ORIGINAL ARTICLE

Regional elevations in microglial activation and cerebral glucose utilization in frontal white matter tracts of rhesus monkeys following prolonged cocaine self-administration

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Abstract

It has been shown that exposure to cocaine can result in neuroinflammatory responses. Microglia, the resident CNS immune cells, undergo a transition to an activated state when challenged. In rodents, and possibly humans, cocaine exposure activates microglia. The goal of this study was to assess the extent and magnitude of microglial activation in rhesus monkeys with an extensive history of cocaine self-administration. Male rhesus monkeys ($N = 4$/group) were trained to respond on a fixed-interval 3-min schedule of food or 0.3 mg/kg/injection cocaine presentation (30 reinforcers/session) for 300 sessions. At the end of the final session, monkeys were administered 2-[^{14}C]deoxyglucose intravenously and 45 min later euthanized. Brain sections were used for autoradiographic assessments of glucose utilization and for microglia activation with [^{3}H]PK11195, a marker for the microglial 18-kDa translocator protein. There were no group differences in gray matter [^{3}H]PK11195 binding, while binding was significantly greater in cocaine self-administration animals as compared to food controls in 8 of the 11 white matter tracts measured at the striatal level. Binding did not differ from control at other levels. There were also significant increases in white matter local cerebral glucose utilization at the striatal level, which were positively correlated with [^{3}H]PK11195 binding. The present findings demonstrate an elevation in [^{3}H]PK11195 binding in forebrain white matter tracts of nonhuman primates with a prolonged history of cocaine self-administration. These elevations were also associated with greater cerebral metabolic rates. These data suggest that white matter deficits may contribute to behavioral, motivational, and cognitive impairments observed in cocaine abusers.

Keywords Cocaine · Self-administration · Microglia · Neuroinflammation · Rhesus monkey · Local cerebral glucose utilization

Introduction

There is a growing body of evidence suggesting that exposure to psychostimulants such as cocaine induces neuroinflammatory responses. Altered levels of pro-inflammatory immune signaling molecules, for example, have been identified in human cocaine abusers (Fox et al. 2012; Levandowski

et al. 2016a) as well as in rodents (Cearley et al. 2011; Clark et al. 2013; Periyasamy et al. 2018) and in vitro models of cocaine exposure (Guo et al. 2015; Northcutt et al. 2015; Periyasamy et al. 2018). Gene expression analysis of the nucleus accumbens in rhesus monkeys with an extensive history of cocaine self-administration identified a cluster of upregulated immune response and inflammation related genes, associated predominately with microglial activation (Vallender et al. 2017).

Microglia are the resident immune cells in the CNS, surveying the environment for the presence of pathogens, cellular debris, and other abnormal or exogenous substances. When challenged by disease, injury, or foreign substances microglia undergo a transition to an "activated" phenotype and secrete a variety of cytokines, chemokines, reactive oxygen species, and other pro-inflammatory substances that initiate an immune response. An increase in

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microglial activation, therefore, can be interpreted as an indicator of neuroinflammation. During chronic pathological states this pro-inflammatory response can be amplified and may be detrimental to surrounding tissue (Perry 2007).

Cocaine has been demonstrated to initiate microglial activation both in vitro and in vivo in mouse striatum (Liao et al. 2016), and activated microglia were elevated in an immunohistochemical analysis of post-mortem tissue from the midbrain of cocaine users (Little et al. 2009). The anatomical scope of each study was restricted, however, and in the rodent study cocaine exposure was very limited. Furthermore, cocaine use in humans is often accompanied by multiple comorbid factors, such as affective disorders and other drug use, including alcohol abuse, many of which are associated with elevated microglial activation (see, for example, He and Crews 2008; Setiawan et al. 2015). In order to overcome these limitations we used a nonhuman primate model of prolonged cocaine self-administration, in which the conditions surrounding intake were carefully controlled, to determine whether cocaine-driven microglial activation would occur in multiple regions throughout the brain. To this end we used receptor autoradiography with [³H]PK11195 to measure the microglial 18 kDa translocator protein (TSPO), at three rostro-caudal levels of the rhesus monkey brain: at levels of the prefrontal cortex (PFC), the precommissural striatum, and the substantia nigra (SN). [³H]PK11195 binding to the TSPO is a marker of activated microglia, and by extension, can be considered an indicator of neuroinflammation (Colonna and Butovsky 2017; Lannes et al. 2017).

The energy demands of activated microglia are greater than those in their quiescent form and in vitro this is reflected by adaptations in their metabolic pathways and greater glucose consumption (Gimeno-Bayon et al. 2014). This finding raises the question of whether microglial activation might have functional metabolic consequences in the brain. Indeed, glucose metabolism, as measured by [¹⁸F]-2-fluoro-2-deoxyglucose (FDG) imaging, and microglial activation, as measured by [¹¹C]PK11195 binding potential, were co-localized and elevated in peri-infarct regions after 7 days of ischemia in rodents (Schroeter et al. 2009). Likewise, in the spinal cord of rats, regional elevations in FDG were anatomically overlaid with immunolabeling of activated microglia following induction of experimental autoimmune encephalomyelitis (Buck et al. 2012). Thus, a second aim of this study was to assess whether any cocaine-induced microglial activation observed would be associated with altered glucose utilization. Therefore, we measured local cerebral glucose utilization (LCGU) using the quantitative 2-[¹⁴C]deoxyglucose (2DG) method (Crane and Porrino 1989; Kennedy et al. 1978; Sokoloff et al. 1977) in tissue sections adjacent to those assessed for [³H]PK11195 binding.

