Circadian variation of the acute and delayed response to alcohol: Investigation of performance, physiological, and biochemical variations.

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Abstract

The overall aim of this thesis was to examine a range of measures, with respect to alcohol administration time, in the same subjects using a controlled experimental design over an extended period of time. Evaluation and comparison of different systems simultaneously was undertaken in order to examine the chronokinetics and chronesthesy of alcohol. The aim of Study 1 was to ascertain whether a low or high dose of alcohol could be detected as interacting with time of day (1300 hr and 1800 hr) on performance of a dual task and subjective sleepiness. It was found that when a high dose of alcohol was ingested at 1300 hr, time off target on pursuit rotor under single and dual task conditions was higher than when alcohol was ingested at 1800 hr. There was no time of day variations in the BAC results. Study 2 aimed to further investigate the time of day effect found in Study 1. The aim of this study was to investigate time of day (1300 hr and 1800 hr) effects on biochemical (melatonin and cortisol) and physiological variables (temperature and heart rate) for no alcohol and alcohol conditions across the blood alcohol curve (0 – 5 hr post alcohol ingestion). Additionally, the pharmacokinetics of alcohol were explored for time of day variations. Results from Study 2 showed performance on a dual task and ratings of subjective states was not dependent on alcohol administration time. In contrast, core body temperature, heart rate and melatonin changes after alcohol ingestion appeared to be dependent on the time alcohol was ingested. Alcohol absorption rate was also found to be dependent on the time of day alcohol was ingested. Study 3, the second phase of study 2, aimed to investigate the delayed effects (5 hr – 14 hr post ingestion) of alcohol ingestion at two times of the day on core body temperature and subjective states. It was found that alcohol had statistically significant effects on the core body temperature and ratings of the subjective state of gregariousness 14 hr post alcohol

ingestion. Of greatest consequence was the finding that regardless of when alcohol was ingested (1300 hr or 1800 hr), core body temperature was increased in comparison to a no alcohol condition from 2330 hr to 0830 hr (sleep phase).

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Table of Contents

Chapter 1
Biological rhythms

Chapter 2
Chronopharmacology

Chapter 3
Alcohol

Chapter 4

Study 1: Effect of alcohol dose and time of day on performance and sleepiness

Chapter 5

Study 2: Acute effects of alcohol at two times of the day on performance, physiology, biochemistry, and subjective states in humans

Chapter 6

Study 3: Delayed effects of alcohol at two times of the day on body temperature, melatonin, cortisol, and subjective states

List of Figures

List of Tables

Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any other institution of tertiary education. Information derived from published or unpublished work of others has been acknowledged in the text and a list of references is given.

3rd Dec 2002
Date

Introduction

> Time is a brisk wind, for each hour it brings something new....but who can understand and measure its sharp breath, its mystery and its design? Therefore the physician must not think himself too important; for over him there is a master – time - which plays with him as a cat with a mouse.
>
> (PARACELUS VON HOHENHEIM'S GERMAN COMMENTARY ON THE APHORISMS OF HIPPOCRATES cited by Gaer Luce, 1971)

Chapter 1

In this chapter, research is presented demonstrating that a human time structure exists. Information is presented on the variety of techniques used to measure circadian rhythms, including, physiological and biochemical measures such as temperature, melatonin, and cortisol. Behavioural and cognitive variations across the day are also explored including a discussion of the cognitive and performance tasks used to measure these. The notion of circadian typology is reviewed and the influence typology has on rhythmic and/or time dependent changes in performance and physiology.

Chapter 2

Research on human chronobiology has important consequences for the investigation of the effects of alcohol on physiology and behaviour. In this chapter the observations of chronopharmacology researchers are examined and findings on the chronokinetics and chronopharmacology of alcohol are critiqued.

Chapter 3

The third chapter is divided into two sections. The first section deals with the pharmacology of alcohol and the factors that can influence the pharmacokinetics and

pharmacodynamics of alcohol, including characteristics of the individual such as age, rate of intake, and whether food was consumed with the alcohol. The remainder of the chapter explores research on the effects of alcohol on commonly used indices of circadian rhythms such as body temperature, melatonin, cortisol and heart rate. Alcohol's effects on human performance tasks are also reviewed. Finally, design and methodological issues relevant to studies of the effects of alcohol on human physiology and performance are considered.

Chapter 4

Chapter 4 presents Study 1. The focus of the study was to ascertain whether time of day could be detected as interacting with alcohol on measures of performance and sleepiness. Further, the study aimed to ascertain whether the measures of performance were sensitive to alcohol and/or time of day changes, and if so, which dose was the most effective in producing these effects.

Chapter 5

Chapter 5 presents study 2. Study 2 examined the acute effects of alcohol at two times of the day on performance, physiology, biochemistry, and subjective states. The focus of this study was to examine a range of measures, with respect to alcohol administration time, using a controlled experimental design over a longer period of time. Evaluation and comparison of different systems simultaneously was undertaken in order to examine the chronokinetics and chronesthesy of alcohol.

Chapter 6

Chapter 6 presents study 3 which followed on from study 2 to explore the delayed effects of alcohol on body temperature and subjective states. The focus of this study was to track changes in core body temperature throughout the sleep phase to the following morning after alcohol ingestion.

Chapter 7

The final chapter discusses major findings from the three studies. The findings are examined within a chronopharmacological framework. The implications of the findings from thesis are discussed along with the limitations. Finally, recommendations are made for future research.

Chapter 1: Biological rhythms

1.1 History of biological rhythms

"To have life is to have rhythmicity" (Brown, 1982, p. 4). The sense of stability and calm experienced by organisms is due to the many inter-relationships among the multitude of biological rhythms. Rhythms are found in the simplest-celled to the most complex organisms. Additionally, rhythmicity can be seen in the annual growing cycles of plants. It is no accident that the rhythms of life correspond to particular natural geophysical oscillations, as the universe exudes rhythmicity (Brown, 1982). In ancient times it was mostly believed that cyclical variations in organisms were the result of cycles in the environment (Touitou & Haus, 1992).

However, it was not until the 18th century that scientific awareness developed about the possible existence of organisms' rhythms independent of direct influence from environmental cycles (Brown, 1982). In 1729 de Marian made the first observations leading to the recognition of the endogenous nature of certain rhythms. de Marian challenged the traditional theory that the heliotrope plant opened and closed its leaves in response to the daily cycle of light and darkness. By observing that the rhythm persisted in isolation from the daily light/dark cycle (L/D), de Marian demonstrated the existence of an endogenous rhythmic process (Brown, 1982; Coren, 1996; Kronauer, 1994).

For the next 200 years work by many investigators suggested the existence of an internal mechanism controlling the daily pattern of leaf movements. However, investigation to determine the mechanisms of these rhythms did not begin until the late 1920s. The Dutch botanist, Kleinhoonte conducted an extensive study of natural and artificial lighting factors affecting the spontaneous daily leaf movements in the

bean plant. Internally driven rhythms were validated and were found to originate from an endogenous mechanism, in contrast to an exogenous synchroniser (Brown, 1982). Research on the alleged endogenous rhythms of organisms was slow. However, since 1960 there has been much research conducted on biological rhythms at all levels, and in all forms of life. To discover more about the endogenous nature of rhythms in humans, researchers adopted a version of de Marian's experimental paradigm. This paradigm is known as the free-run paradigm.

1.2 Free-run paradigm

The recognition of an endogenous component of a rhythm requires the persistence of a rhythm under – as far as feasible – constant conditions removed from any known environmental time cues. The precedent for studying human rhythms in isolation was set by sleep researcher, Kleitman (1939, as cited in Coren, 1996). He and a colleague isolated themselves in Mammoth Cave, Kentucky. The primary objective was to remove themselves from the influence of daily changes in the environment. In the early 1960's, free-run experiments involving humans were initiated at several locations in Europe. Various types of isolation environments were used such as natural caves (e.g. a subterranean glacier, underground caves, an area in Antarctica that was remote from alteration of light/dark (L/D) and social activity) or laboratory isolation facilities. Core body temperature (rectal) and activity were continuously monitored. It was found that under isolation conditions, people do show a circadian rhythm of sleep and wakefulness. Their activities regularly cycled over the course of a day, thus confirming the existence of an internal biological clock (Coren, 1996).

These studies have demonstrated that the internal timer was not exactly synchronised with the 24 hr day. For most people, the day was around 25 hr long. The period of the rhythm usually deviated slightly but consistently from the environmental cycle to which it was normally entrained (synchronised); it thus "free-runs" from the synchroniser cycle (solar day). On the first day of free-running people go to sleep about one hour later than they would normally and rise later each day, the next day they go to sleep another hour later, and so on. After about two weeks, subjects in these studies are about half a day out of synchrony with the outside world. Their sleep and wake times continue to drift as their biological clock is using a day that is one hour longer than the normal day-night cycle (Coren, 1996). It has also been shown that the time estimates of people who participated in isolation studies were vastly underestimated. For example, Siffre (1962, as cited in Gaer Luce, 1972) emerged after 63 days in Scarasson Cavern, reporting that he estimated his time in the cave to be 25 days or less. Moreover, other volunteers report having naps, when in fact they have been asleep for over 6 hr (Gaer Luce, 1972).

More elaborate isolation studies have been undertaken since the 1960s that have incorporated advanced technology, such as EEG monitoring, plasma hormone rhythm measurement, and performance measurement. Such in-depth appraisal of the individuals' physiology renders these studies very expensive, however the wealth of information gathered enables many questions to be answered (Kronauer, 1994).

1.3 Principles of rhythms

Rhythms are either externally imposed, generated internally, or more usually a combination of both (Arendt, 1994). A rhythm represents a regularly recurring oscillation, the repeating unit of which is referred to as a cycle. The periods of the

rhythms have varying ranges, for example a fraction of a second (e.g. the firing of neurons) to years (e.g. population variations) (Arendt, 1994). While the current focus is on circadian[1] rhythms, it is noted that circadian rhythms are only one frequency in a human's multi-frequency biological time structure. The cycles with shorter periods, less than a day, (higher frequencies) are called ultradian (e.g. pulse rate, pulsatile secretion of hormones). The rhythms with longer periods, more than a day (lower frequencies) are called infradian, which include rhythms with periods of about one week (circaseptan rhythms), rhythms with periods of about 30 days (circatrigintan), and rhythms with a period of about one year (circannual) (Arendt, 1994; Touitou & Haus, 1992). Other rhythm terms are illustrated in Figure 1.1 and have been defined in the glossary. Rhythms corresponding to major external environmental rhythms (daily, lunar, annual) are the most prominent; daily rhythms are the most extensively studied rhythms, with most interest lying in human physiology and pathology (Arendt, 1994; Touitou & Haus, 1992). Circadian rhythms of the length of about one day, ranging from 22 – 28 hr have been observed free running at every system level in nearly all plants and animals, from subcellular particles to the mammalian species including humans (Coren, 1996; Kronauer, 1994).

[1] Latin word meaning `about a day`

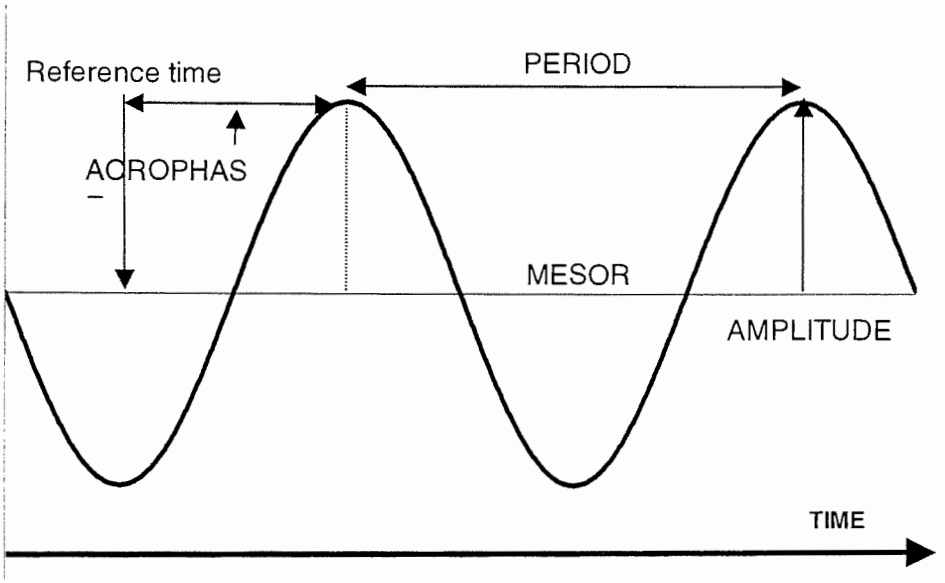

Figure 1.1. Illustration of rhythmic function parameters by cosine curve fitting. (1) MESOR (midline estimator of rhythmicity), (2) AMPLITUDE (one half the peak to trough difference), (3) ACROPHASE (peak time with reference to a time marker) (adapted from Haus & Touitou, 1992).

1.4 Entrainment of rhythms

The reason that our daily cycle does not drift out of agreement with the solar day is due to a mechanism that synchronises the external environment with the internal biological clock. The geophysical cycles of day and night and the social surrounding induce cyclical responses, however they may also function as entraining agents for endogenous rhythms (Monk, 1991; Touitou & Haus, 1992; Wever, 1982).

The environmental oscillator exerting phase control over an endogenous rhythm can be termed synonymously "synchroniser" or "zeitgeber" (German word meaning time giver) (Coren, 1996; Touitou & Haus, 1992). Importantly, as noted by Touitou and Haus (1992) entrainment of an endogenous rhythm can only be to

frequencies that do not deviate too much from its own frequency, that is, each endogenous rhythm can only be synchronised by exogenous cycles within its *range of entrainment* (Monk, 1991; Wever, 1982). In the event that the frequency is unacceptable to the endogenous rhythm, the rhythm will no longer be synchronised but will free-run with its own frequency. However, it may still be modulated by the environmental stimulus (Monk, 1991; Touitou & Haus, 1992;Wever, 1982). The effect of a synchroniser upon an endogenous rhythm is dependent upon the stage of the rhythm when the stimulus is applied. A graph of those effects (phase advance, phase delay) is called a Phase Response Curve (PRC). The PRC for different synchronisers can vary between individuals and across species (Monk, 1991; Touitou & Haus, 1992; Wever, 1982).

1.4.1 Light: The primary zeitgeber

It has been outlined that the reason that internal and behavioural rhythms continue on a 24 hr cycle and do not drift out of agreement with the solar day is the fact that there is a mechanism that synchronises the internal clock with environmental (local) time. It was once believed that humans were not affected by light and that social cues were the strongest zeitgebers. Research over the past two decades on light has demonstrated that light has profound chronobiological effects in humans. It is now generally believed by sleep researchers that light is the primary zeitgeber (Lewy, Sack, & Singer, 1990).

Demonstration that light is a zeitgeber that resets the internal clock has been conducted again through the use of sleep isolation chambers. Light was dimmed for 6 - 8 hr out of each 24 hr period, in order to simulate day and night light levels. Subjects tended to sleep when the light dimmed and awakened sometime after it

brightened. The internal timer appeared to be in synchrony with the light in the environment, instead of reflecting the 25 - hr day with no variation in light levels (Binkley, 1990; Coren, 1996; Gaer Luce, 1972).

Under normal conditions, circadian cycles, such as core body temperature, urine release, and release of hormones and steroids, are all synchronised with one another. For example, nightly sleep is usually preceded by a downward turn in body temperature. Under free-running conditions, internal desynchronisation (defined as a loss of synchronisation between two or more rhythms so that they show independent periods) can occur. Thus, one of the important functions of light as a zeitgeber is to lock all of these cycles together. Light variations, thus, do not only reset the circadian clock that controls sleep and wakefulness; they also reset all of the other circadian cycles in the body (Coren, 1996).

Blind people provide further evidence for light as a synchroniser for the internal timer. Blind individuals, as a group, have very high rates of sleep complaints. Furthermore, most blind people report that their sleep problems are cyclical in nature, occurring in cycles around one month in length (Coren, 1996). This is what would be expected if blind people had a free running internal clock that was an hour longer than 24 hr. (Arendt, Skene, Middleton, Lockley, & Deacon, 1997).

The light/dark cycle may not be the only zeitgeber. Rather, the light/dark cycle may act in combination rhythms of activity and meal times, and other natural behavioural consequences of the light dark cycle. For example it has been demonstrated that appropriately timed exercise can phase shift the circadian temperature rhythm (Eastman, Hoese, Youngstedt, & Liu, 1995). In spite of this, it

has been contended that all of these zeitgebers are much weaker and less reliable than light (Kronauer, 1994).

1.5 Circadian system

1.5.1 Circadian system: Suprachiasmatic nuclei (SCN)

The general understanding is that oscillatory components of the circadian system are enslaved to a dominant pacemaker (Kronauer, 1994). Moreover, free run studies have demonstrated that different variables oscillate with different periods. Typically the sleep/wake rhythm, under the subjects' choice, had an average period of 30 – 50 hr, and the cycle of body temperature had a period of about 24.5 hr. Only a fraction of free-running subjects (less than 30%) exhibit these two rhythmic components, representing internal desynchrony. Most free-running subjects show a single rhythm with a period of about 25.5 hr. These subjects are described as internally synchronised (Kronauer, 1994). Kronauer (1994) intimated that these data suggested "at least a two-oscillator system where the oscillators influence each other and have adequate power in their internal synchrony to offset individual period differences" (Kronauer, 1994, p. 4).

The suprachiastmatic nuclei (SCN) are located in the anterior hypothalamus, just above the optic chiasm. Their total volume is approximately 0.1 mm^3, containing about 20,000 neurons in two major subdivisions. "The ventrolateral portions (which are the major recipients of neural input) contain vasoactive intestinal peptide cells while the dorsal-medial portions contain tightly packed vasopressin cells. The SCN are innervated principally by specialized ganglion cells which have large receptive fields that very likely sample the entire retina in each eye" (Kronauer, 1994, p. 103). A host of experiments involving mainly rats and hamsters has led to

the present understanding that the paired suprachiasmatic nuclei (SCN) are a circadian pacemaker. The neural pathway known as the retinohypothalamic tract (RHT) arises from retinal ganglion cells and terminates in the anterior hypothalamus. In animals, ablation of all visual pathways past the optic chiasm produces a lack pupillary light reflexes and results in behavioural blindness. However, the animals display normally entrained circadian rhythms. On the contrary, lesions that undercut the SCN, ablating retinohypothalamic projections, abolish entrainment. These observations indicate the retinohypothalamic projection is adequate to mediate entrainment (Moore, 1990).

A major objective of circadian rhythm researchers over the past decades has been to identify and localise biological clocks in living tissue (Turek, 1998). It has now been established in mammals that the SCN contains a master circadian clock that regulates most, if not all, endogenously generated circadian rhythms (Miller, Morin, Schwartz, & Moore, 1996). The initial finding that led to the hypothesis that the SCN was a master circadian pacemaker was the discovery that following SCN ablation in rats various circadian rhythms were abolished (Moore & Eichler, 1972). However, loss of circadian function could reflect either a pacemaker function or a relay station function for those rhythms that are abolished after SCN removal (Kronauer, 1994; Moore, 1990; Rietveld, 1992). Nevertheless, there is now substantial evidence supporting the view that the SCN is a circadian pacemaker and that the effects of SCN ablation reflect loss of pacemaker activity. Additionally, there is substantial evidence from a variety of experiments (Klein, Moore, & Reppert, 1991) to demonstrate a central role for the SCN as a 'master circadian pacemaker' in mammals. The first is that isolation of the SCN neurally by knife cuts demonstrated circadian rhythmicity persisted despite isolation. Further, neural

transplantation studies also provide evidence for a pacemaker function of the SCN. If fetal anterior hypothalamus containing the SCN is transplanted into the rostral third ventricle in adult animals rendered arrhythmic by SCN lesions, rhythmicity will be restored. To date there is no convincing data to indicate that any structure outside of the SCN acts as a master circadian pacemaker. There is occasional confusion, particularly with respect to the role of the mammalian pineal gland as a possible circadian pacemaker in mammals. The pineal gland is not capable of generating circadian rhythms, the regulation of melatonin appears totally under the control of circadian neural signals from the SCN, although the pineal melatonin rhythm may regulate other rhythms.

1.5.2 Circadian system: Melatonin rhythm generating system

It is known today that the photoneuroendocrine system is comprised of three key components: (a) an input pathway, that is, photoreceptor cells located in the retina, that transmits information about light levels necessary for entrainment to the light dark cycle; (b) a pacemaker, the SCN contained in the hypothalamus; (c) an output pathway for the expression of overt rhythms, that receive signals from the photoreceptors and the endogenous oscillators and translate them into a neurohormone response (see Figure 1.1) (Arendt, 1994; Korf, Schomerus, & Stehle, 1998).

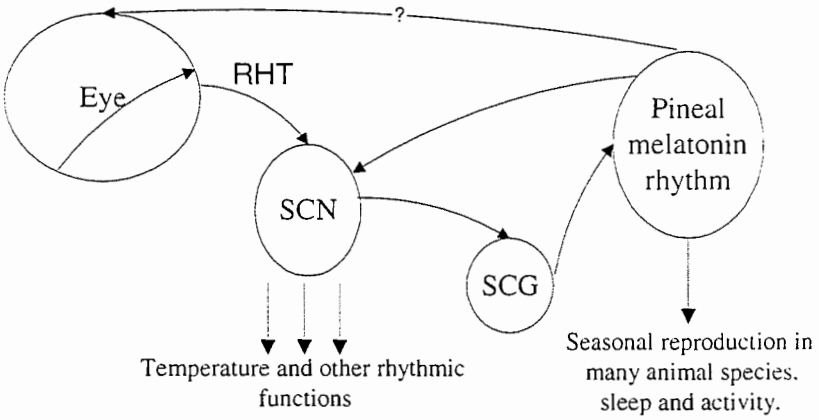

Figure 1.2. The circadian system (adapted from Klein, 1993). Superior cervical ganglion (SCG) is a pair of sympathetic ganglia located in the neck; relay signals to the pineal gland

The pineal gland named because of its similarity in appearance to a pinecone, lies almost in the centre of the human brain (Coren, 1996; Graham & Armstrong, 1995). The pineal gland is interesting in terms of its evolution. It appears to be all that is left of what is referred to as the third eye in some Eastern cultures (Coren, 1996; Graham & Armstrong, 1995). This eye would have been located quite high and in the middle of the head. By far the most important input to the pineal is that of light-dark transitions that indicate photoperiod. Just as ablation of the SCN destroys rhythm generation, the removal or denervation of the pineal destroys the ability to perceive changes in photoperiod and thus also abolishes rhythm generation (Arendt, 1992). In many of the lower animals, such as some fish and reptiles, the pineal gland still serves as a light sensor. In several nonmammalian species (e.g. zebra fish and house sparrow) the pineal holds a complete photoneuroendocrine system, which compromises, photoreceptors, endogenous oscillators, and neuroendocrine effectors (Klein, 1993; Korf et al., 1998). The term melatonin rhythm generating system was established to emphasise the integration between the SCN, eyes, and pineal. Lesions

at any point in the melatonin rhythm generating system dramatically disrupt the rhythm (Klein, 1993).

While the human pineal tends to calcify during development, it does not imply that the gland is functionless or has decreased activity throughout adult life (Arendt, 1992). The mesor and amplitude of the plasma melatonin rhythm are very low during the early stage of life; they rise during the ages of 0 to 3 years and then slowly decline to reach adult levels in the late teens (Arendt, 1992). The decline has been attributed to pubertal development, assuming an anti-gonadotrophic function of the pineal (Arendt, 1992). During adult life melatonin in plasma and aMT6s (melatonin metabolite) in urine remain constant. However, it has been reported that in old age there is a significant decline in the amplitude of melatonin production (Arendt, 1992). This claim has been challenged recently. Research by Zeitzer et al. (1999) found that older subjects exhibited similar melatonin rhythms with similar nocturnal melatonin peaks. Supporting this, Fourtillan et al. (2001) found that the rate of melatonin secretion did not differ between young and elderly subjects. In summary, it appears that melatonin levels are relatively constant throughout adult life although there is some conflict in regards to the melatonin rhythm throughout older age.

1.6 Melatonin: The darkness hormone

The pineal gland manufactures and secretes melatonin rhythmically during the nighttime in response to photoperiodic stimuli and signals from the SCN (Korf et al., 1998; Arendt, 1992). In humans, the direct effect of an increased amount of melatonin in the blood is a state of sleepiness. Daytime melatonin levels tend to be around the limit of detection of most assays less than 10 pg/ml. According to Arendt

(1992) average profiles indicate that the evening rise starts around 2100 hr, with maximum values occurring usually between 0100 hr - 0500 hr, declining to daytime levels by around 1000 hr. While the melatonin rhythm is extremely reproducible in the same individual, there are large inter-individual variations. Thus, if an experimenter were to use a between-subjects design to examine melatonin rhythms, a very large sample of subjects would be needed (Arendt, 1992).

The main biosynthetic steps involved in the production of melatonin have been studied primarily in rodents. Biosynthesis starts with the uptake of circulating tryptophan into pinealocytes. This is then hydroxylated in the 5 position to hydroxytryptophan, which is subsequently decarboxylated to serotonin. According to Korf et al. (1998) the concentration of serotonin in the pineal gland exceeds the amounts of any other tissue except for the raphe nuclei of the midbrain. The next step of biosynthesis involves the formation of N-acetylserotonin catalysed by serotonin-N-acetyltransference (NAT). NAT is usually considered to be the rate-limiting enzyme in melatonin production in view of its large increase in activity at night (Arendt, 1992; Korf et al., 1998).

Many animal species use photoperiod as the primary environmental input for time dependent events. Therefore, from a functional point of view, it seems that the rhythmic production of melatonin is necessary for seasonal reproduction, it influences sleep and activity and modulates the function of the endogenous rhythm generator, which consequently plays a role in physiology and pathology (shift work, jet lag, seasonal affective disorder) (Arendt, 1992; 1994; Korf et al., 1998).

1.7 Circadian rhythm related pathology

1.7.1 Light therapy

Until the 1980s, chronobiologists agreed that light had a relatively minor role in regulating melatonin production and entraining biological rhythms in humans (Hughes & Lewy, 1998). This claim was based on several unsuccessful attempts to suppress nocturnal melatonin and to entrain rhythms with light (Hughes & Lewy, 1998). The report by Lewy, Wehr, Goodwin, Newsome, and Markey (1980) changed this thinking. Lewy et al. (1980) published a report that showed that bright light (2500 lx for 2 hr during the night between 0200 hr and 0400 hr) could suppress human melatonin to daytime levels. Bright light has been shown to have acute phase shifting effects on human circadian rhythms; bright light in the evening produces a phase delay and a phase advance when given in the morning (Aoki, Yamada, Ozeki, Yamane, & Kato, 1998). The amount of light required to suppress melatonin secretion either during the night or at the beginning and end of the night varies considerably from species to species. For example in sheep, hamsters, and monkeys a light intensity of 500 - 600 lux has been reported to suppress melatonin secretion, whereas in rats, as little as two lux is sufficient. An important factor involved in this suppression is the previous exposure to light. Evidence from laboratory animals has shown that prolonged maintenance in light of the intensity of natural light leads to decreased sensitivity (Arendt, 1994).

While some researchers have reported that 500 lux does not suppress nocturnal melatonin, Trinder, Armstrong, O'Brien, Luke, and Martin (1996) suggested that light as low as 250 lux was capable of suppressing melatonin in humans to undetectable levels when the stimulus was applied prior to nocturnal melatonin onset. Recently, it was shown that light intensity as low as 285 lux was

capable of suppressing melatonin, even after melatonin onset (0200 hr to 0400 hr) (Aoki et al., 1998.) Aoki et al. (1998) reported that the minimum light intensity required to suppress melatonin secretion decreased as duration of exposure increased. For example, the minimum light intensity estimated from this study was 285 lux for a 2 hr exposure.

On the basis of the phase shifting effects, bright light exposure has been successful in treating circadian rhythm related sleep disorders. Numerous studies on the biological and therapeutic effects of light in humans have tested bright light as a stimulus for regulating circadian physiology producing therapeutic benefits in people with depression, sleep disorders, menstrual difficulties, as well as problems associated with shift work and jet lag (Hughes & Lewy, 1998; Ross, Arendt, Horne, & Haston, 1995). Similarly, there is evidence to suggest that appropriately timed melatonin administration may also be useful in the treatment of jet lag, shiftwork, and sleep disorders associated with blindness (Arendt et al., 1997).

1.7.2 Melatonin administration

Exogenous melatonin administration provides another option for the treatment of circadian rhythm related pathology. Melatonin is able to phase shift circadian rhythms, including the sleep/wake cycle. It has been confirmed that an acute administration of melatonin (1-5mg) in the early evening acutely lowers body temperature and after administration, the following day, the endogenous melatonin rhythm is advanced by approximately 1 hour (Lewy, 2000). Further field studies show that self-rated jet lag symptoms can be reduced by 50% with appropriately timed melatonin administration (Arendt, 1994; Hughes & Lewy, 1998). One of the advantages of using melatonin is that since it is a naturally occurring hormone, it

often assists in promoting sleep but lets individuals wake without the "hangover" that is common with drugs like barbiturates.

