Searching for intelligence in the sleeping brain: The relationship between adolescent sleep spindles and cognitive performance

by

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Thesis Summary

Sleep spindles are short bursts of oscillatory brain activity during sleep, which have shown positive relationships with measures of intelligence and cognition in adults. Less is known about this phenomenon in adolescence, however. Adolescence is a period of change, with the onset of puberty, development of higher-order cognitive functions and brain maturation coinciding with the pressure to perform well in high school. Adolescents also experience changes to their sleeping patterns, which typically involves later bedtimes, difficulty waking in the morning for school, and an overall loss of sleep. This thesis aimed to add to the literature by investigating the relationship between adolescents' spindles and cognition, and to determine whether the state factor of sleep restriction and the trait factor of development influence this relationship.

Following an introduction to adolescents' sleep patterns and sleep spindles (Chapter 1), a systematic review and meta-analysis (Chapter 2) investigated the relationship between spindles and cognitive performance in adolescent samples. Adolescents' spindles showed moderate positive associations with fluid IQ (r = 0.44), working memory/executive function (r = 0.40) and speed/accuracy (r = 0.33), while full IQ/verbal IQ was not significantly associated (r = -0.05). These findings set up expectations for the following chapters.

A second major aim of the thesis was to determine what happens to adolescents' spindles and their relationship with cognition during sleep restriction, using a dose-response design (5hrs vs 7.5hrs vs 10hrs' time in bed) (n = 34). Firstly, the reliability of adolescent spindle characteristics was established in the control condition (10hrs), with slow spindle amplitude and fast spindle density, duration and amplitude showing good reliability with a single night of sleep recording, while 2 nights were required for slow spindle duration and 4 nights for slow spindle density (Chapter 3). Secondly, adolescents' spindles showed significant changes during severe sleep restriction (5hrs), where fast spindle amplitude

became lower and fast spindle duration became longer (Chapter 4). Lastly, spindles were not significantly associated with cognitive performance at baseline, nor was the change in spindle activity during sleep restriction related to changes in cognitive performance (Chapter 5). A potential protective function of spindles was revealed, however, where adolescents with longer spindles at baseline experienced less deficits to sustained attention during severe sleep restriction. Adolescents' spindles were therefore affected by sleep restriction, however the association between spindles and cognition was unclear.

The third major aim of the thesis was to investigate the developmental changes of spindles in a longitudinal study of early adolescents (n = 20). Fast spindle frequency showed a significant increase across an 18-month period, and slow frequency showed a similar pattern, confirming past findings, however changes to other spindle characteristics were not yet evident (Chapter 6). Again, spindles did not show significant associations with cognitive performance, and the changes in spindles over time were not meaningfully related to changes in cognition over time (Chapter 7). Spindles therefore showed some developmental changes in early adolescence, however were not related to cognition.

Overall, adolescent sleep spindles are impacted by both state (sleep restriction) and trait (development) factors, adding to the current literature and providing theoretical and clinical implications (Chapter 8). The associations between spindles and cognition seen in previous studies, however, were not replicated. Future investigations are encouraged to combine data sets to comprehensively investigate this phenomenon, and longitudinal studies with an extended time-base would add to the understanding of the development of spindles and cognition in adolescence.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Chelsea Reynolds (B. Psych. (Hons.)) 28th November 2018

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List of Publications in Thesis

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List of Refereed Conference Proceedings Resulting from Thesis

Reynolds, C.M., Gradisar, M. and Short, M.A. (2016, March). *Cognitive Performance and Sleep Spindles in Adolescents: A Summary and Preliminary Data from a Sleep Restriction Protocol.* Symposium presentation. The 4th International Pediatric Sleep Association Congress, Taipei, Taiwan.

Reynolds, C.M., Gradisar, M. and Short, M.A. (2016, May). *Cognitive Performance and Sleep Spindles in Adolescents: A Summary and Preliminary Data from a Sleep Restriction Protocol.* Poster presentation. International Conference on Sleep Spindling, Budapest, Hungary.

Reynolds, C.M., Gradisar, M., & Short, M. (2016, September). *Cognitive performance and sleep spindles in adolescents: Preliminary data from a sleep restriction protocol*. Poster presentation. 23rd Congress of the European Sleep Research Society, Bologna, Italy. *Abstract published in Journal of Sleep Research, 25, (Suppl. 1), 2016.*

Reynolds, C.M., Gradisar, M., & Short, M. (2016, October). *Cognitive performance and sleep spindles in adolescents: A review and data from a sleep restriction protocol.* Symposium presentation. 28th Annual Scientific Meeting of the Australasian Sleep Association, Adelaide, Australia.

Reynolds, C.M., Gradisar, M., Coussens, S. & Short, M. (2017, November). *The influence of experimental sleep restriction on cognitive performance and sleep spindles in adolescents.* Symposium presentation. Ninth Biennial Conference on Pediatric Sleep Medicine, Florida, USA.

Reynolds, C.M., Gradisar, M., & Short, M. A. (2018, May). *The relationship between sleep spindles and cognitive performance following sleep restriction and sleep extension in adolescents.* Poster presentation. 2nd International Conference on Sleep Spindling and Related Phenomena, Budapest, Hungary

Reynolds, C.M., Gradisar, M., Short, M. A. (2018, September). *The relationship between adolescents' sleep spindles and cognitive performance following experimental sleep restriction.* Oral presentation. 24th Congress of the European Sleep Research Society, Basel, Switzerland.

Chapter 1.

An introduction to adolescence, sleep and sleep spindles

The Adolescent Years

As children enter adolescence they experience considerable changes in their biology, psychology and in their social world. They firstly experience the onset of puberty, where they see dramatic changes in their physiology (Jensen & Nutt, 2015; Shaffer & Kip, 2013), and are affected by under-developed connections between emotional areas of the brain (the amygdala and limbic system) and the rational, decision-making areas of the brain (the frontal lobes) (Jensen & Nutt, 2015). This often leads to risky behaviour and the ups and downs of emotional reactivity (Jensen & Nutt, 2015). Adolescents also experience difficulty navigating their social world, attributed to self-consciousness over their changing bodies, the beginning of romantic feelings, and the start of learning who they are and their place in the world (Shaffer & Kip, 2013). At the same time, adolescents around the world are pressured to perform well in school (Klinger et al., 2015), with one study in India highlighting the trade-off between adolescents who spend their free time studying and experience lower emotional states, and adolescents who opt for more leisure time but experience higher academic stress as a result (Verma et al., 2002). In high school, adolescents face a particular pressure to plan what they will do in their adult years (Klinger et al., 2015), and in many countries, adolescents' performance in high school dictates their potential career paths. For example, 15-16-year-olds in England complete a General Certificate of Secondary Education that determines their ability to continue to tertiary education, with adolescents identifying this as the most stressful time so far in their lives (Denscombe, 2000), to the point where it can be detrimental to their performance and mental health (Putwain, 2009). In Korea, adolescents go to extreme efforts to do well in university entrance examinations by studying for long hours after school and attending private cramming schools on the weekends (Lee & Larson, 2000). In the current climate, adolescents feel the pressure to keep up with the 24/7 world of social media, often leading to poor sleep quality and low self-esteem (Woods & Scott, 2016).

Overall, there are many factors creating an unfamiliar, high-pressured environment for the average adolescent, and one crucial factor impacting adolescents' functioning and well-being is their sleep.

Adolescent Sleep

Adolescent sleep has been aptly coined the 'perfect storm' (Carskadon, 2011; Crowley et al., 2018), because of the biological and psychosocial factors that pull adolescent sleep patterns into disarray. Adolescents' bedtimes generally drift later, and school-night sleep durations become shorter, inadequately meeting their sleep need, and leading to the typical 'sleepy teen' (Moore & Meltzer, 2008). As children progress through adolescence, the timing of their body clocks, or circadian rhythms, starts to delay, so that their circadian 'night' is later than that of their childhood counterparts, with some experiencing a larger delay than others (see Figure 1.1; Carskadon, 2011; Crowley et al., 2018).

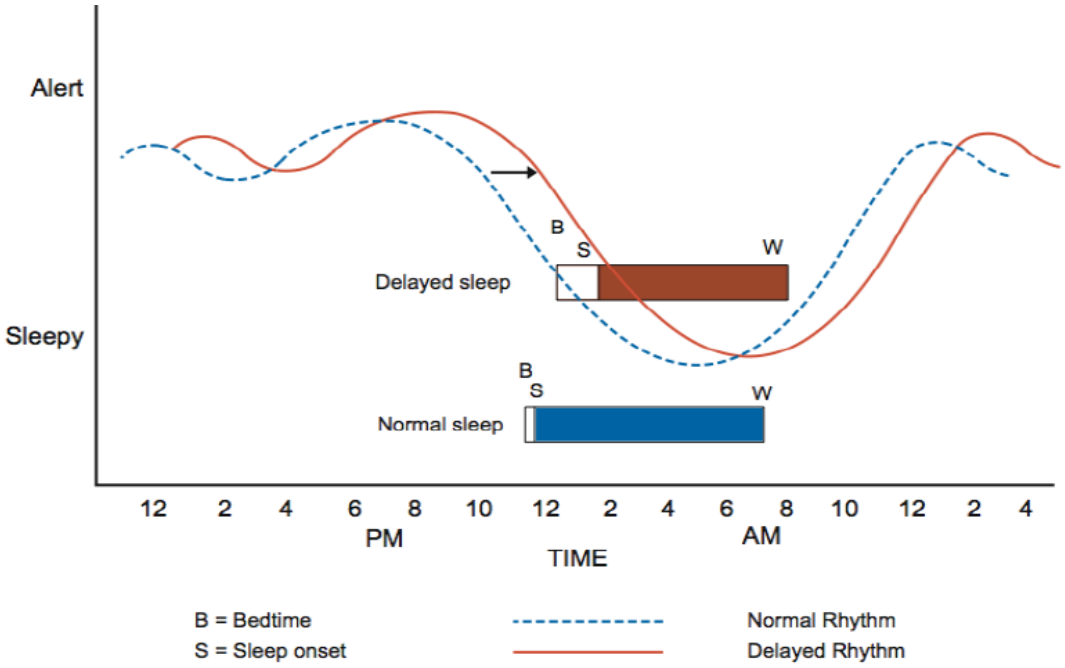

Figure 1.1 Circadian rhythms of normal (blue dotted line) and delayed (red solid line) sleepers. The structure of the alertness curve is identical between groups, however the delayed adolescent's curve is timed later. Horizontal bars indicate the bedtime period, with coloured sections indicating sleep, showing a delay in bedtime (B), sleep onset time (S) and wake time (W) for delayed adolescents. Figure developed by the author.

At the same time, adolescents find themselves less sleepy at their allotted bedtime, due to a slower build-up of sleepiness, or 'homeostatic sleep pressure' across the day (Carskadon, 2011; Taylor et al., 2005). These two processes comprise the classic 'two-process model' of sleep (Figure 1.2), where homeostatic sleep pressure is labelled Process S and the circadian rhythm is Process C (Achermann, 2004; Borbely, 1982; 2016). Both processes interact to prepare the body for sleep and explain how regularity can be achieved in sleep-wake patterns. Changes in both processes encourage adolescents to not only decide to stay up later due to increased alertness, but also to not be physiologically able to fall asleep until a later time than desired (Carskadon, 2011). Adolescents retain the social constraint of an early school start time, however, leading to an overall loss of sleep during the school week, which is often remedied by long sleep-ins on the weekend (Figure 1.1; Carskadon, 2011; Crowley et al., 2018).

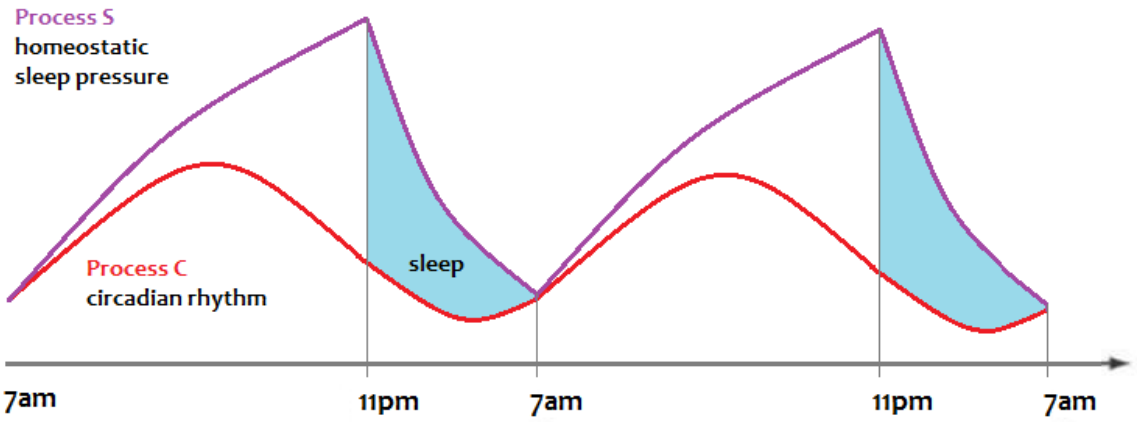

Figure 1.2. The two-process model of sleep (Borbely, 1982). Process S (homeostatic sleep pressure) increases across longer durations of wakefulness and decreases during sleep. Process C (circadian rhythm) drives arousal and oscillates in approximately 24-hour cycles. Sleep is most likely to occur when homeostatic sleep drive is high and when circadian arousal is low (shaded area). Figure developed by author, adapted from Borbely (1982; 2016).

Sleep loss has a myriad of consequences for adolescents' performance and wellbeing, including deficits to their emotional regulation (Chaput et al., 2016), psychosocial health (Owens, 2014; Shochat et al., 2014), learning and academic achievement (Chaput et al., 2016; Curcio et al., 2006; Shochat et al., 2014) and various aspects of cognitive performance, such as sustained attention (Lo et al., 2016; Short et al., 2018). In terms of emotional health, there is believed to be a cyclic relationship between sleep loss and worse mood in adolescents, with pre-sleep worry being a significant predictor of later bedtimes (Bartel et al., 2015), and increased worry and anxiety being a consequence of poor sleep (Shochat et al., 2014). Furthermore, some cognitive domains may be more susceptible to sleep loss than others, with adolescents experiencing clear deficits to sustained attention following experimental sleep restriction (Lo et al., 2016; Short et al., 2018) while at the same time higher-order cognitive areas show less impact from sleep loss (e.g., executive function and declarative memory, Anderson et al., 2009; Kopasz et al., 2010; Voderholzer et al., 2011). There may therefore be certain characteristics of sleep that are protective of the effects of sleep loss on some areas of cognition, which are discussed in **Chapter 5**.

Of note, adolescents maintain a similar sleep need to older children, however their late bedtime preference results in less sleep, rather than a reduction in sleep need as previously believed (Carskadon, 2011). Recent evidence suggests that adolescents require 9.35 hours of sleep for optimal attentional resources (Short et al., 2018), echoing past suggestions of a 9.2-hour sleep need in this age group (Carskadon et al., 1983) and recommendations of 8-10 hours' sleep opportunity for teens (Hirschkowitz et al., 2015). However, young people often achieve less than the required amount of sleep on school nights, with 69% of adolescents in grades 9-12 in the USA reporting less than 7 hours of sleep on school nights (Eaton et al., 2010). This pattern is consistent across the world, for example meta-analytic findings revealed Northern American and Asian adolescents achieve

7.5 hours of total sleep time on average, while European adolescents experience closer to 8.5 hours (Gradisar et al., 2011). This deficit increases with age, with another meta-analysis revealing a linear decline in total sleep time on school nights from 9-10 hours at age 9 to 6.5-8 hours at age 18 (Olds et al., 2010). Again, there was a geographic difference, with Asian and Northern American adolescents achieving less total sleep time in later adolescence (6.5-7.5 hours) than Australian and European adolescents (8.5 hours) (Olds et al., 2010). It is likely, then, that different 'doses' of sleep will have a differing impact on cognitive performance. Along with biological factors influencing later sleep times, adolescents are also influenced by social factors, with technology use a common culprit leading to slightly later bedtimes (specifically internet, phone, computer use and video gaming; Bartel et al., 2015). This 'self-imposed' sleep restriction across the school week in adolescents forms the methodological basis of the experimental sleep restriction study in **Chapters 4** and **5**.

Cognitive development during adolescence

Along with biological changes to sleep, adolescents also experience significant developmental changes in the reorganisation of brain networks, indicative of synaptic pruning and myelination of specific networks (Blakemore & Choudhury, 2006; Feinberg & Campbell, 2010; Steinberg, 2005). In the first few months of life, there is a rapid increase in the number of synapses in the brain, known as synaptogenesis (Blakemore & Choudhury, 2006). This is highly important for early cognitive development, so that infants can process a myriad of stimuli in the world they are getting to know. Synaptic pruning in late infancy and early childhood operates to fine-tune these synapses to focus only on those stimuli that are important in their culture (Blakemore & Choudhury, 2006). Synaptic pruning therefore occurs across childhood, however in adolescence, more dramatic synaptic changes occur in the prefrontal cortex. There is firstly a slight burst of synaptogenesis in the prefrontal cortex in early puberty, followed by a phase of synaptic pruning in late puberty, so that the overall

number of synapses at the end of puberty is lower than that in childhood (Blakemore & Choudhury, 2006; Giedd et al., 2008; Gogtay et al., 2004). The decrease in synaptic density can be seen in magnetic resonance imaging (MRI) through overall decreases in grey matter in adolescent brains (Casey et al., 2005). At the same time, white matter increases, reflecting higher levels of myelination, a process that insulates the axon component of a neuron to allow more efficient transmission of information between synapses (Barnea-Goraly et al., 2005; Casey et al., 2005; Paus, 2005). The *implication* of this complex reorganisation is that the adolescent brain becomes more efficient through the removal of unnecessary synapses, and experiences faster information processing from increased myelination (Blakemore & Choudhury, 2006; Steinberg, 2005). Concurrently, the relative changes in grey and white matter in adolescence are believed to be indexed by changes to adolescent sleep (Feinberg & Campbell, 2010).

This neural network reorganisation has long been theorised to enable adolescents to engage in complex higher-order thinking and prepare for adulthood, for example learning complex tasks such as driving, or improving maturity by engaging in less risk-taking (Blakemore & Choudhury, 2006; Steinberg, 2005). One cognitive ability that improves dramatically across adolescence is executive function, which heavily involves the frontal lobes (Casey et al., 2005; Luna et al., 2010). Executive functions include the capacity to plan and organise tasks and engage flexible thinking styles, as well as to control one's thoughts and behaviour, related to impulse control (Blakemore & Choudhury, 2006). Often adolescents experience a phase of impaired impulse control and sensation-seeking in the period between pubertal onset and full development of executive function skills (Luna et al., 2010; Steinberg, 2005), with risky decision-making being a contributing factor to later bedtimes in adolescence (O'Brien & Mindell, 2005; Reynolds et al., 2015). Some executive functions, such as strategic planning, are solidified in childhood, while other functions of

selective attention, problem solving and working memory show a continued linear improvement from 11 to 17 years of age (Anderson et al., 2001). Working memory involves the capacity to process and manipulate multiple pieces of information concurrently (Luna et al., 2010), and is associated with increased myelination in the frontal lobes between 8 to 18 years of age (Nagy et al., 2004). Working memory skills continually improve across childhood, but during adolescence these skills become more precise, for example the ability to separate salient cues from distractors and monitor one's own performance (Luna et al., 2010). Lastly, fluid intelligence, which includes abilities such as abstract reasoning and solving of novel problems, also improves during adolescence, reaching its peak shortly before the end of high school (Hartshorne & Germine, 2015). Fluid intelligence performance has also been linked with specific brain wave patterns occurring in the frontal lobes during sleep (Bodizs et al., 2014). Furthermore, fluid intelligence and working memory are believed to be intertwined in both their developmental trajectory through adolescence (Fry & Hale, 1996), and their neurological underpinnings in the prefrontal cortex (Kane & Engle, 2002). Considering these cognitive skills are continually evolving across adolescence, it is important to understand *how* adolescents' typical sleep loss might affect performance. Both working memory and fluid intelligence are two key cognitive performance domains investigated alongside sleep in **Chapters 5** and **7**.

Why is sleep important for the adolescent brain?

The developing adolescent brain is especially vulnerable to the effects of sleep loss (Telzer et al., 2013) due to the complex brain-reorganisation occurring across this period, predominantly in the pre-frontal cortex, which is particularly sensitive to sleep deprivation (Harrison & Horne, 2000). Furthermore, sleep deprivation has been implicated in lowering social inhibitions and increasing risky behaviour in adolescents (Beebe, 2011; Owens, 2014; Telzer et al., 2013), which occurs due to the influence of sleep deprivation on prefrontal

cortex functioning (Dahl, 1996; Horne, 1993), possibly due to less recruitment of the dorsolateral prefrontal cortex (Telzer et al., 2013). The alterations to neurological makeup across adolescence are also evidenced in changes to sleep architecture, for example by an overall decrease in slow wave sleep, or 'deep sleep', and an increase in rapid-eye movement (REM) sleep, commonly known as 'dreaming sleep' (Feinberg & Campbell, 2010). Furthermore, within the adolescent period there are considerable individual differences in the timing of changes to sleep, neurological makeup and puberty, meaning this period of vulnerability can extend from late childhood to late adolescence. For example, some subjects show signs of puberty as early as 8 years of age (Kaplowitz et al., 2001; Lee, 1980), which may be a marker for the *start* of adolescence. Some show significant changes to sleeping patterns (Roenneberg et al., 2004) and cortical development (Sisk & Foster, 2004) that cease in the early 20s, which may be a marker for the *end* of adolescence. It is essential, therefore, to investigate both sleep and cognitive ability from early, or even pre- adolescence, until early adulthood to more fully understand the impact of sleep on the developing brain. Considering the potential for sleep loss to negatively impact attention and academic achievement (Curcio et al., 2016; Shochat et al., 2014), it is highly important to understand how sleep impacts on performance in this age group, and what specific aspects of sleep may be involved. Through the detailed measurement of sleeping brain waves in adolescence, it is possible to identify specific brain wave patterns that might be related to cognition. One of these brain waves is of key interest in the present thesis.

How is sleep measured?

Depending what aspect of sleep is of interest and the resources available, there are several different measurements of sleep that can provide valuable information. Large-scale studies often use sleep diaries, on which adolescents record their bedtimes and wake times, sleep onset latency (SOL, the time taken to fall asleep), wake after sleep onset (WASO, the

total time spent awake during the night after sleep onset), total sleep time (TST) and total time in bed (TIB), as well as other relevant information, such as caffeine consumption and light exposure (Moore & Meltzer, 2008). Sleep diaries provide subjective accounts of sleep, accounting for the adolescent's impression of their own sleep, which can result in recall errors, and sleep diaries furthermore do not measure sleep architecture. One objective sleep monitoring method is the use of actigraphy (Acebo et al., 1999), which is often worn on the adolescent's wrist and monitors motor activity for a specified duration (e.g., one or two weeks). Various algorithms analyse movement data from actigraphy to infer sleep and wake (Acebo et al., 1999). A limitation of this device is the potential to over-estimate the time spent awake after falling sleep, for example if an adolescent moves a lot in their sleep (Acebo et al., 1999). A third measurement option is polysomnography (PSG), which involves attaching electrodes to the adolescent's scalp and monitoring sleeping brain waves (electroencephalography, EEG) as well as eye movements (electrooculography, EOG) and muscle tension (electromyography, EMG), which assist in determining what stage of sleep the adolescent is in (Rechtschaffen & Kales, 1968). An example of PSG setup is depicted in Figure 1.3. This method is the 'gold standard' for measuring sleep, as it provides objective, detailed data about a person's sleep (Kushida et al., 2005). In terms of limitations, this method is time-consuming and more financially onerous than sleep diaries and actigraphy, and therefore studies using PSG are often unable to achieve a large sample size. Nevertheless, it is the only method to measure specific aspects of sleep EEG. For research that aims to investigate links between specific characteristics of sleep and cognitive performance, such as the present thesis, PSG is essential.

Figure 1.3. An example of polysomnography (PSG) setup. Electrodes are placed on the scalp to measure brain waves (EEG), next to the eyes to measure eye movements (EOG) and under or on the chin to measure muscle tone (EMG). Image owned by Flinders University.

What does brain activity look like during sleep?

Most brain waves that occur during sleep are a direct result of electrical impulses from pyramidal neurons throughout the cerebral cortex (Bear et al., 2007). EEG brain waves, while specific to each individual, follow common patterns which can be categorised into different states of consciousness: wake, stage 1 (light sleep), stage 2 (intermediate sleep), stages 3 and 4 (deep sleep) and REM (rapid eye movement sleep, commonly involving dreams) (Rechtschaffen & Kales, 1968). Stages 1-4 are also known as NREM or 'non-rapid eye movement sleep'. Waking brain waves are seen on EEG as low amplitude, high frequency oscillations: both beta (~13-25Hz, active wakefulness) and alpha waves (~9-12Hz, relaxed wakefulness) (Landolt, 2011). The transition from wake to stage 1 sleep is evidenced by an increase of theta waves (5-9Hz) and slow rolling eye movements (Landolt, 2011). Stage 2 is indicated by sleep spindles in the sigma frequency range (~12-16Hz) and k-complexes, while stage 3 sees an increase in delta frequency waves (~0.5-2Hz), which make up almost the entirety of stage 4 (Landolt, 2011). Figure 1.4 illustrates typical adolescent PSG during different stages of sleep.

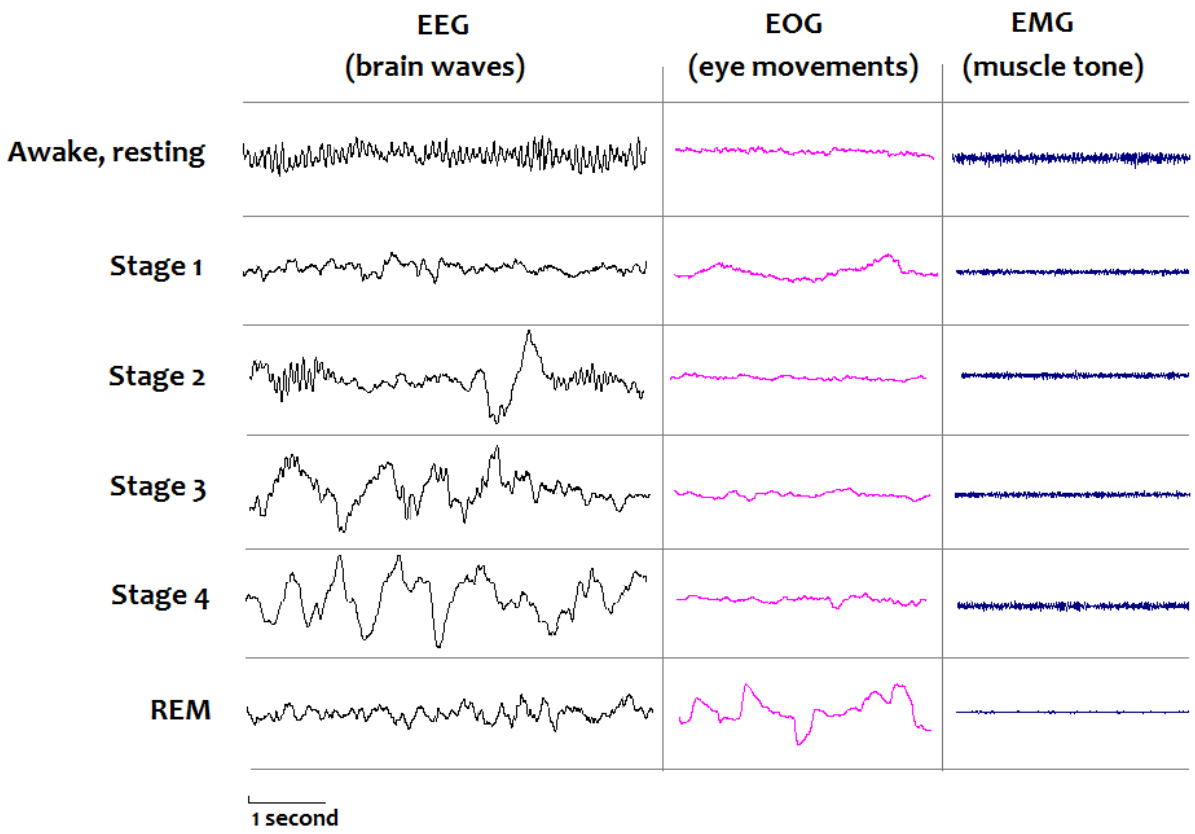

Figure 1.4. Typical adolescent polysomnography patterns from different sleep stages. Figure developed by author, adapted from Williams et al. (1974).

As discussed earlier, adolescents experience a decrease in slow wave sleep and an increase in REM sleep as they transition from childhood through adolescence and into adulthood (Feinberg & Campbell, 2010). An example of adolescent sleep is depicted in the hypnogram in Figure 1.5, which illustrates the amount of time spent in each stage of sleep from sleep onset to offset. In humans, sleep cycles (from light sleep to progressively deeper sleep and into REM sleep) last approximately 90 minutes (Carskadon & Dement, 2011).

Figure 1.5. An example of an adolescent hypnogram, showing the progression through different sleep stages across a single night. Stage 1 sleep is depicted in yellow, stage 2 in green, stage 3 in teal, stage 4 in dark blue and REM in red. Note that stage 4 sleep is included in the standard scoring method utilised in the present thesis (Rechtschaffen & Kales, 1968), however an alternative scoring method (American Academy of Sleep Medicine, Berry et al., 2012) combines stages 3 and 4. Figure developed by author.

What aspects of sleep are important for cognitive performance?

For many decades, researchers have shown that sleep is important for the consolidation of learned information (Diekelmann & Born, 2010; Fogel & Smith, 2011; Maquet, 2001). The strongest indicator of this is in studies that show improved memory performance when the period of time between knowledge acquisition and recall contains an amount of sleep, whether this is just a nap (Mednick et al., 2003) or a full night of sleep (Maquet, 2001). Aspects of REM sleep have been established as important for overnight learning (Stickgold & Walker, 2007), while non-REM sleep has gained traction as a contributing element (Maquet, 2001; Stickgold & Walker, 2007), particularly in the deeper sleep stages, where memory improvement has been linked to hippocampal activity during slow wave sleep (Peigneux et al., 2004). Further from this, researchers have investigated different non-REM EEG characteristics to improve understanding on exactly why sleep might be important for cognitive performance. One contender is the sleep spindle, a hallmark of stage 2 sleep which has become increasingly popular in investigations of sleep-dependent memory consolidation and general cognition (Fogel & Smith, 2011). The present thesis will

focus on the sleep spindle as a potential index for cognitive performance and intelligence in adolescents.

What is a sleep spindle?

Sleep spindles are bursts of oscillatory waveforms during stage 2 sleep, however they are also present in slow wave sleep and sometimes REM sleep. A typical sleep spindle follows a waxing and waning profile and is 10-16Hz in adults (De Gennaro & Ferrara, 2003; Luthi, 2014), however may be slower in early and mid-adolescents (10-14Hz; Gruber et al., 2013; Hoedlmoser et al., 2014; Tarokh & Carskadon, 2010), which is discussed in more detail in **Chapter 6**. Sleep spindles arise from interactions between the thalamic reticular nucleus and thalamocortical neurons, which together make up the 'thalamocortical loop' (Luthi, 2014). The production of sleep spindles begins with interactions between thalamic reticular and thalamocortical neurons, which create excitatory post synaptic potentials on pyramidal neurons, seen on the EEG as spindles (Luthi, 2014). Spindle activity is afterward maintained by signalling from the brainstem, hypothalamus and basal forebrain through the thalamocortical pathways to the cortex (Gruber et al., 2013). An example of adolescent sleep spindles is presented in Figure 1.6.

Sleep spindles can be described in terms of their:

- density (a ratio of the number of spindles per time frame, e.g., spindles per minute),
- duration (length in seconds),
- amplitude (power of the waveform in μV) and,
- frequency (the number of oscillations per second in Hz) (De Gennaro & Ferrara, 2003).

Spindles are often divided into two frequency types: slow (e.g., 10-13Hz) and fast (e.g., 13-16Hz) (De Gennaro & Ferrara, 2003), which show topographical and often

functional differences. For example, slow spindles are seen over the frontal areas of the brain (De Gennaro & Ferrara, 2003) and relate more often to overnight learning (Astill et al., 2014), while fast spindles are seen over central-parietal areas (De Gennaro & Ferrara, 2003) and relate to more complex functions such as fluid intelligence (Bodizs et al., 2014). Sigma power (11-16Hz), measured through spectral power analysis (an analysis of all EEG frequencies), is also used as a proxy for sleep spindle activity (De Gennaro & Ferrara, 2003), however it has several limitations. Firstly, sleep spindles are brief events which encompass many different frequencies, while sigma power estimates only a portion of frequency in the spindle waveform (Tarokh & Carskadon, 2010). Secondly, spectral analysis is unable to distinguish between specific spindle waveforms and background EEG activity (De Gennaro & Farrara, 2003). For these reasons, the experimental chapters in the present thesis (**Chapters 3, 4, 5, 6** and **7**) used specific spindle characteristics of spindle density, duration, amplitude and frequency, and sigma power was not investigated.

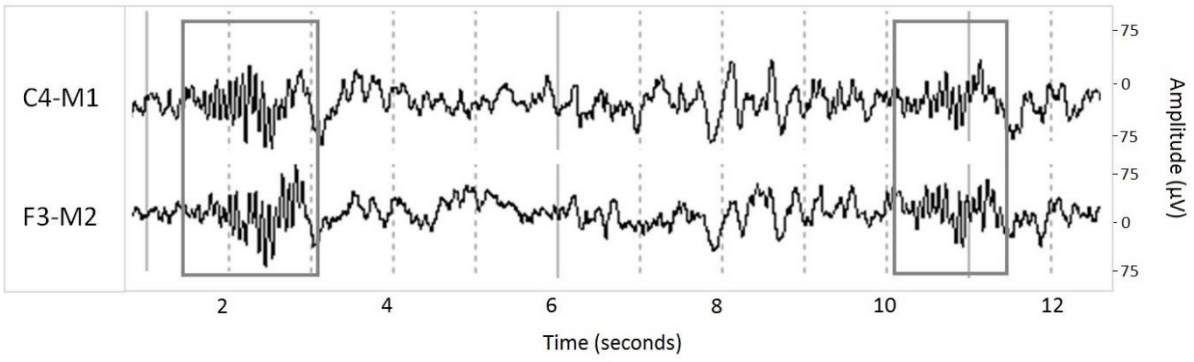

Figure 1.6. Adolescent sleep spindles on EEG derivations C4-M1 (electrode placed in a central position on the skull) and F3-M2 (in a frontal position on the skull). Figure developed by author (Reynolds, Short & Gradisar, 2018 (**Chapter 2**)).

How are sleep spindles measured?

There are several methods of measuring specific spindle characteristics, which are often debated. Many studies have focused on the best method to analyse spindles (Causa et

al., 2010; Huupponen et al., 2007; Warby et al., 2014), with much contention over using automatic or algorithmic analysis compared to manual analysis. Manual analysis of spindles usually involves detailed visual consideration of EEG and firm criteria for spindle detection (e.g. frequency between 11.0-15.9Hz, duration at least 0.5 seconds, sinusoidal envelope; Chatburn et al., 2013), to decipher a true spindle waveform from other EEG activity or artefact (distorted EEG activity, for example from participant movement) (Causa et al., 2010). However, this method requires a technician to manually detect each spindle, which can be time-consuming, leading to fatigue and potential decreased accuracy, and furthermore relies on subjectivity, with inter-rater agreement varying between 80-90% (Nonclercq et al., 2013) and Cohen's kappa of 0.68 (Warby et al., 2014). The use of automatic spindle scoring methods saves considerable time and allows for standardisation of spindle measurement to reduce subjectivity. However, the algorithms used in automatic methods use set criteria that may not apply equally to all individuals (Nonclercq et al., 2013), as spindles have been shown to be markedly consistent within individuals, however vary between individuals (Bodizs et al., 2009; De Gennaro et al., 2005; Werth et al., 1997). One solution to this is a spindle detection method called the Individual Adjustment Method (Bodizs et al., 2009) that considers an individual's spectral profile and uses the individual's frequencies to base detection of specific spindle characteristics. This allows a compromise between the time-consuming nature of manual scoring and the inability of other automated methods to account for individual differences and is therefore the preferred method for spindle detection in the present thesis. The Individual Adjustment Method is discussed in more detail in each experimental chapter.

How do sleep spindles develop?

Along with changes to sleep stages, sleep spindles also change from childhood through adolescence and into adulthood. The first sign of sleep spindles in the human EEG is

typically seen at 2 months of age (De Gennaro & Ferrara, 2003). From 3 months to 5 years, spindles change dramatically and are believed to be an index of neural maturation (De Gennaro & Ferrara, 2003), with one study showing increases in spindle duration, amplitude and sigma power, a decrease in frequency, and no change in spindle density from ages 2 to 5 years (McClain et al., 2016). The emergence of the two types of spindles (slow and fast) is believed to occur in early adolescence (De Gennaro & Ferrara, 2003). In the transition from childhood to adolescence, cross-sectional research indicates spindle frequency typically *increases*, while spindle duration, amplitude and sigma power *decrease* (Jenni & Carskadon, 2004; Kurth et al., 2010; Nader & Smith, 2015; Shinomiya et al., 1999). Furthermore, in studies of spindle generation across the lifespan, spindle duration and amplitude often decrease further, while frequency continues to increase (Martin et al., 2013; Nicolas et al., 2001). Due to the continual changes to sleep spindles across the lifespan, and to the complex brain reorganisation that is indexed in adolescents' sleep EEG (Feinberg & Campbell, 2010), it is difficult to apply findings from adult studies to adolescents. It is therefore prudent to investigate specific spindle makeup in the adolescent period. The development of specific sleep spindle characteristics is the focus of the longitudinal investigation in **Chapter 6**.

Sleep spindles and cognition

Sleep spindles have been associated with a myriad of cognitive functions in many adult studies, including general cognitive ability (Fogel & Smith, 2011; Ujma, 2018), learning (Fogel et al., 2007), memory consolidation (Clemens et al., 2005; Gais et al., 2002; Schabus et al., 2008) and intelligence (Bodizs et al., 2005). Researchers have speculated that the shared brain networks between cognitive processing and spindle generation, particularly the thalamocortical loop, explain the mechanism behind these associations (Fogel & Smith, 2011). These associations are not easily attributed to adolescents, however, as spindle characteristics of adults look different to those in children and adolescents. The associations

between sleep spindles and cognition during adolescence is therefore the topic of key interest in the present thesis.

Thesis Aims

While the relationship between sleep spindles and cognitive performance has been of increasing interest in adult studies, the nature of this relationship is less understood in adolescents. This relationship was therefore examined in the present thesis in a meta-analytic review in **Chapter 2**, with an inclusion of many spindle and cognitive variables, and a wide age range (8-22 years) to allow for a broad overview across the potential start of puberty and the end of changes to adolescent sleeping patterns.

This review supported the presence of an association between spindles and cognition in adolescence, however highlighted several gaps in the research. Firstly, no studies had been conducted to evaluate night-to-night stability of spindle measurements in adolescents and, furthermore, most studies had measured only a single night of EEG – thus, the reliability of their spindle measures was unknown. To address this gap, **Chapter 3** investigated the reliability of spindle measurements over 8 nights from the control condition of an experimental sleep restriction protocol, as explained below.

Secondly, although sleep spindles have been studied in cross-sectional studies during adolescence, there are specific aspects of the adolescent period that have not been considered. As discussed, adolescents experience significant changes to sleeping patterns, often with sleep loss occurring during the school week, potentially impacting their cognitive performance. Past studies have focused on associations between sleep spindles and cognition using an optimal sleep opportunity, often with only one or two nights of sleep monitoring. To address this gap, **Chapters 4** and **5** investigated sleep spindles in a 9-night-long experimental sleep-restriction paradigm, mirroring a typical adolescent school week with optimal sleep at the 'weekends' (2 nights at the start and 2 nights at the end of the study), and restricted sleep

during the ‘school week’, with different doses of time in bed (TIB) given for 5 nights: severe sleep restriction (5 hrs), moderate sleep restriction (7.5 hrs) or a control condition of optimal sleep (10 hrs). **Chapter 4** aimed to investigate changes to sleep spindles alone during sleep restriction, while **Chapter 5** investigated the relationship between sleep spindles and cognitive performance during sleep restriction.

The third gap addressed by the meta-analytic review was the lack of studies addressing the development of sleep spindles, and their relationship to cognition, in a longitudinal fashion. Due to the complex brain reorganisation occurring during this period, it was hypothesised that sleep spindles would show developmental changes during the transition from late childhood to early adolescence. **Chapters 6** and **7** therefore aimed to address this gap by using a longitudinal study of early adolescents, with **Chapter 6** focusing on the development of spindles alone, and **Chapter 7** investigating the relationship between spindles and cognition over time.