Methods

Subjects

Brain tissue from 8 male rhesus monkeys (*Macaca mulatta*) weighing 9.2–12.8 kg at the start of the study was used for these experiments. Monkeys were individually housed in stainless steel cages with access to water ad libitum; animals had physical and visual contact with each other. Their body weights were maintained at ~95% of free-feeding weights. Every effort was made to minimize the number of animals used for the experiments. All procedures were performed in accordance with established practices as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals. In addition, all procedures were reviewed and approved by the Animal Care and Use Committee of Wake Forest University.

Self-administration

All behavioral, surgical and self-administration procedures have been described previously (Beveridge et al. 2006, 2009; Nader et al. 2002). Briefly, monkeys were initially trained to respond on a fixed-interval 3-min schedule of food reinforcement (FI 3-min). After baseline performance had been established, all monkeys were surgically prepared with intravenous catheters, as previously described, and randomly assigned to food control or cocaine groups. Following surgery, control animals ($N = 4$) continued to respond for food, while the remaining monkeys were assigned to the cocaine self-administration group ($N = 4$; 0.3 mg/kg per injection/30 reinforcers per session). Experimental sessions were conducted at approximately the same time each day (Monday–Friday) and continued for a total of 300 sessions. Total lifetime intakes over the ~15 months of cocaine self-administration ranged from 2732 to 2756 mg/kg cocaine. Approximately 2 days prior to the final operant session monkeys were surgically prepared, in the opposite leg to their venous catheter, with a chronic indwelling catheter into the femoral artery for collection of timed arterial blood samples during the 2DG procedure. The 2DG procedure was initiated at the end of the final experimental session, as previously described (Beveridge et al. 2006, 2009).

2-[¹⁴C]deoxyglucose procedure

The 2DG procedure, as adapted for use in the conscious monkey (Kennedy et al. 1978), was initiated at the end of the last session, 2 min following the last cocaine injection or food presentation, with an intravenous pulse of 2.76 MBq/kg 2-deoxy-D-[¹⁴C]glucose

([¹⁴C]2DG; PerkinElmer, Waltham, MA; specific activity 1850–2035 MBq/mmol), followed by a flush of heparinized saline. Timed arterial blood samples were then drawn according to a schedule sufficient to define the time course of the arterial [¹⁴C]2DG and glucose concentrations; all samples were obtained from outside the experimental chamber and without disturbing the monkey. Arterial blood samples were centrifuged immediately. Plasma ¹⁴C concentrations were determined by liquid scintillation spectrophotometry (Beckman Instruments, Fullerton, CA), and plasma glucose concentrations were assessed using a glucose analyzer (Analox Instruments, London, UK). 45 min after tracer injection, the animals were humanely killed with an intravenous overdose of sodium pentobarbital (100 mg/kg), therefore the 2DG procedure was completed while active levels of cocaine were present in the brain and blood.

Tissue processing

Brains were removed rapidly, blocked in three parts, frozen in isopentane (−45 °C), and stored at −70 °C prior to cryosectioning. Coronal sections (20 μm thick) were cut in a cryostat maintained at −20 °C. For the 2DG experiment 4 of every 20 sections were thaw-mounted onto glass coverslips, dried on a hot plate, and apposed to Kodak MinR film (Rochester, NY) for 15–30 days, along with a set of [¹⁴C]methylmethacrylate standards (GE Healthcare, Little Chalfont, UK), previously calibrated for their equivalent ¹⁴C concentration in 20 μm brain sections. Sections for receptor autoradiography were collected on Superfrost Plus slides (Brain Research Laboratories, Newton, MA), desiccated under a vacuum overnight at 4 °C, and then stored at −80 °C until processing for autoradiography.

[³H]PK11195 autoradiography

Binding to the TSPO was determined with [³H]PK11195 by quantitative in vitro receptor autoradiography according to procedures adapted from Mankowski et al. (2003). Sections were pre-incubated at room temperature in buffer (50 mM Tris, pH 7.4) for 20 min to remove any residual cocaine and [¹⁴C]2DG, then incubated at room temperature for 30 min in 1 nM [³H]PK111195 (81.7 Ci/mmol; PerkinElmer) in the presence (non-specific binding) or absence (total binding) of 10 μM unlabeled PK11195. Sections were rinsed 3 times for 5 min in buffer at 4 °C, with a final 30 s dip in ice-cold distilled water. Sections were immediately dried under a stream of cold air and placed on tritium-sensitive film (Kodak MR; GE Healthcare) for 10 weeks in the presence of [³H]methylmethacrylate standards (GE Healthcare).

Densitometry

Autoradiograms were developed in Kodak GBX developer, indicator stop bath, and rapid fix at 68 °C. Quantitative densitometry of autoradiograms was accomplished with a computer-assisted image-processing system (MCID, Interfocus Imaging Ltd., Linton, UK).

[³H]PK111195 autoradiography

Tissue equivalent values of [³H]PK11195 binding (in fmol/mg wet weight tissue) were determined from optical densities using a calibration curve obtained by densitometric analysis of the autoradiograms of [³H] standards. Specific binding was determined by subtracting non-specific binding values from the total binding values, measured in adjacent sections.