1.8 Measurement of circadian rhythms

From the earliest free-run studies on humans, the rhythm of body temperature has been used as an indicator of the endogenous rhythm, essentially because of its stability and ease of measurement. Essentially it has become the benchmark rhythm used in most human circadian rhythm research. Typically, the circadian rhythm of body temperature in humans is measured orally or rectally. In humans, body temperature rises in the morning, reaches its peak in the late afternoon, drops in the evening just before sleep, continues to slowly decrease until about 0400 hr, when it reaches its 24 hr nadir, and then starts to slowly rise again (see Figure 1.2) (Sewitch, 1987). It has amplitude of about 0.4 °C (Cagnacci, Kräuchi, Wirz-Justice, & Volpe, 1997). Body temperature is strongly influenced by both sleep and activity; the act of sleeping lowers body temperature and activity raises temperature. According to Kronauer (1994) the typical daily rhythm of temperature consists of the endogenous rhythm and the cyclic pattern of sleep and wake, also termed the evoked component or *masking* of the endogenous component. Masking of a rhythm has been defined as "alteration of the usual shape and/or parameters of a rhythm due to random or non-random environmental stimuli, persisting for the duration of the stimulus only (without persistent alterations of endogenous components)" (Touitou & Haus, 1992, p. 711). The endogenous and exogenous rhythm components cannot be easily disentangled when studying human subjects living in their natural environments (Haus & Touitou, 1992). The rhythm observed will consist of both components. Masking effects can be minimised through experimental design considerations such

as constant conditions (e.g. isolation from time cues, complete bed rest, controlled food intake, exposure to dim light), however it should be noted that these conditions are quite artificial and thus lack ecological validity (Haus & Touitou, 1992).

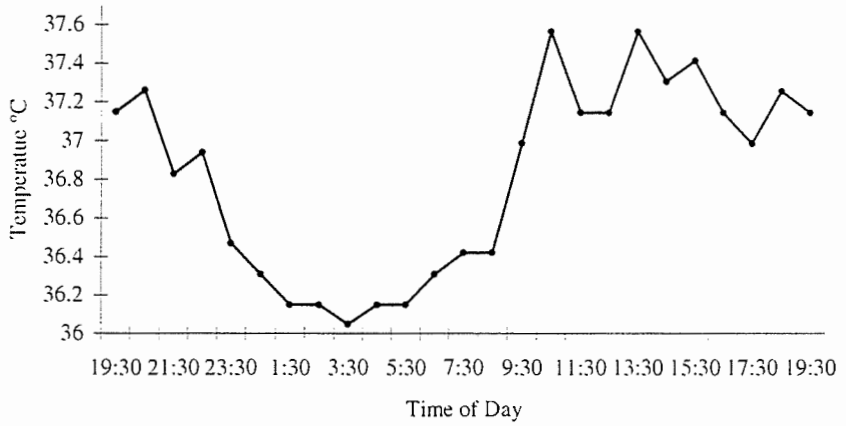

Figure 1.3. The circadian rhythm of body temperature. Data from one subject collected across a 24 hr period, at one-minute intervals, using a rectal temperature probe and data logger. Points on the graph represent one hour core body temperature means.

Other markers such as plasma cortisol, heart rate, and melatonin are also used as indicators of the endogenous rhythm. Interestingly, the nocturnal decline of core body temperature is opposite to the nocturnal rise of melatonin. The peak values of melatonin are related to the core body temperature nadir and the decline of melatonin is followed by an increase in temperature (Cagnacci et al., 1997). However, this temporal relationship between melatonin and core body temperature rhythms does not prove that one rhythm is driving the other (Cagnacci et al., 1997). Nevertheless, both rhythms are driven by the SCN with a major component of the nocturnal fall in core body temperature during sleep being the contribution of endogenous melatonin (Cagnacci, Soldani, Laughlin, & Yen, 1996). Heart rate change is correlated in time with body temperature (Jennings, 1986). Heart rate is usually highest in the late

afternoon; it decreases throughout the night and rises in the morning (Jennings, 1986). Cortisol, on the other hand, peaks in the morning, with lowest levels seen before onset of sleep (Jennings, 1986). Other measures used in circadian rhythm studies include sleep measures. Sleep measures are determined polygraphically and are categorised into sleep stages for analysis (REM and non-REM). As will be shown in the following sections, performance measures are also used in circadian rhythm studies. These tests are usually short in duration and reflect the speed and accuracy with which a task can be performed. Additionally, tests of mood and subjective activation can be associated with the performance variables. Measures taken from all these variables are then plotted as functions of time, and various patterns and interactions are studied, often with fairly complex statistical techniques.

1.9 Chronobiology of mental performance

The notion that an individual's capacity for doing mental or physical work changes throughout the day is a common belief. Understanding of the daily variations in performance is important both theoretically and practically, particularly for work and educational settings. Many different studies have shown that generalisations can be made about the population as a whole, by plotting time of day variations in performance for a particular task.

1.9.1 Arousal model

A misconception regarding circadian performance rhythms is that they reflect circadian body temperature rhythm (Colquhoun, 1971). According to Monk (1992) this misconception originated in the writing of Kleitman who found an association between simple repetitive tasks such as card sorting, and the body temperature

rhythm. Kleitman believed the relationship between body temperature and performance to be a causal one (cited in Monk, 1992). Numerous studies have subsequently been conducted on the relationship between body temperature and performance (Colquhoun, 1971). These studies have found performance to reach a maximum earlier than body temperature. Colquhoun (1971) rejected the notion of a causal relationship between temperature and performance, but argued that with the exception of the post lunch dip in arousal, circadian changes in body temperature parallel those in basal arousal as both are argued to be driven by the same underlying oscillator. Changes in performance are thus attributed to changes in basal arousal (as indexed by body temperature). Increased levels of arousal should lead to increases in performance up to an optimum, beyond which further increases in arousal should result in performance decrements (Petros, Beckwith, & Anderson, 1990). These notions comprise the "arousal model" of circadian performance rhythms.

A major advantage of the arousal model was that it could be used to explain time of day effects in performance measures that did show the same evening peak as one usually obtained in body temperature. Blake (1967) showed that although most of his tasks demonstrated the approximate 2100 hr peak found for body temperature, digit span was unique in showing superior performance at 1030 hr, with performance decreasing over the day. This finding was also confirmed by Baddeley, Hatter, Scott, and Snashall (1970) and Hockey and Colquhoun (1972). Thus, short-term memory (STM) showed the opposite time of day trend in performance to other tasks used by Blake (1967) and other researchers. Using the arousal model, Colquhoun explained the apparent inconsistency between memory tasks by suggesting that for more cognitively complex tasks, such as digit span, optimal arousal levels were reached

sooner in the day (i.e. at a lower arousal level) than was the case for simpler repetitive tasks.

The classic publication by Folkard's (1975) on the time of day effects in logical reasoning demonstrated improvement in performance from 0800 hr – 1400 hr (peak at 1400 hr), in between the peak of STM and that of *immediate processing* performance, and then a decline across the rest of the day. The results were interpreted as indicating that the different functions relating performance efficiency to time of day are due to differences in task demands. This finding changed the emphasis to the mechanisms underlying circadian performance rhythms rather than being solely concerned with the times of the day at which performance was better (Folkard, Knauth, Monk, & Rutenfranz, 1976; Folkard, Monk, Bradbury, & Rosenthall, 1977; Folkard & Monk, 1978; Folkard & Monk, 1979; Folkard, 1979b; Maury & Queinnec, 1992). These studies reflected a tendency to consider arousal to have qualitative effects on the way in which individuals process information. While there are some findings that are inconsistent with the predictions of arousal theory, it is unclear whether the theory should be abandoned or even replaced with a multifactorial arousal theory.

Most studies of time of day effects in mood and performance have examined subjects living in their normal environment (light-dark conditions) and therefore diurnal variations in mood and performance may be susceptible to the masking effects (e.g. sleep timing and duration, activity, and meal times.). Further, the description of diurnal variations in psychological parameters is often restricted to only a few times of day or to a portion of the normal waking period. Additionally, performance measures may show significant practice effects that could mask the true nature, if any, of a diurnal rhythm. While methods can be employed to control for

practice effects, such approaches assume that different participants exhibit similar time of day and practice trends. Additionally, these studies infer that the diurnal trend found in relatively unpractised subjects would generalise to real life situations where individuals perform what are normally highly practised tasks (e.g. reading or driving).

More recently the relationship between core body temperature and psychological performance has been investigated using various chronobiological methodologies including 'constant routine' (Dijk, Duffy, & Czeisler, 1992; Monk et al, 1997). These studies have demonstrated some similarities in the circadian rhythm of core body temperature and various psychological measures. Following prolonged sleep deprivation troughs in temperature and performance have been found to occur in the early morning and within a few hours of one another (Dijk et al., 1992). Although psychological measures and temperature have been claimed to be fairly similar at a general level, closer inspection of the trends often reveals the timing of peaks and troughs in performance measures to differ both from one another and from that of core body temperature. Monk et al (1997) demonstrated that the reliable sinusoidal pattern evident in core body temperature measures were not present in psychological measures, and concluded that performance rhythms are not a expression of temperature changes.

1.9.2 Tasks explored for time of day differences

Memory tasks are the most extensively studied tasks for time of day performance variations. The majority of the memory and time of day studies have been conducted using immediate memory tasks. These studies have been conducted using four types of tasks: digit span, word lists, prose information and working

memory (Folkard, 1982). Variations in findings are apparent due to the different tasks utilised. The studies (Baddeley et al., 1970; Blake, 1967; Folkard, 1979a; Gates, 1916) using digit span have consistently found that immediate memory improves by approximately 0.4 digits early to mid-morning and then decreases by approximately 0.8 digits over the rest of the day to reach a minimum in the evening. Other immediate memory studies investigating time of day effects have used unstructured syntax word lists (Folkard, 1979a; Folkard & Monk, 1979; Maury & Queinnec, 1992). Folkard and Monk (1979) argued that studies in this area have yielded inconsistent results due to times of the day utilised, the modality and rate of presentation, the length of word lists used, and the type of recall required (free or ordered). A third collection of studies has investigated memory for information presented in more of a real life context – prose. The findings from performance trends of prose tasks differ from that of digit span mainly in their failure to show improvement from early to mid-morning. Folkard (1982) put forward the claim that prose tasks may be more difficult than digit span and thus have a lower optimal level of arousal. Alternatively, Folkard (1982) also proposed that given the novelty of a digit span task, the initial rise could reflect a practice effect. It has been suggested that the differences between the tasks could reflect differences in the demands of the task rather than random error.

Research on time of day effects for tasks other than memory have also revealed interesting findings. Payne (1989) investigated time of day effects (nine hourly blocks, 0900 hr – 1700 hr) for time on target on a paced mirror-tracking task. Results revealed a significant linear decline of mean score from 0900 hr – 1300 hr, a sharp post lunch recovery at 1400 hr, and another significant decline from 1400 hr – 1700 hr. Similarly, Lenné, Triggs, and Redman (1998) found time of day variations

for performance on a driving task regardless of sleep deprivation. Under control and sleep deprivation conditions, ability to maintain a stable position on the road and constant speed improved across the day (0800 hr, 1100 hr, 1400 hr, 1700 hr, 2000 hr), with secondary reaction time (RT) also improving. Other studies have examined the interactions between different independent variables and numerous types of tasks in an effort to unravel mechanisms contributing to time of day effects. Examples include - time of day, caffeine, and personality (Revelle, Humphreys, Simon, & Gilliland, 1980), time of day, stress, and impulsivity (Corr & Kumari, 1998), sleep loss and time of day (Heuer, Spijkers, Kiesswetter, & Schmidtke, 1998), and time of day and task characteristics (Craig, Davies, & Matthews, 1987).

1.10 Individual variations of rhythms: Morning and Evening types

Long before the introduction of chronobiology it was noticed that individuals varied in their circadian rhythms. Terms such as *early birds* or *night owls* are demonstrations of this. Kleitman (1939; as cited in Lacoste & Wetterberg, 1993) was the first to acknowledge the recognition of morning and evening types in the scientific arena. Other terms have been used in the chronobiological literature to describe these phenomena. These include poetic terms such as owls and larks, or more scientific terms such as diurnal type, time of day preference, and subjective circadian phase position (Lacoste & Wetterberg, 1993). Morning (M-) and Evening (E-) types are generally regarded as the extremes of a continuum on which the intermediate, Neither (N-), types represent the largest category (Lacoste & Wetterberg, 1993).

Kerkoff (1985) concluded that the M-E dimension was the most important factor related to reliable inter-individual difference in circadian rhythms. Typically,

M-types rise earlier and go to bed earlier and show less variable sleep length and awakening times than E-types. This difference occurs in all physical cycles, including the circadian rhythm of temperature and various hormones. Psychologically, however, the differences in rhythms seem to be much greater. M-types do their best work in the early hours; E-types do their best work in the late hours (Coren, 1996).

It has been suggested that the inter-individual differences could reflect differences with regard to M- and E- types' biological oscillators (Lacoste & Wetterberg, 1993). In support, human temporal isolation studies have found individual differences in circadian rhythm cycles ranging from 16 - 56 hr days (Lacoste & Wetterberg, 1993). Alternatively, differences in circadian phase positions could be the result of the habits of individuals (e.g. work and meal times). Whether morningness-eveningness is endogenous or exogenous in origin is still under investigation. A recent twin study by Hur, Bouchard and Lykken (1998) showed that genetic variability accounted for about 54% of the variance in morningness-eveningness; age accounted for 3% of the variance; and the remaining variance was explained by nonshared environmental influences and measurement error. It has been contended that the two explanations, endogenous and exogenous, may not be mutually exclusive and interactions between the two are definitely possible (Lacoste & Wetterberg, 1993).

Reliable differences in the circadian rhythms of M- and E- types have been found in various behavioural, physiological, and performance measures, and subjective measures such as mood and alertness. The majority of studies have investigated the phase or timing of the rhythms. All studies consistently argue that the maximum value for M-types precedes that for E-types. The difference in phase

position between the two types varies in the range of 1.5 - 3 hr (Lacoste & Wetterberg, 1993; Tankova, Adan, & Buela-Casal, 1994). Morningness-Eveningness has been linked with personality (Mitchell & Redman, 1993; Sexton-Radek & Harris, 1992; Vidaček, Kaliterna, Rodoševic-Vidaček, & Folkard, 1988), substance intake (Ishihara et al., 1985), intelligence and performance differences (Horne, Brass & Pettitt, 1980; Song & Stough, 2000), seasonal adaptation (Lacoste & Wetterberg, 1993), and mood (Kerkoff, 1998). For example, it has been argued that E-types ingest more alcohol, cigarettes, and caffeine than M-types (Ishihara et al., 1985). Further, it has been consistently argued that a positive correlation exists between level of eveningness and degree of extroversion (Horne & Östberg, 1977; Mitchell & Redman, 1993).

However, studies investigating typology differences are conflicting in their results. For example Song and Stough (2000) found no significant interaction between time of testing and Morningness-Eveningnness and cognitive ability (as measured by the Multidimensional Aptitude Battery IQ and Inspection Time task). In contrast, Roberts and Kyllonen (1999) found that E-types had higher intelligence scores, as measured by the Armed Services Vocational Aptitude Battery (ASVAB), than M-types even when they were tested in the morning. For a more comprehensive review see section 1.10.2 outlining individual differences in diurnal variations of performance.

1.10.1 Circadian typology determination

Many self-report instruments have been developed to measure circadian typology, although little published validation of these scales exist. For example, the first English language questionnaire developed by Horne and Östberg (1976) is the

most widely used and referenced measure to determine the circadian typology of subjects, despite its questionable psychometrics (see Smith, Reilly, & Midkiff, 1989). Smith et al. (1989) have also developed a self-report questionnaire, The Composite Scale, to measure morningness. The Composite Scale was developed as a response to weaknesses noted in other scales. This scale was validated using external criteria such as self-reports of sleep length, hours of sleep, and perceived mental alertness. More recently, a re-assessment of the conceptualisation of morningness-eveningness dimension and its measurement was undertaken by Lehtonen and Graham (1998). A more conservative measure, the Circadian Continuum Scale (See Appendix A) has been developed and validated using core body temperature.

1.10.2 M-types and E-types: Differences in performance rhythms

A number of studies have investigated the variations of chronobiological type on the diurnal changes of performance. Further differences between types have been found for long-term memory access (Anderson, Petros, Beckwith, Mitchell, & Fritz, 1991). Consistent with previous literature, performance of M-types decreased across the day, whereas the performance of evening types improved across the day (0900 hr, 1400 hr, 2000 hr). This study highlighted the importance of identifying types when investigating time of day and memory performance. Further, Adan (1991) emphasized the significance of using N-types in research of performance variations across the day. Given that the extremes account for approximately 40% of the population (Adan, 1991), it is important to study the other 60%, N-types, to clarify whether values are intermediate between M-types and E-types. Adan (1991) did find that the N-types were the least dispersed in the parameters studied (visual reaction time and verbal memory) and their values were not intermediate of M- and E-types.

1.11 Summary

The research presented has demonstrated that a human time structure exists. These rhythmic variations can be examined using a variety of measures including, physiological and biochemical measures such as temperature, melatonin, and cortisol. Cognitive and behavioural variations across the day can also be examined using cognitive and performance tasks. It has also been shown that individual variations such as circadian typology should be considered when examining rhythmic and/or time dependent changes as differences have been found in circadian cycles. Research on human chronobiology has important consequences for the investigation of physiology and behaviour and the effects drugs have on these processes.

Chapter 2: Chronopharmacology

2.1 History of chronopharmacology

In historical accounts of ancient Greek naturalists and physicians, environmental cycles and time played a major role in health, disease, and behaviour. For example, there was a strong belief in the moon's influence on the mind, disease, and behaviour. Aristotle believed that children suffered more from epileptic attacks when the moon was full (Oliven, 1943; Kelley, 1942). Similarly, it was generally accepted that the moon intensified the lunatic's condition. Daquin, the French psychiatrist, wrote, "it is a well established fact that insanity is a disease of the mind upon which the moon exercises an unquestionable influence" (Oliven, 1943; Kelley, 1942). Further, the Greek physician, Hippocrates (400 BC), founder of western medicine, wrote, "only a fool tries to heal without considering the orbit of the planets and stars" (Touitou & Haus, 1992).

The first medical degree in chronobiology was awarded to Julien-Joseph Virey in 1814. Recommendations from Virey's doctoral thesis were that research should be focussing on the importance of timing for therapeutic interventions. Similarly, Balfour (1815) suggested treatment should be timed according to the expected next manifestation of the disorder. Balfour based this contention on his work on the rhythm of fevers and in accordance to the lunar cycle (Touitou & Haus, 1992). In 1861 Smith reported a circadian periodicity for time of death, "death occurs more frequently from 0100 hr – 0500 hr than at other hours of the day" (Touitou & Haus, 1992, p. 5). According to Reinberg (1990b) and Touitou and Haus (1992) understanding and appreciation of these earlier reports went mostly

unnoticed, as the principal notions of biological rhythms were not developed until the 1960s.

2.2 Modern medicine: The homeostatic hypothesis

Prior to the development of modern chronobiology it was thought that all processes of living organisms were constant as a function of time (Bing et al., 1997; Lamberg, 1994; Reinberg, 1990a). Medicine has traditionally administered therapeutic agents at specified hours in the day, for example, equal dosing in the morning, at noon, and in the evening, or before or after meals. The homeostatic hypothesis has been offered as the basis of the treatment schedule; that is, the body and consequently drug effects (toxic, pharmacological, and therapeutic) are constant across time. The concept that the body's functions are in a constant steady state throughout the day and night, directs treatment towards maintaining or restoring the steady state (i.e., ensuring a steady state of the drug is in the bloodstream). Researchers conducting chronopharmacology and chronotoxicology experiments have argued this hypothesis is invalid. It has been forwarded that a drug administered in the morning may not have the same pharmacokinetics or efficiency of effect compared to administration in the evening or it could have differing toxicity and side effects at different times of administration (Bing et al., 1997; Lamberg, 1994; Reinberg, 1990a).

2.3 Basis for chronopharmacology: Chronotoxicology

Given the observations of chronobiological researchers it is arguable that the response of an organism to a drug may depend on its biological timing. Rhythms may significantly alter the occurrence or severity of illness symptoms and the

response to diagnostic tests, surgical procedures, drugs and other treatments. The research area termed chronopharmacology investigates the effects of drugs as a function of biological timing, and the effects these drugs have on the endogenous bioperiodicities, including period, mesor, amplitude, and acrophase (Reinberg, 1990a). Early research in this area investigated the responses and changes in biological susceptibility to many agents in lower animals, however, chronopharmacological research was extended to human subjects by Reinberg and Halberg and many others since the 1960s (Reinberg, 1990a; Touitou & Haus, 1992). This area of research has developed rapidly in the last 20 years resulting in the development of a large research literature. Further, technological advances in computers, computer software, and measuring techniques (such as implanted measurement devices) have facilitated the emergence of chronobiology into medicine (Touitou & Haus, 1992).

The biological rhythms in system functions allow researchers to understand why an exogenous agent may not have the same effect on organisms if administered at different times of the day (Cambar & Pons, 1997). Most studies on the chronosusceptibility of organisms have used acute toxic doses of an exogenous agent (Cambar & Pons, 1997). The general aim of these studies was to investigate the influence of administration time on the mortality of animals to toxic agents (Cambar & Pons, 1997). The experiments of Halberg in the 1960s on "hours of changing resistance" began modern chronotoxicology (Bruguerolle, 1992; Reinberg, 1990b). Reinberg, (1990b, p. 14) stated that Halberg demonstrated that "a fixed dose of a potentially noxious agent might kill 80% of mice treated at a certain clock hour but only 20% of the mice treated 12 hr earlier or later". In summary, many reviews have

reported circadian (and circannnual) variations in toxic and pharmacological effects of drugs.

2.4 Chronotherapy

Chronotherapeutics is the synchronisation of medication with the biological rhythms of the disease to optimise the pharmacological effect of a drug and to decrease any potential side effects of the drug (Bing et al., 1997; Bruguerolle, 1992; Reinberg, 1990a). The following review discusses some of the substances and diseases for which clinical chronopharmacology is well documented.

2.4.1 Cardiovascular medications

There is much evidence concerning circadian changes in regulatory mechanisms and functions of the cardiovascular system. This important physiological rhythm was ignored in general and hospital medical practice until recently (Lemmer, 1990a). Data on the chronopharmacology of cardiovascular active drugs demonstrate convincingly that the pharmacokinetics and effects of these drugs display time of day variability in animals, healthy humans, and patients (Black, Smolensky, Johnstone, & White, 1995; Decousus et al., 1991; Lemmer, 1990a; Moore-Ede, 1973; Smolensky & Portaluppi, 1999). It has been demonstrated that patients with thromboembolism infused with heparin at a constant rate over 48 hr showed the greatest anticoagulant effect occurred early at night during each day of the 48 hr infusion; this was the time when most bleeding (undesired effect) occurred (Decousus et al., 1985, as cited in Decousus et al., 1991).

2.4.2 Corticosteroids

Nocturnal worsening of asthma is associated with an intensification of airway inflammation and mediator release (Reddel, Jenkins, & Woolcock, 1999; Reinberg, 1990c; Syabbalo, 1997). Thus, chronotherapy has been directed at treating this aspect of the disease (Goldheim & Schein, 1991; Syabbalo, 1997). The ideal chronotherapy for corticosteroids has been demonstrated to be administration of two thirds of the oral corticosteroids in the morning and the remaining one third administered in the early afternoon for diurnally active patients. This has been demonstrated to improve peak expiratory flow (Bruguerolle 1992; Reinberg, 1990c; Syabbalo, 1997).

2.4.3 Non-steroidal anti-inflammatory drugs (NSAIDS)

According to Labrecque (1990) although most people in the medical field know empirically that the primary symptoms of arthritis (such as pain, inflammation of joints, stiffness) vary across the times of day, there is a lack of systematic studies on the circadian variations of these symptoms (Labrecque, 1990). Additionally, the use of NSAIDs is problematic and many patients have reported that they stopped taking NSAIDs due to the frequent side effects (Labrecque, 1990). Thus, investigations into the chronopharmacology and chronotherapy of NSAIDs have been undertaken with the aim of optimising the efficiency of these drugs (and reducing side effects). These studies have clearly demonstrated that careful selection of the time of administration can optimise the effectiveness of treatment for arthritis with NSAIDs. For example, studies with indomethacin indicated that the chronotolerance (host tolerance) was noticeably better after its evening ingestion (Bruguerolle, 1992; Labrecque, 1990).

2.4.4 Anti-cancer drugs

The treatment of cancer is one of the most impressive examples of chronotherapy (Bruguerolle, 1992). Anticancer drugs are generally very potent and toxic. Consequently, increasing the tolerance to these drugs is a primary objective (Bruguerolle, 1992). According to Lévi (1990) biological rhythms "characterise all processes involved in the malignant transformation of cells, the cellular proliferation of both healthy and tumour tissues as well as their susceptibility to cytotoxic agents and the pharmacokinetics of the chemotherapeutics drugs" (p. 85). Circadian variations of drug pharmacokinetics and pharmacodynamics in humans have been found for several anticancer drugs, including fluoropyrimidines, cisplatin, I-OHP carboplatin, doxorubicin, 6mercapto-purine, and methotrexate (Hrushesky & März, 1992). Studies (Lévi, 1990; Lévi, Zidani, & Misset, 1997; Takane, Ohdo, Yamada, Yukawa & Higuchi, 2000) in these areas have accumulated and revealed promising results for the treatment of cancer. For example, chronoradiotherapy, performed according to the marker of the rhythm of tumour temperature, doubled the two-year survival rate of patients with perioral tumours (Carandente & Halberg, 1991).

2.4.5 Neuropsychotic medication

The majority of the brain's functions go through rhythmic fluctuations during the course of the day. Manic-depressive disorder (bipolar disorder) has been the object of intense chronobiological research (Lemmer, 1990b). Chronobiological and chronopharmacological investigations to date conducted on manic-depressive disorders clearly indicate rhythm disturbances may be causally involved in these disorders. Therapeutic intervention achieved by drugs, light therapy, and sleep

deprivation, to resynchronise rhythms appear to be effective treatments (Arendt, 1993; 1994). Chronobiological and chronopharmacological investigations of other psychological disorders are rare. There is a real paucity of clinical investigations into the notion of whether the side effects of drug treatment (neuroleptics, tranquillisers, antiepileptic) for these disorders can be reduced by modification of time of administration (Lemmer, 1990b). One study did demonstrate support for the use of chronotherapy with neuroleptic drugs. Mazurek and Rosebush (1996) studied the circadian pattern of acute, neuroleptic-induced dystonic reactions and found that over 80% of the episodes of acute dystonia occurred between 1200 hr – 2300 hr suggesting that the therapeutic efficacy of neuroleptics might vary across the day.

2.5 Mechanisms for temporal variations in drug response

Bruguerolle (1992) forwarded that the many examples of temporal changes in drug toxicity and efficiency of effect raises the question of mechanisms through which this occurs. There is growing interest in the underlying mechanisms of the temporal variations in drug effects as previously most studies have been predominately descriptive (Bruguerolle, 1992). The following discussion is adapted from reviews of Reinberg (1990a) and Bruguerolle (1992).

Many different functions of a cell are programmed in time, such as enzyme functions in liver cells, which could influence the drug effect; depending on the stage a biological function is at when the drug reaches the cell (Reinberg, 1991). This explanation is known as chronesthesy, defined as the rhythmic changes in the biological susceptibility of a target biosystem to an agent. The biosystem may involve receptors of target cells (e.g. enzyme system) or organs (e.g. brain or liver), and changes in membrane permeability. In addition to the host, this also includes

tumours and parasites. In other words chronesthesy concerns the temporal variations of the pharmacodynamics of a drug (Bruguerolle, 1992; Reinberg, 1990a).

An alternate explanation is that of chronokinetics. Chronokinetics has been defined as the study of the temporal changes in absorption, distribution, metabolism, and elimination of a drug. Additionally, this includes rhythmic changes in the parameters of Cmax (concentration peak), Tmax (time to reach peak), AUC (area under the curve) and so on. According to Reinberg (1990a) chronokinetic phenomena can be clearly demonstrated for the acute dose administrations of a drug and also for its chronic administration. The mechanisms proposed to underlie changes in chronokinetics involve changes in the biosystems involved in processes such as absorption (e.g. gastric emptying) (Reinberg, 1991). The final possible mechanism proposed is chronergy. This concept represents the "rhythmic change of the response of the organism to a drug (its total effect) according to its chronesthesy and its chronokinetics" (Bruguerolle, 1992, p.115).

2.6 Summary

Chronopharmacological principles and methods have been used to show the importance of biological rhythms to symptom appearance/severity and medication effectiveness for many acute and chronic disorders. Consequently, it has been suggested by some that the homeostatic philosophy of therapeutic management is obsolete, and inappropriate. Accordingly, if biological timing modulates an individual's response to medication, it is plausible that the pharmacokinetics and the response to recreational drugs, such as alcohol would also be dependent on the time of day it was consumed.

2.7 Chronokinetics of alcohol

It is possible that the administration times of an alcohol dose affect the pharmacokinetics of alcohol. The study of the temporal changes in absorption, distribution, metabolism, and elimination of a drug have been termed chronokinetics (Bruguerolle, 1992; Reinberg, 1990a). Much of the work on the chronokinetics of alcohol was done in the late 1970s and early 1980s. For example, Lakatua, Lesar, Zaske, Wargin, and Haus (1984) demonstrated that the absorption of alcohol was faster earlier in the day that is, at 1000 hr compared to 2200 hr, in eleven human subjects. The subjects abstained from alcohol for seven days prior to the experiment. They received an alcohol dose of 0.451 g/kg of body weight after fasting for six hours. Blood samples were taken every 10 min for 1 hr, every 20 min for 2 hr 40 min, at hourly intervals for 4 hr, 4 hr after ingestion, and once 22 hr after ingestion. This study found that alcohol absorption was greater at 1000 hr compared to 2200 hr, which resulted in significant increases in Cmax (maximum concentration) and AUC (area under the curve) and a shorter Tmax (time to maximum concentration) at 1000 hr in comparison to 2200 hr.

