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Effects of Systematic Sleep Fragmentation on Tolerance and Threshold in a Pressure Pain Task: Associations with Sustained Attention

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Dissertation submitted
to the Eberly College of Arts and Sciences
at West Virginia University

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Doctor of Philosophy in
Behavioral Neuroscience

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Sleep

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ABSTRACT

Effects of Systematic Sleep Fragmentation on Tolerance and Threshold in a Pressure Pain Task: Associations with Sustained Attention

Margeaux M. Gray

Purpose: Pain is amplified following partial sleep deprivation. Pain has not been evaluated after sleep interruption, when total sleep time is preserved. Sleep interruption, also called sleep fragmentation, is ecologically relevant because it is caused by common sleep disorders such as Obstructive Sleep Apnea [OSA], Upper Airway Resistance Syndrome [UARS] and Periodic Limb Movements Disorder [PLMD]. The Sleep Continuity Hypothesis posits that the restorative effects of sleep are related to sleep *quality* in addition to *quantity*. With this study, my goal was to evaluate whether sleep fragmentation affected pain threshold and/or tolerance by systematically fragmenting the sleep of otherwise healthy adults.

Methods: Twelve adult female participants without chronic pain or evidence of a sleep disorder underwent a 14-day protocol. Sleep was monitored using actigraphy throughout the study. Participants completed daily morning and evening reaction time tasks to evaluate changes in attention. To measure changes in pain threshold (when a stimulus becomes painful) and tolerance (when a stimulus is no longer tolerable), a pressure-pain task was administered in-lab by a researcher. This test occurred a total of eight times, morning and evening. Participants spent the eighth, ninth and 13th nights in-lab. Night eight was for acclimatization to the research facility [BASE]. To compare pain after experimental sleep fragmentation (every five minutes; [FRAG]) with pain after sham [SHAM], these conditions were assigned pseudo-randomly to nights nine and 13. Three nights of recovery sleep outside the lab occurred between SHAM and FRAG nights.

Results: Sleep interruptions were induced at a rate of 5.2 times per hour, on average, without changing participants' total sleep time. Stage two sleep proportion was higher on fragmentation night. Lapses in vigilance were lower after BASE than other nights. The slowest 10% of reaction times were slower after SHAM than BASE. Overall, reaction time did not reliably differ as a result of fragmentation. Neither pain threshold nor pain tolerance differed as a function of experimental condition.

Conclusions: Systematic sleep fragmentation, particularly of stage two sleep, did not affect reaction time (a measure of sustained attention) or pressure pain (threshold or tolerance). Reaction time was not related to individual-level changes in fragmentation or pain. Future work should aim to establish the minimal fragmentation that engenders a clinical effect (without concomitant hypoxemia) to inform clinical definitions of fragmentation severity.

Support: WVU Office of Academic Affairs Doctoral Student Research Program; WVU Department of Psychology Graduate Student Research Fund

Acronym Legend

AASM - American Academy of Sleep Medicine

ANOVA - Analysis of Variance

EEG - Electroencephalography

IASP - International Association for the Study of Pain

NREM - Non-Rapid Eye Movement

OSA - Obstructive Sleep Apnea

PLMD - Periodic Limb Movement Disorder

PSG - Polysomnography

PVT - Psychomotor Vigilance Task

REM - Rapid Eye Movement

UARS - Upper Airway Resistance Syndrome

VAS - Visual Analog Scale

WVU - West Virginia University

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Effects of Systematic Sleep Fragmentation on Tolerance and Threshold in a Pressure Pain Task:
Associations with Sustained Attention

Overview

Sleep is related to both attention and pain. Sleep restores attention-related performance after sustained wakefulness (Kahn, Fridenson, Lerer, Bar-Haim, & Sadeh, 2014; Kingshott, Cosway, Deary, & Douglas, 2000; Lim & Dinges, 2008; Roca et al., 2012; Short & Banks, 2014; Stepanski, 2002;) and is related to pain sensitivity (Edwards, Almeida, Klick, Haythornthwaite, & Smith, 2008; Khalid, Roehrs, Hudgel, & Roth, 2011; Lautenbacher, Kundermann, & Krieg, 2006). Attentional factors can also affect pain (Chan, Chan, Kwan, Ting, & Chui, 2012; Sprenger et al., 2012). Each of these effects might be discrete and independent. Alternatively, attention could mediate the relation between sleep and pain. A mediated relationship among sleep, attention and pain could point to a physiologically-grounded interrelation among all three. This research sought to identify whether a particular kind of sleep disruption, called fragmentation, affected pressure pain or sustained attention – either distinctly or as a mediated effect on pain through attention.

The goal of this research was to evaluate whether systematic sleep fragmentation changed responses to a pain pressure task. Because little is known about the intermediates of the sleep-pain relationship in general, this study additionally addressed whether sleep fragmentation modulated pain through effects on attention, which pain research identifies as a robust modulator of pain. Hypothetically, a decrease in pain threshold could make those with sleep disturbance more susceptible to hyperalgesia (a condition where ordinarily painful stimuli are perceived as more painful) or even allodynia (when otherwise neutral stimuli are perceived as painful; International Association for the Study of Pain [IASP], 1986), thereby affecting the daily

function and well-being of otherwise healthy individuals who frequently operate under the pressure of insufficient sleep (like hospital care staff; Buxton et al., 2012b). A decrease in pain tolerance could make persistent pain that was once tolerable debilitating, with profound implications for the chronic pain population who appear to report sleep problems more often than the general population (Call-Schmidt & Richardson, 2003; McCracken & Iverson, 2002), especially when the pain condition is fibromyalgia (Theadom, Cropley, & Humphrey, 2007) or the sleep-related complaint is insomnia (Haack et al., 2012; Ohayon, 2005; Wong & Fielding, 2012).

The upcoming sections review information pertaining to this research, beginning with general sleep and pain introductions. Evidence of the relationships between sleep and pain, sleep and attention, and pain and attention follow in greater detail. The theoretical aspects of this research culminate in a synopsis of implications for a sleep-attention-pain trifecta before proceeding to the experimental methodology and outcomes.

Adult Sleep

Sleep is a reversible but necessary cycling of neurological activity that occurs in distinct stages and is commonly measured using noninvasive recording electrodes attached to the head (polysomnography [PSG]; Iber, Ancoli-Israel, Chesson, & Quan, 2007; Kugler, 1991). This section will provide an overview of sleep stages and their typical presentation in adults to familiarize the reader with sleep identifiers that were used in this study.

Adult sleep stages NREM 1-3 [N1, N2 and N3] and REM are partially defined by the frequency (Hz) and voltage recorded by scalp electroencephalography ([EEG] Iber et al., 2007), which reflects the neuronal synchronicity, neuronal orientation relative to the electrode (Kappenman & Luck, 2012), and likely the number of neurons recruited in the underpinning

cortical activity (Feinberg & Campbell, 2010). Other defining physiological features include eye movements and muscle tonus (Iber et al., 2007). Just before sleep onset with eyes closed, alpha (9-11 Hz) activity in the occipital region wanes in amplitude and slows. The transition to N1 occurs when the majority of a 30 second recording interval is in the theta (4-7 Hz) frequency range (Iber et al., 2007). N1 is considered a transitional sleep stage (Carskadon & Dement, 2011) and constitutes 5-10% of total sleep time in healthy adults (Hume, Van, & Watson, 1998). It is not uncommon for someone in the N1 state to continue performing simple tasks (Casagrande, De Gennaro, Violani, Braibanti, & Bertini, 1997; Carskadon & Dement, 1979) or report that they have not yet fallen asleep upon EEG-identified awakening (Bonnet & Moore, 1982). For this reason, the present study protocol was designed to delay sleep interruptions until a deeper sleep stage was attained by participants.

Neuronal firing patterns in the thalamus, to which sensory afferents synapse, change from tonic activity during wakefulness to phasic, burst-firing during sleep. Sleep stage N2 is defined by the presence of cortical activity that is driven by this burst-firing from a thalamocortical and thalamic reticular interplay, which appears in the EEG as sleep ‘spindle’ waveforms (Lüthi, 2013; Steriade, McCormick, & Sejnowski, 1993) although tonic firing has also been implicated in spindle generation (Lee et al., 2013). Identification of spindles and the other waveform that distinguishes N2, the ‘k-complex’ (Iber et al., 2007), were used in this research to time the initiation of sleep fragmentation.

The theta background EEG activity of N2 slows and increases in amplitude as it develops into the delta (0.5-4 Hz) activity characteristic of stage N3 (Iber et al., 2007). Delta activity is also the result of thalamic hyperpolarization (Steriade et al., 1993) but some of the slowest sleep EEG rhythms (<1 Hz) appear to be cortically-derived (Lemieux, Chen, Lonjers, Bazhenov, &

Timofeev, 2014; Timofeev, Grenier, Bazhenov, Sejnowski, & Steriade, 2000; Timofeev & Steriade, 1996). Prior to entry into REM sleep there is generally a shift back to N2 in healthy adults (Carskadon & Dement, 2011). The N1-N2-N3-N2-REM cycling pattern repeats through the sleep period with only slight variation depending upon the time of night (Carskadon & Dement, 2011). Interrupting the cycle typically causes a shift in EEG frequency which, if lasting between three and 15 seconds, is called an ‘arousal’ (Iber et al., 2007). Arousals can develop into awakenings or cause a switch to other sleep stages. EEG arousals evoked by experimental intervention were used to identify successful fragmentation of sleep in this research.

Sleep Disturbance

Disruption of normative sleep can take several different forms mentioned in the introduction: deprivation, restriction and fragmentation (Figure 1). Sleep deprivation is the total loss of a typical sleep period, or about eight hours of sleep ‘debt’ that leads to increased N3 and REM during the next sleep opportunity by altering normal sleep structure (Verma, Radtke, VanLadingham, King, & Husain, 2001). Sleep restriction is a partial loss of sleep, often recurring, that can result in accumulated functional deficits relative to the total amount of sleep missed (Belenky et al., 2003; Van Dongen, Maislin, Mullington, & Dinges, 2003). Sleep fragmentation entails a general preservation of total sleep time but with frequent sleep interruptions. Fragmentation is often measured to evaluate sleep quality (rather than quantity) and, for the purposes of this study, is the most pertinent type of disruption.

Arousals from sleep that occur during experimental or inadvertent fragmentation may or may not result in full awakenings (Bonnet, 1987; Martin, Engleman, Kingshott, & Douglas, 1997). When participants are fully awakened (by requiring a response to a disruptive auditory stimulus) after every minute of non-N1 sleep for two nights, their latency to sleep in a

subsequent morning nap decreases to an extent similar to that induced by two nights of total sleep deprivation even though total sleep time in the fragmentation condition was reduced relative to baseline by only about two hours (Bonnet, 1986). Experimentally evoking arousals after every 30 seconds of non-N1 sleep for two nights, without requiring a full awakening and while preserving total sleep time on the whole, has adverse metabolic effects like reducing insulin sensitivity and glucose effectiveness (Stamatakis & Punjabi, 2010).

One prominent clinical example of sleep fragmentation (also potentially accompanied by restriction) is Obstructive Sleep Apnea [OSA]. OSA is a sleep-related respiratory pathology that occurs when upper airway closure during sleep prevents breathing until chemoreceptors detect an increase in blood acidity (caused by elevated carbon dioxide) – usually culminating with a brief arousal and airway reopening (Zucconi, Oldani, Ferini-Strambi, Calori, Castronovo, & Smirne, 1995). When respiratory events in OSA occur regularly (5-15 per hour without extreme hypoxemia is considered mild for adults; Iber et al., 2007), they decrease sleep continuity and the amount of time in bed spent asleep (Norman, Scott, Ayappa, Walsleben, & Rapoport, 2006). Although a less severe respiratory issue, Upper Airway Resistance Syndrome [UARS] can cause sleep fragmentation equally as concerning as that in OSA (Guilleminault, Stoohs, Clerk, Simmons, & Labanowski, 1992; Philip, Stoohs, & Guilleminault, 1994). Another clinical example is Periodic Limb Movement Disorder [PLMD], in which rhythmic leg movements have the potential to induce arousals from sleep (American Academy of Sleep Medicine [AASM], 2001; Carskadon & Dement, 2011). Among experimental literature, sleep fragmentation is most typically induced by auditory tones (Bonnet, 1986; Kingshott et al., 2000; Martin, Brander, Deary, & Douglas, 1999; Martin, Engleman, Deary, & Douglas, 1996; Philip et al., 1994; Roehrs, Merlotti, Petrucelli, Stepanski, & Roth, 1994; Stamatakis & Punjabi, 2010; Stepanski,

Lamphere, Roehrs, Zorick, & Roth, 1987), vibration (Ferri et al., 2010; Stamatakis & Punjabi, 2010), or otherwise manually by an experimenter, especially if full awakenings are required (Downey & Bonnet, 1987). The present research employed a tactile method of disturbance accompanied by auditory stimulation, the former of which was the more likely to induce arousals (Buxton et al., 2012a). The experimental fragmentation of sleep in healthy individuals circumvents the influence of disorder-related comorbidities on experimental outcomes when clinical populations are used; it creates more consistency in the level of disruption, but has not yet been applied to evaluate changes in pain due to sleep fragmentation.

Sleep and Pain: A Putative Relation

Nociception describes the neurological, sensory-level processing that occurs after a potentially tissue-damaging stimulus is applied. This differs from pain, which has a perceptual basis and may or may not result from a nociceptive stimulus (IASP, 1986). Pain is a somatosensory perception with a threshold (perceiving a stimulus as painful at least half of the time) and tolerance (perceiving a painful stimulus as no longer tolerable) level that are influenced by a variety of factors, including prior sleep (IASP, 1986). For the remainder of this paper, 'pain' will refer to the perception of pain given a stimulus, rather than peripheral nociception or cellular sensitization/potential in neuronal pathways (although there is some evidence of cellular effects in the periaqueductal grey and somatosensory cortex after sleep disruption as well; Gorgoni et al., 2014; Tomim et al., 2015).

It is not clear how nervous system fatigue might affect the processing of potentially painful stimuli, or how it might alter perception of those stimuli. A variety of primary cortical areas contribute to higher-order pain processing: anterior cingulate, somatosensory, and insular (Bastuji et al., 2012; Hu, Valentini, Zhang, Liang, & Iannetti, 2014; Torta et al., 2013). These

cortical regions are differentially active relative to sleep stage (Bastuji et al., 2012), indicating an opportunity for sleep to induce change in pain processing regions during sleep and ultimately influence subsequent pain processing during wakefulness. For example, behavioral responses to thermal nociceptive stimuli are attenuated *during* sleep (just 2.5% of thermal pain stimuli elicit a nocifensive motor response, Lavigne et al., 2000). Further, sleep disruption has an amplifying effect on *subsequent*, evoked pain during wakefulness (discussed later; Lautenbacher et al., 2006). This latter effect was the primary focus of my study.

There is some speculation regarding the causal factors for pain amplification after sleep interference (Karmann, Kundermann, & Lautenbacher, 2014). One line of conjecture implicates the concomitant increase in prostaglandins that occurs with sleep deprivation (Haack, Lee, Cohen, & Mullington, 2009), but it is not clear how this effect of sleep loss might influence the higher-order sensory processing of painful stimuli, which appears to also be affected according to changes in evoked potentials elicited by laser stimuli (at least in the context of hyperalgesia; Ødegård et al., 2014). A change in cellular activity in the somatosensory cortex is apparent after sleep disruption (Gorgoni et al., 2014), and in the periaqueductal gray after sleep modulation (Tomim et al., 2015; Tracey et al., 2002), highlighting the potential role of late processing. Regardless of the route, the sleep-to-pain *causal direction* appears to be the strongest approach (versus pain-to-sleep), based on correlational research comparing sleep duration to pain survey scores (Edwards, Almeida, Klick, Haythornthwaite, & Smith, 2008).

Sleep Disturbance and Pain

In sleep research, painful stimuli have been delivered using thermal (and laser), cold pressor, electrical, mechanical pressure, and (among animals) chemical techniques. Stimulus type can affect afferent nociceptors differently (Dubin & Patapoutian, 2010) and could, in turn,

be differentially susceptible to sleep disruption. Sleep disruption, and specifically REM disruption, appears to influence a rodent's response to noxious electrical (Dametto et al., 2002; Hicks, Moore, Findley, Hirshfield, & Humphrey, 1978) but perhaps not thermal (Asakura, Matsumoto, Ohta, & Watanabe, 1992) stimuli, and evidence for mechanical change appears equivocal (Onen, Alloui, Eschallier, & Dubray, 2000; Ukponmwan, Ruprecht, & Dzoljic, 1984). Aversive thermal (Drewes et al., 2000; Faraut et al., 2015; Khalid, et al., 2011; Kundermann, Sernal, Huber, Krieg, & Lautenbacher, 2004; Onen, Alloui, Gross, Eschallier, & Dubray, 2001; Tiede et al., 2010), cold (Faraut et al., 2015; Kundermann et al. 2004) and mechanical pressure (Arima, et al., 2001; Drewes et al., 2000; Faraut et al., 2015; Lentz, Landis, Rothermel, & Shaver, 1999; Moldofsky & Scarisbrick, 1976; Older et al., 1998; Onen et al., 2001) stimuli administered to humans also yield inconsistent evidence for an impact of sleep on pain depending upon the study paradigm and, particularly, sleep stage-specific deprivation.

Measuring pressure-related pain has translational value because sleep complaints from the clinical fibromyalgia population relate to their pain reports (Theadom et al., 2007) and Fibromyalgia is defined clinically by evaluating joint pressure sensitivity (Wolfe, Ross, Anderson, Russell, & Herbert, 1995). From an empirical perspective, some have suggested that pressure-related pain may be more impacted by sleep disruption than thermal-related pain (Lautenbacher et al., 2006), making it a good target for evaluating pain change relative to a specific type of sleep fragmentation. Outcomes from studies specifically considering the effects of sleep disturbance on subsequent, waking pain are discussed later in more detail.

Both sleep deprivation and sleep restriction increase behavioral sensitivity to painful stimuli (Finan, Goodin, & Smith, 2013; Onen et al., 2001; Tiede et al., 2010). There is clinical evidence that pain amplification also occurs after sleep fragmentation (Khalid et al., 2011). An

individual may have adequate sleep quantity, but if their sleep is fragmented they lose the organizational integrity of sleep, which is constituted by a highly uniform cycling of sleep stages. Fragmentation can decrease sleep continuity, possibly interfering with restoration that is dependent upon sleep organization or deeper sleep stages (Sleep Continuity Hypothesis; Bonnet, 1985 & 1986). Clinical evidence is sourced from clinical populations, however, and in the case of work by Khalid and colleagues (2011), low oxygenation or concomitant sleep restriction may contribute to pain outcomes. This demands further investigation of sleep fragmentation's independent impact on pain, which has not been addressed in the literature. In the following sections I consider the effects of sleep deprivation and restriction on pain each in turn, along with support for the case of fragmentation.

Total sleep deprivation for 40 hours and selective deprivation of a specific sleep stage (REM or N3) decreases tolerance for pressure-related pain, but the effect is recoverable after subsequent sleep (Onen et al., 2001) with some equivocality in evidence that the effect is sleep stage-dependent (Drewes et al., 2000; Lentz et al., 1999; Moldofsky & Scarisbrick, 1976; Older et al., 1998). Nonhuman animal work identifies REM sleep deprivation, rather than loss of deep sleep, as a major cause of hyperalgesia (Lautenbacher et al., 2006). One human study, although correlational, also specifically implicates REM as a mediator of thermal pain processing in healthy adult females (Smith, Edwards, Stonerock, & McCann, 2005). Participants' reports of thermal pain increase after multiple nights of total sleep deprivation (Kundermann et al., 2004). No stage-selective deprivation studies have yet accounted for the loss of sleep time from repeated arousals or poor sleep quality incurred by methods used to deprive participants of those specific sleep states. Therefore, it appears that the general effect of sleep disruption is the more

consistent finding among work relating sleep and pain, rather than stage-specific disruption, at least for humans.

The effects of sleep restriction, usually when nocturnal sleep is limited to 4 hours in experimental inpatient studies, are similar to those of sleep deprivation. Increases in pain correspond to elevated inflammatory biomarkers after 12 days of sleep restriction (Haack, Sanchez, & Mullington, 2007). Just one night of sleep restriction is able to increase pain ratings of thermal, laser stimuli applied to the hand by 30% (Tiede et al., 2010). After two nights of restriction, participants have less tolerance for cold-related pain (Ødegård et al., 2013). The recorded EEG elicited by laser and cold stimuli decreases in amplitude for higher-order processing waveforms during subsequent wakefulness (Ødegård et al., 2013; Tiede et al., 2010). This could be interpreted as attenuated rather than amplified perceptual processing of the nociceptive stimuli or, perhaps, as less consistent processing of stimuli that results in lower evoked potential amplitude.

No research reports pain outcomes after systematically interrupting the sleep of healthy adult humans. Sleep fragmentation increases, but does not induce, mechanical hyperalgesia in mice during wakefulness (Sutton & Opp, 2013). Of note, however, is that many rodent sleep 'fragmentation' paradigms (such as in Sutton & Opp, 2013) do not rule out concomitant deprivation or restriction. These studies also permit compensatory sleep during rodents' light phase. However, a small amount of work has been done working with the human OSA population.

Treated OSA patients can better tolerate pain, indicated by a longer latency to finger withdraw from thermal exposure (Khalid et al., 2011). It takes just two days for their pain tolerance to decrease again with therapy discontinuation. OSA patients usually spend less of

their time in bed actually asleep (reduced sleep efficiency), so the change in their pain may be attributable to intermittent hypoxemia from respiratory events, the effects of sleep restriction, or the effect of sleep fragmentation. To isolate the effect of fragmentation from the other two possibilities, research must simulate the fragmentation of OSA while avoiding overall sleep restriction and must utilize a healthy group of participants to circumvent hypoxemia. These are the goals of the present study.

Sleep and Attention

The total impact of sleep disruption is comprehensive and includes compromised immune function (Vgontzas et al., 2004), altered circadian hormonal and metabolic regulation (Knutson, Spiegel, Penev, & Van Cauter, 2007; Leproult, Copinschi, Buxton, & Van Cauter, 1997), mood dysregulation (Franzen, Siegle, & Buysse, 2008), impaired judgment (Anderson & Platten, 2011) and most notably for the purposes of this proposal, attentional deficits (described in further detail below). Poor vigilance after sleep disruption has a societal consequence because perceived sleepiness and actual vigilance impairment are often dissimilar (Franzen et al., 2008; Van Dongen et al., 2003). This increases the risk of motor vehicle accidents related to drowsy driving. There are several different types of attention (Callejas, Lupiáñez, Funes, & Tudela, 2005) but this section will focus on deficits related to *sustained* attention (also called ‘tonic alertness’) after sleep deprivation, restriction, or fragmentation. The vigilance task used in this research was a measure of sustained attention.