Overall, the present thesis aimed to investigate both the *state* relationship between spindles and cognition (during sleep restriction, **Chapter 5**) and the *trait* relationship (developmental changes, **Chapter 7**), as well as establishing what typical spindles look like in early- (**Chapter 6**) and mid-adolescence (**Chapter 4**). **Chapter 8** presented a summary and discussion of the thesis findings, implications for what these findings mean for the literature on sleep spindles and cognition, and suggestions for future research.

Chapter 2.

Sleep spindles and cognitive performance across adolescence:

A meta-analytic review

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Author Contributions

CR was the primary author of the manuscript, conducted the literature search and review process and conducted all statistical analyses. MS and MG Short, M.A. contributed to revising of the manuscript, confirming of suitable literature and statistical assistance.

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Abstract

Higher sleep spindle activity generally relates to better cognitive performance in adults, while studies in children often show the opposite. As children become young adults, there is rapid brain maturation and development of higher-order cognitive functions, and therefore investigations within this age group may elucidate the relationship between spindles and cognition in this developmental period. Twelve studies published between 2009 and 2016 were identified. Meta-analyses revealed a positive relationship between spindles and cognition overall (r = 0.27), however effects varied depending on cognitive domain. Moderate positive relationships were seen for fluid IQ (r = 0.44), working memory/executive function (r = 0.40) and speed/accuracy (r = 0.33), while full IQ/verbal IQ was not significantly associated (r = -0.05). Meta-regressions indicated cognitive domain and spindle characteristic had a small influence over effect sizes, while age and gender did not have a significant influence. The relationship between spindles and cognition in adolescents is likely influenced by individual neural makeup and brain maturation.

Keywords: sleep spindles; cognitive performance; IQ; children; adolescents; EEG

Sleep spindles are bursts of synchronised oscillatory neural activity seen via electroencephalography (EEG) that occur throughout various stages of sleep and are believed to be indicators of mental efficiency (Fogel & Smith, 2011). Spindles are associated with diverse cognitive functions, including learning and memory (Fogel et al., 2007), intelligence (Fogel & Smith, 2011; Geiger et al., 2011), synaptic plasticity (Urakami et al., 2012) and sleep-dependent memory consolidation (Clemens et al., 2005, Gais et al., 2002; Schabus et al., 2008). Accordingly, the study of spindles and its implications for cognition and intelligence is the focus of increasing research attention. However, the relationship between spindles and cognition in older children and adolescents is less clear, with some studies reporting findings consistent with the adult literature (Bodizs et al., 2014; Geiger et al., 2011), and others finding the opposite relationship (Chatburn et al., 2013; Tessier et al., 2015). The present meta-analytic review will summarise the current empirical literature regarding the relationship between sleep spindles and cognition in adolescents in order to address the gap in the literature regarding this discrete developmental period. Meta-analyses will examine how this relationship might vary according to the cognitive domain measured, how sleep spindles are operationalised, and participant characteristics.

What is the current evidence for the relationship between spindles and cognition?

Sleep spindles are characterised by synchronised bursts of typically 10-16Hz activity (Figure 2.1) that arise from interactions between the thalamic reticular nucleus and thalamocortical neurons, which together make up the 'thalamocortical loop' (Steriade, 2006). Given the role of the thalamocortical network in information processing and encoding during wakefulness (Bear et al., 2007), it is believed that spindle-related activity of the thalamocortical network during sleep is related to cognitive processes such as memory integration, information processing and intelligence (Fogel & Smith, 2011).

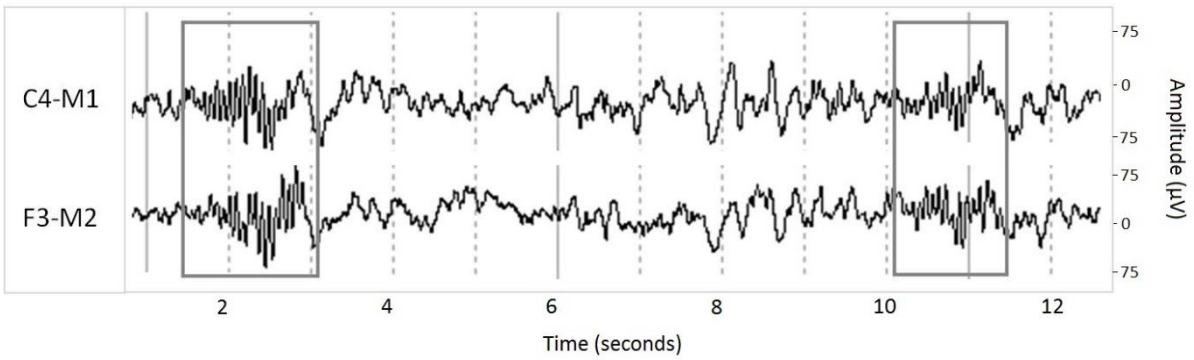

Figure 2.1. Sleep spindles in stage 2 seen over both central (C4) and frontal (F3) EEG derivations. Sleep spindle parameters include total number of spindles per sleep episode, average spindle frequency (Hz), average spindle amplitude, average duration (e.g. seconds), density of sleep spindles (number per time period, e.g. per minute), spectral power in the sigma frequency band (11-16Hz) and an estimated calculation of 'spindle activity' (e.g., mean spindle duration*mean spindle amplitude; Schabus et al., 2004).

In adults, sleep spindle characteristics are positively correlated with cognition and intelligence measures (Bodizs et al., 2005; Fogel et al., 2007; Fogel & Smith, 2011; Schabus et al., 2006; Urakami et al., 2012), providing support for the hypothesis that spindles are related to general mental ability (Anderson, 2005; Luthi, 2014). The associations between spindle activity and cognition, along with their shared brain networks, are consistent with the notion that spindles reflect a process by which memory and learning are consolidated during sleep (Fogel & Smith, 2011). For example, spindles have been implicated in both declarative memory consolidation (i.e., memories of facts) and procedural memory consolidation (i.e., memories of how to do things) through investigations of the hippocampus and thalamocortical oscillations (Fogel & Smith, 2011). Furthermore, the common thalamocortical networks employed in both spindle production and cognition allow speculation of a causal relationship, where normal intellectual development may depend on normal spindle production (Fogel & Smith, 2011). An important question remains, however:

How do spindles and cognition relate during the discrete developmental period of adolescence?

Sleep spindles and cognitive performance across adolescence

While existing research supports a relationship between sleep spindles and cognition in adults, there are several salient reasons why these findings cannot be generalised to youth. Firstly, spindle characteristics change across development from childhood to older adolescence. Spindle duration, density (spindles per time unit) and total number of spindles per sleep period decrease with age, while spindle oscillatory frequency increases over time (Nicolas et al., 2001; Shinomiya et al., 1999). Second, the nature of the relationship between spindles and cognition is not uniform among samples of different ages. For example, in a sample of 3- to 5-year-old children, Kurdziel and colleagues (2013) found memory performance negatively correlated with spindle density, which is opposite to the effect witnessed in adults (Cox et al., 2014; Lafortune et al., 2014). At preschool ages, lower spindle density may be linked to more mature, rather than less mature, brain development (Tarokh et al., 2014), indicating a change in the direction of effect from childhood to adulthood. Given the lack of consensus regarding the relationship between spindles and cognition in young people, it is beneficial for the field to gain a picture of the relationship that might be occurring. Furthermore, an examination of adolescent samples might add to the understanding of this phenomenon across age groups.

Are there factors that moderate the relationship between sleep spindles and cognition?

There are several factors that may influence the relationship between sleep spindles and cognitive performance. One of these is the way spindles are operationalised. For example, previous studies have compared cognitive measures with total spindle number, frequency, amplitude, duration, density, sigma power and ‘spindle activity’ (see Figure 2.1. for more detail). Some have further divided spindles into ‘fast’ and ‘slow’ depending on

relative frequencies (e.g. slow spindles <13Hz, fast spindles >13Hz). This distinction illustrates differences within the spindle-cognition relationship, where slow spindles relate more strongly to procedural performance (Astill et al., 2014) while fast spindles relate to more complex skills such as fluid intelligence (Bodizs et al., 2014).

The relationship between spindles and cognition may also vary according to cognitive measure. For example, some studies have used singular cognitive tests, while others use a cognitive battery such as an IQ test to provide multiple cognitive outcome measures. Original IQ tests grouped cognition under umbrella terms of 'performance intelligence (IQ)' and 'verbal intelligence (IQ)' (Wechsler, 1958). Performance IQ includes cognitive areas of fluid reasoning, spatial processing and visual-motor integration, while verbal IQ includes areas such as verbal comprehension, general knowledge, arithmetic and vocabulary. In adults, total number of spindles and spindle density have shown the strongest relationships with performance IQ tests, while the weaker or non-significant relationships are seen for verbal IQ (Fogel et al., 2007; Nader & Smith, 2003). Lastly, it is possible that age may affect the relationship, with changes across childhood to spindle duration, density and frequency, as discussed, but there may also be gender influences, with effects seen more often in females than males (Ujma et al., 2014). To address this, mean age, age range and gender will be included as moderator variables in the present meta-analysis.

It should be noted that meta-analyses of variables that include four or more studies provide a reliable estimate of a pooled effect, while analyses with less than four studies provide a limited picture. With this in mind, as well as the anticipated heterogeneity in measures, the current meta-analytic review will not be a summation of all presented research, but instead aims to paint a picture of the relationship between spindles and cognition in a field that has yet to reach a consensus. Borenstein and colleagues (2009) suggest that heterogeneity within meta-analysis allows researchers to broaden understanding of patterns of

effects, and this is generally the goal of meta-analysis, rather than synthesising identical studies. Furthermore, the quantitative synthesis of effects, regardless of the number and heterogeneity of studies available, allows us to provide a more accurate and informative picture and thereby reduce the likelihood of researchers applying their own idiosyncratic conclusions (Borenstein et al., 2009; Ioannidis et al., 2008). Nevertheless, there are methods within meta-analysis to address heterogeneity in studies, namely using a random effects model and examining covariates within meta-regression (Ioannidis et al., 2008), which will both be utilised in the present review.

Method

This meta-analytic review was conducted in accordance with the guidelines set out in the preferred reporting items for systematic reviews and meta-analyses statement (PRISMA; Moher et al., 2009).

Literature Search

In December 2016, electronic databases (PsycInfo, PubMed, Web of science) were searched for empirical articles that investigated the relationship between sleep spindles and cognitive performance in young people using a combination of keywords (*sleep spindles, cognit*, adolescen*, children, learning, neurobehav*, performance, intelligence*). Abstracts were examined to gauge key variables and participant age. Where one or both were not expressed, a full-text assessment was performed. Once all keywords and databases were exhausted, additional articles were manually identified in the included studies' reference lists and relevant review articles.

Inclusion Criteria

Included studies examined the relationship between sleep spindles and any cognitive performance measure in healthy young people. Participant age range was eight to 22yrs. The minimum of eight years was decided based on biological research on the minimum pubertal

onset (Kaplowitz et al., 2001; Lee, 1980), while the maximum was based on bio-regulatory evidence which suggests the end of adolescence can reach 22 years of age (Roenneberg et al., 2004), while cortical development can extend into the early twenties (Sisk & Foster, 2004). Furthermore, the World Health Organisation (2011) defines 'young people' as those aged 10-24. A large age range allows for examination across the initial transition from childhood to early adolescence and from late adolescence to early adulthood. Additionally, articles needed to be peer-reviewed, empirical and available in English.

Exclusion Criteria

Articles were excluded if they investigated cognitive performance indirectly (e.g., brain imaging rather than direct cognitive testing). Studies that included participants with psychological or medical disorders were excluded given the disorders' potential confounding impact on sleep and/or cognition (Del Felice et al., 2013; Goder et al., 2008; Spoormaker & van den Bout, 2005). Twelve studies were included (Figure 2.2).

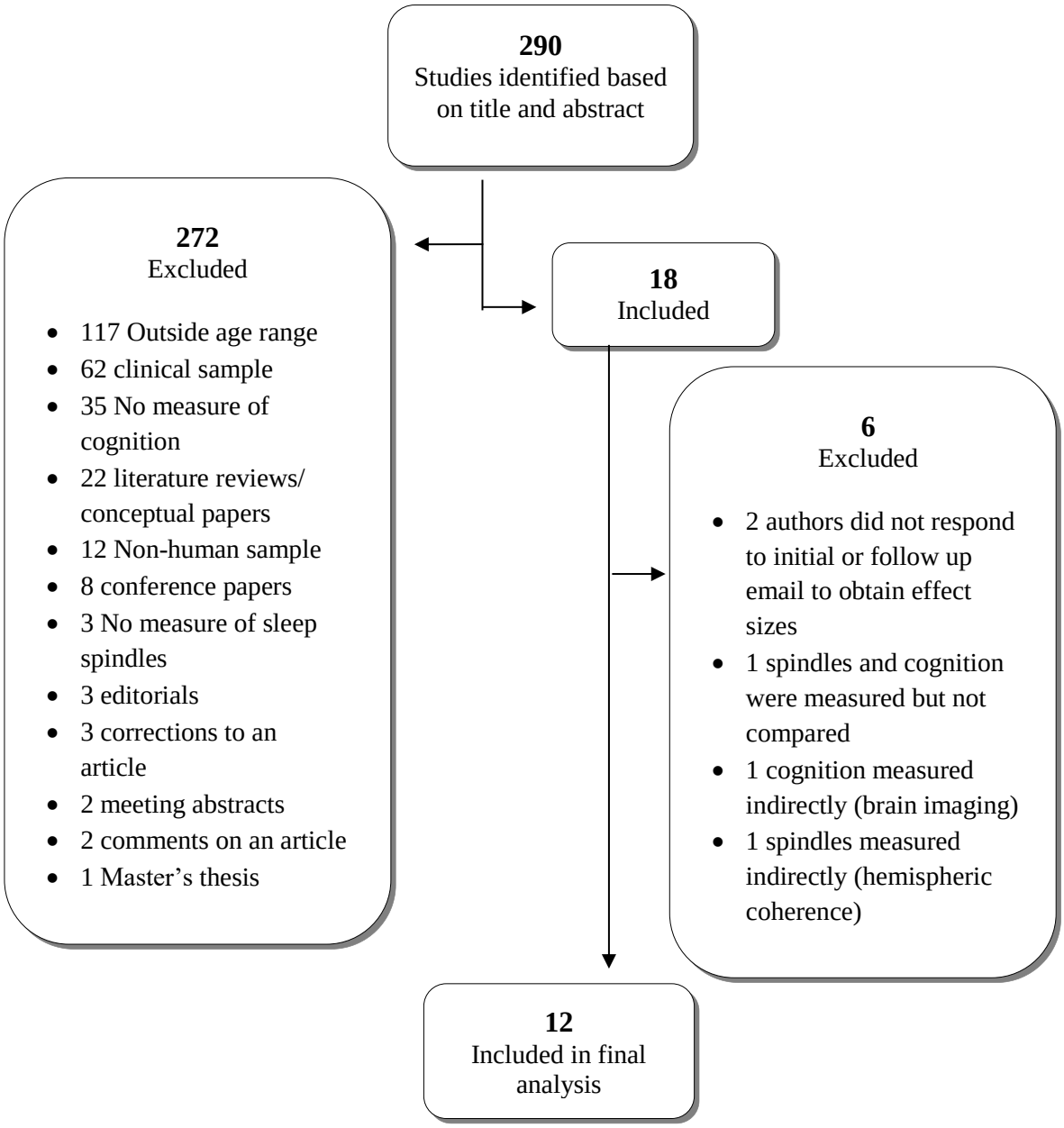

Figure 2.2. Flowchart of studies included and excluded in the literature search

Meta-Analyses

Meta-analyses were conducted using comprehensive meta-analysis (Biostat, Inc., USA). Analyses were performed in accordance with meta-analysis reporting standards (MARS; APA, 2008). Effect sizes from the identified studies were converted to Pearson's *r* correlations and transformed to Fisher's *z*. Where non-significant findings were available in the paper or obtained by contacting the authors, these were incorporated into the meta-

analyses to allow a comprehensive representation of the relationship. Furthermore, for studies which included a sample outside the specified age range, authors were contacted to obtain effect sizes for those participants within the age range, and when successful, these were incorporated. For these reasons, many non-published statistics were included in meta-analyses, and therefore an analysis of publication bias was not performed. Moderator variables including cognitive domain, spindle characteristic, mean age, age range and gender composition (%male) were included in the overall meta-analysis to address heterogeneity between samples and to determine whether the relationship between spindles and cognition varied according to their influence. Random effects were used for the overall effect as this accounts for variation between studies, and applies equal weight to each study regardless of how many effects they contributed to the analysis (Borenstein et al., 2009).

Heterogeneity in spindle characteristics and cognitive variables was anticipated, and therefore subgroup analyses were performed. To further investigate the association between spindles and cognition, a second meta-analysis was conducted to compare effect sizes between cognitive domains. Spindle characteristics were collapsed to allow a focus on cognition. Cognitive measures were grouped into 5 domains: full IQ/verbal IQ, fluid IQ/performance IQ, working memory/executive function and speed/accuracy. Abbreviated IQ tests and general knowledge tests correlate well with full IQ (Furnham & Chamorro-Premuzic, 2006; Wymer, 2003) and were thus grouped under the 'full IQ/verbal IQ' domain. Quantitative and perceptual reasoning measures have shown factor loadings on fluid IQ and performance IQ measures (Kline, 2000), and were thus grouped under 'fluid IQ/performance IQ'. Cognitive tests relating to memory performance were grouped together, and executive function was combined to give a 'working memory/executive function' domain given the strong associations between these constructs (McCabe et al., 2010). Motor speed and accuracy (finger tapping task; Astill et al., 2014) was grouped under the domain

'speed/accuracy' as it has been shown to overlap with processing speed tests, such as the continuous performance task, in older children (Sadeh et al., 2003). A third meta-analysis was conducted with a focus on spindle characteristics, with cognitive measures collapsed. Sleep spindle charactersitcs were spindle duration, density, frequency, peak frequency, amplitude, sigma power, total number and 'spindle activity' SPA (mean amplitude*mean duration).

Two studies analysed sleep over 2 nights (Geiger et al., 2011; Hoedlmoser et al., 2014). Geiger and colleagues (2011) report averaged spectral power across both nights. Hoedlmoser and colleagues (2014) report relationships between spindles and cognition on both baseline and experimental (post-learning) nights, and the correlations for the experimental night were analysed, although it is noted that correlations were almost identical between nights. Although Piosczyk and colleagues (2013) include a daytime nap rather than overnight sleep, the study was nonetheless included given previous research showing similar benefits of naps to overnight sleep in regard to memory consolidation (Mednick et al., 2003) and the lack of evidence that spindle activity is different during naps compared to overnight sleep.

Results

Across the 12 studies, 15 cognitive domains and eight spindle characteristics were examined (see Tables 2.1 & 2.2). Study design characteristics differed between studies regarding where (lab vs home) sleep was measured, spindle definition, spindle detection and what sleep stage(s) were investigated (see Table 2.3). Six studies used an experimental manipulation to explore the relationship under different conditions (e.g. before vs after learning a task; Hoedlmoser et al., 2014; with/without a daytime nap after learning; Piosczyk et al., 2013), and are thus listed as 'quasi-experimental', with one study (Astill et al., 2014) attempting to manipulate spindle production using acoustic perturbation. Six studies used a

cross-sectional design, while none used a longitudinal design. Participant ages ranged from 8-22 years (mean = 13.6 years). Gender was usually balanced (54% males overall; three used males only; one used females only).

Table 2.1 *Cognitive performance domains investigated in each study.*

Author & Year	**Cognitive domain used**														
	Full IQ	Verbal IQ	Abbreviated IQ	General Knowledge	Fluid IQ	Performance IQ	Perceptual Reasoning	Quantitative Reasoning	Working memory	Memory consolidation	Declarative memory	Memory improvement	Executive function	Reading accuracy	Processing speed
Astill et al (2014)															X
Bodizs et al. (2014)[a]					X										
Bruni et al. (2009)														X	
Chatburn et al. (2013)[b]			X	X				X	X				X		
Geiger et al. (2011)[c]	X	X			X				X						X
Hoedlmoser et al. (2014)[c]	X									X	X	X			
Lustenberger et al. (2012)										X		X			X
Nader & Smith (2015)[c]	X						X								X
Piosczyk et al. (2013)											X				
Prehn-Kristensen et al. (2011)										X		X			
Tamminen et al. (2010)										X		X			X
Tessier et al. (2015)[d]	X	X				X									

IQ tests used are:

[a.] Raven Progressive Matrices Test

[b.] Stanford-Binet Intelligence Scale

[c.] Wechsler Intelligence Scale-IV

[d.] Wechsler Intelligence Scale-III

Table 2.2. *Spindle characteristics investigated in each study.*

Author & Year	Sleep spindle characteristic investigated						
	Density	*Amplitude*	*Frequency*	*Total number*	*Duration*	*Sigma Power*	*SPA spindle activity*[a]
Astill et al (2014)	X						
Bodizs et al. (2014)	X	X	X				
Bruni et al. (2009)	X					X	
Chatburn et al. (2013)	X		X	X	X		
Geiger et al. (2011)			X			X	
Hoedlmoser et al. (2014)							X
Lustenberger et al. (2012)						X	
Nader & Smith (2015)	X	X			X		
Piosczyk et al. (2013)						X	
Prehn-Kristensen et al. (2011)	X			X			
Tamminen et al. (2010)	X					X	
Tessier et al. (2015)	X				X	X	

[a] SpA spindle activity = mean spindle duration*mean spindle amplitude

Table 2.3. *Study design characteristics for each study.*

Author & Year	Sample size (%male)	Age range (mean, standard deviation)	Research design	Spindle Definition (Slow; Fast, where applicable)	Where spindles measured		No. nights sleep analysed	Spindle detection method			Stage of sleep investigated	Electrode positions used for spindle analysis
					Lab	Home		Automatic/ semi-automatic	Manual	Spectral Analysis		
Astill et al (2014)	30 (37)	9.9-11.5 (10.7, 0.8)	Quasi-experimental Repeated-measures	9-15Hz (<12; >12)	✓	-	1	✓	-	-	S2, SWS	Fpz, Cz
Bodizs et al. (2014)	24 (50)	15-22 (18, 2.3)	Cross-sectional	9-16Hz	-	✓	1	✓	-	-	S2, SWS, REM	Fp1, Fp2, Fpz, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4, Pz, T3, T4, T5, T6, O1, O2, Oz
Bruni et al. (2009)	16 (38)	8-16 (10.8, SD not given)	Cross-sectional	11-16Hz	✓	-	1	-	✓	✓	S2	Fz, Cz, Pz, Fp1, Fp2, C3, C4, T3, T4, O1, O2
Chatburn et al. (2013)[a]	14 (43)	8.2-12.7 (9.9, 1.34)	Cross-sectional	11-15.9Hz (11-12.9; 13-15.9)	✓	-	1	✓	✓	-	S2	C3, C4
Geiger et al. (2011)	14 (57)	9.5-11.5 (10.5, 1)	Cross-sectional	12-15Hz	✓	-	2	-	-	✓	S2	C3
Hoedlmoser et al. (2014)	54 (54)	8-11 (9.6, 0.8)	Quasi-experimental	11-15Hz	-	✓	2	✓	-	-	S2, SWS	F3, F4, Fz, C3, C4, Cz, P3, P4, Pz, O1, O2
Lustenberger et al. (2012)	15 (100)	18-21 (19.3, 0.8)	Quasi-experimental	12-15Hz	✓	-	1	-	-	✓	S2, SWS	C4
Nader & Smith (2015)	32 (47)	12-19 (15.4, SD not given)	Cross-sectional	11-16Hz (11-13.5; 13.51-16.0)	-	✓	1	✓	✓	-	S2, SWS, REM	C3, C4, Fz, Pz
Piosczyk et al. (2013)	49 (0)	All 16	Quasi-experimental	12-15Hz (12-14; 14-15)	✓	-	1hr nap	-	-	✓	S2, SWS, REM	C3
Prehn-Kristensen et al. (2011)	24 (100)	10-16 (12.8, 0.38)	Quasi-experimental	12-14Hz	✓	-	1	-	✓	✓	S2	C3, C4
Tamminen et al. (2010)	30 (23)	20.3, SD not given	Quasi-experimental	11-15Hz (11-13Hz; 13-15Hz)	✓	-	1	✓	-	-	S2, SWS	F3, F4, C3, C4
Tessier et al. (2015)[b]	13 (100)	8-12 (10.23, 2.01)	Cross-sectional	12-16Hz	✓	-	1	-	✓	✓	S2	Fp1, Fp2, C3, C4

Note. S2 Stage 2 sleep; SWS: Slow wave sleep; REM: Rapid-eye-movement sleep

Subsamples from published data are: [a.] only those aged 8-12 [b.] only typically-developed sample.

Meta-Analyses

Results revealed that across the 12 studies, sleep spindles related to cognition with an overall effect size of $r = 0.27$ ($k = 12$, CI [0.17,0.37], $p < .01$). Meta-regressions were performed to examine the influence of covariates on the overall effect size. Covariates included continuous variables: mean age, age range in years, and gender (% male), and categorical variables: cognitive domain and spindle characteristic. Results showed no demographic covariates significantly moderated the relationship between spindles and cognition (age range: $R^2 = 0.0$, $p > .05$; gender: $R^2 = 0.0$, $p > .05$) although the small variance explained by mean age ($R^2 = 0.04$, $p > .05$) indicates there was small trend for this relationship being stronger among older participants. Spindle detection method was also not a significant predictor ($R^2 = 0.0$, $p > .05$). Cognitive domain ($R^2 = 0.23$, $p < .05$) and spindle characteristic ($R^2 = 0.18$, $p < .05$) significantly moderated the relationship between spindles and cognition, indicating that much of the variability in this relationship across studies is due to the way that cognition and spindles are operationalised.

Two further meta-analyses were performed to investigate the effect of cognitive domain and spindle characteristic on the relationship between spindles and cognition. Figure 2.3 shows a forest plot of the relationships found between spindle characteristics and cognitive performance, grouped by cognitive domain. There was some variability in this relationship, with moderate positive relationships observed for fluid IQ, working memory/executive function and speed/accuracy, while full IQ showed no significant association with sleep spindles (see Table 2.4 for effect sizes). Figure 2.4 shows studies grouped according to spindle characteristics. Moderate positive relationships were seen for spindle frequency, sigma power, total number and spindle activity (SPA), while spindle duration and density were not significantly associated with cognition (Table 2.4). Both spindle amplitude and peak frequency were evidenced by one study each, so overall effects

are not applicable. Furthermore, only spindle density and sigma power each contained four or more studies, and therefore results of other characteristics should be interpreted with caution. Note that both forest plots aggregate the same effect sizes and therefore share the same overall effect size.

Table 2.4. *Meta-analyses of studies relating to sleep spindles and cognitive performance*

Overall	k	n	r	95% CI
	12	1,366	0.27**	[0.17, 0.37]
Cognitive domain				
Full IQ/Verbal IQ	5	350	-0.05	[-0.25, 0.15]
Fluid IQ/Performance IQ	4	169	0.44**	[0.30, 0.57]
Working memory/Executive function	7	586	0.40**	[0.26, 0.52]
Speed /Accuracy	4	247	0.33**	[0.10, 0.53]
Spindle characteristic				
Duration	2	139	-0.12	[-0.37, 0.15]
Density	7	289	0.17	[-0.07, 0.40]
Frequency	2	192	0.36**	[0.14, 0.54]
Peak frequency	1	14	-0.56*	[-0.84, -0.04]
Amplitude	1	24	0.41*	[0.01, 0.70]
Sigma power	5	244	0.49**	[0.27, 0.67]
Total number	3	278	0.33**	[0.17, 0.47]
Spindle activity SPA	1	162	0.41**	[0.23, 0.57]

Note. k =no. of studies; *n* =total no. of participants; *r* =effect size; CI =confidence interval
*p < .05, **p < .01.

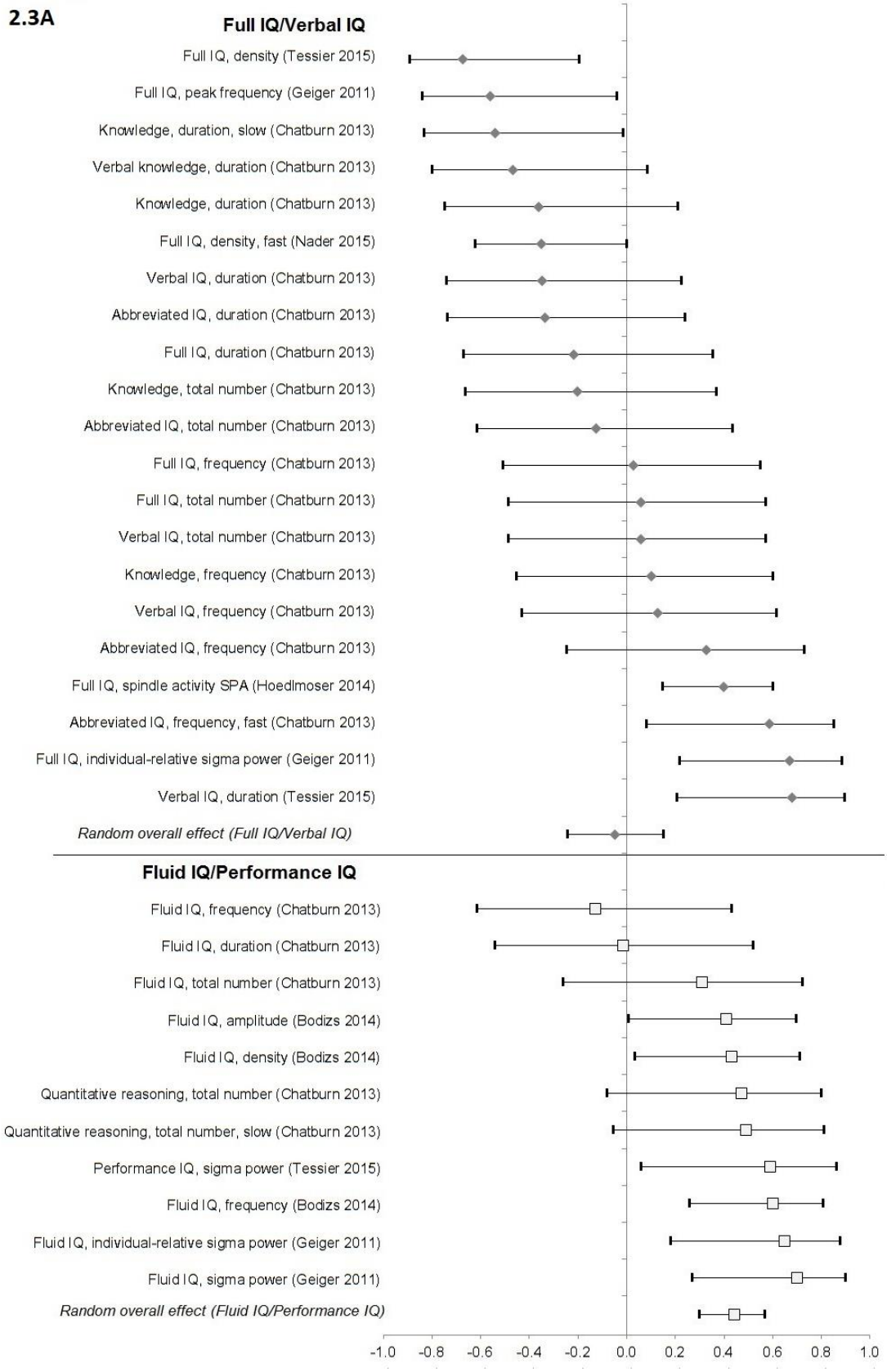

Figure 2.3. Forest plot depicting effect sizes and 95% confidence intervals for the relationship between sleep spindles and cognitive performance. Spindle characteristics are collapsed and results focus on cognitive domains. Symbols indicate the positive or negative effect size, and significant effects are those where the confidence interval does not cross the zero line. Effect sizes are considered small at r = 0.2, medium at 0.35 and large at 0.5 and above. 'Fast' and 'slow' are indicated where separate effects were given for fast and slow spindles. (**A**) plots for Full IQ/Verbal IQ and Fluid IQ/Performance IQ (**B**) plots for Working memory/Executive Function and Speed/Accuracy.

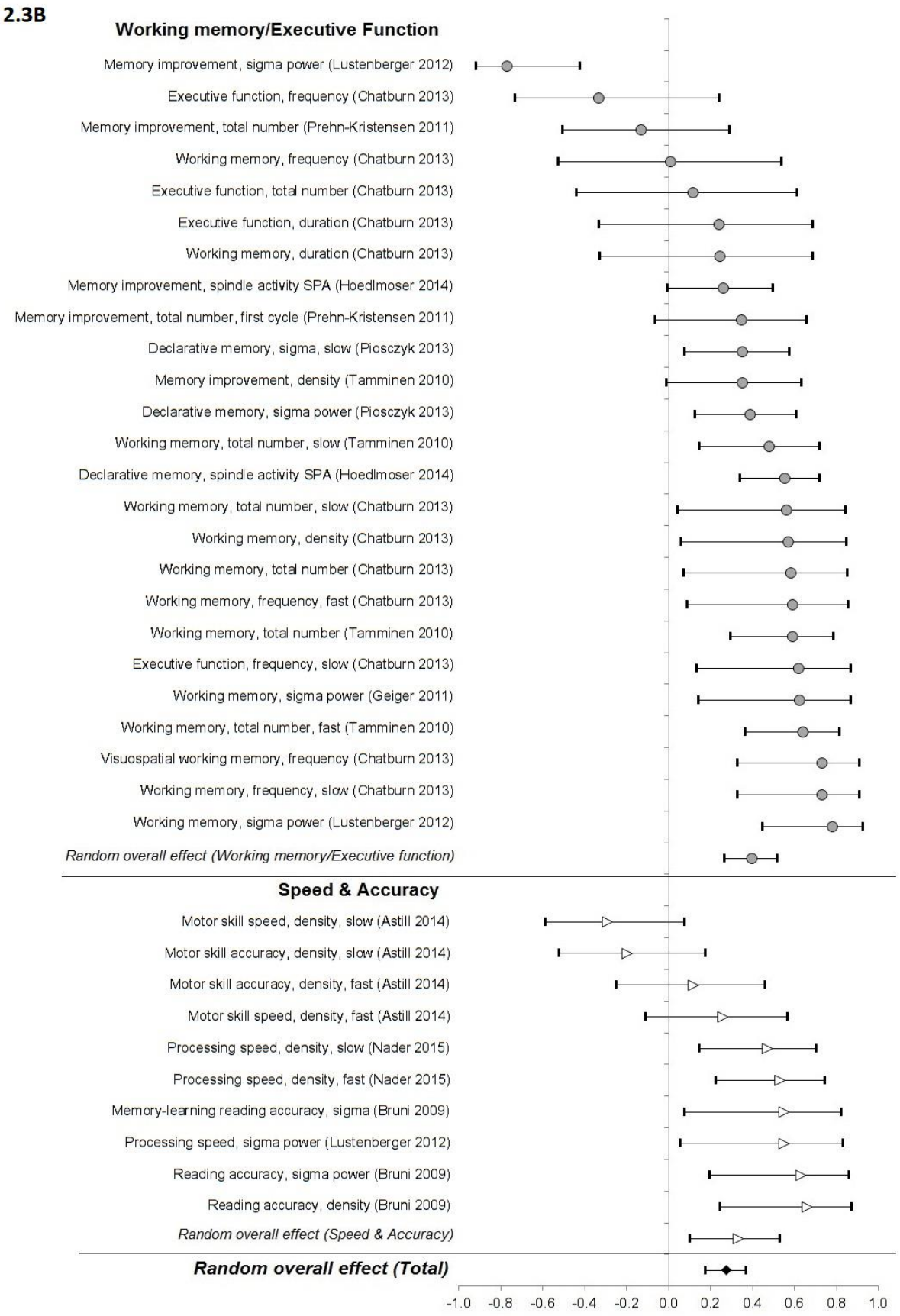

Figure 2.3. (continued)

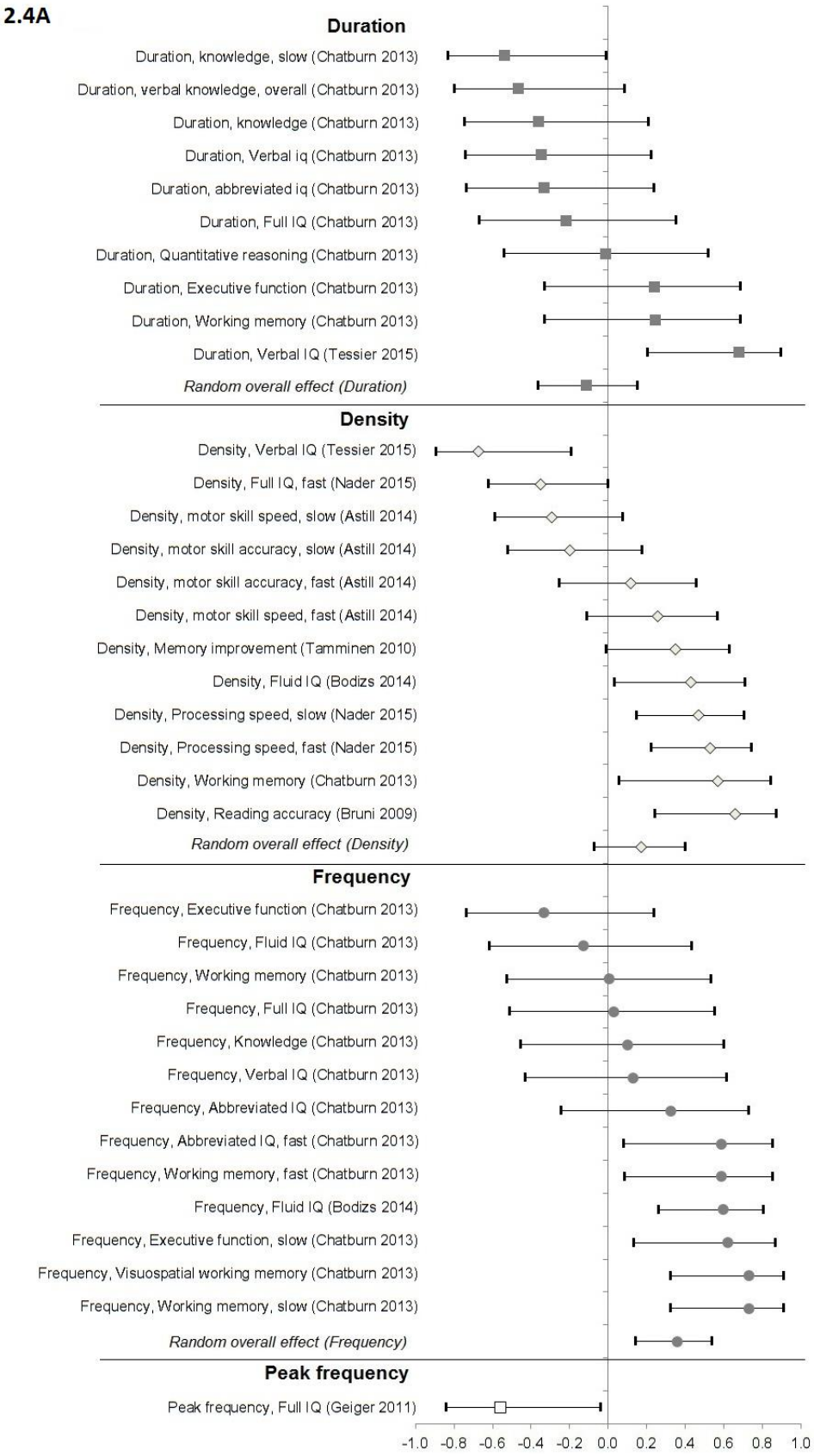

Figure 2.4. Forest plot depicting effect sizes and 95% confidence intervals for the relationship between sleep spindles and cognitive performance. Cognitive measures are collapsed and results focus on spindle characteristics. Diamonds indicate the positive or negative effect size, and significant effects are those where the confidence interval does not cross the zero line. Effect sizes are considered small at r = 0.2, medium at 0.35 and large at 0.5 and above. 'Fast' and 'slow' are indicated where separate effects were given for fast and slow spindles. (**A**) plots for Duration, Density, Frequency and Peak Frequency (**B**) plots for Amplitude, Sigma power, Total number and 'Spindle activity' SPA

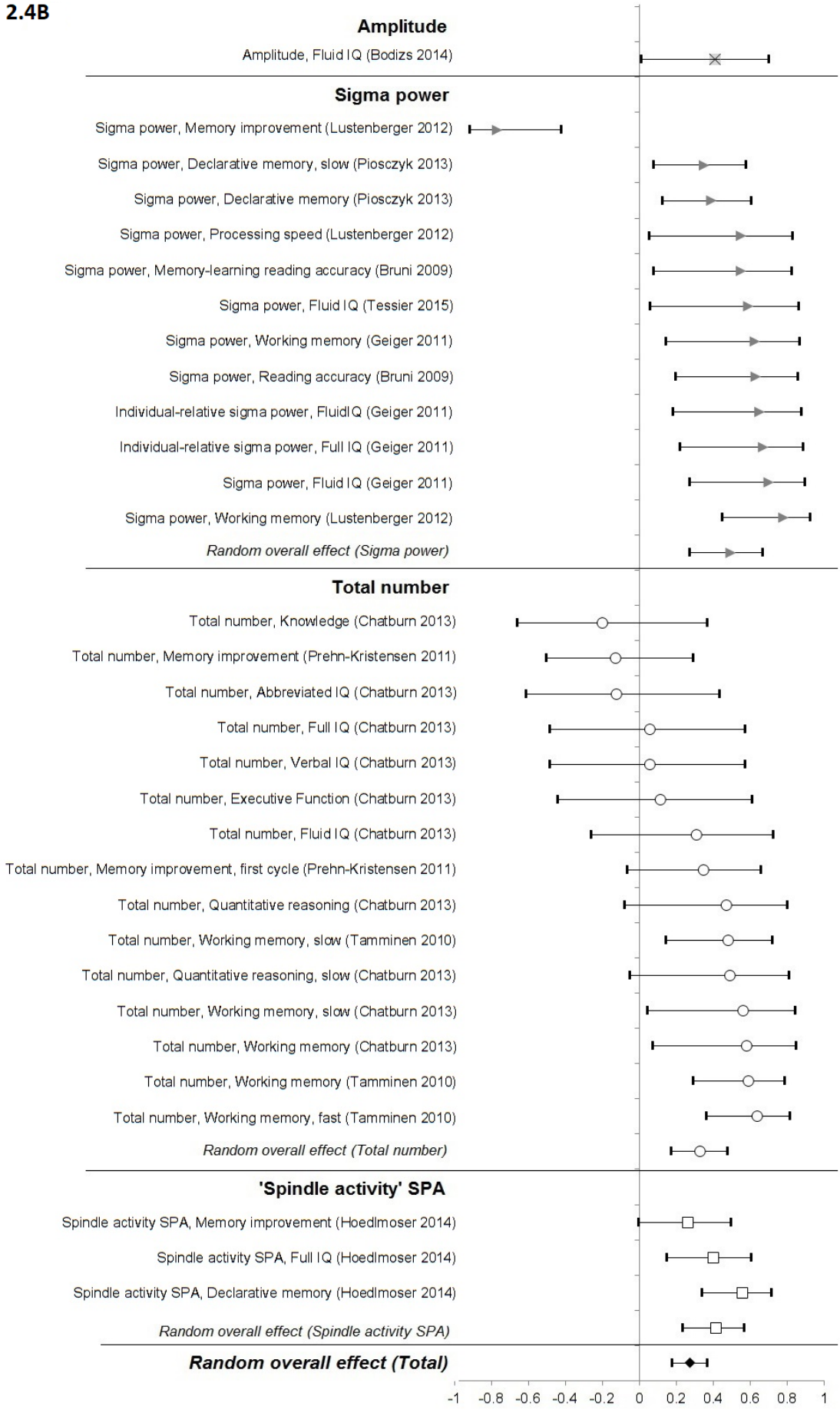

Figure 2.4. (continued)

Discussion

Sleep spindles showed a significant, positive association with cognition in young people, with a small to moderate overall effect size found ($r = 0.27$, $p < .01$). There was a great deal of heterogeneity among the studies, however, as this relationship was not observed consistently across cognitive domains or spindle characteristics. The overall effect size should thus be interpreted with caution, where it cannot summate a set of homogenous studies, but instead paints a picture of the relationship that may be occurring within this developmental period. The small, though non-significant, variance (4%) explained by mean age indicates the effect was slightly stronger in older participants and aligns with previous evidence that less developed brains show different relationships between spindles and cognition than more mature brains (Tarokh et al., 2014). However, considering the heterogeneity in ages included, this small variance might indicate more uniformity across the ages than expected. The diversity among methodologies and selection of cognitive and spindle variables help to explain the high variability among the identified studies.