Further, it has been shown that circadian variation exists in the elimination rates of alcohol (Jones, 1974; Minors & Waterhouse, 1980; Sturtevant, Sturtevant, Pauly, & Scheving, 1978). Sturtevant et al. (1978) administered repeated doses of alcohol approximately four hours apart to five male human subjects. The subjects fasted for 12 hr before receiving the first dose of alcohol. For four days prior to the experiment, subjects adhered to their normal sleep schedules. An oral dose of 0.75 g/kg of alcohol, as 3:1 diluted bourbon whiskey was consumed in 10 min. Each dose thereafter was calculated to raise the BAC to the same reading obtained 1 hr after the initial BAC reading. Venous alcohol levels were examined at 20 min intervals 2 hr

after the first alcohol dose. Disappearance curves exhibited statistically significant circadian variation in four of the five subjects. Minimal elimination rates of alcohol disappearance occurred between 1200 hr and 2000 hr.

Similarly, Minors and Waterhouse (1980) showed a significant circadian variation in the rate of alcohol decline. Eight subjects ingested alcohol (0.8 g/kg of scotch whiskey drunk within 2 min) once per week at one of the six times of the day, 0100 hr, 0500 hr, 0900 hr, 1300 hr, 1700 hr, 2100 hr, over six successive weeks. Throughout the weeks of the experiments, subjects adhered to the sleep schedule, sleeping between 2330 hr and 0730 hr, except when the experiments began at 0100 hr (they did not sleep at all); at 0500 hr they retired at 0030 and woke at 0300 hr, and at 0900 hr when they woke at 0400 hr to consume a meal. Subjects did not drink alcohol in the 24 hr preceding the experiment. Subjects had a meal five hours prior to the commencement of the experiment and fasted thereafter. Urine was collected at regular intervals, (1 hr and 1 min prior to alcohol ingestion, and 20, 40, 60, 80, 100, 120, 150, 180, 240, and 300 min post alcohol ingestion) for alcohol concentration determination. Group cosinor analyses showed circadian variation in the rate of alcohol decline in both male and female subjects, with the acrophases at 0757 and 0244 respectively. The overall acrophase (M and F) occurred at 0501 hr.

Other studies have also reported time of day differences for mean BACs (Lenné, Triggs, and Redman, 1999; Yap, Mascord, Starmers, & Whitfield, 1993). Lenné et al. (1999) found that peak BAC, estimated through breath analysis, was higher at 1200 hr and 1800 hr compared to 2300 hr. Similarly, Yap et al. (1993) found that peak BAC, also estimated through breath analysis, and was highest at 0900 hr compared to 1500 hr, 1700 hr, and 0300 hr. There were no significant

differences between the peak BACs at the other times of the day. These studies will be further explored in the following section.

In opposition to these findings, other studies have not reported significant circadian phase differences in human BAC (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence, Herbert, & Jeffcoate, 1983; Reinberg, 1992[2]), alcohol metabolism enzymes such as alcohol dehydrogenase in rats (Brick, Pohorecky, Faulkner, & Adams, 1984), or time to reach peak BAC, AUC, or elimination rates (Yap et al., 1993).

2.8 Chronopharmacology of alcohol

2.8.1 Animal studies

The most systematic chronopharmacological investigations of alcohol have been conducted on rodents. Time of day effects, or more appropriately in animal research, circadian phase-dependent effects of alcohol toxicity have demonstrated comparatively consistent findings. It is important to note that rodents have a circadian rhythm that is opposite to that of humans: rodents are nocturnal and their activity phase corresponds to the dark phase. According to Bruguerolle (1992) it would appear feasible to extrapolate chronopharmacological results from rodents to humans by inverting the phase, however, it is not always the case as effects are sometimes reported at the same clock hour irrespective of resting period in humans or rodents (Bruguerolle, 1992).

Haus and Halberg (1959) demonstrated a time of day effect in alcohol toxicity in C mice, synchronised in light from 0600 hr – 1800 hr and dark from 1800 hr – 0600 hr, (fixed dose, single injection of alcohol). Separate subgroups of mice

[2] Reinberg (1992) did not report statistics conducted on BAC, although no time of day differences were stated in the published paper.

were injected at 4 hr intervals during a 24 hr period starting at 0800 hr of one day and ending at 0800 hr the next day. Mortality was recorded at 20 min, 1 hr, 4 hr, and 1 week after injection. The percentage of deaths at 4 hr after alcohol injection was reported. The mortality rates from 20 min, 1 hr, and 1 week after alcohol injection were not reported as data was comparable in terms of temporal placement of susceptibility levels to alcohol. Results indicated a 24 hr rhythm for mortality rates from alcohol. The highest death rates were recorded for the subgroup of the mice injected 2 hr after the beginning of the dark phase of the rhythm, 2000 hr, (the animals' activity phase) and the lowest mortality was observed when injected in the light phase (just before the beginning of the rest phase).

Consistent with this observation, that the biological effects of alcohol are greater in the dark phase of the light dark (LD) cycle, Sauerbier (1987) found that when groups of pregnant NMRI mice were injected with either 1 g/kg, 2.5 g/kg, or 5.8 g/kg of alcohol, fetotoxicity of alcohol was found to be related to the dose, the period of gestation, and circadian phase at the time of injection. Fetal damage was found to be significantly greater when the alcohol injection was given in the middle of the dark phase (activity phase) and this effect was most pronounced after injection of 5.8 g/kg of alcohol.

Similarly, Williams, Soliman, and Mizinga (1993) conducted three experiments to study the circadian variation in tolerance to the hypothermic action of alcohol, apomorphine, and nicotine. In the alcohol experiment SAF male mice or Sprague - Dawley male rats were assigned to six groups and injected for three consecutive days during the light phase (1000 hr, 1400 hr, or 1800 hr) or the dark phase (2200 hr, 0200 hr, or 0600 hr) and the degree of hypothermia was measured. It was found that mice repeatedly (3 times) injected with 20% alcohol (3 g/kg IP)

showed varying degrees of hypothermia depending on the time of alcohol injection. After the first dose of alcohol, the hypothermic effect was highest at 1800 hr (light phase) and lowest at 0200 hr (dark phase). Results indicated that tolerance in animals injected at 1800 hr developed after the first injection and for the animals injected at 1000 hr and 1400 hr after the second injection. In contrast, repeated administration of alcohol during the dark phase produced varying results. At 0600 hr (end of the dark phase) the degree of hypersensitivity increased and was apparent after the second injection.

Baird et al. (1998) presented data on circadian phase dependence in the disruption of circadian parameters of the body temperature rhythm following an alcohol injection. Baird et al. (1998), using a mixed factorial design, provided results on the effects of 3 doses (0 saline, 1, and 2 g/kg) of alcohol, administered acutely at 0100 hr, 0700 hr, 1300 hr, 1900 hr within the circadian cycle of a normal 12:12 L/D cycle on daily rhythms of activity and temperature for 99 male Sprague-Dawley rats. Overall the pattern of results was consistent with previous studies (Haus & Halberg, 1959; Sauerbier, 1987; Williams et al., 1993) investigating circadian dependent pharmacology and toxicity of alcohol with mice and rats. Alcohol altered body temperature and activity rhythms differentially depending on the time of day it was administered. It was found that alcohol had significant effects on the acrophase, amplitude, and period; however, mesor was unaffected on temperature and activity rhythms. Alcohol produced dose-dependent significant hypothermic and hypoactive effects when rats were injected at all times, however, the largest hypothermic effect was found at the 1900 hr injection time (dark phase). Alcohol significantly shortened the period of activity rhythms when injected in either 1.0 or 2.0 g/kg doses at 0700 hr and 1300 hr, and also produced period-shortening effects on temperature

rhythms at 1300 hr and 1900 hr. The acrophase of the activity rhythm was significantly phase delayed by a 1.0 g/kg dose at 0700 hr while the acrophase of temperature was significantly phase advanced by a 2.0 g/kg dose at 0100 hr but significantly phase delayed by the same dose at 1300 hr. A significant dose dependent reduction in the amplitude of the body temperature rhythm was observed at 1900 hr administration time.

Given the known dose dependent biphasic action of alcohol, Brick et al. (1984) investigated the circadian variation for both stimulatory and depressant effects of alcohol on a number of dependent variables in male Sprague-Dawley rats (12:12 LD cycle, lights on at 0700 hr). The effects of alcohol and circadian variations found in this study varied with the different measures investigated. Overall though, the most salient depressant effect of alcohol on startle response and gross motor activity occurred during the light phase, however the greatest sensitivity to the stimulative effect of alcohol occurred at the beginning of the dark phase. Further, sensitivity to the hypothermic effect of alcohol was more pronounced at the end of the dark phase. At 0600 hr, alcohol caused a significant decline in body temperature, maximum decline occuring at 60 min post injection. The group injected with alcohol at 1800 hr showed a different thermal response. While basal temperature was 1.2 °C less than the 0600 hr group, no significant differences from controls were found in alcohol treated subjects until 60 min post injection; maximum hypothermia was seen 90 min post injection. There were no significant differences in blood alcohol levels or alcohol dehydrogenase activity across the times utilised in their study, suggesting that differences in response to alcohol observed in the study may not be attributed to varying levels of blood alcohol in the rats. A theory was put forward that circadian phase dependent responses could be attributed to changes in

circadian CNS sensitivity to alcohol, that is chronesthesy, rather than chronokinetics as no significant differences were noted in BACs during the times of day subjects were tested.

2.8.2 Human studies

Most people drink alcohol at the end of the day, rather than at the beginning, with the exception of chronic alcohol users, and possibly shift workers. When people do drink earlier in the day, they often report a more potent effect of alcohol compared to when alcohol has been consumed in the evening or night (Horne & Gibbons, 1991). Despite this common contention, little research, using human subjects, has been conducted on the chronopharmacology of alcohol. While there are some differences in the methods and findings, most of the human studies support the findings of other animal studies. Subjects who ingest alcohol either in the morning or afternoon perform more poorly on behavioural and cognitive tasks and report greater subjective impairment on sleep/mood measures compared to the performance of those that ingested alcohol during the evening. The following is a review of these studies.

Jones (1974) compared the results of two separate alcohol studies to assess time of day differences in cognitive performance. Subjects had a regular night's sleep prior to testing and abstained from alcohol and medication the night before and on the testing day. Subjects were tested from either 1300 hr – 1700 hr or 1700 hr – 2200 hr. Subjects from both studies were given the same dose of alcohol (1.32 ml/kg) and tested on the Ravens Progressive Matrices on the descending limb of the BAC curve at a BAC of 0.09 g/100ml. The subjects who drank in the afternoon showed a significantly greater impairment of scores on the Ravens Progressive

Matrices than subjects who consumed alcohol in the evening. Further, there was a significant difference in alcohol metabolism between afternoon and evening groups, with the afternoon group showing a faster alcohol metabolism. No significant differences were found for peak BACs, time to reach peak, amount of alcohol consumed, or absorption rate.

Similarly, Lawrence et al. (1983), using a between subjects design, investigated changes in psychological functioning (mood inventory, four choice serial reaction time, and logical reasoning), at two times of the day (0900 hr and 1800 hr) in 20 individuals after consuming an alcohol dose (2 ml/kg) over 90 min. Each subject had abstained from alcohol for 24 hr and had fasted for eight hours. Although their were no significant differences in group mean BACs (approximately 0.07 g/100ml BAC peak), subjects reported feeling less coordinated after alcohol in the morning. There were no significant differences between the groups on mean reaction time on the four-choice serial reaction time task, however those studied in the morning made significantly more errors and had significantly more variablity in performance at the 55 min test point. Similarly, the morning group attempted significantly fewer questions on the logical reasoning task, but error was not significantly different between the two groups.

Similarly, Horne and Gibbons (1991) documented the effects of time of day and dose on subjective sleepiness and vigilance performance. Using a double blind repeated measures design, Horne and Gibbons (1991) administered alcohol to eight women (aged between 18 – 23 years, mean weight 54.5 kg) to produce BACs of approximately 0.07 g/100ml, 0.035 g/100ml, or < 0.005 g/100ml with food at 1300 hr or 1830 hr. Subjects all reported afternoon sleepiness at least once per week and were moderate consumers of alcohol (1/2 - 2 units/day). All subjects were Neither

types on the MEQ (Horne & Östberg, 1976). One hour of vigilance testing as measured by the Wilkinson Auditory Vigilance Task (WAVT) was undertaken after consumption of the alcohol. After consumption of alcohol, subjects rated themselves for sleepiness on a half-hourly basis using the Stanford Sleepiness Scale (SSS). There were significant main effects for dose and for time of day on reaction time, hits and sleepiness scores and a significant dose by time of day interaction on reaction time and hits. Horne and Gibbons (1991) concluded that under both alcohol dose conditions there was greater impairment in the afternoon compared to the evening. There were no time of day differences reported for BACs.

Following the same subject and method protocols as Horne and Gibbons (1991), Horne and Baumer (1991) further investigated the time of day effects found in the Horne and Gibbons (1991) study using a driving simulator. Twenty-four women were divided into two time of day groups, tested at, 1310 hr and 1810 hr. Subjects were given a dose of alcohol that peaked just under 0.08 g/100ml (BAC level legal maximum for driving in the UK) or placebo. Subjects engaged in 40 min of monotonous driving using the car simulator. BACs over time were similar, however, the BACs for the afternoon group were slightly higher (although they were not analysed for statistically significant differences). The results from the Stanford Sleepiness Scale indicated that alcohol had greater impact on sleepiness in the afternoon than in the evening. Neither time of day or alcohol affected lateral corrective steering movements, however, alcohol significantly increased the following distance and the variability of this distance, particularly in the afternoon compared to evening.

Reinberg (1992) investigated circadian changes in psychological effects after alcohol ingestion (0.67 g/kg; BACs up to around 0.11 g/100ml) in six healthy human

males (aged 22 - 26 years) synchronised from 0700 hr to 2400 hr. Subjects had fasted for 12 hr preceding the alcohol ingestion time and eight hours post ingestion. Each subject performed four separate tests, one week apart, at 0700 hr, 1100 hr, 1900 hr, 2300 hr, with measurements taken before and after alcohol ingestion, at 15, 30, 60, 90, 120, and 140 min. The measurements taken were self-rated variables (such as inebreity, physical vigour, mood), random number addition test, tempo, eye-hand skill, heart rate, systolic and diastolic blood pressure, peak expiratory flow, oral temperature, grip strength, biochemical measures taken from plasma samples (cortisol, lactic and pyruvic, glucose, and potassium erythrocyte content), and urinary samples (adrenaline, noradrenaline, and 5-hydroxyindolacetic acid). However, statistical analyses were not presented for all of these variables in the published paper. Self-rated intoxication was lowest after ingestion at 1100 hr. Changes in physiological measures, observed in self-rated vigour, tempo, handgrip strength, and blood pressure, did not appear to be dependent on the time at which alcohol was ingested. However, there was a decrease in plasma cortisol at 90 min post ingestion at 0700 hr and an increase in cortisol at 2300 hr, 90 min post ingestion. No change was observed at 1100 hr or 1900 hr. This study suggests that some psychological effects of alcohol are related to the time of ingestion. In contrast to previous studies presented (such as Horne & Baumer, 1991; Jones, 1974), the 2300 hr administration time was the peak time of self rated inebriety and the poorest performance time for the speed of performance of both random number addition and hand-eye skill tests.

Yap et al. (1993) gave 10 male subjects alcohol (0.75 g/kg; peak BACs around 0.10%) at 0900 hr, 1500 hr, 2100 hr, and 0300 hr in order to investigate time of day effects for alcohol. Blood alcohol concentrations were monitored by breath analysis. Dependent variables were measured at 0, 60, and 120 min after alcohol

ingestion. Measurements were made of plasma levels of alcohol, acetalaldehyde, acetate, pyruvate, lactate and cortisol. Additionally, physiological measures of blood pressure, heart rate, and body temperature were taken before and at 60 and 120 min after alcohol administration. The effects of alcohol on behavioural parameters were assessed using a divided attention task (pursuit tracking and visual discrimination) and digit symbol coding. Critical flicker fusion and body sway were also monitored. Visual analogues (0 - 9) were used to assess perceived aspects of intoxication (drowsiness, coordination, ability to concentrate, and perceived level of intoxication). While a significantly higher peak blood alcohol concentration was attained at the 0900 hr session, other time of day differences (such as time taken to reach peak and alcohol elimination rate) did not reach statistical significance. The pharmacokinetics of alcohol were not affected by time of day. Alcohol had clear effects on most of the measures studied, however, besides an increase in body sway at 0900 hr compared to the other times of alcohol administration, time of day variability was not demonstrated for biochemical, physiological, or psychomotor measures while under the influence of alcohol.

More recently, Lenné et al. (1999) investigated the effects of alcohol on driving as a function of time of day and driving experience. Twenty-eight subjects (14 inexperienced drivers and 14 experienced drivers), all "Neither types" according to the Morningness-Eveningness questionnaire attended two sessions at 1200 hr, 1800 hr, 2300 hr, after consuming either 0.70 ml/kg of alcohol or juice. Driving performance was measured using a driving simulator. The simulator recorded the mean and standard deviation of position adopted in the lane, and the mean and standard deviation of speed. A secondary task was also measured while driving. This task measured subjects' reaction time (pressing the foot pedal) to a stimulus

presented at six points on the track. For all sessions subjects drove the simulator for four ten-minute blocks (separated by a ten-minute rest). At all times of the day, driving performance and secondary reaction time was significantly impaired after alcohol ingestion. However, alcohol had a similar influence on performance at all times of the day. Nevertheless, it was found that BAC did vary across the three times of the day being higher at 1200 hr and 1800 hr, than 2300 hr. Driving performance (lateral position and secondary RT) was reduced at 1200 hr compared to 1800 hr and 2300 hr both with and without alcohol. Thus, the researchers concluded that from a practical point of view, accident risk might be slightly elevated for people who drive after consuming alcohol in the middle of the day compared to the late evening or night (Lenné et al., 1999).

A number of explanations for the results of this study were offered by Lenné et al. (1999) based on the fact that the BAC was variable across the day. Firstly, it was suggested that given subjects had a lower BAC at 2300 hr and still produced impairment comparable to the other times of the day, and showed decline in mood at 2300 hr that this might indicate a reduced ability to compensate for the effects of alcohol at 2300 hr. Alternatively, Lenné et al (1999) also proposed that there could be a circadian variation in the sensitivity of performance being highest at 2300 hr. Lenné et al (1999) contended that, otherwise, if sensitivity were constant across the day then it would be expected that the higher BAC peak at 1200 hr would have produced greater impairment than at the other times of the day.

2.9 Summary

The observations of chronopharmacology researchers have indicated that the response of an organism to a drug may depend on its biological timing. Rhythms

may significantly alter the occurrence or severity of illness symptoms, or the magnitude of response to drugs. The results of the alcohol chronokinetic studies have indicated circadian variability for the absorption and metabolism of alcohol. Similarly, research on the chronopharmacology of alcohol has shown that some responses of subjects (human or other animals) to alcohol were dependent on the time of day that alcohol was administered. The most consistent findings of increased hypothermia, increased mortality, and depressed activity occurred when rodents were injected with alcohol during the dark phase (activity phase) of the L/D cycle (Haus and Halberg, 1959; Sauerbier, 1987; Williams et al., 1993; Baird et al., 1998; Brick et al., 1984). Comparatively, this effect has also been observed in some human chronopharmacology of alcohol studies. In some cases alcohol had a differential sedative, performance, and subjective effect in humans according to the time of day it was ingested (Horne & Baumer, 1991; Horne & Gibbons, 1991; Jones, 1974; Lawrence et al., 1983). More pronounced effects, such as performance impairment and increased subjective sleepiness, have been shown when alcohol was consumed during the day compared to when it was consumed in the evening (Horne & Baumer, 1991; Horne & Gibbons, 1991; Jones, 1974; Lawrence et al., 1983). Some studies have not found the same results, for example, Yap et al. (1993) did not find time of day effects for the pharmacokinetics, biochemistry, physiology or psychomotor tasks (dual task and digit symbol coding). In contrast to previous studies, Reinberg (1992) found that administration of alcohol at 2300 hr was the peak time of self rated inebriety and the poorest performance time for the speed of performance of both random number addition and hand-eye skill tests, compared to 0700 hr, 1100 hr, and 1900 hr.

The results obtained from the chronopharmacology of alcohol studies, human and other animals have differed in the methodologies undertaken such as, characteristics of the sample, fasting schedules, doses of alcohol administered, and the time period of measurement. These methodological factors could significantly affect processes such as the absorption and metabolism of alcohol that ultimately affect the impact of the drug.

Chapter 3: Alcohol

3.1 Alcohol

Alcohol has been associated with worship, used in rituals, and has been central to celebration in many cultures throughout time (Lang, 1998). The word alcohol is of Arabian origin, from the word that means "something subtle" (Feldman, Meyer, & Quenzer, 1997). There is evidence that alcoholic drinks existed in prehistoric times, most likely discovered accidentally through the fermentation of honey, grains steeped in water, or fruit juices. Mead, a fermentation product of honey, is regarded as the oldest alcoholic beverage, having existed in the Palaeolithic age, approximately 8000 B.C.E. (Feldman, et al., 1997; Lang, 1998).

Ethyl alcohol (ethanol), the chemical formula being $CH_3 CH_2 OH$, is the chemical name for alcohol, a psychoactive drug that is similar in effect to the sedative-hypnotic compounds, such as barbiturates, antihistamines, and benzodiazepines (Julien, 1998; Giles & Kapur, 1991). Pure ethanol is a colourless, inflammable liquid. It is a simple molecule that is completely miscible in water and can be almost completely absorbed into the bloodstream after ingestion (Giles & Kapur, 1991). Different systems are used to indicate the alcohol concentration in drinks. The most straightforward is the percentage of alcohol by volume (percent v/v) for example, 20 ml of alcohol per 100 ml of the beverage (Giles & Kapur, 1991). Rarely consumed in its pure form, alcohol is found in 10 - 12% concentrations in wines, 3 - 5% in beers, and 30 - 50% in liquors (Feldman, et al., 1997). Table 2.1 lists a number of Australian alcoholic beverages and their respective alcohol contents.

Table 2.1

Examples of Australian alcoholic beverages alcohol content

Type of Alcoholic Beverage	Name	Alc/Vol
Beer	Victoria Bitter	4.9%
Light Beers	Fosters Light Ice	2.7%
Wine	Mount Pleasant Chardonnay (1997)	13.5%
Fortified Wines	McWilliams Family Port Reserve	18%
Spirits	Single Malt Australian Whiskey	40%

3.2 Pharmacokinetics

Two concepts in pharmacology, pharmacokinetics and pharmacodynamics describe the relationship between the dose of the drug taken and the ability of that drug to affect the individual. Pharmacokinetics deals with the way in which the drug is delivered to the body: its absorption, its distribution within the body, its biotransformation and its excretion (Goldstein, 1983; Julien, 1998, Mycek, Harvey, & Champe, 2000).

3.2.1 Absorption of alcohol

"Absorption is the transfer of a drug from its site of administration to the bloodstream" (Mycek et al., 2000, p. 4). Alcohol enters rapidly into blood circulation through the process of diffusion, the drug moves from a region of high concentration to one of lower concentration. Absorption of alcohol occurs mainly across the lining or membrane of the duodenum and jejunum (small intestine) and to a lesser degree from the stomach and large intestine (Agarwal, 1998; Feldman et al., 1997; Mycek, et al., 2000). The time from completion of drinking to peak alcohol concentration in the blood ranges from 30 to 120 min (Agarwal, 1998; Julien, 1998). Gastric absorption is fastest when beverages fairly high in alcohol concentration are

taken on an empty stomach (Feldman et al, 1997; Goldstein, 1983: Julien, 1998;). Concentrations between 15 and 30% (fortified wines) give the fastest absorption (Feldman et al., 1997). With higher concentrations such as undiluted distilled spirits and with lower concentrations such as beer the rate of absorption is slower. Absorption is slower with high concentration beverages because the opening of the pyloric valve between the stomach and the small intestine becomes handicapped, a pylorospasm, so that alcohol is retained in the stomach and eventually absorbed from there (Feldman et al., 1997). Absorption is slower with low concentration beverages, such as beers (3 - 5%) or wine (12%) because it takes longer for alcohol to diffuse out of the solution to the walls of the small intestine (Goldstein, 1983).

People show large variation in alcohol absorption rate. There is even large variation in the same person on different occasions with respect to the absorption of alcohol. It has been suggested that any event that alters the movement of alcohol from the stomach to the small intestine will affect absorption (Goldstein, 1983). Absorption is fastest when the stomach empties quickly (Goldstein, 1983). Food taken with alcohol has a dual effect: it dilutes the alcohol in the gastrointestinal tract and it delays stomach emptying. In addition, plain water, by decreasing the concentration, slows the absorption of alcohol, but carbonated liquids speed it up. The carbon dioxide acts to move everything quite rapidly through the stomach to the small intestine. It is this emptying of the stomach and the more rapid absorption of alcohol in the intestine that gives champagne and sparkling burgundy a faster onset of action than wine (Ray & Ksir, 1993). Figure 3.1 illustrates how food affects the blood alcohol concentration after a dose of alcohol administered orally, either on an empty stomach or with food. It can be seen that the absorption phase is quite different if food is taken with alcohol, the BAC peak is later and lower.

Additionally, the reduction of BACs has been argued to be associated with a reduction in the degree of behavioural impairment (Millar, Hammersley, & Finnigan, 1992). It was found that alcohol significantly impaired dual task performance with impairment significantly reduced for subjects who had ingested food previously. BAC was significantly lower in these subjects. While prior ingestion of food reduced the adverse effects of alcohol, performance was still impaired relative to the no alcohol condition.

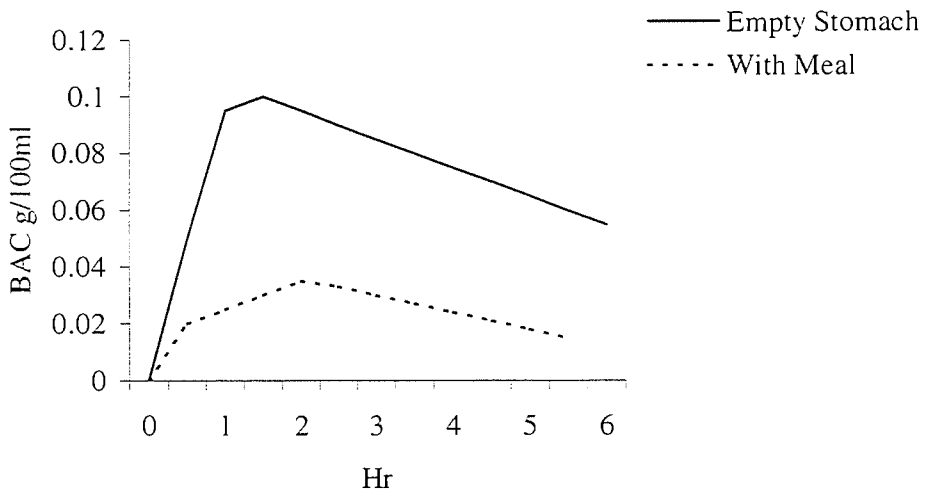

Figure 3.1. Effect of food on the absorption of alcohol using hypothetical values (adapted from Goldstein, 1983, p. 4).

3.2.2 Distribution of alcohol

According to Goldstein (1983) alcohol rapidly diffuses throughout water compartments of the body. When alcohol appears in the blood and reaches the blood-brain barrier it crosses into the brain immediately and with ease. However, alcohol is not very lipid soluble. Concentrations in the body water are about ten times higher than in body fats (Goldstein, 1983). Wherever there is water in the body, alcohol can usually be found in a concentration that depends on the water

content of the tissue. The rate of alcohol distribution into body tissue varies depending on their blood supply. Therefore, the alcohol concentration of the highly vascularized CNS equilibrates rapidly with arterial blood. Thus, during absorption, brain alcohol concentration is much higher than in venous blood. As alcohol slowly diffuses into the large mass of skeletal muscles, the blood level of alcohol falls, reversing the concentration gradient between the blood and brain so that alcohol diffuses out of this organ (Feldman et al., 1997; Goldstein, 1983;).

3.2.3 Metabolism of alcohol

Metabolism refers to the transformation of a substance into other substances (Mycek et al., 2000). The metabolism of alcohol occurs primarily in the liver. After alcohol consumption the majority of the alcohol is metabolised into other substances. However, some (5 - 15%) ingested alcohol is excreted unchanged, mainly through the lungs, sweat, and urine (Feldman et al., 1997; Julien, 1998). While alcohol can be lost from the body as a liquid it can also be excreted as a gas, such as, breath[3]. An amount of alcohol metabolism (up to 15%) is carried out by gastric alcohol dehydrogenase enzyme, located in the lining of the stomach (Julien, 1998). This enzyme metabolises some of the alcohol as it is absorbed across the stomach wall into the bloodstream. It has been suggested that this metabolism can decrease the BAC by about 15% (Feldman et al., 1997; Julien, 1998). An important gender difference has been established in alcohol metabolism. Women have approximately 50% less gastric metabolism of alcohol than men because there is substantially less alcohol dehydrogenase (ADH) activity in the gastric mucosa of women than in men,

[3] Analysis of the breath allows a non-invasive determination of alcohol concentration, for example, using a breathalyser. The principle of breath analysis is based on the fact that alcohol is partly excreted via the lungs and that the exhalation is proportional to the BAC (teWierik, 1991). The partition coefficient between breath and blood has been estimated at 1:2100 to 1:2300 (teWierik, 1991). That is, there is the same amount of alcohol in 1 ml of blood as in 2100 ml of alveolar air.

and the little gastric oxidation that does occur in women is virtually abolished in alcoholic women (Julien, 1998). However, most of the metabolism of the ingested alcohol (approximately 95%) is achieved enzymatically. Alcohol is ultimately oxidized to carbon dioxide and water with the release of seven kilocalories of energy per gram of alcohol (or 200 kcal per oz) (Agarwal, 1998; Gerald, 1981; Goldstein, 1983; Julien, 1998). With most drugs a constant proportion of the drug is removed in a given amount of time, so that with a high blood level the amount metabolised is high. In contrast to this, the amount of alcohol that can be metabolised is constant at about 6 – 8 g/hr (7.5 – 10 ml/hr), regardless of the blood alcohol concentration. If a person consumes alcohol faster than it can be metabolised, the BAC of the individual will get higher after each successive drink (Giles & Kapur, 1991).

