Distal Cooling and Sustained Attention

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ABSTRACT

Introduction: Vigilance is directly related to core and skin temperatures (T_C and T_{sk}). Biological day reflects a high T_C and alertness; night reflects *vice versa*. At rest, T_C is regulated largely by controlling blood flow (and hence T_{sk}) in extremities; their vasodilation strongly predicts reduced vigilance (Raymann et al., 2007b) and faster sleep onset (Kräuchi et al., 1999). In narcolepsy, high daytime extremity temperatures and a smaller distal-to-proximal gradient (DPG) indicates higher sleep propensity (Fronczek et al., 2006b). Cool extremities have been linked observationally to delayed sleep onset in the elderly, and experimentally shown to reduce sleep propensity in narcolepsy (Fronczek et al., 2008). Therefore, the aim of this study was to test the hypothesis that cooling the feet would maintain vigilance during extended wakefulness in healthy adults.

Methods: A randomised cross-over experiment was completed using nine healthy young adult participants with normal sleep patterns. After providing informed consent, and a daytime familiarisation, they undertook three 4-h laboratory sessions in which water-perfused booties were used to provide Mild cooling, Moderate cooling or no cooling (Control). Sessions were in a dimly-lit room, beginning at 2230. Each 30 min consisted of quiet rest interspersed with a 10-min psychomotor vigilance task (PVT), 7-min Karolinska Drowsiness Test (KDT), and ratings of sleepiness, perceived body temperature and thermal discomfort. EEG spectral powers (theta, alpha and beta) were determined within the PVT and KDT. Analyses were by repeated measures ANOVA (α=0.05) with post-hoc contrasts.

Results: Foot temperatures in Control and Mild and Moderate cooling averaged 34.5 ±0.5, 30.8 ±0.2 and 26.4 ±0.1 ^{o}C (all P<0.01). Yet, the upper-limb DPG remained stable (at ~0.3 ^{o}C) regardless of condition (P=0.57). The decline in T_C (~0.35 ^{o}C) was also unaffected by condition (P=0.84), as was vigilance (interaction for response speed: P=0.45). A small and transient reduction in sleepiness was evident with cooling (P=0.046); otherwise sleepiness and vigilance

deteriorated in conjunction with the fall in T_C in each condition (r>0.80). Participants felt cooler throughout both cooling trials, but thermal comfort was unaffected (P=0.43), as were almost all EEG parameters during the KDT. All dependent measures were affected by time.

Discussion and Conclusion: In healthy, young adults, core temperature and vigilance decline during the period of normal sleep onset and early sleep regardless of mild or moderate cooling of the feet, and any effect on sleepiness is small and transient.

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CONTENTS

ABSTRACT..... **i**

ACKNOLEDGEMENTS..... **iii**

ABREVIATIONS..... **viii**

LIST OF TABLES..... **x**

LIST OF FIGURES..... **xi**

1.0 INTRODUCTION..... **1**

2.0 LITERATURE REVIEW..... **5**

2.1 Driving..... 6

2.2 Thermoregulation..... 8

2.2.1 Homeostasis..... 8

2.2.2 Sensation..... 13

2.2.3 Vasomotion..... 20

2.2.4 Arteriovenous Anastomoses..... 25

2.2.5 Summary..... 26

2.3 Circadian Rhythms..... 29

2.3.1 Sleep permissive/ wake promoting factors..... 31

2.3.2 Homeostatic hourglass – Process S..... 33

2.3.3 Central circadian clock – Process C..... 35

2.3.4 Interaction of Process S and Process C..... 36

2.3.5 Circadian temperature rhythm..... 40

2.3.6 Narcolepsy..... 43

2.4 Vigilance..... 44

2.4.1 Psychomotor vigilance..... 45

2.4.2 Validity of the psychomotor vigilance task..... 47

2.5 Sleep onset..... 49

2.6 Electroencephalography and electro-oculogram..... 51

2.6.1 Alpha and theta rhythms..... 53

2.6.2 Age related alpha frequency changes..... 55

2.6.3 Beta rhythm..... 56

2.6.4 10/20 electrode positioning..... 58

2.6.5 The electro-oculogram (EOG)..... 59

2.7 Conclusion..... 60

3.0 METHOD..... 62

3.1 Experimental Protocol..... 64

3.2 Participants..... 66

3.4 Apparatus and Measures..... 67

3.4.1 Psychomotor vigilance task..... 67

3.4.2 Electrophysiological measures (EEG, EOG, ECG)..... 67

3.4.3 Sleep onset staging..... 69

3.4.4 Temperature..... 69

3.4.5 Actigraphy and sleep diary..... 71

3.4.6 Subjective questionnaires..... 71

3.4.7 Hydration status..... 72

3.5 Data Analysis..... 73

3.5.1 Data reduction..... 73

3.5.2 Statistical analyses..... 74

4.0 RESULTS..... 76

4.1 Participant characteristics and compliance..... 76

4.1.1 Screening..... 76

4.1.2 Adherence..... 76

4.1.3 Sleep diaries – sleep onset..... 77

4.1.4 Mood state characteristics and hydration..... 78

4.2 Induced temperatures..... 80
4.2.1 Temperature manipulation..... 81
4.2.2 Foot temperatures..... 81
4.2.3 Upper limb..... 82
4.2.4 Core temperature..... 82
4.2.5 Temperature gradients..... 85
4.2.6 Temperature perception..... 87
4.3 Subjective sleepiness..... 89
4.4 Vigilance..... 91
4.4.1 Reciprocal reaction time..... 91
4.4.2 Variability..... 92
4.4.3 Lapses..... 92
4.4.4 Core temperature and vigilance..... 94
4.4.5 Core temperature and Sleepiness..... 94
4.5 Cognitive arousal..... 96
4.5.1 Karolinska Drowsiness Test..... 96
4.5.2 C4-A1..... 97
5.4.3 O2-A1..... 98
4.6 Heart Rate..... 101
5.0 DISCUSSION..... 103
5.1 Temperature and vigilance..... 104
5.1.1 Temperature effects..... 104
5.1.2 Vigilance and arousal..... 106
5.2 Transient Effects..... 110
5.3 Limitations..... 111
5.3.1 Order effect 111
5.3.2 Laboratory/Equipment set up..... 112

5.3.3 Statistical power…… 112

5.3.4 Electroencephalography…… 113

5.3.5 Temperature…… 114

5.3.6 Vigilance…… 115

5.4 Variable Temperatures across thermistor locations on the feet…… 115

5.5 Practical Application…… 116

5.6 Conclusions and Recommendations for future research…… 117

7.0 REFERENCES…… 120

8.0 APPENDICES…… 133

Appendix A: Participant Information and consent form…… 133

Appendix B: Activity monitoring and sleep diary…… 137

Appendix C: Depiction of participant set up…… 141

Appendix D: Criteria for analysis of the 10 min PVT…… 142

Appendix E: Outline for drowsiness scoring…… 143

Appendix F: Pilot temperatures …… 144

Appendix G: Water perfused booties…… 146

Appendix H: Karolinska Sleepiness Scale…… 147

Appendix I: Thermal Sensation and Thermal Discomfort…… 148

Appendix J: The Brunel mood state questionnaire…… 149

Appendix K: Additional Data…… 150

Appendix L: Participant eight…… 151

ABREVIATIONS

AAS	Ascending Arousal System
ANOVA	Analysis of Variance
AVA	Arteriovenous Anastomoses
BRUMS	Brunel Mood State Questionnaire
CSN	Cold Sensitive Neuron
DPG	Distal Proximal Gradient
ECG	Electrocardiogram
EEG	Electroencephalogram (Scalp abbreviations in Figure 3.3)
EOG	Electro-oculogram
ISI	Inter-stimulus Interval
KDT	Karolinska Drowsiness Test
KSS	Karolinska Sleepiness Scale
LH	Lateral Hypothalamus
N1	Sleep Stage 1
N2	Sleep Stage 2
N3	Sleep Stage 3/4
NREM	Non Rapid Eye Movement Sleep
PCG	Proximal Core Gradient
POAH	Preoptic Anterior Hypothalamus
PSG	Polysomnography
PVT	Psychomotor Vigilance Task
REM/R	Rapid Eye Movement Sleep
RRT	Reciprocal Reaction Time [RRT=1000/RT]
RT	Reaction Time
SCN	Suprachiasmatic Nucleus

SD	standard deviation
SEM	Slow Eye Movement
SWS	Slow Wave Sleep
T_{amb}	Ambient Temperature (dry bulb temperature)
T_C	Core Body Temperature
T_{Foot}	Foot temperature
$T_{Forehead}$	Forehead temperature
T_{Sk}	Skin temperature
TNZ	Thermoneutral Zone
TRP Channel	Transient Receptor Potential Channel
TRPA	Transient Receptor Potential Ankyrin
TRPM	Transient Receptor Potential Melastatin
TRPV	Transient Receptor Potential Vanilloid
T_{sk}	Skin Temperature
USG	Urine Specific Gravity
VLPO	Ventrolateral Preoptic Hypothalamus
WEM	Waking Eye Movement
WSN	Warm Sensitive Neuron

LIST OF TABLES

Page

3.1: *Participant details.*…… 66

4.1: *Sleep hours: sleep onset, waking, total sleep time.* …….. 79

4.2: *Pre and post subjective sleepiness and hydration.* ……..79

4.3: *Ambient and water temperatures.* …….. 81

Appendix D: Table 1: *Criteria for the analysis of 10-min PVT*…….. 142

Appendix E: Table 1: *Outline for significant events in progressing drowsiness*…… 143

Appendix F: Table 1: *Trial cooling protocol for application to Mild and Moderate conditions in main study.* …… 144

LIST OF FIGURES

Page

2.1: Schematic representation of thermoTRP channel activation……..16

2.2: Correlation between skin temperature and resting blood flow velocity…… 26

2.3: Time course of Processes S and C after regular and extended waking periods…….. 29

2.4: Diagrammatic representation of normally entrained endogenous rhythms of core body temperature, melatonin and sleep propensity.…… 40

3.1: Protocol overview…… 62

3.2: Study overview…… 62

3.3: Manipulated testing cycle.…….. 65

3.4: International 10/20 system, electrode positions…….. 68

4.1: average foot, upper limb and core temperature across conditions……83

4.2: Localised foot temperature parameters comprising average foot temperature across conditions……..84

4.3: Time course of individual foot thermistors across conditions……..84

4.4: Upper and lower limb distal proximal gradients, and PCG.……86

4.5: Differences in upper limb gradients between conditions…… 86

4.6: Thermal sensation and thermal comfort…… 88

4.7: Progression of subjective sleepiness. ……..90

4.8: Ceiling effect of Karolinska sleepiness scale. …….. 90

4.9: Change in subjective sleepiness pre and post PVT.…….. 91

4.10: 1 min averages of reciprocal reaction time, and variance across PVTs……93

4.11: Average PVT lapses across conditions. ……95

Page

4.12: Correlation of change in core body temperature and change in RRT……..95

4.13: Correlation of change in core body temperature and subjective sleepiness…….. 96

4.14: Theta, alpha and beta EEG frequencies from C4-A1 derivation across KDTs eyes open and eyes closed. …… 99

4.15: Theta and alpha EEG frequencies from O2-A1 derivation across KDTs eyes open and eyes closed. ……..100

4.16: Change in theta and alpha EEG frequencies between eyes open and eyes closed within each KDT…….. 101

4.17: Change in heart rate across each condition…….102

4.18: Heart rate pre post PVT…….. 102

Appendix C: *Figure 1:* Depiction of participant set up……..141

Appendix F: *Figure 1:* Original study, foot temperatures across warming and cooling conditions. ……..145

Appendix F: *Figure 2:* Collated EEG for peak alpha and beta frequencies pre and post cooling and warming protocols. …….. 145

Appendix G: *Figure 1:* View of internal tubing lining the custom fitted booties…….. 146

Appendix G: *Figure 2:* Custom fitted booties worn. …….. 146

Appendix K: *Figure 1:* Brunel Mood state characteristic of vigour across conditions…….. 150

Appendix K: *Figure 2:* Brunel Mood state characteristic of fatigue across conditions.…….. 150

Appendix L: *Figure 1:* Participant eight core temperature recordings across conditions with comparison to group conditions. …….. 151

Appendix L: *Figure 2:* Participant eights DPG across conditions, compared to the group trend. …….. 151

1.0 INTRODUCTION

Sleep deprivation and its associated mental fatigue may be increasingly prevalent in modern society (Alvarez et al., 2004; Gradisar et al., 2011; Halson, 2014; Olds et al., 2010; Zealand, 2001). A more mechanised industry and more artificial lighting has allowed for 24-hour operation, expanding both work and play hours far into the night (Wright et al., 2013). Fatigue-related incidents are especially concerning in situations of high risk such as shift work or driving (Jones et al., 2010; Lal et al., 2001; Reyner et al., 2012), as the underlying state of sleepiness can lead to serious decrements in performance capabilities (Lal et al., 2002; Loh et al., 2004). Indeed, a high incidence of injury appears to surround tasks of prolonged monotonous nature and operating hours outside of the "biological day" (Jones et al., 2010; Loh et al., 2004). Drowsy driving accounts for ~14% of the New Zealand's fatal road traffic accidents (Transport, 2011b), with shift work-related accidents presumably contributing a similar percentage, especially in sustained operations and during night shifts (Loh et al., 2004). Interventions for fatigue-related incidents, especially the road death toll, appear limited and potentially ineffective with regard to adherence, indicating a greater focus on wakefulness or vigilance promoting methods merits further investigation and insight (Horne et al., 1996; Jones et al., 2010).

Vigilance is characterised by cognitive arousal and attention, with (sustained) attention encompassing the temporal and directional (focal) components (Lal et al., 2001). Vigilance is commonly defined by its antonyms; drowsiness, fatigue, and sleepiness, which are used synonymously to mean the neurobiological process regulating circadian rhythms and the drive to sleep. Mental fatigue is a cumulative process, which first affects the top-down processes (Lorist, 2008), resulting in greater inconsistency during tasks, an inability to bias relevant from irrelevant information, and a general slowing of reaction times (Boksem et al., 2005; Dorrian et al., 2005; Lim et al., 2008; van Dongen et al., 2000).

The waking arousal state is tonically affected by the interaction of both the homeostatic hourglass and the circadian clock – processes S and C, respectively. Process S is the homeostatic

drive to sleep, believed to be influenced by some structure or substance (probably adenosine), which increases sleep propensity as waking time increases. Process C is the circadian influence on sleep and wakefulness controlled by the suprachiasmatic nucleus (SCN) located in the anterior hypothalamus (Jin et al., 1999; Reppert et al., 2002). The SCN is regulated by light input from the retina during the day, and melatonin secretion from the pineal gland during the dark cycle (Cassone et al., 1986). The process S pressure to sleep rises exponentially across the waking day (Moore, 2007; Porkka-Heiskanen et al., 2011; Romeijn et al., 2012a), whereas the process C drive for waking peaks during the biological day and troughs during biological night, enforcing a ~24-hour rhythm entrained to the light-dark cycle (Johnson et al., 1988). In healthy individuals, the additive effects of Processes S and C are evident in the evening, with greater slowing and inconsistency in reaction time, indicative of vigilance decay (Dorrian et al., 2005; Lim et al., 2008).

Waking from sleep is promoted by a group of cells originating in the brainstem that activate the thalamus and cerebral cortex via a network of cell groups known as the ascending arousal system (AAS). Wakefulness is reinforced by the neuropeptide orexin (also known as hypocretin), produced exclusively by a cluster of neurons in the posterior lateral hypothalamus. In patients suffering from narcolepsy (described in more detail in section 2.3.6), the waking state is destabilised due to loss of orexin, thereby allowing their rapid transition between waking and sleeping states (Fronczek et al., 2006b; Overeem et al., 2012).

Process C is linked to cognitive arousal via the AAS, but is also one of the non-thermal factors that modulate core body temperature (T_C (Sciences, 2001)). The circadian oscillation of T_C is driven by the SCN and controlled via systems that alter heat loss and heat gain, with T_C reaching maximum during the day and minimum during the night. The changes in T_C respond to both thermal and non-thermal cues. The extremities also play a large role in modulating T_C through their anatomical specialisations for heat loss; however, it is increasingly evident that the distal skin temperature may independently modulate vigilance and sleepiness (see review by Van Someren, 2006). This modulatory role of the distal skin temperature is further supported

practically by the use of a bedding microclimate to maintain high distal temperatures (~34-35 ^{0}C) when initiating sleep (Van Someren, 2006).

Vigilance is multifactorial, and is often broken into its constituent components, cognitive arousal and sustained attention, which are observable using electroencephalography (EEG) and psychomotor vigilance tasks (PVT), respectively. The EEG is often purported to be the gold standard of tracking vigilance decay, however, the PVT has alternately been described as the 'archetype neurocognitive assay of attention after sleep loss' ((Lim et al., 2008), p. 306). Thus, EEG and PVT each offer important insights into different aspects of vigilance. EEG provides direct recordings of cognitive arousal but is limited by poor signal-to-noise ratios and a lack of anatomical and physiological anchors to particular frequencies (e.g., alpha frequency (Pizzagalli, 2007)) (Callaway, 1966). PVT provides insight into sustained attention and thus functional effects of mental fatigue and drowsiness (Basner et al., 2011; Lim et al., 2008). Sleep deprivation further accentuates fatigue-related changes in PVT and EEG as homeostatic sleep pressure is raised (Lim et al., 2008; Santamaria et al., 1987), and as such, both techniques become more sensitive measures of vigilance and its decay.

Interventions conducted on vigilance have typically involved either attenuating or facilitating its decline. Such interventions have included postural change (Caldwell et al., 2003; Cole, 1989), stimulants such as caffeine and amphetamines (Lim et al., 2008), meal size and satiety (Reyner et al., 2012), and more recently subtle thermal modulation (Fronczek et al., 2008; Kräuchi et al., 1999; Raymann et al., 2007b; Van Someren, 2006). Indeed, some arousal altering drugs may work by way of their effects on the thermoregulatory system (e.g., Atenolol (Van Den Heuvel et al., 1997). Several studies have focussed on facilitating sleep through distal warming, observing either time-on-task degradation, or appearance of sleep stages (Kräuchi et al., 1999; Kräuchi et al., 2008; Raymann et al., 2007a; Raymann et al., 2007b), as demonstrated using the PVT and EEG. Facilitating the evening decline of T_C has been shown to assist sleep onset (Kräuchi et al., 1999). Core cooling and sleepiness has also been demonstrated during the day (Fronczek et al.,

2008; Gilbert et al., 2000). As such, inhibiting the evening-related reduction in T_C response may cause the opposite effect, i.e., maintained vigilance. This possibility remains largely unexplored. (Fronczek et al., 2008)) addressed this very question in a narcoleptic individuals, who have distal temperatures associated with drowsiness (Fronczek et al., 2006b). Subtle ranges of temperature manipulation may offer the most promising findings (Fronczek et al., 2008; Raymann et al., 2005), as manipulations involving whole-body or excessive temperatures have been shown to impair psychomotor function (Enander, 1987), at least partly by their distraction effect (Cheung et al., 2007).

The aim of the current study was to examine the effects of cooling of the extremities – specifically subtle and moderate cooling of the feet – on sustained attention and sleepiness in healthy individuals. Changes in vigilance/arousal state were assessed using EEG and PVT. It was hypothesised that foot cooling would produce greater wakefulness (indicated from EEG) and attenuate the decline in vigilance (indicated from PVT), relative to the normal/control state. Change in the vigilance state was considered to be due to either or both of changes in distal skin temperature per se, or resultant changes in the rate of T_C decline.

2.0 LITERATURE REVIEW

Fatigue due to prolonged mental or physical work precipitates drowsiness and sleep onset. The terms fatigue, drowsiness and sleepiness are used synonymously to describe the reduction in vigilance state (Dinges, 1995). Fatigue, the antonym of vigilance, can be described as a disinclination to continue performing a task, or as an effort-to-reward imbalance, whereby motivation to perform declines with increasing duration and/or difficulty (Brown, 1994; Guyton, 1991; Tops et al., 2004). A myriad of factors influence vigilance and fatigability, many of which are beyond the scope the current review. Of particular interest are the internal regulatory processes S and C, and their associated arousal and thermoregulatory changes; i.e., vigilance and fatigability are tightly related to circadian (Process C) oscillation of core body temperature.

Core temperature approximates 37 ± 1^{0}C (Waterhouse et al., 2005), and is maintained largely by adjusting skin temperature (T_{sk}). In an evolutionary sense, thermoregulation is tied to vigilance to maintain homeostatic functioning when thermoneutrality is threatened (Romeijn et al., 2012a). Core temperature is perturbed by thermal (e.g., heat) and non-thermal (e.g., Process C) factors that change the interthreshold zone within which T_C normally resides. Process C alters core body temperature through adjustments in heat loss and heat gain systems (and hence T_{sk}) across the day. The level of thermal response to environmental temperature can induce changes in vigilance (Fronczek et al., 2008; Kräuchi et al., 2008).

The current study is focussed on maintaining vigilance through modulation (cooling) of foot temperature in the evening. Cooling has been demonstrated to be an effective strategy for vigilance and maintenance of wakefulness in Narcoleptics (Fronczek et al., 2006a; Fronczek et al., 2008). Similarly, findings from an observational study indicate that thermal discomfort from cold extremities relates to difficulty initiating sleep in healthy populations (Kräuchi et al., 2008). Equally, warming the extremities has been shown to decrease vigilance (Raymann et al., 2007b) and sleep onset latency (Kräuchi et al., 1999; Raymann et al., 2007a). Since subtle warming

potently promotes sleepiness responses, does cooling have a similar capacity for altering (increasing) the vigilance state?

2.1 Driving

In 2010, New Zealand had 337 fatal crashes, with 47 (14%) being causally related to fatigue (Transport, 2011a, 2011b). This proportion remained consistent in 2011, at 13% (Transport, 2012). Fatigue is the state of deteriorating vigilance or attention to a sustained task (Eoh et al., 2005), and/or being unable to maintain performance on a task (Dinges, 1995). These situations however, do not necessarily result in sleep. Fatigue has also been shown to raise reaction time (RT) (Caldwell et al., 2003; Dinges et al., 1985; Fronczek et al., 2008; Schier, 2000; Van Dongen et al., 2003) and increase the number of lapses (i.e. RT >500 ms) in psychomotor vigilance tasks (Fronczek et al., 2008; Van Dongen et al., 2003), along with decreasing information processing speed and memory capacity (Eoh et al., 2005; Klimesch, 1999). Reduced task performance occurring with fatigue has been researched thoroughly (Altena et al., 2008; Belyavin et al., 1987; Dinges et al., 1985; Eoh et al., 2005; Fronczek et al., 2008; Lal et al., 2002; Van Dongen et al., 2003). With negative implications of fatigue and drowsiness, it becomes prudent to further assess mechanisms involved and possible methods of attenuation, especially with regard to driving, where the results of raised reaction time and lower information processing have potentially devastating repercussions (Dinges, 1995).

Current regulations in New Zealand prohibit long-haul drivers from exceeding 13 hours of operation, and require 10 hours of rest between work periods (Jones et al., 2010; Transport, 2011a). No such regulation is required for commuters (Jones et al., 2010). Drivers, arguably have good insight into increasing drowsiness and are always aware of sleepiness prior to incidents (Filtness et al., 2012; Horne et al., 1996), yet young everyday commuters have the highest fatigue-related incidence of crashes (Filtness et al., 2012; Reyner et al., 2012; Transport, 2011a,

2011b). Technological advances have allowed the long-haul industry to implement sensors within many cabs to detect sleepiness and fatigue through short and long blinks (e.g., Driver Fatigue Monitor, Hao Nai Industrial Co, China). The current "gold standard" method of preventing fatigue for general commuters involves a power nap (Horne et al., 1996), however worldwide this is found to have minimal adherence (Jones et al., 2010), and may be of limited benefit (Gillberg et al., 1996). Research regarding fatigue prevention focuses on policy change and driving conditions, with few studies investigating the contribution of the driver *per se* (Jones et al., 2010). Currently, the most researched driver-oriented fatigue prevention method is caffeine, for its adenosine inhibiting, and sympathetic stimulating effects (Eoh et al., 2005; Horne et al., 1996; Porkka-Heiskanen et al., 2002; Reyner et al., 1998). However, anecdotal reports have long suggested that cooling of the skin (such as air conditioning) aids driving performance when drowsy.

Previously, cold convective airflow directed at the face has had minimal impact on fatigue prevention while driving (Reyner et al., 1998), however, the distal extremities appear to be more intimately related to heat loss pathways and sleepiness (discussed below), and therefore may offer an alternate method of fatigue prevention. Unfortunately, it is difficult to evaluate fatigue-prevention methods and their underlying mechanisms for use in motor vehicles. Driving simulators have thus been developed and validated as tools to test sustained attention for the laboratory setting (Gillberg et al., 1996). This too has limitations, despite its external validity, due to the difficulty of combining driving simulation with other equipment and recordings, such as the electroencephalogram (EEG). The current study reduced the sustained attention component of drowsy driving to a simple psychomotor vigilance task (Dinges et al., 1985) in conjunction with EEG, to assess the vigilance decrement, as a test of concept.

2.2 Thermoregulation

2.2.1 Homeostasis

Humans, like other mammals, are, homeotherms (Gagge et al., 1996; Refinetti et al., 1992; Romanovsky, 2007). That is, we self-regulate the internal thermal state though various metabolic processes (e.g., basal metabolism, dietary intake, exercise, shivering and non-shivering thermogenesis, and likely brown adipose tissue (Nagashima et al., 2000)) (Gagge et al., 1996; Lv et al., 2007). The T_C is ideally maintained within a tight range of around 37 ^{o}C, subject to oscillations from thermal and non-thermal factors (Mekjavic et al., 2006; Romanovsky, 2007). At the extremes, core body temperatures outside 35-41 ^{o}C cannot be tolerated for long (Taylor et al., 2008a), although, overheating is of greater immediate physiological concern than overcooling (Romanovsky, 2007). The small range of control necessitates multiple regulatory mechanisms to ensure thermal stability. Deviations of ~1 ^{o}C away from neutral T_C are enough to fully activate thermoeffectors (Mekjavic et al., 1989; Taylor et al., 2014). Maintenance of the core within such ranges occurs at the expense of the skin if necessary (Werner et al., 1980). Despite the skin's tight optimal range (30-33 ^{o}C) (Cline et al., 2004; Mekjavic et al., 1989), it can tolerate wide deviations to ~10 ^{o}C and ~50 ^{o}C without damage (See (Werner et al., 1980)). Such deviations in skin temperature involve thickening or thinning of a "shell" layer through fine adjustments of cutaneous and subcutaneous blood flows.

The thermoregulatory centre maintaining core body temperature lies within the preoptic anterior hypothalamus (POAH), and has been well established (Nagashima et al., 2000; Porkka-Heiskanen et al., 2002; Raymann et al., 2005; Raymann et al., 2007b; Romanovsky, 2007; Romeijn et al., 2012a). The POAH contains the largest central concentration of warm-sensitive neurons (WSN) and cold-sensitive neurons (CSN). WSNs are dominant within the POAH (Boulant, 1996), and are most responsive to changes in heating and cooling (Nagashima et al., 2000). Warm- and cold-sensitive neurons are reactive to changes in core and skin temperatures, facilitating many parallel thermoregulatory responses to deviations in skin and core body

temperature. The WSNs have an inhibitory action on CSNs, such that the activity associated with thermal change is actually driven by increases or decreases in WSN activity and their inhibitory action on CSNs (Further discussed in (Van Someren et al., 2002; Van Someren, 2004) and (Romanovsky, 2007)). WSN excitation, occurring with body heating, results in hierarchical recruitment of thermoeffector responses. Whereas, WSN inhibition results in CSN excitation and hierarchical activation of thermoeffector responses associated with cooling. The extent of thermoafferent signalling determines recruitment of thermoeffector response (Romanovsky, 2007; Stevens et al., 1979; Stevens et al., 1974). Such signalling shows graded input from any thermosensitive structure, and spatial summation of thermoafferent feedback for cold and somewhat for warmth (Romanovsky, 2007; Stevens et al., 1979; Stevens et al., 1974).

Previous theories of thermoregulation, encouraged by engineering terminology for thermostats, utilised the idea of a set point (Ranson, 1939; Romanovsky, 2007; Werner, 1980). Although, the belief in a set point has been challenged (Kobayashi et al., 2006; Mekjavic et al., 1989; Mekjavic et al., 1991; Mekjavic et al., 2006; Romanovsky, 2007). The 2001 glossary of terms for thermal physiology (Sciences, 2001) states that the set point refers to the value of a regulated variable that is stabilised at by way of regulatory processes. The integrative control for the thermoregulatory set point was originally suggested to be the hypothalamus (Benzinger, 1961); however, to date, no integration centre for comparing ideal to actual temperatures has been discovered (Nagashima et al., 2000; Romanovsky, 2007). Rather, the system regulating homeostatic temperature ranges appears to be regulated by heat loss and heat gain systems, acting semi-independently but regulating either side of an ideal level (Kobayashi et al., 2006). These two systems appear to operate from within the POAH, with all thermoreceptors ultimately transmitting thermal signals to hypothalamic neurons (Kräuchi, 2002). While WSNs and CSNs within the POAH are likely to be the regulators of the heat gain and heat loss systems, little is known of their projecting pathways (Nagashima et al., 2000). The critical core temperature theory, originating from belief in a set point variable, initially held that deviations away from the set point instigated thermoeffector responses. Resultant significant effector

signalling would drive heat loss or heat gain mechanisms to amend the changes. (Sciences, 2001; Waterhouse et al., 2005).

In Benzinger's series of original studies (Benzinger, 1961a, 1961b, 1963), the term set point was widely used, despite a thermoregulatory 'null zone' of ~0.5 ^{0}C between T_C sweating and shivering thresholds when skin temperature was between 28 ^{0}C and 32 ^{0}C (Mekjavic et al., 1989). The notion of a null zone has been discussed thoroughly in many studies (See (Kingma et al., 2012) for a review). The null zone, formally referred to as the interthreshold zone or thermoeffector threshold zone, is 'the temperature range between two threshold body temperatures, for activation of any thermoeffector responses' ((Sciences, 2001) p.273). Consolidation of the interthreshold zone occurred following an investigation by (Cababac et al., 1977), who observed an interthreshold zone negligible enough to justify the term set point. Other research by Mekjavic and colleagues (Mekjavic et al., 1989; Mekjavic et al., 1991; Mekjavic et al., 2006) also established the effector interthreshold zone. Complications incurred in their first study (Mekjavic et al., 1989) encountered significant methodological errors, which were refined in their second study (Mekjavic et al., 1991). Using a protocol in which T_{sk} was clamped using immersed (28 ^{0}C water) cycling, experimental observation was made of a "null zone" of ~0.5 ^{0}C, as measured in the oesophagus and rectum (Mekjavic et al., 1991). As such, the notion of an interthreshold (null) zone has become increasingly prominent as an explanation of thermoeffector response to thermal cues.

Distinct from the interthreshold zone, the thermoneutral zone (TNZ) is the ambient temperature at which deviations in T_C are controlled by sensible heat loss (discussed later) (Mekjavic et al., 2006; Sciences, 2001). The thermoneutral range is not rigid but alters according to endogenous thermal and non-thermal factors, such as posture or basal metabolic rate, or even clothing (Crawshaw et al., 1975; Sciences, 2001). Wenger et al (1976) observed a lowering of the thermoeffector threshold at night, requiring earlier onset of sweating to maintain the lower circadian-associated T_C. Outside the thermoneutral zone, thermal homeostasis is maintained

through increases in thermoeffector activity (i.e., sweating, non-shivering thermogenesis, and shivering thermogenesis, additional to further changes in vasoconstrictor tone), with graded increases being linearly proportional to the perturbation (Mekjavic et al., 2006; Werner, 2010).

While thermal factor changes (i.e., ambient temperature deviation) obviously lead to thermoregulatory opposition, so too do non-thermal factors, some of which oscillate in modulating T_C (Kräuchi, 2002; Taylor et al., 2008a; Waterhouse et al., 2005). Such non-thermal factors include diurnal fluctuations such as activity-rest states (Refinetti et al., 1992; Sciences, 2001), circadian fluctuations such as hormonal and sleep-wake regulation (Karasek et al., 2006; Kräuchi et al., 1994; Van Someren, 2006; Waterhouse et al., 2005), and ultradian cycles such as the ovulatory cycle (Charkoudian, 2003; Refinetti et al., 1992). Bright light, by way of modulation of such circadian hormone release, produces changes in T_C (Cajochen et al., 2000). Aging also acts as a non-thermal factor due to decreased responsiveness to deviations in T_C, altered surface-to-volume ratios and reduced thermoeffector capacities (i.e., reduced vasomotor tone, etc.) (Raymann et al., 2005; Van Someren et al., 2002). The confounding effects of thermoregulation often mask the non-thermal factors (Kräuchi et al., 1994). For the purpose of the current review, only the circadian-related of the non-thermal factors will be considered.

Daily T_C oscillation is driven by the non-thermal central circadian clock (discussed in *2.3.5 Circadian temperature rhythm*), and serves as an excellent indirect marker of the circadian system timing (Heller et al., 2011; Kräuchi et al., 1994; Refinetti et al., 1992). The oscillation occurs within - and modulates - the thermoneutral zone, with an amplitude of basal T_C fluctuation between ~0.5-1.0 ^{o}C (Aschoff, 1983; Wright et al., 2002). This rhythm is achieved mainly by modifying heat exchange (Waterhouse et al., 2005), which necessitates interaction of thermal and non-thermal systems. Non-thermal factors interact with thermoregulation to either enhance or impair temperature-induced vasomotor activity (Mekjavic et al., 2006). This interaction has been shown in humans performing nocturnal exercise (Wenger et al., 1976).

Thermoregulation is significantly impaired during REM sleep (new terminology, stage R) in humans; and to a lesser extent in NREM sleep (Heller et al., 2011). Many animal studies show reduced responses to thermal cues, or disrupted sleep in order to maintain homeothermy (Heller et al., 2011). Parmeggiani et al (1970) were the first to note reductions in stage R sleep in cats at high and low ambient temperatures, as well as cessation of thermoeffector activity upon onset of REM sleep. In NREM sleep thermoeffector thresholds (for both non-shivering and shivering thermogenesis) are lowered during NREM sleep compared to waking, in both the cold and the heat (Heller et al., 2011). Following investigation of the effect of a range of ambient temperatures (21-34 ^{0}C) on sleep in men, Haskell et al (1981) asserted that thermoregulation is incompatible with stage R sleep. Participants had the highest rate of sleep disruptions at ambient temperatures of 21 ^{0}C, with the least time spent at NREM stages 3 and 4, and in stage R sleep, and the largest sleep onset latency and shortest total sleep. Similar sleep disruptions occurred at 34 ^{0}C (Haskell et al., 1981). Observations on women produced comparable results (Sewitch et al., 1986). However, men selected for their ability to sleep in cool environments, showed no decrease in R sleep in cool compared to thermoneutral conditions (Palca et al., 1986). In summary, sleep substantially reduces the capacity for both heat loss and heat gain (Haskell et al., 1981), profoundly so during R sleep (Heller et al., 2011; Refinetti et al., 1992). The detailed nature of thermoregulation in sleep is complex and is beyond the scope of the current review due to disassociations from the waking state (see (Driver et al., 2000; Heller et al., 2011) for extensive reviews of exercise, thermoregulation and sleep).

The timing, nature and extent of physical activity throughout the day alter the amplitude of T_C oscillation, thereby impacting the sleep-associated nadir. In nocturnal rodents, the use of a running wheel in constant routine dark environments has been shown to phase-advance the rest period of the circadian clock (Yamanaka et al., 2006). Exercise inducing phase modulation is further supported in men, with exercise in the afternoon enhancing sleep, and *vice versa* in the evening (Driver et al., 2000). The placement of sleep enhancing exercise may be in part due to the pyrogenic effects of exercise enhancing the T_C oscillation, however exercise in the evening

has also been shown to assist the heat loss component of the T_C cycle. Yoshida et al (1998) demonstrated 1 h of late evening (20:30-21:30 h) exercise at 50-60% $\dot{V}_{O2MAX}$ significantly reduced sleep onset latency. Tanaka (2001) similarly demonstrated improved sleep onset latencies in elderly individuals following a lunchtime nap (30 min at ~ 1300 h) and exercise in the evening (30 min at ~1700 h). The non-thermal T_C cycle actively promotes heat loss. Torii et al (1995) demonstrated this with significantly higher heart rates and sweat rates in men exercising at 30% and 60% $\dot{V}_{O2max}$ in the evening (2000 h) when compared to the morning (0900 h). These thermoregulatory responses were related to changes in the circadian phase of T_C (oral), with low intensity morning exercise facilitating a much more rapid rise in T_C (Torii et al., 1995). The blunted rise in evening T_C in the mild intensity reflects the lower non-thermal thermoeffector zones (Torii et al., 1995).

Thermal homeostasis is maintained despite thermal and non-thermal factors impinging upon it. Thermal homeostasis is focussed on maintaining T_C within a null zone of ~0.5 ^{o}C. Due to non-thermal factors, this null zone is not static but oscillates throughout the cycle. Temperature can significantly modulate this cycle and thereby impact sleep. The thermal factors can be ambient temperatures or the timing exercise.