Does the relationship between sleep spindles and cognition vary according to cognitive domain?

Full IQ/verbal IQ.

Sleep spindles were not significantly associated with full IQ/verbal IQ ($r = -0.05$). The relationships within this cognitive domain displayed the highest variability between studies, with some reporting significant negative associations and others significant positive associations. Part of this variability may be due to differences in the spindle characteristics analysed. This is seen within studies where both positive and negative relationships were found for one cognitive measurement. For example, Geiger and colleagues (2011) found full IQ was negatively associated with spindle peak frequency, but positively associated with sigma power. When examining individual studies, significant positive relationships were

found between full IQ and sigma power, spindle frequency and 'spindle activity' (Chatburn et al., 2013; Geiger et al., 2011; Hoedlmoser et al., 2014), while negative relationships were found with peak frequency and spindle density (Geiger et al., 2011; Nader & Smith, 2015; Tessier et al., 2015). The relationship between full IQ and spindle duration is less consistent, with both positive and negative relationships seen (Chatburn et al., 2013; Tessier et al., 2015). This finding may reflect the developmental trajectory of sleep spindle duration, with spindles tending to have longer durations in less mature brains (Shinomiya et al., 1999). In children and adolescents, it is speculated that spindle activity is a marker for brain maturation (Astill et al., 2014; Bodizs et al., 2014; Geiger et al., 2011). Furthermore, as spindle frequency and fast spindle density are expected to increase with age, and spindle duration and total number decrease with age (Nicolas et al., 2001; Shinomiya et al., 1999), this may help explain the positive relationship seen for spindle frequency and full IQ (Chatburn et al., 2013), and the negative relationships for density (Nader & Smith, 2015; Tessier et al., 2015). These directions of effect are, however, not consistent across studies and domains, so these explanations are speculative. In addition, full IQ and verbal IQ are not unitary constructs, but rather contain several subordinate constructs, some of which may vary in their relationship to spindles. As such, full IQ and verbal IQ may not be sensitive enough to illustrate inter-individual differences in relation to sleep spindles.

Fluid IQ/performance IQ.

Positive relationships between fluid IQ/performance IQ and spindles were seen in all four studies that examined this relationship (r = 0.44), with spindle amplitude, total number, density and sigma power relating positively, while spindle frequency and duration were less consistent. Relationships between spindle characteristics and fluid intelligence measures are particularly interesting in youth, given these higher-order functions fully develop later in adolescence (Casey et al., 2005) alongside dramatic changes to sleep EEG and brain

reorganisation (Feinberg & Campbell, 2010; Uhlhaas et al., 2009). These separate variables can therefore, in theory, be seen developing together through analysis of spindles and fluid IQ. This also explains why the relationships are more consistently positive, as those young people who have achieved skills in higher-order functions may be those who have more mature brain networks and therefore higher values of certain spindle characteristics.

Working memory/executive function.

Similar to fluid and performance IQ measures, working memory and executive function measures are believed to relate to brain maturation in youth (Casey et al., 2005). Accordingly, the majority of studies found a positive relationship (r = 0.40). The negative relationship between memory improvement and sigma power (Lustenberger et al., 2012) indicates that the more spindle activity a young person showed after learning a task, the smaller the improvement in their recall the next day. This indicates an improvement effect, where those who showed better performance and higher spindle activity had less room for improvement, while those who had lower performance and less spindle activity showed greater improvement. Considering this, along with the overall positive effect size, current studies indicate higher spindle activity is related to better working memory and executive function performance.

Speed and accuracy.

Overall, sleep spindles were positively related to speed/accuracy, with an effect size of r = 0.33. This relationship was observed in all four studies included. There is also evidence that fast, rather than slow, spindles may have stronger links with motor speed and accuracy, as young people with higher fast spindle density may have faster learning and more mature brain networks than those with higher slow spindle density (Astill et al., 2014). Similar to other cognitive domains, processing speed and accuracy improve across the first two decades. A possible explanation is that processing speed is linked with executive functioning which is

developing in adolescence, and higher spindle activity reflects more developed brain networks, and therefore faster and more efficient information processing (Nader & Smith, 2015).

Does the relationship vary according to spindle characteristic?

Cognitive performance was positively associated with spindle frequency (r = 0.36), amplitude (*r* = 0.41), sigma power (*r* = 0.49), total number (*r* = 0.33) and spindle activity 'SPA' (*r* = 0.41), but not spindle duration (*r* = -0.12), density (*r* = 0.17) or peak frequency (*r* = -0.56). However, only spindle density and sigma power each contained four or more studies, and therefore results of other characteristics should be interpreted with caution. These findings highlight the importance of considering the way spindles are operationalised, as the relationship between spindles and cognition varies depending on the spindle characteristic examined. This pattern of results may be partly explained by the parallel developmental trajectories of sleep spindle characteristics and cognition. During adolescence, the total number of spindles and density decreases, while spindles become briefer but with a higher frequency (Nicolas et al., 2001; Shinomiya et al., 1999). During this time cognitive abilities also increase (Casey et al., 2005). These parallel developmental changes make causation difficult to determine from cross-sectional studies of young people. At the time of writing this paper, there were no longitudinal studies examining the relationship between specific spindle characteristics and cognition in this cohort. As well as considering spindles and cognition from a developmental framework, there are likely to be other within- and between-subjects differences that influence cognition. Importantly, the developmental and neurophysiological processes may be confounded in youth. It is possible that in this age group there are both developmental changes to sleep EEG, such as synaptic pruning (whereby less spindles = better cognition; Chatburn et al., 2013), as well as stable neurophysiological relationships in

young people with more completed development (i.e. more spindles = better cognition; Nader & Smith, 2015).

Lastly, there may be other relatively understudied aspects of spindle activity that may explain this relationship. Tarokh and colleagues (2014) investigated cognition and sigma activity (11-16Hz) in a longitudinal study of nine- and 10-year-old children and found no relationship between sigma and cognition at concurrent time points, however improvement in sigma coherence over time was related to the improvement in cognition over time.

In addition to variability in spindle characteristics, the present review examined whether spindle detection method moderated the relationship between spindles and cognition. Indeed, much research has focused on the best way to detect and analyse spindles accurately (Causa et al., 2010; Huupponen et al., 2007). In the 12 studies reviewed, 4 used an algorithm or automatic analysis, 3 used only spectral analysis, 3 used both automatic and manual scoring and 3 used both manual and spectral analysis. Using an algorithm or automatic spindle detection method is appealing, as it greatly reduces the time to analyse each sleep record. In past child and adult studies, researchers have tested various spindle analysis algorithms for their validity and reliability with varying success (Causa et al., 2010; Ray et al., 2010). This has brought debate to the area, as there are clear inter-individual differences in sleep EEG, particularly with spindles, and as such the criteria used within an algorithm may not apply universally (Bodizs et al., 2009). Furthermore, the use of algorithms in young samples has not been extensively researched or validated. It is beyond the scope of this review to speculate which method is best with young samples, and indeed spindle detection method was not a significant covariate, however it is important to keep this in mind when discussing the different relationships seen between studies.

What can we infer about the mechanism underlying the relationship?

The overwhelming consensus from the literature is that spindle activity is likely reflective of both trait cognitive efficiency (Fogel & Smith, 2011) and brain maturation (Bodizs et al., 2014; Tarokh et al., 2014). To illustrate the latter, differences in both cognitive ability and spindle production may be due to a combination of the EEG channels used and the topographical changes to spindle activity across different brain regions during development. For example, decreases in spindle generation in frontal electrode derivations occur during adolescence (De Gennaro & Ferrara, 2003). Therefore, the negative relationship between verbal IQ and spindle density observed in the frontal pole derivation in the study by Tessier and colleagues (2015) may also reflect development. This raises the important question of whether changes to spindles and cognition are causal or whether they develop independently.

In regard to the discrepancy in the direction of the relationship, it may be that in a sample of young individuals there is such heterogeneity in brain development that, for some cognitive areas at least, there may always be mixed findings. Fogel and Smith (2011) propose a 'non-linear U-curve' of the relationship in adults, whereby a high spindle count can occur in those who have poor mental abilities, but also for those who have superior mental abilities, while those who show average intelligence have fewer spindles. This could apply to developing adolescents, where some younger adolescents may have poorer performance concurrently with higher number of spindles compared to their older counterparts (Nicolas et al., 2001; Shinomiya et al., 1999), which provides a developmental explanation. At the same time, those with superior performance may have more active thalamocortical networks and thus more spindles (Fogel & Smith, 2011), providing a trait neurological explanation. As none of the studies in this review were longitudinal, it is not clear whether the relationship can be predicted solely by developmental stage, and perhaps individual cognitive ability is more likely predicted by neural network efficiency.

One aspect which has not been considered in the youth literature is when spindles are measured, when cognition is measured, and whether the timing of these comparisons makes a difference to the relationship. For example, spindles one night may relate to performance earlier that same day, possibly reflecting that day's processing, however they may also relate to performance the *following* day, indicating general network efficiency. The latter indicates a more stable relationship between spindles and cognition, whereas the former indicates spindle activity can change day-to-day. Either way, superior performance can relate to higher spindle activity, but the implications for the underlying mechanism are different. Of the 9 studies that specified timing of measures, 3 analysed spindles immediately following the test, 1 analysed spindles immediately before the test, 1 analysed spindles both before and after a test (the second night was used in this review), 2 analysed spindles in a sleep period which occurred between two tests, and 2 analysed spindles a week or two following cognitive testing. This holds an important implication for the interpretation of the current findings, where effect sizes from studies that measured overnight learning cannot be easily compared to studies that measured general cognitive performance and therefore general network efficiency. More studies are needed to determine whether the underlying neural mechanism of the relationship between spindles and cognition differs when spindles are measured in an overnight learning context compared to a general one. Furthermore, sigma power is known to have good intra-individual night-to-night consistency in both adults (De Gennaro et al., 2005) and young adolescents (Geiger et al., 2011), however, it is unclear whether there is night-to-night stability in specific spindle characteristics in young people. Spindle characteristics may also show alterations after learning (e.g. an increase after a memory task; Schabus et al., 2008), indicating a possible effect of situation on the relationship. This provides an opportunity for future studies to examine the relationship over multiple nights to further investigate how spindles may relate to young people's everyday capabilities.

Implications for learning across adolescence

Considering the results of the present meta-analysis, the overall indication is that higher levels of spindle activity are related to better performance in cognitive areas that are relevant to adolescents' learning, in particular, the higher-order functions of working memory, executive functioning and fluid IQ. This raises the question of how to optimise spindle activity, which has been manipulated with mixed success using methods such as sensorimotor rhythm conditioning (Schabus et al., 2014), acoustic perturbation (Astill et al., 2014), and non-invasive trans-cranial direct current stimulation, which has shown concurrent improvements in frontal spindle production and declarative memory retention (Marshall et al., 2006; 2011). As discussed, slow and fast spindles show different functional roles for cognitive functions, which may be related to dissimilar coupling with slow oscillations and slow waves during slow wave sleep (Molle et al., 2011). For example, slow spindles in the frontal-central region have shown links with heuristic creativity (Yordanova et al., 2017) and NREM dream recall (Marzano et al., 2011). Lastly, there is also the question of whether spindles are affected by restricted or extended sleep, as sleep loss itself impacts on cognition (Lo et al., 2016). Further research is needed in adolescents to increase understanding of how spindles and cognition relate during development, particularly in an age group that, on average, achieves significantly less sleep than is recommended for optimal daytime functioning (Gibson et al., 2006; Lo et al., 2016). Although not examined in this review, it should be noted that sleep spindles have shown associations in childhood beyond cognitive processes, where higher spindle activity has been associated with better coping strategies and prosocial processes (Mikoteit et al., 2012; 2013).

Future research directions

A limitation of this review is the small number of studies included that have investigated spindles and cognition in youth. While meta-analyses were possible given the

number of effects within each study, the bigger picture of the relationship between spindles and cognition in young people contains significant gaps. While many studies have proposed theories as to why spindle activity relates to cognition, none of these are longitudinal, nor did they experimentally link spindle activity with cognition, and causation can therefore not be inferred. More experimental and longitudinal studies are needed to tease out developmental and stable effects and to form stronger conclusions about the overall relationship. The relative frequency of sleep spindles (slow vs fast) may modulate the relationship between spindles and cognition (Astill et al., 2014; Bodizs et al., 2014), however only four of the included studies supplied effect sizes for both slow and fast spindles, so comparisons could not be made, and this warrants further investigation. Furthermore, one study included a nap rather than overnight sleep (Piosczyk et al., 2013), and while spindles during naps certainly related to cognition, it is unknown whether spindle makeup during a nap equates to that in overnight sleep, and this would be worth future exploration. Lastly, there were many cases through the 12 studies where relationships were seen for some cognitive and spindle variables, but not for others. This indicates that the nature of this relationship is not always consistent and further research is needed to understand the underlying aetiology.

Conclusion

The present meta-analytic review indicates that spindle activity is related to various cognitive domains in the discrete developmental period between older childhood and young adulthood, such that higher spindle activity is related to better cognitive performance. The nature of this relationship largely varies depending on the spindle characteristics and cognitive measurements investigated, while meta-regressions indicate demographic factors of age and gender do not have an influence on this relationship. Methodologies are also highly variable between studies, which may influence the conclusions reached by each study and make it difficult to speculate why the same relationships are positive in some studies, yet

negative in others. The field needs uniformity in methods of data collection and analysis to illustrate the true nature of this relationship in the developing adolescent brain. Overall, there are implications that spindle activity relates both developmentally and neurologically to cognitive performance in adolescents, and there remain key questions about the underlying mechanism behind this relationship.

Chapter 3.

Reliability of sleep spindle measurements in adolescents: How many nights are necessary?

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Author Contributions

CR. was the primary author, contributed to data collection and conducted all spindle and statistical analyses. MG and MS contributed to statistical analyses, conceptualisation and manuscript revisions. MS contributed to study design.

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Abstract

Evidence of night-to-night variation in adolescent sleep spindle characteristics is lacking. Twelve adolescents (M = 15.8 ± 0.8yrs, 8 males) participated in a laboratory study involving 9 nights with 10 hours' sleep opportunity. Sleep electroencephalograph was analysed and intra-class coefficients were calculated to determine the reliability of sleep spindles across multiple nights of recording. Slow spindle amplitude and fast spindle density, duration and amplitude characteristics all had acceptable reliability within a single night of sleep recording. Slow spindle density and duration measurements needed a minimum of 4 and 2 nights, respectively, for reliable estimation. Theoretical and methodological implications are discussed.

Keywords: adolescence, sleep EEG, night-to-night variability

Introduction

In adult studies, sleep spindle characteristics and sigma power are believed to show individual trait-like features (Werth et al., 1997; Bodizs et al., 2009), while also showing low night-to-night variation within individuals (Finelli et al., 2001; De Gennaro et al., 2005). To date, no studies have examined night-to-night variation in spindle activity in adolescents. Findings from adult studies cannot be generalised to adolescents due to developmental changes in sleep EEG from childhood to adulthood (Shinomiya et al., 1999). Furthermore, most studies estimate sleep spindles from a single night of sleep EEG. If these estimates are unreliable (i.e., do not reflect individual's typical sleep spindle activity due to night-to-night variability), then resulting analyses may be inaccurate. Considering adolescence is a period of dynamic synaptic pruning and brain reorganisation (Feinberg & Campbell, 2010), it is important to determine if reliable spindle measurements can be obtained from a single night of sleep recording, and, if not, how many nights are needed?

Sleep spindles are commonly measured on several characteristics of the 10-16Hz oscillatory EEG waveform: total number of spindles per sleep episode, average spindle frequency (Hz), average spindle amplitude, average duration (e.g., seconds) and density of sleep spindles (number per time period, e.g., per minute). Spindles have been dichotomised into 'fast' and 'slow,' depending on the relative frequency. The present study investigates night-to-night variability of spindle density, duration and median amplitude, each considered separately for slow and fast spindles.

Method

Participants

Twelve adolescents (mean age = 15.8 ± 0.8yrs, 15-17yrs, 8 males, Tanner stage 4/5; Tanner, 1990) participated, had no self-reported medical/psychological disorders, were not taking medications and had an intermediate chronotype (score of 23-43 on the Composite

Morningness/Eveningness Scale; Smith et al., 1989). Ethics approval was granted by the University of South Australia Human Research Ethics Committee. Parents and adolescents provided written informed consent, and participants received remuneration.

Design

Data from the present study are taken from a larger study (Short et al., 2018). Adolescents in the current sample were the control group, receiving 9 consecutive nights (1 adaptation, 8 experimental) of 'optimal sleep opportunity'; 10 hours' time in bed (21:30-07:30) (Paruthi et al., 2016).

Materials

Polysomnography

Sleep EEG (derivations of Fp1, Fp2, F1, F2, C3, C4 and O1 as well as EOG and EMG, all referenced to contralateral mastoid electrodes (M1 and M2)) was recorded using the Compumedics Grael Sleep System (Melbourne, Australia) at a sampling rate of 512Hz. Adolescents were monitored overnight by an experienced sleep technician.

Sleep spindles

Sleep spindles were analysed using an automated spindle detector, following the Individual Adjustment Method (Bodizs et al., 2009; Bodizs et al., 2014). Slow and fast spindles were defined as those occurring in the strongest power peaks in the slow and fast frequency ranges within each individual's spectral profile. Slow and fast peaks are indicated by zero-crossing points on the profile. The use of separate spindle limits for individuals accounts for trait-like features (Bodizs et al., 2009). In most cases, the zero-crossing points signifying the strongest power peaks were clearly depicted. For some cases, slow frequency peaks were not clearly depicted by zero-crossing points, thus limits were decided by visual inspection of the spectra to determine the range showing the highest power (Bodizs et al., 2009). There were no cases where fast spindle peaks were unclear. EEG channels were

referenced with a calculation of [(M1+M2)/2]. All NREM sleep periods were used to detect spindles. Spindles detected on derivation C3 were preferred for analyses, and C4 used when C3 was unsuitable.

Procedure

Sleep-wake patterns were monitored for 5 nights prior to the study using sleep diaries and actigraphy (MicroMini-Motionlogger, Ambulatory Monitoring Inc., NY, USA). Adolescents were instructed to achieve 10 hours' time in bed to ensure adequate sleep during the week prior to the protocol. Adolescents attended the laboratory for 9 nights during school holidays (Friday 17:00 - Sunday 21:00). Temperature (21 ± 1°C) and light (<50 Lux in wake periods) were controlled and the laboratory was sound attenuated and free of time cues. During wake periods, adolescents were given test batteries and had set meal times. Caffeine consumption was prohibited and adolescents were supervised at all times.

Statistical Analyses

Statistical analyses were performed using SPSS V.22 (IBM, Armonk, NY). Means and standard deviations were calculated per night for each spindle characteristic (Table 3.1). Two-way random-effects models were used to determine the reliability (intra-class correlation coefficient, ICC).

Results

Means and standard deviations indicate pooled estimates were very stable across nights, however, some night-to-night variability was present, as indicated by the ICCs (see Table 3.1). Specifically, slow amplitude, fast density, fast duration and fast amplitude showed either good (ICC = 0.60 - 0.74) or excellent (ICC = > 0.75) reliability with only one night of recording. Slow spindle duration was reliably estimated with a minimum of 2 nights' sleep recording, while slow spindle density reached good reliability with a minimum of 4 nights'

sleep recording. Figure 3.1 illustrates individual spindle characteristics per night, highlighting intra-individual consistency and inter-individual variability.

Table 3.1. *Intra-class Coefficients for Sleep Spindle Parameters*

	Single night estimate based on number of nights							
Spindle parameter	**1-night**	**2-nights**	**3-nights**	**4-nights**	**5-nights**	**6-nights**	**7-nights**	**8-nights**
Density (spindles per minute)								
Slow	0.45	0.29	0.57	0.74*	0.79**	0.84**	0.84**	0.87**
Mean(SD)	7.09(0.3)	6.98(0.4)	7.13(0.4)	7.09(0.3)	7.10(0.3)	7.04(0.3)	7.10(0.3)	7.09(0.3)
Fast	0.78**	0.78**	0.90**	0.93**	0.95**	0.96**	0.96**	0.97**
Mean(SD)	8.00(0.1)	8.00(0.2)	8.00(0.2)	8.00(0.1)	8.00(0.1)	8.01(0.1)	7.99(0.1)	8.00(0.1)
Duration (length of spindles in seconds)								
Slow	0.56	0.74*	0.73*	0.80**	0.85**	0.85**	0.88**	0.91**
Mean(SD)	1.53(0.1)	1.53(0.1)	1.51(0.1)	1.53(0.1)	1.55(0.1)	1.52(0.1)	1.52(0.1)	1.53(0.1)
Fast	0.70*	0.82**	0.92**	0.92**	0.94**	0.94**	0.94**	0.95**
Mean(SD)	1.25(0.01)	1.24(0.01)	1.24(0.01)	1.24(0.01)	1.24(0.01)	1.24(0.01)	1.24(0.01)	1.25(0.01)
Amplitude (amplitude of spindles in μV)								
Slow	0.62*	0.64*	0.79**	0.89**	0.90**	0.90**	0.92**	0.93**
Mean(SD)	4.21(0.3)	4.20(0.7)	4.27(0.5)	4.28(0.4)	4.22(0.4)	4.27(0.4)	4.24(0.3)	4.21(0.3)
Fast	0.79**	0.77**	0.90**	0.94**	0.95**	0.96**	0.96**	0.97**
Mean(SD)	7.18(0.2)	7.28(0.4)	7.29(0.3)	7.24(0.3)	7.15(0.3)	7.14(0.3)	7.15(0.3)	7.18(0.2)

**good reliability, 0.60 - 0.74 **excellent reliability, > 0.75 (Cicchetti, 1994)*
Note: Estimates for the 1-night variability are based on the single-night measures from the 8-night analysis. Mean and SD presented are for the nights included in each analyses. Multiple nights are consecutive combinations (e.g. 2 nights = night 1+2; 3 nights = night 1+2+3, etc.). Spindles are measured in a narrow-band filter, which is amplitude attenuated (Bodizs et al., 2009).

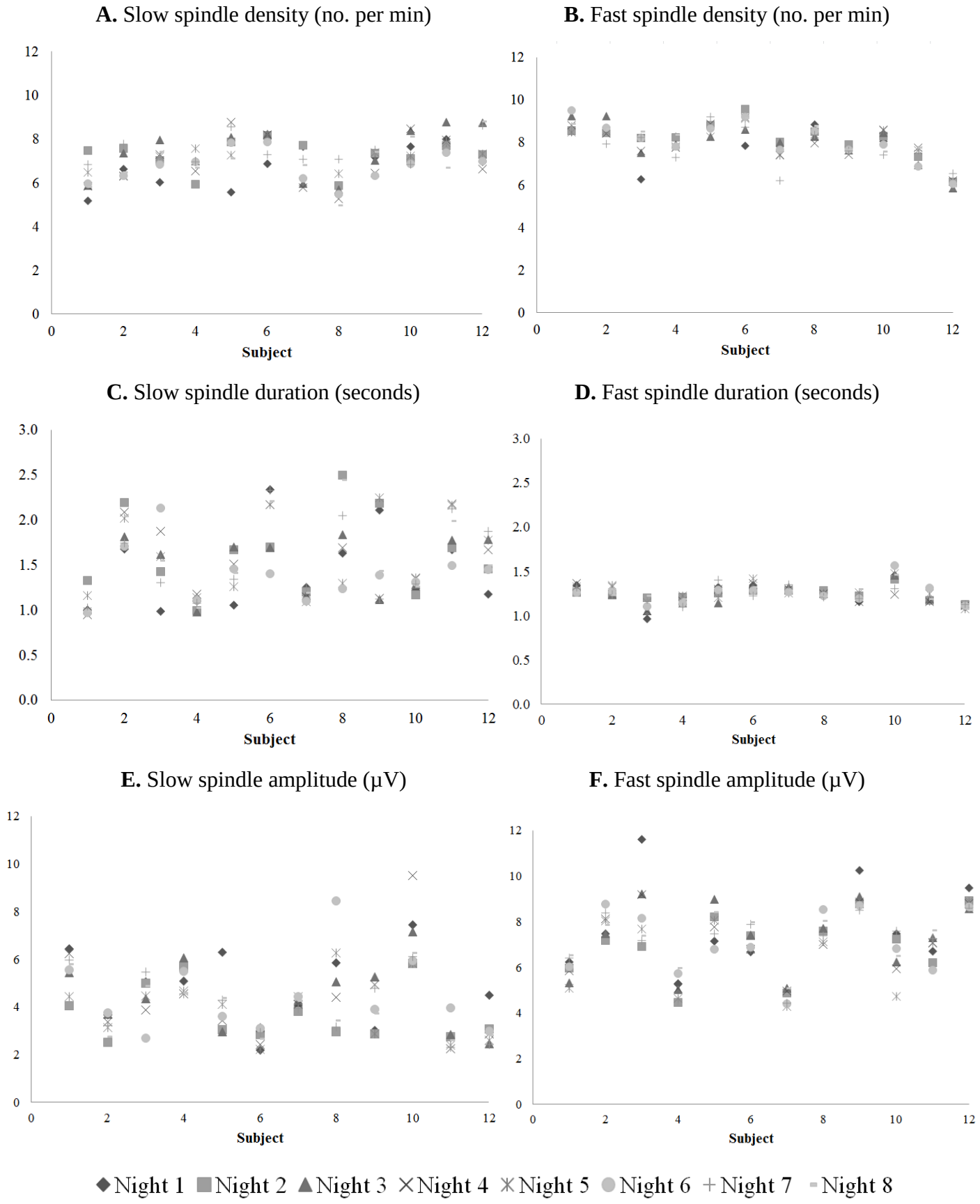

Figure 3.1. Sleep spindle characteristics per subject (*n* = 12) per night.

Discussion

For many spindle characteristics (slow spindle amplitude, fast spindle density, duration and amplitude), a single night of measurement was sufficient to reliably estimate adolescent spindle activity. This was not the case for slow spindle density and duration, which needed a minimum of 4 and 2 nights, respectively. Resultantly, studies including slow spindle density estimates from < 4 measurement nights, and slow spindle duration from only 1 night should be interpreted with caution.

In the present study, there is evidence that the reliability of fast sleep spindle measurement is superior to the measurement of slow spindles. Indeed, the reliability estimate for one night's recording of slow spindle amplitude was 0.62, while ICCs for fast spindle characteristics were higher (all > 0.70). Slow spindles typically yield less prominent spectral peaks (De Gennaro et al., 2005), and in some subjects this peak is entirely absent (Werth et al., 1997). The present detection method may therefore have reduced sensitivity to detect slow, compared to fast, spindles. Past studies have speculated about the lower prominence of slow, compared to fast, spindles and one suggestion is that slow spindles may have a different intra-cortical origin to fast spindles (Timofeev & Chauvette, 2013), however this is still unknown. This nonetheless highlights the importance of examining spindles in a dichotomised fashion, where averaging individual spindle frequency may mask distinct patterns of neural activity, and furthermore highlights the usefulness of considering individual spectra (Bodizs et al., 2009). This may help to explain the large variance in effect sizes in **Chapter 2**, where some studies dichotomised sleep spindles and others combined the two types. It is important to note that the present findings may be limited by small sample size and cannot be generalised beyond adolescent samples. Furthermore, sigma power was not included, which may be a more reliable metric than spindle parameters from an automated detector (Warby et al., 2014).

Examining the raw data (Figure 3.1), fast spindle duration is consistent within individuals across the week, and fast density shows little deviation, however amplitude characteristics appear more varied, with less reliability for slow amplitude, as discussed. The ICC data indicate how many nights are needed for reliable spindle estimates, on average, although for some adolescents a degree of intra-individual variability was present (e.g., subject 3 showed less consistency within fast amplitude estimates across the week than others), and such subjects may need more nights for reliable comparisons between spindle characteristics and performance measures.

Conclusion

Sleep spindle characteristics of slow amplitude, fast density, fast duration and fast amplitude can be reliably estimated in a single night of sleep EEG. Slow spindle characteristics may overall be less reliable for a single night of sleep recording in adolescent samples, particularly for slow spindle density and duration. Future research investigating sleep spindle characteristics would profit from aggregating data across multiple nights of sleep recording to obtain reliable estimates.

Chapter 4.

Sleep spindles in adolescence:

A comparison across sleep restriction and sleep extension

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Author Contributions

CR. was the primary author, contributed to data collection and conducted scoring of sleep EEG, sleep spindle analysis and all statistical analyses. MG contributed to revising of the manuscript and statistical assistance. SC contributed to revising of the manuscript and scoring of sleep EEG. MS contributed to study design, substantial revising of the manuscript and statistical assistance.

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Abstract

The tendency for adolescents to have restricted sleep has been examined in numerous studies, however the impact of sleep restriction on adolescents' neural activity during sleep (measured via electroencephalography, EEG) is less extensively researched, particularly regarding sleep spindles. In this experimental study, 34 adolescents attended a 10-day laboratory study where they received 5 consecutive nights of either 5 hrs, 7.5 hrs or 10 hrs of sleep opportunity, with 1 adaptation, 1 baseline and 2 recovery nights of 10 hrs' sleep opportunity before and after the experimental phase. Both within- and between-subjects' effects were seen for fast sleep spindle characteristics of density, duration and amplitude, and slow spindle amplitude. Overall, when experiencing severe sleep restriction, fast spindles in adolescents were lower in amplitude and longer in duration. Sex differences were also seen for fast spindle amplitude. This investigation adds to the field by investigating specific sleep spindle characteristics in the context of experimentally manipulated sleep. Sleep restriction is highly prevalent among adolescents. These findings indicate that chronic sleep restriction impacts brain activity related to sleep spindles.

Keywords: Sleep spindles, adolescents, sleep restriction, EEG

Introduction

Adolescence is marked by chronic sleep restriction during weekdays, reciprocal longer sleep time on the weekend, and later bedtimes, when compared to children's sleep patterns (Carskadon, 2011; Leger et al., 2012). Both experimental and large-scale epidemiological studies have found that adolescents receive significantly less sleep than is necessary for optimal daytime function (Gibson et al., 2006; Leger et al., 2012; Lo et al., 2016; Fuligni et al., 2017). Changes to adolescent sleep timing have been linked with biological factors, including delayed circadian timing and reduced homeostatic sleep drive in the evening (Taylor et al., 2005; Jenni et al., 2005; Crowley et al., 2007), as well as social and behavioural factors, such as technology use before bedtime (Bartel et al., 2015; Reynolds et al., 2015).

In addition to altered sleep patterns, there are also changes to adolescents' sleep architecture and neural activity which are believed to relate to brain reorganisation (Feinberg & Campbell, 2010; Tarokh & Carskadon, 2010). For example, the delta wave activity that characterises slow wave sleep (SWS) decreases rapidly from ages 12 to 17 years, which is attributed to synaptic pruning (Feinberg & Campbell, 2013). Furthermore, as adolescents develop, the proportion of sleep spent in stage 2 sleep increases while the proportion of slow wave sleep decreases (Carskadon & Dement, 2011). Sleep spindles are a hallmark of stage 2 sleep, with frequencies typically in the range of 11-16Hz (see Figure 4.1). Importantly, spindles are believed to indicate brain maturation and changing cortical connectivity during development (Feinberg & Campbell, 2010). With age, sleep spindles decrease in number, density and duration, yet increase in frequency (Shinomiya et al.1999; Nicolas et al., 2001). Overall EEG power in the spindle frequency range (sigma; 11-16Hz) also decreases in early adolescence (Tarokh & Carskadon, 2010).

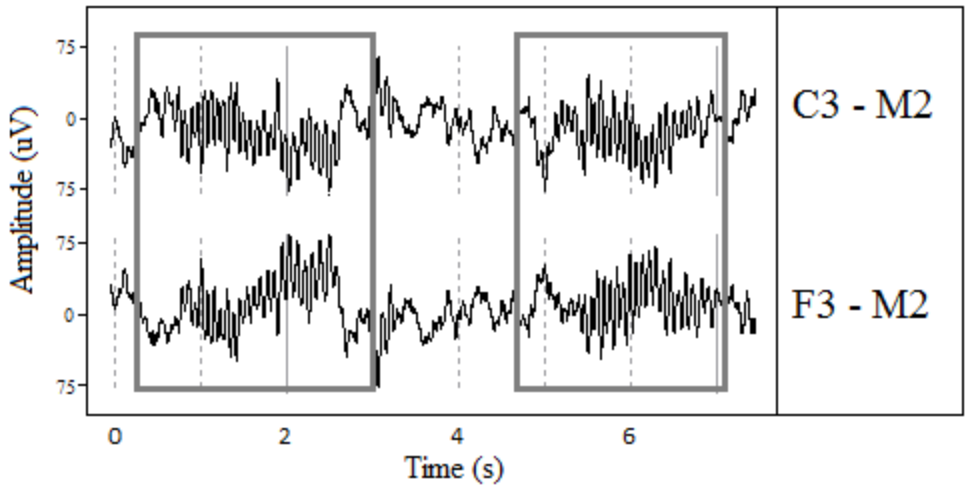


Figure 4.1. Sleep spindles seen over both central (C3) and frontal (F3) EEG derivations in stage 2 sleep in an adolescent (15yrs). Spindles are bursts of synchronized oscillatory neural activity, typically 11-16Hz, with > 0.5 second duration.

Although there is some research on age-related changes to sleep spindle activity (Shinomiya et al., 1999; Nicolas et al., 2001), there is little research that examines whether sleep spindle characteristics change in response to sleep restriction. This is particularly relevant to adolescents, who typically experience restricted sleep across the school week. Sleep restriction leads to reductions in the duration of stage 2 sleep (Agostini et al., 2017), which is where spindles frequently occur (Rechtschaffen & Kales, 1968). While aspects of sleep, such as slow wave sleep, are homeostatically regulated (Borbely & Achermann, 1999), it is unknown whether sleep spindle activity is also homeostatically regulated. As sleep spindles are argued to play an important role in cognitive functions (Fogel et al., 2007; Fogel & Smith, 2011), changes to spindle activity in response to sleep loss may be protective of cognitive functioning.

Sleep spindles can be examined across several characteristics: the number of spindles per sleep episode, average spindle frequency (Hz), average spindle amplitude (μV), average duration of sleep spindles (seconds) and density of sleep spindles (e.g. spindles per minute). Slow (~11-13Hz) and fast (~13-16Hz) spindles are often examined separately, given previous

research indicating they are functionally and topographically distinct, as well as having distinct patterns of neural activation (Schabus et al., 2007). For example, slow spindles are more commonly seen in frontal areas and are more often associated with overnight learning (Astill et al., 2014), while fast spindles are seen in centro-parietal areas and may be linked with more complex cognitive abilities, such as fluid intelligence (Bodizs et al., 2014). Several studies have shown evidence of large inter-individual differences in spindle characteristics in adolescents, indicating *trait*-like properties of sleep spindles, which may reflect an individual's brain anatomy and intellectual abilities (Bodizs et al., 2014; Geiger et al., 2011; Hoedlmoser et al., 2014). However, it is largely unknown whether sleep spindles in adolescents show *state* characteristics, for example, if they change within the individual in response to restricted sleep.

Previous adult studies have shown significant reductions in mean spindle frequency and density of fast spindles (Knoblauch et al., 2003), as well as reduced overall spindle density (Borbely et al., 1981; Dijk et al., 1993), sigma activity (Dijk et al., 1993; Finelli et al., 2001), 'total spindle activity' (amplitude x duration x density; Dijk et al., 1993) and slightly longer spindle duration (Dijk et al., 1993) following 40 hours of total sleep deprivation. Results regarding changes to spindle amplitude following sleep deprivation have been mixed, with one study reporting increased amplitude (Knoblauch et al., 2003) while another found no difference (Dijk et al., 1993). To date, there have been two studies examining spindles and sleep restriction in adults, which found a reduction of activity within the sigma band after two consecutive nights of 4 hours' sleep (Brunner et al., 1990) and after 4 consecutive nights of 4 hours' sleep (Brunner et al., 1993).

To our knowledge, only two studies to date have examined changes to even indirect spindle metrics following sleep loss in adolescent samples, and none have examined spindles directly. Jenni and colleagues (2005) found that, after 36 hours of total sleep deprivation,

power density in the sigma range was reduced for young (M = 12yrs) and older (M = 14yrs) adolescents during recovery sleep. Given the association between sigma and spindle amplitude (Tarokh et al., 2014; Purcell et al., 2017), sleep loss may lead to decreases in spindle amplitude in adolescents. Voderholzer and colleagues (2011) provided adolescents (14 - 16 yrs) with 4 consecutive nights of either 9hrs, 8hrs, 7hrs, 6hrs, or 5hrs sleep opportunity per night, and found less time in bed led to larger increases in delta, theta and alpha activity, but, contrary to Jenni et al. (2005), no change in sigma activity. The reduction in sigma power may be more pronounced with longer time spent awake before sleep (36hrs vs minimum 19hrs, respectively), however the contrary findings may also be impacted by an absence of baseline, separate from adaptation, for comparison in Voderholzer and colleagues' (2011) study. It should be noted, however, that sigma frequency activity cannot be equated with spindle activity, given the range of frequencies within spindles, which spectral analysis cannot take into account. Further, spectral analysis does not provide any information regarding specific spindle parameters, such as spindle density, amplitude or duration (Tarokh et al., 2014). Given the paucity of literature focusing on sleep spindles during sleep restriction in adolescents, it would be highly informative to investigate this, especially given the prevalence of sleep restriction at this developmental stage.