3.3 Factors influencing the effect of alcohol

3.3.1 Age

It has been shown that age can affect BAC (Lucey, Hill, Young, Demo-Dananberg & Beresford, 1999); drink for drink more alcohol will be concentrated in the blood stream of older people than younger people. Most researchers attribute this effect to an age-related reduction in percent body water that results in a smaller volume of distribution (Lucey et al., 1999).

3.3.2 Gender

Body size and gender are additional factors in alcohol diffusion (Lucey et al, 1999; Giles & Kapur, 1991). Generally men tend to be larger than women and have a higher ratio of muscle to fat; thus men have a proportionately greater vascular capacity because fat is not as vascularized as muscle. Therefore, drink for drink:

alcohol will be more diluted in the bloodstream of a man than that of a woman. Further, even for men and women of the same weight, the volume of total body water is lower in women (see TBW formula section 4.7.2). It cannot be stated at this time whether menstrual cycle influences alcohol pharmacokinetics. Talbot and LaGrange (1999) stated that researchers who have investigated the influence of the menstrual cycle on alcohol metabolism have obtained conflicting data.

3.3.3 Other medication

If a person is taking medication, the medication could interact with the alcohol to increase the effects of alcohol. Further, the symptoms of a person who is sick may also be amplified by alcohol. It has been shown that alcohol interacts with a range of psychoactive drugs. For example, benzodiazepines in combination with alcohol have been shown to have additive effects on cognitive and psychomotor performance, and to potentiate sedation (Kerr & Hindmarch, 1998).

3.3.4 The route of administration

The route of administration is primarily determined by the properties of the drug and the therapeutic/recreational objective (e.g. rapid onset of action, long term administration) (Mycek et al., 2000). There are two major routes of drug administration, enteral (e.g. oral, sublingual, rectal) and parenteral (e.g. intravascular, intramuscular, subcutaneous) (Mycek et al., 2000). Other routes of administration include inhalation, intranasal, and topical. The route of administration for alcohol is generally enteral (oral). Consequences for the effect of this route of administration have been outlined (see section 3.2.1).

3.3.5 Rate of intake

The effect of alcohol consumption is largely a balance between absorption rate and the metabolic rate. Some people who drink alcohol ingest it in small doses steadily. These people may not show signs of intoxication as the rate of metabolism keeps pace with the rate of intake. People who drink large doses of alcohol rapidly (binge drinking) will potentially show signs of intoxication as the rate of intake outweighs the rate of metabolism (Feldman et al., 1997).

3.4 Pharmacodynamics of alcohol

"Pharmacodynamics refers to the way in which drugs act and the relationship between the blood concentration of the drug and its effect on the body" (Whelan, 1998, p. 20). The following discussion briefly summarises some of the theories forwarded for alcohol's actions, the types of tolerance that can be acquired when an individual consumes alcohol either acutely or chronically, and finally, related to tolerance, how the environment can influence the effect of alcohol.

3.4.1 Action of alcohol

Alcohol is often thought of as a stimulant, however it is a CNS depressant. The perception of its stimulating effects reflects unrestrained activity because of alcohol-induced suppression of inhibitory control mechanisms (Feldman et al., 1997). While alcohol has substantial effects on many organs, the central nervous system is the most affected, if judged by its almost immediate change in subjective feelings and motor effects (Feldman et al., 1997; Julien, 1998;). As stated, the speed at which these effects occur is due to the complete solubility of alcohol in water, resulting in rapid absorption into the blood and distribution throughout the brain

(Feldman et al., 1997). The physical and psychological effects of alcohol have been shown to be related to the BAC. The BAC and behaviour relationship is described comically in the following quote from Ray (1978, p. 146),

> At less than 0.03%, the individual is dull and dignified
>
> At 0.05%, they are dashing and debonair.
>
> At 0.1%, they may become dangerous and devilish.
>
> At 0.2%, they are likely to be dizzy and disturbing.
>
> At 0.25%, they may be disgusting and dishevelled.
>
> At 0.3%, they are delirious and disoriented and surely drunk.
>
> At 0.35%, they are dead drunk.
>
> At 0.6%, the chances are that they are dead.

Julien (1998) stated that identification of the mechanism of action of alcohol has been and continues to be complex due to alcohol's varying behavioural and neurochemical actions. Further, a hypothesis to explain alcohol's actions using one single neurochemical may also be impossible. Given alcohol is water-soluble, the hypothesis that alcohol acted through a general depressant action on nerve membranes and synapses was forwarded. It was believed that alcohol dissolved in membranes "perturbing" them, similar to anaesthetic action, altering membrane processes such as electrical transmission and release of synaptic transmitter chemicals. In essence, the membrane became more fluid, disrupting its functions (Julien, 1998).

Acute alcohol intoxication exerts a number of effects on cell membrane constituents. Such effects are widespread, reflecting the involvement of almost all areas of the CNS. In contrast, alcohol also influences specific brain regions and cell types (Feldman et al., 1997). The general depressant hypothesis correlates with, and

can explain the properties of alcohol, however many investigations have revealed that alcohol's effects can be highly specific. In the 1990s the general depressant hypothesis was replaced by mechanisms based on specific actions on excitatory (glutamate) and inhibitory (GABA) neurotransmitter systems (Feldman et al., 1997; Julien, 1998).

3.4.2 Tolerance

Alcohol tolerance refers to a reduction in the physiological response to alcohol generally because of prolonged frequent use (Feldman et al., 1997; Vogel-Sprott, 1997). A person developing tolerance to alcohol must drink greater quantities of alcohol in order to produce the same effect previously achieved at a lower level of alcohol consumption (Julien, 1998;Vogel-Sprott, 1997). According to Vogel-Sprott (1997), some researchers believe that the physiological actions of alcohol (and other drugs) contribute to tolerance by triggering the body to produce opposite reactions in order to compensate and restore the body's internal conditions (i.e., homeostasis). The compensatory response is argued to become stronger each time a person uses alcohol or other drugs and to diminish during a period of abstinence. There are two types of tolerance: acute and chronic. Acute tolerance is demonstrated within the time a single dose is cleared from the bloodstream. Whereas chronic tolerance develops after repeated dosing with alcohol and is shown by a lower effectiveness of the same doses given previously. Tolerance can be of two main types, dispositional (metabolic or pharmacokinetic) or functional (pharmacodynamic). These two forms of tolerance involve different mechanisms, however, both require higher levels of alcohol in the blood to maintain the same biological effect. Further, they both have

the same ultimate outcome, that is, the subject appears less intoxicated after alcohol administration (Feldman et al., 1997; Goldstein, 1983).

Dispositional tolerance refers to the outcome of the drug having less of an effect after chronic use because there is less of it at its site of action (Goldstein, 1983). Metabolic tolerance has been attributed to alcohol-induced changes in the absorption, distribution, and excretion of alcohol (Goldstein, 1983; Feldman et al., 1997). Thus, after a particular dose of alcohol, people who are metabolically tolerant would produce BACs that are lower and shorter lasting (Goldstein, 1983; Feldman et al., 1997). According to Goldstein (1983) the level of metabolic tolerance to alcohol is approximately 30 - 50% after experimental alcohol administration in animals and in some experiments it is not acquired. Metabolic tolerance is difficult to demonstrate in humans as its magnitude is small and because other variables have an influence on the liver (such as nutrition) (Feldman et al., 1997; Goldstein, 1983).

A second type of pharmacological tolerance is termed functional tolerance (Goldstein, 1983) or cellular adaptive or pharmacodynamic tolerance (Julien, 1998). "Cellular tolerance differs from metabolic tolerance in that it has a different mechanism and site of action, a different time course, and different dose relationships" (Feldman et al., 1997, p. 646). Cellular tolerance is demonstrated by a shift to the right in the BAC response curve after repeated drug use. Therefore, achieving a given alcohol effect requires a higher BAC obtained by a greater intake of alcohol (Feldman et al., 1997; Goldstein, 1983). This type of tolerance indicates an adaptation of receptors in the brain to the continued presence of the drug (Feldman et al., 1997; Goldstein, 1983; Julien, 1998). A number of theories have been proposed to explain this phenomenon, such as neurons adapt either by increasing the number of receptors or by reducing the sensitivity to the drug,

however it has been contended that none of the theories are completely satisfactory (Feldman et al., 1997; Goldstein, 1983).

Additionally, some researchers (e.g. Siegel and Vogel-Sprott) have studied the situation specificity of tolerance explaining this phenomenon as an example of Pavlovian conditioning. One mechanism that has been hypothesised to explain situation-specific tolerance is that the drug effect evokes physiological mechanisms that tend to counteract it. These adaptive processes become conditioned to environmental cues. The repeated association of the drug effect with the environment leads to a state where the environment itself evokes the adaptive response, so that the drug effect becomes reduced. The classic experiment by Siegel (1975) found that a decrease in analgesia over successive morphine injections (tolerance) was controlled by contextual stimuli (cited in Mazur, 1994). Researchers have interpreted these results in terms of associative learning: when distinctive events reliably precede drug administration, they serve as a signal that provides a basis for expecting the drug. When tolerance is established, this expectation results in anticipatory compensatory reactions to reduce the drug's effect. Similar associative effects have been observed using a pharmacological cue (low dose of alcohol followed by a high dose of alcohol) (Greeley, Lê, Poulos, & Cappell, 1984).

The role of behavioural conditioning processes in the development of tolerance has been identified (Julien, 1998). Tolerance can be demonstrated when a drug such as alcohol has been administered in the context of usual pre-drug cues, but not in the context of alternate cues (Julien, 1998). Besides the physiological mechanisms that may operate in the development of tolerance, there is another form of tolerance, known as behavioural tolerance, which involves practice and learning (Goldstein, 1983). For example, rats that were trained (with and without alcohol) to

perform a specific behaviour in a maze showed progressive improvement in performance while under the influence of alcohol. The group trained without alcohol, but when tested with alcohol did not show improvement (Goldstein, 1983).

Related to behavioural tolerance is state dependent learning, or dissociated learning, that describes the phenomena of learning in one state that is dissociated from learning in another state (Goldstein, 1983). It has been shown that subjects can recall something better when they are in the same state, drug or no-drug state, in which the material was learned.

3.5 Summary

In summary, alcohol is a CNS depressant. Unlike many other psychoactive agents, alcohol does not have a single specific locus of action in the brain. Instead alcohol acts on diverse neural mechanisms influencing several of the neurotransmitter systems as well as endocrine systems. There are a variety of factors that can influence the pharmacokinetics and pharmacodynamics of alcohol, including characteristics of the individual such as gender and age. Similarly the conditions under which the alcohol is consumed can also influence the effect of alcohol such as rate of intake, and whether food was consumed with the alcohol. Finally, tolerance can also impact on the effect of alcohol.

The interest of this thesis is the chronopharmacology of alcohol in humans. It has been indicated that very few studies exploring the chronopharmacology of alcohol using human subjects have taken a systematic approach toward elucidating alcohol's effects on circadian rhythms. These studies have not examined, concurrently, physiology, biochemistry, and performance in the one study, on the same subjects. This is most likely attributed to factors such as the financial cost of

the study, the arduous nature of such studies, and the amount of experimental control necessary in order to explore the phenomenon. The remainder of this chapter explores research on the effects of alcohol on commonly used indices of circadian rhythms such as body temperature, melatonin, cortisol and heart rate. Alcohol's effects on human performance tasks are also briefly reviewed. Finally, design and methodological issues relevant to studies of the effects of alcohol on human physiology and performance are considered.

3.6 Alcohol and body temperature (T_b)

Humans are homeo-therms, that is, they thermoregulate around a given temperature (set point) within the limits of approximately ± 2 °C despite much larger variations in ambient temperature (Geller & Adler, 1990). According to Geller and Adler (1990) a change in body temperature (T_b) is a prominent sign of drug action. It has been stated that if a drug acts by changing the set point, the body can still thermoregulate in a narrow range around the new temperature. In contrast, if a drug affects thermoregulation, then it interferes with the body's ability to maintain a particular core body temperature. At the extreme, an effect known as poikilothermia occurs, that is, the body cannot control temperature and moves toward the temperature of the environment (Geller & Adler, 1990; Myers, 1981).

In order to determine whether a drug affects set point or the ability to thermoregulate a researcher must investigate more than T_b. Other methods proposed have been to determine if the subject will engage in behavioural thermoregulation, such as some tasks that will aid in maintaining a given T_b. For example, Gordon, Fogelson, Mohler, Stead, and Rezvani (1988) investigated the effects of oral administration of alcohol on behavioural thermoregulation and body temperature in

rats using a temperature gradient. The temperature gradient enabled the rats to select a thermal preference. It was found that rats given a relatively high dose, 3.0 g/kg, of alcohol became hypothermic and selected a significantly cooler ambient temperature (T_a) in the temperature gradient. Thus, if an animal moves to a warmer environment because of a drug related decrease in T_b then the set point has not been affected. However, if the animal moves to a colder environment then it is inferred that set point has been altered. Lack of movement indicates loss of thermoregulatory control (assuming that physical ability to respond has remained intact). Similarly, Ritzmann and Tabakoff (1976) found that mice undergoing withdrawal after chronic alcohol consumption were found to be hypothermic if kept in room temperature. Placing the mice in a cold environment (4 °C) exacerbated the hypothermia whereas placing the animals at 34 °C reversed the hypothermia and produced hyperthermia. It was concluded that the set point mechanism and the ability to regulate around this set point were disturbed in animals physically dependent on alcohol.

In contrast to the rigorous animal studies investigating the interaction between the ambient temperature and the temperature response to alcohol (Gordon et al., 1988; Myers, 1981), the human data are minimal. Most studies that have investigated the effect of cold ambient temperature on the human temperature response to alcohol have not recorded a change in body temperature (Fellows, MacDonald, & Bennet, 1984; Martin, Diewold, & Cooper, 1977; Risbo, Hagelsten, & Jessen, 1981). For example, subjects who had consumed alcohol (BACs of 0.09g/100ml, ±0.112) and were immersed in cold water for 20 min (13 °C water temperature) showed no change in body temperature compared to no alcohol conditions (Martin et. al., 1977). Similarly, male subjects who had received 0.5 g/kg of alcohol after an overnight fast at a T_a of 21 °C or 30°C showed no significant

difference in temperature change between the two conditions being 0.18 °C and 0.17 °C, respectively (Fellows et al., 1984). However, Roeggla et al. (1995) found that male subjects who drank 1 L of beverage containing 50 g alcohol or placebo before a 1 hr cold water immersion (20 °C) showed significant mean temperature differences. After immersion, in the alcohol condition, mean temperature decreased by 1 °C whereas mean temperature decreased by 0.66 °C in the placebo condition. The few studies that have investigated subjects' response to exercising in the cold after alcohol ingestion are more consistent than those obtained with subjects who did not exercise (Kalant & Lê, 1991). For example, studies have shown that in all cases when subjects performed either mild or strenuous exercise in ambient temperatures ranging from –5°C to + 15°C that doses of alcohol ranging from 0.3 to 0.8 g/kg produced a significantly greater decline in mean core body temperature than was found in the same or other subjects drinking water or glucose solution (Haight & Keatinge, 1973; Graham & Dalton, 1980).

3.6.1 Site of thermoregulatory control

"Appropriate thermoregulatory responses, necessary for maintenance of normal T_b, result from the integration of central and peripheral thermal information. It is a balancing of heat production and heat loss, through the activation of autonomic nervous system mechanisms and alterations in behaviour" (Geller & Adler, 1990, p. 101 - 102). The hypothalamus is the main thermosensitive structure in the central nervous system. Other thermosensitive structures in the central nervous system include the mid-brain and the spinal cord[4] (Holdcroft, 1980). It is known that a thermoregulatory response can be initiated by temperature stimuli from various parts

[4] The existence of deep body thermosensors outside the CNS is questionable (Holdcroft, 1980)

of the human body and a combination of thermal inputs leads to an integrated response (Holdcroft, 1980).

3.6.2 Methods of measuring body temperature

Temperature can be measured in a number of sites on the body and each has a normal range (Holdcroft, 1980). In humans, the body consists of a warm core, the mean temperature of the tissues that are not directly affected by changes in the temperature gradient through peripheral tissues, and a peripheral region throughout which there are temperature gradients. The true core temperature should be that of the hypothalamus, however this cannot be measured accurately. The temperatures taken in the oesophagus, closed mouth or rectum, give adequate indications of the core temperature and may vary between sites by about 1 °C (Holdcroft, 1980).

3.6.3 Research on the effects of alcohol on temperature

For most people the ingestion of alcohol often results in a visible flushing of the face and extremities (Kalant & Lê, 1991). The flush causes a feeling of warmth, but heat loss from increased sweating may lead to a fall in body temperature. After ingesting large doses of alcohol the temperature-regulating mechanism in the brain becomes depressed and the decline in body temperature can become quite severe. Little research has been conducted on human temperature changes after alcohol consumption (Reinberg, 1992; Yap et. al., 1993). Even less research exists on human temperature changes across the sleep period after alcohol ingestion. A very early study by Mullin, Kleitman and Cooperman (1933) investigated the effects of drinking alcohol (60 to 75cc. of 95% alcohol diluted to a 19% solution) in the late evening on rectal temperature across the sleep period. On control nights an equal

volume of water was drunk instead of the alcohol. All body temperature data was based on 10 alcohol nights interspersed with 12 control nights in three subjects. It was found that rectal temperature dropped to a lower temperature than the control condition during the first half of the night. Following this, during the second half of sleep, temperature remained significantly elevated above the mean control temperature. During the first three hours of sleep the mean rectal temperature on alcohol nights was approximately 0.2 to 0.35 °F lower than control temperature, although it followed the same trend. From the third hour through the rest of the night, temperature of the subjects who had consumed alcohol was 0.1 to 0.4 °F higher than the control curve.

The same effect was found more recently by Danel, Libersa, and Touitou (2001). The circadian rhythm of core body temperature was investigated in nine men, comparing a 26 hr alcohol session and a 26 hr placebo session. In the alcohol session, 256 g of alcohol were administered between 1000 hr (first day) and 1200 hr (second day) to maintain a BAC between 0.05 g/100ml and 0.07 g/100ml throughout the experimental session. In the placebo session only fruit juice was administered. During the experiment (1000 hr to 1500 hr), subjects remained in bed, reading and watching television; they ate standard meals at 0800 hr, 1200 hr, and 1900 hr on the first day, and 0800 hr and 1200 hr on the second day. Lights were off between 2200 hr and 0600 hr. Ambient temperature was maintained from 20 °C to 22 °C. Blood samples were collected every 6 hr for BAC determination. Results showed that the temperature during the alcohol session was significantly higher at night and significantly lower during the daytime at the beginning of the trial compared to the control session. The mean lowest temperature was 0.36 °C higher in the alcohol

session compared to the control session with seven of the nine subjects showing a hyperthermic effect of alcohol at night.

3.7 Research on the effects of alcohol on melatonin

The literature on the effects of acute doses of alcohol on melatonin is limited, particularly in humans. One study using human subjects investigated the inhibition of melatonin secretion by acute alcohol ingestion in humans, and the effect of supplementary glucose on melatonin secretion post-alcohol ingestion (Röjdmark, Wikner, Adner, Andersson, and Wetterberg, 1993). The authors proposed that if alcohol has blood glucose lowering properties it could be expected that melatonin secretion could be reduced, which may return to normal when the intoxication is over, or when glucose is supplemented during alcohol ingestion. Melatonin and glucose levels were measured every 2 hr between 1800 hr and 0800 hr. It was found that repeated oral administration of alcohol at doses (0.34 g/kg; 0.52 g/kg; respective serum alcohol level peaks 13 mmol/L; 25 mmol/L) at 1800 hr, 2000 hr, and 2200 hr resulted in inhibited nocturnal melatonin secretion in healthy humans relative to controls. Greater inhibition of melatonin occurred at the higher alcohol dose and when basal melatonin levels were around their peak. Further, blood glucose levels decreased after alcohol, however, the oral administration of glucose during alcohol consumption failed to normalise pineal function and thus, melatonin secretion.

More studies on the effects of alcohol on melatonin have been conducted using animal subjects. Chik and Ho (1992) examined the effects of alcohol on pineal melatonin synthesis after maximal activation of cAMP synthesis. Cells were treated with alcohol, the duration being 15 min for cAMP accumulation and 4.5 hr for NAT and melatonin determination. Using isolated rat pinealocytes, findings provided a

clear indication that alcohol has an inhibitory effect on pineal melatonin synthesis. Alcohol treatment led to a significant reduction of NE-stimulated NAT and melatonin production in the rat pineal gland. Further, alcohol appeared to have an effect on a post-cAMP step in pineal melatonin synthesis.

In order to investigate the effect of alcohol dependence and withdrawal on melatonin levels, Moss, Tamarkin, Majchrowicz, Martin, and Linnoila (1986) administered alcohol (9 – 11 g/kg) in six to nine divided doses for each 24 hr period to rats (N = 6/condition) in order to maintain intoxication across either one to four days. On each day a group of animals was decapitated at 0300 hr. Pineal glands were removed for analysis of melatonin. It was found that continuous intoxication with alcohol produced a reduction in melatonin levels compared to controls at 0300 hr. No effect was found for duration of treatment, indicating that the reduction in melatonin levels was unrelated to duration of exposure. For the withdrawal study (14-16 hr after the last dose of alcohol), pineal glands were obtained at 1500 hr. No significant differences in melatonin content between alcohol and control groups were revealed for the withdrawal phase (1500 hr). The study found that during the dark phase melatonin concentration was significantly reduced in the alcohol-intoxicated rat compared to either controls or withdrawing animals.

In another rat study, Creighton and Rudeen (1988) used male rats to investigate the effect of alcohol (2 g/kg body weight) injected at 1200 hr and 1600 hr on pineal serotonin NAT activity, norepinephrine, and indoleamine content. Following the second injection, animals were decapitated at 1700 hr, 2100 hr, 2400 hr, 0300 hr, and 0700 hr, and pineal glands were removed for analysis. The results indicated that acute alcohol administration during the daytime significantly delayed the onset of NAT activity. The saline-injected animals had significantly increased

NAT activity by 2100 hr, while the NAT activity of alcohol treated animals was not significantly elevated above daytime levels until 2400 hr. NAT activity in animals remained elevated for both groups between 2400 hr and 0300 hr, declining to near daytime levels by 0700 hr. NAT activity was significantly lower in the alcohol treated animals compared to the saline treated group at 0700 hr. Further, pineal 5HT content was reduced in alcohol treated animals compared to the saline treated animals at 1700 hr and 2100 hr. There was no significant altering of norepinephrine in alcohol groups during the nocturnal phase (there was a significant difference at 0700 hr, norepinephrine remained elevated in the alcohol treated animals compared to the saline treated group).

Even though the previous researchers did not find an altering of norepinephrine by alcohol, the primary argument forwarded for nocturnal alcohol-inhibited melatonin secretion is that there is a down regulation of the pineal beta-receptors in response to increased noradrenergic transmission caused by alcohol administration (Moss et al., 1986; Röjdmark et al., 1993; Schmitz, Sepandj, Pichler, & Rudas, 1996). Given pineal melatonin synthesis is regulated by adrenergic neurotransmission, reduced melatonin secretion may be a reflection of decreased sympathetic outflow (Moss et al., 1986).

One of the reasonable consequences of nocturnal alcohol-inhibited melatonin secretion is disruption to sleep. It is known that melatonin has sleep promoting properties, alcohol in contrast has been shown to disturb sleep patterns: for example, variable reductions in sleep latency and intermittent wakefulness, and effects on total sleep time (Buysse, 1991). Whether this effect is in part due to disruption to melatonin secretion is unknown at this time.

3.8 Research on the effects of alcohol on cortisol

While some investigators have suggested that there is a marked increase in cortisol levels associated with alcohol intoxication and withdrawal compared to controls (Fonzi et al., 1994) other researchers have not found similar results (Yap et al., 1993). It has been suggested that the cortisol response to alcohol could be due to individual differences, such as stress levels (Canals, Colomina, Domingo, & Domènech, 1997). Yap et al. (1993) found a significant reduction in plasma cortisol levels after ethanol consumption, achieving BACs less than 0.11 g/100ml. According to Yap et al (1993) BAC is a good indicator of the direction of the cortisol response after alcohol. If BACs remain below 0.10 g/100ml, cortisol levels will reduce; at higher BACs cortisol levels may increase due to stress. Similarly, Reinberg (1992) found that the cortisol response after alcohol was dependent on the time of day alcohol was ingested. A decrease in plasma cortisol occurred 90 min after alcohol ingestion (dose = 0.67 g/kg body weight) at 0700 hr and cortisol increased 90 min post ingestion at 2300 hr; no change was found at 1100 hr or 1900 hr.

3.9 Research on the effects of alcohol on heart rate

Previous findings have reported that heart rate increases as a result of acute alcohol intoxication (Bruce, Shestowsky, Mayerovitch, & Pihl, 1999; Sayette, 1993; Cohen, Schandler, & Naliboff, 1983; Conrod, Peterson, Pihl, & Mankowski, 1997; Yap et al., 1993). According to Sayette (1993, p. 803) "at low doses (e.g., 0.05 g/kg) heart rate increase has been attributed to a reduction in vagal (parasympathetic) inhibition to the heart...At higher doses (e.g. 0.1 g/kg), however, pulse transmission time decreased. Thus for lower range doses of alcohol used heart rate increase

appears to be a function of parasympathetic inhibition, and at higher doses possibly sympathetic activation as well".

Sayette (1993) stated that the most frequent criticism in the use of heart rate concerns initial values. The law of initial values asserts that physiological responses occur within a certain range of values. Therefore, when initial values approach these limits, the physiological reactivity can be restricted (Sayette, 1993). A number of methodological issues have been highlighted in the literature that mediate the effect alcohol has on heart rate. These include age, gender, factors pertaining to the drinking setting (e.g. dose, time to consume dose, socialness of the setting), time after consumption when heart rate measurements are taken, differences in the time of day when alcohol is consumed, mood, and other drugs that may also affect heart rate. For example, decreases in heart rate as alcohol's effects decline have been noted. As such, conflicting results could be attributed to the time allowed for consumption and absorption and could influence whether heart increases or decreases as a result of alcohol ingestion (Sayette, 1993).

3.10 Tests of performance

Numerous research reports have shown that a sufficient dose of alcohol will impair performance. Alcohol induced impairment at doses up to 0.10 g/100ml have been demonstrated for instance, for driving simulations (Fairclough & Graham, 1999; Liguori, D'Agostino, Dworkin, Edwards, and Robinson, 1999), choice reaction time tasks (CRT) (Maylor, Rabbitt, Sahgal, & Wright, 1987), and divided attention tasks (also referred to as a dual task), consisting usually of a tracking and reaction time test (Kerr, Sherwood, & Hindmarch, 1991; Maylor, Rabbitt, James, & Kerr, 1990; Miles, Porter, & Jones, 1986). The particular versions of these tasks vary, and

there are a variety of tasks that are unique. Many studies have used more than one task to measure performance and often alcohol will affect performance on one task and not another. The reasons for this are difficult to ascertain. Some researchers have suggested that it could be a genuine null effect while others suggest that the task may not be sensitive to the effects of alcohol (Finnigan & Hammersley, 1992).

Several distinctive points emerge from the large body of literature on the effects of alcohol on performance that are of relevance. In a review by Finnigan and Hammersley (1992) on the alcohol's effects on performance it was shown that alcohol induced performance deficits were greatest for those tasks that required complex cognitive functioning, including information processing and decision-making and tasks that divide attention. These types of tasks can allow for detection of alcohol impairment more ethically acceptable BAC levels (0.05 g/100ml-0.08 g/100ml) and the degree of impairment is usually greater than on less complex tasks. There have been many studies that have investigated alcohol's effects using divided attention tasks. A few of these studies have been chosen to demonstrate points of relevance.

In a study conducted by Kerr et al. (1991) investigating the separate and combined effects of social drugs (alcohol, caffeine, and nicotine) on psychomotor performance, subjects were required to perform a number of performance tasks. Subjects performed a divided attention task (tracking and visual detection) as well as, choice reaction time, a short-term memory task, and testing for critical flicker fusion threshold. While using a joystick to keep a moving cursor in alignment with a moving target, subjects also responded to visual stimuli (white lights) presented in the peripheral vision. It was found that alcohol alone (30 g of 80% proof vodka in 200 ml of juice, BAC was not monitored) significantly disrupted the error measure

(mean deviation of the joystick tracking the fixed program) of the tracking task. It was found that alcohol did not impair performance on the other tasks. This study demonstrated how the impairment from alcohol is more salient on complex tasks, such as divided attention as opposed to simpler tasks, for example STM and CRT.

