine addicts.

Long-term cocaine abuse can be effectively modelled pre-clinically in experimental paradigms where rats are trained to self-administer the drug [6]. These models represent a powerful experimental tool to investigate the neuroplastic events associated to long-term voluntary drug-intake at a cellular and behavioural level. However, the exact degree to which these models replicate the neurofunctional and microstructural alterations observed in human neuroimaging studies remains unknown. In order to begin to address this question, here we used Diffusion Tensor Imaging (DTI) [7] to investigate the effect of long-term long-access cocaine self-administration on brain white matter microstructure.

Methods

Experiments were carried out in accordance with Italian regulations governing animal welfare and protection. Protocols were also reviewed and consented to by a local animal care committee, in accordance with the guidelines of the Principles of Laboratory Animal Care (NIH publication 86-23, revised 1985). **Cocaine self-administration:** Male Lister-Hooded rats were fitted with a jugular cannula and trained to self-administer cocaine (or vehicle) under a fixed ratio schedule, 6 h per day, per 5 days followed by 72 h of discontinuation as previously described [8]. Total duration of the experiment was 53 days. Imaging studies were performed 7 days after the last session. **Experimental groups**: Sham (saline self-administration), n=6; cocaine self-administration, n=7. **Animal preparation**: Rats were anaesthetised with halothane and tracheotomised to allow mechanical ventilation under neuromuscular blockade. Left femoral artery was cannulated to allow continuous blood pressure monitoring, and measurement of arterial blood gases. Image acquisition was performed under 1 % halothane anaesthesia **MRI acquisition:** Images were acquired on a Bruker 4.7 T system. High-resolution T2-weighted coronal images of the brain were acquired using a RARE sequence (4cm FOV, 256x256x20 matrix, 1 mm slice thickness, TR/TEeff=5461/72.6; RARE factor 8, α=180). Co-centred images with high white/grey matter contrast were acquired using a segmented (n=4) gradient echo with inversion (MDEFT; TI:1400; TR:6000; TE 4 ms). DTI images were acquired using a segmented (n=4) spin-echo EPI diffusion-weighted sequence (matrix 128x128x20 – 1mm thickness, TR/Teff=3000ms/35.0 m, Diff orientations = 30; α=90; b=1000 s/mm^2). Five acquisitions with negligible diffusion weighting (b=0.6 s/mm^2) were acquired as reference signal. The diffusion tensor was calculated using multivariate linear fitting [7], and eigenvalues were determined to calculate fractional anisotropy (FA). **Data analysis:** individual fractional anisotropy (FA) maps were spatially normalised to a reference stereotaxic atlas and statistical maps of inter-group differences in FA were calculated within the framework of the general linear model using FEAT (FMRI Expert Analysis Tool) Version 5.63, part of FSL, spatial smoothing of 0.7 mm., and cluster correction threshold of 0.05. FA values from anatomical volumes of interest were extracted from a 3D stereotaxic rat template as previously described [9]

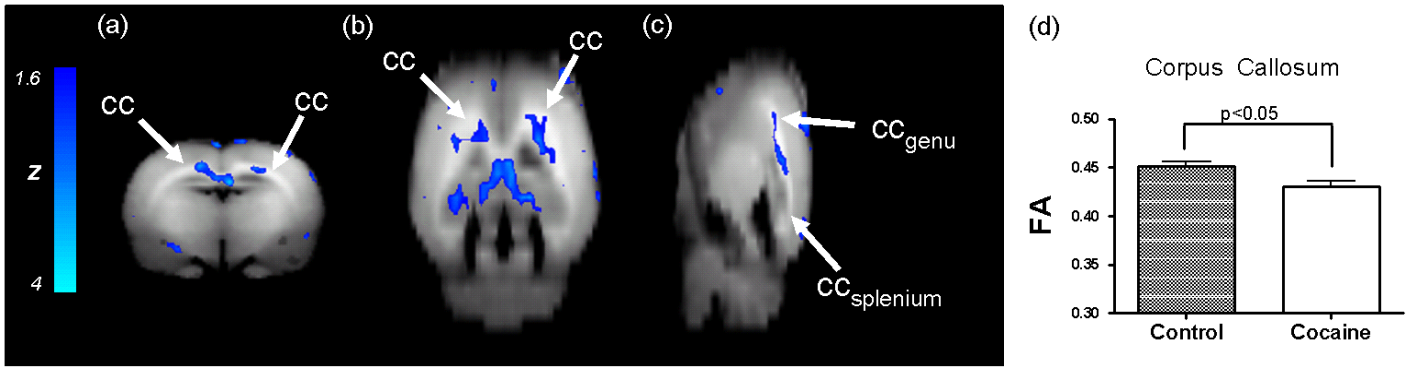

Figure 1 (a-c) Group statistical maps of regions exhibiting reduced FA in rats that self-administered cocaine vs. control (sham) overlaid onto a high white /gray matter contrast map from a representative subject. Blue indicates significantly reduced FA versus control (Z>1.6, CC: corpus callosum). Three perpendicular sections are reported corresponding to coronal (a) horizontal (b) and saggital (c) planes intersecting at the following stereotaxic coordinates (mm): 27.0, -31.0 and -29.5 [9] **(d)** Group comparison of mean FA in the corpus callosum (three-dimensional VOI integration). The difference was statistically significant (p<0.05; one-way ANOVA).

Results

Figure 1 illustrates anatomical location of regions exhibiting statistically significant (Z>1.6; cc= p0.05) alterations in fractional anisotropy (FA) in animals that underwent chronic self-administration of cocaine. Chronic-cocaine intake was associated to focal FA decreases in several areas of the corpus callosum. The effect was most prominent in frontal portions of the structure, although bilateral areas of decreased FA anisotropy were also observed in ventromedial white matter areas. No significant fractional anisotropy *increases* were observed anywhere in the brain (Z>1.6, cc=0.05). The effect was statistically significant on a VOI-basis when the entire corpus callosum was selected (Fig 1, d).

Discussion

Fractional anisotropy is an established structural marker of white matter integrity, with reduced values typically found in conditions that involve neurodegenerative processes such as multiple sclerosis. More recently, significant decreases in white matter FA have been observed in alcohol and cocaine abusers, a result that is though to underlie some of the neurofunctional deficits observed in prefrontal cortical areas of these subjects [4,5,1]. Our results provide evidence of microstructural alterations in the brain white matter of rats that chronically and voluntarily self-administered cocaine over a substantial period of time, a model that is widely used preclinically to mimic cocaine-addiction in humans. These findings corroborate the notion that chronic exposure to cocaine and its metabolites lead to long-term behavioural alterations that are intimately associated to substantial and potentially irreversible microstructural changes in the brain. Our results strengthens the face- and construct-validity of this self-administration model as a tool to investigate the behavioural and neuroanatomical determinants involved in the transition from recreational drug-consumption to the development of addiction.

References [1] Bolla et al., 2000; [2] Goldstein et al., 2004; [3] Volkow al., 2005 [4] Moeller et al., 2005 [5] Ma et al., 2009 [6] Ahmed et al., 1999 [7] Basser et al 1994 [8] Ahmed et al., 2003 [9] Schwarz et al., 2007