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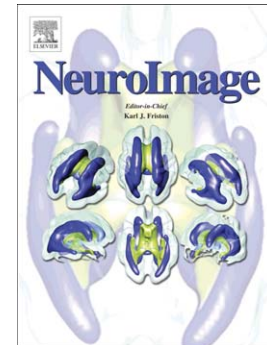
Reduced striatal volume in cocaine-dependent patients

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Title page

Reduced striatal volume in cocaine-dependent patients

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ABSTRACT:

Long-term cocaine consumption is associated with brain structural and functional changes. While the animal literature on cocaine use and dependence has traditionally focused on the striatum, previous human studies using voxel-based morphometry have reported reduced volumes of gray matter in several brain areas, but not in the striatum. Brain magnetic resonance imaging was performed with 20 cocaine-dependent patients and 16 healthy age-, education- and intelligence-matched control men. The cocaine-dependent group had lower gray matter volumes in the striatum and right supramarginal gyrus compared to controls. Within the cocaine-dependent group, years of cocaine use were inversely associated with the volume of the bilateral middle frontal gyrus, left superior frontal gyrus, parahippocampus, posterior cingulate, amygdala, insula, right middle temporal gyrus and cerebellum. These results show that cocaine dependence is associated with reduced gray matter volumes in the target structures of the dopaminergic system. These findings are the first to suggest reduced gray matter in the striatum by means of voxel-based morphometry in human users, thereby linking human results to animal models of addiction. In addition, the relationship between years of use and grey matter volumes in numerous brain regions are consistent with these volume reductions arising as a consequence of the cocaine use.

Key words: cocaine, addiction, voxel-based morphometry, MRI, striatum, amygdala

1. Introduction

Structural neuroadaptations in the central nervous system are related to the changes in behavior and brain function associated with cocaine dependence. Much of the research on these neuroadaptations has focused on dopamine circuitry and particularly on the ventral striatum (Koob, *et al* 1994, White and Kalivas, 1998). For example, animal and human studies have shown altered functional activity in the ventral striatum associated with cocaine consumption (Lyons, *et al* 1996; Porrino, *et al* 2002; Porrino, *et al* 2007; Volkow, *et al* 2006; Risinger, *et al* 2005; Hanlon, *et al* 2009). Chronic effects of cocaine on the striatum have also been reflected in the concentration of dopamine receptors (Volkow, *et al* 1993), dendrites and dendritic spines (Robinson and Kolb, 2004). A central role for the striatum in drug addiction is suggested by its involvement in drug incentive salience processes (Robinson and Berridge, 2003; Robinson and Berridge, 2008), drug-related neuroadaptations (Koob and Moal, 2008), and in the proposed change in the locus of behavioral control from the ventral to dorsal striatum associated with drug-seeking behavior after chronic drug self-administration (see Everitt and Robbins, 2005; Everitt, *et al* 2008). Therefore, theories of addiction and the empirical animal literature lead to the prediction of changes in the striatum in cocaine-dependent patients.

Neurobiological differences related to cocaine addiction at the macrostructural brain level are widespread across the brain as shown by voxel-based morphometry (VBM). Previous studies on the morphometric changes associated with drug use and abuse which applied VBM have reported gray matter (GM) reductions in the volume of the orbitofrontal cortex, the anterior cingulate cortex, the insula, the superior temporal cortex (Matochik, *et al* 2003;

Franklin, *et al* 2002; Lim et al., 2008) and the cerebellum (Sim, *et al* 2007). Although the studies cited above have suggested that structural changes should be found in the striatum, they have not been reported by means of VBM. It is possible that the micro-structural changes observed in animal studies may not be detectable by a macro-structural technique such as VBM. However, the possibility of detecting structural changes in important subcortical structures such as the striatum by means of this non-invasive tool in human studies would prove valuable as it could provide an assay of cocaine's effects while linking the human and animal literatures.

