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Elevated DNA Oxidation and DNA Repair Enzyme Expression in Brain White Matter in Major Depressive Disorder

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Abstract

Background: Pathology of white matter in brains of patients with major depressive disorder (MDD) is well-documented, but the cellular and molecular basis of this pathology are poorly understood.
Methods: Levels of DNA oxidation and gene expression of DNA damage repair enzymes were measured in Brodmann area 10 (BA10) and/or amygdala (uncinate fasciculus) white matter tissue from brains of MDD (n=10) and psychiatrically normal control donors (n=13). DNA oxidation was also measured in BA10 white matter of schizophrenia donors (n=10) and in prefrontal cortical white matter from control rats (n=8) and rats with repeated stress-induced anhedonia (n=8).
Results: DNA oxidation in BA10 white matter was robustly elevated in MDD as compared to control donors, with a smaller elevation occurring in schizophrenia donors. DNA oxidation levels in psychiatrically affected donors that died by suicide did not significantly differ from DNA oxidation levels in psychiatrically affected donors dying by other causes (non-suicide). Gene expression levels of two base excision repair enzymes, PARP1 and OGG1, were robustly elevated in oligodendrocytes laser captured from BA10 and amygdala white matter of MDD donors, with smaller but significant elevations of these gene expressions in astrocytes. In rats, repeated stress-induced anhedonia, as measured by a reduction in sucrose preference, was associated with increased DNA oxidation in white, but not gray, matter.
Conclusions: Cellular residents of brain white matter demonstrate markers of oxidative damage in MDD. Medications that interfere with oxidative damage or pathways activated by oxidative damage have potential to improve treatment for MDD.

Keywords: oxidative stress, DNA, messenger RNA, anhedonia, oligodendrocyte

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Significance Statement

Currently available antidepressants produce remission in only two-thirds of patients with depression, a disorder that affects over 10 million people in the US each year. The proposed study deviates from traditional studies of the pathobiology of depression that focus on systems directly affected by current and less than adequate antidepressants. This study unearths vulnerabilities of oligodendrocytes in brain white matter in major depressive disorder. Oligodendrocytes are critical for the transmission of information from one region of the brain to another. The findings provide a theoretical cellular and molecular basis for brain white matter pathology observed in living patients with depression by in vivo brain imaging. Further study of the affected pathways in oligodendrocytes has the potential to uncover novel targets for drugs that could prevent or reverse white matter pathology in depression, and ultimately improve overall treatment of this disorder.

Introduction

Brain white matter pathology is a feature of several psychiatric diseases, including major depressive disorder (MDD) and bipolar disorder. In living patients, white matter pathology has been intimated by *in vivo* imaging studies, including diffusion tensor imaging (DTI) to ascertain anisotropy and diffusivity in white matter and T2- or proton density weighted images from MRI to measure white matter hyper-intensities or white matter volumes. Abnormalities in DTI, including reduced anisotropy, and elevated numbers of white matter hyper-intensities have been reported in MDD patients (Taylor et al., 2007; Tham et al., 2011). The cellular basis of white matter abnormalities has not been conclusively identified, but is thought to be a result of pathology of myelinating cells (oligodendrocytes), vascular tissues (especially in the elderly), astrocytes, and/or microglia that reside in white matter regions of the brain. In MDD, cellular abnormalities have been reported in white matter obtained postmortem from depressed patients who died by suicide as compared to individuals dying from other medical causes and lacking a psychiatric diagnosis. These abnormalities include alterations in astrocyte morphology (Torres-Platas et al., 2011), reduced myelin staining (Regenold et al., 2007), and aberrations in oligodendrocyte gene expressions and morphology (Szebeni et al., 2014; Rajkowska et al., 2015).

Recently, we discovered shortened telomeres in white matter oligodendrocytes collected from postmortem brains of MDD donors, as compared to age-matched psychiatrically normal control donors (Szebeni et al., 2014). Mechanisms contributing to telomere shortening in other cell types include cell divisions and telomere DNA damage mediated by oxidation events (Von Zglinicki, 2002). Since oligodendrocytes are post-mitotic, we speculated that telomere shortening in oligodendrocytes from MDD subjects may be secondary to oxidative damage of telomere DNA. Evidence of oxidative damage in MDD includes a wealth of support such as elevated biological products of oxidative stress (Forlenza and Miller, 2006; Bilici et al., 2001; Selley, 2004; Sarandol et al., 2006, 2007; Dimopoulos et al., 2008; Yager et al., 2010) and reduced plasma concentrations of free radical scavengers (Peet et al., 1998; Maes et al., 2000; Owen et al., 2005).

The primary targets of reactive oxygen on DNA are oxidation-sensitive guanine nucleotides due to their low ionization potential in comparison to other nucleic acid bases (Kawanishi et al., 2001). Guanine-rich areas of DNA, such as telomere DNA and promoter/enhancer areas of genes, are thereby particularly sensitive to oxidative attack. One of the fastest cellular responses to oxidative damage of DNA is poly(ADP-ribosyl)ation, a post-translational modification of nuclear proteins, catalyzed by the nuclear enzyme poly(ADP)-ribose polymerase-1 (PARP1) (Gibson and Kraus, 2012). In the CNS, PARP1 is ubiquitously distributed in glia and neurons, found predominantly in the nucleus, and exerts a vast array of nuclear effects that influence the process of DNA repair. When DNA is oxidized, 8-oxoG DNA glycosylase (encoded by *OGG1*) excises oxidized nucleotides (Hazra et al., 2001). Gene expression of PARP1 and OGG1 is upregulated by oxidative stress (Liu et al., 2000; Lan et al., 2003; Adaikalakoteswari et al., 2007). Hence, PARP1 and OGG1 are two key mediators of the cellular apparatus for base excision and repair (BER) in response to oxidative attack of DNA.

