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Attention in the Brain Under Conditions of Sub-Optimal Alertness: Neurobiological Effects and Individual Differences

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Attention in the Brain Under Conditions of Sub-Optimal Alertness: Neurobiological Effects and Individual Differences

Abstract

Sleep deprivation (SD) is a prevalent problem in modern society, and one that can have serious adverse consequences for health and safety. Critically, even short periods of SD can lead to relatively large decrements in attention, which may in turn cause an individual to neglect important environmental stimuli. In this thesis, I report the results of three experiments designed to investigate the neural bases of attentional declines under conditions of sleep loss and mental fatigue. In two experiments using arterial spin labeled fMRI, a technique that enables the quantification of absolute levels of cerebral blood flow (CBF), it was found that CBF patterns in the resting brain differed significantly based on arousal levels (Study #1) and prior cognitive workload (Study #2). These findings are a departure from prior neuroimaging studies, which have typically taken neural activity during non-task periods as static and inseparable baseline. In a test of sustained attention, performance declines were observed both following SD (Study #1) and when performing the task for an extended period of time while well-rested (Study #2). These decrements were primarily mediated by hypoactivation in a fronto-parietal attentional circuit. Furthermore, resting baseline levels of cerebral blood flow in the thalamus and prefrontal cortex before the start of the task were predictive of interindividual differences in subsequent performance decline (Study #2). In Study #3, an experiment using standard BOLD fMRI, it was found that performance declines in a test of selective attention following SD were accompanied by reduced functional connectivity between top-down control areas and regions of ventral visual cortex, as well as reductions in activation to targets in object-selective areas. Taken together, these results further our understanding of the neural basis of attention under conditions when this system is taxed beyond its normal limits.

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Attention in the brain under conditions of sub-optimal alertness: neurobiological effects and individual differences

Julian Lim

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In

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To Min Zhi Heng, for being brave for me during the times that I was not.

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Abstract

Attention in the brain under conditions of sub-optimal alertness: neurobiological effects and individual differences

Julian Lim, M.A.
Advisor: David F. Dinges, PhD

Sleep deprivation (SD) is a prevalent problem in modern society, and one that can have serious adverse consequences for health and safety. Critically, even short periods of SD can lead to relatively large decrements in attention, which may in turn cause an individual to neglect important environmental stimuli. In this thesis, I report the results of three experiments designed to investigate the neural bases of attentional declines under conditions of sleep loss and mental fatigue. In two experiments using arterial spin labeled fMRI, a technique that enables the quantification of absolute levels of cerebral blood flow (CBF), it was found that CBF patterns in the resting brain differed significantly based on arousal levels (Study #1) and prior cognitive workload (Study #2). These findings are a departure from prior neuroimaging studies, which have typically taken neural activity during non-task periods as static and inseparable baseline. In a test of sustained attention, performance declines were observed both following SD (Study #1) and when performing the task for an extended period of time while well-rested (Study #2). These decrements were primarily mediated by hypoactivation in a fronto-parietal attentional circuit. Furthermore, resting baseline levels of cerebral blood flow in the thalamus and prefrontal cortex before the start of the task were predictive of interindividual differences in subsequent performance decline (Study #2). In Study #3, an experiment using standard BOLD fMRI, it was found that performance declines in a test of selective attention following SD were accompanied by reduced functional connectivity between top-down control areas and regions of ventral visual cortex, as well as reductions in activation to targets in object-selective areas. Taken together, these results further our understanding of the neural basis of attention under conditions when this system is taxed beyond its normal limits.

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And if tonight my soul may find her peace
in sleep, and sink in good oblivion,
and in the morning wake like a new-opened flower
then I have been dipped again in God, and new-created.

~D.H. Lawrence

Chapter 1

The Cognitive Consequences of Sleep Deprivation

On March 23, 1989, the *Exxon Valdez*, a massive tanker carrying 54.1 million U.S. gallons of crude oil, struck a reef and spilled almost 20% of its cargo into the Prince William Sound. The environmental consequences of this disaster were devastating, affecting over 1,100 miles of the Alaskan coastline. The U.S. Fish and Wildlife service estimated that more than 350,000 birds, 3,500 - 5,500 sea otters and 200 harbor seals were killed as a direct result of the spill (National Oceanographic and Atmospheric Administration, 1992), and the long-term impact of the pollution continued to be felt more than a decade later (Peterson et al., 2003).

The public outcry over this disaster led to an extensive investigation into its causes. In a report issued by the National Transportation Safety Board, the following facts were noted; that "the third mate had *probably had very little sleep the night before the grounding*, and had *worked a stressful, physically demanding day*… at the time of the grounding, he could have had *as little as 5 or 6 hours of sleep in the previous 24 hours.*" (Kolstad, 1990)

At first blush, the attribution of this major disaster, even in part, to such an easily preventable cause may seem nothing less than astonishing. It would appear like a common sense notion that individuals for whom alertness is a critical part of their jobs should be required to obtain sufficient rest and be responsible monitors of their own performance. Research and experience have shown that neither of those things is the case. Furthrmore, the Exxon-Valdez crash is but the tip of the iceberg of a serious and

highly prevalent societal problem. It has been estimated that as many as 40% of Americans currently obtain less sleep than they need for optimal healthy functioning (NSF, 2005), and given that sleep duration is typically underreported in population studies (Silva et al., 2007; Lauderdale et al., 2008), it is possible that this figure is in fact even higher.

Sleep loss is both a cause and a consequence of the massive cultural changes occurring in modern society. The widespread use of artificial light has eliminated our reliance on natural *zeitgebers* (literally, time-givers), and social cues govern our sleep and wake cycles instead. For example, television has been found to account for almost half of the two-hour time period directly preceding sleep onset (Basner and Dinges, 2009). At the same time, globalization and the growing importance of commerce on the Internet have changed the nature of our economy, and transformed many industries into 24-hour operations, requiring many to work against their natural circadian rhythms. Even among non-shift workers, the trend in many industries has been to increase the number of hours demanded of employees, with complementary data showing that work time is the primary activity that has an inverse relationship with sleep time (Basner et al., 2007).

The need to engage in a particular behavior due to external pressure has often led a society to normalize, or even glorify such actions. In American culture, a now-common shibboleth is the notion that "sleep is for the weak", this meme now easily locatable in song lyrics, t-shirts, or college dorm posters. High-powered, successful individuals or heroes are often portrayed in the media as needing little (or no) sleep – Jack Bauer on Fox's "24" being a perfect exemplar of a character on the go for (no surprise) 24 hours at a stretch. This is justification, or even incentive for many to obtain as little sleep as

possible, and pride themselves on the extra hours of productivity and/or recreation obtained.

Several additional circumstances exist that encourage this behavior. First, many short sleepers have habituated to the subjective effects of their reduced sleep schedules, and have convinced themselves that their default level of fatigue is how they "should" feel (Basner and Dinges, 2009). Second, as we will see later in this chapter, many of the cognitive impairments that result from sleep deprivation make little material difference in highly complex tasks, thus creating in individuals a false sense of security in their ability to continue working. Moreover, it is been repeatedly shown that subjects are unaware of the level of their objective impairment on many tasks (Van Dongen et al., 2003); this failure of meta-cognition is a particular hazard when no external monitoring is available. Finally, it is believed by many that caffeine, a legal but fairly potent stimulant, is a panacea against the ills of being sleep deprived. Caffeine is one of the world's most highly traded agricultural commodities (for the most recent data, see http://faostat.fao.org), and over 90% of adults in the United States consume it in some form on a daily basis (Lovett, 2005). However, while caffeine does provide a short-term boost to many cognitive systems after sleep loss (Penetar et al., 1993; Wesensten et al., 2002), it does not dissipate homeostatic sleep need.

The problem of sleep deprivation comes to a head in situations where a failure in attention, memory, or executive control may be highly undesirable, or even fatal. To provide a few examples, the following populations and circumstances have come under scrutiny in the research community in recent years as being most vulnerable to such mistakes.

Medical doctors, in particular interns, are frequently made to work overnight, and in long uninterrupted shifts. Arendt et al. (2005) found that impairments in the performance of doctors following 4 weeks of heavy call was equivalent to impairments associated with 0.04 to 0.05g% blood alcohol content. Conversely, in an intervention study, it was found that eliminating extended work shifts significantly reduced the number of serious medical errors made by medical interns (Landrigan et al., 2004). A meta-analysis of these effects demonstrated that approximately one night of sleep loss leads to a reduction in clinical performance of 1.53 standard deviations – a worryingly large figure (Philibert, 2005). These findings are a particular concern because of the level of trust placed in the medical profession by the public; patients are likely to assume a visibly sleepy doctor still knows what he is doing, while unaware that preventable errors in medicine cost the American public in excess of $17 billion dollars a year (Institute of Medicine, 1999).

It is also known that many sleep-related motor vehicle crashes occur while **drivers** are working (Leger, 1994), which has caused the transportation industry to come under scrutiny for accidents related to sleep loss (for a review, see Horne and Reyner, 1999). Crashes associated with sleepiness have rates and injury severity levels comparable to alcohol-related crashes (Knipling and Wang, 1994), but in the case of larger vehicles, can come at considerably greater financial cost (Mitler et al., 1988). The odds of being in a sleep-related crash are significantly higher in those who work the night shift, or at odd schedules (Stoohs et al., 1994; McCartt et al., 2000; Stutts et al., 2003), yet truck drivers do much of their driving at an adverse circadian phase.

In **military settings**, fatigue and sleepiness also persist as major risk factors for on-the-job errors and failures of judgment. For tactical reasons or other reasons of necessity, many military operations take place under the cover of dark, or for long continuous stretches of time. Although the U.S. military currently stresses the importance of adequate rest time, personnel continue to report fatigue as a problem that affects on-the-job alertness (Caldwell and Gilreath, 2002; Caldwell, 2005). As a consequence, substantial amounts of research have been conducted on the efficacy of pharmacological countermeasures (e.g. amphetamines and modafinil) on performance during military operations (for a review, see Eliyahu et al., 2007).

A point worth highlighting at this juncture is that the ubiquity of sleep loss in these professions is only part of the problem; often, it is the *interaction of sleep loss and environment* that ultimately leads to serious consequences. Specifically, the modern-day workplace is structured such that relatively small errors can lead to huge disasters. In my opinion, there are three key reasons for this – the changing role of technology, the increasing specialization of duties, and our growing reliance on economies of scale. It is worth taking a brief excursion to elaborate on these in turn.

In his book *Things that Make us Smart*, Donald Norman (1994) discusses the "machine-centered orientation" of modern society, in which humans are often seen as inferior because of our imprecision and tendency towards mental lapses. This machine-centered paradigm ignores that fact that machines (as of now, anyway) lack cognitive flexibility and the ability to solve problems creatively, which is the forte of the human mind. Rather than complementing one other, many machines force human users to conform to their modes of operation, and are unforgiving of inevitable human error.

Thus, Norman argues that because of this cultural mindset, technological advancement ironically often leads to more, not fewer errors.

In particular, the way that a machine *represents* a problem may be highly dissimilar to the optimal way a problem could be presented to a human being. Norman provides several examples in his text about how changing the way information is displayed without altering any of its content can greatly alter how well that information is processed and understood. For instance, medical prescriptions provided as a list are much easier to misinterpret than those given in tabular form (with columns representing the time a dose is to be administered) (p 63-65). Moreover, there are differences between groups or even individuals in the representations that they may prefer. When there is a mismatch between a human mind and its interface with a problem, errors are more likely to occur.

Builders of machines and devices often adopt a one-size-fits-all approach to interface design. This invariably leads to cognitive strain on at least a subset of their users. Under conditions of sleep deprivation, when cognitive resources are low, the probability of error when a person deals with information represented in less accessible forms no doubt increases significantly. Our increasing reliance on technology in the workplace is thus a likely contributor to the growing volume of mistakes associated with sleep loss

On a related note, machines now perform highly complicated functions with very little need for human intervention and/or operation. This leads us to my second point: that more and more responsibility in the workplace is falling on fewer and fewer people. In other words, a machine is often a mindless amplifier of human error, compounding a

small lapse in vigilance or judgment into a large catastrophe. This is highly related to the third point – that many modern businesses take advantage of economies of scale in order to increase profit margins. Big equipment – such as the *Exxon Valdez* – affords us the opportunity to make big mistakes.

Cognition – the vital mediator

We turn our attention now to the underlying causes of sleep-deprivation-related errors and mistakes. In broad strokes, this story is relatively straightforward. First, sleep deprivation leads to changes in brain functioning on a cellular level, as well in larger neural systems. These latter system-wide changes are complex, and likely involve interactions among multiple brain regions. As a result, cognitive failures begin to appear, which lead to corresponding behavioral errors. In the present work, we focus on cognition as a crucial mediator in this causal chain, and attempt to better understand how and why it is affected by sleep deprivation.

A broad review of the effects of sleep loss on cognition was undertaken by Durmer and Dinges (2005), and updated recently by Goel et al. (2009). These authors summarize the broad array of neurobehavioral effects produced by sleep loss, which include:

1) Increased instability of attentional performance

2) Cognitive slowing in subject-paced (processing speed) tasks

3) Declines in short-term memory and working memory

4) Other frontal lobe deficits including declines in learning and problem solving, response suppression/inhibition errors, and perseverative errors.

Although these impairments are uncontroversially present after sleep deprivation, many reviewers hesitated to make claims about their relative magnitudes. Moreover, there exist three general schools of thought as to the theoretical framework through which the available data should be interpreted. These viewpoints are summarized below, with the caveat that these theories are not mutually exclusive and certainly amenable to integration.

The controlled attention hypothesis

Many of the early studies on SD and cognition cite novelty and motivation as critical variables in determining performance levels under adverse conditions (Williams et al., 1959; Wilkinson, 1965). These suggestions were made subsequent to the initially paradoxical observations that many highly-demanding cognitive tests are unaffected by short bouts of total sleep deprivation. For example, performance on Baddeley's logical reasoning test is consistently found to be stable, even as sleepiness and impairment in other cognitive domains appear (Smith and Maben, 1993; Magill et al., 2003). These negative findings have prompted the creation of theories such as the "controlled attention" model of Pilcher et al. (2007). In this model, the authors highlight the importance of "bottom-up" task characteristics, arguing that tasks that are monotonous or intrinsically less engaging are more severely affected by SD, due to the fact that greater top-down control is needed to sustain optimal performance on these tests. The authors suggest that tasks be classified based on whether or not they encourage attentive behavior, and hypothesize that tasks that are high on this dimension are affected the least by SD.

The neuropsychological hypothesis

Several reviewers have suggested that sleep deprivation has domain-specific effects on cognition, with particular focus on tasks mediated by prefrontal cortex (PFC) function. Jones and Harrison (2001) and Harrison and Horne (2000) both review the literature on the impact of SD on PFC-oriented tasks, and conclude that these tests provide incremental validity in assessing impairment beyond the consideration of vigilance or sustained attention alone. For example, Harrison et al. (2000) gave young adults a neuropsychological battery following 36 hours of total SD, and found specific impairments on PFC-oriented tests (of temporal memory, verbal fluency, and response inhibition), but not a test of recognition memory. The authors note that the impairments seen are similar to those displayed by healthy, middle-aged (55-64 y) participants, with diminution of PFC function being a known consequence of normal aging. More recently, neuroimaging data have lent further support to this claim; for instance, studies using fMRI (Drummond et al., 2000; Chee and Choo, 2004) have demonstrated hypoactivation in regions of the lateral and medial PFC to a variety of tasks following SD, thus localizing the putative neural basis for the observed behavioral changes.

Proponents of this view interpret these findings as evidence that the impairment seen in many complex cognitive tasks is not merely driven by the failure of more basic cognitive skills; that is to say, PFC-oriented tasks are vulnerable to specific failures that are above and beyond those expected to be caused by low arousal and sleepiness (Harrison et al., 2000). Conceptually, this can be thought of as a neuropsychological model, that is, SD produces a reversible functional lesion in the PFC that is detectable by tests sensitive to these deficits in brain-injured patients. This model provides some

explanatory power in resolving the mixed results in the literature which researchers had previously tried to account for with moderators such as task type, task length, novelty and motivation.

The vigilance hypothesis

Finally, other reviewers have singled out arousal and vigilance as general factors that explain much of the variance in cognitive deficits following sleep loss. Durmer and Dinges (2005) state that "cognitive tasks vary considerably in their sensitivity to sleep loss" (p 120), but remark that reaction-time measures of tasks of attention and vigilance are the predominant instruments used to assay vulnerability to SD. Lim and Dinges (2008) also spotlight vigilant attention as a cognitive process that is consistently and robustly affected by total SD. Finally, Balkin et al. (2008) make the stronger assertion that "the array [of activities affected by sleep loss] is so extensive that it is reasonable to posit that sleep loss exerts a nonspecific effect on cognitive performance" (p 654).

There is strong experimental evidence for these assertions. Tests of sustained attention are not only reliable, but also highly valid in predicting real-world performance and assessing the level of impairment faced by an individual under conditions of fatigue (Dinges et al., 1997; Lim and Dinges, 2008). As discussed below, these tests are also sensitive in tracking both circadian and homeostatic modulations in sustained attention and arousal over the course of several days without sleep (Doran et al., 2001). Finally, models of attention often stress that vigilance and sustained attention are fundamentally important to many higher aspects of cognition, and that these higher processes will

necessarily decline if a subject is not able to sustain a sufficient level of vigilance while performing a task (Sturm et al., 1999; Sturm and Willmes, 2001).

The three models discussed are above not mutually incompatible – one could argue that the controlled attention hypothesis and the vigilance hypothesis merely take different perspectives in explaining the same set of phenomena, and that the neuropsychological hypothesis, while consistent with both these models, accounts for effects above and beyond what may be expected from either. As a result, certain theorists have proposed a more integrative approach in interpreting the available data. For instance, Boonstra et al. (2007), suggest that impairment in the PFC following a period of SD may underlie changes in both executive functioning and attention, stressing the role of the PFC in the interaction between top-down and bottom-up processes.

Meta-analysis

In order to gain a better understanding of the relative size of these effects, Lim and Dinges (in press) conducted a meta-analysis of total sleep deprivation experiments in six different cognitive domains. Data were entered into this analysis if a study involved healthy adults aged 18 and above and included a period of total sleep deprivation not exceeding 48 hours. All studies were classified into six different cognitive domains: simple attention, complex attention, working memory, short-term memory, processing speed, and reasoning/crystallized intelligence. Speed and accuracy variables were analyzed separately, and 5104 individual effect sizes were calculated from these datasets. Results from this meta-analysis are presented in Figure 1.1.
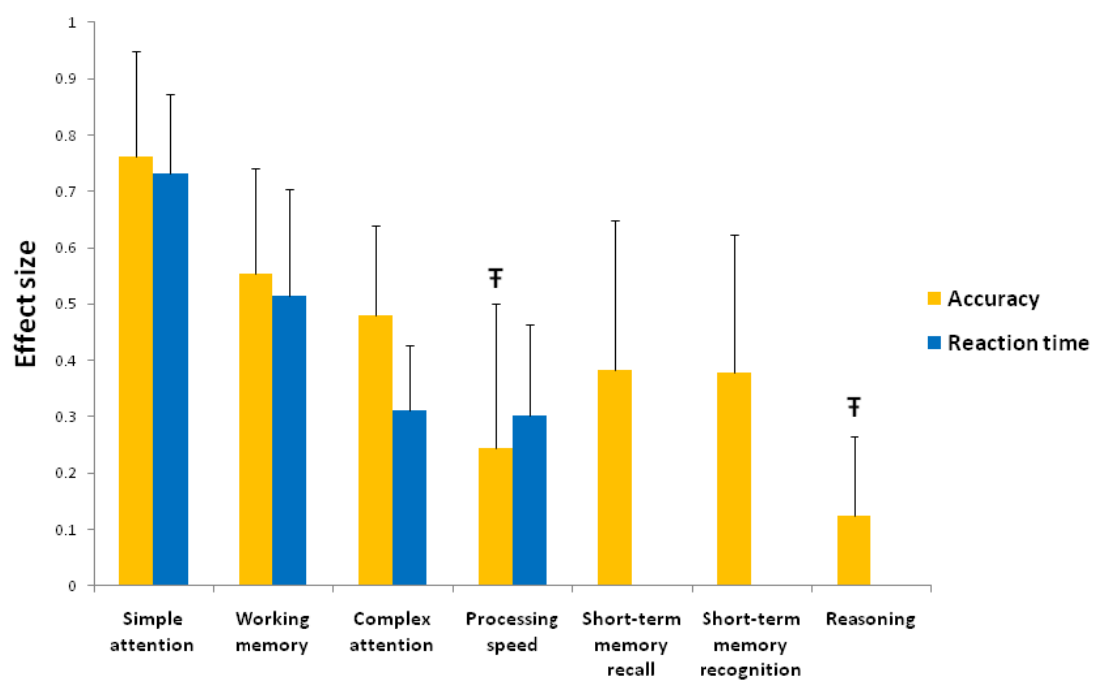
Figure 1.1. Meta-analysis of total sleep deprivation studies assessing accuracy and speed in six different cognitive domains. N = 5104 individual effect sizes. Bars represent 95% confidence intervals. Ŧ = *not* significant at *p* = .05

Table 1.1. Analysis of variance comparing average effect sizes (a) within outcome variable type (accuracy/reaction time) and across cognitive domains; (b) within cognitive domains and across outcome variable type

(a)

Outcome variable	Combined effect size	Variance	Q	df	p-value
Accuracy/lapses	-0.407	0.001	33.94	6	<.001
Reaction time	-0.450	0.001	25.63	3	<.001

(b)

Cognitive domain	Z*	p-value
Simple attention	0.256	N.S.
Complex attention	1.645	N.S.
Working memory	0.555	N.S.
Processing speed	0.292	N.S.

Secondary analyses conducted on this dataset revealed a significant difference in effect sizes across cognitive domains for both speed ($Q(3) = 24.5$; $p < .001$) and accuracy ($Q(5) = 36.8$; $p < .001$) measures, but no differences between speed and accuracy measures within each cognitive domain (Table 1.1).

The findings from this meta-analysis highlighted a number of crucial points. First, it is notable that speed-accuracy tradeoffs were not found in any of the cognitive domains, as suggested by a previous meta-analysis (Pilcher and Huffcutt, 1996) and in the primary literature. More importantly, the results indicate that short-term sleep deprivation does not affect all cognitive processes to a similar degree, with the largest

effect sizes found in speed ($\bar{g}$ = -0.732) and lapses ($\bar{g}$ = -0.776) on tests of simple attention.

The relative speed and magnitude at which deficits in sustained attention accrue suggest that this cognitive module may be primarily responsible for accidents and errors due to sleep loss. While this finding does not take away from the validity of the other models, it also suggests that sustained attention may be the most efficient target when considering prophylactic steps or countermeasures to deal with the cognitive consequences sleep deprivation in the real world. This was the motivation for focusing on sustained and selective attention in the current work. In the next section, I highlight some of the issues surrounding the effects of sleep loss and fatigue on attention in order to give the reader a better understanding of the rationale behind the specific experiments performed.

Attention and sleep loss – a closer look

Numerous influential models of attention have been developed by psychologists, and it is beyond the scope of this thesis to perform a comprehensive review of these. Instead, I provide definitions here of two key terms which are used in the present experiments. *Sustained attention* and *vigilant attention*, both of which have already been alluded to, are used interchangeably in this thesis, and refer to *the capacity of an agent to respond to relevant environmental cues in an accurate and timely manner.* In contrast, the term *selective attention*, which is interrogated in Study #3, refers to the *conscious, enhanced processing of an object, feature, or spatial location in order to more accurately and efficiently respond to the relevant target.*

The behavioral effects of sleep deprivation on vigilant attention are generally well understood, and the remainder of this section focuses on this cognitive module. Here, I briefly recapitulate the evolution of this field over the last half-century, and summarize the current thinking on the topic.