A few human studies on the structural changes associated with cocaine addiction have specifically analyzed brain volume differences in the striatum (Jacobsen, *et al* 2001; Martínez, *et al* 2004) by means of manual volume segmentation. Jacobsen et al. (2001) showed an increased volume of the caudate head and putamen while Martínez et al. (2004) found no such differences. This lack of consensus in human research may reflect methodological and sample characteristic differences between studies. Compared to these previous studies, the present investigation was restricted to a sample of cocaine-dependent males for two main reasons. First, gender considerably influences GM values, even in healthy subjects (Good, *et al* 2001; Giedd, *et al* 1996; Filipek, *et al* 1994; Ahsan, *et al* 2007); therefore limiting analyses to one gender should reduce unwanted variance. Second, and more importantly, gender differences in cocaine dependence have been described in perfusion abnormalities (Levin, *et al* 1994), treatment outcomes (Weiss, *et al* 1997), patterns of abuse (Griffin, *et al* 1989), situations that lead to consumption (Waldrop, *et al* 2007) and the brain's stress response (Li, *et al* 2005). Particularly, it seems that some cocaine-related phenomena, like craving, specifically involve the striatum in males (Kilts,

et al 2004; Kilts, *et al* 2001). Therefore, it seems plausible that cocaine neuroadaptations may depend on gender, and that striatum changes might be more pronounced in males. Therefore, we hypothesized that we would find GM volume changes in those brain areas functionally affected by cocaine addiction which are dependent on dopaminergic neurotransmission, and our main expectations focused on the striatum.

2. Methods and Materials

2.1 Participants

Twenty male cocaine-dependent patients and 16 matched controls participated in this study. The cocaine patients were recruited from the Addiction Treatment Service of San Agustín in Castellón, Spain. The inclusion criteria included cocaine dependence based on the DSM-IV criteria. Control subjects were required to have no diagnosis of substance abuse or dependence. The exclusion criteria for all the participants included neurological illness, prior head trauma, positive HIV status, diabetes, Hepatitis C, or other medical illness and psychiatric diagnoses such as schizophrenia or bipolar disorder. Some patients reported a background of depressive symptoms ($n = 3$), anxiety symptoms ($n = 3$), but never a DSM IV Axis I disorder, and these symptoms were absent at the time they were recruited and tested. The patients reported a history of consumption of other psychoactive drugs without involving dependence (Table 1). The controls and patients groups did not differ in terms of the distribution of smokers (56% controls vs. 65% patients, $p > 0.1$). Cocaine consumption was assessed with a urine toxicology test, which ensured a minimum period of abstinence of two to four days prior to MRI data acquisition. Groups were matched on the basis of age

(mean controls = 33.38 ± 9.17 ; patients = 33.30 ± 6.94), level of education (mean controls = 8.53 ± 1.45 ; patients = 9.20 ± 1.70 years) and general intellectual functioning (mean control = 10.86 ± 2.58 ; patients = 9.55 ± 2.37 ; standardized scores in the Matrix Reasoning Test from WAISIII; 32, Weschsler, 2001); there were no inter-group differences for any of these variables ($p > 0.1$). All the participants were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971; Bryden, 1977). They all signed an informed consent prior to participating in the study.

2.2 MRI imaging

Images were acquired on a 1.5T Siemens Avanto (Erlangen, Germany) with a standard quadrature head coil. Subjects were placed in a supine position in the MRI scanner. A high resolution three-dimensional T1-weighted gradient echo pulse sequence was acquired (TE = 4.9 ms; TR = 11 ms; FOV = 24 cm; matrix = $256 \times 224 \times 176$; voxel size = $1 \times 1 \times 1$ mm).