The Psychomotor Vigilance Test (PVT) is a button-pressing task frequently used in sleep research to evaluate sustained attention (Drummond et al., 2005; Lim & Dinges, 2008; Van Dongen et al., 2003). Participants are instructed to attend to a screen and quickly respond after the appearance of a target symbol. The measure of interest is their latency to respond. Response

latencies exceeding 500 ms (called ‘lapses’) are characteristic of participants after sleep disruption (Basner & Dinges, 2011; Chee et al., 2008; Van Dongen et al., 2003). Attenuation of alpha activity in the EEG (as what occurs at sleep onset) is related to PVT lapses and supports the hypothesis that very brief sleep episodes (“microsleeps”) manifest from sleep debt, in turn degrading sustained attention (Jugovac & Cavallero, 2012). Scoring methods conceptually based on microsleep intrusion also increase the PVT’s measurement sensitivity to attentional lapses (Basner & Dinges, 2011; Chee et al., 2008; Drummond et al., 2005) and these were used to analyze PVT scores as predictors and mediators in this research.

Sustained attention deficits after sleep *deprivation* are evidenced by more psychomotor lapses and longer reaction times versus controls (Doran, Van Dongen, & Dinges, 2001; Drummond et al., 2005; Franzen et al., 2008; Jugovac & Cavallero, 2012). After 16 hours of wakefulness, these deficits are dose-responsive to the duration of ongoing wakefulness (Van Dongen et al., 2003). As such, it is not surprising that cumulative sleep *restriction* also has deleterious effects on tonic alertness measurable by the PVT (Belenky et al., 2003; Dinges et al., 1997; Van Dongen et al., 2003; Vgontaz et al., 2004). Work on sleep deprivation and restriction has made good use of the PVT but the PVT and analogous tests are remarkably absent from published work considering attentional deficit after sleep fragmentation.

Because a literature search produced just three studies that measured sustained attention after sleep *fragmentation* and just one using the PVT specifically (Bonnet, 1985; Insana & Montgomery-Downs, 2010; Martin et al., 1996), a broader overview of the relevant fragmentation work is included here.

A spate of research productivity related to impairment after sleep fragmentation occurred around the 1990s. This body of work utilized a variety of executive function assays and some

other attention-dependent tasks (Bonnet, 1985 and 1986; Bonnet, Berry, & Arand, 1991; Martin et al., 1996; Martin, Brander, Deary, & Douglas, 1999; Philip et al., 1994; Roehrs et al., 1994; Stepanski et al., 1987) but few if any incorporated the PVT. One study evaluating the functional deficits of postpartum women, whose sleep is notoriously interrupted for extended intervals when providing child care, did use the PVT to demonstrate decrements in their sustained attention (Insana & Montgomery-Downs, 2010).

Each of the other two studies that used tasks targeted at sustained attention demonstrated effects of sleep fragmentation on performance (a trail-making task, Martin et al., 1996; a simple reaction time task, Bonnet, 1985). Other work was unable to demonstrate fragmentation effects on attention tasks not targeting tonic alertness *per se* (Bonnet, 1985; Martin et al., 1996; Roehrs et al., 1994) in spite of demonstrating that fragmentation impairs mood (Martin et al., 1996) and shortens latency to sleep onset in clinical tests (Martin et al., 1996; Philip et al., 1994; Roehrs et al., 1994). Performance on one translationally applicable test of vigilance, a Steer Clear driving task, was also not affected by sleep fragmentation (Martin et al., 1996). Increased subjective sleepiness after fragmentation is equivocally reported (Bonnet, 1985; Martin et al., 1996), but subjective sleepiness is not a linear measure of actual impairment (Franzen, et al., 2008; Van Dongen et al., 2003).

Attentional measures that do not target lapses such as those applied in most of the aforementioned work may miss components of attention that are most sensitive to sleep fragmentation. Also, if metrics used for these other measures are based on averages, then they can wash-out attention effects because the majority of response latencies after sleep disruption are normal (Chee et al., 2008). For comparative consistency across methods of sleep disruption,

the PVT was implemented in this research to quantifiably establish whether fragmentation decreased sustained attention.

Pain and Attention

Attention is a well-known modulator of pain. Attentional shifts (orienting attention) correlate with lower retrospective pain rating of electrical shocks (Chan et al., 2012). Mental distraction, using a working memory task (Sprenger et al., 2012) or visual stimulation (Van Ryckeghem et al., 2011), also reduces pain ratings.

Different attentional qualities may each influence pain processing (Chan et al., 2012; Sprenger et al., 2012; Van Ryckeghem et al., 2011), but the mechanisms of attention's influence are not known. Recent work indicated that, specifically for attentional distraction, there is a down-regulation of afferent nociceptive signaling at the dorsal horn of the spinal cord likely caused by the periaqueductal grey opioid system (Sprenger et al., 2012). Not only do exogenous manipulations of attention affect pain but endogenous shifts such as mind wandering also appear related to pain processing networks (Kucyi, Salomons, & Davis, 2013). One way that sleep disturbance, including fragmentation, may be affecting pain is through the attentional system. In fact, the periaqueductal grey brain region (implicated in feedback/regulatory pain processing; Iannetti & Mouraux, 2010; Todd, 2010; Tracey & Mantyh, 2007) which is affected by sleep, as previously noted, is also affected by changes in attention (Tomim, et al., 2015; Tracey et al., 2002). Sleep disruption is also able to diminish the analgesic effects of distraction (Tiede et al., 2010), together suggesting that there may be functional connectivity for an interplay between the sleep and attentional systems that affect pain. The concept of quantifying attention to evaluate its relationship between sleep and pain was first formally advanced in February of 2015 but there is no evidence of it being tested to date in the literature (Faraut et al., 2015).

Intersection of Sleep, Attention and Pain: Statement of the Problem

Sleep and attention are independently known to affect pain. Sleep deprivation and restriction lead to hyperalgesia during subsequent wakefulness but the same effect has not been experimentally established for sleep fragmentation. Some manipulations of attention, especially attentional distraction, are successful analgesics. Sleep has established effects on sustained attentional function and can mitigate the analgesic effects of distraction, implicating an intersection in the processing pathways that link sleep, attention and pain.

The goal of this research was to evaluate whether sleep fragmentation reduced pain threshold and tolerance to determine the importance of sleep continuity in the perception of and nocifensive response to pressure stimuli. This research targeted the effects of sleep fragmentation by systematically interrupting the sleep of otherwise healthy adults while extending their overall sleep opportunity to minimize sleep restriction, and used a behavioral measure of pain threshold and tolerance to a mechanical pressure stimulus for relevance to clinical populations (Rainwater & McNeil, 1991). Sustained attention performance was also measured before and after sleep fragmentation using the PVT to evaluate the extent to which attention participated in the sleep-pain relationship.

Research Questions and Hypotheses

Research Question 1: Does Sleep Fragmentation Worsen Scores or Increase Score Variability on an Attention-Related Task?

Hypothesis 1A. Reaction times after sleep fragmentation will be slower than those after sleep without experimental disruption.

Hypothesis 1B. Vigilance lapses (reaction times > 500 ms) will occur more frequently after sleep fragmentation than after sleep without experimental disruption.

Hypothesis 1C. There will be greater reaction time variability in PVTs administered after sleep fragmentation than after sleep without experimental disruption.

The PVT has been frequently used to measure sustained attention in sleep deprivation and sleep restriction studies but has been less used in prospective fragmentation paradigms. The PVT is sensitive in particular to deficits in sustained attention. I evaluated PVT performance using two metrics specifically sensitive to sleep disruption and that are related to response speed: for Hypothesis 1A, the slowest 10% of reaction times (as an inverse), and for Hypothesis 1B, lapse frequency (Basner & Dinges, 2011). Therefore, the slowest among reaction times after fragmentation were expected to be slower than after undisturbed sleep and the number of lapses after fragmentation were expected to be higher than after undisturbed sleep.

After deprivation and restriction the fastest reaction times can be similar to participants' responses without sleep disruption because sleep pressure is expected to manifest in lapses (Basner & Dinges, 2011). Episodic lapses in attention would also cause increased response time variability. Hypothesis 1C would be supported if reaction time variability increased after sleep fragmentation relative to sleep without experimental disruption.

Research Question 2: Does Sleep Fragmentation Influence Pain Response?

Hypothesis 2A. Latency to indicate pain threshold will be shorter after sleep fragmentation than after sleep without experimental disruption.

Hypothesis 2B. Latency to indicate tolerance will be shorter after sleep fragmentation than after sleep without experimental disruption.

Sleep deprivation and restriction consistently increase next-day pain ratings but no prospective research on fragmentation has included pain evaluation. Patients with OSA, a clinical group with sleep fragmentation and hypoxemia, demonstrate improved thermal pain

tolerance when treated (Khalid et al., 2011). Their improvement suggests that sleep discontinuity may affect pain like other sleep disruptions, but simultaneous recovery of sleep quantity and oxygen perfusion confound any possible inferences about causality without a randomized, controlled paradigm.

Because pain threshold and tolerance are independently defined constructs (IASP, 1986; Rainwater & McNeil, 1991) that may be differentially sensitive to sleep disruption, they were measured separately in this work.

Research Question 3: Is Pain After Sleep Fragmentation Explained by Attention Task Performance?

Hypothesis 3A1. Worsening of sleep fragmentation from a sham-disruption night to a night of experimentally fragmented sleep will correspond to a decrease in reaction time performance between the same nights.

Hypothesis 3A2. Worsening of sleep fragmentation from a sham-disruption night to a night of experimentally fragmented sleep will correspond to increases in pain response between the same nights.

Hypothesis 3B. The relation between sleep and pain changes from sleep without experimental disruption to fragmented sleep will be partially explained by changes in performance on attention tasks between the same nights.

There is convincing evidence reporting changes in pain after attentional manipulation (see Introduction [p.14]). While pain is related to artificial attention tasks and conditions, the relationship between endogenously fluctuating attention and pain was only recently revealed (Kucyi et al., 2013). Because of attention's known sensitivity to insufficient sleep, attention is a plausible path linking sleep disruption and pain. If there are deficits of sustained attention after

sleep fragmentation then the PVT's incremental sensitivity to sleep need (Van Dongen et al., 2003) should reveal them; and further, the extent of these deficits should explain the relation between sleep and pain if sleep's impact on attention is the means by which it influences pain. Considering the known relations between attention and pain, sleep deprivation/restriction and attention, and clinical fragmentation and pain, there arises a potential pathway between sleep and pain through attention (Figure 2).

Methods

Participants

Recruitment and screening. Institutional Review Board [IRB] approval for this research was obtained through West Virginia University ([WVU]; protocol #1508781106). Participants were recruited using West Virginia University (WVU) online websites and other media sources and by word-of-mouth. Participants used the contact information of the sleep laboratory provided in advertising to express interest in research participation. The primary researcher contacted potential participants by phone or email to schedule screening for eligibility. Phone or in-lab screening was used to confirm initial eligibility and potential participants were scheduled for blood pressure evaluation and consent in the WVU sleep research lab. All recruitment, screening and testing were executed by the primary researcher according to WVU IRB approval for the protection of human subjects and all qualifying participants underwent informed consent.

Inclusion and exclusion criteria. Algometer performance is influenced by an interaction between the gender of the researcher administering the task and the gender of the participant (Aslaksen, Myrbakk, Høifødt, & Flaten, 2007; Kállai, Barke, & Voss, 2004). To avoid diluting power by including gender comparisons, all participants were gender-matched to

the test administrator and exclusive data collector (female). Diagnosed attentional deficits, pain-related disorders, or high risk for a sleep disorder (as evaluated by a screening survey; see Appendix A) were disqualifying characteristics. Participants were screened for hypertension in-lab prior to consent and were ineligible if systolic exceeded 140 mmHg or if diastolic exceeded 90 mmHg (O'Brien et al., 2013). These criteria extended to participant baseline evaluation, such that any participant who screened positive for a sleep disorder during the first night of in-lab testing was excused from further participation with partial compensation (Appendix B).

Sleep disorders and pain disorders both increase in prevalence with age (Ohayon, 2002; Ohayon & Roth, 2002; Punjabi, 2008; Wolfe et al., 1995) and the number of arousals from sleep increases with age (Boselli, Parrino, Smerieri, & Terzano, 1998), so it was important to limit age variability in recruitment. Because the goal of this work was to identify whether the pain of healthy individuals was affected by sleep fragmentation, females between the ages of 18 and 30 years were recruited to participate. The research facility's location on a college campus also permitted accessibility to this participant population. Any prescribed use of controlled psychostimulants disqualified an individual from participation because of potential drug interactions with sleep and attention. Women self-reporting that they may be pregnant were excluded because of potential stress to the fetus and hormonal differences.

Study interval restrictions. Participants were asked to refrain from smoking and drinking alcohol within two hours of overnight PSG, with a maximum of two servings of alcohol daily during the 14 day study period, because of known stimulant and alcohol effects on sleep organization (Landolt, Dijk, Gaus, & Borbély, 1995; Yules, Lippman, & Freedman, 1967; Zhang, Samet, Caffo, & Punjabi, 2006). Caffeine and napping, although able to influence circadian regulation and possibly have an impact on test performance, were not restricted

(Buxton, L'Hermite-Baleriaux, Turek, & Van Cauter, 2000; Faraut et al., 2015). This was due to participant safety concerns, because participants were not monitored by research staff while outside the laboratory.

Protocol

After participants were survey-screened for sleep-related disorders and other exclusion/inclusion criteria to determine eligibility (Appendix A) they reported to the sleep laboratory at WVU to be evaluated for hypertension using a sphygmomanometer and, if still eligible, underwent informed consent with IRB-approved materials. Figure 3 (below) illustrates the full protocol.

Phase I. Participants were provided with a protocol schedule that was individualized based on intake data about typical bed and rise times. This schedule included lab appointments, study protocol instructions, and reminders. The protocol began with one week of continuous actigraphy, hereafter referred to as Phase I, during which participants wore the device on their non-dominant wrist and kept a record of their sleep behavior in an electronic diary. Participants were instructed to maintain relatively consistent (± 2 hrs) sleep time as best they were able during Phase I. Participants also completed a daily morning reaction time test (see below).

Phase II. Study Phase II began on the morning of the seventh day and continued through study end. Participants continued using actigraphy and the sleep diary throughout the remainder of testing. Phase II included in-laboratory testing, a three-night break between the second and third lab nights, and a reaction time test on the final morning. Actigraphy data were evaluated on the morning of the seventh day, when participants reported to the lab for the beginning of Phase II, to determine protocol adherence and estimate in-lab sleep opportunity for subsequent Phase II

nights. Testing at the beginning of Phase II prior to any nights in-lab will henceforth be called 'HOME' measures.

On the morning of the seventh day, participants were instructed to report to the lab within one hour after waking. Upon arrival, participants reported their wake time to a researcher. They completed a brief survey (Appendix C) followed by their first sequence of a repetitive three-test series beginning at least 45 mins after their wake time: a cognitive test battery lasting about 20 minutes that was administered on the lab computer (cognitivefun.net), the same reaction time test that had been administered outside of the lab during Phase I, and an algometer pain task (see Measures for details). This test series was repeated on mornings eight, nine and 13 (mornings after an overnight in the lab) and on evenings seven, eight, nine and 13 (the first Phase II evening and each evening after an overnight in the lab). Participants reported to the lab for evening testing about two hours before their scheduled bedtime for administration of the three-test series and instrumentation for polysomnography ([PSG], see Methods). The first Phase II night served to screen participants for sleep disorders and account for first-night effects on sleep organization in a new environment (Curcio, Ferrara, Piergianni, Fratello, & De Gennaro, 2004; Lorenzo & Barbanoj, 2002). Reading forward, this baseline night and related tests will be referred to as 'BASE.'

The first participant was randomized (using the online resource random.org) to the blood pressure cuff-active or sham PSG condition on their second night in-lab and experienced the alternate condition on their third night in-lab. Subsequent participants alternated in their order of sham/fragmentation conditions according to identification number (even/odd). Between these two experimental nights, all participants had three recovery nights at home; this break was designed to mitigate potential, extended effects of a night of sleep fragmentation on a subsequent

sham condition. In this document, the night of sleep fragmentation and associated testing will be called ‘FRAG,’ and the sham condition with its associated testing ‘SHAM.’

The duration of a nocturnal sleep opportunity during BASE, FRAG and SHAM conditions was determined based on the average duration of nocturnal sleep during the full week prior to testing, measured using actigraphy and sleep diaries. Sleep opportunity duration in all in-lab conditions was extended by the time spent awake during in-lab testing on that night, which was measured using EEG wakefulness indicators (e.g., continuous alpha activity) greater than 1.5 minutes in duration (Rechtschaffen & Kales, 1968). Sleep opportunity extension allowed equivalent sleep opportunity (like that successfully implemented by Roehrs et al., 1994) across conditions in the event that extended awakenings were perpetuated by the fragmentation protocol.

The 14-day protocol culminated after PVT on the last morning, after which participants return their actigraphy equipment to the lab. Participants received compensation according to the portions of testing completed up to a potential \$150 (Appendix B).

Measures

Actigraphy. Actigraphy is a method of recording activity levels from weight displacement within a wrist-worn, watch-like piezoelectric accelerometer (Tonetti, Pasquini, Fabbri, Belluzzi, & Natale, 2008). Analog movement sampled at 32 Hz from the non-dominant wrist of participants is digitized, in this case at a 30 second resolution for similarity with PSG intervals (Iber et al., 2007), by a Philips Respironics Mini Mitter Actiwatch-64 device (Philips Respironics, Bend, Oregon). Actigraphy has good sensitivity (97%) relative to “gold-standard” PSG for adult sleep/wake classification (De Souza et al., 2003) and is recommended as an estimate thereof (Morgenthaler et al., 2007). In spite of good sleep identification, a known

limitation of actigraphy in general is its misclassification of wakefulness as sleep (Insana, Glowacki, & Montgomery-Downs, 2011; Sadeh & Acebo, 2002), or low specificity. Particularly low specificity values are obtained from computer autoscoring algorithms (De Souza et al., 2003; Tonetti et al., 2008), so manual scoring was performed for this work. Actigraphy has support as an assay to estimate wakefulness occurring after the onset of sleep [WASO] and total sleep time [TST] despite its limitations in specificity (wake detection; Marino et al., 2013). This study did not rely on actigraphy as a measure of outcome variables in light of these limitations because of the more reliable data available from PSG; rather, actigraphy served to evaluate protocol adherence and reliability of participant data based on its estimates of sleep behavior while not being directly observed in the laboratory.

For the purposes of this study, actigraphy was used to measure sleep time during Phase I. This information was used to determine the time-in-bed necessary for in-lab portions of testing during Phase II, to ensure adequate sleep opportunity relative to a participant's normal schedule, to avoid sleep restriction. Over-estimation of total sleep time by actigraphy during the week preceding in-lab testing even with manual scoring would result in a small amount of additional time in bed for participants in the lab because actigraphy can identify 91% of recorded data accurately as sleep or wake (De Souza et al., 2003). This potential time-in-bed extension was not expected to affect research outcomes because a participant that has fulfilled their sleep need should awaken without intervention and a participant in the fragmentation condition who continues to sleep during this opportunity continued to experience the fragmentation protocol.

Actigraphy data were corroborated using participant self-report in an electronic PDA (Palm Zire 72 with custom software developed by Bruner Consulting, Inc., Longmont,

Colorado). Participants were instructed to log their actigraph-wearing behavior (on/off) in a PDA sleep diary.

Data pre-processing. Raw actigraphy data are depicted as movement per unit time (30 seconds in this study, to correspond to the unit of time typically interpreted in PSG). This record is scored for sleep time based on a combination of zero-movement, 30-second intervals, and a participant's sleep diary entries. The primary researcher time-matched participant sleep onset and wake time reports with recorded movement data and then applied a scoring algorithm to estimate actual sleep onset and wake times. Application of this algorithm involved identifying the first consecutive two minute bout of zero-movement data as sleep onset and the last consecutive two minute bout of zero-movement data as wake time, within the overall window of participant-reported sleep time. The primary researcher worked with participants to fill-in any missing information based on movement data and participant recall.

Polysomnography and sleep fragmentation. PSG entails monitoring a variety of biological features related to sleep for gold-standard sleep classification and identification of sleep disorders, especially sleep-related breathing disorders (Kushida et al., 2005). Minimum signal acquisition includes central and occipital EEG leads, chin and leg electromyography, dual ocular movement recording, electrocardiography, snore and airflow sensors, thoracic and abdominal effort sensors, pulse oximetry, and body position (Kushida et al., 2005); our lab also included frontal EEG. The sleep laboratory environment consists of two participant bedrooms separated by a full bathroom and adjoining technician/reception area. Bedrooms are designed to imitate an at-home sleep environment as best as possible and do not have windows. Red lighting in the rooms allowed participants to rise and use the bathroom without phase-shifting light exposure during the night; researchers entering the room to manage equipment or initiate

the blood pressure cuff protocol used a flashlight or similar device to minimize light exposure. Rooms are equipped with video recording and audio transmission for communication between researchers in the reception area and participants in bedrooms. Sleep data were collected using Embla N7000 REMbrandt software version 9.1 (Natus Medical Inc., Pleasanton, CA).

Procedures. Participants were outfitted with sensors (above) and then connected to the recording interface for the duration of testing. Initial equipment checks, during which the participant responds to a series of commands from research staff (e.g. blinking eyes or flexing feet), were performed at the beginning of each nocturnal recording session to ensure data quality. Participants were permitted to follow typical bedtime routines that did not interfere with study protocol, such as using their phone or computer before sleep onset. The primary researcher monitored participants throughout for participant safety, to correct data collection issues, or to disconnect the participant for restroom access.

For BASE, participants wore the full montage of sensors described above. For FRAG and SHAM nights, sensors used exclusively to evaluate for sleep disorders were not used (because participants had already been screened on BASE) and there was the addition of an ambulatory blood pressure monitor to the bicep corresponding to the participant's non-dominant hand. For ambulatory blood pressure, an attachment used for palpating was adhered to the inner arm over the brachial artery with medical tape and the cuff was positioned over the top.