Early research on sleep loss and attention employed paradigms that were long, with relatively low signal density (e.g. the Mackworth Clock test) (Mackworth, 1948; Mackworth, 1968). Also popular were research on paradigms that either simulated or were identical to real-world tasks. For example, Bergstrom (1972) studied Swedish soldiers as they performed a missile-type tracking task, and subsequently as they engaged in radar-watching over a 40-minute period. Marksmanship is still commonly used today as a dependent variable in studies on sleep loss and stress conducted by the U.S. Army (e.g. Lieberman et al., 2002; Tharion et al., 2003). It is believed that the ecological validity of these tests makes them particularly useful assays of ain individual's level of vigilance. Furthermore, it was thought that tasks of insufficient length and/or high signal density would not induce observable changes in performance among sleep-deprived subjects.

The leading explanatory model of these data at the time was the lapse hypothesis of Williams et al. (1959). This model postulates that performance in the sleep-deprived state is on the most part normal, but is also punctuated by periods of non-responding, or "lapses". Because of the low signal density of the tests used, this model was a promising account of the available experimental data – the most salient feature of these tests was the sleep-deprived subject's complete failure to respond to a random subset of the target stimuli.

In the last quarter of a century, however, the advent of new paradigms and tools to assess sustained or vigilant attention has led to a fundamental change in the way the effects of sleep loss on these functions has been characterized. In particular, the use of tasks of shorter duration and higher signal density, most prominently the Psychomotor Vigilance Test (PVT) (Dinges et al., 1997), led to observations that could not have been made using classic assays of vigilance. The PVT is described in much greater detail in the experiments in Chapter 2 and 3; in brief, the test is typically a ten-minute simple reaction time task in which stimuli appear at random intervals between two and ten seconds. In a review on this topic, Lim and Dinges (2008) point out the four salient features of how sleep deprivation changes the pattern of responding on this task, namely:

1. *Sleep deprivation causes a general, overall slowing of reaction times.*

Average reaction times on the PVT increase in length overall after a period of sleep deprivation (Wilkinson, 1965; Pilcher and Huffcutt, 1996; Beaumont et al., 2001; Doran et al., 2001; Smith et al., 2002). This generalized response slowing is also reflected through a worsening of the fastest 10% of response times on both visual and auditory vigilance tasks (Dinges and Powell, 1989). The increase is independent of the fact that subjects are also "lapsing" (Bjerner, 1949; Williams et al., 1959; Kjellberg, 1977) (defined on the PVT as responding more than 500 ms after the stimulus onset, which is discussed in the following section.

2. Sleep deprivation results in increased errors of omission and commission.

Lapsing, or failing to respond in a timely fashion to a presented stimulus, is a hallmark of the sleep-deprived state (Dinges et al., 1997; Doran et al., 2001). On the PVT, this is defined as any reaction exceeding 500 ms in length. Sleep-deprived individuals experience so-called "microsleeps" and slow eyelid closures (Williams et al., 1959), and these are typically the intervals during which prolonged lapses occur. However, even when subjects are full alert and have their eyes open, these long responses can still occur (Anderson et al.; Johns et al., 2007). Lapses also grow more frequent as cumulative wakefulness increases (Doran et al., 2001; Graw et al., 2004), making this variable a useful outcome measure of the PVT.

Errors of commission, or false alarms, also increase in number during SD, and show the same pattern of circadian modulation as lapses (Doran et al., 2001). The appearance of false alarms may reflect a compensatory response to drowsiness; however, little work to date has been done to investigate this behavior.

3. Sleep deprivation enhances the time-on-task effect

The time-on-task (TOT) effect describes the phenomenon whereby performance worsens across the course of a cognitive task owing to fatigue or other factors (e.g. boredom or diminishing motivation). This decline is usually measured using either the change in reciprocal response speed or number of lapses over time. Originally thought to be present only in tasks of considerable duration (30 minutes or greater), it has since been found that time-on-task decrements are measurable within the first several minutes of performance in sleep-deprived individuals (Gillberg and Akerstedt, 1998).

Sleep deprivation greatly enhances the TOT effect, especially in operations with high cognitive demand (Dinges and Powell, 1988; Doran et al., 2001). However, as hours of wakefulness increase, these time-on-task decrements do not change in a straightforward fashion. Initially, sleep deprivation changes the slope of responses across a task. However, as time awake increases further, the *intercept*, representing the average reaction time of the first few PVT trials, shifts downwards, causing the slope to level off. This change arises when subjects are no longer to compensate for their attentional deficits, even for very short periods of time. Finally, the downward movement of the intercept stops, and the slope of responses once again increases.

4.   Tests of vigilant attention during periods of SD are sensitive to both circadian and homeostatic drives.

Human sleep-wake behavior is most commonly modeled using the two-process model of sleep regulation (Borbely, 1982). This model consists of two interacting components: a circadian process, which is a sinusoidal oscillator with a 24-hour period, and a homeostatic process, which increases exponentially with time awake, and dissipates in a similar exponential fashion. This model was initially used to predict sleep propensity (Borbely and Achermann, 1999), however, it soon became clear that aspects of cognitive function could be forecast using the model in a similar fashion.

Performance on the PVT is affected by both circadian and homeostatic drives (Wyatt et al., 1999; Van Dongen and Dinges, 2000). Figure 1.2 shows PVT data from an 88h total SD paradigm, in which both a steadily increasing linear trend and an oscillating circadian rhythm can clearly be seen. The number of lapses and the slowest (10th

percentile) reaction times are particularly sensitive in tracking this pattern (Graw et al., 2004). Critically, the pattern in this figure informs us that decline in performance over time during SD is not unidirectional – for example, "circadian rescue" can account for better performance in the morning after 48 hours of continuous wakefulness compared to the preceding hours of the night. Additionally, as time awake increases, the homeostatic drive interacts with, and exerts a multiplier effect on the circadian cycle, thus amplifying performance deficits at each circadian nadir (Babkoff et al., 1991).
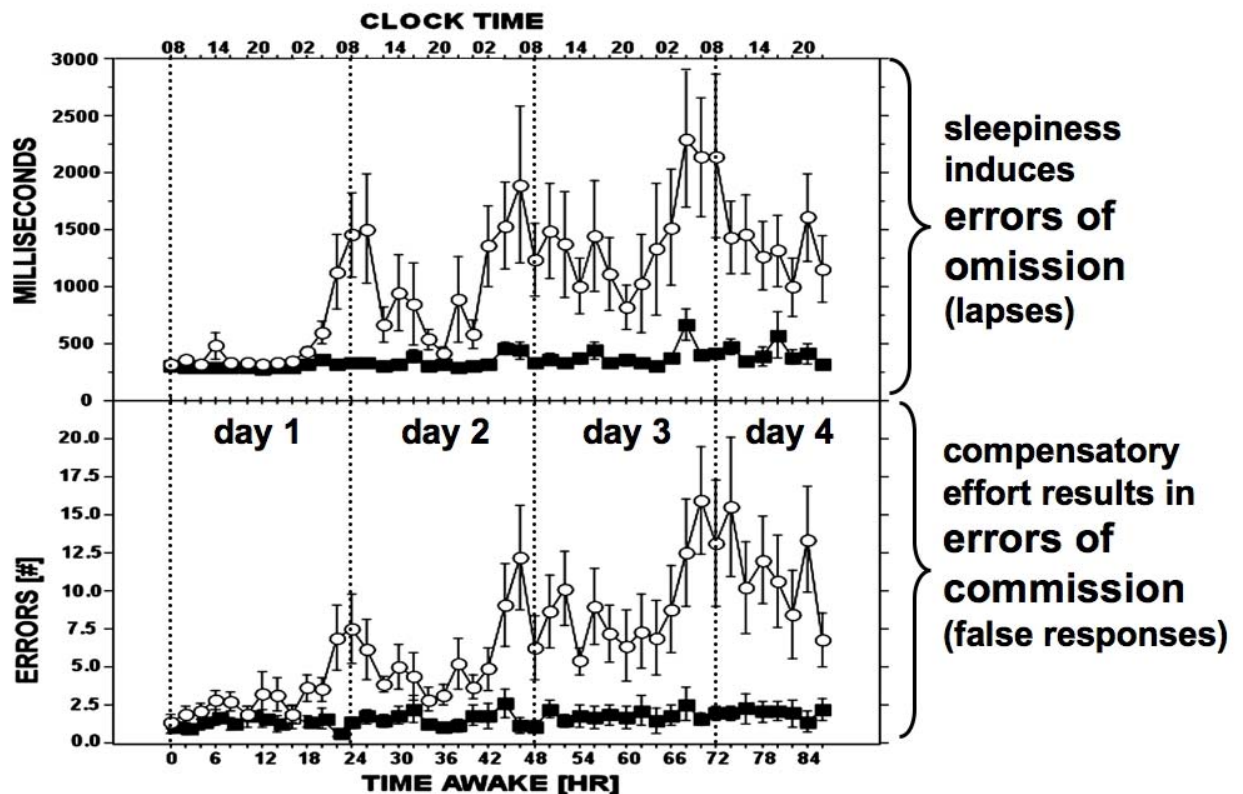
Figure 1.2. Errors of omission and commission are modulated by circadian and homeostatic drives.
White circles represent subjects undergoing 88h of total SD, and black squares represent control subjects (8h time-in-bed).

It has been suggested that gamma curves may be useful in synthesizing information across multiple test bouts of the PVT. This method involves plotting a cumulative distribution function (CDF) of reaction times across multiple tests during a baseline period, as well as the relevant periods of sleep deprivation (Figure 1.3), and then computing a difference function by subtracting one curve from the other. The maximum difference between the two curves (baseline vs. SD) serves as a single coefficient reflecting the level of impairment of an individual. Preliminary data suggest that this is a valid way of classifying individuals as vulnerable or resistant to the deleterious effects of SD (Goel et al., 2007), and that these phenotyping coefficients are stable over periods of time. Difference values also have utility in investigating the sensitivity and specificity of the PVT, for instance, computing the threshold reaction time that maximally discriminates rested individuals from individuals first experiencing sleep-deprivation-related impairment.

Individual differences

A consistent observation in early experiments of sleep deprivation was that there were significant differences in how individuals responded to such a treatment (Wilkinson, 1965; Webb and Levy, 1984), with some subjects showing little to no cognitive deficit after extended periods without sleep and others demonstrating severe impairment after as little as one night of sleep loss. The first well-controlled study to address this evidence quantitatively was conducted by Leproult et al. (2003). The eight subjects in this experiment underwent 2 sessions comprising 27 hours of total sleep deprivation each, during which subjective, objective and EEG recordings were taken. The two objective

tests administered were a selective attention task similar to the Posner cueing paradigm, and a simple reaction time test. These authors found high inter-session correlations in all of these measures, but noted that there was no correlation between objective and subjective measures of impairment. They concluded that "fatigue during a night of total sleep deprivation is a phenomenon involving multiple components that are regulated differently." (R289)

Van Dongen and colleagues (2004) subsequently and more definitively conducted a study to investigate the within- and between-subjects variance components associated with total sleep deprivation. In this experiment, 21 healthy adults underwent three separate 24-hour sleep deprivation periods in a controlled laboratory setting. Measures administered included subjective sleepiness and performance scales, the psychomotor vigilance test, and a number of other cognitive paradigms. Factor analysis revealed a three-factor structure as described above. High intra-class correlations were found for in both objective and subjective measures, and a large amount of the variance (67.5% to 92.2%) in these measures was attributable to stable variations among individuals. The authors concluded that this was evidence for trait-like vulnerability to sleep deprivation among individuals, and suggested that genetic and other biological markers may be found that identify vulnerable and resistant individuals.
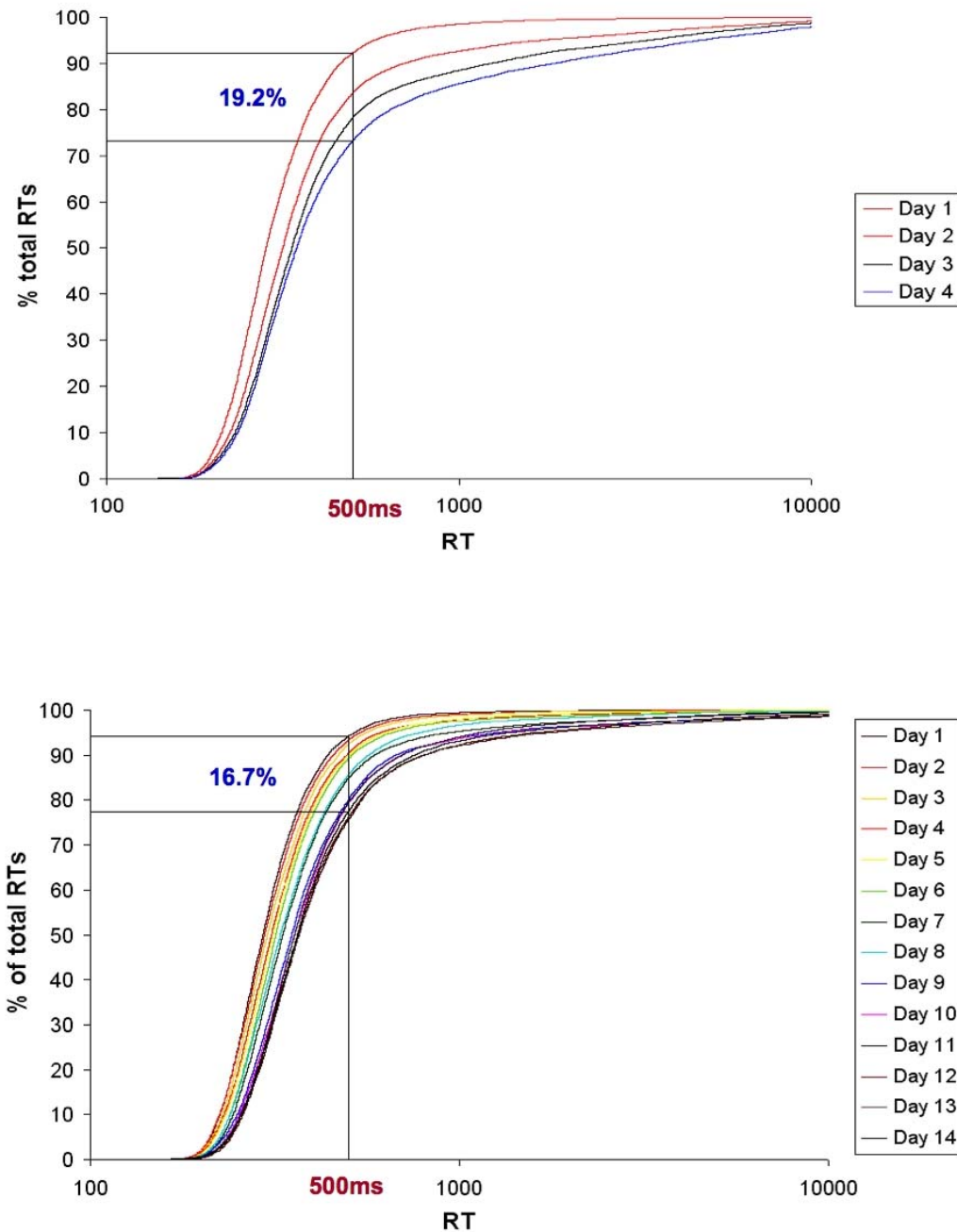
Figure 1.3. Gamma distributions as a way of representing PVT data. All individual reaction times across a period of interest are plotted as a cumulative distribution function. The top panel shows data from an 88h total sleep deprivation (SD) protocol; the bottom panel data from a 14-day partial sleep restriction protocol (4h time-in-bed). One potential use of these curves is to calculate a cut-off point for lapses that produces maximum discriminability between different groups of interest. For example, using a 500ms threshold, there is a 19.2% difference between subjects at baseline and after 88hr SD, and a 16.7% difference between baseline and performance after 14 days of chronic sleep restriction. (Data from Lim and Dinges, 2008)

Indeed, several recent papers have hinted at the putative biological bases of these trait-like differences. In a study using functional magnetic resonance imaging (fMRI), Lim et al. (2007) scanned 19 subjects before and after 24 hours of total sleep deprivation on two separate occasions as they performed a working memory task. These authors found high intra-class correlations in brain activation in task-related areas, and behavior-activation correlations in the intra-parietal sulcus (IPS) in both sessions. Supporting evidence for the importance of the IPS in mediating SD-related deficits has accrued from numerous other fMRI studies, in which this area is consistently found to be hypoactivated following sleep loss (for a review, see Chee and Chuah, 2008)

The discovery of these neurophysiological phenotypes has motivated a search for the gene-behavior associations that they mediate. In particular, interest has centered on several well-conserved candidate genes known to regulate aspects of circadian biology. In two separate human studies, associations were found between cognitive performance and a variable-number tandem repeat polymorphism in the PER3 allele, with homozygotes for a longer variant ($PER^{5/5}$) showing worse early-morning performance on tests of executive functioning (e..g n-back tests) (Groeger et al., 2008), and overall cognitive deficits in the early hours following the melatonin midpoint on a total sleep loss paradigm (Viola et al., 2007). In an fMRI experiment, different patterns of brain activity were obesrved between resilient ($PER^{4/4}$) and vulnerable ($PER^{5/5}$) individuals performing the PVT, with decreased posterior prefrontal activity in the latter subjects, and increased ventral anterior prefrontal in the former group (Vandewalle et al., 2009). Other work has suggested a role for the *Val158Met* polypmorphism of catechol-O-methyltransferase (COMT) (Bodenmann et al., 2009), and polymorphisms of A2A receptor genes (Retey et

al., 2006; Retey et al., 2007) in moderating the pharmacological effects of wake-promoting drugs.

With this background information in mind, I turn now to the work of this dissertation proper. The overarching aim of the studies reported here was to explore the workings of the attentional system under conditions of fatigue due to time-on-task and sleep deprivation. In doing this, I aimed to augment our knowledge on the topics discussed above, specifically the physiological basis of individual differences in response to sleep deprivation, and the relative importance of different aspects of attention in contributing to behavioral deficits. These hypotheses were explored primarily using blood oxygenation level dependent (BOLD) and arterial spin labeled (ASL) fMRI. This general problem was approached using three different paradigms, which are described below:

Chapter 2 reports the results of an experiment aimed at testing the neurobiological correlates of changes in resting activity and sustained attention performance after sleep loss. In this study, 26 subjects underwent ASL fMRI scanning in the well-rested state and after a night without sleep. I found that task-related activity was relatively lower following SD than in the well-rested state in multiple cortical regions; however, baseline levels of CBF paradoxically increased during resting baseline periods before and after the task. This study has implications for other SD-related imaging work in which absolute levels of CBF quantification are not used.

Chapter 3 discusses an experiment on individual differences in well-rested subjects performing the PVT for an extended period of time (20 minutes). Individuals in this experiment differed significantly on their rate of performance decline over the task

period, a phenomenon known as the time-on-task effect. In this study, I found that cerebral blood flow levels in fronto-parietal circuits pre- and post-task acted as markers of objective decline in performance. I also found that pre-task blood flow levels in the thalamus and frontal lobe predicted the subsequent extent of performance decline.

Chapter 4 turns to the effects of sleep loss on selective attention. Twenty-three healthy young adults were scanning using BOLD fMRI while detecting face and house targets in a series of foils. Prior to sleep loss, enhanced activation was observed to targets in the fusiform face area (FFA) and parahippocampal place area (PPA), which are selectively engaged by faces and houses respectively. This enhancement effect was eliminated in the sleep-deprived state. Moreover, functional connectivity between an attentional control region (the intraparietal sulcus) and relevant areas of ventral visual cortex was observed in the well-rested, but not the sleep-deprived state.

Finally, Chapter 5 provides a brief summary of the presented studies, discusses the broader implication of these findings for the field as a whole, and notes the limitations and future directions of the current work.

Chapter 2

Study #2: Resting cortical levels of cerebral blood flow increase following one night of total sleep deprivation

A majority of the neuroimaging work on sleep deprivation (SD) in humans has focused on the effects of total SD on various aspects of cognitive performance (for a review, see Chee and Chuah, 2008). With few exceptions, studies using both PET and fMRI have reported hypoactivation in fronto-parietal attention networks following sleep loss (Drummond et al., 1999; Drummond et al., 2000; Chee and Choo, 2004; Chee et al., 2010), and these changes appear to be most strongly associated with decreases in vigilant attention (Lim et al., 2007; Schmidt et al., 2009). Thalamic hypoactivation is also commonly seen across many cognitive paradigms (Wu et al., 1991; Thomas et al., 2000), and is thought to mediate the interaction between top-down attention and bottom-up arousal (Portas et al., 1998).

One caveat in interpreting these results is that most of the studies cited above employed blood oxygenation level dependent (BOLD) fMRI as their imaging method of choice. BOLD fMRI is a relative measure, and task activity is typically z-transformed relative to a chosen baseline. While this strategy still yields interpretable results pertaining to relative increases and decreases in activity when data from more than one state (i.e. from well-rested and sleep deprived individuals) are entered into a single model, it does not allow for separation of the variance associated with baseline and task-related changes.

Two sources of variance in baseline levels of neural activity are of interest in the context of sleep loss. First, changes in resting activity across state may be an important

marker of homeostatic sleep drive, and by extension have direct implications on the interpretation of task-related effects as reported in the studies cited above. Second, inter-individual differences in the level and distribution of cerebral blood flow (CBF) in both the well-rested and sleep deprived state may contain meaningful information pertaining to alertness and attentional capacity (Lim et al., 2010b). Our focus in this chapter is on the former question.

Converging evidence from several other methodologies has suggested that baseline neural activity may change dynamically over the course of waking and sleep. Findings from electroencephalography (EEG) have indicated that changes in theta and alpha power may reflect homeostatic sleep need. Increases in frontal theta power are commonly found as wake time increases (Cajochen et al., 1995; Caldwell et al., 2004), with some data suggesting that this change correlates positively with subjective sleepiness (Strijkstra et al., 2003).

Only a handful of published studies have investigated baseline cerebral metabolic rates in human subjects associated with differences in circadian phase and homeostatic sleep drive[1]. As one of the few examples, Buysse et al. (2004) studied 13 healthy adults using positron emission tomography (PET) during periods of wakefulness in the morning and evening. Resting brain activity was assessed at these two time points. No significant changes were observed in whole brain glucose metabolism. However, the authors did find differences in relative regional metabolic rates, with increases in the hypothalamus, midbrain and brainstem, and decreases in temporal and occipital cortex, and suggested

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[1] Although studies by Thomas and colleagues (2000) and Wu and colleagues (2006) are occasionally cited as evidence that brain metabolism decreases following SD (e.g. Feinberg and Campbell, 2010), subjects in both of these studies were engaged in a cognitive task at the time of FDG uptake. He importance of this distinction is elaborated on further in the discussion

that these metabolic increases in subcortical structures reflect the heightened circadian alerting effect known to be present in the evening hours (Borbely, 1982).

On a cellular level, it has been observed that prolonged wakefulness leads to higher firing rates of cortical neurons (on the order of a few action potentials per second increase), and that this change is homeostatically regulated by sleep opportunity (Vyazovskiy et al., 2009). Moreover, in rats, extracellular glutamatergic concentration in prefrontal and motor cortex increases during wakefulness and REM sleep and decreases during nREM sleep (Dash et al., 2009). However, there has also been some controversy over how to interpret these data in the light of the findings of Buysse et al. described above (Feinberg and Campbell, 2010), since the authors of this PET study did not find any changes in absolute global cerebral metabolic rate as may be predicted by the animal studies.

Changes in metabolic rates (using cerebral blood flow as a proxy) are detectable using fMRI in human subjects. Our primary goal in this experiment was therefore to augment our understanding of this phenomenon by measuring resting cerebral blood flow (rCBF) levels in a group of subjects before and after exposure to 24 hours of acute total sleep deprivation. We chose to use arterial-spin labeled (ASL) fMRI (Detre and Wang, 2002) in this study for a number of reasons. First, ASL fMRI allows for absolute quantification of cerebral blood flow, thus permitting across-scan comparisons during periods when subjects are not engaged in any cognitive task (in a fashion similar to PET). Second, it has high levels of temporal stability, and is thus highly suitable for assessing tonic differences between subjects and across states. Finally, ASL fMRI is non-invasive,

making it safe and feasible when conducting experiments that use repeated-measures designs.