Our previous work (Szebeni et al., 2014) demonstrating telomere shortening in white matter oligodendrocytes from MDD brain donors strongly implicates elevated levels of DNA oxidation of white matter in MDD. In the present study, we wished to confirm this by directly measuring a common product of DNA oxidation, 8-hydroxy-deoxyguanosine (8OHdG), in white matter from MDD donors. Since nearly all MDD donors studied previously (Szebeni et al., 2014) died by suicide, it seemed important to determine whether biological factors specific to suicidal behavior, rather than depression, might contribute to vulnerabilities to DNA oxidation. To evaluate this, MDD donors that died as a result of suicide or natural medical deaths were included in the present study. To increase the sample size of the non-suicide vs suicide comparison, we also evaluated DNA oxidation in a group of schizophrenia brain donors that died as a result of suicide or natural medical deaths. Given the roles of PARP1 and OGG1 in DNA damage repair, levels of expression of *PARP1* and *OGG1* were evaluated in specific white matter cell populations collected by laser capture microdissection from MDD and psychiatrically normal control brain donors. In addition, white matter DNA oxidation was studied in rats that demonstrated anhedonia following repeated exposure to both social defeat stress and chronic unpredictable stress, to examine DNA oxidation associated with anhedonia in the absence of the many variables that can complicate human postmortem brain tissue studies. The results of this study provide further evidence for oxidative damage of white matter oligodendrocytes in MDD, implicating oligodendrocyte pathology as a contributory factor in the psychopathology of MDD.

Methods

Human Subjects and Tissue Acquisition

The methods for obtaining tissues from psychiatrically characterized brain donors is exactly as described previously (Chandley et al., 2013). Briefly, tissues are obtained at autopsy at the Medical Examiner's Office of Cuyahoga County, OH (USA) using an approved Institutional Review Board protocol. Axis I diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV) (American Psychiatric Association, 1994) were established following structured interviews of next-of-kin of all subjects using the Structured Clinical Interview for DSM Axis I Disorders modified for third-person reporting (First et al., 1996). Table 1 shows demographic information of brain donors.

Table 1. Subject Demographics

Subject Code	Gender	pH	RIN[a]	PMI[b]	Smoker	Toxicology	Axis I Diagnosis	Suicide	Tissue	Assay
Normal control donors										
1 c	M	6.47	7.3	17.0	No	NDD[c]	None	No	UF[e], BA10	Ox[f], GE[g]
2 c	M	6.49	6.9	23.0	No,hx[d]	Norpropoxyphene, propoxyphene, oxycodone	None	No	UF, BA10	Ox, GE
3 c	M	6.28	6.4	17.0	No	None	None	No	UF	GE
4 c	M	6.42	7.4	11.0	No	NDD	None	No	UF, BA10	Ox, GE
5 c	M	6.10	7.1	25.0	No	Propoxyphene	None	No	UF, BA10	Ox, GE
6 c	M	6.95	6.8	19.0	No	NDD	None	No	UF, BA10	Ox, GE
8 c	M	6.76	7.0	11.0	No	NDD	None	No	UF	GE
9 c	M	6.72	6.7	16.0	No	None	None	No	UF	GE
10 c	M	6.88	8.4	17.0	Yes	NDD	None	No	UF, BA10	Ox, GE
11 c	M	6.79	7.6	6.0	No,hx	Lidocaine	None	No	UF, BA10	Ox, GE
12 c	M	6.95	7.2	24.0	Yes	None	None	No	UF	GE
13 c	M	6.80	7.1	17.0	Yes	None	None	No	UF	GE
14 c	M	6.98	7.4	9.0	Yes	NDD	None	No	BA10	Ox, GE
15 c	M	6.78	7.7	21.0	Yes	Ethanol	None	No	BA10	GE
16 c	F	6.57	N/A[h]	32.5	No	NDD	None	No	BA10	Ox
18 c	M	6.58	N/A	22.0	No	NDD	None	No	BA10	Ox
19 c	F	6.59	N/A	21.0	Yes	Brompheniramine	None	No	BA10	Ox
20 c	M	6.71	N/A	24.0	No	Ethanol	None	No	BA10	Ox
MEAN		6.66	7.21	18.5						
SEM		0.06	0.13	1.5						
Donors with Major Depressive Disorder										
1 d	M	6.67	6.9	31.0	No	Ethanol	MDD	Yes	UF, BA10	Ox, GE
2 d	M	6.80	6.8	20.0	Yes	Acetone	MDD	Yes	UF, BA10	Ox, GE
3 d	M	6.48	5.8	18.0	No	CO 83%	MDD	Yes	UF	GE
4 d	M	6.27	7.5	30.0	Yes	CO 75%	MDD	Yes	UF, BA10	Ox, GE
5 d	M	6.84	7.0	11.0	No	EtOH	MDD	Yes	UF, BA10	Ox, GE
6 d	M	6.26	7.5	17.0	No	NDD	MDD	No	UF, BA10	Ox, GE
8 d	M	6.70	6.7	20.0	No	Diphenhydramine	MDD	Yes	UF	GE
9 d	M	6.23	7.0	21.0	Hx	NDD	MDD	Yes	UF	GE
10 d	M	6.91	8.0	18.0	Yes	NDD	MDD	Yes	UF, BA10	Ox, GE
11 d	M	6.32	6.8	20.0	No	Ethanol	MDD	Yes	UF, BA10	Ox, GE
12 d	M	6.20	6.7	30.0	Yes	Codeine	MDD	Yes	UF	GE
13 d	M	6.60	6.6	17.0	No	NDD	MDD	Yes	UF	GE
14 d	M	6.24	6.7	19.2	Yes	Chlorpheniramine	MDD	No	BA10	Ox, GE
15 d	M	6.80	7.2	26.0	Yes	Ethanol	MDD	Yes	BA10	GE
26 d	M	6.50	N/A	14.0	No	Tolnaftate, brucine	MDD	No	BA10	Ox
27 d	M	6.67	N/A	26.0	Yes	Sertraline, carbamazepine	MDD	No	BA10	Ox
MEAN		6.53	6.9	21.1						
SEM		0.06	0.1	1.5						
Donors with Schizophrenia										
16 d	F	5.74	N/A	18.0	No	NDD	SCZ[i]	No	BA10	Ox
17 d	M	6.62	N/A	16.0	Yes	NDD	SCZ	No	BA10	Ox
18 d	M	6.77	N/A	16.0	Yes	NDD	SCZ	No	BA10	Ox
19 d	F	6.58	N/A	6.0	Yes	Amoxapine, loxapine	SCZ	No	BA10	Ox
20 d	M	6.78	N/A	10.0	Yes	Ethanol	SCZ	Yes	BA10	Ox
21 d	M	6.76	N/A	24.0	Yes	NDD	SCZ	Yes	BA10	Ox
22 d	M	6.71	N/A	19.0	No	NDD	SCZ	Yes	BA10	Ox
23 d	M	6.70	N/A	15.0	Yes	Ethanol, cocaine, diphenydramine	SCZ	Yes	BA10	Ox
24 d	M	6.70	N/A	24.0	No	Olanzapine	SCZ	Yes	BA10	Ox
25 d	M	6.55	N/A	12.2	Yes	Olanzapine	SCZ	Yes	BA10	Ox
MEAN		6.59	N/A	16.7						
SEM		0.08	N/A	1.7						

