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Chronic cocaine administration causes extensive white matter damage in brain: Diffusion tensor imaging and immunohistochemistry studies

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ABSTRACT

The effect of chronic cocaine exposure on multiple white matter structures in rodent brain was examined using diffusion tensor imaging (DTI), locomotor behavior, and end point histology. The animals received either cocaine at a dose of 100 mg/kg ($N=19$), or saline ($N=17$) for 28 days through an implanted osmotic minipump. The animals underwent serial DTI scans, locomotor assessment, and end point histology for determining the expressions of myelin basic protein (MBP), neurofilament-heavy protein (NF-H), proteolipid protein (PLP), Nogo-A, aquaporin-4 (AQP-4), and growth associated protein-43 (GAP-43). Differences in the DTI measures were observed in the splenium (scc) and genu (gcc) of the corpus callosum (cc), fimbria (fi), and the internal capsule (ic). A significant increase in the activity in the fine motor movements and a significant decrease in the number of rearing events were observed in the cocaine-treated animals. Reduced MBP and Nogo-A and increased GAP-43 expressions were most consistently observed in these structures. A decrease in the NF-H expression was observed in fi and ic. The reduced expression of Nogo-A and the increased expression of GAP-43 may suggest destabilization of axonal connectivity and increased neurite growth with aberrant connections. Increased GAP-43 suggests drug-induced plasticity or a possible repair mechanism response. The findings indicated that multiple white matter tracts are affected following chronic cocaine exposure.

1. Introduction

Cocaine is a potent stimulant drug of abuse that can result in behavioral and neurochemical changes in preclinical studies (Stankeviciute et al., 2013). In humans, cocaine abuse is also associated with changes in brain structure and function (Moeller et al., 2005; Poon et al., 2007; Ma et al., 2009). Earlier neurochemical studies on the effects of cocaine abuse focused on the brain gray matter (GM) structures. For example, it was shown that myelin proteins are significantly decreased in the nucleus accumbens, which is implicated in addiction (Kovalevich et al., 2012).

Recent studies also found that cocaine affects white matter (WM) integrity. Diffusion tensor imaging (DTI) studies showed evidence of structural alterations in cocaine users in the corpus callosum (cc) (Lim et al., 2002; Moeller et al., 2005, 2007; Ma et al., 2009; Lane et al., 2010). These impairments seen on DTI appear to be associated with high levels of impulsivity, poor cognitive control, and mental flexibility (Moeller et al., 2005). Human studies, based on DTI-derived transverse or radial diffusivity (RD) measurements, have also suggested that chronic cocaine use may be associated with compromised myelin integrity (Moeller et al., 2007), consistent with mRNA and histology findings (Bannon et al., 2005).

Our controlled studies investigated if these changes in the DTI measures observed in humans can be replicated in the rodent model of cocaine exposure and determined the pathological underpinnings using immunohistochemistry (Narayana et al., 2009). In our earlier study, based on the effects seen in human cocaine users, we focused only on the cc and demonstrated significant changes in the DTI measures and altered expression in myelin basic protein (MBP) and neurofilament-heavy (NF-H) following chronic cocaine exposure. Previous studies also found similar changes in these proteins in the nucleus accumbens following cocaine administration (Kovalevich et al., 2012). Based on the mRNA studies, a robust and consistent decrease in the expression of myelin-related genes, including MBP, proteolipid protein (PLP), and myelin-associated oligodendrocyte basic protein (MOBP), was observed in the nucleus accumbens following cocaine exposure (Albertson et al., 2004). Some of the published studies also implicated other proteins such as Nogo-A and growth-associated protein-43 (GAP-43). Nogo-A is a protein that has a central role in the inhibition of axonal growth (Chen et al., 2000; GrandPre et al., 2000). Nogo-A is highly expressed in oligodendrocytes and in some neurons in the hippocampus, motor neurons, and

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dorsal root ganglia (Huber et al., 2002; Schwab, 2002; Meier et al., 2003; Gil et al., 2006; Trifunovski et al., 2006). Nogo-A plays a role in restricting plasticity in the central nervous system (McGee et al., 2005). Aquaporin-4 (AQP-4) is an integral membrane pore protein that mediates the movement of water (Preston and Agre, 1991) and has been demonstrated to play a role in regulating extracellular cocaine-induced dopamine and glutamate release in brain (Li et al., 2006).

