# Sleep and Symptomology after Spinal Cord Injury

by

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#### **1.0 Proposal Introduction**

Traumatic spinal cord injury (TSCI) is a condition that has widespread impact across age, ethnic and socioeconomic backgrounds and commonly the result of motor vehicle accidents, falls or workplace hazards. TSCI is a serious condition with consequences on health-related quality of life, but also disparaging financial implications. Historically, TSCI commonly occurred in young adults, however, the median age= 42.5 years, illustrates the recent trend in increased incidence among middle and older adults (Mestre, Alkon, Salazar, Ibarra, 2011). With an incidence of 17,000 new cases annually, a prevalence of approximately 240,000-250,000 persons living with SCI, and improvement in life expectancy, there is a greater aging TSCI population with motor and sensory impairment (Jain, Ayers, Peterson, Harris, Morse, O'Connor, & Garshick, 2015; Noonan, Fallah, Park, Dumont, Leblond, Cobb, & Noreau, 2014) and increased risk for secondary health complications (Jensen, Truitt, Schomer, Yorkston, Baylor & Molton, 2013). According to the literature secondary health complications occur as a result of the primary cause of disability, are more prevalent in people with disability, and are associated with immune impairment and chronic inflammation (Molton, Terrill, Smith, Yorkston, Alschuler, Ehde, & Jensen, 2014; Allison & Ditor, 2015). With dependence on the spine for posture, shock absorption, and maintaining a

protective covering of the spinal cord and the network of nerves and communication pathways between the brain and the periphery, the need to improve patient health outcomes and reduce functional impairments of symptoms post TSCI is a critical issue that needs to be addressed.

Sleep of adequate duration and good quality is required for health and human behavior. The regulatory activities of sleep are critical for brain performance, healing and repairing of cells in the body, as well as other regulatory functions of the metabolic and cardiovascular systems (Brain Basics, NINDS, 2017). Sleep is in part governed by genetics and includes 1) sleep need (daily amount of sleep required to feel rested); 2) homeostatic regulation of sleep (the response to sleep deprivation); and 3) circadian regulation of sleep (how the occurrence of sleep is timed relative to the 24-hr cycle) (Cirelli, 2005). Each of these regulation mechanisms have different responsibilities in sleep activity but their roles are not exclusive of each other. An imbalance in one mechanism impacts the function of the others. Particularly in victims of TSCI, sleep disturbances have significant implications on short- and long-term outcomes, such as cord repair and recovery, symptom experience (Almutary, Douglas, & Bonner, 2016), and health related-quality of life (Crocker & Sehgal, 2010); however, a review of the literature demonstrates that there is a dearth of research on sleep disturbance not related to sleep disordered breathing in patients with TSCI.

While there is little literature specific to TSCI, disturbed sleep pattern is a common symptom that correlates with other symptoms across populations (Allison & Ditor, 2015). Sleep disturbance is a common complaint among persons with chronic pain and in SCI populations (Alshuler, Jensen, Sullivan-Singh, Borson, Smith, & Molton, 2013; Gioannocaaro, Moghadam, Pizza, Boriani, Maraldi, Avonic, et al, 2013). This is an important association because it could explain common biological pathways shared by sleep and other symptoms; moreover, lack of sleep is associated with poor pain tolerance, depressive symptoms and anxiety (Almutary, Douglas, & Bonner, 2016). Understanding that sleep plays a major role in healing, repair, and reconciling mental processes, in the general population, there is an even more critical need to establish this link in the period following TSCI. Thus, having a better understanding of sleep and other symptoms within the context of TSCI as well as increased comprehension of the underlying mechanism likely shared by inflammation and the co-occurring symptoms of sleep, pain, anxiety

and depression can support development of the best strategies to manage symptom burden and improve capacity for life-long management of TSCI.

This study will use an innovative, objective, EEG based continuous monitoring system, to assess sleep duration, sleep efficiency and sleep architecture in patients who recently suffered a TSCI, in addition to subjective symptom reports of impaired sleep. The use of an epigenomic approach is particularly promising for this project because it allows the dynamic nature of sleep and symptom phenotypes to be addressed using measures that can be similarly dynamic (i.e. DNA methylation). Candidate genes regulated by DNA methylation and important to sleep regulation (PER2) and inflammation (IL-1 beta) will specifically be explored.

#### **1.1 Specific Aims**

**Specific Aim 1**: Phenotype sleep and describe co-occurring symptoms in TSCI patients during the in-hospital rehabilitation phase of recovery.

Hypothesis- During the in-hospital rehabilitation period, patients will experience sleep disturbance and also report one or more co-occurring symptom including pain, anxiety, and/or depression.

**Specific Aim 2**: Evaluate the ability of DNA methylation profiles for candidate genes in the inflammatory and sleep regulation pathways to explain variation in sleep disturbance and cooccurring symptoms in TSCI patients during the in-hospital rehabilitation phase of recovery.

Hypothesis- DNA methylation profiles indicative of a pro-inflammatory state and sleep disturbance will be related to variability in sleep and co-occurring symptom phenotypes.

#### 1.2 Background

Sleep is a complex phenomenon that impacts cognitive function, emotional well-being, and biological processes required for healthy functioning. Sleep plays a critical role in neuron communication and waste removal of toxins that accumulate in the brain during wakefulness, particularly for neurologic disorders and diseases such as spinal cord injury and traumatic brain injury (Brain Basics, NINDS, 2017; Borbely, Daan, Wirz-Justics, Deboer, 2016). Sleep is usually divided into "sleep architecture" that is classified as non-rapid eye movement (NREM) and rapid eye movement (REM). NREM sleep is further categorized as N1 (transition from wake to sleep), N2 (light, frequently non-restorative sleep), and N3 (deeper, physical restorative "slow wave" sleep. REM sleep is required for mental restoration including consolidation of learning and mood. Impaired sleep architecture has far reaching implications on recovery and repair of the spinal cord and neural plasticity. When sleep is disturbed, the consequences are substantial affecting the performance of every organ system. Likewise, environmental factors, such as stress, and temperature also play a role in sleep regulation and the biochemical response to the need for sleep.

TSCI is characterized by two phases of injury, the primary injury is the mechanical damage to the spinal cord, and the secondary injury is the sequelae of biochemical changes that impact homeostasis regulation and the cellular bionetwork of every organ system (Hausmann, 2003; Hunter, 2012; Fogelberg, Leland, Blanchard, Rich, & Clark, 2017). Inflammation is initiated after acute TSCI and continues into the chronic period and significantly impacts biological changes central to symptom and secondary health complication development. It is believed that systemic inflammatory response targets inflammatory responsive neurons in the central nervous system that produces co-occurrence of symptoms (Cleeland, Bennett, Dantzer...2003; Ma, Fang, Liu, & Zhou, 2015). While there has been some progress in understanding the biological mechanisms that occurs

in the secondary phase there remains a gap in the literature that focuses on the biology of symptom genesis and bio-behavioral response with a particular focus on sleep (Gioannocaaro, Moghadam, Pizza, Boriani, Maraldi, Avonic, et al, 2013). This project aims to further explore one source of individual biologic variability, gene methylation, in the relationship between sleep and co-occurring symptoms after TSCI. Given the impact TSCI has on every organ system, symptom management remains a huge issue in this population. With unique interruptions to sleep such as spasticity, bladder management, and breathing related sleep interference; sleep fragmentation and sleep-wake dysregulation, can pose significant challenges on the sleep health in TSCI.

Sleep dysfunction in TSCI has been found highly prevalent, in individuals with tetraplegia (C1-8) and some individuals with paraplegia (T1-L5) at least 1 year post injury, or in chronic TSCI (Budh, Hulting, & Lundeberg, 2005). Typically studies of the sleep experience in TSCI, are based on patient self-report and are often over or under estimates of perceived adequacy of sleep, as participant perspectives are influenced by age, level of injury, and co-presence of other symptoms. Barriers or predictors of impaired sleep include pain, anxiety, emotional distress, spasticity, bladder management, and repositioning; these findings are primarily by self-report, in the chronic phase (>1 year post injury) (Avluk, Gurcay, Gurcay, Karaahment, Tamkan, & Cakci, 2014; Thijssen, Eijsvogels, Hesse, Ballak, Atkinson, & Hopman, 2010).

Much of the literature speaks to sleep disordered breathing (SDB), an umbrella term for conditions related to pauses in breathing accompanied by snoring, such as sleep apnea, that results in interference in daytime activities and daytime fatigue. SDB is a very common, condition in in the TSCI population, given the baseline of poor chest wall compliance and reduced inspiratory/expiratory function (causing hypoventilation and hypercapnia), as a result of unopposed parasympathetic system promoting airway narrowing, changes in neck circumference and weakened motor innervation of diaphragm and intercostal muscles (Sankari, Vaughan, Bascom, Martin, & Badr, 2019; Gioannocaaro, Moghadam, Pizza, Boriani, Maraldi, Avonic, et al, 2013; Chiodo, Sitrin, & Bauman, 2016). However, it should be noted that despite the growing research on SDB in this population there still remains mixed findings. Two studies showed preliminary evidence that individuals with tetraplegia suffer impaired sleep quality as a result of poor core body temperature control (Thijssen, Eijsvogels, Hesse, Ballak, Atkinson, & Hopman, 2010) as well as, a reduction or absence of melatonin (Scheer, Zeitzer, Ayas, Brown, Czeisler, & Shea, 2006), however limitations of these studies were small sample sizes (n=24; n=5) that findings cannot be generalized. There has been no previous study of the genetic influence on sleep in this population during the subacute period. Despite the fragmented findings of sleep dysfunction in TSCI, there is clear evidence in non-TSCI that poor sleep health is linked to many chronic health issues and therefore needs to be addressed in this population to improve health-related quality of life and decrease further secondary health complications.

There is a growing body of evidence related to omic variability associated with symptoms and quality of life reports by those with chronic illness (Cashion & Grady, 2015). Epigenetics, is defined as changes outside of DNA sequence (environmental influence) that can contribute to micro and macro mechanistic processes that create phenotypic diversity within individuals and populations (Jin, Li, & Roberston, 2011; Aran, Toperoff, Rosenberg, & Hellman, 2011; Moore, Le, & Fan, 2013). One primary epigenetic process is DNA methylation. DNA methylation occurs as a result of the addition (or removal) of a methyl group to where cytosine bases occur on DNA, and plays a critical role in maintaining gene expression patterns through cell divisions (Aran, Toperoff, Rosenberg & Hellman, 2011; Moore, Le, & Fan, 2013). Many proteins are effectively prevented from high-affinity interaction with their cognate DNA recognition sequences when those loci are methylated (Shimbo and Wade, 2016). Methylation causes reduced transcription and reduced protein levels, when unmethylated, CpG island sites are open to transcription and expression (Fessele & Wright, 2018). Predominantly in the brain, it is believed there are greater levels of DNA methyltransferases (Dnmts), which are responsible for initiating the transfer of the methyl group to the cytosine/guanine sequence, and this has significance for individuals suffering tissue injury, such as TSCI, as improper DNA methylation can yield severe secondary health and behavioral outcomes (Moore, Le, & Fan, 2013). While all epigenetic mechanisms could be entertained for this study, DNA methylation was chosen over other epigenetic mechanisms because data from the literature supports dynamic changes in DNA methylation post injury. Focusing on DNA methylation over DNA polymorphisms was primarily due to the larger number of participants needed to conduct a polymorphism-based study versus DNA methylation.

# **1.3 Scientific Premise**

It is clear across oncology literature that symptoms do not occur singularly, but often in the presence of other symptoms or contribute to the development of other symptoms (Miaskowski & Aouizerat, 2007). Similarly, in TSCI depression and chronic pain are often reported together in quality of life (QoL) surveys, and increased pain is associated with increased depression, decreased social functioning, increased disability, and decreased perceived health (Revell-Hunter, 2011). It is reasonable to postulate that symptoms, such as pain, mood disturbance, and sleep, derived from central nervous (CNS) and peripheral nervous systems (PNS) control will likely co-occur, as the CNS and PNS are regulated by brain function. Changes, whether pathological (mechanical trauma)

or neurochemical (inflammation/immune), or both will likely result in variability related to the mechanisms that underlie these symptoms.

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Inflammation occurs post TSCI in the acute, subacute and chronic phases of progression and inflammation is linked to depression, neuropathic pain, immunosuppression, adrenal insufficiencies, and metabolic disorders, likely as a result of a complex bidirectional communicatory pathway between the inflammatory response and immune impairment (Bowes & Yip, 2014). Specifically, immune impairment (elevated serum/blood antibodies) and chronic inflammation (elevated serum/blood pro-inflammatory cytokine levels) were found in individuals both symptomatic and asymptomatic for secondary health conditions after TSCI (Allison & Ditor, 2015). This increase in circulating cytokines, yields an increase in stress response genes such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interlukin-6 (IL6), interlukin-1 alpha (IL1- $\alpha$ ), which contribute to cell surface receptors for pain, inflammatory response to tissue injury and apoptosis. According to Zhang & An (2009), IL-1beta has heightened expression following trauma and is associated with pathological pain states (pg. 28). Despite some gains in understanding the biological mechanisms that underlie TSCI, there is still much largely unknown.

DNA methylation is a dynamic marker that can be analyzed in blood samples overtime to identify changes that are associated with the presence of objective and subjectively reported symptom profiles including sleep disturbances, pain, anxiety and depressive symptoms. It was believed that a single gene regulated sleep, however, it is now recognized that contributions to sleep-wake and circadian systems are the result of multiple genes. Cells maintain a 24hr rhythm that involves molecular feedback loops, that influence transcription of several circadian genes such as Cryptochrome (Cry) 1& 2, and Period (Per) 1 & 2, which result in changes to sleep amount and time of sleep onset (Crocker & Sehgal, 2010). Moreover, in the presence of increased stress

response, sleep-wake and circadian genes likely, experience maladaptive activation or inactivation of regulatory mechanisms and transcription resulting in dysfunctional sleeping patterns and poor sleep quality; this impacts sleep but also related biobehavioral sleep functions clinically linked to symptom expression. Having a good understanding of the transcriptional response of cells involved in sleep regulation is critical to targeting common gene expression patterns and their underlying regulatory mechanisms that could illuminate targets for early interventions to improve sleep and symptom experience.

#### 1.4 Scientific Gaps in Knowledge

While much of the research around TSCI is aimed at improving outcomes through surgical intervention and cord regeneration, there is a paucity of studies that have investigated sleep, other symptoms and the biology that is likely shared by coexisting symptoms (Barsevick, 2007; Cashion & Grady, 2015; Cleeland, Bennett, Dantzer, et al, 2003; Miaskowski & Aouizerat, 2007). Particularly in the subacute rehabilitation phase of recovery there are several endogenous and exogenous influences on sleep quality that also impact recovery and healing and are believed to influence the homeostatic regulation of sleep and the subsequent development of other symptoms. Few if any studies have approached the multifactorial changes after acute TSCI through omics approaches or epigenetic changes (i.e. methylation) of genes related to symptom expression during the acute or subacute phase of recovery in human populations. However, it is difficult to assess biological changes in the presence of spinal shock and/or neurogenic shock, which immediately follows primary injury; the subacute period provides a stable environment to observe variation that reflects the new baseline for individuals post-injury and a valuable target for improving quality of

life. Given the variability within TSCI, epigenetics offers an innovative way to explain variability in symptoms experienced and their severity. This research will fill this gap and move the science forward by objectively and subjectively characterizing the sleep experience of TSCI patients and correlate those with other symptom profiles, while examining a potential common biological mechanism connecting these symptoms.

# **1.5 Innovation**

Innovation of this project lies in the novelty of the timeframe under study, cutting edge technology to measure sleep, and inclusion of biological mechanisms to understand variability in sleep in this patient population. No studies of TSCI in the subacute window of time have used real time monitoring of sleep architecture. Self-reports of sleep problems only alert practitioners to the concern of a problem and do not offer an individualized understanding of where in the activity of sleep problems are occurring, as well as how this is affecting or being affected by other co-occurring symptoms. The EEG focused Sleep Profiler, maintains the foundational mechanism found in polysomnography (PSG), which is the gold standard in identifying sleep disturbances. In comparative studies, the Sleep Profiler and standard polysomnography EEG data were scored by a board-certified neurologist and the algorithm auto scoring software, resulting in similar percent agreement (wake stage=.92; N1=.89; N2=.78; N3=.86; REM=.85) (Finan, Richards, Gamaldo, Han, Leoutsakos, Salas, & Smith, 2016). The Sleep Profiler overcomes the challenge of ecological disruptions and care related needs limiting traditional PSG and limitations of absence of movement (paralysis) and inability to measure sleep staging, in actigraphy monitoring. We are able to

compare results of self-report to real time objective changes in sleep quality and biological examination.

By approaching this study using the NIH symptom science model, we are able to use biological and clinical data to characterize sleep and symptom experience in TSCI into a phenotype; then apply epigenetic methodologies to illuminate targets for future therapeutic interventions (Hausmann, 2003). Despite the advances in surgical and pharmacologic interventions in the immediate management of TSCI, and the resultant increased survival and survivorship, few studies focus on improving health related and/or overall quality of life from a symptom management focus. The challenge of recovery and rehabilitation for affected persons is ongoing. The innovation of this study has the potential to be the impetus of change in TSCI care by 1.) increasing sleep disturbance and co-occurring symptom awareness and recognition during rehabilitation/recovery in this population 2.) generating findings that will support future studies aimed at therapeutic interventions; 3.) laying the groundwork for omics driven clinical application at the bedside. Taking steps to improve symptom management in the TSCI population will improve quality of life facilitating return to engagement with family, community and society at large, and decrease rehospitalizations, complications and fiscal burdens to the healthcare and family/caregiver system.

## **1.6 Design and Methods**

The proposed study will utilize an observational prospective study design, including objective and self-reported sleep data, extensive symptom phenotype data and biological marker (DNA methylation) data to identify correlation between sleep and co-occurring symptoms and

explore a plausible biological mechanism for such interactions in the subacute rehabilitation phase (30 days post TSCI). While there will be repeated assessments of subjective sleep, pain, and DNA methylation (day 1 and day 2), objective sleep analysis will focus only on time point two. According to the sleep literature, night one of a sleep study is considered "acclimation" to the test equipment and the assumption is that sleep patterns will not reflect that of a "normal" night sleep; thus, night one is not typically considered in the final analysis (Finan, Richards, Gamaldo, Han, Leoutsakos, Salas, et al, 2016). However, given the exploratory nature of this pilot, if it is determined there is not a significant different in night one to night two findings, both nights may be included in the final analysis.

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#### 1.6.1 Setting and sample

We will recruit participants through the University of Pittsburgh Model System on Spinal Cord Injury (UPMS-SCI) at Mercy Rehabilitation Institute. This site provides care for individuals 0-15 years post TSCI including quadriplegic (complete and incomplete) and paraplegic (complete and incomplete) neurologic levels of injury. Participants are on average 49.7 (SD 19.9; range 15-94) years old, male (77.7%) and female (22.3%), and primarily Caucasian (81.4%) and African-American (17.3%). The selection of participants for this study is a convenience sample from the UPMS-SCI, which offers demographic heterogeneity (ethnicity, age, sex, level of injury, socioeconomic status) based on model system criteria.

#### 1.6.1.1 Inclusion/Exclusion Criteria

We will enroll newly presenting UPMS-SCI participants using the following inclusion criteria: 1) aged >18 years; 2) newly diagnosed ( $\leq$ 30 days) TSCI classified with neurologic level

(cervical, thoracic, and lumbar); 3) American Spinal Cord Injury Association (ASIA) motor and sensory score (A, B, or C); 4) able to read/speak English. Exclusion criteria include: 1) history of neurological disorders; 2) cognitive impairment (history or current diagnosis).

Sleep apnea was not included in the exclusion criteria. While the literature is clear that obstructive sleep apnea is more prevalent in older individuals (Jensen et al, 2013), the literature around sleep disordered breathing in TSCI remains mixed. While there are many studies examining SDB, the predominant patterns vary based on cervical versus thoracic injury; with tetraplegia being a risk factor for central sleep apnea while, paraplegia is a risk factor obstructive sleep apnea (Sankari, Bascom, Chowdhuri, & Badr, 2014). SDB is believed to develop in the subacute phase of recovery and peaking at 3 months to 1 year from initial injury, but may change over time from initial injury into the chronic phase (Tran, Hukins, Geraghty, Eckert, & Fraser, 2010; Sankari, Bascom, Oomman, & Badri, 2014). Therefore, using sleep apnea as exclusion criteria would significantly decrease study participation, as higher-level injuries generally are most susceptible to poor sleep and equally to SDB, but may not be a factor in a later phase of recovery. Moreover, individuals with injury are not screened for sleep disorders as a routine of care, so there may be participants that have a co-morbid existence of sleep apnea, while others may be developing SDB undiagnosed (particularly in the subacute period). This would pose significant challenges on inclusion/exclusion criteria. We acknowledge this will impact findings as a limitation in this exploratory pilot.

