# Supplementary Material

## Cocaine dependence: A fast-track for brain ageing?

Karen D. Ersche, P. Simon Jones, Guy B. Williams, Trevor W. Robbins, and Edward T. Bullmore

Participants

We recruited 120 individuals, aged 18-50 years. Half of the sample met the DSM-IV criteria for cocaine dependence; the remainder had no history of substance misuse disorder or major psychiatric disorder. All participants were in good physical health and were psychiatrically evaluated using the Structured Clinical Interview for DSM-IV[1] and completed the National Adult Reading Test[2] as an estimate of verbal IQ. Participants were excluded if they had a lifetime history of a psychotic disorder, any serious medical disorder or traumatic head injury, or any contra-indications to MRI. The cocaine-dependent individuals were non-treatment seeking and recruited from the local community by advertisements and word-of-mouth. On average, cocaine-dependent individuals had been using cocaine for 10 years (±7.1 standard deviation [SD]), starting at the age of 21 years (±5.7 SD), and they were actively using cocaine, as verified by positive urine screens for cocaine on the day of scanning. One cocaine user was prescribed mirtazapine, two were prescribed benzodiazepines, and one regularly used over-the-counter paracetamol. Fifty cocaine-dependent individuals also met DSM-IV criteria for nicotine dependence, sixteen for alcohol dependence, eleven for cannabis dependence, and four for heroin dependence. The majority in the cocaine group were smoking cannabis regularly (68%) and many also consumed other drugs sporadically (ecstasy 28%, amphetamines 18%, hallucinogens 15%, benzodiazepines 11%, and opiates 7%).

The healthy volunteers were partly recruited from the GSK healthy volunteer panel, and partly by advertisement in the local community. They were screened for drug and alcohol abuse but none met criteria for abuse or dependence; none of them reported taking prescribed or illicit drugs on a regular basis. Seventeen percent of this sample reported recreational cannabis use in the past, 7% were occasional tobacco smokers, and 36% had smoked tobacco in the past. Urine sampled on the day of scanning was negative for standard illicit substances.

Demographic data were analyzed using two independent sample t-tests and chi-square analysis for categorical data implemented in the Statistical Packages for the Social Sciences (SPSS 19, IBM). Statistical tests were two-tailed and an effect was deemed significant at $P<0.05$. The study protocol received ethical approval from the Cambridge Research Ethics Committee and written informed consent was obtained from all participants prior to study enrolment.

MRI scan acquisition and processing

MRI scans were acquired at the Wolfson Brain Imaging Centre, University of Cambridge, with a Siemens Magnetom 3T Tim Trio scanner (www.medical.siemens.com) with a magnetization-prepared rapid acquisition gradient-echo (MPRAGE) sequence (176 slices; 1mm thickness, TR=2300 ms, TE=2.98ms, TI=900 ms, flip-angle=9°, FOV=240x256mm). Images were screened by a neuroradiologist for normal appearance.

Grey matter volume maps of each participant were produced and analysed using FSLVBM (http://www.fmrib.ox.ac.uk/fsl/fslvbm/index.html, Version 4.1), which incorporates brain extraction, segmentation and registration to a standard space. To correct for local expansion or contraction, the registered partial-volume images were modulated by division with the

Jacobian of the registration warp field. The modulated segmented images were then smoothed with an isotropic Gaussian kernel with full width half maximum (FWHM)=2.3mm. The smoothed grey matter maps were statistically analysed using CamBA software, version 2.3.0 (http://www-bmu.psychiatry.cam.ac.uk/software/).

For statistical inference, we used permutation methods and spatially extended statistics with nominal type I error control and greater sensitivity than voxel-based metrics[3]. First, we investigated the effect of age on grey matter volume for each group separately by regressing grey matter volume at each voxel on the individual's calendar age. We then imported the total grey matter volumes for each individual into SPSS to compare the slopes of the correlations between global grey matter volume and age between the two groups. In a second step, we investigated the age-by-group interaction effects at voxel level by fitting the general linear model (GLM) with age and group as independent factors in the ANOVA design. The P-value for significance was adjusted to control for multiple comparisons so that the expected number of false positive clusters in each analysis was less than one. The cluster-wise probability threshold for significance in the whole-brain analysis was P = 0.001. Finally, we examined the effects of the duration of cocaine abuse on grey matter volume at each voxel within the group-by-age interaction. To verify that our findings were not confounded by effects associated with the duration of tobacco, cannabis and alcohol use, we repeated the regression analyses for these three drugs. The age-by-group interaction remained significant when in a separate analysis the 16 individuals with co-morbid alcohol dependence were excluded from the analysis.

**Acknowledgements**

The authors would like to thank the participants for taking part in the study and staff both at the GlaxoSmithKline Clinical Unit Cambridge and at the Wolfson Brain Imaging Centre for their dedicated support throughout the study.

**References**

1 First MB, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. (SCID-I/NP). New York: Biometrics Research, New York State Psychiatric Institute; 2002.

2 Nelson HE. National Adult Reading Test Manual. Windsor (UK): NFER-Nelson; 1982.

3 Suckling J, Bullmore E. Permutation tests for factorially designed neuroimaging experiments. *Hum Brain Mapp* 2004;**22**:193-205