Similarly, Maylor et al. (1990) investigated the effects of alcohol (peak BAC achieved = 0.773 g/100ml) and extended practice on divided attention performance, as measured by tracking and an auditory detection task. The tracking task required subjects to use a joystick to keep a cross as near to the centre of a moving target as possible. The auditory detection task required subjects to respond, using a footswitch, to an unpredictable tone every few seconds. Under divided attention conditions, both tasks were performed concurrently. It was found that tracking accuracy improved with practice under single task conditions without alcohol. Tracking accuracy improved with practice and was impaired under divided attention conditions however it was unaffected by alcohol, under both single and dual conditions. The effects of the three factors, alcohol, practice, and divided attention were more obvious in the speed of signal detection. Performance on this task was impaired by alcohol, improved with practice, and impaired under divided attention conditions. The effect of alcohol did not decrease with practice and was greater under divided attention conditions compared to the single condition. This study demonstrates the relevance of using a divided attention task to study the effects of alcohol, as performance on the tracking task, single or dual condition, was unaffected by alcohol. However, reaction time on the detection task was impaired under the alcohol condition, particularly in the dual condition.

Also using a dual task and a within-subjects design, Miles et al. (1986) explored the interactive effects of alcohol and mood. The dual task was conducted

for 15 min (divided into three 5 min epochs). The mood condition involved subjects watching two 30 min films under alcohol (mean BAC = 0.0367 g/100ml) or placebo conditions. One film was humorous and the other, a documentary (neutral condition). After viewing a film, subjects performed the dual task. They were required to use a joystick to match the movement of a target (a square box) with the movement of the cursor (also a square box) while responding to a digit detection task presented in the boxes of the primary task. The primary tracking task was not sensitive to the mood of the subject. However, both alcohol and time epoch of performance independently impaired tracking performance. With each successive epoch tracking performance declined. Tracking performance was not affected by the interaction between mood and alcohol. However, correct reaction times on the digit detection showed interaction effects between alcohol and mood. Individuals who consumed alcohol and watched the documentary, performed worse on the digit detection component of the dual task. This study did not test subjects on the tasks independently; this does not allow an assessment of dual task scores relative to single task scores to be made (Maylor et al., 1990). It has been recommended (Maylor, et al., 1990) that when using a dual task a performance baseline, which is testing under single conditions, should be used to assess alcohol's effects under dual task conditions.

3.11 Effects of alcohol on performance across time

The second study of this thesis examined performance after alcohol consumption over a three and half hour period. While the literature on the acute effects of alcohol on performance is vast, the research on the residual effects of alcohol (or hangover) on performance is minimal and the few studies conducted report inconsistent findings. Thus, several studies were selected to demonstrate these

inconsistencies. Some of the research has investigated residual effects of alcohol as a function of time, repeating testing at intervals across the BAC curve.

Millar, Finnigan, and Hammersley (1999) found that performance impairment on psychomotor tasks was maintained across a two-hour session despite the decline in BAC from a peak of 0.08 g/100ml over the same period. Subjects also supported this evidence by indicating a perception of reduced ability to perform across the two-hour period. The methodological procedure of repeated testing was examined as a potential cause of the residual impairment. Millar et al. (1999) found repeated testing (testing at 20 min intervals for 2 hr) resulted in significantly more impairment on a secondary reaction time task and sustained attention when compared with testing at only 1 hr and 2 hr from ingestion. However alcohol did not interact with these performance conditions, indicating that residual impairment is not an artefact of a repeated testing methodology.

Other studies, such as those of, Chait and Perry (1994), Finnigan, Hammersley, and Cooper (1998), and Lemon, Chesher, Fox, Greeley, & Nabke, (1993) have been specifically designed to examine the "morning after" hangover effect on performance. These studies found no evidence for impaired performance the morning after alcohol ingestion. For example, Finnigan et al. (1998), using a within subjects design, found that ingestion of alcohol up to 0.10 g/100ml BAC had little effect on psychomotor performance (vigilance, dual task, probed memory recall) the morning after ingestion. Similarly, Lemon et al. (1993) found no evidence for an impairing effect of alcohol 12 hr after consumption on any of the behavioural tasks examined. The tasks examined were a dual task, simple reaction time, Mackworth clock, and self-ratings. The authors proposed that the night's sleep might have helped to ameliorate any hangover effects. In the same way, Chait and

Perry (1994) found little evidence for next day effects after marijuana or alcohol or the combination of both drugs. The alcohol treatment resulted in some subjective effects the following morning: POMS fatigue scores were increased, and subjects' reports of clumsiness and tiredness the morning after alcohol ingestion were increased. The total dose of alcohol administered during the evening (alcohol was administered at two points in the night 2 hr apart) achieved a BAC level of 0.88 g/100ml after the second drink. There was no evidence of behavioural impairment on the psychomotor and cognitive tasks undertaken by the subjects the day after the dose.

Investigators in the aviation industry have done much work in order to research the hangover effect of alcohol given the considerable safety implications regarding pilot performance. Several studies have now accumulated (Collins, 1980; Collins & Chiles, 1980; Morrow, Leirer, & Yesavage, 1990; Yesavage & Leirer, 1986) on hangover effects on the performance of pilots. Morrow et al. (1990) found hangover impairment varied depending on the abilities measured. Overall flying performance was impaired 8 hr after drinking, to a level of 0.10 g/100ml BAC, however, radio communication performance was significantly impaired for only 2 hr post drinking. Communication and overall performance was just as impaired 2 hr post drinking as it was at the 0.10 g/100ml BAC, although mean BAC was 0.56 g/100ml at the 2 hr testing point. These findings are consistent with those of Yesavage and Leirer (1986) who found evidence for impairment 14 hr after consuming alcohol to produce a peak BAC of 0.10 g/100ml. Using a within-subjects design, 10 navy pilots were required to fly two simulated flights under control and hangover conditions. For the control condition, the pilots drank no alcohol for 48 hr before undertaking the simulation. The hangover condition required the pilots to fly

14 hr after drinking alcohol. Performance was significantly worse on three of the six variance measures (heading error for take-off and landing and vertical distance from glidescope during landing) and one of the six performance measures (average yaw).

However other investigators have not found impairment in performance the "morning after" alcohol. Collins (1980) examined pilots' performance on a two dimensional tracking task under static and dynamic conditions at ground level and simulated altitude of 12,000 feet, 8 hr after alcohol. It was hypothesised that altitude might interact with hangover malaise to result in performance decrements eight hours post drinking. At BAC peak subjects had obtained a mean of 0.091g/100ml. Testing at this point revealed performance deficits, however in the morning there were no significant differences between control and alcohol conditions. Subjects did rate the degree of hangover higher and mood as poorer in the morning following alcohol, even though tasks were unaffected.

Collins and Chiles (1980) used the same group of pilots and repeatedly tested them on a Multiple Test Performance Battery and a tracking task (static and dynamic) before drinking (1945 hr), after drinking (midnight), and the following morning (0800 hr). Vodka (non-congener) or bourbon (congener) was ingested to achieve an average BAC of 0.093 g/100ml. Eight hours later, BACs declined to 0.007g/100ml for the vodka drinkers and 0.005 g/100ml for the bourbon drinkers. Alcohol impaired performance acutely at the midnight test, however no adverse effects were found for performance when subjects were tested in the morning. While subjects stated significant hangover symptoms, increased fatigue, anxiety, sleepiness, and less vigour, the morning after alcohol, no measurable performance hangover effects were found. Further, there were no statistically significant congener differences (bourbon compared to vodka).

In summary, residual performance impairment has been demonstrated for up to 2 hr post alcohol consumption, even though subjects' BACs have declined over that time. Similarly, it appears that hangover effects are apparent, as subjects have reported changes in mood and feeling states although accompanying performance effects are usually observed only on the more complex tasks, such as performance on flight simulators.

3.12 Overall summary

The evidence and importance of the interaction between circadian rhythms and drugs has been demonstrated. When the circadian effect is applied specifically to alcohol, many studies, using human and rat subjects, have noted that the time alcohol is administered can significantly affect the response to alcohol. This effect can be elucidated using the explanations outlined by chronopharmacologists such as Reinberg (1990b) and Bruguerolle (1992). These mechanisms have been termed, chronokinetics, chronesthesy, and chronergy. Chronokinetics and chronesthesy refer to how alcohol interacts with a subject's physiology according to the time alcohol has been administered. Chronergy is a broader approach, to determine the influence of alcohol on the subject as a whole. While rat studies have made more attempts to address these mechanisms, very little systematic research toward elucidating alcohol's direct effects on circadian rhythms in humans have been conducted. Most of the studies investigating the chronopharmacology of alcohol using human subjects have examined performance and subjective states with respect to time of day. Only two studies have examined performance, physiology, and biochemisty concurrently. None of the studies reviewed have assessed alcohol and time of day interactions for melatonin. Similarly, these studies have not investigated the delayed effect of

alcohol at different alcohol administration times. Thus, the overall aim of this thesis was to examine a range of measures, with respect to alcohol administration time, in the same subjects using a controlled experimental design over a longer period of time. Evaluation and comparison of different systems simultaneously was undertaken in order to examine the chronokinetics and chronesthesy of alcohol. The measures used were indicators of circadian rhythms, rectal temperature, melatonin, cortisol, and heart rate. The performance measure, a dual task, was used as dual tasks have been suggested to be the most sensitive to alcohol's effects (of the commonly used tasks). BAC was measured using a breathalyser in order to assess the pharmacokinetics of alcohol, and as such determine the chronokinetics of alcohol. Importantly, the results from this thesis provide a clearer understanding of the interplay between alcohol and circadian rhythms, with respect to the pharmacokinetics of alcohol and the physiological and biochemical changes due to time of day.

Chapter 4: Study 1: Effect of alcohol dose and time of day on performance and sleepiness

4.1 Introduction

It has been shown that circadian rhythms can modulate the response to alcohol in humans. Generally, alcohol has been shown to produce more impairment, as measured by subjective reports or performance tasks, when it was consumed during the early activity phase (Horne & Baumer, 1991; Horne & Gibbons, 1991; Jones, 1974; Lawrence et al., 1983). Performance on cognitive tasks or a driving simulator, after alcohol (BACs up to 0.10 g/100ml) was more impaired after alcohol was consumed in the morning (0900 hr) or afternoon (1300 hr) than in the evening (evening times ranging between 1800 hr – 2200 hr) (Horne & Baumer, 1991; Horne & Gibbons, 1991; Jones, 1974). Similarly, subjective states such as sleepiness and perceived incoordination were also rated more negatively in the morning (0900 hr) or afternoon (1300 hr) than in the evening (evening times ranging between 1800 hr – 2200 hr). Additionally temporal changes have been found for the pharmacokinetics of alcohol. For example, Lakatua et al. (1984) demonstrated that absorption was faster after alcohol ingestion at 1000 hr compared to 2200 hr in human subjects. Further, circadian variations in the elimination of alcohol have also been noted. Sturtevant et al. (1978) found minimal elimination of alcohol occurred between 1200 hr and 2000 hr. Other studies have also reported time of day differences for peak BAC (Lenné et al. 1999; Yap et al. 1993). In contrast, other studies have not found significant circadian variation in human BAC (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence et al., 1983; Reinberg, 1992).

Other studies have not found the same time of alcohol administration variations on performance and subjective states. Yap et al. (1993) did not find time of day effects on pharmacokinetics, biochemical, physiological, or psychomotor measures. However, they did find that the BAC peak and body sway was significantly higher at 0900 hr compared to the other three times of the day 1500 hr, 2100 hr, and 0300 hr. Similarly, Reinberg (1992) investigated circadian changes on speed of random number addition, eye-hand skill test, and self rated inebreity after alcohol ingestion at 0700 hr, 1100 hr, 1900 hr, 2300 hr. In contrast to previous studies, the 2300 hr administration time was the peak time of self-rated inebriety and the poorest performance time for the speed of performance of both random number addition and hand-eye skill tests. More recently, Lenné et al. (1999) investigated the effects of alcohol on driving as a function of time of day and driving experience at 1200 hr, 1800 hr, and 2300 hr, after consuming either alcohol or juice only. While Reinberg's study found that performance was lowest at 2300 hr, Lenné et al. (1999) found that driving skills were at optimum levels in the late evening. BAC did vary across the three times of the day being highest at 1200 hr. Alcohol had a similar influence on performance at all times of the day. Although, it was stated that given driving performance was reduced at 1200 hr compared to 1800 hr and 2300 hr, both with and without alcohol, from a practical point, accident risk may be slightly elevated for people who drive after consuming alcohol in the middle of the day compared to the late evening or night (Lenné et al., 1999). Additionally, it is possible that the reduction in driving performance at 1200 hr (both with and without alcohol) could be due to coinciding with the beginning of the post lunch dip in physical and mental performance. The addition of alcohol during this time (post lunch dip) may elevate the accident risk.

Studies of the chronopharmacology of alcohol in humans have been previously outlined in section 2.8, and are also summarised in Table 4.1, with regard to the times of the day alcohol was administered, doses of alcohol administered, dependent measures, and design issues. Various measures have been employed to investigate time of day influences on the alcohol performance response such as driving simulators (Horne & Baumer, 1991; Lenné et al., 1999), dual task, digit symbol coding (Yap et al., 1993), choice RT and logical reasoning (Lawrence et al., 1983). There does not appear to be any real consistency for which tasks show time of day variations in the effect of alcohol, as time of day variations have been found using a variety of tasks.

Furthermore, deciding which task/s to use to examine time of day variations in alcohol's effects on performance should also include consideration of the tasks used to examine the chronobiology of performance. Many studies have shown time of day variations in performance using a variety of tasks. Due to the different pattern of performance shown when using STM tasks to investigate the chronobiology of performance in comparison to other tasks, memory tasks have been the most extensively studied tasks among the time of day studies. Nevertheless research on time of day effects for tasks other than memory have also revealed interesting findings. For example, Payne (1989) investigated time of day effects for time on target on a paced mirror-tracking task. Results revealed a significant linear decline of mean score from 0900 hr – 1300 hr, a sharp post lunch recovery at 1400 hr, and another significant decline from 1400 hr – 1700 hr. Similarly, Lenné et al. (1998) found time of day variations for performance on a driving task regardless of sleep deprivation. Likewise, the tasks used to examine alcohol's effects on performance should also be considered. Unlike the chronobiology and chronopharmacology

research, alcohol's effects on performance have been repeatedly demonstrated using dual tasks. A dual task has been shown to be more sensitive to the effect of alcohol at lower doses of alcohol (Kerr et al., 1991; Maylor et al., 1990).

Most human chronopharmacology of alcohol studies reviewed have employed measures of subjective states, such as the Stanford Sleepiness Scale (SSS) (Horne & Baumer, 1991; Horne & Gibbons, 1991) or Visual Analogue Scales (VAS) (Lenné, et al., 1999; Reinberg, 1992; Yap et al., 1993). In some studies, time of alcohol ingestion was shown to influence subjective states, such as sleepiness, perceived incoordination, and inebriety. Alcohol had a greater impact on subjective states either in the morning or the afternoon compared to the evening (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence et al., 1983), although, Reinberg (1992) found that self-rated inebriety was higher at 2300 hr.

The doses of alcohol used in studies investigating the chronopharmacology of alcohol varied from doses administered to achieve BACs of 0.035 g/100ml to 0.11 g/100ml. The lowest dose administered in the study Horne and Gibbons (1991) was used to examine the interaction between alcohol dose and time of day. There were differences between the low and high doses of alcohol. Performance and sleepiness scores were more impaired at the higher dose. Additionally, the dose by time of day interaction was significant for reaction time and hits indicating that impairment was greater in the afternoon at the higher dose.

Related to the dose of alcohol, is the issue of a control group to compare alcohol's effects on the dependent measures. Only one study has incorporated a separate control condition (Lenné et al. 1999). Most studies have been interested in the alcohol and time of day interaction rather than alcohol's effects on performance per se. The reviewed studies have differed in other aspects of design such as fasting

schedules (varying from 2 hr to 12 hr), samples size (ranged from 6 to 80 subjects), and most studies have used a within subjects design, two studies used between subjects. The characteristics of the sample, such as gender have also differed between the studies. The study by Lenné et al. (1999) was the only study to include male and female subjects; the other studies have used either male or female subjects. Similarly, only three studies (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lenné et al., 1999) have screened for circadian typology, with all subjects reported as Neither types. Circadian typology was not considered in the other four studies (Jones, 19774; Lawrence et al., 1983; Reinberg, 1992; Yap et al., 1993), although, Reinberg (1992) used subjects that were synchronised for diurnal activity between 0700 hr – 2400hr.

The number of times of the day that alcohol was administered has also varied between studies. Four studies have used two times of the day, one study used three times of the day, and two studies have used four times of the day. Similarly, the times that alcohol was administered also differed between studies. Most consistently, the studies have used a "middle of the day" time (1100 hr – 1300 hr) and evening time (1700 hr – 1900 hr). Significant time of day variations in performance and subjective effects has been found between these times of alcohol administration.

In summary, several investigators have demonstrated that alcohol has a differential sedative, performance, and subjective effect in humans according to the time of day it is ingested. It has been shown by some researchers that when alcohol was ingested in the morning or afternoon subjects' BACs can be inflated, they can feel sleepier, and their performance can be more impaired than when alcohol was ingested in the evening. However, these studies do differ in the methods and designs

utilised, such as alcohol doses and dependent measures. Thus, it is important to conduct a study that also tests these methodological parameters. It is necessary to establish whether the measures of performance are sensitive to alcohol and/or time of day variations, and if so, what dose of alcohol is most effective in producing these effects.

Table 4.1

Summary of studies reviewed exploring the circadian variation of the effect of alcohol on performance and subjective states.

Study	M/F	CR. Type	N	Dose	Approx BAC g/100ml	Fast	Design	DVs	ToD hr	Significant ToD effects
Jones (1974)	M	NA	80	1.32ml/kg 95% ethanol	0.10	≥ 3hr	B	BAC, Raven's Progressive Matrices	1300-1700; 1700-2200	Performance ↓ 1300 hr
Lawrence et al. (1983)	M	NA	20	2ml/kg 37.5% Vodka	0.07	8hr	B	BAC, Mood inventory, 4-choice serial reaction time, Logical reasoning, self perceived co-ordination.	0900; 1800	↑ CRT Errors, ↑ variability in performance, and ↓ no. of questions attempted at 0900 hr.
Horne & Gibbons (1991)	F	N	8	Four. Two, and zero units Vodka	0.07 and 0.035	2hr	W	BAC, Wilkinson Auditory Vigilance Task (WAVT), SSS	1300; 1830	↓ Performance & ↑ sleepiness at 1300 hr.
Horne & Baumer (1991)	F	N	12	Four and zero units Vodka	0.07	2hr	W	BAC, Car simulator, SSS	1310; 1810	↓ Performance & ↑ sleepiness at 1300 hr.
Reinberg (1992)	M	NA	6	0.67g/kg Ethanolemia	0.11	12hr	W	BAC, VAS, random number addition test, tempo, eye-hand skill, heart rate, blood pressure, peak expiratory flow, oral temperature, grip strength, plasma variables, urinary variables	0700; 1100; 1900; 2300	↑ Self rated inebriety at 2300 hr; ↓ performance at 2300 hr

Yap et al. (1993)	M	NA	10	0.75g/kg Vodka 37.5%	0.10	8 h	W	Acetate, Acetaldehyde, ethanol, lactate, pyruvate, cortisol, divided attention, digit symbol coding, body sway, critical flicker fusion, blood pressure, heart rate, and temperature, VAS.	0900; 1500; 2100; 0300	↑ BAC peak at 0900 hr, ↑ body sway at 0900 hr.
Lenné et al. (1999)	M/F	N	28	0.70ml/kg Vodka	0.05	4hr	W	Driving simulator, VAS, BAC	1200; 1800; 2300	↑ BAC peak at 1200 hr. Performance varied across the day, no interaction with alcohol.

Legend

M/F	Gender of subjects used in the study
CR. Type	The circadian typology of subjects in the study. A 'N' indicates neither types. A `NA' indicates circadian typology was not considered in the study.
N	Sample size
Fast	Number of hours subjects fasted prior to consuming alcohol.
Design	The experimental design of the study is indicated by a 'W' for within-subjects and a 'B' for between subjects.
DVs	The dependent variables measured in the study
ToD hr	Times of day alcohol was administered.
Significant ToD effects	Time of day variations in the response to alcohol noted in dependent variables.

4.2 Aim and hypotheses

The aim of this study was to ascertain whether time of day could be detected as interacting with alcohol on measures of performance and sleepiness. A further aim was to ascertain whether the measures of performance were sensitive to alcohol and/or time of day changes, and if so, which of the doses were most effective in producing these effects. It was hypothesised that performance would be more impaired and sleepiness would be greater in the afternoon compared to the evening. Further, these effects would be more pronounced under the higher dose condition. Similarly, it was hypothesised that after an equivalent dose of alcohol, the BACs registered in afternoon conditions would be higher than those obtained in the evening.

Method

4.3.1 Ethics approval

James Cook University Human Ethics Committee approved the current study (Ethics approval H927).

4.4 Design

A 2 (dose) x 2 (time of day) within-subjects design was used for this study. The two target BAC levels were 0.05 g/100ml and 0.10 g/100ml and the two times of the day when alcohol was ingested were 1300 hr and 1800 hr. The dose and time of day conditions were counterbalanced for all subjects. There was at least one week between each testing session with each subject assigned to either a Friday or Saturday for testing, to minimise day of the week interferences. The study was conducted over a four-month period, March – July 2000.

Table 4.2

Design of study 1

	Time of Day	
Target BAC g/100ml	1300 hr	1800 hr
0.05		
0.10		

4.4.1 Absence of no alcohol condition

A separate no alcohol condition was not used in this study. It was not the interest of this study to compare performance under no alcohol and alcohol conditions but rather to determine whether a low or high dose of alcohol would interact with times of administration.

4.4.2 Alcohol administration times

The times, 1300 hr and 1800 hr, were chosen for the investigation of alcohol's effects because these times have been used in previous experiments (Horne & Baumer, 1991; Horne & Gibbons, 1991; Jones, 1974; Lenné et al., 1999) and have produced significant time of day variations in performance and subjective states. Given this, a comparison of results could be undertaken. The times of day selected were also times that allowed generalisation to the times of the day when people drink alcohol socially (lunch and pre-dinner).

4.5 Setting

All experimental sessions were conducted in the Psychology Research Laboratory at James Cook University. Measurement of performance utilising

computer tasks took place in individual testing rooms. The testing rooms were sound proofed, with a bench adjacent to one wall. Subjects sat in front of a Studio display 17 inch Macintosh monitor with resolution set to 85 MHz at 640 x 400 dpi (thousands of colours). Subjects were seated approximately 80 cm from the computer monitor, with the monitor at a comfortable viewing height.

4.6 Subjects

Twelve individuals (10 males and 2 females) were recruited from the student population at James Cook University and the Cairns community. They were aged between 18 and 39 years (M = 24, SD = 7.6), their weights ranged between 53 and 121.5 kg (M = 74.70 and SD = 20.38), and their heights ranged between 161.5 and 188 cm (M = 178.7 and SD = 7.58). They were non-smokers, free from drugs and/prescribed medication, and moderate drinkers, at the time of testing. They drank, on average, two days/week (SD = 0.74) having an average of 7 standard drinks on these occasions (SD = 2.31). All subjects were `Neither' types[5] (M = 108.75, SD = 17.3) on the Circadian Continuum Scale, Cronbach's alpha for the Circadian Type factor was computed to be .91 (Lehtonen & Graham, 2000) (see Appendix A). Volunteers were excluded from the study if they (see Appendix C for screening instrument):

- Used other recreational drugs regularly (more than once/month).
- Had a history of excessive alcohol use (not being able to go 2 days without an alcoholic drink).
- Were receiving counselling/treatment for personal or alcohol problems from psychologist/psychiatrist/counsellor.

[5] All subjects were typed according to the CCS. Given this was a new instrument, its concurrent validity to the Smith et. al. (1989) Composite Scale was verified in five subjects (see Appendix B for data).

- Were receiving medical treatment, or taking prescription or non-prescription medication.
- Had a history of low alcohol consumption (this included teetotallers, people with a history of not having consumed alcohol in the previous 4 weeks, and people who cannot consume, without becoming ill, 5 - 7 standard drinks within 1 - 2 hours). To be included in the study subjects were required to be moderate drinkers. Moderate drinker was defined as drinking at least two days/week and no more then five days/week and having at least two drinks/drinking session.
- Could not refrain from cigarette smoking or caffeine for 3 hours without signs of withdrawal[6].
- Were pregnant or attempting to fall pregnant
- Were older than 45 years of age.

Subjects who were suitable for the experiment received a `preparation instructions' sheet (Appendix D). This form gave subjects instructions about the fasting schedule, refraining from alcohol and drugs, and transportation requirements. A written consent form was signed prior to the experimental sessions (Appendix E).

4.7 Materials

4.7.1 Breathalyser

Blood Alcohol Concentration (BAC) was estimated from breath alcohol concentration using a Dräger Alcotest 7410 ^{Plus} Breathalyser. The measurement range of this breathalyser was 0.00 to 0.300 %. The measurement accuracy of

[6] While this study allowed subjects to be smokers all subjects in this experiment were non smokers.

readings from 0 – 0.1% was ± 0.0005%. For reading greater than 0.1 % the accuracy was ± 5% of the measured value. The breathalyser was recalibrated according to technical instructions (every six months).

4.7.2 Alcohol

Alcohol was administered in the form of Smirnoff Vodka 37.5% alc/vol. The reason for using vodka is that the alcohol content is greater than beers or wines. Consequently, to reach the target BAC, the amount of vodka to be consumed is less than the amount of beer or wine that would be required. Further, vodka has fewer congeners than many other spirits, which is thought to decrease the frequency and severity of a hangover. The reduced congeners render vodka a relatively flavourless drink and thus acceptable to most individuals. The vodka used in this experiment was diluted with fresh orange juice in the ratio of 3:1 by volume.

The dose of alcohol was calculated per litre of body water (see Equation 1). Total Body Water (TBW) refers to the sum of the volumes of the individual components of body water. As stated (chapter 3), the volume of distribution of alcohol is affected by the body composition and that relates to factors such as age, weight, and gender. Separate formulae are used for males and females for the estimation of TBW (Watson, Watson, & Batt, 1981). The formula for females does not include age as a component. For example a woman, who weighed 60 kg and had a height that measured 165 cm would have a TBW of (0.247 x 60 + 0.107 x 165) -2.1 = 30.375 L. On the other hand, a male who weighed 60 kg and was 165 cm tall, and 40 years of age would have a TBW of (0.338 x 60) + (0.107 x 165) + (-0.0952 x 40) + 2.45 = 36.557 L.

The dose of alcohol was calculated to achieve peak BACs of approximately 0.05 g/100ml and 0.10 g/100ml. An additional 30 ml of vodka was added to the calculated dose as a pilot study (N = 16) (see Appendix E for these data) and other studies (such as Friel, Logan, O'Malley, & Baer, 1999) have shown that the dose of alcohol calculated using the formula underestimates the dose needed to achieve the targeted peak, particularly in the high dose condition.

(1)

$$\text{Dose of Alcohol} = \left(\frac{\left(\frac{\text{TargetBrAC} \times \text{TBW}}{0.08} \right) + \left(\frac{75 \times \text{Time}}{60} \right)}{0.789} \right) \times \left(\frac{100}{\text{Alc/Vol}} \right)$$

$$\text{TBW} \begin{array}{l} \text{Males} = (0.338 \times \text{Weight}) + (0.107 \times \text{Height}) + (-0.0952 \times \text{Age}) + 2.45 \\ \text{Females} = (0.247 \times \text{Weight}) + (0.107 \times \text{Height}) - 2.1 \end{array}$$

4.7.3 BAC recording form

A form was used to chart BAC recordings and other information that may influence BAC such as food last eaten and at what time this food was consumed. Information regarding the subjects' gender, weight, height, and age was also recorded on this form to be used in the TBW formula to determine the dose of alcohol needed to reach the targeted BACs. The time the subjects started and finished drinking, and the time they started the experimental tasks were also recorded on this form (see Appendix G).

4.7.4 Stanford Sleepiness Scale (SSS)

The SSS was used to measure subjects' subjective alertness and sleepiness throughout the testing sessions. The scale has seven points (1 = active, vital, wide awake; 2 = functioning at a high level, not at peak; 3 = relaxed, not at full alertness,

responsive; 4 = a little foggy, let down; 5 = fogginess, slowed, starting to lose interest; 6 = sleepiness, woozy, fighting sleep; 7 = struggling to remain awake) (Appendix H). While there is no psychometric information for this scale, it is a scale commonly used in the literature (Horne & Baumer, 1991; Horne & Gibbons, 1991).

4.7.5 Computerised behavioural measures

Based on the findings of the review of tasks used in chronobiology, chronopharmacology and alcohol and performance research, a dual task was used to examine performance. Tasks were presented, and responses recorded by Power Macintosh G3 computers. The following is an outline of each of the tasks used in the experiment. The order of presentation of tasks was counterbalanced across subjects.

4.7.5.1 Auditory detection task

Subjects used the keyboard space bar to respond to a random 1000 Hz tone presented using Psyscope 1.2.4 delivered via headphones. There were seven intervals, 2000 ms, 3000 ms, 4000 ms, 5000 ms, 6000 ms, 7000 ms, and 8000 ms that were chosen randomly by the program and then replaced back into the list once used. Subjects had 5 s to respond to the tone before the program would self terminate, meaning that it would stop the current trial and move on to the next trial. Reaction time to each tone presentation was recorded. The task was run for 120 s, which was equal to approximately 26 trials.

4.7.5.2 Pursuit rotor task

The MacLaboratory for Psychology motor skills task (Chute, 1994) was used. The parameters were set to a star shape track in a counter-clockwise cursor direction.

The trial time was 15 s, with an inter-trial interval of 5 s, and cursor speed 30 mm/s. The subjects received no feedback during trials for example, trial count, distance travelled, or a sound to indicate that the cursor was off the target. Subjects used the Power Macintosh G3 keyboard mouse to maintain the pursuit cursor on target (see Figure 4.1). The computer mouse speed was set to slow. The time off target, in seconds, was computed for 15 trials.