[a] RNA integrity number generated by the Agilent Bioanalyzer 2100.
[b] Postmortem interval.
[c] No drugs detectable.
[d] History.
[e] Uncinate fasciculus.
[f] DNA oxidation assay.
[g] Gene expression assay.
[h] Not applicable to donors that were used for only DNA oxidation studies.
[i] Schizophrenia.

All diagnoses were active at the time of death. Psychiatrically normal control, MDD and schizophrenia donors were matched as closely as possible for age, gender, postmortem interval (PMI), tissue pH and smoking history. Two control donors had current alcohol abuse at the time of death while four MDD subjects had comorbid alcohol abuse disorder and one MDD subject had comorbid alcohol dependence. In addition, two donors that had schizophrenia had active alcohol abuse. Control donors died as a result of cardiovascular disease (11), pulmonary emboli (2), homicide (2), aneurysm (1), crushing impact (1), and pancreatitis (1). MDD donors died as a result of suicide (14), cardiovascular disease (3), and gunshot wound (1). Donors that had schizophrenia died as a result of cardiovascular disease (2), pulmonary disease (1), homicide (1) and suicide (6). The range of ages was 17 to 82 y for control donors (47.9 ± 4.1 y), 16 to 86 y for MDD donors (50.5 ± 5.0 y), and 16 to 78 y for schizophrenia donors (39.7 ± 5.1 y). All demographic details for each donor are not included in Table 1 to protect the identity of donors. Two frozen brain areas from the right hemisphere were obtained for study, BA10 including its white matter and the amygdala containing the uncinate fasciculus.

Measurement of 8OHdG

Frozen human right BA10 tissue was cut on a cryostat microtome to 50 μm thick sections and then white matter was punch-dissected using a 3.5 mm trephine dissection tool at -20°C. White matter from rat prefrontal cortex was similarly punch-dissected with a 1.5 mm trephine at the level of Plate 9 (Paxinos and Watson, 1986). Genomic DNA was isolated from 10 punches from each species using the QiaAmp DNA microkit (Qiagen, Valencia, CA). Levels of 8OHdG were measured from the DNA isolated from white matter tissue using a commercially available ELISA (JaICA, Shizuoka, Japan).

Immunohistochemistry for Laser Capture Microdissection

Frozen tissue sections (10 μm) cut on cryostat microtome at -20°C were prepared for laser capture microdissection as previously described (Ordway et al., 2009), including rapid immunohistochemical staining for 2',3'-cyclic nucleotide 3' phosphodiesterase (CNPase; Abcam, Cambridge, MA) at 37°C and glial fibrillary acidic protein GFAP (GFAP; Thermo Fisher Scientific, Waltham, MA) at 22°C. Following immunostaining, 500 oligodendrocytes or 500 astrocytes were captured onto CapSure Macro caps within 2 h for each sample using a ArcturusXT (Thermo Fisher Scientific, Waltham, MA) and incubated at 42°C for 30 min in RNA lysis buffer immediately following capture.

RNA Purification and End-Point PCR

Total RNA extraction, cDNA synthesis and endpoint PCR was performed as previously described (Szebeni et al. 2014). Genes used in endpoint PCR included three reference genes (glyceraldehyde-3-phosphate dehydrogenase, *GAPDH;* 18S ribosomal 1 RNA, *RNA18S1*; ubiquitin C, *UBC*), identification genes for each cell type (*CNP*; *GFAP*) and target genes (*PARP1, OGG1*), see Supplementary Table S1 for more information including primer sequences and NCBI accession numbers. All experiments were performed in triplicate using an Agilent 2200 Tapestation Instrument (Agilent Technologies, Santa Clara, CA).

Laboratory Animals

The use of animals was approved by the University Committee on Animal Care. Male Sprague-Dawley rats (Envigo, Inc., Indianapolis, IN, USA) weighing 225–250 g upon arrival served as 'intruders' in the social defeat paradigm. Animals were individually housed and were provided enrichment per the NIH Guideline for the Care and Use of Animals. Rats were housed on a 12 h on/12 on off light/dark cycle in a climate-controlled vivarium.

Female Rat Fallopian Tube Ligature

Fourteen female Sprague-Dawley rats (Envigo, Inc., Indianapolis, IN) weighing 175–199 g upon arrival were allowed 6 days of habituation to animal colony conditions. Rats then underwent surgery to ligate the fallopian tubes (Aygen et al., 2002), and then were allowed 7 days to recover before being mated with a male rat.

Social Defeat Stress (SDS)

Sixteen male Long-Evans hooded rats ('resident') were each mated with a female Sprague-Dawley (ligated) rat for a 7-day period. On the eighth day, the female was removed, and an 'intruder' rat was placed into the cage for a 5 min period. In all cases, the resident established dominance over the intruder. The interaction was stopped when a rat demonstrated a supine posture for up to 10 s, had received up to 10 attack bites, or 5 min had elapsed. SDS was performed daily for 10 consecutive days, and was performed between 0900 and 1000 h. Controls were handled by the experimenter each day during this same time period, but were not exposed to SDS.

Chronic Unpredictable Stress (CUS)

CUS was performed after SDS on the same day, but at random times either during the day or evening. Unpredictable stressors included a 10-min cold (18°C) or a 15-min warm swim (24°C) that was conducted in a tank 61 cm in diameter, a tilted cage for 24 h, restraint stress for 30 min, or 1 h of shaking/crowding. These different stressors were randomly arranged, given during either the light or dark cycle and given each day for 10 consecutive days. All rats were exposed to each of these 5 stressors twice during the 10-day period. Controls were handled by the experimenter each day during the same time period but not exposed to the stressor.