The present study addresses many of the limitations in the current literature by examining sleep restriction from both a within-subjects (comparing baseline and recovery sleep to restricted sleep) and between-subjects (comparing between doses of sleep restriction) perspective. It is expected that during severe sleep restriction, spindle density will decrease, as witnessed in past adult studies of sleep deprivation (Borbely, et al., 1981; Dijk et al., 1993). Additionally, spindle amplitude is expected to decrease with sleep restriction, given its relation to sigma power (Tarokh et al., 2014; Purcell et al., 2017), which decreases with sleep loss in adults (Brunner et al., 1990; Finelli et al., 2001) and adolescents (Jenni et al., 2005).

Spindle duration is also expected to become longer with sleep restriction, given similar findings with adult studies of sleep deprivation (Dijk et al., 1993).

Method

Participants

34 adolescents (mean age = 15.9 ± 0.9yrs, 20 males) participated in the study. Participants were recruited from newsletters in secondary schools in South Australia and through referrals from other participants. Included adolescents were aged 15-17 years, late or post-pubertal (Tanner stage 4 or 5; Tanner, 1990), had no self- or parent-reported medical or psychological disorders, and were not taking medications. Participants had an intermediate chronotype (a score of 23-43 on the Composite Morningness/Eveningness Scale; Smith et al., 1989), which is important given recent evidence of lower spindle amplitude and intensity (duration x amplitude) in adolescents with morning preference (Merikanto et al., 2017). All adolescents had a habitual sleep duration longer than 8 hours, which is within the normative range for Australian adolescents (Short et al., 2013). Ethics approval was granted by the University of South Australia Human Research Ethics Committee. Parents and adolescents provided written informed consent, and adolescents received remuneration for their time.

Design

Adolescents received one of three sleep 'doses' during the experimental period: 5hrs (n = 12, 6 males), 7.5hrs (n = 10[1], 6 males) or 10hrs (control condition, n = 12, 8 males) of time in bed for 5 consecutive nights (Sunday to Friday, see Figure 4.2). Adolescents were allocated to each sleep dose in blocks, with 4 adolescents per group receiving the same sleep dose, and were blinded to their condition. A 5hr sleep opportunity was chosen as the most severe amount of sleep restriction to determine the impact of severe sleep loss on adolescents' functioning. Previous experimental research has shown sleep restriction to 4 or 5

[1] Two adolescents withdrew during the 7.5-hour condition.

hours' sleep opportunity increases subjective and objectively tested sleepiness in children and adolescents (Fallone et al., 2001; Lo et al., 2016; Randazzo et al., 1998), and yet some adolescents and young people in western societies regularly obtain between 4 and 6.5 hours of time in bed (Lund et al., 2010; Eaton et al., 2010). A 7.5hr sleep opportunity reflects the average time adolescents in western societies spend in bed on school nights (Lund et al., 2010; Gradisar et al., 2011), while a 10hr sleep opportunity was given as a control condition to allow for optimal sleep opportunity (Carskadon et al., 1981). Wake times were kept consistent at 7:30am (Short et al., 2013) and bedtime adjusted accordingly. The adaptation night, baseline night and two recovery nights consisted of 10hrs' time in bed. This design mimicked the sleep restriction that adolescents might experience on an average school week, with sleep restriction commonly occurring on school nights and sleep-ins occurring on the weekend (Crowley et al., 2007; Carskadon et al., 2011; Owens, 2014; Lo et al., 2016).

Materials

Polysomnography

Sleep recordings were conducted in the sleep laboratory at the Centre for Sleep Research (Adelaide, South Australia) using the Compumedics Grael Sleep System (Melbourne, Australia) at a sampling rate of 512Hz. The present study used EEG derivations of Fp1, Fp2, F1, F2, C3, C4 and O1, all referenced to contralateral mastoid electrodes (M1 and M2), as well as EOG and EMG. Adolescents were monitored overnight by an experienced sleep technician. Sleep recordings were scored according to the classification developed by Rechtschaffen and Kales (1968) by two independent technicians with high inter-rater reliability ($\kappa = 82.5$).

Sleep spindles

Sleep spindle analyses were conducted with an automated spindle detector, following the Individual Adjustment Method (IAM) developed by Bodizs and colleagues (2009).

Spindle analyses were separated for slow and fast spindles. Slow- and fast-spindle limits were decided on an individual basis, where zero-crossing points on a spectral profile indicated the highest spectral peaks for both slow and fast spindles. The use of individually-designated spindle limits accommodates the trait-like features of spindles, which are commonly different between individuals (Bodizs et al., 2009), while having low night-to-night variation within each individual (Werth et al., 1997; Urakami et al., 2012). The zero-crossing points, indicating the strongest fast- and slow-spindle peaks on the spectral profile, were clearly depicted for most participants. For 25% of cases, the slow frequency peaks were not clearly depicted, and visual inspection of spectra was carried out to determine the range that showed the highest power (Bodizs et al., 2009). Fast spindle peaks were consistently clear. EEG channels were referenced to mastoids with a calculation of [(M1+M2)/2], rather than the typical FpZ reference. FpZ is preferentially active for slow spindles, and thus it can dampen slow spindle densities in frontal derivations and increase them in posterior derivations (Bodizs et al., 2009). Furthermore, spindles are measured in a narrow-band filter, which proportionately reduces the processed amplitude signals. Spindle analyses are reported for both slow and fast spindle density, duration and amplitude. All NREM sleep stages were used to detect spindles. Spindle parameter definitions from the Individual Adjustment Method (Bodizs et al., 2009) are applied as follows in the present study:

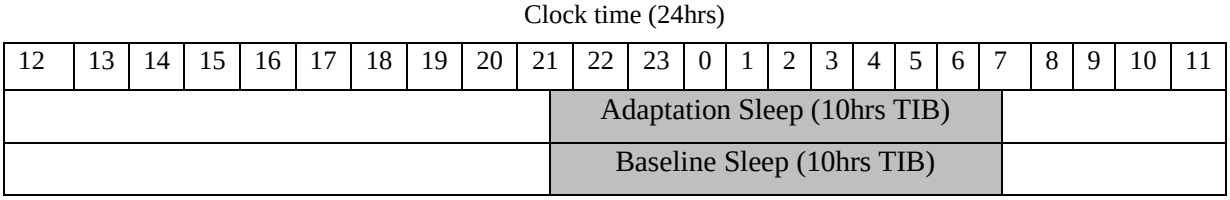
- *Spindle density:* the total number of spindles per minutes of non-REM sleep (Stage 2+Stage 3+Stage 4).
- *Spindle duration:* the average duration (seconds) of the segment of each spindle that meets the individually-based peak amplitude criteria for > 0.5 seconds.
- *Spindle amplitude:* the average amplitude taken from the midpoints of all detected spindles, using the rectified signals.

Procedure

Sleep-wake patterns were monitored for 5 nights prior to the laboratory stay using sleep diaries, activity watches (Actigraphy; MicroMini-Motionlogger, Ambulatory Monitoring Inc., Ardsley, NY, USA) and with telephone calls morning and night to confirm adherence to set bedtimes (between 21:30 – 22:00) and wake times (between 07:00 – 07:30)[2]. Adolescents were instructed to achieve 9 to 10hrs' time in bed to ensure adequate sleep during the week prior to the experimental protocol. Adolescents attended the sleep laboratory for 9 nights during the South Australian school holiday period, from 5pm on a Friday night until 9pm on the Sunday night the following week. The sleep laboratory was temperature and light controlled, sound attenuated and free of time cues. During wake periods, adolescents were given test batteries and had set meal times. Caffeine consumption was prohibited throughout the study. During free periods between testing, adolescents engaged in board and card games, craft activities and watched films. Adolescents were supervised at all times to prevent accidental napping. Figure 4.2 outlines the study protocol.

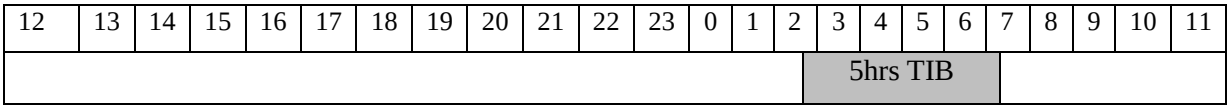
[2] One adolescent was excluded from the study due to non-adherence to these times.

Days 1 - 2: Adaptation and Baseline (n = 34):

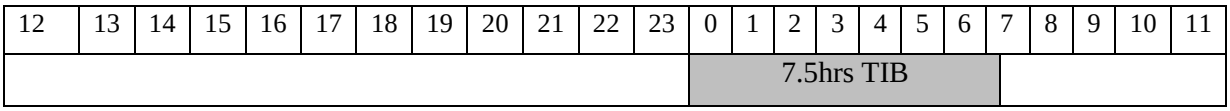


Days 3 - 8: Experimental phase

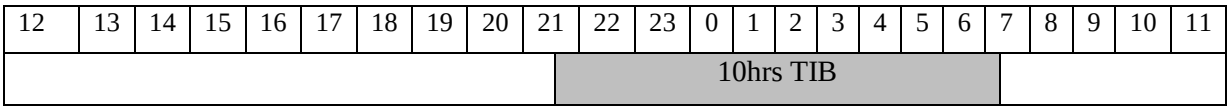
Severe sleep restriction (n = 12):



***or* Moderate sleep restriction (n = 10)**:



***or* Control condition (n = 12)**:



Days 9 - 10: Recovery (n=34):

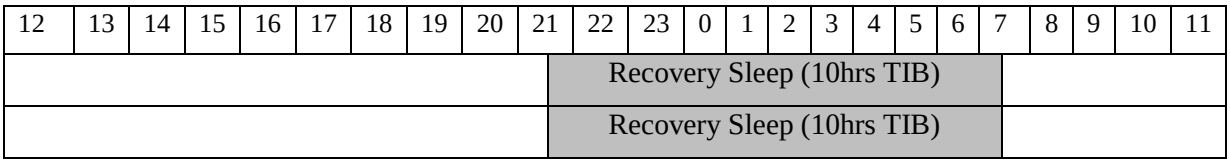


Figure 4.2 Sleep restriction study protocol. Shaded bars indicate the sleep opportunity for the full sample at baseline and recovery, and during the experimental phase for the three sleep 'doses': 5hrs, 7.5hrs or 10hrs TIB (time in bed). Unshaded areas indicate wake time.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics Version 22 (IBM Corp., Armonk, NY). Linear mixed model (LMM) analyses were conducted to test differences in sleep spindle parameters across study phases, appropriately accounting for within- and between-participant variance (Van Dongen et al., 2004). The adaptation night was eliminated from analyses to account for the first-night effect (Rechtschaffen & Verdone,

1964). Resultantly, the baseline phase consisted of night 2, the sleep restriction phase consisted of the average of the 5 experimental nights, and the recovery phase consisted of the average of both recovery nights. All models specified a random effect of participant ID. Models specified both slow and fast spindle density, duration and amplitude as dependent variables, with fully saturated models (all main and interaction effects) for study phase (baseline, experimental phase, recovery), sleep dose condition (5hrs, 7.5hrs, 10hrs TIB), and sex. Sex was included in the analyses, given previous research showing a sexual dimorphism of spindle activity (Carrier et al., 2001; Huupponen et al., 2002). Sex was only a significant contributor to analyses of fast spindle amplitude, and it was therefore removed from subsequent analyses for all other spindle characteristics. Pairwise post-hoc analyses with least significant differences (LSD) were conducted to further investigate significant main and interaction effects. The data for between-subjects post-hoc analyses were equated at baseline to control for baseline group differences when making comparisons, while original values were used for study phase comparisons within each sleep dose.

Results

Sleep Characteristics

Sleep parameters of the 34 adolescents during the experimental phase are presented in Table 4.1. The total sleep time achieved was close to the time in bed for all conditions, thus confirming the manipulation (Short et al., 2018). Total minutes of sleep stages for each condition during the experimental phase are shown in Figure 4.3.

Table 4.1 *Sleep parameters for the experimental phase for the three sleep 'doses', with sleep stages presented as a proportion of total sleep*

Sleep Dose	TST (min)	SOL (min)	SE%	S1%	S2%	S3%	S4%	REM%
5 hrs	288.5	3.5	96.5	3.1	34.5	8.5	33.8	20.1
7.5 hrs	426.2	4.9	94.8	4.0	44.1	9.1	23.0	19.9
10 hrs	536.7	24.5	89.4	6.1	44.3	9.6	18.2	21.8

Note. TST = Total sleep time in minutes, SOL = sleep onset latency in minutes, SE = sleep efficiency (percentage of time in bed spent asleep), S1 = stage 1 sleep, S2 = stage 2 sleep, S3 = stage 3 sleep, S4 = stage 4 sleep, REM = rapid eye movement sleep.

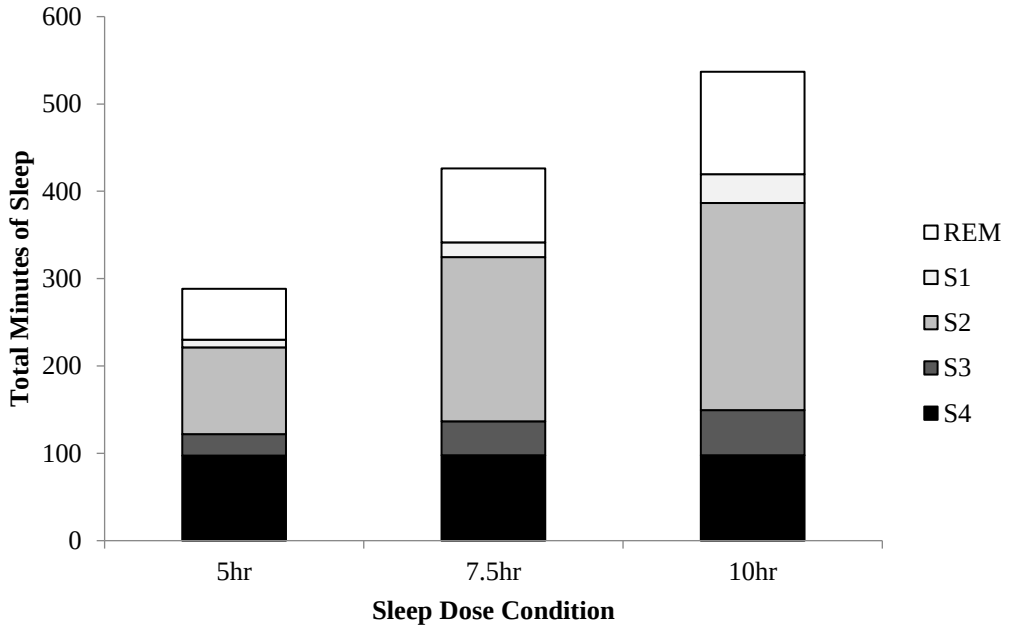


Figure 4.3. Total minutes of stages of sleep in each sleep restriction condition (5hr, 7.5hr and 10hr), for the experimental phase (excluding baseline and recovery sleep). REM = rapid eye movement sleep, S1 = stage 1 sleep, S2 = stage 2 sleep, S3 = stage 3 sleep, S4 = stage 4 sleep.

Sleep Spindle Characteristics

Descriptive statistics for the spindle parameters are provided in Table 4.2 (for each night of the study) and Table 4.3 (for study phase). Results of the inferential test are given in

Table 4.4 and the change in spindle characteristics across study phase is displayed in Figure 4.4.

Table 4.2. *Mean values (standard deviations) for each spindle parameter (density (no. per minute), duration (seconds), amplitude (μV)) for each night.*

		BL2	**SR1**	**SR2**	**SR3**	**SR4**	**SR5**	**RC1**	**RC2**
5hr	Slow density	7.49	7.24	7.52	7.36	7.51	7.47	7.59	7.12
		(1.25)	*(1.45)*	*(0.92)*	*(0.80)*	*(1.25)*	*(0.93)*	*(0.75)*	*(1.34)*
	Fast density	7.50	7.57	7.87	7.46	7.71	7.55	7.99	7.71
		(0.93)	*(1.10)*	*(0.70)*	*(1.20)*	*(0.95)*	*(0.91)*	*(0.86)*	*(1.15)*
	Slow duration	1.53	1.53	1.49	1.53	1.31	1.42	1.40	1.40
		(0.44)	*(0.44)*	*(0.33)*	*(0.35)*	*(0.19)*	*(0.32)*	*(0.41)*	*(0.41)*
	Fast duration	1.13	1.18	1.24	1.31	1.34	1.31	1.27	1.20
		(0.12)	*(0.14)*	*(0.16)*	*(0.29)*	*(0.26)*	*(0.27)*	*(0.18)*	*(0.19)*
	Slow amplitude	4.23	3.97	3.81	3.70	4.33	4.65	4.10	4.28
		(1.99)	*(2.17)*	*(2.10)*	*(1.97)*	*(1.84)*	*(2.15)*	*(1.72)*	*(2.06)*
	Fast amplitude	7.24	6.16	5.77	5.78	5.75	5.30	6.23	6.80
		(3.24)	*(2.12)*	*(2.10)*	*(2.19)*	*(1.87)*	*(1.69)*	*(2.58)*	*(2.27)*
7.5hr	Slow density	7.12	7.04	7.36	7.00	7.26	7.31	7.25	7.31
		(0.81)	*(0.98)*	*(0.99)*	*(0.45)*	*(1.05)*	*(0.80)*	*(0.90)*	*(1.0)*
	Fast density	7.63	7.70	7.52	7.49	7.24	7.39	7.99	7.83
		(0.85)	*(0.89)*	*(0.77)*	*(0.82)*	*(1.46)*	*(0.82)*	*(0.64)*	*(0.93)*
	Slow duration	1.45	1.42	1.44	1.46	1.40	1.44	1.37	1.44
		(0.42)	*(0.43)*	*(0.40)*	*(0.37)*	*(0.33)*	*(0.33)*	*(0.32)*	*(0.45)*
	Fast duration	1.22	1.18	1.16	1.22	1.20	1.24	1.25	1.18
		(0.15)	*(0.15)*	*(0.16)*	*(0.21)*	*(0.21)*	*(0.24)*	*(0.11)*	*(0.14)*
	Slow amplitude	4.45	4.41	4.29	4.44	4.23	4.10	4.71	5.15
		(1.87)	*(2.06)*	*(1.71)*	*(2.30)*	*(1.74)*	*(1.83)*	*(2.05)*	*(2.07)*
	Fast amplitude	6.36	6.23	6.28	6.22	6.49	6.39	6.56	6.96
		(1.95)	*(1.46)*	*(1.89)*	*(1.44)*	*(1.24)*	*(1.63)*	*(1.93)*	*(1.80)*
10hr	Slow density	6.70	7.27	7.43	6.96	7.11	6.76	7.48	7.03
		(0.92)	*(0.71)*	*(1.12)*	*(1.16)*	*(0.65)*	*(0.73)*	*(0.63)*	*(1.03)*
	Fast density	7.83	8.17	7.99	8.00	8.00	8.07	7.83	8.13
		(0.93)	*(0.83)*	*(0.95)*	*(0.82)*	*(0.83)*	*(0.96)*	*(0.90)*	*(0.87)*
	Slow duration	1.44	1.63	1.48	1.58	1.61	1.40	1.54	1.59
		(0.45)	*(0.46)*	*(0.34)*	*(0.44)*	*(0.45)*	*(0.31)*	*(0.46)*	*(0.48)*
	Fast duration	1.23	1.25	1.23	1.23	1.27	1.25	1.24	1.25
		(0.14)	*(0.07)*	*(0.11)*	*(0.10)*	*(0.11)*	*(0.12)*	*(0.09)*	*(0.09)*
	Slow amplitude	4.69	4.41	4.29	4.44	4.23	4.10	4.71	5.15
		(1.63)	*(1.17)*	*(1.44)*	*(1.97)*	*(1.31)*	*(1.62)*	*(1.32)*	*(1.24)*
	Fast amplitude	7.58	6.98	7.29	7.12	6.77	7.12	7.15	7.39
		(1.97)	*(1.40)*	*(1.56)*	*(1.46)*	*(1.67)*	*(1.44)*	*(1.38)*	*(1.12)*

Note: BL = baseline; SR = sleep restriction/experimental phase, RC = recovery

Table 4.3 *Mean values (standard deviations) for each spindle parameter (density (no. per minute), duration (seconds), amplitude (μV)) for each study phase (baseline, experimental phase, recovery), and effect sizes for within-subjects phase differences.*

		BL	**SR**	***Effect (d) BL vs SR***	**RC**	***Effect (d) SR vs RC***
5hr	Slow density	7.49	7.42	***0.07***	7.36	***0.07***
		(1.25)	*(0.81)*		*(0.93)*	
	Fast density	7.50	7.63	***0.15***	7.85	***0.25***
		(0.93)	*(0.82)*		*(0.91)*	
	Slow duration	1.53	1.43	***0.28***	1.41	***0.07***
		(0.44)	*(0.23)*		*(0.35)*	
	Fast duration	1.13	1.27	***0.88****	1.24	***0.16***
		(0.12)	*(0.19)*		*(0.18)*	
	Slow amplitude	4.23	4.09	***0.07***	4.19	***0.05***
		(1.99)	*(1.82)*		*(1.82)*	
	Fast amplitude	7.24	5.74	***0.56****	6.51	***0.36****
		(3.24)	*(1.86)*		*(2.36)*	
7.5hr	Slow density	7.12	7.20	***0.11***	7.28	***0.10***
		(0.81)	*(0.65)*		*(0.87)*	
	Fast density	7.63	7.54	***0.20***	7.82	***0.40****
		(0.85)	*(0.78)*		*(0.61)*	
	Slow duration	1.45	1.44	***0.03***	1.35	***0.31***
		(0.42)	*(0.31)*		*(0.27)*	
	Fast duration	1.22	1.20	***0.12***	1.21	***0.07***
		(0.15)	*(0.17)*		*(0.12)*	
	Slow amplitude	4.45	4.29	***0.09***	4.93	***0.33****
		(1.87)	*(1.87)*		*(1.98)*	
	Fast amplitude	6.36	6.32	***0.02***	6.76	***0.27****
		(1.95)	*(1.43)*		*(1.85)*	
10hr	Slow density	6.70	7.11	***0.49***	7.25	***0.19***
		(0.92)	*(0.75)*		*(0.70)*	
	Fast density	7.83	8.05	***0.25***	7.98	***.08***
		(0.93)	*(0.84)*		*(0.84)*	
	Slow duration	1.44	1.54	***0.26***	1.57	***0.08***
		(0.45)	*(0.31)*		*(0.45)*	
	Fast duration	1.23	1.25	***0.17***	1.25	***0.01***
		(0.14)	*(0.09)*		*(0.08)*	
	Slow amplitude	4.69	4.18	***0.35***	4.06	***0.09***
		(1.63)	*(1.28)*		*(1.26)*	
	Fast amplitude	7.58	7.06	***0.30****	7.27	***0.16***
		(1.97)	*(1.41)*		*(1.21)*	

Note: BL = baseline; SR = sleep restriction/experimental phase, RC = recovery, d = Cohen's d: small 0.2, medium 0.5, large 0.8
**p < .05*

Table 4.4. *Main effects and interaction of sleep dose (5hr, 7.5hr, 10hr), study phase (baseline, experimental phase, recovery) and sex (male, female) on sleep spindle variables*

	F-value	df	p	Post-hoc comparisons
Slow density (no. per min)				
dose	0.90	2,33	.42	
phase	0.85	2,232	.43	
dose*phase	0.98	4,232	.42	
Fast density (no. per min)				
dose	0.55	2,33	.58	
phase	3.36	2,232	**.04***	BL<RC, SR<RC
dose*phase	1.76	4,232	.14	
Slow duration (seconds)				
dose	0.30	2.33	.75	
phase	0.29	2,232	.75	
dose*phase	1.36	4,232	.25	
Fast duration (seconds)				
dose	0.17	2,33	.84	
phase	2.77	2,232	.07	
dose*phase	3.26	4,232	**.01***	5hr: BL<SR, BL<RC 7.5hr and 10hr: no differences *Dose comparison:* SR & RC: 5hr > 7.5hr & 10hr
Slow amplitude (μV)				
dose	0.15	2,32	.86	
phase	2.06	2,232	.13	
dose*phase	1.99	4,232	.10	
Fast amplitude (μV)				
dose	0.74	2,29	.49	
phase	12.92	2,226	**<.01***	BL>SR<RC
sex	1.42	1,29	.24	
dose*phase	4.64	4,226	**<.01***	5hr: BL>SR<RC 7.5hr: SR<RC 10hr: BL>SR *Dose comparison:* SR: 5hr < 7.5hr
dose*sex	0.12	2,29	.89	
phase*sex	3.54	2,226	**.03***	Males: BL>SR<RC Females: BL>SR
dose*phase*sex	4.55	4,226	**<.01***	Males: 5hr: BL>SR<RC 7.5hr: BL>SR<RC 10hr: no differences Females: 5hr: BL>SR, BL>RC 7.5hr: BL<SR, BL<RC 10hr: BL>SR

*$p < .05$; BL = baseline, SR = sleep restriction/experimental phase, RC = recovery

*Note: Dose comparisons for significant dose*phase interactions were conducted for change from baseline values.*

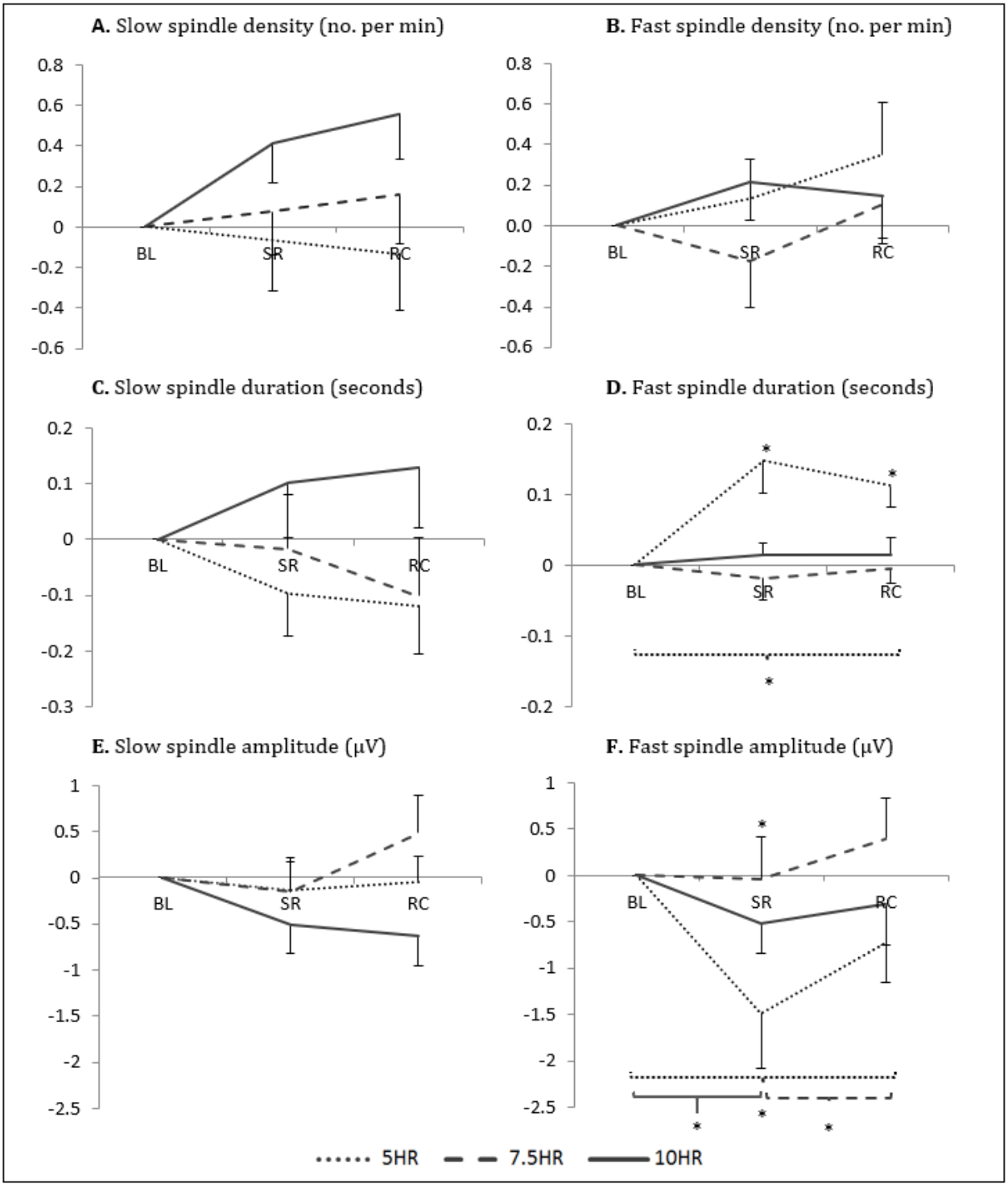


Figure 4.4. Spindle characteristics across the week, formatted as change from baseline (mean and standard error). Results are presented by study phase (BL = baseline: 1 night, SR = sleep restriction/experimental phase: average of 5 experimental nights, RC = recovery: average of 2 nights). Bracketed asterisks below each graph (*{) indicate significant within-subjects' differences between study phases. Brackets are dotted in the same style as the condition they refer to. Singular asterisks above each graph (*) indicate significant group differences.

Spindle Density

There were no main or interaction effects seen for slow spindle density in the present study (Figure 4.4A). There was, however, a significant main effect of study phase on fast spindle density across all groups, where density increased at recovery compared to both baseline and the experimental phase. There were no significant differences between sleep doses (Figure 4.4B).

Spindle Duration

There were no main or interaction effects seen for slow spindle duration in the present study (Figure 4.4C). There was a significant interaction between sleep dose and study phase for fast spindle duration. The 5hr condition showed an increase in fast spindle duration from baseline to the experimental phase which did not recover to baseline levels (Figure 4.4D). Across conditions, the duration of sleep spindles was significantly longer in the 5hr condition compared to the 7.5hr and 10hr conditions, for both the experimental phase, and recovery.

Spindle Amplitude

There were no significant main or interaction effects of sleep dose or study phase on slow spindle amplitude (Figure 4.4E). There was a two-way interaction between sleep dose and study phase on fast spindle amplitude, where amplitude was lower during severe sleep restriction (5hrs) than at baseline, and did not recover (Figure 4.4F). There was a significant three-way interaction between sleep dose, study phase and sex for fast spindle amplitude. For males, there was a significant interaction between sleep dose condition and study phase, where fast spindle amplitude decreased during sleep restriction compared to baseline and recovery for both the 5hr and 7.5hr conditions, however, this was more prominent in the 5hr condition (Figure 4.5A). There were no significant differences across study phases for males in the 10hr condition. For females, there was also a significant interaction between sleep dose and study phase (Figure 4.5B), where fast amplitude significantly decreased during

experimental phase compared to baseline for the 5- and 10hr conditions, with the 5hr condition not returning to baseline levels at recovery, while fast amplitude significantly increased from baseline during the experimental phase and recovery for the 7.5hr condition.

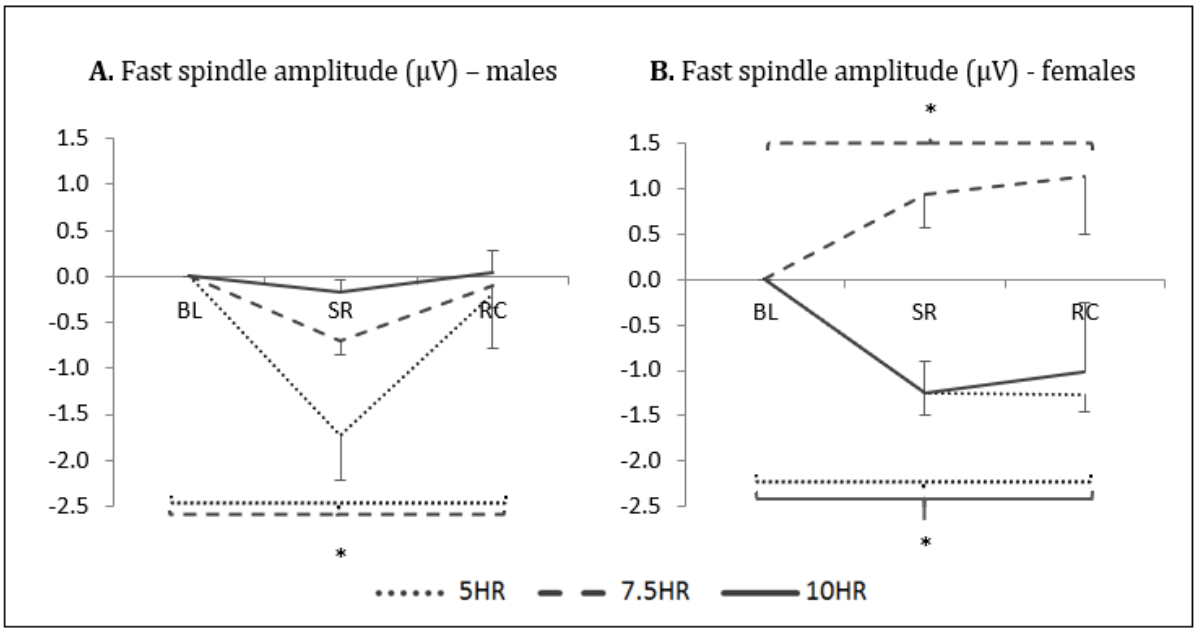


Figure 4.5. Fast spindle amplitude across the week, formatted as change from baseline (mean and standard error), for males (**A**) and females (**B**). Results are presented by study phase (BL = baseline: 1 night, SR = sleep restriction/experimental phase: average of 5 experimental nights, RC = recovery: average of 2 nights). Bracketed asterisks (*{) indicate significant within-subjects' differences between study phases. Brackets are dotted in the same style as the condition they refer to.

Discussion

The present study aimed to address gaps in the literature on adolescent sleep restriction and sleep spindles using a dose-response paradigm. Adolescents typically experience sleep restriction in the school week. While this is known to affect some aspect of the sleep EEG, little is known about the effect of sleep loss on sleep spindles. In the present study, adolescents' sleep spindle characteristics were affected by sleep restriction, however the nature of this effect was not consistent between conditions or across all spindle

characteristics. When adolescents experienced severe sleep restriction (5 hrs' sleep opportunity), fast spindles had a lower amplitude and longer duration. This aligned with expectations that spindle amplitude would decrease with sleep restriction, given its relation to sigma power (Tarokh et al., 2014; Purcell et al., 2017), which decreases with sleep loss in adults (Brunner et al., 1990; Finelli et al., 2001) and adolescents (Jenni et al., 2005), although this effect was not seen for slow spindle amplitude. An important question arising from the present findings is, why might fast spindle amplitude and duration be affected by sleep restriction? Firstly, a reduction in sleep spindle activity (sigma power, density, amplitude and 'total spindle activity' (amplitude x duration x density)) and concurrent increase in slow wave activity are hypothesised to relate to reduced activation of the thalamocortical circuit after sleep deprivation, indicative of sleep homeostasis (Dijk et al., 1993). The increase in slow wave activity following sleep loss may in fact suppress spindle activity. Because slow waves and spindles are both produced in the thalamocortical network, this trade-off between slow wave and spindle activity might suggest that sleep loss affects the thalamocortical system as a whole. Further research could investigate this hypothesis in more depth.

While previous research has shown inter-individual differences in sleep spindle characteristics, indicating that sleep spindles can be trait-like, these results indicate that there is also a state aspect to some sleep spindle characteristics. Specifically, some characteristics change in response to restricted sleep. In particular, fast spindle activity was seen to be more sensitive to sleep loss than slow spindles. Fast spindles are believed to be more important than slow spindles for maintenance and encoding of the thalamocortical system (Fogel et al., 2007), as well as learning and complex cognitive abilities (Urakami et al., 2012; Bodizs et al., 2014). For example, Bodizs and colleagues (2014) found higher fast spindle amplitude related to better fluid intelligence in adolescents. The reduced fast spindle amplitude following severe sleep restriction in the present study may therefore be linked to deficits in

cognitive function. To date, there have been no studies investigating associations between fast spindle duration and cognition in adolescents, which is an area for future research.

Along with changes to sleep spindle characteristics during severe sleep restriction, there were interesting effects seen for the other conditions. Overall, those in the 7.5hr condition showed no difference in spindle characteristics from baseline to sleep restriction. This is interesting, as it implies the sleep opportunity typical of western adolescents (Lund et al., 2010; Gradisar et al., 2011) does not affect sleep spindles following baseline 'optimal' sleep opportunity (Carskadon et al., 1981). The only effect seen for this sleep dose was an increase in fast spindle amplitude from sleep restriction to recovery. Perhaps an increase in fast amplitude on return to 10hrs' sleep opportunity implies some homeostatic mechanism. The overall impression is that a reduction to 7.5hrs' sleep opportunity does not lead to a detriment, but sleep extension to 10hrs may lead to some benefit, at least for a short time. A full week of 10hrs' sleep opportunity, however, led to a decrease of fast spindle amplitude. There may be an optimal sleep opportunity somewhere between 7.5hrs and 10hrs for stable sleep spindle production. Overall, there was not a clear dose-response effect of sleep restriction on sleep spindle production, apart from males' fast spindle amplitude. To date, no other studies have examined the effect of these longer sleep opportunities on spindles in adolescents, and these interpretations are therefore speculative.

While there is mounting evidence that sleep restriction impacts cognitive functioning in areas such as full-scale IQ (Geiger et al., 2010), fluid IQ (Geiger et al., 2010) and memory (Randazzo et al., 1998, Geiger et al., 2010, Vriend et al., 2012) the mechanisms behind these effects are not fully elucidated. If sleep restriction affects those sleep spindle characteristics known to be associated with cognitive functioning, it is possible that this is one potential mechanism. Certainly, future investigations should examine the relationship between cognition and spindle characteristics in the context of sleep restriction.

Overall, the changes in spindles were not consistent within sleep spindle characteristics or for each of the sleep dose conditions. The current body of research in this area is limited, so definitive conclusions cannot be readily made. It was expected that in the 5hr condition, spindle density would decrease, as witnessed in past adult studies of sleep deprivation (Borbely, et al., 1981; Dijk et al., 1993), however, there were no clear changes to sleep spindle density in the current adolescent sample. Additionally, the 5hr group showed an increase in fast spindle duration compared to the other groups, similar to adult findings of increased spindle duration following 40-hour total sleep deprivation (Dijk et al., 1993). No studies to date have reported changes to adolescent spindle duration during sleep loss, and these data may be a first to indicate that spindle duration becomes longer with severe sleep restriction in this age group, at least for faster frequencies. It should be noted that measures of slow spindle density and duration in adolescents have been found to lack single-night reliability (Reynolds et al., 2018 (**Chapter 3**)), and the present findings for those parameters should thus be interpreted with caution.

In terms of sex differences, males experienced a larger decrease in fast spindle amplitude compared to females, and this was a dose-response effect. Notably, severe sleep restriction (5hrs) led to a significant decrease of fast amplitude for both sexes, and females showed an inability to recover to baseline levels compared to males, even though the magnitude of females' amplitude deficit was smaller. Past adult research found gender differences in the relationship between spindles and intellectual performance, where females showed positive relationships between fast spindle amplitude and intelligence, while males showed non-significant negative relationships (Ujma et al., 2014). Combined with our small finding, this may indicate differing functionality of spindles between sexes, and further investigation of the implications of sleep loss on male and female performance in adolescent samples is warranted. However, it should be noted that sample sizes of each sex in each dose

were small (5hr: *n* = 12, 6 males; 7.5hr: *n* = 10, 6 males; 10hr: *n* = 12, 8 males), and these findings require confirmation with a larger sample.

A key strength of this study was the experimental manipulation of sleep duration across three "doses" of sleep. Past research into sleep spindle activity in response to sleep restriction has been conducted primarily with adults, and with a single dose of restricted sleep or sleep deprivation. The present study also examines several spindle parameters across both fast and slow spindles. There are several gaps, therefore, that have been addressed with this research. Of note, the present findings cannot be generalised beyond adolescent samples.

Notwithstanding barriers of scheduling adolescents in holiday periods, and needing considerable funding and resources, a future improvement on this research would be to use within-subjects' comparisons for dose-response effects, rather than between-subjects comparisons as used in this study, which introduces individual differences when making comparisons across conditions. Nonetheless, the use of change from baseline values for between-subject comparisons adjusted for individual differences at baseline to help alleviate this limitation. Future research would also profit from including more baseline nights in order to have reliable estimates of slow spindle density and duration from which to base findings (Reynolds et al., 2018 (**Chapter 3**)). Furthermore, it is likely that the 5hr condition would show a higher stage 2 spindle density reduction than the other doses given the overall loss in total sleep time and stage 2 reduction. A limitation of the present study is that varying total sleep durations were used across the three experimental groups, which in itself may impact the overall spindle variables. For example, both spindle frequency and sigma power (for slower frequencies) increase across the night (Campbell & Feinberg, 2016; Purcell et al., 2017). Sigma power is furthermore believed to show a reciprocal relationship with slow wave activity across the night, where slow wave activity decreases and sigma power increases (De

Gennaro & Ferrara, 2003). Future studies would therefore benefit from comparing across a NREM duration common to all doses.