A researcher started the sleep fragmentation protocol for *both* SHAM and FRAG nights after a participant reached and sustained (without arousal) stage N2 sleep for at least one, 30 second frame. Stage N2 onset was defined using the AASM's adult sleep scoring standards, which is the appearance of a spindle or K-complex in the EEG lasting at least a half-second (Iber et al., 2007). The researcher entered the study room and manually initiated an ambulatory blood

pressure monitor already attached to the sleeping participant's non-dominant arm. Inflation was programmed to occur in five-minute intervals. In this study the primary goal of cuff inflation was to interrupt sleep, which is a known iatrogenic effect of nocturnal monitoring (67% of inflations produce arousal, Davies, Jenkins, & Stradling, 1994; Degaute, Van de Borne, Kerkhofs, Dramaix, & Linkowski, 1992; Schwan & Eriksson, 1992).

The first reading was expected to cause an arousal from sleep for both SHAM and FRAG conditions. After a successful reading, the researcher discontinued the pre-programmed ambulatory monitor's readings *only in SHAM* and recorded the rest of the night without fragmentation. One exception to this was in the event of participant awakening for longer than 10 min or getting up to use the bathroom during testing. In this case, the first reading was taken again after a participant's next uninterrupted 30 second frame of stage N2, then was discontinued as before. The goal of a first reading was to simulate fragmentation on a non-fragmented night and minimize participant awareness of condition. A successful trial of an ambulatory monitor in our research lab confirmed arousal-producing effects. Some individual variability in the sensitivity of participants' sleep to cuff activation was expected (as has been demonstrated in auditory disturbance work; Dang-Vu, McKinney, Buxton, Solet, & Ellenbogen, 2010), and this was taken into account by considering changes in fragmentation between SHAM and FRAG conditions in specific analyses.

Data pre-processing. Sleep and sleep fragmentation data were processed prior to use in analyses. Researchers blinded to experimental condition identified arousals from sleep and the sleep stage of each 30-second recording interval according to AASM standards (Iber et al., 2007). All PSG information except what was necessary to remove for initial blinding to the experimental condition (i.e. oximetry-related information) was used to determine sleep stages

and wakefulness. The proportion of sleep stages relative to total sleep time in general was calculated and used to evaluate for stage-specific differences that may have been inadvertently produced by the protocol. Shifts of ≥ 3 seconds to a higher frequency in the EEG (particularly alpha) out of stable and sustained sleep, lasting at least 10 seconds, constituted a neuro-cortical “arousal,” with the additional contingency of a noticeable EMG tone increase to determine an arousal during REM (during which alpha activity is not atypical; Iber et al., 2007).

After the initial scoring of EEG data for arousals while blinded to participant and condition, arousals were further categorized into cuff-associated and cuff-unassociated events while un-blinded. Arousals occurring within 10 seconds of the cuff inflation start (according to the oximeter plethysmograph trace’s initial waning) through 10 seconds of the cuff release (according to the oximeter plethysmograph trace’s waxing back to initial amplitude) were considered cuff-associated. These 10-second extensions were selected as cutoffs because of noise generated by the cuff during inflation/deflation.

From sleep scoring information, a “fragmentation index” was calculated for statistical comparisons: the number of arousals from sleep was indexed to the total sleep time in hours to produce a metric of *overall* arousals per hour, regardless of arousal source. A similar metric, which I will refer to as “spontaneous index,” was calculated to reflect the frequency of arousals that did not meet cuff-associated criteria; spontaneous index was calculated per hour of sleep overall and was used to evaluate the impact of sleep disruption that was not directly manipulated in supplemental analyses.

To evaluate the ambulatory blood pressure cuff’s efficacy in producing arousals, each inflation that occurred during sleep was categorized as associated with an arousal or not (using the same 10 second criteria as those used above). This separate categorization was also

necessary because certainly not all neurocognitive arousals were associated with inflations and the overall difference in arousal index across Phase II nights could have been diluted or otherwise affected by changes in the spontaneous arousal rate. Further, it is possible that exogenously induced arousals have a fundamentally different effect on sleep restoration than do endogenously sourced, spontaneous arousals.

Inflations that were deemed “associated” with arousals were further evaluated for their association with a particular sleep stage or stage change (to a “lighter” stage). Cuff inflations related to arousals were also considered “related” to the sleep stage prior to the arousal. Specifically, the epoch during which the majority of a cuff inflation occurred was identified and the stage of the epoch preceding that inflation epoch was considered the “related” stage. If an inflation spanned two epochs fairly equally, then the epoch preceding the epoch where the inflation began was considered the “related” stage. An inflation was considered associated with a stage *change* if the epoch following an inflation was in a lighter stage than the inflation epoch or, if inflation spanned two epochs, if it ended in a different stage than it began. Like the possible fundamental difference between induced and spontaneous arousals, it is possible that arousals related to a specific sleep stage have a different impact on restoration than arousals related to other sleep stages, thereby motivating the calculation of “stage-related” arousal metrics. These more nuanced metrics that were not originally proposed for this study were used in a series of supplemental analyses (labeled as such in the Results).

Psychomotor vigilance and cognitive battery. For this study, an electronic tablet was programmed to administer a 10-min PVT (Bruner consulting, Longmont, CO; Roach, Dawson, & Lamond, 2006) and participants tapped the touch-sensitive screen to respond rather than pressing a button. When testing in the laboratory, a 20-min electronic cognitive battery

(cognitivefun.net) consisting of four, five-minute individual tasks that are typical in sustained attention or working memory research (auditory reaction time, Jung, Ronda, Czeisler, & Wright, 2011; Shelton & Kumar, 2010; Eriksen flanker test, Eriksen, 1995; Sanders & Lamers, 2002; reverse Corsi block task, Berch, Krikorian, & Huha, 1998; Kessels, 2008; and a 2-back task, Kane, Conway, Miura, & Colflesh, 2007) preceded the PVT because attentional lapses are sensitive to the ‘time on task effect,’ or an increase in lapse frequency with increasing exposure to monotonous testing or ongoing PVT duration after sleep disruption (Basner & Dinges, 2011; Doran et al., 2001).

General effects of latency after awakening on performance (called ‘sleep inertia’) are greatest within two hours of awakening (Hoffman et al., 2005; Jewett et al., 1999). Furthermore, there are circadian effects on the PVT (Monk et al., 1997). To minimize the effects of sleep inertia, participants waited at least one hour after their final awakening to complete the PVT in-lab and were advised to also do so during Phase I acclimatization with the PVT.

Procedures. When testing in-lab, participants completed the cognitive battery on a desktop computer while being video-recorded in a separate, back room of the sleep research laboratory. A researcher read a consistent explanation of the cognitive battery to participants at the beginning of each session as a reminder of testing content and response method (in some tests responses were on the keyboard, while others used the mouse), stopping after each of the four test explanations to permit questions or practice at the discretion of the participant. For continuous cognitive tasks (auditory reaction time and reverse Corsi block task (three unit length)), the researcher initiated testing and waited for the five-minute test period to elapse before helping the participant to switch tasks. For cognitive tasks presented at intervals (Eriksen flanker and the 2-back), the researcher initiated the first interval and then waited quietly at the

back of the room to re-start testing upon interval completion; intervals were repeated until the five minute test period had elapsed. After cognitive task completion, participants completed their PVT on the tablet.

To begin each PVT session, participants selected an icon on the main screen of the tablet. They answered four questions related to their perceived sleep duration and quality on the preceding night before reaction time testing began. After answering the final question, an image identical to the response-soliciting image (a target) appeared on the screen and participants were prompted to tap the screen to begin the PVT. Participants were instructed to respond to each appearance of the target image by tapping once, as fast as possible after the target appeared. If a participant responded before the target image appeared, a “too early” warning was displayed before the next target. If a participant waited to respond more than 1000 ms after a target image appeared, a “too late” warning was displayed before the next target. The test program closed automatically after the full test session was complete (10-minute duration).

Data pre-processing. Reaction time metrics for the PVT that are sensitive to sleep disturbance were calculated from raw PVT data for subsequent statistical comparison; specifically, the inverse of the slowest 10% of responses, and the proportion of lapses (responses > 500 ms; Basner & Dinges, 2011; Van Dongen et al., 2003). Errors of commission were defined as responding faster than 200 ms after appearance of the target cue (Basner & Dinges, 2011), including responses before cue appearance, and were excluded.

It is necessary to quantify the extent of attentional effects after fragmentation because of individual variability in the magnitude of sleep’s impact on attentional performance (Van Dongen, Baynard, Maislin, & Dinges, 2004). The PVT permits this quantification because of its sensitivity to the effects of sleep disruption on attention (Basner & Dinges, 2011). Therefore, a

difference score for PVT between FRAG and SHAM nights was used in the correlation analyses for Hypothesis 3A.

Pain assessment. Pain measurement devices come in several varieties; the device used here, an algometer, specifically assesses pressure-pain at a distal extremity (the fingers). I utilized an administration technique for the pressure algometer that was developed by other researchers to permit measurement of both pain threshold and pain tolerance in an escape paradigm (Rainwater & McNeil, 1991; details below).

Procedures. Algometer testing was performed in the same, quiet, in-laboratory environment across conditions. Research personnel escorted participants to the lab, which neighbored the sleep laboratory (in the same research wing) and where distractions, although likely minimal, would have been consistent across testing. The primary researcher was trained by staff of the lab that originally developed the device (Rainwater & McNeil, 1991) to provide as much behavioral consistency across administration conditions as possible.

The device is designed to apply pressure to a focal point between the first and second knuckles. At the start of the pain task participants underwent one practice round with a weight (750 mg) on the index finger of their non-dominant hand, which corresponded to the arm exposed to ambulatory blood pressure monitoring on SHAM and FRAG nights. After practice, participants completed testing on each of their ring and middle fingers. Participants were instructed to provide a report of their anticipated maximum pain on the Visual-Analog Scale (VAS) before testing began, their current level of pain while their finger was in the device without weight (baseline), and every 30 seconds throughout a testing session. Two different weights (500 g and 1000 g), similar in appearance, were individually placed atop a platform that rested on the participant's finger. The test administrator started a manually operated timer and

placed a weight on the device. The duration of a participant's voluntary exposure to weight pressure was recorded, along with their subjective pain ratings every 30 seconds on the visual-analog scale (where 0 represents *no pain* and 100 represents *the worst pain imaginable under the circumstances*; Bijur, Silver, & Gallagher, 2001; Bird & Dickson 2001). Participants indicated the time when pressure-induced discomfort became painful (threshold) and the test continued until a participant indicated that they no longer wished to tolerate the pressure (tolerance) by touching designated signs. The weight was immediately removed from the apparatus once a participant indicated that the pain was no longer tolerable. Applied pressure also affects circulation to the distal extremity, so each session was limited to three minutes of weight exposure to prevent tissue damage (Rainwater & McNeil, 1991). The algometer task was administered eight total times; twice (AM/PM) after the last night of sleep at home, twice (AM/PM) after an acclimatization night in-lab, and twice (AM/PM) after each experimental night (SHAM or FRAG) in-lab.

A briefing script for the algometer task is included as Appendix D. This standardized script permitted participant-observed demonstration of the device on the researcher and also a participant trial session prior to data collection to minimize and standardize effects of anxiety or fear (Dougher, Goldstein, & Leigt, 1987). Appendix E contains the order of hand/finger/weight testing that was applied in each session. This specific order of weight presentation was selected to ensure data collection on each hand (cuff-associated and unassociated), with each test weight presented once to each hand and with each weight presented consistently to the same finger. Therefore, within each test, a participant was exposed to weights five times; three times on their non-dominant hand and twice on their dominant. Because this research used a repeated-measures design, the presentation order was the same in all eight algometer tests to allow

consistent comparison across conditions. The effects of weight and hand-dominance were considered in statistical analyses.

Data pre-processing. The algometer task was video-recorded and scored by an assistant researcher blind to condition. Differences in algometer latencies between SHAM and FRAG were calculated for use in correlation and mediation analyses between pain/attention/sleep, but raw scores were used in repeated-measures comparisons.

Analyses

Data were analyzed using SPSS statistical software (version 24; IBM Corp., Armonk, NY). Data acquired for each measure were evaluated for distribution normality (skew and kurtosis) and outliers exceeding three standard deviations (Osborne & Overbay, 2004).

Transformations were applied to the data as necessary to achieve a distribution shape that was appropriate for analysis, from least-to-most extreme (square root, inverse, and logarithmic). An alpha of $< .05$ was the threshold for statistical significance. Pairwise comparisons were carried out pending a significant omnibus ANOVA. Effect size for ANOVA (η_p^2) used the convention small = .01, medium = .06 and large = .14; effect size for post-hoc *t*-tests (Cohen's *d*) used the convention small = 0.2, medium = 0.5 and large = 0.8 (Aron, Aron, & Coups, 2006).

Results

Descriptive

Excluded participants. Seventeen participants enrolled in the study between November 2015 and May 2016 and 12 completed participation. Four participants withdrew at various stages after consenting: one did not respond to attempts to contact her after enrollment, two had repeated scheduling conflicts, and one was uncomfortable with the PSG monitoring equipment. One participant was excluded by the investigators from further participation after exceeding the

cutoff for periodic leg movements per hour (> 5) during baseline PSG. This participant was given an example screenshot of recorded events and, consistent with the approved IRB protocol, contact information for local clinical sleep testing facilities.

Withdrawn/excluded vs. included participants. Years of education and annual household income did not differ ($p = .198$, $p = .560$, respectively) between withdrawn/excluded and included participants (note that income required a square root transformation to meet parametric requirements; also note that participants supported by their parents were asked to include their parents' income in their annual household income value). Age differed significantly between these groups ($p = .043$): withdrawn/excluded participants ($M = 21.5$, $SD = 2.0$) were significantly younger than those who completed participation ($M = 23.8$, $SD = 2.0$).

There were no significant differences in marital status/living situation (Fisher's Exact = .294) or handedness (Fisher's Exact = 1.000) between withdrawn/excluded participants (all of whom were single and one of whom was left-handed) and completed participants (information in next section and Table 1). All 17 participants self-identified as female. Enrollment of 6% of participants who were Black/African-American initially made the sample consistent with the ethnic diversity of students enrolled at West Virginia University's main campus (4% as of Fall 2014, West Virginia Higher Education Planning Commission); however, all participants who completed the study identified as White (100%; Fisher's Exact = .294).

Included sample. From this point forward, "participants" will refer to only the sample of 12 who completed study participation. All included participants were right-handed and self-identified as female. All but one were single. Their rounded median household income was \$34,000 (which is lower than the rounded 2015 national average of \$57,000; US Census Bureau,

2016), and they were educated for 17.2 years on average. The average participant age was 23.8 years. Additional demographic details are provided in Table 1.

For two participants, a different brand of ambulatory blood pressure monitor was used during SHAM. The cuff of this monitor was applied in a similar way as the monitor used for FRAG, but had a built-in attachment used for palpating instead of a separately applied attachment. Two participants requested that their Phase II start date be shifted forward by one day after beginning Phase I and one participant requested that their final Phase II night be shifted forward by one day during their three-night ‘break’ period. The protocol was adjusted to accommodate all of these requests because additional Phase I time was not expected to affect study outcomes and additional washout time would likely have improved rather than hindered any necessary sleep recovery. In cases where study nights were shifted, data from the days adjacent to subsequent in-lab testing were used.

Participants’ Total Sleep Time and Opportunity

Actigraphy-recorded time and opportunity. The sleep period was identified using actigraphy data and was behaviorally corroborated using the sleep diary for all except one participant. This participant wore the actigraph but did not accurately adhere to the protocol; the actiwatch was removed at night between her sleep diary entries, which were maintained on her personal device and entered retrospectively in the PDA. Upon further analysis of her actigraphy data, the activity time recorded was consistent with retrospective diary entries. This consistency inspired confidence in the most relevant factor estimated by actigraphy – sleep opportunity or time-in-bed – with the limitation that periods of awakening during the night as recorded by actigraphy could not be accounted for in her estimated sleep time like they were for other participants. Thus, her data were maintained and she continued participation. Her data were not

extreme or outlying relative to other data. For all participants who reported at least one daytime nap during Phase I, either on their diary or in person, actigraphy-verified nap time was included in the estimate of that study day's total sleep time (TST). All participants provided diary information for all seven nights, although some diary entries were recorded retrospectively ($19.9\% \pm 9.7\%$, not including the aforementioned participant with all retrospective diary entries; these entries were flagged in the electronic diary). In the event of unclear or missed diary nights, this information was requested in person at the beginning of study Phase II.

Participants' average actigraphically-recorded TST during Phase I (at-home testing) was 7 hr 44 min (464 min; ± 39 min), which exceeds the AASM's and Sleep Research Society's minimum recommendation for healthy adult sleep duration (Watson et al., 2015). There was a significant difference between Phase I average and TST on nights in the lab (omnibus repeated-measures ANOVA; $p = .038$, $n^2 = .22$; Figure 4). Post-hoc pairwise evaluations indicated that average Phase I TST was significantly shorter than BASE ($p = .028$, $d = 0.53$) and FRAG nights ($p = .002$, $d = 0.56$) and marginally shorter than SHAM night ($p = .056$, $d = 0.40$). Participants therefore did not experience sleep restriction in the lab and possibly had extended sleep on Phase II nights, according to actigraphy. Conversely, participants may have had some sleep restriction at home. It is more likely, however, that actigraphy overestimated in-lab sleep time by mis-categorizing WBSO or WASO as sleep given that poor specificity (wake detection) is a known limitation of actigraphy (Insana, Glowacki, & Montgomery-Downs, 2011; Sadeh & Acebo, 2002).

Polysomnography-recorded time and opportunity. Within-subjects t -tests were used to compare nights two and three in the lab in an evaluation of condition-related order effects on sleep. Recall that the FRAG and SHAM conditions were counter-balanced across nights two and

three to avoid an impact of order, which was not apparent, on test outcomes. Neither sleep time nor opportunity differed based on night in the lab regardless of test condition ($p = .432$ and $p = .590$, respectively).

A comparison of sleep across BASE, SHAM and FRAG nights was performed using data from PSG (Suppl. Figures 1-2). Neither TST nor sleep opportunity differed significantly across nights (within-subjects ANOVA TST $p = .367$; sleep opportunity $p = .406$), so any changes observed in outcome measures are not accounted for by changes in sleep time in the lab.

Participants' Sleep Quality and Architecture

Neither sleep quality nor architecture variables differed based on night in the lab regardless of test condition: sleep efficiency ($p = .392$), awakening index ($p = .764$), neurocortical arousals ($p = .611$), neurocortical arousals unassociated with cuff inflation ($p = .306$), proportion of N1 ($p = .408$), proportion of N2 ($p = .205$), proportion of N3 ($p = .091$), or proportion of REM ($p = .765$). Therefore, any differences observed between SHAM and FRAG conditions should be related to the cuff activity rather than the night number relative to BASE.

Sleep quality. Sleep Efficiency was also similar across the three night conditions (BASE/SHAM/FRAG; omnibus $p = .103$; Suppl. Figure 3) despite significant differences in the frequency of awakenings (omnibus $p = .011$, $n^2 = .34$) and neurocortical arousals (omnibus $p < .0001$, $n^2 = .61$; Figures 5-6). Post-hoc pairwise tests revealed that participants had significantly more frequent awakenings during FRAG than in SHAM ($p = .012$, $d = 1.18$) and significantly more frequent neurocortical arousals in FRAG than in either SHAM ($p < .001$, $d = 1.16$) or BASE ($p < .001$, $d = 1.14$), regardless of the source of those awakenings or neurocortical arousals.

Note, however, that sleep efficiency, as a variable, is distorted due to the sleep extension protocol. Efficiency is calculated as TST relative to sleep opportunity (or time in bed), which is not impacted by micro-arousals. An extension of sleep opportunity to compensate for wakefulness, as was applied here, can artificially influence sleep efficiency relative to what it might have been without extension. Similar efficiency across conditions does, however, indicate similar proportionality between sleep and sleep opportunity during Phase II.

Spontaneous arousal index did not differ ($p = .052$; Suppl. Figure 4), although this outcome was marginal. Spontaneous index trended *less* frequent on FRAG, however, supporting the notion that changes in the overall proportion of micro-arousals across conditions (which were higher in FRAG than in either BASE or SHAM) were the result of experimentally-induced arousals, rather than an increase in the frequency of spontaneous or environmentally-induced arousals on FRAG night. Importantly, this lower trend in spontaneous arousals on FRAG night could potentially dilute evidence of cuff-related disturbance if arousals are only quantified by an overall change in frequency, as they are discussed above.

Rather than explaining sleep disruption as an overall change in arousals that includes both cuff-associated and unassociated arousal frequency, individual cuff inflations that occurred during sleep were identified, determined cuff-associated or unassociated, and evaluated for association with a sleep stage shift (see Methods for details). Of all cuff inflations during sleep, 44.3% ($SD = 9.7\%$) caused arousal on average and 28.0% ($SD = 7.9\%$) preceded a sleep stage shift to a lighter stage or to wake. After standardizing these inflations to participants' TSTs, the average arousal-inducing inflation index was 5.2 per hour ($SD = 1.3$), or just above the minimum disruption rate that might be expected for an individual with mild OSA (Iber et al., 2007). The distribution of arousal-inducing inflations was not even across sleep stages, however, as might be

expected given known arousal threshold differences between N3 and other sleep stages (Rechtschaffen, Hauri, & Zeitlin, 1966). Stage-specific differences are addressed below.

Sleep architecture. Proportions of stage N1, N3 and REM sleep did not differ significantly across conditions ($p = .192$, $p = .081$ and $p = .304$, respectively; Suppl. Figure 5). Only the omnibus ANOVA for N2 indicated a significant difference ($p = .030$, $n^2 = .273$; Figure 7). Post-hoc pairwise tests revealed a significantly greater proportion of N2 sleep on FRAG than on SHAM ($p = .037$, $d = 0.66$). Average REM proportion was similar to normative values (20-25%) for a young adult age group on all nights and there was slight variation from normative ranges (2-5%, 45-55% and 13-23% for N1, N2 and N3, respectively; Carskadon & Dement, 2011) among other stages on specific nights (see Table 2 for details).