Sucrose Preference

Sucrose preference was performed the final three days of stress induction (days 8–10), using a procedure based on that of D'Aquila et al (1997). Animals were given two bottles on their cages between the 1900 and 2100 h on each day that it was performed (the first 2 h of the dark cycle) with one bottle containing tap water and the other containing 0.8% sucrose. Amounts of sucrose consumed were calculated as percentages of the total amount of fluid consumed during the 2 h period on each of the 3 days of testing.

Social Interaction Test

The social interaction test was given 24 h after the last social defeat stress on day 11 of behavioral testing. The interaction test was conducted exactly as previously described (Brown et al., 2011).

Statistical Analysis

A Grubb's test was used to remove statistical outliers from each dataset prior to analyses. Statistical analyses were performed as indicated using IBM SPSS Statistics (version 23.0) and data were graphed using GraphPad Prism (version 5.0b, GraphPad

Software, La Jolla, CA). For analysis of variance, a Bonferroni-correction was applied to limit Type I error in multiple post-hoc comparisons. All data are expressed as mean ± standard error of the mean.

Results

DNA Oxidation in Human BA10 White Matter

Levels of 8OHdG in BA10 white matter were significantly impacted by the psychiatric status of brain donors ($F=20.9_{[2,30]}$, p=2.1e-6). Using age as a covariate did not alter the outcome of the analysis. Post hoc comparisons revealed robustly elevated levels of 8OHdG in MDD donors compared to psychiatrically normal control donors, but also significantly elevated 8OHdG levels in schizophrenia donors (Figure 1A). Since many of the MDD donors and schizophrenia donors died as a result of suicide, the possibility that oxidation of white matter DNA might be related to suicide rather than psychiatric disease was evaluated by grouping the two psychiatric illnesses together and comparing those that died as a result of suicide to those who had non-suicide deaths. This comparison was made with the caveat that schizophrenia donors, particularly those that may have suicided during an acute psychosis, are behaviorly very different from MDD donors of whom none had a history of psychosis. Levels of 8OHdG in white matter in suicide brain donors were not significantly different from 8OHdG levels in non-suicide brain donors, but 8OHdG levels were significantly elevated in both suicide and non-suicide psychiatric groups compared to the normal control group (Figure 1B). Suicide vs non-suicide was also evaluated within each psychiatric diagnosis, albeit the sample sizes for these comparisons were much smaller. Comparison of 8OHdG levels in MDD donors dying as a result of suicide (n=6, 1.96 ± 0.12 ng/ml) to those MDD donors that died of natural/medical causes (n=4, 2.10 ± 0.21 ng/ml) were not significantly different ($t_{[8]}=0.61$; p=0.56). Likewise, 8OHdG levels in schizophrenia donors dying as a result of suicide (n=6, 1.70 ± 0.11 ng/ml) was not significantly different from schizophrenia donors that died of natural/medical causes (n=4, 1.71 ± 0.08 ng/ml; $t_{[8]}=0.12$; p=0.91).

PARP1 and OGG1 Gene Expression in Human BA10 Glia

Expression levels of *PARP1* and *OGG1* were measured in oligodendrocytes and astrocytes collected by laser capture microdissection of BA10 white matter from MDD donors and normal control donors. Glia were also laser captured from a second white matter region (uncinate fasciculus) that was too small for measurement of DNA oxidation. We had previously observed indirect evidence of DNA damage in uncinate fasciculus (Szebeni et al., 2014). Multivariate ANOVA revealed a significant group effect on *PARP1* expression in the two white matter regions ($F=41.0_{[4,9]}$; $p<1x10^{-5}$). Using age, RNA integrity (RIN), pH, and PMI as covariates in the analysis did not alter the outcome of the analysis. Post-hoc Bonferroni adjusted comparisons of MDD to control donors showed statistically higher expressions of *PARP1* in oligodendrocytes in uncinate fasciculus and BA10, and in astrocytes only in BA10 white matter (Figure 2A and 2B). Similarly, a significant group effect (control vs MDD) was observed for the expression of *OGG1* ($F=22.6_{[4,10]}$; $p<1x10^{-4}$). As for *PARP1* expression, using covariates in the analysis did not alter the outcome of the analysis. Post-hoc comparisons demonstrated significantly elevated *OGG1* expression in oligodendrocytes in both uncinate fasciculus and BA10 white matter (Figure 2C). However, no significant difference in levels of OGG1 expression was observed in astrocytes from the two white matter regions (Figure 2D). In the samples collected for laser capture microdissection, there were not a sufficient number of MDD donors who had not died as a result of suicide to statistically evaluate the potential contribution of suicide to gene expression changes. However, for oligodendrocytes collected from uncinate fasciculus, where there were two non-suicide donors, *PARP1* and *OGG1* gene expression levels in both non-suicide MDD donors were higher than the levels of *PARP1* and *OGG1* gene expression levels of every control donor.

The potential that *PARP1* and *OGG1* expression levels were linked to DNA oxidation levels was examined. As noted in Methods, DNA oxidation levels were determined in a subset of brain donors for which gene expressions were measured, i.e. 6–8 donors per group. In these small samples, there were no significant correlations between DNA oxidation levels in BA10 white matter and gene expression levels of either BER enzyme in oligodendrocytes or astrocytes from BA10 white matter