2.3 Image processing

Image processing was conducted with the voxel-based morphometry toolbox (Structural BrainMapping Group, Department of Psychiatry, University of Jena, <http://dbm.neuro.uni-jena.de/vbm/vbm5-for-spm5/>) implemented in Statistical Parametric Mapping (SPM5) running on Matlab® 7.0. VBM combined tissue segmentation, bias correction and spatial normalization into a unified model (Ashburner and Friston, 2005; Rosario et al., 2008). The parameter settings were: warp frequency cutoff to 25 mm, warping regularization light to

(0.001), a thorough clean up of segmentations and a 1-mm³ voxel size resolution for normalization. Iteratively weighted hidden Markov random fields (HMRF) were applied to improve the accuracy of tissue segmentation (Cuadra et al., 2005) by removing isolated voxels which were unlikely to be a member of a certain tissue class and closing hole in the clusters of connected voxels of a certain class, resulting in a higher signal to noise ratio of the final tissue probability maps (Koutsouleris et al., 2010). We did a visual inspection to check the accuracy of the segmentation in those areas where the distinction between gray and white matter is known to be problematic (e.g., thalamus and basal ganglia). Otherwise, default parameters were used (Rosario et al., 2008). Each subject's brain was normalized to the tissue probability maps provided by the International Consortium for Brain Mapping (ICBM, <http://www.loni.ucla.edu/Atlases/>). **These transformations involve a first linear transformation and a second nonlinear shape transformation.** Segmented GM images were modulated to restore tissue volume changes after spatial normalization. The GM images modulation was performed by multiplying the voxel intensities by the Jacobian determinant of the spatial transformation matrix derived from normalization. Modulated GM segmented images were corrected for nonlinear warping only (<http://dbm.neuro.uni-jena.de/vbm/segmentation/modulation/>), making correcting for total intracranial volume of the individual unnecessary (see Scorzin et al., 2008). Thus, global brain volume effects were removed from the data to allow inferences on local GM volume changes. An 8-mm FWHM Gaussian kernel was applied to the segmented GM maps.

2.4 Statistical and Image analyses

Group differences were evaluated in an ANCOVA analysis with age as the covariate. An absolute threshold mask of .1 was used to restrict the analysis to gray matter tissue, thus equaling the use of an implicit mask. Statistical significance was defined at a statistical threshold at the voxel level of $p < 0.001$ (uncorrected for multiple comparisons), and at an extent threshold of $p < 0.05$, corrected for multiple comparisons with family-wise error (FWE). To ensure the validity of cluster-level statistics on smoothed data, a nonisotropic smoothness correction was applied (Hasayaka, *et al* 2004). This correction is related to a methodological issue about how smoothness on VBM data depends on the underlying anatomy in order to apply a cluster extent threshold (Ashburner and Friston, 2000). Thus, Random Field corrections for multiple dependent comparisons based on the extent statistic need the smoothness of the residuals to be spatially invariant throughout the brain (Mechelli, *et al* 2005). This fact was solved by means of a nonstationary cluster extent correction (as implemented at <http://dbm.neuro.uni-jena.de/vbm/non-stationary-cluster-extent-correction/>) which adjusts the sensitivity of the test in accordance with image smoothness by means of a nonstationary permutation test (Hasayaka, *et al* 2004). Lastly, a limited set of exploratory correlations was performed between drug-use variables and the observed volumetric effects.

3. Results

3.1 Group differences

Whole brain voxelwise analyses with an age-adjusted ANCOVA showed significantly reduced GM in the left striatum (x, y, z MNI coordinates = -5, 2, -1; T-value = 4.46,

$p < 0.001$; cluster size = 833) and the right supramarginal gyrus (x, y, z MNI coordinates = 50, -60, 32, T-value = 5.20, $p < 0.001$; cluster size = 1086) for the cocaine group. Figure 1 charts voxel means for these clusters revealing the percentage of volume change between controls and patients was 14.8% for the striatum and 12.1% for the supramarginal gyrus. Opposite contrasts between groups (patients > controls) revealed no significant areas of increased GM volume in patients. Correlation analyses found no significant relationship between GM volumes in these two regions and the age of first cocaine use and years of cocaine use. Whole-brain voxel-wise regression analyses based on these same variables were then conducted and were thresholded at $p < 0.001$ (uncorrected) and cluster-corrected with a threshold at $p < 0.05$. The age of first cocaine use variable (mean = 19.60, SD = 5.97, ranging from 14 to 40) showed no positive or negative association with brain volume in any region. On the other hand, the years of cocaine use variable (mean = 13.2, SD = 5.97, ranging from 2 to 21 years) inversely related to local GM volumes in bilateral middle frontal gyri, left superior frontal gyrus, parahippocampus, posterior cingulate cortex, bilateral amygdala, parietal operculum (insula), right middle temporal gyrus and cerebellum (culmen) (t-values ranged from 5.95 to 4.53, see Table 2 and Figure 2).