While there remains some ambiguity on the distinction between what acute, subacute, and chronic phases of recovery, the general consensus is, acute is minutes to hours; subacute is days to weeks; and chronic is months to years (Fehlings, Tetreault, Wilson, Kwon, Burns, Martin, & Harrop, 2017). Rehabilitation begins around the subacute period (1-2 weeks post-TSCI) and this

is the timeframe we aim to capture in order to identify targets for early intervention. We have specifically included adults 18 and over, to cast a wide net in characterizing these concepts over the full TSCI population.

#### 1.6.2 Variables

The variables in aim 1 include depressive symptoms, anxiety, and pain. Symptom data for anxiety, depressive symptoms, and pain are collected by the University of Pittsburgh Model System on Spinal Cord Injury (UPMS-SCI) upon admission using their Form I and hence will not increase participant burden. Characterization of depressive symptoms will be obtained using the Patient Health Questionnaire (PHQ-9): a valid and reliable (Cronbach's  $\alpha$ =0.89) 9-item screening instrument to assess the presence of a depressed mood, with assigned scores ranging from 0-3 (0=not at all, 3=nearly every day) on 9 items with a total severity score of 0-27; scores of 5, 10, 15, and 20 represented mild, moderate, moderately severe, and severe depression, respectively (Whooley, Avins, Miranda, & Browner, 1997; Kroenke, Spitzer, & Williams, 2001). Anxiety is assessed as self-reported diagnosis by a health professional prior to admission; "Have you ever been told by a health professional that you had post-traumatic stress disorder (PTSD) or generalized anxiety disorder (GAD)" with 0=no, 1=yes, PTSD, 2=yes panic disorder, 3=GAD, 4=first diagnosis unknown, 7=declined to participate. The UPMS-SCI measures pain, using the pain rating scale, scoring pain from 0-10 (0=no pain, 10=worse possible pain) with vital signs. Pain data will be collected through chart review, and measured as a continuous variable. Depressive symptoms and anxiety for this study will be examined as nominal binary variables measured as "yes", a positive depression screen/anxiety diagnosis is present, or "no", negative depression screen/no anxiety diagnosis.

Sleep is being measured both objectively and subjectively. The sleep profiler, is an EEG based objective measure of sleep and records brain wave activity through frontal leads, where autoscoring software measures alpha, beta, theta, and delta waves that are associated with periods of sleep and wake stages (Keenan & Hirshkowitz, 2017). Subjective sleep is measured by way of the consensus sleep diary to be compared to what is reported and what is seen on the EEG report. The Consensus sleep diary similar to a likert scale, will have an ordinal level of measure. The sleep diary is a valid and reliable tool for measuring nightly subjective sleep (Maich, Lachowski, & Carney, 2018). Participants, or an assistant, will complete the diary daily during the 48-hour collection period, providing both quantitative (time to sleep; sleep amount; number and duration of awakenings) and qualitative (restfulness, impact to daytime function) sleep evaluation (Carney, Buysse, Ancoli-Israel, Edinger, Krystal, Lichstein, & Morin, 2012). EEG sleep data, has a ratio level of measurement, generated from autoscoring software, based on quantity, looking at sleep onset; sleep amount; time in each sleep cycle; and number and duration of awakenings. The Sleep Profiler by Advanced Brain Monitoring<sup>™</sup>, will obtain objective sleep data for 2 consecutive nights to measure sleep duration, sleep efficiency, sleep latency, arousals, wake after sleep onset, and sleep architecture. Sleep architecture findings will include the percentage of time stage in wake stages N1, N2, N3, and REM. These variables relate to sleep wake cycle and sleep disturbances.

The Sleep Profiler will acquire, record, transmit, and display electroencephalogram (EEG), electrooculogram (EOG), electrocardiogram (ECG), and/or electromyogram (EMG) signals, with optional accelerometer, acoustical, and photoplethysmographic signals. The Sleep Profiler has been cleared by the FDA for use on adults. The hardware has been tested for compliance with overall safety, environmental safety for home use, and electronic emissions and immunity requirements. All components in contact with the skin have met the medical safety standards for

biological hazards including those for irritation, sensitization and cytotoxicity. The Sleep Profiler will be placed around the head of the patient by the applicant/PI. The electrodes will be placed and the headband will be adjusted to head for appropriate fit and the comfort of the participant. Device will be initiated and begin data collection for 48 hours. At the conclusion of the study, profiler software will autoscore the results of the sleep data collected. After use, the Sleep Profiler will be cleaned and disposables removed and replaced by applicant/PI. Data will be downloaded into the software portal system. A secure SSL protocol (2048-bit) is used to encrypt when transferring data over the internet. As an added step, all data will be collected, transferred and stored using only de-identified information (unique study ID) further decreasing breach of personally identifiable information.

#### 1.6.3 Sample DNA extraction, and methylation data collection

For aim 2, methylation profiles of the two candidate genes being examined are continuous variables with ratio level of measurement. One 3 ml EDTA tube of blood will be collected by the applicant, coinciding with sleep data collection when the Sleep Profiler is applied on 2 consecutive evenings. Blood will be delivered to Dr. Conley's laboratory in the School of Nursing and plasma and buffy coat removed and stored at -80°C. DNA will be extracted from the buffy coat using a simple salting out procedure (Qiagen Corp). The DNA will be stored in 1X TE buffer at 4°C. Methylation data collection will be conducted using EpiTect Methyl II PCR Assays (Qiagen Corp). This methodology enables the study CpG island methylation of individual genes and disease or pathway-focused gene panels without bisulfite modification. It relies on differential cleavage of target sequences by two different restriction endonucleases whose activities require either the presence or absence of methylated cytosine in their respective recognition sequences. The relative

amount of DNA remaining after each enzyme digest is quantified by real-time PCR, delivering reliable calculation of the methylation status of individual genes and the methylation profile across a gene panel.

#### **1.6.4 Descriptive Statistics**

Detailed descriptive statistics for phenotype sleep disturbances including both objective and subjective measures and co-occurring symptoms including pain, anxiety and depression will be reported using standard descriptive summaries (mean, standard deviation for continuous variables and frequency, percentage for categorical variables) and graphical techniques (e.g., histograms, scatter plots).

#### **1.6.5 Data Screening Procedures**

The data analysis will be performed using STATA for Macintosh software (version 15, StataCorp LLC, College Station, TX). Data quality diagnostics will be examined prior to aimspecific analyses, to identify outliers or invalid data, examine data distributions, identify patterns of missing data, and evaluate relationships between the variables. Preliminary analyses will be conducted for potential confounder/covariates. Known confounders include level of injury (cervical, thoracic, lumbar), neurologic classification of injury (ASIA scale), age, and sex. Other potentially relevant confounders include comorbidities (i.e. sleep disordered breathing), and race/ethnicity. Standard descriptive statistics will be computed for all independent, dependent, and potentially confounding/covariate data and graphs will be generated. Identified outliers will be checked for accuracy, amended, and retained in analyses (and their influence will be explored). Data transformations will be performed as required. If assumptions are severely violated, alternate, robust procedures will be considered. Missing data will be carefully evaluated for pattern of missingness, bias or experimental error and imputation methods, such as regression substitution (given small sample size), will be used and reported.

#### **1.6.6 Data Analysis Procedure**

Initially for aim 1, univariate analysis/bivariate association between sleep disturbance and symptoms will be assessed using descriptive statistics. Using frequencies, paired t-tests, and contingency tables, we will be able to observe patterns in the findings to explore the relationship between sleep disturbance and symptoms. Similar statistical analysis strategy described for aim 1 will be used for aim 2, between sleep disturbance and DNA methylation profiles representing the inflammatory state (pro or normal), and between symptoms and DNA methylation profiles representing the inflammatory pathway will be assessed. Because this proposed project is a pilot study, summary statistics (i.e., means and standard deviations) will be emphasized rather than hypotheses testing. Moreover, given the novel use of the sleep profiler, consideration for case study analysis, may best support our exploratory pilot analysis. Characterization through multiple case studies allows for between subject comparison and visual inspection of changes in trends, means, and levels (i.e. DNA methylation) over time.

#### **1.6.7** Sample Size Justification

The sample size for this study will be 8-10. This sample size is selected based on the number of participants that can be recruited by the applicant based on current recruitment rates for

the UPMS-SCI, providing opportunity to witness variability in phenotype and epigenomic data (aim 1 and 2), and is congruent with sample sizes investigating objective sleep disorders in the literature (Fogelberg, Leland, Blanchard, Rich, & Clark, 2017; Gioannocaaro, Moghadam, Pizza, Boriani, Maraldi, Avonic, et al, 2013). The sample size was not selected based on power analysis but for the purpose of collecting adequate phenotype and epigenomic data for this pilot work, providing research experience, and providing data that can support the next phase of investigation. Moreover, the sample size will support attainment of research skills as the PI will be hands on conducting each step of this study from recruitment, consenting, data collection, DNA methylation and phenotyping and analysis. The UPMS-SCI admits approximately 80-90 subjects per year, meeting our inclusion criteria, which is more than adequate to support this sample size (NIDDLR, 2016).

#### 1.7 Potential Limitations of Proposed Study

While this study has many strengths through tapping into an ongoing cohort study of spinal cord injury patients it does present some limitations. One limitation is that we are using the symptom data collection for anxiety, depressive symptoms and pain collected through the UPMS-SCI. This is to reduce subject burden, future studies would require using tools with established reliability and validity for prospective assessment of these symptoms. Moreover, sample size as previously noted is modest and not selected based on power analysis, but feasibility. Given the variability of phenotypes within TSCI, a larger sample would be ideal but given the exploratory pilot nature of this study is comparable to other studies in the literature. Lastly, co-morbid sleep disordered breathing was not considered exclusionary criteria for this study, however, will be

reported in the findings, if appropriate and recommendations will be made on how to best approach this in future studies.

#### **2.0 Proposal Changes**

# 2.1 Candidate Gene CHRFAM7A

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Prior to the DNA methylation data collection, it was determined that an IL-1B assay was not available, thus it was replaced with another biologically relevant candidate gene in the antiinflammatory pathway. Alpha 7 nicotinic acetylcholine receptor duplicate 7 gene CHRFAM7A was chosen. CHRFAM7A, a hybrid gene, that results from partial duplications in the cholinergic receptor nicotinic alpha 7 gene (CHRNA7) and the family with sequence similarity 7 alpha gene (FAM7A) is expressed in both the brain and periphery, and plays an important role in cognition and the immune system (Sinkus, Graw, Feedman, Ross, Lester, Leonard, 2015). These genes represent the nicotinic acetylcholine (ACh) superfamily, whose main function is to transmit signals for ACh at neuromuscular junctions and in the peripheral and central nervous system (Wang, Yu, Ochani, Amella, Tanovic, et al, 2003). Although discovery was over twenty years ago, only recently has more research emerged but its function remains largely unknown. One study, examined how genetic polymorphisms of the CHRFAM7A gene contributed to functional outcomes after SCI, revealing the functional polymorphism represented by a 2 base pair deletion (del2bp) contributed to increased circulating pro-inflammatory cytokines, as well as higher pain score reports in the subacute period of injury (Huang, Kabbani, Brannan, Lin, Theiss, et al, 2019). They further noted that carriers of the del2bp polymorphism have a pro-inflammatory phenotype. Given the biological relevance of this gene to symptoms and patient outcomes after SCI and the availability of an assay to assess DNA methylation level, it was selected to replace IL-1B.

# 2.2 Anxiety Variable

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The symptom of anxiety was initially included in the symptoms to be analyzed. However, only 1 participant had an indication of anxiety, thus it was not included in the final analysis due to lack of variability in this variable.

# 2.3 Sleep Profiler™

Reliability and validity analysis were not part of the original proposal of this project. However, given this availability of resources to conduct this type of assessment by certified polysomnography technicians and to strengthen the findings of this study an evaluation of the autoscored software was initiated. It was determined that some of the records were truncated, rendering some of the studies with variable completeness of overnight sleep data. Given this variability of accuracy, the profiler studies presented will focus only on individuals with the most complete data.

#### 3.0 Data Based Manuscript: Sleep and Symptomology after Spinal Cord Injury

Sleep and Symptomology after Spinal Cord Injury

Letitia Yvette Graves, PhD

University of Pittsburgh, 2019

# **3.1 Abstract**

Problem: Sleep dysfunction after traumatic spinal cord injury (TSCI) is highly prevalent. However, it has not been characterized beyond self-report of a perceived problem with sleep disordered breathing (SDB) being most commonly investigated. Poor sleep has significant implications for reduced health-related quality of life, diminished neuroplasticity, and long-term functional outcomes.

Methods: This exploratory study utilized an observational prospective study design, collecting objective EEG based sleep data, self-report sleep diary, symptom data and biological marker (DNA methylation) data to characterize sleep and co-occurring symptoms in the subacute rehabilitation phase (30 days post TSCI) over two consecutive nights.

Results: Sample (N=9) were mostly male (75%), Caucasian (89%) with an average age of 45.7 years (SD=19.6; range=18-71 years). Over half of the TSCI diagnoses were incomplete paraplegia (56%) with ASIA scores of D. Sleep diary reports were variable among participants, however, consistently reported fragmented sleep, underestimates of sleep onset latency (SOL) and wake after sleep onset (WASO), and overestimates of TST. Sleep Profiler summaries scored all

participants as having abnormal sleep patterns based on sex and age norms. Sleep architecture, revealed high N1 percentages, and low N3 and REM stages of sleep. SE, was poor across all participants with no one achieving >85%. Overall, most participants had PHQ-9 severity scores ranging from 2-4, indicating no depression. Pain tended to be moderate to high (>5/10) in most participants on both nights. DNA methylation data was variable across individuals for both genes; however descriptively there were no consistent relationships with sleep variability.

Conclusions: The findings of this study support that sleep after TSCI during the subacute rehabilitation phase is significantly impaired based on subjective and objective measures of sleep. The co-occurring impact of depression and pain on sleep was not clear given the likely confounding effects of demographic variables. This study lays a foundation to characterize sleep after SCI and what is considered "normal" that reflects the unique challenges that accompany specialized populations such as those with neurologic injury.

## 3.2 Background

Spinal cord injury (SCI) is a complex condition with challenges related to treatment options aimed at improving patient outcomes. Symptom burden and the context in which symptoms occur, is equally challenging. The mechanisms that contribute to symptom expression after SCI are variable and not well understood. Following the primary injury to the spine there is a secondary cascade of biochemical changes that often contributes to further injury. A major result is a robust systemic inflammatory response that overtime can have deleterious effects on recovery (Yilmaz, Turan, & Keles, 2014; Allison & Ditor, 2015; Noller, Groah, & Nash, 2017). It has been accepted that between two similar neurologic injuries, functional outcomes will vary in that no two persons with SCI will look exactly the same from a biological, symptomatic, or functional perspective; however this also provides the optimal opportunity to direct efforts toward precision health as a means of individualizing treatment. Phenotypic variation within a condition, is central to understanding how to best approach inter-individual differences that can significantly impact quality of life and overall health related outcomes.

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Sleep after TSCI is largely studied in the context of sleep disordered breathing (SBD), however, studies that collectively assess subjective, objective, and biological sleep measures are sparse. Sleep quality describes what is "good" versus "bad" sleep and subjective report lends itself to this type of qualitative assessment (Hoey, Fulbrook, & Douglas, 2017; Ohayon, et al, 2017). However, subjective report of sleep has its limitations, particularly in the presence of cognitive impairment, and/or the co-presence of other symptoms such as pain and/or depression. Such symptoms can bias perceptions of sleep and perpetuate "rumination" or worry and paradoxically contribute to worse sleep (Carney, Edinger, Meyer, Lindman, & Istre, 2006). Objective measures offer the ability to quantify sleep in a way that questionnaires and or diaries alone cannot and often work in tandem with subjective instruments. They also support sleep staging, which provides real-time brain wave biometrics that illustrates how much time an individual spends in non-rapid eye movement (NREM) and rapid eye movement (REM) stages of sleep.

Biological assessment of sleep elucidates the underlying mechanisms that contribute to the regulation of sleep and further explains what is being observed on objective measures and gives context to the perception of subjective reports (i.e. the potential influence of other symptoms). Given that sleep is a brain-based activity, disease or injury to the brain and surrounding structures will most likely suffer from sleep disturbances more significantly. The neurochemicals and

hormones involved in the regulation of sleep and wakefulness, such as acetylcholine, dopamine, gamma-aminobutyric acid (GABA), glycine, hypocretins (orexins) and immune regulatory molecules are also involved with motor, sensory, metabolism, and hormonal functionality (Zeitzer & Mignot, 2005). Moreover, there is a body of evidence that sleep is associated with the innate immune system functioning, and inflammation and inflammatory signaling are activated by sleep loss (Irwin & Opp, 2017). This has significance in individuals after TSCI due to biochemical changes that occur secondary to injury and the subsequent autonomic dysfunction that contributes to impaired regulation of immune and hormone activity that could potentially increase the ongoing chronic inflammatory state that characterized TSCI. Understanding the impact of neurochemicals that influence the timing, structure, and/or depth of sleep, as well as their role in inflammatory signaling activation and the homeostasis of related symptomology can potentially improve interventions targeting recovery and symptom expression.

Inflammation occurs in response to injury, infection, and disease, in an effort to decrease harmful effects and maintain homeostasis. However, as is the case with SCI, prolonged inflammation can yield a series of changes where pro-inflammatory signaling begins to direct its effects on healthy tissues and cells leading to further "secondary" damage in places like the central nervous system where repair and regeneration are needed (Allison & Ditor, 2015; Yilmaz, Turan, & Keles, 2014). Literature also supports this chronic, ongoing, pro-inflammatory state is linked to symptom expression, such as depression and pain (Allison & Ditor, 2015; Maes, Berk, Goehler, Song, & Anderson, et al, 2012; Noeller, & Groah, Nash, 2017; Zhang & An, 2007). More recently, research has focused on the co-existence of symptoms, noting that the presence of one symptom often acts as a catalyst for the development or worsening of other symptoms (Armstrong, 2003; Barsevick, 2016; Miaskowski & Aouizerat, 2007). It is then, not unreasonable to consider that
mood disturbances and other neuro related symptoms such as sleep disturbance are interconnected, however, this relationship has not been established after TSCI, nor has there been an examination of this relationship in the context of a shared common biological mechanism.

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In considering how individuals are responding to the impact of TSCI, it equally important to consider the diversity of underlying biological mechanisms on injury and disease susceptibility. As previously noted, TSCI is a condition with unpredictable outcomes, in that, no two injuries will have the same deficits or recovery trajectory; genetic diversity can explain some of the differences in these features. Genetic variation describes the change in the DNA sequence that makes individuals unique and recognizes that information in DNA interacts with the environment producing variability in traits among individuals (Templeton, 2019). This environmental alteration of DNA describes the concept of epigenetics, or changes outside of the DNA sequence that contributes to mechanistic process variability within individuals and populations (Aran, Toperoff, Rosenberg & Hellman, 2011; Jin, Li, & Robertson, 2011; Moore, Le, & Fan, 2013). Epigenetic processes, such as DNA methylation plays a critical role in maintaining gene expression patterns and regulating transcriptional activity. Therefore, improper methylation resulting from pre or post injury genetic variation can yield severe secondary health complications.

We have chosen two candidate genes, period 2 (*PER2*) and human-specific  $\alpha$ 7-nicotinic acetylcholine receptor (*CHRFAM7A*) to explore biological underpinnings of sleep, depression and pain. PER2, is one of the core clock genes that functions as a transcription repressor forming a negative feedback loop with cryptochrome 1 and 2 (CRY 1 & 2). PER2 proteins transport CRY1 and CRY2 into the nucleus with appropriate circadian timing, and contribute directly to repression of clock-controlled target genes through interaction with several classes of RNA-binding proteins, helicases and others transcriptional repressors (genecards.org).

CHRFAM7A, a hybrid gene that results from partial duplications in the cholinergic receptor nicotinic alpha 7 gene (CHRNA7) and the family with sequence similarity 7 alpha gene (FAM7A) is expressed in both the brain and periphery. It plays an important role in cognition and the immune system (Sinkus, Graw, Feedman, Ross, Lester, Leonard, 2015). These genes represent the nicotinic acetylcholine (ACh) superfamily, whose main function is to transmit signals for ACh at neuromuscular junctions and in the peripheral and central nervous system (Wang, Yu, Ochani, Amella, Tanovic, et al, 2003). Although discovery was over twenty years ago, its function remains largely unknown. One study examined how genetic polymorphisms of the CHRFAM7A gene contributed to functional outcomes after SCI, revealing the functional polymorphism represented by a 2 base pair deletion (del2bp) contribution to increased circulating pro-inflammatory cytokines, as well as higher pain score reports in the subacute period of injury (Huang, Kabbani, Brannan, Lin, Theiss, et al, 2019). They further noted that carriers of the del2bp polymorphism had a pro-inflammatory phenotype.

