Altered DNA Methylation Patterns Associated With Clinically Relevant Increases in PTSD Symptoms and PTSD Symptom Profiles in Military Personnel

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Abstract

Military personnel experience posttraumatic stress disorder (PTSD), which is associated with differential DNA methylation across the whole genome. However, the relationship between these DNA methylation patterns and clinically relevant increases in PTSD severity is not yet clearly understood. The purpose of this study was to identify differences in DNA methylation associated with PTSD symptoms and investigate DNA methylation changes related to increases in the severity of PTSD in military personnel. In this pilot study, a cross-sectional comparison was made between military personnel with PTSD ($n = 8$) and combat-matched controls without PTSD ($n = 6$). Symptom measures were obtained, and genome-wide DNA methylation was measured using methylated DNA immunoprecipitation (MeDIP-seq) from whole blood samples at baseline and 3 months later. A longitudinal comparison measured DNA methylation changes in military personnel with clinically relevant increases in PTSD symptoms between time points (PTSD onset) and compared methylation patterns to controls with no clinical changes in PTSD. In military personnel with elevated PTSD symptoms 3 months following baseline, 119 genes exhibited reduced methylation and 8 genes exhibited increased methylation. Genes with reduced methylation in the PTSD-onset group relate to the canonical pathways of netrin signaling, Wnt/Ca⁺ pathway, and axonal guidance signaling. These gene pathways relate to neurological disorders, and the current findings suggest that these epigenetic changes potentially relate to PTSD symptomology. This study provides some novel insights into the role of epigenetic changes in PTSD symptoms and the progression of PTSD symptoms in military personnel.

Keywords

PTSD, DNA methylation, biomarkers, military

Posttraumatic stress disorder (PTSD) is a trauma/stress-related disorder that is initiated by psychologically distressing events such as combat or physical assault (Hughes & Shin, 2011; Xue et al., 2015). Among the more than 2 million U.S. military personnel who deployed to Iraq and Afghanistan, approximately 153,000 were diagnosed with PTSD after returning from military duty (Fischer, 2014; Fisher, 2014). Costs of treatment over 2 years for combat-related PTSD are estimated to be around US\$2 billion (Cesur, Sabia, & Tekin, 2013). PTSD symptoms, including intrusion, avoidance, negative alterations in cognition and mood, and alterations in arousal and reactivity, can last for months or even years after an individual's return from deployment (American Psychiatric Association 2013). PTSD is therefore associated with dramatic reductions in health-related quality of life that have longlasting impacts on all area of health and well-being (Bush, 2014).

Previous studies have investigated the role of genes in PTSD symptoms, demonstrating associations between these symptoms and genes related to serotonergic, dopaminergic systems, and the hypothalamic–pituitary–adrenal axis (Almli, Fani,

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Figure 1. Study design. Participants were grouped by score on the Post-traumatic Stress Disorder Checklist–Military Version (PCL-M) at both time points assessed. In the longitudinal analysis, changes to methylation patterns in the posttraumatic stress disorder (PTSD)-onset group between time points (B and E) were analyzed and then compared with changes in the control group between time points (A and D). In the cross-sectional analysis, methylation patterns of all participants who met PTSD criteria at the follow-up time point (E and F) were compared to controls at follow-up (D).

Smith, & Ressler, 2014; Rampp, Binder, & Provencal, 2014). The expression of these genes is modulated by many epigenetic mechanisms. DNA methylation, a major epigenetic mechanism for regulating gene expression, occurs by the covalent transfer of a methyl group to the five position of cytosine residues of DNA by DNA methyltransferases, which results in differential gene activity (Robertson, 2005). Canonically, increased DNA methylation (hypermethylation) in the promoter region of a gene reduces gene expression, whereas reduced DNA methylation (hypomethylation) increases gene expression.

Various lifetime experiences may alter DNA methylation profiles, which then affect downstream expression of genes (Rampp et al., 2014). Traumatic events from deployment or war could cause DNA methylation changes, which could then affect PTSD symptoms (Almli et al., 2014). In military personnel, increased methylation of *NR3C1-1F* promoter was related to more severe symptoms of PTSD (Yehuda et al., 2015), and decreased methylation of the *FKBP5* gene exon 1 (*FKBP51*) promoter region was associated with recovery from PTSD after psychotherapy (Yehuda et al., 2013). Methylation of *SKA2* was associated with more severe PTSD symptoms and with cortical stress reactivity in veterans (Boks et al., 2016; Sadeh et al., 2016).