4. Discussion

We provide the first evidence of gray matter (GM) volume reduction in the striatum of cocaine-dependent patients by means of VBM as well as a volume reduction in the right supramarginal gyrus. The detection of striatal volume reduction in the cocaine group in this report may be explained by sample characteristics or methodological improvements. For example, unlike prior studies that applied this same image-analysis technique

(<http://dbm.neuro.uni-jena.de/vbm/>) to substance-dependence individuals (Tanabe et al., 2009), we applied an iterative weighting of a Hidden Markov Random Field to improve tissue segmentation (see Cuadra et al., 2005). Furthermore, we used a slightly lower spatial smoothing (8 mm in our case as opposed, for example, to 12 mm in Tanabe et al., 2009). In fact, when we applied a bigger spatial smoothing kernel (12-mm FWHM), the between-group structural differences disappeared (data not reported). This suggests that the choice of smoothing kernel and its relationship to the size of the structure under study can impact on the sensitivity of the analysis.

The striatum is identified as a key target structure in cocaine addiction. Volume differences in a given brain area related to drug use may affect functional activation as measured by brain BOLD patterns (Aron and Paulus, 2007). Thus, the cell numbers in a structure as reflected by its volume measure would be important for capillary recruitment associated with brain activity (Makris, *et al* 2004), suggesting a reduced functionality in an area of reduced volume. In line with this idea, different fMRI studies have shown cognitive (Garavan, *et al* 2008), motivational (Goldstein, *et al* 2007; Garavan, *et al* 2000) and motor (Hanlon, *et al* 2009) deficits in cocaine dependent patients which directly related with either striatum functionality or other dopamine-mediated regions. On the other hand, the right supramarginal gyrus function has been related to executive functioning and craving in this population (Garavan, *et al* 2000). Furthermore, early reports suggest GM recovery in the right supramarginal gyrus after cocaine abstinence (Connolly, *et al* 2009).

We did not find regions of significantly increased GM in the cocaine group compared with controls. In contrast, other previous studies report a quantitative volumetric increase of the GM volume in the caudate and putamen of cocaine addicts (Jacobsen, *et al* 2001) or a lack

of differences (Martínez, *et al* 2004) by means of ROI-based methods. Jacobson et al. (2001) used volumetric measures by manually outlining the caudate head and body as a single structure, while our analyses were based on voxel-wise statistics. Their cocaine and control group samples were equal in numbers of males and females, but differed in terms of the racial ratio (Caucasian-African American) whereas the present sample contained only Caucasian males. Jacobsen and colleagues reported that race was apparently important for the effect of cocaine dependence on caudate volume because when this variable was regressed out, the group difference was no longer significant. Furthermore, Jacobsen et al. (2001) reported a mean 2-week period of abstinence in the cocaine group; however, we cannot assume abstinence of more than two to four days given the sensitivity of the urine test (Vearrier et al., 2010). Therefore, methodological and sample differences may explain the apparent contradiction between the present results and the small extant literature.