Inflations associated with a particular sleep stage were evaluated for their success in causing neurocortical arousal (see Methods). Not surprisingly, the highest sleep disturbance success rate ($M = 68.1\%$, $SD = 40.9\%$) was when inflations occurred during the transitional sleep stage N1 (Carskadon & Dement, 2011), although this stage occupied the lowest proportion of sleep time. Stage N2 inflations were related to arousals 56.7% ($SD = 12.3\%$) of the time and standardized to a rate of 6.7 times ($SD = 1.7$) per hour. During stage N3, which has the highest arousal threshold (Rechtschaffen et al., 1966), interruption occurred for 25.8% of inflations ($SD = 12.3\%$) or only 3.8 ($SD = 2.2$) times per hour. Inflations during REM interrupted sleep 35.1% ($SD = 17.2\%$) of the time or 4.5 ($SD = 2.2$) times per hour. Observe that for all non-transitional sleep stages the cuff-associated arousal index was higher than the overall (both spontaneous and cuff-associated) change in arousal index across conditions, possibly related to the lower-trending frequency of spontaneous arousals on FRAG. If there is no meaningful difference in the impact of induced and spontaneous arousals of sleep restoration, then spontaneous arousals may

interfere with the ability to detect behavioral changes related to associated arousals, if they exist. Therefore, a series of supplemental analyses (not originally proposed) considering pain and reaction time utilized spontaneous arousal data as a random effect across conditions in the event that induced-arousal differences might have been masked in proposed comparisons.

Research Question 1: Does Sleep Fragmentation Worsen Scores or Increase Score Variability on an Attention-Related Task?

Preliminary analyses. A preliminary comparison between morning (AM) and evening (PM) scores was performed to determine time-of-day differences in each of the attention-related measures: frequency of attention lapses, slowest reaction times, and reaction time variability. The three AM scores from each participant in each condition (BASE, SHAM and FRAG) were averaged to produce one representative AM score; the same technique was applied to PM scores. One participant's PVT data from a PM measure were lost due to technological error; this participant was represented by two rather than three scores in the average used for preliminary analyses.

Lapses were converted to a proportion out of total reaction trials in a session (not including false starts). The lapse proportion (%lapses) required a square root transformation to meet parametric assumptions (slightly more than 3 times the standard error for positive skew and slightly leptokurtic). The slowest 10 percent of responses were averaged and the inverse of this average was used in analyses (1/slowest 10%; the inverse is conventionally applied to this metric, Basner & Dinges, 2011). The variance across individual response latencies (excluding false starts) did not require further transformation.

Within-subjects *t*-tests did not reveal time-of-day differences between AM and PM on any PVT metric (%lapses $p = .870$, 1/slowest 10% $p = .596$, variance $p = .191$). Because AM

and PM measures did not differ, and to maintain equal weight of data from each participant in the proposed comparisons across conditions (BASE/SHAM/FRAG), only morning scores were used in analyses of hypothesized outcomes. Results from preliminary PVT analyses are presented in Table 3.

Hypothesis 1A. Reaction times after sleep fragmentation (the cuff-active condition) will be slower than those after normative sleep (the cuff-sham condition). The slowest responses differed after BASE, SHAM and FRAG (omnibus $p = .028$, $n^2 = .28$). Responses were significantly faster after BASE than after SHAM ($p = .016$; $d = 0.44$; Figure 8), but fragmentation did not appear to impact the slowest reactions independently because SHAM and FRAG did not differ.

Hypothesis 1B. Vigilance lapses (reaction times > 500 ms) will occur more frequently after sleep fragmentation than after normative sleep. The frequency of lapses in vigilance also differed significantly across the three conditions (omnibus $p = .006$, $n^2 = .38$). Specifically, there were more frequent lapses after FRAG than after BASE ($p = .007$, $d = 0.37$) and after SHAM than after BASE ($p = .033$, $d = 0.28$). FRAG and SHAM did not differ significantly in their effect on %lapses (Figure 9). The effect sizes for differences in %lapses after BASE versus other Phase II conditions were small, reflected in the large error variance in this metric. However, without a significant difference in %lapses between SHAM and FRAG conditions, it is unlikely that neocortical arousals were the precipitating factor of %lapse changes.

Hypothesis 1C. There will be greater reaction time variability in PVTs administered after sleep fragmentation than after normative sleep. Variability in response times did not differ across conditions (omnibus ANOVA $p = .071$; Figure 10). The hypothesized increase in

response time variation after FRAG was not supported, however a large omnibus effect size in an unexpected direction draws attention to the substantial variability in response times after the SHAM condition. Additional details about PVT comparisons are available in Table 4.

Supplemental analyses.

Impact of sleep time. A follow-up analysis considered whether PVT lapses or the slowest 10% of latencies might have been affected by possible differences in sleep time, given the possibility of longer duration sleep in the laboratory according to actigraphy. Paired-samples *t*-tests between PVT scores after sleep at home (the first morning of Phase II) and those after BASE sleep did not reveal performance differences (see Supplemental Table 1). This outcome supports the notion that reaction times were not impacted by a potential difference in TST between Phase I and Phase II, if actigraphy accurately represented a longer TST in the laboratory.

Consideration of spontaneous arousals. Because spontaneous arousals affected overall sleep interruption rate and therefore may have interfered with an impact of induced arousals on outcome measures, they were considered as a covariate. Different spontaneous arousal rates occurred in each condition within each participant, however. To account for this, a mixed-model analysis in SPSS (MIXED; IBM Corp., 2016; IBM Corp. 2002; Peugh & Enders, 2005) was used in which a fixed effect compared across BASE, SHAM and FRAG conditions while permitting random intercepts and slopes for individual participants because of the repeated-measures design. FRAG condition was set as the point of comparison, given that my objective was to detect differences resulting from experimentally induced disruption relative to lab acclimatization or sham-disruption. Model structure was first designated as Unstructured (different variances and covariations at each measurement time) and, if correlation matrix values

were similar and Information Criteria did not significantly differ between the Unstructured model and one with Compound Symmetry (using a Chi-square test of the -2 Restricted Log Likelihood), then the simpler (symmetric, constant variances and covariations) model was selected. In cases of a significant difference, the better model fit was selected. Spontaneous arousals were then included as a random effect to covary for differing frequencies across conditions. Restricted Maximum Likelihood Estimation (rather than Maximum Likelihood Estimation; Harville, 1977) was used because of the small sample size. Table 5 presents a summary of supplemental, mixed model outcomes for PVT.

Compound Symmetry fit the data significantly better than an Unstructured model when evaluating differences in 1/Slowest 10% across conditions ($\chi^2(11.26, 4); p = .024$), so covariance was kept constant across condition pairings. Before accounting for spontaneous arousals, it appeared that BASE responses may have been faster than after FRAG ($p = .021$) and SHAM responses appeared marginally slower than after FRAG ($p = .067$). Neither potential difference between conditions persisted after accounting for spontaneous arousals, however ($p = .306$ vs. BASE; $p = .104$ vs. SHAM), which is similar in outcome to repeated-measures ANOVA.

Lapse frequency was square root transformed to accommodate normal distribution assumptions (as above, in ANOVA). The fit of Unstructured and Compound Symmetry models did not differ ($\chi^2(1.62, 4); p = .805$), so covariance was kept constant across condition pairings. After accounting for spontaneous arousals, %Lapses was significantly higher (by an estimated 7.6%) after FRAG than after BASE (similar to outcomes of repeated-measures ANOVA; $p = .006$). As with ANOVA, %Lapses between FRAG and SHAM did not differ ($p = .508$).

Variance in lapses was square root transformed to accommodate normal distribution assumptions (as above, in ANOVA). The two model structures did not differ when evaluating

for response variability ($\chi^2(1.84, 4); p = .766$), so covariance was kept constant across condition pairings. The potentially higher variability in reaction times after SHAM than FRAG before accounting for spontaneous arousals ($p = .045$) was sustained after accounting for them ($p = .008$). This difference was an apparent trend between SHAM and FRAG in the previous ANOVA, but did not reach significance. Response variance did not differ between BASE and FRAG ($p = .593$), which is consistent with ANOVA outcomes.

Research Question 2: Does Sleep Fragmentation Influence Pain Response?

Preliminary analyses. Preliminary analyses were conducted to determine differences between factors in the algometer task: different weights, hands, or times of testing. In the task, the 500 g (light) weight was always used on the ring finger and the 1000 g (heavy) weight on the middle finger, and both weights were applied to each hand (dominant or non-dominant) in a particular session. Weight responses were measured at two times (AM/PM) and after each Phase II condition (BASE, SHAM and FRAG). These measurements produced a total of 24 pain scores that were related to Phase II nights for each participant. These 24 scores were used in preliminary analyses.

The first preliminary analysis tested whether weight differences needed to be accounted for as a factor in the main analyses, presumably because participants are able to discern different weights and may respond to them differently or because different fingers have different weight sensitivities. Because the same weight was always placed on the same finger, all scores related to a particular finger (12) were averaged for a paired-samples comparison evaluating threshold and a paired-samples comparison evaluating tolerance.

There was a significant difference in both threshold and tolerance based on weight, as evidenced by different algometer response latencies. Participants took longer to reach pain

threshold with the lighter weight (ring finger; $p = .002$, $d = 1.19$) and they tolerated the lighter weight longer ($p < .001$, $d = 1.62$; Suppl. Figure 6). Large effect size outcomes for this preliminary analysis indicate a potentially robust contribution to pain measures, indicating a need to maintain separation between weights in the main analyses.

A similar preliminary comparison between hands (dominant or non-dominant) was used to evaluate whether hand dominance, or potentially whether the hand related to blood pressure cuff placement (always on the non-dominant arm), was related to pain scores. Both hands were exposed to both weights (heavy and light) in each algometer session so that, despite differences in weight response, any overall differences in response between hands would be balanced.

Scores related to a particular hand (12) were averaged for a paired-samples comparison evaluating threshold and a paired-samples comparison evaluating tolerance. Threshold scores required a square root transformation because of slight leptokurtosis.

Participants responded to pressure-pain differently depending upon the hand that was tested, for both threshold and tolerance. Participants took longer to reach pain threshold ($p = .012$, $d = 0.55$) and tolerated weight longer ($p = .047$, $d = 0.52$) when it was placed on their dominant hand (no cuff; Suppl. Figure 7). Medium effect size outcomes for this preliminary analysis emphasized the need to maintain separation between hand scores in the main analyses.

Scores related to AM and PM test times (12 each) were compared after averaging across other variables, but no differences were observed in these preliminary analyses for either pain threshold or tolerance (Suppl. Figure 8). Therefore, AM and PM algometer scores from each participant were averaged for the main analyses. Results from preliminary algometer analyses are presented in Table 6.

The differences observed between weights (fingers) and between hands in preliminary analyses were incorporated into the two, originally planned within-subjects ANOVAs (one for pain threshold and one for tolerance) across BASE, SHAM, and FRAG conditions. Tests were adjusted to 3x2x2 (condition x weight x hand) factorial, within-subjects ANOVAs. Algometer scores were all square root transformed because of select threshold and tolerance scores with large positive skew. Sphericity assumptions were violated for the condition (BASE/SHAM/FRAG) main effect ($p = .037$, $\epsilon = .674$) and the condition*weight interaction ($p = .020$, $\epsilon = .648$) on pain threshold values, so a Greenhouse-Geisser correction was used for interpreting threshold test outcomes.

As shown in the preliminary analyses, the difference in algometer response between weights was confirmed by a significant main effect on threshold ($p < .001$, $n_p^2 = .76$) and on tolerance (Figure 11; $p < .001$, $n_p^2 = .79$). Latency to reach threshold and tolerance was consistently longer for the lighter weight. The main effect of hand on algometer response was also significant for both threshold ($p = .005$, $n_p^2 = .53$) and tolerance ($p = .002$, $n_p^2 = .59$). Latency to reach threshold and tolerance was consistently longer for the dominant hand.

Hypothesis 2A. Latency to indicate pain threshold will be shorter after the fragmentation condition than the normative condition. There was not a main effect of Phase II condition on algometer threshold ($p = .293$), therefore the latency to reach pain threshold overall was not affected by Phase II condition. Further, no threshold interactions were statistically significant ($p = .419$ condition*weight, $p = .233$ condition*hand, $p = .085$ weight*hand, and $p = .122$ condition*weight*hand). Details about each outcome are available in Table 7. Weight (finger) and hand (dominance) did not impact threshold differently depending upon BASE, SHAM, or FRAG conditions (Figure 11). Without a difference in algometer

responses between SHAM and FRAG, even after accounting for weight and hand factors, there was unlikely an effect of FRAG on pain threshold.

Hypothesis 2B. Latency to indicate tolerance will be shorter after the cuff-active condition than the cuff-inactive condition. Overall weight tolerance was not affected by Phase II condition (main effect $p = .138$); neither were there statistically significant tolerance interactions ($p = .185$ condition*weight, $p = .398$ condition*hand, $p = .083$ weight*hand, and $p = .222$ condition*weight*hand; Table 7). Weight (finger) and hand (dominance) did not impact tolerance differently depending upon BASE, SHAM, or FRAG conditions (Figure 12). Similar to threshold, there was unlikely an effect of FRAG on pain tolerance because algometer responses between SHAM and FRAG did not differ, even after accounting for weight and hand factors.

Supplemental analyses.

Impact of sleep time. A follow-up analysis considered whether pain threshold or tolerance might have been affected by possible differences in sleep time, given the possibility of longer duration sleep in the laboratory according to actigraphy. Phase I algometer threshold and tolerance (also the first morning of Phase II) were compared to those after BASE. This analysis evidenced *longer* latencies to pain threshold and tolerance after Phase I than after BASE – the opposite direction of effect that might be expected in the event of at-home sleep restriction (Faraut et al., 2015; Ødegård et al., 2015); see Supplemental Table 2).

Consideration of spontaneous arousals. A similar approach to supplemental analyses was used for pain outcomes as was used for PVT. Because covariates used in repeated-measures ANOVA (hand and weight) did not reveal any interaction with pain threshold or tolerance, suggesting a similar impact on pain scores across conditions, they were not included in the mixed

model. Spontaneous arousals affected overall sleep interruption rate and therefore may have interfered with any impact of induced arousals on outcome measures, so they were considered as a covariate. A mixed-model analysis was used in which a fixed effect compared across BASE, SHAM and FRAG conditions while permitting random intercepts and slopes for individual participants because of the repeated-measures design. FRAG condition was set as the point of comparison. Model structure was first designated as Unstructured and, if correlation matrix values were similar and Information Criteria did not significantly differ between the Unstructured model and one with Compound Symmetry (using a Chi-square test of the -2 Restricted Log Likelihood), then the simpler (symmetric) model was selected. In cases of a significant difference, the better model fit was selected. Spontaneous arousals were then included as a random effect to covary for differing frequencies across conditions. Restricted Maximum Likelihood Estimation was used because of the small sample size. Table 8 presents a summary of supplemental, mixed model outcomes for pain threshold and tolerance.

As in ANOVAs, threshold scores were square root transformed to comply with assumptions of a normal distribution. An Unstructured model did not fit the data differently than one with Compound Symmetry, so the simpler, symmetric model was used ($\chi^2(8.26, 4); p = .083$). Although data converged on the model that included spontaneous arousals as a random effect, failure to reach positive values in the Hessian matrix prevented accurate estimation of covariance parameters. The variance estimate for the covariance of spontaneous arousals was approximately zero, suggesting that there was not enough variability in spontaneous arousals to reasonably justify its inclusion in the model. Fixed effects estimates were unchanged from the Compound Symmetry model without the covariate (given the negligible impact of the covariate) and indicated no significant differences between FRAG and BASE ($p = .230$) or FRAG and

SHAM ($p = .465$); these outcomes are similar to the lack of a condition main effect in the factorial ANOVA.

An Unstructured model did not fit the data differently than one with Compound Symmetry when evaluating tolerance, so the simpler, symmetric model was used ($\chi^2(6.54, 4); p = .162$). Not surprisingly considering the congruence of threshold and tolerance measures, a similar Hessian fit error occurred when spontaneous arousals were included as a covariate. Although data converged on the model, failure to reach positive values in the Hessian matrix prevented accurate estimation of covariance parameters. The variance estimate for the covariance of spontaneous arousals was approximately zero, suggesting that there was not enough variability in spontaneous arousals to reasonably justify its inclusion in the model. Fixed effects estimates were unchanged, and indicated no significant differences between FRAG and BASE ($p = .387$) or FRAG and SHAM ($p = .412$); these outcomes are similar to the lack of a condition main effect in the factorial ANOVA.

Research Question 3: Is Pain After Sleep Fragmentation Explained by Attention Task Performance?

Hypothesis 3A1. Worsening of sleep fragmentation from SHAM to FRAG will correspond to decrements in reaction time metrics from SHAM to FRAG. Change scores between SHAM and FRAG were used to compare overall sleep fragmentation (ΔFrag) to changes in vigilance lapses ($\Delta\%\text{lapses}$) and slowest reaction times ($\Delta 1/\text{slowest } 10\%$). Change calculations were organized so that a positive change value reflected more frequent lapses in FRAG and slower responses in FRAG (however recall that the $1/\text{slowest } 10\%$ metric is inverse, so slower responses in FRAG will yield a negative change). As with the PVT analyses in the above section regarding Research Question 1, only AM change scores were used because one

participant was missing an evening PVT score. Distributions met parametric assumptions. Neither correlation between ΔFrag and either $\Delta\%\text{lapses}$ or $\Delta\text{l/slowest } 10\%$ was statistically significant ($p = .510$ and $p = .489$, respectively). A summary of ΔFrag -PVT correlation outcomes is presented in Table 9.

Hypothesis 3A2. Worsening of sleep fragmentation from SHAM to FRAG will correspond to increases in pain metrics from SHAM to FRAG. Change scores between SHAM and FRAG were used to compare ΔFrag to changes in pain threshold ($\Delta\text{Threshold}$) and tolerance ($\Delta\text{Tolerance}$). Change calculations were organized so that a positive change value reflected a faster threshold or tolerance response in FRAG. Recall that multiple algometer samples were measured from each participant (across fingers, hands, times of day, and conditions; 24 scores). Because no interactions were observed between condition and fingers or hands in prior analyses, all of a participant's AM scores (four; two for fingers and two for hands) were averaged within each condition to compute change scores for use in algometer correlations. Neither correlation between ΔFrag and $\Delta\text{Threshold}$ or $\Delta\text{Tolerance}$ was statistically significant ($p = .188$ and $p = .204$, respectively). A summary of ΔFrag -algometer correlation outcomes is presented in Table 9.

Supplemental analyses. The ΔFrag metric represents an overall change in sleep fragmentation, regardless of the source of that fragmentation (cuff-associated or spontaneous). There is a possibility that induced arousals have a unique impact on sleep restoration that endogenously-sourced arousals do not that is overlooked in this metric. Therefore, to evaluate the relation between algometer score changes and induced arousals specifically, additional correlations were performed. These supplemental correlations also included evaluations of

specific sleep stages that were disrupted by cuff inflation. All supplemental metrics met normal distribution assumptions according to similar limits set for other metrics in this study.

The $\Delta\%$ lapses metric did not significantly correlate with the frequency of cuff-associated arousals ($p = .720$), nor with the frequency of said arousals disrupting a particular sleep stage (N2 $p = .500$; N3 $p = .666$; REM $p = .577$). Outcomes were similar for $\Delta 1$ /slowest 10%, which was not significantly related to the cuff-associated arousal index ($p = .836$), N2 arousal index ($p = .692$), N3 arousal index ($p = .155$), or REM arousal index ($p = .798$).

Neither Δ Threshold nor Δ Tolerance were significantly related to the frequency of cuff-associated arousals (Δ Threshold $p = .593$; Δ Tolerance $p = .805$). There were also no stage-specific correlations between Δ Threshold and associated arousal index (N2 $p = .660$; N3 $p = .421$; REM $p = .775$) or Δ Tolerance and associated arousal index (N2 $p = .603$; N3 $p = .590$; REM $p = .603$). Taken together, it does not appear that any unique association between cuff-associated arousals and reaction time or pain measures was masked by changes in spontaneous neurocortical arousals. Additional details from supplemental correlations are available in Suppl. Table 3.

Hypothesis 3B. The relation between sleep and pain changes from SHAM to FRAG will be partially explained by changes in performance on attention tasks from SHAM to FRAG. To test a Baron and Kenny (1986) simple mediation model where mediator M explains the relation between predictor variable X and outcome variable Y (in this case, where changes in attention (M) explain the relation between changes in sleep fragmentation (X) and pressure pain (Y)), statistical assumptions ('causal steps') presuppose a sizeable effect between both X and M as well as X and Y in order to follow with a logistic regression for mediation. No significant relation between sleep fragmentation and attention was observed; neither was there an observed

relation between sleep fragmentation and pressure pain. Therefore, a standard Baron and Kenny (1986) approach is not warranted given that presupposed relations do not exist between variables.

Four supplemental evaluations of indirect effects rather than complete mediations, two testing threshold and two testing tolerance with either % lapses or 1/10% slowest responses in the model, were performed using the statistical software SPSS macro PROCESS (Hayes 2012; Hayes, 2009; Preacher & Hayes, 2004). This approach operates under more lenient contingencies (Hayes, 2009) than the Baron and Kenny (1986) assumption that a significant total effect between X and Y must be pre-existing. Models were bootstrapped to 5000 iterations and evaluated against confidence intervals not held to homoscedastic distribution assumptions (Hayes, 2009; Hayes 2012). There was not a statistically significant change in the pain variance explained by sleep fragmentation (not statistically significant in the c or c' pathways) after a Sobel test (Baron & Kenny, 1986) when either attention metric was included in the model. Details about a, b, c and c' pathways are presented in Table 10. Non-significant correlation and indirect effect test outcomes between SHAM and FRAG conditions suggest that there were neither changes in pain related to individual participants' susceptibility to sleep fragmentation nor changes in pain related to individual variabilities in whether sleep fragmentation manifested in attention changes.

Structural Equation Modeling (SEM) did not provide a reasonable approach to data analysis in this study because of sample size requirements. To attain the power (at least 80%) to detect an indirect effect of attention (M) using an ideal measure (without error) would require at least 70 participant change scores (Wolf, Harrington, Clark, & Miller, 2013). Evaluating effects using SEM was beyond the scope of this pilot study.

Discussion

This prospective, pseudo-randomized, controlled experimental protocol successfully induced cuff-associated sleep fragmentation in healthy adult female participants by an average of 5.2 interruptions per hour. It accomplished fragmentation without compromising participants' total sleep opportunity or total sleep time in the FRAG condition, relative to BASE and SHAM. This fragmentation rate reflects an arousal rate that might reasonably be induced by mild OSA, in which respiratory events often leading to micro-arousal or full arousal from sleep occur at least five times per hour (up to 15; Iber et al., 2007).