Given the dearth of research conducted in patients who have sustained a TSCI related to characterizing sleep, particularly using objective measures of sleep, evaluation of co-occurring symptoms, and potential mechanisms of sleep dysfunction after TSCI, this exploratory project was conducted.

#### 3.3 Methods

# **3.3.1** Study design and participant information

This prospective observational pilot study was conducted on an in-patient rehabilitation unit for individuals recovering from SCI. Participants were recruited from an ongoing research study through the University of Pittsburgh, SCI Model System. To be eligible for this study, participants had to be enrolled in the model system prior to being consented. Institutional Review Board (IRB) approval was obtained for the collection of EEG based sleep measures, self-reported sleep, and biospecimens all collected at two time points covering two consecutive nights. Inclusion criteria for this study, participants had to be: 1) 18 years of age or older; 2) have sustained a traumatic SCI within the last 30 days; 3) be able to speak and read English. Exclusion criteria included those: 1) not enrolled in the SCI model system; 2) under 18 years of age; 3) time from initial injury exceeded 30 days; 4) cognitive impairment that rendered individuals decisionally impaired.

#### **3.3.2 Sleep Data Collection**

#### **3.3.2.1** Consensus Sleep Diary

Sleep diaries have been regarded as the "gold standard" for subjective sleep assessment (Carney, Buysse, Ancoli-Isreal, Edinger, Krystal, et al, 2012). Subjective sleep was collected using an adapted version of the Core Consensus Sleep Diary (CSD), which included items from the Expanded Consensus Sleep Diary for Morning (CSD-M) and Evening (CSD-E), plus one Likert scale rating item on mental alertness (0=very poor, 1=poor, 2=fair, 3=good, 4=very good) and one

on physical energy (0=very poor, 1=poor, 2=fair, 3=good, 4=very good). With a total of three versions, each consists of "core" items that measure sleep onset latency (SOL), wakefulness after initial sleep onset (WASO), total sleep time (TST), total time spent in bed (TIB), sleep efficiency (SE), and sleep quality or satisfaction (Carney, Buysse, Ancoli-Isreal, Edinger, Krystal, et al, 2012). The expanded CSD versions include daytime activity such as caffeine intake, napping and dozing and rating of restfulness (0=not at all rested, 1= slightly rested, 2=somewhat rested, 3=well rested, and 4=very well rested) and sleep quality (0=very poor, 1=poor, 2=fair, 3=good, 4=very good) (Appendix G).

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#### 3.3.2.2 Sleep Profiler<sup>TM</sup>

The Sleep Profiler<sup>™</sup> employs a 3 frontal sensor electroencephalogram (EEG) method of collecting brain wave activity, that records SE, TST, SL, sleep staging (sleep architecture), and cortical arousals/awakenings (Figure 1). The Sleep Profiler<sup>™</sup> has been validated as a reliable tool in healthy sleeping adults, compared to the gold standard of objective sleep, polysomnography (PSG) (Finan, Richards, Gamaldo, Han, & Leoutsakos, et al, 2016). However, no studies have examined reliability and validity in neuro impaired populations, such as TSCI. This device was placed on participants for two nights between the hours of 5:00pm and 6:30pm, respectively and removed in the morning between the hours of 7:00am and 8:00am. During off periods when participants were not wearing the device, it was charged in preparation for night two. At the conclusion of the second night, EEG data was downloaded from device and autoscoring software generated a sleep report (Figure 2). Appendix A describes how each stage is characterized and approximations of normal time spent in each stage. Appendix B defines how the Sleep Profiler defines variable measures.



Figure 1 Image of Sleep Profiler

#### Sleep Profiler PSG2 Study Report Summary

Patient Name	4 percent, 3 percent	Study Ordered by	Sample Physicia
Date of Night 1	01 Jan 2010	Date of Birth	7 Jun 1967
		Study Type	Diagnostic

CLINICAL HISTORY: 49 year old male with questionnaire responses that suggest sub-threshold insomnia (Insomnia Severity Index = 12), sub-threshold daytime sleepiness (Epworth Sleepiness Score = 10), moderately severe depression (PHQ-9 score = 15), and mild anxiety (GAD-7 score = 7). The patient has a history of high blood pressure. The patient reported that he sometimes takes the prescription sleeping aid Lunesta (Eszopicione). He sometimes consumes caffeine in the afternoon or evening. The patient has a BMI of 27 and a 13.5-inch neck; and reported that he sometimes snores, rarely wakes up choking, and rarely has witnessed apneas. He has high predicted risk of having OSA.

STUDY FINDINGS: The patient underwent a one-night overnight type II polysomnography.

NIGHT ONE - Sleep Disordered Breathing: Based on total sleep time of 3.0 hours and 3% desaturation criteria, the overall apnea-hypopnea index (AHI) was 21.8, supine AHI was 54.5, and non-supine AHI was 1.6. The NREM AHI was 16.2, REM AHI was 55.4, respiratory disturbance index (RDI) was 31.0, and the respiratory event related arousal index (RERA) was 9.2. The oxygen desaturation index (ODI) was 22.4, the lowest respiratory event related desaturation was 90.0%, and the SpO2 was less than 90% for 0.0 minutes, and less than 88% for 0.0 minutes.

<u>Sleep Architecture:</u> The recording time of 4.4 hours and total sleep time of 3.0 hours resulted in a sleep efficiency of 69.3%. The patient fell asleep 21 minutes after the lights were turned off, started stage N3 22 minutes later, and entered his first REM cycle 87 minutes later. He sleep 14.3% of the night in stage N1, 11.3% in light N2, 39.3% in total N2, 32.1% in slow-wave sleep (stage N3), and 14.3% in REM.

Awakenings and Arousals: The patient was awake for a total 59 minutes after initially falling asleep. He experienced an average of 7.7 awakenings ≥ 30 sec and 2.5 awakenings ≥ 90 sec per hour of sleep. He experienced an average of 20.8 cortical arousals, 76.6 autonomic activations, and 4.9 movement arousals per hour of sleep.

Other: He slept 38.1% of the night supine, while snoring 15.7% of the night above 40 dB and 9.8% above 50 dB.

Abnormal Sleep Patterns: The patient's total sleep time, sleep efficiency, percentage of time in stage REM and N1, and awakening index were outside age/gender-matched normative ranges.

CLINICIAN COMMENTS: Diagnosis: The findings are consistent with moderate positional obstructive sleep apnea

Therapy: Nasal Continuous Positive Airway Pressure (CPAP) or positional therapy.

Other recommendations: Follow-up with the referring physician recommended to discuss the results and the next steps in the treatment plan.

Signature:	Samole Physician	Date:	15 Feb 2017
signature.	Sampa Mysician	Date.	10 160 201

Study Review: The overnight EEG and other signals have been reviewed by Sample Physician, MD.

Normative comparison data obtained from

Nonnorte Comparison battownshife'n fund. Sleep and Reported Daytime Sleepiness in Normal Subjects: the Sleep Heart Health Study, Walsleben JA, Kapur VK, Newman AB et al. Sleep 2004; 27(2):293-8 A WASO Sub-Group Analysis of a 6-month Study of Eszopicione 3mg, Krystal et al. Sleep Medicine 2012; 13:691-696.

Traceability:056d77c9-e5db-4052-bd31-ab4dc23a8b25\_\_\_\_20151216\_121806\_0000014000029

#### Figure 2 Sample Autoscored Report Sleep Profiler

# 3.3.3 Other Symptom Data Collection

#### 3.3.3.1 Depression

Depression was measured using the PHQ-9 (Figure 3), which has been shown adequate in accurately screening for depression in TSCI populations (Tulsky, Kiasala, Kalpakjian, Bombardier, Phlig, et al, 2015). Participants were asked to rate how often over the past 2 weeks, they were bothered by the problems presented in nine questions related to mood, sleep, energy, concentration, and other feelings or events. Response scores range from, 0 ("not at all") to 3

("nearly every day"), with a 27 as the highest possible score, yielding a finding of major depressive

syndrome.

FORM I INTERVIEW	System ID Patient N	umber		_			
Interviewer, ask only if participant is 18 years or olde experiences these feelings or events	er. Collect data from participant (	only. 'Both	nered by' is	intended to i	dentify whe	ther the par	ticipant
The next questions ask about how often have you	u been bothered by the followi	ng proble	ems in the	past 2 week	s.		
+! PHQ-9 PATIENT HEALTH QUE	ESTIONNAIRE	Not At All	Several days (1 – 7 days)	More than half the time (8 – 12 days)	Nearly every day (13 – 14 days)	Declined	Unkn
+! 50. Little interest or pleasure in doing thing. If not sure, ask 'did you lose interest or pleasure in thin unsure, 'Did you have to force yourself to do things?' Yo "DON'T CARE", "NO MOTIVATION", "WITHDRAWN".	s? Would you say gs you usually enjoy?' If still ou may accept words like:	<b>0</b>	<b>1</b>	<b>2</b>	□3	<b>7</b>	<b>9</b>
+! 51. Feeling down, depressed or hopeless?		<b>0</b>	□1	□2	□3	□7	□9
+! 52. Trouble falling or staying asleep, or slee	ping too much?	0	1	<b>2</b>	□3	□7	<b>9</b>
+! 53. Feeling tired or having little energy?		<b>0</b>	<b>1</b>	<b>2</b>	□3	□7	<mark>9</mark>
+! 54. Poor appetite or overeating?		0	1	<b>2</b>	□3	□7	9
+! 55. Feeling bad about yourself – or that you yourself or your family down?	are a failure or have let	<b>0</b>	□1	<b>2</b>	□3	□7	<mark>_9</mark>
+! 56. Trouble concentrating on things, such as watching television?	s reading the newspaper or	0	1	□2	□3	□7	<b>9</b>
+! 57. Moving or speaking so slowly that other Or the opposite – being so fidgety or restless the around a lot more than usual?	people could have noticed? hat you have been moving	0	<b>1</b>	<b>2</b>	□3	<b>7</b>	<b>9</b>
+! 58. Thoughts that you would be better off de	ad or of hurting yourself?	0	<b>1</b>	<b>2</b>	□3	□7	<b>9</b>
59. MAJOR DEPRESSIVE SYNDROME*							
60. SEVERITY OF DEPRESSION*							

Figure 3 National Spinal Cord Injury Statistical Center Form I [PHQ-9] (2016-2021)

# 3.3.3.2 Pain

Pain data was collected from pain screening using the numeric rating scale (NRS) recorded in patient charts. The NRS using a 0 ("no pain") to 10 ("worst possible pain") self-report of pain intensity, is a validated measure of pain that is widely used across clinical settings (Jensen, Turner, Romano, & Fisher, 1992; Krebs, Carey, & Weinberger, 2007). Pain was assessed with vital signs, which occurred at the beginning of the morning shift (approx. 7:00am). If pain was not present at that time, no additional pain assessment was done. If individuals reported pain and received pharmacologic intervention, they were reassessed within the hour and/or per patient-initiated

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report. The total number of pain reports were recorded over the two days of study participation, with only the highest level of pain per day being retained for analysis (Table 7).

#### 3.3.4 Blood Collection and DNA extraction

Blood was obtained using peripheral venipuncture, except in cases where a peripherally inserted central catheter (PICC) access was available. At two time points, night 1 and night 2, one 3mL EDTA purple top tube and one 2.5mL PAXgene tube (PreAnalytix; Qiagen/BD Company) was collected. EDTA tubes were spun at 2500 RPM for 5 minutes in the centrifuge, plasma was removed and the buffy coat was placed in a 50mL tube with lysis solution (40mL of ammonia chloride and 5mL ammonium bicarbonate). After 20 minutes at room temperature, tubes are then spun again for 20 minutes at 2500 RPM. Supernatant was removed and remaining pellet was suspended in 1mL of freezing solution and placed in the -20 freezer, until batch DNA extraction could be completed. The PAX gene Blood RNA Tube was stored upright in the refrigerator  $(2-8^{\circ})$ for 24 hours, transferred to -20 °C for 24 hours and then maintained for long-term storage at -80 °C. DNA was extracted from the buffy coat using a salting out procedure: 500µL of Proteinase K solution and 200µL 10% SDS was added to 15mL conical tubes that were sealed and placed in a 37°C rotating incubator overnight to digest. Following digest, 1mL of NaCl was added before centrifuging at 2500 RPM for 15 minutes. Absolute alcohol (EtOH) was then added until DNA precipitated out of the solution and was removed. After EtOH was removed and evaporated, 1mL of TE buffer was added to each tube and placed in incubator to dissolve DNA into buffer for storage. The PAX gene Blood RNA tubes will remain in storage for future gene expression studies.

#### 3.3.5 DNA Methylation Procedure

EpiTect Methyl II PCR single gene arrays (Qiagen Inc., Germantown, MD) were used to collect data for a priori selected candidate genes, PER2 and CHRFAM7A. Each sample was measured in duplicate and duplicates were averaged for the daily value. The method employed by this system is based on detection of the remaining input DNA after cleavage with a methylationsensitive and/or methylation dependent restriction enzyme. The enzymes digest unmethylated and methylated DNA respectfully in separate reactions, followed by real-time polymerase chain reaction (PCR) quantification using primers that border the region of interest. To perform restriction digest, a reaction mix without enzymes was prepared using RNase/DNase-free water and 5x restriction digestion buffer (26 µL). Digestion reactions include a mock (no enzymes), a methylation sensitive (MSRE), methylation dependent (MDRE), and both (MSRE and MDRE) to compare the amount of relative fractions of methylated and unmethylated DNA. After digestion, enzyme reactions are mixed directly with PCR master mix and dispensed into a 96-well PCR array plate containing pre-aliquoted primer mixes. Real-time PCR was carried out using specified cycling conditions and raw values were available in an Excel spreadsheet, which automatically calculated the relative amount of methylated and unmethylated DNA fractions (Figure 1).

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Figure 4 EpiTect Methyl II PCR Procedure

#### 3.3.6 Data Analysis

Case reports were constructed to fully characterize sleep and report methylation status in each participant, and support the emergence (if any) of trends of similarities and differences within and between participants. Using SPSS for Mac (version 25, IBM Corp., Armonk, NY) and Microsoft Excel for Mac (version 16.25, Microsoft® 2019) descriptive statistics were generated including demographics, frequency tables, and supporting charts and graphs. Data was assessed for missingness and missing values were either due to nonresponse or partial data as a result of participant withdrawal. Two participants withdrew from the study after night one, thus yielding only partial results. Data imputation would not be appropriate due to the extent of variability and

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sample size. Given the exploratory nature of this pilot and modest sample size inferential statistics could not be supported here.

# **3.4 Results**

# 3.4.1 Participant Demographics

The sample (N=9), (Table 1) was mostly male (75%), Caucasians (89%) with an average age of 45.7 years (SD 19.6; range 18-71 years). Over half of the spinal level injuries were thoracic (56%) with ASIA scores mostly level D. Each participant was assigned a case report number 1-9, and their full individual case reports can be found in Appendix E.

Case #	Age	Sex	Race/Ethnicity	SCI Level	ASIA Score	Time since injury
1	18	Male	Caucasian	L1	С	16 days
2*	58	Male	African American	C1	D	25 days
3*	27	Female	Caucasian	Т9	D	10 days
4*	71	Male	Caucasian	C3	D	23 days
5	38	Male	Caucasian	<b>T8</b>	D	28 days
6*	53	Female	Caucasian	T11	D	17 days
7*	26	Male	Caucasian	Τ7	В	24 days
8*	71	Female	Caucasian	C2	D	19 days
9*	50	Male	Caucasian	<b>T10</b>	Α	14 days

Table 1 Participant Demogram	phics
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Abbreviations: SCI=spinal cord injury; ASIA=American Spinal Injury Association. Participants 2 and 9 withdrew after night 1. (\*)

Indicates participants taking Melatonin supplement.

# 3.4.2 Subjective Sleep Diary

Sleep diary reports (Table 2; Figures 5-9) were variable among participants, however, consistently there were reports of fragmented sleep. Getting into bed was reported as early as 12:00 noon and as late as 12:00 am (question 1); however, the actual attempts to fall asleep, ranged from 9:00 pm to 1:00 am, with most generally between 10:00-11:00pm (question 2). Rising times were as early as 4:30am and as late as 8:00am (question 6). Reported SOL, ranged from 5 minutes to 60 minutes (question 3). Awakening after initial falling asleep was reported as low as no awakening and as high as 5 times throughout the night, with total WASO periods, lasting 15 minutes to 60 minutes. After accounting for SOL, and WASO, which was summed and subtracted from TSP, total sleep duration ranged from 1.75 hour (105minutes) to 9.5 hours (570 minutes). All but one participant endorsed taking at least one nap on the first evening, with nap times that ranged from 20 min to 2 hours. However, only one person reported a nap on day two for 2 hours.



Figure 5. Reported Bedtimes

Abbreviations: TIB=time in bed; TTS=time to sleep; M1=morning 1; M2=morning 2

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Table 2 Subjective Sleep Diary Morning

Study

Case

		2000						
Report #	Age	Night	SOL	AWKG	TST	WASO	# of Naps	Length of Nap
1	18	1	10min	3-4 times	320 min	60 min	1	120 min
1	18	2	20min	3 times	400 min	30 min	1	90min
2	58	1	N/A	N/A	N/A	N/A	N/A	N/A
3	27	1	65min	3 times	375 min	30 min	1	45 min
3	27	2	75min	3 times	330 min	30 min	0	No naps
4	71	1	30 min	0 times	350 min	0 min	1	20 min
4	71	2	120min	N/A	N/A	N/A	0	No naps
5	38	1	15min	3 times	105 min	30 min	0	No naps
5	38	2	N/A	0 times	375 min	0 min	0	No naps
6	53	1	5min	2 times	480 min	35 min	1	30 min
7	26	1	60min	5 times	255 min	45 min	1	20 min
7	26	2	30 min	2 times	420 min	15 min	0	No naps
8	71	1	60min	0 times	570 min	N/A	2	No naps

8	71	2	N/A	N/A	565 min	0 min	0	No naps
9	50	1	30 min	5 times	540 min	30 min	3	30-40 min
9	50	2	120min	0 times	540 min	0 min	0	No naps

Abbreviations: M1= morning 1; M2=morning 2; TIB=time in bed; TTB=time to bed; SOL=sleep onset latency; AWK=awakening; WASO=wake after sleep onset; TST=total sleep time;

OOB=out of bed; QLTY=quality; REST=restful; 'm'=minutes; 'a'=am; 'p'=pm; See Appendix G for full diary questions.

On morning one, 22% reported their quality of sleep as "poor" and 44% reported their sleep as "fair"; however, on morning two 44% of participants reported sleep quality as "good" and only one person reported their sleep as "fair. When asked about "how refreshed did you feel when you woke up for the day" on morning one, 33% reported feeling "slightly rested" or "somewhat rested" for the day. One person reported "not at all rested" and another reported feeling "well rested". On night two 33% of participated reported feeling "somewhat rested", while, 22% reported feeling "well rested". Questions on mental alertness and physical energy/fatigue, had higher rated responses. On night one, 33% rated their mental alertness as "very good" or "good" and 22% as "fair"; on night two, 33% again rated their mental alertness as "very good" or good". No one rated below good on night two. When asked "how would you rate your physical energy", 33% rated "fair", and 22% rated "poor" on night one. One person reported both "fair" and "good", one person rated "very good" and another rated "good". On night two, all but one person rated physical energy as "good".





Abbreviations: M1=Morning 1; M2=Morning 2; See Appendix G for full diary questions.



Figure 7 Subjective Feeling of Restfulness

Abbreviations: M1=Morning 1; M2=Morning 2; See Appendix G for full diary questions.

# Figure 8 Subjective Alertness



Abbreviations: E1=Evening 1; E2=Evening 2; See Appendix G for full diary questions.



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**Figure 9 Physical Energy/Fatigue** 



Abbreviations: E1=Evening 1; E2=Evening 2; See Appendix G for full diary questions.

# 3.4.3 Objective EEG-Sleep Profiler

Sleep profiler results are based on autoscoring software report summaries. Additional analysis was made to evaluate accuracy and completeness of records by certified sleep polysomnography technicians. Therefore, only three of the nine cases are reported here that best represent accurate and complete data. Findings are categorized into groups by sleep time, SE, sleep architecture, latencies and sleep continuity, which includes awakenings (Table 3). According to summary reports all participants were scored as having abnormal sleep patterns based on sex and age norms. Only one participant (case 5) achieved the recommended 8 hours of sleep. SE, was poor across all participants with no one achieving >85%. SOL ranged from 25 minutes to as high as 200 minutes. Sleep continuity was significantly fragmented, with awakenings >30 as frequent as 3.5 times and awakenings >90seconds as 10.7 times. WASO times were also significantly prolonged ranging from 209 min (approx. 3.5 hours) to 276 min (4.6 hours). Sleep architecture (Table 4), revealed high N1 percentages, and low N3 and REM stages of sleep.