2.2.2 Sensation

Thermosensation is a modality of the skin (Schepers et al., 2010). Thermosensitive receptors drive thermoeffector function by providing input of both state (static) and change (dynamic) of internal and external environments (Taylor et al., 2008a). Receptors located in the core are primarily warm sensitive, while those located in the skin are predominantly cold sensitive (Romanovsky, 2007; Werner, 2010). This reflects the asymmetrical nature of core temperature, residing close to the upper temperature limit of survival while comparatively far from the lower limit (Romanovsky, 2007; Taylor et al., 2008a). In thermoneutral environments, cutaneous cold thermoreceptors display a tonic firing pattern to code for the cutaneous temperature state

(Boulant, 1996; Schepers et al., 2010), while hypothalamic thermal pacemaker cells display burst firing to code for brain temperature (Pierau, 1996).

The high prevalence of cold relative to warm thermoreceptors in the skin is more relevant to the thermoregulatory system in combatting change in core temperature. Both cold and warm receptors display a bell-shaped curve of activation with regard to their respective temperature ranges. Cold receptors display maximum discharge in the range of 20-30 ^{0}C (Boulant, 1996; Pierau, 1996; Schepers et al., 2010; Van Someren et al., 2002), while warm receptors display maximum discharge between 40-45 ^{0}C (Schepers et al., 2010; Taylor et al., 2008b; Van Someren et al., 2002). Beyond these receptor firing ranges, supplemental (e.g., high threshold cold receptors; see (Schepers et al., 2010)) and pain receptors are active.

Cold receptors are located within the dermal/epidermal border at ~150 μm from the surface, and conduct primarily along myelinated Aδ fibres. Warm receptors are located slightly deeper in the dermal layer and conduct signals along unmyelinated C-fibres. Cold receptor activity is transduced much more rapidly to the POAH. As such, peripheral thermoregulation is further sensitised to cooling. Peripheral thermoafferents travel via the anterolateral, spinothalamic pathways (Pierau, 1996). Centrally, the core thermoreceptors are arranged so as to detect brain temperature oscillations, particularly fluctuations toward brain warming (Romanovsky, 2007). Central thermoreceptors are arranged horizontally with dendrites located medially at the 3rd ventricle and laterally at the medial forebrain (Taylor et al., 2008a), but can be found all over the brain (Nagashima et al., 2000). The orientation of central thermoreceptors optimises detection of cerebrospinal fluid temperature and the temperature of blood supply flowing around the brain. This relationship between warm central and cold peripheral thermoreceptors, allows for efficient detection of the most likely adverse thermal challenges, from internal and external sources, respectively.

Two effector systems are driven by thermo-sensitivity: autonomic and perceptual/ alliesthesial sensation. Autonomic thermo-sensitivity describes the physiological responses

whereas alliesthesial thermo-sensitivity describes 'the changed perception of a given peripheral stimulus resulting from the stimulation of internal sensors' ((Sciences, 2001), p.247). These two effector systems are differentially driven between core and skin, and between skin sites, as shown in both closed loop and open loop settings (Cotter et al., 1996; Cotter et al., 2005; Crawshaw et al., 1975; Greenspan et al., 1993; Simmons et al., 2008). The ratio between the core and skin in driving autonomic thermoeffectors is ~9:1 (Taylor et al., 2008b), however, this differs between autonomic effectors. For example, the T_C: T_{sk} ratio is between 6:1 and 20:1 in driving the sweating response (Nadel et al., 1971; Wyss et al., 1974), and 3:1 to 5:1 for metabolic heat production(Cheng et al., 1995; Mekjavic et al., 1986). The ratio for behavioural/alliesthesial effector stimulus is closer to 1:1 (Bulcao et al., 2000). The greater contribution of the skin to the behavioural stimulation minimises the energetically costly metabolic/autonomic responses brought about through core body stimulation. Despite their typically smaller relative contribution, skin receptors constitute a primary input for both autonomic and alliesthesial thermoregulation (Crawshaw et al., 1975), especially in stable environments (Caldwell et al., 2014).

Clarification of the thermosensitive basis of thermoreceptors has come with the discovery of TRP (Transient Receptor Potential) channels (Boulant, 1996; Romanovsky, 2007; Schepers et al., 2010). TRP channels are non-specific cation channels that are divided into six subfamilies dependent on their receptivity (Figure 2.1) (Romanovsky, 2007). Within these subfamilies, TRPM8 (melastatin) and A1 (ankyrin) are responsive to cold, and TRPV (vanilloid) and some additional M channels are responsive to warm (Romanovsky, 2007). Together these channels (TRPM, TRPA, TRPV) are referred to as thermoTRP channels (Romanovsky, 2007; Schepers et al., 2010). Collectively TRPV4 and TRPM4 & 5 appear to be focal for response to innocuous cool stimuli, whereas TRPV3, 1 & 2 respond to increasingly warm stimuli (Romanovsky, 2007; Schepers et al., 2010). The arrangement of these thermoreceptive channels is such that individually they cover modest temperature ranges, but overlap in activation to cumulatively cover a large active temperature range (Romanovsky, 2007). Activation of any TRP channel

results in inward ion currents such that resting membrane potential is depolarised (Romanovsky, 2007). In response to cold stimuli (menthol), Reid et al (2001) observed this inward ion flux to be predominantly calcium mediated. ThermoTRPM8 is selectively expressed within CSNs (Boulant, 1996). Inhibition of K^+ channels, as a result of cooling has also been observed, which facilitates the depolarisation and signalling of cold-sensitive thermoreceptors (Boulant, 1996).

Mammalian thermoreceptors are specialised at detecting innocuous temperatures impinging on the skin (Boulant, 1996). This is reflected in the convergence of thermoTRP channels sensitised in these ranges (Romanovsky, 2007; Schepers et al., 2010). When innocuous change in thermal state is detected, receptors respond dynamically by transiently, but vigorously increasing firing rate (Pierau, 1996; Schepers et al., 2010). The firing rate of thermoreceptors during temperature change is as much as 5-10 times greater than at steady state (Hensel, 1982). The threshold for detection appears to sensitize with increased *rate* of cooling as well (Greenspan et al., 1993), although some postulate that this is ambiguous (Crawshaw et al., 1975). Following initial receptor responses, thermoreceptors rapidly adapt to new steady states (Zhang et al., 2010c). Beyond innocuous ranges, however, thermosensors do not adapt (Schepers et al., 2010).

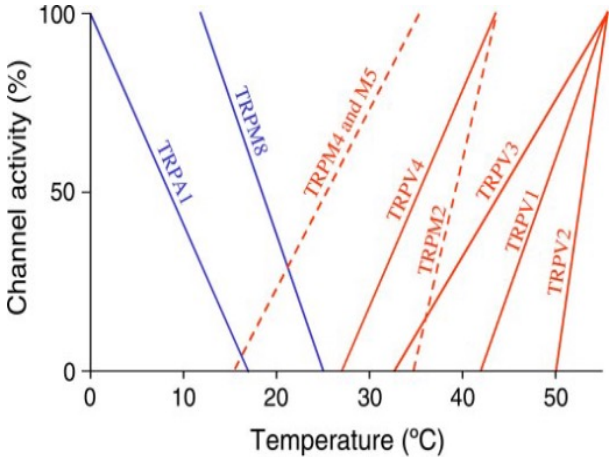


Figure 2.1: Schematic representation of the dependence of the activity of the cold activated (blue) and warm activated (red) thermoTRP channels. From Patapoutian et al (2003)

Detection of thermal change is dependent on the location and areal extent of stimulation; e.g., the face, hands and feet have greater receptor densities, improving their sensitivity. Spatial summation can also reduce the threshold and increase detectability and the perceived intensity of a particular temperature (Greenspan et al., 1993). Peripheral thermosensitivity is better at

detecting *change* more than actual temperature (See (Kobayashi et al., 2006) for comparator theory). Cline et al (2004) argued that humans are 'poor estimators of their own thermal state' (p.2302). Cline's (2004) assertion is corroborated by Greenspan et al (1993), who noted that individuals are more apt at recognising temperature decrease than detecting static coolness *per se*, following an experiment in which the majority of participants failed to complete cooling staircases due to *rates* of cooling being perceptually unbearable. Behavioural responses to changes in the thermal environment also appear to be bimodal (Humphreys et al., 1999), with adjustments consistently overshooting thermoneutral (Cline et al., 2004). Arens et al (2006a, 2006b) and Zhang et al (2010a, 2010b, 2010c) have demonstrated in both uniform and non-uniform environments that individuals responded inordinately to onset of cooling or heating in both local and whole-body manipulation settings. Bimodal, exaggerated behavioural stimulation serves to protect the body against potentially damaging temperatures (Weiss et al., 1961), especially at body locations most prone to environmental influence.

The thermal sensation associated with sudden change in temperature is likely due to the magnitude of the dynamic thermoreceptor response mentioned above (Arens et al., 2006b; Zhang et al., 2010a). The overshoot to temperature change is more pronounced for thermal comfort than for thermal sensation, and for *local* than *overall* thermal comfort (Arens et al., 2006b; Zhang et al., 2010c). Despite an overshoot for thermal comfort, thermal sensation displays a roughly linear relationship to skin temperature in the range 29 ^{0}C and 34 ^{0}C (Zhang et al., 2010c). Outside this range, thermal sensation disassociates from skin temperature, exaggerating the perceived change (Zhang et al., 2010c). As a whole, thermal comfort thresholds outside the neutral range are highly dependent upon whole-body thermal sensation/state and comfort, size of the affected area and the rate at which warming or cooling is occurring (Cotter et al., 1996; Greenspan et al., 1993; Van Someren et al., 2002; Wang et al., 2007).

Of all skin regions, the face has the greatest alliesthesial and autonomic sensitivity (Arens et al., 2006a; Cotter et al., 1996; Cotter et al., 2005; Crawshaw et al., 1975; Simmons et al., 2008);

this is more apparent in cooling than warming (Cotter et al., 2005; Zhang et al., 2010c). The face's sensitivity is in part due to the predominance of non-convergent thermal afferents –a great number of which are still unaccounted for- which is not present to the same extent in other regions (Cotter et al., 2005; Nagashima et al., 2000; Romanovsky, 2007). It is the distribution of thermoafferents that determines the dominance of whole-body thermal sensitivity and comfort (Arens et al., 2006b; Cotter et al., 1996). Head temperature appears to be critical for whole-body thermal comfort (Arens et al., 2006a), and cooling the head appears to be the best method of reducing thermal discomfort associated with heat, irrespective of core body temperature changes (Simmons et al., 2008). In uniform ambient heat stress, the head is also less comfortable than the rest of the body, prompting corrective behaviour (Arens et al., 2006a). Arens et al's (2006a, 2006b) appraisal of the head's role in whole-body thermal sensation and comfort corroborates studies such as Cotter et al (1996) and Simmons et al (2008). Simmons et al (2008) argue facial cooling is more efficient at dissipating heat, reducing thermal strain, and improving comfort than body areas of equal size (i.e., 10% of total body area (Dubois et al., 1916)).

The head and face have major thermoafferent and thermoefferent roles also for autonomic thermoregulation. Heating the face generates powerful sudomotor responses (Cotter et al., 2005); presumably facial cooling would elicit a similarly powerful shivering response. The large vascular supply in conjunction with specialised skin on the face (discussed later) also facilitates vasomotor and sudomotor-mediated cooling (Cotter et al., 2005). This may be why in warm environments (i.e., 32 ^{0}C), the head and face become significantly warmer than the rest of the body excepting the hands (Arens et al., 2006a) and toes (Love, 1948; Werner et al., 1980). Cooling the head in hot environments can blunt the core temperature rise by up to 50% (Simmons et al., 2008) despite seemingly counter intuitive suppression of sweating, which is 2-5 times more powerful than other skin areas (Cotter et al., 2005). While Cotter et al (2005) agree that facial cooling can be used to optimally reduce thermal discomfort, they indicate that the extremities are better for alleviating heat storage, in part due to their minimal autonomic drive (Cotter et al., 1996).

The hands and feet have large perceptual representations to provide early awareness of temperature change; furthermore, the hands are more sensitive to temperature changes than the feet (Cotter et al., 2005; Taylor et al., 2014). The distance of the extremities from the core makes them most sensitive to environmental change. However, the sensitivity of the extremities does not necessarily translate to autonomic thermoregulation (Cotter et al., 1996; Crawshaw et al., 1975), as this would be counterproductive to maintaining thermal homeostasis in active humans. The extremities undergo the greatest temperature fluctuation of all body areas (Greenspan et al., 1993; Werner et al., 1980), which may be instrumental in the feet's high thresholds for temperature recognition and low thermosensitivity (Crawshaw et al., 1975; Greenspan et al., 1993). Conversely, the hands appear to have significantly lower coolness thresholds than other limb segments observed (arm, leg, foot) (Greenspan et al., 1993). At local skin temperatures of ~26 ^{0}C thermal sensations are of slightly cool and neutral for the feet (Arens et al., 2006b); this suggests that discomfort threshold would occur at much lower temperatures. The hands reach their discomfort threshold at 20.7 ^{0}C (Candas et al., 2007) . The apparently attenuated thermal sensation of the feet can be beneficial for studies conducting subtle manipulations as they do not easily induce thermoregulatory or behavioural responses, however, this may yet be detrimental for sleep onset (See 2.3 Circadian Rhythms).

The extremities are able to have some impact whole-body thermal discomfort. In observations of whole-body and local thermal sensations and comfort, (Arens et al., 2006a) observed that overall perception is dominated by the one or two most uncomfortable locations. In hot ambient situations where both the body and the head are hot, then the temperature of the head has been demonstrated to dominate thermal comfort; likewise in warm situations with warm whole-body and segments, cooling the feet is able to remediate the thermal discomfort (Arens et al., 2006b). In cool environments (T_{Amb}=20^{0}C), the greatest increase in local and overall thermal comfort is produced by warming the feet as opposed to the neck or head (Arens et al., 2006b) (i.e., by warming the coolest region).

The magnitude of thermal sensation differs depending on the body location undergoing the thermal stimulus and the type of receptor activated at that particular temperature. This is in regard to both the sensitivity of the receptor as well the dominant thermoafferent feedback it delivers; autonomic or alliesthesial. The extremities are most heavily influenced by the environment and therefore are most capable of providing perceptual information, to minimise the need for physiological effector responses. If sufficient thermoafferent feedback arrives to drive an autonomic thermoeffector response, however, the vasomotor response is the most energetically efficient thermoeffector.

2.2.3 Vasomotion

In humans, there are essentially three autonomic effector categories for thermoregulation: metabolic heat production, sweating and active vasodilation, and release of vasoconstriction (Werner, 2010). Various behavioural mechanisms act in synergy with these autonomic effectors. The systems of efferent control of effector organs can be similar, as for active vasodilation and sweating (Charkoudian, 2003), or brown adipose tissue and skin vasculature (Romanovsky, 2007), but are often functionally independent of each other (Charkoudian, 2003; Nagashima et al., 2000; Romanovsky, 2007). The thermoeffector systems often operate in parallel, in response to perturbations (actual or impending) of the T_C. Almost all thermal afferents converge at the POAH, which also drives thermoeffectors, though complete pathways are not yet fully understood (Nagashima et al., 2000). Incomplete knowledge of integrative and efferent pathways applies to all thermoeffectors.

Within TNZs, T_C is modulated solely through vasodilation and vasoconstriction to control sensible heat loss. At rest, these thermally induced changes are restricted to the cutaneous compartment (Caldwell et al., 2014; Edholm et al., 1956); these are presumably via withdrawal of vasoconstrictor tone. Neural pathways for vasomotion are known to transmit directly to the medulla, through the medial forebrain bundle (Nagashima et al., 2000; Romanovsky, 2007). Within the medulla, there appears to be a functional separation in vasoconstriction and

vasodilation pathways. Experimental studies have identified two medullary areas differentially responsive to heating or cooling signals (Kanosue et al., 1999); these are the caudal projection from the lateral hypothalamus to the reticular formation, and the ventral tegmental area. The anatomical divisions in heat loss and heat gain pathways supports current theory of thermoregulatory control. Nagashima et al (2000) postulate that WSNs likely send excitatory signals to the vasodilatory heat loss pathways and inhibitory signals to the vasoconstrictory heat gain pathways.

Efferent pathways are largely inhibitory (Romanovsky, 2007). As such, thermoeffector activation requires disinhibition of tonically inhibited neurons. Upon activation of such neurons, excitatory signals typically flow through sympathetic ganglionic pathways (Folkow, 1955). Evidence indicates that dilatory pathways also use sympathetic nerves (Taylor et al., 2014), especially those supplying vessels within glabrous skin. The existence of sympathetically active vasodilation has been apparent since the 1930's (Grant et al., 1938; Lewis et al., 1931). However, vasodilatory control is not organised solely through the ganglionic pathway, as shown in lesion studies in mammals (Folkow, 1955). Current evidence indicates that vasodilation is mediated through co-transmission in sympathetic cholinergic nerves (Charkoudian, 2003). The exact mechanisms of active vasodilation are still elusive despite many proposed mechanisms. Some possible contributors that have been proposed include acetylcholine, nitric oxide, vasoactive intestinal peptide and/or substance P (Charkoudian, 2010).

Changes in vasomotor tone are driven largely by central sympathetic outflow, via either cholinergic or adrenergic transmitting fibres. In normothermia, central modulation is largely via sympathetic adrenergic nerves modulating vasoconstrictor tone (Charkoudian, 2003). Disinhibition of this sympathetic adrenergic drive is termed passive dilation (Caldwell et al., 2014; Taylor et al., 2014). Within the TNZ, neural input is constantly changing vasomotor and venomotor tone, leading to highly unstable skin temperatures (Taylor et al., 2014; Werner, 2010) as observed by Kräuchi et al (1994). Indeed, within normothermic environments,

cutaneous blood flow fluctuates more often than it does at extremes. Fagrell (1985) observed periodic fluctuations in the capillaries of 6-10 cycles/min. Similarly, Wang et al (2007) observed frequent fluctuations in finger temperature of ~1-2 ^{o}C, occurring at 2-5 min intervals; presumably to alter T_C heat exchange.

The control of skin vasomotion is determined by central (neural outflow and hydrostatic pressure) and local factors (e.g., nitric oxide, and endocrine adrenaline) – aside from the fact that local T_{sk} is also part of the thermoafferent drive. While local factors are powerful, they cannot abolish or dominate the central drive (Taylor et al., 2014). The degree of local and central control differs between skin locations. With heating, Kingma et al (2012) observed blood flow increases of ~29-fold in the hands in the heat, but only ~3-fold increases in the forearm. Conversely, in cold environments, the head reduces heat loss by 17%, the arms and legs by 25% and the hands and feet by 50% (Kingma et al., 2012). Such differences can be attributed to differing skin types, as the degree of central control, and presence of specialised vessels (AVA's; discussed later) differs widely. The control of skin vasculature from both central and local factors in conjunction with specific vascular adaptation (AVAs) allows for the extravagant ranges in skin blood flow.

The ratio of control in the hands and feet is dominated by central thermoefferents with minimal local modulatory control. This means that while local factors can –and do– sensitise regional vessels in the extremities they do so to a lesser extent due to the control exhibited by the central thermoeffectors. The volar surfaces (palmar and plantar aspects) are innervated solely by adrenergic constrictor tone (Caldwell et al., 2014; Taylor et al., 2014). In non-glabrous skin (on dorsal aspects of hands/feet), passive dilation, brought on through withdrawal of constrictor tone is accompanied by active (perhaps cholinergic) dilation. Active dilation is responsible for 80-95% of the flow increase in non-glabrous skin (Taylor et al., 2014).

In cold environments (e.g., 10 ^{o}C), vasoconstriction of the limbs is maximal in lightly clothed individuals at rest, leading to greater environmental influence. Even in moderate air

temperatures (e.g., 20 ^{o}C) cold-induced vasoconstrictor tone can be maximal (Taylor et al., 2008b). The inter-regional differences in local skin temperatures can be as great as 17 ^{o}C (foot to forehead), whereas in warm environments (40 ^{o}C) the difference between the core and skin temperatures and also between skin regions is as small as ~2 ^{o}C, due to changes in vasomotor control (Werner et al., 1980). The sympathetic adrenergic outflow, acts on arterial vasculature (arterioles and AVAs) and also the veins. The reservoirs in veins lie distally (i.e., legs and feet); as such, constriction of veins reduces these reservoirs and has an amplifying effect of central constrictory input.

The hands and feet have specific morphological characteristics allowing them to serve as excellent radiators (Charkoudian, 2003; Taylor et al., 2014), while also being capable of significant withdrawal of heat to insulate the core. The bones, in the hands and feet, act as heat sinks, while minimal internal muscle that is also relatively inactive makes for minimal local heat production. Large surface area-to-volume coefficients in the hands and feet also maximise heat loss capacity; i.e., fingers have coefficients of 2.2, whereas proximal skin sites have coefficients of ~1.0 (Aschoff et al., 1958). Compared to proximal segments of similar size, the hand has a surface to mass ratio 4.1 – 5.2 times greater (female – male), while the foot is 2.9 -3.2 times greater (Taylor et al., 2014). Such morphological and physiological (e.g., high AVA and sweat gland densities) characteristics make the hands and feet the best capable sites for heat dissipation, yet they represent 4.4 – 4.6% and 7.1 – 7.4%, respectively, of total body surface area (Kingma et al., 2012; Taylor et al., 2014).

The hands and feet are well supplied by arteries. The hand receives flow directly from the ulnar and radial arteries, which progress into the superficial and deep palmar arches (Standring, 2008). The palmar arches join at the digital branches, to supply the finger (Standring, 2008). The foot is supplied by the medial plantar and lateral plantar arteries, which progress behind their respective malleolar prominences, and by the *dorsalis pedis*, progressing along the dorsal aspect of the foot (Standring, 2008). Along the dorsal aspect, the *dorsalis pedis* forms the medial and

lateral tarsal arteries, and the arcuate artery and first dorsal metatarsal artery (Standring, 2008). Branching from these are the arteries supplying the toes. Of the plantar arteries, the medial plantar artery supplies the foot muscles and medial skin, while the lateral plantar artery supplies the heel and lateral sole, before progressing deep and forming the plantar arch with the *dorsalis pedis* (Standring, 2008). The plantar digital arteries supply the toes (Standring, 2008). Blood flow to the feet is consistently lower than flow to the hands (Caldwell et al., 2014).

Deep and superficial veins, as well as a more superficial venous plexus, drain the hands and feet. Such redundancy allows for the withdrawal of blood flow from superficial to deep areas, "thickening" the shell against heat loss. The veins, being capacitance vessels, can store 70-80% of total blood volume in the distal regions in TNZs (Taylor et al., 2014). It is primarily by constriction of these drainage vessels, in the cold, that reduces the heat offload from the distal extremities (Taylor et al., 2008b). In addition, within the limbs the proximity of the arteries to the veins (arguably) allows for a counter current heat exchange to occur, which further helps to limit heat loss (Taylor et al., 2014).

Ambient cold generates vasoconstriction beginning in the extremities and progressing proximally as required to minimise heat exchange with the environment. Ambient heat produces a much more generalised vasodilation. At rest in TNZs, skin blood flow is ~0.250 L/min (~0.35 L in males; Taylor et al., 2014), and can range from close to 0 L/min to 6-8 L/min under extreme thermal stress (Charkoudian, 2003; Hardy et al., 1938; Rowell, 1970). The minimum blood flow required to support cutaneous tissue has been estimated to be ~0.8 mL/100 mL/ min (Abramson, 1965). Basal blood flow at the hands is ~6.7 mL/100 mL/min, while at the feet it is ~2.8 mL/100mL/min (in males (Caldwell et al., 2014)). This volume is sufficient to allow for the appropriate rate of sensible heat loss (~1.5 W/kg) in a resting state (Charkoudian, 2003).

The changes in vasomotor tone alter the interactions of heat loss between the core and skin, and the skin and environment. The skin serves as a shell layer to protect the core, to its detriment if necessary. The skin temperature typically resides lower than the core, effectively

providing a gradient for heat to flow from the core to skin and finally environment. Distal-proximal temperature gradients (DPGs) are impeded by reduced cutaneous blood flow, despite larger gradients, whereas dilation facilitates DPG by increasing the skin-environment gradient, making heat removal more permissible. In wide ranges of ambient temperatures the shell typically comprises 10-20% of the human body, however, when cold stressed, the shell layer increases as much as 30-40% (Gagge et al., 1996). The thickening of the shell is due to the gradual withdrawal of cutaneous flow (Raymann et al., 2007b; Romeijn et al., 2012a; Waterhouse et al., 2005).

2.2.4 Arteriovenous anastomoses

The arteriovenous anastomoses (AVAs) are densely located in regions most capable of heat offload (as alluded to above) and are controlled solely by central sympathetic adrenergic input (Charkoudian, 2003). The majority of glabrous skin contains AVAs, however AVAs are most densely packed in acral skin. Examples of acral skin include the nose, ears, palms and soles of the feet (Gagge et al., 1996; Taylor et al., 2008b). In such regions, anastomoses lie relatively deep to the papillary capillaries, and serve as shunts to the poorly insulated venous plexus (Hales, 1985). The comparatively large diameters allow for the vastly greater flow (AVAs have ~25-125 μm, compared to the typical cutaneous capillaries of ~10 μm); this equates to roughly 10 000 times greater blood flow (Poisseuilles Law; (Taylor et al., 2014)).

At thermal neutrality, anastomoses display some vasomotor tone; constricting approximately three times per minute (Kingma et al., 2012). In the cold, AVAs are continuously maximally constricted; however, when heating is applied AVAs dilate *passively* at ~34 ^{0}C (Figure 2.2) (Fagrell et al., 1977; Gagge et al., 1996). The AVAs are primarily responsible for the alterations in the DPG and associated alterations in T_C heat loss. This is especially apparent in response to the non-thermal circadian rhythm (discussed in *2.3.5 Circadian temperature rhythm*) (Van Someren, 2006).

As stated above, the hands and feet are specialised for heat offload, and in conjunction with anatomical/morphological adaptations, also have a rich cutaneous vascularity that reflects this – between 40-70 vessels/mm^2 (Taylor et al., 2014). The most complex and longest anastomoses reside with in the soles and palms, with between 30 and 200 AVAs/cm^2 (Taylor et al., 2014). At rest, basal hand blood flow, when normalised for segmental surface areas is more than three times greater in the hands (550 mL/m^2/min) than in the rest of the body (Taylor et al., 2014). Increases in blood flow in the hands and feet (among other acral skin sites) are facilitated by AVAs. By shunting such large volumes of blood to the venous plexus, the hands and feet are able to effectively radiate off vast quantities of heat into the environment (Gagge et al., 1996; Hales et al., 1978).

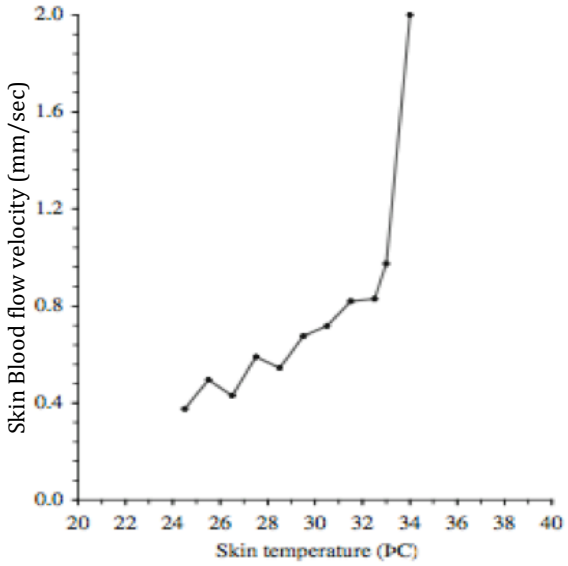


Figure 2.2: Correlation between skin temperature and resting blood flow velocity (CBV) in one nailfold capillary of a healthy 37-year-old man. Figure and legend extracted from (Fagrell et al., 1977), who investigated the effect of skin warming on skin blood flow

2.2.5 Summary

Thermoregulation is a large and complex system, of which only the relevant aspects are discussed here. Thermoregulation attempts to maintain homeostasis of core temperature in response to perturbations from thermal and non-thermal factors (i.e., the perturbations on T_C are ultimately thermal, but modulating influences on thermoregulation can be non-thermal). One's thermoregulatory state is transduced by core and skin thermoreceptors, which provide differing autonomic and perceptual drive based on location (e.g., the T_C is a powerful autonomic driver, whereas hands and feet provide high perceptual drive for localised thermal sensations but perhaps not for behavioural thermoregulation, and the face provides strong drive for all of

these). Autonomic, and to a lesser degree, perceptual drivers, induce thermoeffector responses in opposition to the direction of change.

Altered vasomotor tone is the primary *physiological* thermoeffector response. Vasomotion is capable of producing fluctuations in cutaneous blood flow from 6-8 L/min (Rowell, 1970) to ~0 L/min (minimal volume specific flow in hands and feet is approximately 0.8 mL/100 mL/min; (Abramson, 1965)), allowing more heat to be mostly convected and radiated from the extremities when blood flow is high, while being capable of minimal heat loss (but also potential damage to the extremities) at low flow. Thermoregulation is still incompletely understood, particularly with regard to the transfer of heat from the core to the limb segments, especially the feet. The temperature profiles of the extremities are of particular interest within the sleep field.

Discomfort thresholds of the extremities are widely recognised in regard to sleep onset (Haskell et al., 1981; Kräuchi et al., 2008; Palca et al., 1986; Raymann et al., 2005, 2008; Sewitch et al., 1986). The extremities are highly susceptible to cooling due to their strong vasoconstriction, location and lack of heat generating capacity. The thermoregulatory contribution of the extremities in uniform and transient environments has been elucidated by Arens et al (2006a, 2006b) and Zhang et al (2010a, 2010b, 2010c), and in particular by the recent review by Taylor et al (2014). However, research on the extremities has previously focussed on the hands (Candas et al., 2007; Cline et al., 2004; Taylor et al., 2014), with few studies on the feet. Studies exploring temperature profiles of the extremities have found differential responses to interventions (e.g., to sleep deprivation (Romeijn et al., 2012b)), such that upper limb responses cannot be assumed to apply to the lower limbs. Thermal discomfort has provided insight into the role of the extremities in sleep onset and, clinically, in sleep difficulties involving vasospastic syndrome (Flammer et al., 2001), thermal discomfort of cold extremities (Kräuchi et al., 2008), aging and insomnia (Raymann et al., 2007a; Raymann et al., 2007b), and narcolepsy (Fronczek et al., 2008).

Thermal control of distal extremities has been further implicated with sleep. An elegant study by Kräuchi et al (1999) identified a causal relation of foot temperature on sleep onset latency. Broadly stated: 'vasodilation of distal extremities was the best predictor of the body's readiness for sleep' (Kräuchi et al., 1999, p.37). Furthermore Romeijn et al (2012b) sleep deprivation study revealed sustained circadian rhythmicity in foot temperature cycles across day and night, while the hands lost rhythmicity. The loss of whole-body circadian temperature rhythmicity reciprocated dramatic sleep deprivation-induced changes in arousal levels to sustained attention tasks (Romeijn et al., 2012b). Such findings warrant more attention in regard to their contribution to thermoregulatory knowledge per se, and with regard to sleep knowledge. An increasing number of sleep research studies are addressing thermoregulatory aspects, and through the relation between temperature and arousal, have focussed on facilitating sleep. Unfortunately, there has been almost no research into the possible *value* of low extremity temperatures in preventing sleep and maintaining arousal. Fronczek et al (2008) have utilised core warming and peripheral cooling to induce reciprocal increases in vigilance and sleep onset latency in narcoleptics (further discussed in *2.3.6 Narcolepsy*). Fronczek et al's (2008) findings have not been applied to a healthy population. The present thesis is that lower-limb extremity cooling would also be applicable for interrupting the thermoregulatory profile associated with sleep onset, and hence help in maintaining vigilance in healthy individuals.

Subtle temperature manipulation is often overlooked for more aggressive temperature manipulation strategies. Subtle, or innocuous, temperature manipulation affects local vasomotor tone, without impinging substantially on thermal sensation. Thermal sensation of the extremities also does not (necessarily) implement autonomic change (Arens et al., 2006; Cotter et al., 2005; Simmons et al., 2008), therefore in subtle ranges, local vasomotor tone is the primary means of innocuous cold defence. In healthy populations, thermoafferent input would be expected to override non-thermal inputs in driving thermoregulatory response, even as sleep pressure accumulates. However, non-thermal input appears to modulate the thermoregulatory effector thresholds, such that the TNZ oscillates according to the circadian rhythm of T_C (See

(Heller et al., 2011; Wenger et al., 1976; Yamanaka et al., 2006)). In turn, thermal factors appear to impair circadian processes (Kräuchi, 2007), or exacerbate underlying circadian or thermovascular issues (e.g., narcolepsy, insomnia, vasospastic syndrome). Non-thermal factors rely on the healthy function of AVA's to allow circadian oscillation of T_C, yet local temperatures less than 30 ^{0}C induce vasoconstriction of AVAs.

2.3 Circadian Rhythms

The most overt and highly studied circadian rhythm is the sleep-wake cycle, including vigilance and drowsiness (Oken et al., 2006). In 1916 *encephalitis lethargica*, studied by Von Economo, sparked interest in hypothalamic pathology directly related to the disruption of sleep-wake regulation (Moore, 2007; Von Economo, 1931). The two-process model of sleep regulation was introduced by Borbely in 1982 (Borbely, 1982), and pervades through all models addressing regulation of fatigue and performance (Achermann, 2004). The two-process model describes the interaction of the timing of alternation between the sleep and wake cycles (Beersma, 1998). The two processes that comprise the model are the homeostatic and circadian aspects of sleep and waking, termed process S, and process C, respectively (Borbely, 1982). The homeostatic hourglass, process S interacts with the circadian clock, Process C, to regulate the pressure to sleep (Figure 2.3) (Porkka-Heiskanen et al., 2002; Romeijn et al., 2011; Tononi et al., 2006). These two aspects, as described originally by Von Economo lie primarily within the

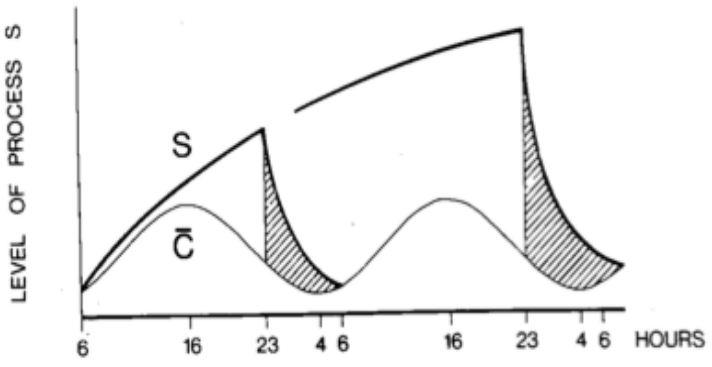


Figure 2.3: Time course of Processes S and C after regular and extended waking periods. From (Borbely, 1982). Shaded area represents sleep and recovery of Process S.

hypothalamus. Specifically, the ventrolateral preoptic hypothalamus (VLPO) is the primary site for the homeostatic hourglass and the suprachiasmatic nucleus (SCN) is the site of the central circadian clock (Moore, 2007; Saper et al., 2005). Von Economo originally described the anterior hypothalamus and the posterior hypothalamus aptly as the sleep centre and the wake centre (Moore, 2007; Saper et al., 2005).

The two-process model refers not only to the processes individually instituted by the homeostatic and circadian processes, but more importantly, their interaction and integration (Achermann, 2004); for example, the flip-flop switch inhibiting the VLPO and facilitating the ascending arousal system (AAS), which switches rapidly to aid sleep by allowing the VLPO to inhibit the AAS (Gaus et al., 2002). Originally proposed in rats, the two-process model 'postulates that… process S rises during waking and declines during sleep, and interacts with process C' (See Figure 2.3) ((Borb et al., 1999), p.560). Process C is independent of waking and sleeping but regulates the duration of each according to modulation via the light-dark cycle. A further process representing sleep inertia, and the interaction between Processes S and C, was instituted to the original model to effectively create a three-process model that appears to be more reflective of human diurnal cycles (Achermann, 2004). This third component was used to further describe the ultradian dynamics, of which sleep inertia is one component (Borbely, 1982). The ultradian dynamics help to encompass the two sleep cycles, R and NREM, but also the time course of daytime vigilance brought about through the interaction between the homeostatic and circadian processes (Borbely, 1982).

Both the homeostatic hourglass and the central circadian clock are the two primary sites of oscillation that influence sleep-wake regulation. Saper et al (2001; 2005) put forward a model proposing that the hypothalamus acts as the "sleep switch" allowing sharp transitions between the sleep and waking states. The switch, known as the "flip-flop" switch, alternates between the wake-promoting ascending arousal system that activates "wake-active" neurons during awake periods, and the "sleep-active" neurons in the POAH, more specifically the VLPO area. This

switch is stabilised by orexin/hypocretin neurons in the lateral hypothalamus, the loss of which results in narcolepsy (See *2.3.6 Narcolepsy*). The physiological alterations between waking and sleeping states occur alongside facilitative behavioural cues. Such behavioural factors are called sleep-permissive or wake-promoting factors, and include light intensity (Burgess et al., 2001; Karasek et al., 2006), posture (Raymann et al., 2007a; Romeijn et al., 2012a), temperature change (Fronczek et al., 2008; Raymann et al., 2007a; Romeijn et al., 2011) and dietary intake (Fronczek et al., 2008; Reyner et al., 2012).