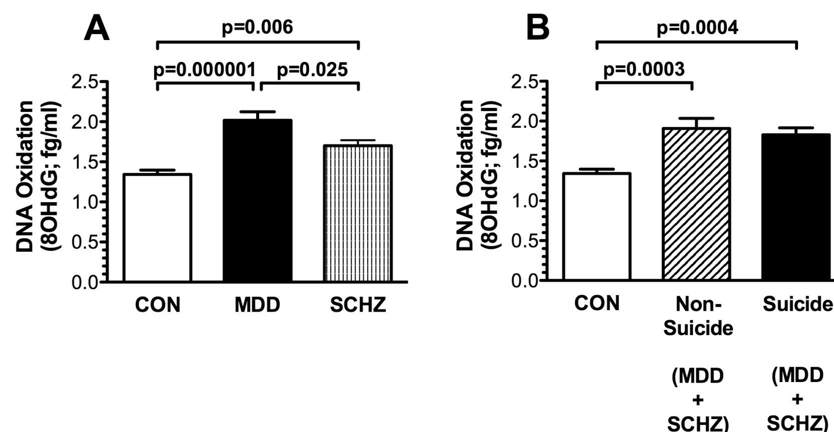

Figure 1. Measurement of 8-hydroxydeoxyguanosine (8OHdG) immunoreactivity in homogenates of BA10 white matter from psychiatrically normal control donors (CON, n=13), major depressive disorder donors (MDD; n=10) and schizophrenia donors (SCHZ; n=10). The two psychiatric groups include suicide and non-suicide causes of death in panel A. The same data appears in panel B with psychiatric groups divided according to cause of death, i.e. suicide (n=12 consisting of 6 MDD and 6 SCHZ donors) and non-suicide (n=8 consisting of 4 MDD and 4 SCHZ donors). P values for group comparisons that reached statistical significance are noted.

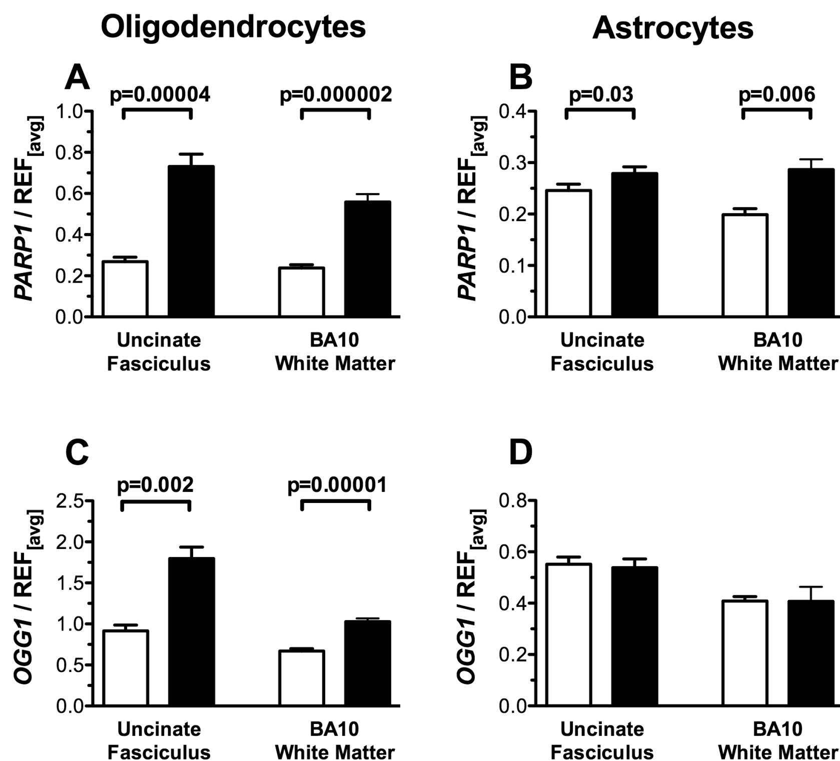

Figure 2. Expression levels of BER enzyme genes, *PARP1* (panels A and B) and *OGG1* (panels C and D) in laser captured oligodendrocytes (panels A and C) and astrocytes (panels B and D) from BA10 white matter of psychiatrically normal control donors (white bars) and MDD donors (black bars). P values for group comparisons that reached statistical significance are noted.

when considering data from both study groups (Supplementary Table S2).

Potential correlations between *PARP1* and *OGG1* expression levels were also evaluated. Here, Pearson correlations were computed after grouping together data from the two brain regions, examining oligodendrocytes and astrocytes separately. Data from control and MDD donors were also examined separately because levels of both mRNAs were significantly different comparing the two donor groups. Levels of *PARP1* expression significantly correlated with *OGG1* expression levels in both oligodendrocytes and astrocytes, and in both control and MDD donors (Figure 3).

Evaluation of Potential Modifiers of DNA Oxidation and DNA Repair Enzyme Gene Expression in Human Postmortem Brain Samples

The potential impact of several demographic and biological variables on DNA oxidation and gene expression was evaluated. Levels of DNA oxidation modestly correlated with age in the control group (r=-0.56, p=0.048). However, DNA oxidations levels did not significantly correlate with age in the psychiatric groups, whether considered separately (MDD, r=-0.28, p=0.33; schizophrenia, r=0.34, p=0.33) or when MDD and schizophrenia donors were grouped together (r=-0.34, p=0.14). To examine a potential effect of smoking on DNA oxidation, known smokers and non-smokers were compared regardless of psychiatric status. DNA oxidation levels were not significantly different comparing smokers to non-smokers ($t=0.42_{[27]}$, p=0.967). Likewise, the potential effect of chronic alcohol exposure was examined by grouping all alcohol abuse/dependence donors regardless of psychiatric status (n=9) and comparing to those without alcohol abuse/dependence (n=24). As such, alcohol exposure had no significant effect on white matter DNA oxidation levels ($t=-0.748_{[31]}$, p =0.46).

Factors such as age, tissue pH, PMI, and RIN were considered as possible modifiers of gene expression data. None of these factors were significantly correlated with *OGG1* or *PARP1* expression levels in oligodendrocytes collected from either the uncinate fasiculus or BA10 white matter (Supplementary Tables S3 and S4). The effect of cigarette smoking on *PARP1* and *OGG1* expression was evaluated by comparing smokers to nonsmokers regardless of psychiatric status. There was no significant effect of smoking on levels of *PARP1* and *OGG1* gene expression (*PARP1*: $F=1.35_{[8,20]}=0.35$; *OGG1*: $F1_{[8,20]}=1.142$, P=0.38). Comparisons of age, RIN, PMI, and RIN were made for all group comparisons. There were no significant differences in group comparisons for any of these variables for DNA oxidation measurements (Supplementary Table S5). Likewise, there were no significant differences between groups for these variables for the gene expression measurements, with the exception of a modest difference in mean PMI values (p=0.04; Supplementary Table S6). There were only four female donors available for study, two control donors and two age-matched schizophrenia donors. Tissues from these subjects were used to measure DNA oxidation in BA10 white matter (see Table 1). As observed comparing all schizophrenia donors to control donors, DNA oxidation levels were higher in female schizophrenia donors (1.74 and 1.90 fg/ml; average 1.82 fg/ml) as compared to female control donors (1.00 and 1.29 fg/ml; average 1.14 fg/ml), although the sample size is too small to permit a statistical comparison.