Case #	Age	SOL	Awakenings (>90 secs)	Awakenings (>30 secs)	TST	WASO	Sleep Efficiency
1_1	18	78 min	3.0	10.5	384 min	257 min	53.30%
5_0	38	25 min	3.5	10.7	486 min	209 min	67.4%
6_0	53	200 min	3.4	8.1	240 min	276 min	33.70%

Table 3 Sleep Profier [SOL/WASO/Continuity/TST/SE]

Case Report number '#\_0' indicates night one; '#\_1' indicates night two; See appendix B for measurement defininitions.

#### **Table 4 Sleep Profiler Architecture**

Case Report #	Age	Sex	Wake	Stage N1	Stage N2	Stage N3	Stage REM
1_1	18	М	46.7%	21.7%	40.4%	25.7%	12.2%

5_0	38	М	32.6%	28.3%	52.7%	18.3%	0.7%
6_0	53	F	66.3%	18.4%	67.9%	0.4%	13.3%

Case Report '#\_0' indicates night one; '#\_1' indicates night two; See Appendix A for staging characteristics

# 3.4.4 CSD & Profiler

The results of the diary and the profiler were compared on SOL, number of awakenings, TST and WASO. Metrics reveal similar findings with respect to awakenings (>90 seconds) (Table 5). However, diary SOL and WASO were often underestimated, while diary TST was overestimated when compared to profiler. The awakenings >30 secs are related to cortical arousals where the brain is active but not enough to transition an individual to an awake state. While there is no meaningful way of comparing this on a sleep diary, it does give context to the profiler WASO and TST. It also elucidates the brain activity that contributes to sleep fragmentation.

## Table 5 Combined CSD and Sleep Profile Results

						AWKG Profiler	AWKG Profiler					
Case		Study	SOL	SOL	AWKG	(>90	(>30	TST	TST	WASO	WASO	SE
Report #	Age	Night	Diary	Profiler	Diary	secs)	secs)	Diary	Profiler	Diary	Profiler	Profiler

5	38	1	15 min	25 min	3 times	3.5	10.7	105 min	486 min	30 min	209 min	67.4%
6	53	1	5 min	200 min	2 times	3.4	8.1	480 min	240 min	35 min	276 min	33.7%

Abbreviations: SOL=sleep onset latency; AWKG=awakenings; TST=total sleep time; WASO=wake after sleep onset; SE=sleep efficiency

3.4.5 Symptom Data

Table 6 Symptom I	Data
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Case Report #	Age	Sex	Depression (Y/N)	Pain
1_0	18	М	Yes	10
1_1	18			9
2_0	58	М	No	2.5
3_0	27	F	No	8
3_1	27			8
4_0	71	М	No	5
4_1	71			5
5_0	38	М	No	8

5_1	38			8
6_0	53	F	No	10
7_0	26	М	No	7
7_1	26			7
8_0	71	F	No	0
8_1	71			0
9_0	50	М	Yes	8
9_1	50			0

Note: Pain scores are based on highest score reported on each day

# 3.4.5.1 Depression

Overall, most participants had severity scores ranging from 2-4, indicating no depression. Two participants had severity scores >4 indicating "mild" depression (Table 7). These same two cases, however, indicated a pre-injury diagnosis of depression. All but one participant indicated "feeling down, depressed or hopeless" (question 2) at least several days; and similarly reported having trouble falling or staying asleep or sleeping too much" (question 3) at least several days or nearly every day. Only one participant had a diagnosis of major depressive disorder (MDD).

Table 7 PHQ-9 Results

_																	Pre
Case #	٨٥٩	Sov	Race	٨٢١٨	01	02	03	04	05	06	07	08	09	MDS	SUS	Categ	inj Dv
π	Age	JEX	Nace	11	QI	QL	Nearly	4	<u> </u>	<u> </u>	Q/	QD	<u> </u>	WIDS	505	categ	
				ASIA	Several	Several	every	Several	Several	Not at	Several	Not	Not				
1	18	М	CC	C	days	days	day	days	days	all	days	at all	at all	0	8	Mild	Yes
				C1													
				ASIA	Several	Several	Several		Not at	Not at		Not	Not				
2	58	М	AA	D	days	days	days	Not at all	all	all	Not at all	at all	at all	0	3	No D	No
				Т9													
				ASIA		Several	Several	Several	Not at	Not at		Not	Not				
3	27	F	CC	D	Not at all	days	days	Days	all	all	Not at all	at all	at all	0	3	No D	No
				C3			Causanal	Coursel		NI-4-4		N1 - +	Net				
1	71	N 4	<u> </u>	ASIA	Not at all	Not at all	Several	Several	NOT at	NOT at	Not at all			0	2		No
4	/1	IVI	LL.	D TO	NOLALAI	NOLALAII	Moro	Days	dli	dll	NOLALAI	at all	at all	0	Z	NOD	NO
						Several	than %	Several	Not at	Not at		Not	Not				
5	38	М	CC	D	Not at all	davs	the days	Davs	all	all	Not at all	at all	at all	0	4	No D	No
				- T11				20,0	un			ut un		Ū			
				ASIA		Several	Several		Not at	Several		Not	Not				
6	53	F	CC	D	Not at all	days	days	Not at all	all	days	Not at all	at all	at all	0	3	No D	No
				T7													
				ASIA		Several	Not at		Not at	Not at	Several	Not	Not				
7	26	Μ	CC	В	Not at all	days	all	Not at all	all	all	days	at all	at all	0	2	No D	No
				C2		- ·											
-		_		ASIA		Several	Several		Not at	Not at	Several	Not	Not	-	-		
8	71	F	CC	D	Not at all	days	days	Not at all	all	all	days	at all	at all	0	3	No D	No
				110	More	Coursel	Not of	Coursel	Not of	No.	More	NI-L	NIST				
_	50	N 4	<u> </u>	ASIA	than ½	Several	Not at	Several	Not at	Not at	than ½	NOT	NOT	2	~	Mild	Vac
9	50	IVI		A	the days	udys	all	udys	dli	dii	the days	atali	at all	2	6	iviliu	res

Responses are on a 4 point scale indicating degree of severity; items are rated 0(not at all) to 3 nearly every day. Total Score ranges from 0-27. Interpretive guidelines as a severity measure: 1-4= no depression; 5-9=mild depression; 10-14=moderate depression; 15-19=moderately severe depression and 20-27=severe depression. (Kroenke, Spitzer, & Williams, 2001).

# Abbreviations: CC=Caucasian; AA=African American; MDS=Major Depressive Syndrome; SDS=Severity Depression Score; Categ=Category; PreInjDx=Preinjury

diagnosis

# 3.4.5.2 Pain

Pain was also variable, with some individuals having frequent high ratings of pain, while others having only a single report or no reports of pain. Only the highest reported pain score was retained each day as some individuals had multiple pain scores in one day and some did not have any or one (Table 6).

Case Report #	Age	CHRFAM-7A Methylated	PER2 Methylated
1_0	18	94.42%	94.40%
1_1	18	Failure	Failure
2_0	58	92.88%	92.57%
3_0	27	92.80%	68.87%
3_1	27	0.06%	59.01%
4_0	71	73.40%	2.49%
4_1	71	98.94%	97.47%
5_0	38	94.55%	93.07%
5_1	38	Failure	Failure
6_0	53	0.08%	12.23%
7_0	26	Failure	Failure
7_1	26	Failure	Failure
8_0	71	0.05%	1.86%
8_1	71	N/A	N/A

# Table 8 DNA Methylation Results

9_0	50	Failure	Failure
9_1	50	0.15%	7.56%

#### **3.4.6.1 CHRFAM7A**

Methylation data was found to be variable from night to night and between genes (Table 8). Three participants had partial results where detection failed with both genes on either night one or night two. CHRFAM7A, was found to be highly methylated in four participants on night one. Of those same 4 participants, night two methylation was low or results failed. Case 7 had failed results on both nights. The remaining participants had low methylation or failed results.

#### 3.4.6.2 PER 2

PER2 was also highly methylated on night one and less on night two (Table 8). Four participants had high methylation in both genes on night one, but not on night two. Case 7 was an unreliable sample, as neither methylated nor unmethylated DNA was detected.

#### **3.4.6.3** Sleep, Depression, Pain and Methylation

Case 1, was the only individual that had high levels of methylation, positive depression screen, high levels of pain, increased awakenings, low TST and normal SOL but only on night one. Similarly, case 9, had low methylation, MDD, high level of pain, increased awakenings, perceived high TST and prolonged SOL on one night. All other participants did not have positive depression screens, but still had significantly poor sleep. Pain was variable across participants but overall reflected a moderate to high rating for most.

# **3.5 Discussion**

The findings here are consistent with sleep focused research in the TSCI population, that sleep is impaired by self-report. This study also confirms that sleep is impaired using objective measures. The first aim of this study was to phenotype sleep and describe co-occurring symptoms after TSCI. Determining individual phenotypes and constructing an inventory of impaired sleep patterns after SCI is a starting place to eventually develop tailored interventions and treatments for these patients.

Collecting subjective sleep-wake patterns and behavior is considered the first step in assessing sleep and typically done through the use of sleep diaries (Natale, Leger, Bayon, Erbacci, Tonetti, et al, 2015). Overall, participants completed the sleep diary without difficulty and the design of the consensus sleep diary supported both quantitative and qualitative measures of the sleep experience. Interestingly, there was incongruity in reported perceptions of sleep quality, restfulness, mental alertness, and physical energy. It would be anticipated that with lack of restfulness and poor sleep quality, mental alertness and physical energy would decrease. However, participants that described their sleep as fair or poor and/or indicated a lack of feeling rested, rated mental alertness and physical energy as good or very good. Without a separate measure of daytime sleepiness, such as the Epworth Sleepiness Scale (ESS), or daytime function such as, the Pittsburgh Sleep Quality Index (PSQI) it is difficult to pinpoint exactly what participants felt was poor about their sleep or how their functional perception is more optimistic than their view of restfulness. One thought is the responses on physical and cognitive function could be based on how well they did or did not do in therapy that day, given these questions are supposed to be answered in the evening before bed; while questions of restfulness are answered in the morning upon rising. A typical day on an in-hospital rehabilitation unit has a wide range of variability. Therapy regimens are tailored

to individuals based on injury and patient goals; thus, what is considered "typical" in terms of activity, time spent in bed, and other activities of daily living will differ between participants and even day to day within participants. It was also noted that between night one and night two most responses reflected an improved perception of sleep satisfaction. The use of the Sleep profiler may have influenced responses, as participants may have been adjusting to the monitoring device. Typically, night one is considered an acclimation or "first night effect" and not reflective of normal sleep pattern and is removed from analysis (Shrivastava, Jung, Saadat, Sirohi, & Crewson, 2014). However to support characterization, both nights were retained in this analysis. It could also indicate that other variables were at play such as age, sex, neurologic level of insult, or presence of other symptoms.

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Age is known to influence sleep quality with or without injury. Studies in individuals without SCI have increased sleep latency, percentage of lighter stages of sleep (N1 & N2), and wake after sleep onset as they age, however, unique to individuals after TSCI is the acceleration of aging on joints and organ systems (Jensen, Hirsh, Molton, Bamer, 2009). Thus, middle to older adults are thought to biologically age beyond their chronological age, placing them at higher risk for experiencing impaired sleep. Ohayon, et al, 2017, suggests that for all age groups, a sleep latency period of <15 minutes is a good index for good sleep quality; while >60 min is considered poor sleep. Findings from this study support that older adults likely experience increased sleep latency and spend more time in lighter stages of sleep, although results remain variable. In this sample there were two individuals aged 71. Of these two participants, case 4, had one night of normal sleep latency (>60 min) both nights. Thus, no definitive conclusion can be drawn.

Gender differences after TSCI are recognized but understudied due to the low percent of women in the TSCI population. In non-injured populations, sleep, pain and depression have been found to manifest differently in women than their male counterparts. Here women make up 25% of participants. It is challenging to separate what is gender specific over age, co-morbid conditions, or injury level. Among the women, case 3, who is 27 years of age, had the lowest SE over both nights than case 6, who is 53 years of age and case 8 who is 71 years of age. In observing case 3 among all participants, she had the lowest SE. However, in examining SOL, case 8 had the longest latency among all participants. It should be noted that each woman had a significant number of comorbid conditions and/or past history compared to male participants, which included, chronic low back pain, fibromyalgia, night terrors, and generalized anxiety disorder. So while, the sample size does not support such inferences, gender differences appear to make a contribution to the overall symptom experience after TSCI and should be considered in all studies in this population.

Several studies that have examined secondary conditions after spinal cord injury, have inferred that sleep disturbances have a correlation with neurologic level of injury. Earlier studies found that having cervical level injuries is more likely to impact circadian rhythm of core body temperature and is associated with decreased or absence in melatonin production (Thijssen, Eijsvogels, Hesse, Ballak, Atkinson, & Hopman, 2011; Scheer, Zeitzer, Ayas, Brown, Czeisler, & Shea, 2006). Studies examining subjective report of sleep disturbance, also found that cervical level injuries more often report poor sleep than lower level injuries (Spong, Graco, Brown, Schembri, & Berlowitz, 2015). Of the three cervical level injuries observed in this sample, case 8, with a C2 ASIA D injury, had the highest SOL of all participants; however, did not seem to perceive her sleep as poor based on diary reports. The findings from this study do support that cervical level injuries do experience poorer sleep than other injury levels. It should also be noted

that this sample primarily comprised of ASIA D functioning participants, thus they have retained more motor and sensory function than those with A, B, or C level scores; which likely had an impact on findings.

Similar to injury level, the co-presence of other symptoms such as pain and depression have been found to negatively impact sleep after TSCI. Pain is the symptom most consistently associated with poor subjective sleep quality (Avluk, Gurcay, Gurcay, Budh, Hulting, & Lundeberg, 2005; Karaahmet, Tamkan, Cakci, 2014; Widerstrom-Noga, Felipe-Cuervo, Yezierski, 2001). However, it is not always clear if pain interferes with sleep or poor sleep worsens severity of pain. The findings here suggest that sleep is impaired regardless of the presence of pain. Using threshold cut-offs to categorize pain as low, moderate, and high based on the NRS (1-4=low, 5-7=moderate, 8-10=high) most participants experienced moderate to high pain (scores of 5-10). Some participants did not have recorded pain scores; however, this may be due to assessment schedules of the rehab unit and not necessarily indicative of total absence of pain. Pain is assessed daily, in the morning with vital signs, unless patient receives pharmacologic intervention, which would then yield more frequent reassessments. It was also noted anecdotally that some individuals who did not complain of pain were premedicated with Tylenol before therapy and would have likely impacted pain reports.

Comparably, depression is commonly associated with poor sleep in injured and non-injured populations. However, given that two participants had mild depression and one had a diagnosis of MDD, this study cannot confirm or refute this assertion as it relates to individuals with TSCI. Of interest in this study, is the results of the PHQ-9 found most participants had no depression, although some of those individuals were just below the severity score of mild. Moreover, many of the questions that addressed somatic concerns, such as questions 3 and 4 (Figure 3), 55% of the

participants answered "several days" and/or "more than half the days". Despite the decreasing stigma around mental health it is still more likely that physical ailments (fatigue, poor appetite, pain) prompt individuals to present to clinics where depression is found to be the source underlying those complaints (Kaplakijan, Toussaint, Albright, Bombardier, Krause, & Tate, 2009; Kroenke, 2003; Sime, VonKorff, Piccinelli, Fullerton, & Ormel, 1993). Therefore, it is important to consider if the PHQ-9, although reliable and valid, is the best tool to evaluate depression in this population, as symptoms could be more somatic than psychological.

Using a combination of subjective and objective measurement is considered the most comprehensive approach to appraising sleep (Buysse, Ancoli-Israel, Edinger, Lichstein, & Morin, 2006). However, very few studies, have assessed real-time objective measures of sleep outside of the in-hospital sleep lab in individuals with TSCI. This makes the use of the Sleep Profiler in this patient population, a novel method of objective sleep measure. While polysomnography (PSG) is considered the "gold standard" in objective sleep measurement, it is costly and can pose environmental challenges for older adults, as well as individuals with mobility impairments (Lyster, Choi, Yeh, Imes, Johansson, & Chasens, 2015). Actigraphy, while accepted as an alternative to PSG, is evaluated using accelerometry; determining sleep and wake periods are by motion (wake) and no motion (sleep), although some newer versions are now endorsing the ability to stage sleep (deZambotti, Cellini, Goldston, Colrain, & Baker, 2019). The use of actigraphy in TSCI populations has limitations. Depending on neurologic level of insult, absence or decreased motor ability may inappropriately capture periods of inactivity as sleep based on software algorithms thresholds. Moreover, biometric data such as heart rate and skin temperature that is also utilized by actigraphy monitors, would not adequately capture these measures for some individuals with TSCI, given the poor thermoregulation control and decreased peripheral vascular

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tone, (resulting in a low resting heart rate) in higher level injuries. For these reasons, actigraphy was eliminated as an option for this study. This does not, however, exclude actigraphy for activity focused studies as a viable option.

Using the adapted, expanded version of CSD-M/CSD-E in combination with the Sleep Profiler, many of the same metrics that are meaningful to gaining a global assessment of sleep quality were present in both. This offered a means of comparing self-report to objective findings. When comparing diary reports to Profiler results, SOL was often underestimated. Participants often reported less time to fall asleep than what was measured by the Profiler. Case 1 underestimated SOL reporting 20 min, but recorded by the Profiler as 74 min (>1 hour), as did case 6 who reported 5 min, but was recorded as 200 min (3 hours) by the profiler. Awakenings of >30 seconds by the profiler, describes the transition from sleep to wake and back to sleep after initial sleep onset (Advanced Brain Monitoring, 2013). These numbers were very similar to the diary reports of awakenings, however awakenings of >90 seconds also describes this same transition but for longer than 1 min and was found to be abnormal based on age/sex norms. Remarkably, while most often participants underestimated SOL and WASO metrics. Normal healthy adults are recommended to have 7-8 hours of sleep per night (Ohayon et al, 2017), however, profiler findings revealed that only 1 individual (case 5) achieved 8 hours of sleep; the remaining participants achieved 3 or less hours of sleep. The discrepancy between self-report and objective measure is recognized in the literature, however, underscores the significance of a critical need for objective sleep assessment both in research and clinically after TSCI.

Sleep architecture describes the percent of time spent in each stage of the sleep cycle, with typical approximations for adults as 5% spent in N1, 50% in N2, 20% in N3 and 25% in REM sleep (Shrivastava, Jung, Saadat, Sirohi, & Crewson, 2014). Cases 1, 5, and 6 spent nearly 20% of

sleep in the N1 stage. N2, is where most sleep occurs and a low percentage may be related to sleep fragmentation, obstructive sleep apnea-related arousals, or more time spent in other sleep stages; whereas higher percentages are associated with age related changes and/or medication effects (Shrivastava, Jung, Saadat, Sirohi, & Crewson, 2014). Comparing arousals and N2, those with low percentages in N2, also had an increased number of arousals. Given the high prevalence of sleep disordered breathing (SDB) in this population, and subsequent development found to be during the rehabilitation period in general, it would not be surprising if the two were related. However, a larger sample would be necessary to confirm these inferences.

N3, or "slow wave sleep" is a deeper sleep, thought to play a role in cerebral restoration, metabolic clearance systems of the brain, and recovery in humans (Levendowski, Ferini-Strambi, Gamaldo, Cetel, Rosenberg, et al, 2017; Roth, 2009). When N3 sleep exceeds norms, it is thought to be the result of rebound or recovery sleep, from sleep deprivation; while low N3 is associated with side effects of certain medications (Shrivastava, Jung, Saadat, Sirohi, & Crewson, 2014). Case 6, was observed to have very low N3, (0.4%), however this participant was taking a significant number of medications for co-morbid conditions, that likely contributed to this low. The other two participants fell into normal or near normal percentages. REM sleep, like N3, is also impacted by medication and amount can be dependent upon severity of sleep deprivation (Shrivastava, Jung, Saadat, Sirohi, & Crewson, 2014). None of these participants achieved normal REM percentages. REM sleep remains ambiguous in its role within sleep. Some literature describes REM as a dream state, others note that this stage serves as a critical time for the brain to consolidate memories, and "file" cognitive processes from wake states as well as, perform cellular "cleanup" (Vyazovskiy & Delogu, 2014). Based on these, presumed roles, neurologically injured populations with decreased or absent REM can suffer significant cognitive and attention deficits.
The second aim of this pilot was to evaluate the ability of DNA methylation profiles of candidate genes in the inflammatory and sleep regulation pathways to explain variation in sleep disturbance and co-occurring symptoms in TSCI patients during the in-hospital rehab phase of recovery. It is well known that both inflammation and sleep are regulated in part by genetics and that DNA methylation plays a significant role in phenotypic variability, causing impairments in transcription factor binding and silencing of gene expression (Moor, Le, & Fan, 2013). The impact of these epigenetic changes on symptom expression after TSCI has not been studied.