There are also questions remaining about the implications of this research, which cannot be answered without investigation of performance outcomes (e.g., working memory and IQ). Future studies should examine the effect of sleep restriction on spindle activity in the context of cognitive performance. This is also relevant given the emerging body of research into the relationship between sleep spindles and cognition during adolescence (Geiger et al., 2011; Astill et al., 2014; Bodizs et al., 2014, Hoedlmoser et al., 2014).

Conclusion

There were significant changes in sleep spindle activity during severe sleep restriction to 5 hrs' sleep opportunity per night, where fast spindles became lower in amplitude and longer in duration compared to baseline. There were not, however, clear differences in slow spindle characteristics or fast spindle density. Adolescent sleep spindle activity appears to be affected by severe sleep restriction, and the nature of this effect warrants further investigation.

Chapter 5.

How does sleep loss affect sleep spindles and cognitive performance?

An experimental sleep restriction study in adolescents

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Author Contributions

CR. was the primary author, contributed to data collection and conducted scoring of sleep EEG, sleep spindle analysis and all statistical analyses. MG and MS contributed to revising of the chapter and statistical assistance. MS contributed to study design.

Abstract

The present study investigates the associations between adolescents' sleep spindle activity and cognitive performance and determines whether experimental sleep restriction has an impact on this relationship. Thirty-four adolescents (15-17yrs; M = 15.9 ± 0.9yrs, 14 females) participated in a 10-day laboratory study. Adolescents were allocated to one of three sleep 'doses': 5hrs, 7.5hrs or 10hrs of time in bed, for 5 consecutive nights, with one adaptation, one baseline and two recovery nights of 10hrs' time in bed either side. Working memory (operation span task), fluid intelligence (letter sets and number series tasks) and sustained attention (psychomotor vigilance task (PVT) lapses > 500ms) were assessed. Spindle and cognitive variables were not associated at baseline, contrary to expectations and past research. Fluid intelligence improvements across the study were associated with increased slow spindle amplitude for the 7.5hr condition (r = .84, p < .003), but not the other sleep dose conditions, which showed a performance ceiling effect, and indicate that sleep restriction did not influence this relationship. Working memory improved across the study, but was not related to spindle characteristics. Participants with longer baseline fast spindle durations trended to be protected from sustained attention lapses during severe (5hr: r_s = -0.55, p = .06) and moderate (7.5hr: r_s = -0.48, p = .16) sleep restriction. Previously reported static associations between sleep spindles and cognitive performance were not replicated. Changes to spindles and cognition during sleep restriction were not associated, although fast spindle duration may be protective of attentional losses.

Keywords: adolescence, sleep loss, EEG, cognition

Introduction

Adolescence is a developmental period in which sleeping patterns change dramatically, typically involving a reduction in total sleep time and concurrent impairments in daytime functioning (Fuligni et al., 2017; Leger et al., 2012; Lo et al., 2016). Sleep loss in adolescents can lead to poor concentration, emotional dysregulation, daytime tiredness, decreased motivation and poor academic performance (Chaput et al., 2016; Curcio et al., 2006; Shochat et al., 2014). Changes to the timing of adolescent sleep result from circadian and homeostatic changes conducive to delayed sleep timing (Carskadon et al., 2011; Jenni et al., 2005). Biological factors are compounded by psychosocial factors such as pre-bedtime technology use (Bartel et al., 2015; Hale et al., 2018; Reynolds et al., 2015), pre-sleep worry (Bartel et al., 2015; Danielsson et al., 2014) and homework completion (Gaina et al., 2005; Short et al., 2011). Considering the impact of sleep loss on adolescents' functioning, it is important to investigate the effect that sleep loss has on cognitive performance, and the mechanism through which this occurs. One potential mechanism is sleep spindles.

Sleep spindles are typically 11-16Hz electroencephalographic (EEG) waveforms seen in intermediate sleep (stage 2) (De Gennaro & Ferrara, 2003). These waveforms are created through oscillatory firing within the network connecting the thalamus to the cortex, known as the thalamocortical loop (De Gennaro & Ferrara, 2003). Because the thalamocortical network is integral to information processing during wakefulness, it is likely that sleep spindles reflect a mechanism through which memories are consolidated during sleep (Fogel & Smith, 2011). Spindles have been shown to correlate with cognitive performance in adult samples (Fogel & Smith, 2011) and more recently in adolescents (Geiger et al., 2011, Tessier et al., 2015, Bodizs et al., 2014), with two recent meta-analyses identifying small-to-moderate associations. In adolescents, positive associations were seen between overall sleep spindle characteristics and fluid intelligence (r = 0.44), working memory/executive function (r =

0.40), and cognitive speed and accuracy (r = 0.33), but not with full IQ (r = -.05) (Reynolds et al., 2018 (**Chapter 2**)). In another meta-analysis including a broader age range (4-79 years), only spindle amplitude (combined with sigma power) evidenced a significant association with general cognitive ability (r = 0.15) (Ujma, 2018). Notably, all studies meta-analysed were cross-sectional. It is therefore unclear whether the sleep loss seen across a typical school week has an impact on this relationship. An analysis of sleep spindle activity and cognitive performance in the context of experimental sleep restriction that simulates school week sleep loss (i.e., 5 consecutive days of restricted sleep) may elucidate a mechanism through which sleep loss affects academic performance.

Sleep spindles can be examined across several characteristics, including spindle density (number of spindles per minute), spindle duration (in seconds) and spindle amplitude (in Hz) (De Gennaro & Ferrara, 2003). These spindle characteristics are often analysed separately for slow and fast spindles, depending on relative frequencies, which have been shown to have functional differences. For example, slow spindles, seen predominantly in frontal derivations, show stronger associations with visual learning (Bang et al., 2014), declarative memory retention (Marshall et al., 2011) and heuristic creativity (Yordanova et al., 2017); while fast spindles, more commonly seen in central derivations, are linked to memory as well (Bodizs et al., 2008), but also more complex functions such as fluid intelligence (Bodizs et al., 2014) and learning ability (Lustenberger et al., 2012). In a previous paper, we found severe sleep restriction to 5hrs' time in bed for 5 consecutive nights led to longer fast spindle duration and a decrease in fast spindle amplitude in adolescents (Reynolds et al., 2018 (**Chapter 4**)). These findings are consistent with past studies of reduced sigma power following sleep restriction (Jenni et al., 2005, Voderholzer et al., 2011), which is associated with spindle amplitude (Tarokh et al., 2014). It is possible that each spindle characteristic (density, duration and amplitude) has a different function for cognitive

performance. Overall, both spindle density and amplitude have shown mostly positive associations with cognitive performance, while spindle duration has shown mostly negative relationships (Reynolds et al., 2018 (**Chapter 2**)). Notably, slow spindles are typically related to memory functions, while slow spindle amplitude is not affected by sleep loss (Reynolds et al., 2018 (**Chapter 4**)). If each spindle characteristic is differentially affected by sleep restriction, and these spindle characteristics are known to have different relationships to cognition, it is possible that experimental manipulation of spindles may reveal a mechanism through which some cognitive functions are impaired by sleep loss, while others are not. Further, spindles have been shown to protect against external noise disruptions to sleep (Dang-Vu et al., 2010), while at the same time improving overnight learning (Clemens et al., 2005). Thus, sleep spindles may have some protective effect on the loss of cognitive abilities through sleep restriction.

The present study will extend previous research by examining the adolescent spindles-cognition relationship using experimental sleep restriction in a 'dose-response' paradigm. In this study, cognitive performance measures that have shown moderate relationships with spindle characteristics will be examined (fluid intelligence and working memory; Reynolds et al., 2018 (**Chapter 2**)), as well as sustained attention, which has shown cumulative deficits from sleep restriction (Lo et al., 2016; Short et al., 2018), however has not been examined in relation to sleep spindles to date. Working memory has also shown deficits from experimental sleep restriction in adolescents (Lo et al., 2016), and fluid intelligence may be impaired by shorter sleep (< 6hrs) in young adults (Hicks et al., 1978) and by poor sleep quality in normal-sleeping adolescents (8hrs' habitual sleep duration; Johnston et al., 2010). Baseline associations are expected to be positive between working memory and fluid intelligence and spindle measures (Reynolds et al., 2018 (**Chapter 2**)), while the relationship

between spindles and cognition during dosed sleep restriction is examined in an exploratory nature, given the present study is the first to examine this relationship in this paradigm.

Method

Participants

34 adolescents (mean age = 15.9 ± 0.9yrs, 14 females) were recruited from South Australian school newsletters, provided informed consent and were remunerated for their time. Included adolescents were 15-17 years of age, late or post-pubertal (Tanner stage 4 or 5; Tanner, 1990, self-reported questionnaire: Petersen et al., 1988), and were free of medical and psychological disorders and medications (self-report). Participant chronotype was intermediate (scoring 23-43 on the Composite Morningness/Eveningness Scale; Smith et al., 1989), thus avoiding adolescents with strong morning preference, who experience decreased spindle amplitude and intensity (duration x amplitude; Merikanto et al., 2017). The study had ethics approval from the University of South Australia Human Research Ethics Committee.

Design

Adolescents attended the sleep laboratory at the Centre for Sleep Research (Adelaide, South Australia) for 9 nights (Friday 5:00pm until the following Sunday 9:00pm) during the South Australian school holiday period. Adolescents were unable to wear timekeeping devices and were thus unaware of the time during the study, and were also blinded to their condition.

Figure 5.1 outlines the study protocol. Adolescents received one adaptation and one baseline night, each with 10hrs' time in bed, and then entered the experimental phase for 5 consecutive nights. Adolescents were allocated to one of three sleep 'doses': 5hrs (n = 12, 6 females), 7.5hrs (n = 10, 4 females) or 10hrs (control condition, n = 12, 4 females) of time in bed. Two recovery nights of 10hrs' time in bed followed. Wake times on all days were kept consistent at 7:30am, and bedtime adjusted accordingly. This design mimicked the sleep

restriction that adolescents might experience in an average school week, with sleep restriction typically occurring during the school week and potential for sleep-ins on the weekend (Crowley et al., 2007; Carskadon et al., 2011; Lo et al., 2016).

Days 1-2: Adaptation and Baseline (n=34):

Clock time (24hrs)

12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11
										Adaptation Sleep (10hrs TIB)												W	P
				F						Baseline Sleep (10hrs TIB)												W	P

Days 3-8: Experimental phase

Severe sleep restriction (n=12):

12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11
															5hrs TIB							W	P

***or* Moderate sleep restriction (n=10)**:

12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11
												7.5hrs TIB										W	P

***or* Control condition (n=12)**:

12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11
												10hrs TIB										W	P

Days 9-10: Recovery (n=34):

12	13	14	15	16	17	18	19	20	21	22	23	0	1	2	3	4	5	6	7	8	9	10	11
				F						Recovery Sleep (10hrs TIB)												W	P
										Recovery Sleep (10hrs TIB)												W	P

Figure 5.1 Sleep restriction study protocol. Shaded bars indicate the sleep opportunity for the full sample at baseline and recovery, and during the experimental phase for the three sleep 'doses': 5hrs, 7.5hrs or 10hrs TIB (time in bed). Unshaded areas indicate wake time. W = working memory task (operation span task), F = fluid intelligence task (Letter Sets and Number Series Tasks), P = psychomotor vigilance task (PVT).

Materials

Polysomnography.

Polysomnographic recordings were conducted using the Compumedics Grael Sleep System (Melbourne, Australia) at a sampling rate of 512Hz. EEG derivations of Fp1, Fp2, F1, F2, C3, C4 and O1, all referenced to contralateral mastoid electrodes (M1 and M2), were used in the present study, as well as EOG and EMG. Adolescents were monitored overnight by an experienced sleep technician. Sleep recordings were scored according to standard sleep stage criteria (Rechtschaffen & Kales, 1968) by two independent technicians with high interscorer concordance ($\kappa = 82.5$).

Sleep spindles.

Sleep spindles in all NREM stages (2, 3 and 4) were analysed using an automated spindle detector, following the Individual Adjustment Method developed by Bodizs and colleagues (2009). Spindle characteristics of spindle density (number per minute of NREM sleep), spindle duration (length of the spindle in seconds) and amplitude (amplitude of the spindle midpoint, μV) were determined for each individual, and were divided into slow and fast spindles depending on individually-based peak frequencies. A spectral profile is generated for each individual sleep record, following rectification and smoothing of the filtered EEG signal (Bodizs et al., 2009). Slow and fast spindles are detected in the lowest and highest frequency peaks, respectively (mean slow frequency = 10.99 ± 0.72Hz; fast = 12.99 ± 0.41Hz). Individually-based spindle detection accounts for the trait-like features of spindles that differ between individuals (Bodizs et al., 2009) as well as the low night-to-night variation within each individual (Reynolds et al., 2018 (**Chapter 3**); Urakami et al., 2012). For 25% of sleep records, the slow peak was unclear, and limits were manually placed around the segment with the visibly highest power (Bodizs et al., 2009). Spindle characteristics analysed in the present study were fast spindle density, duration and amplitude, and slow

spindle amplitude. Slow spindle density and duration were excluded from analyses due to high night-to-night variability in these measures (single-night intraclass coefficients < 0.60; Reynolds et al., 2018 (**Chapter 3**)). It is important that there is not too much night-to-night variability in each spindle characteristic, as this would mean a single night's sleep recording (i.e. the single baseline night in this study) would not reflect the adolescent's typical spindle activity, and resulting analyses may therefore be inaccurate. EEG derivation C3-M2 was used for analyses, and C4-M1 was used when C3 was not suitable.

Cognitive Performance Measures

Working memory: Operation span task.

The operation span task (Ospan; Turner and Engle, 1989) was used to assess working memory capacity and processing abilities, which have been shown to relate positively to spindle activity in adolescents (Reynolds et al., 2018 (**Chapter 2**)). The present study used a computerized version of the test (Unsworth et al., 2005). Adolescents were required to complete a mathematical equation whilst memorising unrelated letters that appeared at the end of each equation. For example, adolescents were asked to consider "(5 x 8) + 2 = 40", respond that the equation was either 'true' or 'false', and were then presented with the letter "G." After a series of equation-letter pairs, adolescents were asked to recall as many letters as they could in the order of presentation. There were two practice trials and 15 test trials of 3 to 7 randomised equation-letter pairs. The final score followed the 'Absolute Ospan' scoring method, which is the sum of all perfectly recalled sets (Unsworth et al., 2005)[3]. The operation span task was given each day at 10am (Figure 5.1). The automated operation span task correlates well with other tests of working memory capacity, has good internal consistency (alpha = .78) and holds good test-retest reliability (.83) (Unsworth et al., 2005).

[3] If a participant correctly recalled 4 letters in a set size of 4, 5 letters in a set size of 5, but only 3 letters in a set size of 6, then their absolute ospan score is 9 (4+5+0) (Unsworth et al., 2005).

Fluid Intelligence: Letter Sets and Number Series tasks.

The letter sets (Ekstrom et al., 1976) and number series (Thurstone, 1938) tasks were given together as a battery to measure fluid intelligence components of inductive and deductive reasoning, which have been shown to relate positively to spindle activity in adolescents (Geiger et al., 2011; Bodizs et al., 2014; Tessier et al., 2015). For the letter sets task, adolescents were shown 5 groups of letters in strings of either 2, 3 or 4 letters long. Four of these groups followed a logical rule while one group did not, and adolescents were asked to circle the pair or group of letters that was the odd one out, e.g. "Circle the pair of letters that is the odd one out: JK, WX, FG, NM or CD"**.** The original test consisted of 18 test items, and this was split into 2 halves of 9 randomly allocated items so that adolescents completed one half at baseline and the second half following sleep restriction. For the number series task, adolescents were shown a series of 7 numbers that follow a logical rule, and had to provide the next number in the sequence, e.g. "What number comes next in the following sequence? 2, 5, 8, 11, 14, 17, 20, ___". Similar to the letter sets task, the number series task of 21 items was split into 2 randomly selected halves (one item excluded), with one half at baseline and the other following sleep restriction. The letter sets and number series tasks were given together on Days 3 and 8, both at 16:00 (Figure 5.1). Internal consistency for these measures is good, with Cronbach's alpha ranging from .91-.92 for letter sets and .85-.86 for number series (Csapo, 1997; Johnston et al., 2010).

Sustained Attention: Psychomotor Vigilance Task (PVT).

The psychomotor vigilance task was given to measure sustained attention and reaction time, at 3-hourly intervals across each day of the study (Figure 5.1). Adolescents were given a hand-held device (PVT-192; Ambulatory Monitoring, Ardsley, NY), including a small display and a response button. Adolescents were instructed to concentrate on the display and press the response button when a stimulus appears. Stimuli were presented at intervals of 2 to

10 seconds, throughout a test bout of 10 minutes. The present study used mean PVT lapses (reaction times > 500 milliseconds), indicating the adolescents' level of sustained attention, which is a sensitive indicator of sleep loss and is implicated in higher-order cognitive functions (Lim & Dinges, 2008). The PVT upholds excellent validity and reliability (Lim & Dinges, 2008) and has been used in past adolescent sleep studies (Wolfe et al., 2014; Lo et al., 2016). PVT trials analysed in the present study took place at 11:30am (Figure 5.1).

Statistical Analyses

All statistical analyses were conducted using IBM SPSS Statistics Version 22 (IBM Corp., Armonk, NY). The adaptation night was eliminated from analyses to account for the first-night effect (Rechtschaffen & Verdone, 1964). Baseline associations between spindle and cognitive variables were tested using non-parametric correlations (Spearman's rho), due to non-normal distribution of baseline cognitive performance data (skewness (standard error) = OpSpan: -0.84 (0.40); Letter Sets: -1.21 (0.40); Number Series: -1.21 (0.40); PVT: 1.26 (0.41)). In all cases, baseline sleep from night 2 of the study was analysed with the cognitive measure the following day (day 3 of the study), with the aim to provide a reflection of the adolescents' general network efficiency, rather than comparing cognition to spindles the night after the test, which may instead reflect that day's processing (Reynolds et al., 2018 (**Chapter 2**)). To adjust for multiple comparisons, the Bonferroni correction was applied for a set of 16 comparisons (4x spindle variables, 4x cognitive tests; 0.05/16 = .003), and the cut-off for statistical significance was thus reduced to $p < .003$. Linear mixed model (LMM) analyses were conducted to examine whether sleep restriction had an impact on the relationship between sleep spindles and cognitive performance. For LMM analyses, both spindle and cognitive variables were computed as a 'change from baseline' value, using night 2 for spindle variables, and day 3 for cognitive variables, as baseline. The analyses therefore excluded this baseline night, as all values equalled zero. The recovery phase was included in

analyses given past findings that indicate the deficits to spindle amplitude and duration (Reynolds et al., 2018 (**Chapter 4**)) and psychomotor vigilance (Short et al., 2018) during severe sleep restriction do not fully recover. The phases compared in the LMMs, therefore, were the sleep restriction phase, which consisted of the average of the 5 experimental nights, and the recovery phase, which consisted of the average of both recovery nights. All nights were included for working memory and sustained attention tasks. For the Letter Sets and Number Series tasks (fluid intelligence) which were given once at baseline and once during the experimental phase, only the last experimental night was included, as this related to the fluid intelligence battery the following day. Fluid intelligence analyses, therefore, only included the experimental phase. All models specified a random effect of participant ID. Models specified operation span task, Letter sets task, Number Series task and PVT lapses (all as change from baseline) as dependent variables, with fully saturated models (all main and interaction effects) for study phase (experimental phase, recovery) and sleep dose condition (5hrs, 7.5hrs, 10hrs TIB). Spindle characteristics of fast spindle density, duration and amplitude, and slow spindle amplitude (all as change from baseline) were added as covariates. Pairwise post-hoc analyses with least significant differences (LSD) were conducted to further investigate significant main and interaction effects.

Results

Sleep manipulation

Examination of total sleep time (TST) per condition during the experimental phase confirmed the manipulation (5hr: 289 minutes; 7.5hr: 426 minutes, 10hr: 527 minutes). See Supplementary Table S5.1 for descriptive statistics of sleep quality and quantity, spindle and cognitive variables.

Baseline associations between sleep spindles and cognitive performance

Correlation analyses revealed no significant associations between cognitive performance and spindle characteristics at baseline for the full sample, contrary to the expectations that working memory and fluid intelligence would be positively related to spindle variables as in past research (Reynolds et al., 2018 (**Chapter 2**)) (Table 5.1).

Table 5.1. *Correlation coefficients (p values) for sleep spindle and cognitive performance variables at baseline for the full sample (n=34).*

	Correlation coefficient r_s *(p)*
Operation Span Task (working memory)	
Fast density	.18 (.30)
Fast duration	.004 (.98)
Fast amplitude	-.17 (.35)
Slow amplitude	.20 (.26)
Letter Sets Task (fluid IQ)	
Fast density	.30 (.08)
Fast duration	.24 (.18)
Fast amplitude	.14 (.43)
Slow amplitude	.29 (.10)
Number Series Task (fluid IQ)	
Fast density	.05 (.79)
Fast duration	-.01 (.96)
Fast amplitude	.13 (.46)
Slow amplitude	.05 (.78)
Psychomotor Vigilance Task (sustained attention)	
Fast density	-.12 (.51)
Fast duration	-.07 (.72)
Fast amplitude	.15 (.41)
Slow amplitude	-.04 (.83)

Note. Correlations use Spearman's rho (r_s). Effect sizes are considered small at r = 0.1, medium at 0.3 and large at 0.5 and above (Cohen, 1988). Significance is corrected to $p < .003$, as per the Bonferroni correction.

Changes in sleep spindles and cognitive performance during sleep restriction

Working memory

Working memory scores improved in all groups across the experimental and recovery nights, $F(2,236) = 17.29$, $p < .001$. There was no relationship between working memory and changes in spindle characteristics and, furthermore, changes in working memory scores did not differ between sleep dose conditions.

Fluid Intelligence

Similar to working memory findings, fluid intelligence was not affected by sleep loss, and performance either remained the same or improved between baseline and sleep restriction. There was a significant main effect of sleep dose on change in fluid intelligence scores for the letter sets task, $F(2,28) = 7.76$, $p = .002$, where participants in the 7.5hr condition showed significantly greater improvements in letter sets performance than those in either the 5hr or 10hr conditions. This appeared to be due to the latter 2 conditions performing extremely well at baseline and thus there was a ceiling effect in these conditions. A similar pattern of more improvements in the 7.5hr condition occurred for the number series task, however the main effect of sleep dose in this case did not survive the Bonferroni correction ($p < .003$), $F(2,28) = 3.73$, $p = .04$. There was a significant 2-way interaction between sleep dose and change in slow spindle amplitude on change in letter sets scores, $F(2,28) = 8.05$, $p = .002$. Specifically, improvements on the letter sets task were significantly associated with increases of slow spindle amplitude, however this was only seen for participants in the 7.5hr condition ($r_s = .84$, $p = .0026$) and not the 5hr ($r_s = .09$, $p > .05$) or 10hr ($r_s = -.16$, $p > .05$) conditions (Figure 5.2).

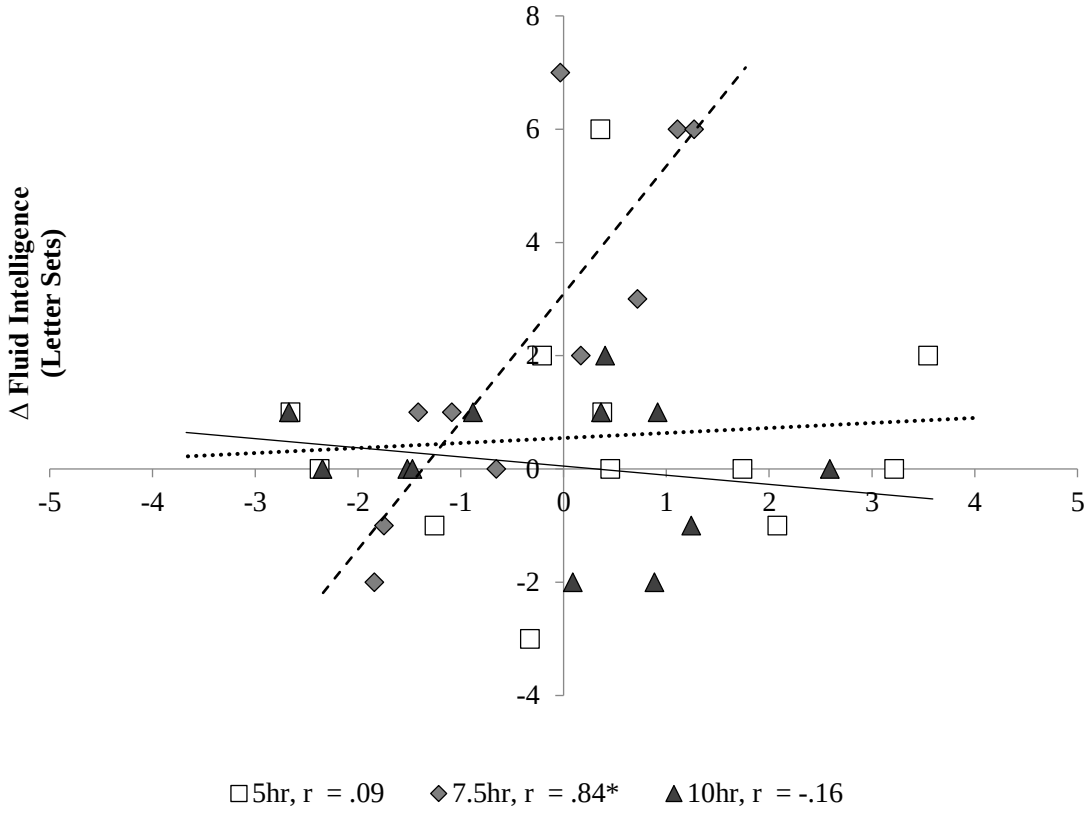

Figure 5.2. Differences between sleep doses (5hr, 7.5hr or 10hrs' time in bed) for the association between change in fluid intelligence (letter sets task) and slow spindle amplitude. Trendlines are dotted for 5hr, dashed for 7.5hr, and solid for 10hr conditions. *Correlations are significant at $p < .003$ (Spearman rank-order correlations)

Sustained Attention

Sustained attention showed an effect of sleep loss in this study, as reported previously (Short et al., 2018) [4]. Sleep spindle characteristics showed no relation to changes in sustained attention across the experimental or recovery phases. Considering sustained attention was the only cognitive domain affected by sleep loss, secondary analyses were performed to investigate whether there was a protective function of baseline spindle activity on changes to sustained attention during sleep restriction, given past research indicating spindles may be

[4] Sustained attention showed a clear dose-response, with the highest deficits seen in the 5hr condition, moderate deficits with 7.5hrs' time in bed, and the least deficits in 10hrs' time in bed (Short et al., 2018).

protective of sleep (Dang-Vu et al., 2010) and concurrently improve overnight learning (Clemens et al., 2005). Only those spindle characteristics that changed during sleep restriction were examined (fast spindle amplitude decreased and duration increased; Reynolds et al., 2018 (**Chapter 4**)). No significant associations were found, however strong effect sizes nonetheless emerged. During severe (5hr) and moderate (7.5hr) sleep restriction, baseline fast spindle duration was negatively correlated with PVT lapses during sleep restriction (5hr: r_s = -0.55, p = .06; 7.5hr: r_s = -0.48, p = .16), where higher lapses (i.e. worse performance) were associated with shorter duration of spindles. This indicates that for adolescents whose spindles were initially shorter, their sustained attention became worse during sleep restriction. Put differently, adolescents who had longer spindles at baseline experienced fewer deficits in sustained attention during sleep restriction. Fast spindle amplitude, however, was not related.

Discussion

Do sleep spindles relate to cognitive performance at baseline?

None of the cognitive variables related to spindle characteristics at baseline in the present study, contrary to past research. The absence of a baseline relationship between working memory and spindle characteristics in this study is particularly surprising, given the large number of studies showing this relationship across samples of young people (see Reynolds et al., 2018 (**Chapter 2**) for a review). However, it is possible that the present body of research has been influenced by heterogeneity in the specific working memory tasks used, as well as spindle detection methods, which greatly differ between most investigations of spindles and cognition (Reynolds et al., 2018 (**Chapter 2**); Ujma, 2018). Certainly, in the present study, performance on the operation span task did not relate to spindle characteristics, and this task is, to date, exclusive to the present investigation of spindles and cognition. Nonetheless, the present findings, including the lack of relationship for fluid intelligence at baseline, question the robustness of the overall phenomenon. However, it is noteworthy that

the letter sets task (fluid intelligence) in the present study had the most consistent effect sizes with spindle variables, all of which were small-to-moderate (fast density: $r = 0.30$, $p = .08$; fast duration: $r = 0.24$, $p = .18$; fast amplitude: $r = 0.14$, $p = .43$; slow amplitude: $r = 0.29$; $p = .10$). This is similar to meta-analysed effect sizes, where fluid intelligence had a moderate relationship with overall spindle characteristics ($r = 0.44$; Reynolds et al., 2018 (**Chapter 2**)) and general cognitive ability showed a small relationship ($r = 0.15$; Ujma, 2018). With a sample size of 34, only large correlations are likely to reach significance, and there is growing belief that sleep spindles may be able to explain a small, though consistent, amount of variance in cognitive abilities, which can be strengthened through collating and meta-analysing findings (Ujma et al., 2018). Cognitive abilities may rely on several neural mechanisms, and perhaps the thalamocortical system shows evidence of just one neural network through which sleep EEG may be connected to cognition (Ujma et al., 2014).

Is the relationship between spindles and cognition influenced by sleep restriction?

Overall, spindle characteristics were not related to cognitive variables across different doses of sleep restriction. Both working memory and sustained attention showed performance change across the week (improvements for working memory, and dose-response deficits to sustained attention; Short et al., 2018), and neither of these changes were influenced by sleep spindle characteristics. Only fluid intelligence improvements, as measured by the letter sets task, showed any relationship to spindle activity, and this was only for one spindle variable (slow spindle amplitude). Those in the 7.5hr condition showed better performance improvements and a concurrent strong positive association between improved fluid intelligence and higher slow amplitude across the study ($r = .84$), while the 5hr and 10hr conditions showed less improvement, and did not relate ($r = .08$ and -.21, respectively). This association is limited by the ceiling effects of the 5hr and 10hr conditions and relatively lower baseline performance of the 7.5hr condition, which furthermore prevents the present

study from detecting a dose-response effect. There may be an indication that when there is room for improvement in fluid intelligence performance, this is related to the amplitude of slow spindles, supporting previous findings of a relationship between fluid IQ and spindle activity (Bodizs et al., 2014). This requires confirmation with a larger sample size, however (7.5hr condition: n = 10).

The strong association between longer fast spindle duration at baseline and fewer sustained attention deficits during sleep restriction (5hr: r_s = -0.55; 7.5hr: r_s = -0.48), albeit not meeting significance with the present sample size, presents an interesting implication. Considering spindles become longer during severe sleep restriction (Reynolds et al., 2018 (**Chapter 4**)), spindle length may be protective of attentional deficits during sleep loss. This bears the question, why is spindle duration, in particular, protective of attention? The first explanation comes from the sleep-maintenance role of spindles (Dang Vu et al., 2010), where longer spindles may increase the likelihood of remaining asleep, therefore optimising sleep duration and consequently optimising cognitive faculties. A second explanation comes from a hypothesis about brain maturity. Past findings suggest that spindle duration is longer in childhood and shortens in adolescence (Nader & Smith, 2015; Scholle et al., 2007), suggesting those with longer baseline spindles in the present study may have been less developed than those with shorter spindles. The present findings hold an interesting similarity to a recent theory that less development may protect from cognitive performance deficits following sleep loss (Astill et al, 2012). In their meta-analysis, Astill and colleagues found sleep duration was not related to attention in children, as would be expected from adult literature, and posed a theory that children are somewhat protected from sensitivity to sleep loss due to insufficiently developed neuronal networks. Their argument was that certain networks (e.g. the default mode network and frontoparietal network) are essential for modulating attention in adults, however these are not developed in children, so children are

therefore not vulnerable to sleep-related deficits to those networks (Astill et al., 2012). The same could be true in the present study, where adolescents with longer spindles may have had less developed networks, and concurrently less sensitivity to attention deficits from sleep restriction. The present associations were likely underpowered, however, and this novel finding should be explored in future investigations.

Lastly, there were no effects seen in the recovery phase of the sleep restriction protocol, indicating that the past findings of incomplete recovery of sustained attention (Short et al 2018), and fast spindle amplitude and duration (Reynolds et al. 2018 (**Chapter 4**)), following severe sleep restriction were not related. Aside from the lack of consistent relationships between spindles and cognition, the absence of an effect of sleep loss on working memory adds to the literature showing sleep curtailment is not always associated with deficits in complex cognitive areas for adolescents (Anderson et al., 2009; Kopasz et al., 2010; Voderholzer et al., 2011). The preservation of slow wave sleep may help to explain this resilience (Voderholzer et al., 2011).

Future directions

The present findings indicate that there are minimal influences of sleep spindles on changes in cognitive performance across multiple days, and certainly there was no relationship between sleep spindles and any effects of sleep loss. Although our study included a larger sample (n = 34) than several other studies investigating spindles and cognition in older children and adolescents (Bodizs et al., 2014, n = 24; Chatburn et al., 2013, n = 27; Geiger et al., 2011, n = 14; Lustenberger et al., 2012, n = 15), the dose-response design, while a key strength of this study, resulted in smaller numbers in each condition. Nonetheless, the relationship between adolescent spindles and cognition has not yet been examined in the context of experimental sleep restriction that simulates restricted sleep across the school week, and the present study is a first to investigate this effect. Replication with a larger

sample size would be beneficial to confirm the effects. Similarly, the present sample size did not allow for sex comparisons, however this is a potential area for future research, given an indication from past findings that females show a stronger connection between fluid intelligence and spindle activity (Bodizs et al., 2014; Ujma et al., 2014). Lastly, the present study indicated that adolescents' fluid intelligence and working memory did not appear to be affected by sleep loss, with the latter contradicting a previous adolescent sleep restriction study that found deficits to working memory (verbal n-back tasks) after 7 consecutive nights of 5 hours' sleep opportunity (Lo et al., 2016). This could be explained by a sample difference, where participants in Lo et al.'s (2016) study had a lower nightly sleep duration in the week preceding the experimental protocol than the current study (approximately 8hrs vs 9.5-10hrs, respectively). Past research has indicated that participants who extend their sleep durations before sleep restriction experience a reduced impact of sleep loss (Rupp et al., 2009). Considering adolescents in the present study had an optimal time in bed of 10 hours (Carskadon et al., 1981) both in the week preceding the study and in the baseline nights, they may have experienced a buffer against deficits from sleep loss for working memory performance. This inconsistency should be examined further in experimental, laboratory-controlled studies such as the present study, with standardised cognitive measures, to confirm these effects and improve on the heterogeneity in the literature (de Bruin et al., 2017).

Conclusion

Overall, there was an indication in the present study that the relationship between adolescents' sleep spindles and cognitive performance is not influenced by sleep loss. Previous static relationships between spindle and cognitive variables (fluid IQ and working memory) could not be replicated. Furthermore, sustained attention and spindle activity were not related, although fast spindle duration may be protective of attentional losses during sleep restriction. In the absence of a ceiling effect, which was only the case in the 7.5hr condition,

there was a strong positive relationship between fluid intelligence improvements and increased slow spindle amplitude. The varying findings for separate spindle and cognitive variables in the present study provide support for heterogeneity in the literature (Reynolds et al., 2018 (**Chapter 2**)). Lastly, the lack of dose-response for the relationship between fluid intelligence and spindle variables indicates that these associations are immune to sleep loss, which, to date, had not been tested.

Supplementary Table S5.1. *Sleep parameters, sleep spindle characteristics, and cognitive performance scores (standard deviations) for baseline (BL), experimental/sleep restriction (SR) and recovery (RC) phases for the three sleep 'doses'.*

	Sleep Dose								
	5 hrs			7.5 hrs			10 hrs		
	BL	SR	RC	BL	SR	RC	BL	SR	RC
Sleep parameters									
TST (min)	550.4 (23.1)	288.5 (6.6)	565.8 (16.7)	542.4 (18.5)	426.2 (17.3)	538.3 (39.9)	539.0 (24.1)	536.7 (28.0)	520.0 (30.2)
SOL (min)	26.5 (21.4)	3.5 (3.5)	11.2 (10.8)	19.2 (14.2)	4.9 (4.7)	10.2 (8.9)	17.2 (8.7)	24.5 (16.9)	34.4 (21.2)
SE%	91.8 (3.9)	96.5 (2.0)	94.9 (2.8)	90.4 (3.0)	94.8 (3.8)	89.8 (6.6)	90.0 (4.0)	89.4 (4.7)	86.5 (5.0)
S1 (min)	32.3 (12.8)	8.8 (5.5)	21.4 (6.8)	23.0 (5.5)	16.7 (7.7)	29.6 (12.9)	30.0 (22.5)	32.7 (14.7)	33.3 (14.2)
S2 (min)	262.0 (28.3)	99.4 (18.3)	276.0 (23.9)	263.5 (36.2)	188.3 (27.0)	252.7 (31.6)	255.6 (32.7)	237.3 (38.5)	224.6 (45.0)
S3 (min)	41.4 (18.4)	24.6 (11.6)	42.1 (18.6)	44.4 (17.4)	38.6 (15.8)	50.9 (21.1)	49.0 (16.1)	51.8 (18.0)	53.7 (22.5)
S4 (min)	87.7 (19.4)	97.4 (19.0)	87.5 (18.5)	98.5 (19.9)	97.9 (18.4)	94.1 (17.4)	97.5 (25.9)	97.7 (29.7)	95.2 (30.7)
REM (min)	127.0 (27.4)	58.1 (19.1)	138.8 (19.2)	113.1 (28.1)	84.8 (18.4)	111.1 (25.2)	107.0 (22.1)	117.3 (22.1)	113.3 (23.1)
Spindle characteristics									
Fast Density (no. per min)	7.5 (0.9)	7.6 (1.0)	7.8 (1.0)	7.6 (0.8)	7.5 (1.0)	7.9 (0.6)	7.8 (0.9)	8.0 (0.9)	8.0 (0.9)
Fast Duration (seconds)	1.1 (0.1)	1.3 (0.2)	1.2 (0.2)	1.2 (0.1)	1.2 (0.2)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)	1.2 (0.1)
Fast Amplitude (μV)	7.2 (3.2)	5.7 (2.0)	6.5 (2.4)	6.4 (1.9)	6.3 (1.5)	6.8 (1.8)	7.6 (2.0)	7.1 (1.5)	7.3 (1.2)
Slow Amplitude (μV)	4.2 (2.0)	4.1 (2.0)	4.2 (1.9)	4.4 (1.9)	4.3 (1.9)	4.9 (2.0)	4.7 (1.6)	4.2 (1.5)	4.1 (1.3)
Cognitive performance									
Operation Span Task (working memory)	43.1 (11.1)	49.5 (16.6)	53.7 (17.6)	39.6 (15.6)	48.4 (15.1)	57.4 (12.3)	41.2 (18.9)	45.8 (17.9)	47.8 (15.5)
Letter Sets (fluid IQ)	7.33 (2.4)	7.92 (2.1)	-	4.7 (3.3)	7.0 (1.2)	-	7.8 (1.0)	7.9 (0.9)	-
Number Series (fluid IQ)	7.42 (1.2)	8.2 (1.2)	-	6.3 (2.0)	7.2 (1.2)	-	7.3 (1.4)	6.8 (1.4)	-
Psychomotor Vigilance Task (PVT) (sustained attention)	0.7 (0.9)	6.6 (7.7)	4.9 (5.5)	1.6 (1.9)	3.3 (3.2)	2.8 (2.2)	1.8 (2.2)	2.6 (2.8)	3.1 (3.4)

Note. TST= Total sleep time in minutes, SOL= sleep onset latency in minutes, SE= sleep efficiency (percentage of time in bed spent asleep), S1= stage 1 sleep, S2= stage 2 sleep, S3= stage 3 sleep, S4= stage 4 sleep, REM= rapid eye movement sleep. Values are averaged for each phase, so that baseline consists of baseline night 1, experimental/sleep restriction consists of the average of the 5 experimental nights, and recovery consists of the average of the 2 recovery nights. Letter Sets and Number Series tasks were given once at baseline and once at sleep restriction (no test at recovery).

Chapter 5.B.
Additional information from the sleep restriction study of Chapters 4 and 5

In the preceding Chapters 4 and 5, the spindle characteristics of investigation were spindle density, duration and amplitude, separated for slow and fast. Following completion of these chapters, an additional characteristic of mean central spindle frequency was available. At this stage, the frequency values were not added into the already completed chapters for analysis, however the values themselves are informative for Chapters 6 and 7. Thus, the mean values for slow and fast spindle frequency for the adolescent sample from Chapters 4 and 5 can be found below. Note that these values are presented for the baseline night (following adaptation, as in the preceding chapters), rather than during sleep restriction, which will be investigated in a future paper.