Finally, the potential relationship between length of illness and DNA oxidation and DNA repair enzyme gene expression was evaluated. No significant correlation between length

of illness and levels of DNA oxidation were observed for MDD brain donors (Supplementary Table S7). Likewise, there were no significant correlations between length of illness and expression levels of *PARP1* or *OGG1* (Supplementary Table S7).

Psychological Stress-Induced Depressive Behavior in Rats

Rats were exposed daily to two classical psychological stressors, social defeat and unpredictable stress, for 10 days. The rational for a double stress paradigm was to reduce potential for habituation to stressors. Anhedonia was quantified in stressed and non-stressed control rats using the 2-bottle sucrose preference task over the last three days of the stress paradigm (days 8–10). Social interaction was assessed the day after the last stressor (day 11). A repeated measures ANOVA revealed that rats exposed to stress demonstrated a significant reduction in preference for sweetened water across the three test days (Figure 4A; $F=28.9_{[1,26]}$, $P=1.3 \times 10^{-5}$). Likewise, time spent in the interaction zone was also significantly reduced in stressed rats (Figure 4B; $t=2.59_{[26]}$, $P=0.016$). DNA oxidation in white matter from the prefrontal cortex from these rats, measured two days following the last stressor, was significantly elevated in stressed rats as compared to unstressed controls (Figure 4C; $t=3.7_{[14]}$, $P=0.002$). In contrast, no significant change in levels of DNA oxidation was observed in prefrontal cortical gray matter comparing stressed to control rats (Figure 4C; $t=0.045_{[14]}$; $P=0.96$).

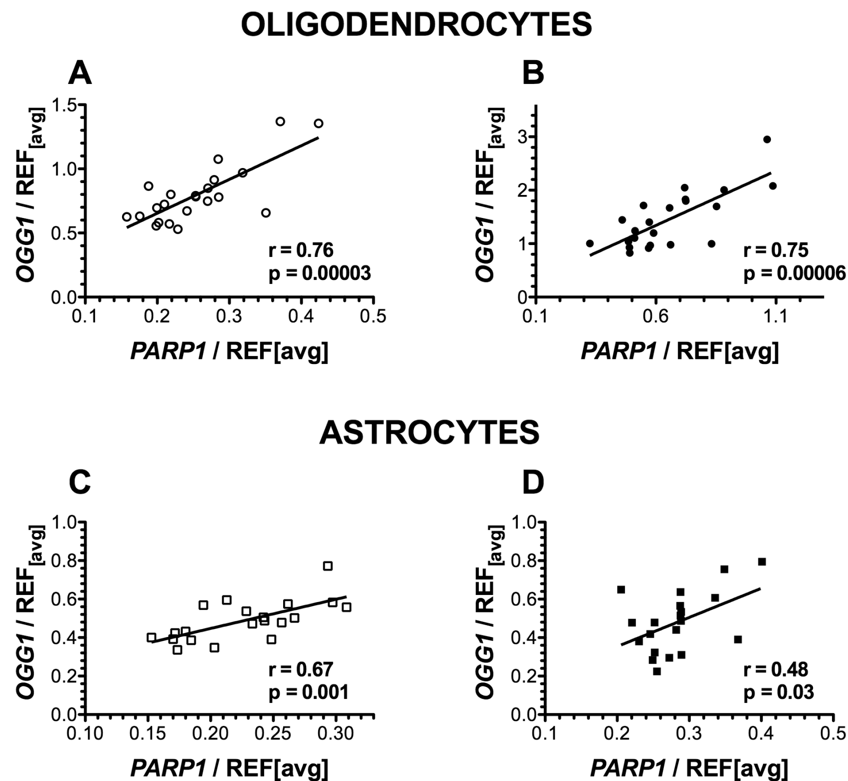

Figure 3. Relationship between expression levels of *PARP1* and *OGG1* in white matter glia in normal control (panels A and C) and MDD (panels B and D) donors. Each graph shows gene expression levels in cells laser captured from both uncinated fasciculus and BA10 white matter for each group of subjects. Pearson correlations and p values are shown.

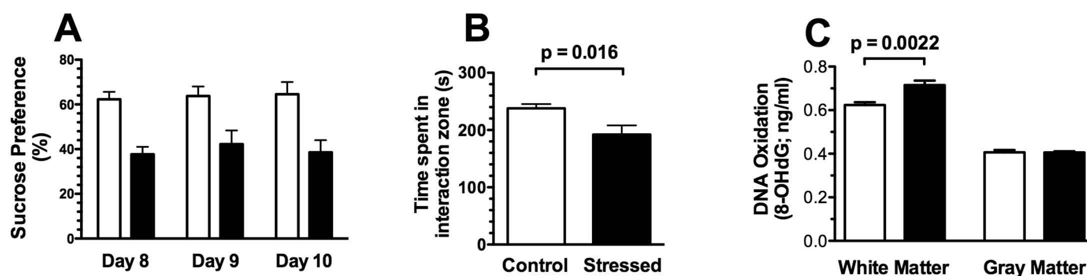

Figure 4. The effect of repeated exposure to social defeat stress plus unpredictable stress on sucrose preference in the two-bottle choice task (A), time spent in the interaction zone (B), and DNA oxidation levels (C) measured in prefrontal cortical white and gray matter of rats. For each graph, white bars indicate handled control rats and black bars indicate stressed rats. Sucrose preference was measured over the last three days (day 8–10) of the stress paradigm (N=14 rats per group). Repeated measures ANOVA revealed a significant effect of stress on sucrose preference across the three days ($p = 1 \times 10^{-5}$). Interaction zone times were measured on the 11th day, 24 h after the last social defeat session (N=14 rats per group). DNA oxidation levels were measured two days after the last stressor (day 12) in 8 rats from each of the same two groups of rats used for the two-bottle choice task and interaction zone measurements. Statistically significant comparisons are shown above the bars for panels B and C.