2.3.1 Sleep permissive/ wake promoting factors

Behavioural actions modulate circadian and homeostatic processes to permit or prevent changes in vigilance and sleep. Such actions can moderate the tonic changes in the sleep-wake cycle, brought about by Processes S and C. These factors are crucial to allowing or withholding the changing of state. Some such conditions found to alter the degree of tonic change include cold (Haskell et al., 1981; Palca et al., 1986; Sewitch et al., 1986), heat (Haskell et al., 1981; Kräuchi et al., 1999; Liao, 2002; Raymann et al., 2005), immediate danger (Yerkes et al., 1908), and posture (Caldwell et al., 2003; Cole, 1989).

As a wake-promoting factor, light intensity is instrumental in generating sleep onset illnesses, due to suppression of melatonin with increased light intensity. The effect of lighting is further compounded as working hours are increased into the night, whereby people spend more time in upright, seated postures as a function of lighting, further disrupting sleep onset behaviour. Light intensity modulates the circadian process C, through direct synapses transmitted via the retinohypothalamic tract to the SCN (Arendt et al., 2005; Brzezinski, 1997; Carpentieri et al., 2012); essentially instigating or inhibiting night-time melatonin secretion (further discussed in *2.3.4 Interaction of Process S and Process C*). By removing artificial lighting (e.g., 2 weeks of camping) the circadian sleep wake system and light-dark cycle can resynchronise (Wright et al., 2013). By resynchronising these systems, Wright et al (2013) were able to improve both sleep

onset and morning vigilance; essentially restoring the timing of wake promoting and sleep permissive lighting.

As light can modulate sleep onset and maintenance significantly, it is apparent that temperature may also modulate sleep onset and maintenance (Kräuchi et al., 1999; Raymann et al., 2005; Van Someren, 2004). As alluded to above (in *2.2.1 Homeostasis*), either side of an ideal (e.g., ~29 ^{0}C; (Haskell et al., 1981)), ambient temperature becomes an inadvertent wake promoting factor. This has been evidenced across a range of temperatures in both men and women, by Haskell et al (1981) and Sewitch et al (1986), but also indicated by Kräuchi in a questionnaire of a Swiss population (Kräuchi et al., 2008). Typical bed-time behaviour minimises temperature variation by using bedding to generate a microclimate, ensuring sleep-permissive temperatures (Goldsmith et al., 1968).

Similarly, skin temperature has long been considered an indicator of sleep preparedness and as such, a sleep permissive factor (Fronczek et al., 2006b). The association between core body and distal temperature flux, initiating sleep onset, has now been largely elucidated, and causal implications of temperature on sleep have been both debunked experimentally (Fronczek et al., 2008; Kräuchi et al., 1999; Kräuchi et al., 1994; McDonnell et al., 2014; Raymann et al., 2005, 2008) and reviewed thoroughly (Aschoff, 1983; Kräuchi, 2007; Kräuchi et al., 2010; Refinetti et al., 1992; Romeijn et al., 2012a; Van Someren et al., 2002; Waterhouse et al., 2005) (discussed in *2.3.5 Circadian temperature rhythm*). As argued by Liao (2002) and Romeijn et al (2012a), body temperature, core and skin, are sleep permissive and/or a wake promoting factors. The behavioural adjustments in the evening serve to facilitate the oscillation in core body and skin temperatures, and thereby enhance their sleep permissive role. More importantly, it is argued that circadian cycles can be entrained by skin temperature fluctuations (Romeijn et al., 2012a).

The crucial role of sleep permissive or wake promoting conditions in coding for tonic neuronal change is often overlooked but very important to altering or maintaining the arousal state (Romeijn et al., 2012a). There is a myriad of factors that are able to significantly alter the

arousal state; among these are postural changes, light intensity, and thermal status. Pharmacological methods are also capable of altering arousal state; however, these aspects are beyond the scope of the current study. Thermal factors are the most important to the current study, with skin temperature being manipulated. Phasic cues such as lighting or temperature serve to alter the underlying rhythms of process S and C through their tight neurological input with the critical areas of sleep-wake regulation.

2.3.2 Homeostatic Hourglass – Process S

The reason for sleep is still unknown; however its restorative effects are obvious (Saper et al., 2005). Process S, was derived from observations of slow wave sleep (SWS), in which SWS, represented by electroencephalographic delta power (0.5-4 Hz), rose during initial NREM phases of sleep in response to longer prior waking periods (Borb et al., 1999; Borbely, 1982). SWS power then reduced across the duration of sleep. Process S reduces as a function of both R and NREM sleep, making its decline slow and disjointed (Achermann, 2004; Borbely, 1982). Studies debunking NREM and R sleep components of Process S found that there is a specific rebound of any of the sleep states to selective deprivation (Borbely, 1982). For instance, following 40 h of sleep deprivation, SWS was observed to rebound, but R sleep wasn't (Nakazawa et al., 1978). R sleep does, however, produce a rebound to specific R sleep deprivation (Dement, 1960). Although, rebound has also been demonstrated to specific deprivation to stage 4 (Agnew et al., 1964; Agnew et al., 1967). The association between waking hours and rebound SWS appears to be predominantly associated with NREM sleep (Borbely, 1982), however it appears all levels of sleep are important. The rise in potency of Process S across the day demonstrates an exponentially saturating sleepiness to be remediated by SWS (Figure 4) (Beersma, 1998).

Substances known to raise this sleep pressure are defined as sleep factors. A sleep factor is a substance that accumulates in the brain across waking until it reaches a threshold, whereby sleep is induced (Porkka-Heiskanen et al., 2002). Many sleep factors have been proffered that

may additionally serve as sleep factors, such as synaptic homeostasis (Tononi et al., 2006). However only relatively recently has a causative substrate been largely accepted; namely, adenosine (Porkka-Heiskanen et al., 2011). There are likely many sleep factors that are unknown and likely to be very complicated (Moore, 2007; Porkka-Heiskanen et al., 2002). Adenosine accumulates during wakefulness and decreases during sleep and is thus considered the most likely sleep factor/substance (Moore, 2007; Porkka-Heiskanen et al., 2011).

Importantly, the areas in the brain with which adenosine is most implicated are the basal forebrain and the thalamus; both key neuronal groups involved in the ascending arousal system. For example, the inhibitory effect of adenosine on the basal forebrain may play a large contributory role in the flip-flop switch (Saper et al., 2005). Local adenosine administration to the POAH area in rats has also been shown to induce sleep, while across sleep duration in cats, adenosine levels in the basal forebrain and thalamus decrease by 75-80% (Porkka-Heiskanen et al., 2002). As a sleep factor, the rise and fall in adenosine levels in the basal forebrain are specific to this neuronal population (Porkka-Heiskanen et al., 2011). Adenosine has therefore been proposed as the mechanism driving this switch to inhibition of arousal through its action on both the basal forebrain and the VLPO (Lim et al., 2008). The interaction of adenosine with the VLPO is further noted in Section *2.3.4 Interaction of Process S and Process C.*

Process S tracks the internal needs of the body, driving greater sleep propensity with progressing wakefulness. It also modulates the rest-activity cycle through its cortical inhibition (Porkka-Heiskanen et al., 2002). This obviously has a large associative effect on vigilance in the evening, as the pressure to rest and sleep incrementally builds across the day. Individuals involved in mentally stressful tasks, especially across the biological night display a much greater rate of decline, and loss of attention. This loss of attention is in part due to build up of sleep pressure across days, as the sleep restriction limits the time for sleep factors to return to baseline. Moore (2007) identified 'sleep homeostasis' by which sleep propensity is modulated by

preceding sleep amounts to provide an average "reference level" of sleep. If sleep is deferred the sleep drive augments propensity, whereas excessive sleep supresses sleep propensity.

Over a day, the drive to sleep also undergoes fluctuations as its association with process C serves to minimise or modulate its potency (Figure 3). Reyner et al (2012) observed one such component, known as the bi-circadian or 'post lunch' dip. This dip, while not overly significant, was apparent in drivers who had consumed a heavy meal, after only 30 min of driving. The bi-circadian dip is a function of decreased process C arousal drive rather than any increase in Process S. Another dip in vigilance comes with the evening change in Process C from arousal to sleep, allowing process S to drive sleep onset. This change leads to significant declines in vigilance, as process S is no longer challenged.

2.3.3 Central Circadian Clock – Process C

While process S regulates the internal sleep-wake environment, process C regulates according to the external environment. Process C fluctuates in a roughly 25-hour frequency, but is entrained to the 24-hour cycle through diurnal fluctuations in light intensity (Jin et al., 1999; Johnson et al., 1988; Reppert et al., 2002). In this way, Process C regulates sleep and waking by constraining Process S sleep propensity. The SCN serves primarily as pacemaker and integrator, by processing the various diurnal signals (e.g., light) and signalling the sleep and waking changes (Moore, 2007; Saper et al., 2005). While other pacemakers have been found to exist, for instance one in the eye, and another related to timing of dietary intake, as well as individual clock neurons (Bass et al., 2010; Raymann et al., 2007a; Raymann et al., 2007b; Romeijn et al., 2012a), these are hierarchically governed by the SCN and do not appear to, in and of themselves, relate to regulation of sleep timing (Beersma, 1998; Burgess et al., 2001; Romeijn et al., 2012a).

Process C regulates the timing of onset and cessation of sleep and hormonal functions –in particular pineal melatonin and orexin/hypocretin (Hurwitz et al., 2004; Lim et al., 2008; Pandi-Perumal et al., 2008) - though it does not determine the function of these processes (Achermann,

2004). Process C also regulates the sleep-wake cycle, of which vigilance and drowsiness are components. The change in the sleep-wake cycle is instigated by neuronal (ascending arousal drive; see below) and hormonal (pineal melatonin) changes (Cagnacci et al., 1992; Cassone et al., 1986; Jin et al., 1999). The sleep-wake cycle is also modulated by, and modulates, the T_C cycle. The state of vigilance/drowsiness is tightly related to core body heat fluctuation. The circadian rhythm of T_C is very robust. In modified daily cycles (~26-hours, or no light), circadian rhythm desynchronisation occurs within a minor range, but retains its internal synchronisation of physiological functions (Aschoff, 1983). In this way, the two-process model has been applied to the understanding of the temporal profile of neurobehavioural functioning across days of sleep deprivation (Van Dongen et al., 2003). During the biological "day" T_C is at its peak, while biological night occurs with T_C declining into its nadir (Van Someren, 2006; Waterhouse et al., 2005). The oscillation in T_C is a highly reliable tool by which to assess the timing of the central circadian clock, especially with regard to the transition period between sleeping and waking states.

2.3.4 Interaction of Process S and Process C

The balance between waking and sleeping states relies on the interactions of the main hormones and neurotransmitters of Processes S and C. The predominant drivers for circadian change are two hormones orexin and melatonin, whereas the proffered driver of homeostatic change is adenosine. Figure 2.3 best illustrates the interaction between Process S and Process C, as they offset each other across the day and facilitate over the night, with the "flip-flop" switch allowing the transition between the waking and sleeping states.

Orexin/hypocretin secreting neurons, within the lateral hypothalamus (LH), stabilise the waking state through axonal projections spreading diffusely throughout the cortex (Hassani et al., 2009; Sakurai et al., 2010). They project into a ventral pathway - progressing anteriorly into the prefrontal cortex and spreading posteriorly - which aids cortical activation and facilitates thalamic inputs (Saper et al., 2005). The thalamic pathway stems from reticular activating

neurons (Saper et al., 2005). Together these two pathways produce the AAS, which excites many neuronal populations and allows smooth functioning of the cortex (Moore, 2007; Saper et al., 2005). Orexin neurons are most excited during active waking and progressively down regulate as stimuli decrease (Sakurai et al., 2010). During NREM sleep, orexin neurons are inactive, with burst firing occurring as an individual progresses through R phases of sleep (Lee et al., 2005).

Orexin stabilises the ASS, and offsets the rise in adenosine (Sakurai et al., 2010). The "wake-active" neurons of the LH interact with the homeostatic VLPO by mutually inhibitory projections, the potency of which determines the direction of the aforementioned "flip-flop" switch to either waking or sleeping state (Saper et al., 2001). The VLPO overcomes this inhibition in the evening, "triggering" the flip-flop switch (Lim et al., 2008; Saper et al., 2005). Adenosine, as mentioned above, is thought to be the main contributor to VLPO disinhibition by directly reducing GABAergic input from cholinergic axonal cells from the midbrain neuronal populations (the lateral coeruleus) (Porkka-Heiskanen et al., 2011; Saper et al., 2001). Once the VLPO inhibition is overcome, its GABAergic projections up-regulate. The VLPO projections send inhibitory signals to many areas of the hypothalamus, and brainstem areas at the origin of the ascending reticular activating system, namely the monoaminergic nuclei, the raphe and the lateral coeruleus (Moore, 2007; Sakurai et al., 2010).

Concurrent with destabilisation of the AAS, release of pineal melatonin is stimulated indirectly by Process C (Arendt et al., 2005; Karasek et al., 2006). Melatonin stimulates vasodilation and T_C drop, either through its role on cardiac autonomic activity, or through direct activation in the vasculature (Cardinali et al., 1998). Pineal melatonin could be considered an antagonist to orexin, being secreted at night, and facilitating sleep onset processes (Arendt et al., 2005; Burgess et al., 2001; Carpentieri et al., 2012; Hassani et al., 2009). When melatonin secretion is stimulated, orexin is typically inhibited; however these are only associative changes (Hassani et al., 2009).

Since its discovery, melatonin has been found to have a number of roles within the body including interaction with free radicals, the immune system and the gastrointestinal tract (Carpentieri et al., 2012). It is also secreted for these different roles by many organs, however the pineal synthesis and secretion of melatonin appears to be the most pervasive throughout the body during nightly secretion (Karasek et al., 2006; Pandi-Perumal et al., 2008). Eighty percent of its secretion is at night (Karasek et al., 2006). During the day, bright light (>600 Lux) inhibits melatonin synthesis; however even within continuous darkness, melatonin synthesis exhibits a near-24-hour cycle (Brzezinski, 1997). Conversely, substantial evidence also supports an acute suppression of evening melatonin synthesis in response to bright light (>5000 lux) exposure (Cajochen et al., 2000; 2005; Wright et al., 2000). As bright light entrains the SCN, it follows that SCN-induced melatonin release is also entrained by light as a function of this. The loss of light-entrainment of the circadian clock is exhibited in disorders/disabilities causing "free running" sleep wake cycles such as in complete blindness, where melatonin secretion, among various other processes, is not entrained to the regular 24-hour cycle (Brzezinski, 1997; Karasek et al., 2006).

Nightly onset of melatonin synthesis and release begins after dark, peaking between 2-4 am (or 12 pm-3 am (Carpentieri et al., 2012; Karasek et al., 2006)), during the midpoint of sleep (Brzezinski, 1997; Hurwitz et al., 2004; Pandi-Perumal et al., 2008). Onset and offset of melatonin appears to typically occur between 9-10 pm and 7-9 am, respectively (Cagnacci et al., 1992; Hurwitz et al., 2004; Karasek et al., 2006); however the duration of melatonin release is also altered according to duration of night and coding for length-of-day (Arendt et al., 2005; Pandi-Perumal et al., 2008).

Light inhibits melatonin through neural activation of paraventricular nuclei and sympathetic activation via SCN pathways (Carpentieri et al., 2012). The threshold for inhibition is 200-400 lux with maximal inhibition occurring at 600 lux (Brzezinski, 1997). Maximal waking light exposure in a constructed environment is ~900 lux (Wright et al., 2013), well above the maximal

inhibition of melatonin. The retinal light influx causes synchronisation of the SCN to the light-dark cycle (Pandi-Perumal et al., 2008). The light-dark cycle co-varies with T_C and melatonin change, however the T_C cycle appears to have a stronger correlation to melatonin secretion than that of the light-dark cycle (see Figure 2.4) (Burgess et al., 2001; Cagnacci et al., 1992; Karasek et al., 2006). It is believed that melatonin could in fact serve as an internal synchroniser able to stabilize or reinforce rhythms (Karasek et al., 2006). As stated by Karasek et al (2006) "biological day" corresponds to a lack of melatonin secretion accompanied by an increase in T_C and a decrease in waking theta activity, whereas the "biological night" melatonin secretion is associated with decreased T_C and increased waking theta activity (Figure 2.4).

The relationship between melatonin and T_C appears to be causative, with (Burgess et al., 2001) observing a rise in foot temperature 30 min after administration of an oral melatonin load, followed an hour later by a drop in T_C (Figure 2.4). This effect is also observable with daytime administration of melatonin (Hurwitz et al., 2004). Similarly, night-time administration of atenolol, suppressing melatonin synthesis, also reduces the decline in T_C; infusion of melatonin, offsetting the atenolol restores normal reductions (Van Den Heuvel et al., 1997). Nocturnal melatonin secretion accounts for ~40 % of the drop in T_C (Cagnacci et al., 1992). There appears to be a lot of support for direct modulation of the cardiac autonomic activity, however Burgess et al (2001) argues with his findings using synthetic melatonin administration that melatonin does not directly interact with the autonomic system but rather acts on receptors in the heart and periphery. Melatonin receptors have been identified within coronary arteries, ventricles and systemic arteries (Pandi-Perumal et al., 2008). As such, pineal melatonin synthesis is determinative on cutaneous vasodilation and subsequent DPG development or abolition (Kräuchi et al., 1997; Pandi-Perumal et al., 2008). The effect of melatonin on the systemic circulation is linked to thermoregulation and appears to lower blood pressure through vasodilation (Pandi-Perumal et al., 2008). Collectively these interventions and observations support the notion that melatonin is largely responsible for facilitating sleep onset.

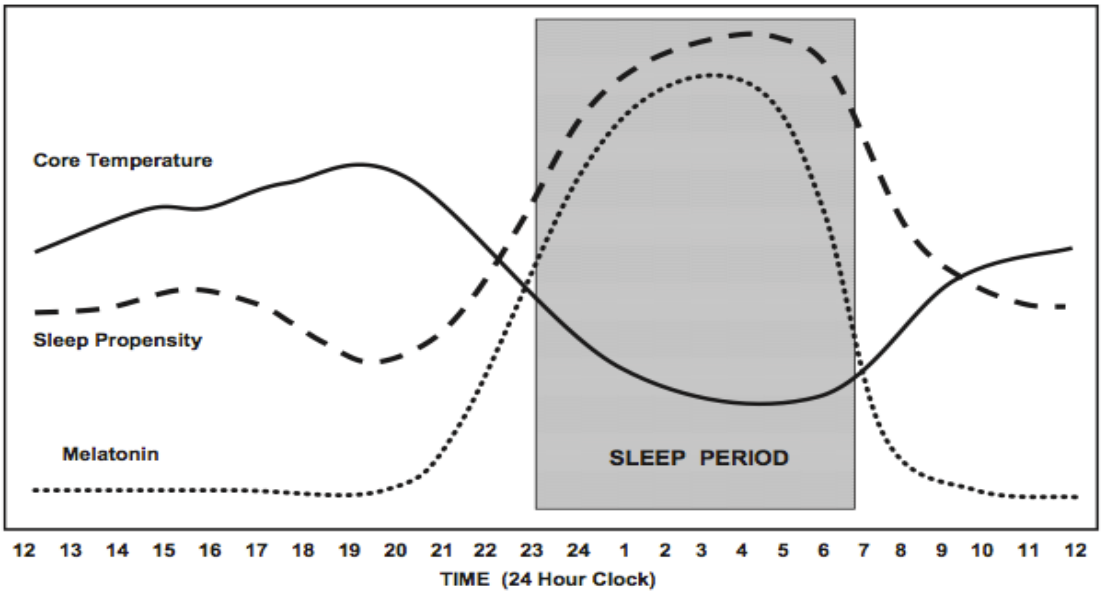


Figure 2.4: Circadian rhythm, with associated melatonin and sleep propensity cycles. From Lack et al., 2008

2.3.5 Circadian temperature rhythm

Temperature fluctuation, while not directly controlled by the central circadian clock, is modulated by direct neuronal connections between the SCN and POAH, via a rostral projection of the SCN (Moore et al., 2002). The synapsing of the POAH with the SCN allows for the circadian fluctuation in T_C (Figure 2.4). Tonic up-regulation of SCN firing stimulates WSN firing, which has been demonstrated to spontaneously increase at sleep onset (Raymann et al., 2007a). Circadian oscillation of T_C occurs through modifications in heat loss, which is stimulated by WSN excitation (Kräuchi et al., 1994). T_C variation only occurs within normothermic ranges as a function of SCN input, with sleep onset occurring around the maximum rate of T_C decline (Kräuchi et al., 1999; Murphy et al., 1997). Neural stimulation of evening heat loss occurs with hormonal fluctuations such as melatonin, which, along with increases in skin temperature contribute to the associated rhythm of T_C and sleep (Arendt et al., 2005; Cagnacci et al., 1992). A causal relationship between skin temperature and sleep onset has gained evidential support (Raymann et al., 2005; Van Someren et al., 2002; Van Someren, 2004). Skin temperature rises as a function of vasodilation, allowing the T_C to decline. In dilating the distal blood vessels, skin temperature forms an inverse relationship with T_C. It is currently debated whether it is the level of distal skin temperature that

is essential (Fronczek et al., 2006b; Raymann et al., 2005)or the gradient that is induced (Fagrell, 1985; Kräuchi et al., 1999).

While the cycles of sleep and waking are intimately related with the rhythm of T_C (Van Someren, 2006), they are not directly causative (Aschoff, 1983). The nature of the relationship has been challenged by constant routine protocols preventing sleep, whereby T_C continues to oscillate, but at a reduced rate (~50%) (Waterhouse et al., 2005). Gillberg et al (1982), further demonstrated T_C independence from rest-activity cycles, by prompting sleep at different times of day, and thereby different phases of the T_C rhythm. Sleep during the biological day, causes a drop in T_C across the first hour of sleep, whereupon it realigns with the normal rhythm across sleep duration (Gillberg et al., 1982). The typical rhythm of T_C peaks between 2-8 pm and troughs at 5 am (Figure 2.4).

The role of maximal rate of decline in T_C on sleep onset was first determined by (Murphy et al., 1997), in young and old individuals by using a disentrainment study design. Across three days of time-cue isolation, participants typically went to sleep ~60 min following the maximum rate of T_C decline (Murphy et al., 1997). This relationship could be overridden given enough motivation. Kräuchi et al (1999; 1994) expanded on the temperature and sleep relationship by demonstrating that the degree of dilation of blood vessels in the hands and feet, and subsequent DPG development are the best physiological predictors of rapid sleep onset. Raymann and colleagues further demonstrated the role of raising skin temperature on inducing sleep, in a series of studies (Raymann et al., 2005, 2007a, 2008; Raymann et al., 2007b). With use of a water-perfused thermosuit, Raymann et al (2005), demonstrated 26% faster sleep onset latencies with subtle manipulation (0.8 ± 003 ^{o}C) of proximal skin sites throughout the day. This has been further applied in an ecologically valid setting, using bed socks to promote distal warming and a beneficial DPG for sleep onset (Raymann et al., 2007a). Even more recently, (McDonnell et al., 2014) has added to the growing literature in a bed rest study involving effects of hypoxic verses normoxic conditions. They observed persistence of the non-thermal

mechanism mediating evening vasodilation, regardless of induced vasoconstriction (McDonnell et al., 2014).

A number of factors are capable of impinging on T_C and impairing sleep onset. Cooling may perhaps be one such factor, as sleep-related processes appear to be difficult to initiate in susceptible individuals. In a questionnaire of a Swiss population thermal discomfort of cold extremities was correlated with difficulty initiating sleep (Kräuchi et al., 2008); this has previously been observed (as mentioned above *2.2.1 Homeostasis*) across a range of ambient temperatures, with both cold (21 ^{0}C) and hot (34 ^{0}C) ambient temperatures disturbing sleep onset (Haskell et al., 1981).

Furthermore, susceptibility to ambient temperature may be sex specific, with women typically having a lower distal temperature (~3 ^{0}C) (Candas et al., 2007), and therefore suffering greater complaints of thermal discomfort and difficulty initiating sleep (Kräuchi et al., 2008). Additionally, the effects of aging appear to generate similar difficulties initiating sleep, which are improved by distal warming (Raymann et al., 2007a). Declining synthesis of melatonin (from ~65 years) further exacerbates the effects of aging on the circadian rhythm of T_C (Brzezinski, 1997; Pandi-Perumal et al., 2008; Raymann et al., 2007a; Van Someren et al., 2002).

In summary, the rhythm of T_C is highly correlated to the circadian sleep wake rhythm. Indeed, the rhythm of T_C appears to be causative in healthy sleep patterns, more so through the fluctuations in skin temperature and its role on T_C. Both hormonal and neuronal non-thermal factors maintain and entrain the T_C cycle, which in turn influence the rate of sleep onset and the depth of sleep. Conversely, minimised fluctuations in T_C or thermal factors generating vasoconstriction appear to reduce sleep propensity. This disruption may have a role in influencing maintenance of vigilance, as evidenced by the altered thermoregulation and sleep-wake physiology observed in narcoleptics (Fronczek et al., 2008).

2.3.6 Narcolepsy

'Narcolepsy is clinically characterised by excessive daytime sleepiness and cataplexy' (Fronczek, 2006, p.1444). Narcolepsy reflects a dysfunction of the orexin/hypocretin system, with ~95% of narcoleptic patients having very low levels of hypocretin-1 in cerebrospinal fluid (Overeem et al., 2012). The loss of "normal" levels of orexin leads to the destabilisation of the waking state, as the flip-flop switch becomes destabilised (Saper et al., 2001). Without the input provided by orexin, the flip-flop switch triggers rapid changes in sleep-wake states across the day, typified in narcolepsy.

The relation between temperature and sleep has been established, both with evidential causation of distal skin temperature and sleep onset (Fronczek et al., 2006; Kräuchi et al., 1999; Raymann et al., 2005), and with synaptic input between the SCN and POAH (Wright et al., 2002). As orexin is an active component of the circadian clock, low levels may ultimately contribute to decreased distal sympathetic constrictor tone, which is indicated by altered DPGs (Fronczek et al., 2006). The relation between orexin deficiency and decreased sympathetic tone has been demonstrated in orexin knockout mice (Kayaba et al., 2003). Fronczek and colleagues explored the relationship between distal temperatures and sleepiness in narcoleptics in a series of studies (Fronczek et al., 2006a; Fronczek et al., 2006b; Fronczek et al., 2008). In comparing daytime sleep propensity between narcoleptics and healthy individuals, Fronczek et al (2006b) observed a relationship between high distal skin temperature and rapid sleep onset during a Multiple Sleep Latency Test. When focussing on vigilance Fronczek et al (2006a) experimentally manipulated both T_C and T_{Sk} to restore normal daytime temperatures; this was achieved through ingestion of hot drinks and use of a water perfused suit. By ingesting a hot drink, the participants were better able to raise vigilance (~24%), and by cooling the skin sleep propensity was reduced. Fronczek et al's (2006; 2008) studies serve to further demonstrate the role of temperature on sleep and indicate the permissiveness of cooling as a technique for maintaining wakefulness.

2.4 Vigilance

Vigilance contrasts sleep, but forms a more complex relationship with T_C. The vigilance state reflects cortical excitation and maintenance of attention (Klimesch, 1999; Lal et al., 2002). Some factors involved in altering neuronal excitability are the duration of waking state (Process S) (Klimesch, 1999; Porkka-Heiskanen et al., 2002; Romeijn et al., 2012a; Tononi et al., 2006), time on task (Fronczek et al., 2008; Lal et al., 2002), and satiety (Reyner et al., 2012). Vigilance is greatest during the circadian phase of increased T_C, and declines with core temperature. As the relationship between T_C and the circadian clock are entwined, the core and skin temperature phase relationship can therefore be used to infer these underlying vigilance processes (Raymann et al., 2007b; Romeijn et al., 2012a). Indeed this has been observed with narcoleptics as aforementioned (Fronczek et al., 2006b). The daily rhythm in temperature fluctuation results in a reciprocal fluctuation in vigilance (Fronczek et al., 2008; Romeijn et al., 2012a).

Vigilance appears to be directly related to thermal and non-thermal temperature changes within the TNZ. A causal relationship has been observed between the circadian T_C peak and cognitive task proficiency (Wright et al., 2002); additionally, the 'post lunch' bi-circadian dip in T_C appears to result in reductions in vigilance (Reyner et al., 2012). Further facilitating the circadian decline of T_C in the evening (distal skin temperature ~34 ^{o}C, compared to ~31 ^{o}C) appears to generate greater declines in vigilance (Raymann et al., 2007b). Beyond TNZs of T_C-rhythm, both cooling and heating appears to exhibit an inverted-U relationship (Cheung et al., 2007; Raymann et al., 2007b). In a cold pressor test (2-3 ^{o}C water), Patil et al (1995) observed raised alertness but decreased short term memory. Similarly, Cheung et al (2007) found that mild whole-body cooling (T_{water}≈18-25 ^{o}C, ΔT_{re}=0, -0.5, -1.0 ^{o}C), via water immersion, led to performance impairment due to distraction, but greater cold did not relate to worse performance. This led to the suggestion that cooling *per* se, rather than temperature, is distracting.

The most dramatic changes in vigilance are seen in the evening, as sleep is physiologically facilitated. If sleep is deprived, the greater Process S-associated sleep propensity produces greater vigilance decline (Basner et al., 2011). A number of studies use sleep deprivation as a means to generate greater sensitivity in vigilance measures (Van Dongen et al., 2003).

2.4.1 Psychomotor vigilance

Psychomotor vigilance is a state of arousal and attentiveness to tasks (Lal et al., 2001; Warm et al., 2008), or simply sustained attention (Oken et al., 2006). Maintaining attention to a task is energy demanding and requires considerable cognitive processing dedicated to maintaining vigilance (Lorist, 2008; Warm et al., 2008). The ability to maintain attention is specifically affected by mental fatigue – fatigue from prolonged cognitive processing (Boksem et al., 2005; Dinges, 1995; Lal et al., 2001). The psychomotor vigilance task (PVT) is the best measure of assessing mental fatigue associated with increasing time-on-task (Wright et al., 2002). Psychomotor vigilance decay has been observed with temperature manipulation (Fronczek et al., 2008; Raymann et al., 2007b), with changes to body posture (Caldwell et al., 2003), and bright light exposure (Cajochen et al., 2005; Kaida et al., 2006), following sleep deprivation (Van Dongen et al., 2003), and with more complex tasks such as driving or flying (Dinges, 1995; Gillberg et al., 1996; Horne et al., 1995; Schier, 2000).

Attention allows us to, firstly, bias incoming information so as to privilege relevant information for achieving goals, and secondly, actively ignore irrelevant information capable of interfering with goal performance/completion (Boksem et al., 2005). Attention shares many common characteristics with arousal, however where arousal refers to non-specific activation of the cortex, attention includes cognitive processing (Oken et al., 2006). In a resting situation, neurologically, an individual can still be vigilant and alert without the stressors requiring attention. When task demands are applied, individuals are forced to direct attention and considerable cognitive processing to maintain vigilant attention in readiness to respond; this is fatiguing and stressful (Warm et al., 2008). The top down processes involved in coordinating

accurate timing of activities are most affected by fatigue, which result in a loss of performance efficiency (Lorist, 2008).

The majority of research defines vigilance by its declination (Oken et al., 2006). There are many terms used to define the deterioration of vigilance, as outlined in Chapter 1, and while sleepiness is more precise, fatigue is more inclusive of the spectrum of processes surrounding decreased alertness and performance (Dinges, 1995; Horne et al., 1995; Jones et al., 2010). Fatigue, in the context of the current study, can be described as the transitory period between awake and sleep (Lal et al., 2001), marked by reduced efficiency and general unwillingness to continue (Grandjean, 1979, 1988). Unwillingness to continue a task can be described as an effort/reward imbalance, resulting in loss of motivation and feelings of fatigue (Tops et al., 2004).

Monotonous tasks are prone to generating fatigue. During a monotonous driving task, Brown (1994) observed a gradual withdrawal of attention as task duration increased. During driving of a monotonous looped track, Schier (2000) observed withdrawal of attention and fatigue as participants became familiar to the track. This withdrawal of attention from the task, due to familiarity or loss of motivation, generates errors in performance with the loss of efficiency. Within PVT tests, familiarisation to the task is minimised, allowing fatigue to be observed separately from familiarity; these are observed as lapses or increased reaction times as the task progresses (Fronczek et al., 2008; Raymann et al., 2007b; Van Dongen et al., 2003).

As alluded to above, tonic factors such as sleep deprivation, circadian rhythm phase, environmental factors and the individual's characteristics, also impinge on the vigilance state and fatigability (Boksem et al., 2005; Eoh et al., 2005; Lal et al., 2001; Transport, 2011a). The input of tonic factors is minimised by constant routine protocols, which maintain constant conditions. The constant routine protocol was designed by Mills et al (1978) and was modified by Czeisler et al (1986). The constant routine protocol, minimises extraneous factors not directly required for the observances of the study; e.g., Kräuchi et al (1994) maintained constant

ambient temperature and humidity, light levels, motor activity, vigilance state and food intake to unmask the endogenous circadian temperature rhythms.

On top of constant routine protocols sleep deprivation is often used to sensitise tests. Oken et al (2006) warned that many studies might be negatively affecting motivation, by inducing sleep deprivation. Sleep deprivation artificially raises fatigue and alters motivation, and may therefore be creating an unrealistic conflict. Van Dongen et al (2003) and Oken et al (2006) argue that sleep deprivation is a common occurrence in today's society; as such, inducing further sleep deprivation may reduce the validity of vigilance studies. However, the high prevalence of sleep deprivation in driving accidents may be better understood with further sleep deprivation.

In summary, the definitions encompassing vigilance and fatigue vary, however the causative factors remain consistent. Fatigue appears to comprise a number of agreed characteristics, including: Loss of efficiency and alertness; difficulty maintaining attention and filtering irrelevant information; as well as a loss of motivation. Each characteristic of fatigue describes the deterioration of vigilance. Vigilance is the ability to maintain sustained attention, without noticeable decline in efficiency. Maintaining vigilance is costly, and drowsiness impacts the top-down processes most.

2.4.2 *Validity of the psychomotor vigilance task*

The PVT is the most widely used measure of behavioural alertness (Basner et al., 2011). It provides an excellent marker of attentional deficit in most states (i.e. sleep deprivation, following stimulants, circadian measures) (Lim et al., 2008). The PVT is a portable device that contains a brief, ecologically valid and reliable task that is almost devoid of learning effects (Basner et al., 2011; Lim et al., 2008; Loh et al., 2004). In essence the PVT provides a series of simple reaction time tasks, supplied at a random inter-stimulus interval (ISI), which requires sustained attention. Due to its high signal-load ratio, 10 min allows collection of a large amount of information without it being too onerous (Lim et al., 2008). When shortened or lengthened,

no further information is gleaned, and more often sensitivity is lost due to loss of motivation, or lack of data (Lim et al., 2008; Loh et al., 2004).

The PVT is made specific to vigilance, through the random alternation between 2 and 10 s, in the ISI. A reaction time that is longer than standard occurs when an individual is unprepared for the stimulus. As more trials are performed an individual's capacity for continuous, concentrated attention is revealed (Wilkinson et al., 1982). As stated above, the cognitive load applied to maintain vigilance is onerous and increases with fatigue. The ISI is the stressor with regard to maintenance of vigilance, as its inconsistency requires the individual undergoing testing to maintain a state of readiness to respond. This allows the PVT to be sensitive to small changes in attentional function (Lim et al., 2008).

The metrics utilised to understand the effect of experimental conditions or time-on-task on vigilant attention, are typically assessed as reaction time, or lapses. Lapses are denoted by reaction times of >500 ms. These are considered errors of omission, where the individual has failed to maintain vigilance, resulting from distraction or micro-sleeps (Basner et al., 2011; Lim et al., 2008). Lapses are most commonly observed in sleep deprivation studies, in which the PVT has demonstrated its ability as the 'archetype neurocognitive assay of attention after sleep loss' (Lim et al., 2008), p.306). Van Dongen et al (2003) found that across 14-days of sleep restriction, lapses differed significantly between those restricted to 4, 6 and 8 hours per night. Moreover, Lim et al (2008) demonstrated that sleep deprivation and associated sleep pressure incrementally adds to lapses, with the greatest number of lapses corresponding to the summation of circadian drop in T_C and arousal, and homeostatic pressure to sleep. The combination of circadian cycle and pressure to sleep compound the effect of fatigue and significantly alter the number of lapses brought on by attention loss.

Reaction time (often expressed as reciprocal reaction time [RRT=1000/RT] (Fronczek et al., 2008)), the other metric obtained from the PVT, also serves to elucidate attentional change. Reaction time increases with time-on-task. The RRT serves to emphasise response speed in the

normal to fast response domain, thereby avoiding influence of long lapses (Basner et al., 2011). A worsening RRT decreases toward zero. As such RRT provides a performance indicator of the level of alertness preceding stimulus. Fronczek et al (2008) observed significant declines in RRT as a function of time-on-task. In conjunction with a steady increase in the number of lapses, and reduction in response speed, fatigue also produces more frequent errors of commission/omission, and more variability in RRT with increasing time-on-task and sleepiness (Basner et al., 2011; Lim et al., 2008). These fatigue-related performance declines are often exaggerated further by using sleep deprivation to demonstrate larger effects, via the additional sleep pressure.