Although DNA methylation is associated with PTSD in military personnel and veterans, much less is known regarding the role of methylation in the onset of PTSD or increases in symptom severity at the whole genome level. To address this, in the present pilot study, we examined a cohort of military personnel returning from deployment and hypothesized that changes in DNA methylation would relate to clinically relevant increases in PTSD symptoms over a 3-month period.

Materials and Method

Participants

This pilot study was a case–control comparison between 2 time points based on PTSD symptom change among active-duty

personnel (Figure 1 illustrates the study design). The institutional review board approved this study prior to data collection, and we obtained written informed consent from all participants. This sample was part of a larger military cohort recruited from a sleep clinic at Madigan Army Medical Center (Mysliwiec et al., 2013). Participants were active-duty military personnel who had returned from deployment to Afghanistan (Operation Enduring Freedom) or Iraq (Operation Iraqi Freedom) within the prior 18 months and were seeking care for sleep disturbances. Exclusion criteria included (1) history of drug or alcohol abuse in the previous year, (2) current severe medical conditions requiring chronic treatment (e.g., cancer, diabetes, HIV, and autoimmune disorders) or a severe psychiatric condition (i.e., schizophrenia, bipolar disorder), and (3) severe neurological disorders (e.g., multiple sclerosis, seizure disorders, and history of stroke). Because PTSD in military populations often co-occurs with combat injuries like traumatic brain injury (TBI) and comorbid psychological disorders such as depression, we did not exclude subjects with these conditions.

For the longitudinal analysis, we compared changes in DNA methylation from baseline to 3-month follow-up between participants whose symptoms did not meet the criteria for PTSD at baseline but had worsened to meet those criteria by follow-up (PTSD onset) and participants whose symptoms did not meet the criteria for PTSD at either time point (no-PTSD controls). For the cross-sectional analysis, we compared methylation patterns at follow-up between all participants whose symptoms met the criteria for PTSD at follow-up (PTSD) and whose symptoms did not meet these criteria at follow-up (no-PTSD controls).

Clinical Symptoms Measurement

We measured PTSD symptoms using the PTSD Checklist Military Version (PCL-M). Possible score ranges from 17 (lowest severity) to 85 (highest severity), with a score of

50 or more indicating a clinical diagnosis of PTSD as determined by a structured clinical interview. In the present study, we conformed to the military standard that a score of 50 or higher is a diagnostic indicator of PTSD and grouped participants based on this 50-point cutoff. We measured depression symptoms using the Quick Inventory of Depressive Symptomatology (QIDS; Rush et al., 2003). We administered the PCL-M and QIDS at both baseline and follow-up. Participants identified TBI via self-reporting on the Warrior Administered Retrospective Casualty Assessment Tool (Terrio, Nelson, Betthausen, Harwood, & Brenner, 2011).

Genome-wide DNA Methylation Profiling Using MeDIP-seq

We collected peripheral blood at baseline and follow-up from nonfasting participants. Genomic DNA was extracted from whole blood using QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. To obtain the methylated DNA, 2.5 μ g of isolated genomic DNA was fragmented down to 250 bp using an M220 Focused-ultrasonicator (Covaris, Woburn, MA), then 1 μ g fragmented DNA was processed with MethylMiner Methylated DNA Enrichment Kit (Invitrogen, Carlsbad, CA), which resulted in selection, via immunoprecipitation, of DNA fragments containing methylated CpG. Enriched methylated-DNA fragments (10 ng) were used for a DNA library preparation with the TruSeq Nano DNA kit (Illumina, San Diego, CA). The HiSeq 2500 system was used to sequence with a paired-end method, where both ends of a DNA fragment are sequenced, with 101 base pairs read from each end of the fragment with TruSeq Rapid SBS kit (Illumina) for the sequencing of the DNA library. Sequencing resolution allows identification of regions that are methylated, which were mapped to the gene level during the analysis. Macrogen Corp. (Rockville, MD) performed DNA library preparation and sequencing.