The inflammatory/immune response after acute TSCI is known to persist after initial injury into chronic phases and indefinitely (Noeller, Groah, & Nash, 2017). Acetylcholine receptors are highly expressed in immune cells and are said to control immune function, through an inhibition of pro-inflammatory cytokine production (DeJonge & Ulloa, 2007). Thus, CHRNA7, serves an important function for all individuals, but particularly after TSCI in reducing pro-inflammatory cytokine production. The literature is clear that pro-inflammatory cytokines increase the risk of developing secondary health conditions such as sepsis, respiratory infections, pain and depression (Allison & Ditor, 2015). Understanding that DNA methylation, silences gene expression, the findings of this study would indicate that individuals that were hypermethylated in the CHRFAM7A gene, have a largely decreased ability to depress pro-inflammatory cytokine production. However, it should be noted that over time, hypomethylation can cause genomic instability and cell transformations that could be equally significant depending on tissue type and length of time gene remains in this state, particularly in older adults (Kullis & Esteller, 2007). With over half of the sample (55%) as highly methylated, on at least one night and one individual (case 4), showing increased methylation on both nights, likely indicates a state of sustained proinflammation. Given the dynamic nature of methylation profiles to change overtime, almost half

(44%) of the sample had either low methylation or failed results on night two, however this does not necessarily indicate that inflammation was absent. The change in methylation levels are likely in response to changes in disturbed sleep between night one and night two. As previously noted, most participants had improved sleep (albeit abnormal) on night two than on night one. The methylation variability finding support the variability seen in the sleep results, as well as the depression and pain findings with case 1, demonstrating high levels of methylation on night one, positive for mild depression and had pain scores of 10/10 and 9/10. Several studies have linked immune regulation to circadian rhythm, with upregulation of cytokines controlled by the master circadian regulator, and thus expression and suppression is reflective of circadian oscillations (Scheiermann, Kunisaki, & Frenette, 2013). Sleep duration (or total sleep time) has also been linked to inflammation in adults, with increased cytokine production triggered by sleep loss (Irwin & Opp, 2017). The findings of this study do point to inflammation and sleep having a cyclical relationship. IL-1B and TNF-alpha have been identified in several studies as having enhanced expression following trauma and peripheral nerve injury (Zhang & An, 2007). It is also, known that pain is directly related to pro-inflammatory cytokine production and suggests that pain also follows circadian oscillations (Zhang & An, 2007).

Having support for impaired sleep from a self-report and EEG based standpoint, examining the epigenetics of sleep could further characterize phenotypic variation by capturing underlying mechanisms that influence disturbed sleep in this population. PER 2, one of three orthologs of the period gene, has a fundamental role in transcription regulation of clock component proteins and robust expression is necessary for the maintenance of circadian rhythms (Fan, Chen, Li, Yongluo, Chen, et al, 2014). Thus decreased expression could have significant effects on circadian rhythm control. The findings of this study observed that over half (67%) of the participants had high levels of PER 2 methylation on at least one night, also had prolonged SOL and WASO, frequent awakenings, and poor SE. Moreover, given the circadian regulation on immune cells, restricted expression of circadian sleep genes, as a result of methylation theoretically could contribute to inefficient regulation of pro-inflammatory cytokine production, further worsening sleep patterns and quality.

Age and sex, have been found to influence cellular changes in methylation. A longitudinal study of older adult men, using DNA methylation in blood samples, found decreased methylation as age increased (Bollati, Schwartz, Wright, Litonjua, Tarantini, et al, 2009). Case 8, participant is a 71 year of age, female whose methylation results on night 1 were low (CHRFAM7A=.05% and PER2=1.86%), also had the highest SOL on both nights. However, it should be noted that lifestyle factors were not accounted for (i.e. smoking history; diet; activity) which could also influence methylation changes over time. Interestingly, case 4, who is also 71 years old, male, did have increased methylation on CHRFAM7A (73.4% and 98.9%) on both nights and increased methylation level on PER 2 (2.5% and 97.4%) on night two only. It is not clear why one had increased methylation and the other did not. Sex, has been studied as a contributor to methylation changes and may vary based on cell type, as to which sex will express higher levels. Since there are only one of each sex in this age range a conclusion cannot be inferred, but does indicate the need for further evaluation of sex on methylation in TSCI studies.

#### **3.6 Conclusions**

The findings of this study support that sleep after TSCI is significantly impaired. Observations could not lend to the categorization of phenotypes across the sample due to size and heterogeneity but each individual represented a potential phenotype that may be better differentiated in a more powered sample. Moreover, the influence from demographic features (age, sex, level of injury) have significance on findings and may represent common phenotypes within population. This study lays a foundation to characterize sleep after SCI and what is considered "normal" that reflects the unique challenges that accompany specialized populations such as those with neurologic injury. There was variability in methylation and variability in sleep measures and that in this small sample size there can be some relationships (like those mentioned above) that can be made between DNA methylation of these genes and sleep phenotype. Depression was not found to be prevalent in this sample, but given the positive responses to questions that focus on somatic manifestations associated with depression, further examination is warranted. Pain was present and tended to be moderate to high in most participants, but its co-occurring impact on sleep and depression, given other potential confounding variables was not clear. Acknowledging the novelty of the sleep profiler's ability to measure sleep in the same way as PSG and the high frequency of sleep disordered breathing, using this instrument to the fullest extent of its capabilities may also help support a more comprehensive assessment of sleep in this population.

#### **3.7 Limitations**

The most significant limitation of this pilot was the sample size. With a modest sample there was no power to utilize inferential statistics; moreover, with the extreme variability within and between participants findings should be interpreted with caution. Two participants, case 2 and case 6, withdrew from the study after night one and thus have one night of EEG results and biomarker results. Case 2 did not complete their sleep diary and thus subject sleep report was not

available. Case 4 and case 8 have partial sleep diaries, with both nights of EEG result. Biomarker data was unable to be collected on night 2 for case 8. Therefore, findings while summative are not comprehensive.

This study took place on an in-patient rehabilitation unit, however, tracking of potential environmental contributions to sleep disruption such as a noise, light, and care activities (i.e. repositioning; bladder management) were not possible. Tracking these variables are recognized as important to the monitoring of sleep to give context to prolonged latency and/or periods of wake after sleep onset. It should be noted that very few instruments have been psychometrically evaluated as reliable and valid instruments in TSCI populations neither the Sleep Profiler, nor the Consensus diary, have been validated in TSCI populations. Moreover, while the aim was to decrease participant burden, more than one subjective sleep instrument should be utilized when evaluating sleep quality.

Medication lists were reviewed for sleep aides and potentially insomnia promoting medications. Several participants were prescribed melatonin at bedtime, which could have impacted findings. Although a limitation in this study, it is unclear if the use of melatonin as a sleep aid, actually improved sleep or if there was a placebo effect. The two participants that did not receive melatonin had better sleep compared to other participants that did. Melatonin has known anti-inflammatory properties, in addition to its sleep enhancement which theoretically could be beneficial in this population, more studies are needed to confirm this. It may have also impacted the methylation findings.

Although heterogeneity was the desired composition of the sample, the wide age range of participants did not allow for a meaningful evaluation of the age-related influences on sleep, mood, and methylation profiles, particularly in the older adults of this study. Likewise, injury levels were

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fairly similar, with incomplete paraplegia, which is not representative of the experience in higher level injuries with complete motor and sensory deficits.

#### **3.8 Future Directions**

This pilot study provides evidence that effort should go into replication, in a larger sample, with validated measures of sleep and other symptoms. In addition, future studies could include environmental variables such as light, noise, and care routines to determine their level of influence on sleep variability during the in-hospital rehabilitation phase of recovery. Given the collection of an additional Paxgene tube (now stored in freezer) at the same time of this analysis, the effect of methylation on transcriptional activity could be examined in this sample. While the findings support much of the literature about impaired sleep and the co-occurrence of other symptoms questions still remain and new questions have emerged. One next step would be to re-evaluate these same participants in six months to one year and compare findings in a more chronic phase of recovery. Another question is how well is depression being measured with the PHQ-9 in this population? This could lead to a study about examining depression using valid SCI designed scales compared to the PHQ-9. Other steps could include psychometric evaluation of the expanded CSD, as well as, a secondary evaluation of autoscoring software of the Sleep Profiler with PSG trained technicians, as validation of these tools in TSCI populations. Considering that a number of participants were on Melatonin supplementation, a future study could look at efficacy on sleep related outcomes.

## Appendix A Sleep Staging and Stage Characteristics

	Normal	Age/Gender	
	Percentage	Distinctions	Stage Characteristic
Non-REM	5%	All age groups;	<ul> <li>"Light" sleep</li> </ul>
Stage 1 (N1)			<ul> <li>Transitionary phase from wakefulness to sleep; easy arousal</li> <li>Considered a direct measure of daytime alertness and the subjective refreshing sleep quality</li> <li>Prolonged time in this period is associated with frequent arousals</li> </ul>
Non-REM Stage 2 (N2)	50%	All age groups;	<ul> <li>Predominates the sleep stages</li> <li>Follows N1 and reoccurs throughout the night</li> <li>Low percent may be associated with         <ul> <li>obstructive sleep apnea related arousals</li> <li>o sleep fragmentation</li> </ul> </li> </ul>
Non-REM Stage 3 (N3)	20%	All age groups;	<ul> <li>Considered "deep" sleep; also referred to as slow wave sleep (SWS)</li> <li>Increased time in this stage is seen during rebound sleep</li> <li>Decreased time in stage is related to medication side effects (benzodiazepines, TCA's, barbiturates)</li> <li>Episodes of night terror sleep walking; sleep talking are associated with this stage.</li> </ul>

REM	25%	Older adults may	<ul> <li>Considered the "dream" stage</li> </ul>
		have 4-5% less than young/middle	<ul> <li>Cycles every 90 to 120 min throughout the night with progressively increasing periods.</li> <li>Suppresses movement (paralysis)</li> </ul>
		adults	

Cited from Ohayon et al, (2017); Shrivastava, Jung, Saadat, Sirohi, & Crewson (2014)

## Appendix B Sleep Profiler<sup>™</sup> Auto-staging Report Definitions

## **REPORT TERMINOLOGY**

## DEFINITION

SLEEP TIME	Recording time minus wake time		
SLEEP EFFICIENCY	Sleep time divided by recording time		
% TIME WAKE	Hours of valid wake divided by hours of sleep time		
% TIME STAGE REM	Hours of valid REM divided by hours of sleep time		
% TIME STAGE N1	Hours of valid stage 1 sleep divided by hours of sleep time		
% TIME STAGE N2 (TOTAL)	Hours of valid stage 2 sleep divided by hours of sleep time		
% TIME N3	Hours of valid stage 3 sleep divided by hours of sleep time		
SLEEP ONSET LATENCY	Elapsed time from the start of recording until the start of the first three consecutive non-Wake epochs at the start of the night		
WASO	Wake after sleep onset sums all minutes the patient was awake after sleep onset until the end of the record		
AWAKENINGS >30 SECONDS	Number of occurrences (lasting 30 seconds or longer) of a transition from sleep to awake and back to sleep subsequent to sleep onset.		
AWAKENINGS >90 SECONDS	Number of occurrences (lasting 90 seconds or longer) of a transition from sleep to awake and back to sleep subsequent to sleep onset.		

Reported from the Sleep Profiler Portal Technical Manual copywrite by Advanced Brain Monitoring Inc.

# Appendix C Key Terms and Definitions

ASIA score	American Spinal Injury Association Impairment scale describes the cumulative score from a systematic neurological examination of dermatomes (motor) and myotomes (sensory) on both the left and right side of the body. Grades assigned based on these findings. See appendix D, for grades and degree of impairment. Reference: International Standards for Neurological Classification of Spinal Cord Injury, 2000.
DNA methylation	The addition of a methyl group to cytosine and adenine residues in DNA that leads to the epigenetic modification of DNA and the reduction of gene expression and protein production.
Phenotype	Defined as the state of an organism resulting from interactions between genes, environment, disease, molecular mechanisms, and chance.
Acute/subacute rehabilitation period	This period begins with admission to hospital and stabilization of the patient's neurological state and is a 6-12 wk bed period.
Sleep architecture	Describes sleep patterns as the percentage of time in spent in sleep stages.
Transcription	The first step in gene expression. It involves copying a gene's DNA sequence to make an RNA molecule.
Epigenetics	Process that alters gene activity without changing the DNA sequence and leads to modifications that can be transmitted to daughter cells.
EEG	Monitoring method to record electrical activity of the brain. It is typically noninvasive, with the electrodes placed along the scalp. EEG measures voltage fluctuations resulting from ionic current within the neurons of the brain.

Polysomnography	Used to assess sleep architecture and characteristics, as well as diagnose sleeping disorders and titrate positive away pressure treatment. The "gold standard" in sleep evaluation and guidelines are set by the American Association of Sleep Medicine (AASM).
Buffy coat	The fraction of an anticoagulated blood sample that contains most of the white blood cells and platelets following density gradient centrifugation of the blood. The buffy coat is used to extract DNA from the blood of mammals.
Hypermethylation	An increase in the epigenetic methylation of cytosine and adenosine residues in DNA
Hypomethylation	A decrease in the epigenetic methylation of cytosine and adenosine residues in DNA
First night effect	Occur as a combination of the change in environment and the sleep equipment in which sleep can be negatively impacted by sleep monitoring
Reverse first night effect	Refers to a pattern in which the individual sleeps better on the first PSG night in comparison to remaining PSG nights.

A=Complete	No motor or sensory function is preserved in the sacral segments S4–S5
B=Incomplete	Sensory but no motor function is preserved below the neurological level and includes the sacral segments S4–S5
C=Incomplete	Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3
D=Incomplete	Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more
E=Normal	Motor and sensory function are normal

Retrieved from https://www.orthobullets.com/spine/2006/spinal-cord-injuries

#### **Appendix E Case Summaries**

#### E.1 Case Summary 1

This participant is an 18-year-old Caucasian male, who sustained an L1 burst fracture and spinal compression, as a result of a fall. His ASIA score is a C, indicating incomplete motor function preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3. On night one, reported one nap that lasted approximately 2 hours, and rated his mental alertness and physical energy as "fair". However, on night 2 reported one nap that lasted approximately 1.5 hours, and rated mental alertness and physical energy as "good. Total sleep time on night 1 was self-reported as about 7.5 hours, however, EEG activity revealed a total sleep time of 3.5 hours. On night 2, total sleep time was self-reported as 8 hours, while EEG activity report showed a total of sleep time of 6.5 hours. It should be noted that time in bed was longer on night one, getting into bed at 5:00pm, but not preparing for sleep until 12midnight; while on night 2, getting into bed time was reported as 9:00pm, and preparing to go to sleep was around 11:00pm. Participant was not prescribed melatonin, nor were there any other medications that were found to impact sleep or contribute to insomnia symptoms. Patient does have a history of depression and does endorse high pain over both days.

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#### E.2 Case Summary 2

This 58-year-old African-American male, was diagnosed with a central cord injury as a result of a fall. Central cord injuries tend to have more upper extremity motor loss and weakness, than lower extremities. Participant only completed one night of the study using the profiler and did not complete his sleep diary. He withdrew after the first night. Chart review revealed one report of pain as a 2.5 out of 10 and depression screen indicated no depression. Blood was retained for methylation analysis and both genes were found to be highly methylated. Received Melatonin supplement at bedtime.

#### E.3 Case Summary 3

Case 3 is a 27-year-old, Caucasian female, with a history of chronic back pain, which resulted in the surgery that led to her SCI. She was diagnosed with a T9 ASIA D, as a result of post-operative complications from her surgery. She is ambulatory, as ASIA D grading indicates motor function is preserved below the level of the injury and more than half of the key muscles are normal. Pain scores are reported as high 8/10 on both days. No depression reported. Self-reports 3 awakenings both nights. Reported time to bed was between 9:45pm and 10:00pm, with an actual attempt to go sleep around 11:00pm. Sleep latency was observed around 1.5 hrs on both nights. Reports only 1 nap on night 1 for 45 min. Describes sleep quality as fair to good and reports feeling somewhat (night 1) to well rested (night 2). Rates mental and physical alertness as very good. Complete EEG data was not available for this participant. Methylation levels on night 1 was

high in both genes, but night 2 revealed low methylation of CHRFAM7A and partial methylation of PER2. Received Melatonin supplement at bedtime.

#### E.4 Case Summary 4

Case 4 is a 71-year-old Caucasian male, with a C3 ASIA D, secondary to a motor vehicle accident. Pain scores are reported as high 5/10 on both days. No depression reported. Sleep diary, revealed no reports of awakening on night 1 and night 2 data was not available. Reported time in bed was "noonish", with an actual attempt to go sleep around 11:00pm. Sleep latency was observed around 30 minutes on both nights. Reports only 1 nap for 20 min. Describes sleep quality as good and reports feeling somewhat rested on both nights. Rates mental alertness as good and physical energy as fair. Complete EEG data was not available for this participant. Methylation levels were high in CHRFAM7A genes on both nights, whereas PER2 levels were low on night 1 and high on night 2. Received Melatonin supplement at bedtime.

#### E.5 Case Summary 5

Case 5 is a 38-year-old Caucasian male, with a T8 ASIA D injury as a result of motor vehicle accident. Pain scores are reported as high 8/10 on both days. No depression reported. Self-reports 3 awakenings on night 1 and 1 on night 2, which is consistent with his sleep profiler report of awakenings >90 seconds. Reported time to bed was 12 midnight, with an actual attempt to go sleep around 1:00am on night 1 and time to bed and attempt to go to sleep was 1:00am. Sleep

latency was observed around 20 minutes on night 1 and 8 minutes on night 2. Denies taking naps on either night. Describes sleep quality as poor (night 1) to good (night 2) and reports feeling slightly rested (night 1) to well rested (night 2). Rates mental alertness as very good and physical energy as very good/good. Methylation levels on night 1 was high in both genes, but failed in both genes on night 2. Did not receive melatonin at bedtime.

#### E.6 Case Summary 6

This 53-year-old Caucasian, woman is diagnosed with a T11 ASIA D, as result of a standing fall from a hypoglycemic episode. Participant withdrew after night one, as she reports being unable to tolerate wearing the profiler. She has a history of falls; mitochondrial myopathy, encephalopathy, lactic acidosis and stroke syndrome (MELAS); and Fibromyalgia. She endorsed a high sensitivity to pain. Pain scores are reported as high 10/10 on night 1. No depression reported. Reports awakening twice on night one. Sleep profiler report shows 3.4 awakenings >90 seconds. Reported time to bed was 9:00 pm, with an actual attempt to go sleep around 10:00pm. Sleep latency was observed around 200 minutes (~3 hours). Reports only 1 nap for 30 min. Describes sleep quality as fair and reports feeling somewhat rested. Rates mental alertness as very good and physical energy as good. Methylation levels were low for both genes. Despite receiving melatonin supplement at bedtime, still had difficulty with falling asleep and maintaining sleep.

#### E.7 Case Summary 7

This 26-year-old Caucasian male, suffered a fall and was diagnosed with a T7 ASIA B. Has a history of seizure disorder and non-Hodgkin's lymphoma. Pain scores are reported as high 7/10 on both days. No depression reported. Self-reports five awakenings on night 1 and two on night 2. Reported time to bed was 7:00pm, with an actual attempt to go sleep around 11:00pm on night 1 and time to bed at 8:00pm and attempt to go to sleep was 10:00pm. Sleep latency was observed at 172 minutes on night 1 and 64 minutes on night 2. Report 1 nap for 20 minutes. Describes sleep quality as poor (night 1) to good (night 2) and reports feeling slightly rested (night 1) to somewhat rested (night 2). Rates mental alertness as good and physical energy as good/fair. Complete EEG data was not available for this participant. Methylation levels failed on both genes on both nights. Reported that he had just begun antibiotic therapy for a UTI, and was experiencing fever chills on night one. Did receive melatonin supplement at bedtime.

#### E.8 Case Summary 8

Participant is a 71-year-old Caucasian female, with a C2 ASIA D, as a result of a fall. Has history of anxiety, epilepsy, and night terrors. No reported pain on either day. No Depression. Denies awakenings however, sleep profiler reports 7 awakenings >90 seconds on both nights. Reported time to bed was between 9:00pm and 9:30pm, with an actual attempt to go sleep around 9:30pm. Sleep latency was observed around 4 hours on both nights. Reports 2 naps with no report on length of nap. Describes sleep quality as very good and reports feeling well rested (night 1). Rates mental alertness as good/very good and physical energy as poor (day 1) and good (day 2). Complete EEG data was not available for this participant. Methylation levels on night 1 was low in both genes, on night 1 and specimen was unable to be obtain on night 2. Received melatonin supplement at bedtime.

#### E.9 Case Summary 9

This 50-year-old male, was diagnosed with T11 ASIA D, as a result of fall. Pain scores are reported as high 8/10 on one day. Has a past medical history of depression and bipolar disorder. Self-reports five awakenings on night 1 and none on night 2. Reported time to bed was 6:00pm, with an actual attempt to go sleep around 10:00pm on night 1 and time to bed 6:00pm and attempt to go to sleep was 9:00pm on night 2. Sleep latency was observed around 2 minutes on night 1 and 29 minutes on night 2. Reports 3 naps about 20-30 min long on day 1. Describes sleep quality as fair (night 1) to good (night 2) and reports feeling not at all rested (night 1). Rates mental alertness as fair/ good and physical energy as poor/fair. Complete EEG data was not available for this participant. Methylation levels on night 1 failed, and levels were low in both genes on night 2. Received melatonin supplement at bedtime.