2.5 Sleep onset

In the transition from fully alert to sleep onset, a series of clearly observable and reliable processes occur (Chokroverty et al., 2005). These changes are predominantly founded in EEG, with marked increases of slow wave theta activity, and alpha anteriorisation, signalling sleep onset (Caldwell et al., 2003; Klimesch, 1999; Oken et al., 2006). As an individual progresses further into sleep, sleep spindles occur, denoting sleep N2 (Chokroverty et al., 2005). Stages 1 and 2 are now termed N1 and N2 respectively (Iber et al., 2007). Although N1 sleep precedes N2, it is difficult to detect due to relapse into and out of the waking state. As such, some studies define sleep from more than one consecutive occurrence of 30 s of N1 sleep, or N2 sleep with the first occurrence of a sleep spindle to ensure certainty of sleep onset (Fronczek et al., 2008). Although sleep onset is defined differently between studies, epochs in N1 or entry into N2 sleep are always among criteria (Fronczek et al., 2008; Kräuchi et al., 1999). Stage 3 and 4 sleep are characterised by increasing frequency and depth of delta (slow) waves. However stages 3 and 4 are no longer recognised as distinct states, but are merged as slow wave sleep, or termed N3 sleep, according to changes in the American Academy of Sleep Medicine guidelines (Iber et al.,

2007). Typically, a subject enters stage R sleep; after slow wave sleep. R sleep is characterised by mixed frequency low voltage EEG, loss of muscle tone and bursts of rapid eye movement.

Awareness of drowsiness occurs after physiologically observable (e.g., EEG) changes (Santamaria et al., 1987). A patient lapsing into N1 often has no recollection of sleep onset, and when queried are often adamant that no sleep onset occurred (Horne et al., 1995). In conjunction with this, subjects with increasing drowsiness become increasingly less responsive to external stimuli (Oken et al., 2006). This reflects a worrying state of awareness, as a decline in reaction time and lapses are common occurrences in drowsiness (Dinges, 1995). Filtness et al (2012) observed good insight into drivers' increasing sleepiness. However this insight appears to be limited, as Van Dongen et al (2003) observed no difference in perceptual awareness in sleepiness between individuals restricted to 4 and 6 hours of sleep, despite a marked differences in vigilance measures. As such, electrophysiological changes provide a more direct measure of sleepiness progression, as opposed to reliance in perception.

The waking state is characterised by small amplitude, fast frequency brain wave activity predominantly involving beta and gamma activity with interspersed alpha frequency. As an individual's state of alertness decreases the frequencies are increasingly infiltrated by alpha, and theta activity (Chokroverty et al., 2005). The waking state includes ~50% observable alpha activity, mixed with beta activity (Chokroverty et al., 2005). As sleep onset occurs, alpha power reduces, and theta and delta power increases (Klimesch, 1999). In cases of sleep deprivation participants may forgo traditional entry into sleep through N1 and N2, and progress straight into R if the pressure to sleep is high (Chokroverty et al., 2005). When N1 does occur, EEG recordings typically show very low amplitude, fast frequency with at least 15% theta frequency and the appearance of delta subsuming alpha frequency (Chokroverty et al., 2005). N2 sleep is easily definable, as it becomes predominantly theta activity and delta, interspersed with sleep spindles (Chokroverty et al., 2005).

Recognition of sleepiness occurring from waking state to sleep onset shows a number of reliable events that allow the sleep specialist to be sure of sleepiness and finally, sleep onset. This is demonstrated by the slow increase of alpha frequency in the waking state due to reducing arousal, followed by infiltration of theta frequency, reductions in alpha, and finally the disappearance of alpha frequency and introduction of delta. These particular, reliable events are discernible only with EEG, and contribute to the understanding of differences in classifications between sleepiness and fatigue.

2.6 Electroencephalography (EEG) and the electro-oculogram (EOG)

EEG is perhaps the most direct method of determining the biological decay in vigilance in humans (Horne et al., 1995; Lal et al., 2002), and remains the standard methodology for assessing behavioural state (Moore, 2007). The dominant polysomnographic techniques used for determining vigilance and drowsiness are EEG, EOG and ECG, as they are highly responsive to changes in waking vigilance state, and provide insight into underlying neuronal excitability (Chua et al., 2012; Eoh et al., 2005; Lal et al., 2002; Schier, 2000). EEG power is affected by both phasic and tonic changes, such as opening and closing the eyes, or circadian rhythms (Klimesch, 1999). Phasic electro-ocular and cardiographic changes with fatigue and drowsiness are consistent and reliable, with an observable change in blink type/rates and eye movements, and large reductions in heart rate with fatigue progression. EEG records the general neuronal excitability across the scalp through the use of electrodes placed over anatomical landmarks denoting specific areas of the brain as set by the international 10/20 EEG settings (Chokroverty et al., 2005; Himanen et al., 2000; Niedermeyer, 1999). The various synchronisations and desynchronisations of neuronal activity can be measured as a range of various frequencies, which can change depending on electrode positioning (Ruehland et al., 2011). As such, electrode positioning directly over sites of interest (i.e. O1/O2 for visual tasks) maximise the signal-noise

ratio and provides more specific information of change in neuronal activity. By altering electrode positioning to the constraints of the task, EEG is better able to register changes in vigilance-related EEG.

EEG is a minute signal, easily masked by noise due to scalp muscle activity or the environment (Niedermeyer, 1999). Studies often use EMG placement on the chin and/or EOG to clarify phases in the EEG signal (Hakkinen et al., 1993; Santamaria et al., 1987). Electrodes placed more laterally or inferior/posterior (i.e., T7, T8, O1, Oz, O2), are also exposed to more muscular noise than are midline electrodes (McEvoy et al., 2000). EEG also presents high inter-individual variability, with variations such as skull thickness and cerebrospinal fluid flow affecting the signal received (Klimesch, 1999; Niedermeyer, 1999). However, test-to-retest reliability has been validated in relation to vigilance (PVT) and working memory tasks (McEvoy et al., 2000). In conjunction with the anatomical changes, variability can present within the specific frequencies (Klimesch, 1999). The observed discrepancies in synchronising and desynchronising powers are reflective of high inter-individual variability inherent in EEG (McEvoy et al., 2000; Niedermeyer, 1999; Santamaria et al., 1987). Indeed, Chokroverty et al (2005) noted that a small percentage of patients present no alpha activity, with their dominant frequency presenting within the beta frequency range.

Electroencephalography is used in both clinical and research settings. Research utilises EEG to determine vigilance state (Filtness et al., 2012; Lal et al., 2002; Reyner et al., 2012; Schier, 2000), memory capacity (Klimesch, 1999; Ray et al., 1985a, 1985b), or cognitive performance and intelligence (Boksem et al., 2006; Klimesch, 1999), along with aforementioned insights into onset of sleep. EEG is often used to document the transition from waking state to sleep (Chokroverty et al., 2005), or from relaxed to attentive states (Filtness et al., 2012; Klimesch, 1999; Lal et al., 2002). Changes in cognitive state are often interpreted through brain wave frequency and amplitude. The most commonly observed frequencies are delta (0.5-4 Hz), theta (5-7 Hz), alpha (8-13 Hz), and beta (15-35 Hz). Traditionally, delta frequency was ignored due to

the susceptibility of the low frequency to be influenced by artefacts and noise (Lal et al., 2002). Theta, alpha and beta frequencies are used predominantly to assess drowsiness, vigilance, pressure to sleep, and attentional focus through observation of power changes in each band (Burgess et al., 2001; Filtness et al., 2012; Klimesch, 1999; Schier, 2000).

2.6.1 Alpha and Theta rhythms

Alpha rhythm is the dominant frequency on the human scalp (Klimesch, 1999), and is generated from the posterior portion of the brain (Klimesch, 1999; Niedermeyer, 1999). In healthy adults, alpha typically has an amplitude between 10 and 45 μV (Pizzagalli, 2007). Alpha amplitude is best observed over parietal and occipital regions in quiet waking, with eyes closed, and tends to progress anteriorly in fatigue (Chokroverty et al., 2005; Klimesch, 1999; Pizzagalli, 2007; Teplan, 2002). Alpha activity is particularly sensitive to changes in state (i.e., opening closing eyes, change in attention, wake to sleep state) (Klimesch, 1999; Schier, 2000) and is abolished by alerting stimuli and tasks (Teplan, 2002).

The physiological role of alpha remains largely unknown (Pizzagalli, 2007), despite many attempts to define it. Wide speculation upon the physiological basis of alpha includes, among other explanations, reflection of brain maturity, and cognitive and memory performance (Eoh et al., 2005; Klimesch, 1999). Alpha synchronisation has been suggested to reflect cortical inactivity (Pizzagalli, 2007). Indeed, alpha attenuation occurs when alertness decreases into drowsiness (Pizzagalli, 2007), however this is also somewhat due to slowing of frequencies (Klimesch, 1999; Pizzagalli, 2007). During actual task performance, alpha power is typically depressed or desynchronised relative to resting states (Klimesch, 1999); this is referred to as "alpha blocking" (Chokroverty et al., 2005; Niedermeyer, 1999; Pizzagalli, 2007). The suppression of alpha rhythms due to attentional demands (Filtness et al., 2012; Pizzagalli, 2007; Schier, 2000), or even by light influx (Klimesch, 1999), and can last from seconds to cessation of the stimulus (Niedermeyer, 1999). In prolonged repetitive tasks alpha synchronisation occurs when attentional demands reduce. Schier (2000) observed a return of alpha synchronisation

toward the end of monotonous, repetitive simulated driving (lap 5 of 6) as participants lost attentiveness to the task.

Furthermore, understanding of the physiological basis of alpha has been complicated by emerging evidence of alpha sub-bands, heavily advocated by (Klimesch, 1999). Klimesch (1999) and others (Cooper et al., 2003) have argued that alpha power is also highly correlated to speed of cognitive processing in the form of reaction time. The power of a specific frequency is generally determined through the peak frequency within a range (i.e., the peak frequency in the alpha band is generally 10.89 Hz). It has been postulated that an individual with a faster alpha frequency will consistently respond faster than an age-matched individual of slower alpha frequency (e.g., a peak frequency of 9.49 Hz compared to 10.89 Hz) (Klimesch, 1999). Although, task-related shifts in alpha power observed in the majority of research appear not to be related to intelligence or speed of processing (Klimesch, 1999).

Theta power is often studied in conjunction with alpha. Similar to alpha power, theta can be separated into subcategories, which clouds its definition. During wakefulness, two types of theta have been described (Schacter, 1977). The first, frontal midline theta appears to be associated with focussed attention, whereas the second has widespread scalp distribution and is linked to drowsiness and impaired information processing (Pizzagalli, 2007). Klimesch (1999) notes the former theta synchronises upon task onset, reflecting a greater attentive state. However, in a study observing the effect of orthostatic change on vigilance, Caldwell et al (2003), ascribing to the latter theta category found the general rise in power as task duration continued, to be reflective of increasing inhibition of CNS excitation, indicative of low arousal. Caldwell et al's (2003) notion is supported by Lim et al (2008), who suggest that theta activity is related to the homeostatic drive to sleep.

Physiologically, theta activity has been implicated with the septo-hippocampal system (Pizzagalli, 2007). Theta power can be recorded differentially in numerous other limbic regions (e.g., anterior cingulate cortex, medial septum) (Pizzagalli, 2007). The different sources of theta

power may explain the sometimes conflicting response to task demands between recording sites. Theta power has been speculated to subserve a gating function on the information processing flow within the limbic systems (Vinogradova, 1995).

Theta and alpha bands in particular reflect cognitive and memory performance (Eoh et al., 2005; Klimesch, 1999), while beta band indicates increased processing demands (Eoh et al., 2005; Pizzagalli, 2007; Prinzel et al., 2003). Alpha and beta rhythms appear to have an inverse relationship such that when beta power increases alpha decreases (Schier, 2000). Alpha power desynchronises with task onset, as alpha band synchronicity represents generalised neuronal activity unrelated to attention (Klimesch, 1999). This has led researchers to argue that alpha power reflects external events, whereas beta reflects intrinsic events (Ray et al., 1985b). In light of conflicting synchronisations/ desynchronisations occurring within frequency bands, authors must be careful to define the frequency pattern to which they are ascribing their specific correlations.

2.6.2 Age related alpha frequency changes

The alpha frequency does not appear to preside as the dominant power over the scalp until 12 years of age at earliest, and gains dominance at ~16 yrs old, whereupon it develops predominance over the posterior cranium (Klimesch, 1999). Prior to 12 years of age, studies conducted on alpha power have noted that a theta peak at ~4.5 Hz, appears equal to an alpha peak at ~9 Hz (Klimesch, 1999). As adolescent's age, the theta peak decreases along with a residing waking delta frequency, and alpha continues to rise (Klimesch, 1999). After rising to dominances by ~16 yrs, alpha frequency then begins to slow with aging, progressively reducing from perhaps as early as 30 years of age and definitively from 50 years of age (Klimesch, 1999) (such that the frequency slows from ~10.89 Hz, to ~8 Hz (Kopruner et al., 1984)). Kopruner et al (1984) found a linear relationship between mature alpha frequency and aging.

The progressive slowing of alpha frequency may not be related to a loss of cognitive power, but rather due to other neurological issues (Klimesch, 1999). The slowing alpha frequency means that the traditional alpha range residing between 8-13 Hz misses a dominant portion of the lower alpha band in elderly individuals. This slowing down is also interpersonally variable such that determining new alpha bands must be either widened to encompass variability, or determined individually (Klimesch, 1999). In conjunction with temporal changes, spatial changes also take place in aging individuals, with a shift from parietal and occipital dominance toward a temporal lobe shift. As a consequence, EEG studies focussing on aging populations outside the 20-30 year old range must focus electrode placement from parietal and midline, to temporal to encompass this shift in spatial alpha activity (Chokroverty et al., 2005).

The age related changes in alpha activity –both frequency and power –alter the EEG activity away from the traditional alpha range of 8-13 Hz. This 'traditional' range can only be truly said to hold true for a relatively limited age range across the span of most individual's lifetime. As such, the range between full maturation of the alpha rhythm at ~16 yrs, and its degradation at ~30 yrs, provides researchers a relatively clear –albeit limited – cross-section to observe healthy populations.

2.6.3 Beta Rhythm

The beta frequency in adults is predominantly focussed in the frontal and central regions of the cranium and interspersed with alpha frequencies in the posterior (Chokroverty et al., 2005). Beta power typically increases at task onset as attentional demands are focussed and processing demands increase, while it decreases when fewer cognitive resources are required to maintain adequate performance (Eoh et al., 2005; Schier, 2000). In situations of long, sustained attention or sleep deprivation, fatigue inertia is also associated with beta power reduction (Lal et al., 2002). Fatigue inertia is the process of increasing pressure to sleep as a result of Process S mechanisms (Klimesch, 1999; Marzano et al., 2011). Beta indicates the change of vigilance, and brain activity in the prefrontal lobe (Lim et al., 2008).

Beta activity is arguably the most useful EEG discriminator of worsening vigilance. Belyavin et al (1987) found beta power to be the greatest indicator of attention and arousal, and this has since been supported by Schier (2000) and Oken et al (2006). When attentional demands are present beta power synchronises, responding to the task demands on sensory cortical areas; beta is especially prevalent when participants engage working memory tasks. Prior to Belyavin and Wright (1987), studies such as Townsend et al (1979) found the decrease in beta frequency prior to stimulus presentation to be a better marker than theta frequency, despite more consistency found in the alpha and theta frequencies (Oken et al., 2006). However EEG shows great inter-individual variability as described by Niedermeyer (1999). This variation seen between frequency sensitivity may be due to differing task constraints as opposed to variable EEG. For example, tasks requiring sustained attention with working memory will produce greater beta activity than a task simply requiring visual cues (Lim et al., 2008; Oken et al., 2006). However, despite changes in task demands, McEvoy et al (2000) found EEG to be highly stable and reliable during performance of all of their tasks (PVT, and easy versus hard working memory tasks).

Beta rhythm is used to determine alertness and arousal; the two main components of vigilance (Eoh et al., 2005; Lim et al., 2008). As such, beta rhythm is not typically present during rest with eyes closed, but appears over frontal sites and spreads posteriorly when tasks are performed. As task duration increases, beta activity tends to reduce due to decreased attention and lower arousal (Prinzel et al., 2003). Beta activity is most strongly associated with change in vigilance (Lim et al., 2008). Schier (2000), noted that as task duration increased participants required less working memory to maintain task performance, which was reflected as a decrease in beta and a rise in alpha frequency. As the pressure to sleep increases, beta power drops out, until sleep onset. If sleep is prevented, however, beta activity appears to reflect the brain activity focussed on maintaining the waking state (Marzano et al., 2011). Upon onset of a novel task, beta activity synchronises in response to attentional demands. Following initial synchronisation, beta power declines as task proficiency increases, requiring less attention. A late peak in beta activity,

seen in states of sleepiness is often attributed to the greater focus on maintaining the waking state (Niedermeyer, 1999; Schier, 2000).

2.6.4 *10-20 EEG electrode positioning*

The 10-20 system for electrode positioning was originally designed by Jasper (1958). The majority of studies observing change in the waking state use at least four electrodes encompassing various sites over the scalp (e.g., C3-M2, C4-M1, O1-M1, O2-M2) (Chokroverty et al., 2005). Other studies will use 8-10 electrodes to encompass areas pertaining to abnormalities in EEG frequencies (Chokroverty et al., 2005). Electrode positioning is in reference to anatomical landmarks denoting the lobe beneath, and is recognised as the standardised system for observing EEG (Chokroverty et al., 2005). Electrode positions correspond to anatomical features of the brain, such as lobes (e.g., O1, O2), with odd and even numbers representing the left and right hemispheres, respectively.

The choice of electrode positioning is important as it allows localisation of specific rhythms from cortical sites involved in any given task (e.g., beta rhythm located over fronto-central regions, alpha predominance over occipital regions) (Madrid et al., 2010). EEG positions commonly used include O1/O2 due to the nature of most tasks involving visual stimuli. For example, Belyavin et al (1987) recorded from posterior positions (P3-O1, P4-O2) measuring theta, alpha and beta change, during a visual vigilance task. When memory tasks are included within such tests frontal electrodes are used to measure excitability from the prefrontal lobe encompassing the working memory (Klimesch, 1999). Schier (2000) found F4 to be the most sensitive site for assessing working memory, as commonly accepted (Klimesch, 1999).

Ray et al (1985b) found no hemispheric bias in an intake verse rejection task, but observed differential representations of attentional, cognitive and emotional factors, in terms of EEG frequency and site. However, right hemisphere dominance has been demonstrated through observation of cerebral blood flow (Deutsch et al., 1987). Despite this finding, similar activation

occurs bilaterally across the scalp, with the elicited left hemisphere only slightly less active compared to the right (Mesulam, 1986; Schier, 2000). Vigilance tasks engage a variety of systems including working memory, visual, and motor responses, and lead studies to record from multiple sites. These sites typically cover a range from frontal sites to occipital (Chokroverty et al., 2005).

Electroencephalographic activity is variable according to the cortical site being assessed (Gillberg et al., 1996). EEG is also dependent on the task demands and degree of pressure to sleep (Caldwell et al., 2003). As such electrode placement and stimulation must be very carefully performed to ensure that interpretation of the waveforms can be assessed accurately and validly. For example, in an experiment by Caldwell et al (2003), theta synchronisation was argued to be associated with cognitive decline, as delta and theta activity had previously been associated with prolonged wakefulness, and reduction of arousal (Gillberg et al., 1996; Lal et al., 2002). Conversely, other studies have noted that theta synchronisation is associated with increasing task demands (Eoh et al., 2005; Klimesch, 1999). As such, the paradigm or approach to the interpretation becomes important. However, before these approaches can be made, the electrode positioning is the most important consideration.

2.6.5 Electro-oculogram (EOG)

The electro-oculogram is commonly used as a reference when focussing on frontal EEG sites, and provides insightful measures in its own right. It is typically recorded from the outer canthus, 1cm inferior, and 1 cm lateral to the cornea on the left eye, and 1cm superior, and 1cm lateral to the cornea on the right eye (Hakkinen et al., 1993). There are three main categories of blinks: waking eye movements (WEM), slow eye movements (SEM), and REMs (Chokroverty et al., 2005). WEMs are associated with blinks and saccadic eye movements, whereas SEMs are recorded consistently in the horizontal axis at onset of N1 sleep. The change in blinks appears to display a consistent change with fatigue, providing meaningful inference of declining sustained attention (Stern, 1994).The efficacy of EOG is further supported by Lal et al (2002), who

displayed a change from initial fast blinks and WEM toward increasing slow blinks and no eye movement as their monotonous driving task progressed, indicating a loss of attention and fatigue advancement. Santamaria et al (1987) also argue that EOG provides significant observational changes as fatigue progresses. EOG provides clear indices of fatigue during continuous tasks, such as driving (Schier, 2000; Stern, 1994). EOG when coupled with EEG can strengthen the findings by directly correlating eye movement and blink duration at a particular time with EEG bands seen (Santamaria et al., 1987). This allows greater insight into the cause of the apparent bands observed.

2.7 Conclusion

Peripheral vascular tone is highly controlled, with the two main controlling factors being thermoregulation and circadian neuronal rhythmicity. When maintained in a normothermic environment, the core body and skin temperatures will undergo reciprocal inverse fluctuations as a result of the circadian rhythm. Hormonal and neural mediation by the SCN accounts for the non-thermal modulation of the T_C cyclic rhythmicity. Evening core nadir is achieved through vasodilation of specialised skin blood vessels (AVA's) as a result of melatonin synthesis, parasympathetic and POAH innervation, seemingly to facilitate a DPG capable of generating maximal rate of heat loss - and through this - sleep onset (Kräuchi et al., 1999). The rise of distal skin temperature also appears to independently facilitate sleep onset. Phasic external perturbations that oppose this circumstance, such as foot cooling in the evening, will result in vascular responses inverse to that facilitating sleep onset (Fronczek et al., 2008; Kräuchi et al., 2008; Raymann et al., 2005). Despite the role of circadian rhythms, homeostatic pressure to sleep amplifies any sleep facilitative process following extended waking hours, regardless of phase in biological day or night. Since extended (late evening) waking appears to result in continuing decline in core temperature with continuing robust circadian fluctuation, the effect of

relatively passive wake-promoting temperature modulation may be outweighed by the aggressive sleep permissive metabolic and neuronal factors such as adenosine, and/or synaptic density (Hurwitz et al., 2004; Porkka-Heiskanen et al., 2002; Tononi et al., 2006)

Since skin temperature is highly influenced by the environmental temperature, and as noted by Haskell et al (1981), thermoregulation is incompatible with sleep, it is therefore integral to further explore the impact resulting from thermal modulation within the physiological ranges of thermoregulatory responses. As previously observed in sleep research, manipulating skin temperature can reduce sleep onset latency (Kräuchi et al., 1999; Raymann et al., 2005, 2007a; Romeijn et al., 2012a), or vigilance can be maintained (Fronczek et al., 2008; Raymann et al., 2007b). The only study that appears to have directly attempted to maintain vigilance through subtle temperature manipulation, was performed on narcoleptics, with Fronczek et al (2008) greatly altering daytime sleepiness and vigilance with temperature manipulation within the normothermic range. As narcoleptic cycles resemble the sleep onset patterns of normal individuals, it could be postulated that temperature manipulation may override circadian and homeostatic hourglass processes. Maintenance of vigilance has been of critical importance within driving oriented research, however, as of yet, peripheral cooling appears not to have been investigated as a method for maintaining vigilance in the normal healthy population. More importantly, the investigation of subtle systematic distal cooling has not yet been performed in a controlled setting.

Thus, while warming has been explored for various measures, cooling, as a wake-promoting factor appears to have received almost no consideration despite its widespread relevance. The current study aims to examine such effects.

3.0 METHOD

The purpose of the current study was to examine the effect of distal lower limb (foot) cooling on the evening vigilance decline. The aim was to prevent evening distal temperature rise. The associated effect on T_C decline may have an important role in sleep propensity, however given the multifaceted nature of sleep physiology outlined previously (Chapter 2), manipulating skin temperature was deemed sufficient. A partially blinded, cross over design was utilised, in which healthy participants undertook a psychomotor vigilance task during three evening sessions with different extents of temperature manipulation of their feet, in a temperate, controlled thermal environment (~25 ^{0}C, Humidity: 33%). The protocol overview is detailed schematically in Figure 3.1. As a component of the experimental design, participants were expected to maintain a sleep diary and wear an accelerometer for the purpose of monitoring prior (to test night) sleep wake circadian rhythm.

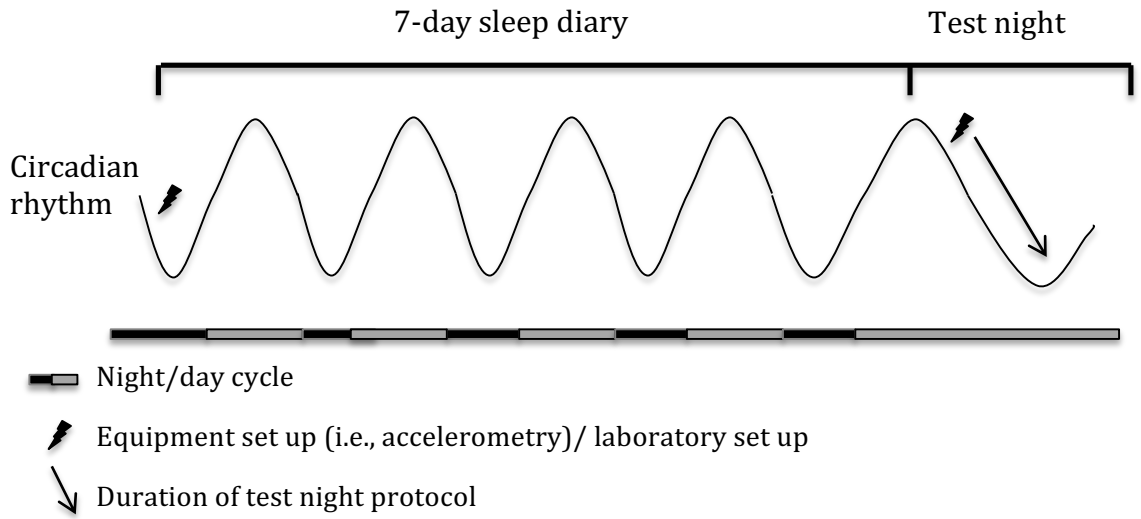
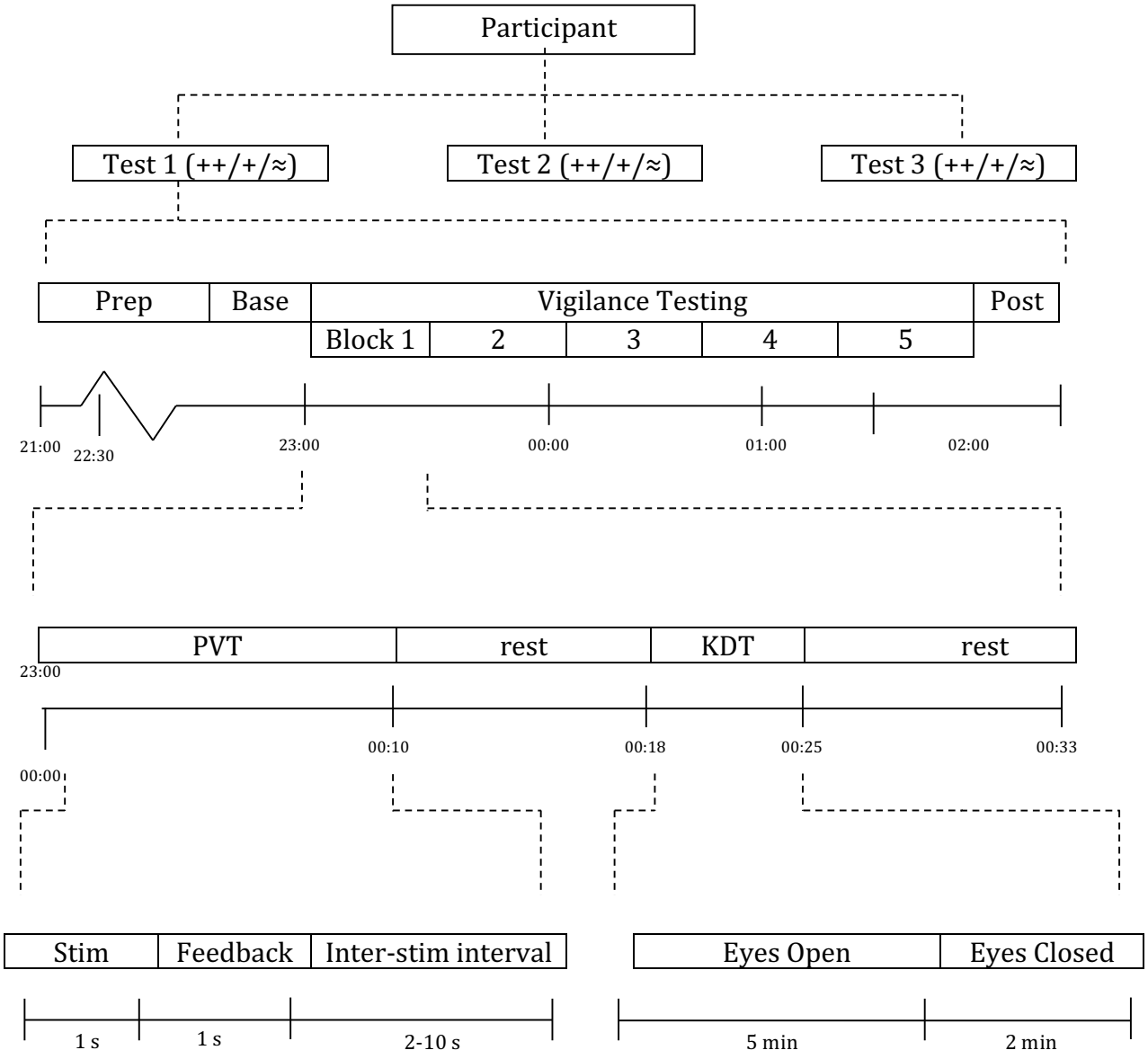


Figure 3.1: Protocol overview

The experimental design is detailed schematically in Figure 3.2. Environmental temperature and humidity were maintained using an environmental chamber (School of Physical Education Sport and Exercise Sciences, University of Otago, NZ). The three test nights were separated by 7 days of normal sleep, with the exception of one male who was delayed a fortnight in his third trial due to sickness. Females participated in the follicular phase (days 4 to 12) of their

menstrual cycle allowing one month between sessions. One female experienced a lapse in her cycle due to excess training, and therefore participated in the zone based upon her previous two cycles. This study was approved by the Otago Human Ethics Committee (Protocol 13/087).

Figure 3.2: *Study overview*



++ Moderate condition
+ Mild condition
≈ Control condition

3.1 Experimental protocol

Familiarisation: A familiarisation session was conducted to introduce the participants to the equipment and procedure of the test night. All familiarisation sessions took place during daytime. Participants underwent a full set up of EEG electrodes and performed one 2-min practice PVT followed by a full 10-min PVT trial. Participants were urged to respond as quickly as possible to the task. No thermistors were fitted in the familiarisation. Participants were also screened for age, height and mass; these characteristics of the cohort are shown in Table 3.1. Sleep/wake cycles were monitored both objectively, using accelerometry (Actical accelerometer, Mini-Mitter Co., Inc., Bend, OR), and subjectively using a sleep diary for six days prior to testing. Actigraphic data were assessed and analysed using a custom-made Matlab programme.

Testing sessions: On the evening of testing, participants arrived at 21:00 to undergo a status assessment (Brunel questionnaire, hydration status [USG], subjective sleepiness [KSS]). Mood state was assessed prior to going to the bathroom to insert the rectal thermistor and providing urine for hydration. Once seated (~21:15), participants were served 250 mL of a 749 kJ chocolate milk drink (Calci-Strong chocolate milk, Meadow Fresh, NZ), while EEG (including ECG and EOG) electrodes and thermistors were fitted. During set up, participants were played a podcast (TED Radio Hour, and The Joe Rogan Experience).

Following set up, participants were afforded ~15 min quiet reading of chosen materials (novel or academic literature) prior to the 22:30 start time. The testing procedure took place in a dim room lit from behind through a window by lighting of the adjoined room (See Appendix C for photos of setup). Participants, forbidden from facing the light source, received 0.25 nW/m^2 in their field of view. Participants wore earplugs throughout the test to minimise extraneous noise.

Baseline data consisted of a PVT (at 22:30), followed by a question, "how sleepy do you feel?" Participants then performed the KDT, before being asked four questions: "How does the

temperature of specifically your feet feel?" "Are you comfortable with this?" "How does the temperature of the rest of your body feel?" "Are you comfortable with this?"

Participants then commenced one of the three thermal conditions: moderate cooling of the feet (25 ^{0}C), mild cooling of the feet (30 ^{0}C) or, un-manipulated/ control (~35 ^{0}C). Thermal manipulations were via custom-made water-perfused booties, as described in *Apparatus and Measures* below. Manipulation began with statement "ten-minutes of manipulation; you may read". Following initial reduction of temperatures participants began vigilance testing (~23:00); depicted in Figures 3.2 and 3.3. Feet were kept at their assigned temperature for the duration of testing.

Participants were assessed for vigilance, sleepiness, and cognitive arousal using electroencephalography, and thermal perceptions using the validated tests and scales described in *Apparatus and Measures*, at timings shown in Figure 3.2. In brief, alternating vigilance and drowsiness tasks were separated by 8 min rest periods, during which participants read quietly (Figure 3.3). The subjective questionnaires were asked between the PVT and the KDT. The PVT, KDT and associated rest and subjective questionnaires comprised a single block. PVT was repeated five times, while KDT was repeated six, with the extra KDT performed immediately after the 10-min manipulation period.

Participants were monitored constantly (visually and using electrophysiolographic variables) for signs of sleep onset. At any stage in the evening where sleep onset appeared imminent participants were roused after ~5 s with the statement "eyes open, please". During the PVT this interval was typically shorter (~2-4 s). Participants most commonly suffered from sleep onset during the eyes closed KDT, at which, they were allowed to drift for the duration of the period (2 min).

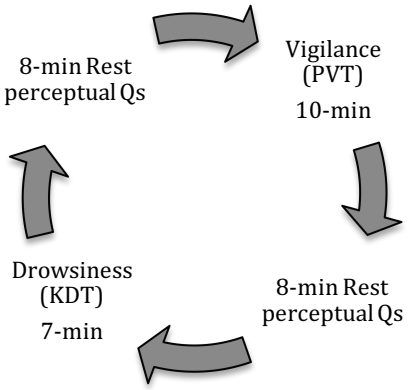


Figure 3.3: Manipulated testing cycle

3.2 Participants

Nine healthy participants (6 males, 3 females), with no sleep complaints and normalised sleep rhythms, completed the study (of a total 13 recruited and screened). Contraindications to participation in the study included the uses of vaso-active drugs or sleep medications, or participants displaying tendencies toward late night behaviour (i.e., frequently up later than 12am). Participants complaining of broken up sleep and excessive daytime sleepiness were also excluded. Participants were not screened for abnormal EEG rhythms prior to testing. Expectations of sleep/wake cycle and alcohol/caffeine intake were discussed with participants. Expectations restricted only participants' alcohol consumption and sleep patterns 48 hours before the test night. Adherence to these restrictions was checked by questioning before beginning the night session.

Table 3.1: *Participant details*

	Number	Age	Height (cm)	Mass (kg)	BMI (kg/m^2)
Male	6	19.2 ± 0.8	180.03 ± 5.96	80.40 ± 9.11	24.75 ± 1.81
Female	3	21.3 ± 2.1	161.80 ± 5.90	59.77 ± 5.93	22.82 ± 1.64

3.4 Apparatus and Measures

3.4.1 Psychomotor Vigilance Task

Vigilance was measured using the Psychomotor Vigilance Task (PVT-192, Ambulatory Monitoring Inc., New York, USA), originally designed by Wilkinson et al (1982) and validated for vigilance assessment by Dinges et al (1985) and for sleep deprivation by Basner et al (2011). The PVT assesses sustained attention across a relatively brief period (10 min). It was performed while seated, with the device resting in participants' hands and against the table at an approximate distance of 40 cm from the face. The task consists of a simple 4-digit counter that appears and increments in milliseconds, to which participants respond by tapping the button with their right thumb. The stimuli appear at random intervals of 2-10 s (see Appendix D). Participants were reminded to respond as quickly as possible prior to beginning the baseline test. The number of lapses (RTs >500 ms or a missed response) per 10 min task was counted as a measure of performance decrement, indicative of behavioural awareness (Van Dongen et al., 2003). Reaction times were represented as the Reciprocal of Response Time (RRT=1000/RT), to provide a normative distribution, and minimise bias within valid responses (Caldwell et al., 2003; Fronczek et al., 2008). Thus, consistently larger numbers with few lapses indicate better vigilance. Averages were calculated per minute to quantify the typical performance decline across the course of the time-on-task (Fronczek et al., 2008).