Statistical Analysis

To compare demographic and clinical characteristics between participants with PTSD and the no-PTSD controls and between participants with PTSD onset during the study and the no-PTSD controls, we employed two-tailed χ^2 tests and t tests using SPSS Version 22.0 (SPSS Inc., Armonk, New York). We compared symptom inventory scores between groups using independent samples t tests and TBI using χ^2 test. We considered differences to be statistically significant when $p < .05$.

We assessed base call quality on FASTQ files, which is a raw data format that contains unaligned reads with a quality score for each base call, using FASTQC Version 0.10.1. Reads were aligned to the reference human genome of hg19 from UCSC using a short read aligner, BWA Version 0.7.8. Peaks were called using MACS Version 2.1.0.20140616 to identify DNA regions that were methylated. For identification of differentially methylated DNA regions between the participants with PTSD and the control group in the cross-sectional analysis as

well as baseline and follow-up in the longitudinal analysis, we used the DiffBind (Version 2.6.6) software package. The binding affinity matrix was generated by counting sequenced reads overlapping intervals in peak sets. A differential binding affinity analysis was employed to identify statistically significant differences in methylated peak intervals between groups with a false discovery rate (FDR) = 0.10. We used a higher FDR cutoff than the conventionally used value of 0.05 to reduce the false-negative rate considering this pilot study's small sample size. To identify molecular pathways related to the differentially methylated genes as well as top-gene networks implicated in these gene-regulation changes associated with PTSD, we used QIAGEN's Ingenuity Pathway Analysis (IPA, QIAGEN, Venlo, Limburg, the Netherlands). (Details of these analyses are provided in the Online Supplementary Appendix.)

Results

Demographic and Clinical Characteristics

Longitudinal analysis. Our longitudinal analysis included PTSD-onset participants ($n = 4$), whose PCL-M scores increased between the baseline and follow-up assessments to above the 50-point threshold (mean = 46.25, standard deviation [SD] = 2.9 at baseline; mean = 59.25, $SD = 3.5$ at follow-up), with an increase of >10 points (Δ mean = 13, Δ SD = 1.63) in each participant and no-PTSD control participants ($n = 6$). Participants in this analysis were an average of 39.0 years old ($SD = 7.6$), were male, and were 50% Caucasian. No-PTSD control subjects had PCL-M scores ≤ 25 at both time points (mean = 23.33, $SD = 1.6$; mean = 22.67, $SD = 1.6$, respectively). There were no significant differences between groups in demographic characteristics including age, body mass index, race, education, medication use, and the number of deployments. Significantly more participants reported a previous TBI in the PTSD-onset group (100% in PTSD-onset group and 16.7% in controls, $p = .003$). PCL-M and QIDS scores were significantly higher in the PTSD-onset group as compared to the no-PTSD controls at both time points.

Cross-sectional analysis. At the time point assessed for this analysis (3-month follow-up), PTSD participants ($n = 8$) had a PCL-M score ≥ 50 (mean = 60.38, $SD = 5.5$), while no-PTSD controls ($n = 6$) had a PCL-M score less than or equal to 25 (mean = 22.67, $SD = 1.6$). Demographic and clinical characteristics of the participants included in the cross-sectional analysis ($n = 14$) are shown in Table 1. There were no significant differences in the demographic characteristics between the two groups. However, the PTSD group had significantly higher scores on both the PCL-M and the QIDS at baseline and follow-up.

Differentially Methylated Genes

Longitudinal analysis. We observed significant alterations in methylation from baseline to the 3-month follow-up in participants in the PTSD-onset group, while in the control group only

Table 1. Demographic and Clinical Characteristics for the PTSD and no-PTSD Control Groups.

