Drug and Alcohol Dependence 137 (2014) 143–147

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Short communication

Regionally-specific alterations in myelin proteins in nonhuman primate white matter following prolonged cocaine self-administration

Hilary R. Smith, Thomas J.R. Beveridge, Michael A. Nader, Linda J. Porrino*

Department of Physiology and Pharmacology, Center for the Neurobiology of Addiction Treatment (CNAT), Wake Forest School of Medicine, Winston Salem, NC 27157, USA

ARTICLE INFO

Article history:
Received 25 November 2013
Received in revised form 10 January 2014
Accepted 20 January 2014
Available online 30 January 2014

Keywords:
Cocaine
Self-administration
White matter
Myelin basic protein
Proteolipid protein
Monkey

ABSTRACT

Background: Neuroimaging studies of cocaine users have demonstrated white matter abnormalities associated with behavioral measures of impulsivity and decision-making deficits. The underlying bases for this dysregulation in white matter structure and function have yet to be determined. The aim of the present studies was to investigate the influence of prolonged cocaine self-administration on the levels of myelin-associated proteins and mRNAs in nonhuman primate white matter.
Methods: Rhesus monkeys ($N = 4$) self-administered cocaine (0.3 mg/kg/inj, 30 reinforcers per session) for 300 sessions. Control animals ($N = 4$) responded for food. Following the final session monkeys were euthanized and white matter tissue at three brain levels was processed for immunoblotting analysis of proteolipid protein (PLP) and myelin basic protein (MBP), as well as for *in situ* hybridization histochemical analysis of PLP and MBP mRNAs.
Results: Both MBP and PLP immunoreactivities in white matter at the level of the precommissural striatum were significantly lower in tissue from monkeys self-administering cocaine as compared to controls. No significant differences were seen for either protein at the levels of the prefrontal cortex or postcommissural striatum. In addition, no differences were observed in expression of mRNA for either protein.
Conclusions: These preliminary findings, in a nonhuman model of prolonged cocaine self-administration, provide further evidence that compromised myelin may underlie the deficits in white matter integrity described in studies of human cocaine users.

1. Introduction

Recent investigations have demonstrated alterations in the structural integrity and density of white matter in the brains of human cocaine users (Hanlon et al., 2011a; Lane et al., 2010; Moeller et al., 2005; Romero et al., 2010). These deficits have been associated with impaired behavioral measures of impulsivity and decision-making (Lane et al., 2010; Moeller et al., 2005), and correlate with duration of cocaine use (Lim et al., 2008). Cocaine-associated disruptions in functional connectivity have also been demonstrated (Hanlon et al., 2011b; Kelly et al., 2011; Ma et al., 2012; McHugh et al., 2013). Taken together, these reports suggest that compromised white matter integrity may contribute to the disruptions in connectivity and executive control typically observed in chronic cocaine abusers.

Despite the compelling evidence from human studies, however, the questions of both a causal relationship between cocaine use and altered white matter integrity, as well as the pathological processes underlying these deficits, remain largely unanswered. These questions are especially challenging given that white matter alterations have been observed in several conditions which frequently accompany cocaine abuse, such as depression and anxiety (Dolan et al., 1990; Wang et al., 2012), tobacco use (Hudkins et al., 2012; Paul et al., 2008), and alcohol abuse (de la Monte, 1988; Monnig et al., 2012). Rodent studies, however, have begun to address the issue of causality, with reports of dysregulated myelin-related proteins (Kovalevich et al., 2012; Narayana et al., 2009) and modification of myelin-related genes (Nielsen et al., 2012) following cocaine exposure, as well as deficits in white matter integrity, as measured by diffusion tensor imaging (DTI; Narayana et al., 2009).

DTI studies in cocaine-dependent subjects have shown reduced white matter integrity primarily in anterior portions of the corpus callosum and frontal fiber tracts (Ma et al., 2009; Moeller et al., 2005). The availability of callosal tissue from a monkey

* Corresponding author at: Department of Physiology and Pharmacology, Center for the Neurobiology of Addiction Treatment (CNAT), Wake Forest School of Medicine, Winston Salem, NC 27157-1083, USA. Tel.: +1 336 7168590; fax: +1 336 7168501.
E-mail addresses: lporrino@wakehealth.edu, rnewsome@wakehealth.edu (L.J. Porrino).