3.4.2 Electrophysiological measures (EEG, EOG, ECG)

Cognitive arousal was measured using electroencephalography at seven scalp locations: F3, F4, C3, C4, Pz, O1, and O2, and referenced to M1, M2 (A1 and A2 on Figure 3.3), and Fpz according to the international 10/20 system. Bipolar EOG was fitted to the outer canthi of the eyes 2 cm above and lateral to the right eye and 2 cm below and

lateral to the left eye, according to the protocol of Hakkinen et al (1993). A three-lead ECG was also applied and referenced into the EEG/EOG system. All scalp sites were inked before cleaning sites and fixing the electrodes. Electrodes were not fixed in place but instead held firm by a head wrap. Prepping sites involved abrasion of the skin site (NuPrep, Weaver & Co, Colorado, USA), followed by cleaning with an alcohol swab (Alcohol Preps, Tyco Healthcare, Mansfield, USA). Saline paste (10/20 Paste, Weaver & Co, Colorado, USA) filled the gold cup electrodes for conductivity. Acceptable impedance levels were <5 kΩ. Electroencephalography was recorded continuously, with markers indicating onset of cognitive tests. The test-retest reliability of EEG has been validated (McEvoy et al., 2000).

Cognitive arousal was assessed using the KDT (Akerstedt et al., 1990). The KDT involves 5 min eyes open followed by 2 min eyes closed. During the eyes open section participants fixated upon a non-moving point (e.g., drawing pin) approximately 1 m in front of them. Frequencies commonly associated with the KDT are theta (4-7 Hz) and alpha (8-12 Hz). All electrophysiological signalling was compiled in the polysomnography (PSG) system (Compumedics E-series, Compumedics Ltd, Victoria, Australia). Within the software used, sample rates for EEG, EOG, and ECG were 256 Hz. High (0.3 Hz) and low (70 Hz) band pass and notch (50 Hz) filters were also applied. Later analyses used Fast Fourier Transform and power spectral analysis to derive the frequencies of interest.

Electrocardiographic recordings of the R-R interval were observed to obtain a highly reliable and valid measure of the sleep onset associated heart rate decline. This was performed using two lead ECG added to the PSG software. ECG was analysed as change over both the test session as each PVT within sessions.

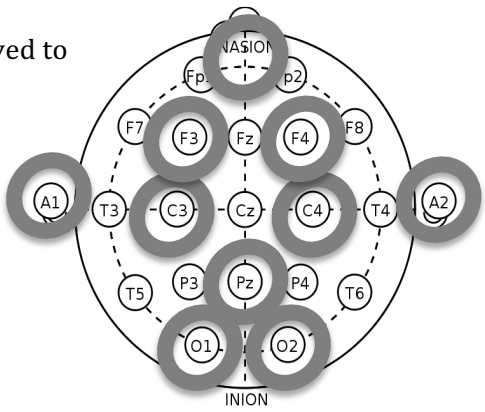


Figure 3.4: International 10/20 system. Scalp locations used are circled.

3.4.3 Sleep onset/staging

Sleep scoring was based upon the Reichtshaffen-Kales criteria (Rechtschaffen et al., 1968) for analysing sleep stages using EEG and modified for drowsiness using (Santamaria et al., 1987) definitions for drowsiness zones (See Appendix D for outlines). Stage wake (W) was defined as containing low voltage, mixed frequencies, including predominantly alpha band. The drowsiness periods between wakefulness and NI sleep are not well defined, but the current study used the guidelines provided by (Santamaria et al., 1987). Specifically, in following from waking into drowsiness alpha slows, as fatigue progresses beta frequency disappears, alpha continues to slow losing amplitude, then becomes more diffuse, progressing anteriorly. Finally, alpha amplitude minimises or disappears into N1 sleep. With eyes closed, alpha dominates ~50%, with low voltage, high frequencies present. N1 presents low voltage, mixed frequency with theta frequency rising in power and appearance of occasional vertex sharp waves. N2 was recognised as including at least one K complex but less than 20% delta frequency. Participants were not expected to progress into sleep stages beyond N1, however clarification was beneficial during eyes closed KDT period.

3.4.4 Temperature

Core temperature was measured using a flexible, sterile thermistor (Mallinckrodt 400 general purpose, Mallinckrodt Medical Inc. St Louis, USA) placed in the rectum prior to equipment set up each night. Participants were instructed on the insertion of their own thermistor (10 cm past the anus). Each participant reused his or her own rectal thermistor for each of the test nights, following its disinfection. Factory calibration was assumed for thermistors. As thermistors were reused within participants, absolute accuracy was less important.

Skin temperature was recorded at nine skin sites: dorsal and ventral aspects of the first distal phalanx of the foot, mid-plantar aspect of the foot, 1st tarsometatarsal joint, mid lateral calf, mid anterior thigh, chest (at xiphisternum), mid anterior forearm, palmar surface of the hand, first distal phalanx, and the forehead. Temperature was recorded from insulated skin thermistors (EU thermistors, Grant Instruments Ltd, Cambridge, UK), taped to the skin.

All temperature data were collected using a portable data logger (2020 Squirrel, Grant Instruments, Cambridge, UK) in 1 min intervals. Ambient dry bulb and water temperatures were also measured throughout. Distal-proximal gradient was derived from skin temperatures. Distal sites were separated into upper limb (forearm, palm, and hand), and lower limb (toe pad, toe top, foot arch, foot 1st MT, and calf). Proximal skin sites for the gradient included: thigh, chest and forehead.

Temperature manipulations were based upon pilot data (Appendix F). Manipulation was achieved through use of custom-built water-perfused booties (See appendix G). These booties were custom fitting, and lace up with elastic lining the outside of the arch to ensure contact of water-perfusion tubes through the mid foot. These were perfused with cooled water in the manipulated sessions, such that the temperature modulations achieved reached approximately -5 ^{o}C and -10 ^{o}C less than the evening distal temperature of 35 ^{o}C. DPGs associated with distal temperatures were separated between upper and lower limbs. An urn served to provide a reservoir for the circulating water, and the means by which water temperature was altered. The water temperature required to maintain constant skin temperatures were variable and had to be constantly reassessed. Temperature of the water was monitored before it entered the booties.

3.4.5 Actigraphy and sleep diary

Each participant's sleep-wake cycle was measured using both accelerometry and sleep diaries (Sleep diary and activity monitoring provided in Appendix B). The specialised accelerometers used were worn against the skin around the waist using belts (Actical, Philips Respironics, USA). The accelerometers were designed to be worn at all times (e.g., including shower). If removed for longer than 5-10 min, participants were expected to record time off and time back on as well as reason for removal/activity while not worn. Accelerometry was recorded for the week prior to testing, in conjunction with the 7-day sleep diary. Accelerometry was used to ensure validity of sleep wake cycle and to accompany the 7-day sleep diary. The data were analysed for standard sleep-wake variables using the count-scaled algorithm incorporated within Matlab script (Galland et al., 2012). The 7-day sleep diary included: start date, bedtime, and wake up time. 48 hours prior to testing participants were expected to adhere to normal sleep times.

3.4.6 Subjective questionnaires

Subjective measures were assessed either side of each PVT, with sleepiness recorded immediately pre and post. Sleepiness was recorded using the 9-point Karolinska Sleepiness Scale (KSS): 1= extremely alert, 2= very alert, 3= alert, 4= rather alert, 5= neither alert nor sleepy, 6= some signs of sleepiness, 7= sleepy, no effort to stay awake, 8= sleepy, some effort to stay awake, 9= very sleepy, great effort to keep awake, fighting sleep (Appendix H). The KSS has been validated against objective physiological measures, specifically that of the KDT (Akerstedt et al., 1990; Kaida et al., 2006; Putilov et al., 2013).

Perceived temperature was recorded using a modified 13-point Thermal Sensation Scale: 1= unbearably cold, 2= extremely cold, 3= very cold, 4= cold, 5= cool, 6= slightly

cool, 7= neutral, 8= slightly warm, 9= warm, 10= hot, 11= very hot, 12= extremely hot, 13= unbearably hot (Cotter et al., 2005). Thermal sensation was augmented by the thermal discomfort scale: 1= comfortable, 2= slightly uncomfortable, 3= uncomfortable, 4= very uncomfortable, 5= extremely uncomfortable (Appendix I). The questions were asked at the beginning of each rest period, "how does the temperature of your body feel?" At baseline, adjusted baseline, and post, participants were also asked to separate the sensation of their feet from the rest of the body; "how does the temperature of specifically your feet feel?" "How does the rest of your body feel?" Extended from the scales designed and validated by Gagge et al (1996). Thermal sensation and discomfort questionnaires are highly validated to reflect underlying thermoregulatory changes (Candas et al., 2007; Cline et al., 2004; Kräuchi et al., 2008), but can be prone to skew (e.g., focus of perception on other body regions) and are often temporally delayed (Cline et al., 2004; Cotter et al., 2005).

Mood state was assessed by the Brunel Mood State questionnaire (BRUMS; Appendix J) (Terry et al., 1999). The BRUMS assesses changes in participants' current state (Lal et al., 2002). There are 24 adjectives comprising six elements of mood (anger, confusion, depression, fatigue, tension, and vigour). For the sake of brevity and specificity only the constituents of vigour and fatigue were analysed. The BRUMS (Appendix J) was asked upon entry into the lab, and post-test, to assess emotional status of the participants for potential confounding factors. Participants were asked to rate how they feel right now on a five-point Likert scale. Scales were anchored to verbal cues of: "not at all", "a little", "moderately", "quite a lot" and "extremely".

3.4.7 Hydration Status

Hydration status was measured pre- and post-test as urine specific gravity, using a portable refractometer (Atago, Astra, Zeneca Pty Ltd, Japan). A USG below 1.020 (range:

1.001-1.025) denotes euhydration. Above 1.030 indicates the beginning of dehydration. No participant needed to empty their bladder during any of the tests.

3.5 Data analysis

A priori determination of sample size was not performed in the current study. A sample size of 14 was initially selected based upon (a) the much higher statistical power achieved with a repeated measures design, and (b) prior research addressing a similar question obtaining statistically significant findings with ~8 participants (ranging from n=7-16) (Caldwell et al., 2003; Fronczek et al., 2008; Krauchi et al., 1994; Raymann et al., 2005). Due to time and participant constraints this was lowered to nine, following prolonged difficulty with recruitment and retention of prospective participants (e.g., recruitment was undertaken for a duration of 12 months).

3.5.1 *Data Reduction*

Missing data were interpolated when required (this was less than 10% of the data). Specifically, coefficient of variation was checked, and if sufficiently small (i.e., <10%) participant data were interpolated by averaging the difference of adjacent data points. Data sets were analysed primarily in SPSS (SPSS, IBM, New York, USA), and Prism (Prism, GraphPad Software, California, USA).

Prior to analysis, PVT reaction times were converted into Reciprocal reaction time (RRT=1000/RT). Due to the random ISI there was various numbers of responses. As such, responses were condensed into 1 min blocks. Lapses (responses >500 ms) were removed from RRT analysis.

Electroencephalography required significant data processing prior to statistical analyses. Derivations of significant components of EEG were made using Matlab. Analysed channels were minimised to C4-A1, O2-A1. From the continuous recording, data were minimised into seven blocks of KDT for each channel. KDT's were visually scanned for artefacts and, where problematic, were removed from analysis. Blinks were automatically removed in Matlab using an algorithm along with artefacts. KDT data were further minimised into 15 s epochs (20 for eyes open and 8 for eyes closed) using the Hanning cosine window. A Fast Fourier Transform was then performed to derive power frequencies from each block. Of these blocks Absolute power density (uV^2) was calculated for theta and alpha and beta frequency bands over derivations: C3, C4, O1, and O2. Data was further reduced by averaging the 15 s epochs into whole trial periods (KDT1 eyes open, KDT1 eyes closed, etc.); where by the repeated-measures ANOVA could be applied.

3.5.2 Statistical Analyses

Where sphericity was violated, as determined by Mauchly's test, degrees of freedom were corrected using Greenhouse-Geisser estimates so as to determine significance ($P<0.05$). The analyses that required correction were: Foot locations, Thermal discomfort, KSS, RRT, and relation of T_C and RRT.

Two-way and three-way repeated measure ANOVA was used for most analyses. Two-way ANOVA was used to assess order effect in sleep patterns of the 6-days preceding each session. Contrasts were made to observe differences between six nights and two nights before session, to assess differences between normal sleep patterns and adherence to sleep recommendations. Pairwise comparisons with Bonferroni correction were carried out. Temperature data were also analysed using a two-way ANOVA, to assess the effects of conditions on various temperatures (individual foot measures, core, upper limb, DPG, PCG). Other measures such as ECG, and subjective scales were

analysed using a two-way ANOVA. A three-way ANOVA (3 x 6 x 10) was used to assess the effect of thermal conditions on PVT outcome parameters (RRT, lapses). Subjective questionnaires were also analysed as two-way ANOVA, but with ordinal variables. EEG was analysed using two-way ANOVA.

4.0 RESULTS

Results are presented in a sequence progressing from standardisation variables (e.g., compliance and sleep diary) to foot temperature (i.e., the independent variable) and associated body temperature, then vigilance, and electroencephalography. Relations between physiological and perceptual measures are reported last. Data are mean ± S.D. unless stated otherwise, with 95% confidence intervals for comparisons of interest.

Variability bars (e.g., SD) are omitted from some figures for clarity, and because they would typically illustrate between-participant variability whereas the variability of major interest is the within-subject variability (of the mean difference between conditions).

4.1 Participant characteristics and compliance

4.1.1 Screening

Of the participants screened, two completed a single night of testing. Both these participants were females and suffered bouts of extreme sleepiness during the test, such that they were unable to complete a single PVT without prompts. Both participants subsequently expressed an unwillingness to perform another test, and were therefore removed. One further screened participant professed to be epileptic and was taking vasoactive drugs, while another was obese and suffered from sleep apnoea, so both were excluded, leaving n=9 who completed all testing.

4.1.2 Adherence

Adherence to caffeine and alcohol restrictions was checked verbally at the beginning of each testing session. Of those not complying fully: P1 consumed caffeine at midday of her Control test day; P5 consumed caffeine at 9 am of his Moderate test day; and P6,

before noon. P6 also consumed one beer the night before each test. Visual checking of their data produced no obvious outlier data.

4.1.3 Sleep diaries – sleep onset

Sleep diaries and accelerometers were provided to ensure "normal" healthy sleep-wake cycles occurred before test nights. Participants were not expected to comply completely to regimented sleep times, particularly well ahead of their test night. Participants that suffered a week of poor sleep (e.g., assignment due, or sickness) were deferred until a suitable date. Compliance to regular sleep onset schedules was variable between as well as within participants. Sleep data comprise accelerometry and sleep diaries. Complete sets of accelerometry and diaries were obtained from three and six participants, respectively. Seven participants provided a complete record for at least one of these methods of daily sleep monitoring. Resulting sleep data are comprised of an average of both accelerometry and sleep diaries due to the poor adherence of subjects to filling out their diaries and wearing the accelerometer. Table 4.1 (p.79) summarises normal sleep behaviour in all groups and the variability is described in more detail below.

Bedtime: A trend toward earlier bed times occurred across the six testing days ($F_{(5,30)}$=2.363, P=0.064), but not differentially between conditions ($F_{(2,12)}$=1.77, P=0.211). As there was no interaction effect, it is evident that there was no significant displacement of bedtime between regulated and unregulated nights (i.e., day 1 verse day 5 or 6). On the two nights before testing (days 5, and 6) participants got to sleep on average at 23:22 (hh:mm) (± 41 min) and 22:45 (± 43 min), whereas six nights out from testing (day 1) participants went to sleep on average at 23:43 (± 49 min).

Waking: There was no interaction effect on wake up time ($F_{(10,60)}$=0.97, P=0.482). The mean wake up time on the day of testing was 08:21 (± 42 min). The large between-subject variability reflects earlier typical wake up time of the rowers in the group.

Duration: Sleep duration averaged 8.7 ± 0.2 hours per night, with no difference between conditions ($F_{(2,12)}$=0.11, P=0.894). Sleep duration ranged from a minimum of 5 h on one occasion and ~6 h for several participants, to a maximum of 12 h for two participants on one occasion. Control was the most consistent condition for sleep hours, whereas Mild and Moderate both tended to show increased sleep hours between days 5 (8.10 ± 1.29 h, 8.3 ± 1.6 h, respectively) and 6 (9.6 ± 1.66 h, 9.8 ± 1.2 h, respectively); this did not show an order effect.

Timing: Six participants adhered to the recommendation of going to sleep before midnight throughout the six days preceding a test night, but the other three participants still accrued an equivalent (unpaired *t*-test: P=0.21) total duration of sleep in this period. Within the two days before test night, where the recommendation was expected to be adhered to, two participants did not conform to sleep onset times, but had similar total sleep times (group: 8.8 h; n=2: 8.7 h).

Order effects: No order effects were evident across the three pre-trial periods for sleep onset time ($F_{(2,12)}$=0.49, P=0.368), wake up time ($F_{(2,12)}$=0.11, P=0.896), or total sleep time ($F_{(2,12)}$=0.44, P=0.652).

4.1.4 Mood state characteristics and hydration

Mood state and hydration were tested before and following each test. The elements of mood analysed from the BRUMS were vigour and fatigue because of their relevance in this study. Vigour declined ($F_{(1,8)}$=44.70, P<0.0001); not differentially so between conditions ($F_{(2,16)}$=2.31, P=0.131), but there was a main effect of condition ($F_{(2,16)}$=5.01, P=0.021). Specifically, vigour was higher in Control than in Mild (Appendix K: Figure 1).

Fatigue increased significantly across the test ($F_{(1,8)}$=40.13, P<0.0001), but not differentially (interaction: $F_{(2,16)}$=1.99, P=0.168), and was not different between conditions ($F_{(2,16)}$=0.56, P=0.584; illustrated in Appendix K: Figure 2).

Participants entered the test hydrated and ended the test mildly hypohydrated according to USG values (Table 4.2)

Table 4.1: *Bed time, waking time and total sleep time (TST) determined from the 7-day sleep diary and accelerometry.*

Condition	Bed time (± min)	Waking time (± min)	TST (hours (± min)
Control	23:05 ±125	08:21 ± 139	8.70 ± 0.40
Mild	23:44 ± 132	08:11 ± 146	8.66 ± 0.59
Moderate	23:08 ± 141	08:09 ± 132	8.64 ± 0.76

Note: Data are compiled from both measures, as recorded from 7 of the 9 participants due to poor adherence to reporting in the diary and wearing the accelerometer. Where both measures were obtained concurrently, the data appeared to be comparable (not tested formally).

Table 4.2: *Subjective sleepiness and hydration in testing sessions*

	Pre	Manipulated	Post
Subjective Sleepiness			
Control	6.8 ± 1.1	7.2 ± 1.1	8.3 ± 0.7
Mild	6.4 ± 2.1	6.2 ± 1.5	8.6 ± 0.7
Moderate	7.3 ± 1.2	6.6 ± 1.7	8.3 ± 0.8
USG	1.019 ± 0.002		1.026 ± 0.004

Note: "Manipulated" refers to the first measures following initial 10 min manipulation period. Sleepiness data are from the Karolinska sleepiness scale (KSS), which has a range from 1 – "extremely alert" to 9 – "Very sleepy, great effort to stay awake, fighting sleep". USG is urine specific gravity. Data are the means and SD for n=9.

In the Control condition, P1 suffered a vasovagal episode following the final PVT. The participant's data for this condition are retained because they otherwise showed no atypical responses in any dependent variable up to this point (i.e., for T_C, heart rate, perceptions, PVT or EEG).

4.2 Induced Temperatures

The ambient temperature in the environmental chamber was equivalent between conditions; averaging 24.4 ± 1.1 ^{O}C. Relative Humidity was set at 33% (Set point ratio 8.1). Both ambient and water temperature for each of the three conditions are represented in Table 4.3. Temperature did not deviate significantly across the evening.

Foot temperatures at baseline were 35.1 ± 0.3 ^{O}C. Participant 8 displayed a T_C rhythm (shown in Appendix L) contrary to all other participants and the expected pattern for this time of day (see Figure *2.4 Circadian temperature rhythm*). This unusual T_C rhythm was thought to not be caused by errors in experimental design (i.e., timing of menstrual cycle) or technical problems (e.g., moisture accumulating in the electrical junction). Her temperature data have been omitted from the mean, but her other results retained because her response data for all dependent measures were checked against those of the remaining participants and were not outliers.

Table 4.3: *Ambient and water temperatures of associated conditions.*

Condition	T_{amb} (^{o}C)	T_{water} (^{o}C)		
		Baseline	Manip (i)	Manip (f)
Control	24.46 ± 0.24	19.92 ± 0.21	18.87 ± 0.07	22.70 ± 0.01
Mild	24.57 ± 0.17	20.46 ± 0.28	13.52 ± 0.28	22.00 ± 0.15
Moderate	24.16 ± 0.30	20.29 ± 0.24	3.21 ± 0.31	16.37 ± 0.32

Note: Manip (i) refers to the period immediately following 10 min reduction of foot temperature to assigned condition, while Manip (f) refers to the end of the manipulation and test period.

4.2.1 Temperature manipulation

Foot temperature (Figure 4.1) was manipulated by controlling urn temperatures (Table 4.3), with water flow through the booties turned on following baseline recordings. Cooling protocol (water temperature) varied between participants depending on their foot temperature susceptibility to the manipulation.

4.2.2 Foot Temperatures (Figures 4.1a, 4.2 and 4.3)

Foot temperature declined to a different extent between conditions (interaction effect: $F_{(40, 280)}$=39.58, P<0.0001) (Figure 4.1). Contrasts revealed that differences from baseline were significant by the ~30th minute for the mild condition ($F_{(1,7)}$=32.90, P=0.001), and by the 20th minute for the Moderate condition. The rate of skin cooling across minute 20 through to minute 40 was approximately doubled between Mild and Moderate cooling (Figure 4.1a: slope = -0.14 ^{o}C/min verses, -0.26 ^{o}C/min). Foot temperature was thereafter maintained at 30.8 ± 0.2 ^{o}C, and 26.4 ± 0.1 ^{o}C, for Mild and Moderate cooling conditions, and at 34.5 ± 0.5 ^{o}C in the un-manipulated Control condition (Figure 4.1a). Larger variability evident in Control is due to lower foot temperatures in some participants (P6: 32.19 ± 1.00 ^{o}C; P8: 29.27 ± 1.26 ^{o}C).

Foot temperature differed significantly between thermistor location and condition (Interaction: $F_{(6,42)}$=13.79, P<0.0001) (Figure 4.2). Temperatures on the foot became as much as 5.4 ± 0.6 ^{o}C (Moderate) below those of the toe (Figure 4.3). Temperatures did not differ significantly between different toes (difference: 0.05 ^{o}C, P=1.00), or between different locations on the foot itself (difference: 0.17 ^{o}C, P=1.00).

4.2.3 Upper limb

Upper limb temperatures remained in the range 34-35 ^{o}C for all conditions. Although ANOVA showed a clear difference in response between conditions (Interaction: $F_{(40,280)}$=2.064, P<0.0001; Figures 4.1b, and 4.5a), contrasts did not reveal the source of these differences.

4.2.4 Core temperature

Baseline T_C's were 36.94 ^{o}C (±0.22), 36.91 ^{o}C (±0.32), and 36.91 ^{o}C (±0.30) for Control, Mild and Moderate, respectively. T_C declined across the evening, displaying no differential trends (Figure 4.1c). The rate of T_C decline was on average -0.002 ± 0.00015 ^{o}C/min across all conditions, such that final T_C was approximately 36.5 ± 0.1 ^{o}C. There was no interaction effect on T_C ($F_{(40, 280)}$=0.768, P=0.843). T_C did not differ significantly between conditions ($F_{(2, 14)}$=0.188, P=0.831), however it decreased across the test ($F_{(20, 140)}$=27.77, P<0.0001).

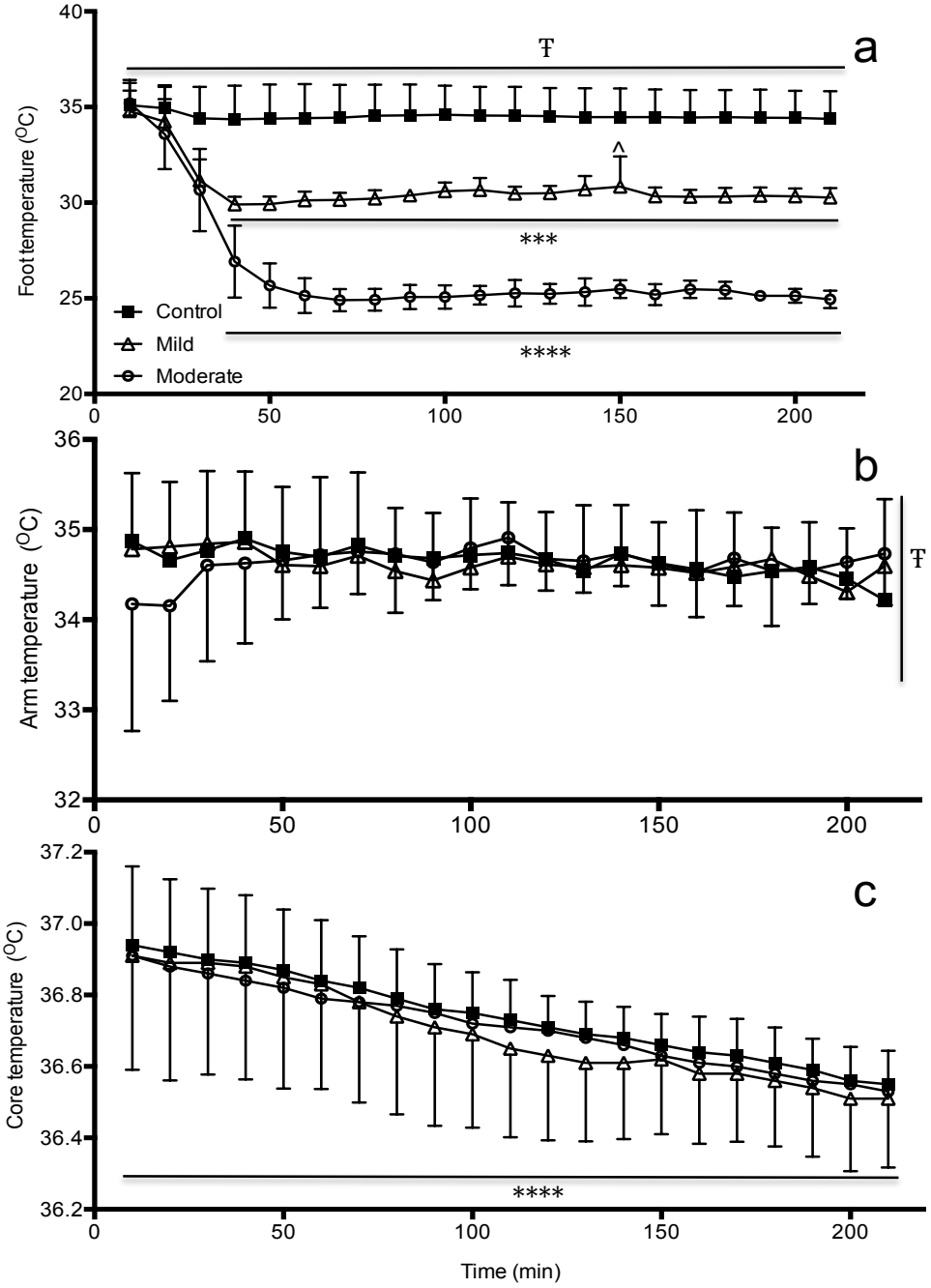


Figure 4.1: Average foot (a), average upper limb (b) and core (c) temperatures across the three conditions; Control (■), Mild cooling (Δ) and Moderate cooling (O). Time is measured in blocks of 10 minutes. Data are mean and SD for eight participants.

* = P<0.05, ***=P<0.001 ****= P<0.0001, Ŧ= Significant interaction for both LL and UL.

^ - Increased variability observed at the 150th minute in Mild is due to P4 foot temperatures rising across 20-minutes to the physiologically "normal" evening temperature. This experimental error was quickly remediated following the 150th minute.

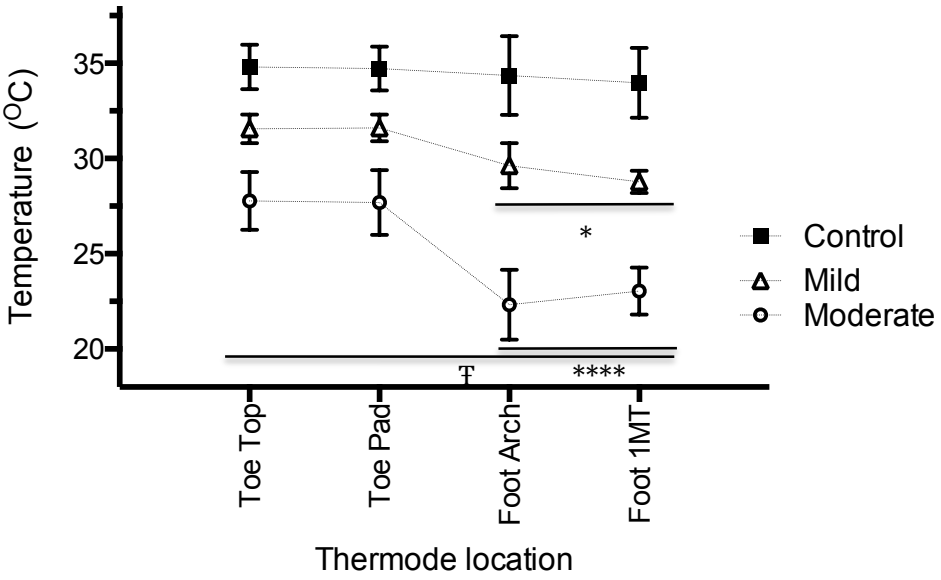


Figure 4.2: Localised foot temperature parameters comprising average foot temperature across conditions: Control; Mild cooling; and Moderate cooling. Data are mean and SD for eight participants.

Note: Thermistor locations are: TP= toe pad, TT= toe top, FA=foot arch, FM=1st tarsometatarsal joint.

* = P<0.05, ***=P<0.001 ****= P<0.0001, Ŧ= Significant interaction

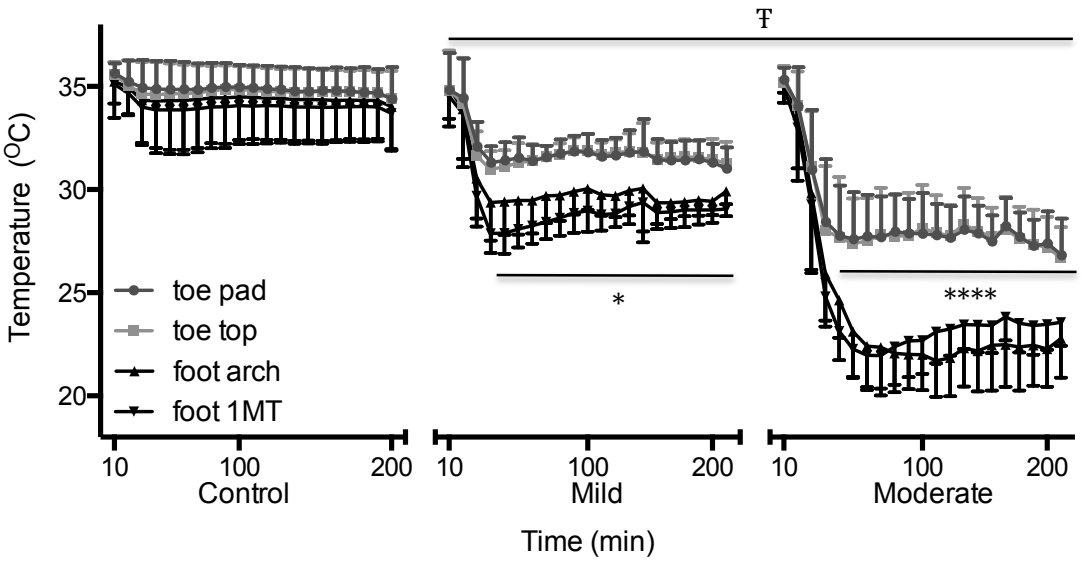


Figure 4.3: Temperature across time and conditions of four individual foot thermistors comprising average foot temperature. Data are mean and SD for eight participants.

* = P<0.05, ****= P<0.0001, Ŧ= Significant interaction

4.2.5 Temperature Gradients

The distal-proximal gradient (DPG) for the lower-limb was measured between the manipulated distal site (four thermistors comprising average foot, as well as calf), minus proximal sites (thigh, chest, and forehead; Figure 4.4a). The upper limb DPG was calculated as finger, hand and forearm minus aforementioned proximal sites. Baseline DPG was slightly positive, 0.71 ± 0.44 and 0.32 ± 0.07 for the lower and upper limbs respectively. The lower limb gradient altered in accordance with foot cooling, whereas the upper limb gradient remained unchanged throughout the trial (Figure 4.4a). Specifically, the upper limb DPG showed no significant time effect ($F_{(20,80)}$=1.65, P=0.062), or an interaction effect ($F_{(40,160)}$=0.95, P=0.566), but a significant condition effect ($F_{(2,8)}$=7.30, P=0.016). The lower limb DPG showed an interaction between condition and time ($F_{(40, 280)}$=46.53, P<0.0001), reflecting the Mild gradient becoming significantly larger than in Control by the 30th minute ($F_{(1,7)}$=104.61, P<0.0001), after which it stabilised at 4.05 ^{o}C larger (C.I.: 2.65, 5.45), and larger in Moderate than Control by the 20th minute, after which it stabilised at 8.6 ^{o}C (CI= 7.32, 9.81).

The proximal skin-to-core gradient (PCG) differed significantly as a function of condition and time ($F_{(40,280)}$=1.67, P=0.010) (Figure, 4.4b). Despite apparent differences in variability illustrated in Figure 4.4b, sphericity was not violated ($\chi^2(2)$=0.284, P=0.883). Contrasts revealed that there was no significant effect within Mild condition, but that Moderate differed significantly from Control by the 30th minute ($F_{(1,7)}$=7.67, P=0.028). The PCG was always negative (Control: -2.90 ± 0.07, Mild: -2.6.10 ± 0.11, Moderate: -2.49 ± 0.12), but Moderate condition was slightly less so. The difference from Control was -0.20 (C.I.: -0.53, 0.13, P=0.287) for Moderate, whereas Mild was -0.08 (C.I.: -0.46, 0.30, P=1.000).

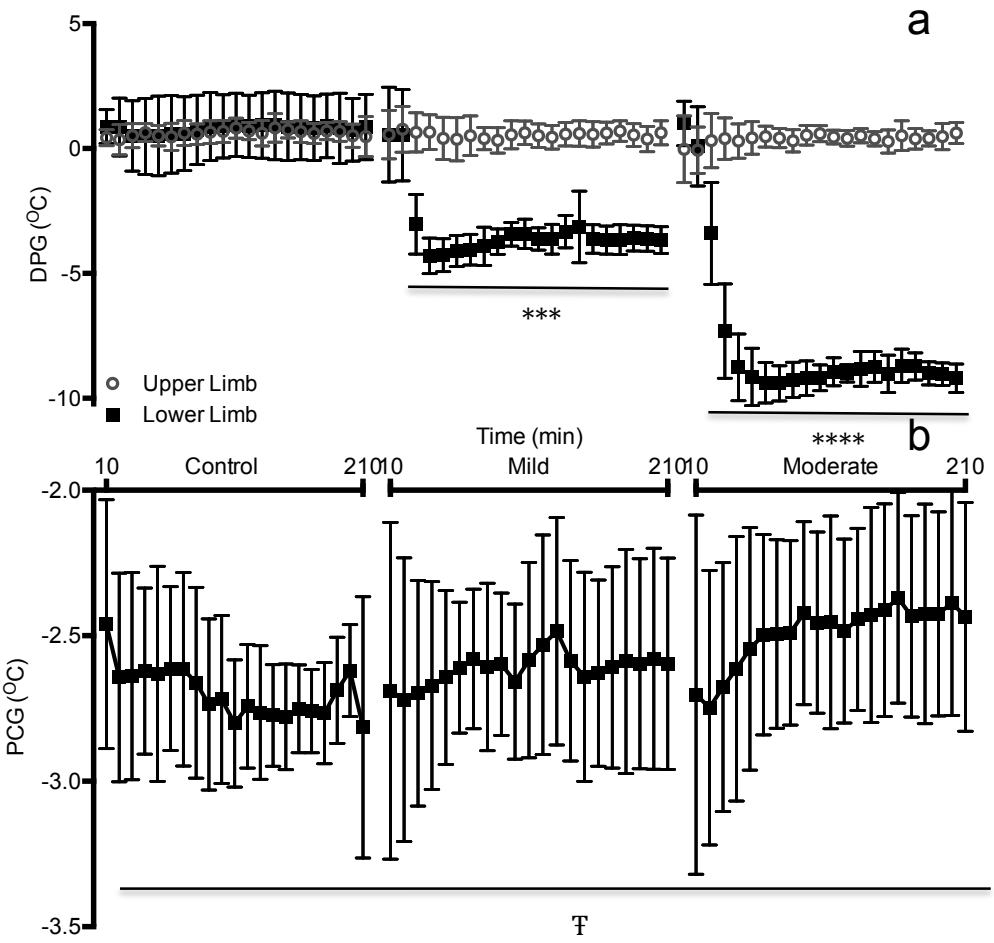


Figure 4.4: (a) Upper (O) and Lower limb (■) distal proximal gradients (DPG) across the three conditions: Control; Mild cooling; and Moderate cooling. (b) Proximal to core gradients (PCG) across the three conditions. Data are mean and SD for eight participants.

Note: Each data point represents a ten-minute average.