As indicated above, our previous study focused only on DTI and MBP and NF-H expressions in the cc in chronic cocaine administration (Narayana et al., 2009). However, based on some of the published studies referenced above, we hypothesized that chronic cocaine administration affects multiple WM structures, besides the cc, and a number of proteins. Therefore, the main purpose of the studies described in this article was to investigate the effect of chronic cocaine exposure on (1) all the brain WM structures using DTI; (2) the expressions of multiple WM proteins including MBP, PLP, NF-H, Nogo-A, GAP-43, and AQP-4 using immunohistochemistry; and (3) motor behavior. A further goal of these studies was to relate the DTI changes to altered expressions in various proteins in rodents to gain an insight into cocaine-induced pathological changes. In these studies we acquired DTI data with high spatial resolution (isotropic voxel of 0.27 mm) for reduced partial volume averaging.

2. Methods

2.1. Cocaine administration

Eight-week-old male Sprague-Dawley rats in the weight range of 280–300 g (289 g ± 12.18 g) at baseline were included in this study. The animals were divided into the following two groups: (1) cocaine at a dose of 100 mg/kg ($N=17$) and (2) saline-treated controls ($N=19$). Cocaine dissolved in saline or saline alone (control animals) was continuously infused for 28 days through an Alzet osmotic minipump (Model 2ML4, Durect Corp., Cupertino, CA, USA), implanted subcutaneously as described previously (Narayana et al., 2009). The infusion pump was filled with 2 ml of 400 mg/ml of cocaine or 0.9% saline, which served as the control. The pump delivery rate was 2.5 μl/h. The pump was primed 24 h before implantation by warming in a tube filled with saline at 37 °C in a water bath.

2.2. Magnetic resonance imaging (MRI) acquisition

All MRI scans were performed at baseline (before implantation of the infusion pump) and weekly thereafter for a period of 1 month. Thus, each animal was scanned at five time points.

For MRI, animals were anesthetized with isoflurane at an induction dose of 4% and were placed on a custom-designed Plexiglas bed that was inserted into the magnet bore; the animals were secured with ear and tooth bars. During the scans, the animals were anesthetized with 2% isoflurane in a mixture of air (68%) and oxygen (30%) delivered by a rodent ventilator (Harvard Apparatus, South Natic, MA, USA) through a nose cone. The respiratory rate and rectal temperature were monitored throughout the experiment with a physiologic monitoring unit (Model 1025, SA Instruments, Stonybrook, NY, USA). A pulse oximeter (Model 570-100, SA Instruments, Stonybrook, NY, USA) was used to monitor the heart rate and oxygen saturation levels. An airflow warmer activated by a temperature probe (Model 11007B, SA Instruments) maintained the body temperature at 36 °C.

A 7T/30 USR MRI scanner (Bruker BioSpin, Karlsruhe, Germany) with an actively shielded, water-cooled gradient coil system (Model BGA12; 116-mm diameter) capable of producing maximum gradient amplitude of 400 mT m^{-1} with 80 μs rise time was used for image acquisition. The scanner operating system was ParaVision PV5.1. The vendor-supplied birdcage radiofrequency (RF) coil (Bruker BioSpin, Karlsruhe, Germany) with 72-mm internal diameter and 112-mm effective length was used for transmission. An in-house designed and remotely tunable receive-only RF coil (15-mm diameter) was placed over the head of the animal and fixed to the Plexiglas bed. As a quality assurance procedure, before each study, using a spin echo sequence, a homogenous water phantom doped with $NiCl_2$ was used to measure the signal-to-noise ratio (SNR) with the macro Auto_snr, which is part of the Bruker scanner software. In this method, the mean signal was measured in a region of interest (ROI) of 3 by 3 voxels around the brightest voxels. The noise at the edges and corners of the image was measured. The lowest value (noise) in any of these locations was determined. The SNR was computed as mean signal/noise. In order to account for the effect of acquisition parameters such as slice thickness, bandwidth, and number of averages, the Auto-snr routine calculates the SNR per unit volume as:

$$SNR/mm^3 = SNR \times acqfactor \times voxel\ factor$$

where voxel factor = 1/(volume in mm^3) and acqfactor = $[256 \times 5.12/(acqsize \times acqtime)]^{-1}$, where acqtime is the sampling time in the frequency encoding direction and acqsize is the number of phase encoding steps. The acqfactor is the normalization factor that is calculated for standard acquisition of bandwidth of 50 kHz and 256 complex points (20 μs/pt), and 256 × 256 (read × phase) matrix size. This method yields SNR that is independent of the acquisition parameters and allows comparison across different scanners and with different acquisition parameters. Even though in the current study all the acquisition parameters were kept the same at different time points, we preferred using this method of computing SNR since it is calculated automatically and avoids any possible operator bias. To convert this to the commonly defined SNR in an image, the values reported here should be divided by 7.28.