N = 34, mean age = 15.9 ± 0.9yrs (range 15-17yrs), 14 females

Slow spindle frequency: mean = 10.99Hz, standard deviation = 0.72Hz

Fast spindle frequency: mean = 12.99Hz, standard deviation = 0.41Hz

While Chapters 4 and 5 dealt with mid-adolescents' (age 15-17yrs) sleep spindles during sleep restriction, Chapters 6 and 7 will examine the developmental trajectory of spindles in a longitudinal study, starting at age 10. It is therefore helpful to have an idea of the mid-adolescents' spindle frequency values as a point of comparison for the frequency values (along with density, duration and amplitude) of the younger cohort in the following two chapters.

Chapter 6.

Sleep spindles across pre-adolescence:

A longitudinal investigation

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Author Contributions

CR. was the primary author, contributed to study design and data collection and conducted scoring of sleep EEG, sleep spindle analysis and all statistical analyses. MG and MS contributed to revising of the chapter, statistical assistance and study design.

Abstract

Adolescence involves significant cortical development and altered sleep patterns, with sleep spindles being one facet of EEG that may reflect these changes. Past studies are largely cross-sectional in design or investigate EEG power spectra exclusively, with scarce evidence of changes to specific spindle characteristics in the transition from childhood to adolescence. The present longitudinal study addresses these gaps by investigating spindle characteristics of 20 pre-adolescent boys every 6 months over an 18-month period (mean age at baseline = 10.3 ± 0.4yrs). Participants attended the sleep laboratory for one night of sleep monitoring every 6 months, during which slow and fast spindle characteristics of density, duration, amplitude and frequency were investigated. Fast spindle frequency significantly increased over the four time points, and slow frequency followed a similar trend, supporting previous findings, however no other spindle characteristic showed significant variation. Developmental changes in spindle characteristics may appear later in adolescence, aligning with expected synaptic pruning and network refinement, while early adolescence shows only small changes.

Keywords: Sleep spindles, adolescents, longitudinal, development, EEG

Introduction

Adolescence is a discrete developmental period in which significant changes occur to sleep timing and sleep quantity (Carskadon, 2011), as well as brain function and connectivity (Sisk & Foster, 2004). Sleep timing is expected to delay due to circadian rhythm delays and a slower build-up of sleep pressure across the day, while sleep quantity decreases due to delayed sleep timing and psychosocial factors, e.g., early school start times (Carskadon, 2011). These changes begin at around 9yrs of age and continue to 18-20yrs, largely involving increases in white matter and decreases in grey matter which are believed to indicate synaptic pruning and network refinement (Gogtay et al., 2004; Giedd et al., 2008; Hagmann et al., 2010; Paus et al., 1999; Peters et al. 2012; Sisk & Foster, 2004). The brain reorganisation that occurs in this developmental stage is argued to result in changes to sleep architecture, particularly an overall decrease in slow wave sleep and an increase in stage 2 sleep (Feinberg & Campbell, 2013). Changes to brain makeup during adolescence can be seen through altered electroencephalography (EEG) power (Feinberg & Campbell, 2013; Whitford et al., 2007). More recently, individual differences in white matter have been implicated in the makeup of sleep spindles (Bodizs et al., 2014, Piantoni et al., 2013). Specifically, individuals with higher sleep spindle power and density have higher white matter axial diffusivity in several areas of the brain, including the thalamus (Piantoni et al., 2013), which is responsible for spindle production (Steriade, 2006). Considering adolescents experience dramatic changes to grey and white matter through synaptic pruning, it is likely that spindle characteristics will reflect these changes.

In addition, sleep spindles have been implicated in a myriad of functions including protection from external disruptions to sleep (Dang Vu et al., 2010), synaptic plasticity (Urakami et al., 2012) memory improvements (Clemens et al., 2005; Gais et al., 2002; Hoedlmoser et al., 2014; Lustenberger et al., 2012; Prehn-Kristensen et al., 2011; Schabus et

al., 2008) and higher order functions which are developing in adolescence, such as fluid intelligence (Bodizs et al., 2014; Chatburn et al., 2013; Geiger et al., 2011; Tessier et al., 2015) and executive function (Chatburn et al., 2013). It is therefore important to firstly understand how sleep spindles change across adolescence as they may reflect the cognitive abilities of this developmental age group.

Sleep spindles are oscillatory waveforms commonly seen in stage 2 sleep, and less frequently in slow wave sleep, with a typical frequency of 11-16Hz in adults (De Gennaro & Ferrara, 2003). Spindles are commonly investigated in terms of their density (number per minute), duration (length in seconds), amplitude (μV) and frequency (Hz) (De Gennaro & Ferrara, 2003). Spindle activity is also estimated through EEG power in the sigma band (11-16Hz) (De Gennaro & Ferrara, 2003). Often, spindles are dichotomised into 'slow' and 'fast' spindles, depending on the relative frequencies (e.g. slow ~11-13Hz; fast ~13-16Hz). Slow spindles are more commonly seen in frontal areas, and fast spindles in centroparietal areas (De Gennaro & Ferrara, 2003). The dichotomisation of spindles is increasingly valued, given their differing topography, functionality, and developmental trajectory (Astill et al., 2014; Bodizs et al., 2014; Marshall et al., 2011). As spindle frequency in children and adolescents is often slower than that of adults (Nicolas et al., 2001), individually-based frequency limits are thus preferred to provide more accurate estimates of spindle characteristics (Bodizs et al., 2009). The occurrence of spindles in the human EEG is seen by 2 months of age and changes dramatically in the first 5 years of life (McClain et al., 2016; Tanguay, 1975). Spindle characteristics remain relatively stable during middle childhood, until large changes occur once again during adolescence (Clawson et al., 2016; Shinomiya et al., 1999). However, we still do not know about changes in spindle characteristics in the transition from late childhood to early adolescence.

Spindle makeup in the developmental period of adolescence has become an area of increasing interest in the past decade, although methods and findings are significantly varied. Of the studies investigating an impact of age on spindle activity in older childhood and adolescence, most have been cross-sectional (Bodizs et al., 2014, Chatburn et al., 2013, Jenni & Carskadon, 2004; Gruber et al., 2013; Hoedlmoser et al., 2014; Kurth et al., 2010; Nader & Smith, 2015; Nicolas et al., 2001; Scholle et al., 2007; Shinomiya et al., 1999), and only three studies to date have been longitudinal. Two of these looked at spectral analysis (Campbell & Feinberg, 2016; Tarokh & Carskadon, 2010), which does not take into account the range of frequencies within spindles or provide information about specific spindle characteristics (Tarokh et al., 2014). The third, published only recently, measured children's spindles at two intervals, spaced 7 years apart (8-11-year-olds at baseline and 14-18-year-olds at follow up) and focused on spindle density and frequency (Hahn et al., 2018). Despite differing methodologies across these studies, there are several similarities in findings. The *frequency of spindles* appears to increase across older childhood and adolescence in most studies (Campbell & Feinberg, 2016; Hahn et al., 2018; Jenni & Carskadon, 2004; Kurth et al., 2010; Purcell et al., 2017; Scholle et al., 2007; Shinomiya, 1999; Tarokh & Carskadon, 2010) and has been seen to increase over the lifespan (Nicolas et al., 2001), with the exception of one study that found spindle frequency was negatively correlated with age in a sample of 7-11-year-olds (Gruber et al., 2013). *Spindle duration*, on the other hand, often decreases during adolescence (Nader & Smith, 2015; Scholle et al., 2007) and across the lifespan (Martin et al., 2013; Nicolas et al., 2001). These findings, again, are opposite to Gruber and colleagues' (2013) sample, who showed higher spindle duration with higher puberty scores. *Spindle amplitude* (Nader & Smith, 2015; Shinomiya et al., 1999) and *sigma power* are closely related, and both decrease during adolescence (Jenni & Carskadon, 2004; Shinomiya et al., 1999; Tarokh & Carskadon, 2010), as well as over the lifespan (Martin et al., 2013). Findings

for spindle density are less clear and appear to be discrepant for slow and fast frequencies. Fast spindles appear to increase in number during adolescence (Bodizs et al., 2014; Nader & Smith, 2015), while slow spindles decrease (Nader & Smith, 2015). Other research has shown that overall spindle density increases across childhood and adolescence until early adulthood, and subsequently decreases (Purcell et al., 2017), indicating a likely fluctuation in adolescent samples that might explain discrepant findings. Once in adulthood, spindle density decreases over the lifespan (Martin et al., 2013; Nicolas et al., 2001).

Of note, some investigations have found no influence of age on some aspects of older children's and adolescents' spindle activity (Bodizs et al., 2014; Chatburn et al., 2013; Kurth et al., 2010). Overall, there are similar findings for many aspects of spindle makeup across adolescence, however findings are not always consistent, and this may be attributed in part to differences in ages sampled. Dramatic changes in EEG have been shown to occur in the transition into adolescence (Clawson et al., 2016; Feinberg & Campbell, 2013; Whitford et al., 2007). Furthermore, spindles are known to have strong trait-like characteristics within individuals (Reynolds et al., 2018 (**Chapter 3**)), while being highly variable between individuals (Bodizs et al., 2009). Cross-sectional studies may thus be limited by the noise introduced by different ages, and therefore less able to identify developmental changes compared to longitudinal studies.

In order to improve our understanding of the developmental trajectory of spindle activity during adolescence, the present study will investigate spindle activity using a longitudinal design. Furthermore, the present study will examine spindle activity in the transition from childhood to adolescence, commonly known as the pre-adolescent period (Corsaro, 2017). It is expected that, with increasing age, spindle frequency will increase (e.g. Campbell & Feinberg, 2016; Hahn et al., 2018; Tarokh & Carskadon, 2010), while decreases will be seen for spindle duration (Martin et al., 2013; Nader & Smith, 2015; Nicolas et al.,

2001; Scholle et al., 2007) and amplitude (Jenni & Carskadon, 2004; Martin et al., 2013; Nader & Smith, 2015; Shinomiya et al., 1999; Tarokh & Carskadon, 2010). Spindles will be examined in a dichotomised fashion (slow vs fast), and, as per previous findings, fast spindle density is expected to increase (Bodizs et al., 2014; Nader & Smith, 2015), while slow spindle density is expected to decrease (Nader & Smith, 2015). Spindle frequency, amplitude and duration have not been separated into slow and fast frequencies in previous studies of age-related changes in adolescents and will therefore be examined in an exploratory nature.

Method

Participants

20 pre-adolescent boys participated, with laboratory participation taking place at four time points over an 18-month period (T1: mean age = 10.3 ± 0.4yrs; T2: mean age = 10.9 ± 0.4yrs; T3: mean age = 11.4 ± 0.4yrs; T4: mean age = 11.9 ± 0.3yrs). The baseline age of 10 years accounts for the potential onset of puberty in boys, which can begin between 10-12 years of age (Walvoord, 2010), and has been indicated as a minimum age from which developmental changes can affect sleep (Carskadon et al., 1993). Females were excluded from the study due to the influence of menstrual cycles on sleep (Driver & Baker, 1998) and spindle frequency (Ishizuka et al., 1994) and to reduce the impact of more variable, and potentially earlier, pubertal onset in girls (Kaplowitz et al., 2001). Participants were recruited from newsletters in primary schools in South Australia, through advertisements on social media, and through referrals from other participants. Interested parents gave screening information via a telephone interview using the Sleep, Medical, Education and Family History form. Included participants at baseline were 10 years of age or within 2 months of turning 10 or 11, had no parent-reported medical or psychological disorders and were not taking medications. Participants were healthy sleepers, with no evidence of difficulties initiating or maintaining sleep, excessive daytime sleepiness, snoring, sleep disordered

breathing or parasomnias. All participants had a typical sleep duration longer than 9 hours, which is within the recommended range for school-aged children (6-13yrs) (Hirshkowitz et al., 2015), showed evidence of stable sleep patterns (<1hr difference in sleep times between weeknight and weekends), and had a similar chronotype to other healthy school-aged children (Werner et al., 2010), as measured by sleep diaries (free day sleep midpoint: 02:17 ± 34 mins). Ethics approval was granted by the Flinders University Social and Behavioural Research Ethics Committee. Parents and participants provided written informed consent and participants received remuneration for their time.

Design

Participants attended the sleep laboratory for sleep monitoring once every 6 months. A 6-month time period was chosen to capture potentially rapid changes in sleep timing and puberty in adolescence. Sleep studies were held on weekends during the school term to maximise consistency in routines. Participants received one night of 10 hours' time in bed (TIB), which allows for optimal sleep opportunity (Carskadon et al., 1981). The timing of the sleep period was kept constant (bedtimes at 21:30, wake times at 07:30).

Materials

Polysomnography

Sleep was recorded using ambulatory Compumedics Somte PSG systems (Version 2; Compumedics Ltd., Victoria, Australia) connected via Bluetooth wireless transmission to allow real-time sleep monitoring, at a sampling rate of 200Hz. The present study used EEG derivations C3, C4, F3, F4, O1 and O2 referenced to contralateral mastoids (M1 and M2), as well as electrooculogram (EOG) and electromyogram (EMG). Participants were monitored by an experienced sleep technician. Sleep was scored according to standardised criteria (Rechtschaffen and Kales, 1968).

Sleep spindles

Sleep spindle characteristics were scored using the Individual Adjustment Method (Bodizs et al., 2009), which generates each participant's spectral profile to provide individual-specific power peaks in the slow and fast frequency ranges from which to base spindle detection. This addresses the strong reliability of spindle characteristics within individuals (Reynolds et al., 2018 (**Chapter 3**)) as well as the trait-like features of spindles that vary between individuals (Bodizs et al., 2009). For 8 participants, the slow frequency peak was not clearly depicted on the spectral profile, similar to previous adolescent studies (Jenni & Carskadon, 2004; Reynolds et al., 2018 (**Chapter 3**); Shinomiya et al., 1999), and in these cases the range showing the highest power was visually estimated on which to base slow spindle detection. There were no cases where fast spindle peaks were unclear. Non-REM stages 2, 3 and 4 were used to detect spindles. Spindle analyses were based on the C3-M2 derivation, and when this was not suitable, C4-M1 was used. Spindle characteristics included spindle density (the total number of spindles per minutes of non-REM sleep), spindle duration (the average duration (seconds) of each spindle), spindle amplitude (the average amplitude taken from the midpoints of all detected spindles, using the rectified signals) and spindle frequency (the average frequency of detected spindles), all of which were separated for slow and fast spindles.

Puberty

The Pubertal Development Scale (PDS; Carskadon & Acebo, 1993) was used as a self-report measure to estimate pubertal development. The scale includes 5 items that assess physical components of development, e.g. "Have you begun to grow hair on your face? a) not yet started, b) has barely started, c) has definitely started, d) growth seems complete or e) don't know". The total score of 3 of the 5 items (body hair growth, facial hair growth and voice change) is created to give a 'Puberty Category Score', and this is converted to an

equivalent Tanner stage (Tanner, 1990; Carskadon & Acebo, 1993). The PDS was administered at each time point during the weekend laboratory stay.

Procedure

Participants and their parents were given a tour of the sleep laboratory prior to the weekend stay to familiarise them with the environment, bedrooms and equipment, with an aim to reduce potential first-night effects (Rechtschaffen & Verdone, 1964). Sleep-wake patterns were monitored for 2 weeks prior to the laboratory study using sleep diaries and activity watches (Actigraphy; MicroMini-Motionlogger, Ambulatory Monitoring Inc., Ardsley, NY, USA), with 1 week of typical sleep patterns and 1 week of a stabilised sleep schedule with 10 hrs' TIB (21:30-07:30) to ensure adequate sleep during the week preceding the study. Participants attended the Flinders University Sleep Laboratory for one weekend "sleep camp" (Friday 17:00 – Sunday 12:30) at each 6-month time point. The Friday night's sleep data (21:30-07:30; 10 hrs' TIB) are investigated in the present study. The sleep laboratory was sound-attenuated, light (<20 lux) and temperature (23°C ± 1°C) controlled, and free of time cues. During wake periods, participants completed cognitive testing as part of another study, had set meal times and had free periods where participants played video and board games and watched films. Participants were supervised at all times.

Statistical Analyses

Statistical analyses were performed using IBM SPSS Statistics Version 22 (IBM Corp., Armonk, NY). Linear mixed model analyses (LMM) were conducted to test for changes in sleep architecture and spindle characteristics over the four time points of the longitudinal study (T1, T2, T3 and T4), accounting for within- and between-participant variance (Van Dongen et al., 2004). Due to the time constraints of the thesis, only 12 participant's data for Time 4 were able to be included. Nevertheless, LMM is not dependent upon having a complete data set and takes missing data into account (Van Dongen et al.,

2004). All models specified a random effect of ID, dependent variables of slow and fast spindle density, duration, amplitude and frequency, with fully saturated models (main effects and interactions) for time (T1, T2, T3, T4). Puberty was also included as a covariate in the model to determine whether there was an influence of puberty on spindle variables, which has been found in past research (Gruber et al., 2013; Jenni & Carskadon, 2004; Shinomiya et al., 1999). Where puberty did not show significant main effects or interactions with spindle variables, it was removed from subsequent analyses. Pairwise post-hoc analyses with least significant differences (LSD) were performed to further examine significant main effects.

Results

Participant Characteristics

Pubertal status showed an initial increase from baseline to the second time point, and puberty scores remained constant between the 2nd, 3rd and 4th time points, $F(3,49) = 3.74$, $p = .02$. Overall scores indicated the adolescents met criteria for an equivalent Tanner stage 1 at baseline (pre-pubertal: a Puberty Category Score of 3; Carskadon & Acebo, 1993), and Tanner stage 2 (early puberty) at all other time points (score of 4) (mean puberty scores: T1 = 3.90 ± 1.52; T2 = 4.90 ± 1.52; T3 = 4.89 ± 1.20; T4 = 4.92 ± 1.51). Chronotype, as measured by the sleep mid-point on free days, did not differ significantly over time, $F(3,47) = 2.36$, $p = .08$, but suggested a small delay of 17 mins at time 4 (T1 = 02:17 ± 34 mins; T2 = 02:16 ± 31 mins; T3 = 02:15 ± 30 mins, T4 = 02:32 ± 43 mins).

Sleep Characteristics

Sleep characteristics for the 20 pre-adolescents are presented in Table 6.1. LMMs revealed that none of the parameters for sleep quality changed significantly across the three time points: total sleep time (TST), $F(3,49) = 2.17$, $p = .10$; sleep onset latency (SOL), $F(3,49) = 1.98$, $p = .13$; wake after sleep onset (WASO), $F(3,49) = 2.07$, $p = .12$; sleep efficiency (SE), $F(3,49) = 2.63$, $p = .06$). Similarly, the proportion of sleep stages did not

differ significantly across the three time points: stage 1, $F(3,51) = 0.74$, $p = .53$; stage 2, $F(3,49) = 1.74$, $p = .17$; stage 4, $F(3,49) = 0.70$, $p = .56$; REM, $F(3,50) = 0.82$, $p = .49$, except for stage 3 sleep, which was significantly lower at T1 compared to T3, $F(3,51) = 3.16$, $p = .03$. The total minutes of each stage of sleep are shown in Figure 6.1.

Table 6.1. *Mean sleep parameters (standard deviations) for each time point, with sleep stages presented as a percentage of total sleep*

Time	TST (min)	SOL (min)	WASO (min)	SE (%)	S1%	S2%	S3%	S4%	REM%
T1 (baseline)	514.1 (44.6)	27.7 (14.0)	53.4 (38.8)	86.4 (7.4)	6.1 (2.4)	37.9 (10.8)	9.9 (3.8)	24.8 (8.7)	21.0 (3.3)
T2 (6 months)	501.9 (38.0)	31.2 (19.1)	65.9 (38.8)	83.8 (6.4)	6.8 (3.4)	38.0 (8.4)	10.0 (3.1)	25.0 (6.9)	20.2 (3.7)
T3 (12 months)	496.2 (38.1)	28.2 (13.6)	74.8 (43.2)	82.8 (6.4)	5.9 (3.4)	34.4 (8.8)	12.9 (5.6)	27.0 (5.6)	19.9 (4.8)
T4 (18 months)	515.9 (55.5)	22.6 (15.0)	60.1 (55.9)	86.2 (9.3)	5.4 (3.4)	34.6 (7.6)	12.2 (3.6)	26.5 (7.4)	21.3 (3.9)

Note. TST = Total sleep time, SOL = sleep onset latency, WASO = time spent awake after sleep onset, SE = sleep efficiency (percentage of time in bed spent asleep), S1 = stage 1 sleep, S2 = stage 2 sleep, S3 = stage 3 sleep, S4 = stage 4 sleep, REM = rapid eye movement sleep.

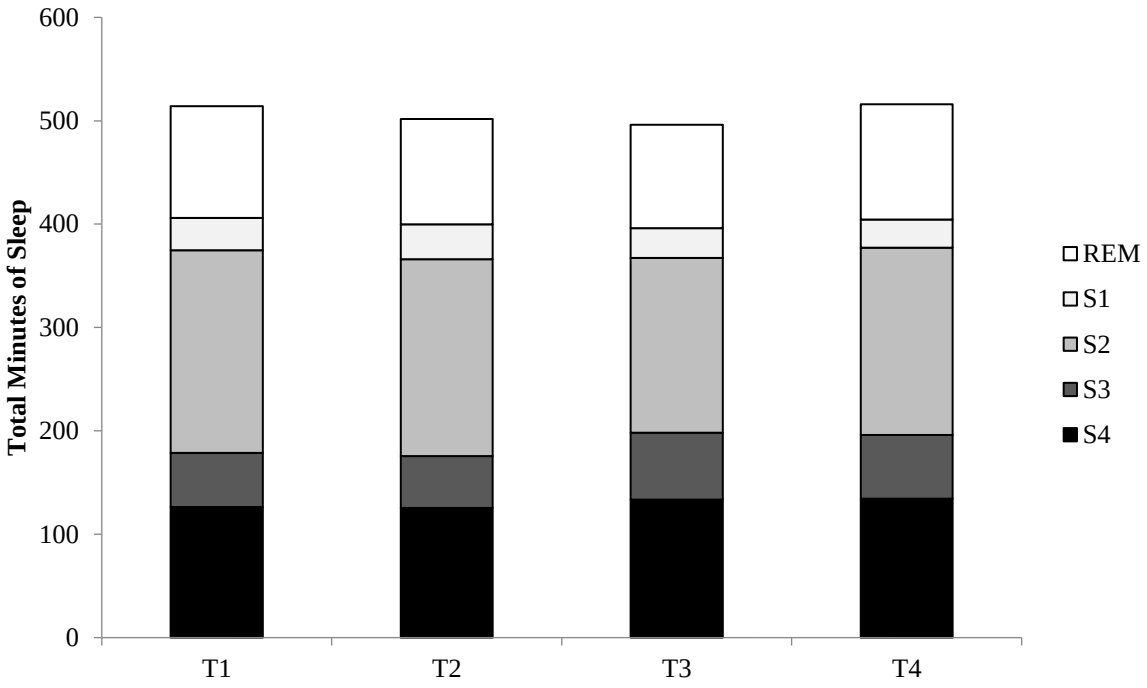


Figure 6.1 Total minutes of stages of sleep at each time point (T1 (baseline), T2 (6 months), T3 (12 months), T4 (18 months)). REM= rapid eye movement sleep, S1= stage 1 sleep, S2 = stage 2 sleep, S3 = stage 3 sleep, S4 = stage 4 sleep.

Sleep Spindle Characteristics

A graphical representation of the changes in spindle characteristics over time is presented in Figure 6.2, and inferential statistics are presented in Table 6.2. Exact values for each spindle characteristic can be found in Supplementary Table S6.1. Puberty did not have a significant influence on spindle characteristics and was therefore removed from analyses. There was a significant main effect of time on fast spindle frequency, where frequency became higher over time, as expected. There was a non-significant trend (p = .14) for slow spindle frequency to also increase over time. A graphical depiction of changes in slow and fast spindle frequency for each individual at each time point can be found in Supplementary Figure S6.1 There were no significant main effects of time on any other spindle characteristics, contrary to expectations.

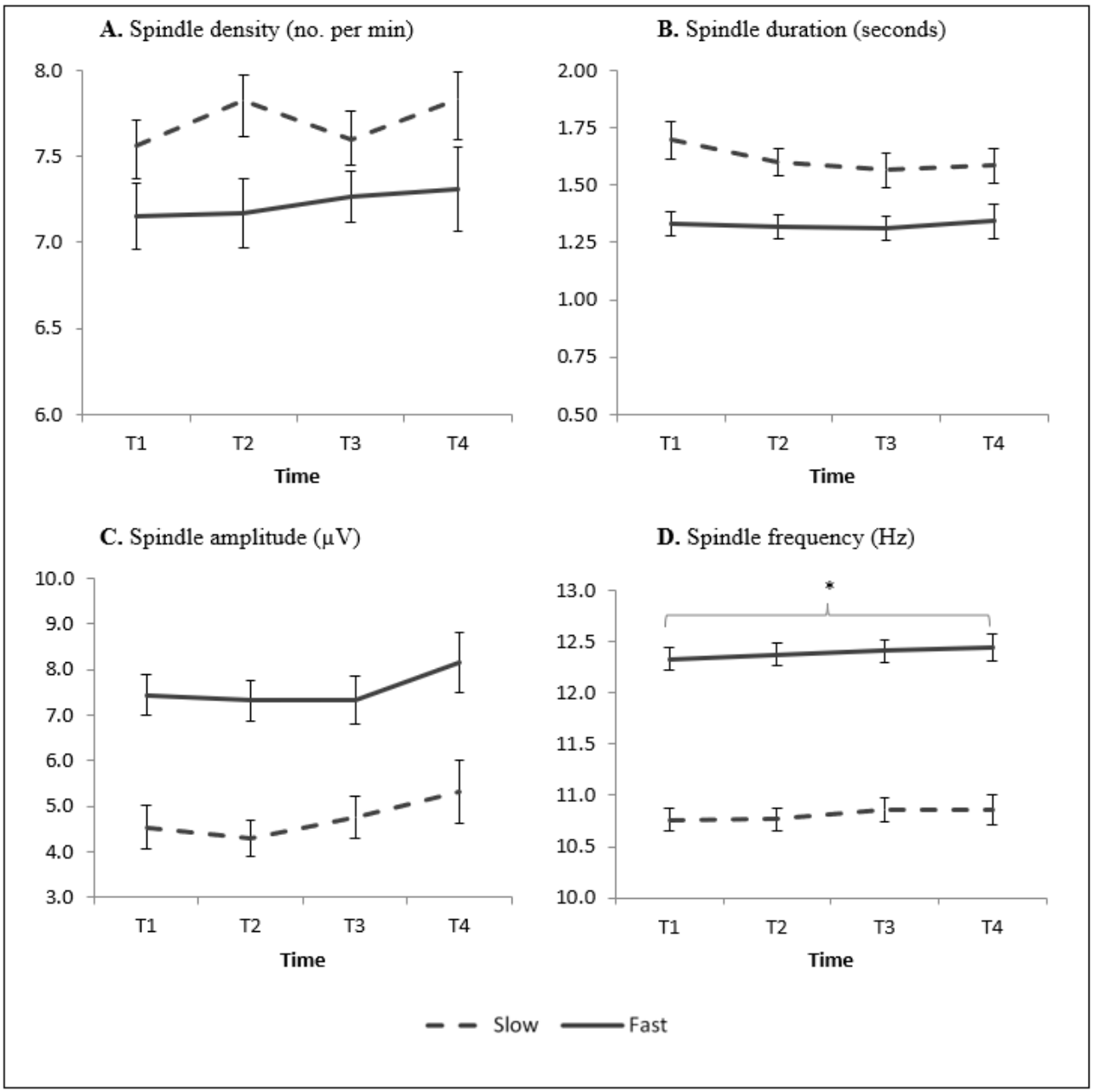


Figure 6.2. Spindle characteristics (mean and standard error) across the four time points (T1 (baseline), T2 (6 months), T3 (12 months), T4 (18 months))

Table 6.2. *Main effects of time (T1 (baseline), T2 (6 months), T3 (12 months), T4 (18 months)) on sleep spindle characteristics.*

	F-value	df	p	Effect size (*d*) for time: T1-T4	Post-hoc comparisons
Slow density (no. per min)					
time	1.19	3,48	.32	0.46	
Fast density (no. per min)					
time	0.26	3,49	.85	0.19	
Slow duration (seconds)					
time	1.31	3,49	.28	0.34	
Fast duration (seconds)					
time	0.07	3,48	.98	0.04	
Slow amplitude (μV)					
time	1.27	3,48	.30	0.34	
Fast amplitude (μV)					
time	0.11	3,48	.95	0.34	
Slow frequency (Hz)					
time	2.02	3,45	.13	0.20	
Fast frequency (Hz)					
time	6.69	3,48	**.001***	0.24	T1 < T2 < T3 < T4

Note: *d* = Cohen's d: small 0.2, medium 0.5, large 0.8
*$p < .01$

Discussion

Overall, sleep spindle characteristics did not change considerably in the young adolescent period. Fast spindle frequency was the only characteristic to show a significant change across the 18 months of the study, which was in line with expectations and past research (e.g. Campbell & Feinberg, 2016; Jenni & Carskadon, 2004; Kurth et al., 2010; Purcell et al., 2017; Tarokh & Carskadon, 2010). The mean values correspond with an increase in fast spindle frequency of about 0.1Hz per year. Although it was a small effect (*d* = .24), this provides initial support for the theory that spindle makeup starts to change in this age group, potentially related to increases in white matter (Bodizs et al., 2014; Piantoni et al., 2013), during myelination and synaptic pruning that is believed to occur during adolescence (Feinberg & Campbell, 2010; 2013). This is in line with neuroimaging research which shows

adolescent males experience significant increases in white matter, but a slower decrease in grey matter during adolescence, compared to their female counterparts (Giedd, 2008). The current findings present an interesting question: Why might fast spindle frequency be the first characteristic to show developmental changes? Past studies that have also found increases in spindle frequency with development have theorised that this may indicate maturation of the thalamocortical network (Jenni & Carskadon, 2004; Tarokh & Carskadon, 2010) or occur due to changes in the threshold for calcium spikes in the thalamus (Nicolas et al., 2001), yet it is still unclear why spindle frequency might be the first characteristic to change, or in fact whether that finding is specific to the present study. A more recent study suggested changes in spindle frequency from childhood to adolescence might be the underlying mechanism for observed relationships between spindle density and memory consolidation (Hahn et al., 2018), so perhaps changes in spindle frequency drive subsequent changes in other spindle characteristics. This presents an interesting opportunity for future research to determine the neurological underpinning and functional implication of developmental changes to spindle characteristics.

Looking at the raw data, three of the spindle characteristics appeared to have a consistent linear change as expected from past research, with non-significant small-to-medium effects: slow spindle frequency increased over time (d = .20) (Jenni & Carskadon, 2004; Kurth et al., 2010; Nicolas et al., 2001; Shinomiya, 1999; Tarokh & Carskadon, 2010), while slow duration decreased (d = .34) (Martin et al., 2013; Nader & Smith, 2015; Nicolas et al., 2001; Scholle et al., 2007), and fast spindle density increased (d = .19) (Bodizs et al., 2014; Nader & Smith, 2015). The implication from the present findings is that early adolescence may not involve as robust changes to spindle makeup as initially expected and, perhaps, these changes are more likely to be seen in mid-to-late-adolescence. Indeed, younger samples have found no evidence of an effect of age on spindle activity (Chatburn et

al., 2013: age range 4-12yrs; Hoedlmoser et al., 2014: 8-11yrs), and in fact sometimes show the opposite to the broader literature (Gruber et al., 2013: 7-11yrs, spindle frequency decreased with age). Past adolescent studies with older samples, on the other hand, have found more consistent associations between age and spindles or sigma power (Bodizs et al., 2014; Jenni & Carskadon, 2004; Nader & Smith, 2015; Tarokh & Carskadon, 2010), indicating that sleep spindles may indeed be "trait-like markers of maturity" (Astill et al., 2014, p. 9), in mid-to-late-adolescence. For young adolescents in the present study, fast spindle frequency was the strongest indicator of maturation, and it remains to be seen when and how the other spindle characteristics change. On visual inspection of the individual participants' developmental trajectories for slow and fast spindle frequency (Supplementary Figure S6.1), there appears to be more inter- and intra-individual variation in slow, compared to fast, frequency. This echoes earlier findings where adolescents' fast spindle characteristics showed more consistency within individuals compared to slow characteristics (Reynolds et al., 2018; **Chapter 3**).

Considering the present findings did not indicate robust changes to spindle characteristics, the mean spindle values for other adolescent samples were considered as a point of comparison to illustrate a potential trajectory. Figure 6.3 presents the current study's spindle frequency findings alongside other mean values in the literature. Firstly, the present study's baseline value for fast spindle frequency (12.3Hz) was identical to that of pre-pubertal children (mean age = 11yrs) in Jenni and Carskadon's (2004) study, while post-pubertal adolescents (mean age = 14yrs) had a fast spindle frequency of 12.9Hz (Jenni & Carskadon, 2004). The present study's adolescents at time 4 (mean age = 11.9yrs) had a fast spindle frequency of 12.4Hz, and, using the corresponding increase of 0.1Hz per year, are expected to have a fast spindle frequency of 12.7 by 14 years of age, seeming to align with the expected trajectory. Studies of older adolescents fit with this same increasing trajectory

(Figure 6.3; Bodizs et al., 2014; Shinomiya et al., 1999), as well as our previous study of 15-17-year-olds (**Chapter 5.B**, fast spindle frequency during optimal sleep opportunity (10hrs' time in bed) was 12.99Hz). For the studies that considered an overall spindle frequency, this too aligned with the present study's trajectory, but also grouped with relatively faster frequencies (Figure 6.3), confirming that fast spindles are often more prominent than slow spindles (Astill et al., 2014; Jenni & Carskadon, 2004; Reynolds et al., 2018 (**Chapter 3**)). Of note, studies using 'overall' frequencies are more numerous than those comparing slow and fast frequencies, illustrating the paucity of studies that dichotomise spindle frequencies, which presents several issues, as described in the next section. Interestingly, the presently observed minimal changes in spindles over time are parallel to findings from recent cross-sectional (Purcell et al., 2017) and longitudinal (Campbell & Feinberg, 2016) research, which also found minimal changes in the respective study periods and can be found in Figure 6.3. For further descriptive purposes, the spindle characteristics that showed a consistent linear trajectory in the present study (fast spindle density and slow spindle duration) were compared to other mean values in the literature. Fast spindle density for the present sample was expected to continue its linear increase from 7.3 spindles per minute at time 4 to 7.8 spindles per minute by 15 years of age (an increase 0.1 fast spindles per year), which aligns with Reynolds and colleagues' (2018 (**Chapter 3**)) 15-17-year-old adolescents' fast density of 8.0 spindles per minute and Bodizs and colleagues' (2014) fast density of 8.11 in 15-22-year-olds. Similarly, slow spindle duration at time 4 in the present study (1.58 seconds), is expected to continue a linear decrease of 0.3 seconds per year, aligning with Reynolds et al. (2018 (**Chapter 3**); 1.53s) and Bodizs et al. (2014; 1.39s). It is difficult to compare mean spindle values between studies that have used different spindle detection methods, as the criteria for spindle detection can influence the resulting values (Causa et al., 2010;

Huupponen et al., 2007), and the above comparisons are therefore for illustrative purposes only.

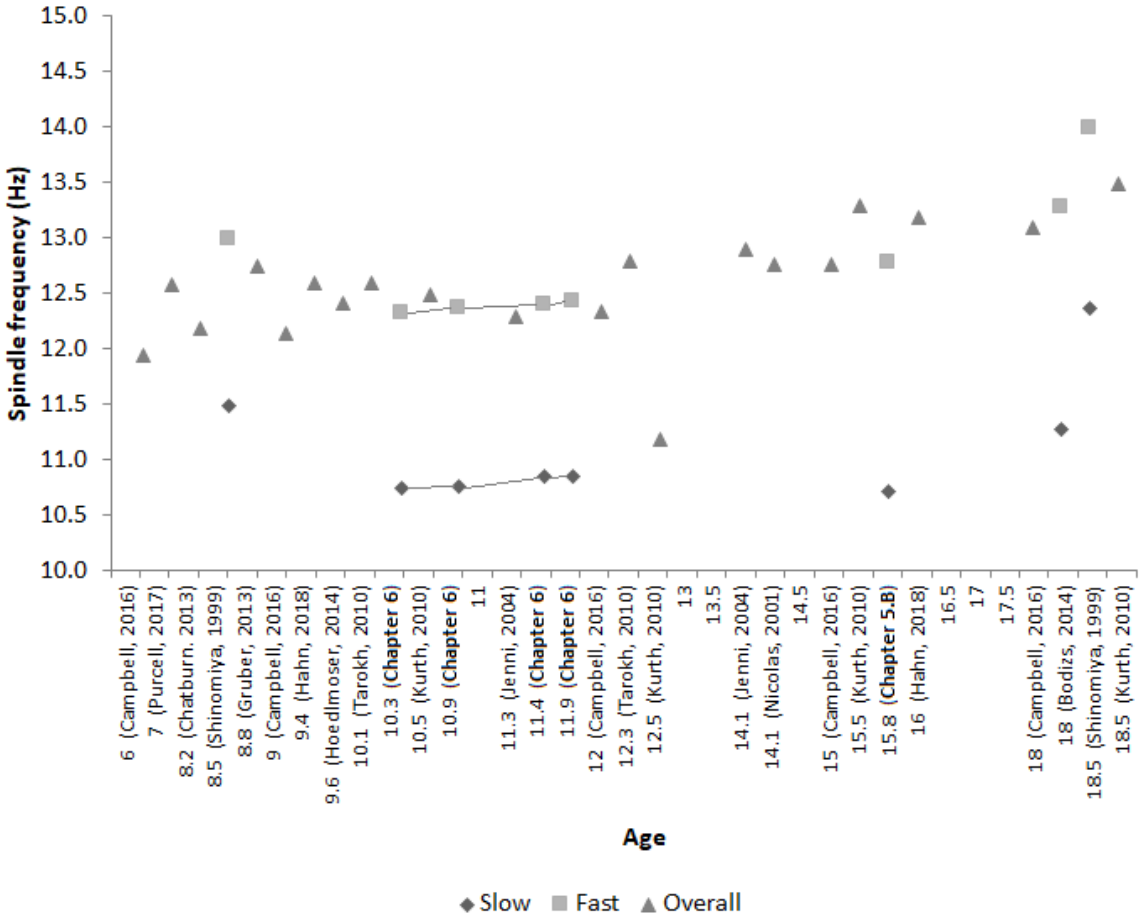


Figure 6.3. The trajectory of changes in spindle frequencies from the present study and other values in the literature (separated for slow and fast where available and presented as 'overall' when a single frequency measure was provided), from middle childhood to late adolescence. The present longitudinal study's findings are joined, while other cross-sectional studies' findings are single symbols. Gaps are left for ages where frequency estimates are not included. Note mean frequency values from other studies were estimated when a figure or range was provided rather than specific values. Campbell and Feinberg (2016) included measurements in cohorts at approximately 6-month intervals, and are presented for every 3 years.

The dichotomisation into slow and fast spindles in the present study was a key strength, as there have been clear differences between the two types in past research in terms of topography and functionality (Astill et al., 2014; Bodizs et al., 2014; Marshall et al., 2011).

Of relevance to the present study, the frequencies of slow and fast spindles in adolescents appear to be much lower than in adults. This means that the typical limits placed on slow and fast spindles for spindle detection, largely driven by adult studies (e.g. slow ~11-13Hz; fast ~13-16Hz; De Gennaro & Ferrara, 2003) may overestimate the number of slow spindles and underestimate the number of fast spindles in an adolescent sample. To illustrate, the present study used a spectral profile to identify the strongest power peaks for each individual (IAM method; Bodizs et al., 2009). On average, slow peaks were found at 10.8Hz (time 1) – 10.9Hz (time 4), while fast peaks were seen at 12.3Hz (time 1) – 12.4Hz (time 4). According to typical criteria (slow ~11-13Hz; fast ~13-16Hz), many of the present adolescents' slow spindles would be missed, and many fast spindles would be incorrectly considered 'slow'. This could explain why some child and adolescent studies have found so few 'fast' spindles. For example, Nader and Smith's (2015) 12-19-year old sample had a fast spindle density of 1.38 per minute (compared to the present study's fast densities of 7.1 (T1) - 7.3 (T4)), with fast spindle limits of 13.51-16.0Hz. Similarly, Hahn and colleagues (2018) found a fast spindle density of approximately 1.5 per minute in 8-11-year-olds with fast spindle limits of 13-15Hz, while Chatburn and colleagues (2013) found a fast spindle density of only 0.3 per minute in a sample of 4-12-year-olds, with fast spindle limits of 13-15.9Hz. For studies that provided an overall spindle frequency, either as the exclusive frequency measure or in addition to separating for slow and fast, the mean frequency of the strongest peak was closer to 12Hz or 13Hz (Chatburn et al., 2013; Gruber et al., 2013; Hahn et al., 2018; Hoedlmoser et al., 2014; Jenni & Carskadon, 2004; Kurth et al., 2010; Nicolas et al., 2001; Tarokh & Carskadon, 2010), indicating older children's and adolescents' frequencies are more likely to fall into the 'slow' side of the typical dichotomy. If anything, this highlights the importance of detecting spindles using individually-based limits, due to the impracticality of applying adult-based slow and fast spindle criteria to children and adolescents, and further due to the

variation of spindle characteristics between individuals (Reynolds et al, 2018 (**Chapter 3**); Bodizs et al., 2009). This also presents a case for the minimum spindle frequency limit being lowered to 9 or 10Hz for child and adolescent samples, notwithstanding careful consideration of individual alpha frequencies (within the range of 7-11Hz in adolescents; Jenni & Carskadon, 2004; Kurth et al., 2010; Tarokh & Carskadon, 2010), which is beyond the scope of the present study to investigate.