Another discrepancy between the present and previous results (e.g., Makris, *et al* 2004; Sim, *et al* 2007) is the relative scarcity of between-group GM volume differences in other brain areas. In light of the sizeable numbers of regions that showed significant relationships between years of cocaine use and volume, we surmise that the variability within the cocaine group that drove these correlations served to reduce the statistical power to detect between-group effects. These structures (the amygdalae, the lateral prefrontal cortex, the insula and the posterior cingulate) have all been implicated in cocaine addiction and craving (Grant et al., 1996; Bonson et al., 2004). Cognitive control-related activation in the lateral prefrontal cortex and the posterior cingulate regions have been identified as predictors of treatment outcome in a sample of cocaine dependent patients (Brewer et al., 2008), the structural changes associated with the insula are in agreement with the role this region may play in

interoception and awareness in addiction (Naqvi et al., 2007; Garavan, 2010; Volkow et al., 2010) while reduced GM volume in the amygdalae of cocaine addicts has previously been reported by Makris et al. (2004). In contrast to these regions showing a relationship between volume and years of use, the striatum showed no such relationship but did show a significant between-group difference. This suggests either a neuroadaptation that occurs relatively early in use (i.e., one that does not continue to deteriorate with continued years of use) or a pre-existing condition that might predispose to cocaine abuse or addiction (Nader and Czoty, 2005; Dalley, *et al* 2007). Future longitudinal studies that assess the emergence of structural volume changes in the striatum as an effect of cocaine exposure will better characterize these relationships. Likewise, it would be useful to know the functional correlates of the reduced striatal volumes of the patients. Although the present study did not include a psychometric psychological/cognitive assessment, the reduced volumes are plausibly related to the deficits that are well known in cocaine addicts, such as impulsivity or compulsivity.

As far as we are aware, this study is the first to analyze the structural volumetric differences of a male-only cocaine group; an earlier study (Franklin *et al* 2002) focused on structural gray matter concentration or density in male cocaine-dependent patients. Whereas the gray matter concentration measure used by Franklin and colleagues reflected the proportion of gray matter in relation to all other tissue types within each voxel, the present study employed a volumetric measure which is optimal for identifying regional differences (the modulation step compensated for spatial expansion or shrinkage when warping to a standard space) (Good et al., 2001)). The volumetric measure is thought to reflect volume differences since the relative concentration of gray matter, coded in intensities of voxels

(the concentration measure), is corrected by the relative spatial transformations before and after normalization allowing inferences about volume (Mechelli et al., 2005). Otherwise, restricting the present study to males had the advantage of maximizing the homogeneity of the cocaine group, this selection limits the scope of the present results. Further research will test whether gender differences contribute to the structural changes associated with cocaine addiction. The screening for alcohol and cannabis consumption habits was not considered in the control group, and may involve structural effects on the cohorts as reported by previous studies (Lorenzetti et al., 2010; Mechtcheiriakov et al., 2007; Fein et al., 2006). In the case of cannabis and amphetamine, as they involved a small group of participants of our already small cocaine group, we excluded them from the control group and repeated the analyses. Excluding the patient who reported amphetamine use did not change the results. Moreover, excluding the four cannabis users from the cocaine group ($n=16$) did not significantly change the statistical significance value of the difference ($p<0.001$, uncorrected), but slightly lessened the statistical significance of the cluster extent ($p<0.052$, FWE-corrected). The reduced effect of excluding these four patients from the cocaine group may be due to a similar unknown use of cannabis in the control group, or more probably to the effect of losing degrees of freedom in the statistical model. Other limitations of the study relate to the colinearity between the years of cocaine use and age variables in the cocaine group. Logically, older individuals had been consuming cocaine for more years than younger ones. One solution for the colinearity effects would be to recruit a cohort of cocaine addicts of similar ages, but of a wide range of years of cocaine use. That said, all areas with the exception of the insula continued to show a significant relationship to years of cocaine use when age was included in a multiple regression analysis. Another matter for consideration was the size of the isotropic Gaussian kernels applied in this study.