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**Appendix F Consent Form** 



School of Nursing

3500 Victoria Street Pittsburgh, PA 15261 Fax: 412-624-2401

## CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH STUDY

#### **Sleep after Spinal Cord Injury**

Principal Investigator: Letitia Graves, MSN, RN, Predoctoral Fellow Telephone: 412-436-9083 Email: lyg10@pitt.edu

Co-Investigators: Yvette Conley, PhD, FAAN, Professor Eileen Chasens, PhD, RN, FAAN, Associate Professor Gwen Sowa, MD, PhD, Associate Professor Amanda Harrington, MD, Assistant Professor Karen Greenwald, BSN, RN, Clinical Coordinator Dianxu Ren, PhD, Associate Professor

**Source of Support:** National Institutes of Health (T32NR009759) Robert Wood Johnson Foundation

#### Why is this research being done?

Spinal cord injury (SCI) is a condition that is often accompanied by impaired sleep. The purpose of this study is to examine sleep after a SCI and investigate the biological causes for variation in sleep after SCI.

The long-term goal of this study is to provide healthcare providers with the tools they need to improve diagnosis and treatment of sleep and other symptoms in spinal cord injuries.

#### Who is being asked to take part in this study?

You are being asked to participate in this study because you have indicated you are interested in this additional study about sleep. This study will enroll a total of 20 participants who are also participating in SCI Model System research.

#### What procedures will be performed for the purpose of this study?

If you agree to participate in this study, you will undergo the following procedures that are not part of your routine medical care:

- Sleep data will be collected by use of a sleep EEG monitor (measures electrical brain activity) that will be worn around the head like a headband. The monitor will take real time measurement of your sleep patterns. The EEG sleep data will be collected over 48 continuous hours. During the 48hr period, your reported sleep experience will also be collected using a sleep diary, reporting your sleeping and waking time and related activities.
- 2. Approximately 1 tablespoon of blood will be collected every day for two days for the purpose of evaluating genes and gene products. Blood will be obtained from indwelling intravenous tubes (catheters) if available, or using peripheral venipuncture (i.e. arm, hand). All efforts will be made to obtain a sample when other routine laboratory tests are taken.
- 3. We will link the data obtained about sleep and from the above samples to data collected through the SCI Model System.

We will be collecting DNA samples for future testing of genes and gene products that may be involved in SCI or recovery from SCI; however, we cannot at this time, tell you exactly what genes or gene products will be tested. If you agree to participation in the research project, your biological sample and genetic material will be maintained in a -80°C freezer in Victoria building under the control of the principal investigator of this research project and will be maintained indefinitely. Stored specimens and other data may be made available without identifiers (name; date of birth etc.) to investigators not listed on the consent form. We are also requesting your authorization or permission to review your past, current and future medical records to use your previous records in addition to the results of the research procedures for this study. We will obtain the following information: medication administration records (medications for sleep or that significantly affect sleep), demographic information, level of injury, and vital sign data. This identifiable medical record information will be made available to members of the research team for an indefinite period of time. This authorization is valid for an indefinite period of time. However, you can always withdraw your authorization to allow the research team to review your medical records by contacting the investigator listed on the first page and making the request in writing.

You will not be provided with the results of this research study since the sleep and genetic data cannot yet be interpreted or applied in a clinically relevant manner.

The length of participation in this study is for 3-4 days (72-96hrs).

## What are the possible risks and discomforts of this research study?

**<u>Risk of blood collection</u>**: Some persons eligible for this study have indwelling, intravenous tubes (catheters) that are placed for standard care. If available, blood will be obtained from these catheters. These catheters have a risk for infection. The risk is not increased by the collection of these samples. All efforts will be made to collect these samples to coincide with routine blood tests. A rare risk infection of these catheters from sampling (occurs in less than 1% or less than 1 out of 100 patients). If no IV access is available, blood will be collected through peripheral venipuncture. The risks of taking blood include pain, a bruise at the point where the blood is taken, redness and swelling of the vein and infection, and a rare risk of fainting. Again all efforts will be made to collect these samples to coincide with routine blood tests.

<u>**Risks of genetic testing**</u>: In this study, all genetic data are stored without personal identifiers.

Your genetic sample will be stored with a study ID number (unique number containing no personal information) attached to it. Information linking these ID numbers to your private information (name, date of birth etc.) is stored in a separate secure location. Every effort is made to ensure that participant confidentiality is maintained, however, it is possible that a breach of confidentiality may occur during study participation. All investigators are trained in HIPAA policies and procedures and sign confidentiality agreements. The risks associated with gene studies include the potential for a breach of confidentiality which could affect future insurability, employability, or reproduction plans, or have a negative impact on family relationships and/or result in paternity suits or stigmatization. Your participation in this research study might, in the future, include whole genome sequencing.

In addition, there is a new Federal law, called the Genetic Information Nondiscrimination Act

(GINA), that generally makes it illegal for health insurance companies and group health plans to use genetic information in making decisions regarding your eligibility or premiums. GINA also makes it illegal for employers with 15 or more employees to use your genetic information when making decisions regarding hiring, promoting, firing, or setting the terms of employment. This new Federal law does not protect you against genetic discrimination by companies that sell life, disability, or long-term care insurance.

**<u>Risks of Sleep Diary:</u>** There is a risk of fatigue or frustration when completing the sleep diary. You can take a break or stop at any time without full completion of the diary. You have the right to refuse participation.

#### Who will pay if my loved on is injured as a result of taking part of this research study?

University of Pittsburgh Investigators and their associates who provide services at the UPMC recognize the importance of your voluntary participation in their research studies. These individuals and their staffs will make reasonable efforts to minimize, control and treat any injuries that may arise as a result of this research. If you believe that you are injured as a result of the research procedures being performed, please contact immediately the Principal Investigator listed on the first page of this form. Emergency medical treatment for injuries solely and directly related to your participation in this research study will be provided to them by the hospitals of UPMC. It is possible that UPMC may bill your insurance for the cost of this emergency treatment, but none of these costs will be charged directly to you. If your research-related injury requires medical care beyond this emergency treatment, you will be responsible for the costs of this follow-up care unless specifically stated below. There is no plan for monetary compensation. You do not, however waive any legal rights signing this form.

#### What are possible benefits from taking part in this study?

It is unlikely that you will receive any direct benefit as a result of your participation in this research study. Benefits to society include enhancing our means of detecting a biomarker in the blood sample of a person with a spinal cord injury to determine the severity of symptoms and predict outcomes. Information obtained from this study may help physicians, in the future, judge how severe symptoms are, if treatments need to be adjusted and may also predict outcomes in patients with a spinal cord injury.

#### Will my insurance provider be charged for participation in the research study?

None of the services and/or procedures, such as blood sampling or completion of outcome questionnaires that occur for the purpose of research during this study will be billed to your insurance. If you receive a bill, or believe that your health insurance has been billed in error for something that is part of the research study, you will be instructed to notify a member of the research team or UPMC Patient Billing Services.

There is no charge for participating in this research study. Research chemical analyses and genetic testing charges are the responsibilities of the investigator. All other exams are routine medical care, and will be billed in the standard fashion and become your responsibility. There will be a one-time participation compensation of \$25.00, that will be distributed in the form of a gift card at completion of the study. This gift card will be issued through the University of Pittsburgh system. It can be used as a debit/credit card for purchases, or the card balance can be converted to a lump sum similar to an ATM withdrawal.

Your biological sample or genetic material may lead, in the future, to new inventions or products. If the research investigators are able to develop new products from the use of your biological sample or genetic material, there are currently no plans to share with you any money or other rewards that may result from the development of the new product.

#### Who will have access to the information I provide during this study?

Any information about you obtained from this research study will be kept as confidential (private) as possible. You understand that any information about you obtained from this research will be kept strictly confidential and stored in locked files at the School of Nursing. Only authorized study investigators and staff will be allowed to look at these files. You will not be identified by name in any publication of research results unless you sign a separate form giving permission (release). In addition to the investigators listed on the first page of this authorization (consent) form and their research staff, the following individuals will or may have access to identifiable information related to your participation in this research study:

Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review your identifiable research information for the purpose of monitoring the appropriate conduct of this research study.

Authorized representatives of the sponsor of this research study, the National Institutes of Health, will review and/or obtain identifiable information related to your participation in this research study for the purpose of monitoring the accuracy and completeness of the research data. While the study sponsor understands the importance of maintaining the confidentiality of your identifiable research information, the UPMC and University of Pittsburgh cannot guarantee the confidentiality of this information after it has been obtained by the study sponsor.

Authorized representatives of UPMC hospitals or other affiliated health care providers may have access to identifiable information (which may include your identifiable medical information) related to your participation in this research study for the purpose of (1) fulfilling orders made by the investigators, for hospital and health care services (e.g.

laboratory tests, diagnostic procedures) associated with research study participation; (2) addressing correct payment for tests and procedures ordered by the investigators; and/or (3) for internal hospital operations (i.e. quality assurance).

In unusual cases, the investigators may be required to release identifiable information (which may include your identifiable medical information) related to your participation in this research study in response to an order from a court of law. If the investigators learn that you or someone with whom you are involved is in serious danger or potential harm, they will need to inform, as required by Pennsylvania law, the appropriate agencies.

Stored specimens and other data may be made available without identifiers to investigators not listed on the consent form. This information may be identifiable. We will be happy to provide you with a list at your request.

#### Is my participation in this study voluntary?

Your participation in this research study is completely voluntary. Whether or not you provide your consent for participation in this research study will have no effect on your current or future relationship with the University of Pittsburgh.

#### May I withdraw, at a future date, my consent for participation in this research study?

You may withdraw, at any time, your consent for your participation in this research study, to include the use and disclosure of your identifiable information for the purposes described above. (Note, however, that if you withdraw your consent for the use and disclosure of your identifiable medical record information for the purposes described above, you will also be withdrawn, in general, from further participation in this research study.) If you choose to withdraw from this study, all data collected prior to the date of withdrawal will be continued to be used, unless you request we destroy it.

To formally withdraw your consent for participation in this research study, you will provide a written and dated notice of this decision to the principal investigator of this research study at the address listed on the first page of this form.

Your decision to withdraw your consent for participation in this research study will have no effect on your current or future medical care at a UPMC hospital or affiliated health care provider or your current or future relationship with a health care insurance provider. You will receive stand medical care regardless of your participation in this study.

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### VOLUNTARY CONSENT

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions, voice concerns or complaints about any aspect of this research study during the course of this study, and that such future questions, concerns or complaints will be answered by a qualified member of the research team. I understand that I may always request that my questions, concerns or complaints be addressed to the investigators listed on the front page of this form. At any time, I may also contact Human Subjects Protection Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668) to discuss problems, concerns, and questions; obtain information; offer input; or discuss situations in the event that the research team is unavailable.

By signing this form, I consent to participate in the is research study and provide my authorization to share my medical records with the research team. A copy of this form will be given to me.

Participant's Name (print)

Participant's SignatureDate

WitnessDate

### **CERTIFICATION OF INFORMED CONSENT**

I certify that I have explained the nature and purpose of the **Sleep after Spinal Cord Injury** research study to the above-named individual, and I have discussed the possible risks and potential benefits of participation in this research study. Any questions the individual has about his/her participation in this research study have been answered, and the physicians and research staff associated with the University of Pittsburgh, School of Nursing will always be available to address future questions as they arise.

I further certify that no research component of this protocol was begun until after this consent form was signed.

Printed Name of Person Obtaining ConsentRole in Research Study

#### Signature of Person Obtaining ConsentDate

#### **Appendix G Consensus Sleep Diary (Adapted)**

#### **General Instructions**

What is a Sleep Diary? A sleep diary is designed to gather information about your daily sleep pattern.

How often and when do I fill out the sleep diary? It is necessary for you to complete your sleep diary every day. If possible, the sleep diary should be completed within one hour of getting out of bed in the morning.

What should I do if I miss a day? If you forget to fill in the diary or are unable to finish it, leave the diary blank for that day.

What if something unusual affects my sleep or how I feel in the daytime? If your sleep or daytime functioning is affected by some unusual event (such as an illness, or an emergency) you may make brief notes on your diary.

**Will answering these questions about my sleep keep me awake?** You should not worry about exact times; you should not watch the clock.

**Comments** If you have anything that you would like to say that is relevant to your sleep feel free to write it in the designated spaces.

*Item Instructions* Use the guide below to clarify what is being asked for each item of the Sleep Diary.

Date: Write the date of the morning you are filling out the diary.

1. What time did you get into bed? Write the time that you got into bed. This may not be the time that you began "trying" to fall asleep.

2. What time did you try to go to sleep? Record the time that you began "trying" to fall asleep.

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3. How long did it take you to fall asleep? Beginning at the time you wrote in question 2, how long did it take you to fall asleep.

*4. How many times did you wake up, not counting your final awakening?* How many times did you wake up between the time you first fell asleep and your final awakening?

5. In total, how long did these awakenings last? What was the total time you were awake between the time you first fell asleep and your final awakening. For example, if you woke 3 times for 20 minutes, 35 minutes, and 15 minutes, add them all up (20+35+15= 70 min or 1 hr and 10 min).

6. What time was your final awakening? Record the last time you woke up in the morning.

7. What time did you get out of bed for the day? What time did you get out of bed with no further attempt at sleeping? This may be different from your final awakening time (e.g. you may have woken up at 6:35 a.m. but did not get out of bed to start your day until 7:20 a.m.)

8. How would you rate the quality of your sleep? "Sleep Quality" is your sense of whether your sleep was good or poor.

9. How restful or refreshed did you feel when you woke up for the day? This refers to how you felt after you were done sleeping for the night, during the first few minutes that you were awake.

10. *How many times did you nap or doze?* A nap is a time you decided to sleep during the day, whether in bed or not in bed. "Dozing" is a time you may have nodded off for a few minutes, without meaning to, such as while watching TV. Count all the times you napped or dozed.

11. In total, how long did you nap or doze? Estimate the total amount of time you spent napping or dozing, in hours and minutes. For instance, if you napped twice, once for 30 minutes and once for 60 minutes, you would answer "1 hour 30 minutes." If you did not nap or doze, write "N/A."

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12. How many caffeinated drinks did you have? Enter the number of caffeinated drinks you had with one drink of coffee or tea = 6-8 oz; while for caffeinated soda or energy drink one drink = 12 oz. <u>Many drinks are larger than this size, if so, increase the number accordingly.</u>

13. What time was your last caffeinated drink? If you had a caffeinated drink, enter the time of day in hours and minutes of your last drink. If you did not have a caffeinated drink, write "N/A" (not applicable).

14. How would you rate your mental alertness? Please rate from "very poor" = mentally sluggish to "very good"= mentally alert.

15. How would you rate your physical energy? Please rate from "very poor" = physically exhausted to "very good"= physically energetic.

Consensus Sleep Diary – **Morning** (Please Complete Upon Awakening) ID:

Today's Date	Sample 8/22/13
1. What time did you get into bed?	10:15 pm
2. What time did you try to go to sleep?	11:30 pm
3. How long did it take you to fall asleep?	55 min.
4. How many times did you wake up, not counting your final awakening?	6 times
5. In total, how long did these awakenings last?	2 hours 5 minutes
6. What time was your final awakening?	6:35 am
7. What time did you get out of bed for the day?	7:20 am
8. How would you rate the quality of your sleep?	□Very Poor ☑Poor □Fair □Good □Very Good

	□Not at all rested	
9 How rested or refreshed did you feel when you wake-up for	☑Slightly rested	
9. How rested or refreshed did you feel when you woke-up for	□Somewhat rested	
the day?	□Well-rested	
	□Very well-rested	
Comments (if applicable)	I have a cold.	

Today's Date	Sample		
	8/22/13		
10. How many times did you nap or doze?	1		
11. In total, how long did you nap or doze?	15 min.		
12. How many caffeinated drinks (coffee, tea, soda, energy	2 drinks		
drinks) did you have?			
13. What time was your last caffeinated drink?	3:00 pm		
	□Very Poor		
	⊠Poor		
14. How would you rate your mental alertness?	□Fair		
	□Good		
	□Very Good		

	□Very Poor
	⊠Poor
15. How would you rate your physical energy?	□Fair
	□Good
	□Very Good
Comments (if applicable)	I have a cold.

## Appendix H Published Manuscript Symptom Science: Advocating for the Inclusion

of Functional Genetic Polymorphisms

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Sleep Profiler homepages.rommands.pdf	Plus Size Swi RoseGal.com Differer	ce beic Regression	Spiritual Soulurch Listings Amazo	on.com:DVDs & more Whol	lesale Loholesale.com	»
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JOURNALS PERMISSIONS	Institutional Rep	oositories: I	nformation for SAG	E Authors and	Users	
Process for Requesting Permission	Green Open Access: subscription journal articles deposited in institutional repositories					ies
Guidelines for SAGE Authors						
Reusing Open Access and SAGE Choice Content	Information for Authors Authors of articles published in subscription journals may share and reuse their article as outlined on the Guidelines for SAGE Authors page and stated in their signed Contributor Agreements.					
Posting to an Institutional Repository (Gold OA)						
Posting to an Institutional Repository (Green OA)	Under SAGE's Green Open Access policy, upon the article being accepted for publication, the Accepted Version of the article may be posted in the author's institutional repository.					
License Information for CHORUS	For information about funding agency Open Access policies and ensuring compliance of agency-funded articles, see our Funding bodies, policies and ensuring compliance page.				odies, policies	
JOURNAL ARTICLE REPRINTS						
ACCESSIBILITY	Information for Users of the Institutional Repository					
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#### Appendix I Impaired Sleep After Spinal Cord Injury Manuscript [Under Review]

#### **Introduction**

Adequate sleep quality is a fundamental requirement for health and wellbeing in healthy adults (Ohayon, et al, 2017). Sleep quality refers to how well one sleeps and is generally characterized by the convergence of quantitative and qualitative features including sleep onset (falling asleep), sleep maintenance (staying asleep) and the self-report of daytime sleepiness. Sleep problems such as sleep disordered breathing (SBD) and sleep-related movement disorders are common in persons with SCI and are significant risk factors for impaired sleep quality (Giannoccaro et al. (2012). However, in persons with SCI additional concomitant factors beyond sleep disorders can negatively influence sleep quality, such as pain, depression, neurogenic bladder and bowel impairment, pressure sores, and respiratory compromise (Stricksek, et al., 2017). This has significant implications for persons with SCI, particularly during the rehabilitation phase of recovery. Sleep plays a role in neuron communication needed for neuroplasticity, hormone regulation, and metabolic function, which in a state of dysfunction could impede rehabilitation goals and functional outcomes (Allison & Ditor, 2015).

The purpose of this integrative review was to 1) appraise the methodologic approaches for evaluating sleep quality in adults with SCI, and 2) identify SCI-related factors influencing sleep patterns (e.g. the level of injury [tetraplegia/paraplegia]). This review was guided by Whittemore and Knafl's methodologic approach (2005) for integrative review, which includes problem identification, literature search, data evaluation, data analysis, presentation of discussion, and conclusions. Integrative reviews utilize qualitative and quantitative empirical and theoretical publications to provide a comprehensive understanding of a particular phenomenon or healthcare problem (Hopia, Latvala, & Liimatainen, 2016; Whittemore & Knafl, 2005).

#### **Background to the Problem**

Spinal cord injury (SCI) is highly prevalent, with an estimated worldwide annual incidence between 250,000 and 500,000 new SCI cases (WHO, 2018). In the United States, there are 282,000 individuals currently living post-SCI injury and approximately 17,000 incident cases of SCI annually (University of Alabama, 2016). Care for individuals with SCI is complex, requiring intensive respiratory, musculoskeletal, neurologic, renal and integumentary management. This complex care creates considerable economic burden to the healthcare system with the total direct cost of care for a single patient with SCI ranging from \$350,000 to over \$1 million during the first year of injury (University of Alabama, 2016). In addition to the costs of SCI care, because individuals with SCI are most likely to be affected during prime working years, such individuals are often unable to fully return to the workforce, compounding the societal economic burden of SCI. As the coordinator and care manager for complex treatment plans aimed at decreasing long term negative health consequences and improving community reintegration (Association of Rehabilitation Nurses, 2007), it is crucial that rehabilitation nurses assimilate the best evidence around complex care of individuals with SCI into their practice.