The present study was one of the first to examine spindle characteristics in a longitudinal nature and added to the present body of research, which is largely made up of cross-sectional studies. Of note, at the time of conceptualising the study, collecting and analysing data, there was no other longitudinal study of specific spindle characteristics, with Hahn and colleagues' (2018) investigation being published after completion of the current study. There are differences which nonetheless allow the current study to add knowledge to the field, however. Firstly, Hahn and colleagues measured samples 7 years apart, while the present study used more concise intervals of 6 months. Secondly, the use of an individually-adjusted algorithm for detecting adolescents' spindles in the present study may provide more distinct measurements of slow and fast spindles, as discussed earlier. The nature of a longitudinal investigation, however, presents several limitations. Firstly, the sample size was small (N=20) and analyses may have been underpowered, even though this was larger than another longitudinal investigation of adolescent spindles (Tarokh & Carskadon, 2010; N = 14). Nonetheless, the size of changes was small (e.g. average 6-month increase in fast spindle density of 0.07 spindles per minute), indicating that even with a larger sample, there may not yet be much movement in spindle characteristics at this age. The second limitation is the time-scale of the longitudinal design, where the young adolescents remained in early puberty at the end of the 18-month study and showed little pubertal change in the last year. Decreases of slow wave sleep during adolescence have been attributed to synaptic pruning and are

implicated in pubertal onset (Feinberg & Campbell, 2010; 2013), however the present data show slow wave sleep did not differ across 18 months and, if anything, the proportion of stage 3 sleep slightly increased over the study. The absence of alteration to sleep architecture with increasing age aligns with Gruber and colleagues' (2013) younger sample (7-11yrs). Similarly, chronotype did not change in a meaningful way over the four time points and is not expected to delay until 12-13 years (Koscec et al., 2013) or until early-to-mid puberty (Tanner stages 2 and 3; Carskadon et al., 1993). Again, changes to sleep are likely not evident until later in adolescence and, furthermore, brain reorganisation may not be prominent in early adolescence, or at least not enough to be reflected on sleep EEG. Notwithstanding the difficulty of maintaining a laboratory study with a paediatric sample over several years, further studies would benefit from including a larger sample and extending the time-course of the present longitudinal investigation, ideally until at least 18 years of age. Lastly, more reliable spindle estimates would be achieved from using several nights of sleep monitoring, as adolescents' slow spindle density and duration require a minimum of 4 and 2 nights, respectively, to reach acceptable within-subject reliability (intraclass coefficient > 0.60; Reynolds et al., 2018 (**Chapter 3**)). This would likely require laboratory monitoring during school holiday periods, rather than during weekends of the school term, as in the present study. Overall, as this is one of the only longitudinal studies of adolescent spindle characteristics to date, and is the first to investigate spindle changes over concise intervals, the magnitude of intra-individual change in specific spindle parameters remains to be seen and warrants further investigation.

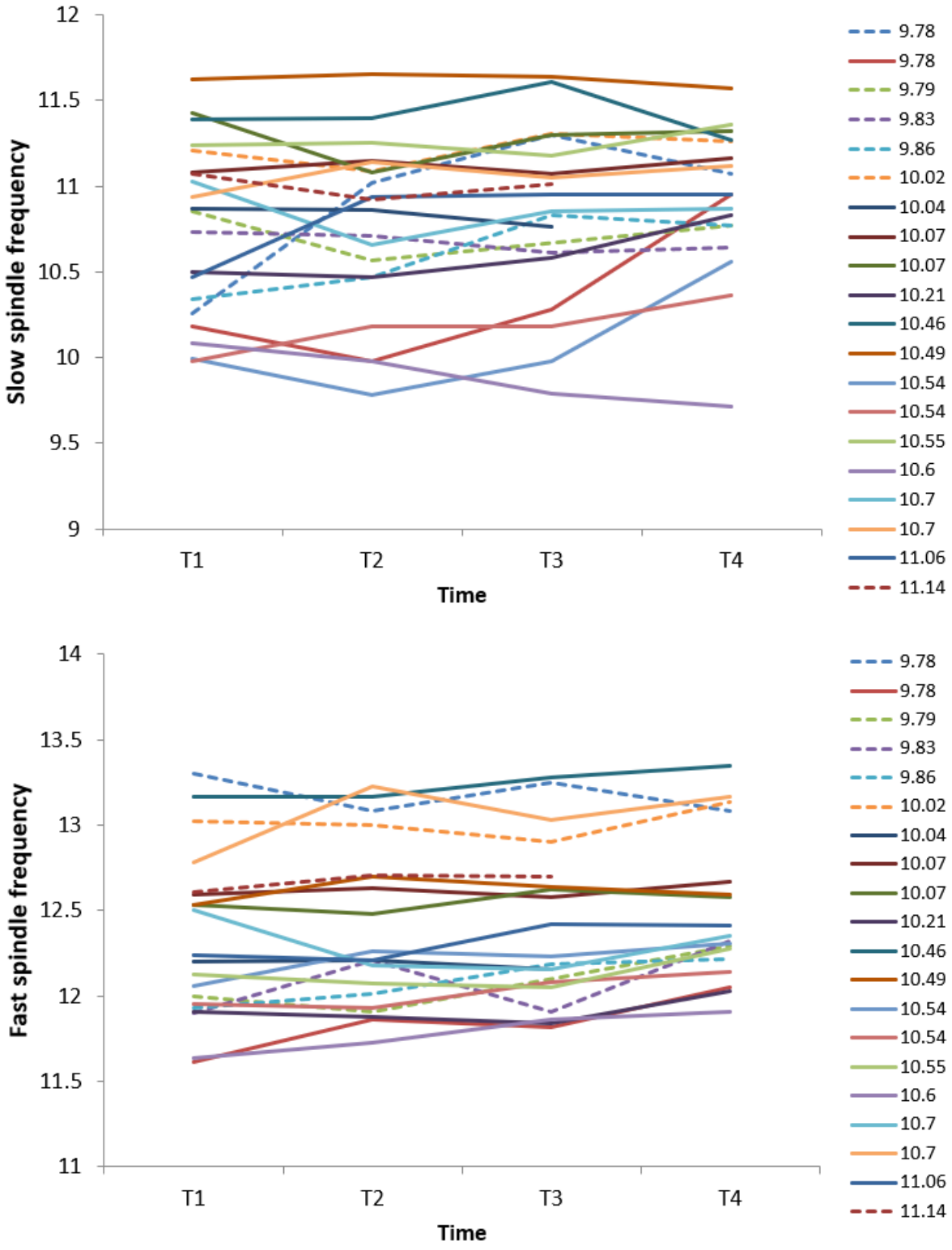
Conclusion

The present study addressed a significant gap in the literature on sleep spindle changes across early adolescence by using a longitudinal design and focusing on specific spindle characteristics over concise intervals. In the 18 months of the pre-adolescent period,

fast spindle frequency increased significantly, and slow spindle frequency followed a similar pattern, aligning with the expected trajectory based on prior research. Other spindle characteristics, however, did not change. This is likely influenced by the age of the participants, where larger changes in spindle characteristics are not expected to arise until mid-to-late adolescence, when further network refinement and synaptic pruning is likely to occur.

Supplementary Table S6.1. *Means (standard deviations) for each sleep spindle characteristic for each time point of the longitudinal study.*

Time	Density		Duration		Amplitude		Frequency	
	Slow	Fast	Slow	Fast	Slow	Fast	Slow	Fast
T1	7.56	7.15	1.69	1.33	4.53	7.44	10.76	12.33
(baseline)	(0.15)	(0.19)	(0.08)	(0.05)	(0.48)	(0.43)	(0.11)	(0.11)
T2	7.82	7.17	1.60	1.32	4.31	7.31	10.77	12.37
(6 months)	(0.15)	(020)	(0.06)	(0.05)	(0.40)	(0.44)	(0.11)	(0.11)
T3	7.60	7.27	1.56	1.31	4.77	7.32	10.86	12.41
(12 months)	(0.17)	(0.15)	(0.07)	(0.05)	(0.46)	(0.52)	(0.12)	(0.11)
T4	7.84	7.31	1.58	1.34	5.31	8.15	10.86	12.44
(18 months)	(0.15)	(0.24)	(0.08)	(0.07)	(0.69)	(0.65)	(0.14)	(0.13)



Supplementary Figure S6.1. Slow and fast spindle frequency for each subject (n = 20) per time point. Coloured lines depict each subject's age at baseline.

Chapter 7.

How does the relationship between sleep spindles and cognition change over time? A longitudinal study of pre-adolescents.

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Author Contributions

CR. was the primary author, contributed to study design and data collection and conducted scoring of sleep EEG, sleep spindle analysis and all statistical analyses. MG and MS contributed to revising of the chapter, statistical assistance and study design.

Abstract

Sleep spindles have been implicated in cognitive functioning across many age groups, however the nature of the relationship in childhood is less clear, and often opposite, to that seen in adolescents and adults. The developmental trajectory of the relationship between spindles and cognition was therefore tested in the present longitudinal study of emerging adolescents. Twenty pre-adolescent boys (mean age at Time 1 = 10.3 ± 0.4yrs) participated in an 18-month study, with sleep spindles characteristics (density, duration, amplitude and frequency, separate for slow and fast) and cognitive performance (working memory and fluid intelligence) measured in a sleep laboratory once every 6 months. Spindle parameters and cognition did not show cross-sectional associations at any time point, contrary to past findings, and while working memory and fluid intelligence improvements were related to changes in slow and fast spindle frequency and slow density, these did not show consistent or meaningful patterns. The relationship between sleep spindles and cognition may be unstable in early adolescence due to the complex brain reorganisation in this developmental period.

Introduction

The developmental stage of adolescence involves significant changes to brain formation (Hagmann et al., 2010; Peters et al. 2012), sleep architecture (Feinberg & Campbell, 2013) and spectral power seen through electroencephalography (EEG) during sleep (Feinberg & Campbell, 2013; Whitford et al., 2007). Sleep spindles within an adolescent's EEG profile also change, where typically spindle amplitude, density and duration decrease (Jenni & Carskadon, 2004; Martin et al., 2013; Nader & Smith, 2015; Nicolas et al., 2001; Scholle et al., 2007; Shinomiya et al., 1999; Tarokh & Carskadon, 2010), while spindle frequency increases (Campbell & Feinberg, 2016; Hahn et al., 2018; Jenni & Carskadon, 2004; Kurth et al., 2010; Nicolas et al., 2001; Shinomiya, 1999; Tarokh & Carskadon, 2010). Furthermore, adolescence involves the development of higher-order cognitive functions (Casey et al., 2005; Luna et al., 2010; Sternberg & Downing, 1982, Wright et al., 2008), for which changes in the brain are implicated, such as increases in white matter and decreases in grey matter, indicative of synaptic pruning (Gogtay et al., 2004; Hagmann et al., 2010; Sisk & Foster, 2004). Such changes are further believed to be indexed by changes to sleep EEG (Feinberg & Campbell, 2010; 2013). The relationship between sleep spindles and cognitive performance across this transitional period may shed light on ways in which brain development underpins cognitive development.

What is currently known about the relationship between sleep spindles and cognition in children and adolescents?

A recent meta-analysis found that adolescents' sleep spindle characteristics have a small-to-moderate positive correlation with cognitive performance in the domains of fluid IQ (r = 0.44), working memory and executive function (r = 0.40) and speed and accuracy (r = 0.33) (Reynolds et al., 2018 (**Chapter 2**)), which aligns with findings in adult studies (Bodizs et al., 2005; Fogel et al., 2007, Schabus et al., 2008). In children, however, the few studies

published report mixed findings, with some showing positive associations between spindles and performance (Doucette et al., 2015; Geiger et al., 2011; Ujma et al., 2016), while others found no relationship between some spindle variables and cognitive performance (Chatburn et al., 2013; Gruber et al., 2013; Kurdziel et al., 2013). In one study comparing age cohorts, there was an association between memory consolidation and spindle count in adults but not in children (Wilhelm et al., 2012). In other cases, opposite associations are seen between children and older adolescents. In preschool-aged children, spindle density was negatively associated with immediate memory performance, and positively associated with performance improvement (Kurdziel et al., 2013), while the opposite has been found for immediate and improved memory performance and sigma power in older adolescents (Lustenberger et al., 2012). Similarly, higher spindle frequency is associated with *worse* fluid reasoning (Gruber et al., 2013) and working memory (Chatburn et al., 2013; Gruber et al., 2013) in children, yet with *better* fluid reasoning in older adolescents (Bodizs et al., 2014). This implies that there may be a reversal in the direction of the effect at some stage during adolescence, at least for some cognitive and spindle variables.

Of note, studies that investigate different cognitive and spindle variables are difficult to compare, given the relationship between spindles and cognition varies considerably depending on the cognitive task used and spindle characteristic measured (Reynolds et al., 2018 (**Chapter 2**)). Accounting for the large changes to brain network formation (Hagmann et al., 2010; Peters et al. 2012; Sisk & Foster, 2004) and sleep EEG (Feinberg & Campbell, 2013) during early adolescence, it is also difficult to compare different age groups within cross-sectional studies. For example, young adolescents experience a slight increase in synaptic density in the prefrontal cortex, while older adolescents experience more dramatic synaptic pruning (Blakemore & Choudhury, 2006), which is believed to be indexed on sleep EEG (Feinberg & Campbell, 2010). If spindles are potentially associated with brain network

reorganisation (Bodizs et al., 2014) then younger and older adolescents will show considerable differences in spindle parameters and combining these ages may therefore lead to unreliable findings. Furthermore, the developmental trajectory of the relationship between sleep spindles and cognition is thus far unknown. The only longitudinal study to compare spindles to behavioural outcomes in children to date found that higher spindle density at 5 years of age predicted better prosocial behaviour at 9 years of age (Mikoteit et al., 2018). This finding may extend to general cognitive ability and more complex cognitive domains in adolescents. Considering the extensive neurological changes that occur during adolescence, it would be highly informative to investigate associations between spindles and cognition in the developing adolescent brain. The present study therefore aims to investigate the relationship between spindles and cognition in a longitudinal protocol.

How do sleep spindle characteristics change between childhood and adolescence?

In a previous paper, we found that spindle frequency was the first spindle characteristic to show significant developmental change between 10 and 12 years of age, where fast spindle frequency became faster with development, and slow spindle frequency showed a similar trend (**Chapter 6**). This aligned with past findings in both cross-sectional (Jenni & Carskadon, 2004; Kurth et al., 2010; Purcell et al., 2017; Scholle et al., 2007; Shinomiya, 1999; Tarokh & Carskadon, 2010) and longitudinal (Campbell & Feinberg, 2016; Hahn et al., 2018; Tarokh & Carskadon, 2010) investigations. Along with changes to spindle makeup and brain networks, adolescents also experience significant improvement in higher-order cognitive functions, including executive function (Blakemore & Choudhury, 2006; Steinberg, 2005), working memory (Luna et al., 2010) and fluid intelligence (Hartshorne & Germine, 2015). Furthermore, these improved cognitive functions are associated with decreases in synaptic density and increased myelination in frontal lobes (Steinberg, 2005). It is hypothesised, therefore, that changes to cognitive performance over time would be

associated with changes in spindle frequency. Worth noting is that spindle density, duration and amplitude did not significantly change in our previous study (**Chapter 6**), contrary to past findings suggesting all three characteristics would decrease with development (Nader & Smith, 2015; Scholle et al., 2007; Shinomiya et al., 1999). Nonetheless, these parameters have shown associations with various cognitive domains in adolescents (Reynolds et al., 2018 (**Chapter 2**)), and are expected to show at least static, if not dynamic, associations with cognitive performance in the present study. Given the inter-individual variation of slow and fast spindle characteristics in mid-adolescence (Reynolds et al., 2018 (**Chapter 3**)) as well as topographical and functional differences of slow and fast spindles (Astill et al., 2014; Bodizs et al., 2014; De Gennaro & Ferrara, 2003; Marshall et al., 2011), the two types will be examined separately.

Method

Participants

Twenty pre-adolescent boys participated in a longitudinal study (mean age at baseline = 10.3 ± 0.4yrs). Included participants were free of medical or mental health concerns at baseline, did not take medications, and had no parent-reported problems with sleeping (e.g., snoring, sleep disordered breathing, difficulty initiating or maintaining sleep, parasomnias). Participants had a typical sleep duration of 9-11 hours, which aligns with recommendations for school-aged children (Hirshkowitz et al., 2015). Female pre-adolescents were excluded from the study due to the potential impact of menstrual cycles on sleep (Driver & Baker, 1998) and spindle frequency (Ishizuka et al., 1994). Participants were pre-pubescent at baseline (Tanner stage 1; Tanner, 1990), and in early-puberty at all other time points (Tanner stage 2), as estimated by the Pubertal Development Scale (Carskadon & Acebo, 1993). Ethics approval was granted by the Social and Behavioural Research Ethics Committee of Flinders

University. Parents and participants provided written informed consent after a tour of the sleep laboratory, and participants received remuneration per data collection time point.

Design

Pre-adolescents stayed in the sleep laboratory for one weekend every 6 months, over an 18-month period. The first night of sleep was used in the present study, during which participants were given a 10 hour sleep opportunity, aligning with optimal sleep given in previous studies (Carskadon et al., 1981; Reynolds et al., 2018 (**Chapter 4**)). Time in bed was kept constant between 21:30 and 07:30. The target baseline age of 10 years was chosen as a minimum for pubertal onset in boys (Carskadon et al., 1993; Walvoord, 2010). Cognitive testing took place the day after the sleep period when spindles were analysed, which aimed to investigate general network efficiency, as opposed to investigating cognition the day before the sleep period, which may reflect that day's processing (Reynolds et al., 2018 (**Chapter 2**)).

Materials

Polysomnography

Sleep EEG was measured with ambulatory Compumedics Somte PSG systems (Version 2; Compumedics Ltd., Victoria, Australia), at a sampling rate of 200Hz. Somte units were connected via Bluetooth wireless transmission to provide real-time monitoring of participants' sleep, conducted by an experienced sleep technician. EEG derivations of C3, C4, F3, F4, O1 and O2 referenced to contralateral mastoids (M1 and M2), electrooculogram (EOG) and electromyogram (EMG) were used in the present study. Standardised scoring criteria were used for sleep staging (Rechtschaffen and Kales, 1968).

Sleep Spindles

Sleep spindles were detected and analysed using the Individual Adjustment Method (Bodizs et al., 2009). A spectral profile is generated for each individual, and zero-crossing points indicate the strongest power peaks in relative slow and fast frequencies for each

individual. This method accounts for trait-like features of spindles that are similar within individuals, but diverse between individuals (Bodizs et al., 2009; Reynolds et al., 2018 (**Chapter 3**)). C3-M2 was used for spindle analyses, and when this was not suitable, C4-M1 was used. The present study used slow and fast spindle characteristics of density (number of spindles per minute of non-REM sleep), spindle duration (the average duration (seconds) of each spindle), spindle amplitude (the average amplitude taken from the midpoints of all detected spindles, using the rectified signals) and spindle frequency (the average frequency of detected spindles).

Cognitive Performance Measures

Fluid Intelligence

Fluid intelligence was measured with the matrix reasoning task (Wechsler Intelligence Scale for Children – 4th Edition (WISC-IV), Flanagan & Alfonso, 2017; and Wechsler Adult Intelligence Scale – 4th Edition (WAIS-IV), Lichtenberger & Kaufman, 2009), letter sets task (Ekstrom et al.,1976) and number series task (Thurstone, 1938), which examine inductive and deductive reasoning skills. Fluid intelligence has shown positive associations with spindle characteristics in adolescents (Geiger et al., 2011; Bodizs et al., 2014; Tessier et al., 2015).

For the *matrix reasoning* task, participants were shown a series of images in a matrix, with one blank square of the matrix that contained a question mark, and five separate images underneath the matrix. Participants were asked to identify which of the five images would fit in the blank square. Items from the WISC-IV and WAIS-IV were combined to optimise the number of test items for the length of the longitudinal study. The original tests had a combined total of 66 items, which were randomly separated into 7 separate tests (9 items per test, with 5 WISC-IV items and 4 WAIS-IV items per test, and 3 excluded overall), with one test for each of 7 potential time points (randomly allocated), of which 4 were completed in the present study. Items increased in difficulty, as per the original tests. The matrix reasoning

task has shown good evidence of internal consistency (WISC-IV: *internal consistency* = .89; Williams et al., 2003, WAIS-IV: *split-half reliability* = .90; Lichtenberger & Kaufman, 2009) and reliability for multiple testing (WISC-IV: *stability coefficient* = .77; Williams et al., 2003, WAIS-IV: *test-retest reliability* = .74; Lichtenberger & Kaufman, 2009).

In the *letter sets task,* participants were asked to consider 5 groups of letters in strings of either 2, 3 or 4 letters long. Four groups followed a logical rule (e.g. alphabetical), while one group did not, and participants needed to identify the group of letters that did not follow the pattern. For example, participants are instructed, "*Circle the pair of letters that is the odd one out: JK, WX, FG, NM or CD*". The original task of 18 items was divided into two 9-item measures, with one version given at the second time point, and the other given at the third time point (randomly allocated). The *number series* task involved similar pattern recognition, where participants were shown a series of 7 numbers in a logical sequence, and were asked to provide the next number in the sequence, e.g. "*What number comes next in the following sequence? 2, 5, 8, 11, 14, 17, 20,* ___". The original task consisted of 21 items and was divided into two halves (one item removed), similar to the letter sets task, with both halves randomly allocated. The letter sets and number series tasks have been administered this way in a previous study comparing spindles and cognition in adolescents (**Chapter 5**). It was therefore decided that the test would be given in the same two halves, and the test took place at time points 2 and 3 to reduce the potential impact of a first-study effect from time 1. The letter sets and number series tasks possess good internal consistency (Cronbach's alpha = .91-.92 and .85-.86, respectively; Csapo, 1997; Johnston et al., 2010).

Fluid intelligence tests were given at a consistent time of 1pm on the Saturday of each study weekend, with the letter sets and number series tasks being combined with the matrix reasoning task to form a test battery at Times 2 and 3 (Figure 7.1). The proportion of correct responses was used for the overall fluid intelligence score for each test.

Working Memory

The operation span task (Ospan; Turner and Engle, 1989) was used to measure working memory, which has shown positive associations with adolescent spindle activity (Chatburn et al., 2013; Hoedlmoser et al., 2014; Lustenberger et al., 2012; Piosczyk et al., 2013; Prehn-Kristensen et al., 2011). The computerised version of the test was used in the present study (Unsworth et al., 2005), given once at each time point at a consistent time of day (11:00; Figure 7.1). In this task, participants were required to solve mathematical equations while memorising a series of randomised letters. Participants were presented with a mathematical equation and were then asked if it was 'true' or 'false' (e.g., (5 x 8) + 2 = 40, TRUE or FALSE), and were then presented with a letter (e.g., L). After a randomly-distributed number of trials (ranging from 3-7), participants were asked to recall all letters in the series, in the order they were presented. The task involved two practice trials and 15 test trials. The 'Absolute Ospan' scoring method was used for their overall working memory score, which is the sum of all perfectly recalled sets (Unsworth et al., 2005).

T1 (baseline) n = 20

Time (24hrs)

17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arrive at lab					10hrs' sleep opportunity													W		F1			

T2 (6 months) n = 20

17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arrive at lab					10hrs' sleep opportunity													W		F1, 2 & 3			

T3 (12 months) n = 19[5]

17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arrive at lab					10hrs' sleep opportunity													W		F1, 2 & 3			

T4 (18 months) n = 12

17	18	19	20	21	22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Arrive at lab					10hrs' sleep opportunity													W		F1			

Figure 7.1. The study protocol over the four time points of the longitudinal study. W = Working memory task (operation span task). F = Fluid intelligence task (1 = Matrix Reasoning, 2 = Letter Sets, 3 = Number Series).

Statistical Analyses

IBM SPSS Statistics Version 22 (IBM Corp., Armonk, NY) was used to conduct all analyses. Basic associations between spindle and cognitive variables at each time point were tested with correlation analyses. To adjust for multiple comparisons for the correlation analyses, the Bonferroni correction was applied for a set of 24 comparisons (8x spindle variables, 4x cognitive tests; 0.05/32 = .002), and the cut-off for statistical significance was thus reduced to $p < .002$. Linear mixed model (LMM) analyses were conducted to examine whether the relationship between spindles and cognitive performance changed over time. 'Change from baseline' values were computed for these analyses, so that the first time point

[5] One participant was unable to attend T3 due to illness, however remained in the study at T4.

became the baseline value for spindle variables and working memory scores, while the second time point was used as baseline for the letter sets and number series tasks. Analyses therefore excluded the respective baseline time points, as all values equalled zero. Participant ID was specified as a random effect in all models. Dependent variables were cognitive scores from the operation span task, matrix reasoning, letter sets task and number series task (all as change from baseline). Covariates included slow and fast spindle characteristics of density, duration, amplitude and frequency (all as change from baseline). LMMs for the operation span task and matrix reasoning task had fully saturated models (all main and interaction effects) for time. Further significant main and interaction effects were investigated using pairwise post-hoc analyses with least significant differences (LSD). The letter sets and number series tasks were given twice during the study (T2 and T3), meaning that there was only one time point for 'change from baseline', and therefore those LMMs only included the main effect of change in each spindle characteristic, and did not include time as a factor. Puberty was not included as a covariate, as there was not a significant relationship between changes in spindle activity and puberty scores over time, as evidenced in a previous paper (**Chapter 6**).

Results

Associations between sleep spindles and cognitive performance

Correlation coefficients for associations between all spindle and cognitive variables at each of the four time points are presented in Table 7.1. None of the associations between spindle and cognitive variables reached significance after the Bonferroni correction for multiple comparisons ($p < .002$). Furthermore, very few associations (n = 10 of 96 total associations) reached a moderate effect size ($r > 0.30$, Cohen, 1988), and these did not reveal a consistent pattern.

Table 7.1. *Correlation coefficients (p values) for sleep spindle and cognitive performance variables at each time point.*

	Correlation coefficient *r (p)*			
	T1 n = 20	T2 n = 20	T3 n = 19	T4 n = 12
Operation Span Task (working memory)				
Slow density	0.07 (.78)	**0.33 (.16)**	-0.18 (.46)	**0.64 (.03)**
Slow duration	**0.30 (.19)**	-0.07 (.78)	-0.07 (.79)	-0.12 (.72)
Slow amplitude	-0.24 (.32)	-0.13 (.59)	0.10 (.69)	0.09 (.79)
Slow frequency	-0.19 (.42)	0.18 (.45)	0.05 (.85)	**0.34 (.28)**
Fast density	-0.21 (.38)	**-0.42 (.07)**	0.03 (.89)	-0.16 (.63)
Fast duration	-0.16 (.50)	-0.20 (.41)	0.10 (.70)	**-0.53 (.08)**
Fast amplitude	0.05 (.84)	-0.08 (.75)	-0.02 (.95)	0.09 (.79)
Fast frequency	-0.27 (.25)	0.10 (.67)	-0.17 (.49)	**0.34 (.28)**
Matrix Reasoning (fluid IQ)				
Slow density	0.22 (.36)	<0.01 (>.99)	-0.13 (.59)	0.29 (.36)
Slow duration	0.03 (.91)	0.25 (.30)	-0.02 (.92)	-0.18 (.58)
Slow amplitude	-0.03 (.91)	-0.15 (.52)	0.04 (.87)	**0.30 (.35)**
Slow frequency	0.19 (.43)	0.13 (.60)	0.03 (.90)	**0.34 (.28)**
Fast density	-0.29 (.22)	0.09 (.70)	-0.27 (.26)	-0.21 (.51)
Fast duration	0.05 (.84)	0.15 (.52)	**-0.44 (.06)**	-0.15 (.63)
Fast amplitude	0.16 (.50)	-0.10 (.68)	0.09 (.73)	0.24 (.46)
Fast frequency	0.13 (.59)	-0.21 (.37)	0.11 (.65)	0.10 (.75)
Letter Sets Task (fluid IQ)				
Slow density	-	-0.06 (.79)	0.15 (.54)	-
Slow duration	-	0.28 (.24)	0.08 (.75)	-
Slow amplitude	-	<0.01 (>.99)	0.04 (.86)	-
Slow frequency	-	**0.46 (.04)**	0.18 (.45)	-
Fast density	-	0.12 (.62)	-0.05 (.85)	-
Fast duration	-	0.13 (.59)	-0.28 (.25)	-
Fast amplitude	-	0.15 (.54)	0.08 (.73)	-
Fast frequency	-	0.22 (.36)	0.22 (.38)	-
Number Series Task (fluid IQ)				
Slow density	-	-0.04 (.86)	0.03 (.92)	-
Slow duration	-	-0.18 (.45)	0.07 (.77)	-
Slow amplitude	-	0.11 (.64)	0.09 (.73)	-
Slow frequency	-	0.23 (.32)	0.21 (.38)	-
Fast density	-	0.17 (.47)	0.04 (.87)	-
Fast duration	-	0.07 (.78)	-0.04 (.89)	-
Fast amplitude	-	0.02 (.94)	<0.01 (>.99)	-
Fast frequency	-	0.10 (.68)	0.15 (.54)	-

*Note. Letter Sets and Number Series tasks were only given at T2 and T3. Effect sizes are considered small at r = 0.1, medium at 0.3 and large at 0.5 and above (Cohen, 1988). Correlations of a medium effect or higher are **bolded**. Significance is corrected to p < .002, as per the Bonferroni correction.*

Changes in sleep spindles and cognitive performance over time

Working memory

Working memory performance improved significantly over time, $F(3,49) = 4.63$, $p = .006$, where performance improved slightly from T1 to T2, was stable between T2 and T3 and showed a large improvement at T4. LMM analyses revealed that changes in both slow and fast spindle frequency were associated with changes in working memory performance. There was a two-way interaction between change in slow frequency and time on working memory performance, where the relationship between change in slow frequency and change in working memory varied over time, $F(2,31) = 5.61$, $p = .008$, however none of the static associations in change scores at each time point were significant (T2: $r = 0.26$, $p = .27$; T3: $r = -0.37$, $p = .12$; T4: $r = 0.26$, $p = .42$). There was a main effect of change in fast spindles on change in working memory performance, $F(1,45) = 4.27$, $p = .045$, indicating an overall association between change scores, however the correlation was not significant ($r = -0.12$, $p = .31$). No other spindle variables were related to working memory change over time.

Fluid Intelligence

Fluid intelligence remained relatively stable over time, with a small but significant improvement for the number series task, $F(1,19) = 4.97$, $p = .04$, and no significant change seen for the letter sets task, $F(1,19) = 0.29$, $p = 0.60$, or the matrix reasoning task, $F(3,50) = 1.43$, $p = .25$. For matrix reasoning, there was a significant two-way interaction between change in slow spindle density and time, $F(2,32) = 3.53$, $p = .04$, where improved performance on the matrix reasoning task was not related to changes in slow density at either T2 ($r = 0.05$, $p = .84$) or T4 ($r = 0.10$, $p = .76$), however was strongly related to lower slow spindle density at T3 ($r = -0.55$, $p = .02$). Changes in letter sets and number series performance were not related to changes to any spindle characteristics over time.

Discussion

The present study revealed that, for the most part, sleep spindles were not significantly associated with cognitive performance during emerging adolescence using the current sample, nor did this relationship develop over time. In regard to the static associations between spindles and cognition at each time point, there was a remarkable lack of consistency in size and direction of effect for all of the correlations. Although there was an indication that changes in both slow and fast spindle frequency might be related to changes in cognitive performance over time, once the associations between change scores were examined, these too did not show a clear pattern. Increases in fast spindle frequency may have been related to less changes in working memory performance, possibly indicating that adolescents who had more of an increase in fast spindle frequency did not show as much working memory improvement, however the static association from the mixed model was a small and non-significant effect (r = -0.12, p = .31). The pattern of change for the associations between slow spindle frequency and working memory was yet more confusing, with the direction of effect changing at each time point. Similarly, fluid intelligence improvements (matrix reasoning task) were significantly related to decreases in slow spindle density at only the third time point (T3), but not at T2 or T4. Overall, the associations do not seem conceptually logical or meaningful.

There are two factors that might explain the present findings. Firstly, the complex brain reorganisation that is occurring in early adolescence (Feinberg & Campbell, 2010; 2013), as well as the development of complex cognitive skills (Casey et al., 2005, Luna et al., 2010; Wright et al., 2008) might create instability in the possible associations between spindles and cognition in this young adolescent cohort. White and grey matter changes have been linked with cognitive performance (Draganski et al., 2004; Jung & Haier, 2007; Gur et al., 1999), and at the beginning of adolescence, there is first an increase of synaptogenesis

(i.e. increased grey matter) in the prefrontal cortex followed by more dramatic synaptic pruning by the end of adolescence (i.e. decreased grey matter) (Blakemore & Choudhury, 2006; Gogtay et al., 2004). The adolescent brain therefore sees an increase - and then decrease - in neural substrates of learning within a relatively short space of time. This may help to explain why there were dramatic changes in the direction of effect in associations between spindles and cognition in the present study. This study might then be evidence of instability for associations between spindles and some cognitive tasks in young adolescents, while these associations are stronger in childhood (Chatburn et al., 2013; Gruber et al., 2013; Kurdziel et al., 2013) and in older adolescence (Bodizs et al., 2014; Lustenberger et al., 2012). If this were the case, one interesting direction for future research would be to extend longitudinal investigations from late childhood into later adolescence or even early adulthood, to investigate changes in the spindle and cognition relationship over a longer time-frame.

The second possibility is that, in spite of many studies attesting to the fact that spindles are an index of cognitive performance in many samples (see meta-analyses in Reynolds et al., 2018 (**Chapter 2**) and Ujma, 2018), this relationship may not be as consistent across cognitive performance measures as previously thought. Further from this, there may be too many complexities in the associations between neural substrates and observable cognition to make firm conclusions with the tools we have available, particularly in the case of the sleep spindle. In this case, it may be time to return focus to more in-depth EEG studies to improve understanding of these links in the brain. Recent attention has been given to the synchrony of sleep spindles with other sleep rhythms (Luthi, 2014), with the coupling of spindles to slow waves and hippocampcal sharp-wave ripples another proposed indicator of learning (Molle et al., 2009; 2011). When a spindle aligns with the up-state of a slow oscillation, this often aligns with the induction of a hippocampal sharp-wave, and this

co-occurrence appears to create an environment in which memory is consolidated (McDevitt et al., 2017). Correlations between cognitive performance and spindles in this circumstance appear to be more consistent than with spindles occurring out-of-phase with the slow wave up-state (McDevitt et al., 2017). Associations between spindles and cognition have also been found when spindles are experimentally manipulated. For example, in adults, zolpidem has been shown to increase spindle density and subsequently improve memory consolidation over and above other sleep factors (Mednick et al., 2013), while a selective norepinephrine reuptake inhibitor has been shown to improve motor memory via enhanced spindle density (Rasch et al., 2009). Non-pharmaceutical interventions, such as closed-loop stimulation (delivering acoustic tones during oscillatory activity), have also resulted in increased spindle density and this is associated with better memory retention (Ngo et al., 2013). A similar technique in a child sample (Astill et al., 2014), was not able to influence spindle characteristics, however (Astill et al., 2014), indicating there is more research needed to investigate appropriate experimental manipulations of spindles in paediatric cohorts. These experimental techniques nonetheless aim to overcome the pitfalls of working with static correlation methods and, notwithstanding ethical and practical considerations, may point toward future methods to better understand the spindle and cognition relationship across the adolescent period.

The present study was ambitious in its longitudinal laboratory-controlled study of a paediatric cohort and was one of the first investigations of the associations between specific sleep spindle characteristics and cognitive performance within-subjects over time. As with many laboratory-based sleep EEG studies, it is difficult to achieve a large sample size, however the present study (n = 20) is comparable to, if not an improvement on, other child and adolescent studies comparing cognition to spindles and sigma power (e.g. Doucette et al., 2015: n = 10; Geiger et al., 2011: n = 14; Tarokh & Carskadon, 2010: n = 14). Nonetheless,

an increased sample would provide more confirmation and clarity to the findings, and replication would be necessary. The present study's methods could be improved by collecting two nights of sleep EEG on a weekend, rather than one night. Unfortunately, this was not possible in the present study, which was part of a larger investigation using the second night for a separate research question. It is also worth noting that the final 8 participants' data were not yet collected for T4 at the time of writing the present chapter, and if included, this may have changed the findings. On inspection of individuals' mean spindle values, those final 8 participants had consistently lower spindle amplitudes over T1-T3 than the 12 subjects that had complete data at T4, which adds emphasis to the need for a larger sample to control for such individual differences. There is increasing importance placed on collaboration between sleep research teams to combine findings to more reliably evaluate the true associations between spindles and cognition with larger sample sizes (Ujma, 2018). Lastly, investigations using several spindle characteristics (e.g. density, duration, amplitude and frequency in the present study) run the risk of multiple comparisons, and future studies could benefit from a more focused investigation of one or two characteristics. Spindle frequency appears to be the first characteristic to show developmental changes in adolescence (**Chapter 6**) and may be a candidate for exclusive focus in future investigations.

Conclusion

In the present early adolescent sample, slow and fast sleep spindle characteristics of density, duration, amplitude and frequency did not show clear relationships with working memory or fluid intelligence. Changes in these variables over time were also not meaningfully associated. The absence of clear associations may be related to the instability of brain networks due to the reorganisation of white and grey matter during early adolescence. Future studies would benefit from longer-term investigations across early and later adolescence to confirm the nature of this relationship during this complex developmental period.

Chapter 8.

General Discussion

Summary of findings

The present thesis set out to investigate the relationship between sleep spindles and cognition in an adolescent population, and to establish whether this relationship changes during experimental sleep restriction and over a longitudinal time period. In **Chapter 1**, a broad overview of adolescents and their sleeping patterns was provided, and the key player of this thesis, the sleep spindle, was introduced. It was firstly established that sleep is an important aspect of adolescents' lives, not just because of the extreme changes in sleep and brain development in this period, but because of the impact that sleep may have on their functioning and wellbeing alongside the academic pressure of their secondary schooling. It was proposed that the sleep spindle may be a mechanism through which we can improve our understanding of the links between sleep and cognitive performance. In **Chapter 2**, emphasis was placed on the emerging popularity of correlating sleep spindle activity with cognitive performance, with meta-analytic results indicating that adolescents exhibit strong positive correlations between spindles and cognitive domains of fluid IQ (r = 0.44), working memory and executive functioning (r = 0.40), and speed and accuracy (r = 0.33), while full IQ was not significantly related to spindles (r = -0.05). This meta-analytic review informed the expectations of the subsequent chapters looking at spindles and cognition, with mid-adolescents in **Chapter 5** and young adolescents in **Chapter 7**. The review also left two main questions unanswered: 1. What happens to the relationship between spindles and cognition when adolescents are sleep restricted? and 2. How does this relationship develop over time? In other words, the present thesis examined both the *state* (sleep restriction) and *trait* (development) aspects of the relationship between spindles and cognition in adolescents.

In order to establish the impact of sleep restriction on sleep spindles and cognition in adolescents, it was first necessary to establish the night-to-night stability of adolescent sleep spindles (**Chapter 3**) and secondly, to establish what happens to sleep spindles alone during

sleep restriction (**Chapter 4**). For 15-17-year-old adolescents - and likely for extensions beyond this age group - a single night of sleep monitoring was deemed adequate for reliable measurements of slow spindle amplitude and fast spindle density, duration and amplitude. Slow spindle density required four consecutive nights, however, while slow spindle duration required two. For all fast spindle characteristics and slow spindle amplitude, then, any change in spindle activity during sleep restriction could be deemed due to the restriction itself, rather than to instability in the spindle characteristic. Indeed, **Chapter 4** established that severe sleep restriction (5hrs of time in bed) led to significant increases in fast spindle duration and decreases in fast spindle amplitude. The functional significance of these alterations to spindle characteristics was examined in **Chapter 5**. Although the change in spindle activity was not related to changes in cognitive performance across multiple days of sleep restriction, there was an indication that adolescents who had longer fast spindle duration at baseline were protected from the effects of sleep restriction on sustained attention for both the severe (5hr) and moderate (7.5hr) sleep restriction conditions. Our initial expectations of a static relationship between spindles and cognition at baseline, however, were not supported. There was a small indication that slow spindle amplitude might be related to fluid intelligence improvements, however the lack of consistent patterns in spindle-cognition associations indicated that the expected relationship may not be that strong in this age group.