Local cerebral glucose utilization

Optical density measurements for each structure were made in a minimum of eight brain sections. Measurements were made bilaterally and averaged across hemispheres. Tissue ¹⁴C concentrations were determined from the optical densities and a calibration curve obtained by densitometric analysis of the autoradiograms of the calibrated standards. Glucose utilization was then calculated using the operational equation of the method (Sokoloff et al. 1977), local-tissue ¹⁴C concentrations, the time course of the plasma [¹⁴C]2DG and glucose concentrations, and the appropriate kinetic constants (Kennedy et al. 1978). Because of differences in the baseline levels of glycemia in some animals, the lumped constant was adjusted appropriate to the glucose levels according to procedures based on previous work (Kennedy et al. 1978; Porrino et al. 2004; Schuier et al. 1990; Suda et al. 1990). Nomenclature and localization of white matter tracts were based on the work of Schmahmann and Pandya (Schmahmann and Pandya 2006), and those of gray matter brain regions were based on the rhesus monkey brain atlas of Paxinos et al. (2000). Sections were chosen for analysis based on proximity to sections processed for [³H]PK111195 receptor autoradiography.

Statistical analysis

The assessments of levels of [³H]PK11195 binding to the TSPO and cerebral glucose utilization in the brain were conducted at three different levels of the brain, which corresponded approximately to Figs. 24 (PFC level), 36 (striatal level), and 72 (SN level) of Paxinos et al. (2000). Levels of [³H]PK11195 binding or glucose utilization were analyzed using two-way analyses of variance (ANOVA): drug treatment X brain region, with brain region as a repeated

measure. These analyses were followed by pre-planned Student's *t* tests when a significant interaction was present. In addition, effect size (Cohen's *d* corrected for small sample sizes) and power were calculated for those differences that achieved statistical significance. Statistical significance was considered as $p < 0.05$ where greater than 75% power was observed.

In order to determine whether the increases in [³H] PK11195 binding were reflected in the effects in LCGU,

Table 1 [3H]PK11195 binding in gray matter of food-reinforced and cocaine-exposed animals. Binding is expressed as fmol/mg wet weight tissue

Gray matter structure	Food controls $N = 4$	15 months cocaine $N = 4$
Prefrontal level		
Dorsolateral PFC	117.23 ± 11.8	87.71 ± 11.7
Orbital PFC	87.16 ± 10.8	93.98 ± 11.7
Ventromedial PFC	108.20 ± 13.3	94.18 ± 12.7
Cingulate gyrus	93.74 ± 4.2	90.89 ± 15.2
Striatal level		
Dorsolateral caudate	95.43 ± 5.4	98.52 ± 4.1
Dorsomedial caudate	115.38 ± 6.4	125.43 ± 9.9
Ventromedial caudate	113.63 ± 6.0	112.58 ± 7.7
Dorsal putamen	94.05 ± 4.1	95.87 ± 5.0
Ventral putamen	91.50 ± 3.2	105.08 ± 8.6
Nucleus accumbens	101.05 ± 9.4	97.45 ± 5.8
Substantia nigra level		
Mediodorsal thalamus	64.47 ± 6.7	56.11 ± 12.4
Ventrolateral thalamus	58.54 ± 4.8	48.83 ± 12.1
Substantia nigra	55.76 ± 9.3	55.84 ± 14.6
Hippocampus	81.67 ± 14.9	68.67 ± 11.5
Entorhinal cortex	88.72 ± 15.1	73.03 ± 7.8

Pearson product-moment correlations were carried out for brain structures at the striatal level where two-way ANOVA of binding densities detected a significant interaction between treatment group and brain level. Correlations were conducted in control and cocaine groups combined.

Results

[³H]PK11195 binding

[³H]PK11195 binding levels in gray matter of food-reinforced and cocaine self-administration animals are described in Table 1. Levels of [³H]PK11195 binding in the brains of control monkeys (Fig. 1, left panel) were consistent with a previous study of simian immunodeficiency virus encephalitis, in which binding in normal gray matter was greater than that in white matter (Mankowski et al. 2003). Levels of gray matter [³H]PK11195 binding to the TSPO in control monkeys ranged from 56 to 117 fmol/mg wet weight tissue, with greatest levels in the PFC and striatum, and lower densities in more caudal brain areas. Baseline binding levels in the white matter were approximately half of that in gray matter, with densities ranging from 23 to 63 fmol/mg. [³H]PK11195 binding densities in control white matter were greater at the PFC level than at the levels of the striatum and SN.