Discussion

The present study demonstrates elevated levels of DNA oxidation in prefrontal cortical (BA10) white matter from brain donors with an active MDD at the time of death, as compared to psychiatrically normal control donors. The elevated DNA oxidation in MDD was accompanied by increased levels of expression of the DNA damage sensor gene *PARP1* and the DNA repair gene *OGG1* in white matter oligodendrocytes. Moreover, elevated DNA oxidation was observed in prefrontal cortical white matter from rats demonstrating anhedonia after repeated exposure to psychological stress. These novel findings provide evidence that oxidative damage to white matter may contribute mechanistically to white matter pathologies that have been described (Tham et al., 2011) in imaging studies of living MDD patients. Furthermore, DNA oxidation in white matter in MDD may be linked to psychological stress-induced oxidative stress that yields damage to vulnerable cell populations in the brain.

The brain is susceptible to damaging effects of ROS, primarily due to a very high rate of metabolism and oxygen consumption, but also because of specific biochemical reactions (Connor and Menzies, 1996) that generate free radicals. During periods of elevated ROS, such as in oxidative stress, DNA and other cellular constituents such as RNA, proteins and lipids are susceptible to oxidation. Interestingly, numerous studies link MDD to elevated oxidative stress conditions, but the majority of this evidence arises from measurements of oxidative stress-related indices in blood components. For example, elevated levels of lipid peroxidation products (Bilici et al., 2001; Selley, 2004; Sarandol et al., 2006; Dimopoulos et al., 2008; Yager et al., 2010), DNA oxidation (Forlenza and Miller, 2006), and shortened telomeres (Simon et al., 2006; Kinser and Lyon, 2013; Verhoeven et al., 2013), have been measured in blood components of MDD patients. In addition, reduced plasma concentrations of free radical scavengers (Maes et al., 2000, 2011; Owen et al., 2005) have also been found in MDD. Fewer studies have examined indices of oxidative processes in the brains of MDD subjects. Che et al. (2010) demonstrated elevated levels of oxidized RNA in hippocampal neurons using postmortem tissue from MDD, bipolar disorder and schizophrenia brain donors. DNA oxidation was also examined in hippocampal neurons, but showed only a trend for an elevation in MDD donors. Kim HK et al. (2014) reported elevated oxidation and nitration of proteins in prefrontal cortices from bipolar disorder and schizophrenia brain donors, but no significant effects were observed in prefrontal cortex of MDD donors. To date, no study has investigated the potential role of oxidative damage to white matter in MDD, other than the work by our lab (Szebeni et al., 2014). Hence, the present study is the first of two studies to implicate oxidative damage to white matter in the pathology of MDD.

Myelinating oligodendrocytes are the predominant cell type in brain white matter. Among cell types in the brain, oligodendrocytes are known to be uniquely vulnerable to oxidative stress, with extensive lipid membranes being a primary target of free radicals (Kim and Kim, 1991; Thorburne and Juurlink, 1996), increased oxidative load due to lipid production for myelination, relatively low glutathione reductase enzyme activity (Juurlink et al., 1998), and high levels of iron (Connor and Menzies, 1996; Thorburne and Juurlink, 1996), a pro-oxidant. Previously, we found the first preliminary evidence of oxidative damage to white matter oligodendrocytes in the brain in MDD (Szebeni et al., 2014). In this previous study, oligodendrocytes, but not astrocytes, laser captured from two white matter regions (uncinate fasciculus and BA10) demonstrated shorter relative telomere lengths in MDD brain donors as compared to psychiatrically normal control subjects. These initial findings led us to speculate that elevated DNA oxidation may be responsible for the shorter telomeres in oligodendrocytes from MDD donors. The present findings demonstrating elevated levels of 8OHdG in white matter from BA10 of MDD brain donors, support the hypothesis that shorter telomeres in white matter oligodendrocytes in MDD is a result of elevated DNA oxidation in MDD.

Elevated DNA oxidation was observed in prefrontal cortical white matter but not gray matter in rats exposed to repeated psychological stress. Differential sensitivity of various brain regions to oxidative damage is known. For example, the hippocampal CA1 region of the brain is more vulnerable to oxidative stress than the hippocampal CA3 region or the cerebral cortex (Wang et al., 2009). The apparent lack of elevated DNA oxidation in the gray matter from stressed rats may be a result of the fact that the fraction of all cells that are oligodendrocytes in white matter is much greater than that fraction in gray matter. In humans, glia considerably outnumber neurons (approximately 15 to 1) in cortical white matter, whereas numbers of glia and neurons are more similar in cortical gray matter (approximately 1.4 glia to 1 neuron) (Azevedo et al., 2009). Considering that oligodendrocytes are uniquely susceptible to oxidiatve stress, DNA oxidation may be occurring in gray matter oligodendrocytes but it is not detectable because of a signal dilution by other unaffected glia and neurons.

Since oligodendrocytes are normally susceptible to oxidative damage, elevated levels of DNA oxidation observed in oligodendrocytes in MDD relative to normal controls suggest that susceptibility to oxidative damage is worsened in MDD. Gene expressions of four antioxidant genes were observed to be low in MDD oligodendrocytes as compared to oligodendrocytes from normal control subjects (Szebeni et al., 2014). Some of these genes are downregulated by glucocorticoids (Pereira et al., 1995; McIntosh et al., 1998; Schmidt et al., 2005), suggesting that elevated stress hormones in MDD may contribute to enhanced susceptibility of oligodendrocytes to oxidative damage in MDD. Low levels of circulating antioxidants in MDD could conceivably also contribute to enhanced oxidative damage to normally susceptible oligodendrocytes. Low blood levels of antioxidants, e.g. vitamin E, glutathione, coenzyme Q, and ascorbic acid, have been reported in depressed patients (Maes et al., 2011). However, the impact of varying diets on levels of circulating antioxidants is difficult to assess in studies involving human postmortem brain tissues.