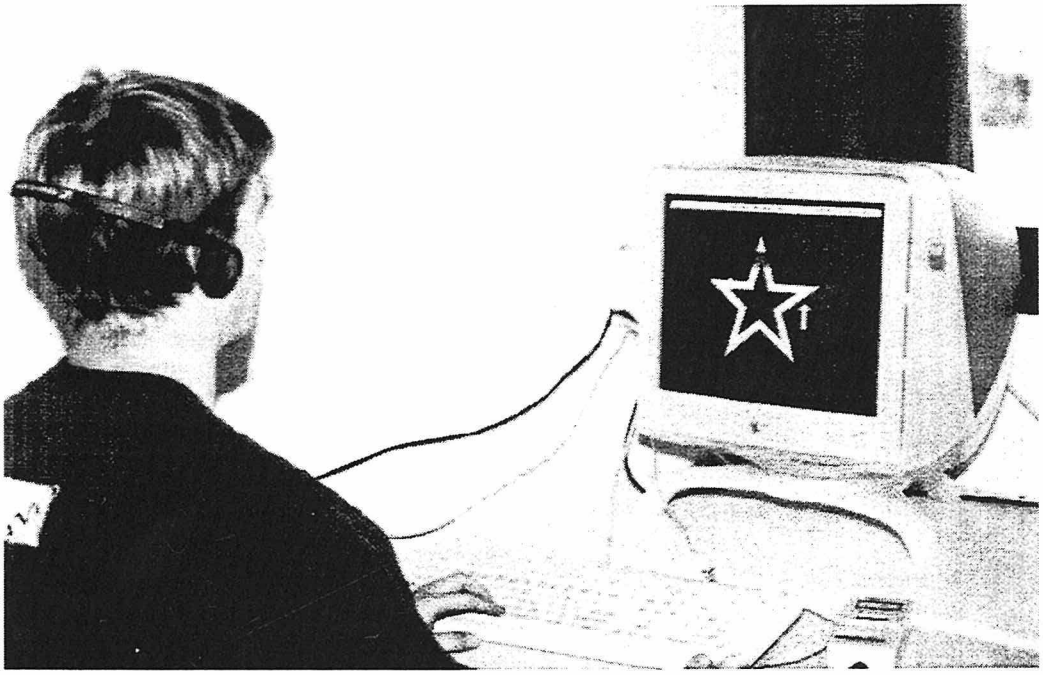

Figure 4.1. A subject performing the dual task. The tracking task required the subject to keep the cursor, presented as an animated mouse, within the target as the target circle automatically moved around the star in a counter-clockwise direction. The subject was also required to respond, using the space bar on the keyboard, when an auditory tone was presented.

4.7.5.3 Dual task (concurrent pursuit rotor and auditory detection)

Subjects were required to perform the pursuit rotor and detection tasks simultaneously. They were instructed to give equal attention to both tasks. The dual task was performed for 15 trials as calculated by the pursuit tracking task. The secondary task, auditory detection, was set to run for 290 s. Time off target and reaction time were recorded.

4.7.6 Weighing scales and height measurement

Soehnle digital domestic scales were used to weigh each subject. Height was determined using a tape measure fixed to the laboratory wall.

4.8 Procedure

Subjects were required to arrive 1 hour prior to the testing time, having had a light meal[7] three hours earlier and no food or drink (water permitted) following the light meal. On arrival, subjects were weighed and their height measured in order to determine the appropriate alcohol dosage, using the TBW formula, to reach the target BAC. A breath test was then taken to ensure that there was no alcohol in the blood. Detailed task instructions and demonstrations were then given (Appendix I). Task order was counterbalanced across testing sessions.

Subjects undertook practice of the performance tasks without alcohol in order to become familiar with the task. Practice consisted of trials on all three tasks. The subjects practiced the pursuit rotor task until "time off target" scores on at least 7 trials (out of 15 trials) were zero and the remainder of the trials had time off target

[7] A light meal was defined as nothing heavy that would sit in their stomach such as meat pies, burgers, hot chips. Foods given as examples to eat included fruit, salad, crackers.

scores less than 0.5 s. Thus, practice on the trials ranged from a minimum of 30 trials to a maximum of 90 trials at the first training session and a minimum of 15 trials and a maximum of 60 trials for the following practice sessions.

The practice session on the auditory detection task was set to the same duration as the experimental test of 120 s. Practice on the dual task consisted of 5 trials on the pursuit tracking task (10 s trials with a 4 s inter-trial interval) while the auditory detection task was set to run for 60 s concurrently. Subjects then rated their level of sleepiness on the SSS after completion of the practice session without alcohol.

Subjects were given 30 min to consume their alcohol. Subjects were asked to pace drinking equally over the allotted 30 min. Subjects did not observe preparation of the beverages. Subjects were required to provide a breath sample[8] before the beginning of each experimental task used. Results of breath testing carried out throughout the training and experimental sessions were not disclosed to subjects until completion of the study. Before each breath test subjects were required to rinse their mouths with water. This was carried out to prevent the possible inflation of the recording by residual alcohol in the saliva. Subjects rated their level of sleepiness on the SSS prior to performing the experimental tasks. The 15 min of computerised testing began after the desired BAC was reached on the ascending limb of the BAC curve (M = 30 min, SD = 6.15 min for the high dose and M = 21 min, SD = 5.14 min for the low dose). If subjects had not reached the target BAC by 30 min for the low dose or 40 min for the high after completion of drinking they performed the experimental tasks regardless of their BAC level. Following the test session,

[8] The blowing time to provide the sample varied from 4 s to 12 s depending on the intensity of the breath.

subjects were provided with a meal and videos whilst waiting for their BACs to return to zero.

On the day of the experiment subjects were asked not to drive/ride a vehicle to or from the university. Transportation was provided. Subjects were required to remain within the laboratory facilities until their BAC reading returned to zero. Subjects were also informed that they could sign a waiver of release (Appendix J) if they wanted to leave the university before their BAC returned to zero. However, this was not permitted until their BAC was below 0.05 g/100ml (the legal limit for driving). Subjects were transported home at the completion of each test session. Debriefing was undertaken at the end of the four sessions. Subjects were thanked for their involvement and paid AUD $100.

4.9 Data analysis

During the analysis of the data from this experiment it was noticed that subjects performance data, particularly for the pursuit rotor task, from the first two experimental sessions was significantly more impaired than the their 3rd and 4th experimental sessions. This was interpreted as a practice effect and indicated that the practice prior to the experimental sessions was not adequate to reach peak performance. Subjects were achieving time off target scores of less than 500 ms for most trials in the first two sessions; however, in the 3rd and 4th sessions a score of zero was obtained on most trials. In order to reduce this practice effect, two more experimental sessions were conducted. These sessions were replications of the subjects' first and second sessions. These data are presented at the end of the results section of this chapter with a summary of the effect of practice on findings without additional practice.

Prior to analysis, data were screened for accuracy, missing values, and fit between distributions and assumptions of multivariate analysis. No missing values were found. Means and standard deviations were calculated for the data collected in this study and analysed using repeated measures ANOVAs. An alpha level of .05 was used for all statistical analyses. Repeated measures ANOVAS were used to analyse performance and SSS data and planned orthogonal polynomial contrasts were used to analyse BAC data. All statistical analyses are reported in Appendix Q.

Results

4.10.1 BAC results

The means and standard deviations for the BACs obtained in the four experimental sessions are shown in Table 4.3.

Table 4.3

Means and between subjects standard deviations for BAC for the four alcohol conditions measured at the beginning of each task.

		1300 hr			1800 hr		
Dose		Block 1	Block 2	Block 3	Block 1	Block 2	Block 3
Low	*M*	0.044	0.045	0.044	0.044	0.046	0.048
	SD	± 0.011	± 0.012	± 0.012	± 0.013	± 0.011	± 0.013
High	*M*	0.069	0.076	0.076	0.071	0.073	0.073
	SD	± 0.016	± 0.017	± 0.013	± 0.014	± 0.014	± 0.013

Planned orthogonal contrasts with an analysis of trends were conducted on the BAC data. As would be expected, there was a significant difference between the two dose conditions, $F(1,11) = 73.21$, $p < 0.01$. There was no significant linear or quadratic tends across time, $F(1,11) = 1.12$, $p = .31$, $F(1,11) = 4.47$, $p = .06$. However, there was a significant linear dose by time post ingestion interaction, $F(1,11) = 7.06$, $p = .02$. At both doses, BACs increased across test blocks when alcohol was ingested, however the rate of change was greater in the high dose condition (see Figure 4.2).

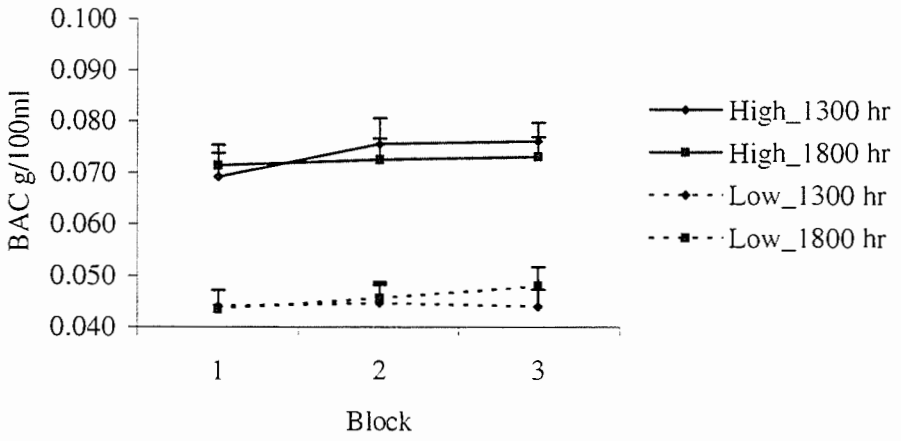

Figure 4.2. Mean BAC for the four conditions measured at the commencement of each task. Points indicate mean BAC; the vertical bars represent the standard errors of the means.

4.10.2 Analysis of psychomotor performance

Means and standard deviations are given for the results of the four tasks in Table 4.4. The performance on each task was analysed using a 2 (dose) x 2 (time of day) repeated measures ANOVA.

Table 4.4

Means and between subjects standard deviations for performance results on the four tasks

		1300 hr		1800 hr	
Task		Low	High	Low	High
Detection (ms)	*M*	263.99	293.37	274.10	289.47
	SD	± 29.96	± 35.05	± 36.83	± 33.45
Pursuit (s)	*M*	121.64	273.66	211.58	141.16
	SD	± 72.86	± 149.82	± 184.77	± 115.02

DT – Det (ms)	*M*	351.90	397.67	375.17	373.91
	SD	± 29.96	± 35.05	± 36.83	± 33.45
DT – Pursuit (s)	*M*	151.10	418.70	274.00	163.40
	SD	± 94.95	± 293.60	± 309.30	± 101.30

4.10.2.1 Dose effects

There was a significant difference between performance under the low and high dose conditions on the auditory detection task under single task conditions, $F(1,11) = 16.56$, $p < 0.01$, and dual task conditions, $F(1,11) = 8.49$, $p = .01$. Performance was significantly more impaired under the high dose condition than the low dose condition for these tasks (see Figure 4.3). The effect of dose was not significant for the pursuit rotor task under single or dual task conditions, respectively, $F(1,11) = 3.0$, $p = .11$; $F(1,11) = 1.8$, $p = .21$.

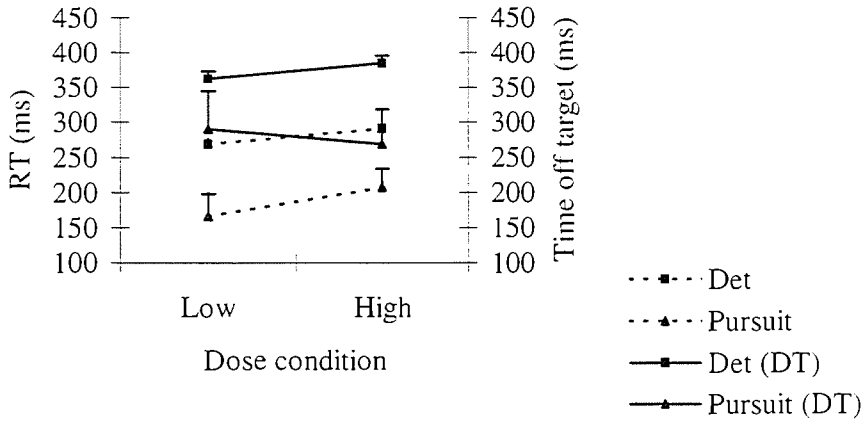

Figure 4.3. Mean time off target (ms) under low and high dose conditions for the pursuit rotor dual task for single and dual conditions and mean reaction time (ms) under low and high dose conditions for the auditory detection under single and dual conditions. Vertical bars represent standard errors of means.

4.10.2.2 Time of day effects

There were no significant main effects for time of day on any of the tasks (all $p's > .10$).

4.10.2.3 Interaction between dose and time of day

The interaction between dose and time of day was significant for the pursuit rotor task under single and dual task conditions, respectively, $F(1,11) = 15.22$, $p < .01$; $F(1,11) = 9.74$, $p = .01$. Subjects performed more poorly after ingesting a high dose of alcohol at 1300 hr compared to when they ingested the high dose of alcohol at 1800 hr on the pursuit rotor single task condition, $t(11) = 2.36$, $p = .04$, and dual task condition, $t(11) = 3.35$, $p < .01$. It appeared that after ingesting a low dose of alcohol that subjects' time off target was higher in the evening, however this was not statistically significant for either task condition, respectively, $t(11) = -1.78$, $p = .10$; $t(11) = -1.60$, $p = .14$.

4.10.2.4 Error for auditory detection task

Errors were defined as a false alarm (making a response when one was not required) or a miss (not making a response when one was required). Errors made by subjects were minimal. Table 4.5 displays the percentage of errors made while performing the auditory detection (single and dual conditions). Using a 2 (dose) x 2 (time of day) repeated measures ANOVA, analysis of the dual task error data was conducted[9]. No statistically significant effects were found for dose of alcohol, time of day, or the interaction between dose and time of day (all $p's > .10$).

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[9] Analysis of the error for the single condition was not undertaken given that the overall mean error for this task was 0.5%.

Table 4.5

Percent errors (with standard deviations) for the four test conditions on the auditory detection tasks (single and dual conditions, calculated as number of errors/number of trials (23 for auditory detection and 55 for the DT auditory detection).

Dose	Time of day	A/Det		DT – A/Det	
		M	*SD*	*M*	*SD*
Low	1300	0.7	± 1.6	0.5	± 1.2
	1800	1.1	± 3.8	1.3	± 2.2
High	1300	0.3	± 1.2	1.8	± 3.9
	1800	0	± 0	2.3	± 3

4.10.3 Stanford Sleepiness Scale (SSS) scores

The means and standard deviations for the scores obtained on the SSS pre and post alcohol are shown in Table 4.6.

Table 4.6

Means and between subjects standard deviations for scores on the SSS for each test condition on arrival (pre alcohol) and post alcohol.

Dose	Time of day	Pre alcohol		Post alcohol	
		M	*SD*	*M*	*SD*
Low	1300	2.33	± 0.07	2.67	± 0.07
	1800	2.58	± 0.07	2.5	± 0.90
High	1300	2.58	± 0.79	3.17	± 0.09
	1800	2.42	± 0.79	3.17	± 0.83

SSS scores for each dose condition were analysed using a 2 (dose) x 2 (time of day) x 2 (pre-post alcohol) repeated measures ANOVA. There was no statistically significant effect of alcohol (pre compared to post alcohol), $F(1,11) = 2.83$, $p = .12$.

Moreover, there was no difference between the high and low dose of alcohol of sleepiness ratings, F (1,11) = 3.95, p = .07. There was no statistically significant difference in sleepiness ratings between the times of day alcohol was administered, F (1,11) = 0.18, p = .90. Further, there were no statistically significant interactions between any other factors.

4.10.4 Performance data with additional practice

The following results are the performance of subjects after six experimental sessions, only those data from the last four sessions are analysed. Means and standard deviations are given for the results of the four tasks in Table 4.7. The performance data on each task were analysed using a 2 (dose) x 2 (time of day) repeated measures ANOVA.

Table 4.7

Means and between subjects standard deviations for performance results on the four tasks after additional alcohol training.

		1300 hr		1800 hr	
Task		Low	High	Low	High
Detection (ms)	*M*	280.71	296.06	276.53	299.59
	SD	± 36.20	± 42.14	± 31.07	± 36.61
Pursuit (s)	*M*	173.07	167.23	130.60	171.99
	SD	± 183.23	± 245.90	± 107.48	± 149.51
DT – Det (ms)	*M*	360.33	399.37	362.53	401.763
	SD	± 23.11	± 50.74	± 41.26	± 61.26
DT – Pursuit (s)	*M*	131.62	141.44	275.87	177.12
	SD	± 113.30	± 125.90	± 206.95	± 126.66

4.10.2.1 Dose effects

There were statistically significant main effects for dose for performance on the auditory detection task, $F(1,11) = 5.55$, $p = 0.04$, and under the dual condition for both components of the task, pursuit rotor and auditory detection tasks, $F(1,11) = 6.90$, $p = .02$, $F(1,11) = 11.87$, $p < .01$, respectively. Performance was significantly more impaired under the high dose than the low dose condition for these tasks (see Figure 4.4). The main effect of dose was not significant for the pursuit rotor task single condition, $F(1,11) = 0.20$, $p = .66$.

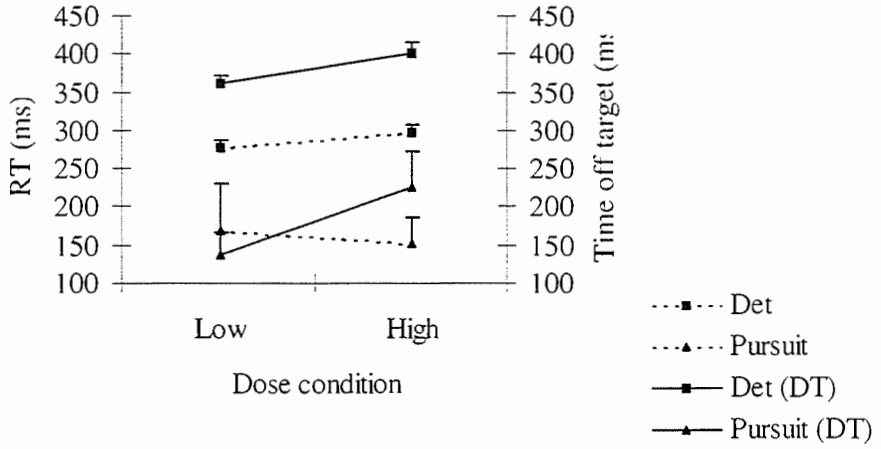


Figure 4.4. After additional alcohol training. Mean time off target (ms) and mean reaction time (ms) under low and high dose conditions for the pursuit rotor and auditory detection tasks under single and dual conditions. Vertical bars represent standard errors of means.

4.10.2.2 Time of day effects

There were no significant main effects for time of day on any of the tasks (all p's > .10).

4.10.2.3 Interaction between dose and time of day

The interaction between dose and time of day was significant for performance on the pursuit rotor task under dual task conditions, F (1,11) = 6.92, p = .03. The pattern of results was the same as that prior to the additional practice under alcohol conditions. The interaction between dose and time of day was not significant for pursuit rotor and auditory detection tasks, single conditions, F (1,11) = 1.70, p = .22, F (1,11) = 0.29, p = .60, or the auditory detection component of the dual task, F (1,11) < 0.01, p = .99.

4.10.2.4 Error for auditory detection task

The percentages of errors made while performing the auditory detection (single and dual conditions) are displayed in Table 4.8. Using a 2 (dose) x 2 (time of day) repeated measures ANOVA, analysis of the dual task error data was conducted[10]. While it appeared that, under dual task conditions, most errors were made in the high dose afternoon condition, no statistically significant effects were found for dose of alcohol, time of day, or the interaction between dose and time of day (all $p's > .10$).

Table 4.8

Percent errors (with standard deviations) for the four test conditions on the auditory detection tasks (single and dual conditions), calculated as number of error/number of trials (23 for auditory detection and 55 for the DT auditory detection) after additional alcohol training.

Dose	Time of day	A/Det		DT – A/Det	
		M	*SD*	*M*	*SD*
Low	1300	2.2	(± 3.8)	0.8	(± 1.4)
	1800	0	0	1.8	(± 3.2)
High	1300	0	0	3.5	(± 4)
	1800	0	0	2.3	(± 3.2)

4.10.5 Summary of the effect of practice on the performance data Study 1

It is evident that undergoing the additional experimental conditions had little effect on the subjects' performance. The difference in performance between the low

[10] Analysis of the error for the single condition was undertaken given that error was obtained in only one condition

and high dose conditions on the auditory detection task under dual conditions was apparent prior to the additional alcohol sessions. The additional training increased the effect of dose, as there was a difference between the low and high dose noted on the pursuit rotor task under dual task conditions as well as the auditory detection task. In contrast, the time of day and dose interaction seen on the pursuit rotor task under single and dual conditions prior to training was only significant for the dual task after the additional training. In summary, the additional experiments increased the dose effect to include the other component of the dual task, pursuit rotor and dampened the effect time of day effect to only the pursuit task under dual task conditions.

Discussion

4.11.1 BAC

While a statistically significant difference was found between the registered BACs after the low and high doses of alcohol, time of day differences for subjects' BACs were not detected. In contrast, previous studies have reported significant effects for the chronokinetics of alcohol. For example, Lakatua et al. (1984) demonstrated that the absorption of alcohol was faster earlier in the day. In addition, it has been shown that circadian variation exists in the elimination rates of alcohol (Jones, 1974; Sturtevant et al., 1980). Similarly, time of day differences for peak BACs have also been reported (Lenné et al., 1999; Yap et al., 1993). However, other studies have not reported significant circadian phase differences in human BAC (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence et al., 1983; Reinberg, 1992; Yap et al., 1993). It was not possible to gauge extensive pharmacokinetic data from this study as BACs were only monitored for the duration of the performance

testing, that is, 15 min. It is noted that while subjects were asked to fast prior to arrival at the laboratory, subjects had consumed differing types and amounts of food three hours earlier. Consequently, subjects' stomach contents differed across sessions (as measured by the questions on BAC measurement sheet, food last consumed and time since this was consumed). This is problematic as contents of the stomach can influence alcohol absorption and elimination processes and thereby could have influenced the BACs of individuals and their relative performance (Millar et al., 1992). This factor could have contributed uncontrolled error in the measurement of differences between test conditions.

4.11.2 Dose

Subjects reached their targeted BACs in the low dose condition and peaked at 0.08 g/100ml for the high dose condition. There were significant performance differences between the high and low dose conditions on the auditory detection task under single and dual task conditions. Performance was more impaired in the high dose condition compared to the low dose condition. These results support those of Horne and Gibbons (1991) who also found significant dose effects (four compared to two units of vodka) for reaction time, and percent hits.

4.11.3 Time of day

The interaction between time of day and dose was significant for performance on the pursuit rotor task under single and dual task conditions. Performance was more impaired after a high dose of alcohol in the afternoon compared to the evening. Performance was the same at both times of the day after a low dose of alcohol. Other researchers have noted similar findings. Jones (1974), Horne and Baumer

(1991), Horne and Gibbons (1991), and Lenné et al. (1999) found that alcohol related performance impairment was increased after consuming alcohol earlier in the day compared to later in the day. In contrast, Yap et al. (1993) did not find performance shifts after alcohol ingestion at 0900 hr, 1500 hr, 2100 hr, 0300 hr. It is noted that the time of day variation could be spurious as it was not seen on the pursuit rotor task under single task conditions when subjects had additional training with alcohol.

4.11.4 SSS

Subjects' scores on the SSS revealed no statistically significant effects. Sleepiness was rated as similar pre and post alcohol consumption, and at both doses of alcohol, and at both times of the day alcohol was consumed. Similarly, there were no significant interactions. These results are in contrast to previous studies that have used the SSS. Horne and Gibbons (1991) found dose and time of day main effects, and a dose and time of day interaction in the level of sleepiness. Comparatively, Horne and Baumer (1991) also found time of day effects for sleepiness using the SSS. A number of possible explanations for the non-significant findings in the current study can be offered. While the chronobiological time for the ingestion of alcohol was similar in the current study to both Horne and Baumer (1991) and Horne and Gibbons (1991), the times and number of replications the SSS was administered differed. Horne and Gibbons (1991) administered the SSS at the completion of testing and then at 30 min intervals for the following two hours. Horne and Baumer (1991) administered the SSS ten minutes after the completion of driving and after a further 60 min and 90 min. Thus, it is possible that administration times of the SSS and the number of replications could have influenced time of day effects for subjective sleepiness. For example, subjects may have become fatigued or bored

from engaging in the performance tasks and over the more extended period of time. In the current study sleepiness was measured prior to task performance, that is, at peak BAC. Changes in the levels of sleepiness due to alcohol, dose of alcohol, and time of day may not have been detected, as levels of sleepiness may need to be measured over a longer period of time, although there is no published evidence to support this claim. Similarly, given sleepiness levels were measured at peak BAC, alcohol may have been having more a stimulatory effect than depressant effect on subjects.

4.11.5 Conclusions

This study allowed for a test of the sensitivity of measures of performance to alcohol and/or phase changes and a determination of which dose of alcohol was most effective in producing these effects. It was found that reaction time was slower at the high dose of alcohol compared to the low dose of alcohol on the auditory detection An additional aim of Study 1 was to explore the dose of alcohol at which the effects of time of day were most evident. The time of day effect found on the pursuit rotor task, single and dual task conditions was noted at the high dose of alcohol. Horne and Gibbons (1991) found similar effects; performance was poorest after alcohol ingestion in the afternoon with a general worsening with increasing alcohol dose.

It was hypothesised that after an equivalent dose of alcohol, that BACs registered in the afternoon would be higher than those obtained in the evening. This hypothesis was not supported. It was contended that in order to detect possible circadian phase differences in the pharmacokinetics of alcohol that some of the methodology of this study needs to be improved, such as a longer BAC collection period and control over subjects stomach contents. Similarly, it was hypothesised

that subjects would rate their level of sleepiness to be greater after ingestion alcohol in the afternoon, particularly after a high dose of alcohol. This hypothesis was not supported. Likewise, subjects' did not feel sleepier after a high dose of alcohol compared to a low dose of alcohol. In conclusion, there was a time of day variation found at the high dose of alcohol for performance on the pursuit rotor task. Time off target increased substantially after the high dose of alcohol in the afternoon compared to the evening. This trend was in line with the results of other human chronopharmacology research such as Jones (1974), Horne and Baumer (1991), Horne and Gibbons (1991), and Lenné et al. (1999). In summary, this study showed that there is a potential time of day variation evident after a high dose of alcohol, on pursuit rotor performance that warrants further investigation.

Chapter 5: Study 2: Acute effects of alcohol at two times of the day on performance, physiology, biochemistry, and subjective states in humans

5.1 Introduction

Research on the chronopharmacology of alcohol has shown that the response of subjects (human or animal) to alcohol can be dependent on the time of day that alcohol is administered. The most consistent findings, such as increased hypothermia in rats, or performance impairment and increased sleepiness, occur when alcohol is given during the activity phase of the subject (i.e., morning to afternoon in humans; dark phase for rats) (Baird et al., 1998; Brick et al., 1984; Haus and Halberg, 1959; Horne & Baumer, 1991; Jones, 1974; Lenné et. al, 1999; Sauerbier, 1987; Williams et al., 1993).

Very few human studies investigating the chronopharmacology of alcohol have tested changes in physiological and biochemical variables. Of the human chronopharmacology studies reviewed, only two studies included physiological and biochemical measures (Reinberg, 1992; Yap et al., 1993). Reinberg (1992) investigated circadian changes in the physiological measures of self-rated vigour, tempo, handgrip strength, and blood pressure. These measures were found not to be dependent on the time at which alcohol was ingested, although not all statistical analyses of these data were presented in the published paper. A decrease in plasma cortisol at 90 min post alcohol ingestion was found at 0700 hr and an increase in cortisol at 2300 hr, 90 min post ingestion. No change was observed at 1100 hr or 1900 hr. This study did not include a separate control condition. Rather, baseline data for all variables was recorded prior to alcohol ingestion.

Similarly, Yap et al. (1993) found that alcohol had clear effects on most of the physiological measures studied, however, circadian variations were not

demonstrated for the dependent variables while under the influence of alcohol. The dependent variable of body sway was the exception. Increased body sway was noted at 0900 hr compared to the other times of the day alcohol was consumed. Prior to the administration of alcohol, there was a significant time of day effect found in cortisol (highest levels at 0900 hr and lowest levels at 2100 hr). Similarly, there was a significant time of day effect on oral temperature prior to alcohol administration. Pre-alcohol readings at 0300 hr and 0900 hr were significantly lower than those at 1500 hr and 2100 hr. Further, while obtaining a significantly higher peak blood alcohol concentration at 0900 hr, other time of day differences (such as time taken to reach peak and alcohol elimination rate) did not reach statistical significance. Like Reinberg (1992), Yap et al. (1993) did not include a separate control condition. Pre alcohol recordings for the variables were taken to be used as a no alcohol comparison.