Two-way ANOVA of [³H]PK11195 binding in the gray matter revealed that there were no significant group differences in the levels of binding between food-reinforced and cocaine self-administration animals at the three levels analyzed, although there were significant regional differences in the degree of binding at the striatal ($F_{(5,25)} = 8.70$, $p = 0.0001$) and midbrain regions ($F_{(4,24)} = 9.54$, $p = 0.0001$).
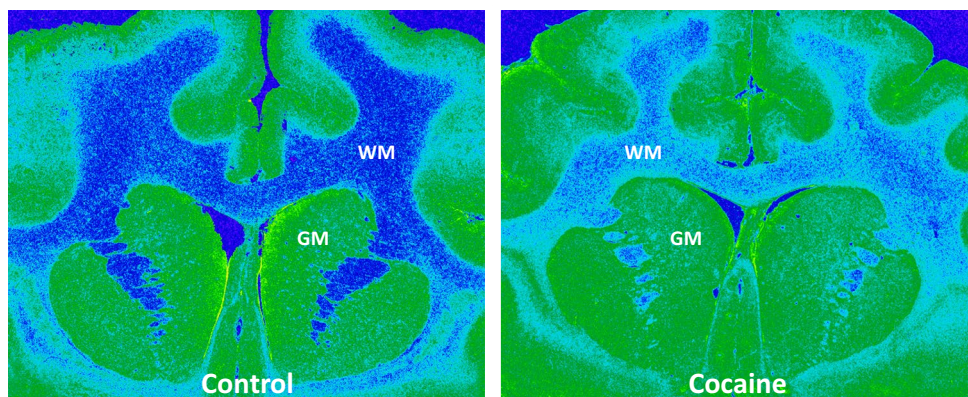

Fig. 1 Effects of prolonged cocaine self-administration in nonhuman primate white matter fiber tracts. Shown are representative color-transformed autoradiograms of [³H]PK11195 binding to the microglial TSPO in coronal sections at the level of the precommissural striatum in brains of monkeys following 15 months of exposure to food reward (left panel) or cocaine self-administration (0.3 mg/kg/injection; 30 reinforcers per session) (right panel). [³H]PK11195 binding site densities were selectively elevated in white, but not gray matter. *GM* gray matter, *WM* white matter

Table 2 [^{3}H]PK11195 binding in white matter of food-reinforced and cocaine-exposed animals

White matter structure	Food controls $N=4$	15 months cocaine $N=4$	Power	Cohen's d
Prefrontal level				
Superior longitudinal fasciculus 1	58.25 ± 3.7	61.95 ± 5.7		
Superior longitudinal fasciculus 2	55.85 ± 3.9	63.03 ± 6.6		
Corpus callosum	54.19 ± 3.1	58.06 ± 4.9		
Cingulum bundle	59.60 ± 4.1	73.15 ± 5.4		
Fronto-occipital fasciculus	53.94 ± 5.0	59.13 ± 5.5		
Extreme capsule	62.95 ± 2.6	66.83 ± 6.9		
Uncinate fasciculus	58.84 ± 4.3	66.35 ± 7.2		
Striatal level				
Superior longitudinal fasciculus 1	36.63 ± 1.7	49.57 ± 2.6**	98%	1.17
Superior longitudinal fasciculus 2	34.00 ± 1.5	46.78 ± 1.7***	100%	1.63
Superior longitudinal fasciculus 3	43.63 ± 1.3	55.42 ± 4.4		
Corpus callosum	35.69 ± 1.6	46.71 ± 2.4**	97%	1.09
Cingulum bundle	36.11 ± 3.2	57.51 ± 4.9*	96%	1.04
Fronto-occipital fasciculus	40.09 ± 2.6	51.13 ± 2.2*	90%	0.92
Striatal bundle	22.53 ± 3.0	34.12 ± 3.4		
Muratoff bundle	50.84 ± 3.9	65.77 ± 2.7*	88%	0.90
Internal capsule	36.58 ± 1.7	47.80 ± 2.0*	99%	1.21
Extreme capsule	60.84 ± 2.5	65.12 ± 5.5		
Uncinate fasciculus	51.87 ± 3.4	75.43 ± 5.4*	96%	1.20
Substantia nigra level				
Superior longitudinal fasciculus 1	38.43 ± 4.1	36.02 ± 6.6		
Superior longitudinal fasciculus 2	39.79 ± 2.3	36.10 ± 5.6		
Superior longitudinal fasciculus 3	41.35 ± 6.5	40.61 ± 7.1		
Corpus callosum	40.36 ± 4.4	41.51 ± 5.4		
Cingulum bundle	42.77 ± 6.5	40.89 ± 7.8		
Fronto-occipital fasciculus	43.18 ± 3.7	41.72 ± 7.1		
Internal capsule	31.04 ± 4.2	35.86 ± 5.3		
Extreme capsule	47.42 ± 7.3	51.26 ± 10.2		
Middle longitudinal fasciculus	42.71 ± 4.9	46.88 ± 7.7		
Inferior longitudinal fasciculus	38.67 ± 3.6	42.64 ± 5.3		
Parabrachial bundle	22.59 ± 5.0	29.75 ± 4.5		

Binding is expressed as fmol/mg wet weight tissue. Two-way repeated-measures ANOVAs were followed by post hoc Student's t tests. Asterisks denote significant differences from food controls, *$p<0.05$, **$p<0.01$, ***$p<0.005$. Significance at $p<0.05$ was only accepted when accompanied by a power value of at least 75%. Effect size measured with post hoc Cohen's d tests for the difference between two means, corrected for small sample size. $d \geq 0.5$ considered medium effect size, $d \geq 0.8$ considered large effect size