The observations of elevated gene expression of the BER enzymes, PARP1 and OGG1, in white matter oligodendrocytes provide additional evidence of enhanced DNA oxidation in these cells in MDD. Gene expression of both of these enzymes is induced by conditions of oxidative stress (Liu et al., 2000; Lan et al., 2003; Adaikalakoteswari et al., 2007) presumably as a compensatory mechanism to enhance BER. The correlation of *PARP1* and *OGG1* expression levels in both glial cells from all donors in the present study is consistent with a coordinated response of these cells to DNA oxidation. This correlation of *PARP1* and *OGG1* expression levels has also been observed in dysplastic cells from cancer patients (Dziaman et al., 2014). Extensive activity of PARP1 depletes nicotinamide adenine dinucleotide (NAD+) and ATP that are essential to generate PAR polymers and cells can die from energy loss (Berger, 1985; Ha and Snyder, 1999; Fouquerel and Sobol, 2014). In a mature myelinating oligodendrocyte, NAD+ and ATP are critically important to myelin lipid synthesis for the myelin sheath that encloses segments of neuronal axons.

Hence, it is conceivable that elevated PARP activity, in response to oxidative damage to DNA in oligodendrocytes could deplete cellular energy supplies in MDD and interrupt important functions of these cells, particularly those that are highly energy dependent and critical to normal neurotransmission.

While PARP1 and OGG1 are major components of the BER apparatus, both enzymes are also involved in other cellular processes. PARP is a highly complex protein that engages in several cellular functions, including regulation of transcription through modifying chromatin structure, angiogenesis, mitochondrial energy metabolism and cell division (Martínez-Bosch et al., 2016). Of particular relevance to the study of MDD are PARP1 interactions with many transcription factors involved in regulation of inflammatory gene expression such as nuclear factor-*kappa* B (NF-KB) (Oliver et al., 1999). In fact, poly(ADP-ribosyl)ation by PARP1 is a necessary step in the activation of NF-KB induced by DNA damage (Stilmann et al., 2009). Recent evidence also suggests that PAR chains, once cleaved from proteins poly(ADP-ribosy)lated by PARP activity, act as damage associated molecular patterns that drive inflammation (Krukenberg et al., 2015). Likewise, recent evidence demonstrates that mice deficient in OGG1 have impaired immune responses, and that repair of oxidized DNA also results in an OGG1-dependent activation of NF-KB (Aguilera-Aguirre et al., 2014). Hence, these studies demonstrate that oxidation of oligodendrocyte DNA could be an initial step in the activation of inflammatory cascades that could contribute to further damaging effects on these and surrounding cells, including further generation of ROS by activation of microglia. Interestingly, elevated numbers of activated microglia in postmortem brain white matter from depressed suicide victims have been reported (Torres-Platas et al., 2014). Collectively, these findings contribute to growing evidence of a role of inflammation in MDD (Swardfager et al., 2016), and studies demonstrating that anti-inflammatory compounds have therapeutic utility in the treatment of depressive disorders (Fond et al., 2014).

Only one previous study has reported a relationship between DNA oxidation and anhedonia in rats. Sigwalt and coworkers (2011) exposed rats to dexamethasone daily for 21 days to induce anhedonia as measured by the sucrose preference test. Dexamethasone-treated animals demonstrated significantly elevated DNA oxidation in the CA1 of the hippocampus, an area of the brain containing neurons known to particularly susceptible to oxidative damage (Wilde et al., 1997; Wang et al., 2005). In the present study, repeated exposure to social defeat and chronic unpredictable stress resulted in an elevation of DNA oxidation of white matter, as well as anhedonia. While oxidative damage to white matter cells alone may be insufficient to produce anhedonia, other susceptible brains cells such as the hippocampus CA1 neurons may be similarly negatively affected by psychological stress-induced oxidative damage. Regardless of the specific cellular cause of anhedonia, elevated white matter DNA oxidation in anhedonic rats are consistent with white matter findings in human MDD donors, reducing the likelihood that DNA oxidation in human MDD donors is secondary to other confounding variables (smoking, drug exposures, environmental toxin exposures, dietary variables) associated with human postmortem tissue studies.

There are several limitations of the present study that must be taken into consideration with regard to conclusions drawn from the data. The sample sizes of the present study are relatively small and findings need to be replicated in a larger group of human brain donors. While oxidative stress can regulate the expression of *PARP1*, the PARP1 enzyme activity is acutely regulated by binding to damaged DNA that increases its activity by >500-fold (Martínez-Bosch et al., 2016). Hence, while elevated *PARP1* and *OGG1* expressions were observed in MDD, PARP1 or OGG1 enzymatic activities have not been measured. PARP activity would presumably be a difficult undertaking given detrimental effects of the postmortem interval on enzyme activities. Additionally, whether elevated gene expressions are accompanied by increases in protein is not known. It is noted that PARylation is rapidly degraded by poly(ADP-ribose) glycohydrolase (PARG) resulting in rapid downregulation of PARP1 activity (Davidovic et al., 2001). Given that, changes in PARP1 activity associated with disease may be difficult to observe in postmortem human tissues or in blood cells from living patients.

Numerous psychiatric and neurological disorders are associated with elevated white matter hyper-intensities and other indicators of white matter pathology. The cellular bases of these *in vivo* findings have not yet been clarified. Glia are abundant in white matter and abnormalities in glia morphology, cell number and gene and protein expression have been described in the postmortem human brain from MDD brain donors. The present findings provide additional evidence of white matter pathology in MDD, and draw attention to potential molecular mechanisms that may be involved in the etiology of white matter pathology. Elevated BER activity in oligodendrocytes, secondary to oxidative damage to these susceptible cells, may drive other deleterious energy-depleting and/or inflammatory processes that further contribute to oligodendrocyte dysfunction. Given the critical role of oligodendrocytes in facilitating neurotransmission, disruption of oligodendrocyte function could play a critical role in the development of depression. Searching for compounds that could inhibit pathways activated by elevated BER activity has the potential to unearth novel antidepressant mechanisms.

Supplementary Material

Supplementary data are available at *International Journal of Neuropsychopharmacology* online.

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Statement of Interest

None.

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