The lack of time of day dependent changes in physiology and pharmacokinetics in the studies conducted by Reinberg (1992) and Yap et al. (1993) could be due to the relatively short testing periods used in these studies. Reinberg's study measured physiology and biochemistry alcohol-related changes before and 15, 30, 60, 90, 120, and 140 min post alcohol ingestion and thereafter at 4 hr intervals during a 24 to 36 hr period. Reinberg (1992) reported that they examined over these time frames although only the data for 90 min post alcohol ingestion was explored in the published paper. Likewise, Yap et al. (1993) measured alcohol related changes in physiology and biochemistry before and 60 and 120 min post alcohol ingestion. While most animal studies investigating the chronopharmacology of alcohol track changes in body temperature across many hours, even days (Baird et al., 1998; collected temperature for 21 days), there is a lack of comparative research in humans.

It has been found that when large doses of alcohol are ingested the temperature-regulating mechanism in the brain becomes depressed and the fall in body temperature can become quite dramatic. This is supported by chronopharmacological studies using rats as subjects (it should be noted that rats are behaviourally active in the dark hours and sleep during the light hours). Significantly increased hypothermia has been found in rats when they were injected with alcohol during the dark phase in comparison to the light phase (Baird et al., 1998; Brick et al., 1984; Williams et al., 1993). Similarly, alcohol induced changes in body temperature have been shown to vary as function of time of day in humans (Reinberg, Clench, Aymard, Galliot, Bourdon, & Gervais, 1975, cited in Reinberg, 1992; O'Boyle, 1994). In contrast, Yap et al. (1993) did not find a circadian variation in oral temperature changes after alcohol ingestion. To date there is very little research on the effects of alcohol on the body temperature of human subjects and even less has been conducted on the circadian variation of this response particularly across the sleep phase.

If alcohol impacts on physiological systems over an extended periods of time it is reasonable to expect that performance could show different impairment over time. Most of the studies examining the chronopharmacology of alcohol using human subjects have measured performance and subjective states across time, up to about 120 min post alcohol consumption. Research investigating the delayed effects of alcohol on performance have shown that performance can be impaired for many hours despite the decline in BACs (e.g Millar et al., 1999). Some of the research has investigated residual effects of alcohol as a function of time, repeating testing at intervals across the BAC curve, while others have investigated typical hangover effects on performance impairment. Performance impairment is difficult to

demonstrate after acute alcohol intoxication, however, residual performance impairment has been demonstrated for up to 2 hr post alcohol consumption, even though subjects' BACs have declined over that time. Similarly, it appears that hangover can be apparent as subjects have reported changes in mood and feeling states although accompanying performance effects are usually observed only on the more complex tasks, such as performance on flight simulators. Thus, it is important to explore performance impairment over a more extended period of time and examine whether the time of alcohol consumption interacts with the time since alcohol was consumed.

Similarly, in order to systematically investigate circadian variations in the response to alcohol, circadian markers such as body temperature and melatonin should be included as dependent variables. Measures of melatonin have not been included in any chronopharmacology of alcohol studies to date, although, nocturnal melatonin levels have been monitored in animals and humans after acute and chronic alcohol ingestion/injection (e.g., Moss et al., 1986; Röjdmark, et al., 1993). Röjdmark et al. (1993) measured melatonin (and glucose) levels every 2 hr between 1800 hr and 0800 hr with repeated oral administration of alcohol (0.34 g/kg; 0.52 g/kg) at 1800 hr, 2000 hr, and 2200 hr. Results showed inhibited nocturnal melatonin secretion in healthy humans relative to controls.

Some researchers have proposed that time of day related changes in alcohol effects are related to circadian phase of the target tissues, chronesthesy (Lenné et al., 1999; Reinberg, 1992). Alternatively, time of day dependent alcohol effects could be related to changes in alcohol pharmacokinetics, chronokinetics. For example, Lakatua et al. (1984) demonstrated that the absorption of alcohol was faster earlier in the day that is, at 1000 hr compared to 2200 hr in eleven human subjects. Further, it

has been shown that circadian variation exists in the elimination rates of alcohol (Jones, 1974; Minors & Waterhouse, 1980; Sturtevant, Sturtevant, Pauly, & Scheving, 1978). Other studies have also reported time of day differences for mean BACs suggesting possible differences in absorption or volume of distribution (Lenné et al, 1999; Yap et al., 1993). In opposition to the findings presented, other studies have not reported significant circadian phase differences in human BAC (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence et al., 1983; Reinberg, 1992).

While research on the mechanisms mediating circadian dependency in the actions of alcohol and related changes to physiology have been explored using animals (e.g. Baird et al., 1998), comparative studies using humans are very few. The main reasons for the lack of human research are that, while it is possible to keep rats under constant conditions (L/D cycles) and know their drug history, prior tolerance and dependence, diet and nutritional history, behavioural learning history, it is impossible, ethically and practically, to completely control all of these variables in human subjects. Furthermore, using human subjects to collect this type of data is laborious and expensive and places restrictions and inconveniences on subjects and their behaviour for lengthy periods of time. Nevertheless, observations found in rats should be investigated in humans, even though these studies cannot be as tightly controlled as those of the animal studies.

To date, alcohol has been shown to have time dependent effects on the physiological measures of temperature (O'Boyle, 1994; Reinberg et al, 1975 cited in Reinberg, 1992) and cortisol (Reinberg, 1992). Time of day dependent effects for heart rate have not been demonstrated, although alcohol ingestion has been shown to increase heart rate. To date melatonin has not been included in any chronopharmacology of alcohol study, most likely due to the fact melatonin onset

does not occur until about 2100 hr. More research has been conducted on the time dependent effects of alcohol on performance and subjective states. This research has shown that alcohol appears to have more pronounced effects on performance and subjective states when it is ingested during the subjects' active phase.

It has been stated that the chronopharmacology studies, to date, do not generally include a separate control condition. Rather, these studies take baseline measurements of variables prior to alcohol ingestion. The only study (Lenné et al., 1999) that has included a control condition investigated time of day changes in performance rather than physiological measures. To further elucidate apparent time dependent effects of alcohol, the inclusion of a control condition seems necessary in order to explore any physiological and performance shifts across time due to ingesting alcohol at different times of the day.

5.2 Aims and hypotheses

The aim of this study was to examine change across an extended period of time on a range of measures, as a function of alcohol administration time. Evaluation and comparison of different systems was undertaken simultaneously in order to examine the chronokinetics and chronesthesy of alcohol. The measures used were commonly employed indicators of circadian rhythms, rectal temperature, melatonin, cortisol, and heart rate. The performance measure, a dual task, incorporating pursuit rotor and auditory detection, was used as dual tasks have been shown to be among the most sensitive to alcohol's effects (of the commonly used tasks). BAC was measured using a breathalyser as an indicator of the pharmacokinetics of alcohol, and as such, to investigate the chronokinetics of alcohol.

The following hypotheses were tested in Study 2.

- This hypothesis is based on the trend found in Study 1 and previous evidence found in other studies such as Horne and Baumer (1991), Horne and Gibbons (1991), and Jones (1974). It is hypothesised that after ingesting alcohol, performance on the behavioural tasks will decrease for both time of day conditions, however, performance will be poorer under the time of day condition, 1300 hr. Performance is hypothesised to improve across time, within testing session, due to practice and/or acute tolerance. Finally, given the literature on the chronobiology of performance, performance under the no alcohol condition is hypothesised to be significantly different at 1300 hr compared to 1800 hr. The direction of the difference is not specified as previous research has found conflicting results.
- Given the research on alcohol-induced hypothermia in animals, it is hypothesised that, after ingesting alcohol, rectal temperature will decrease for both time of day conditions, however, the decrease is hypothesised to be of greater magnitude for the 1300 hr condition (which equates to the activity phase of humans). Core body temperature is expected to be at similar levels at these times of the day. The decrease in body temperature after alcohol would be expected to be of greater magnitude after the 1300 hr administration time as body temperature will be increasing towards its peak after administration of alcohol at 1800 hr, suggesting a reduced response range for the evening alcohol condition. It is hypothesised that under the no alcohol condition body temperature will decline across time, particularly for the 1800 hr condition, due to the evening fall in body temperature.

- It is hypothesised that heart rate will be slightly higher in the afternoon condition compared to evening condition given the normal circadian pattern of heart rate. Heart rate will increase after alcohol for both times of day conditions, with a greater magnitude of increase predicted at 1800 hr. Based on the law of initial values, given that heart rate is lower at 1800 hr the increase in heart rate after alcohol would be expected to be higher at this time of day compared to 1300 hr. Under alcohol conditions it is hypothesised that heart rate will follow the same trend across time as the BAC curve. Under no alcohol conditions heart rate will be fairly stable across the same period.
- Peak BAC is hypothesised to be higher for the 1300 hr condition compared to the 1800 hr condition. While Study 1 did not find a higher peak for BAC at 1300 hr compared to 1800 hr, other researchers have reported a higher peak for morning and afternoon alcohol consumption compared to evening alcohol (Lenné et al., 1999). BAC will be measured for a longer period of time in this study to investigate absorption and elimination rates more thoroughly. It is hypothesised that time of day differences in these processes will be evident. The shape of the BAC curves will be that found in other studies, an increase in BAC to peak about 45 - 60 min following alcohol ingestion, with a following decline in BAC across time.
- It is expected that there will be a significant difference in melatonin levels between the two time of day conditions as average profiles indicate that daytime levels tend to be <10 pg/ml. The evening rise is initiated around 2100 hr, with maximum values occurring usually from 0100 hr - 0500 hr, declining to daytime levels by around 1000 hr. Given this it is hypothesised that higher melatonin levels will be produced in the evening condition

compared to the afternoon condition. Previous research has shown that alcohol ingestion has an inhibitory effect on melatonin levels. However, these studies have used different times of the day for alcohol ingestion, different doses of alcohol, and melatonin was usually measured only after melatonin onset. The inhibition of melatonin by alcohol may be more readily detected in the 1800 hr condition particularly at the last sampling time. Although, given the differences in the methods of previous studies to the current study the times for sampling melatonin in this study may not be ideal for the detection of inhibition of melatonin.

- It is hypothesised that time of day differences will be evident in cortisol under no alcohol conditions (the normal circadian variation is a peak in the morning with lowest levels seen before onset of sleep). Yap et al. (1993) stated that generally, alcohol decreases cortisol levels, however it has been stated that at high doses (>0.1% BAC) cortisol levels will increase due to stress. Thus, it is hypothesised that alcohol will decrease cortisol levels. Time of day variation in this response may be evident. Reinberg (1992) found a decrease in plasma cortisol at 90 min post ingestion at 0700 hr and an increase in this cortisol at 2300 hr, 90 min post ingestion; however, no change in cortisol levels was observed at 1100 hr or 1900 hr. In contrast, Yap et al. (1993) did not find a time of day variation in the cortisol change after alcohol ingestion.
- It is hypothesised that VAS ratings of the physical and cognitive effects will indicate more negative effect after alcohol ingestion. However, VAS ratings of how social subjects feel will be rated more positively in the short term. Further, it is hypothesised that these ratings will indicate a greater subjective impact of alcohol at 1300 hr. Under no alcohol conditions, ratings may

indicate negative effects (decline in gregariousness and cognition) across time due to the lengthy testing procedure.

Method

5.3.1 Ethics approval

The James Cook University Human Ethics Committee approved the study (Ethics Approval H927).

5.4 Design

A within subjects design was used to study the effects of alcohol on performance, physiology, and biochemistry (see Table 5.1). A no alcohol control condition was included in this study. The target BAC for the alcohol condition was 0.10 g/100ml. The two times of the day were 1300 hr and 1800 hr. The dose, time of day condition, and order of presentation of performance tasks were counterbalanced for all subjects. There was at least one week between each testing session for each subject. The study was conducted over a three-month period, November 2000 – January 2001.

Table 5.1

Design of study 2 (2 x 2 x 3 within subjects) for behavioural performance measures. The test blocks correspond to the amount of time since alcohol ingestion.

		Time of Day	
Target BAC (g/100ml)	Test Block	1300 hr	1800 hr
0.00	30 min		
	120 min		
	210 min		
0.10	30 min		
	120 min		
	210 min		

5.4.1 Alcohol administration times

The times of the day utilised for testing were the same as those used in the previous study, 1300 hr and 1800 hr. These times were chosen based on their social applicability (e.g. lunch time drinks and evening drinks after work). Further, these times are the most commonly used in the research literature on the chronopharmacology of alcohol, thus enabling comparison of findings (see Table 4.1).

5.4.2 Dose of alcohol

The use of 0.10 g/100ml target BAC was selected given the results found in Study 1, that is, the detection of the time of day effect in performance impairment.

5.4.3 Control condition

A control condition is necessary, in order to be able to separate out time of day and alcohol effects on performance. Chronobiology studies have indicated that physiology, biochemistry, mood, and performance can vary across the day, thus a control condition was incorporated into this study to assess whether time of day effects exist without alcohol for the measures under investigation. This condition was a no beverage condition to explore the normal circadian patterns of measures. Subjects were told when they would receive alcohol.

5.5 Subjects

Twelve individuals participated in the study (four males and eight females). Subjects were recruited from the student population of James Cook University. They were aged between 18 and 40 years (M = 25.1, SD = 6.9), their weights ranged between 52 and 80.5 kg (M = 69.7 and SD = 10.7), and their heights ranged between

163 and 182 cm (M = 170.1 and SD = 7.4). At the time of testing, all subjects were free from drugs or prescribed medication, and were moderate drinkers. They drank, on average, 2 days/week (SD = 1) having an average of 4.3 standard drinks on these occasions (SD = 2.3). All subjects reported drinking only at nighttimes. It was a requirement of this study that subjects were `Neither' types[11] on the Circadian Continuum Scale (Lehtonen & Graham, 2000) (see Appendix A) (M = 111.7, SD = 12.6).

Identical to Study 1, volunteers were excluded from the study if they:

- Used other recreational drugs regularly (more than once/month).
- Had a history of excessive alcohol use (not being able to go 2 days without an alcoholic drink).
- Were receiving counselling/treatment for personal or alcohol problems from psychologist/psychiatrist/counsellor.
- Were receiving medical treatment, or taking prescription/non-prescription medication.
- Had a history of low alcohol consumption (this included teetotallers, people with a history of not having consumed alcohol in the previous 4 weeks, and people who cannot consume, without becoming ill, 5-7 standard drinks within 1-2 hours). To be included in the study subjects were required to be moderate drinkers. Moderate drinker was defined as drinking at least two days/week and no more then five days/week and having at least two drinks/drinking session.

[11] All subjects were typed according to the CCS. Given this was a new instrument, its concurrent validity to the Smith et. al. (1989) Composite Scale was verified in seven subjects (see Appendix B for data).

- Could not refrain from cigarette smoking or caffeine for 3 hr without signs of withdrawal[12].
- Were pregnant or attempting to fall pregnant
- Were older than 45 years of age.

Subjects who were suitable for the experiment received the `preparation instructions' sheet (Appendix D), and a written consent form to sign prior to experimental sessions (Appendix K).

5.6 Setting

The testing environment was the same as that utilised in Study 1, that is, the Psychology Research Laboratory of James Cook University.

5.7 Materials

5.7.1 Breathalyser

Blood Alcohol Concentration (BAC) was estimated from breath alcohol concentration using a Dräger Alcotest 7410 ^{Plus} Breathalyser. The measurement range of this breathalyser was 0.00 to 0.300 %. The measurement accuracy of readings from 0 – 0.1% was ± 0.0005%. For readings greater than 0.1 % the accuracy was ± 5% of the measured value. The breathalyser was recalibrated according to technical instructions (every six months).

[12] All subjects in this study were non smokers.

5.7.2 Alcohol

As in Study 1, alcohol was administered in the form of Smirnoff Vodka 37.5% alc/vol. The dose of alcohol was calculated per litre of body water (see Equation 1 section 4.7.2) and chosen to achieve a peak BAC of 0.10 g/100ml. An additional 30 ml was added to the dose calculated for each subject as previous studies have noted that the formula underestimates the dose needed to achieve a desired BAC (Friel et al., 1999). The underestimation has been stated to be that of approximately one standard drink (Maylor, Rabbitt, & Connolly, 1989). For example if the formula calculated that a particular subject required 220 ml of vodka to achieve a BAC of 0.1 g/100ml, then the subject received 250ml.

5.7.3 Food snacks

During the course of conducting Study 1 it was noticed that subjects' compliance to fasting conditions was low. For example, some people would eat heavy foods one hour prior to arrival and others would not have eaten for six hours prior to arrival. To facilitate compliance for Study 2, snacks were provided for the subjects before all four experimental sessions. The snack consisted of a small muesli bar, three small biscuits with cheese, and a snack size container of preserved fruit. This snack was to be eaten three hours prior to the alcohol administration time. For example for the 1300 hr session, the snack should be eaten at 1000 hr. No food or drinks (except water) were to be consumed after this time.

5.7.4 BAC recording form

The same BAC recording form used in Study 1 was used in this study (see Appendix G). The form was used to record information such as, food last eaten,

what time this food was consumed, and information regarding the subjects' gender, weight, height, and age to be used in the determination of alcohol dose.

5.7.5 Visual analogue scales

Given the possible lack of sensitivity of the SSS to detect time of day differences in sleepiness, visual analogue scales were used to detect changes in subjective states. The subject was presented with a solid line that lay between two descriptors. They were asked to place a mark at the point on the line that represented their experience. A major advantage of visual analogue scales is that they are potentially very sensitive (DeVellis, 1991). Another advantage of visual analogue scales is that when they are used repeatedly over time it is difficult for subjects to remember previous responses (unless they chose an end point) in comparison to other types of scales (DeVellis, 1991).

Study 2 was designed to track changes over 210 min, with repeated testing, thus a subject would probably remember that they had chosen a 3 on a 5-point likert scale more readily than where they had placed a mark along a line. However, visual analogue scales have been criticised (DeVellis, 1991) for possible differences between subjects' interpretation of the line space as related to values on the continuum. For example, a mark placed at a specific point along a drunkenness visual analogue scale by one subject may not mean the same thing to another subject given the fact that drunkenness can be evaluated on many dimensions, such as decreased inhibitions, drowsiness, sensory-motor impairment etc. However, it could be argued that this criticism is not relevant when changes within the same individual across different conditions are being investigated. Interpretation of the line space would remain relatively constant within the same individual over time and across

experimental conditions. The anchor points on the visual analogue scales used for this study were based on the clinical symptoms often experienced after consuming a moderate to high dose of alcohol (see Appendix L). The final question on the visual analogue scale asked subjects to estimate their BAC. This was not rated using a VAS. The subjects were asked to write down their estimated BAC.

5.7.6 Weight and height measurements

Soehnle digital domestic scales were used to weigh each subject and a tape measure was fixed to the laboratory wall to measure the subjects' height.

5.7.7 Computerised behavioural measures

The three tasks, auditory detection, pursuit rotor, and dual task (auditory detection and pursuit rotor) used in Study 1 were incorporated into Study 2. These tasks were presented and responses recorded utilising the equipment and procedures as that used in Study 1, with the exception of the computer mouse. In study 1, while every attempt was made to keep the trackball mouse and tracking surface clean, subjects indicated that the mouse would sometimes stick due to dirt build-up on the trackball component of the mouse. Therefore the trackball mouse was replaced with an infrared mouse. Subjects in study 2 did not indicate tracking problems using an infrared mouse. For a description of tasks, see section 4.7.5.

5.7.8 Heart rate (Electrocardiogram - ECG)

The MacLab Bio Amp and the associated software, Chart Version 3.5, were used to measure heart rate. Disposable electrodes were placed onto the subjects' body upon arrival and the patient leads (supplied with the bio amp) were snapped

onto the appropriate electrodes when testing was required. The positive electrode was positioned on the left side of the body, approximately 15 cm under the armpit. The negative electrode was attached in the same position on the right side of the body, and the earth was positioned in the area around the sternum. ECG was measured at three testing blocks and concurrently measured while performing a computer task. A one-minute recording was conducted prior to the commencement of the task and on completion of the task to be used as baselines in the event that performing the task had an influence on heart rate. Given the known sensitivity of heart rate to changes in activity, subjects were informed that the equipment was responsive to movement therefore subjects were instructed to remain seated and stationary during the collection of the heart rate data. Observation of subjects throughout experimental procedures was also undertaken to ensure subjects adhered to this protocol.

5.7.9 Core body temperature

Core body temperature was measured at one-minute intervals using portable T-Tec electronic data loggers. One-minute intervals were chosen as this interval setting allowed the logger to have a memory capacity of at least 33 hr recording time. The subject inserted a disposable rectal probe, and attached the other end of the probe into the data logger. Detailed written instructions were provided to subjects (Appendix N). The temperature data recorded was downloaded onto a windows platform computer by connecting the electronic data logger to the computer (T-Tec provided the computer software to enable the download of information). The data logger batteries were replaced at the beginning of the study having a lifetime of eight months. The ambient temperature in the laboratory during the experimental sessions

(6 hr) was 23 °C with a variation of 1 °C, although the ambient temperature could not be controlled once subjects left the laboratory.

5.7.10 Cortisol and melatonin collection

Subjects were asked to collect approximately 2 ml of saliva in a test tube. The method used to collect the saliva was a non-stimulated and non-timed method. Subjects were required to give two specimens at each experimental test block (0, 30 min, 120 min, 210 min) for replicate determination if necessary. It took subjects 5 – 10 min to provide the saliva specimens. Given the fasting requirements of the experiments, subjects refrained from eating and drinking during the sampling period. After alcohol consumption and consumption of the meal after test block 120 min, subjects rinsed their mouths with water and waited five minutes prior to collection. Light intensity was controlled for throughout both time of day conditions to eliminate any effects on melatonin levels due to light. Thus, for both afternoon and evening experimental sessions the lighting environment was constant (250 – 500 lux that of normal room lighting). Given the length of experimental sessions and the effects of alcohol, it was not practical to control for subjects' posture. Nevertheless, subjects were instructed to not lie down or sleep throughout the testing sessions. This was strictly monitored. Once collected, samples were immediately stored in a freezer (-20 °C). Samples were packed in dry ice and transported by air courier to be analysed in the biochemistry laboratory at the School of Psychological Science, LaTrobe University.

Salivary melatonin was assayed by a direct RIA (radioimmunoassay) using reagents from the Buhlman Saliva RIA Melatonin kit. Prior to analysis previously frozen salvia was defrosted and centrifuged at 6 °C for 5 min at 2060 x g and

immediately sampled. The kit procedure was modified slightly. Saliva samples (in duplicate), quality controls and standards (all 200 uL) were all incubated overnight (20 hr) with 50 uL anti-melatonin and 50 uL I^{125}-labelled melatonin, at 4 °C. Solid phase bound second antibody (50 uL) was then added to all tubes and incubated for a further 15 min at 4 °C. All tubes had 1 ml cold nanopure water added, and then were centrifuged for 2 min at 2000 x g at 4 °C. The supernatant was aspirated and then the tubes counted for 2 min on a gamma counter. Non Specific Binding was 6.61%. The within assay CV (using in-house Quality Controls) is 15.75% and 14.31% for 3.48 pg/ml and 17.21 pg/ml, respectively. The between assay CV is 26.21% and 15.16% for 3.48pg /ml and 17.21 pg/ml, respectively. Twenty zero standard replicates were assayed in a single run. The minimum detectable concentration of melatonin in 400 ul incubation buffer was calculated to be 0.2 pg/ml (0.9 pmol/ml) by subtracting two standard deviations of averaged zero standard duplicates from the counts at maximum binding and intersecting this value with the standard curve obtained in the same run.

Salivary cortisol was assayed by a direct RIA using reagents from the Orion cortisol coated tube kit. Prior to analysis previously frozen saliva was defrosted and centrifuged at 6 °C for 5 min at 2060 x g and immediately sampled. Standards (1.0 – 100 nmol/L or 0.36 ng/ml-36.25 ng/ml) were serial diluted in 0.1 M Tris-HCl, pH 7.4, 0.2% BSA. Standards were diluted from a 2000 nmol/L standard that was supplied by Orion and reconstituted in 0.5ml de-ionised distilled water. Saliva samples, quality controls and standards (all 150 uL) were all added to the polyclonal (rabbit) cortisol antibody coated tubes in duplicate. All tubes were then incubated with 500uL I^{125}-labelled cortisol for 30 min in a 37 °C water bath. The tubes were decanted and washed once with 1 ml distilled water. The tubes were counted for one

minute on a gamma counter. Non Specific Binding was 0.6%. The within assay CV (using in-house Quality Controls) is 10.93%, 5.87% and 1.55% for 0.98 ng/ml, 3.19 ng/ml and 6.24 ng/ml, respectively. The between assay CV is 20.35%, 8.38% and 13.66% for 0.98 ng/ml, 3.19 ng/ml and 6.24 ng/ml, respectively. The sensitivity of the kit as defined by Orion is 0.8 nmol/L (0.29 ng/ml).

5.8 Procedure

The procedure was similar to that utilised in Study 1. Subjects were required to arrive one hour prior to the testing time of day. On arrival, a breath test was taken to ensure that there was no alcohol in the blood. None of the subjects had a positive breath reading on arrival for any of the sessions in this experiment. Subjects were weighed and their height measured in order to determine the appropriate alcohol dosage to reach the target BAC of 0.10 g/100ml. Subjects began logging rectal temperature, saliva samples were collected, and the visual analogue scales were completed. Detailed task instructions and demonstrations were then given (Appendix I).

To become familiar with the tasks, subjects undertook practice of the performance tasks without alcohol. The subjects practiced the pursuit rotor task until "time off target" scores on a minimum of seven of the 15 trials were below 0.5 s and the time off target for the remaining eight trials was less than 1 s. Thus, practice on the pursuit rotor task ranged from a minimum of 30 trials to 90 trials at the first training session and a minimum of 15 trials to a maximum of 30 trials for the following experimental sessions. Subjects' practice session on the auditory detection task was set to the same time as the experimental test of 120 s. Practice on the dual task consisted of five trials on the pursuit tracking task (10 s trials with a 4 s inter-

trial interval). The auditory detection component of the task was set to run for 60 s concurrently. Practice on behavioural tasks was undertaken at the beginning of all experimental sessions.

The measurement of the dependent variables utilised in this study was conducted at several time points (see Table 5.2). The performance measures and heart rate were taken at 30, 120, and 210 min after completion of alcohol ingestion. The saliva samples for determination of neuro-hormones were collected on arrival, 30, 120, and 210 min after alcohol consumption. The inclusion of the arrival collection was to cross reference baseline readings with the control condition at the same time of day. Breath readings were taken every 15 min. This gave a total of 14 readings/alcohol session. Rectal temperature was recorded for 16 hr (1700 hr-0900 hr) for the evening condition and 21 hr for the afternoon condition (1200 hr – 0900 hr). This recording time was chosen to acquire data during the sleep phase after drinking and the beginning of the light phase (study 3). While it would have been advantageous to acquire more body temperature data it was believed that due to the already existing demands of the experimental protocol asking subjects to log rectal temperature for longer (i.e. into the following activity phase) may have hindered recruitment.

Subjects were given the allocated dose of alcohol, calculated using the TBW formula, to be consumed over 30 min. Subjects were specifically instructed to pace drinking equally over the allotted 30 min. Subjects did not observe preparation of the beverages. Breath alcohol testing was conducted every 15 min beginning from the completion of alcohol ingestion. The results were not disclosed to subjects until completion of the four experimental sessions. Before breath testing subjects were required to rinse their mouths with water. This was carried out to avoid errors in

recording due to residual alcohol in the saliva. At the completion of alcohol ingestion saliva samples were collected, visual analogue scales filled out, and testing on the computerised tasks was undertaken. The order of the tasks was counterbalanced for each subject to reduce possible order effects. The same procedure was undertaken at 120 and 210 min after alcohol ingestion. After the 210 min block subjects were provided with a meal (toasted sandwich for the afternoon conditions or pasta for the evening conditions[13]). Between the testing blocks subjects watched television or talked with each other.

Subjects were informed that, ethically, they should remain at the university under the supervision of the researchers until their BAC returned to zero. However, subjects were also informed that they could sign a waiver of release if they wanted to leave the university before their BAC returned to zero. By the end of the third testing block (210 min post alcohol ingestion) most subjects' BACs were below 0.05 g/100ml, however if their BAC was still above 0.05 g/100ml they were not permitted to sign the waiver. At the completion of each experimental session, subjects were transported home. As subjects were to log rectal temperature throughout the sleep phase and respond to the VAS in the morning, they were asked to relax for the remainder of the evening and to go to bed as normal[14]. Subjects were asked not to drink alcohol or engage in any strenuous activity. Debriefing was undertaken at the end of the four sessions. Subjects were thanked for their involvement and paid AUD $100.

[13] The meals were different for the afternoon and evening conditions however both meals were predominately the same size and both were carbohydrate based.

[14] Seven of the twelve subjects reported going to sleep between 2330 hr and 0130 hr and waking between 0700 hr and 0900 hr. The other five subjects did not report their sleep and wake times.

Table 5.2

The test block at which dependent variables were measured and the corresponding time of day.

Time of Day (hr)	1200hr 1700hr	1300 – 1330 1800 – 1830	1400 – 1430 1900 – 1930	1530 – 1600 2030 - 2100	1700 - 1730 2200 - 2230
Block	0		30	120	210
DVs measured	VAS Melatonin Cortisol Temperature logging begins	Drink alcohol over 30 min	VAS Melatonin Cortisol Performance Heart rate	VAS Melatonin Cortisol Performance Heart rate	VAS Melatonin Cortisol Performance Heart rate

5.9. Data analysis

Prior to analysis, data were screened for accuracy, missing values, and fit between distributions and assumptions of multivariate analysis. The handling of missing data was only necessary for temperature logging data. Data were inspected visually for missing values and replaced by a linear interpolation procedure. Periods of missing data were no longer than 5 minutes for any one condition. There was no missing data for the other dependent variables. Means and standard deviations were calculated for the data collected in this study and analysed using planned orthogonal polynomial contrasts and trend analysis, as the purpose of trend analysis is to find a mathematical expression to relate changes in the dependent variable to changes in the independent variables (Keppel, 1982). An alpha level of .05 was used for all statistical analyses.