[^{3}H]PK11195 binding to the microglial TSPO in white matter of food-reinforced and cocaine self-administration animals is shown in Table 2 and Figs. 1 and 2a. At the levels of the PFC and SN there were significant main effects of brain region (PFC: $F_{(6,36)} = 6.80$, $p = 0.0000$; SN: $F_{(10,60)} = 13.74$, $p = 0.0000$), but not of group, and no significant region x group interaction. At the level of the striatum, however, there were significant main effects of brain region ($F_{(10,60)} = 36.35$, $p = 0.0000$) and group ($F_{(1,6)} = 20.92$, $p = 0.0038$), and an interaction between region and group ($F_{(10,60)} = 2.21$, $p = 0.0292$). Multiple comparisons revealed that [^{3}H]PK11195 binding was significantly greater in cocaine self-administering animals as compared to food controls in 8 of the 11 white matter tracts measured at the striatal level. The greatest elevations in binding densities were observed in the cingulum bundle (59%, $p = 0.0107$) and uncinate fasciculus (45%, $p = 0.0104$). In addition, binding was greater than control in the superior longitudinal fasciculus, divisions 1 (35%, $p = 0.0063$) and 2 (38%, $p = 0.0013$), corpus callosum (31%, $p = 0.0088$), fronto-occipital fasciculus (28%, $p = 0.0181$), internal capsule (31%, $p = 0.0054$), and Muratoff bundle (29%, $p = 0.0200$).
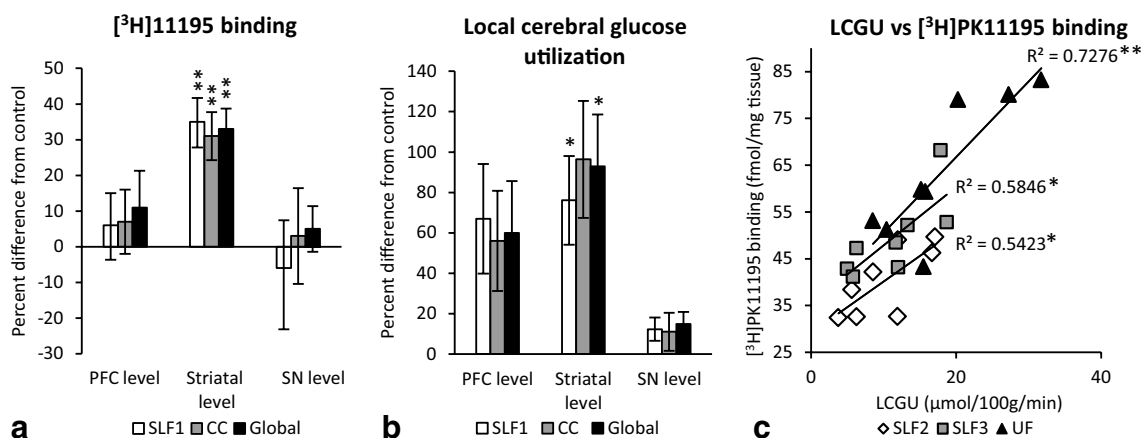
Fig. 2 Regionally specific effects of prolonged cocaine self-administration on [³H]PK11195 binding in white matter tracts of the nonhuman primate (**a**). Binding to the microglial TSPO (fmols/mg wet weight tissue) was selectively elevated in white matter tracts of cocaine-exposed animals at the level of the precommissural striatum, while no differences from control were observed at either the level of the prefrontal striatum or that of the substantia nigra. **$p < 0.01$ different from food control; two-way analysis of variance followed by post hoc Student's t tests for multiple comparisons. Significance at was only accepted when accompanied by a power value of at least 75%. Regionally specific effects of prolonged cocaine self-administration on LCGU in white matter tracts of the nonhuman primate (**b**). Local cerebral glucose metabolism (μmol/100 g/min) was elevated in white matter of cocaine-exposed animals at the level of the precom-

missural striatum, while no significant effects were seen at the levels of the prefrontal cortex or the substantia nigra. *$p < 0.05$ different from food control; two-way analysis of variance followed by post hoc Student's t tests for multiple comparisons. Significance was only accepted when accompanied by a power value of at least 75%. Correlational analyses of the relationship between [³H]PK11195 binding densities and local cerebral glucose utilization in white matter tracts at the level of the precommissural striatum (**c**) *$p < 0.05$, **$p < 0.01$. Global measurements for graphs **a** and **b** were averages of all the white matter data points for a given brain level. *CC* corpus callosum, *LCGU* local cerebral glucose metabolism, *SLF1* superior longitudinal fasciculus division 1, *SLF2* superior longitudinal fasciculus division 2, *SLF3* superior longitudinal fasciculus division 3, *SN* substantia nigra, *UF* uncinate fasciculus

Local cerebral glucose utilization

Functional metabolic activity in white matter of food control and cocaine-exposed animals, as measured with the 2DG method, is shown in Table 3 and Fig. 2b. At the prefrontal and nigral levels there were main effects of brain region (PFC: $F_{(1,6)} = 8.47$, $p = 0.0000$; SN: $F_{(10,50)} = 14.71$, $p = 0.0000$), but no effects of group and no interactions.

Two-way ANOVA of white matter LCGU at the striatal level revealed significant main effects of brain region ($F_{(10,60)} = 22.50$, $p = 0.0000$) and group ($F_{(1,6)} = 8.20$, $p = 0.0286$), as well as an interaction between region and group ($F_{(10,60)} = 2.34$, $p = 0.0211$). Multiple comparisons of LCGU in white matter at the striatal level revealed profoundly elevated LCGU in the superior longitudinal fasciculus, divisions 1(79%, $p = 0.0278$), 2 (97%, $p = 0.0360$), and 3 (115%, $p = 0.0135$), striatal bundle (107%, $p = 0.0389$), internal capsule (111%, $p = 0.0394$), extreme capsule (80%, $p = 0.0277$), and uncinate fasciculus (99%, $p = 0.0289$).