***=P<0.001 ****= P<0.0001, Ŧ= Significant interaction for PCG

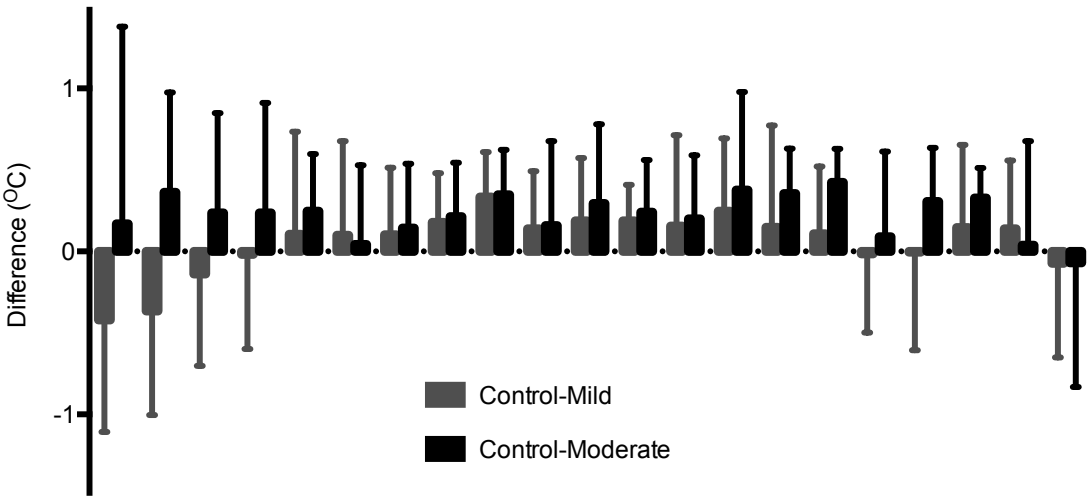


Figure 4.5: Difference in Mild and Moderate upper limb gradients from Control.

Note: A positive difference reflects a more negative DPG. Each data point represents a ten-minute average as mean and SD for eight participants.

4.2.6 Temperature Perception

Participants' thermal sensation showed a significant interaction between condition and time ($F_{(22,154)}$=2.52, P=0.001). Participants began the test with a thermal sensation of "warm" (9.1 ± 0.0), with an associated thermal discomfort of "slightly uncomfortable" (1.9 ± 0.2) (Figure 4.6). Following manipulation, thermal sensation underwent an initial decrease in all conditions. It decreased below Control for both Mild and Moderate ($F_{(1,7)}$=25.74, P= 0.01; $F_{(1,7)}$=21.13, P=0.002) with sensations of "slightly cool" (6.5 ± 0.9) and "cool" (5.5 ± 1.3), respectively, by min 30 and remained lower than in Control thereafter (P<0.05). The initial sensation of cooling in Mild and Moderate became sensations of neutrality (7.6 ± 0.4; 7.1 ± 0.4), representing a difference of 1.2 scores (C.I.: -0.3, 2.8, P=0.107), and 1.7 scores (C.I.: 0.1, 3.3, P=0.039), respectively (Pairwise comparison).

Thermal discomfort was not differentially altered by the foot cooling interventions (Interaction effect: $F_{(22,154)}$=1.04, P=0.425; Figure 4.6). There was also no main effect of condition ($F_{(2,14)}$=1.63, P=0.242) or time ($F_{(11,77)}$=1.55, P=0.133) on thermal discomfort. Participants ranked Control as most uncomfortably warm.

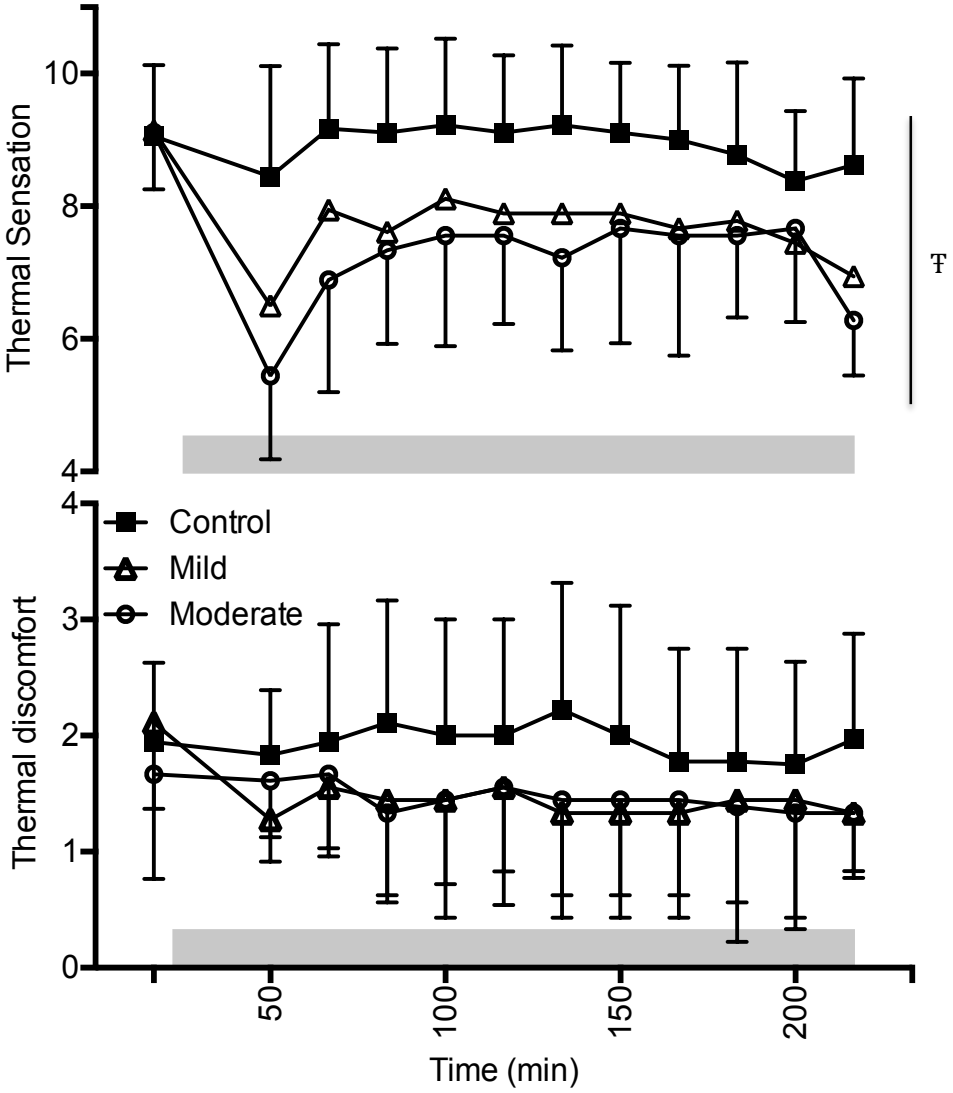


Figure 4.6: Whole-body thermal sensation and thermal discomfort across the test duration for each condition: Control, Mild and Moderate. Data are mean and SD for nine participants.

Note: The grey area indicates the manipulation period. Thermal sensation ranges from 1- "unbearably cold" to 13- "unbearably hot". Thermal discomfort ranges from 1- "comfortable" to 5- "extremely uncomfortable".

Ŧ= Significant interaction for thermal sensation

4.3 Subjective sleepiness

Subjective sleepiness rose steadily during testing irrespective of condition (Figure 4.7). The subjective sleepiness score (KSS) was prone to a ceiling effect, with a number of participants reaching maximum early in the test (Figure 4.8). For example, one participant (P9, mild condition) reached maximum sleepiness by baseline measures, progressing from KSS scores of 5 on entry to 9. Adjusting for those that reached ceiling early produced no change to the progression of sleepiness.

Progression of subjective sleepiness was significantly affected by duration of testing ($F_{(13,104)}$=28.91, P<0.0001), and despite a trend for this to interact with condition, it was not statistically significant ($F_{(26,208)}$=1.45, P=0.080). The trend for an interaction is presumably due to apparent early reduction in KSS scores within the two intervention conditions (Figure 4.7). Participants entered the lab at 21:00 feeling “rather alert” (4.7 ± 0.4); KSS then rose 2 (± 0.6) scores by baseline.

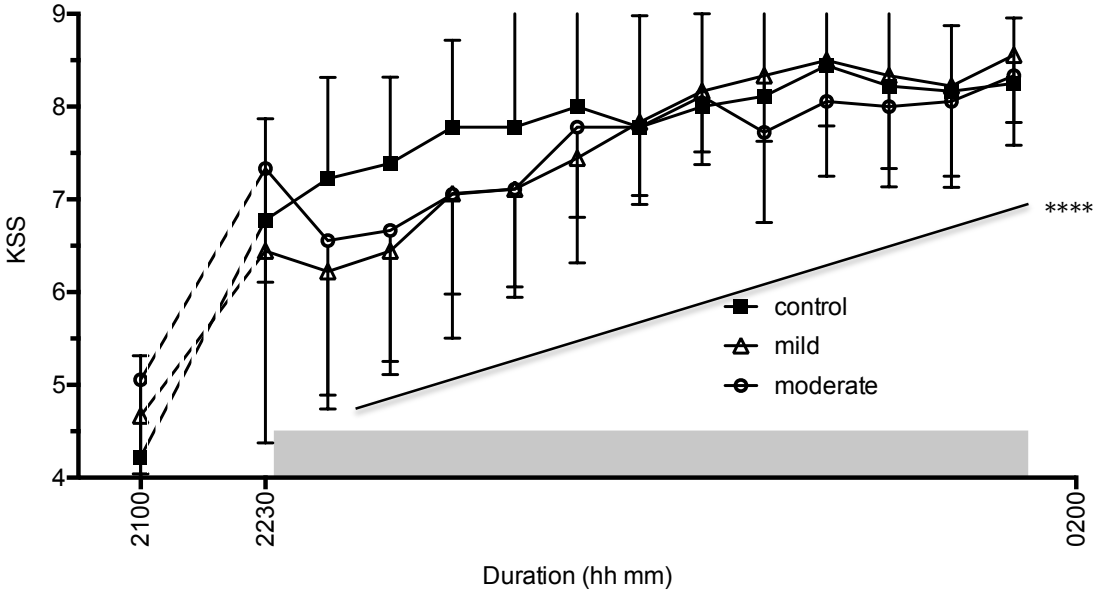


Figure 4.7: Progression of subjective sleepiness across testing for each condition: Control, Mild, and Moderate.

Note: The slashed line indicates equipment set up. The grey area indicates onset of manipulation. 2100 is entry to lab, while 2230 is baseline measures. 0200 is cessation of the test. KSS ranges from 1 to 9. Data are mean and SD for nine participants.

****= P<0.0001 effect of time

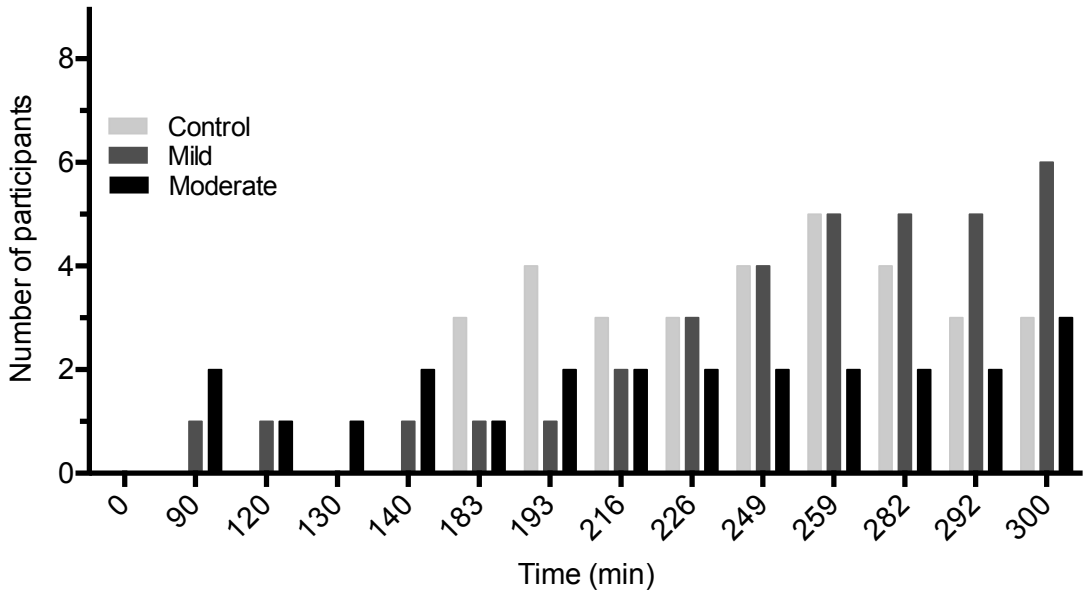


Figure 4.8: Number of participants reaching maximal subjective sleepiness rating (9– "very sleepy, greater effort to keep awake, fighting sleep") during testing, in each condition: Control, Mild, and Moderate. The numbers do not necessarily reflect the same participants remaining at maximal rating across time. Data are mean for eight participants.

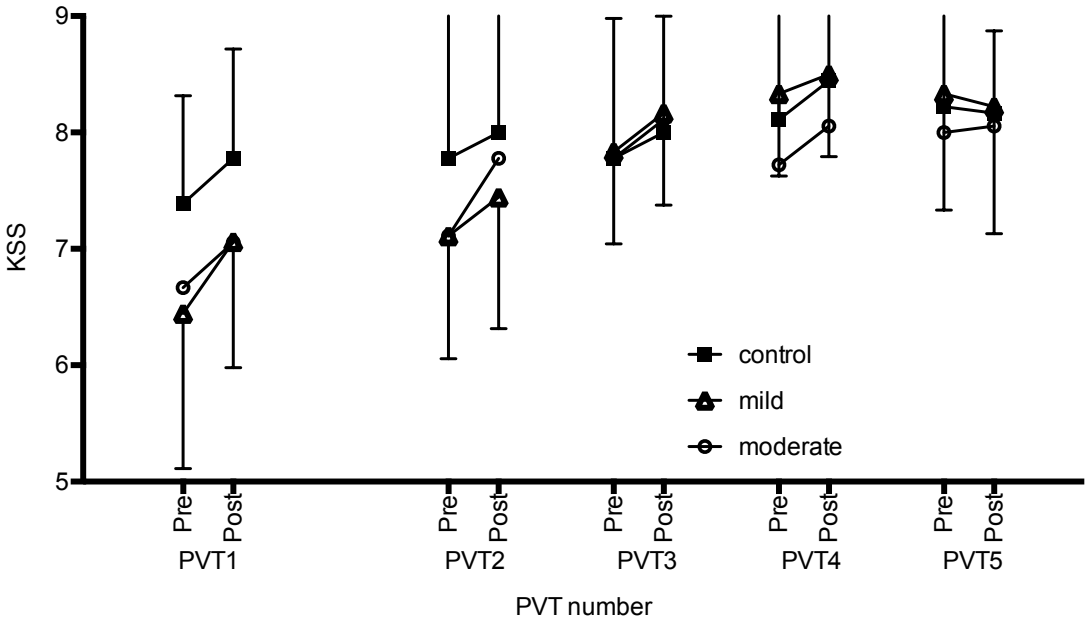


Figure 4.9: Change in subjective sleepiness score pre and post PVT across the night for each condition. Data are mean and SD for eight participants.

Note: The KSS ranges from 1– "extremely alert" to 9– "Very sleepy, great effort to stay awake, fighting sleep".

4.4 Vigilance

Eight (of the nine) participants were included in the PVT analysis; P7 was, omitted due to missing baseline recordings, while a missing baseline in Control for P1 and P5 were estimated as the average of their other two baseline recordings. There was no order effect on PVT ($F_{(2,14)}$=1.97, P=0.177).

4.4.1 Reciprocal reaction time

Reaction times were converted to reciprocal reaction time (RRT). The RRT did not respond differently between conditions across the session ($F_{(90,630)}$=0.98, P=0.448) (Figure 4.10); nor did it show main-effect differences between condition effects (Interaction: condition and test; $F_{(10,70)}$=0.75, P=0.550; Interaction: condition and time;

$F_{(18,126)}$=1.07, P=0.391). While RRT deteriorated during each test (main effect) this deterioration became more pronounced with progression across the evening (interaction of test and time: $F_{(45,315)}$=1.88, P<0.0001). Grouping of the different conditions remained tight with minimal difference (grand mean: 3.54 ± 0.12).

Reciprocal reaction time significantly decreased across the succession of PVTs ($F_{(9,63)}$=11.95, P<0.0001). The slope of decrease across the duration of a test was greatest in the baseline with mean decline of -0.60 ± 0.005 RRT/min. Following within test slopes were no less than -0.036 RRT/min. Mild condition typically had the greatest rate of decline once manipulation had begun, with Control condition having the smallest slope.

4.4.2 Variability

All conditions produced high variability in RRT (Figure 4.10a/b). Variability was greatest in Control (Figure 4.10 b); however, despite this apparent difference, there was no interaction effect ($F_{(10,70)}$=0.72, P=0.703) or condition ($F_{(2,14)}$=1.16, P=0.342) on variability. Variability of RRT fluctuated greatly minute-to-minute as well; again, this fluctuation was most apparent in the Control condition.

4.4.3 Lapses

Lapses were classed as reaction times >500 ms. Figure 4.11 indicates the collated number of lapses that occurred in consecutive tests undergoing each condition of manipulation. The mean number of lapses was 8 (± 2) per test. There was no interaction effect ($F_{(10,80)}$=1.30, P=0.299), nor any condition effect ($F_{(2,16)}$=0.812, P=0.462), or test effects ($F_{(5,40)}$=0.93, P=0.388).

A number of lapses my have been due to actual sleep onset. This is indicated by consecutive lapses producing large spikes in numbers of lapses. For example, P6

producing 46 lapses in their Control baseline (KSS rating: 8 – "sleepy, some effort to stay awake"). These spikes in lapses typically occurred late in the PVT (i.e., 8-9th min) and had a skewing effect on the mean.

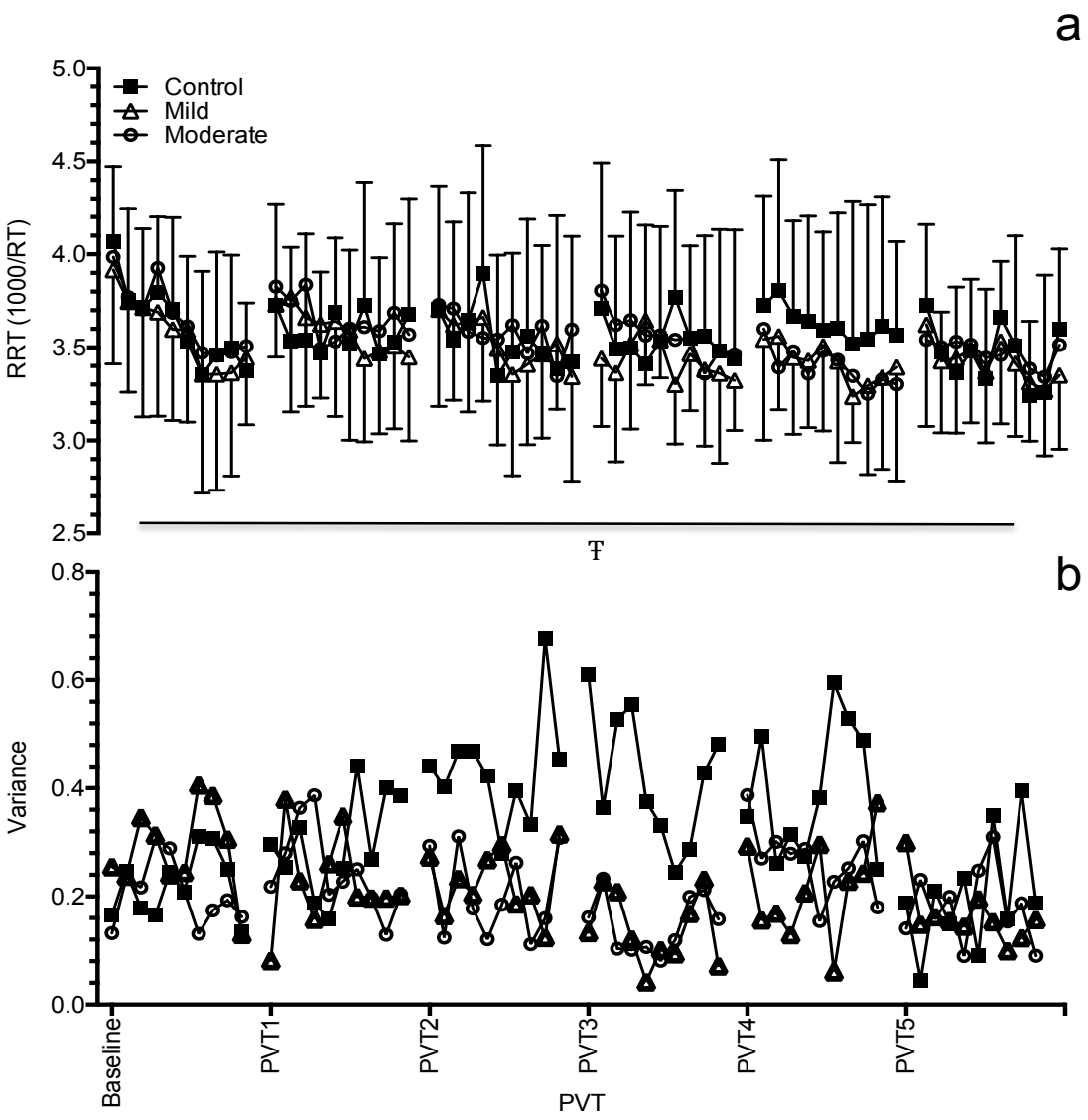


Figure 4.10: PVT reciprocal reaction times (a) and variance (SD) (b) across six PVTs between conditions: Control (■), Mild (Δ), and Moderate (O). Data are mean and SD for eight participants.

Note: Each PVT contains ten 1min averages of the reciprocal reaction times

Ŧ= Significant interaction between test and time for RRT

4.4.4 Core temperature and Vigilance

The correlation between core temperature and RRT is displayed in Figure 4.12. RRTs were similar at baseline with a maximum difference less than one (unit: 1000/RT). Similarly, there was minimal difference between core temperatures (T_C difference<0.1 ^{o}C).

There was no interaction effect ($F_{(10,70)}$=0.51, P=0.875), and no main effects of condition ($F_{(2,14)}$=0.02, P=0.978) or PVT ($F_{(5,35)}$=2.87, P=0.92). Manipulation produced an initial, insignificant rise in reaction time in Mild. Moderate condition had the greatest slope in RRT (-0.19), with Control following (-0.12), and Mild having the smallest slope (-0.1). Change in PVT correlated well with change in T_C, with -0.85, -0.81, -0.85, Control, Mild and Moderate conditions, respectively.

4.4.5 Core temperature and Sleepiness

The relation between T_C and subjective sleepiness (KSS) is displayed in Figure 4.13. There was a significant interaction effect between condition and time ($F_{(24,192)}$=1.59, P=0.046). This interaction effect is apparent in the dynamic response to cooling conditions. Mild subjective sleepiness decreased by 0.2 (unit: KSS scores; range 1-9) and showed an immediate significant difference from baseline, contrasted with Control ($F_{(1,8)}$=8.00, P=0.022). Moderate decreased initially by 0.8 but became significantly different from baseline, in contrast to Control, at ~20 min (Figure 4.13, point 3: $F_{(1,8)}$=7.10, P=0.029); Control KSS rose continually across the test. The initial dip in subjective sleepiness was 0.2 and 0.8 scores in Mild and Moderate, respectively. Change in sleepiness correlated well with change in T_C with 0.90, 0.96, ad 0.85, for Control, Mild, and Moderate conditions, respectively.

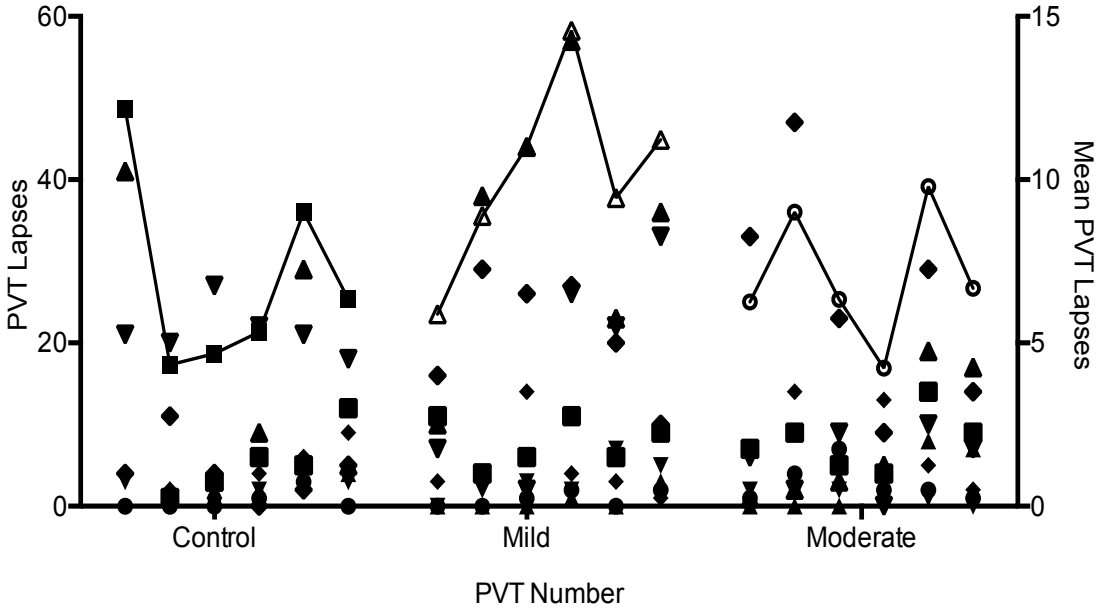


Figure 4.11: PVT lapses (RT>500 ms) across cumulative waking. Data are mean and SD for eight participants.

Note: P6 Control baseline of 41 lapses was omitted.

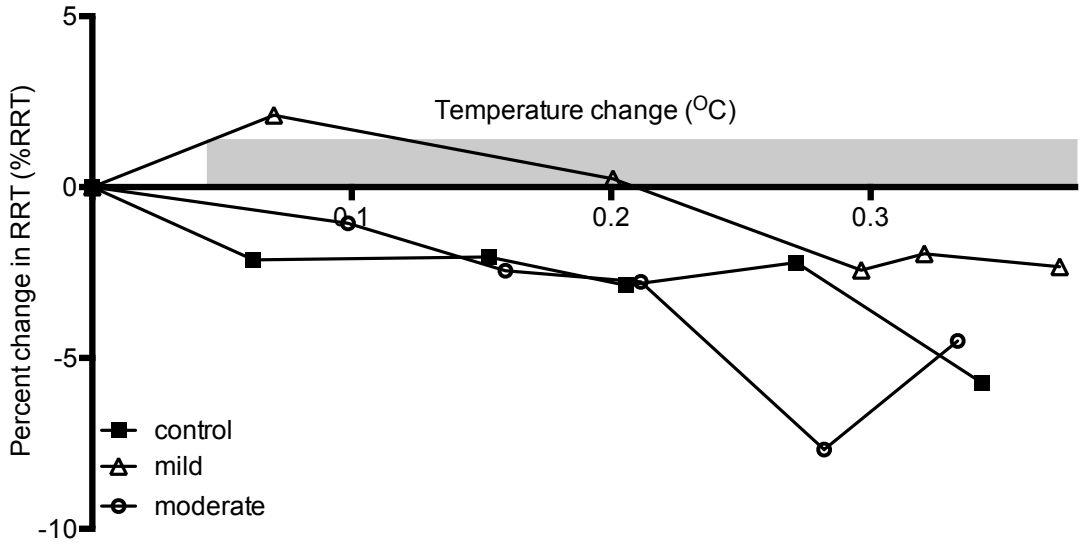


Figure 4.12: Relation of change in core body temperature and percentage change in Reciprocal Reaction Time. Each RRT point represents a 10-min PVT. Data are mean for seven participants. SD omitted due to loss of clarity of trend.

Note: grey indicates manipulated period

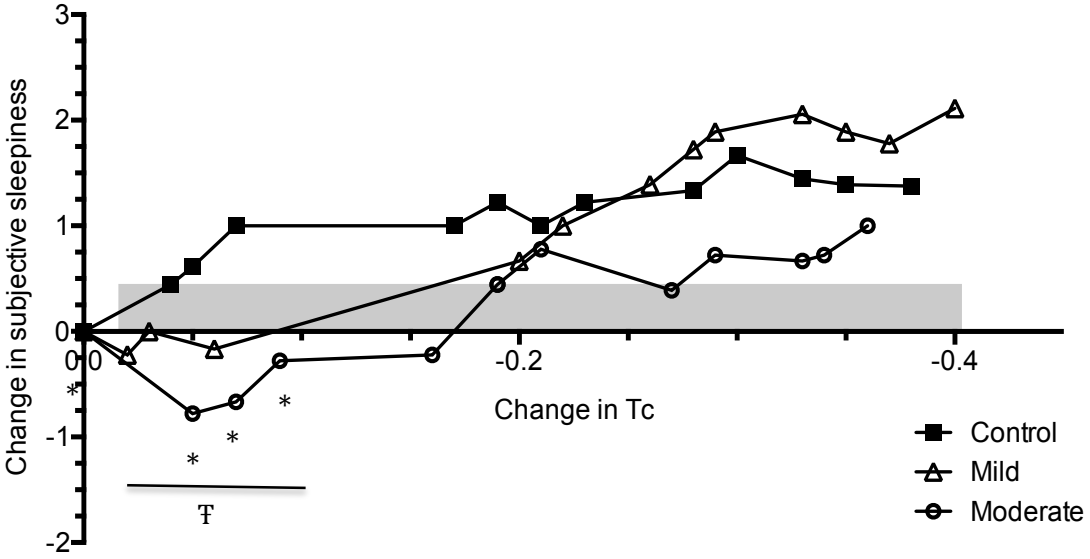


Figure 4.13: The correlation between change in and change in subjective sleepiness (KSS; range: 1-9). Data are mean for nine participants, SD omitted from figure due to misrepresentation of within participant variability.

Note: grey indicates manipulated period

* = P<0.05, Ŧ= Significant interaction

4.5 Cognitive arousal

Issues in electrode conductance generated errors in output powers; with theta power the most vulnerable to noise. Six participants were included in C4-A1 (referential electrode placement) derivation powers, and eight participants were included in O2-A1. Power frequencies were within their normative ranges. Of the remainder, Coefficient of Variation was too high to interpolate figures. Analyses for C4 were based on four participants, and O2 was based upon seven participants.

4.5.1 Karolinska Drowsiness Test

Seven KDTs –including baseline- were tested, with epochs reduced to a single eyes open power and a single eyes closed power. These were further analysed for both C4 and O2 derivations, to observe stable progression of specific frequencies across the

evening (Figures 4.14 and 4.15). Additionally, comparison of the change spectral power from eyes-open to eyes-closed was observed for both theta and alpha frequencies (Figure4.16).

Theta and alpha powers showed the greatest change in the eyes closed condition, while beta showed the greatest change across eyes open tests. Across the eyes closed tests, theta power decreased, and alpha power increased in C4, while theta power increased and alpha power decreased in O2.

4.5.2 C4-A1

Figures 4.14 and 4.16: a/b represent the sum of interactions between test eyes open and eyes closed at the right central (C4) electrode derivation for each condition. Theta power displayed a significant effect of interaction ($F_{(12,36)}$=2.20, P=0.034) during eyes-open. Contrasts and comparisons could not identify the source of difference. Eyes-closed theta power showed no interaction ($F_{(12,36)}$=1.12, P=0.377), and no effects of condition ($F_{(2,6)}$=0.02, P=0.980) or test ($F_{(6,18)}$=0.99, P=0.463).

Alpha power, eyes-open showed no interaction between condition and test ($F_{(12,36)}$=1.49, P=0.174), or of either condition ($F_{(2,6)}$=1.11, P=0.389) or test ($F_{(6,18)}$=0.80, P=0.581). Similarly, alpha eyes-closed showed no effects of interaction ($F_{(12,60)}$=1.09, P=0.386), or of condition ($F_{(2,10)}$=0.73, P=0.506), although a highly significant decrease occurred across testing ($F_{(6,30)}$=5.652, P=0.001).

Beta power indicated no interaction effects, for eyes open and eyes closed states (interaction: $F_{(12,60)}$=0.65, P=0.794; $F_{(12,60)}$=1.45, P=0.168), however displayed a significant eyes open trend across tests ($F_{(6,30)}$=4.31, P=0.003) and indicated a trend during eyes-closed across tests ($F_{(6,30)}$=2.14, P=0.078).

The change in theta power between eyes open and eyes closed states demonstrated no interaction ($F_{(10,70)}$=0.72, P=0.703), nor an effect of condition ($F_{(2,14)}$=1.16, P=0.342) or test ($F_{(5,35)}$=0.78, P=0.572). There was no interaction occurring in alpha ($F_{(12,60)}$=0.76, P=0.687) or beta frequencies either ($F_{(12,48)}$=0.786, P=0.662). Although alpha frequency indicated an effect across tests ($F_{(6,30)}$=2.80, P=0.028).

4.5.3 O2-A1

Figure 4.15 and 4.16:c/d represent the sum of interactions between test eyes open and eyes closed at the right occipital (O2) electrode derivation for each condition. Theta power displayed a highly significant difference across tests ($F_{(6,36)}$=10.84, P<0.0001), but no interaction in eyes open ($F_{(12,72)}$=1.12 P=0.340), while eyes closed showed no effect (interaction: $F_{(12,72)}$=0.407, P=0.957). The difference in eyes-open theta power appears to be due to a significant increase in power between baseline and the seventh KDT (difference: -5.84, C.I.: -9.86, -1.82, P=0.003). Similarly, alpha power displayed no interaction effect for either eyes open ($F_{(12,72)}$=0.77, P=0.679) or eyes-closed ($F_{(12,72)}$=1.07, P=0.402). However, eyes-closed showed a significant reduction in alpha power as an effect across tests ($F_{(6,36)}$=5.91, P=0.0002).

The change in theta power between eyes-open and eyes-closed showed significant differences in both tests ($F_{(6,42)}$=3.06, P=0.014) and condition ($F_{(2,14)}$=3.90, P=0.045), but no interaction between the two ($F_{(12,84)}$=1.05, P=0.416). Similarly, alpha power showed a significant effect of tests across the night ($F_{(6,30)}$=3.75, P=0.0067), with initial large increases in alpha power upon closing eyes, mellowing by the third KDT. No other trend was apparent (interaction: $F_{(12,60)}$=0.63, P=0.807).

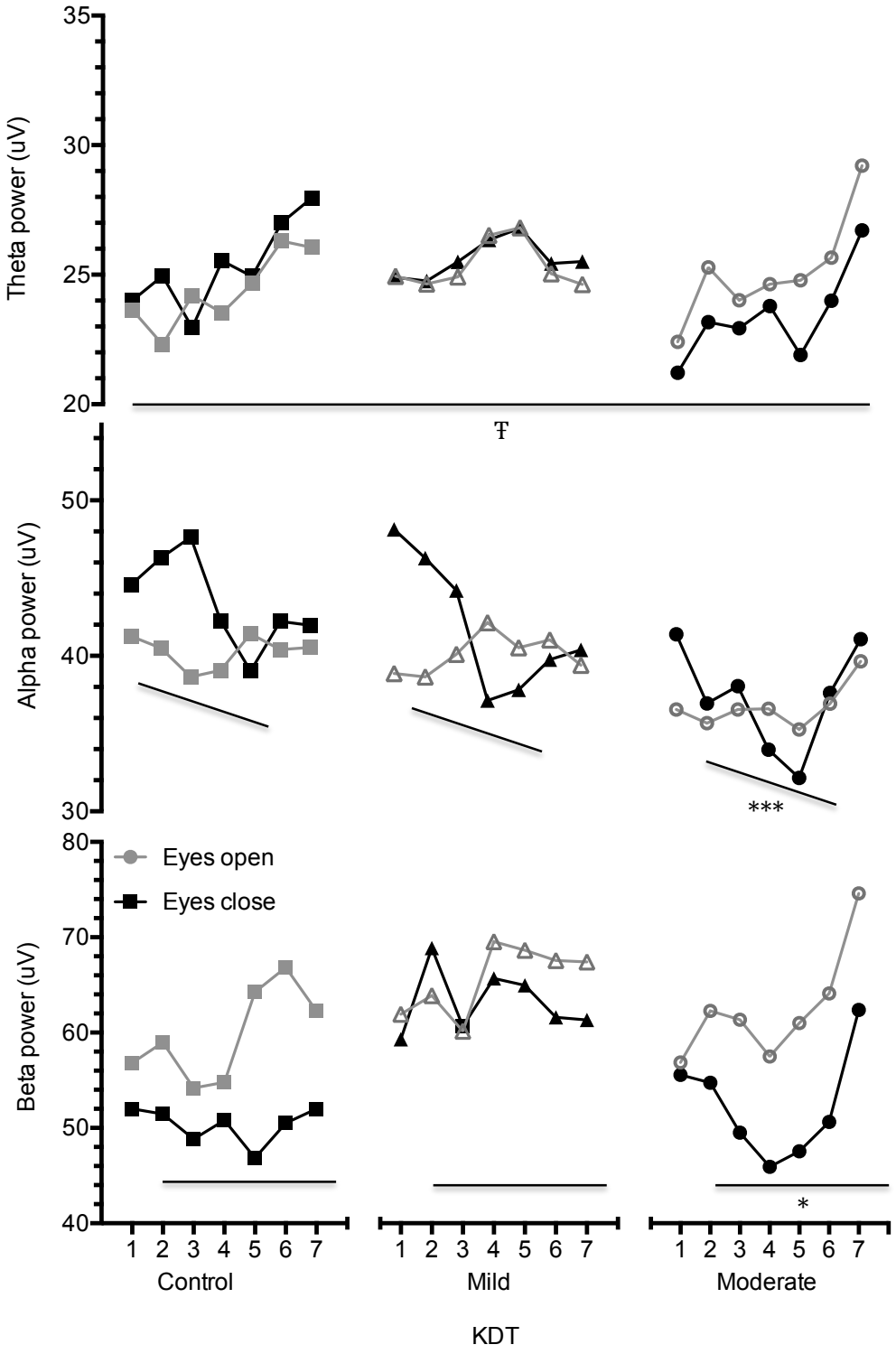


Figure 4.14: EEG spectral power changes across KDTs and conditions, and between eyes open and eyes closed. Data are mean for four participants. Standard deviation omitted due to high inter-individual variability inherent in EEG.