Usually, smoothing kernels in VBM studies range from 8 to 12 mm (Ashburner, 2009). It is likely that the use of a reduced kernel in our dataset favored the detection of significant effects in smaller sized regions (e.g., striatum) (Mechelli et al., 2005). Likewise, smoothing compensates for the imperfect registration of spatial normalization, and it is necessary to apply bigger kernels for less intersubject accuracy during normalization (Ashburner et al., 2009). This effect was seen to be particularly important for a region like the striatum, in which systematic ventricles dilation and, consequently, misregistration led to a significantly reduced volume of the striatum in a sample of patients with Herpes Simplex Encephalitis (Gitelman et al., 2001). However, other studies on samples with no systematic dilation of the ventricles have reported differences in the striatum between a patient group and a matched control group (e.g., Lázaro et al., 2009; Huey et al., 2009). Replicating these results is the best approach to compensate this tradeoff.

In conclusion, we report reduced GM volume in the striatum, a target structure in cocaine addiction, dependent behavior and motivation, but also in the supramarginal gyrus. Likewise, another set of cortical and subcortical structures, such as the amygdalae, the insula and dorsolateral prefrontal cortex, were seen to have volume reductions related to years of cocaine exposure. All these structural changes associated with cocaine addiction seem to merge in the striato-cortico-limbic circuitry linked not only to addiction, but also to the wider set of disinhibitory disorders. Although causal relationships are very difficult to determine in human studies, the significant relationship between years of use and reduced GM volumes are consistent with these volumetric effects arising from the cumulative exposure to cocaine or the concomitant lifestyle (e.g., stress) that accompanies prolonged drug use (Yücel, *et al* 2008) .

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Table/Figure Legends

Table 1. Descriptive scores for frequency of cocaine consumption and consumption patterns of other psychoactive substances without abuse in the cocaine-dependent group (n=20). /

Figure footnote: n = number of subjects at the time of their first visit to the clinic.

Table 2: Brain regions in which gray matter volume reduction was associated with years of cocaine use in the patient sample.

Figure 1: Regions of reduced gray matter volume in cocaine-dependent patients compared to matched controls.

Footnote: Golden gray matter volume maps are shown overlapped on T1 template from MRIcroN.

Image t-value bar represents statistical t-contrast values for significant voxels within the image. Left is left. Charts represent the mean gray matter volumes of those voxels within the clusters which show a significant difference from a confirmatory ROI analysis.

Figure 2: The related **mean-centered volume** of the bilateral amygdala with the years of cocaine abuse variable and other regions-related volumes. The X-, Y- and Z-related MNI coordinates for each coronal, sagittal and axial slice, respectively.

Table 1. Descriptive scores for frequency of cocaine consumption and consumption patterns of other psychoactive substances without abuse in the cocaine-dependent group (n=20).

	n	Percentage
Daily frequency of cocaine consumption	8	40%
Frequency of cocaine consumption: 1-2 days/week	4	20%
Frequency of cocaine consumption: 3-5 days/week	6	30%
Frequency of cocaine consumption: weekends	2	10%
Cigarettes	13	65%
Alcohol	10	50%
Cannabis	4	20%
Amphetamines	1	5%
Heroin	0	0%
Other psychoactive substances	1	5%

Table 2: Brain regions in which gray matter volume reduction was associated with years of cocaine use in the patient sample.

BRAIN REGION	HEMISPHERE	MNI	T –	Cluster
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		COORDINATES	value	size
Middle frontal gyrus	R	49, 19, 25	5.95	702
	R	38, 27, 41	5.87	686
	R	30, 58, 24	5,81	1764
	L	-41, 32, 31	5.39	951
Superior frontal gyrus	R	25, 41, 40	5,48	859
Amygdala	L	-18, 2, -17	5.41	1083
Posterior cingulate	L	-11, -49, 40	5.16	655
Amygdala/parahippocampus	R	23, -5, -24	4.97	1274
Uncus	R	31, 7, -26	4.67	1498
Middle temporal gyrus	L	-47, -72, 25	4.54	770
Insula	L	-43, -18, 1	4.53	700
Cerebellum	R	32, -38, -33	4.80	1121