Methods

31 healthy adults (mean age = 30.9 (6.7), 16 male) participated in this study, which was conducted in the Clinical Translational Research Center of the Hospital of the University of Pennsylvania. Of these, 5 subjects were eventually excluded for non-compliance (e.g. excessive head motion in the scanner, poor behavioral performance) for a final sample size of 26. Participants were selected from a pool of subjects who had previously undergone a partial sleep deprivation protocol in our laboratory. All subjects provided written, informed consent, and were financially compensated for their time.

Prior to the study, subjects were asked to attend a screening session to ensure that they met all inclusion criteria. All invited participants were between 21 and 45 years of age, were right-handed, had a body mass index of within 15% of normal, had normal sleep patterns, and were not extreme morning or evening types based on the Horne-Ostberg Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976). We eliminated any subjects who:

1. Had participated in shift work, transmeridian travel or had an irregular sleep/wake routine in the 60 days prior to the study;

2. Had a sleep disorder, as determined by medical history, actigraphy, pulse oximetry and baseline polysomnography;

3. Had a history of mania or psychosis;

4. Had current depression as determined by the Beck Depression Inventory;

5. Showed evidence of alcohol or drug abuse in the past year based upon history and a urine toxicology screen;

6. Were current smokers;

7. Had an acute or chronic debilitating medical condition or major Axis I psychiatric illness, based on history, a physical exam, and blood and urine screens;

8. Had a regular caffeine intake of more than 250 mg per day.

Screening sessions were conducted a minimum of three days before the commencement of the study. To ensure compliance with sleep instructions during this interim period, subjects were provided with an actigraph (Actiwatch-64, Mini Mitter, Oregon, USA), which they were told to wear at all times until the conclusion of the experiment. Actigraphy data were downloaded and checked at the beginning of the in-laboratory stay. Sleep history for all subjects fell within our pre-allowed range (6.5-8.5 hours time in bed; morning awakening between 0600 and 0900).

Study runs consisted of a 96-hour (four-day, four-night) in-laboratory stay. Subjects were continuously monitored in a semi-isolated living area, and had contact with only nurses, research staff, and other subjects during this time. They were given meals at regular, pre-specified hours, as well as snacks *ad libittum*, within reasonable limits. Meals contained no caffeinated products, turkey or bananas. During their free time, subjects were permitted to watch television, converse with the study monitors or play games. They were not allowed to do any physically or cognitively demanding tasks, work on laptops, or leave the study venue.

Figure 2.1 is a schematic of the experimental paradigm. All subjects came into the lab at 1700h on study day 1, and were given a 10-hour sleep opportunity on their first

night in the protocol. Starting from day two, and throughout the study, subjects performed neurobehavioral testing every two hours. During the protocol, subjects underwent two fMRI scans, one in the resting state, and one after approximately 24 hours of total SD. The order of scans was counterbalanced and randomized across the experimental group. All scans were conducted between 0800h and 1000h, and lasted approximately 45 minutes for each subject. The scanning procedure is described in detail below. Subjects who were sleep deprived on night two were given a recovery opportunity of 12 hours time-in-bed the following night to return them to a fully-rested state. For their safety, those who were sleep deprived on night four were given several hours of sleep just prior to being released from the experiment. There was no subsequent follow-up once the in-lab stay was over.
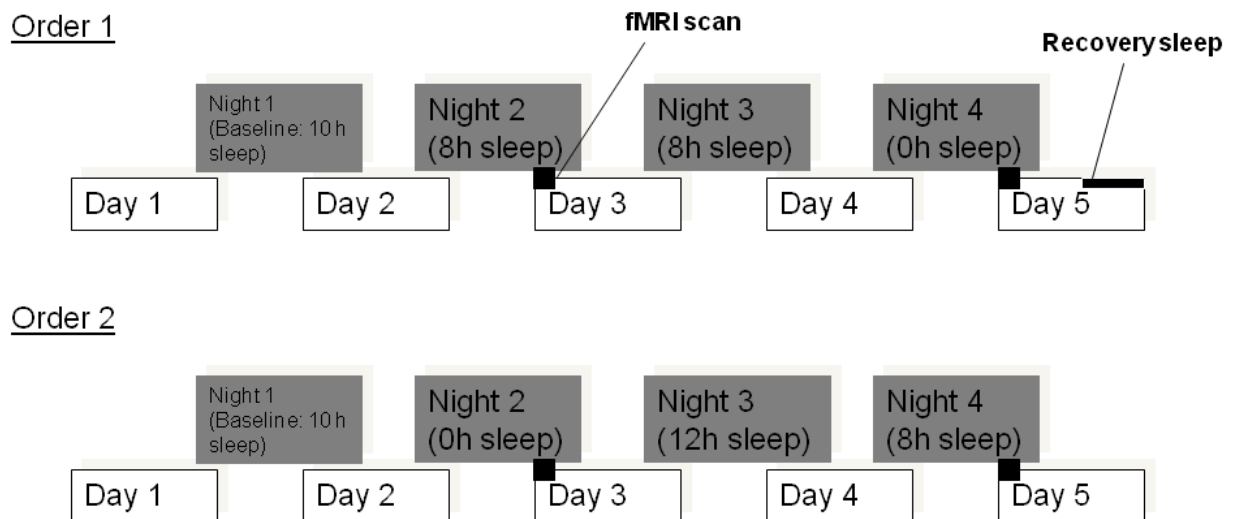
Figure 2.1. Schematic of the experimental paradigm

fMRI paradigm

During both fMRI scans, subjects were administered a 10-minute version of the

psychomotor vigilance test (PVT) (Dinges et al., 1997), a sustained attention task based

on visual reaction time that is highly sensitive to the circadian and homeostatic influences

on cognitive decrements due to sleep loss (Doran et al., 2001; Dorrian et al., 2005; Lim

and Dinges, 2008). A Windows-compatible version of the PVT was used to display the

stimuli (Pulsar Informatics, Philadelphia, PA). Subjective sleepiness ratings were

collected before and after each PVT run using the Karolinska Sleepiness Scale (KSS) as

well as 9-point visual analogue scales on which subjects answered the question "How

sleepy are you?" Subjects were asked to focus their attention on a red, rectangular box

subtending 2 x 1.3 degrees of visual angle in the middle of a black screen, and monitor

that space for the appearance of a millisecond counter, which appeared at random

intervals ranging from 2-10s. They were instructed to stop the counter as quickly as

possible with a button press, after which they would be able to view their reaction time.

Subjects were also instructed to avoid anticipating the stimuli so as not to register "false

starts", or responses when no stimulus was present on the screen. In a standard test, an

auditory alert is issued after 30 seconds of non-response. However, as we did not want

subjects to drift into sleep for extended periods of time in the scanner, we gave a verbal

alert ("Please respond to the stimuli") to any subject unresponsive to the PVT for 15s at a

time.

fMRI scanning procedure

MRI data were collected on a 3.0T Trio whole body scanner (Siemens, Erlangen, Germany) using an 8-channel array coil. For perfusion fMRI scans, a pseudo-continuous arterial spin labeling (pCASL) sequence which is a variant of that developed by Hernandez-Garcia et al. (2004) was used. To determine the optimal positioning of the image bounding box, as well as to maximize tagging efficiency of the RF pulse, we collected one or more short baseline scans to visually inspect signal quality. Using the appropriate parameters, alternating label and control images were then acquired in a superior-inferior direction using a gradient-echo, echo-planar imaging sequence. Sixteen slices (7mm, 1.4 mm gap) were collected using the following parameters: repetition time (TR) = 4s, echo time (TE) = 17 ms, labeling time = 1.5s, flip angle = 90°, FOV = 220 x 220 mm^2, matrix = 64 x 64. A delay of 1s was inserted after the end of the labeling pulse and before image acquisition in order to reduce transit artifacts. We collected 150 acquisitions over the 10-minute PVT bout. Before and after administration of the PVT, we acquired two resting scans, consisting of 60 acquisitions each (4 minutes), during which subjects were asked to lie still with their eyes open (R_1 and R_2). Finally, a high-resolution T1-weighted, 3D anatomical image was acquired using a magnetization-prepared rapid gradient echo sequence (TR = 1,620 ms; inversion time = 950 ms; TE = 3 ms; flip angle = 15°; 160 contiguous slices of 1.0 mm thickness; FOV = 192 x 256 mm^2; matrix = 192 x 256).

fMRI data analysis

fMRI data were preprocessed using VoxBo (www.voxbo.org) and analyzed with

Statistical Parametric Mapping software (Wellcome Department of Cognitive Neurology,

Institute of Neurology, London). All images were realigned to correct for head motion

during each run, and epochs of head movement greater than the width of one voxel were

removed. Images were co-registered to each subject's high-resolution anatomical MRI,

and smoothed in space with a 3-dimensional, 10mm FWHM Gaussian kernel. Perfusion

images were generated with an in-house subtraction script that performed simple pairwise

subtractions of alternating images. These were then normalized to standard space using

the Montreal Neurological Institute template brain. To eliminate noise associated with

insufficient transit time to brain tissue, we removed the 2 inferior-most brain slices of all

functional acquisitions. All sequences: the pre-task resting period (R_1), post-task resting

period (R_2) and PVT performance (PVT) were converted to an absolute cerebral blood

flow (CBF) image series based on a single compartment CASL perfusion model (Wang

et al., 2005).

Using all functional data, we computed a whole-brain voxel-wise general linear

model to compute the following contrasts: main effect of state on each sequence, (PVT -

R_1) in each state and (R_1 - R_2) for each state. This model included an adjustment for

global CBF differences. Thresholds were set at $p < .05$ (FDR corrected across the whole

brain) or $p < .001$ (uncorrected) when the former threshold was deemed too conservative,

and cluster size larger than 20 voxels.

We conducted region-of-interest (ROI) analysis on the following areas: right

middle frontal gyrus (MFG), inferior parietal lobe (IPL), anterior cingulate cortex (ACC),

occipital cortex bilaterally, left supplementary motor cortex (SMC) and the thalamus.

Regions were chosen based on a priori hypotheses concerning associations with PVT

performance (Drummond et al., 2004), as well as data from the ASL study of the PVT

reported in the next chapter (Lim et al., 2010b). The SMC was chosen as a neutral area of

comparison. We defined these regions based on areas that were activated in the overall

contrast of PVT vs. rest (or in the contrast of state for regions that were not task-

relevant). Quantitative CBF values were extracted for R_1, R_2 and the PVT for each of

these areas, and 2-way analysis of variance (ANOVA) conducted on these values.

<center>Results</center>

Behavioral results

Mean (SD) reaction time during the in-scanner PVT bout in the well-rested state

was 302.2 (26.9) ms. In this state, subjects were generally able to perform the task well,

as indicated by the small number of lapses (responses > 500 ms) (2.5 (3.6)). As expected,

sleep deprivation caused a marked decline in these basic PVT performance variables,

with slower median reaction times ($t_{25} = 4.52$; $p < .001$), and a greater number of lapses

($t_{25} = 6.47$; $p < .001$). Because the PVT trial time was held constant at 10 minutes,

subjects viewed significantly fewer stimuli in the SD than the RW state ($t_{25} = 3.8$; $p <$

.01). Finally, subjective sleepiness as measured by the KSS was higher after sleep

deprivation than in the well-rested state, as expected, both before ($t_{25} = 7.82$; $p < .001$)

and after the scan ($t_{25} = 9.32$; $p < .001$).

Task-related activation

Performing the PVT resulted in relatively higher levels of cerebral blood flow in the middle frontal gyrus and inferior frontal cortex bilaterally, right superior parietal cortex and inferior parietal lobe, anterior cingulate cortex, the thalamus and the insula (p < .005, corrected) (Figure 2.2, Table 2.1). Some deactivation was observed in striate cortex. Analyzing the task effects by state, it was observed that cortical activation within these areas was relatively higher when subjects were well-rested.
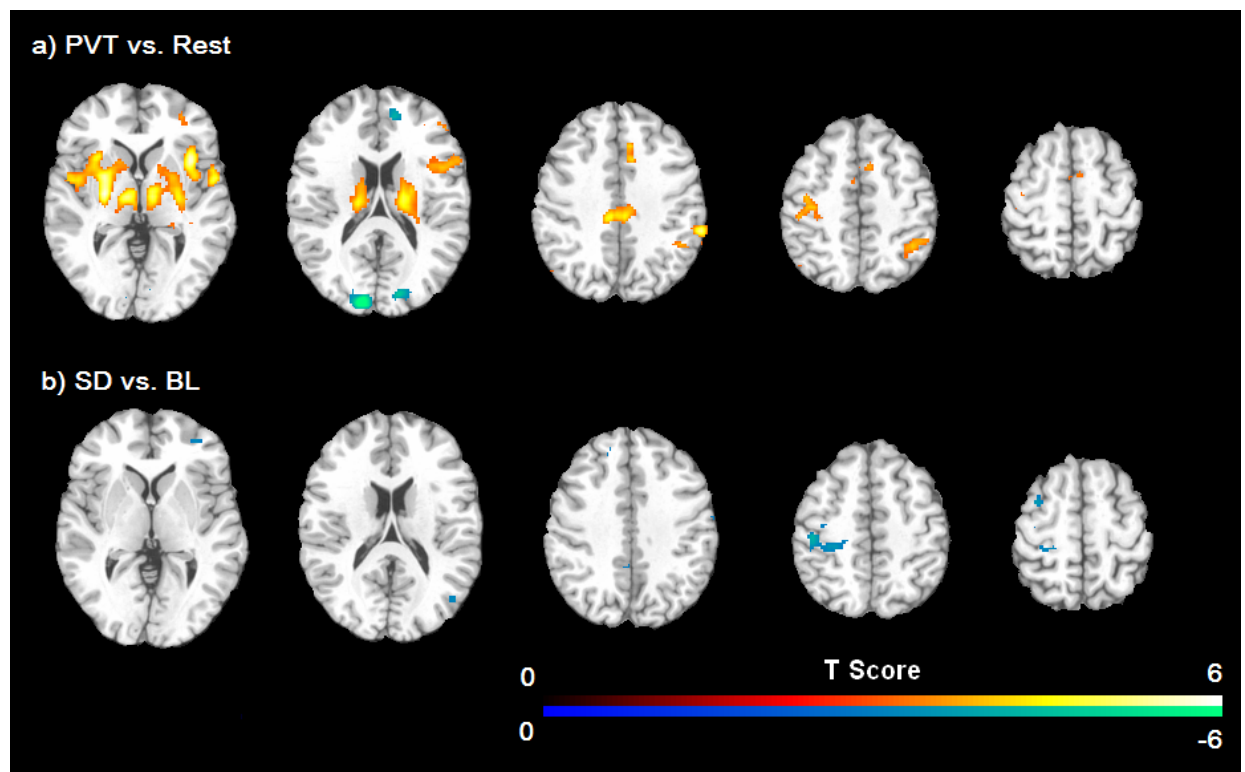
Figure 2.2. Effects of state and PVT performance on cerebral blood flow. The color bar in the bottom right represents the *t*-score scale. a) Compared to rest, PVT performance caused significant activation in bilateral thalamus, basal ganglia, cingulate, parietal, prefrontal, and left sensorimotor cortices, and CBF deactivation in occipital and medial prefrontal cortices. b) Sleep deprivation significantly decreased PVT activation in left sensorimotor, left premotor, right occipital, and right prefrontal cortices during task performance. Thresholds were $p < .05$ and $p < .001$ (uncorrected) respectively.

Table 2.1. Peaks of activation and deactivation to PVT performance combining conditions across state

	Cluster Size	MNI Coordinates			Peak z	Peak p (corrected)
		x	y	z		
a) CBF increases						
B. thalamus, insula, putamen	7106	-14	-14	8	6.10	<.001
		-28	4	-4	5.74	.001
		-14	-14	8	5.61	.001
B. cingulate	760	-2	-26	32	5.15	<.001
		4	-12	28	3.30	.019
R. parietal cortex	893	60	-38	40	5.09	<.001
		60	-48	30	4.35	.001
L. middle frontal cortex	112	-38	40	26	4.96	<.001
B. anterior cingulate cortex/supplementary motor area	626	6	20	42	4.40	.001
		-6	4	48	4.29	.001
L. sensorimotor area	341	-42	-16	56	4.34	.001
L.angular gyrus	80	-48	-64	48	4.11	.003
R. middle frontal cortex	223	44	34	10	3.92	.004
		42	40	26	3.70	.007
	101	30	48	4	3.63	.009
L.parietal cortex	93	-54	-40	30	3.33	.018
b) CBF decreases						
B. Occipital	184	16	-92	18	6.56	<.001
	22	12	-86	18	6.08	.006

The threshold was set at $p < .05$ (corrected)

State-related changes in resting activity

During the pre-task resting phase (R_1), 24 hours of sleep deprivation led to significantly increased regional CBF in several cortical areas, including bilateral occipital areas, the left anterior cingulate cortex, and left sensorimotor cortex (Figure 2.3; Table 2.2). In the post-task resting phase (R_2), many of these changes were attenuated. Moreover, thalamic activity was relatively lower in R_2 during SD than BL.

Table 2.2. Peaks of regional CBF increase during the pre-task resting period after sleep deprivation

	Cluster Size	MNI Coordinates			Peak Z	Peak P (corrected)
		x	y	z		
CBF increases						
R. occipital cortex	117	40	-60	-4	5.13	.015
	53	32	-78	-6	4.73	.015
L. sensorimotor cortex	390	-34	-26	54	4.93	.015
	31	-28	-8	52	4.56	.015
	136	-60	-8	28	4.55	.015
L. anterior cingulate cortex	22	-14	16	28	4.42	.015
L. occipital cortex	24	-44	-70	2	4.18	.017

The threshold was set at $p < .05$ (corrected)

State-related changes in task activity

In the direct comparison of PVT activity across states, there was greater activity in the insula, and less activity in the thalamus bilaterally in SD compared to BL (Figure 2.3.)
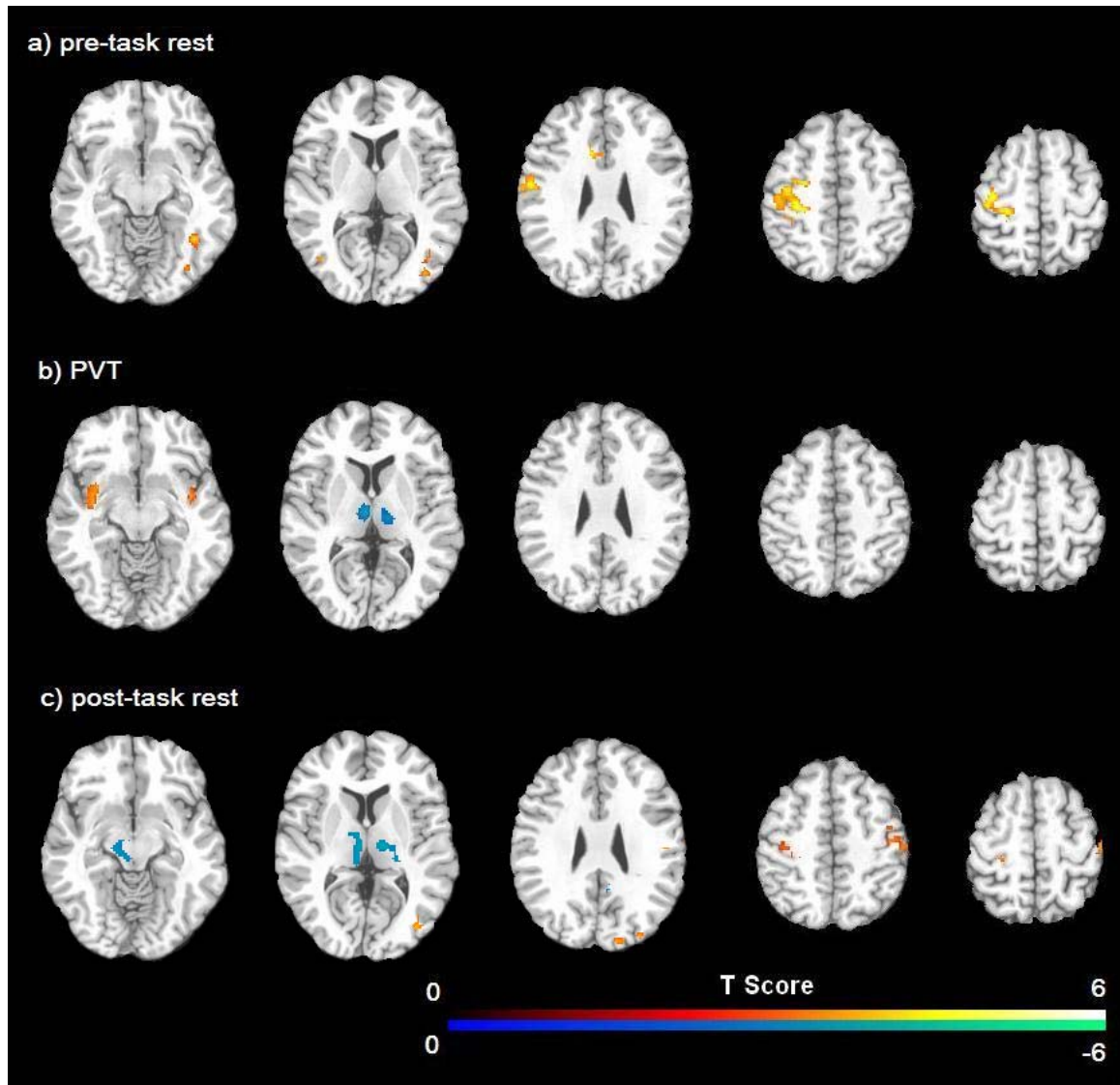
Figure 2.3. Effects of state on blood flow during rest periods and PVT performance. The color bar in bottom right represents the *t*-score scale. a) Sleep deprivation (SD) significantly increased regional CBF in left sensorimotor, left ACC, and bilateral occipital cortices at pre-task rest, b) increased regional CBF in bilateral insula but decreased CBF in the thalamus during PVT performance, and c) significantly increased regional CBF in bilateral sensorimotor,right occipital, and decreased CBF in the thalamus at post-task rest.

Quantitative CBF analyses

Global CBF values are presented in Figure 2.4. A 2-way ANOVA conducted on these variables indicated a significant effect of task performance ($F_{2,24} = 10.32$, $p < .001$), but not of state ($F_{1,25} = 1.58$, *n.s.*). Post-hoc comparisons indicated that this difference was due to significantly lower values during the PVT scan during SD compared to R_1 and R_2. The interaction effect was non-significant ($F_{2,24} = 1.82$, *n.s.*). Across both resting periods and the PVT scan, global CBF values were higher in the sleep deprived than in the resting state. Averaging across the two resting scans, the effect size of this change on baseline levels of activity was 0.19.
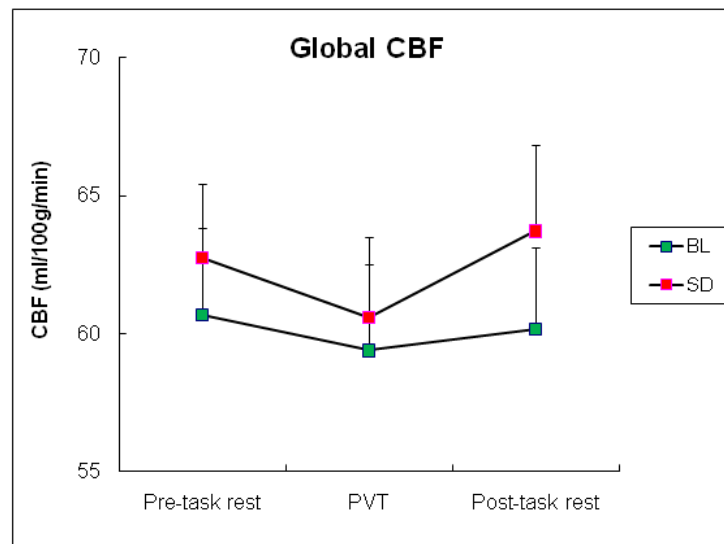
Figure 2.4. Global cerebral blood flow (CBF) values during the task and two resting periods. BL = baseline; SD = sleep deprivation

In order to visualize the relative contributions of task and state to CBF changes, we calculated difference maps based on absolute CBF on a voxel-wise basis, without using any inferential statistical test (Figure 2.5). Values are only displayed on gray

matter. As compared to performing the PVT, sleep deprivation more consistently elevated blood flow in wide areas of cortex, including right MFC, ACC, and right IPL. In contrast, increases due to PVT performance were more pronounced, but localized to areas associated with sustained attention and arousal; decreases were also seen in areas of the default mode network (Raichle et al., 2001).