0376-8716/$ – see front matter © 2014 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.drugalcdep.2014.01.015

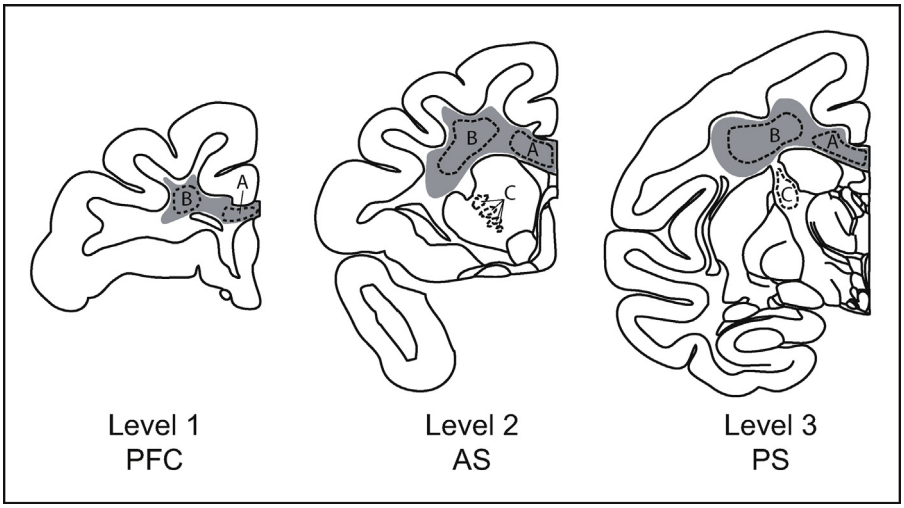

Fig. 1. Schematic of representative monkey brain levels selected for white matter analysis. Gray shaded areas represent the approximate extent of white matter collected for western blot analysis. Outlined areas represent regions of interest analyzed for MBP and PLP mRNA expression. A, corpus callosum; B, corona radiata; C, internal capsule. *Abbreviations*: PFC, prefrontal cortical level; AS, anterior (precommissural) striatal level; PS, posterior (postcommissural) striatal level.

study of prolonged cocaine self-administration, a model closely homologous to human abusers, made possible a preliminary investigation into the impact of cocaine exposure on white matter, while eliminating the need to covary cocaine use with other confounding factors common in human studies. The aim of these studies, therefore, was to determine the influence of extended cocaine self-administration on levels of myelin-associated proteins and mRNAs in dorsal subcortical white matter tracts. White matter tissue from three rostro-caudal brain levels was processed for immunoblotting analysis of the two most abundant proteins in myelin, proteolipid protein (PLP) and myelin basic protein (MBP), as well as for *in situ* hybridization histochemical (ISHH) analysis of their corresponding mRNAs.

2. Materials and methods

2.1. Subjects

A total of 8 male rhesus monkeys (*Macaca mulatta*) served as subjects. All procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were reviewed and approved by the Animal Care and Use Committee of Wake Forest University.

2.2. Cocaine self-administration

Details of the surgical and self-administration procedures have been described previously (Beveridge et al., 2005, 2009; Nader et al., 2002), with the exception of the cumulative duration of exposure. Briefly, monkeys were trained to respond under a fixed-interval 3-min (FI-3-min) schedule of food reinforcement until stable performance was obtained. Animals were then randomly assigned to food-reinforced ($N = 4$) or cocaine-reinforced ($N = 4$) groups and continued to respond under an FI-3-min schedule for either food or cocaine (0.3 mg/kg per injection). Experimental sessions continued for a total of 300 sessions (2750 mg/kg total cocaine intake); sessions ended after 30 reinforcers were delivered. Following the final session animals were humanely euthanized with an overdose of pentobarbital (100 mg/kg, i.v.).