Correlations

In order to investigate further the overlap of effects in white matter at the striatal level between our two measures, Pearson product-moment correlation coefficients were computed to assess the relationship between functional metabolic rate

and [³H]PK11195 binding density in white matter tracts at the striatal level of the brain, where two-way ANOVA revealed a significant interaction of group and brain level. As shown in Fig. 2c, there were positive correlations between the two variables at the striatal level, specifically in the superior longitudinal fasciculus, divisions 2 ($r = 0.7444$, $p = 0.0342$), and 3 ($r = 0.7649$, $p = 0.0270$), as well as in the uncinate fasciculus ($r = 0.8529$, $p = 0.0071$).

In summary, following 15 months of cocaine self-administration, [³H]PK11195 binding levels in gray matter were unaltered as compared to controls (Table 1). Elevations in both [³H]PK11195 binding to the TSPO, a putative marker of inflammation (Table 2; Fig. 1), and rates of glucose utilization (Table 3) in white matter tracts were restricted to the level of the anterior striatum, while [³H]PK11195 and LCGU at the remaining two levels analyzed were not different from those in food-control animals (Tables 2, 3; Fig. 2a, b). Furthermore, [³H]PK11195 binding levels and LCGU in white matter were positively correlated at the striatal level (Fig. 2c).

Discussion

The present study demonstrates an elevation in [³H]PK11195 binding to the TSPO (a marker of activated microglia) within the forebrain of nonhuman primates with a prolonged

Table 3 Functional metabolic activity in white matter of food-reinforced and cocaine-exposed animals

White matter structure	Food controls $N = 4$	15 months cocaine $N = 4$	Power	Cohen's d
Prefrontal level				
Superior longitudinal fasciculus 1	10.07 ± 2.3	16.77 ± 2.7		
Superior longitudinal fasciculus 2	10.15 ± 2.8	16.37 ± 2.8		
Corpus callosum	8.12 ± 2.7	12.64 ± 2.0		
Cingulum bundle	9.45 ± 2.5	15.10 ± 2.5		
Fronto-occipital fasciculus	8.38 ± 2.4	13.51 ± 2.5		
Extreme capsule	10.40 ± 2.6	17.07 ± 2.4		
Uncinate fasciculus	9.46 ± 2.1	14.26 ± 2.1		
Striatal level				
Superior longitudinal fasciculus 1	8.44 ± 1.2	$14.87 \pm 1.9^*$	82%	0.82
Superior longitudinal fasciculus 2	7.08 ± 1.7	$13.79 \pm 1.9^*$	97%	0.76
Superior longitudinal fasciculus 3	7.27 ± 1.6	$15.09 \pm 1.7^*$	94%	0.99
Corpus callosum	6.53 ± 1.9	12.83 ± 1.9		
Cingulum bundle	7.92 ± 1.7	14.72 ± 2.1		
Fronto-occipital fasciculus	6.62 ± 1.7	12.92 ± 1.8		
Striatal bundle	6.55 ± 1.9	$13.58 \pm 1.9^*$	76%	0.74
Muratoff bundle	7.27 ± 1.7	13.64 ± 1.8		
Internal capsule	8.68 ± 2.0	$18.31 \pm 3.0^*$	75%	0.75
Extreme capsule	10.26 ± 1.4	$18.02 \pm 2.3^*$	82%	0.82
Uncinate fasciculus	12.41 ± 1.7	$23.74 \pm 3.6^*$	82%	0.82
Substantia nigra level				
Superior longitudinal fasciculus 1	9.84 ± 2.9	11.05 ± 0.6		
Superior longitudinal fasciculus 2	9.60 ± 2.9	10.37 ± 0.6		
Superior longitudinal fasciculus 3	10.59 ± 2.8	11.25 ± 1.0		
Corpus callosum	10.21 ± 2.9	11.34 ± 1.0		
Cingulum bundle	11.75 ± 2.8	13.08 ± 0.8		
Fronto-occipital fasciculus	9.42 ± 3.0	10.34 ± 0.6		
Internal capsule	10.76 ± 3.2	12.20 ± 1.0		
Extreme capsule	16.33 ± 3.2	21.97 ± 3.1		
Middle longitudinal fasciculus	12.41 ± 41	14.52 ± 1.0		
Inferior longitudinal fasciculus	12.03 ± 3.6	12.68 ± 0.6		
Parabrachial bundle	12.18 ± 4.6	11.80 ± 1.1		

Rates of LCGU are expressed as μmol/100 g/min. Two-way repeated-measures ANOVAs were followed by post hoc Student's t tests. Asterisks denote significant differences from food controls, $^*p < 0.05$. Significance at $p < 0.05$ was only accepted when accompanied by a power value of at least 75%. Effect size measured with post hoc Cohen's d tests for the difference between two means, corrected for small sample size. $d \geq 0.5$ considered medium effect size, $d \geq 0.8$ considered large effect size

history of cocaine self-administration (Table 2; Figs. 1, 2a). The observed effects were of a very restricted nature, such that they were confined to white matter tracts; no elevations in binding densities were seen in any of the gray matter structures measured (Table 1). In addition, the effects were observed in only one of the three levels analyzed; they were confined to the level at which the precommissural striatum is present (Figs. 1, 2a).