To quantify these changes, we used functionally-defined ROIs to extract CBF values from relevant regions based either on the contrast of task or state, and conducted 2-way ANOVA on each of these sets of values.

Discussion

While neuroimaging studies have primarily interrogated the effects of total sleep deprivation on brain activity during a cognitive task, this is to our knowledge the first experiment to explore *in vivo* the effects of total SD on both task-related *and* resting neural activity. Broadly speaking, our results demonstrate that the activation due to task performance while sleep deprived is diminished compared to being well-rested. However, small but widespread increases in both absolute and relative CBF were paradoxically observed during periods of rest. We believe that these results are pertinent to the ongoing debate regarding the theory of synaptic homeostasis (Tononi and Cirelli, 2003, 2006), and may clarify some of the apparent contradictions in the literature raised by Feinberg and Campbell (2010).
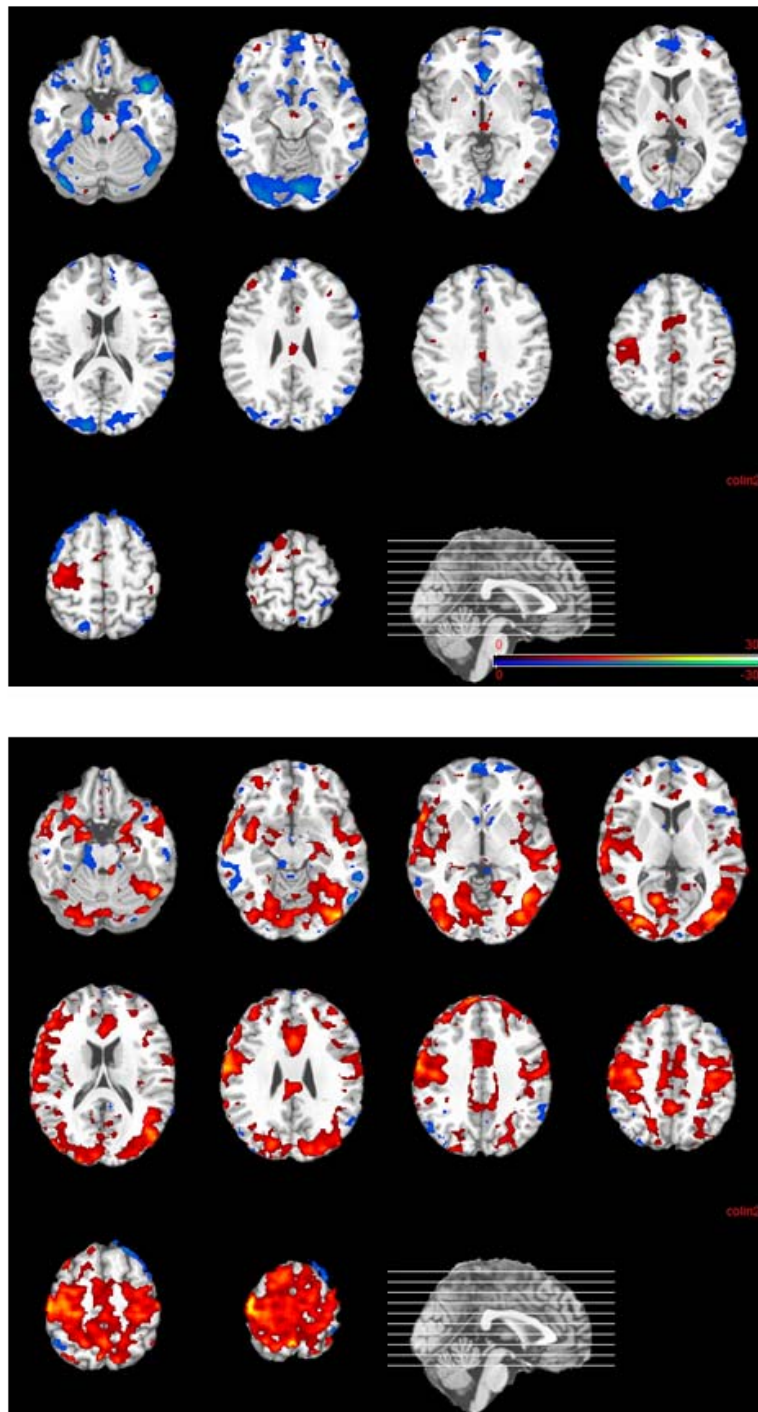
Figure 2.5. Absolute CBF differences in grey matter voxels. Scale (depicted in the top panel) is in ml/100g/min a) The top panel depicts the subtraction of absolute CBF values in R1 from the PVT in the well-rested state. b) The bottom panel depicts the subtraction of absolute CBF values in R1 during the resting state from R1 values in the sleep-deprived state.
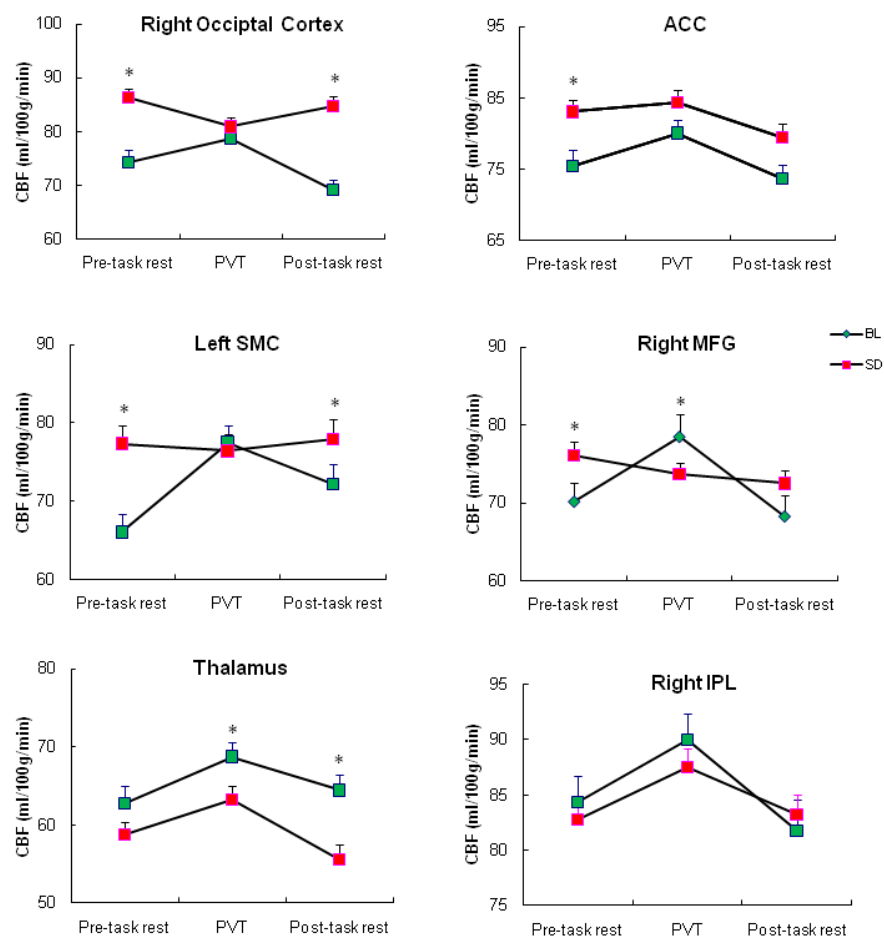
Figure 2.6. Relative cerebral blood flow values extracted from functionally defined regions-of-interest. ACC = anterior cingulate cortex; SMA = supplementary motor area; MFC = middle frontal cortex; IPL = inferior parietal lobe

Table 2.3. *F*-values for 2-way analysis of variance for regions depicted in Figure 2.6.

Region	*F*-value		
	State effect	**Task effect**	**State x task interaction**
Right occipital cortex	16.20***	2.51	5.52*
Left supplementary motor cortex	6.95*	7.78**	10.39**
Right middle frontal gyrus	0.43	8.66**	5.50*
Thalamus	17.06***	23.60***	3.85*
Right inferior parietal lobe	0.32	22.13***	2.73
Anterior cingulate cortex	9.19**	7.81**	0.65

$* p < .05; ** p < .01; *** p < .001$

Cortical cerebral blood flow shows relative decreases following SD during task performance

In the well-rested state, PVT performance elicited activation in a fronto-parietal circuit known to mediate sustained attention, as well as the insula, thalamus and basal ganglia. These results replicated those of a recent experiment using the same paradigm (Lim et al., 2010b), and agree with well-established findings from other similar tasks (Cabeza and Nyberg, 2000). Following SD, we observed relatively less activity in these areas during periods of task performance, suggesting that the function of the attentional system was compromised. We note that in the middle frontal gyrus, this effect was pronounced enough to create the cross-over interaction seen in Figure 2.6. These findings are fully consistent with other SD paradigms, in which hypoactivation in dorsolateral prefrontal cortex (DLPFC) and the superior parietal lobe has been seen across a wide variety of tasks (Chee and Chuah, 2008).

Resting global CBF show non-significant increases after SD

Analysis of global CBF values revealed a small but non-significant change associated with state, and a significant effect of task. Two prior PET studies have shown a global increase of 17.5% in one case (after one night of total SD) (Braun et al., 1997) and a non-significant 6% decrease in the evening compared to the morning. In comparison, we found a non-significant 4.6% increase in global CBF averaged across resting baseline periods, that is, when subjects were merely maintaining a state of wakeful alertness without performing any particular task.

These results are consistent with the synaptic homeostasis theory, which would predict global increases in CBF following a period of SD, and which we discuss in greater detail below. However, the fact that this increase is at best quite small ($d = 0.19$) is also consistent with what is known about the metabolic function of the cerebral cortex. The energy expenditure of the cortex is high compared with other organs, accounting for 20% of resting metabolism despite comprising only 2% of human body weight (Kety, 1957; Sokoloff, 1960). Furthermore, a vast majority (>90%) of this energy is consumed in the generation of action potentials, resting potentials, and postsynaptic (largely glutamatergic) transmission (Attwell and Laughlin, 2001), which are the putative bases for regional CBF changes as detectable by fMRI. Common wisdom has been that global levels of cerebral metabolism must be tightly regulated in order to maintain neural functioning, since even short periods of ischemia or anoxia can lead to severe and irreversible brain damage. Therefore, although increases due to synaptic potentiation are present and observable, there may be a counteracting force on these increases due to a

physical metabolic ceiling. This claim is substantiated by the observation that increases in extracellular glutamate and baseline firing rates reach a plateau after several hours in awake rat neocortex (Dash et al., 2009; Vyazovskiy et al., 2009).

Resting regional CBF increases after SD

We observed significant cortical increases in relative CBF across state in left sensorimotor areas, anterior cingulate cortex, and bilateral occipital regions. These areas were dissimilar to those reported by Buysse et al. (2004) when comparing differences in CBF patterns between the morning and the evening. However, this is also to our knowledge the first demonstration of such increases *in vivo* using ASL fMRI after a full night of sleep loss. The areas in which significant state-related increases were observed were also different from those involved in PVT performance, which prompted us to generate the absolute difference maps in Figure 2.5. These maps revealed the extensive cortex-wide increases that did not reach significance in the general linear model. Our findings thus suggest that CBF is redistributed when subjects are engaged in task performance in the sleep-deprived state, with a complex pattern of reductions and compensation occurring across this attentional network.

Figure 2.6 and Table 2.3 illustrate this pattern change. Within the regions that showed a significant main effect of task (ACC, right MFG, right IPL, thalamus), only two of these (the thalamus and ACC) showed a significant effect of state. These effects operated in different directions -- the thalamus was significantly hypoactivated following SD, consistent with its role as an arousal center, while the ACC was more active overall, in line with its role as a basic area of attentional monitoring. Furthermore, we observed a

significant interaction between task and state in the thalamus and right MFG. Inspection of the activity pattern in Figure 2.6 indicates that this was because resting levels of CBF during SD in prefrontal cortex were already elevated relative to baseline, presumably to maintain tonic levels of alertness. This may have limited the degree of further CBF increase possible when the brain was challenged with an attentionally-demanding task.

Implications of changes in resting CBF patterns

If there is a genuine difference in CBF between states, some consideration must be given to the interpretation of BOLD data collected during sleep deprivation paradigms. Specifically, it is conceivable that a global z-transformed baseline using data from two states may be significantly higher than the actual baseline in the well-rested state, and lower than the actual baseline in the sleep-deprived state in certain cortical regions. This may subsequently lead to an underestimate of the true effect size when data from both states are entered into the same model, as well as an increased risk of Type II error. It is noteworthy, however, that this finding also increases the likelihood that state-related differences in activation found in previous fMRI work are more likely to be due to real effects.

`   Two early PET studies (Wu et al., 1991; Thomas et al., 2000) are sometimes cited as examples of how cerebral metabolic rate decreases rather than increases after a night without sleep. However, subjects in these experiments were engaged in a cognitive task during the glucose uptake period (a serial subtraction task and a continuous performance test respectively). Thus, the widespread decreases observed in the thalamus and across the cortex in these studies were probably reflective of task-related hypoactivation, as

have been observed in most subsequent studies using fMRI, as well as the current data. Confidence in this conclusion is strengthened by the fact that both Thomas et al. (2000) and Wu et al. (1991) found positive correlations in their datasets between behavioral performance and glucose metabolism.

In contrast, evidence from two separate PET studies that did measure glucose metabolism at baseline levels revealed different findings. Buysse et al. (2004) scanned subjects using PET at rest in the morning and the evening, and found relative metabolic increases in brainstem and midbrain structures, and relative decreases in right middle temporal and medial occipital areas. In a study of metabolic activity during sleep and in the period immediate after waking, Braun et al. (1997) found that activity during pre-sleep wakefulness was higher than during post-sleep wakefulness in DLPFC, temporal and occipital cortex, and in the cerebellum and pons. No regions showed greater activity following the night of sleep.

Taken together with our data, these findings suggest that neural patterns of CBF differ after SD based on whether a subject is engaged in a cognitively demanding activity or simply trying to maintain a state of wakefulness. It has already been hypothesized that decreased fronto-parietal activity may reflect task disengagement (Chee et al., 2008, Chee and Tan, in press) due to a weakening of top-down connectivity with regions of sensory processing (Chee et al., 2010, Lim et al., 2010a). Our data support this hypothesis by demonstrating SD-related hypoactivation in these canonical areas. However, a more complex pattern of activity was found across the thalamus, ACC and frontal cortex which may better explain the functional relevance of each of these areas.

These data suggest that the increases in CBF (and presumably synaptic activity) relative to rest when a subject is not engaged in a task are consistent with the synaptic homeostasis hypothesis of Tononi and Cirelli (2006). Data supporting this hypothesis indicate that synaptic potentiation increases with time awake, and that sleep is necessary to selectively downscale these weights so as to improve an organism's performance, as well as keep neuronal energy expenditure at a sustainable level. Slow wave activity (SWA) during sleep is believed to reflect this homeostatic function (Borbely, 1982; Dijk et al., 1987; Werth et al., 1996). According to this theory, the overall increases in CBF observed during resting periods in our paradigm reflect this synaptic potentiation, and support the model that tonic rates of neuronal firing increase as homeostatic sleep pressure grows (Vyazovskiy et al., 2009).

A complementary account of the data relates to the state instability hypothesis of Dinges et al., (1997), which was proposed to account for the patterns of behavior observed on sustained attention tests following periods of SD (Durmer and Dinges, 2005; Lim and Dinges, 2008). This theory suggests that the withdrawal of bottom-up monoaminergic (e.g. noradrenergic) mechanisms in the sleep-deprived state necessitates greater top-down cortical activity in order to maintain normal levels of arousal. Thus, even when subjects are at "rest", compensatory mechanisms are still needed to prevent involuntary lapsing into microsleeps. If this is the case, greater relative CBF across the cortex during resting periods, particularly in prefrontal areas may also reflect this compensatory drive.

Conclusion

The data presented here constitute evidence that sleep deprivation increases both global and regional cerebral blood flow in resting, alert human subjects, an issue which is currently being debated in the literature. Furthermore, these data suggest that task engagement causes a redistribution of CBF that is dissimilar to what is observed in the well-rested state. These findings enrich our understanding of the effects of sleep loss on baseline levels of activity in the human brain, and provide support that one of the functions of sleep is to homeostatically regulate resting levels of neuronal firing.

Chapter 3

Study #2: Imaging the effects sustained cognitive activity: An ASL perfusion study of the time-on-task effect

In the previous chapter, we saw that resting levels of cerebral blood flow in certain regions change following a period of sleep deprivation, and posited that this may have some relation to state instability and attention. In the current study, we aim to test this hypothesis more directly by exploring the relationship between resting levels of CBF and subsequent performance on the psychomotor vigilance test (PVT).

Sustaining attention to a taxing cognitive task often comes at a cost, known in the literature as the "vigilance decrement", or "time-on-task effect" (Mackworth, 1948, 1968). Behaviorally, this effect manifests itself in failures in target detection (Davies and Parasuraman, 1982), also known as "lapses" (Dinges and Powell, 1989), increasing reaction times (Boksem et al., 2005) and escalating reaction time variability, as well as an increasing sense of subjective fatigue over the course of a mentally challenging task. Although this effect was documented early in the psychological literature, it has not been the subject of much investigation in the field of neuroimaging, and the neural correlates of the phenomenon are not well understood.

The real world consequences of fatigue and the time-on-task effect are serious and pervasive. For example, many accidents involving truck drivers and medical personnel have been attributed at least in part to sleepiness, fatigue and lapses in vigilant attention (Landrigan et al., 2004; Arnedt et al., 2005). Because of this, the time-on-task effect has been of special interest to researchers in multiple disciplines who seek to understand the effects of brain function on human work capacity. In particular, the emerging field of

neuroergonomics aims to study the neural basis of cognitive and physical work in order to optimize mental functioning (Parasuraman, 2003; Parasuraman and Wilson, 2008). Viewed through the framework of neuroergonomics, the time-on-task effect arises because the workload associated with tasks requiring vigilance is high, and consumes mental resources that cannot immediately be replenished (Warm et al., 2008). This stands in contrast to traditional views of this phenomenon, which attributed the decrement to boredom or motivational decline (Frankmann and Adams, 1962; Mackworth, 1968).

Logically, neural regions associated with time-on-task effects should correspond with areas active during optimal engagement of attention. As discussed in the previous chapter, studies of attention using positron emission tomography (PET) have implicated a fronto-parietal network associated with successful task performance, including the anterior cingulate cortex (ACC), right middle and inferior prefrontal cortex, right inferior parietal regions, and the thalamus (Kinomura et al., 1996; Paus et al., 1997; Coull et al., 1998; Sturm et al., 1999). These regions can be differentiated into areas of "bottom-up" attention, or detecting signals that are intrinsically alerting, and "top-down" attention, including processes such as biasing attention towards specific signal features, inhibiting unwanted distractions from the task, or modulating attention via motivation (Sarter et al., 2001; Corbetta and Shulman, 2002; Sarter et al., 2006). It is thought that the inferior parietal cortex, temporal-parietal junction and the thalamus provide bottom-up input, while dorsolateral prefrontal cortex and ACC perform top-down functions under conscious control (Kinomura et al., 1996; Foucher et al., 2004; Fan et al., 2005).

There have been relatively few studies on neurobiological mechanisms underlying the time-on-task effect. Coull et al. (1998) compared tasks of selective and non-selective

attention, and found time-on-task effects exclusively in the latter. These effects were accompanied by decreased regional cerebral blood flow (rCBF) in the right inferior parietal cortex and inferior frontal cortex. Although both selective and non-selective tasks activated this fronto-parietal network, and showed no significant difference when averaged over time, activation to the selective task was preserved across the run, suggesting top-down modulation was involved in maintaining performance. The authors hypothesized that decreasing activation in the non-selective task was due either to habituation or diminished attentional resources. In another experiment, Paus et al. (1997) studied subjects performing a 60-minute continuous auditory vigilance task and reported decreased CBF in the thalamus, which was related to diminishing levels of arousal. Right-lateralized areas of the frontal, parietal and temporal cortex also showed decreasing activity over the course of the task, which were related to shifts in task strategy from controlled to automatic processing. However, both of these studies were analyzed at the group level (over time), and did not examine the relationships between brain activity and behavioral performance or inter-subject variability.

One of the challenges in carrying out neuroimaging studies of sustained attention arises from methodological limitations. Early studies such as those identified above have typically employed PET to measure absolute levels of cerebral blood flow. However, PET has poor temporal and spatial resolution, and cannot provide information about brain activity to individual events within a task block. Blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) can overcome some of these problems, yet it is limited by the poor sensitivity to track slow neural activity changes over a time scale longer than a few minutes (Aguirre et al., 1997; Aguirre et al., 2002).

As short tests mostly fail to elicit significant decrements in human performance over time, it is critical to select an imaging method capable of measuring slow varying neural activity with a high-level of spatial and functional resolution.

Using magnetically labelled water in arterial blood as a diffusible tracer, arterial spin labeling (ASL) perfusion fMRI (Detre and Alsop, 1999) provides a non-invasive imaging method of quantifying cerebral blood flow (CBF) at task-free resting baselines (Rao et al., 2007b; Rao et al., 2007a) as well as during the performance of cognitive tasks (Wang et al., 2005; Kim et al., 2006; Olson et al., 2006; Rao et al., 2007c). ASL perfusion imaging also offers reliable measures of CBF with excellent reproducibility over long time periods (Aguirre et al., 2002). These features suggest that ASL perfusion fMRI may provide a highly suitable method for imaging the time-on-task effect over long durations. The present study, therefore, used ASL perfusion fMRI to examine the neural correlates of the time-on-task effect during the performance of a highly-demanding attentional task -- the psychomotor vigilance test (PVT). As described in the previous chapter, the PVT is a simple reaction time task in which subjects are instructed to respond as rapidly as possible to a single stimulus presented at short, random intervals. We selected this test as our measurement instrument for a few reasons. First, it has been shown that the PVT is a simple, reliable and highly sensitive task for measuring attentional and performance deficits due to fatigue (Dorrian et al., 2005; Lim and Dinges, 2008). Second, the PVT is free of aptitude and leaning effects which may confound the effects of TOT. Finally, the attentional requirements in the PVT are undiluted by elements of selectivity such as spatial orientation or executive decision-making. As stimulus saliency is held constant throughout the task, the maintenance of optimal

performance on the PVT is almost completely mediated by top-down processes. These task features allowed us to isolate the time-on-task effect and minimize the confounding effects of differing visual stimuli, aptitude, strategy shifts or learning. In this current study therefore, we aimed to characterize neural activity associated with time-on-task decline not only during the continuous performance of PVT but also at resting baseline periods before and after the 20-minute task.

Materials and Methods

Participants

Fifteen healthy undergraduate and graduate students (8 male, mean age = 23.2 ± 3.6 yrs) from the University of Pennsylvania participated in this study. Written consent was obtained from all participants according to the University of Pennsylvania Institutional Review Board. Each participant was paid $35 in compensation for their effort and time.

Prior to enrolment in the experiment, all participants were screened to ensure that they had no history of chronic physical or mental illness, were right-handed, and did not habitually consume more than 250 mg of caffeine a day. Participants who qualified for enrolment were instructed to obtain between 6.5 and 8 hours of sleep during the 2 nights prior to the experiment, and not to consume caffeine, alcohol, or any other psychoactive substances during the 24 hours before the study. We took special care to control for baseline levels of sleepiness, as the PVT is typically used in the context of sleep

deprivation experiments (Dorrian et al., 2005; Durmer and Dinges, 2005; Lim and Dinges, 2008), and is exquisitely sensitive to the sleep propensity of the test-taker.