Characteristic	Total (n = 14)	PTSD ^a (n = 8)	No-PTSD ^b (n = 6)	χ^2/t	p Value
Age (years), M (SD)	35.07 (8.9)	32.25 (8.9)	38.83 (8.1)	1.423	.180
Sex (males), n (%)	14 (100.0)	8 (100.0)	6 (100.0)		
Race (White) n (%)	6 (46.2)	4 (50.0)	2 (33.3)	0.737	.592
Education (years), M (SD)	14.14 (2.1)	14.38 (3.2)	13.83 (1.0)	−0.453	.658
BMI (kg/m ²), M (SD)	30.63 (3.7)	30.20 (4.2)	31.23 (3.2)	0.520	.613
Medication use, ^c n (%)	8 (57.1)	5 (71.4)	3 (50)	0.627	.592
Number of deployments, M (SD)	2.21 (1.2)	1.75 (1.0)	2.83 (1.2)	1.835	.091
mTBI diagnosis, n (%)	9 (64.3)	8 (100.0)	1 (16.7)	10.370	.003
PCL-M, M (SD)					
Baseline	40.36 (16.6)	53.13 (8.7)	23.33 (1.6)		<.001
Follow-up	44.21 (19.8)	60.38 (5.5)	22.67 (1.6)	−16.117	<.001
QIDS, M (SD)					
Baseline	11.14 (5.5)	15.13 (3.0)	5.83 (2.6)		<.001
Follow-up	11.43 (5.3)	15.38 (2.3)	6.17 (2.6)	−7.024	<.001

Note. BMI = body mass index; mTBI = mild traumatic brain injury; QIDS = Quick Inventory of Depressive Symptomatology; PCL-M = PTSD Checklist–Military Version; PTSD = posttraumatic stress disorder.

^aPTSD group had PCL-M scores ≥ 50 at follow-up. ^bNo-PTSD control group had PCL-M scores ≤ 25 at follow-up. ^cMedications included antidepressants, antipsychotics, benzodiazepines, nonbenzodiazepines, narcotics, and prazosin.

Table 2. Top Genes With Decreased Methylation From Baseline to Follow Up in the PTSD-Onset Group.

Gene Symbol	Description	Adjusted p Value (FDR)	Fold Change
<i>ROCK1P1</i>	Rho-associated, coiled-coil containing protein kinase I pseudogene 1	.02223	6.41446
<i>EPPK1</i>	Epiplakin 1, mRNA	.02632	6.09928
<i>NFATC1</i>	Nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 1, transcript variant 2	.04530	5.73130
<i>USP41</i>	Ubiquitin-specific peptidase 41	.04053	5.55615
<i>JAKMIP3</i>	Janus kinase and microtubule interacting protein 3	.03204	5.46414
<i>INTS1</i>	Integrator complex subunit 1	.03204	5.38736
<i>SLC12A7</i>	Solute carrier family 12 (potassium/chloride transporters), member 7	.04528	5.34616
<i>COL6A1</i>	Collagen, type VI, $\alpha 1$	.04530	5.30660
<i>KIAA1530</i>	KIAA1530	.02223	5.30433
<i>CSNK1G2</i>	Casein kinase I, $\gamma 2$	.05454	5.30192

Note. PTSD-onset group had scores on the PTSD Checklist–Military Version (PCL-M) < 50 at baseline (i.e., no diagnosis of PTSD) and ≥ 50 at follow-up (i.e., diagnosis of PTSD). FDR = false discovery rate; PTSD = posttraumatic stress disorder.

one nonoverlapping region showed statistically significant DNA methylation changes between time points. There were 124 regions, which corresponded to 119 genes, that significantly decreased in methylation from baseline to follow-up in the PTSD-onset group (see Table 2 for top 10 genes, full gene list in Online Supplemental Table 1). The top canonical pathways, according to IPA analysis, for the genes that decreased in methylation included netrin signaling, Wnt/Ca⁺ pathway, and axonal guidance signaling (Table 3). Conversely, there were eight genes that significantly increased in methylation between baseline and follow-up in the PTSD-onset group (Table 4). As

Table 3. Ingenuity Pathway Analysis (IPA) Canonical Pathways of Differentially Methylated Genes in the PTSD-onset Group.