Recently, it was shown that cocaine is able to activate microglia and induce a pro-inflammatory response via its interaction with the microglial toll-like receptor 4 (TLR4) complex (Northcutt et al. 2015), which detects endogenous and foreign substances or pathogens and initiates the cascade of events in microglia that activates the central immune response (Akira and Takeda 2004; Fernandez-Lizarbe et al. 2009). With chronic activation microglia can become primed, such that subsequent challenges induce exaggerated immune responses (Perry 2007). It seems possible then, that with chronic exposure, recurrent binding of cocaine to the microglial TLR4 complex may initiate abnormal activation and heightened secretion of cytokines, chemokines and other components of the pro-inflammatory cascade; an idea that is consistent with reports of increased expression of components of the innate immune response following cocaine administration (Clark et al. 2013; Vallender et al. 2017).

Oligodendrocytes, the myelin-producing cells of the CNS, are particularly susceptible to oxidative and nitrosative stress, excitotoxic damage, and inflammatory cytokines (for review, see Benarroch 2009), and compromised oligodendrocytes are associated with dysmyelination or demyelination in a number of pathologies (see, for example, Barnett and Prineas 2004; Miguel-Hidalgo 2018; Miyamoto et al. 2013; Tanaka et al. 2003). Repeated exposure to cocaine, then, may function as a chronic stressor, and prime resident white matter microglia for heightened immune signaling, resulting in an environment toxic to oligodendrocytes, and culminating in white matter degeneration. Indeed, rodent studies have reported white matter deficits following cocaine exposure (Kovalevich et al. 2012; Narayana et al. 2009, 2014; Nielsen et al. 2012), and deficits in the major myelin constituents proteolipid protein (PLP) and myelin basic protein (MBP) were previously demonstrated in the corpus callosum and dorsal white mater of the same cocaine-exposed animals used for the current studies; furthermore, these white matter deficits were confined to the same rostro-caudal level at which we observed elevated [^{3}H]PK11195 binding densities and glucose utilization in the current investigation (Smith et al. 2014).

Consistent with the idea that the white matter deficits present following cocaine exposure are associated with an inflammatory response, there are numerous studies of aging, schizophrenia, traumatic brain injury, multiple sclerosis, and alcohol use disorder, to name a few, which have also demonstrated a role for neuroinflammation in the compromised white matter integrity and cognition that accompany these conditions. For example, Bettcher and colleagues (2014) showed that, in humans, white matter deficits associated with normal aging correlated with levels of inflammatory cytokine interleukin-6, and in a study of aging rats, the normal decline in myelin proteins was accompanied by an increase in astroglial and microglial activation (Xie et al. 2013). A clear association has been demonstrated between neuroinflammation and white matter pathology in schizophrenia (Najjar and Pearlman 2015). Likewise, in a rodent model of traumatic brain injury, delayed corpus callosum white matter degeneration followed a similar progressive time course as, and was regionally co-localized with, the appearance of inflammatory markers (Glushakova et al. 2014). Finally, ethanol-treated TLR4 knockout mice exhibited far fewer myelin deficits than wild-type mice, indicating that neuroinflammation may underlie the white matter damage observed following ethanol exposure (Alfonso-Loeches et al. 2012).

The current data raise an important question: why is [^{3}H]PK11195 binding elevated selectively in white matter? There is compelling evidence that microglia are not a homogeneous population of cells, rather there are subpopulations of microglia that differ in protein expression (Carson et al.

2007; de Haas et al. 2008; Doorn et al. 2015; Kawahara et al. 2009; Ren et al. 1999; Schmid et al. 2002; Wirenfeldt et al. 2005), morphology (Kuwabara et al. 2003; Lawson et al. 1990), and regional distribution (Carson et al. 2007; de Haas et al. 2008; Lawson et al. 1990; Ren et al. 1999). Microglia in white matter are less abundant than, and are morphologically different from, those in gray matter (Lawson et al. 1990). Furthermore, Anderson and colleagues (2007) found that expression of TIM-3, a pro-inflammatory receptor expressed by cells of the innate immune system, was enriched in white matter microglia, but absent in gray matter. The present findings, then, may reflect the activation of a subset of microglia in white matter with a phenotype that is more sensitive to the presence of cocaine than that of their gray matter counterparts.

The restricted nature of these effects is in keeping with a previous post-mortem study of poly-drug abusers, which also demonstrated that, within the forebrain, activation of microglia occurred selectively in white matter (Buttner and Weis 2006), suggesting that the mechanism underlying this aspect of the specificity of effects may be common to multiple drugs of abuse.