Task parameters

The psychomotor vigilance test (Dinges et al., 1997) was used as the sustained attention task in this study. This test has already been described in detail in the previous chapter. The length of a standard PVT administration is 10 minutes; however, in order to elicit a greater time-on-task effect and increase between-subject variability in performance, we imaged subjects as they underwent a 20-minute PVT bout. In order to boost motivation, we emphasized to subjects the importance of giving their best effort "throughout the task", without any reference to the strenuous nature of the test.

We extracted the following variables from each test as a measure of overall level of performance: median reaction time (RT), standard deviation of reaction times and number of lapses (RT > 500ms). To assess the time-on-task effect, we divided the PVT bout into 4-minute quintiles and obtained the median RT in each of those time bins, as well as computing the percentage change in reaction times from the first to the last quintile for each subject.

Directly before and after administration of the PVT, subjects were asked to rate their subjective fatigue on a 9-point visual analogue scale. To control for other potential confounds, we also obtained data on recent sleep quality and history using the Pittsburgh Sleep Quality Index (PSQI) (Buysse et al., 1989), baseline levels of sleepiness using the Epworth Sleepiness Scale (ESS), and a scale containing questions where subjects were asked to provide a subjective rating of their own performance.

Data acquisition

Functional imaging was conducted on a Siemens 3.0T Trio whole-body scanner (Siemens AG, Erlangen, Germany), using a product 8-channel array coil. All fMRI scans were conducted in the Hospital of the University of Pennsylvania, between 1400 and 1700h. Before entering the magnet, subjects were given a brief 1-minute opportunity to practice performing the PVT. As the PVT is a highly challenging task with negligible learning effects (Dorrian et al., 2005; Lim and Dinges, 2008), we did not allow participants to practice the task for a lengthier period of time.

A pseudo-continuous ASL sequence (Hernandez-Garcia et al., 2004; Wu et al., 2007) was used for the perfusion scan. Arterial spin labeling was implemented with a series of selective RF pulses (Hanning window, peak/average RF, amplitude B=5.3/1.8µT, duration=500µsec) applied at 9cm beneath the center of the imaging slices. Interleaved images with and without labeling were acquired using a gradient echo-planar imaging (EPI) sequence. The tagging/control duration was 1.77s. A delay of 1s was inserted between the end of the labeling pulse and image acquisition to reduce transit artifact. Acquisition parameters consisted of the following: FOV = 22cm, matrix = 64x64, TR = 4s, TE = 17ms, flip angle = 90°. Sixteen slices (7 mm thickness with 1.4 mm gap) were acquired in an inferior to superior direction in sequential order. The perfusion scanning protocol of PVT performance lasted 20 minutes, and consisted of 300 acquisitions. An additional two 4-minute perfusion scanning protocols consisting of 60 acquisitions were collected while subjects were at rest before and after the PVT to assess resting baseline CBF levels. During these resting times, subjects were asked to relax, keep their eyes open and stay awake. After the functional scans, high-resolution T1-

weighted anatomic images were obtained using a 3D-MPRAGE sequence (TR = 1620

ms, TI = 950 ms, TE = 3 ms, flip angle = 15°, 160 contiguous slices of 1.0 mm).

Data analysis

Functional imaging data processing and analyses were carried out with Statistical

Parametric Mapping software (SPM2, Wellcome Department of Cognitive Neurology,

UK implemented in Matlab 6, Math Works, Natick, MA). In house SPM add-on scripts

(online at http://www.cfn.upenn.edu/perfusion/software.htm) developed by two authors

(H.R. and J.W.) were used to quantify CBF values and reconstruct CBF maps for

perfusion analysis.

For each subject, functional images were first realigned to correct for head motion,

co-registered with the anatomical image, and smoothed in space using a three-

dimensional, 6-mm full width at half maximum (FWHM) Gaussian kernel. The perfusion

weighted image series was then generated by pair-wise subtraction of the label and

control images, followed by conversion to an absolute CBF image series based on a

single compartment CASL perfusion model (Wang et al., 2004). Thus, the resulting CBF

data sets contained 210 acquisitions (30 acquisitions for pre-task resting baseline, 150

acquisitions for PVT, and 30 acquisitions for post-task resting baseline) with an effective

TR of 8s. One mean CBF image was generated for every 4-minute perfusion scan for

each individual subject. Thus, each subject had one CBF image for pre-task resting

baseline, 5 CBF images for the PVT, and one CBF image for post-task resting baseline.

These CBF images were normalized to a 2 x 2 x 2 mm^3 Montreal Neurological Institute

(MNI) template using bilinear interpolation and then entered into the whole brain voxel-

wise general linear model (GLM) analysis using the PET model in SPM. The GLM

model included an adjustment for global CBF differences. Contrasts were defined to

compare the PVT with rest baselines (PVT vs. Rest), the post-task resting baseline with

the pre-task resting baseline (Rest2 vs. Rest1), and the last four-minute quintile of PVT

with the first four-minute quintile of PVT (PVTq5 vs. PVTq1). For the contrasts of PVT

vs. Rest and Rest2 vs. Rest1, activation clusters were identified at a significance level of

$p < 0.05$ (FDR corrected across comparisons for the whole brain) and cluster size larger

than 30 voxels. For the contrast of PVTq5 vs. PVTq1, no area survived the whole-brain

correction and a small volume correction ($p < 0.05$) was applied to regions in the right

fronto-parietal network.

Region of interest (ROI) analysis was also conducted to calculate quantitative

regional CBF changes and discover whether regional CBF changes were correlated with

behavioral time-on-task deficits. These ROIs were determined *a priori* to be the brain

regions comprising the fronto-parietal network that mediates sustained attention,

including the thalamus, anterior cingulate cortex (ACC), right middle frontal gyrus

(MFG), and right inferior parietal lobe (IPL) (Kinomura et al., 1996; Paus et al., 1997;

Coull et al., 1998; Sturm et al., 1999). In addition to the functional ROIs which were

defined by the activation clusters from the contrast between the PVT and resting

baselines, we also used structure-based ROIs which were independent of the PVT

activations to further confirm the relationship between CBF activity and behavioral

performance changes. The structure-based ROIs were defined from an automated

anatomical-labeling ROI library (Tzourio-Mazoyer et al., 2002). Quantitative CBF

changes in each voxel of each ROI were averaged and read out by the Marsbar toolbox

(Brett et al., 2002), which provides routines for region of interest analysis

(http://marsbar.sourceforge.net). Finally, correlation analyses were performed between

performance changes and quantitative regional CBF values (after adjusting for global

CBF differences) in these ROIs during the pre-PVT resting baseline.

Results

Behavioral data

Mean (SD) reaction time (RT) to the PVT across all subjects was 293.5 (28.3) ms.

Participants were attentive to the task, as shown by the relatively small number of lapses

(reaction times > 500ms) committed overall (mean = 2.1, SD = 3.0). Performing the PVT

elicited a clear time-on-task effect, with most participants showing steadily increasing RT

over the course of the run (Figure 3.1a). One-way analysis of variance (within subject

repeated measures) of mean RT in the four-minute quintiles revealed a significant effect

of time-on-task (Figure 3.1a, $F_{4,70} = 2.34$, $p < .05$). Tukey's post-hoc comparisons

showed significant differences between reaction time in the first and last quintile only (p

< .05). However, there was no significant effect of time-on-task on the standard deviation

of RT over the run ($F_{4,70} = 0.50$, n.s.), suggesting that this slowing was not confounded

with a change in the stability of the attentional system.

Time-on-task vulnerability was quantified by calculating the percentage change in

mean reaction times from the first to last quintile of the PVT for each subject. These

values ranged from -0.2% to 30.7%. There were noticeable inter-individual differences in

the extent of this vulnerability. Figure 3.1b shows the RT time series of 4 subjects over

the 20-minute PVT, with 2 subjects (s1 and s4) demonstrating little increase in reaction times ($< 3\%$) from the first to the last minute of the PVT, and the other 2 subjects (s2 and s3) showing marked performance decrements ($> 15\%$).

Subjects reported being mentally fatigued by performing the PVT, with subjective ratings on the 9-point fatigue scale increasing significantly from pre- to post-task (Figure 3.1c, $p < .001$). There was no significant correlation between objective performance decline and either increases in self-rated fatigue (Figure 3.1d, $r = 0.06$, n.s.), or levels of fatigue prior to engaging in the task ($r = 0.33$, n.s.). Sleep quality (as measured by the PSQI), subjective performance ratings, and ratings on the ESS were not significant predictors of time-on-task decline (data not shown), and were not subject to any further analysis.

Quantitative CBF maps

With the exception of a single subject who showed poor labeling and abnormally low global CBF values, and who was excluded from further analysis, all individual quantitative CBF maps for the task and resting baseline scans were of high quality. As an example, the quantitative CBF image from a representative subject is illustrated in Figure 3.2. Perfusion in brain regions was visualized with good sensitivity, and clear contrast between gray and white matter was observed in the perfusion intensity. The mean whole brain (global) CBF values of remaining 14 subjects (mean ± SD, in mL/100g/min) were 67.0 ± 12.0, 65.8 ± 12.2, and 70.3 ± 13.7 for the pre-PVT resting period, the PVT, and the post-PVT resting period, respectively. There were no global CBF differences between the pre-PVT resting baseline and post-PVT resting baseline or between the PVT and the

resting baselines, nor correlations between global CBF changes and RT changes (all *p* >

.1).
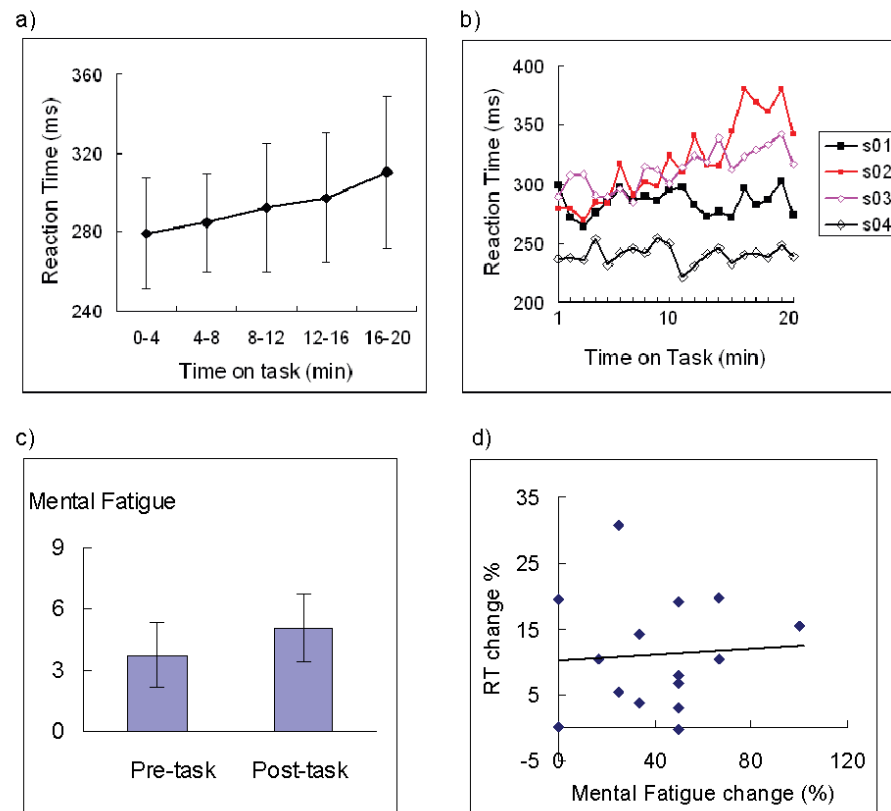
Fig 3.1. Behavioral data from the 20-minute PVT and subjective scales. a) Means and standard deviations of reaction time (RT) from the first to the last 4-minute quintiles; b) RT time series of four subjects from the first minute to the last minute of the 20-minute PVT. Note the robust individual differences in the rate of RT increases. c) Mean mental fatigue scales reported by subjects before and after the PVT. Note both RT and mental fatigue scales showed significant time-on-task effects. d) Self-reported mental fatigue scale changes showed no correlation with RT changes (r = 0.66, p > .9)
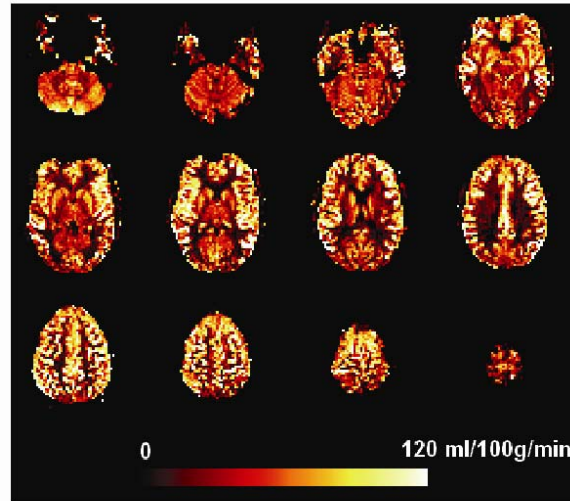
Figure 3.2. Quantitative CBF map from a representative subject

PVT task-related CBF changes

Compared to the resting baseline periods, continuous performance of the PVT increased CBF in middle frontal gyrus and inferior frontal cortex (MFG/IFC), right, right inferior parietal lobe (IPL), bilateral supplemental motor area/anterior cingulate cortex (SMA/ACC), bilateral basal ganglia/insula, and left sensorimotor cortex (Figure 3.3a, Table 3.1). We also observed slight CBF activation in the left MFG/IFG. However, no CBF change was seen in the thalamus. The PVT also induced significant deactivation (CBF decreases) in bilateral occipital cortex and precuneus/posterior cingulate cortex (PCu/PCC).

Quantitative CBF analysis on the functional ROIs revealed that regional CBF increased 8.4% (SD = 5.1%, $p < .001$) in the right MFG, 6.7 % (SD = 6.4%, $p = .002$) in the right IPL, and 7.3% (SD = 5.7%, $p < .001$) in the ACC during the PVT comparing to the baseline. However, no correlations were found between RT changes and these

regional task-induced activations in the attentional network. Quantitative CBF analysis

on the independent structural ROIs confirmed that there were no correlations between RT

changes and regional CBF activations in the right MFG and right IPL, but showed

significant correlations between RT changes and CBF changes in the thalamus and ACC

(both $p < .05$). However, these correlations disappeared (both $p > .1$) when the outlier

(RT change = 30.7%) was removed.

Table 3.1. Brain areas showing significant activation (CBF increases) and deactivation (CBF decreases) to the PVT compared to resting baseline.

Region	Cluster Size	MNI Coordinates			Peak Z	Peak p (corrected)
		x	y	z		
(A) CBF increases						
R. middle frontal cortex, inferior frontal cortex, insula, putamen	3104	42	44	2	5.42	.001
		36	14	2	5.25	.001
		32	36	20	4.76	.002
B. supplementary motor area, anterior cingulate cortex	655	8	8	48	4.71	.002
		-4	4	50	4.66	.002
L. sensorimotor cortex	574	-34	-22	50	4.60	.002
		-56	2	18	4.46	.003
		-50	-16	48	4.24	.004
L. insula, putamen	325	-30	14	6	4.25	.004
R. anterior cingulate cortex	157	8	26	26	3.93	.008

Table 3.1 (cont'd)

Region	Cluster Size	MNI Coordinates			Peak Z	Peak p (corrected)
		x	*y*	*z*		
L. middle frontal cortex	66	-24	32	22	3.61	.014
		-34	36	22	3.33	.025
R inferior parietal lobe	127	60	-40	42	3.42	.021
		54	-48	48	3.02	.044
(B) CBF decreases						
B. occipital	3234	12	-84	6	6.56	<.001
		-12	-82	8	6.08	<.001
L. posterior cingulate cortex	196	-6	-56	46	4.72	<.001

The threshold was set as whole brain FDR-corrected $p < 0.05$. L. left, R. right, B. bilateral.

CBF changes in resting baseline after task

Compared to the pre-task resting baseline, post-task resting baseline scans showed robust deactivation (CBF decreases) in bilateral ACC, MFG, superior temporal cortex (STC), and right IPL/cuneus (Figure 3.3b; Table 3.2). The MFG deactivation overlapped with the PVT activated MFG/IFG regions, but the ACC deactivation was more anterior and inferior, and the right IPL deactivation was more posterior and inferior to the PVT activated regions, respectively. Quantitative CBF analysis on the functional ROIs revealed that regional CBF decreased significantly in the right MFG ROI (mean = -5.4%, SD = 6.5%, $p = .008$). Although CBF decreases in the ACC (mean = -1.4%, SD = 10.1%) and right IPL (mean = -2.9%, SD = 11.2%) did not reach significance (both $p > .1$), CBF

changes in these three functional ROIs all showed significant correlations with RT

changes during the 20-minute PVT (all $p < .05$, Figure 3.4a). Quantitative CBF analysis

on the independent structural ROIs confirmed significant correlations between RT

changes and regional CBF deactivations in the ACC, right MFG and right IPL (Figure

3.4b). These results indicated that better-preserved performance over the 20-minute PVT

(smaller RT increases) was associated with smaller CBF decreases in the fronto-parietal

network after the task.

CBF changes during the 20-minute PVT

No CBF differences were found when comparing the first quintile to the last

quintile of PVT using a whole brain corrected threshold. Using a more liberal threshold

of uncorrected $p < .001$ and cluster-size larger than 30 voxels, regional CBF showed

significant decreases in bilateral posterior cingulate cortex (PCC), right MFG, right SMA,

and right STC from the first to the last quintile of PVT (Figure 3.3c; Table 3.2). The right

MFG deactivation overlapped with the PVT activated MFG/IFG regions and it survived

the small volume correction using a structure-based ROI of MFG. Quantitative CBF

analysis on the functional ROIs revealed that regional CBF decreased 12.8 % (SD =

11.1%, $p = .001$) in the right MFG with no significant CBF changes in other functional or

structural ROIs, and no correlation between regional CBF changes and RT performance

changes.
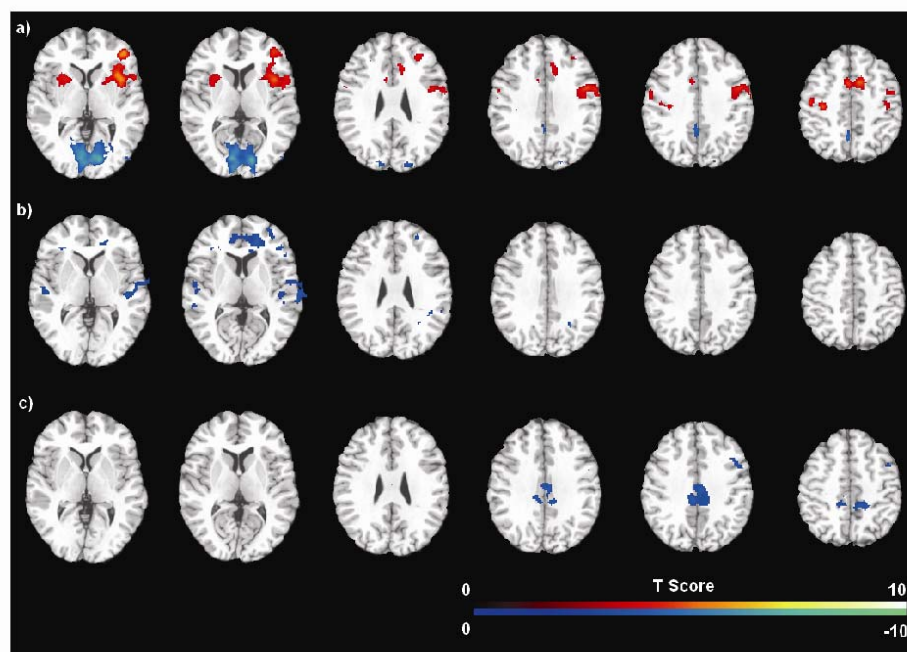
Figure 3.3. Brain activation associated with task performance and time-on-task. Brain areas associated with a) the comparison of PVT versus resting baselines; b) the comparison of post-task resting baseline versus pre-task resting baseline, and c) the comparison of the last quintile of PVT versus the first quintile of PVT. The threshold of display was set as FDR or small volume corrected p < .05.

Table 3.2. Brain areas showing significant CBF decreases for the post-task resting baseline comparing to pre-task resting baseline (Rest2 – Rest1) and for the last quintile of the PVT comparing to first quintile of the PVT (PVTq5 – PVTq1).

Regions	Cluster Size	MNI Coordinates			Peak Z	Peak P (corrected)
		x	*y*	*z*		
(A) CBF decreases: Rest2 – Rest1						
R. anterior cingulate cortex, middle frontal gyrus, inferior frontal cortex	1008	18	48	10	5.12	.005
		2	42	14	5.02	.005
		18	38	6	4.98	.005
L. superior temporal cortex	313	-54	-22	0	4.56	.006
		-52	-34	10	4.29	.009
R. inferior parietal lobe/cuneus	82	26	-56	28	4.48	.007
L. anterior cingulate cortex	70	-12	44	12	4.40	.008
R. superior temporal cortex	477	62	-32	8	4.12	.011
L. middle frontal gyrus	46	-36	46	6	4.05	.013
(B) CBF decreases: PVTq5 – PVTq1						
B. posterior cingulate cortex	565	-6	-32	40	4.23	.010
		2	-18	36	4.12	.011
R. middle frontal gyrus	105	44	10	46	3.81	.03

Thresholds were set as whole brain FDR corrected p < 0.05 and small volume corrected p < 0.05, respectively. L. left, R. right, B. bilateral.
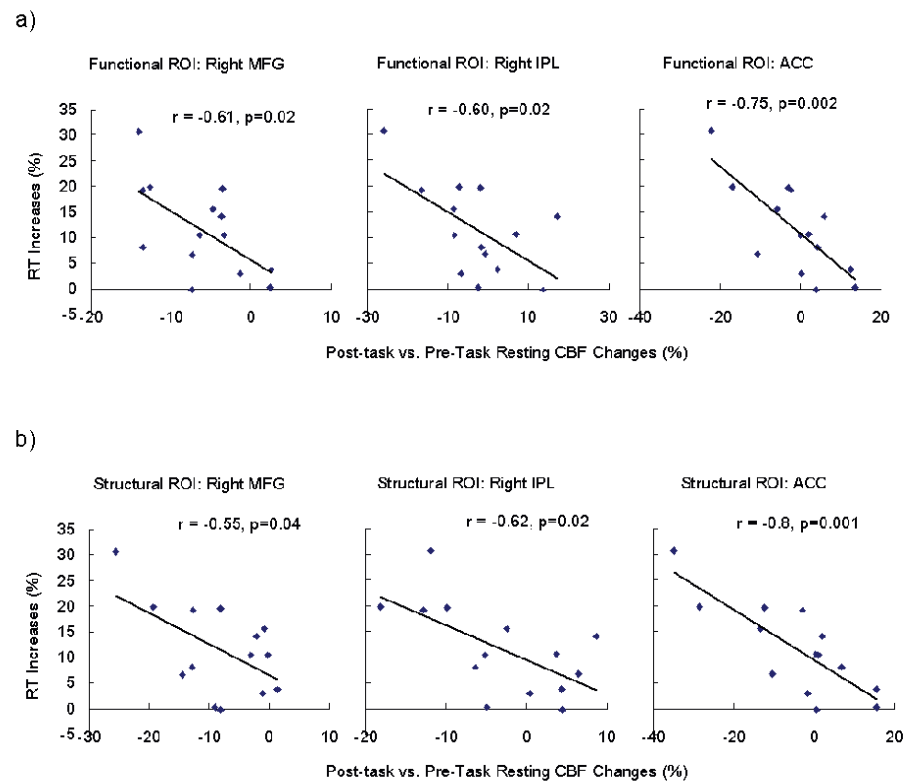
Figure 3.4. Behavior-activation correlations. Region of interest (ROI) analysis on the functional (a) and structural ROIs (b) showing that RT increases (%) during the PVT negatively correlated with post-task regional CBF changes in the ACC, right MFG, and right IPC.