The second major study of the thesis focused on the longitudinal development of sleep spindles (**Chapter 6**) and their relationship with cognitive performance (**Chapter 7**) in emerging adolescents. The overall impression from both longitudinal chapters was that sleep spindle characteristics, and their relationship with cognition, do not change considerably in early adolescence. In an 18-month period starting at age 10yrs, fast spindle frequency showed a consistent, significant increase, in line with expectations and frequency values from other studies (Jenni & Carskadon, 2004; Kurth et al., 2010; Shinomiya, 1999; Tarokh &

Carskadon, 2010). Slow spindle frequency followed a similar, yet non-significant, trajectory, however other spindle characteristics (density, duration and amplitude) did not show any clear changes at this time. Concurrently, the static and developmental associations between spindles and cognition in this age group were considerably murky. At this early period of adolescence, however, there are significant changes to brain makeup (Casey et al., 2005; Feinberg & Campbell, 2010) that might be creating instability in the proposed relationship. The overall findings from both sleep restriction and longitudinal studies nonetheless call into question the strength of the relationship between spindles and cognition in adolescence.

Contribution of thesis findings to what we know

The investigation of sleep spindles has been on the rise in the last 20 years, with many researchers investigating the spindle itself, in terms of its neurological makeup and origin (De Gennaro & Ferrara, 2003; Luthi, 2014), and others investigating its relationship to not only cognition (for reviews and meta-analyses see Feinberg & Campbell, 2010; Reynolds et al., 2018 (**Chapter 2**); Ujma, 2018), but a myriad of other functions, including sensory gating and sleep-maintenance (Dang Vu et al., 2010; Schabus et al., 2012), synaptic plasticity (Luthi, 2014; Urakami et al., 2012), sleep misperception (Normand et al., 2016) and, when abnormal, are associated with neurological disease, particularly schizophrenia (Ferrarelli et al., 2010; Kuula et al., 2018; Merikanto et al., 2018). The discrete period of adolescence, however, has been relatively neglected in the spindle literature, with only 14 studies identified at the beginning of this thesis (**Chapter 2**) that directly investigated the associations between adolescents' spindles and cognition (12 studies qualified for inclusion in the meta-analysis). To this end, the present thesis aimed to add to this knowledge base by providing firstly a meta-analysis of the available studies on adolescent spindles and cognition, and secondly to use strong research designs (i.e., laboratory experimental manipulation, longitudinal investigations) to investigate specific spindle characteristics and

their functional significance for adolescents. The following sections will focus on specific areas where the thesis findings have added to our knowledge of sleep spindles during adolescence.

Developmental aspects of sleep spindles

The present thesis was able to add valuable information to the field for the developmental trajectory of sleep spindles in adolescence. Previous cross-sectional studies have established that spindle characteristics alter across the lifespan, with duration and amplitude showing an overall decrease, while frequency shows a consistent increase (Jenni & Carskadon, 2004; Kurth et al., 2010; Nader & Smith, 2015; Martin et al., 2013; Nicolas et al., 2001; Shinomiya et al., 1999). Studies examining the development of spindle density show discrepant findings (Bodizs et al., 2014; Nader & Smith, 2015), possibly due to an increase in spindle density during adolescence followed by decrease in early adulthood (Purcell et al., 2017). Considering adolescents experience a multitude of brain network reorganisation (Blakemore & Choudhury, 2006; Casey et al., 2005; Feinberg & Campbell, 2010), it is of high interest to investigate these changes on sleep EEG, particularly for the sleep spindle, as it is proposed to relate to cognition (Fogel & Smith, 2011). At the time of completing this thesis, only three studies had investigated longitudinal changes to spindle activity in adolescence, the first two of which focused on sigma power (Campbell & Feinberg, 2016; Tarokh & Carskadon, 2010), which is not able to decipher true spindle characteristics from other EEG activity (Tarokh & Carskadon, 2010). The third was published only recently (Hahn et al., 2018), and focused on specific spindle characteristics of density and frequency. In their study, Hahn and colleagues measured adolescents' spindles and overnight memory consolidation in two 4-day sleep studies, spaced 7 years apart (baseline mean age = 9.44 ± 0.79 yrs (range 8-11 yrs), follow up = 15.97 ± 0.87 yrs (range 14-18). Over this time, adolescents experienced an increase in fast spindle density from baseline to the 7-year follow

up, and this developmental increase was associated with better memory consolidation at the follow up. They also found an overall increase in frequency, consistent with our findings in **Chapter 6**. Their study investigated a longer time-base, with measurements spaced 7 years apart, while our study measured adolescents' spindles and cognition at 6-month intervals over an 18-month period to capture detailed changes that might occur. The findings from **Chapter 6** therefore add valuable information about longitudinal changes in specific spindle characteristics across concise measurement points in the early adolescent period, and furthermore include duration and amplitude measurements. As illustrated in **Chapter 6**, the mean fast spindle frequency values at each time point of the study matched the increasing trajectory laid out by other cross-sectional studies. This confirms that spindle frequency is increasing with age, and furthermore provides evidence from a controlled laboratory setting that frequency increases within-subjects.

Spindles as a protective function of sleep

Another interesting addition to the literature from the present study is the indication that spindles may be protective of sustained attention losses during sleep restriction (**Chapter 5**). Specifically, adolescents with longer spindles experienced less of an increase in PVT lapses over consecutive days of sleep restriction compared to adolescents with shorter spindles. In past studies, sleep spindles have been shown to protect sleep maintenance. For example, Dang Vu and colleagues (2010) tested the alerting effects of small tones played during sleep, and found subjects were more likely to stay asleep if they were producing a spindle than if they were not. This has been evidenced in earlier studies (Ehrhart et al., 1981; Naitoh et al., 1982, Steriade, 1991), and in more recent acoustic perturbation studies (Dang Vu et al., 2011; Schabus et al., 2012). Furthermore, higher spindle density may be protective of stress-related sleep disturbances in young people (Dang Vu et al., 2015). These findings and those in **Chapter 5** align with the hypothesis that spindles hold a sensory-gating

mechanism to maintain sleep in order to optimise cognitive faculties (Parrino & Vaudano, 2018). Notably, a more recent study found that spindles were affected by traffic noise during sleep in young and older adults, but did not appear to protect sleep maintenance (Rudzik et al., 2018), indicating there are limits to this hypothesis. Further to this, another recent study found a person's sleep may be protected by spindles when they are faced with familiar stimuli (e.g., the sound of a familiar voice), while less familiar stimuli (e.g., the sound of an unfamiliar voice) are more likely to elicit a cortical response regardless of the presence of a spindle (Blume et al., 2018). The 'sleep-protective' function of spindles may therefore be moderated by the salience of external stimuli during sleep. Further research is warranted to better understand this phenomenon and replication with a larger sample is necessary to confirm the effects for adolescents in **Chapter 5**. Nonetheless, the notion that the protective sleep-maintenance role of spindles may occur in adolescents adds importance to improving their sleep quality to enhance the opportunity for optimal spindle production, and potentially better cognitive and emotional functioning (Dang Vu et al., 2015).

Reliability of spindle measurements in young samples

The present thesis also adds helpful guidance to the measurement of sleep spindles in future adolescent research. In **Chapter 3**, the reliability of spindle measurements was established, with a single night of sleep monitoring sufficient for reliable estimations of slow spindle amplitude and fast spindle density, duration and amplitude, and 2 and 4 nights needed for measurements of slow duration and density, respectively. This informs future studies on how many consecutive nights of sleep monitoring they would need to establish adequate reliability of their chosen spindle characteristics, which can only strengthen confidence in their findings. Furthermore, this investigation added to the knowledge base that spindles are consistent within individuals (De Gennaro et al., 2005; Finelli et al., 2001), yet divergent between individuals (Bodizs et al., 2009; Werth et al., 2007). Although slow spindle density

and duration were retained in **Chapter 4** due to reviewer preference in the resulting publication (Reynolds et al., 2018), the lack of reliability for these characteristics was nonetheless emphasised in the discussion of **Chapter 4**. These characteristics were, however, removed from analysis in **Chapter 5** comparing spindles to cognition during sleep restriction to focus analyses on only those characteristics likely to be reliable. Finally, all spindle characteristics were retained for the young adolescent sample in **Chapters 6** and **7** due to the potential inability of findings to translate from mid-adolescence to early-adolescence (due to the above-described significant changes in spindle makeup between childhood and adolescence). The reliability of spindle characteristics in early-adolescence, however, warrants confirmation in multi-night studies.

Spindle frequencies of young samples

A further developmental addition from the present thesis' findings is the frequency values of both slow and fast spindles for young- and mid-adolescents. In **Chapter 6** the average frequency of young adolescents' slow and fast spindles was 10.76Hz and 12.33Hz at T1 (mean age = 10.3yrs), respectively, which increased to 10.86Hz and 12.44Hz at T4 (mean age = 11.9 yrs). In **Chapter 5.B**, the slow and fast spindle frequencies for mid-adolescents (mean age = 15.9yrs) were 10.99Hz and 12.99Hz. The mean values from both of these studies fell short of the typical adult spindle frequencies of ~12Hz for slow and ~14Hz for fast (Luthi, 2014), and were in the lower margins for more liberal classifications (slow: 9-12Hz and fast: 12-15Hz; Molle et al., 2011). Furthermore, as noted in **Chapters 3** and **4**, not all adolescents had a clearly distinct slow spindle peak on the spectral profile, which has also been observed in adult samples (De Gennaro et al., 2005; Warby et al., 2014, Werth et al., 1997). In fact, some researchers speculate that slow spindles may have a different intra-cortical origin to fast spindles (Timofeev & Chauvette, 2013). Nonetheless, the slower overall spindle frequencies in young samples bears strong methodological considerations for future

studies, especially for those dividing spindles into the respective slow and fast frequencies for subsequent analyses, or for those using set frequency criteria in automatic algorithm methods for spindle detection. As discussed in **Chapter 6**, these set criteria may have impaired past studies' detection of slow and fast spindles in child samples, so that some fast spindles were mis-represented as slow spindles by a lower frequency limit that was set too high (e.g., Chatburn et al., 2013; Hahn et al., 2018; Nader & Smith, 2015). To illustrate, Figure 8.1 compares spindle density values between past studies and the values in the present thesis, similar to Figure 6.3 for frequency values in **Chapter 6**. Of note, frequency limits in the spindle detection process were remarkably different across studies, leading to a diverse array of density values. While Hahn and colleagues' (2018) frequency measures across their longitudinal study line up beautifully with those in the present thesis (see Figure 6.3 in **Chapter 6**), spindle density values are not comparable to those in **Chapter 6**, as they used set frequency criteria (slow: 11-13Hz, fast: 13-15Hz), in contrast to our adjustment for individual frequencies. This likely explains the discrepancy in the number of spindles detected by each method (e.g. our fast density at a mean age of 10.3yrs was 7.15 spindles per minute, compared to Hahn and colleagues' fast density of approximately 1.5 at 9.4yrs). Future studies that use similar methodologies could compare similar trajectories as in **Chapter 6** for specific spindle characteristics of density, duration and amplitude to consolidate findings across this age group.

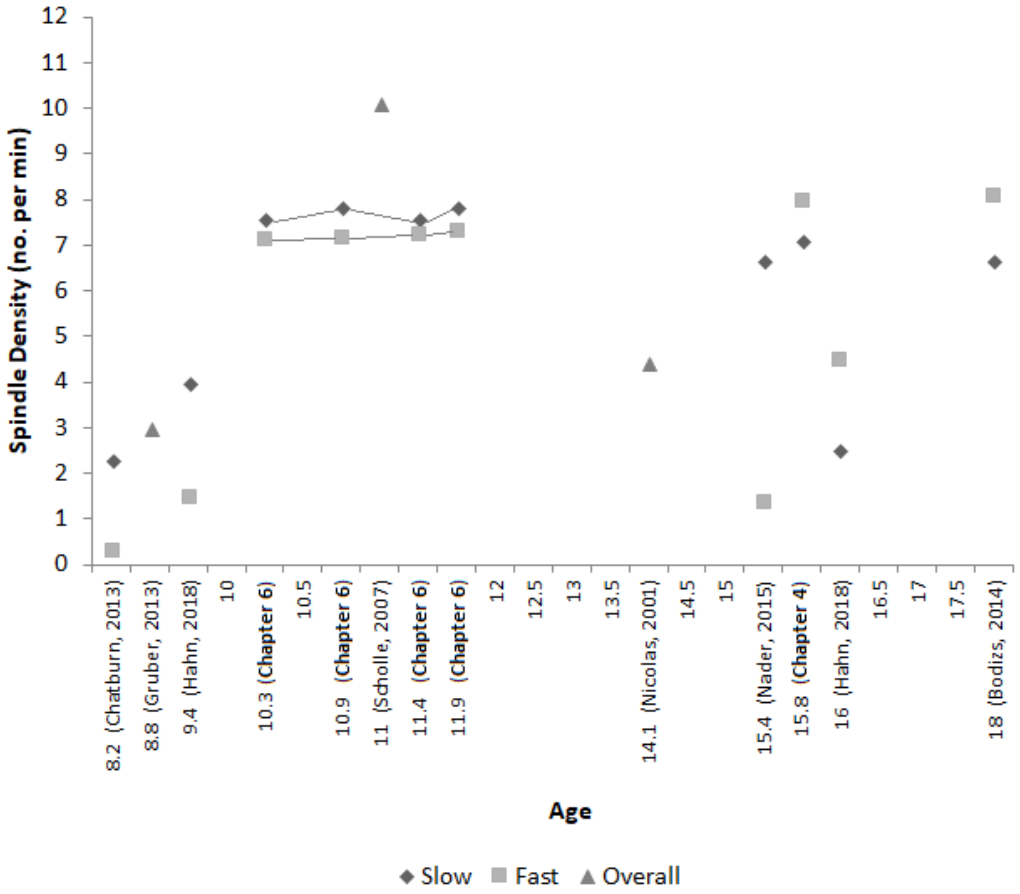

Figure 8.1. The trajectory of changes in spindle densities from the present thesis and other values in the literature (separated for slow and fast where available and presented as 'overall' when a single frequency measure was provided), from middle childhood to late adolescence. The findings from the present thesis' longitudinal study (**Chapter 6**) are joined, while other cross-sectional study's findings, including those from **Chapter 4**, are single symbols. Gaps are left for ages where density estimates are not included. Note mean density values from other studies were estimated when a figure or range was provided rather than specific values.

The present thesis strengthens the knowledge base that slower overall frequencies are present in children and adolescents, as evidenced in previous research (Chatburn et al., 2013; Gruber et al., 2013; Hahn et al., 2018; Hoedlmoser et al., 2014; Jenni & Carskadon, 2004; Kurth et al., 2010; Nicolas et al., 2001; Tarokh & Carskadon, 2010), and justifies the need to

consider individual frequencies when performing spindle detection (Bodizs et al., 2009). The Individual Adjustment Method (Bodizs et al., 2009) used in the present study is not the only automatic spindle detection method that takes individual frequencies into account. A recently released open-access spindle detection program, SpiSOP (used in Klinzing et al., 2016; Rudzik et al., 2018 and others), takes individuals' central frequencies on a spectral profile into account in the spindle detection process. Such methods add improvement to the field of literature on spindle detection considering the widely acknowledged individual differences in these waveforms (Bodizs et al., 2009; Buckelmuller et al., 2006; Reynolds et al., 2018 (**Chapter 3**); Werth et al., 2007). The evidence provided in this thesis supports these methods heading into future research on child and adolescent samples.

What do we now know about spindles and cognition?

The final main addition to the current field of literature is the absence of strong relationships between sleep spindles and cognition in **Chapters 5** and **7**. Considering the strong expectations based on the meta-analytic review (**Chapter 2**), this finding was surprising, and bears considerable theoretical deliberation. The absence of a consistent spindle-cognition relationship in this thesis might point to a new way of thinking about correlational analyses between complex neural substrates and observable cognition. The theoretical considerations of the nature of the relationship between spindles and cognition are discussed in the following sections.

Theoretical implications for the spindles-cognition relationship

The influence of type of task

One of the main take-home messages from the present thesis is that sleep spindle characteristics did not show significant, static associations with higher-order cognitive functions, specifically fluid intelligence and working memory performance. Perhaps spindles do not show consistent relationships with higher-order cognitive performance, contrary to the

results of the meta-analysis in **Chapter 2**. In theory, more mature adolescents would be expected to have higher white matter connectivity (Schmithorst et al., 2005), which is not only related to spindle makeup (Bodizs et al., 2014; Piantoni et al., 2013) but to better performance in the three main cognitive domains in the present thesis: fluid intelligence, working memory and sustained attention (Klarborg et al., 2013; Privado et al., 2014). Sustained attention did in fact show a relationship with spindle duration in **Chapter 5**, where those with fast spindles of longer durations at baseline were protected from sustained attention losses during sleep restriction. Those adolescents were proposed to be *less* mature neurologically, however, as spindle duration decreases with development (Martin et al., 2013; Nader & Smith, 2015; Nicolas et al., 2001). The presence of a protective function for less mature adolescents was based on a previous theory that the networks that are important for sustained attention in adults are not completely developed in children, and therefore children escape the vulnerability of that particular network to sleep loss (Astill et al, 2012). These theories are conflicting, however, as those with more mature networks (i.e., shorter spindle durations) should have had superior sustained attention performance, but this did not occur. Fluid intelligence improvements were also implicated in increases of slow spindle amplitude in **Chapter 5**, however this was a dynamic, rather than a static association, where changes in fluid intelligence scores were positively correlated with changes in slow spindle amplitude. Furthermore, fluid intelligence did not appear related to maturation in the longitudinal study in **Chapters 6** and **7**, contrary to expectations and the theory that better performance is related to brain maturation, as indexed by changes in spindles. One explanation put forth in **Chapter 7**, but which also applies to **Chapter 5**, is that the concurrent complex brain reorganisation (Feinberg & Campbell, 2010; 2013) and development of higher-order cognitive functions during adolescence (Casey et al., 2005, Luna et al., 2010; Wright et al., 2008) creates instability in possible associations between spindles and cognition in this age

group - masking the true nature of the phenomenon. Sustained attention was initially measured in the longitudinal study, however due to issues with PVT device reliability, it was not able to be included in the analyses. It would be an interesting avenue for future research to investigate the relationship between spindles and sustained attention across development when brain restructuring is starting to occur, and to determine if the protective function of spindle duration occurs in the younger age group as well.

Considering higher-order cognitive functions did not show consistent relationships in the present thesis, there may be other cognitive areas that are more reliably related to spindles. Overnight memory consolidation, for example, might be a domain that shows more consistent findings (Clemens et al. 2005; Hahn et al., 2018; Hoedlmoser et al., 2014; Lustenberger et al., 2012; Schabus et al. 2004). In general, subjects are provided with a learning task (e.g., word pairs; Hoedlmoser et al., 2014), their sleep that night is measured, and their recall performance is tested the next day. This method solves the problem discussed in **Chapter 2** of equating studies that vary considerably in the time between sleep recording and cognitive performance measurement. As a reminder, of the 12 included studies in the meta-analysis, only 9 specified when spindles and cognition were measured. Of these 9 studies, 3 analysed spindles immediately after the test, 1 analysed spindles immediately before the test, 1 analysed spindles both before and after the test, 2 analysed spindles in the sleep period between learning and recall, and 2 analysed spindles a week or two after testing. If learning creates new connections in the brain, and this is consolidated during sleep and reflected in spindle activity (Diekelmann & Born, 2010) then the optimal environment in which to test the effect of learning on the brain is when learning occurs immediately before sleep, with the test for improvement immediately after sleep. Accordingly, in tests of overnight learning, the measurement of spindles is placed specifically in the sleep period between learning and recall (Clemens et al. 2005; Hahn et al., 2018; Hoedlmoser et al., 2014;

Lustenberger et al., 2012; Schabus et al. 2004), thereby creating a more reliable time-base to relate spindles to cognition. This methodological consideration warrants focus moving forward, where studies should apply similar durations of time between sleep recording and cognitive tests. Furthermore, if overnight learning is more easily associated with spindles due to the closer proximity in time of measurements, then associations between spindles and higher-order functions would be harder to detect, given these abilities develop over several years during adolescence and into young adulthood (Casey et al., 2005; Hartshorne & Germine, 2015; Luna et al., 2010). This may help to explain the lack of relationships seen between spindles and higher-order cognitive functions (fluid IQ and working memory) in the present thesis. It should be noted, however, that studies of associations between spindles and memory consolidation also show discrepancies (McDevitt et al., 2017). For example, some report slow spindles are more often related to memory consolidation than fast spindles (Holz et al., 2012), while others report the opposite (Hahn et al., 2018; Saletin et al. 2011; van der Helm et al. 2011) or find no relationship (Ackermann et al. 2015). Most likely, there will always be debate and conflicting findings when attempting to determine neural correlates of cognition through brain activity during sleep considering the complex underpinnings of this relationship. However, having a methodological focus for when to measure spindles and cognition, and a suggestion that memory consolidation might be a better cognitive domain of focus, will help consolidate findings within the field.

Other EEG patterns that might be related to cognition

Despite doubts about how strongly spindles are related to cognition, there are clearly elements of sleep that are important for cognitive performance, and indeed, this forms the theoretical justification of the investigations between spindles and cognition (Diekelmann & Born, 2010). However, if spindles are not a strong contender, or at least only a part of the puzzle, what other factors are important? We can return to two main hypotheses of

information consolidation during sleep to determine what else might be involved. The first is the synaptic homeostasis hypothesis of sleep (Diekelmann & Born, 2010; Tononi & Cirelli, 2006). In this model, information that is processed during wakefulness results in a net increase of synapses in the brain. Overnight, these new synapses are integrated into the existing network, and synapses which are no longer important are removed, known as 'synaptic downscaling' (Diekelmann & Born, 2010). Slow wave sleep is believed to relate to synaptic downscaling, whereby slow waves reach maximal amplitude at the start of the night, coinciding with higher synaptic density, and show a subsequent decrease in amplitude later in the night, coinciding with lower synaptic density (Tononi & Cirelli, 2006). For this reason, slow oscillations have been a popular EEG candidate for reflecting consolidation during sleep (Stickgold et al., 2007), and experimental manipulation of slow waves has been shown to improve memory (Marshall et al., 2006), similar to experimental manipulation of spindles (Mednick et al., 2013; Ngo et al., 2013; Rasch et al., 2009) as described in **Chapter 7**. The exact role of the slow oscillation in synaptic downscaling is not well-evidenced, however (Diekelmann & Born, 2010), and correlation analyses will likely experience similar shortcomings as per the spindle research. Nonetheless, this hypothesis is another justification of why overnight memory consolidation may be more reliably associated with sleep EEG than higher-order cognitive functions which likely consist of longer-term synaptic reorganisation compared to a single night for simpler declarative memory tasks.

The second hypothesis of sleep-related memory consolidation is the system consolidation hypothesis of sleep (Diekelmann & Born, 2010). In this hypothesis, several correlates of brain activity are involved: slow waves, spindles and hippocampal sharp-wave ripples. The synchrony of spindles and hippocampal ripples, coupled with the up-state of a slow wave, are proposed to drive the consolidation of information into the cortex (Diekelmann & Born, 2010). As put forth in **Chapter 7**, the coupling of spindles to slow

waves is one area that seems to hold promise for showing associations with cognition (Helfrich et al 2018; Luthi, 2014; McDevitt et al., 2017; Molle et al., 2009; 2011). Furthermore, the uncoupling of spindles and slow waves in older adults has been linked to forgetting and degeneration in the frontal cortex (Helfrich et al., 2018). Again, memory consolidation appears to be a more consistent cognitive performance domain than higher-order cognitive functions. However, the coupling of spindles to slow waves is not always linked to memory (Klinzing et al., 2016), and once again more research is needed to discover more about this phenomenon and in what scenarios it is most likely to be observed. The importance of considering all effects, especially those that disconfirm the hypothesised relationships between spindles and cognition, is another strong future recommendation from the present thesis. Nonetheless, there are clearly areas of interest in sleep EEG and cognition research that go beyond a basic spindle-cognition correlation and may add a deeper understanding of the memory-consolidation function of sleep.

The presence (and absence) of spindle-cognition relationships in the literature

An important acknowledgement in light of the lack of clear associations between spindles and cognition in the present thesis is the fact that these are not the first investigations to fail to replicate this phenomenon. This echoes past scepticism of replication within the psychological and biomedical research fields (Ferguson & Heene, 2012; Ioannidis, 2005). Considering several studies included in the meta-analysis in **Chapter 2** used a neurobehavioural battery or multi-domain intelligence test (Chatburn et al., 2013; Geiger et al., 2011; Hoedlmoser et al., 2014; Nader & Smith, 2015; Piosczyk et al., 2013; Tessier et al., 2015), yet reported only a few, mostly significant, correlations, there is likely an element of publication bias influencing the research field. Due to the addition of unpublished effect sizes from personal correspondence with authors of several included studies, it was not plausible to perform analyses of publication bias in **Chapter 2**, however a recent meta-analysis with a

broader age range (Ujma, 2018) found an indication of publication bias for studies reporting significant associations between general cognitive ability and fast spindle density and amplitude. The tone of this thesis began with a strong expectation for replicating the relationships seen between spindles and cognition in many years of research in the field. Unfortunately, this relationship proved to be more elusive than initially anticipated and requires a reconsideration of the observability of this relationship. Considering the disconnect between strong initial expectations and null findings in the present thesis, a future suggestion is for researchers to report all effects, regardless of significance, and for transparency include an accessible dataset with their publication, so that future meta-analyses will provide a fully comprehensive overview of this relationship. Additionally, a more efficient solution would be to focus future studies on the spindle characteristics and cognitive variables that are more theoretically plausible and thus more likely to show associations. As discussed earlier, one candidate for a cognitive domain focus is overnight memory consolidation. The optimal spindle characteristic, however, is at present unknown, and until more is understood about the functional differentiation between spindle density, duration, amplitude and frequency, all characteristics warrant equal attention.

Clinical implications

Spindles in the face of sleep restriction

The present thesis established that sleep restriction to 5hrs of time in bed resulted in changes to adolescents' sleep spindles (**Chapter 4**). Specifically, fast spindle amplitude became higher, and fast spindle duration became longer. While fast spindle amplitude was not related to changes in cognitive performance, fast spindle duration was. Adolescents with longer fast spindle durations at baseline appeared to be protected from deficits to sustained attention during sleep restriction (**Chapter 5**). An implication of this finding, as described in **Chapter 5**, is that adolescents who have less mature brain networks, and resultantly longer

spindles, may be resilient to the effects of sleep restriction on this relatively basic cognitive function. Could this imply that young adolescent brains preserve functioning during complex brain reorganisation? The theory that spindles are protective of sleep maintenance (Dang Vu et al., 2010; Schabus et al., 2004), as described earlier, suggests some merit to this speculation, particularly when adolescents are restricted to 5hrs of time in bed and need to maintain sleep to optimise cognitive faculties; longer spindles would increase the likelihood of staying asleep. This is particularly important for young people who regularly achieve as low as 4 - 6.5 hrs of sleep (Lund et al., 2010). This also bears cultural considerations, as adolescents globally achieve insufficient sleep durations on school nights (below 8 hrs), with Asian and North American adolescents achieving lower school night sleep durations than European and Australasian adolescents (Gradisar et al., 2011; Short et al., 2013), the lowest of which was 6.6 hrs in 17-19-year-old Hong Kong students (Chung & Cheung, 2008). Certainly, sustained attention was impaired in a dose-response manner in the sleep restriction study used in the present thesis, as reported in another paper (Short et al., 2018), with the highest deficits seen with 5hrs' time in bed, moderate deficits with 7.5hrs, and the least deficits with 10hrs. These findings show that adolescents' sleeping brain waves are impacted by sleep restriction and enhance recommendations that increased sleep durations (between 8 - 10 hrs; Hirshkowitz et al., 2016) will optimise adolescents' daytime functioning. Clinically, adolescents can attain improvements in sleep quality and quantity through psychological treatments (Dewald-Kaufmann et al., 2013; Richardson et al., 2018). For example, adolescents who experience Delayed Sleep-Wake Phase Disorder, where they have difficulty falling asleep and waking at socially appropriate times and often experience sleep loss as a result, have been treated successfully with light therapy (Richardson et al., 2018), showing improvements in sleep timing, sleep onset latency and total sleep time. Interestingly, the most effective components of treatment in Richardson and colleagues' randomised controlled trial

may have been changes in behavioural patterns regarding sleep and psychoeducation about sleep and circadian rhythms, indicating these elements could be widely applied to adolescents to improve sleep. Improvements in sleep timing following treatment have been further evidenced to improve adolescents' cognitive performance, particularly for visual-spatial processing (Dewald-Kaufmann et al., 2013). Of note, working memory and fluid intelligence were not impacted by even severe sleep restriction in **Chapter 5** (5hrs' time in bed), indicating that some cognitive functions may be nonetheless preserved in the face of sleep loss, as has been reported in past studies (Anderson et al., 2009; Kopasz et al., 2010; Voderholzer et al., 2011), and potentially due to the relative preservation of slow wave sleep (Voderholzer et al., 2011) as seen in **Chapter 4**.

Sleep spindles across development

The present thesis established that in the developing young adolescent, sleeping brain waves change. The longitudinal study in **Chapter 6** was one of the first to examine adolescents' specific spindle characteristics in a longitudinal setting, with the only other longitudinal study to our knowledge (Hahn et al., 2018), confirming our finding of increasing spindle frequency with development. In **Chapter 6**, fast spindle frequency was the only spindle characteristic to show a significant change, where frequency became consistently faster with development, and slow spindle frequency followed a similar pattern, indicating spindle frequency might be the first characteristic to change noticeably in early adolescence. In terms of neurodevelopment, it is worthwile to consider the potential neural basis spindle characteristics to help understand the pattern of findings. Intracranial EEG research has suggested that spindle frequency and density may be driven by thalamic hyperpolarisation (Andrillon et al., 2011). These spindle parameters are furthermore related to reciprocal changes in slow wave activity, where stronger thalamic hyperpolarisation is presumed to coincide with high slow wave activity, which in turn supresses thalamic neurons that are

responsible for spindle production (Andrillon et al., 2011). The speculation arising from this is that developmental changes to thalamocortical cells are reflected in at least the increase in spindle frequency seen in this thesis. Spindle density did not show a clear developmental trajectory, however based on this speculation, it may be the next characteristic to show developmental changes. It would be interesting for future research to determine, firstly, the likely neural bases of spindle duration and amplitude and, secondly, investigate how this might be expressed across adolescent development. Alongside the increase in spindle frequency in **Chapter 6**, adolescents showed improvement in working memory and in one measurement of fluid intelligence in the early years of adolescence (**Chapter 7**), supporting past findings (Hartshorne & Germine, 2015; Luna et al., 2010). The improvement in these higher-order cognitive functions was not, however, related to the increase in spindle frequency in this cohort, or changes in any other spindle characteristic. Admittedly, the changes in frequency were relatively small (0.1Hz increase on average per year, $d = 0.24$). Two implications from this are that either the young adolescent brain is not changing significantly enough to be indexed on spindles, or that the improvements in working memory and fluid intelligence are better observed through other methods (e.g., decreases in grey matter and increases in white matter through imaging techniques; Barnea-Goraly et al., 2005; Casey et al., 2005; Paus, 2005). Regardless, sleep spindles do appear to be changing in early adolescence and, particularly as they are affected by sleep restriction (**Chapter 4**), add encouragement for improving adolescents' sleep.

State vs trait aspects of sleep spindles

One of the driving aims of the present thesis was to examine the *state* aspects of spindles alongside *trait* aspects, and what this means for the relationship with cognitive performance. Considering the overall findings, there was stronger evidence that adolescents' sleep spindles alter during sleep restriction (*state*), compared to the extent to which they

changed with development (*trait*). Spindles were more meaningfully altered by severe sleep restriction (**Chapter 4**), with significant increases in fast spindle duration decreases in fast amplitude in an acute time setting, than by development (**Chapter 6**), where only a small increase in fast spindle frequency occurred, albeit consistent with the literature. This distinction may not be surprising, however, given it was established in **Chapter 3** that, for certain spindle characteristics and under controlled conditions, spindles are remarkably consistent within individuals. The longitudinal study of **Chapters 6** and **7**, although conducted during a potentially fragile period of early adolescence, was likely a more controlled setting than that of dosed sleep restriction in **Chapters 4** and **5**. Perhaps spindles are more likely to be affected by *state* than *trait* factors. Furthermore, although cognitive performance was not consistently related to spindle characteristics throughout the present thesis, there were more meaningful relationships seen during sleep restriction (**Chapter 5**), where increased fast spindle duration may have been protective of sustained attention deficits, supporting the theory of a sleep-protective function of spindles (Dang Vu et al., 2010; Schabus et al., 2004). In contrast, no meaningful relationships were seen between both spindle and cognitive development over time (**Chapter 7**). Taken together, the overall thesis findings suggest that *state* aspects of the environment are more influential than *trait* factors for the relationship between adolescents' spindles and cognition.

Limitations and directions for future research

While the experimental and longitudinal studies in the present thesis had comparable sample sizes to other laboratory-based studies of paediatric samples (n = 34 and 20, respectively), there is nonetheless a need for larger sample sizes when dealing with complex neural substrates of cognition that may only ever show small effect sizes (Ujma, 2018). One solution to this is to encourage laboratories to collaborate and combine sleep data to provide larger datasets from which to analyse potential associations (Ujma, 2018). In order to

facilitate reliability of this method, however, it would firstly be essential to establish standard methods for detecting and analysing spindles, given the discrepancy between different methods (Warby et al., 2014). As described earlier, considering the individual variation in spindle makeup (Bodizs et al., 2009; Buckelmuller et al., 2006; Reynolds et al., 2018 (**Chapter 3**); Werth et al., 2007) and the significant changes from childhood to adolescence (**Chapter 6**; Jenni & Carskadon, 2004; Kurth et al., 2010; Nader & Smith, 2015; Martin et al., 2013; Nicolas et al., 2001; Shinomiya et al., 1999) it is becoming clearer that an individualised approach optimises accurate detection of spindles and should be used in collaborative research. Furthermore, the laboratory-controlled environment is preferred for sleep monitoring, as several factors can impact sleep (e.g., light: Cajochen, 2007; ambient temperature: Joshi et al., 2016), cognitive performance (e.g., time spent awake before testing: Dijk et al., 1992; Vetter et al., 2012; prior sleep duration: de Bruin et al., 2017) and spindles (e.g., body temperature: Schmitt et al., 2002). Establishing standard procedures, similar to the strong designs used in the present study, will help to confirm reliability of effects seen. Consideration should also be given to electrode topography and the effect that the chosen EEG sites in the present study (C3-M2 as a first preference, C4-M1 as the alternate) may have had on the resulting relationships, given there is topographic variation in the relationship between spindles and cognition (Bodizs et al., 2014; Fogel & Smith, 2011), as well as topographic changes in spindle density as children develop into adolescents (Hahn et al., 2018). Given the large number of spindle and cognitive variables included in each study of the present thesis, it was not feasible to include varied topographical locations as an additional variable. This may therefore be an area for future in-depth focus when looking at topographical variability in the relationship between spindles and cognition in the developing adolescent.

The relationship between sleep and cognition may be too complex to understand through small bursts of activity recorded on EEG, and indeed changes in sleep EEG may only account for a small amount of variance in cognitive performance. This warrants a more comprehensive consideration of the other areas of sleep that are related to cognition. Cognitive performance may be influenced by more simple variables in the everyday life of adolescents, for example, the amount of sleep the night before the test and general sleepiness (for reviews, see de Bruin et al., 2017 and Dewald et al., 2010), and likely the time spent awake before the test (based on adult findings; Dijk et al., 1992; Vetter et al., 2012). Certainly, sustained attention is impacted by sleep restriction (Short et al., 2018, Lo et al., 2016), and working memory may be affected by sleep restriction for some tasks (Lo et al., 2016), although not for the operation span task used in the present thesis (**Chapter 5**). Furthermore, there can be circadian effects on performance, where testing at different times of the day can lead to dramatic differences in performance (Short et al., 2018; Lo et al., 2012). Certainly, the present study tried to control for circadian factors by using a consistent time of the day for testing and assigning a consistent sleep duration preceding the sleep studies. However, endogenous melatonin profiles can differ considerably between individuals, particularly as circadian rhythms are altering during adolescence (Carskadon, 2011). Interestingly, one's own circadian rhythm can be a determinant of short-term memory performance (Johnson et al., 1992; Wright et al., 2002), which cannot be easily controlled in the laboratory setting. Research has also shown a circadian modulation of spindles, where spindle frequency is often lowest at the nadir of core body temperature (De Gennaro & Ferrara, 2003; Dijk et al., 1995; Wei et al., 1999). This would be an interesting addition to a future study, where changes in spindle characteristics could be compared to changes in circadian rhythms across development. There were in fact proxy measurements taken for core body temperature for the young adolescents in **Chapter 6** and **7** as part of another project,

which presents an interesting opportunity for future research on circadian changes and spindles, albeit beyond the scope of the present thesis.

The longitudinal study in the present thesis (**Chapters 6** and **7**), while achieving successful retention of a paediatric cohort in an 18-month laboratory-based study, was only able to capture a snapshot of the early adolescent years. The time course of puberty differs between individuals (Kaplowitz et al., 2001) and is often linked to brain maturation (Sisk & Foster, 2004) as well as sleep changes (Carskadon, 2011) and spindle production (Tarokh & Carskadon, 2010). Our investigation likely captured the start of puberty for some, but others may not have reached pubertal onset, which can occur between 10-12 years for males (Walvoord, 2010) (at T4 the mean age was 11.9yrs and 2 of these 12 subjects were still prepubescent (Tanner Stage 1; Tanner 1990)). Furthermore, changes to sleep patterns and brain network reorganisation can extend into the early 20s (Roenneberg et al., 2004; Sisk & Foster, 2004). A direction for future research is therefore to aim for longer-term developmental studies to investigate more fully the transition from childhood to adolescence and into adulthood and the changes in spindle makeup that occur in this time. It would be ideal, however, to continue short intervals between testing (e.g., 6 months, as in the present study) to capture any rapid developmental changes to sleep that may occur in the adolescent.

Finally, emphasis has been placed on individual differences in spindle makeup (Bodizs et al., 2009; Werth et al., 1997; Reynolds et al., 2018 (**Chapter 3**)), and one factor that did not receive much attention in the present thesis was sex, where males and females can experience differences not only in spindle makeup, but in the relationships between spindles and cognition. In **Chapter 4** the response of males and females to severe sleep restriction (5hrs' time in bed) varied, with males experiencing a more severe decrease in fast spindle amplitude than females. A sexual dimorphism for the relationship between spindles and cognition has been seen in the past, with females showing stronger associations than

males (Bodizs et al., 2014; Ujma et al., 2014). Furthermore, as discussed earlier, white matter is believed to index spindle activity (Bodizs et al., 2014; Piantoni et al., 2013), and cognitive performance (Klarborg et al., 2013; Privado et al., 2014), and associations between white matter and intelligence are stronger in females than males (Gur et al., 1999; Haier et al., 2005; Ryman et al., 2016). Gender was initially included in **Chapter 5**, however due to the small sample sizes that resulted from the dose-response design (total *n* of 34 was reduced to *n* = 12, *n* = 10 and *n* = 12 for the 5hr, 7.5hr and 10hr groups, respectively), analyses that were further split for gender were not statistically plausible. Furthermore, only boys were included in the longitudinal study of this thesis due to the impact of sex differences on sleep, but also on puberty, which was a key factor of interest in another research question. However, this presents an interesting area for future research, particularly in a longitudinal setting.

Conclusion

The present thesis demonstrated that an adolescent's sleep spindle characteristics change as a function of *state* (sleep restriction) and *trait* (development) factors. During sleep restriction, adolescents' sleep spindles became higher in amplitude and longer in duration - with longer durations potentially acting as a protective mechanism for sustained attention performance. Developmentally, young adolescents experience increases in fast spindle frequency, and are expected to experience changes in other spindle characteristics over a longer time-course. The expected static relationships between sleep spindles and cognition, based on the initial meta-analytic review, were not replicated in either sleep restriction or longitudinal studies. The complexity of the hypothesised relationship is considered a potential barrier to detecting these associations, and there is likely to be publication bias in the current literature. Nonetheless, sleep is clearly important for cognitive performance in all ages, particularly for the developing adolescent, where their academic performance can determine the range of vocational pathways. Spindles may not be as consistently related to higher-order

cognitive functions as expected, and studies investigating overnight memory consolidation might hold more promise. Future studies are encouraged to combine findings to allow a more comprehensive overview of the relationship between spindles and cognition in adolescence.

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