Interaction effect (Ŧ) of theta only present in eyes closed, no other interactions present.

Time effect (***=P≤0.001, *=P<0.05) only present in eyes closed alpha and beta.

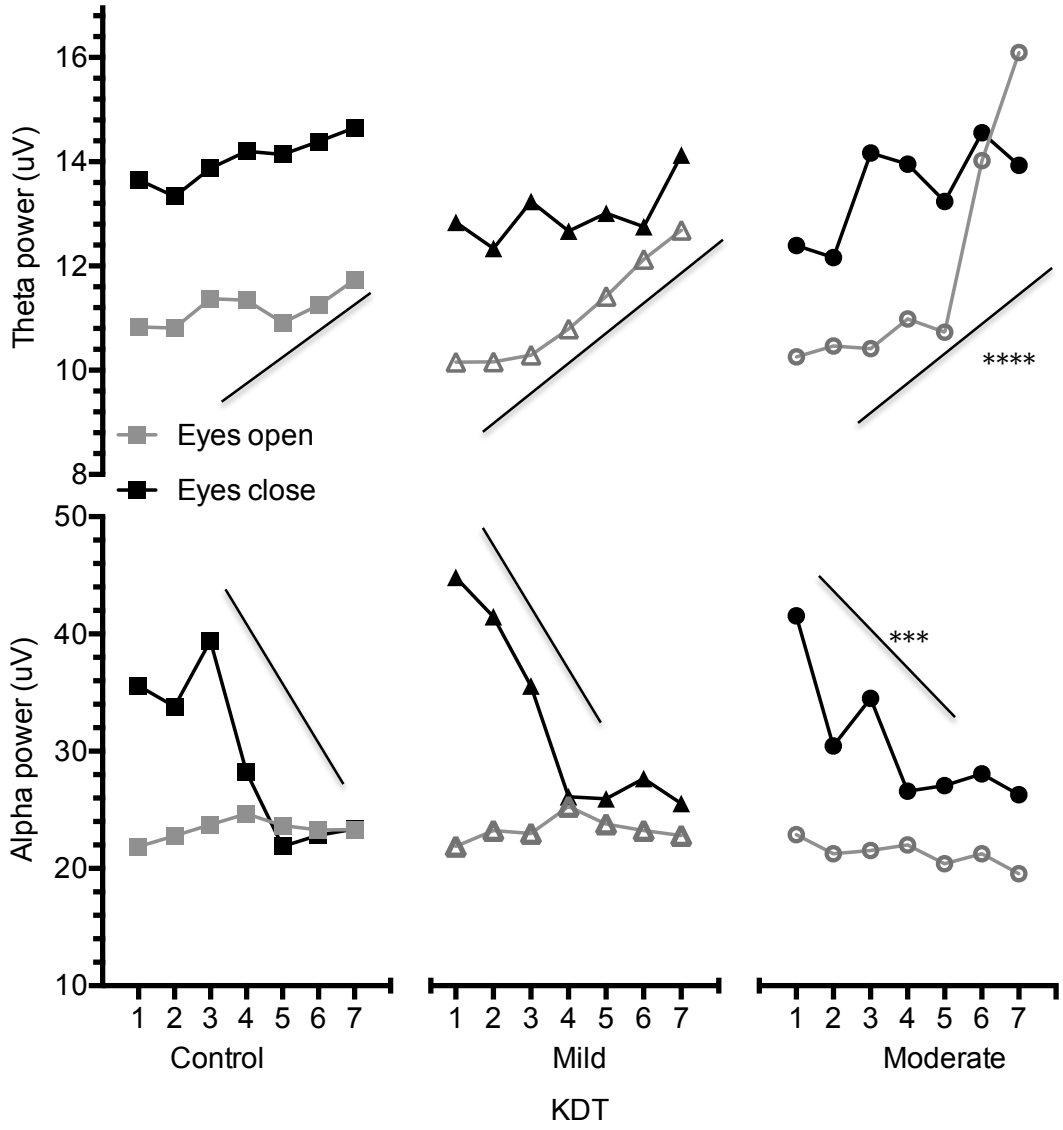


Figure 4.15: EEG spectral power changes across KDTs and conditions, and between eyes open and eyes closed. Data are mean for six participants. Standard deviation omitted due to high inter-individual variability inherent in EEG.

Time effect (****=P<0.0001, ***=P<0.001) present in theta eyes open and alpha eyes closed.

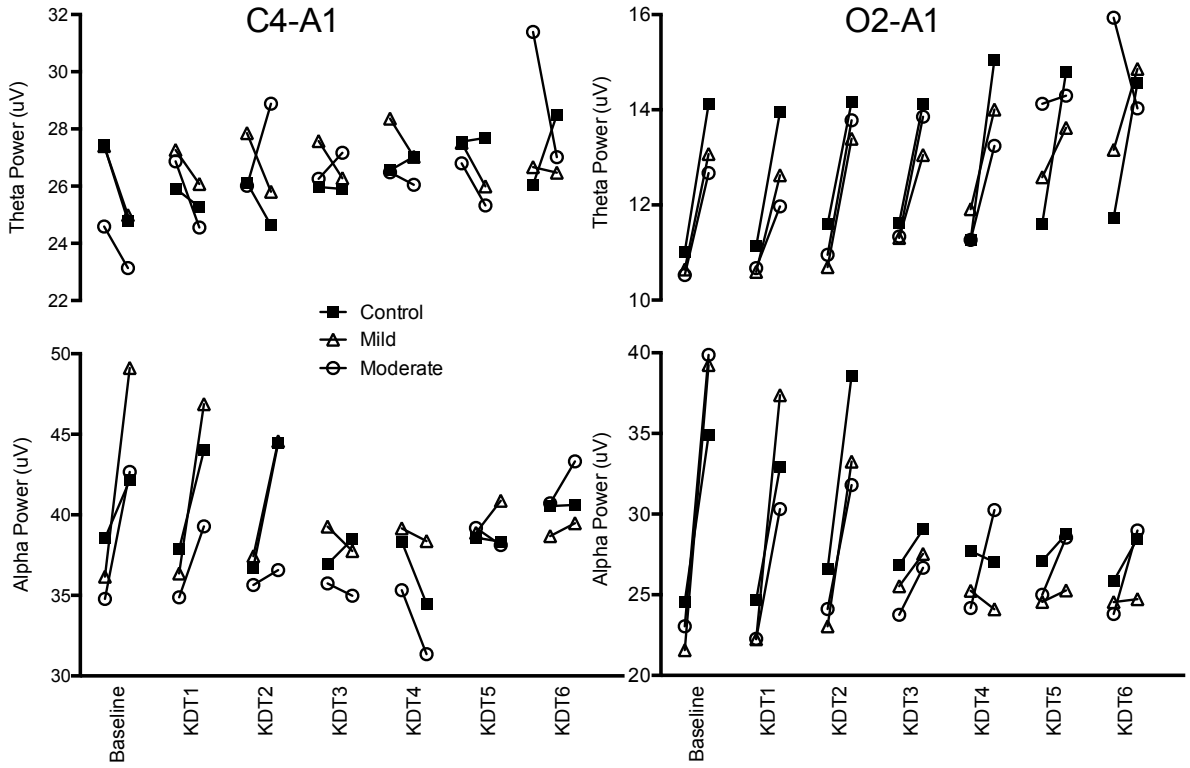


Figure 4.16: Change in EEG spectral power from eyes open to eyes closed across conditions. Theta power is represented in the top figures and alpha in the lower. Origin of frequencies stated above respective figures as channels C4 and O2. Data are mean for eight participants. Standard deviation omitted due to high inter-individual variability inherent in EEG.

4.6 Heart Rate

Heart rate measures are derived from ECG, recorded across the night of testing. Further analyses of in depth cardiographic changes were not made due to technical difficulties extracting the data. Heart rate declined significantly across the duration of the night in all conditions ($F_{(26, 208)}$=7.412, P<0.0001) (Figure 4.15). There was no difference between conditions ($F_{(52,416)}$=0.92, P=0.631). Heart rate declined in all but Control PVT3 and PVT5. There was no interaction between condition and heart rate associated with PVT ($F_{(10,80)}$)0.57, P=0.830).

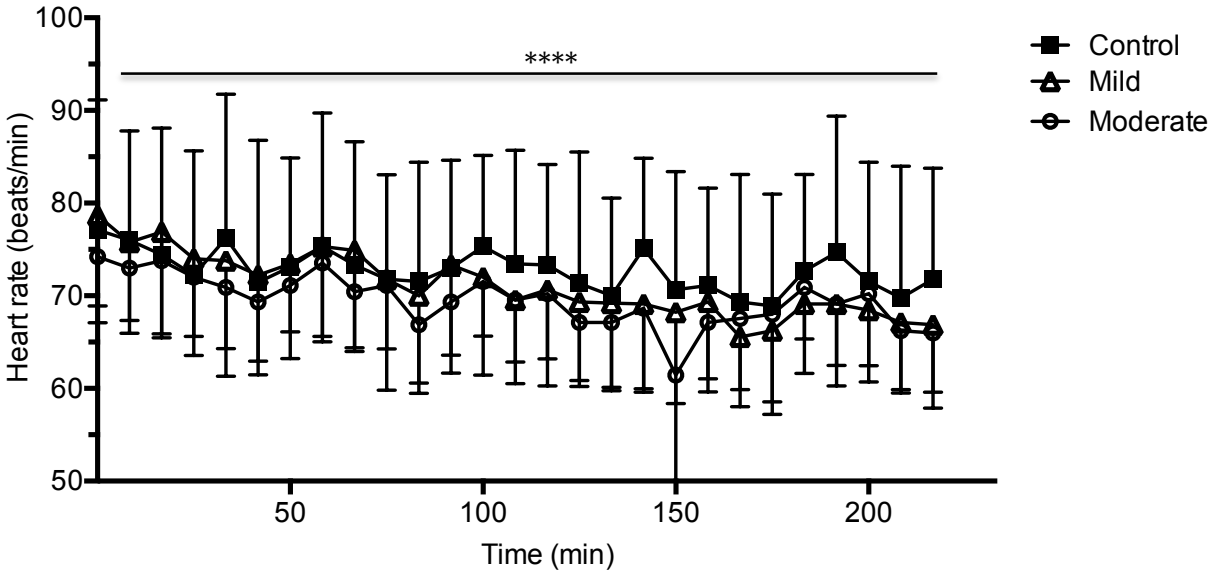


Figure 4.17: Heart rate decline (± SD) across the night for each condition. Data are mean and SD for nine participants.

****=P<0.0001 main effect of time

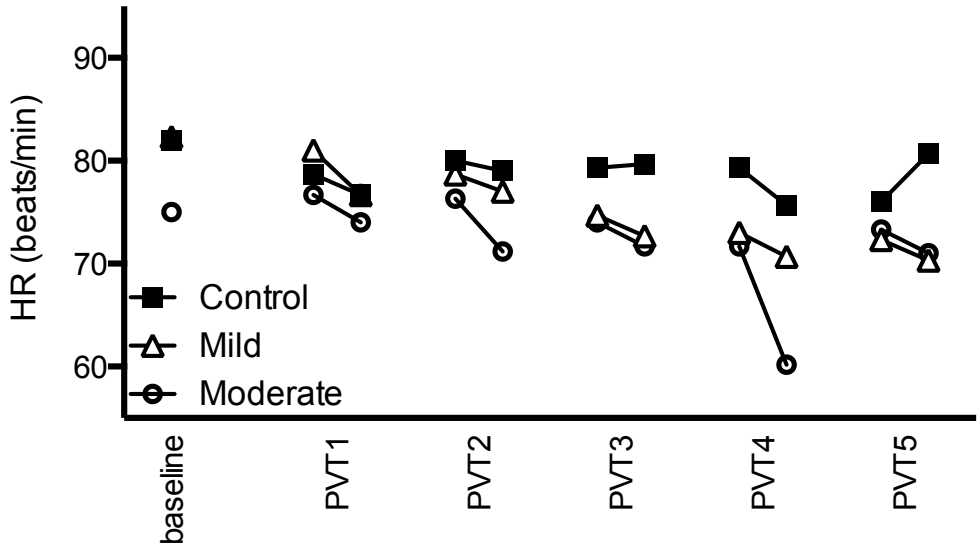


Figure 4.18: Heart rate change pre and post PVT performance for each condition. Data are mean for nine participants.

5.0 DISCUSSION

Several lines of evidence implicate a role for distal temperature in evening vigilance changes; recent research tends to have explored effects and benefits of facilitating a rise in distal skin temperature in populations that struggle with sleep onset (insomniacs (Raymann et al., 2007a), the elderly (Raymann et al., 2007a), and poor sleepers (Refinetti et al.)), be it from the source (Orexin secreting cells, melatonin deficiencies) or from effectors (vasculature). The effect of both spontaneous and induced rises in skin temperature on impairing vigilance and promoting sleep has been thoroughly demonstrated as beneficial (Fronczek et al., 2008; Kräuchi et al., 1999; Raymann et al., 2005; Romeijn et al., 2012b; Van Someren, 2006). The inherent role of cold extremities in acutely preventing sleep onset – while seemingly obvious and intuitive – appears to have been ignored in all but three studies: one exploring subtle temperature manipulations in narcoleptics (Fronczek et al., 2008); another observing apparent trends of thermal discomfort due to cold extremities leading to difficulty initiating sleep (Kräuchi et al., 2008); and a third applying cool air directed at the face as an in-car countermeasure to fatigue (Reyner et al., 1998).

The purpose of the current study was to similarly focus not on body temperature manipulations facilitating sleep, but those that acutely inhibit sleep. Therefore, the effect of distal temperature manipulation (specifically cooling) on vigilance markers was examined, in nine healthy volunteers, across the duration of natural sleep onset time. The hypothesis was that Mild and Moderate cooling would incrementally attenuate the decline in core temperature and improve vigilance parameters, or that vigilance would be improved by the skin cooling in itself. The main finding was that, in apparently healthy individuals, T_C and vigilance –measured as sustained attention (PVT) and cognitive arousal (KDT)- declined irrespective of significant reductions in distal skin temperature. Therefore, in contrast to significant effects on vigilance and sleep onset latencies due to subtle distal temperature manipulations observed in clinical populations (insomniacs and narcoleptics), the current healthy participants buffered even Moderate distal cooling (to ~25 ^{0}C) with robust circadian drives progressing toward sleep.

5.1 Temperature and vigilance

5.1.1 Temperature effects

The feet were cooled successfully, to 30.8 ^{0}C and 26.5 ^{0}C degrees in Mild and Moderate, respectively, and maintained at 34.5 ^{0}C in Control (Figure 4.1a). However, T_C remained unaffected by condition, declining from 36.9 ^{0}C to ~36.5 ^{0}C. This decline in T_C was highly correlated with the decline in sustained attention (Figure 4.12; correlation > 0.80 across conditions) and subjective sleepiness (KSS Correlation > 0.80 across conditions), but not cognitive arousal (EEG correlations < 0.50). The circadian decline of T_C therefore appears to be capable of buffering moderate localised thermal cooling (of the periphery anyway). Increased vasomotor tone in the lower limbs perhaps increased to completely offset the local heat extraction, such that the T_C profile was unaffected.

The functional thermal interdependence between the core and distal skin is well known and understood. The T_C is modulated by thermal and non-thermal input from the SCN to the thermoregulatory centre of the POAH (Moore et al., 2002), such that non-thermal factors alter the interthreshold zone by which thermoefferent responses are modulated (Mekjavic et al., 2006) (See 2*.2.1 Homeostasis*). Heat loss becomes dominant in the evening, raising distal skin temperature through centrally-mediated dilation of AVAs, as shown by Kräuchi et al (2000). The temperature of distal skin follows an inverse pattern to that of the core, while proximal skin oscillates in unison with the core. In the current study, consistent with Kräuchi et al's (2000) findings, the Control condition followed the described trend, with non-thermal cutaneous dilation already driving heat loss from the core (distal temperatures: ~34 ^{0}C).

The DPG reflects the underlying relation between the skin and core, and between heat loss and heat gain pathways in thermoregulation. Typically, heat gain pathways dominate across the day, while heat loss pathways dominate across the night (See section *2.3.5 Circadian temperature rhythm*). The evening heat loss minimises the DPG as the extremities offload heat radiantly and

convectively aided by dilation of AVAs in the volar surfaces of the hands and feet (Kräuchi et al., 1999). However, a small DPG is more difficult to attain in cooler circumstances (Werner et al., 1980), as indicated in the context of sleepiness within this study (Figure 4.4: Mild and Moderate), and inferred by Kräuchi et al (2008). As the feet (and hands) provide high local thermal perceptual sensation but minimal autonomic drive (Cotter et al., 2005; Simmons et al., 2008), individuals are made aware of cooling but do not invoke autonomic responses. Within Mild and especially in Moderate, distal skin temperature reduction produced observable vasoconstriction indicated by reductions in DPG and increases in PCG, as implied by others (Fronczek et al., 2006b; Kräuchi et al., 1999; Raymann et al., 2005; Romeijn et al., 2012b; Van Someren, 2006). The decrease in foot temperature enlarged the associated DPGs to -4 ^{0}C and -9 ^{0}C, in Mild and Moderate (Figure 4.4a), in contrast to a positive DPG of 0.8 ^{0}C in the Control condition.

Local skin temperature manipulation has been previously demonstrated to alter local blood flow (Fagrell et al., 1977). Local factors sensitise the vessels to efferent constrictor activity, increasing the DPG. Caldwell et al (2014) demonstrated that the feet are reactive to cooling only when the whole-body is in a heat loss state (hyperthermia $T_C \approx 39$ ^{0}C). The contribution of local factors is unclear in the current study because participants were clearly less heat stressed than in Caldwell et al's (2014) study, but were in a heat-loss phase of their circadian rhythm. As the T_C decline continued regardless of condition, the cooling may have simply facilitated heat loss.

Vasoconstriction has been assumed rather than measured in the current project, and is further assumed to be mediated by local effects because no increase in DPG was evident in the upper limb (Figure 4.4; $T_{sk} \sim 34$ ^{0}C, DPG: 0.7 ± 0.1). However, it cannot necessarily be assumed that an autonomically-mediated vasoconstriction in the feet would be evidenced by a similar profile in the upper limb, because previous studies have shown that these gradients can demonstrate opposing effects in sleep deprived subjects (Romeijn et al., 2012b). In a study of the effect of sleep deprivation on the vascular profile, Romeijn et al (2012b) identified disassociated upper and lower limb temperature gradients. The upper-limb heat loss profile was attenuated following

sleep deprivation whereas the lower-limb heat loss gradient was enhanced. The current findings contrast with those of Romeijn et al (2012b). While Romeijn et al's (2012b) reasoning may apply to the current study, it is equally likely that any constriction was reactive to cooling. Lower limb cooling could only reasonably affect upper limb temperatures if other, more autonomically-active areas instigated a central thermoregulatory response. As such, it is unsurprising that the feet did not induce a change especially in Mild because they have been previously been established to have low thermosensitivity for autonomic (sweating) responses (Cotter et al., 2005).

In summary, the inferred local vasoconstriction of the feet did not alter the heat loss profile of the core. Energetically, heat extraction may have further facilitated the observed decline in T_C, in a reciprocal manner to generated local constriction (Figure 4.1c). However, as T_C declined regardless of condition, manipulations appeared ineffective in instituting a generalised thermoregulatory response.

5.1.2 Vigilance and arousal

As T_C remained unaffected by distal cooling, it is unsurprising that vigilance also therefore remained unaffected, at least based on the rationale discussed in Section 2.4. The current study may therefore provide support for the association of vigilance with T_C (unless local perceptual effects are important but were too small to have shown up despite cooling to ~25 ^{o}C). The relationship between body temperature and vigilance/arousal state is, however, complex with the extremities undoubtedly playing a large role. The extremities may provide a means by which core body can lose heat (via DPG) (Kräuchi et al., 1999), or may be important as an independent factor altering vigilance/arousal (Van Someren 2006; Raymann 2005, 2007). The current study does not support or refute either position. Despite moderate localised cooling, vigilance demonstrated no overall effect. This may be predicated by the aforementioned T_C change, or may indicate the lack of stimulus at the feet to drive a perceptual distraction effect.

Evidence of the causal role of distal skin temperature on sleepiness in the evening has been demonstrated (discussed in *2.3.5 Circadian temperature rhythm*). Perhaps most relevant to this study, cooling the hands and feet delayed sleep onset latencies by ~24% in narcoleptics, although this effect was tested within a single PVT comparison only, during the daytime (Fronczek et al., 2008). The altered temperature regulation in narcoleptics is comparative to healthy individuals in the evening and is considered to increase their sleep propensity (Fronczek et al., 2006b). The current study did not generate significant reaction time, or latency changes between conditions; however, limiting manipulations to the feet only may have been the contributing factor. Greater spatial manipulation produces greater associated thermoafferent drive (Crawshaw et al., 1975). Including the hands may have generated sufficient cooling to impair the circadian heat loss characteristics. Where distal T_{Sk} manipulations have been used to alter the arousal state –be it for vigilance or sleep induction – subtle manipulations have proven effective in susceptible populations (e.g., narcoleptics, insomniacs), however, moderate manipulations have proved distracting. The distraction effect must not be overlooked, if it maintains arousal. While a potential detriment in complex tasks, distraction is potentially beneficial to maintain wakefulness if in simple vigilance tasks. The distraction effect may furthermore be beneficial in healthy populations, as raising vigilance through thermal modulation of the circadian rhythm appears difficult. Inhibiting the heat loss profile of T_C appears to be relatively difficult, and even moderate cooling does not appear to attenuate T_C.

Overall, distal cooling in the present study resulted in only transient changes in sleepiness (discussed in 5.2 Transient effects). While reaction time supported the decay in sustained attention, this was not reinforced by response variability or lapses, which both remained equally high across the night. Sleepiness rose steadily across the evening, as indicated by subjective sleepiness (Figure 4.7) and EEG parameters. Subjective sleepiness saturated in the majority of participants (Figure 4.8). EEG did not support any initial manipulation effect as observed in subjective sleepiness, however the association between subjective sleepiness or performance is

less apparent when sleepiness varies within normal limits (from “alert” to some “signs of sleepiness”) (Akerstedt et al., 1990). As any transient effect occurred within normal ranges of subjective sleepiness, it is unsurprising that such a modest response was not observed within EEG.

Cognitive arousal (indicated by theta and alpha frequencies) declined across the trial, irrespective of condition, for all markers except C4 and O2 theta (which showed a condition*time interaction: $P<0.05$ and $P<0.0001$, respectively). These markers showed larger increases in theta power in Moderate. Theta power is widely acknowledged to indicate *decreasing* alertness and impaired information processing (Pizzagalli, 2007; Caldwell et al., 2003; Lal and Craig, 2001; Klimesch, 1999), indicating that participants became more fatigued by the Moderate condition. This may be supported by a trend for PVT to decline more strongly in Moderate: 7% worse by PVT4, albeit this effect was unclear ($P= 0.92$).

Alpha power did not indicate any trends of increasing drowsiness within eyes open; however, sleepiness progressed with eyes closed (Figure 4.15). During relaxed wakefulness alpha power synchronises upon eye closure (Klimesch, 1999); this is also true for early onset drowsiness (Figure 4.16) (Santamaria & Chiappa, 1987; Gilberg & Akerstedt, 1982). Alpha synchronisation early in the session was indicative of early drowsiness, however the power of subsequent eyes-closed alpha powers declined rapidly. Typically, alpha power fails to synchronise and eyes-closed alpha power reduces as drowsiness progresses (Pizzagalli, 2007; Santamaria et al., 1987). Alpha power reductions also occur along with frequency slowing (Pizzagalli, 2007); this may have caused some of the later increases in theta power in the present study. Similar to the observations of Akerstedt et al (1990) and further supported by Lal et al (2002), it is likely that the participants were rapidly falling asleep during eyes closed by mid test, and not presenting the typical alpha increase; evidenced by rapid declines in alpha power upon eye closure later in the session (such as Figure 4.16: KDT4). Gilllberg and Akerstedt (1990) suggested that alpha power might have been so rapidly replaced by theta that their 1 min resolution would have failed to display the

change; this may have also been the case in the current study. Participants progressed from initial relaxed (early) drowsiness, into middle, and maybe late drowsiness to sleep onset, by mid session. Following this initial dip, participants may have undergone a rebound in drowsiness, as indicated by C4 alpha powers. The associated increased drowsiness supports the changes in subjective sleepiness and performance measures across the night.

In summary, despite significant distal cooling, T_C, vigilance and sleepiness remained largely unaffected. Contrary to previous indications of improved vigilance and wakefulness with temperature modulation in narcoleptics, the current study achieved no prolonged effects from distal cooling. Certain considerations need to be highlighted in comparing these two populations, and studies. Firstly, narcoleptics have greater room for improvement, as they should be awake and alert during the day – when the protocols have been implemented – as such manipulations *restore* them to normative temperature levels. The current study aimed to *impair* normative temperature levels, at a time when tonic pressures were instigating sleep. Secondly, circadian rhythms in narcolepsy are weakened and therefore likely susceptible to manipulation, whereas the current study's population was young and healthy with noticeably robust rhythms. Distraction effects of cooling perceptually dominant skin regions similarly proved ineffective in generating a prolonged rise in vigilance or wakefulness. Finally, the narcoleptic population was only tested in response to acute measures (a single PVT), whereas the current study observed the prolonged response to cooling over three hours (five PVTs). The difference in study outcomes could be determined by the duration of observation, whereby the initial, dynamic thermoreceptor firing induces greater perceptual awareness, and eventually adapts. The current study observed a dynamic effect that may have produced improved vigilance, similar to Fronczek et al's (2008) study, which in Moderate may have indicated a distraction effect from cooling. EEG further reflected worsening cognitive arousal and increasing sleepiness.

5.2 Transient effects

A changing ambient temperature causes cutaneous cold-sensitive thermoreceptors to produce a dynamic thermoafferent firing that is five- to ten-fold above that of static thermal states (as discussed in *2.2.2 Sensation*) (Hensel, 1982). The perceptual stimulus associated with the dynamic thermoreceptor response follows a similar profile (Arens et al., 2006a, 2006b; Zhang et al., 2010a, 2010b, 2010c). In the present study, subjective sleepiness was significantly reduced (Figure 4.13), while effects on vigilance were possible but unclear during the dynamic phase of Mild and Moderate cooling (Figure 4.12). Steady state temperature was attained after ~10 min in both cooling conditions, with the initial rate of cooling being twice as fast in Moderate than in Mild. Associated overall/whole-body perceptual responses were also marked in this period, with sensation declining from 'warm' (~9) to 'cool' (~6.5), and 'cold' (~5.5.) for Mild and Moderate, respectively (Figure 4.6). As the manipulated temperatures were not severe and remained stable following the initial decline, participants thereafter maintained thermal comfort no more than 'slightly uncomfortable'.

The observed trend in sleepiness was similar to Reyner et al (1998) observed responses to in-car countermeasures, and in fact similar to Fronczek et al's (2008) acute findings. Reyner et al (1998), using cold air (10 ^{0}C) directed at the face, observed insignificant decreases in subjective sleepiness (KSS) of slightly larger to those in the current study's Moderate cooling condition (2 KSS scores (Reyner & Horne 1998), compared with 0.8) but of similar duration (~15 minutes compared with ~20 minutes, respectively). Reyner & Horne's (1998) 2-point drop in subjective sleepiness was presumably due to the dynamic thermoreceptor response, made more powerful by the face's high perceptual and autonomic thermosensitivity. Reyner et al's (1998) study was conducted during the afternoon and still produced subjective sleepiness levels of ~7 "sleepy, no effort to stay awake" (KSS range: 1-9).

5.3 Limitations

5.3.1 Order effect

Within the current study, order effect was a large concern, considering the degree of motivation-related measures. According to Tops et al (2004), motivation is intrinsically related to mental fatigue, in which an imbalance in the effort-reward relationship decreases motivation, resulting in mental fatigue. The boredom associated with the current protocol was anticipated to generate a large order effect due to loss of motivation in subsequent trials. To this end, a crossover design minimised the systematic impact of an order effect. Tasks were also carefully chosen to minimise learning or motivation effects. The PVT has been validated in mentally fatiguing, and sleep deprived situations to serve as a highly sensitive marker of sleepiness and vigilance decline with minimal learning effects (Dinges, 1995; Basner & Dinges, 2011). The PVT has been utilised as a vigilance measure in a variety of studies to demonstrate efficacy of standing (Caldwell et al., 2003), temperature manipulation (Fronczek et al., 2008, Raymann et al., 2005; 2007a, 2007b), and sleep restriction (Van Dongen et al., 2003). Similarly, EEG is often used in parallel with PVT as a gold standard measure of sleepiness, and vigilance decline (Caldwell et al., 2003; Dorrian et al., 2005; Fronczek et al., 2008; Raymann et al., 2007b; Van Dongen et al., 2003). The KDT has been repeatedly used as a standardised technique within EEG, often in conjunction with KSS (Cajochen et al., 2000; Gillberg et al., 1982; Greneche et al., 2008; Kaida et al., 2006). Among the different fields, both EEG and PVT have been argued to be the best measure of drowsiness/fatigue/sleepiness (EEG, see (Horne & Reyner, 1996); PVT see (Lim et al., 2008)). Fortunately, PVT, perhaps the main dependent measure, did not show an order effect of session ($P=0.177$).

Careful standardisation in line with constant routine protocol standards was also attempted, for example, in minimising noise, light, postural change, social interaction, and knowledge of time; all of which have been demonstrated to influence performance and arousal (Mills et al., 1978;

Kräuchi et al., 1999). Sleep patterns were also monitored for variability and order effects, and standardised by asking participants to control sleep patterns 48 hours before testing. Participants were initially screened for normalised sleep patterns, and on average, went to sleep at 23:40 and slept ~8 h. All participants maintained a normal sleep pattern as assessed from accelerometry and sleep diaries (albeit with only a modest proportion of data obtained). No order effect was apparent in the sleep data obtained.

5.3.2 Laboratory/Equipment set up

Distraction and arousal both may have been present in the outcomes as a result of interference from the examiner. Many studies currently conducted on vigilance maintain a computer interface, and severely limit examiner input. This is to minimise alterations to inter-test protocol timelines, and experimental error but importantly to reduce the verbal input which may otherwise confound outcomes. The current study, while attempting to minimise human input used an examiner to verbally cue participants into subjective questionnaires. The examiner also remained present to prompt participants to wake up throughout testing, which is not uncommon (e.g., (Fronczek et al., 2008)), but remains a barrier.

Another issue was maintenance of cooling. As only rudimental technology was used in the cooling protocols, temperatures had to be constantly assessed and adjusted manually. This resulted in the occasional error (See Figure 4.1a: Mild condition, minute 150). However, in conjunction with experimental error, the lack of thermostat meant ice was required to cool the circulating water. Dumping ice into the urn produced unnecessary noise, which, despite participants wearing earplugs, was audible.

5.3.3 Statistical power

There was some indication toward an effect of distal cooling on vigilance. The effect in RRT was not statistically significant (P=0.875; effect size =0.27), whereas the KSS showed a reliable effect of cooling (P=0.046), albeit with modest effect size (0.17). A lack of power arising from a

small sample size is an obvious limitation. The nature of such studies –multiple testing nights of long duration, and invasive procedures – makes recruitment, retention and adherence of participants difficult. Increasing sample size is not an easy task; however, it may be essential to determine the nature of the relationship between alertness and body temperature, and to further delineate the efficacy of cooling in increasing alertness and reducing the risk of accidents. Future studies approaching such research will have bear in mind the barriers to such designs. As a similar dynamic effect of cooling on has been previously observed with 16 participants (Reyner et al., 1998) it is reasonable to suggest that such numbers may have been required to further delineate the differences observed between the levels of the statistical analyses – Control, Mild, and Moderate. Despite this, promising trends indicate a more in-depth study into the effect of cooling on vigilance may be worthwhile.

5.3.4 Electroencephalography

Characterising frequencies as far as specific recording sites, and filtering, transforming and analysing methods are hugely varied. These differences are convoluted and intimidating for those entertaining using EEG as a technique. A lot of methods are now out-dated, however it was cumbersome and difficult to assess which. The current study utilised defined boundaries for traditional theta (4-7 Hz), alpha (8-1 Hz), and beta (13-30 Hz) frequency bands, however there is mounting evidence for Hz-Hz power analyses (Pizzagalli, 2007), or individually defined frequency bands (Klimesch, 1999), which may refute or enhance the current observed EEG outcomes. Clinical EEG provides excellent, current best practice guides for observing and sleep staging EEG criteria, however an equivalent is difficult to find in quantitative EEG; especially quantitative EEG related to drowsiness. The current study utilised guidelines from Santamaria and Chiappa (1987).

Debunking correct methodologies proved time consuming and difficult. This was made considerably so without correct technical software to perform data reduction, spectral analyses and, noise/artefact scanning and rejection. Time consuming techniques limited the scope and versatility of the EEG analysis, an otherwise highly powerful measure that is arguably

underappreciated outside its clinical setting. Matlab was utilised for data reduction. While a highly versatile and powerful program, Matlab requires considerable time to input code for interpretation of data, as well as much greater involvement to understand and learn to use. Additionally, it is difficult to ensure valid outcomes from Matlab coding. As such utilising a Matlab specialist allowed for the input and output of EEG data, at the cost of versatility in EEG spectral analyses

Additional unused electrodes became redundant as time and software proved insufficient to accomplish additional analyses. Additionally, as analyses provided generated no effect, further analyses would have proved cumbersome without adding to the knowledge. Frontal derivations were originally proposed to analyse cognitive arousal during PVT tasks however the collected frontal PVT data was omitted from analyses for the aforementioned reason.

5.3.5 Temperature

The degree of thermal clamping was difficult due to equipment limitations allowing at best 1 ^{o}C of deviation from intended values. Coupled to this problematic control was an interesting physiological uncoupling of foot temperatures while cooling was occurring. Werner et al (1980) observed upon cooling that a large divergence in temperature range occurred, with ~17 ^{o}C of difference between skin temperatures ($T_{foot} - T_{forehead}$) in a cold environment (T_A=10 ^{o}C), when compared to a total core to skin difference of ~2 ^{o}C when under ~50 ^{o}C ambient load. However, they did not observe the variation of change within the feet alone, nor did Love (1948) while observing blood flow changes in the feet across hot and cold conditions. In the current study, under moderate cooling (T=25 ^{o}C), the range in foot temperatures was as great as ~9 ^{o}C, with the foot arch decreasing to temperatures ~19 ^{o}C and ventral toe resting at ~29 ^{o}C. This variability of foot temperatures created considerable difficulty when attempting to gain a reliable average foot temperature.

5.3.6 Vigilance

Vigilance is known to be stressful and fatiguing (Warm et al., 2008). However, fatigue was aimed to be exacerbated in sleep permissive settings at normal bed time. A number of participants were anticipated to drop into early stages of sleep onset (Stage 1, and Stage 2), however for some participants this occurred in the extreme such as to lead to their removal. The argument for removing participants (original P2 and P9 [these participants were replaced]) was the loss of validity due to continuous interaction with the experimenter (i.e., continually rousing the participants from slumber). Original P9 in her second PVT (post intervention) in the moderate trial resulted in her asleep after 5-min duration, requiring continuous rousing upon each stimulus. While she reflects a certain population of the experiment, the validity of her test was considered to be altered.

As with P9 who rapidly reached the ceiling effect in his Mild condition, participants' subjective sleepiness oscillated throughout the night. Although, Figure 4.7 shows a general progression of sleepiness across the night, Figure 4.8 shows the number of participants skewing this trend to be much greater in Control and Mild conditions than in Moderate, with over half participants in Control and Mild reaching ceiling by 300 minutes, with Mild condition showing a subsequent decline. Moderate condition maintained two participants having reached ceiling by 190 minutes until the final measure where upon 3 subjects reached KSS score of 9 (Figure 4.8).