The etiology of impaired sleep quality during the rehabilitation phase of recovery in persons with SCI is multifactorial. However, most studies evaluating sleep quality in persons with SCI have done so only in the context of the presence of a sleep disorder including SDB (Chiodo, Sitrin, & Bauman, 2016), movement disorders (i.e. restless leg syndrome [RLS], and periodic leg movement [PLM]) (Proserpio, et al, 2015; Kumru, et al, 2015). A review by Sankari, Vaughan, Bascom, Martin, & Badr (2019) found that in persons with SCI, SDB was significantly associated
with poor sleep quality. However, to the best of our knowledge, there has been no comprehensive integration of the multidimensional factors that can contribute to sleep quality in persons with SCI. Given the expanded appreciation of the role of sleep during recovery from acute and chronic disease, a comprehensive examination of factors associated with impaired sleep quality, as well as an evaluation of assessment tools used to evaluate sleep is needed in persons with SCI.

# Literature Search

For this review, a computer-assisted search of online databases (PubMed, CINHAL, Web of Science) was systematically completed by two independent reviewers for relevant publications. Consultation with a medical librarian yielded search terms under the medical subject headings [MESH] "spinal cord injury" combined with "sleep," "sleep quality," "sleep patterns" or "sleep wake disorders." Given the limited nature of studies on sleep quality and sleep patterns after SCI, we included all articles meeting criteria regardless of publication date, to provide the broadest possible characterization of the topic.

An a priori determination was made to include experimental studies, observational studies and qualitative studies. Articles were excluded if 1) title, abstract or key words did not include one or all of the search terms; 2) sample was less than 18 years of age when the SCI occurred; 3) the article was non-data based [i.e. letters to the editor, commentaries, or reviews]; 4) sleep in adults with SCI was not the dependent variable, and 5) studies where sleep disorders (i.e. sleep apnea or restless leg syndrome/periodic limb movement) were the primary focus. A total of 14 articles were left for secondary reduction as outlined in the PRISMA diagram [Figure 1]. One article was excluded because it was a pre-clinical study and therefore did not meet the criterion of adult human studies. The other was excluded after further review because it was an intervention clinical trial where treatment of sleep apnea was the primary focus. Grey literature and conference abstracts were not examined in this review. After removing duplicates, abstracts were screened in detail for inclusion criteria and those not meeting criteria were removed. Full texts of the remaining publications were reviewed in detail and assembled our final sample of articles.

## **Data Evaluation**

The quality of each study included in the review was evaluated for its strengths and weakness. Each article was read in detail by two independent reviewers and then discussed to extract key data elements, identify the thematic focus of the article, and evaluate based on adequate and appropriate sample size and description of the data collection methods. Five of the studies had large (N> 150) sample sizes; the remaining studies had relatively small sample sizes and lacked power analyses to determine if they were adequate to avoid type 2 errors. The majority of the studies depended solely on subjective appraisal of sleep quality. Only four of the studies collected data using objective measures of actigraphy or polysomnography to evaluate sleep, (Adey, Bors, & Porter, 1968; Scheer, Zeizter, Ayas, Brown, Czeisler, & Shea, 2006; Thijssen, Eijsvogels, Hesse, Ballak, Atkinson, & Hopman, 2010; Verheggen, et al, 2018) and only two studies provided an evaluation of sleep in individuals with SCI using both subjective and objective measures (Verheggen et al, 2018; Spong, Graco, Brown, Schembri, & Berlowitz, 2015).

## Methodology Used to Assess Sleep Quality

Table 2 reports each of the sleep instruments utilized and whether they have been psychometrically evaluated in an SCI population. The National Sleep Foundation (NSF) organizes sleep quality as consisting of several sleep continuity variables, including, number of awakenings > 5minutes; wake after sleep onset; sleep latency; and sleep efficiency (Ohayon, et al, 2017). The most precise way to capture these variables is through use of objective measurements such as

polysomnography (PSG) or actigraphy, which supports real time quantifiable measurement of brain wave activity and/or other biometric data related to the activity of sleep. Subjective measurement using questionnaires such as the Basic Nordic Sleep Questionnaire (BNSQ), the Karolinska Sleepiness Scale (KSS), and the Pittsburgh Sleep Quality Index (PSQI) also capture these same variables, using recall from the individual. Our review found 6 articles that utilized one or more of these scales to describe sleep quality (Biering-Sorensen, Biering-Sorensen, & Hilden, 1994; Akerstedt & Gillberg, 1990); Buysse, Rynolds, Monk, Berman, & Kupfer, 1989). Distinctively, subjective instruments include aspects of the sleep experience that cannot be extracted from objective measures, such as self-report of daytime sleepiness, daytime function, and subjective well-being. One of the limitations of the sole use of questionnaires is patient recall, especially for individuals in a rehabilitation setting where cognitive abilities may be impaired.

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# **Results**

MESH terms resulted in 145 articles from PubMed; 84 articles from CINAHL; and 60 articles from the Web of Science for a total of 289 publications. After removing articles that were duplicates (n=20) and were not available in English (n=3) there were 266 articles for preliminary review by title and abstract [Figure 1]. After screening title and abstract, 254 articles were excluded, leaving a final sample of 12 articles.

Table 1 presents the final sample of articles, detailing the study design, instrument used, sample size, age and gender distribution, location and severity of spinal injury, and main findings. The twelve studies are organized by the following foci: (1) the sleep experience; (2) predictors of impaired sleep; and (3) biological mechanisms impacting sleep-wake and circadian rhythm.

### The Sleep Experience

A retrospective study by Spong et al, 2015 of adults with tetraplegia, found that individuals with SCI experience significantly worse (p<.05) subjective sleep problems (i.e. difficulty in sleep maintenance, increased wake after sleep onset, worse sleep quality, higher use of medications for sleep, worse daytime sleepiness, increased symptoms of sleep apnea [snoring and pauses in breathing] and reported lower quality of life than their able bodied counterparts (Spong, et al, 2015). A strength of this study was the use of the BNSQ, a sleep instrument with good test-retest reliability in a previous psychometric evaluation in persons with SCI (Biering-Sorensen et al, 1994). Limitations of this study were the observational design without the use of objective measures for the evaluation of sleepiness, sleep-wake patterns, or sleep apnea.

Fogelberg, et al, 2016 study found that, compared with the general population results of these same instruments, individuals with SCI and multiple sclerosis (MS) reported worse sleep. Subjective instruments in this study included the medical outcomes study sleep scale (MOS-S), the patient reported outcomes measurement information system sleep disturbance short form (PROMIS-SD), and PROMIS sleep-related impairments short form (PROMIS-SRI). The strengths of this study are the inclusion of psychometric properties of each sleep evaluation tool, given the difficulty of measuring sleep in special populations and the overall paucity of reliable and valid sleep instruments in SCI populations. Additionally, participants were recruited through shared databases with the Northwest Regional SCI Model System at the University of Washington or the Shepard Center, Virginia Crawford Research Institute, which supported a larger sample size. A weakness of this study was lack of description or differentiation made on level or severity of SCI, which the literature appears to recognize as a contributing factor on sleep experience.

Participants in the Fogelberg and colleagues, (2017) study, described their sleep difficulties (i.e. difficulty in falling asleep, maintaining sleep, or obtaining restorative sleep), barriers to

achieving sufficient high-quality sleep (i.e. medical treatments and care activities that disturbed sleep, beds and environments that were not conducive to sleep, [pain and anxiety]), and the impact of sleep disturbances on their daily function (i.e. difficulty with meeting daily occupational obligations, daytime fatigue). Two weaknesses of Fogelberg's secondary analysis of community-dwelling adults with SCI study are that the original study design did not directly ask questions about sleep and that the number of participants (N=20) was not designed to reach "saturation" where no new information is obtained on factors related to sleep in persons with SCI. The primary strength of this study in individuals with SCI, was that it described factors that impact sleep from their viewpoint.

Therefore, in spite of inconsistency in assessment tools, the three study's findings consistently endorse a poor sleep experience resulting from sleep fragmentation (i.e. frequent interruptions), and difficulty in maintaining sleep or getting back to sleep once awakened (Spong, et al, 2015; Fogelberg, et al, 2016; Fogelberg, et al, 2017). Each study provides supporting evidence that persons with SCI, in general, perceive having more difficulties with sleep and overall poorer quality of life, compared to an able-bodied population. However, lack of consistency in instrument selection, heterogeneity and modest size of the samples ultimately impacts generalizability of the findings.

### Predictors of Impaired Sleep

Predictors of impaired sleep offer an understanding of the contributing factors that lead to actual or perceived sleep interference. Five studies evaluated the predictors of impaired sleep, the most common predictors were related to the presence of pain, depression and anxiety.

Widerström-Noga, et al. (2001) surveyed (N=217) community-dwelling adults with traumatic SCI and chronic pain to examine how chronic pain interfered with their activities of

daily living. Data found that 38% of respondent's experienced frequent pain interfering with initiating sleep (3 nights to every night/per week) and 40% experienced pain interfering with maintaining sleep (3 nights to every night/per week). Two predictors of difficulty in initiating sleep include a high average pain intensity (p<0.01) and an increased number of descriptors respondents used when describing their pain (p<0.001). Difficulty in maintaining sleep had four predictor variables: male sex (p<0.05), older age at time of injury (p<0.05), anxiety (p<0.01) and high average pain intensity (p<0.001). A limitation of this study was the lack of psychometric validation of the survey tool used to measure frequency of sleep interference and the lack of objective measures of sleep.

Budh and colleagues (2005) used a cross-sectional descriptive study to compare the quality of sleep in persons with SCI with and without pain (N=191). Participant composition included, individuals with tetraplegia (n=51), paraplegia (n=78), complete injury (n=57) incomplete injury (n=129) unknown (n=5) were divided into three groups based on the timing and severity of pain: those with "no pain" (n=50), "intermittent pain" (n=42), or "continuous pain" (n=99). Pain scoring was done using a 100-mm visual analogue scale (VAS) with anchors of 0 (no pain) to 100 (unbearable pain). Sleep was measured using the BNSQ and the Hospital Anxiety and Depression questionnaire evaluated mood. Difficulty in initiating sleep, difficulty in maintaining sleep, morning and daytime sleepiness, and poor sleeping quality was significantly worse in participants in the continuous pain group compared to participants in the "no pain" or the "intermittent pain" groups (all p-values <.05). Data also revealed that persons with tetraplegia reported a trend toward poorer quality of sleep than paraplegics (p=0.051), persons with incomplete injuries reported poorer overall sleep quality than those with complete injuries (p=0.045). Anxiety was the main predictor of poor sleep quality among three of the six variables assessed, while pain intensity was

most predictive of perceived sleep over the last 3 months, and depression was most predictive if responders reported early rising and excessive sleepiness in the morning. Strengths of this study were the use of the BNSQ instrument previously, a valid and reliable instrument in SCI populations, evaluation of potential gender differences, and the in-depth analysis of the effect of pain on sleep in a relatively large sample. Limitation of this study was the lack of objective measures of sleep.

A study by Jensen, Hirsh, Molton, and Bamer (2009) compared sleep in communitydwelling adults with SCI, with able-bodied adults with chronic illness and adults from general population, individuals with SCI had significantly worse sleep. The study examined the association of chronological age, duration of SCI, age at SCI, time since SCI, SCI level, and whether the SCI was complete or incomplete with the severity of impaired sleep quality, using the MOS-S questionnaire. While duration and age of onset of SCI were not statistically significant, younger (25-44 years) chronological age was a significant predictor of increased sleep difficulty when compared to the older respondents (>65 years). Data from Jensen's study agreed with previous findings that SCI was associated with increased risk for impaired sleep (LaVela et al, 2012; Widerstrom-Noga et al, 2001; Carskadon & Dement, 2017; Gianccoro et al, 2013; Chiodo, Sitrin, & Bauman, 2016; Proserpio et al, 2015). Strengths of the study are the large sample size; and while the MOS-S is a validated instrument to measure sleep disturbances, it has not been psychometrically evaluated in SCI populations. A limitation to the study was the cross-sectional design and the use of only a subjective sleep measure. The authors noted that the potential effect of additional untested variables (i.e. pain) may have had contributed to the finding of worse sleep quality in younger adults with SCI.

LaVela et al. (2012) examined impaired sleep, patient characteristics and health conditions associated with increased risk of mortality and comorbidities in a sample of veterans (N=622) with either SCI or various spinal cord disorders. A cross-sectional survey included a single question querying dysfunctional sleep, defined as "trouble sleeping or regular insomnia" and coded as either "yes" or "no". Almost half of the respondents (49%) reported dysfunctional sleep; those respondents with dysfunctional sleep were significantly (p<.05) more likely to be white, have risky health behaviors (current smoker, problems with alcohol consumption), and have an increased risk for health conditions (asthma, COPD, weight gain). A strength of the study was the large sample that specifically examined veterans with SCI. Limitations of the study are the cross-sectional design that precludes determining causality, and that sleep was not measured with any objective measure. In addition, the use of a mixed sample of SCI with patients with other spinal cord disorders and the use of a single dichotomous question regarding sleep difficulties limited the interpretation of the results.

Avluk, Gurcay, Gurcay, Karaahment, Tamkan, and Cakci (2014) performed a small (N=44; paraplegic n=28, tetraplegic n=18), cross-sectional study in patients with traumatic SCI to analyze the relationship between chronic pain, functional status, depression and sleep. There was strong reliability and validity of the Turkish versions of the questionnaires for pain (Multidimensional Pain Inventory-Spinal Cord Injury [MPI-SCI), depression [Hamilton Rating Scale for Depression]), and sleep quality (Pittsburgh Sleep Quality Index [PSQI]). Greater chronic pain severity was associated with worse depression and sleep quality (r= .487, r=.312, respectively, p<.05). Two strengths of this study were the use of reliable and valid versions of the instruments (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and the evaluation of the association between

pain, depression and sleep. Limitations of the study are the small sample size, cross-sectional design and the lack of objective measures of sleep.

## Biological Mechanisms impacting Sleep-Wake and Circadian Rhythm

Four of the studies examined biological mechanisms that regulate sleep patterns and sleep quality. The use of electroencephalogram (EEG) to examine wave patterns of the brain during sleep and gene biomarkers associated with regulation of sleep have been an area of increasing interest, as they offer a partial understanding of the changes that impact sleep after SCI. From these studies it is clear there are biological influences, such as fluctuations in hormone production (i.e. melatonin) and homeostatic thermostat regulation that are important components of sleep regulation at the cellular level.

An early study by Adey, Bors, and Porter, (1968) examined EEG sleep pattern after high cervical lesions on 17 participants. Participants were categorized into two groups corresponding to the time from initial injury with the "early post-lesion" group being from 1-2 years after the initial SCI (n=10) and "late post-lesion" being 2.5-14 years after the initial SCI (n=7). Average sleep duration was reduced to about five hours in the early post-lesion group. In the late postlesion group, "light sleep" constituted 78% of total sleep, indicating that "intermediate" or "deep sleep" was not achieved or in very small time frames. There were wide variations in individual participants in the amount of REM sleep in both early and late post-lesion groups, but the mean REM duration was 7-9% of total sleep time, which is less than able-bodied middle-aged persons where REM accounts for 15-20% of total sleep time (Carskadon & Dement, 2017). Sleep duration in persons with lower SCI level lesions was not different from able-bodied norms (7-8 hours); participants with high cervical SCI lesions who had a reduced total sleep duration of 4-5 hours. The strength of this study was the novelty of head based objective sleep measure in patients with SCI. Limitation of this study was the validity of the findings as there was conflicting data results that put into question the rigor of the study.

Scheer, et al, (2006) conducted a secondary case-control study of data from the Zeitzer, Ayas, Shea, Brown, Czeiler, (2000) study that found an absence of melatonin in persons with tetraplegia. The Scheer study using both wrist actigraphy and in-laboratory PSG sleep study to compared sleep efficiency between subjects with cervical SCI (n=3) who had an absence of nocturnal melatonin, thoracic SCI subjects (n=2) with normal melatonin levels, and healthy controls (n=10). Sleep efficiency and total sleep duration were significantly decreased in the cervical SCI patients (p=0.04). There was no significant difference between the thoracic SCI patients and the able-bodied controls in sleep efficiency. REM sleep latency was prolonged in subjects with cervical SCI compared to subjects with thoracic SCI (p=0.04). A strength of this study is the use of objective sleep measures. The findings are limited by the small sample size of subjects with cervical or thoracic SCI and an uncertainty whether use of wrist actigraphy is a valid measure in persons with cervical SCI (Spivak, Oksenberg, & Catz, (2007).

Similarly, Verheggen et al. (2012) examined melatonin levels in saliva over a 24hr period in a sample of individuals with motor and sensory complete tetraplegia (n=6), paraplegia (n=9) and a control group of able-bodied persons (n=10). Melatonin normally increases during the evening in response to dim-light and typically remains high during the night. In Veheggen's study, melatonin levels remained constant in persons with cervical level injuries (tetraplegia). This is different when compared to low level injuries (paraplegia) or with able-bodied controls (p=0.001) who demonstrated a normal circadian pattern. Results of this study adds to the study by Thijssen and colleagues (2010) on impaired core body temperature rhythmicity in persons with tetraplegia with both studies suggesting that the circadian timing and initiation of sleep are directly affected in persons with high SCI. The strength of this study is that it used both subjective and biomarker measures of sleep.

Thijssen et al. (2010) examined circadian alterations in sleep and thermoregulation in SCI patients. The study compared 24hr core body temperature (Tcore) in able-bodied controls (n=8) to persons with high cervical injuries (n=8) and to persons with low thoracic injuries with motor and sensory complete lesions (n=7). The three groups were not significantly different from each other in terms of the timing of their sleep-wake cycles by self-report. There was a circadian variation that showed significant difference among tetraplegics when compared with paraplegics and able-bodied controls. During the daytime, tetraplegics with cervical level lesions were able to maintain normal Tcore; however, Tcore was poorly controlled at night during the sleep period, where a natural decrease in Tcore was absent, resulting in circadian regulation disruption. This is different from able-bodied controls, and paraplegics who reflected able-body cycles, where Tcore regulation demonstrated typical 24-hour variation (early evening peak followed by rapid decline and subsequent plateau during sleep). The strength of this study was the use of actigraphy to objectively measure the timing of sleep and of core body temperature in able-bodied persons and those with SCI. A limitation of the study was the small number of persons in each group.

#### Discussion

All of the reviewed studies concluded that persons with SCI experience poorer quality sleep than their able-bodied counterparts. The studies suggest that sleep in persons with SCI is not only impacted by the care required by a patient with SCI but that there may be direct biological mechanisms that can disrupt central sleep and circadian processes, especially in persons with a high level spinal injury. Data suggest that the level of the SCI lesion may contribute to the differences in subjective and objective findings that were observed across the reviewed articles. Persons with cervical and high thoracic SCI lesions tended to experience a more difficult sleep experience than those persons with lower thoracic lesions, as persons with paraplegia tend to exhibit responses similar to able-bodied persons regarding biological factors (circadian rhythmicity). There remains large heterogeneity in predictors between individuals with tetraplegia and those with paraplegia, such pain and depression, thus, the examination of impaired sleep in SCI may benefit from using a symptom based approach to characterize symptom cluster profiles (Crawford, Chirinos, Iurcotta, Edinger, Wyatt, Manber, & Ong, 2017).

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None of the studies examined the effect of sleep quality interventions on recovery after SCI; this leaves a significant gap in knowledge about the effect of improved sleep may have on functional outcomes in persons with SCI, especially during the rehabilitation phase of recovery when early interventions have the potential for greater long-term improvements. Future research is key to support the development of effective interventions in persons with SCI (e.g. mixed study design with objective and subjective measures, identifying specific drivers of difficulties with sleep, examination of evidenced based sleep interventions used with other populations based on identified concerns such as cognitive behavioral treatment for insomnia). In addition, utilization of the National SCI database may support larger samples in studies with SCI patients. In the current review, only two studies used the SCI Model System database. The SCI Model System, consisting of 14 SCI Centers of Excellence across the United States, holds the largest database of SCI data through the National Spinal Cord Injury Statistical Center (University of Alabama, 2016). The Model System collects data on individuals with SCI throughout the lifetime of those enrolled, tracking demographic and recovery outcomes. The use of large data sets to mine data in a database that has been collected over decades as is possible with the SCI data, is an untapped resource with the potential to make a major impact in SCI rehabilitation and move the science

forward. While, there is little information collected on sleep quality by the SCI Model System; this provides an opportunity for sleep researchers to expand the database by adding to the longitudinal data on sleep quality.

Across the reviewed studies, various instruments to measure sleep quality in the SCI samples were used without report of the psychometric reliability and validity of these instruments. Of the instruments identified in this review (Table 2), only two were found to be reliable and valid in patients with SCI. Future studies need to include with both subjective and objective measures that have been found reliable and valid in the measurement of sleep in SCI patients. The use of common data elements has been of increasing interest across clinical studies has the potential to benefit both the clinical and research communities, as they support rapid and efficient study start-up and enriched data sharing and aggregation using standard definitions and forms; as well as common outcome measures that may be relevant across disease states (Biering-Sorensen, et al, 2015).

#### Limitations

Limitations of this review includes that the articles reviewed were often older articles, beyond a 5-year timeframe. This highlights the dearth of sleep quality related research in this population. The review searched three databases for data-based studies, however, widening our search may have yielded more articles. Grey literature, abstracts and conference proceedings that describe works in progress not yet been published, was excluded.