Correlations between pre-task resting CBF and performance decline

Interestingly, quantitative CBF analysis of pre-task resting CBF in the functional ROIs revealed that resting CBF activity in the right MFG before PVT performance was predictive of the extent to which subjects' performance declined over the course of the 20-minute task. Lower regional CBF in the right MFG at pre-task resting baseline was associated with smaller reaction time increases during the PVT (Figure 3.5a, $r = 0.69$, $p = .007$). Quantitative CBF analysis on the independent structural ROIs confirmed significant correlations between performance decline and right MFG resting CBF (Figure

3.5b, $r = 0.64$, $p = .01$), and further revealed that pre-task resting CBF activity in the thalamus was also predictive of the reaction time increases during the PVT (Figure 3.5c, $r = -0.59$, $p = .03$). Removing the outlier did not change these results.
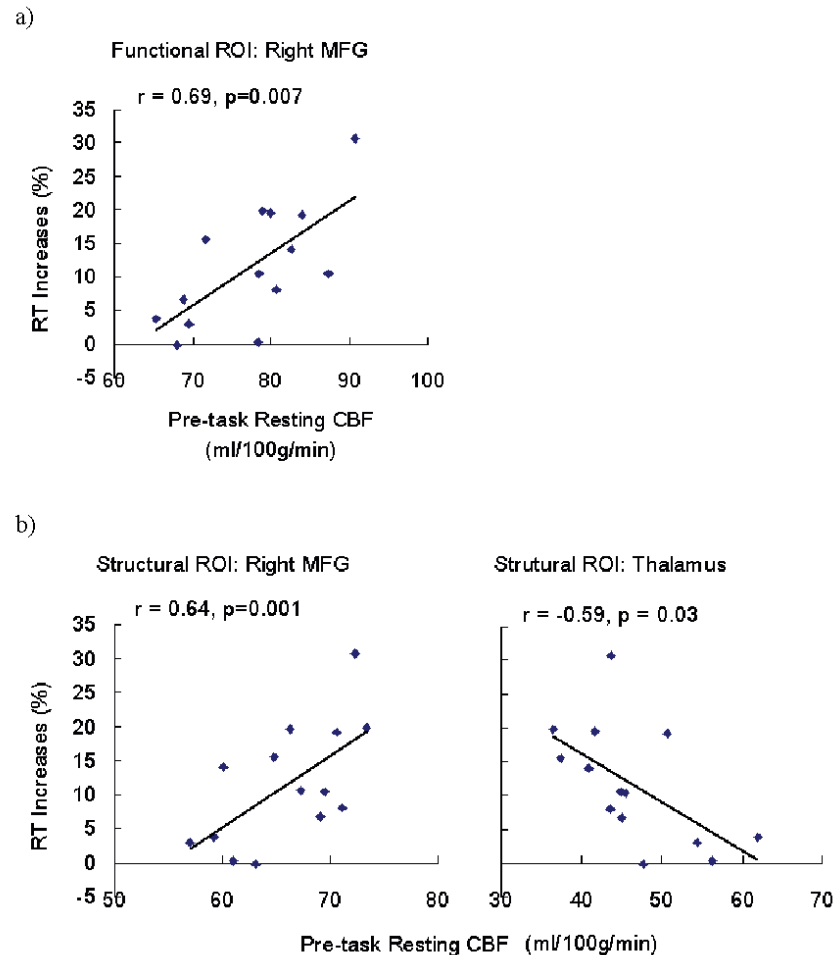
Figure 3.5. Region of interest (ROI) analysis on the functional (a) and structural ROIs (b) showing that pre-task resting CBF in the right MFG and thalamus predicted RT increases (%) during the PVT.

Discussion

In this experiment, we induced time-on-task deficits by asking subjects to perform the psychomotor vigilance test for 20 minutes. As expected, subjects reacted significantly

slower to target stimuli as the task proceeded and reported higher fatigue ratings after the task. Additionally, we observed a substantial amount of inter-individual variation in the degree to which subjects showed this effect. While some participants were able to perform relatively consistently over the course of the 20-minute period, others showed markedly worse performance by the end of the test.

No significant correlation was observed between self-reported mental fatigue changes and objective performance decline. Intuitively, one would imagine that the better-preserved performance is over time, the greater ones subjective sense of fatigue. Our data suggest that this is not the case. This finding is similar to the results from experiments by Leproult et al. (2003) and Van Dongen et al. (2003), in which there was no significant association between subjective and objective measures of alertness in a group of sleep-deprived subjects. Our finding further extended these results by demonstrating a similar dissociation in well-rested subjects, indicating that subjects may not be aware of the degree of their objective impairment after a period of high cognitive workload.

Although the PVT has been widely used for the accurate neurocognitive assessment of performance during sleep loss and the dynamic expression of waking neurobehavioral integrity, there have been relatively few studies on the brain mechanisms mediating PVT performance when compared to other attentional tasks. The only two neuroimaging studies on the PVT to date both employed an event-related BOLD fMRI design (Drummond et al., 2005); Schmidt et al., (2009) and contrasted the hemodynamic responses of fast and slow (or average) reaction times to uncover the areas associated with optimal task performance. While this approach yielded some interesting results, it

was not a method applicable to studying the process of sustained attention per se. Timely responding on a task of sustained attention may be determined by neural activity antecedent to stimulus onset, something which was not captured by the event-related analysis. Instead of focusing on phasic event-related responses, it is arguable that tonic activity throughout the task block is a more meaningful marker of the level of vigilance displayed by a subject. While low-frequency artifacts in the BOLD spectrum make such a design impossible, ASL perfusion imaging is able to overcome this problem by canceling low-frequency noise through pair-wise subtraction of tagged and control images. Our current study of the PVT therefore overcomes the limitations associated with analysis of phasic events, and gives us greater confidence that regions of activation are associated specifically with sustained attention.

Our ASL fMRI data revealed that performing the PVT engaged a right-lateralized fronto-parietal network of regions, which are reported regularly in tests requiring vigilance and continuous performance (Cabeza and Nyberg, 2000). Similar activation patterns have been reported in a variety of studies employing comparable attention tasks and different neuroimaging techniques, including monkey electrophysiology (Colby et al., 1996), human PET (Corbetta et al., 1993; Nobre et al., 1997; Coull et al., 1998) and fMRI (Fan et al., 2005; Ogg et al., 2008). More interestingly, our results revealed significant associations between behavioral performance decline and regional CBF activity in the sustained attention network. Specifically, an individual's ability to preserve better performance over time (i.e., demonstrate less RT increase over the 20-minute task) was associated with smaller CBF deceases in right MFG, right IPC and ACC from pre-task to post-task baseline and less thalamic and ACC CBF activation during PVT

performance. Furthermore, pre-task resting CBF activity in the thalamus and right MFG predicted subsequent performance decline, with better-preserved performance being associated with lower pre-task resting CBF in right MFG and higher resting CBF in the thalamus. These key points of interest are discussed below:

Performance decline correlates with CBF decreases from pre- to post-task rest

The first key finding of this study is that brain fatigue in the right fronto-parietal network persists after a period of heavy mental work and that subjects' ability to maintain baseline levels of performance over time was correlated with CBF decrease from pre- to post-task resting activity in this attentional network. Areas in this network have long been established as important in the effortful maintenance of sustained attention (Lewin et al., 1996; Cabeza and Nyberg, 2000). However, our data extended the classic findings in two important ways:

First, we clearly showed that the residual effects of performing a fatiguing task on brain activity can be detected by ASL perfusion fMRI. Regional activity at rest following task completion decreased in the anterior cingulate cortex, middle prefrontal gyrus and inferior parietal cortex, suggesting that the brain does not immediately "reset" itself following task performance and cognitive resources depleted during demanding tasks can not be restored within a 4-minute post-task window. Second, we showed that larger RT increases were associated with greater post-task CBF decreases, indicating that subjects' ability to better preserve performance may depend on the stability of activity in the attentional system.

The CBF changes associated with time-on-task in our experiment are consistent with those found in previous studies (Paus et al., 1997; Coull et al., 1998), which showed decreases in cortical activation over time. In both of these paradigms, however, decreases in activation were suggested as implying shifts to automaticity. In contrast to oddball paradigms, the PVT demands rapid and timely responding to randomly presented stimuli, and it is not possible for subjects to adapt to the PVT by shifting from controlled to automatic processing and still maintain optimal performance. We thus reject strategy shifts as a theoretical framework for understanding the changes observed in this experiment. Instead, we posit that there is a physiological ceiling to how well one can perform, and that the speed at which these scarce attentional resources get depleted varies widely from person to person, and depends on the demands of the task an individual is engaged in.

One possible alternative account of the data is that declines in performance and brain activity are dictated largely by decreasing motivation. Altering motivation by providing monetary incentives has been shown to modulate the time-on-task effect in college students (Tomporowski and Tinsley, 1996). It seems unlikely, however, that differences in motivation could entirely account for the substantial amount of variability in performance shown in our study. All of our subjects volunteered for the study and were paid and treated identically, and were only required to perform a simple task with no element of motivation and reward. Nevertheless, certain individuals may "try" harder than others on tasks, based (for example) on individual differences in personality traits, and part of the variance captured in this experiment may reflect effort rather than physiological capacity. Future work is needed to test this hypothesis.

Resting baseline activity predicts subsequent performance decline

Another key finding from the present study was that resting CBF activity in the thalamus and right middle frontal gyrus prior to task onset was predictive of the extent to which subject's performance eventually declined. This result is analogous to various findings in the research on sleep deprivation suggesting that baseline activity is associated with cognitive vulnerability to periods of sleep loss. For example, Mu and colleagues (2005) found in an fMRI study that sleep-deprivation resilient individuals had a significantly greater number of activated voxels globally than sleep-deprivation vulnerable individuals when performing a working memory test. Similarly, Caldwell et al. (2005) found that, in a group of well-rested pilots, global fMRI activation (and activation in the left posterior parietal cortex) to a Sternberg working memory task was correlated with subsequent performance measures on a flight simulator after they were sleep deprived.

Why would pre-task resting activity correlate with the level of vulnerability when the attentional system is challenged? We posit that differing levels of resting thalamic activity reflects individual differences related to the amount of sensory filtering the brain performs while in its baseline state. The thalamus has been implicated in worsening performance due to sleep deprivation (Thomas et al., 2000), and as a mediator of attention between different arousal states (Portas et al., 1998). It is conceivable, therefore, that its default state of activity should have some bearing on its efficiency during tasks that require its engagement. Similarly, the prefrontal cortex, an area with known connections to the thalamus, acts in concert with this region to carry out the early filtering of irrelevant information. Our data suggest that individuals for whom this system

operates less efficiently at baseline may be less effective in the early selection of relevant information (reflected by lower baseline thalamic activity), and thus require greater top-down control (reflected by higher prefrontal activity) during later processing stages. Hence, when an additional load is placed on this neural system (as in the case of an attention-demanding task), subjects who are already performing near capacity have no spare neural resources, and thus show the greatest deficits in performance.

Although it may be argued that resting levels of activity could reflect pre-existing levels of sleep debt, we contend that this was not the case, as we controlled for sleep history prior to the scanning session. We found that subjects were regular sleepers as assessed by the PSQI, and neither scores on this questionnaire nor the Epworth Sleepiness Scale correlated with brain activity in any of the attentional regions. We posit therefore, that the observed variability in resting-state activation may reflect a fundamental individual difference trait. Recent perfusion studies showing resting baseline CBF correlating with habitual emotion regulation scores (Abler et al., 2008) and working memory capacity (Beschoner et al., 2008) also support this hypothesis.

Taken together, our main findings highlight the potential importance of resting-state CBF in assessing the degree of taxation on the brain's attentional system, a possibility that has been overlooked by the vast of previous neuroimaging studies for which pre- and post-task activity is taken merely as inseparable "baseline". Future work is needed to determine the time of course of recovery following a period of sustained activity, that is, how long the brain takes to return to its default resting state.

Task-induced activation did not correlate with performance decline

In contrast to the resting-state data, CBF changes during the task in the attentional network regions did not correlate with individual differences in performance decline. This may be due to the mixed CBF signal of both tonic and transient activity changes in this network detected by ASL perfusion while subjects are engaged in a cognitive task. In other words, phasic activity to stimulus responses may have activated regions that overlap with the attentional network (Drummond et al., 2005), thus adding noise to the tonic signal obtained from these areas in our analysis. In general, therefore, the magnitude of activation in perfusion imaging datasets acquired during cognitive task performance should be interpreted with caution, as it may reflect the contribution of multiple sources of variance.

Conclusion

This study demonstrates the utility of ASL perfusion imaging in revealing the neural network associated with tonic activity to a highly demanding sustained attention task. Our results demonstrate the critical role of the right fronto-parietal network in mediating time-on-task effects, and that these effects persist, and can be measured in the immediate aftermath of a period of heavy cognitive workload. Moreover, our findings reveal that differences in neural activity between the resting periods before and after the demanding task can act as markers of cognitive fatigue, and thus contain useful information that has heretofore been neglected in the neuroimaging literature. Most interestingly, pre-task levels of CBF in the thalamus and right middle frontal gyrus appear to be meaningful predictors of subsequent time-on-task decline. These results may

help identify neural "risk factors" for accidents and errors due to prolonged task

performance and lead to greater safety and work efficiency in populations where fatigue

is an ever-present problem.

Chapter 4

Study #3: Sleep deprivation impairs object-selective attention through reduced fronto-parietal connectivity

In studies #1 and #2, our focus was the study of the effects of sleep deprivation and fatigue on vigilant attention and its neural correlates. In the current chapter, we turn to the effects of sleep deprivation (SD) on selective attention, which refers to the ability to focus cognitive resources on particular locations, objects, or features to the exclusion of irrelevant distracters. Existing studies on selective attention in the setting of sleep deprivation have yielded somewhat mixed results (Horowitz et al., 2003; Versace et al., 2006; Santhi et al., 2007; Mander et al., 2008; Tomasi et al., 2009; Chee et al., 2010). One reason for this variability is that deficits in selective attention can accrue from a combination of sources (Horowitz et al., 2003; Jennings et al., 2003) which may not be dissociable using behavioral methods alone. In comparison, studying the neural substrates of attention using fMRI provides added dimensions along which to tease apart the contributions of specific deficits in selective attention from the dominant, non-specific effect of vigilance declines.

In the well-rested state, selective attention results in the biasing of sensory processing in favor of the attended stimulus over competing distracters (Desimone and Duncan, 1995). This leads to topographically specific increases in neuronal firing rate (Reynolds and Chelazzi, 2004; Maunsell and Treue, 2006) and MR signal in sensory cortex (Beck and Kastner, 2009). Behavioral studies evaluating the effect of SD on selective attention suggest that despite an overall decline in response speed, feature-based

visual search (Horowitz et al., 2003) and alerting may be relatively preserved (Versace et al., 2006).

Deficits in selective attention are likely to arise from a reduction in the strength of top-down biasing of information-processing in the sensory cortex. In support of this hypothesis, several functional neuroimaging experiments have shown that sleep deprivation in humans often results in reduced activation of the dorsal fronto-parietal attention network (Drummond et al., 2004; Chee and Chuah, 2007; Lim et al., 2007; Chee et al., 2008; Tomasi et al., 2009). Crucially, however, these findings do not differentiate the effects of sleep deprivation on selective attention from other forms of attention as all forms generally recruit similar cognitive control areas. A useful alternative approach to identifying deficits in selective attention is to examine their downstream effects, for instance the influence of top-down biasing signals on activity in functionally differentiated and spatially dissociable sensory regions (Gazzaley et al., 2005; Yi and Chun, 2005).

In a recent experiment, subjects viewed picture quartets containing alternating faces and scenes with instructions to attend to faces, scenes, or both. In this paradigm, sleep deprivation reduced functional connectivity between the intraparietal sulcus (IPS) and the parahippocampal place area (PPA) (Chee et al., 2010). However, while there was a main effect of state on PPA activation, modulation of PPA activity by attention was relatively preserved. Since the stimuli were presented in a regular and predictable order and timing, subjects could be thought of as being cued to respond to the target stimuli. Cues have been shown to ameliorate the effect of sleep deprivation on selective attention

(Jennings et al., 2003; Versace et al., 2006), which may account for the preserved modulation of PPA in this prior study.

To investigate this hypothesis, we studied the effect of sleep deprivation on the functional anatomy of selective attention using a task that did not provide subjects with a prior alerting cue. We predicted that in addition to decreased activation in fronto-parietal control areas, we would also uncover reduced biasing of activation in the PPA to relevant stimuli. We additionally anticipated a reduction in connectivity between cognitive control regions and ventral visual cortex in the sleep-deprived as compared to the well-rested state.

Materials and Methods

Twenty-seven undergraduates from the National University of Singapore were recruited for this within-subject study through advertisements on a campus website. From this original pool, two were removed from analysis due to excessive head-motion in the scanner, one was excluded based on near-chance performance in both states, and another was excluded on the basis of image problems, giving a final sample of $N = 23$ (12 male; mean age = 21.3 years, SD = 1.4 years). All subjects were right-handed, had no history of chronic physical or psychiatric disorders, or long-term medication use. They had regular sleep schedules and slept between 6.5-8 hours a night based on self-report, and were not extreme morning chronotypes as assessed by a modified Horne-Ostberg Chronotype Questionnaire (Horne and Ostberg, 1976).

Upon entering the study, subjects visited the lab for a briefing to practice the experimental task and to collect an Actiwatch (Actiwatch, Philips Respironics, USA) that

they were instructed to wear at all times until the conclusion of the experiment. Subjects were also issued sleep diaries on which they were to record the onset and offset of all sleep bouts. Sleep history was checked prior to each of the fMRI scanning sessions, and participants who did not comply with a regular sleep schedule (> 6.5 hours of sleep/night; sleep time no later than 1:00 AM; wake time no later than 9:00 AM) were excluded.

At least five days after the briefing, subjects returned to the laboratory for the first of two experimental sessions. In the rested wakefulness (RW) condition, subjects reported to the lab at approximately 7:30 AM. After filling in a questionnaire to assess their subjective level of sleepiness (the Karolinska Sleepiness Scale), they underwent an fMRI scan during which they performed a task involving selective attention to two different classes of stimuli: faces and houses (see fMRI procedures below for detailed description). Anatomical scans were also acquired during this time. fMRI scanning in the RW state typically began at about 8:00 AM. In the sleep deprivation (SD) condition, subjects reported to the lab on the evening prior to their fMRI scan. Subjects' actigraphy records were used to confirm they had awakened at their regular time on that day, and had not taken any daytime naps. Subjects remained awake overnight in the laboratory under the constant supervision of a research assistant. They were permitted to engage in light recreational activities, but were not allowed to smoke or consume caffeine. Every hour, participants performed the Psychomotor Vigilance Test and rated their subjective sleepiness using the Karolinska Sleepiness Scale. In the SD condition, subjects underwent an fMRI scan as in the RW condition, but at 6:00 AM. The order of scanning sessions was counterbalanced across subjects (RW session first; N = 12) to minimize potential order confounds. Sessions were separated by at least one week, so that subjects

undergoing the SD session first had sufficient time to fully recover from the effects of sleep loss.

Ethics statement

Permission to conduct this study was granted by the Singapore General Hospital IRB, and all subjects provided written informed consent prior to participation. Subjects were financially compensated for their time. The individual providing the example face in Figure 4.1 provided written informed consent for the publication of this image.

Experimental paradigm

Subjects were shown blocks consisting of 6 novel targets (grayscale images of three faces and three houses) and 30 scrambled images that were of approximately equivalent luminance as the target pictures (Figure 4.1). Equal numbers of male and female faces bearing neutral expressions were presented. Target stimuli were randomly interleaved with the scrambled images such that the interval between two targets ranged between 10s and 14s (mean = 12s). The interstimulus interval for presentation varied randomly between 0.5s and 3.5s (mean = 1.75s), except after the appearance of a target, when it was held constant at 2s. This was to allow subjects adequate time to respond before the next stimulus onset.

At the start of each block, an instruction screen lasting 2s was presented to the subject, informing them to either attend to faces, attend to houses, or passively observe the stimuli. This was followed by a further 2s delay before the first stimulus appeared. In each of the 'attend' conditions, subjects were instructed to respond to the target by

pressing a button with the right hand. In the 'observe' condition, subjects simply viewed the stimuli without making any response (Figure 4.1). Thus, in the "attend to face" blocks, attend face (AF) and ignore house (IH) events were generated, and in "attend to house" blocks, attend house (AH) and ignore face (IF) events were generated. Observe face and observe house (OF and OH) events were generated in the blocks where stimuli were passively observed. fMRI runs consisted of 4 blocks of fixation (20s) interleaved with 3 task blocks (77s). Subjects performed 6 runs in total (all possible permutations of the task blocks) during each scanning session.

Finally, at the end of the RW session, subjects were scanned while they viewed blocks of faces and houses; data from these scans served as functional localizers that allowed us to identify the fusiform face area (FFA) and parahippocampal place area (PPA) for each individual subject (Saxe et al., 2006). Functional localizers consisted of eight stimulus blocks interleaved with nine fixation blocks, and lasted 6 minutes and 16 seconds each. Each stimulus block comprised either 18 faces or 18 houses, presented at the rate of 1 per second.
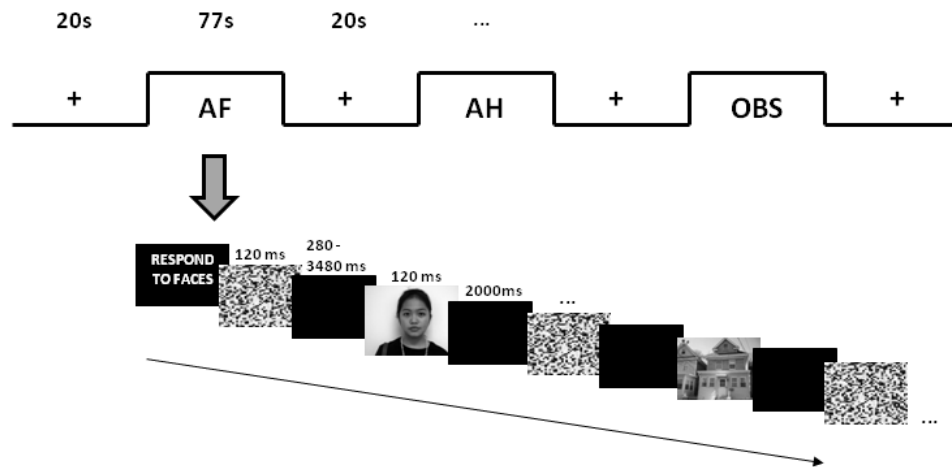
Figure. 4.1 Schematic of the object-selective attention task. Three faces and three houses were presented during every task block. Inter-stimulus intervals varied randomly after each scrambled image, and were held constant at 2000ms following each target. Subjects performed 6 task runs during each scanning session. AF = attend and respond to faces; AH = attend and respond to houses; OBS = passive observation of houses and faces.

Image acquisition

MR imaging was conducted using a 3T Siemens Tim Trio scanner (Siemens, Erlangen, Germany) fitted with a 12-channel head coil. Participants viewed stimuli through a set of MR-compatible LCD goggles (Resonance Technology, Los Angeles, USA) and responded using their right index finger via a MR-compatible button box. Performance was continually monitored by a research assistant who noted all lapses and eye closures (through use of an eye tracking device). Subjects were prompted to attend to the task through an intercom system when they failed to respond to two consecutive trials, or when epochs of eye closure exceeded 3 seconds. Functional images were collected using a gradient echo-planar imaging sequence (TR: 2000ms; TE: 30ms; flip angle: 90°; field-of-view: 192x192 mm; matrix size: 64x64). Twenty-eight 3-mm axial

slices aligned to the intercommisural plane and covering the whole brain were acquired.