The hypothesis that systematic sleep fragmentation at an induced disruption rate similar to that which might occur in cases of mild OSA would negatively affect reaction time performance and pain perception was not supported. Although differences across BASE, SHAM and FRAG conditions were observed, these differences were not consistent with the observations necessary to conclude that fragmentation had an adverse impact on either measure. To implicate fragmentation, differences would need to be observed between *both* FRAG and BASE (indicating that any effects were not solely related to the in-lab test environment) *and* between FRAG and SHAM (indicating that any effects were not solely related to wearing an inactive blood pressure cuff or to other test factors consistent between the two conditions).

PVT analyses evidenced differences between BASE and SHAM (for the slowest reaction times and the frequency of lapses), between BASE and FRAG (for the frequency of lapses), and between FRAG and SHAM (for response variability with supplemental analyses); taken together, these outcomes did not satisfy the pattern of differences necessary to implicate fragmentation as causal for any individual reaction-time variable. Given that pain analyses did not reveal any differences in threshold or tolerance across BASE, SHAM or FRAG and that indirect effects

from planned mediations were not significant, the hypothesis that pain could be explained by sustained attention (PVT) relative to sleep fragmentation was also not supported.

I will begin by discussing the specific results of preliminary, planned and supplemental analyses of outcome measures in the context of the experimental protocol. Then, in light of the lack of support for hypotheses, I will follow with a consideration of the experimental protocol's success and limitations on a broader level. Finally, I will extend those broader implications to the results to draw overall conclusions from this research.

PVT Outcomes

I elected to compare only morning PVT scores between conditions given the similarity between AM and PM scores, and so that I could include data from all participants (recall that one was missing PM data). That PM scores did not differ from AM despite known increases in homeostatic sleep drive as the duration of wakefulness increases (Borbély, 1982) deserves interpretation. The lack of difference between AM and PM may have resulted from the behavioral flexibility that my protocol afforded participants, who were permitted ad-lib use of caffeine outside the lab (therefore most likely affecting evening testing given that participants completed morning tests before leaving the lab, although reminders were provided that, as a stimulant, caffeine may interfere with ability to fall asleep in the lab). Participants also had ad-lib exercise, meal timing/content, light/darkness exposure, and even napping (although neither actigraphy nor sleep diaries evidenced that any participant napped on days following BASE, SHAM or FRAG) – all factors that can affect the circadian or homeostatic regulation of sleep (Atkinson, Edwards, Reilly, & Waterhouse, 2007; Buxton, L'Hermite-Baleriaux, Turek, & Van Cauter, 2000; Buxton, Lee, L'Hermite-Baleriaux, Turek, & Van Cauter, 2003; Landolt et al., 2004; Schibler, Ripperger, & Brown, 2003).

The largest impact of these combined effects was likely on tests administered in the evening (which occurred about one and one-half hours prior to participants' typical bedtime), when the homeostatic sleep drive should also have had its largest effect on outcomes (Borbély, 1982). That participants had this behavioral flexibility before their evening lab visits could have introduced considerable variability in the evening PVT scores, complicating what would otherwise have been the most sensitive time to detect sleep pressure by the test battery. This protocol limitation would have required considerably more manpower (to supervise participants full-time) and funding (to adequately compensate participants for the burden of 14 continuous days in the lab) to prevent, but future work might consider including a postprandial, afternoon measurement when cortisol is low (corresponding to a low wake-promoting circadian drive at this time) to capture lapses (Borbély, 1982).

Ultimately, for hypothesis tests, only morning PVT scores were compared. Although less sensitive to homeostatic sleep pressure, these morning evaluations would likely have been the most reliable and well-controlled of the PVT measures obtained. Morning measures were also outside of the most sensitive window for sleep inertia effects, as controlled by the researcher (Hoffman et al., 2005; Jewett et al., 1999).

There were fewer lapses and faster reaction times among participants' 10% slowest responses after BASE relative to SHAM. This outcome is highly unanticipated, as heavier instrumentation on BASE was expected to interfere with sleep more than on SHAM. Additionally, a reduction in sleep quality is typical on the first night in-lab (BASE) relative to subsequent nights (the first-night effect; Curcio et al., 2004; Lorenzo & Barbanoj, 2002). Taken together, one might expect these factors to negatively impact performance after BASE relative to SHAM. It is not clear why SHAM PVT would not have achieved at least a similar performance

as BASE PVT, especially because sleep comparison metrics did not differ between the two conditions.

One might posit that sleep *quality* in the lab was consistently poorer than *quality* at home regardless of condition (BASE, SHAM or FRAG). This could have caused nonrestorative sleep across the first two nights in the lab and therefore worsened sleep on participants' second night; however if true, this potentially cumulative deficit should have been at least equally present for SHAM and FRAG night relative to BASE because of counter-balancing, if not further amplified when FRAG followed BASE. My data support this interpretation for lapses, which were also less frequent after FRAG than BASE, but not for the slowest responses (which did not differ). Supplemental analyses comparing PVT scores after sleep at home (the first morning of Phase II) to those after BASE sleep are also inconsistent with the notion that sleep quality at home was meaningfully better than in the lab, as there were no performance differences. The unclear causal factor in the differing PVT scores between BASE and SHAM inspires future work that considers possible explanations for poorer performance after the SHAM than the BASE condition.

Variability in reaction times did not differ in ANOVA, but there appeared to be a higher-trending variability after SHAM relative to the other conditions. A post-hoc power analysis indicated that the omnibus ANOVA for response variance across conditions achieved 96% power. A supplemental mixed model analysis that accounted for individual differences across repeated measures did reach significance, however. The mixed model outcome is consistent with the trend from ANOVA and, if interpreted together, leads to the conclusion that variability across reaction times after SHAM was higher than after FRAG.

Ultimately, any PVT differences implicated BASE as unique relative to SHAM or FRAG or only indicated a difference between FRAG and SHAM when FRAG performance appeared to be better. The most consistent differences in reaction time metrics highlighted SHAM performance as the poorest. Therefore, fragmentation of 5.2 times per hour on average did not appear to uniquely impact PVT performance.

Pain Outcomes

As might be expected from a group of participants capable of perceiving weight differences, an overall difference between responses to the heavy and light weight were observed. There was a shorter latency to threshold and tolerance under the heavy weight (1000 g) relative to the light weight (500 g), which is consistent with other pressure algometer literature (Kyle, McNeil, Weinstein, & Mark, 2009). The possibility that weights were perceived differently because of finger presentation (middle or ring) cannot be ruled-out as a confound, however, because weights were consistently applied to the same finger (although evenly across hands) in this work. Concern for different weight perception across ring, middle and index fingers is not evident among literature specific to the pressure algometer utilized here, although other work did utilize a counter-balanced exposure across test fingers (comparing either middle and index or middle and ring fingers after an initial exposure to weight on either the ring or index finger, respectively; Kyle et al., 2009). Regardless of whether the difference between weights observed here was the result of finger placement or the ability to discern between weights, the premise that participants respond differently to the algometer task under different testing circumstances was corroborated and any effects of this difference on algometer outcome variables was accounted for by including it as a factor in subsequent assessments of threshold and tolerance.

Known order effects of weight presentation in pressure algometer literature are related to perception of the lighter weight. Specifically, tolerance latency is shorter for the light weight when it is presented after a heavy weight than when it is presented after another light weight (Kyle et al., 2009). Effects of order were not observed for the heavy weight in the same study. The protocol for weight presentation in this study controlled for the possibility of order effects by creating consistent weight-preceding conditions for all latency values used in analyses. That is, weights were always presented in the following order and to the same finger (although on different hands): light (750 g; initial exposure not used for data), heavy, light, heavy, light. Note that light-weight exposures were always preceded by a heavy weight (and heavy-weight exposures were always preceded by a lighter weight, although this is not necessarily a concern based on the literature) to control for an impact of presentation order.

Preliminary analyses also indicated a difference in pain threshold and tolerance between participants' dominant and non-dominant hands, such that latency was always longer on their dominant. Recall that weights were applied to each hand the same number of times and to the same fingers on each hand. Two plausible interpretations of this outcome are first, that the dominant hand has a lower sensitivity to pressure pain than the non-dominant, and/or second, that the local mild intermittent hypoxemia produced by the blood pressure cuff on the non-dominant arm increased pressure pain sensitivity in that hand, but not the contralateral one.

The first of these two interpretations seems the most reasonable given a lack of interaction effect between condition (BASE/SHAM/FRAG) and hand. If, hypothetically, the hand difference were the result of cuff activity, then algometer scores after BASE and SHAM should be unaffected on the non-dominant hand, producing an effect only after FRAG night. This outcome was not supported, leaving the first interpretation as the most plausible. Extant

pressure algometer literature tends to average across, rather than explicitly evaluate, differences that might exist across hands of participants (Kyle et al., 2009; Vowles, McNeil, Sorrell, & Lawrence, 2006).

Similar to PVT, time of day differences did not appear to impact algometer scores on the whole. Evening algometer evaluations would have suffered the same limitations described for PVT.

Neither pain threshold nor tolerance were altered by the sleep fragmentation increase, as evidenced by a lack of test condition main effect or interaction between test condition and other influential variables (weight or hand). This lack of effect on pain is consistent with fragmentation's lack of effect on PVT between SHAM and FRAG conditions, but is inconsistent with the differences observed between BASE and SHAM for PVT metrics. Therefore, the unknown factor engendering PVT change between BASE and SHAM does not appear to affect pain threshold or tolerance in a similar way.

One possibility for the lack of difference in pain outcomes between SHAM and FRAG is that fragmentation was only presented for a single night in this paradigm. Other work has demonstrated a pain change after two nights' sleep disturbance, but not necessarily after a single night (Ødegård et al., 2013). It is plausible that more than one night of consecutive disruption is necessary to achieve measurable effects on pain. Alternatively, average fragmentation of 5.2 times per hour may be insufficient to produce pain effects despite its consistency with the average rate of respiratory events in mild OSA. One might argue that fragmentation without concomitant hypoxemia may be insufficient to impact pain at any fragmentation frequency. These data at least permit concluding that *local* intermittent hypoxemia paired with an average interruption rate of 5.2 times per hour, and fragmentation of 5.2 times per hour alone, are both

inadequate to produce an effect on pain (because the non-dominant hand was, in fact, exposed to intermittent hypoxemia when the cuff restricted blood flow to the periphery during inflations). Finally, although data were collected from participants within just a few days in this protocol, differences in estrogen and the menstrual cycle that were not controlled for may have affected pain measures (Amandusson & Blomqvist, 2013; Riley, Robinson, Wise & Price, 1999).

Although not relevant to hypotheses in this study, there were notably large effect sizes on tests of interaction between hand and weight for both threshold and tolerance. Referring to Figures 11 and 12, the lighter weight appeared to reveal differences between hands for both threshold and tolerance more readily than did the heavy weight. This could indicate a floor effect in latency to pain response (or escape behavior) for the heavy weight, perhaps suggesting that responses to the light weight in the algometer task were more revealing of pain changes in general.

Correlation and Mediation Outcomes

There was no evidence of a relation between overall fragmentation change and attention; neither was there a relation between overall fragmentation and pain measures. Supplemental analyses were used to consider whether cuff-associated fragmentation was a better predictor of PVT and algometer scores than the change in overall fragmentation, given that overall fragmentation includes spontaneous arousal rates along with induced arousal rates. Correlations remained non-significant for all reaction time and pain change scores.

Change scores that captured individual variation in overall fragmentation between SHAM and FRAG in mediation analyses, when evaluated relative to change in reaction time or pain metrics, revealed that overall fragmentation was related to neither outcome independently. Indirect effect sizes similar to total effect sizes occurred only when %Lapses was used to

estimate sustained attention (Table 10); in these tests the total effects were not significant and Sobel tests did not indicate a significant change. Therefore, there was no evidence of an indirect effect of attention in any model and attention also did not appear to suppress a relation between fragmentation and pressure pain. These outcomes lead to the conclusion that an indirect effect, in which sleep disruption might influence pain perception via an impact on sustained attention, was not supported here.

Protocol Strengths and Limitations

From a clinical perspective, there are not established fragmentation criteria for what constitutes “good-” or “poor-” quality sleep, and interpretation of changes in sleep quality in the clinic are more typically related to self-report of improved daytime sleepiness or by comparing an individual’s fragmentation between treatments. Because there is clinical evidence that sleep quality improvement associated with treating OSA also improves pain (Khalid, et al., 2011), sleep interruption rates relative to the frequency of respiratory events serve as a next-best approximation. Therefore, without otherwise defined ranges of fragmentation valence, fragmenting sleep at least as often as respiratory events might interrupt sleep in mild OSA (at least five and up to 15 times per hour) confers, at a minimum, clinically translational meaning. Our cuff-induced fragmentation index of 5.2 per hour reached this benchmark.

Participants were evenly enrolled across the presentation order of SHAM and FRAG conditions (each order of presentation $n = 6$) and there were not order effects of condition on sleep- or pain-related measures. The number of awakenings, which may or may not have been brief, were also more frequent in the FRAG than in the SHAM condition. Therefore, outcomes of this study reflect an impact of overall “mild” sleep fragmentation independent of overall sleep restriction or a lack of recovery from the FRAG condition. Although outcomes might be

explained by the frequency of either brief or more extended arousals, work by Stepanski and colleagues (1984) indicates that even brief, transient awakenings are related to increases in sleepiness.

At the level of individuals, there were some participants whose interruption rate was less frequent than the average (the lowest cuff-associated fragmentation index was 3.7). However, all participants did experience a fragmentation rate of 4.9 or higher during stage N2 sleep ($M = 6.7$, see Results). Fragmentation of other stages varied and was particularly low for N3 ($M = 3.8$). It is unsurprising that fragmentation was more effective in some sleep stages than others (as is evident when auditory disruption is applied, Buxton et al., 2012a). Therefore, the conclusion that 5.2 cuff-induced interruptions per hour on FRAG was not sufficient to produce an impact on PVT or pain measures should be refined to specify that interruptions predominantly affected stage N2. Supplemental analyses (see Results) of the relation between stage-specific cuff-related fragmentation rate and changes in PVT or algometer scores from SHAM to FRAG did not implicate any particular stage as related to changes that did occur. Although other work has aimed to interrupt N3 or REM exclusively, these stage proportions were often compromised when fragmentation was successful (for N3, Older et al., 1998; for both REM and N3, Onen, et al., 2001). This limits interpretation about fragmentation's effects on pain because of possible restriction/deprivation, so they are more appropriately considered in the next paragraph (concerning quantity rather than quality).

In this work all stages except N2 retained similar proportionality across BASE, SHAM and FRAG; N2 occupied a *greater* proportion of sleep time on FRAG. However, extant literature does not inspire suspicion of N2-related effects on pain. Stages N3 and REM have been advanced as the most plausible contributors to pain change in the sleep restriction and

deprivation literature (Drewes et al., 2000; Lentz, et al., 1999; Moldofsky & Scarisbrick, 1976; Older et al., 1998; Onen et al., 2001). Changes in pain measures were still observed in work where N2 proportionality was sustained, (Lentz, et al., 1999; Onen, 2001), suggesting that N2 change is not *necessary* to observe a pain impact. This evidence does not mean that N2 restriction or deprivation is not *sufficient* to produce an effect, but selective deprivation of N2 is likely impossible to implement given normative sleep architecture. Nonetheless, this study indicated an increase rather than a decrease in the proportion of N2 on FRAG, making an impact of N2 restriction as a result of the FRAG protocol irrelevant.

Worth noting, however, is that greater %N2 may have occurred at the expense of non-significant decreases in REM and N3. Post-hoc power for the omnibus analysis of N3 was 96%; this power is great enough to conclude that increases in sample size would probably not yield a significant outcome in N3 change despite a trending reduction in N3 on FRAG relative to SHAM (whether related to N2 changes or otherwise). Still, there exists the possibility that study outcomes might have been influenced by an additive effect of non-significant N3 and REM changes, especially given that evidence of an independent impact of N3 proportion on subsequent pain is equivocal in the stage-selective restriction/deprivation literature (Arima, et al., 2001; Drewes et al., 2000; Lentz et al., 1999; Older et al., 1998; Onen et al., 2001). Taken in the context of this study's test outcomes, non-significant cumulative effects of %N3 and %REM change on FRAG did not impact PVT or pain scores (which did not differ across test conditions).

While the cuff-associated arousal rate was adequate for my experimental intentions, the overall arousal rate (regardless of arousal source, whether spontaneous or cuff-associated) was on average 3.5 interruptions per hour. This was a statistically significant change on the whole, but the overall arousal rate is below the lowest clinical threshold for mild OSA. If cuff-

associated and spontaneous arousals are not qualitatively unique in their impact on sleep restoration, then an overall difference of 3.5 interruptions per hour may not have been adequate to produce differences in behavioral response between SHAM and FRAG.

Spontaneous arousals present difficulty in experimental design because, unlike cuff-associated arousals, they are not readily manipulated. For example, spontaneous arousals that differed from night-to-night may have affected the utility of ANOVA to detect differences across conditions in this study. Spontaneous arousals were lower on FRAG than SHAM, so despite successful fragmentation of sleep with the blood pressure cuff on FRAG, the overall fragmentation rate was more similar across nights than the protocol intended. This limitation was addressed by including the spontaneous arousal rate as a random effect on the participant-level in supplemental, mixed-model analyses to try and specifically target the impact of cuff-associated arousals. Mixed model outcomes were consistent with those from ANOVA.

Participants had a shorter sleep time according to actigraphy during Phase I relative to Phase II in-lab nights. This difference raises concern that participants might have arrived at the lab for testing already sleep-restricted, causing a floor effect in the outcome measures on BASE. However, multiple considerations reduce the plausibility of this interpretation. Namely, a 7-hour, 44-minute sleep time on average (± 39 min), as identified by actigraphy during Phase I, reflects typical sleep time for a healthy, young adult population (Carskadon & Dement, 2011). Further, the proportions of sleep stages that typically rebound when there is sleep recovery opportunity (stages N3 and REM; Carskadon & Dement, 2011; Verma, 2001), were similar to normative proportions for young adults (Carskadon & Dement, 2011) on BASE night according to PSG, and did not differ from proportions on SHAM or FRAG.

A more compelling explanation exists for lower actigraphy-defined TST during Phase I than on lab nights: poor specificity. As noted in the Measures section, actigraphy does a fair job of correctly identifying sleep (sensitivity), but a poor job of correctly identifying wake (specificity; Marino et al., 2013). It is not surprising that participants might take longer to fall asleep or have a lower sleep efficiency in a foreign environment and that this time, or the time when participants are still during equipment checks, might have been misclassified by actigraphy as sleep. In supplemental analyses (see Results), similar PVT scores after at-home sleep relative to after BASE, and lower pain scores after at-home sleep than after BASE (in the opposite direction than might be predicted in the event of at-home sleep restriction), suggest it is unlikely that differences in actigraphy-defined TST reflect actual sleep differences between Phase I and BASE, SHAM or FRAG. If actigraphy was an accurate TST estimate for both at-home and in-lab conditions, its impact does not appear to have manifested in a predictable way to have an impact on PVT or pain measures.

The outcome of similar sleep efficiency (SE) across test conditions is initially counter-intuitive, given differences that were observed in fragmentation and awakenings. Note however that sleep efficiency, as a metric, is distorted due to the sleep extension protocol. Efficiency is calculated as TST relative to sleep opportunity (or time in bed), which is not impacted by brief arousals. An extension of sleep opportunity to compensate for epochs scored as wakefulness, as was applied here, would dilute the effect of that wakefulness and artificially inflate sleep efficiency relative to what it might have been without extension.

Neurocortical arousals can occur spontaneously (endogenously), after a sleep-disorder related event (Mesquita et al., 2012), or in response to the environment (for example, in response to blood pressure cuff inflation; Davies, et al., 1994; Degaute, et al., 1992; Schwan & Eriksson,

1992). The increase in arousal frequency during FRAG relative to SHAM and BASE appeared to result from an increase in exogenously-induced, cuff-related arousals, rather than from an overall increase in spontaneous or other environmentally-induced arousals. Spontaneous arousals during FRAG did not significantly differ in frequency from SHAM or BASE, suggesting that the fragmentation increase was in fact related to the cuff disturbance. If I interpret the statistical trend, arousals unrelated to cuff inflation trended *less* frequent during FRAG.

Although arousal threshold decreases with ongoing sleep (Rechtschaffen, Hauri, & Zeitlin, 1966), the Sleep Continuity Hypothesis (Bonnet, 1986) suggests that sleep does not begin to confer restoration unless that ongoing sleep occurs in uninterrupted intervals of at least 10 mins (Bonnet, 1986). In this study, an arousal rate more frequent than every 10 mins on average was achieved for stage N2, the sleep stage also occupying the majority of TST. That N2 interruption at this rate, and of this micro-arousal type, did not produce measurable effects on the PVT is inconsistent with the Sleep Continuity Hypothesis's extrapolation to stage N2 specifically, and to the majority of sleep. Alternatively, habituation to the cuff inflation may have occurred, potentially rendering the blood pressure cuff less likely to cause sleep interruption as the night progressed (Arima et al., 2001; Downey & Bonnet, 1987; Philip et al., 1994). However, arguments about the rate of interruption necessary to manifest as a behavioral change remain equivocal, as others have used rates of three or five minutes to interfere with sleep restoration (Levine, Roehrs, Stepanski, & Zorick, 1987). Although this research was conceived from the perspective of the Sleep Continuity Hypothesis, if segments of sleep under five minutes (or shorter than the most frequent interruption in this study attempted) confer restoration then the cuff inflation interval applied here may not have been adequate to achieve an effect.

Additionally, alternative vigilance measures like the tracking task may be more sensitive than the PVT in detecting any effects of fragmentation on performance if present, given recent evidence of differential sensitivity of the PVT to microsleeps and vigilance lapses (Buckley, Helton, Innes, Dalrymple-Alford, & Jones, 2016). Finally, it is plausible that restorative processes facilitated by sleep are affected in a fundamentally different manner by sleep continuity (quality) and sleep quantity, rendering PVT a sensitive measure for sleep quantity loss but less sensitive to micro-arousal sleep quality interference.

This pilot study, in which sleep fragmentation translatable to mild OSA (particularly for stage N2) was combined with intermittent peripheral hypoxemia to evaluate the impact on pressure-pain, revealed no changes in pain or sustained attention. Neither did it indicate a relation between pain and fragmentation with sustained attention as a conduit. These outcomes deserve follow-up to refine the pilot protocol and expand the results to more frequent interruption rates that reflect moderate or severe OSA clinical thresholds. Future work might consider combining blood pressure cuff fragmentation with adjustable auditory fragmentation to *both* achieve higher rates of interruption *and* retain the ability to segregate changes in pain between hands affected or unaffected by intermittent local hypoxemia.