## **Conclusion and Implication for Rehabilitation Nurses**

In conclusion, patients with SCI have a high prevalence of impaired sleep quality when compared to able-bodied samples. Due to small sample sizes, heterogeneity of the type of SCI (tetraplegia vs paraplegia) across studies, and the lack of objective measures to assess sleep quality,

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further study of sleep in SCI is warranted. Emerging evidence suggests that pain and caregiving activities negatively influence sleep in persons with SCI; however, the sleep mechanisms that underlie this relationship remain unclear. None of the studies examined the effect of interventions to improve sleep on recovery from SCI. Impaired sleep after SCI may have critical significance for the management of secondary health conditions as well as the quality of life in this medically vulnerable population.

There remains a substantial gap in the understanding of the impact of sleep quality on recovery from SCI. More importantly, our review reveals a lack of current understanding of optimal application of the little extant knowledge of sleep quality in SCI to improve clinical care for this complex population for a more comprehensive approach to SCI management. The review identified that adding scheduled melatonin doses may improve sleep during the inpatient rehabilitation phase. Care delivery approaches such as standardized sleep assessment, and establishing pre-bed sleep hygiene routines, and clustering of care were identified to allow uninterrupted sleep time may also be beneficial. However, more research is needed to identify additional empirically supported interventions to improve sleep quality. Given advances in technology, the ability to share large data repositories, and advances in symptom science, there exists a considerable window of opportunity to meaningfully address this research and care gap.

#### References

- Adey, W.R., Bors, E., Porter, R.W. (1968). EEG sleep patterns after high cervical lesions in an. Archives of Neurology, 19, 377-838.
- Akerstedt, T., Gillberg, M. (1990). Subjective and objective sleepiness in the active individual. *International Journal of Neuroscience*, 52, 29-37.
- Allison, D.J., Ditor, D.S. (2015). Immune dysfunction and chronic inflammation following spinal cord injury. *Spinal Cord*, *53*, 14-18. Doi: 10.1038sc.2014.184
- Association of Rehabilitation Nurses. (2007). The rehabilitation staff nurse role description (brochure). Retrieved from https://rehabnurse.org/about/position-statements/role-of-the-nurse.
- Avluk, O.C., Gurcay, E., Gurcay, A.G., Karaahment, O.Z., Tamkan, U., Cakci, A. (2014). Effects of chronic pain on function, depression, and sleep among patient with traumatic spinal cord injury. *Annals of Saudi Medicine*, 34(3):211-216. Doi:10.5144/0256-4947.2014.211.
- Biering-Sørensen, F., Biering-Sørensen, M., Hilden, J. (1994). Reproducibility of Nordic sleep questionnaire in spinal cord injured. *Paraplegia*, *32*, 780-786.
- Biering-Sørensen, F., Alai, S., Anderson, K., Charlifue, S., Chen, Y.,...Jakeman, L. (2015). Common data elements for spina cord injury clinical research: A national institute for neurological disorders and stroke project. *Spinal Cord*, 53(4), 265-277. Doi:10.1038/sc.2014.246.
- Budh, C.N., Hultling, C., Lundeberg, T. (2005). Quality of sleep in individuals with spinal cord injury: A comparison between patients with and without pain. *Spinal Cord*, 43, 85-95. Doi:10.1038/sj.sc.3101680.

- Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research*, 28(2),193–213.
- Carskadon, M. A., Dement, W. C. (2017). Normal human sleep: An overview; Principles & practice of sleep medicine, 6<sup>th</sup> ed. M. Kryger, T. Roth, W. C. Dement ed. P.15-24. Philadelphia: Elsevier.
- Chiodo, A.E., Sitrin, R.G., Bauman, K.A. (2016). Sleep disordered breathing in spinal cord injury: A systematic review. *The Journal of Spinal Cord Medicine*, *39*, 374-382.
- Crawford, M.R., Chirinos, D.A., Iurcotta, T., Edinger, J.D., Wyatt, J.K., Manber, R., Ong, J.C. (2017). Characterization of patients who present with insomnia: Is there room for a symptom cluster-based approach? *Journal of Clinical Sleep Medicine*, *13*(7), 911-921.
- Fogelberg, D.J., Hughes, A.J., Vitiello, M.V., Hoffman, J.M., Amtmann, D. (2016) Comparison of sleep problems in individuals with spinal cord injury and multiple sclerosis. *Journal of Clinical Sleep Medicine*, 12(5), 695-701.
- Fogelberg, D.J., Leland, N.E., Blanchard, J., Rich, T.J., Clark, F. C. (2017). Qualitative experience of sleep in individuals with spinal cord injury. *Occupational Therapy Journal of Research*, 37, 89-97.
- Gianccoro, M.P., Moghadam, K.K., Pizza, F., Boriani, S., Maraldi, N.M., Avoini, P., Morreale, A., Liguori, R., Plazzi, G. (2013). Sleep disorders in patients with spinal cord injury. *Sleep Medicine Reviews*, 17, 399-409. http://dx.doi.org/10.1016/j.smrv.2012.12.005.
- Hopia, H., Latvala, E., Liimatainen, L. (2016). Reviewing the methodology of an integrative review. *Scandinavian Journal of Caring Science*, *30*(1), 662-669. Doi: 10.1111/scs.12327.

- Jensen, M.P., Hirsh, A.T., Molton, I.R., Bamer, A.M. (2009). Sleep problems in individual with spinal cord injury: Frequency and age effects. *Rehabilitation Psychology*, 54(3), 323-331. Doi:10.1037/a0016345
- Kumru, H., Vidal, J., Benito, J., Barrio, M., Portell, E., Valles, M., Flores, C., Santamaria, J. (2015). Restless leg syndrome in patients with spinal cord injury. *Parkinsonism Related Disorder*, 21, 1461-1464. https://doi.org/10.1016/j.parkreldis.2015.10.007
- LaVela, S.L., Burns, S.P., Goldstein, B., Miskevics, S., Smith, B., Weaver, F.M. (2012).
  Dysfunctional sleep in persons with spinal cord injuries and disorders. *Spinal Cord*, 50, 682-685. Doi: 10.1038/sc.2012.31
- Ohayon, M., Wickwire, E.M., Hirshkowitz, M., Albert, S.M., Avidan, A., Daly, F.J., Dauvilliers, Y.,...Vitiello, M.V. (2017). National sleep foundations sleep quality recommendations:
  First report. *Sleep Health*, *3*, 6-9. https://doiorg.pitt.idm.oclc.org/10.1016/j.sleh.2016.11.006
- Proserpio, P., Lanza, A., Sambusida, K., Fratticci, L., Figerio, P., Sommariva, M.,...Nobili, L. (2014). Sleep apnea and periodic leg movements in the first year after spinal cord injury. *Sleep Medicine*, 16, 59-66. http://dx.doi.org/10.1016/j.sleep.2014.07.2019
- Sankari, A., Vaughan, S., Bascom, A., Martin, J.L., Badr, M.S. (2019). Sleep-disordered breathing and spinal cord injury: A state-of-the-art review. *Chest*, 155(2), 438-445. Doi: https://doi.org/10.1016/j.chest.2018.10.002.
- Scheer, F.A., Zeitzer, J.M., Ayas, N.T., Brown, R., Czeisler, C.A., Shea, S.A. (2006). Reduced sleep efficiency in cervical spinal cord injury; association with abolished night time melatonin secretion. *Spinal Cord*, 44, 78-81. Doi:10.1038/sj.sc.3101784

- Spivak, E., Oksenberg, A., Catz, A. (2007). The feasibility of sleep assessment by actigraph in patients with tetraplegia. *Spinal Cord*, *45*(1), 765-770. doi: 10.1038/sj.sc.3102040
- Spong, J., Graco, M., Brown, D.J., Schembri, R., Berlowitz, D.J. (2015). Subjective sleep disturbances and quality of life in chronic tetraplegia. *Spinal Cord*, 53:636-640. Doi: 10.1038/sc.2015.68
- Stricksek, G., Ghobrial, G., Wilson, J., Theofanis, T., Harrop, JS. (2017) Complications in the management of patients with spine trauma. *Neurosurgery Clinics of North America*, 28(1), 147-155
- Thijssen, D., Eijsvogels, T., Hesse, M., Ballak, D., Atkinson, G., Hopman, M. (2010). The effects of thoracic and cervical spinal cord lesions on the circadian rhythm of core body temperature. *Chronobiology International*. 28,146-154.
- University of Alabama at Birmingham (2016). National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance. Birmingham, AL: https://www.nscisc.uab.edu/Public/Facts%202016.pdf
- Verheggen, R., Jones, H., Nyakayiru, J., Thompson, A., Groothuis, J., Atkinson, G., Hopman, M., Thijssen, D. (2018). Complete absence of evening melatonin increase in tetraplegics. *FASEB Journal*, 26(7), 3059-3064.
- Whittmore, R., Knafl, K. (2005). The integrative review: updated methodology. *Journal of Advanced Nursing*, 52, 546-553.
- Widerstrom-Noga, E.G., Felipe-Cuervo, E., Yezierski, R.P. (2001). Chronic pain after spinal injury: interference with sleep and daily activities. Archives Physical Medicine Rehabilitation, 82, 1571-1577

- WorldHealthOrganization.Spinalcordinjury.http://www.who.int/mediacentre/factsheets/fs384/en/.Accessed March 30, 2018.
- Zeitzer, J.M., Ayas, N.T., Shea, S.A., Brown, R., Czeiler, C.A. (2000). Absence of detectable melatonin, preservation of cortisol, thyrotropin rhythms in tetraplegia. *Journal of Clinical Endocrinology and Metabolism*, 39(1), 2189-2196.

# **Bibliography**

- Almutary, H. Douglas, C., & Bonner, A. (2016). Multidimensional symptom clusters: an exploratory factor analysis in advanced chronic kidney disease. *Journal of Advanced Nursing*, 72(10), 2389-2400. doi: 10.1111/jan.12997
- Alschuler, K.N., Jensen, M.P., Sullivan-Singh, S.J., Borson, S., Smith, A.E., Molton, I.R. (2013). The association of age, pain, and fatigues with physical functioning and depressive symptoms in persons with spinal cord injury. *Journal of Spinal Cord Medicine*
- Armstrong, T. (2003). Symptom experience: A concept analysis. *Oncology Nursing Forum 30*(4), 601-605. doiL 10.1188/03.ONF.601-606
- Avluk, O.C., Gurcay, E., Gurcay, A.G., Karaahment, O.Z., Tamkan, U., Cakci, A. (2014). Effects of chronic pain on function, depression, and sleep among patient with traumatic spinal cord injury. *Annals of Saudi* Medicine. 34(3), 211-216. Doi:10.5144/0256-4947.2014.211.
- Barsevick, A.M. (2007). The concept of symptom cluster. *Seminars in Oncology Nursing 23*(2), 89-98. doi: 10.1016/j.soncn2007.01.009
- Bollati, V., Schwartz, J., Wright, R., Litonjua, A., Tarantini, L., Suh, H., Sparrow, D., Vokonas, P., Baccarelli, A. (2009). Decline in genomic DNA methylation through aging in a cohort of elderly subjects. *Mechanisms of aging and development*, 130(4), 234–239. doi:10.1016/j.mad.2008.12.003
- Borbély, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: A reappraisal. *Journal of Sleep Research*. https://doi.org/10.1111/jsr.12371
- Bowes, A.L., Yip, P.K. (2014). Modulating inflammatory cell responses to spinal cord injury: all in good time. *Journal of Neurotrauma* 31(1):1753-1766. doi: 0.1089/neu.2014.3429
- Brain Basics: Understanding Sleep. *National Institute of Neurologic Disorders and Stroke*. Patient and Caregiver Education. Retrieved from https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Understanding-Sleep.
- Budh, C.N., Hultling, C., Lundeberg, T. (2005). Quality of sleep in individuals with spinal cord injury: A comparison between patients with and without pain. *Spinal Cord.* 43, 85-95. Doi:10.1038/sj.sc.3101680.
- Carney, C. E., Buysse, D. J., Ancoli-Israel, S., Edinger, J. D., Krystal, A. D., Lichstein, K. L., & Morin, C. M. (2012). The Consensus Sleep Diary: Standardizing Prospective Sleep Self-Monitoring. *Sleep*, 35(2), 287–302. http://doi.org/10.5665/sleep.1642

- Cashion, A.K., Grady, P.A. (2015). The National Institutes of Health/National Institutes of Nursing Research Intramural Research Program and the Development of the NIH Symptom Science Model. *Nursing Outlook*. 63(4), 484-487. doi:10.1016/j.outlook.2015/03/001.
- Cirelli, C. (2005). Sleep-Wake Mechanisms: Genetics. *SRS Basics of Sleep Guide 1<sup>st</sup> ed.* Sleep Research Society, Westchester, IL.
- Cleeland, C.S., Bennett, G.J., Dantzer, R., et al, (2003). Are the symptoms of cancer and cancer treatment due to shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. *Cancer* 97(11), 2919-2925. doi: 10.1002/cncr11382.
- Crocker, A., Sehgal, A. (2010). Genetic analysis of sleep. *Genes & Development* 24(1). 1220-1235. http://www.genedev.org/cgi/doi/10.1101/gsd1913110.
- Finan, P. H., Richards, J. M., Gamaldo, C. E., Han, D., Leoutsakos, J. M., Salas, R., Irwin, M.R., Smith, M. T. (2016). Validation of a Wireless, Self-Application, Ambulatory Electroencephalographic Sleep Monitoring Device in Healthy Volunteers. *Journal of Clinical Sleep Medicine*, 12(11), 1443–1451. https://doi.org/10.5664/jcsm.6262
- Fogelberg, D.J., Leland, N. E., Blanchard, J., Rich, J.T., and Clark, F.A (2017). Qualitative experience of sleep in individuals with spinal cord injury. *Occupation, Participation and Health.* 37(2). 89-97. Doi:10.1177/15394449217691978
- Gioannocaaro, M.P., Moghadam, K.K., Pizza, F., Boriani, S., Maraldi, N.M., Avonic, P., Morreale, A., Liguori, R., Plazzi, G. (2013). Sleep disorders in patients with spinal cord injury. *Sleep Medicine Reviews*. 17(1). 399-409. Htt://dx.goi.org/10.1016/j.smrv.2012.12.005
- Hausmann, O. (2003). Post-traumatic inflammation following spinal cord injury. *Spinal Cord* 41(1), 369-378.
- Huang, W., Kabbani, N., Brannan, T., Lin, M.K., Theiss, M.M., Hamilton, J.F., Ecklund, J.M., Conley, Y.P., Vodovotz, Y., Brienza, D., Wagner, A.K., Robbins, E., Sowa, G.A., Lipsky, R.H. (2019). Association of a functional polymorphism in the CHRFAM7A gene with inflammatory response mediators and neuropathic pain after spinal cord injury. *Journal of Neurotrauma* [ahead of print]. Doi: 10.1089/neu.2018.6200
- Hunter, P. (2012). The inflammation theory of disease. *European Molecular Biology Organization Reports 13*(1), 968-970. doi: 10.1038/embor.2012.142
- Irwin, M.R., Opp, M.R. (2017). Sleep health: Reciprocal regulation of sleep and innate immunity. *Neuropsychopharmacology* 42(1), 129-155. Doi: https://doiorg.pitt.idm.oclc.org/10.1038/npp.2016.148
- Jain, N. B., Ayers, G. D., Peterson, E. N., Harris, M. B., Morse, L., O'Connor, K. C., & Garshick, E. (2015). Traumatic spinal cord injury in the United States 1993–2012. JAMA 313(22), 2236–2243. http://doi.org/10.1001/jama.2015.6250

- Jensen, M.P., Turner, J.A., Romano, J.M., Fisher, L.D. (1999). Comparative reliability and validity in chronic pain intensity measures. *Pain* 83(2), 157-162.
- Kalpakjian, C.Z., Toussain, L.L., Albright, K.J., Bombardier, C.H., Krause, J.K., Tate, D.G. (2009). Patient health questionnaire-9 in spinal cord injury: An examination of factor structure as related to gender. *Journal of Spinal Cord Medicine*, 32(2), 147-156.
- Keenan, S., Hirshkowitz, M. (2017). Sleep Stage Scoring. In Kryger, M., Roth, T., Dement, W.C. Editors (Eds.), Principles and Practice of Sleep Medicine (6<sup>th</sup> ed; pp.1567-1575). Philadelphia: Elsevier Inc.
- Krebs, E. E., Carey, T. S., & Weinberger, M. (2007). Accuracy of the pain numeric rating scale as a screening test in primary care. *Journal of general internal medicine*, 22(10), 1453–1458. doi:10.1007/s11606-007-0321-2
- Kroenke, K., Spitzer, R. L., & Williams, J. B. W. (2001). The PHQ-9: Validity of a Brief Depression Severity Measure. *Journal of General Internal Medicine*, 16(9), 606–613. http://doi.org/10.1046/j.1525-1497.2001.016009606.x
- Landry, G. J., Best, J. R., & Liu-Ambrose, T. (2015). Measuring sleep quality in older adults: a comparison using subjective and objective methods. *Frontiers in aging neuroscience*, 7, 166. doi:10.3389/fnagi.2015.00166
- Levendowski, D.J., Ferini-Strambi, L., Gamaldo, C., Cetel, M., Rosenberg, R., Westbrook, P.R. (2017). The accuracy, night-to-night variability, and stability of frontopolar sleep electroencephalography biomarkers. *Journal of Clinical Sleep Medicine*, 13(6), 791-803. http://dx.doi.org/10.5664/jcsm.6618
- Ma, Y., Fang, J., Liu, H., Zhou, L. (2015). Increased HDAC3 and decreased miRNA-130a expression in PBMCs through recruitment HDAC3 in patients with spinal cord injury. *International Journal of Clinical and Experimental Pathology* 8(2), 1682-1689. www.ijcep.com
- Maich, K.H.G., Lachowski, A.M. & Carney, C.E. (2018) Psychometric Properties of the Consensus Sleep Diary in Those With Insomnia Disorder, Behavioral Sleep Medicine, 16:2, 117-134, DOI: 10.1080/15402002.2016.1173556
- Mestre, H., Alkon, T., Salazar, S., & Ibarra, A. (2011). Spinal cord injury sequelae alter drug pharmacokinetics: An overview. *Spinal Cord*, 49(1), 955-960. Doi: 10.1038/sc.2011.58.
- Miaskowski, C., Aouizerat, B.E. (2007). Is there a biological basis for the clustering of symptoms? Seminars in Oncology Nursing 23(2), 99-105. doi: 10.1016/jsoncn2007.01.008
- National Institute on Disability, Independent Living and Rehabilitation Research. (2016). Annual Statistical Report for the Spinal Cord Injury Model Systems. Birmingham, AL: National Spinal Cord Injury Statistical Center.

- Noonan, V. K., Fallah, N., Park, S. E., Dumont, F. S., Leblond, J., Cobb, J., & Noreau, L. (2014). Health Care Utilization in Persons with Traumatic Spinal Cord Injury: The Importance of Multimorbidity and the Impact on Patient Outcomes. *Topics in Spinal Cord Injury Rehabilitation*, 20(4), 289–301. http://doi.org/10.1310/sci2004-289
- Revell Hunter, S.M. (2011). Symptom Clusters in Traumatic Spinal Cord Injury: An Exploratory Literature Review. Journal of Neuroscience Nursing 43(2), 85-93. Doi: 10.1097/JNN.0b13e31820c2533
- Sankari, A., Vaughan, S., Bascom, A., Martin, J.L., Badr, S. (2019). Sleep-disordered breathing and spinal cord injury: A state-of-the art review. *Chest*, 155(2), 438-445. Doi: https://doi.org/10.1016/j.chest.2018.10.002.
- Scheer, F.A., Zeitzer, J.M., Ayas, N.T., Brown, R., Czeisler, C.A., Shea, S.A. (2006). Reduced sleep efficiency in cervical spinal cord injury; association with abolished night time melatonin secretion. *Spinal Cord* 44, 78-81. Doi:10.1038/sj.sc.3101784
- Shrivastava, D., Jung, S., Saadat, M., Sirohi, R., & Crewson, K. (2014). How to interpret the results of a sleep study. *Journal of community hospital internal medicine perspectives*, 4(5), 24983. doi:10.3402/jchimp.v4.24983
- Spinal Cord Injury Facts and Statistics (2017). Retrieved from http://www.sci-infopages.com/facts.html
- Thijssen, D. Eijsvogels, T., Hesse, M., Ballak, D., Atkinson, G., Hopman, M. (2010). The effects of thoracic and cervical spinal cord lesions on the circadian rhythm of core body temperature. *Chronobiology International.* 28, 146-154.
- Whooley, M. A., Avins, A. L., Miranda, J., & Browner, W. S. (1997). Case-Finding Instruments for Depression: Two Questions Are as Good as Many. *Journal of General Internal Medicine*, 12(7), 439–445. http://doi.org/10.1046/j.1525-1497.1997.00076.x