Pathway	p Value	Overlap
Netrin signaling	.00111	7.7% (3/39)
G protein signaling	.00259	3.7% (4/109)
Wnt/Ca ⁺ pathway	.00315	5.4% (3/56)
Huntington's disease signaling	.00711	2.2% (5/229)
Axonal guidance signaling	.00783	1.6% (7/434)

Note. Posttraumatic stress disorder (PTSD)-onset group had scores on the PTSD Checklist–Military Version (PCL-M) < 50 at baseline (i.e., no diagnosis of PTSD) and ≥ 50 at follow-up (i.e., diagnosis of PTSD).

Table 4. Genes With Increased Methylation From Baseline to Follow Up in the PTSD-Onset Group.

Gene Symbol	Description	Adjusted p Value (FDR)	Fold Change
<i>ANKRD30BL</i>	Ankyrin repeat domain 30B-like	.08837	5.5588
<i>AK125749</i>	cDNA FLJ43761 fis, clone TEST12048109	.03204	4.3178
<i>GRK4</i>	G protein-coupled receptor kinase 4	.04710	3.9876
<i>LRPAP1</i>	LDL receptor related protein associated protein 1	.04710	3.9381
<i>NCS1</i>	Neuronal calcium sensor 1	.04530	3.8335
<i>PCYOX1</i>	Prenylcysteine oxidase 1	.07389	3.6540
<i>ZFH3</i>	Zinc finger homeobox 3	.06857	3.7169
<i>GJC1</i>	Gap junction protein gamma 1	.07342	3.3321

Note. PTSD-onset group had scores on the PTSD Checklist–Military Version (PCL-M) < 50 at baseline (i.e., no diagnosis of PTSD) and ≥ 50 at follow-up (i.e., diagnosis of PTSD). FDR = false discovery rate; PTSD = posttraumatic stress disorder.

mentioned above, one unique gene interval in the intronic region of *FAM178B* had significantly increased methylation between baseline and follow-up in the controls only.

Cross-sectional analysis. In all PTSD subjects at follow-up, 10 genes were significantly hypermethylated compared to controls at follow-up, including *METTL16*, *TMEM242*, and *PNPLA7*, and 18 genes were significantly hypomethylated, including *MYO1D*, *FLJ43860*, and *DLGAP1* (see Online Supplemental Tables 2 and 3 for full list and details).

Discussion

In this pilot study, we have reported the novel finding that PTSD onset in military personnel is related to changes in DNA methylation as well as additional findings congruent with previous reports that DNA methylation profiles are associated with PTSD when compared to non-PTSD controls. Specifically, DNA methylation changed in participants who developed PTSD over a 3-month period in pathways related to axon guidance. In our cross-sectional analysis comparing PTSD to control subjects, a number of genes were differentially methylated, including ubiquitin-specific protease 17 (*USP17*). These pilot findings provide novel insights into the role of epigenetics in PTSD symptoms in a young cohort of military personnel and may ultimately provide targets for future research on DNA methylation and PTSD.

Of the genes that were differentially methylated between baseline and follow-up in participants whose PTSD symptoms worsened over time, the majority (93.7%) exhibited decreased methylation including netrin-5, zinc and ring finger 3 (*ZNRF3*), and frizzled class receptor 9 (*FZD9*). Netrin-5 is of interest because members of the netrin family are essential for synaptic plasticity, primarily through the regulation of growth-cone signaling (Horn et al., 2013; Ziel & Sherwood, 2010). According to the mouse models, netrin-5 is expressed in the adult hippocampus and corpus callosum and is involved in neurogenesis (Yamagishi et al., 2015). Since PTSD is associated with hippocampal volume and function changes (Fragkaki, Thomaes, & Sijbrandij, 2016), this finding suggests that netrin-5 may be related to these pathological changes. *ZNRF3*, an E3 ubiquitin-protein ligase, regulates the Wnt signaling pathway by mediating the ubiquitination of Wnt receptors including receptors in the frizzled family (Jiang, Charlat, Zamponi, Yang, & Cong, 2015; Peng et al., 2013). *FZD9* is one such receptor protein involved in Wnt signaling. It is crucial for hippocampal development, and its continued expression in the adult hippocampus (Zhao et al., 2005; Zhao & Pleasure, 2004) suggests that *FZD9* contributes to neural plasticity. These pilot findings suggest that altered methylation patterns of genes in the Wnt signaling network may be associated with PTSD symptoms.