2.3. Tissue processing

After euthanasia, brains were removed, flash-frozen, and stored at −80 °C. Brains were cut in a cryostat at −20 °C in the coronal plane into 20 μm sections onto electrostatically charged slides.

White matter samples for western blot analysis were collected by scraping corona radiata and corpus callosum from frozen slide-mounted sections. This procedure was carried out in a freezer at −80 °C, and specimens remained frozen until they were thawed for crude protein homogenate preparation. Slides for both western blotting and ISHH were selected at 3 rostro-caudal brain levels (Fig. 1) which correspond to Figures 24 (PFC), 37 (precommissural striatum) and 60 (postcommissural striatum) of the rhesus monkey atlas of Paxinos et al. (2000).

2.4. Western blotting

Samples were homogenized in ice-cold water (20 μL/mg) with the addition of protease inhibitors. Homogenates were centrifuged at 16,000 × *g* at 4 °C for 10 min to remove insoluble material. The supernatant was collected and protein concentrations were determined by Pierce BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA). For electrophoresis, samples were diluted 1:1 with sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, and 62.5 mM Tris–HCL, PH 6.8). Samples (15 μg protein) were denatured at 70 °C for 10 min, separated by 15% SDS polyacrylamide gel electrophoresis, and transferred to PVDF membrane (Bio-Rad; Hercules, CA). Membranes were blocked in 50% blocking buffer (Li-Cor; Lincoln, NE)/50% PBS for 1 hr at room temperature, incubated in primary antibodies against MBP (1:2000; Millipore, Billerica, MA) or PLP (1:1000; Millipore) in blocking buffer/0.1% Tween-20 overnight at 4 °C, washed (PBS, 0.05% Tween-20), incubated in IRDye® 680RD goat anti-mouse secondary antibody (Li-Cor) for one hour, and washed again. Membranes were then scanned and bands were analyzed using an Odyssey® infrared imager (Li-Cor). Membranes were also probed with an antibody against β-actin (1:5000; Abcam, Cambridge, MA) as a loading control. Data are expressed as a ratio of the protein of interest and the corresponding density of β-actin.

2.5. In situ hybridization histochemistry

For ISHH, ^{35}S-labeled antisense oligonucleotide probes were used to hybridize to MBP and PLP mRNAs in the subcortical white matter. Probes complementary to published sequences for PLP (exon 2, bases 274–312; NCBI accession number NM_000533.3) and MBP (exon 1, bases 92–136; NCBI accession number NM_001025081.1) were synthesized by the DNA Synthesis Core Laboratory of Wake Forest School of Medicine. Probes were hybridized to tissue as previously described (Letchworth et al., 1999). In brief, probes were 3′-labeled with α-^{35}S-deoxyadenosine triphosphate (1200 Ci/mmol; PerkinElmer, Waltham, MA) using terminal deoxynucleotidyl transferase (Promega, Madison, WI). Sections were incubated with ~6.0×10^6 cpm of labeled probe in 300 μl hybridization buffer overnight at 37 °C, washed, dried, and apposed to Kodak Biomax MR film (Fisher Scientific, Pittsburgh, PA) for 5 days in the presence of ^{14}C microscale standards (GE Healthcare, Pittsburgh, PA). Densitometric analysis was carried out using a computer-assisted image-processing system (MCID; Interfocus Imaging, Cambridge, UK).

Data were averaged across three adjacent tissue sections per level. Regions of interest were corpus callosum, corona radiata, and internal capsule. Values for each region were determined from optical densities compared to calibrations of ^{14}C standards, and converted from ^{14}C nCi/mg of tissue to disintegrations per minute (dpms) of ^{35}S/mg tissue using ^{35}S brain paste standards, as previously described (Letchworth et al., 1999; Miller, 1991).

2.6. Statistical analysis

For both immunoblotting and ISHH studies differences between cocaine-treated and control groups were determined by Student's *t*-tests with significance set at $p < 0.05$, corrected for multiple comparisons, and carried out with SPSS software (Version 18.0; IBM, New York).