Localized shimming over the brain was performed using a Bruker-supplied Field Map with adjustments up to third order shims followed by fine tuning of the first and second order shims using the PRESS sequence (echo time (TE) = 20 ms, repetition time (TR) = 2500 ms, TE1 = TE2 = 10 ms, 2048 points with Hermite RF pulses) to consistently achieve water line width of less than 0.1 ppm (full width at half maximum). A spin echo sequence with TE = 10 ms and TR = 5000 ms was used to finely adjust the 90° RF pulse power manually over the central 1-mm axial slice (field of view (FOV) = 80 mm × 80 mm, matrix = 128 × 128). The same geometry was used for all other acquisitions with different sequences. For visualizing any anatomical lesions, images were obtained using the dual echo 2D Rapid Acquisition with Relaxation Enhancement (RARE) sequence with the following parameters: number of slices = 28, slice thickness = 0.5 mm, FOV = 35 mm × 35 mm, matrix = 256 × 256, effective TEs = 26 and 78 ms, TR = 5000 ms, Hermite pulse, RARE factor = 4, spectral bandwidth = 60 KHz, number of dummy scans = 2, number of averages = 2. The total scan time was 10 min 40 s.

Diffusion-weighted images (DWIs) were acquired with a segmented (four shots) 3D echoplanar imaging (EPI) sequence, using an icosahedral encoding scheme with bipolar gradients along 42 encoding directions (Madi et al., 2005). The other acquisition parameters were voxel size = 0.27 mm × 0.27 mm × 0.27 mm, FOV = 34.56 mm × 20.00 mm × 9.45 mm, matrix = 128 × 74 × 35, TE = 24.7 ms, TR = 500 ms, Hermite excitation RF pulse (pulse width = 3.6 ms, flip angle = 90°), bandwidth = 250 kHz, number of averages = 1, and partial FT acceleration = 1.5. The EPI scan parameters included double sampling, automatic trajectory adjustment (that also turns on the navigator echoes), and regridding based on the trajectory. The diffusion parameters were b-value = 800 s/mm^2, diffusion gradient duration = 5 ms, diffusion gradient separation = 10 ms, and number of averages without any diffusion gradient (b_0 images) = 9. The total scan time was 1 h.

2.3. MRI analysis

The RARE images were mainly used for visualizing anatomical lesions. Other than the routine processing that is a part of the scanner recon software, no additional processing was performed on these images.

An in-house developed pipeline written in IDL software (Exelis Visual Information Solutions, Boulder, CO, USA) was used for automatically processing the DWIs. The pipeline included eddy current correction with the program 'eddy_correct', part of the FSL package (Smith et al., 2004), filtering noise and smoothing the data (Hahn et al., 2010), extracting brain using a semi-automatic method, and linear and nonlinear registration with automatic image registration (AIR; Woods et al., 1998a, 1998b) to a template for group analysis. The unregistered images were exported to DtiStudio (Jiang et al., 2006), and the parametric maps of fractional anisotropy (FA), mean diffusivity (MD), RD, and longitudinal diffusivity (LD) were generated for each animal.

Two levels of analysis were performed: (1) voxel based analysis of FA, and (2) ROI analysis. The voxel based analysis was performed using SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/) to identify voxels that differ in FA between controls and cocaine animals. Since the only purpose of the SPM8 analysis was to guide in the placement of the ROIs, we did not correct the SPM8 results for multiple comparisons. The final results were based on the ROI analysis. The ROIs were drawn around voxels that showed changes in the t-maps. Even with high isotropic spatial resolution of 0.27 mm, because of the small structure of the scc and gcc, we used relatively small ROIs to minimize partial volume averaging effects. The positioning of the ROIs was based on the 3D anatomy. The ROIs were drawn manually on each slice for each animal by the same person, who was blind to the treatment. Given the experience of this person (about 15 years of experience in rat neuroanatomy and rodent brain MRI), it is unlikely that the operator bias in the ROI placement is significant. However, such a bias cannot be completely ruled out. Since the slice orientation varied slightly from animal to animal, the ROI size varied slightly (±2 pixels) to minimize partial volume averaging. For descriptive purposes, the means and standard deviations of the parametric maps of the DTI measures within each ROI were calculated in each animal's native space using the ROI Manager of ImageJ (National Institute of Mental Health, Bethesda, MD, USA). Regions that did not show