At lower levels of fragmentation such as that applied in this study, a power-spectral analysis of delta (N3) could be a more sensitive evaluation of sustained or mounting sleep need as a result of nonrestoration than the PVT (Ødegård et al., 2015). Power-spectral analysis does not capture impacts on sustained attention that are important for translating functional impacts of fragmentation to daily life, however.

Although not easily translatable to the population diagnosed with Fibromyalgia (and perhaps not as sensitive to sleep disruption; Lautenbacher et al., 2006), the use of a transdermal

electrical pain stimulus might be considered in future work. Although a pressure stimulus is consistent with the dolorimetry used in Fibromyalgia pressure-point evaluation, it is limited by its subjective nature. Repeated presentation of transdermal electrical stimuli might be combined with the monitoring of event-related potentials to achieve both an objective measurement and a measurement potentially more sensitive and consistent than is obtained from the pressure algometer.

In conclusion, sleep deprivation/restriction is a well-known hyperalgesic (Edwards et al., 2008; Lautenbacher et al., 2006). There is clinical evidence that sleep fragmentation also amplifies pain (Khalid et al., 2011). Improved tolerance for pain after OSA therapy is evidence that pain is affected by either the sleep fragmentation, sleep restriction, or hypoxemia that characterize OSA (Khalid et al., 2011; Zucconi *et al*, 1995). By systematically fragmenting the sleep of a healthy group while extending their overall sleep opportunity to minimize restriction, this research isolated the effect of a “mild” but clinically translational sleep fragmentation on pain. Amplified pain perception like that observed after sleep restriction or deprivation was not reproduced by an induced sleep fragmentation of 5.2 times per hour on average, by an overall fragmentation increase of about 3.5 times per hour, or by induced sleep fragmentation of N2 sleep at a rate of 6.7 times per hour on a single night when total sleep, N3, and REM sleep time were maintained. Poorer reaction time performance after SHAM than after BASE did not appear related to sleep variables measured in this study, and participants did not demonstrate changes in pain concomitant with these observed changes in attention.

This pilot work contributes to a limited literature about sleep fragmentation’s effect on pain and to an expanding literature relating sleep and pain more broadly, by considering local hypoxemia and achieving fragmentation without a negative impact on stages suspected to most

strongly contribute to the sleep-pain relationship. It is among the first work to use PVT as a means of incrementally evaluating parametric changes in attention to the relation between sleep and pain. Future work building from this pilot should aim to accumulate evidence that can inform clinical standards of fragmentation frequency and that can translate to clinical populations readily. It should also focus on determining the variance in pain change accounted for uniquely by local hypoxemia, systemic hypoxemia, or sleep fragmentation.

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Table 1

<i>Participant Demographics</i>							
	Frequency (%)		Mean (SD)		<i>t</i>	<i>p</i>	Fisher's Exact
	Withdrawn/ Excluded	Included	Withdrawn/ Excluded	Included			
Female	5 (100)	12 (100)					N/A
Income (in thousands)			\$37.5 (\$63.3)	\$34.4 (\$32.4)	0.60	.560	
Right-Dominant	4 (80)	12 (100)					.294
Age			21.5 (2.0)	23.8 (2.0)	2.21	.043	
Race (White)	4 (80)	12 (100)					.294
Education (in years)			15.9 (1.9)	17.2 (1.8)	1.35	.198	
Marital Status (Single)	5 (100)	11 (92)					1.000

Table 2

In-lab polysomnography tested across conditions, within-subjects (N=12)

<u>Sleep Feature</u>	<u>Pairwise Comparisons</u>	Base <i>M (SD)</i>	Sham <i>M (SD)</i>	Frag <i>M (SD)</i>	<i>F</i>	<i>p</i>	<i>n</i> ² or <i>d</i>
Sleep Opportunity (min)		492.4 (43.1)	490.2 (54.7)	500.8 (43.8)	0.94	.406	.079
Total Sleep Time (min)		429.5 (52.5)	444.3 (40.0)	426.3 (56.8)	1.05	.367	.087
Sleep Efficiency (%)		87.5 (9.7)	91.0 (5.4)	85.9 (9.5)	2.53	.103	.187
Awakenings (/hr)		3.0 (1.1)	2.3 (1.1)	3.5 (1.0)	5.64	.011	.339
	Frag vs. Base					.087	0.53
	Frag vs. Sham					.012	1.18
	Base vs. Sham					.111	0.59
Neurocortical Arousals (/hr)		10.3 (2.9)	10.2 (3.0)	13.7 (2.9)	17.20	<.0001	.610
	Frag vs. Base					<.001	1.14
	Frag vs. Sham					<.001	1.16
	Base vs. Sham					.845	0.04
Spontaneous Arousals (/hr)		10.3 (2.9)	10.2 (3.1)	8.8 (2.1)	3.40	.052	.236
N1 (%)		8.6 (4.5)	7.0 (2.9)	9.1 (3.6)	1.78	.192	.139
N2 (%)		44.7 (4.2)	42.9 (6.2)	47.0 (6.2)	4.14	.030	.273
	Frag vs. Base					.104	0.43
	Frag vs. Sham					.037	0.66
	Base vs. Sham					.164	0.34
N3 (%)		24.1 (7.2)	26.4 (9.7)	22.3 (8.0)	2.82	.081	.204
REM (%)		22.7 (4.7)	23.8 (4.4)	21.5 (6.1)	1.26	.304	.103

Table 3

Preliminary, paired-samples t-tests of PVT metrics between test times, averaged across conditions.

PVT Metric	AM M(SD)	PM ^a M(SD)	<i>t</i>	<i>p</i>	<i>d</i>
%Lapses	10.89 (15.59)	9.55 (12.44)	0.17	.870	0.01
1/Slowest 10%	.00187 (.00045)	.00191 (.00033)	0.55	.596	0.10
Variance ^b	8590.82 (7810.64)	6500.75 (4970.61)	1.39	.191	0.33

^aOne participant was represented by the average of 2 rather than 3 scores due to missing data.

^bVariance of each participant's individual PVT responses in a given session, excluding false starts.

Table 4

Within-subjects ANOVA of each morning-measured (AM) PVT metric across conditions

AM PVT Metric	Pairwise Comparisons	Baseline M(SD)	Sham M(SD)	Fragmentation M(SD)	<i>F</i>	<i>p</i>	<i>n</i> ² or <i>d</i>
%Lapses		9.03 (16.02)	11.01 (13.85)	12.63 (17.27)	6.62	.006	.38
	Base vs. Sham					.033	0.28
	Base vs. Frag					.007	0.37
	Sham vs. Frag					.316	0.10
1/Slowest 10%		.00198 (.00050)	.00177 (.00047)	.00186 (.00045)	4.23	.028	.28
	Base vs. Sham					.016	0.44
	Base vs. Frag					.200	0.25
	Sham vs. Frag					.109	0.20
Variance ^a		7496.53 (8151.08)	10692.15 (9426.02)	7583.78 (7366.19)	3.00	.071	.21

^aVariance of each participant's individual PVT responses in a given session, excluding false starts.

Table 5

Mixed effects models (compound symmetry) and parameter estimates of PVT measures, covaried for spontaneous arousals

Model and Specifications	Levels	Parameters	F	p	Wald Z	t	p	Estimate	
								Fixed	Covariance
1/Slowest 10% ^a	3	2	4.32	.026					
Fixed (BASE/SHAM/FRAG)									
BASE vs FRAG						1.05	.306		
SHAM vs FRAG	3	2				1.70	.104		
Repeated (Subject)	1	1			2.19		.029		1.77 E-7
Random (Spontaneous Arousals)	8	6			0.47		.641		
Total									
%Lapses ^{a,b}									
Fixed (BASE/SHAM/FRAG)	3	2	5.72	.012					
BASE vs FRAG						3.16	.006	-7.58	
SHAM vs FRAG						0.68	.508		
Repeated (Subj)	3	2			2.26		.024		4.22
Random (Spontaneous Arousals)	1	1			0.26		.797		
Total	8	6							
Variance ^{a,b}									
Fixed (BASE/SHAM/FRAG)	3	2	5.16	.014					
BASE vs FRAG						0.54	.593		
SHAM vs FRAG						2.91	.008	20.39	
Repeated (Subj)	3	2			2.19		.029		1367.10
Random (Spontaneous Arousals)	1	1			0.59		.558		
Total	8	6							

^aSymmetric covariance structure

^bSquare root transformed

Table 6

Preliminary, paired-samples t-tests of algometer threshold or tolerance between test times, hands, or weights, averaged across conditions and other covariates not in question.

Covariate with Levels	Threshold M(SD)	<i>t</i>	<i>p</i>	<i>d</i>	Tolerance M(SD)	<i>t</i>	<i>p</i>	<i>d</i>
Time								
AM	36.0 (26.3)				57.9 (26.2)			
PM	35.1 (30.0)	0.75	.468	0.03	56.9 (29.5)	0.36	.723	0.04
Hand								
Dominant	43.3 (33.3)				65.1 (32.1)			
Nondominant	27.8 (24.8)	3.00	.012	0.55	49.6 (27.7)	2.23	.047	0.52
Weight								
Light (Ring)	54.6 (42.1)				83.2 (40.0)			
Heavy (Middle)	16.5 (16.8)	4.18	.002	1.19	31.5 (20.6)	5.58	<.001	1.62

Table 7

Condition x Weight x Hand (3x2x2) within-subjects ANOVAs for algometer threshold and tolerance

Threshold Variables by Condition					Threshold Interactions				
Weight	Hand	Condition			Variable Combinations	F	p	n _p ²	G-G Epsilon
		Base M(SD)	Sham M(SD)	Frag M(SD)					
Light	Dominant	78.7 (60.7)	62.0 (52.1)	61.0 (49.8)	C*W ^a	0.80	.419	.07	.648
	Nondominant	39.2 (40.1)	35.3 (24.4)	51.3 (56.3)	C*H	1.56	.233	.12	
Heavy	Dominant	18.9 (18.9)	17.2 (14.6)	21.7 (25.9)	W*H	3.58	.085	.25	
	Nondominant	13.5 (14.2)	13.2 (13.3)	14.5 (15.9)	C*W*H	2.32	.122	.17	
Threshold Main Effects									
	Weight	Hand	Condition ^a						
F	33.94	12.15	1.27						
p	<.001	.005	.293						
n _p ²	.76	.53	.10						
G-G Epsilon					.674				
Tolerance Variables by Condition					Tolerance Interactions				
Weight	Hand	Condition			Variable Combinations	F	p	n _p ²	G-G Epsilon
		Base M(SD)	Sham M(SD)	Frag M(SD)					
Light	Dominant	113.0 (55.8)	91.6 (50.0)	92.5 (51.0)	C*W	1.82	.185	.14	
	Nondominant	65.5 (36.8)	60.7 (34.1)	76.0 (55.7)	C*H	0.96	.398	.08	
Heavy	Dominant	36.2 (22.1)	34.5 (18.4)	37.0 (30.8)	W*H	3.64	.083	.25	
	Nondominant	27.5 (18.8)	26.9 (19.4)	26.9 (19.8)	C*W*H	1.61	.222	.13	
Tolerance Main Effects									
	Weight	Hand	Condition						
F	41.31	15.62	2.33						
p	<.001	.002	.121						
n _p ²	.79	.59	.18						
G-G Epsilon									

^aMauchly's test of sphericity was significant ($p < .05$); values reflect a Greenhouse-Geisser correction of the degrees of freedom.

Table 8

Mixed effects models (compound symmetry) and parameter estimates of pain measures, covaried for spontaneous arousals

Model and Specifications	Levels	Parameters	<i>F</i>	<i>p</i>	Wald <i>Z</i>	<i>t</i>	<i>p</i>	Covariance Estimate
Threshold ^{a,b}								
Fixed (BASE/SHAMFRAG)	3	2	1.89	.175				
BASE vs FRAG						0.64	.526	
SHAM vs FRAG						1.27	.219	
Repeated (Subject)	3	2			2.28		.023	4.35
Random (Spontaneous Arousals)	1	1						0 ^c
Total	8	6						
Tolerance ^b								
Fixed (BASE/SHAMFRAG)	3	2	1.48	.250				
BASE vs FRAG						0.88	.387	
SHAM vs FRAG						0.84	.412	
Repeated (Subject)	3	2			2.26		.024	660.83
Random (Spontaneous Arousals)	1	1						0 ^c
Total	8	6						

^aSquare root transformed

^bSymmetric covariance structure

^cHessian matrix was not positive after model convergence, see text

Table 9

Correlation between changes from SHAM to FRAG conditions and overall arousal rate.

Variable	Change M(SD)	Δ Threshold		Δ Tolerance		Δ % Lapses		Δ 1/Slowest 10%	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i> ^a	<i>p</i>
Δ Arousal Index	3.48 (2.71)	.188	.559	.204	.524	-.211	.510	.222	.489
Threshold	-5.25 (15.01)								
Tolerance	-4.69 (14.76)								
% Lapses	-1.61 (4.33)								
1/Slowest 10%	0.00009 (0.00019)								

^aBecause of the inverse metric, a negative correlation with Arousal Index would support the hypothesized relation between variables.

Table 10

Mediation analyses using changes in attention (%lapses or 1/slowest 10%) as mediating factors (M) between changes in sleep fragmentation (X) and changes in pain (Y; threshold or tolerance).^a

Model	Path	R ²	t	p	Sobel	
					Z	p
	a	.161	-1.21	.253	.626	.531
	b		-0.95	.368		
	c	.035	0.47	.646		
	c'		0.08	.942		
	Indirect	.034				
	a	.007	0.31	.760	.239	.811
	b		1.23	.251		
	c'		0.44	.671		
	Indirect	.013				
		b		-0.97		
c		.042	0.61	.554		
c'			0.08	.938		
Indirect		.041				
		b		1.37	.205	.249
	c'		0.57	.581		
	Indirect	.016				

^aUsing nonparametric confidence intervals and 5000 bootstrap iterations.

Supplemental Table 1

Within-groups comparison of PVT variables after the first Phase II measure (HOME) and BASE

Variable	Measurement Time		<i>t</i>	<i>p</i>	<i>d</i>
	Home M (SD)	Base M (SD)			
PVT (AM)					
%Lapses ^a	9.60 (16.96)	9.03 (16.02)	0.45	.664	0.07
1/slowest10%	0.00198 (.00044)	0.00198 (.00050)	0.05	.961	0.01

^aLapse values required a square root transformation to meet parametric assumptions (high skew and kurtosis), but are reported without transformation here.

Supplemental Table 2

2x2x2 Repeated-measures factorial ANOVA comparing Algometer latencies (in seconds) after the first Phase II measure (HOME) to after BASE in-lab.

Pain measure and time		Hand and Weight Covariates (M, SD)			
		Dominant		Nondominant	
		Heavy	Light	Heavy	Light
Threshold ^a	HOME	21.1 (11.1)	86.3 (65.6)	20.5 (19.0)	43.8 (34.6)
	BASE	18.9 (18.9)	78.7 (60.7)	13.5 (14.2)	39.2 (40.1)
Tolerance ^a	HOME	51.8 (32.9)	122.0 (58.2)	42.8 (31.1)	85.0 (45.9)
	BASE	36.2 (22.1)	113.0 (55.8)	27.5 (18.8)	65.5 (36.8)
Threshold Outcomes					
Effect	Type	<i>F</i>		<i>p</i>	<i>n_p²</i>
Main	Condition	5.39		.040	.33
Interact.	Cond.*Weight	0.54		.479	.05
Interact.	Cond.*Hand	0.26		.623	.02
Tolerance Outcomes					
Effect	Type	<i>F</i>		<i>p</i>	<i>n_p²</i>
Main	Condition	5.25		.043	.32
Interact.	Cond.*Weight	1.13		.310	.09
Interact.	Cond.*Hand	0.49		.501	.04
Interact.	Cond.*Weight*Hand	0.71		.417	.06

^aBoth threshold and tolerance values required a square root transformation to meet parametric assumptions (high skew and kurtosis), but are reported without transformation here.

Supplemental Table 3

Correlation between changes from SHAM to FRAG conditions and cuff-induced arousal rate only.

Variable	Change M(SD)	Threshold		Tolerance		% Lapses		1/Slowest 10%	
		<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r^a</i>	<i>p</i>
Induced Arousal Index Overall	5.25 (1.33)	.172	.593	.080	.805	.116	.720	-.067	.836
Induced Arousal Index N2	6.67 (1.72)	.142	.660	.168	.603	.216	.500	-.128	.692
Induced Arousal Index N3	3.80 (2.26)	.257	.421	.173	.590	-.139	.666	.438	.155
Induced Arousal Index REM	4.50 (2.34)	-.092	.775	-.167	.603	.179	.577	-.083	.798
Threshold	-5.25 (15.01)								
Tolerance	-4.69 (14.76)								
% Lapses	-1.61 (4.33)								
1/Slowest 10%	0.00009 (0.00019)								

^aBecause of the inverse metric, a negative correlation with Arousal Index would support the hypothesized relation between variables.

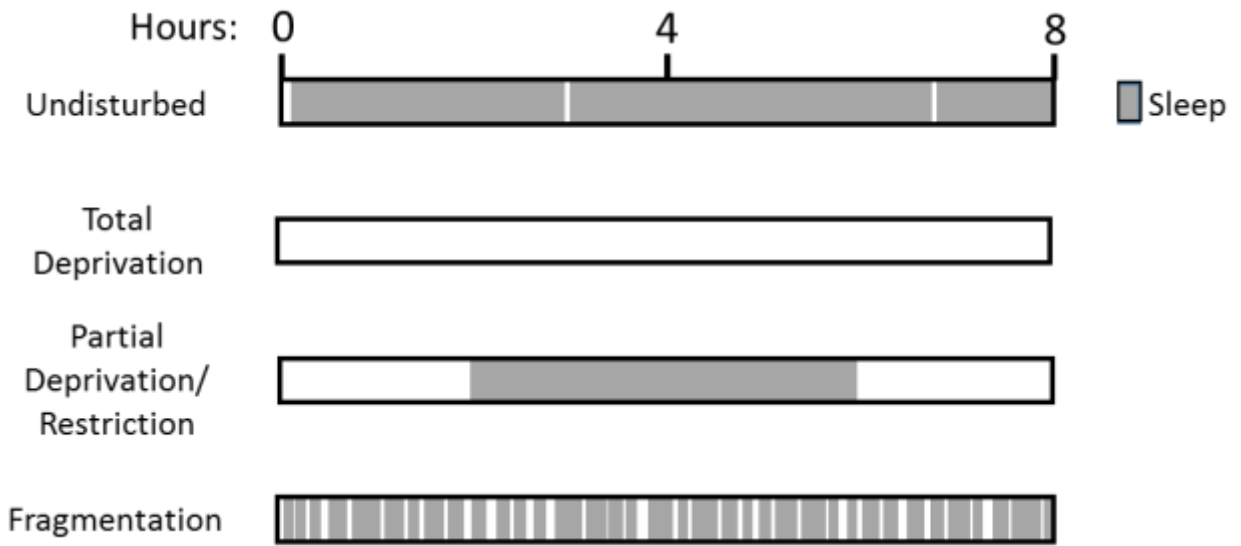


Figure 1. Types of sleep disturbance. Sleep time is represented by shaded regions within an eight hour interval.

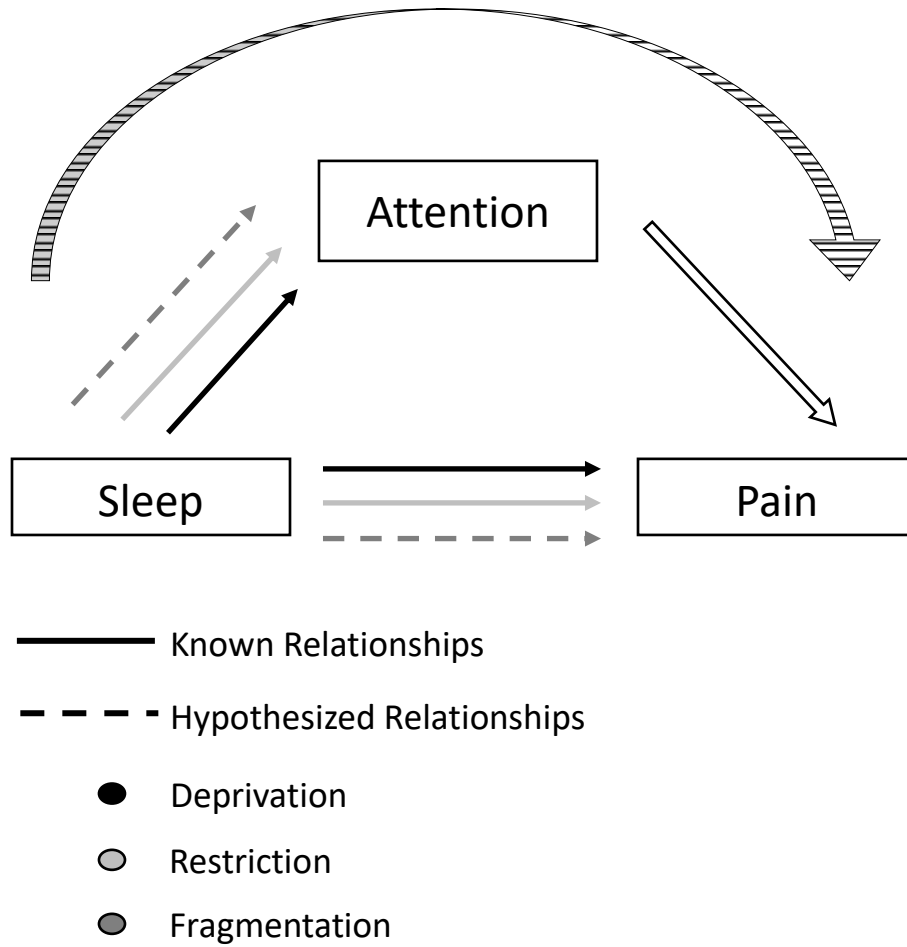


Figure 2. Theoretical model. Attention refers to attention measured by psychomotor vigilance testing. Solid lines represent relationships with support from the extant literature. Dashed lines represent relationships hypothesized in the present work. Types of sleep disturbance are considered individually.