Our findings suggest that changes in methylation contribute to PTSD onset and implicate genes related to altered activation of axonal guidance. Axonal guidance signaling, which functions through interaction with netrin signaling and the Wnt signaling pathways, plays important roles in neural plasticity through direction of neuronal growth cones (Fortress & Frick, 2016; Guthrie, 2007; Maguschak & Ressler, 2011). Disruption to the Wnt signaling pathway has been linked to psychological disorders, including depression and anxiety (Freese, Pino, &

Pleasure, 2010), and to the onset of PTSD in a preclinical model (Maguschak & Ressler, 2012). The connection between Wnt signaling and memory formation, the Wnt signaling pathway's role in neuroplasticity, and localization of Wnt signaling molecules in the hippocampus suggest that this pathway may direct some of the neuronal changes associated with PTSD. Given the changes in neural volume observed in patients with PTSD (Morey et al., 2012), particularly in the hippocampus and amygdala, it may be that differential regulation of axonal guidance and related signaling pathways affect neurological alterations and should be explored further as a potential mechanism of PTSD pathology.

Our findings in the present pilot study also suggest that differential methylation of ubiquitin-specific proteases is associated with both increases in PTSD symptoms over time and differences between PTSD and control subjects. Ubiquitin activity is essential for the degradation of proteins that accumulate within neurons and compromise functioning and integrity. *USP17*, which codes for the deubiquitinating enzyme USP17 and was significantly hypermethylated in the PTSD group, regulates the nuclear function and stability of interleukin 33 (Ni et al., 2015), which is highly expressed in the brain and known to have a critical role in the pathogenesis of Alzheimer's disease (Fu et al., 2016). Studies have also demonstrated the effect of the ubiquitin pathway in neurodegenerative diseases and brain injury, showing that sufficient regulation is essential for neuronal protection and recovery (Atkin & Paulson, 2014). Thus, the present pilot data suggest that ubiquitin pathways are implicated in PTSD and should be further investigated.

The current pilot study has inherent limitations that temper the conclusions that we can draw from these data. The sample was small and consisted of relatively young males in the military. As such, statistical limitations in the cross-sectional analysis make it difficult to confirm that differences in methylation between groups were not driven in part by comorbid conditions such as depression symptoms or self-reported TBI or confounded by individual patient characteristics. We utilized an inventory of PTSD symptoms rather than a clinical diagnosis. While the PCL-M approximates a PTSD diagnosis and allows quantification of changes in symptom severity over time, it is not synonymous with a clinical diagnosis of PTSD. MeDIP-seq does not provide high enough resolution to identify which specific CpG are methylated; instead, it provides several hundred base pair regions of DNA that contain methylated CpG. We extracted methylated DNA from whole blood to improve clinical biomarker applications; therefore, cell heterogeneity needs to be considered in future DNA methylation studies to form conclusions about specific mechanisms contributing to PTSD symptoms. Nonetheless, the longitudinal analysis we performed in the current study in conjunction with the symptom measurement is a unique strength because it allowed exploration of the association between gene methylation and changes to PTSD symptomology.

Our findings suggest that differential methylation of genes is associated with the development of PTSD symptoms over time

and implicate the Wnt and axonal guidance pathways as well as ubiquitin genes. These results provide a starting point for future research to determine biomarkers of PTSD and understand the role of DNA methylation as a regulatory mechanism of PTSD.

The data in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO series accession number GSE93673 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE93673>).

Author Contributions

Christiana Martin and Young-Eun Cho contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Hyungsuk Kim contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Sijung Yun contributed to acquisition and analysis, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Rebekah Kanefsky contributed to interpretation, drafted the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Hyunhwa Lee contributed to conception and design, drafted the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Vincent Mysliwiec contributed to acquisition, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Ann Cashion contributed to interpretation, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. Jessica Gill contributed to conception, design, acquisition, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was supported by the Intramural Department of the National Institutes of Health, National Institute of Nursing Research. This study was also funded, in part, by the Center for Neuroscience and Regenerative Medicine (Grant 60855).

Supplemental Material

Supplementary material for this article is available online.

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