Another intriguing question raised by these data is why these effects are restricted to the level of the brain at which the precommissural striatum is present. This area is the locus of cocaine's primary rewarding and behaviorally stimulating effects, and it is this level at which its most profound cerebral metabolic effects (Porrino et al. 2002, 2004) and dopamine system dysregulation in the gray matter occur (Letchworth et al. 2001; Nader et al. 2002; Staley and Mash 1996). It is well beyond the scope of this descriptive investigation, however, to suggest a mechanistic relationship between the primary effects of cocaine within a specific brain region and an upregulation in microglial activation restricted to its adjacent white matter. The current findings are consistent, however, with imaging studies which have pointed to a greater vulnerability of predominantly frontal, compared to caudal, white matter of human cocaine-dependent individuals (Bell et al. 2011; Hanlon et al. 2011a; Lim et al. 2002; Lyoo et al. 2004; Moeller et al. 2005, 2007; Romero et al. 2010; but see; Lane et al. 2010).

Compared to their ramified counterparts, activated microglia consume a greater amount of glucose (Gimeno-Bayon et al. 2014), therefore we hypothesized that LCGU in monkeys exposed to prolonged cocaine self-administration would be elevated, specifically in the white matter tracts in which microglial activation had been observed. Indeed, in contrast to the previously measured reductions in functional activity in gray matter structures throughout the monkey brain following extended cocaine self-administration in these (unpublished observations) and other nonhuman primates (Beveridge et al. 2006; Porrino et al. 2004), LCGU was profoundly elevated, as compared to controls, in most of

the same white matter fiber bundles in which [³H]PK11195 binding was increased (Table 3; Fig. 2b). As was the case with binding to the TSPO, these effects were confined to the level of the precommissural striatum; in fact the degree to which these effects were superimposed upon each other was striking, and correlations between [³H]PK11195 binding and levels of LCGU at the level of the precommissural striatum revealed a significant relationship between microglial activation and glucose metabolism in white matter tracts (Fig. 2c).

In contrast to the current findings, Narendran et al. (2014) reported a lack of increased microglial activation in human cocaine abusers, as measured by [¹¹C]PBR28 positron emission tomography. This investigation, however, focused only on the gray matter of the mesolimbic dopamine system (where the present study also failed to see microglial activation); white matter tracts were not part of the analysis. Furthermore, the subjects had been abstinent for a minimum of 14 days, so the time-point chosen may have contributed to the negative results. In contrast, for the present investigation the dependent measures were assessed at a time-point when behaviorally active levels of cocaine were present in the brain. Indeed, human studies have suggested that there is at least partial recovery of immune function relatively quickly following cessation of both cocaine (Levandowski et al. 2016b) and alcohol use (Garcia-Marchena et al. 2017; Yen et al. 2017). Likewise, in rodents, microglia have been demonstrated to switch from an activated state to a quiescent phenotype during withdrawal from a repeated binge model of ethanol exposure (Zhao et al. 2013).

Thomas and colleagues (2004) also reported that cocaine failed to elicit microglial activation in the mouse brain, however this study used a low dose of cocaine, and animals received only 4 non-contingent injections of drug, 2 h apart; a dosing regimen that was perhaps insufficient to engender a robust microglial response. Activated microglia have been observed in post-mortem tissue from cocaine users (Little et al. 2009), and since then rodent investigations have also demonstrated elevated microglial activation elicited by cocaine exposure, both in vivo and in vitro (Guo et al. 2015; Liao et al. 2016; Lopez-Pedrajas et al. 2015; Periyasamy et al. 2018).

It should be pointed out that under certain circumstances astrocytes, as well as microglia, can express the TSPO (Kuhlmann and Guilarte 2000; Maeda et al. 2007; Rojas et al. 2007). In most cases, however, binding of [³H]PK11195 has been demonstrated to label predominantly microglia, and various investigations have shown that TSPO expression is associated more closely with selective markers for activated microglia (Bonsack et al. 2016; Myers et al. 1991; Raghavendra Rao et al. 2000; Stephenson et al. 1995). Consistent with the present findings, Mankowski and colleagues (2003) reported strong correlations between [³H]PK11195 densities and microglial markers in white, but not gray matter

of simian immunodeficiency virus-infected monkeys with encephalitis.

One limitation of this study is that causation cannot be inferred from these data, which are strictly associative. Their strength, however, lies in the carefully controlled conditions in which the cocaine self-administration was undertaken, and the fact that each of the two data sets was generated in the same nonhuman primates, in adjacent brain sections. A further limitation is the small number of animals in each group, a problem often inherent in studies using nonhuman primates. It is likely that with a larger sample size, regions at the level of the prefrontal cortex may have achieved significance, particularly in respect to the LCGU findings. However, to avoid the likelihood of Type I errors any trends were not included in this report.

In summary, the present study demonstrates elevated microglial activation which is associated with an apparent vulnerability of frontal white matter tracts following prolonged cocaine self-administration. These data suggest that microglial activation and subsequent neuroinflammation might be a contributing factor to the white matter deficits (Bell et al. 2011; Hanlon et al. 2011a; Lane et al. 2010; Lim et al. 2002; Lyoo et al. 2004; Ma et al. 2009; Moeller et al. 2005, 2007; Romero et al. 2010), altered functional connectivity (Cisler et al. 2013; Hanlon et al. 2011b; Kelly et al. 2011; Ma et al. 2014; McHugh et al. 2013; Zhang et al. 2014), and, ultimately, the behavioral, motivational, and cognitive impairments (Ersche et al. 2011; Goldstein and Volkow 2011; Hanlon et al. 2011b; Hester and Garavan 2004; Lane et al. 2010; Levandowski et al. 2016a; Moeller et al. 2005) typical of cocaine abusers.

Compliance with ethical standards

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