		24-hr Time																								
Day/Night		100	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000	2100	2200	2300	2400	
(N/A)/1									h	(eq)																
1/2									P																	
2/3									P																	
3/4									P																	
4/5									P																	
5/6									P																	
6/7									P																	
7/8									C	P	a											C	P	a	*	
8/9									e	C	P	a											C	P	a	~ or #
9/10									e	C	P	a											C	P	a	
10/11									P																	
11/12									P																	
12/13									P																	~ or #
13/14									e	C	P	a											C	P	a	
14/(N/A)									P	(eq)																

Legend:

□	Actigraphy (estimated sleep)
■	Actigraphy (estimated wake)
P	Survey paper, electronic survey, and 10min PVT (after 1hr of wake time, on tablet)
■	PSG in-lab; *Screening and Acclimatization, ~Sham, #Fragmentation
e	PSG Extension (as needed per wake time during testing)
a	Algometer
(eq)	Equipment pickup/return
h	Algometer habituation at Consent visit (flexible time)
C	Test battery (~20min Cognitive) for Time on Task effect on upcoming PVT

Figure 3. Fourteen day study protocol with estimated data collection times. Symbols are qualified in the legend.

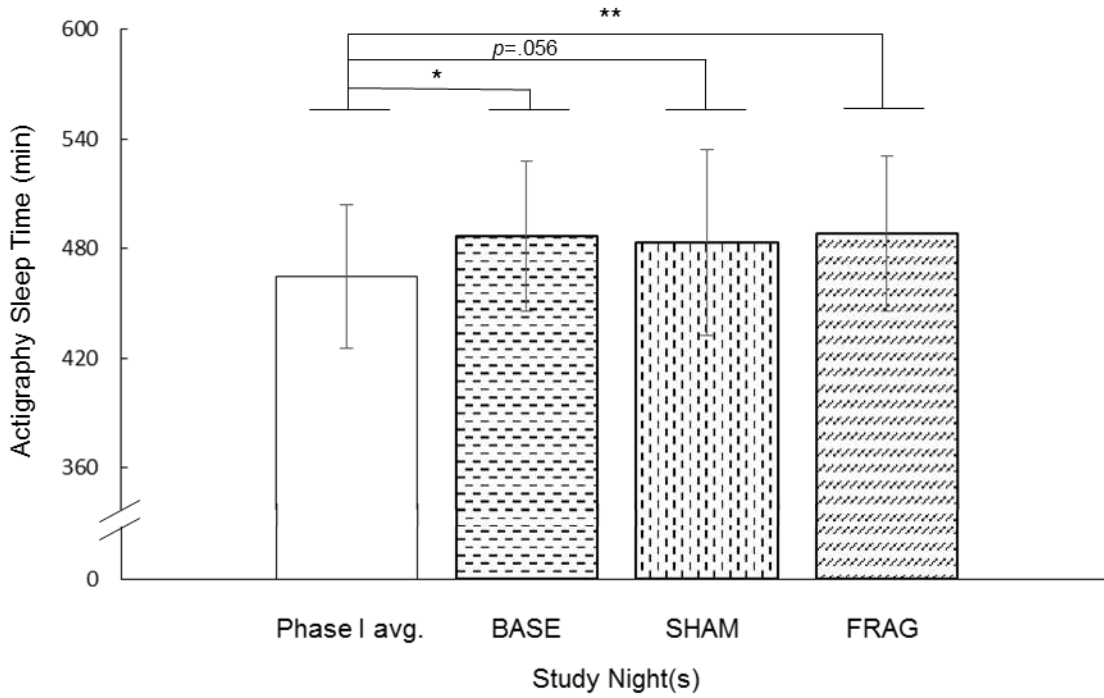


Figure 4. Total sleep time (TST; min), using actigraphy. Error bars are standard deviations.

“Phase I avg.” is the average of seven study Phase I nights; BASE, SHAM, and FRAG bars are individual Phase II nights. According to post-hoc analyses following a significant omnibus ANOVA, the Phase I average TST was significantly lower than TST on the SHAM and FRAG nights. * $p < .05$, ** $p < .01$.

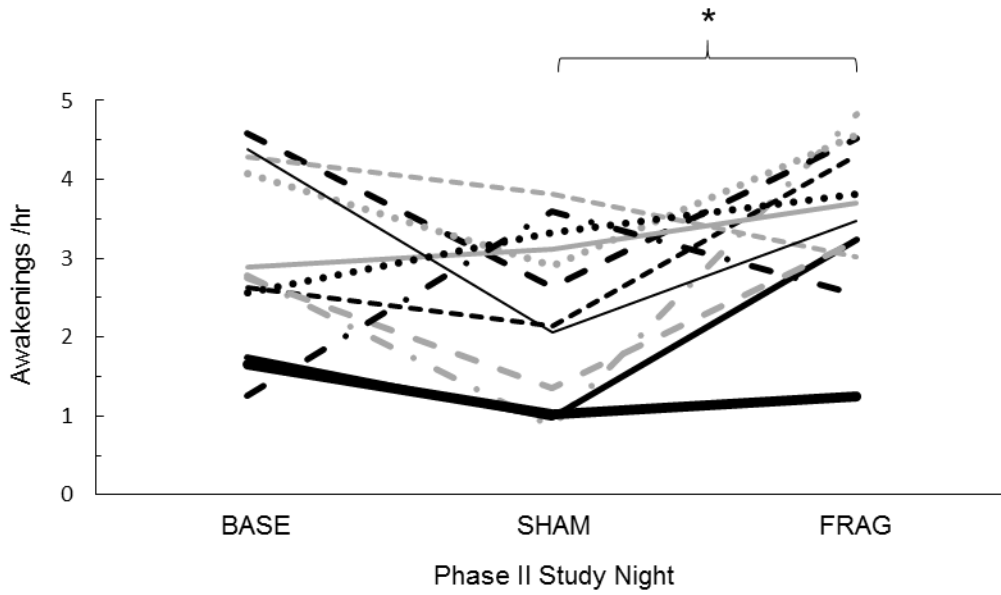


Figure 5. Frequency of awakenings (indexed per hour), using PSG. Each line represents one participant. After a significant omnibus ANOVA, there were more frequent awakenings during FRAG than during SHAM. $*p < .05$.

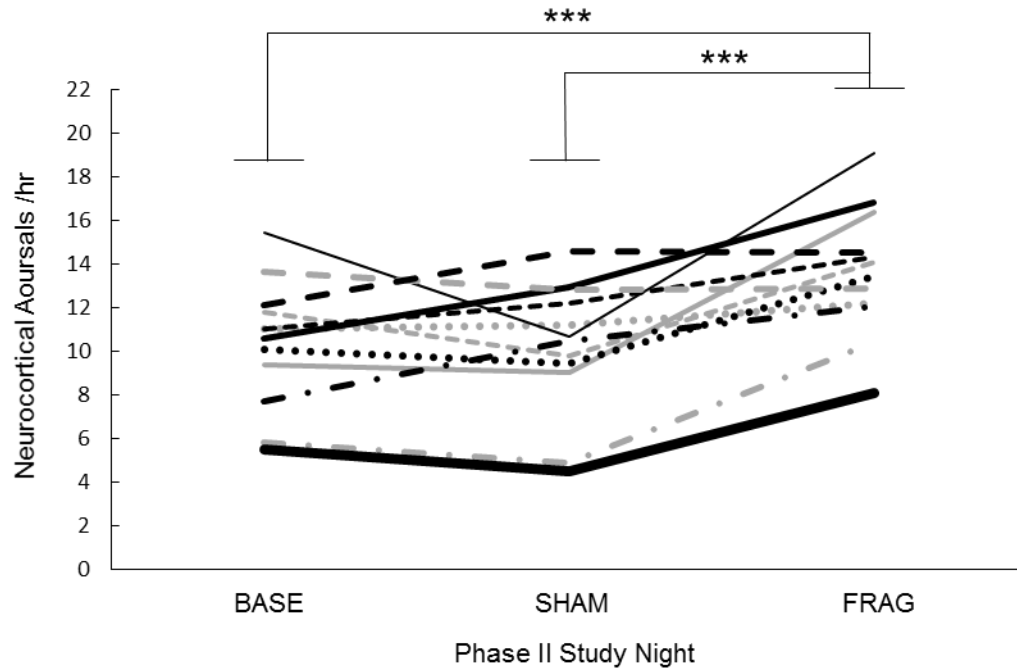


Figure 6. Frequency of neurocortical arousals (indexed per hour), using PSG. Each line represents one participant. After a significant omnibus ANOVA, there were more frequent arousals during FRAG than during either BASE or SHAM. *** $p < .001$.

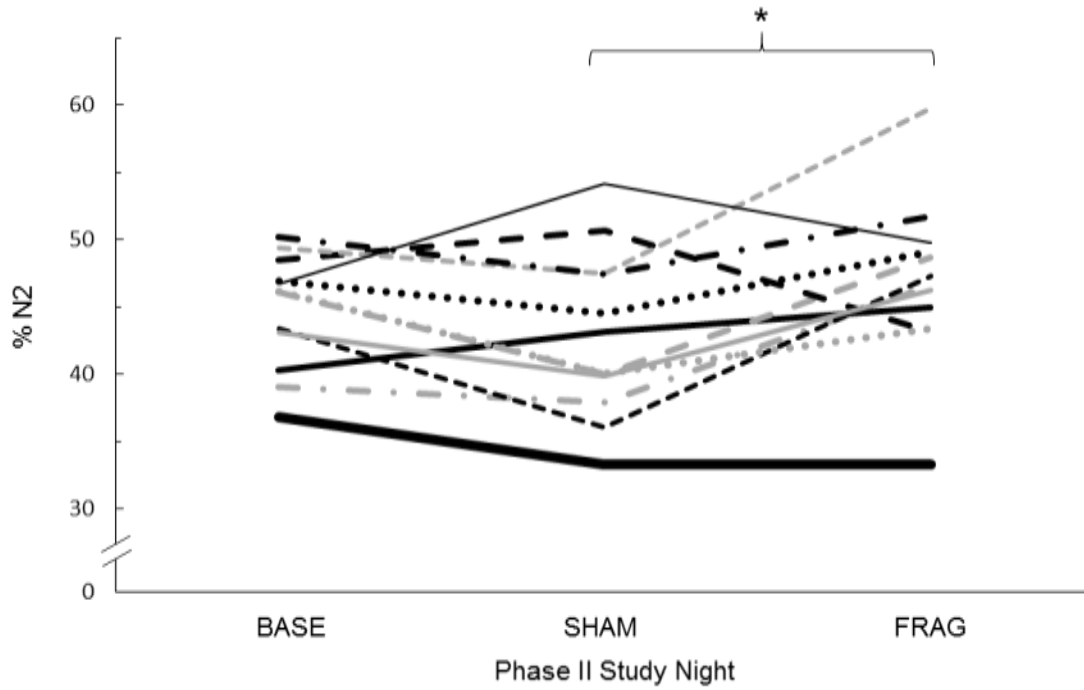


Figure 7. Proportion of NREM stage two sleep. Each line represents one participant. An omnibus ANOVA was significant across Phase II nights; %N2 was significantly higher on FRAG than on SHAM. $*p < .05$.

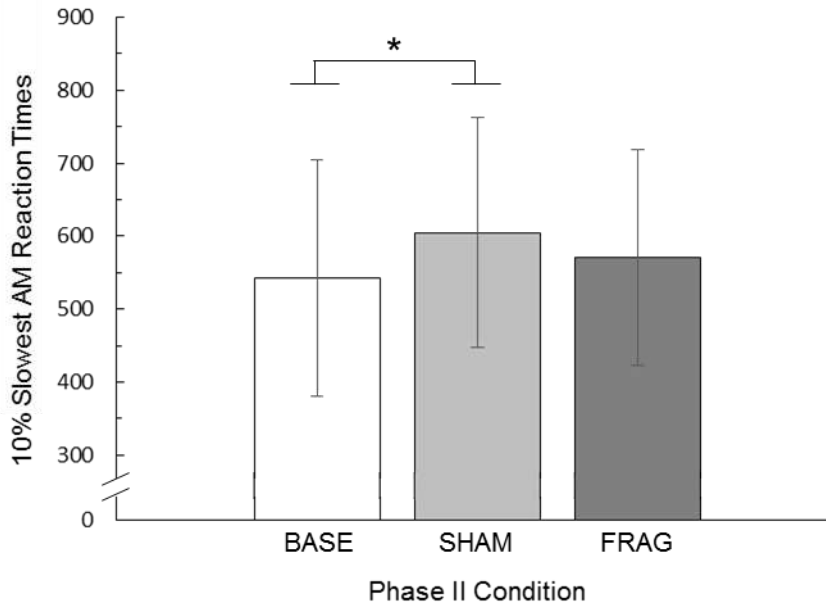


Figure 8. Slowest 10% of reaction times on the morning PVT after each Phase II condition.

Metric scores of $1/\text{Slowest } 10\%$ were converted back to raw scores using $1/(1/\text{Slowest } 10\%)$ for ease of interpretation. Error bars are the standard deviation. The 10% slowest responses were significantly faster after BASE than SHAM. $*p < .05$.

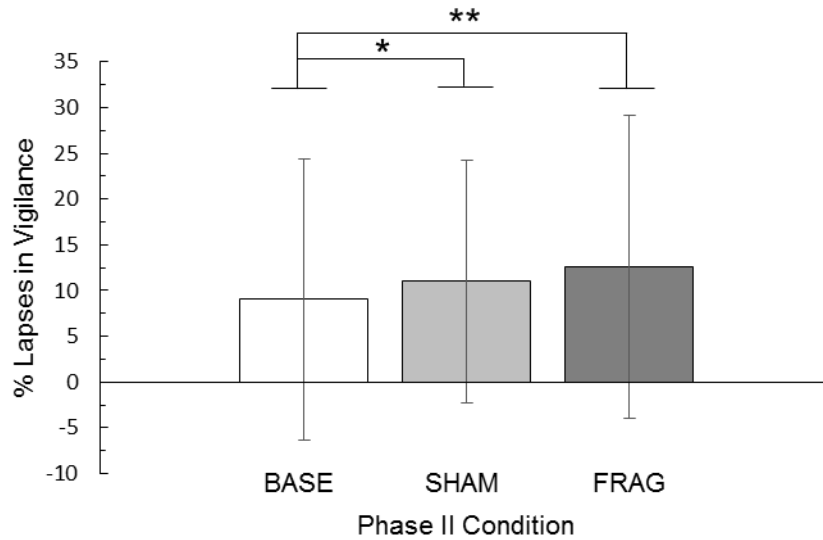


Figure 9. Proportion of lapses (> 500 ms) in vigilance on the morning PVT after each Phase II condition. Error bars are the standard deviation. After a significant omnibus ANOVA, lapses were more frequent after FRAG than after BASE and after SHAM than after BASE. $*p < .05$, $**p < .01$.

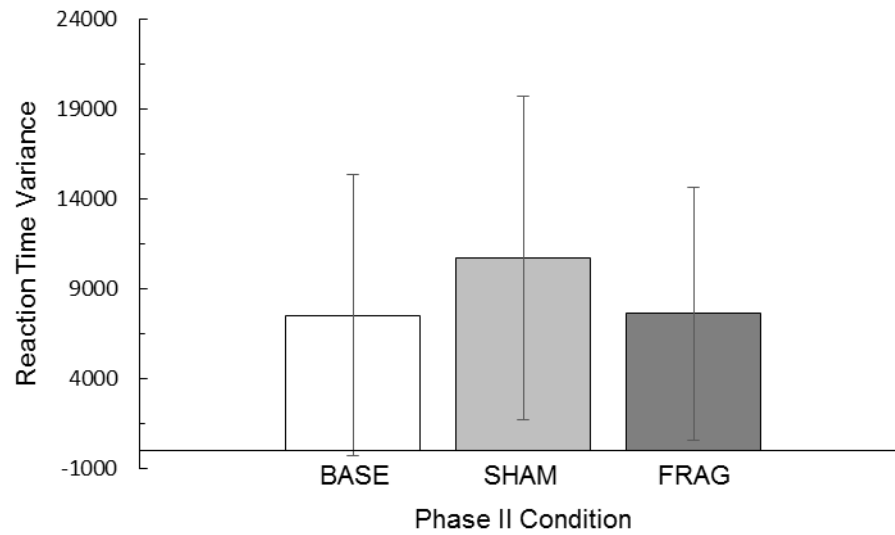


Figure 10. Variance in reaction times within the morning PVT after each Phase II condition.

Error bars are the standard deviation. Reaction time variability did not significantly differ after Phase II nights.

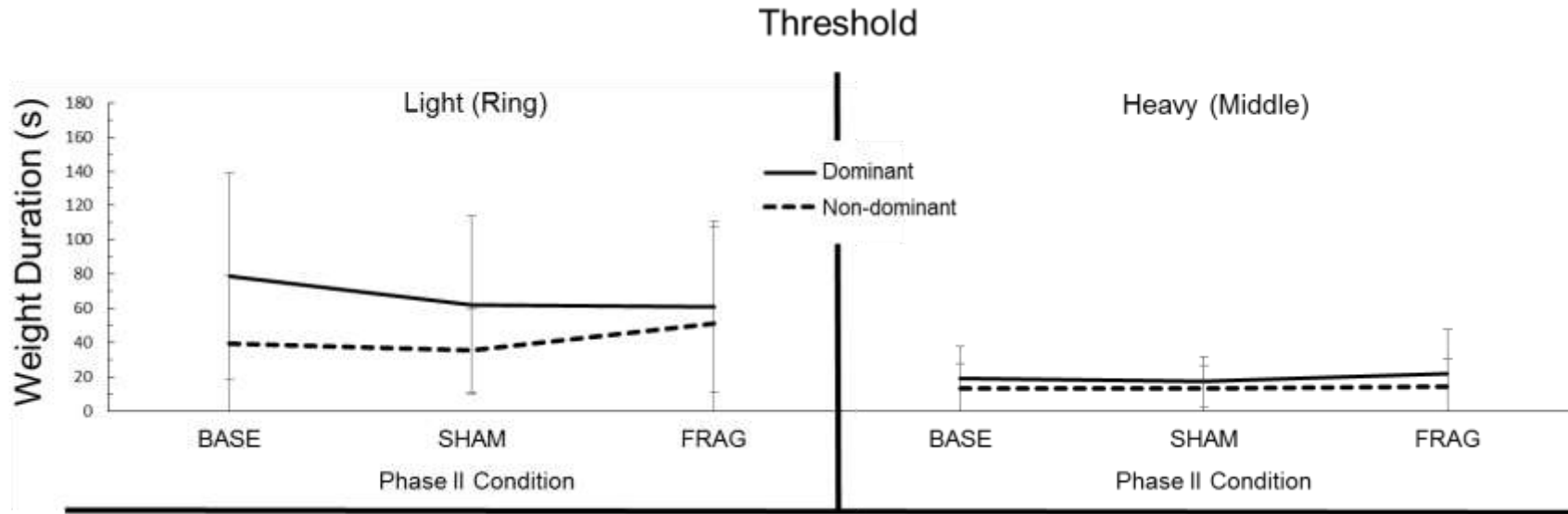


Figure 11. Algotometer threshold latencies, separated by weight and hand across Phase II conditions. Error bars are standard deviation.

There was neither a main effect of condition nor an interaction of weight and/or hand with condition on threshold latency.

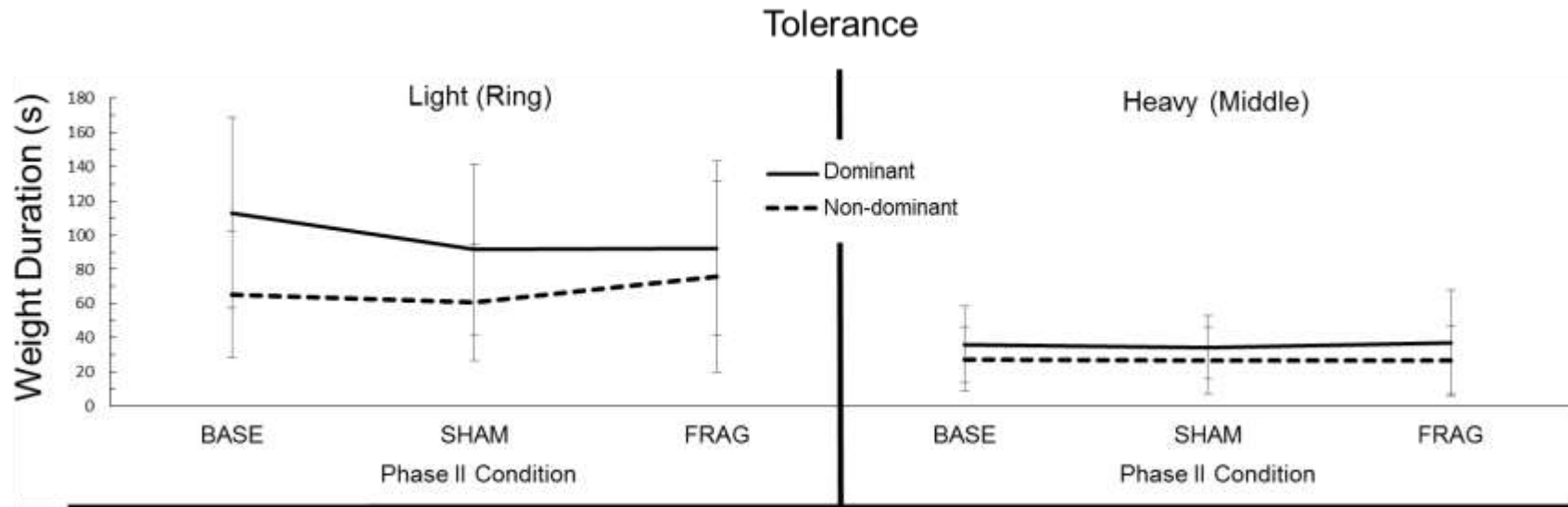
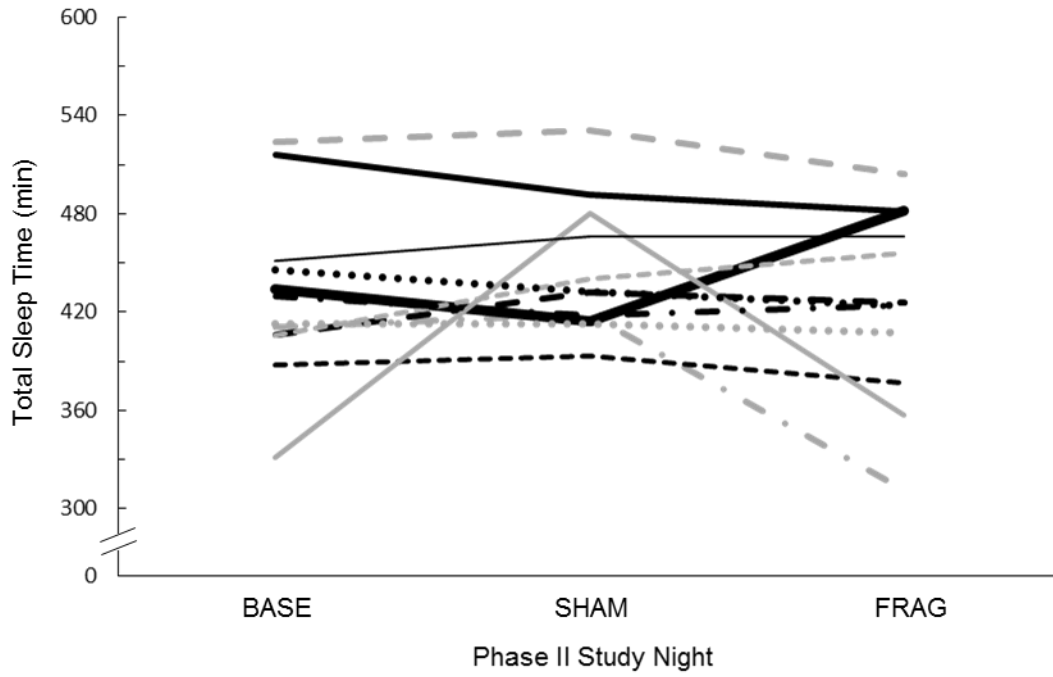