Directly following the functional data collection, a high-resolution T1 coplanar image

was acquired. Finally, a high-resolution 3D MPRAGE sequence was obtained so that

anatomical images could be normalized into common stereotactic space.

Image preprocessing and analysis

MRI data were analyzed using Brain Voyager QX version 1.10.1 (Brain

Innovation) and Matlab R13 (Mathworks). Functional images were aligned across

scanning runs to the first image of the final run. Intrasession image alignment to correct

for motion was performed using the first acquisition of the final functional run as the

reference scan. Interslice timing differences within each functional acquisition were

corrected using cubic spline interpolation. We performed Gaussian filtering in the spatial

domain by applying an 8mm FWHM smoothing kernel. Linear signal drift, and signals of

lower than 3 cycles/functional run were removed. Finally, all images were registered to

their respective individual 3D high-resolution T1 anatomical image, and normalized to

Talairach space (Talairach and Tournoux, 1988).

Functional imaging data were analyzed using a general linear model with 13

predictors in an event-related analysis. Twelve of these predictors were created with a

2x2x3 model using all combinations of state (RW/SD), stimulus type (house/face) and

trial type (attend/observe/ignore). We modeled events by convolving a stick function with

a double-gamma, canonical hemodynamic response. Only correct 'attend' responses were

analyzed. A thirteenth predictor was created to model all lapses (non-responses within 2s)

in each state; these events were not subsequently analyzed any further. As we did not

want to include periods of data that included frequent microsleeps, runs in which there were > 50% of undetected targets were not entered into the model. We excluded 14 out of 288 runs (4.9%) from the analysis for this reason.

In order to identify cognitive control regions activated above threshold by selective attention to houses as well as faces, we computed the conjunction of two contrasts: attend house (AH) vs. baseline and attend face (AF) vs. baseline in the RW state. To control for Type I error, voxels were processed using an iterative cluster size thresholding procedure (Goebel et al., 2006) that considered the spatial smoothness of functional imaging data when generating activation maps based on a corrected cluster threshold ($p < .05$). Subsequent to this, a voxel-level threshold of at least $p < .001$ (uncorrected) for t maps was applied.

To characterize state-related differences in control region activation during task performance, we compared activation within a 10x10x10mm cube of voxels surrounding the peak voxels obtained from the conjunction analysis described above in addition to running an ANOVA-based analysis. The frontal and parietal regions selected from the conjunction analysis have previously been identified as important areas involved in selective attention (Serences et al., 2004; Chee et al., 2010). These ROIs were then interrogated to evaluate the relative magnitude of activation for attend, ignore and observe conditions across the two states. All secondary statistical tests were conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL).

Analysis of object-selective attention within the ventral visual cortex was ROI-based. The PPA and FFA were defined by a separately conducted localizer scan

performed for each individual as described previously. PPA ROIs comprised a 10x10x10mm cube of voxels that surrounded the one voxel showing maximum difference in activation between house and face blocks. We focused our analysis on the PPA as it has been shown to yield more discriminating and spatially more consistent, selectivity data (Gazzaley et al., 2005; Yi and Chun, 2005; Chee et al., 2010). Furthermore, because there was no hemispheric asymmetry of PPA activation, activation magnitude data for all conditions— AH (attend house), IH (ignore house) and OH (observe house) — were obtained from both the left and right PPA and averaged. Activation magnitude across trial type and state was evaluated using paired t-tests. We opted not to use analysis of variance (ANOVA) as we had specific a priori hypotheses, and because some of the comparisons in the 2-way ANOVA would not have been meaningful (e.g. AH_{RW} vs OH_{SD}).

Psychophysiological interaction (PPI) analysis (Friston et al., 1997) was performed by extracting the time series of activation from a 10mm cubic region around the peak voxels identified by the conjunction of AH vs. baseline and AF vs. baseline contrasts within the left intraparietal sulcus (IPS; Talairach co-ordinates: -27, -58, 37) as well as the left inferior frontal gyrus/insula (Talairach co-ordinates: -36, 11, 4). We selected these regions due to their known involvement in biasing object-based attention, and for consistency with a companion study (Chee et al., 2010).

To carry out PPI analysis, we used a linear model with three predictors: the time course of activity in the seed ROI, a task predictor coding for activity within task blocks (AH vs. IH or AH vs. OH) and a PPI term. To construct the PPI term, the deconvolved time-course of the relevant seed region was multiplied with a vector containing the

psychological variables of interest. This product was then re-convolved with a canonical

hemodynamic response function (Gitelman et al., 2003). The coefficient of this third,

interaction term, is the one of interest in PPI analyses. Statistical maps of functional

connectivity for each state were computed by conducting two-tailed, one sample t-tests

on parameter estimates of the PPI (RW and SD) thresholded at $p < .05$.

To evaluate the robustness of the findings, we compared PPI in the AH vs. IH as

well as AH vs. OH contexts as both comparisons evaluate object-selective attention.

Results

Behavioral data

In the RW state, subjects were able to perform the task accurately with high hit

rates (mean = 91.0%, SD = 11.0%) and low rates of false alarms (mean = 4.1%, SD =

4.6%). After sleep deprivation, there was a significant decline in the percentage of hits

($t_{22} = 5.30$, $p < .001$); however, there was no significant change in the percentage of false

alarms, and reaction times were not significantly different across state (Table 4.1). There

were no significant differences in performance accuracy observed between face and

house detection blocks in either state.

Brain activation associated with selective attention

Brain regions activated as a result of attending to houses as well as faces included

the intraparietal sulcus (IPS) and inferior parietal lobes bilaterally (BA 40), left inferior

frontal gyrus, right middle frontal gyrus, anterior cingulate cortex (Table 4.2), the

thalamus, anterior areas of the frontal lobe (Figure 4.2) as well as the ventral visual

cortex.

Table 4.1. Behavioral data from the selective attention task (N = 23).

Behavioral variable	RW	SD	*t value*
Hits (%)	91.05 (10.98)	75.48 (17.13)	5.30*
False alarms (%)	4.11 (4.57)	4.95 (5.09)	-0.63
Mean reaction time (ms)	574.08 (82.97)	593.48 (83.99)	-1.24
Subjective sleepiness	4.65 (1.78)	8.40 (0.71)	-10.1*

Data were collapsed across Attend House (AH) and Attend Face (AF) blocks. Subjective sleepiness was measured using the Karolinska Sleepiness Scale.

$* p < .001$

Attending to houses elicited greater activation than ignoring houses in the left IPS

(t_{22}=2.72, $p < .05$), left inferior frontal regions (t_{22}=6.83, $p < .001$), anterior cingulate

cortex and the thalamus (ACC: t_{22}=7.61, $p < .001$; thalamus: t_{22}=6.47, $p < .001$; Figure

4.3). Similar modulation of attention in the three cognitive control regions as well as the

thalamus was observed when attending to faces as opposed to ignoring or observing them

(Figure 4.S1). In subsequent analyses, we focused on the effect of attending to houses

because of the clearer effects of attention on PPA activation as described in previous

studies (Gazzaley et al., 2005; Yi and Chun, 2005; Chee et al., 2010).

After a normal night of sleep (RW), attending to houses resulted in greater activation in the PPA in both contrasts of interest AH vs. IH (t_{22}=2.36, $p < .05$) and AH vs. OH (t_{22}=3.14, $p < .01$). After correcting for the two comparisons, the former contrast dropped just below the level of statistical significance ($p = .056$). Nevertheless, effect sizes for these comparisons were in the moderate to large range ($d = 0.57$ and 0.68 respectively). To verify that this effect was not spurious, we repeated the analysis using the PPA peak in the group map for reference instead of an individually selected PPA ROI. This resulted in finding significant AH vs. IH ($t = 2.99, p = .006$) and AH vs. OH ($t = 3.25, p = .004$) contrasts in RW, which would have survived Bonferroni correction. AH vs. IH and AH vs. OH comparisons in SD around this voxel failed to reach statistical significance ($t = 0.25, p = .81$ and $t = 1.36, p = .19$ respectively).

Effects of sleep deprivation on activation

SD reduced activation in the left inferior frontal ROI (t_{22}=2.50, $p < .05$) and left IPS (t_{22}=2.41, $p < .05$; Figures 4.3 and 4.4) in the attend conditions but did not affect activation in the anterior cingulate (t_{22}=0.41, *n.s.*) or the thalamus (t_{22}=0.23, *n.s.*). These regions also appeared when probing for a main effect of state using an ANOVA approach (Figure 4.4). The biasing effect of attention on PPA activation evident during RW was significantly attenuated following SD (Figure 4.5). Paired t-tests between AH vs. IH and AH vs. OH in the SD condition were not significant at the $p < .05$ level (effect sizes: $d = 0.18$ and -0.01 respectively). Moreover, there was a significant effect of state when comparing activation in the AH condition relative to baseline (t_{22}=3.93, $p < .001$).
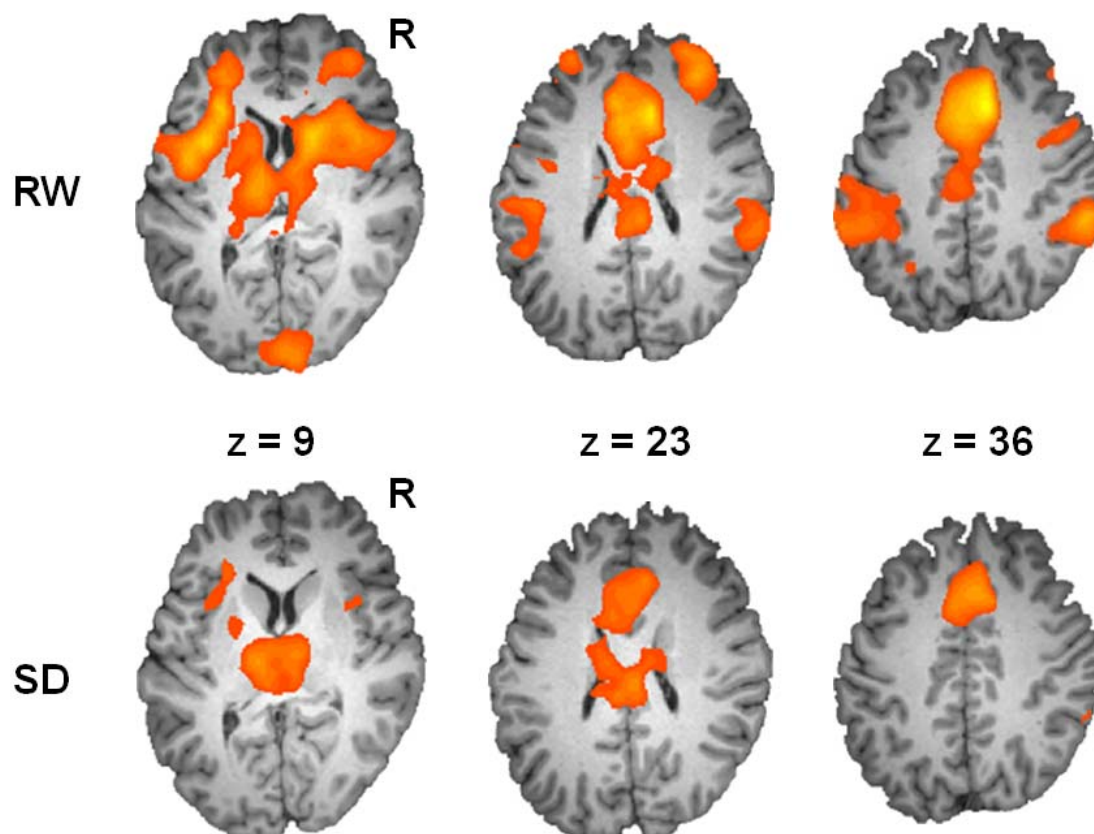
Figure 4.2 Effect of selective attention task on brain activation Brain regions showing significant activation in the conjunction of Attend House (AH) vs. baseline and Attend Face (AF) vs. baseline conditions ($p < .001$, uncorrected). The top panel depicts activation during rested wakefulness (RW), and the bottom panel depicts activation after approximately 24h of total sleep deprivation (SD).
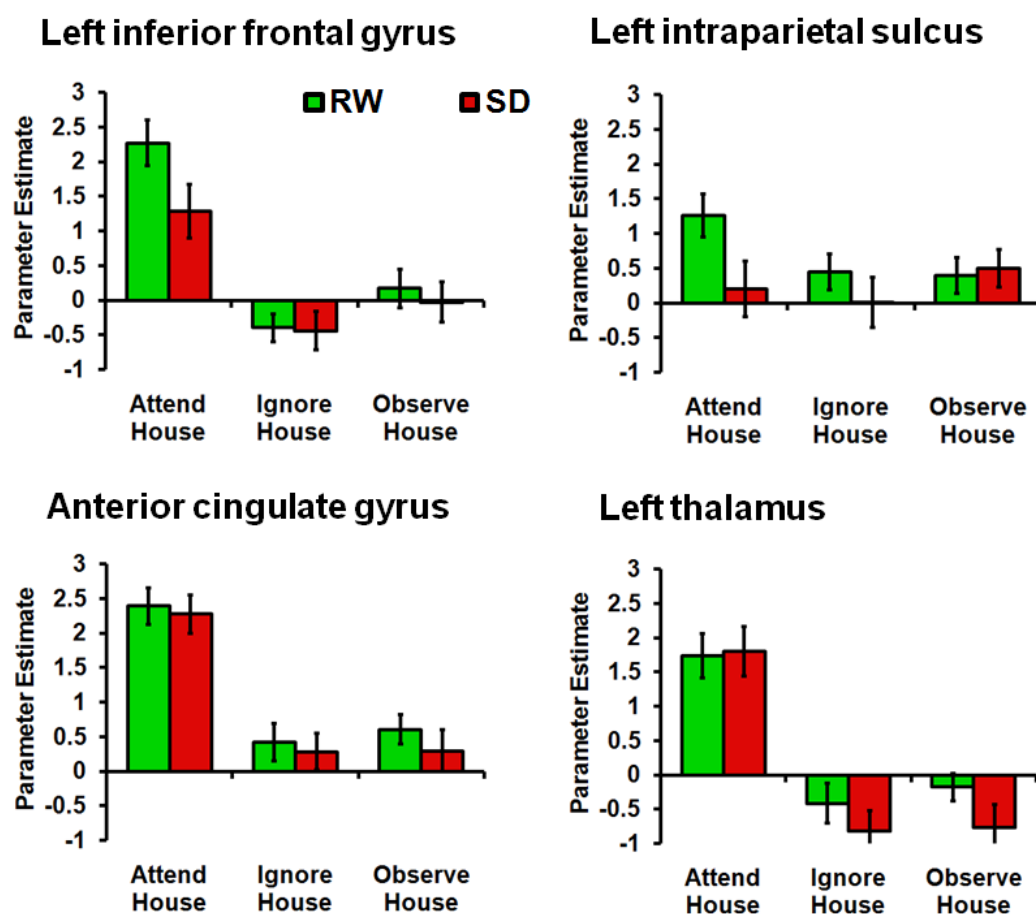
Figure 4 3. Parameter estimates of activation for the house conditions in areas associated with arousal and attention Parameter estimates for each condition and state associated with the left inferior frontal gyrus (IFG), left intraparietal sulcus (IPS), anterior cingulate cortex (ACC), and left thalamus. Significant state-related differences were observed in the left IFG and IPS, but not in ACC or the thalamus.
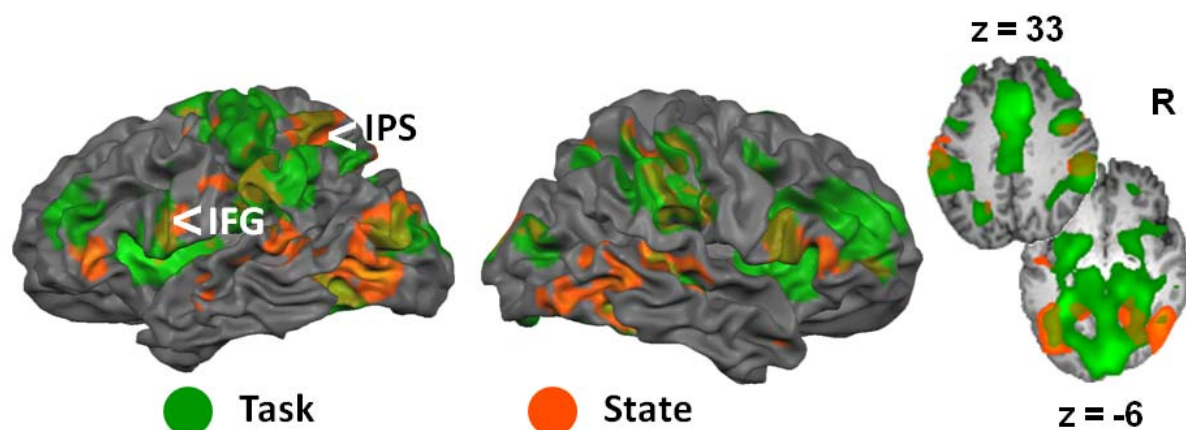
Figure 4.4 Effect of sleep deprivation on activation associated with selective attention for houses
Brain regions that showed a significant effect of state on activation in the Attend House (AH) vs. baseline contrast ($p < .001$ uncorrected; in orange). This finding was similar to the main effect of state obtained using an ANOVA analysis. For comparison, the regions showing the effect of task are overlaid in green and the overlap between regions showing task and state effects are in an intermediate color. IPS = intraparietal sulcus; IFG = inferior frontal gyrus.
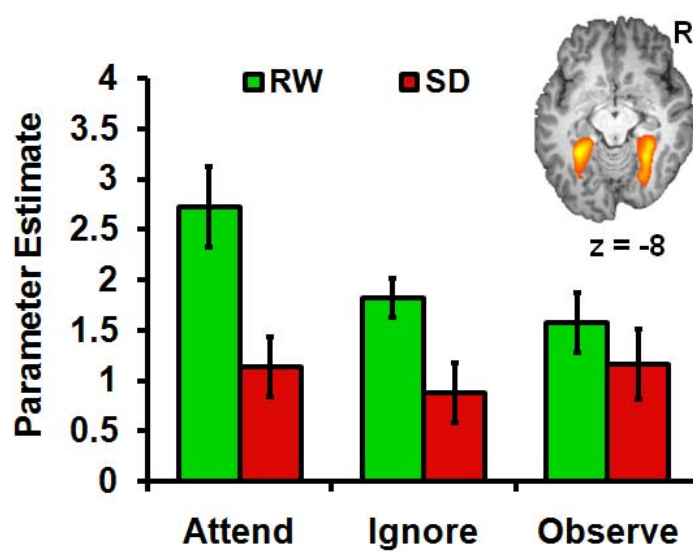
Figure 4.5 Effects of sleep deprivation and attention on parahippocampal place area (PPA) activation
In the rested (RW) state, attention to houses (AH) resulted in significantly greater PPA activation compared to ignoring (IH) or observing (OH) houses. However, this attention biasing was lost during SD.

Table 4.2. Talairach coordinates of activation peaks in regions potentially mediating cognitive control identified by the conjunction of Attend House (AH) vs. baseline and Attend Face vs. baseline trials ($p < .001$ uncorrected).
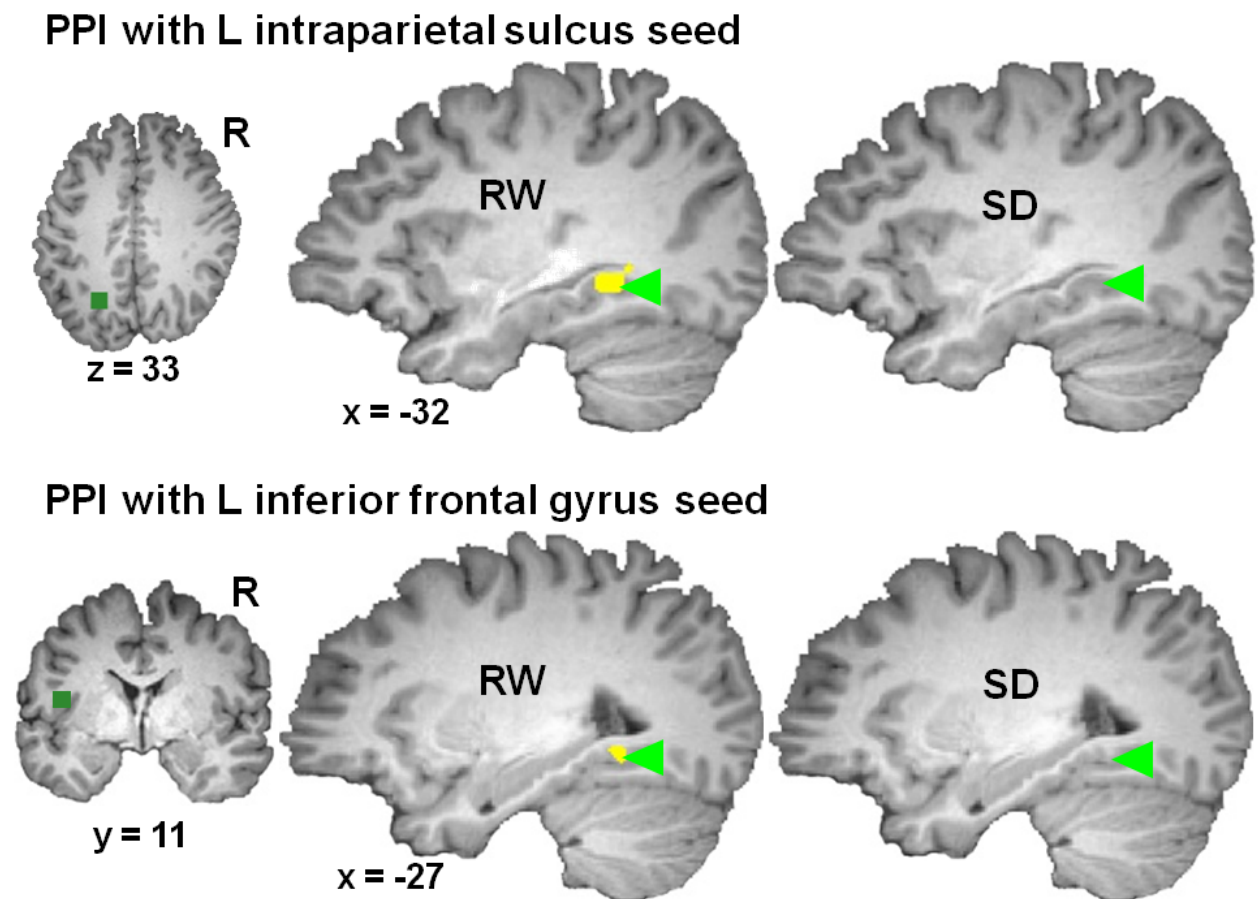
Region	BA	Talairach coordinates			t value	
		x	**y**	**z**	**RW**	*SD*
L intraparietal sulcus	7/40	-27	-58	37	4.48**	1.06
R intraparietal sulcus	7/40	33	-58	43	4.69***	2.69*
L superior frontal gyrus	10	-24	47	5	3.10**	1.21
R superior frontal gyrus	10	30	50	22	4.65***	3.73**
R middle frontal gyrus	46	24	44	-5	3.74***	2.61*
L inferior frontal gyrus	13	-36	11	4	4.97***	4.01**
Anterior cingulate cortex	32	-9	11	43	5.37***	4.57***

BA = Brodmann's area   * $p < .05$ ** $p < .01$ *** $p < .001$

Psychophysiological interaction (PPI) analysis

Whole-brain PPI analysis revealed significant connectivity between the seed voxels in the left IPS and the PPA bilaterally during RW (AH vs. IH: $t_{22}=4.77$, $p < .001$; AH vs OH: $t_{22}=3.34$, $p < .01$) but not following SD (AH vs. IH: $t_{22}=1.52$, *n.s.*; AH vs OH: $t_{22}=1.31$, *n.s.*; Table 4.3, Figure 4.6, Figure 4.S2). Using a paired t-test, the direct comparison of PPI values across state for the PPA was significant only for AH vs. IH (AH vs. IH: $t_{22}=1.88$, $p < .05$, 1-tailed; AH vs. OH: $t_{22}=0.73$, *n.s.*). A separate PPI analysis evaluating connectivity between the left inferior frontal gyrus /insula and other brain areas found significant interaction between the left frontal seed and the PPA following a night of normal sleep (AH vs. IH: $t_{22}=2.67$, $p < .05$; AH vs OH: $t_{22}=3.31$, $p <$

.01) but not following SD (AH vs. IH: t_{22}=1.05, *n.s.*; AH vs OH: t_{22}=0.48, *n.s.*; Table 4.3, Figure 4.6, Figure 4.S2). Comparing the PPI across state for the PPA, we found a significant difference in the AH vs. IH comparison (AH vs. IH: t_{22}=2.69, $p < .05$; AH vs. OH: t_{22}=1.27, *n.s.*).