5.4 Variable Temperatures across thermistor locations on the feet

Cooling was somewhat heterogeneous across the foot; the arch cooled more than the toes, more so in Moderate (~5 ^{0}C difference) than in Mild (~2 ^{0}C difference). The blood supply to the toes arises from the *dorsalis pedis*, which divides into, among others, the first dorsal metatarsal artery (Standring. 2008). The first dorsal metatarsal artery supplies the first and second toes. The cooling applied to the feet was assumed to cause vasoconstriction and thereby restrict cutaneous

blood flow. While nervous control of the feet differs between volar and dorsal skin regions, cooling at below TNZs generates generalised adrenergic vasoconstriction across the whole foot. The observed differences were not divisible by the volar and dorsal skin regions which are expected to provide regional differences to local cooling due to their differing neural control (Taylor et al., 2014). While volar and dorsal regions are differentially controlled, both surfaces receive generalised adrenergic input upon introduction to cooling.

The observed differences in the current study were considered to be very unlikely to have been due to design of the water-perfused booties (see Appendix E for photos). The spacing of tubing was consistent, and condensed as the front of the sock folded around the toes. The arch was elasticated to provide better contact through the mid foot. The tubing flowed around the ankles and over the lateral dorsal foot before progressing into the sole at the heel and progressing forward over the toes and back over the dorsal aspect of the foot before flowing back to the heel, over the medial foot, and out at the posterior aspect of the ankle. If heat exchange with the water were responsible for the differences in toe temperature, then the arch thermistor would also be affected.

The differential thermal changes observed in the feet are divergent from the expected thermoregulatory response. The input of non-thermal heat loss from the SCN may be responsible for the discrepancy in foot temperatures, however despite the confidence in the water perfused booties, they cannot be ruled out as an influencing factor. If the observed differences in foot temperature across locations is a real effect, then it would be comparatively easy to assess, and therefore worth investigation.

5.5 Practical application

In light of the current findings, subtle to moderate cooling of the feet does not appear to increase vigilance. Greater cooling proves inadequate for other reasons; moderate cooling is

distracting (Cheung et al., 2007), as such, although cooling increases alertness it is likely to distract individuals away from task demands. Greater cooling also diminishes dexterity, by which a functional response may also be hampered; this is also a reason for not using hands as a method of cooling. Previous experimentation directing cool air to the face has proven similarly inadequate for improving performance in attentional tasks, despite the greater perceptual and autonomic thermoafferent drivers (Reyner & Horne, 1998). Caffeine and power naps remain the best-proven sleepiness inhibitors (Horne & Reyner, 1996); with standing verses sitting (Caldwell et al., 2003) and exposure to bright light (Cajochen et al., 2003; Kaida et al., 2006; Czelsier et al., 1986) also providing means for reducing drowsiness.

Conversely, in an era of increasing sleep disturbances, and reduced sleep duration, the results of the current study are encouraging in that healthy young adults maintain temperature rhythms related to sleep onset, regardless of moderate exposure to cold at localised regions (i.e., bare-feet on a cold evening). Provided the rest of the body is warm, even moderately cold feet (T_{sk}~25 ^{o}C) have no effect on T_C decline, despite considerable vasoconstriction occurring in the lower limbs. While considerable difficulty initiating sleep has been correlated with cold extremities (Kräuchi et al., 2008), the current study indicates that this may be less of an issue in healthy young populations.

In summary, although peripheral warming has a facilitative effect on sleep initiation, the implication that cooling by association may reduce sleep propensity appears tenuous. Rather, thermoregulation associated with sleep pressure appears capable of resisting cooling in healthy young adults.

5.6 Conclusions and Recommendations for future research

It is widely accepted that reducing the DPG by warming the extremities improves sleep onset latency and worsens vigilance. This association relies on the relationship between T_C and arousal

state, mediated through skin temperature change. Larger perturbations in T_{Sk} might also be expected to have distraction-inducing effects from thermal perceptions arising from T_{Sk} itself. Despite the numerous lines of evidence indicating relationships between vigilance and body temperature, the current study was unable to support or refute any influence of the distal extremities or associated gradients on T_C or arousal state. Warming extremities can apparently influence T_C and arousal decline, however unlike in Fronczek et al's (2008) narcoleptic population, cooling did not appear to perform the inverse function in healthy subjects for the reasons described above (*5.1.2* summary). Cooling the distal extremities during the circadian phase of non-thermal heat loss did not improve vigilance and produced only mild, transient improvements in subjective sleepiness. As stated prior, the factors that may be responsible for this is that cooling may have assisted heat loss despite local constriction factors and blood flow reductions from AVA constriction, however it cannot be ruled out that momentum may have already instigated sufficient heat loss to render the protocol insufficient. It appears that healthy individuals may be more capable of buffering moderate perturbations in distal extremities, without affecting the evening decline in temperature, than the populations used in comparative studies.

Despite the largely null findings currently, there is still a compelling basis for future research surrounding the interaction between vigilance/wakefulness and temperature, with ramifications for easily applicable strategies. As stated in the discussion, the effect of distraction cannot be overlooked as a potential means to maintain vigilance or wakefulness across extended periods. Distraction from temperature manipulation would perhaps be best applied by manipulations of the distal extremities, which have high perceptual thermoafferent drive, at temperatures residing close to thermal discomfort thresholds (e.g., T_{Sk} ~20 ^{o}C). Cheung et al (2007) demonstrated the distraction effect from mild (T_{Sk} ~23 ^{o}C; T_C normal) whole-body temperature manipulation, and Kräuchi et al (2008) has indirectly inferred that the effect of foot and hand cooling around the thermal discomfort threshold improves wakefulness – or at least impairs sleep induction.

However, minimally distracting temperature manipulations would be more appropriate where vigilance is required for complex tasks; this may be achievable through manipulations of T_C that do not evoke or direct awareness to the thermal manipulations.

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7.0 APPENDICES

Appendix A: Participant information and consent form

Reference number: 13/087

April 2013

PARTICIPANT INFORMATION SHEET

Distal Skin Temperature and Cognitive Performance

Thank you for expressing an interest in this project. Please read the following information carefully before deciding whether to participate in the study. If you have any questions, please do not hesitate to ask.

Aim of the Study:

To examine whether cognitive function is affected by temperature of the feet. This study is being undertaken for a Masters of Physical Education research project, under the supervision of lecturers within the Schools of Physical Education and Medicine. The research will take place in the School of Physical Education (Laboratory B01 or 113, 55 Union St West).

Type of Participants Needed:

Healthy young adults (18-30 years) residing in Dunedin, who are willing to stay up late on three nights to perform a monotonous psychomotor vigilance (sustained, prolonged reaction time) task while also having their brain function and body temperature measured (see below).

What Will The Participants Be Doing?

If you are willing to participate, you will need to attend the lab on up to four occasions; one being a familiarisation session of ~1 hour duration, and then 2 or 3 data collection sessions of up to 3 hours each (spaced one week apart). The data collection sessions will *begin at ~10 pm, thereby requiring you to stay up ~2-3 hours later than usual, to perform a monotonous mental function task for ~40 minutes* on each occasion. On each of these days you will need to avoid exercise and caffeinated and alcoholic drinks from midday, and follow the same dietary regime (including hydration). You will be reminded of these requests.

During each session you will have the electrical activity of your brain (EEG) and eyes (EOG) recorded using electrodes attached via a cap and glued to your scalp, your heart's electrical activity (ECG) recorded from electrodes attached to your skin, and your body temperature measured using electrical thermometers worn on your skin and *within your rectum*. You will need to insert the thermistor; it is sterile, flexible and disposable, and should not be uncomfortable. Periodic assessment will be made of your mental functioning (performance on a vigilance task), perceived temperature, thermal comfort and mood state. The task will involve a prolonged sustained reaction time performance task, which will be performed while the temperature of your feet is altered.

Benefits of this Research:

Mental function and thermoregulation are linked, but the nature of these links is not yet well understood. Our research is focussed on improving society's knowledge of how strongly and in which direction relations exist between distal skin temperatures (esp. the feet), mental function and arousal state. Some immediate benefits of this knowledge are in helping provide the evidence for recommendations aimed at reducing sleep deprivation, and conversely, in preventing sleep onset (e.g., in reducing fatigue-related road deaths).

Risks and Benefits to the Participant

There are no discernible, known risks or benefits of participation, other than:

- The social and perhaps transient physical discomfort of rectal temperature measurement;

- The inconvenience and discomfort of remaining up late and the prolonged task;
- Sleep-deprivation on the day following each testing session, thereby increasing your risk of impaired vigilance (e.g., *higher likelihood of having a motor vehicle accident, or concentrating in lectures or other work*) and impairing your immune function (e.g., *possibly higher susceptibility to upper respiratory tract infection*);
- Mild discomfort of foot cooling.

The recording equipment carries no risk or discomfort.

You will be informed of the results of this project, and their practical implications, following completion of the data collection and analysis.

Data Information Required

- Name and phone number: For contact reasons.
- Age and body mass: For participant characteristics purposes.
- Usual sleep patterns (via a 7-day sleep diary).
- Heart rate, core body and skin temperatures, brain and eye electrical activity, sustained reaction time performance and perceptions of mood and temperature. This information is required to examine the physiological, perceived and performance-related effects of increasing and decreasing foot temperature.

All data obtained will be used solely for the purposes described above. Results of this project will be included in a Master's degree thesis (which will be available in the University of Otago Library) and may be published in the scientific literature, but any data included will not be linked to a specific participant. Your data will be assigned a personal identification number to ensure anonymity in both the analysis and documentation of results. The data obtained in this study will only be available to the MPhEd student (Mr Ryan Sixtus), Assoc Prof Jim Cotter, Assoc Prof Barbara Galland and Dr Jon Shemmell.
The data collected will be securely stored in such a way that only those mentioned above will be able to gain access to them. Data obtained as a result of the research will be retained for at least 5 years in secure storage. Any personal information held on participants (name, age, body mass, phone number) will be destroyed late in 2013 whereas measurement/response data will be retained for at least five years following possible publication of the report.

Can Participants Change their Mind and Withdraw from the Project?

You may withdraw from participation in the project at any time and without any disadvantage to yourself of any kind.

Contact Information

Please feel free to contact us at any time with questions and concerns you may have about participating in this research study.

Assoc Prof Jim Cotter
School of Physical Education
Phone: 479-9109
email: jim.cotter@otago.ac.nz

Mr Ryan Sixtus
School of Physical Education
Phone: 479-8991
email: sixry294@student.otago.ac.nz

If you wish to contact an independent person regarding any aspect of your participation, please contact: Professor Douglas Booth, Dean, School of Physical Education, Otago, Phone: 479-8995

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph. 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Reference number: 13/087
April 2013

CONSENT FORM FOR PARTICIPANTS
Distal Skin Temperature and Cognitive Performance

I have read the Information Sheet concerning this project and understand what it is about. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage.
I know that:

1. My participation in the project is entirely voluntary;

2. I am free to withdraw from the project at any time without any disadvantage;

3. The data will be destroyed at the conclusion of the project but any raw data on which the results of the project depend may be retained in secure storage for up to five years to assist in checking the accuracy of this research or developing future research, after which they will be destroyed;

4. I will be required to follow sleep and dietary restrictions on up to four occasions;

5. I will have my mood, heart rate profiles, cognitive performance, deep body and skin temperatures, and brain and eye muscle activity measured on up to four occasions, three of which are late at night, approximately 1 week apart;

6. I will not be receiving any reward or compensation for my participation in this study.

7. The results of the project may be published and will be available in the University of Otago Library (Dunedin, New Zealand) but every attempt will be made to preserve my anonymity.

I agree to take part in this project.

....
(Signature of Participant) (Date)

This study has been approved by the University of Otago Human Ethics Committee. If you have any concerns about the ethical conduct of the research you may contact the Committee through the Human Ethics Committee Administrator (ph. 03 479 8256). Any issues you raise will be treated in confidence and investigated and you will be informed of the outcome.

Appendix B: Activity monitoring and sleep diary

Activity Monitoring and Sleep Diary

Please start filling out the table below on the first night before you go to sleep and then as soon as you wake in the morning, and continue to do so for the duration of the study. It would be great if you could attempt to maintain a continuous sleep time between weekdays and weekends for the duration of the study to ensure you are well rested for the protocol in the evening of the seventh day.

Some notes that may help:

Alcohol: Excessive alcohol intake is **not advised** regardless, however if you feel you must, **it takes 48 hours to normalise sleep patterns following a "late night out"**, as such we would ask you to **do so within the first three days** of your sleep diary and just record the time you went to sleep.

Caffeine containing drinks: energy drinks, soft drinks like coke and Pepsi, coffee, tea (there are decaffeinated options).

Physical activity: If you do exercise regularly, then it may be advisable to have an easy/off day on the night of the experiment, as the late night will considerably fatigue you anyway. This is more a concern when you are heading home following the lab.

Hydration levels: adequate hydration prior to the test can be gained through intake of a good glass of water, or juice with the evening meal. We ask that you also have a good dinner before coming in because the test will require a few extra hours of time out of your day/night.

If you could **wash your hair** prior to coming in that will save a lot of abrasion necessary to lower impedance.

Date	**Time to bed**	**Time out of bed**
Day one:	Starting date:	
Day two:		
Day three:		
Day four:		
Day five: **Please stop caffeine intake until experiment night**		
Day six:		
How well do you feel you slept? If Badly please contact me 0273220250		
Extra day:		
Day seven:	**Experimental night** **Please maintain hydration, and refrain from alcohol and caffeine today**	

Additional notes:

What have you eaten tonight??? AND when???
What are you wearing??? (e.g., sweaters and warm hoodie)

Second Week	Starting date:	
Day one:		
Day two:		
Day three:		
Day four:		
Day five: **Please stop caffeine intake until experiment night**		
Day six:		
How well do you feel you slept? If Badly please contact me 0273220250		
Extra day:		
Day seven:	**<u>Experimental night</u>** **Please maintain hydration, and refrain from alcohol and caffeine today**	

REMEMBER TO EAT AND WEAR WHAT YOU DID THE FIRST TIME ☺

third Week:	Starting date:	
Day one:		
Day two:		
Day three:		
Day four:		
Day five: **Please stop caffeine intake until experiment night**		
Day six:		
How well do you feel you slept? If Badly please contact me 0273220250		
Extra day:		
Day seven:	**<u>Experimental night</u>** **Please maintain hydration, and refrain from alcohol and caffeine today**	

REMEMBER TO EAT AND WEAR WHAT YOU DID THE FIRST TIME ☺

Thank you for participating.

Appendix C: Participant setup

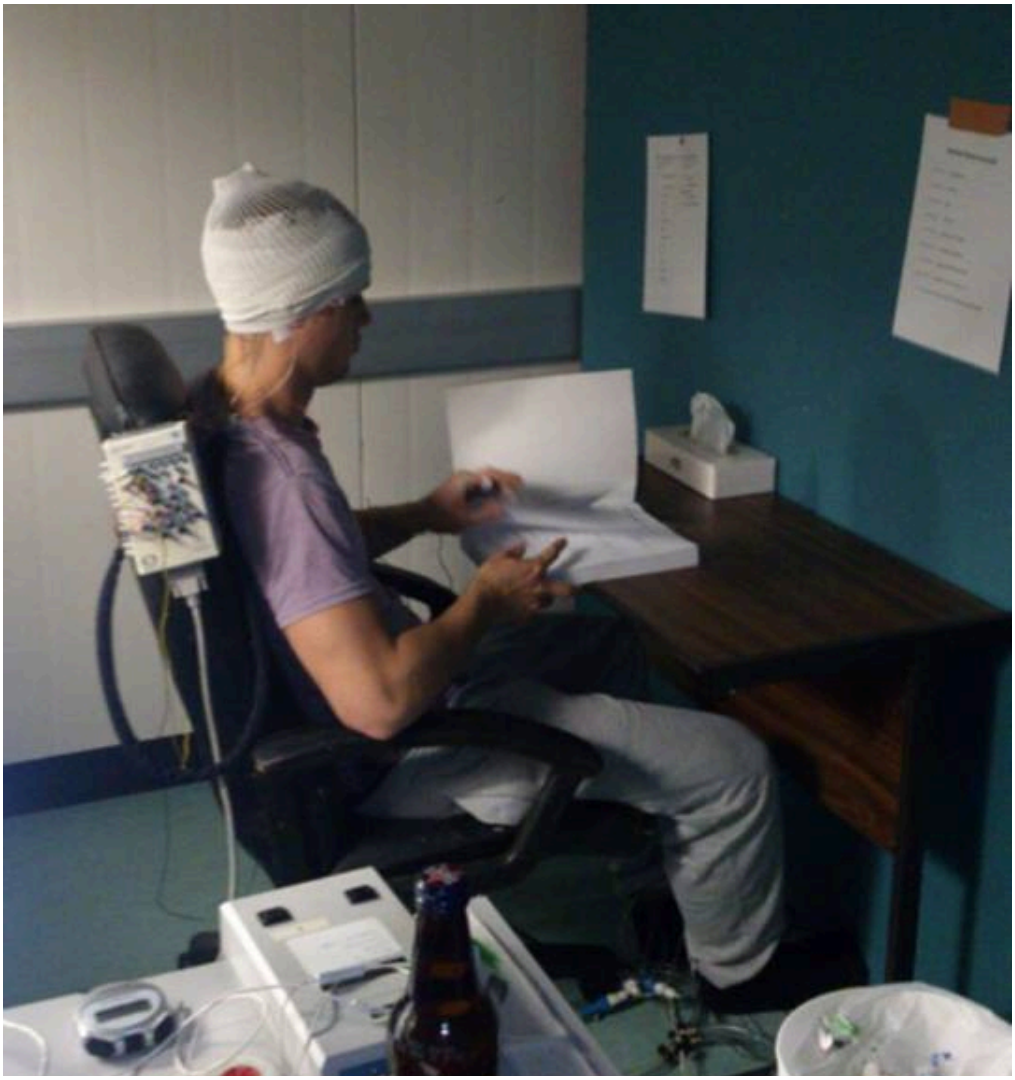


Figure 1: Depiction of participant set up from view of experimenter.

Appendix D: Criteria for analysis of the 10 min PVT.

Table 1: *Criteria for the analysis of the 10-min PVT. Extracted from Basner and Dinges, 2011 (Basner et al., 2011)*

Table 3—Criteria for the analysis of the 10-min PVT

Standard outcomes	Mean 1/RT[1] and number of lapses
Stimulus	Visual millisecond counter in rectangular box
Test duration	10 min (the tests stops with the last response after an elapsed total time of 10 min)
Inter-stimulus interval	2-10 s (defined as the period between the last response and the appearance of the next stimulus)[2]
Feedback	The response time is displayed for 1 s. This period is part of the next inter-stimulus interval.
Errors of commission (false starts)[4]	Responses without a stimulus or response times < 100 ms.[3] „FS" is displayed for 1 second (this period is part of the next inter-stimulus interval).
Errors of omission (lapses)	Response times ≥ 500 ms[3]
Time out	The millisecond counter times out after 30,000 ms without a response. "OVERRUN" is displayed for 1 second (this period is part of the next inter-stimulus interval) and a sound is played back to alert the subject. The stimulus is counted as valid, i.e., as a lapse with a response time of 30,000 ms.
Button fail-to-release[4]	"BUTTON" is displayed after the response button has not been released for 3 s and a signal is continuously played back until the button is released. The new inter-stimulus interval starts once the button is released.
Wrong key press[4]	"ERR" is displayed for 1 s if the wrong response key is pressed (this period is part of the next inter-stimulus interval). If the wrong key was pressed prematurely "FS/ERR" is displayed instead of "ERR."

RT, response time.
[1]Individual raw RTs [in ms] are first transferred to response speed by calculating 1000/RT. These response speeds are then averaged.
[2]In the PVT used in the total and partial sleep deprivation studies, we randomly drew full second inter-stimulus intervals (ISI), i.e., 2, 3, 4, 5, 6, 7, 8, 9, or 10 s. This completely random process may introduce variance in the number of stimuli between test bouts, where bouts with a low number of stimuli will be biased towards longer ISIs. In a revised version of the test, a block randomization technique is used that guarantees that the number of stimuli is similar between bouts without losing the random component of ISIs.
[3]For auditory stimuli, this threshold would have to be lower.
[4]These events do not count as valid stimuli, and thus they do not contribute to the calculation of mean 1/RT.

Appendix E: Outline for drowsiness scoring

Table 1: *Outline for significant events in progressing drowsiness*

TABLE 1. *Changes in ocular movements and EEG during progressive drowsiness*[a]

State	Eye motion	SEM	EOG	Excursion	Velocity	Frequency	EEG
Awake (eyes open)	Blinks	N	Y	H	H	4/10 s	Awake
Awake (eyes closed)	Mini-blinks	N	Y	M/H	H/M	5–10/10 s	Alpha M/absent Alpha maximum
Early drowsiness	SFIEM	N	N	L	H	5–30/10 s	Alpha maximum Drowsy alpha
Middle drowsiness	SFREM	M	Y/N	L	H/L	3–8/s	Drowsy alpha Absent alpha, slow
Late drowsiness	SEM	—	Y	M/H	L	<0.5/s	Absent alpha, slow

[a]See text (I. Relationship Between Ocular Patterns and EEG) for explanation. In SEM and EOG columns, Y/N refers to presence or absence of activity. L, M, and H indicate low, medium, and high. Alpha M indicates alpha of moderate amplitude compared to maximum observed. Drowsy alpha is a change in amplitude, distribution, or frequency of alpha consistently associated with drowsiness. Slow refers to delta and theta activity.

Appendix F: Pilot temperatures

Table 1: *Trial cooling protocol for application to Mild and Moderate conditions in main study.*

		Tpad	Ttop	Farch	F1mt	average	water	difference
	pre	31.2	30.8	32.45	30.95	31.35	21.6	9.75
	in boot	34.1	33.35	33.55	32.65	33.41	21.65	11.76
	0	32.45	32.3	32.05	31	31.95	22.15	9.80
cooling 30	5	31.7	31.6	31.35	30.25	31.23	22.4	8.83
	10	31	31.05	30.75	29.65	30.61	22	8.61
	15	30.4	30.45	30.3	29.25	30.10	22.25	7.85
	20	29.95	30	29.9	28.9	29.69	22.85	6.84
	25	29.95	30	29.7	28.9	29.64	23.6	6.04
	30	30.6	30.55	29.7	29.15	30.00	24.75	5.25
	35	31.05	30.95	29.75	29.45	30.30	24.95	5.35
	40	30.85	30.8	29.65	29.45	30.19	25	5.19
cooling 25	45	29.2	29.1	28.4	28.05	28.69	16.9	11.79
	50	27.25	27.35	27.15	26.35	27.03	14.45	12.58
	55	25.9	26	26.45	25.25	25.90	14.15	11.75
	60	25	25.1	25.9	24.6	25.15	14.65	10.50
	65	24.25	24.4	25.55	24.25	24.61	15.35	9.26
	70	23.75	23.85	25.4	24.1	24.28	17.55	6.73
	75	23.5	23.55	25.4	24.25	24.18	17.85	6.33
	80	23.5	23.5	25.5	24.45	24.24	20.5	3.74
	85	23.8	23.7	25.7	24.9	24.525	21.2	3.33
	90	23.9	23.8	25.75	25.1	24.6375	21.35	3.29

Original pilot study consisted of a simulated driving task, with EEG comprising arousal markers, while undergoing fan directed cooling. Testing took place over two hours starting at 23:30. Four participants took part. Cooling in the original study consisted of two phases: Foot bath, either warm () or cool (), followed by wearing warm socks or directed fan blowing over ice (Figure 1). Cooling methods were deemed to be inadequate for testing the hypothesis, which led to the above pilot testing of a more sustained, and reliable method. Much of the EEG input was lost due to noise (likely electrostatic and mechanical, as well as due to novice protocol), but an indication is provided in Figure 2.

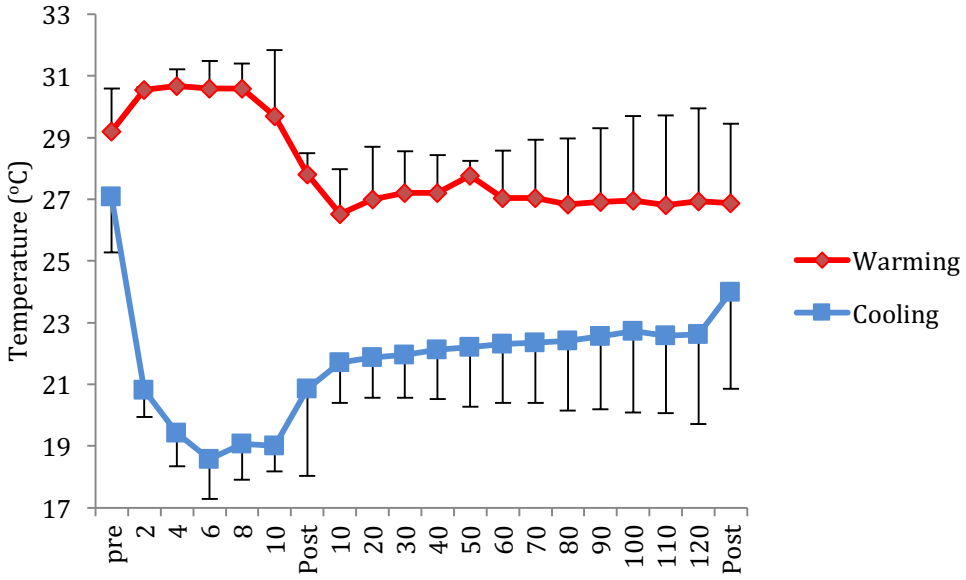


Figure 1: Original study foot temperature manipulations for both cooling and warming protocols.

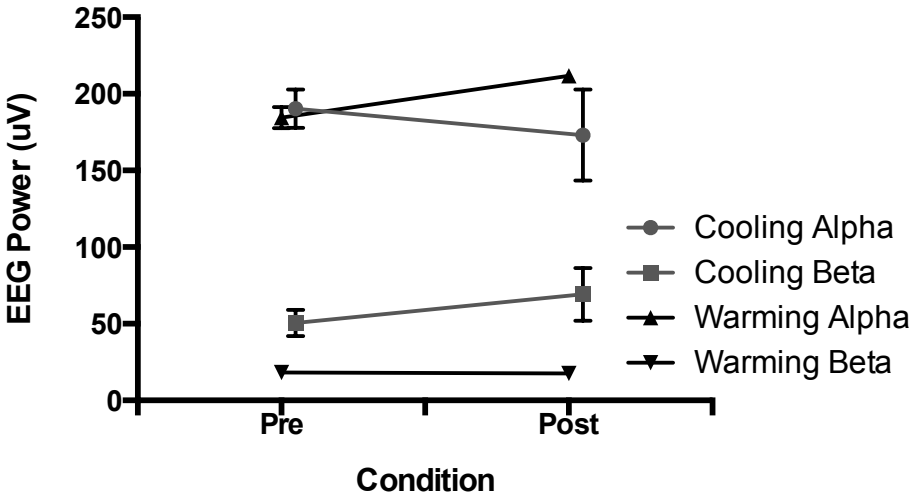


Figure 2: Collated EEG (referential electrode derivations F4, FCz, O2 to A1), of both peak alpha (8-12 Hz), and peak beta (13-30 Hz) powers Pre- and Post-test, for both warming and cooling protocols.

Appendix G: Water perfused booties



Figure 1: View of internal tubing lining the custom fitted booties.



Figure 2: Custom fitted booties worn.

The water perfused booties opened out to reveal inner tubing (Figure 1) and while worn (Figure 2). Laces and white patch on medial border are elasticated (indicated by red arrow).

Appendix H: Karolinska Sleepiness Scale

Karolinska Sleepiness Scale (KSS)

1 ========== extremely alert

2========== very alert

3========== alert

4========== rather alert

5========== neither alert nor sleepy

6========== some signs of sleepiness

7========== sleepy, no effort to stay awake

8========== sleepy, some effort to stay awake

9========== very sleepy, great effort to keep awake, fighting sleep

Appendix I: Thermal sensation and thermal discomfort scales

PERCEIVED TEMPERATURE SCALE

"How does the temperature of your Body [feet] feel?"

1	**Unbearably cold**
2	**Extremely cold**
3	**Very cold**
4	**Cold**
5	**Cool**
6	**Slightly cool**
7	**Neutral**
8	**Slightly warm**
9	**Warm**
10	**Hot**
11	**Very hot**
12	**Extremely Hot**
13	**Unbearably hot**

THERMAL DISCOMFORT SCALE

"How comfortable do you feel with the temperature of your body?"

1	**Comfortable**
2	**Slightly uncomfortable**
3	**Comfortable**
4	**Very Uncomfortable**
5	**Extremely uncomfortable**

Appendix J: The Brunel Mood State questionnaire

The Brunel Mood scale

Below is a list of words that describe feelings. Please read each one carefully. Then cross the box that best describes HOW YOU FEEL RIGHT NOW. Make sure you answer every question.

Name …….. Date __/__/2009

		Not at all	A little	Moderately	Quite a bit	Extremely
1	Panicky……..	( )	( )	( )	( )	( )
2	Lively……..	( )	( )	( )	( )	( )
3	Confused……..	( )	( )	( )	( )	( )
4	Worn out……..	( )	( )	( )	( )	( )
5	Depressed……..	( )	( )	( )	( )	( )
6	Downhearted……	( )	( )	( )	( )	( )
7	Annoyed……..	( )	( )	( )	( )	( )
8	Exhausted……..	( )	( )	( )	( )	( )
9	Mixed-up……..	( )	( )	( )	( )	( )
10	Sleepy……..	( )	( )	( )	( )	( )
11	Bitter……..	( )	( )	( )	( )	( )
12	Unhappy……..	( )	( )	( )	( )	( )
13	Anxious……	( )	( )	( )	( )	( )
14	Worried……	( )	( )	( )	( )	( )
15	Energetic……..	( )	( )	( )	( )	( )
16	Miserable……..	( )	( )	( )	( )	( )
17	Muddled……..	( )	( )	( )	( )	( )
18	Nervous……	( )	( )	( )	( )	( )
19	Angry……..	( )	( )	( )	( )	( )
20	Active……..	( )	( )	( )	( )	( )
21	Tired……..	( )	( )	( )	( )	( )
22	Bad tempered……..	( )	( )	( )	( )	( )
23	Alert……..	( )	( )	( )	( )	( )
24	Uncertain……..	( )	( )	( )	( )	( )

How much sleep have you had in the last 24 hours? .....hours ..... minutes

Appendix K: Additional data

Mood states, vigour and fatigue

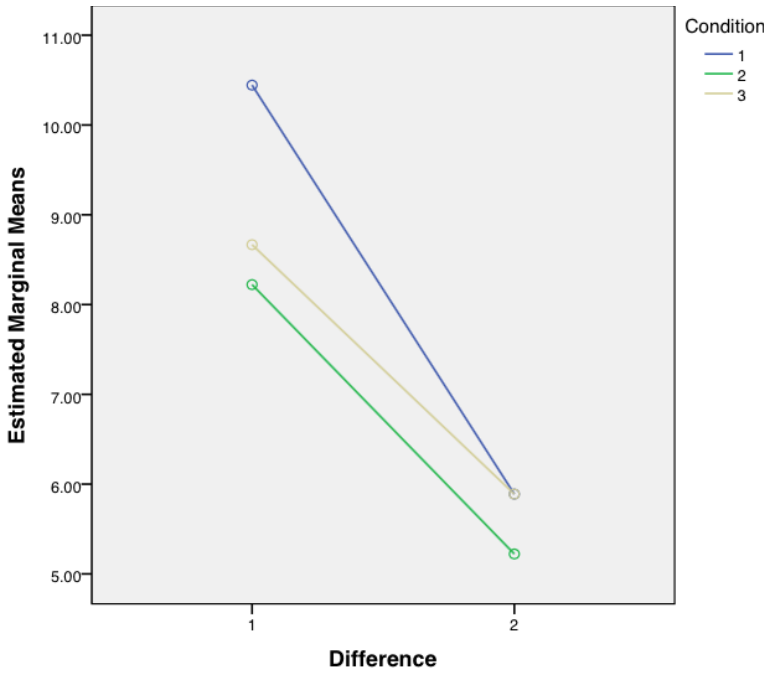


Figure 1: BRUMS mood characteristic: Vigour pre post. Condition 1=Control, 2=Mild, 3=Moderate

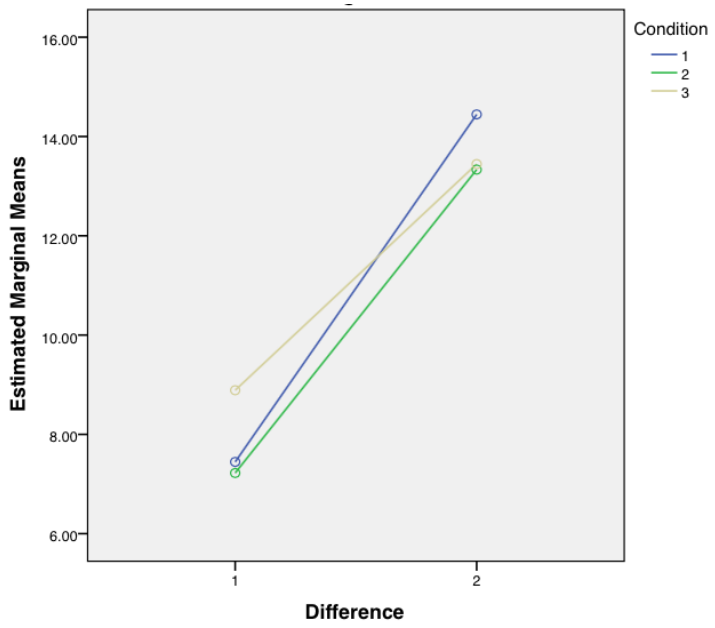


Figure 2: BRUMS mood characteristic: Fatigue pre post. Condition 1=Control, 2=Mild, 3=Moderate

Appendix L: Participant eight

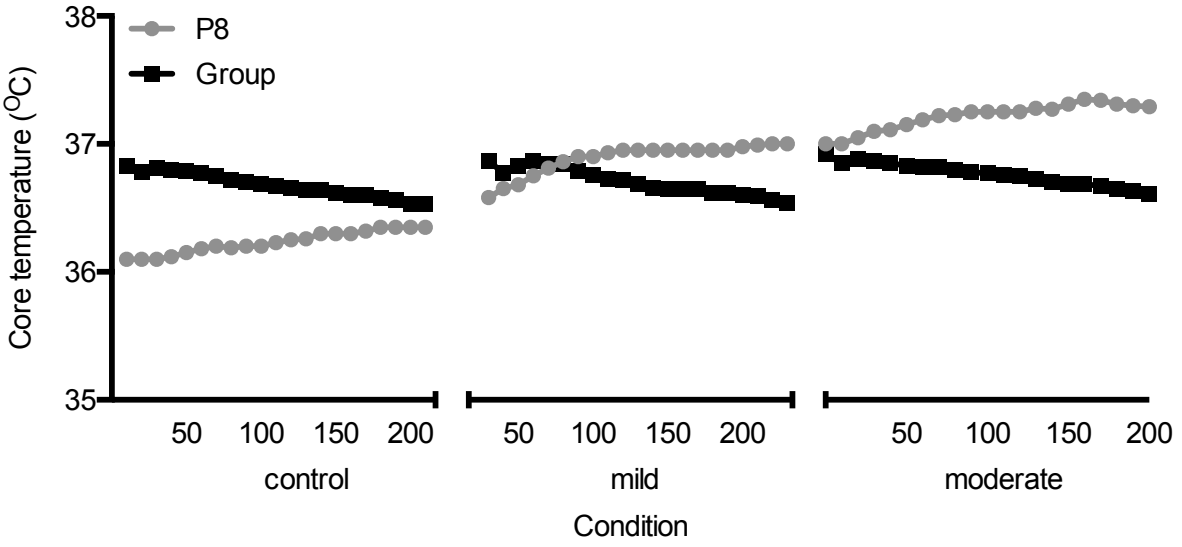


Figure 1: Participant eight core temperature across conditions, compared to the group trend.

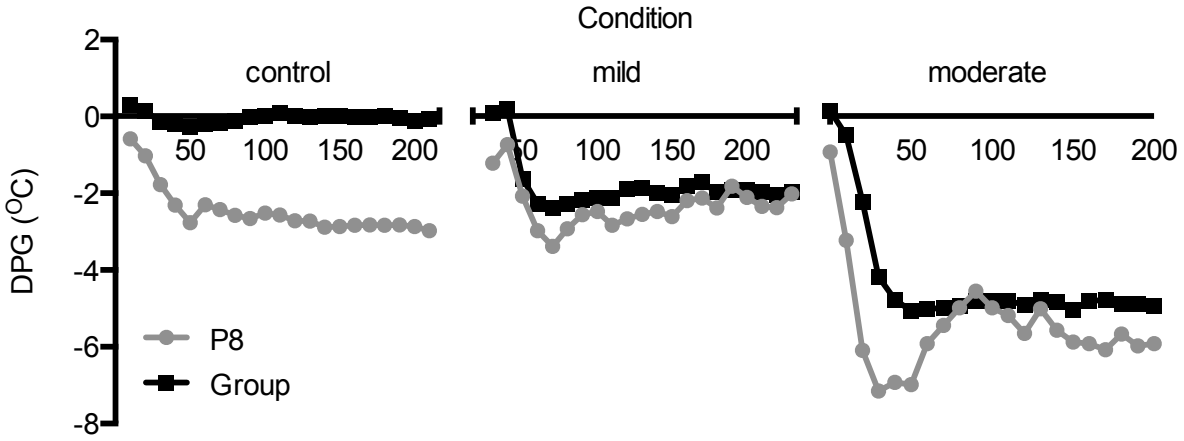


Figure 2: Participant eight DPG across conditions, compared to the group trend.