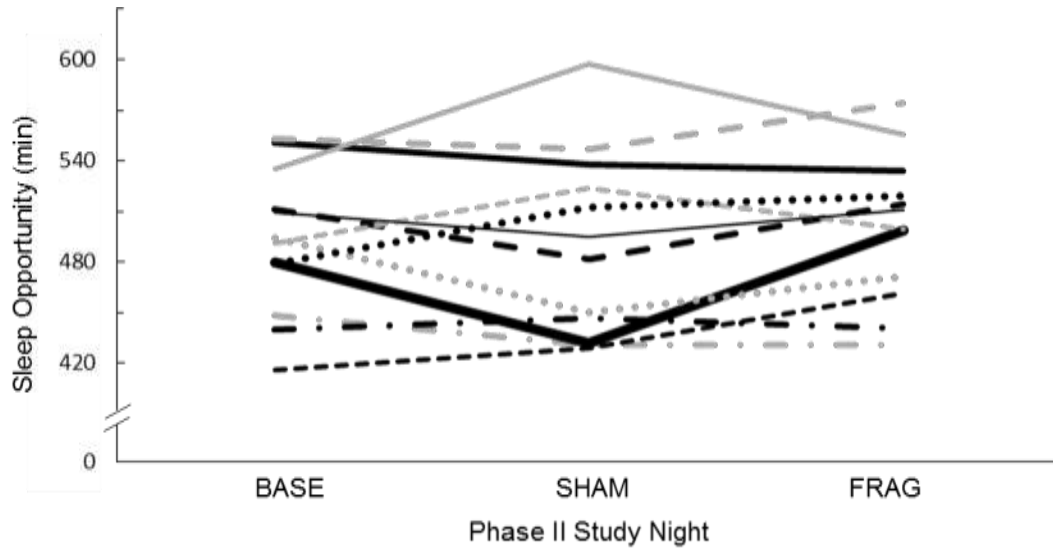
Figure 12. Algometer tolerance latencies, separated by weight and hand across Phase II conditions. Error bars are standard deviation.

There was neither a main effect of condition nor an interaction of weight and/or hand with condition on tolerance latency.

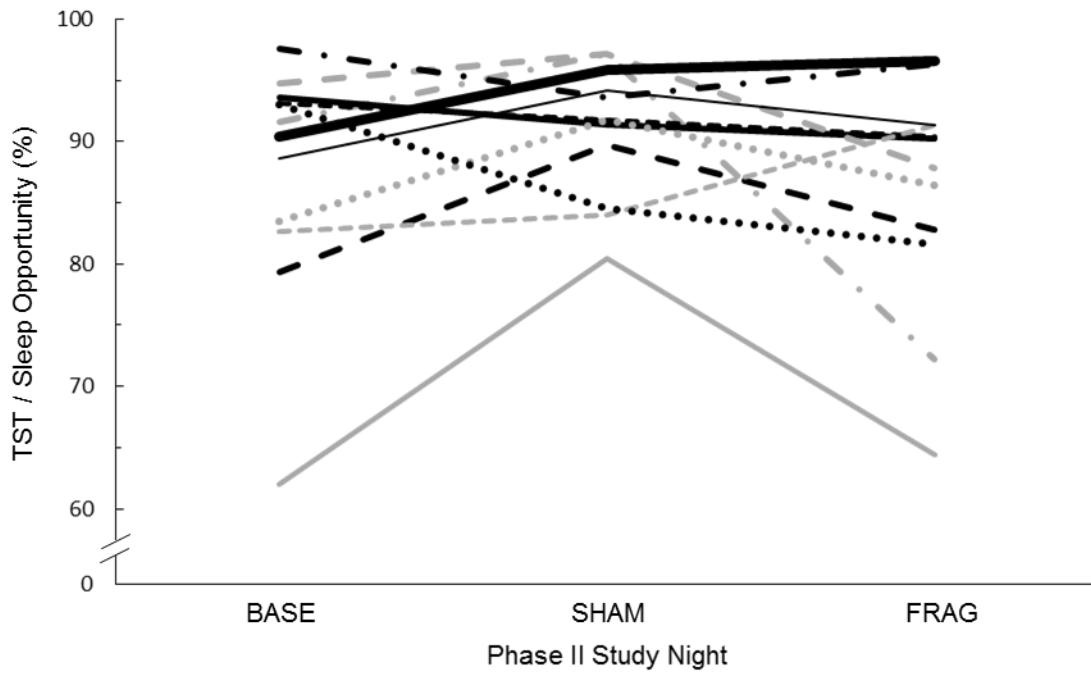


Suppl. Figure 1. Total sleep time (TST; min), using PSG. Each line represents one participant.

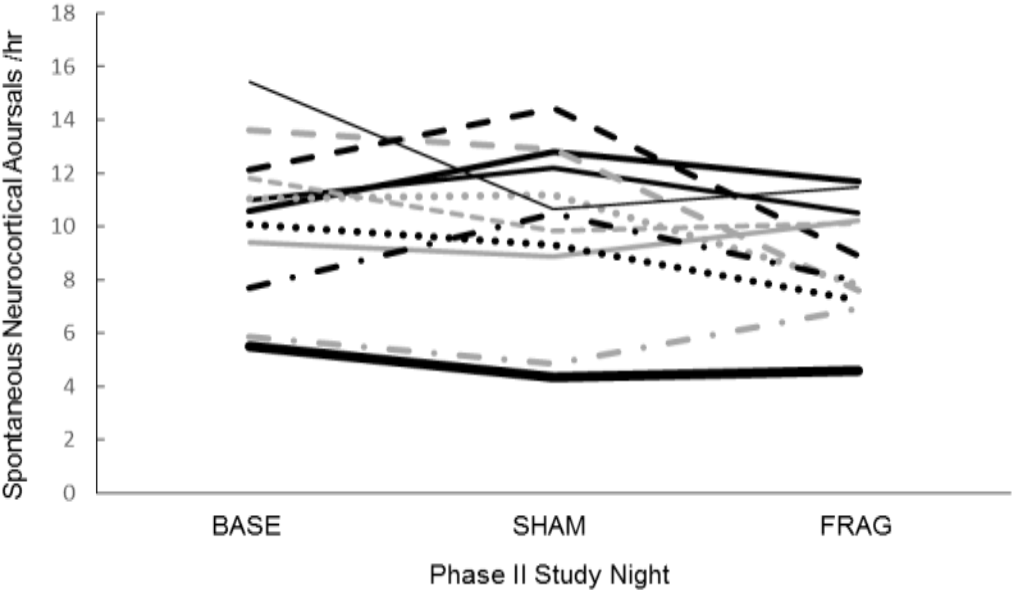
TST did not differ across Phase II nights.



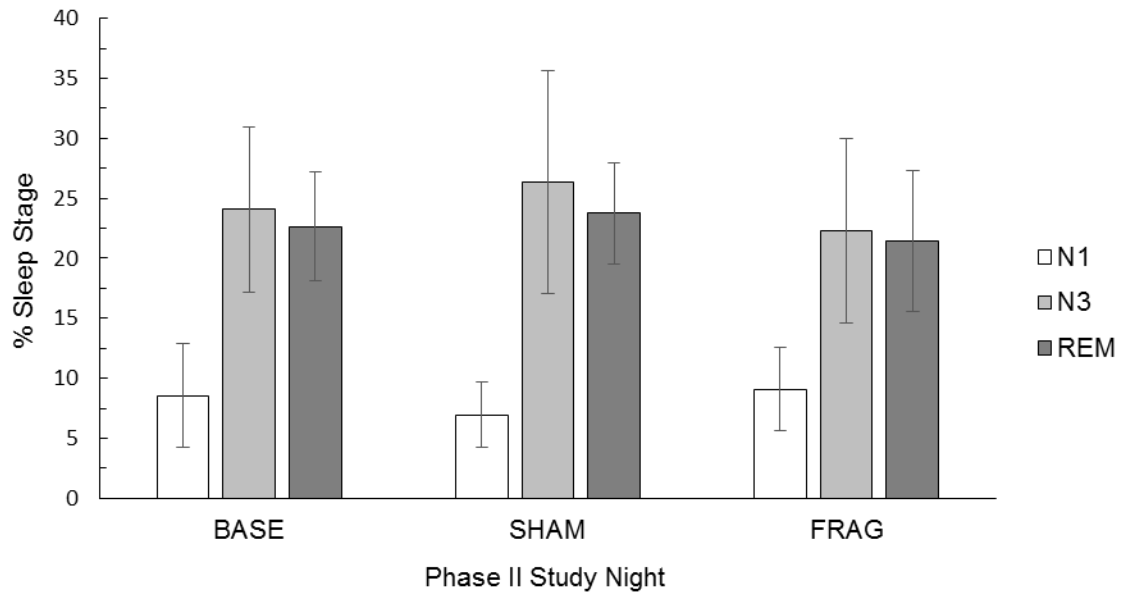
Suppl. Figure 2. Sleep opportunity (min), using PSG. Each line represents one participant. Sleep opportunity did not differ across Phase II nights.



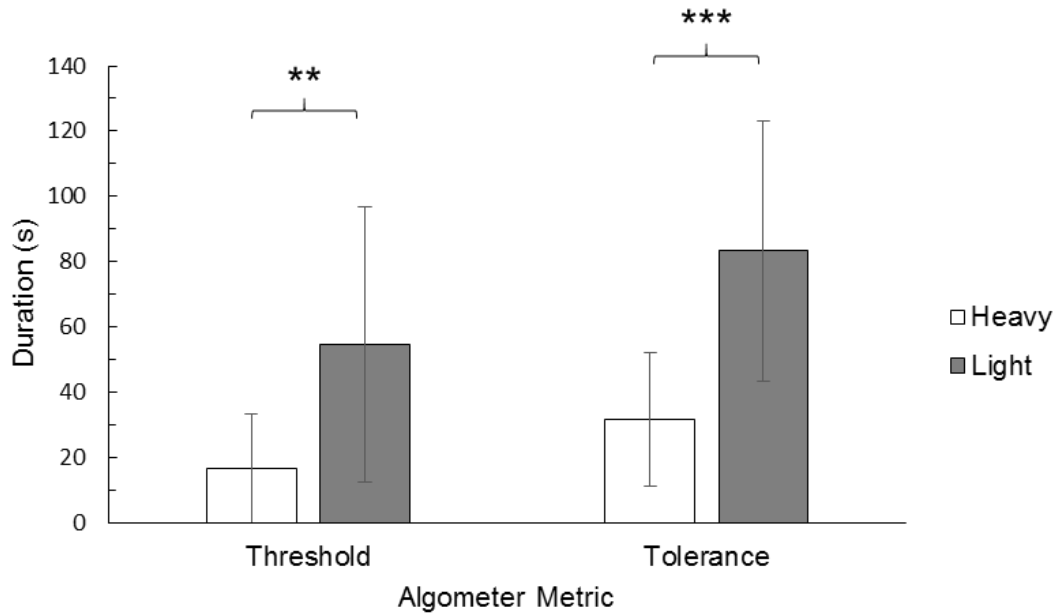
Suppl. Figure 3. Sleep efficiency (%), using PSG. Each line represents one participant. Sleep efficiency did not differ across Phase II nights.



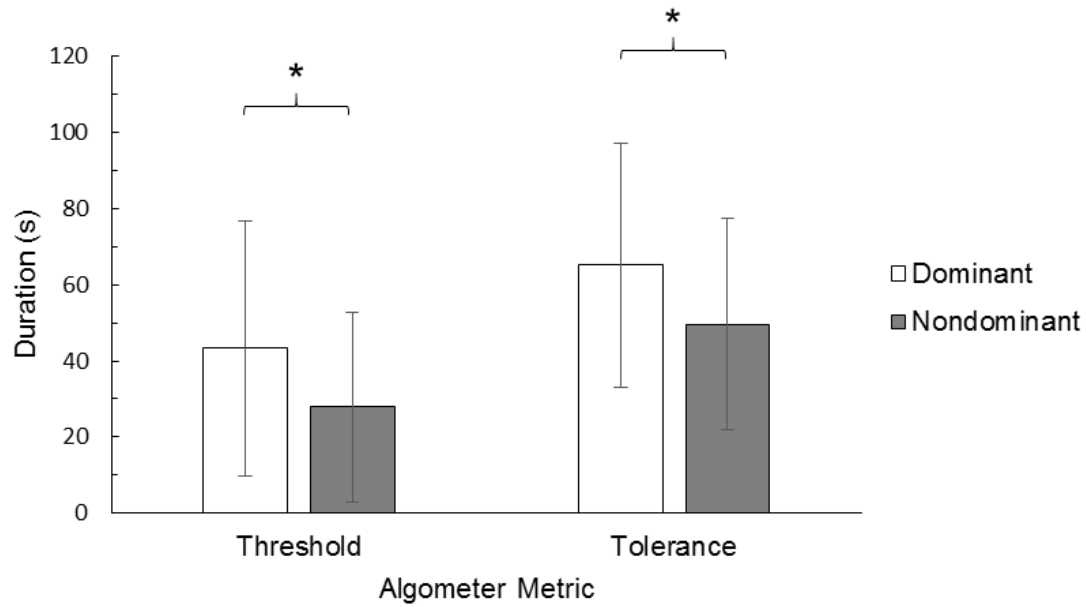
Suppl. Figure 4. Frequency of spontaneous arousals (indexed per hour), using PSG. Each line represents one participant. Spontaneous arousal frequency did not differ across Phase II nights.



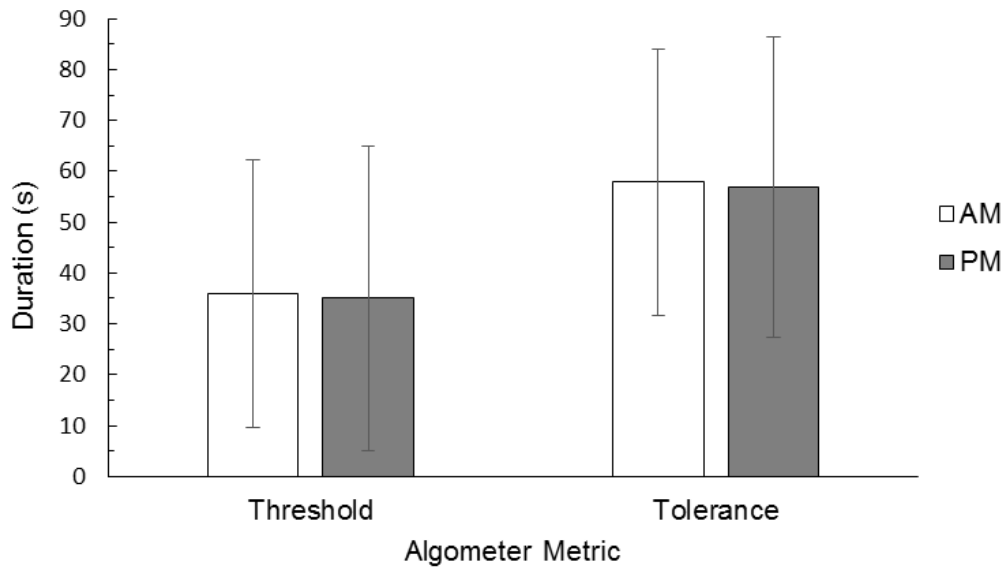
Suppl. Figure 5. Proportion of sleep stages. Stages NREM 1, NREM 3, and REM, represented here with different shaded bars, did not differ across Phase II nights. Error bars indicate standard deviation.



Suppl. Figure 6. Algometer threshold and tolerance latencies based on weight (finger). Each participant was represented by an average of 12 algometer latencies related to a particular weight (finger), regardless of Phase II experimental condition. Error bars are the standard deviation. Participants took longer to reach threshold during light weight trials and tolerated the lighter weight longer. $**p < .01$, $***p < .001$.



Suppl. Figure 7. Algometer threshold and tolerance latencies based on hand (dominant or non-dominant). Each participant was represented by an average of 12 algometer latencies related to a particular hand, regardless of Phase II experimental condition. Error bars are the standard deviation. Participants took longer to reach threshold and tolerated weight for longer in dominant-hand trials. $*p < .05$.



Suppl. Figure 8. Algometer threshold and tolerance latencies based on measurement time (morning or evening). Each participant was represented by an average of 12 algometer latencies related to a particular time, regardless of Phase II experimental condition. Error bars are the standard deviation. Neither threshold nor tolerance differed based on measurement time.

Appendix A

Screening Questions

Not used as data; to determine study eligibility only

- Age?
- Female gender?
- Read and understand English?
- Pregnant?
- Diagnosed or Treating: Hypertension? Sleep disorder? Congestive heart failure or chronic obstructive pulmonary disease? Pain-related disorder? Attentional deficit?
- Unmanageable visual or auditory impairment?
- Prescribed psychostimulant use?
- Pain killer use (e.g. prescribed opioids or over-the-counter non-steroidal anti-inflammatory drugs)?
- BMI \geq 35 (or height/weight to determine BMI)?
- Observed apneas during sleep?
- Regular snoring? (How badly?)
- Daytime drowsiness? Falling asleep unexpectedly or in atypical situations?
- Sleep-onset paralysis?
- Muscle weakness when laughing or surprised?
- Crawling sensation or discomfort in legs, nocturnal kicking, or need to regularly move legs for comfort?
- Regular difficulty falling asleep (>30min) or staying asleep? Worry about being able to fall asleep or racing thoughts at night?
- Travel > 2 time zones outside Eastern within the past month?
- Any sensory or motor deficits in hands?

Appendix B

Participant Compensation

Actigraphy Compensation \$10

Total compensation for study initiation but first week noncompliance (failure to wear the actigraph or consistently enter watch on/off or sleep/wake times in the PDA) ends here.

Baseline PSG Compensation \$20

Total compensation if a participant tests positive in-lab for a sleep disorder ends here.

Testing PSG Compensation \$40 /night

Total of \$80 for completion of two tests.

Algometer Compensation \$10 /test

Total of \$40 for completion of four tests.

Total participant compensation after study completion: \$150

Appendix C

Survey Administered Before Each Segment Spent in Lab

1. Did you take any pain medication between your last lab visit and now? _____

If yes, what type? _____

If yes, what quantity (e.g. milligrams)? _____

If yes, at what time did you take the medication? _____

2. Did you travel more than 2 time zones outside the Eastern zone between your last lab visit and now? _____

3. How many servings of alcohol did you consume in the past day? (If you did not consume alcohol, write "N/A")

4. At what time today did you last use nicotine? (If you do not use nicotine, write "N/A")

_____AM/PM

5. At what time today did you last consume caffeine? (If you did not consume caffeine, write "N/A")

_____AM/PM

At that time, how many servings did you consume? _____

What type of caffeinated food/beverage? _____

Appendix D

Algometer Briefing Script

“This is an algometer pressure device. It will be used to apply pressure to four of your fingers.”

Indicate the ring and middle finger of each hand. Then, demonstrate the following:

“You will place your hand on this platform. One finger will rest between these two bars. I will gently tighten the screws on either side of your finger to keep it aligned but you will still be able to remove your finger from the device if desired. Your other fingers will rest on top of the device.”

“This is the platform that will rest on top of your finger. Go ahead and feel this piece of plastic that will be touching the top of your finger, between your first and second knuckles.”

Allow the participant to touch the platform. Continue demonstrating the following:

“Then, I will gently place a weight on top of this platform. It will feel uncomfortable, and at some point the pressure will become painful. When it does become painful, touch this yellow sign. When the pressure becomes too painful and you want to stop the session for that finger, touch this red sign. Do not worry about trying to withstand any pain you may experience, just act naturally. Remember, touch the yellow sign first when the pressure begins to hurt, and then the red sign second when the pressure is too painful to continue.”

End the demonstration.

“What questions do you have about the algometer or the procedure just explained to you?”

Appendix E

Order of Weights in the Algometer Task

Testing					
Presentation Order:		Weights:		Fingers:	
1 B, D, M		B	1000g	R	Ring
2 S, C, R		S	500g	M	Middle
3 B, C, M					
4 S, D, R		Hand:			
		C	Corresponds to arm with cuff (Nondominant)		
		D	Corresponds to arm without cuff (Dominant)		

Beck's Candied Fruit Cake

1½ lb pitted dates

1 lb candied pineapple

1 lb whole candied cherries

2 c sifted flour

2 tsp double-acting baking powder

½ tsp salt

4 eggs

1 c granulated sugar

2 lb pecan halves

1. Grease and flour small foil loaf pans
2. Preheat oven to 275°F
3. Cut dates and pineapple into pieces and place into large bowl; add cherries
4. Sift flour, baking powder, and salt over fruit
5. Mix with hands until well coated
6. Beat eggs with mixer until frothy; gradually add sugar until well blended, then add to fruit mixture and mix well
7. Add pecans and mix with hands until well coated
8. Pack into pans, pressing and rearranging fruit to fill empty space
9. Bake 1¼ to 1½ hours at 275°F
10. Remove from oven and let stand on cake rack to cool for 5 min, then remove pans and allow additional cooling time before turning right-side up

Makes 7-8 small loaves

Wrap air-tight and store in a cool place (keeps for several weeks) or freeze