Figure 4.6 Psychophysiological interaction (PPI) in the rested and sleep deprived state Connectivity analysis was performed using seeds in the left intraparietal sulcus (IPS; Talairach co-ordinates: -27, -58, 37) and left inferior frontal regions (Talairach co-ordinates: -36, 11, 4) (seed regions represented by green squares). Each map represents the conjunction of regions showing significant PPI in the Attend House (AH) vs. Ignore House (IH) and AH vs. Observe House (OH) conditions (threshold $p < .05$).

Table 4.3. Parietal and frontal seed regions showing psychophysiological interaction with the PPA (Talairach co-ordinates shown) under different task conditions.

Seed region	Contrast	Talairach coordinates of PPA region showing PPI			t value	
		x	y	z	RW	SD
L parietal (-27,-58,37)	AH > IH	-33	-44	-8	4.77***	1.52
	AH > OH	-35	-41	-4	3.34***	1.31
L inferior frontal gyrus (-36,11,4)	AH > IH	-27	-48	-8	2.67*	1.05
	AH > OH	-24	-46	-6	3.31**	0.48

Seeds for this analysis were in left parietal and left inferior frontal regions. *t* values denote the significance of the PPI term determined separately for each state

* *p* < .05   ** *p* < .01   *** *p* < .001

Discussion

Three key findings were of interest in the present study. First, we found that sleep deprivation attenuated connectivity between the IPS and the PPA when selective attention for houses was engaged, replicating our previous report (Chee et al., 2010). Secondly, SD eliminated the biasing effect of attention on PPA activation. Finally, the reduction in fronto-parietal and PPA activation in the sleep deprived state supports the notion that performance decline in the selective attention task may be caused by both specific deficits in selective attention as well as non-specific changes in sustained attention as reported in previous imaging studies (Portas et al., 1998; Chee et al., 2010)

Although inter-individual differences in vulnerability to sleep deprivation (Frey et al., 2004; Van Dongen et al., 2004) can partially explain the differences in behavioral performance reported in various studies, another factor to consider is the extent to which

the cognitive function of interest is actually affected by SD. Speed and accuracy of performance are almost always modulated by several subcomponents within a given cognitive task (Miyake et al., 2000). For example, when evaluating visual search in the setting of sleep deprivation, it was found that search speed did not decrease with increasing search set size (Horowitz et al., 2003). Instead, SD-related response slowing was uniform across search set size suggesting that a non-search-related factor was responsible for performance decline. Along similar lines, an experiment intended to study visual short term memory revealed imaging changes that implied a deficit in attention and/or visual processing rather than in memory capacity (Chee and Chuah, 2007). Finally, a meta-analysis of behavioral changes induced by sleep deprivation indicated that the effect sizes associated with decrements in non-specific processes such as vigilance or sustained attention are relatively large (Philibert, 2005) when compared to other more complex tasks.

Although imaging studies can shed light on functional neuroanatomy, studies that focus their analysis on top-down control regions, which include prefrontal and parietal areas, typically do not decompose total activation into the relative contributions of component cognitive processes (Slagter et al., 2007). However, by assaying activation in spatially differentiated regions in the ventral visual pathway that are the targets of object-selective attention (O'Craven et al., 1999; Gazzaley et al., 2005; Yi and Chun, 2005), we were able to determine how object-selective attention was affected by sleep deprivation.

Sleep deprivation reduces connectivity between the parietal/frontal and ventral visual areas

In a related study (Chee et al., 2010), it was suggested that functional connectivity might be a useful technique to detect deficits in object-selective attention. The current results use an event-related design to provide converging evidence for this claim.

In order to reveal a state-related change in PPI, MR signal in the 'target' area has to show consistent trial-by-trial differences in co-variation of signal with that of the seed region involving both task and non-task related aspects of the signal. This represents a different aspect of how attention might modulate BOLD signal (as opposed to the more intuitive demonstration of selectivity in PPA activation as a function of attention).

Sleep deprivation affects attention-biased changes in PPA activation in the absence of a stimulus cue

In contrast to the related study (Chee et al., 2010), subjects in the current experiment were unable to predict whether they would encounter a house or a face picture. We posit that this may explain why SD interacted with attention to modulate PPA activation in the present work.

The presence of a valid cue significantly reduces response time in experiments evaluating spatial attention (Posner, 1980). In sleep-deprived persons, availability of a neutral or valid cue has been shown to afford preserved performance whereas invalid cues result in delayed responses. It has been postulated that the alerting (warning) effect of a cue, as opposed to re-orienting, is relatively preserved in sleep-deprived persons (Versace et al., 2006).

Orienting recruits the parietal lobe (Fan et al., 2005) and patients with parietal lobe lesions show deficits in performance during invalid and uncued trials (Posner et al., 1987). Coincidentally, reduced task-related activation of the dorsal parietal region is a frequent finding in sleep-deprived persons (Chee and Chuah, 2007; Lim et al., 2007; Chee et al., 2008; Chuah and Chee, 2008; Tomasi et al., 2009). In contrast, alerting recruits the thalamus (Fan et al., 2005) whose activation is often relatively preserved in multiple experiments evaluating attention following SD (Portas et al., 1998; Tomasi et al., 2009; Chee et al., 2010).

The availability of a valid cue may benefit behavior (Jennings et al., 2003; Versace et al., 2006). When a cue is not available, as in the case of the present experiment, selective attention may deteriorate during SD, accompanied by a corresponding failure in the modulation of PPA activation. We acknowledge that the framework we have appealed to was originally used to explain behavior in the context of spatial attention (Posner, 1980). However, the parsimony of the present and prior findings indicates that the framework may also apply to object-based attention.

Changes across state in task-related activation

In addition to the changes in PPI and in PPA activity modulation, sleep deprivation also resulted in significant reductions in activation across conditions in inferior frontal regions, IPS and ventral visual cortex. These state-related changes in activation are consistent with prior studies from our laboratory on visual short-term memory (Chee and Chuah, 2007; Chuah and Chee, 2008), working memory (Lim et al., 2007) and lapses of attention (Chee et al., 2008). These changes in activation are thought

to relate to a loss of sustained attention or a general visual processing resource that cuts across multiple tasks.

We posit that in experiments where sustained attention is a major contributor to the behavioral effect, state-related changes in activation will correlate with the corresponding change in behavior (Lim et al., 2007; Chuah and Chee, 2008). On the other hand, activation-behavior correlations may not be found for tasks in which both sustained and selective attention contribute variance to the final outcome, as in the case of our two selective attention studies (Chee et al., 2010).

Conclusion

Using a novel imaging paradigm and an analysis strategy that focused on the ventral visual cortex, we were able to dissociate the brain activation changes that reflect how sleep deprivation influences selective attention from task-independent changes in brain activation that involve cognitive control and higher visual areas. For selective attention tasks, reductions in connectivity between cognitive control regions and relevant visual areas appear to be a consistent feature of neural activity following SD. Finally, the absence of a cue in the present paradigm could explain the loss of the biasing effect of attention on PPA activation in sleep-deprived persons.
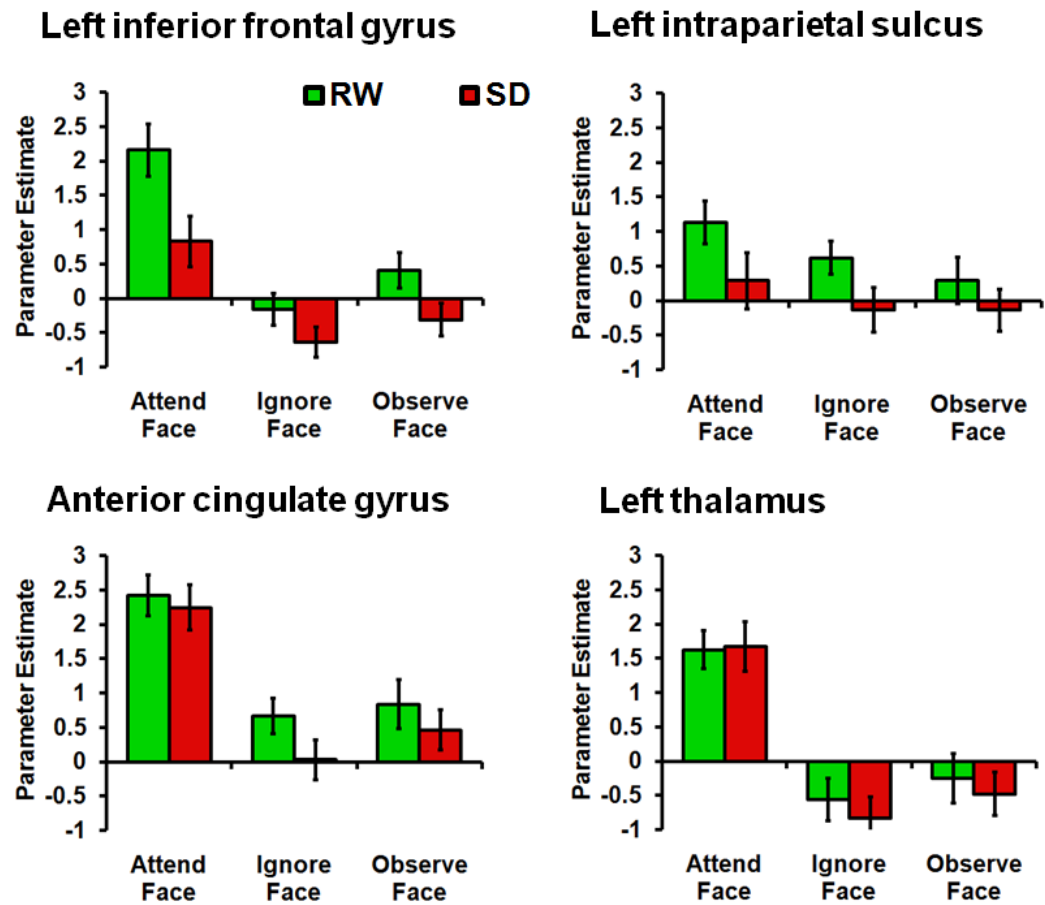
Figure 4.S1 Parameter estimates of activation for faces in areas associated with arousal and attention Parameter estimates for each condition and state in the left inferior frontal gyrus (IFG), left intraparietal sulcus (IPS), left thalamus and anterior cingulate cortex (ACC) for the three conditions attend to face, ignore face, and observe face. Significant state-related differences were observed in the left IFG and IPS, but not in ACC or the thalamus, mirroring the results for the house conditions in Fig. 4
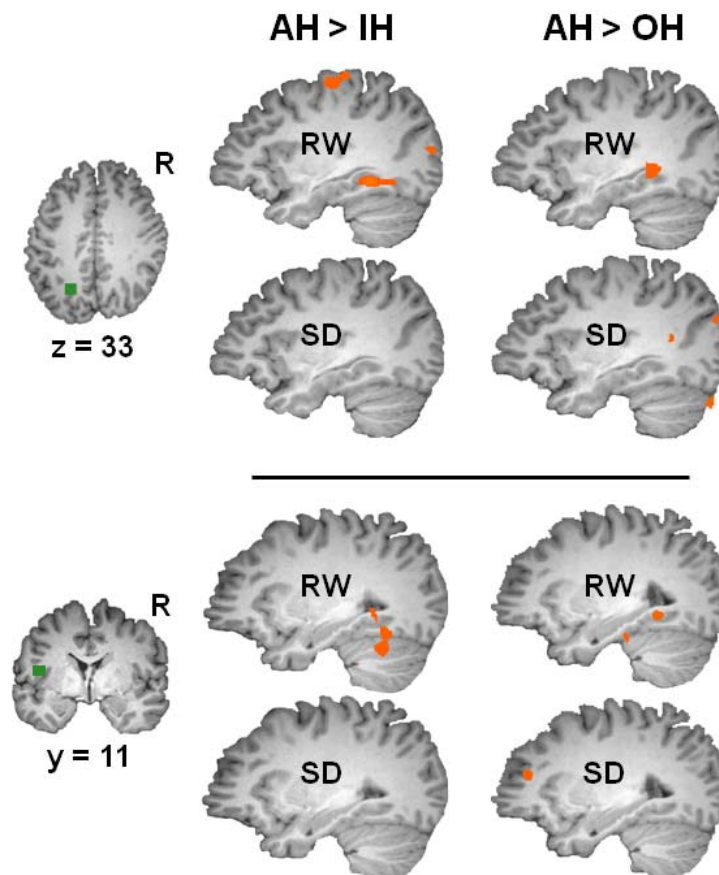
Figure 4.S2 Psychophysiological interaction related to the specific PPI contrasts and state
Connectivity analysis was performed using seeds in the left IPS (top panel; Talairach co-ordinates: -27, -58, 37) and left inferior frontal regions (bottom panel: Talairach co-ordinates: -36, 11, 4). Each map represents regions showing significant PPI in the AH vs. IH and AH vs. OH conditions (threshold $p < .05$) and in each state (RW, SD).

Chapter 5

Synthesis and general discussion

The central motif among the three studies presented in this thesis was the exploration of the workings of the attentional system under conditions when it is taxed beyond its normal limits. We were motivated to conduct these studies through the observation that humans frequently push themselves beyond these limits, and that adverse real-world consequences have been found to ensue. Our goal, therefore, was to investigate several of the neural mechanisms that underlie such cognitive failures, as well as try to better understand the nature of individual differences in vulnerability to sleep loss and fatigue. The work presented here allows us to draw several broad conclusions.

Neuroimaging data are useful endophenotypic markers of vulnerability to fatigue

Previous fMRI studies have yielded data suggesting that behavioral changes following SD correlate with activation decreases in task-related brain regions (Chee and Choo, 2004; Lim et al., 2007), and that neural activity in the rested state may be predictive of how vulnerable an individual is to SD (Caldwell et al., 2005; Mu et al., 2005). These studies had several limitations that indicated the need for additional work. First, several of these datasets had relatively small sample sizes. Second, all studies had subjects engage in a task during the period of scanning. As discussed in Chapter 2, there may be large differences between brain activation during times of cognitive engagement and tonic resting activity.

By using arterial spin labeled fMRI in studies #1 and #2, we were able to obtain absolute measures of cerebral blood flow in the brain under conditions of both rest and cognitive engagement. In both of these paradigms, resting brain activity in prefrontal areas predicted the subsequent level of decline in behavioral performance[2]. Given the robustness of this finding, I propose that *baseline levels of cerebral blood flow in right middle frontal gyrus/dorsolateral prefrontal cortex reflect a fundamental individual difference trait that marks the attentional "capacity" of the brain*. This hypothesis relates to several theoretical models of cognition and attention that have already been proposed. Warm et al. (2008) have reviewed the literature of vigilance in the context of neuroergonomics and conclude that vigilant attention is hard mental "work" that puts a drain on finite cognitive resources. The findings also relate to Yaakov Stern's theory of cognitive reserve (2009), which attempts to explain why equivalent brain pathology between individuals can lead to widely disparate changes in behavior and functioning. Per cognitive reserve theory, there are individual differences in the level of spare "resources" in the human brain, of which the variations in attentional capacity we have found may be an example.

The presence of a measurable marker of attentional capacity raises a number of ethical issues. Assuming we can measure this trait with a high enough level of fidelity, is it permissible or even desirable to select candidates for positions based in part on the neural signature of their ability to maintain high levels of attention? What privacy concerns may arise if such practices become commonplace? For that matter, what are the ethics of enhancing this trait, through (for example) deep brain stimulation or, in the

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[2] These findings were not reported for study #1; see the "Future directions" section below

future, gene therapy? These issues have been taken up by neuroethicists (for reviews, see

Illes et al. (2003) and Farah (2005)), and remain a pressing concern as our ability to

measure the correlates of cognition and personality in the human brain continues to

improve.

Hypoactivation related to sleep loss across a variety of cognitive tasks has the strongest relationships to declines in sustained attention

In a reproducibility experiment using a Sternberg-like working memory task, Lim

et al. (2007) noted that the most robust inter-session correlations between two nights of

sleep deprivation were in response variability and its relationship to activity in the

intraparietal sulcus. These data suggested that even when not performing a task explicitly

testing sustained attention, this cognitive module accounts for much of the reliable

variance over time.

Sustained attention is often thought of as a more fundamental aspect of attention,

and a prerequisite for the proper functioning of higher cognition (Sturm and Willmes,

2001; Lim and Dinges, 2008). In this hierarchical formulation, specific aspects of

attention (i.e. executive or selective attention) cannot maintain their full integrity when

sustained attention is unstable or compromised. Since the sustained attention system is

greatly destabilized following sleep loss (Doran et al., 2001), the implication is that

neural and behavioral changes to *any task* in the sleep deprived compared to the resting

state will reflect some admixture of general and (if any) task specific changes. Given the

magnitude of vigilance changes compared to those of other tasks (Lim and Dinges, in

press), we might expect that a large portion of the variance in neurophysiological change may correspondingly be associated with worsening sustained attention.

The three studies that comprise this thesis lend support to this point. While correlations were found between behavior and brain activation in studies 1 and 2, no such relationships were found in study 3, despite the overall meaningfulness of the findings. This suggests that selective attention declines may have been contingent upon more general tonic declines in vigilance. Moreover, in study 3, we found large effects in the fronto-parietal attentional circuit that were task-independent, thus putatively associated with general attentional declines.

In the introduction, I discussed several theories of sleep deprivation and cognition, and indicated that the vigilance hypothesis was most relevant when considering real world impact, because the effect sizes associated with vigilance declines are large and are seen early in periods of SD. These data not only provide robust support for this hypothesis, but also suggest that reversing the sustained attention deficits may ameliorate the deficits in many other cognitive domains. This hypothesis may be tested by conducting experiments with a variety of neuropsychological tests, and by conducting principal components or factor analyses on cognitive performance after administration of a countermeasure to sleepiness.

Both task-related activity and resting activity are important in understanding the sleep-deprived brain

A critical finding from study #1 was that resting brain activity and task-related activity exhibit different patterns of change after one night of total sleep loss. In general, relative resting CBF increases in the cortex following SD, whereas relative CBF during

task performance decreases. These findings may help resolve the apparent contradiction in the literature between the synaptic homeostasis theory (Tononi and Cirelli, 2003), and the consistent observations of task-related hypoactivation in experiments using PET and fMRI (Chee and Chua, 2008). I have discussed this point more extensively in study #1

Most fMRI studies over the past 2 decades have by necessity considered across- or within-session resting periods as inseparable "baseline". The findings of studies #1 and #2 suggest that meaningful information may be gleaned from these periods of rest. For a start, resting activity is not the same in the pre-task and post-task period, which suggests that averaging these periods (as is done in many BOLD fMRI studies) may in fact be adding error variance to the model. Moreover, individual differences in baseline levels of CBF may artificially dampen or amplify the effects from each subject, again adding noise to group activation data. Further work is necessary to determine whether corrections should be made to account for these baseline variations, and if so, what the appropriate methods might be.

Limitations

The three studies reported contained a small number of limitations that may be addressed by future work. I highlight the most important among these here.

First, we used neither EEG nor eye-tracking in studies #1 and #2 (conducted in the Hospital of the University of Pennsylvania) when subjects were undergoing their resting scans. Thus, we had no way of confirming that subjects were awake and keeping their eyes open during this time. This was less of a concern when subjects were not sleep-deprived; during the SD scans, we increased our emphasis on the need to stay awake and

alert, and ensured that subjects were at least awake and alert at the conclusion of the scan. In future work, however, we recommend that online eye-tracking be conducted (as in study #3) so that subjects can be alerted in the case of extended periods of eye closures.

Our findings in studies #1 and #3 were not designed to assess the relative contributions of circadian and homeostatic effects (Borbely, 1982). One could imagine a more ambitious version of study #1, in which fMRI scans are collected at multiple time points during the day in order to obtain estimates of variance associated with each of these two processes. Indeed, preliminary findings from imaging studies conducted in the evening (i.e. when circadian compensation is acting maximally to counteract homeostatic sleep drive) after 36 hours of total SD suggest that task-related activity is fairly similar to that obtained 12 hours earlier (Chee et al., 2006). The changes observed in studies #1 and #3 reflect a combination and interaction of circadian and homeostatic influence (with homeostatic influence presumably predominating, since scans were conducted at approximately the same circadian phase). However, it is a limitation in these studies that we cannot make a more precise claim about the contributions of each of these effects.

Finally, ASL is hampered by having relatively low temporal resolution, which makes it difficult to study event-related activity (i.e. phasic changes at the onset and response to targets) using this technique. We collected concurrent BOLD contrast data in studies #1 and #2 as subjects performed the PVT; however, analyses of these data (not reported) did not yield meaningful results. In the future, it is likely that improvements in scanning sequences may allow for ASL scans with a shorter repetition time (TR), or that can be optimized for higher signal-to-noise rations in concurrently collected BOLD. Studying both tonic and phasic information in tandem will allow for richer datasets that

are more informative about moment-to-moment fluctuations in attention, and their correlation with neural signals.

Future directions

A primary aim of the data set collected in study #1 was to investigate whether inter-individual differences in brain activation at rest could predict vulnerability to sleep deprivation. This analysis is not reported in this dissertation, and is currently being prepared for publication. In summary, resting levels of activity in the thalamus and prefrontal cortex correlate with a phenotypic measure of vulnerability to sleep deprivation obtained by aggregating performance on a sustained attention test over a night without sleep. Follow-up analyses on these data should detail the association of this vulnerability with slow-wave energy in subsequent recovery sleep, as well as provide more fine-grained detail on the brain regions that have the greatest predictive power if applied to the phenotyping process.

Future work in this area should also involve a continued search for the genetic polymorphisms that moderate the individual differences we have observed. As discussed in the introduction, promising candidate genes include PER3, COMT and adenosine receptor alleles, among others (Viola et al., 2006, Goel et al., 2009). It has already been demonstrated that individuals with different versions of the PER3 allele demonstrate major difference in cortical activation patterns when performing the PVT while sleep deprived (Vandewalle et al., 2009). Follow-up work on these initial findings should employ larger sample sizes, and if possible prescreen subjects for less frequent genetic

polymorphisms so as to maximize study power for the detection of group level differences.

The findings in study #2 should motivate the continued exploration of the effects of mental fatigue on the human brain. Establishing the reliability and validity of this finding is important. Furthermore, several open questions remain. It is not known what the time course of recovery is from these fatiguing effects; that is to say, the time it takes after a period of heavy cognitive work before CBF patterns return to their baseline levels. Also of interest are the dose-response effects of cognitive workload on neural activity, and what the best mathematical fit to these data are. Finally, it is worth investigating whether known or novel countermeasures have any effect on the CBF patterns we have observed, and if so whether these changes may mediate their beneficial effects.

Conclusion

As the pace of development quickens worldwide, sleep deprivation and fatigue will continue to be salient and pressing concerns for health and safety in the workplace and other arenas. It is therefore imperative that we devote sufficient resources to understanding the behavioral and physiological consequences of working under these challenging conditions. The findings in this thesis represent a small piece of this large and important puzzle by strengthening the hypothesis that sleep deprivation exerts its effects primarily on sustained attention. I have also built the case that baseline differences in cerebral blood flow are important in understanding the amount of attentional reserve a human brain possesses at a given moment in time. I hope that these novel results spur further research and progress in the field, and eventually see applications in the design of

countermeasures and other devices aimed at reducing the risk and harm associated with

sleep loss.

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