The abbreviations used to represent conditions are as follows NAA (no alcohol, afternoon), NAE (no alcohol, evening), AA (alcohol, afternoon), AE (alcohol evening). The testing blocks indicate the times at which measures were taken after alcohol ingestion (or control condition) - 30 min, 120 min, and 210 min.

Results

5.10 Physiological measures

5.10.1 BAC

The BAC data was analysed using planned orthogonal polynomial contrasts and trend analysis. There was no significant time of day difference in BAC between the 1300 hr and 1800 hr conditions, $F(1,11) = 0.034$, $p = .86$. As would be expected for a BAC curve, there were significant trends across time. Planned contrasts indicated that the linear, $F(1,11) = 227.24$, $p < .01$, quadratic, $F(1,11) = 32.88$, $p < 0.01$), and cubic, $F(1,11) = 23.47$, $p < .01$, trends were significant. As would be expected for a BAC curve, there was an increase initially, a brief plateau, and then a decline. The interaction between time of day and time post alcohol ingestion was also significant. This interaction was linear, $F(1,11) = 6.65$, $p = .03$ (see Figure 5.2); illustrating that while the decline is similar for both curves, prior to 75 min post ingestion, the 1800 hr condition had a higher BAC at the first time point and appeared to obtain a higher peak higher. However, paired t-tests between afternoon and evening on the first four points revealed no significant differences in BAC between the two times of the day (all p values > .06).

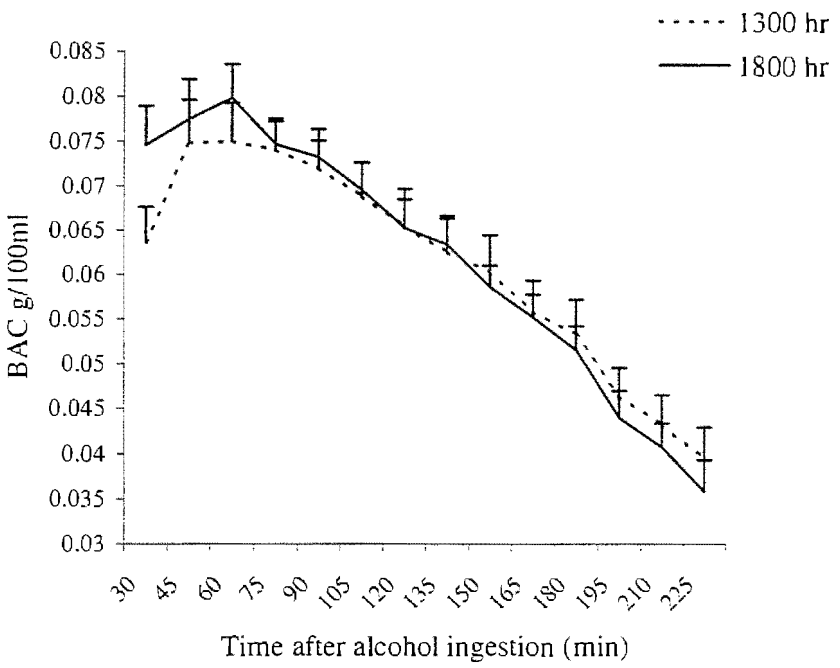


Figure 5.1. Mean BAC curves for afternoon and evening alcohol sessions across the testing session. Vertical bars depict standard error of means.

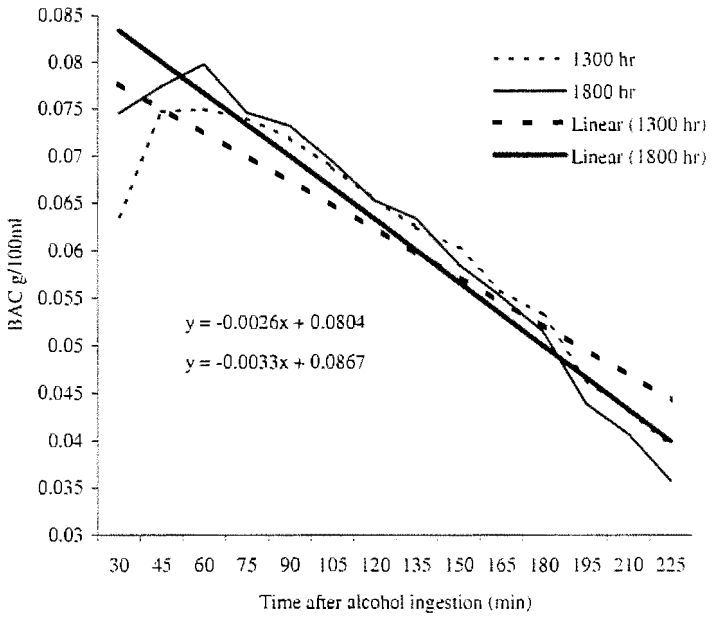


Figure 5.2. Linear trend for the afternoon and evening BAC curves with respective regression equations.

5.10.2 Core body temperature

Figure 5.3 shows the mean core body temperature for the four experimental conditions and respective solar time. The data have been averaged into 1 hr means (60 data points). For the first six points of each temperature curve, the subjects were in the laboratory for experimentation, which enabled control over activity and ambient temperature. After this time (5 – 14 hr) they were in their homes. These data are analysed and discussed in study 3 (Chapter 6).

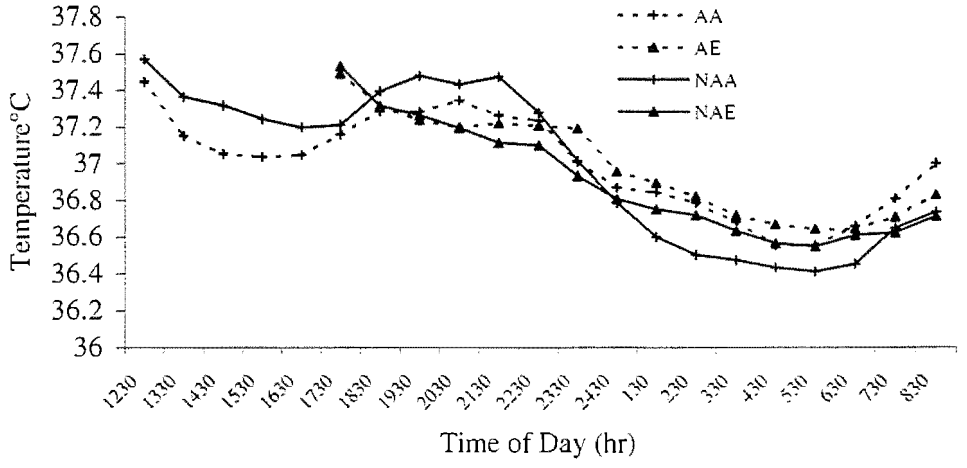


Figure 5.3. Core body temperature (°C) for the four conditions for the total logging time.

Time after alcohol ingestion was determined from the completion of drinking (1330 hr or 1830 hr) to the mid-point of a block of rectal temperature data. For example 2 hours post alcohol ingestion was calculated as 1330 hr to 1530 hr. The corresponding solar time for time after alcohol ingestion for the time of day conditions is presented in Table 5.3.

Table 5.3

Time after alcohol ingestion and relative solar times for the afternoon and evening alcohol conditions.

Time post alcohol ingestion (hr)	Afternoon	Evening
-1	1200 –1300 hr	1700 – 1800 hr
0	1300 – 1400 hr	1800 – 1900 hr
1	1400 – 1500 hr	1900 – 2000 hr
2	1500 – 1600 hr	2000 – 2100 hr
3	1600 – 1700 hr	2100 – 2200 hr
4	1700 – 1800 hr	2200 – 2300 hr

The results from the logging of core body temperature from pre (-1 hr) to post (4 hr) ingestion of alcohol for the four conditions are illustrated in Figure 5.4 and 5.5. To investigate whether core body temperature was equivalent prior to alcohol consumption, a 2 (dose) x 2 (time of day) ANOVA was conducted on the data at -1. The main effects and interaction were not statistically significant (all p values > .3). This indicated that there were no significant differences between conditions at this point. The means and standard deviations for the four conditions up to 4 hr post ingestion are presented in Table 5.4.

Table 5.4

Means (± standard deviations) (°C) core body collapsed across time, alcohol condition, and time of day.

	Condition			
	AA	NAA	AE	NAE
M	37.15	37.28	37.32	37.25
SD	(± 0.28)	(± 0.25)	(± 0.27)	(± 0.31)

The data from each time of day were analysed using planned contrasts and trend analysis. Due to an incorrect logging procedure resulting in loss of a large amount of data, the results of subject 8 were excluded from the analysis. Further, a logger failed to record data for subject 4 in the alcohol afternoon condition as such the data collected for their other three conditions were removed from the analysis, reducing the sample to 10 subjects. Any missing values were replaced by interpolation between nearest adjacent points (standard procedure used in circadian rhythm studies). No more than a 5 min period was replaced for any one subject per condition.

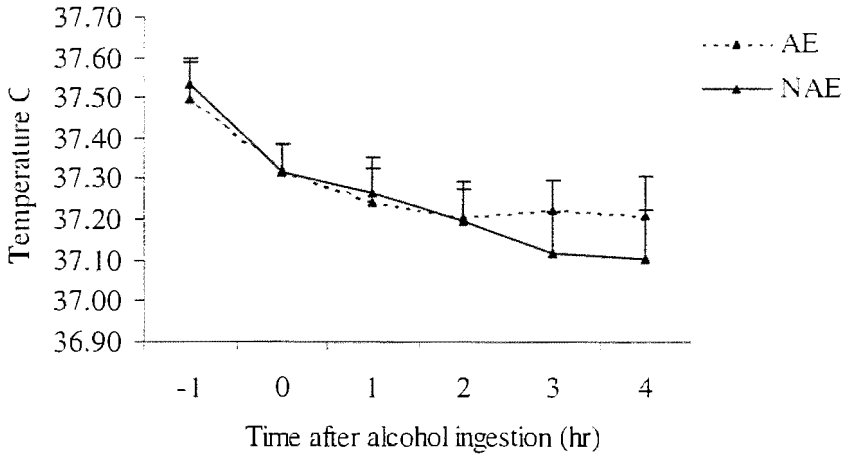
Overall analysis - There was no significant effect of time of day, $F(1,9) = 0.61$, $p = .46$, or alcohol $F(1,9) = 2.00$, $p = .19$. As would be expected, there was a significant change in body temperature across time, for both linear and quadratic trends respectively, $F(1,9) = 24.24$, $p < .01$; $F(1,9) = 125.58$, $p < 0.01$). The analysis was separated out into the two times of day to determine the effect of alcohol at each time of day condition.

In the afternoon, the body temperature of subjects under the alcohol condition was clearly lower than under the control condition, $F(1,9) = 6.39$, $p = .03$. As expected there were significant trends across time post ingestion, linear and

quadratic, $F(1,9) = 27.58$, $p < .01$; $F(1,9) = 48.88$, $p < .01$. The interaction between dose and time was not significant, $F(5,45) = 1.10$, $p = .36$. In contrast to the afternoon temperature analysis, there was no significant alcohol effect on temperature in the evening, $F(1,9) = 0.13$, $p = .73$. As expected there were trends in body temperature across time, linear and quadratic, $F(1,9) = 10.50$, $p = .01$; $F(1,9) = 14.18$, $p < .01$. Like the afternoon condition, the interaction between dose and time was not significant, $F(5,45) = 1.19$, $p = .33$. The linear trends were due to the decline in body temperature from −1 to 0 as removal of the first hour of body temperature removes the significant linear trend. The increased body temperature for the first hour of measurement was most likely the result of insertion of the rectal probe.

Afternoon BAC g/100ml	0.075	0.065	0.053

Figure 5.4. Change in core body temperature (°C) across time for the alcohol and control conditions in the afternoon. Vertical bars depict standard error of means. BAC is indicated for the first three hours of measurement.



Evening BAC g/100ml	0.079	0.065	0.051

Figure 5.5. Change in core body temperature (°C) across time for the alcohol and control conditions in the evening. Vertical bars depict standard error of means. BAC is indicated for the first three hours of measurement.

5.10.3 Heart rate

Table 5.5

Means and between subjects standard deviations for heart rate as a function of dose, time of day, and block, with corresponding BAC (g/100ml).

		1300			1800		
Dose		30	120	210	30	120	210
0	*M*	73.39	69.73	75.06	67.85	64.76	73.15
	SD	(± 12.20)	(± 10.52)	(± 11.94)	(± 13.03)	(± 12.75)	(± 13.22)
0.10	*M*	79.05	77.06	83.68	82.31	78.94	79.91
	SD	(± 9.47)	(± 13.29)	(± 12.98)	(± 11.90)	(± 14.12)	(± 11.91)
BAC		0.064	0.065	0.043	0.075	0.065	0.041

The data for heart rate were collected over seven minutes, 1 min pre and post the performance task, and 5 min during the performance task at the three test blocks (30 min, 120 min, and 210 min). These data were collapsed into one-minute blocks. The data were analysed using planned orthogonal contrasts and trend analysis. The heart rate of the subjects under the alcohol condition was significantly higher than under the control condition, $F(1,11) = 58.79$, $p < .01$. No time of day differences in heart rate were revealed between the 1300 hr and 1800 hr conditions, $F(1,11) = 2.27$, $p = .16$. However, the interaction between dose and time of day was significant, $F(1,11) = 6.83$, $p = .02$. Heart rate for the no alcohol condition was higher in the afternoon condition compared to the evening condition, however heart rate was the same for the alcohol conditions regardless of time of day. In addition, there were significant linear and quadratic trends in heart rate change across test blocks, $F(1,11) = 8.67$, $p = .01$, $F(1,11) = 32.07$, $p < .01$. The heart rate of subjects increased from 30 min to 210 min post alcohol ingestion with a decrease in heart rate at 120 min. There was a significant linear trend in heart rate change across the seven minutes of measurement, $F(1,11) = 15.56$, $p < .01$.

The only other significant interaction was the three-way interaction between dose, time of day, and block. This was a significant linear interaction, $F(1,11) = 13.18$, $p < .01$. At 1300 hr and 1800 hr under the alcohol condition heart rate significantly increased compared to the no alcohol condition. However, under the no alcohol condition, heart rate was lower at 1800 hr than at 1300 hr. At 1300 hr under both the no alcohol and alcohol condition heart rate declined from 30 min to 120 min and increased at 210 min, to be higher than the 30 min mean. However, at 1800 hr under the alcohol condition, heart rate declined across time, while the no alcohol condition had the same block pattern as the 1300 hr time of day, heart rate declined

from 30 min to 120 min and increased, strongly at 210 min post alcohol ingestion. Anticipating completion of testing might have aroused subjects and therefore increased heart rate at 210 min post alcohol ingestion. Analysis excluding the 210 min testing block removed the significant 3-way interaction, while maintaining the significant main effects of dose, test block, and the interaction between dose and time of day (see Appendix Q).

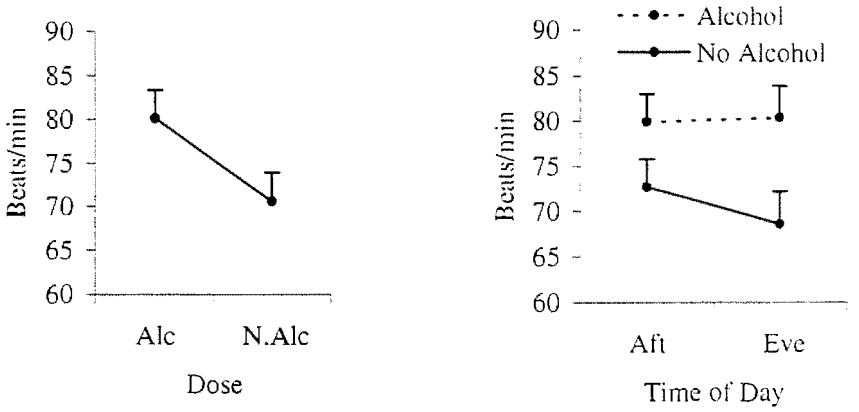


Figure 5.6. (a) Mean heart rate for the alcohol and no alcohol conditions. *(b)* Mean heart rate for alcohol and no alcohol conditions at each time of the day. Vertical bars depict standard error of means.

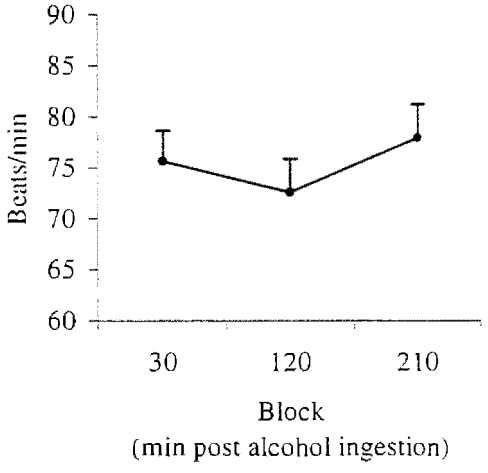


Figure 5.7. Mean heart rate across the three testing blocks. Vertical bars depict standard error of means.

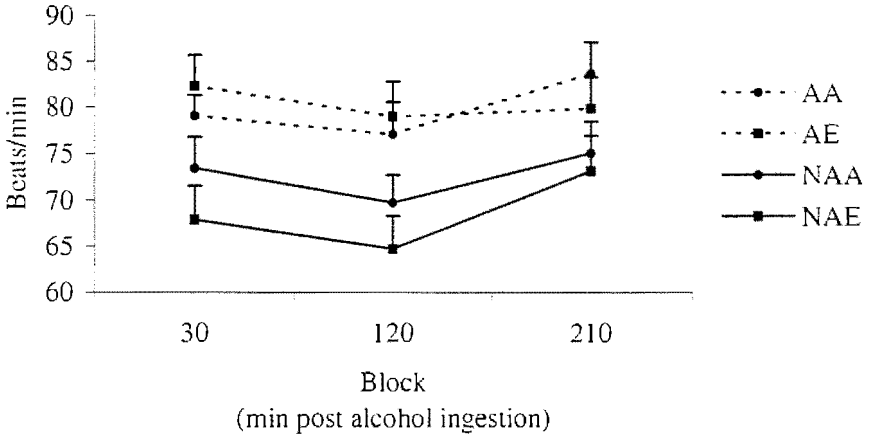


Figure 5.8. Mean heart rate across testing blocks as a function of dose and time of day.

5.10.4 Summary of physiological results

There was no significant time of day difference for the BACs achieved at 1300 hr and 1800 hr conditions. However, the interaction between time of alcohol administration and time post alcohol ingestion was significant. This interaction indicated a time of day variation in the rate absorption. The body temperature of subjects under the alcohol condition in the afternoon was significantly lower than under the no alcohol condition after alcohol ingestion in the afternoon. A decrease in body temperature was not found for the evening condition. Finally, the heart rate of the subjects under the alcohol condition was significantly higher than under the control condition. Similarly, the interaction between dose and time of day was significant. Heart rate for the no alcohol condition was higher in the afternoon

condition compared to the evening condition; however mean heart rate was the same for both of the alcohol conditions regardless of time of day.

5.11 Performance measures

The error data obtained from the auditory detection task are presented first as these data have an influence on the reaction time data.

5.11.1 Error: Auditory detection

Errors were minimal on the auditory detection task particularly under the single task condition. However, one subject had a 32% error rate in the single task condition. Under dual task conditions the mean error rate was 2% and the same subject had an error rate of 22%. While the reaction time scores of this subject were in the range of the other subjects due to the high error rates the reaction time data should be interpreted with some caution. Table 5.6 outlines the error rates (%) for each condition and the corresponding standard deviation for the auditory detection task under dual task conditions excluding the subject with the high error rate. These data were not analysed as the subject with high error would need to be excluded and therefore the counterbalanced design of this study would be compromised. As specified, the reaction time data of the subject with the high error rate were in the range of other subjects, as such the reaction time data were analysed.

Table 5.6.

Percent error and between subjects standard deviations under the dual task condition by each alcohol and time of day condition.

Condition		Block		
		30	120	210
NAA	*M*	1.4	1.8	2
	SD	(± 2.3)	(± 2.3)	(± 2.2)
NAE	*M*	1.3	0.8	1.3
	SD	(± 1.4)	(± 1.9)	(± 1.4)
AA	*M*	4.5	1.8	1.5
	SD	(± 7.9)	(± 2.2)	(± 2.3)
AE	*M*	2.5	3	1.8
	SD	(± 2.8)	(± 3.6)	(± 3.3)

5.11.2 Performance: Reaction time and time off target

Planned orthogonal polynomial contrasts with trend analyses were used to analyse the performance data. Table 5.7 outlines the means and standard deviations for the three tasks, auditory detection, pursuit rotor, and the dual task (auditory detection and pursuit rotor) for each time point and time of day.

Table 5.7

Means and between subjects standard deviations for the performance on four tasks, auditory detection and pursuit rotor alone, and the auditory detection and pursuit rotor as a dual task with the corresponding BAC (g/100ml).

			Condition					
			1300			1800		
			Block					
Task	Dose		30	120	210	30	120	210
Detection	0	*M*	267.84	289.11	294.09	292.45	293.87	306.27
		SD	29.65	37.46	40.40	50.67	51.74	53.10
	0.1	*M*	310.02	308.38	308.43	310.32	315.22	323.03
		SD	53.64	65.17	51.11	69.42	48.47	66.29
Pursuit	0	*M*	470.77	419.68	554.69	325.98	316.41	363.73
		SD	423.67	448.67	648.92	277.38	262.63	310.91
	0.1	*M*	1131.31	821.97	604.57	1090.34	801.08	815.62
		SD	1189.53	608.20	511.44	950.92	791.42	832.97
DT – Det	0	*M*	370.59	371.63	388.45	352.92	363.91	383.07
		SD	57.45	58.79	94.23	42.15	44.60	72.21
	0.1	*M*	423.68	411.08	390.55	431.00	410.30	403.92
		SD	103.52	82.88	60.19	88.28	69.23	69.41
DT – Pursuit	0	*M*	568.54	471.73	608.22	447.39	410.81	479.06
		SD	476.79	471.58	557.45	281.63	238.45	349.52
	0.1	*M*	1196.77	968.84	819.10	1185.59	890.00	658.13
		SD	1075.15	774.90	690.25	869.13	760.93	686.03
BAC			0.064	0.065	0.043	0.075	0.065	0.041

5.11.2.1 Dose

There was a statistically significant effect of alcohol on all performance tasks, auditory detection, $F(1,11) = 9.9$, $p < .01$, auditory detection under the dual condition $F(1,11) = 20.06$, $p < .01$, pursuit rotor, $F(1,11) = 12.74$, $p < .01$, and pursuit rotor under the dual condition $F(1,11) = 14.15$, $p < .01$. Performance was more impaired under the alcohol condition than the control conditions for all tasks. The alcohol effect is illustrated for the auditory detection and pursuit rotor tasks

under single task conditions in Figure 5.9 and under the dual task conditions in Figure 5.10.

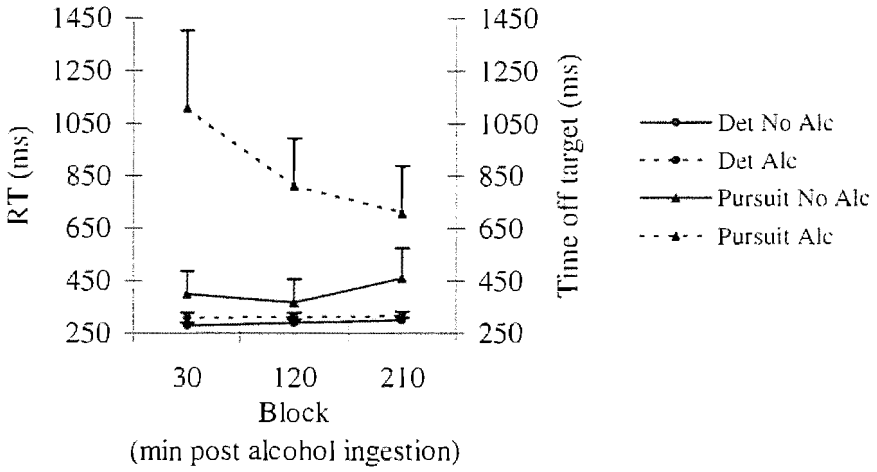


Figure 5.9. Mean reaction time (ms) and mean time off target (ms) for the performance tasks (single condition) under alcohol and no alcohol conditions across test blocks. Vertical bars depict standard error of means.

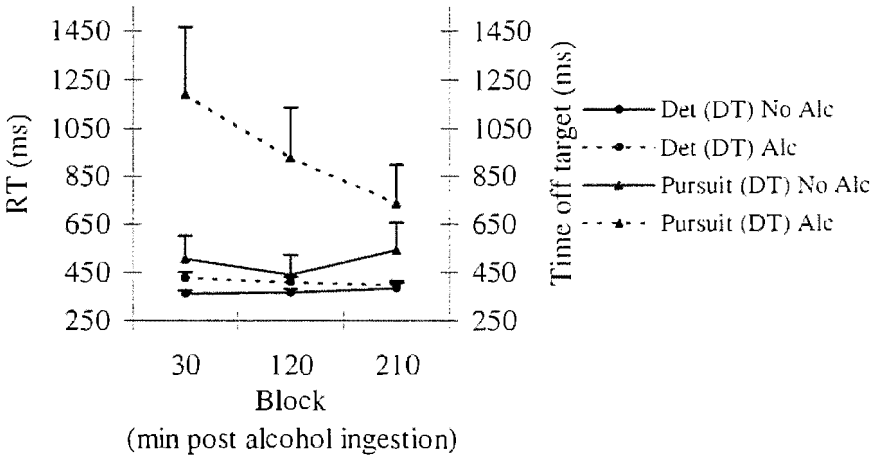


Figure 5.10. Mean reaction time (ms) and mean time off target (ms) for the performance tasks (dual condition) under alcohol and no alcohol conditions across test blocks. Vertical bars depict standard error of means.

5.11.2.2 Time of day

There was no statistically significant effect of time of day on any of the tasks (all p values > .10).

5.11.2.3 Block

There was no statistically significant effect of block for any of the performance tasks (all p values > .10).

5.11.2.4 Interactions

There were no statistically significant interactions between factors (all p values > .10) except for a significant linear interaction between dose and block for the auditory detection task under the dual condition, $F(1,11) = 8.75$, $p = .01$. Performance on the auditory detection task, dual task condition, improved across time under alcohol conditions, whereas performance declined across time under control conditions.

Given the greater error rate of subject 8, the analyses were conducted replacing the reaction time and time off target data of subject 8 with the mean score of two subjects who had been tested under the same counterbalanced conditions. The results of these analyses were comparable to those presented above.

5.11.3 Comparison of the high dose condition in Study 1 to the alcohol condition in Study 2 (block 1)

Comparisons were made between the performance results from the high dose condition in Study 1 to the alcohol condition at the 30 min block in Study 2.

Comparisons of means and standard deviations for each of the tasks are outlined in Table 5.8.

Table 5.8

Comparison of performance data for each time of day condition; the high dose in Study 1 compared to the alcohol condition, block 1, in Study 2.

	Study 1		Study 2	
	1300	1800	1300	1800
Detection (ms)	293.37	289.47	310.02	310.32
	(± 35.05)	(± 33.45)	(± 53.64)	(± 69.42)
Pursuit (s)	273.66	141.16	1131.31	1090.34
	(± 149.82)	(± 115.02)	(± 1189.53)	(± 950.92)
DT – Det (ms)	397.671	373.91	423.68	431.00
	(± 35.05)	(± 33.45)	(± 103.52)	(± 88.28)
DT-Pursuit (s)	418.70	163.40	1196.77	1185.59
	(± 293.60)	(± 101.30)	(± 1075.15)	(± 869.13)

From these data it can be seen that overall the performance of subjects in Study 1 was less impaired than that of subjects in Study 2, particularly on the pursuit rotor task. Subjects in Study 1 had slightly faster reaction time and were more accurate on the pursuit rotor task. Further, the data in Study 1 were less variable. The better performance of subjects in Study 1 could be attributed to a number of factors. Firstly, subjects 1 in study had more practice under alcohol conditions, four alcohol experimental sessions compared to two experimental sessions. Although, subjects in study 2 were required perform three repeats of the tasks within each session.

The primary differences in the samples were mainly gender proportions. Although this is an unlikely explanation for the differences in performance impairment as the TBW formula aims to control for factors such as age, gender, height and weight. Another explanation for the increased performance impairment in study 2 compared to study 1 is the change in equipment. It was noted that the computer mouse, used for tracking, was changed from a trackball mouse in study 1 to an infrared mouse in study 2. The goal of exchanging the mouse was to decrease tracking errors resulting from a "sticky" mouse. However, it is proposed that changing the mouse could have actually had the opposite effect. To overcome any resistance caused by dirt build up on the trackball of the mouse, subjects in study 1 could have finely tuned their motor movement. Alternatively, and perhaps in addition to, the infrared mouse may have actually been more sensitive to movement and therefore subjects could have actually had poorer tracking due to an increased range of movement in the mouse. Thus, the change in the computer mouse may account for the more impaired performance and the lack of time of day variation in pursuit rotor performance after alcohol ingestion in study 2.

While the change in equipment is a potential explanation for the lack of replication of the finding in study 1, it is also possible the effects under investigation are not robust.

Table 5.9

Comparison of sample characteristics and BAC results for Study 1 and Study 2.

Sample Characteristics	Study 1		Study 2	
No. of Subjects	12		12	
Males	10		4	
Females	2		8	
Age	26 (7.4)		25.1 (6.9)	
Height	178.7 (7.58)		170.1 (7.4)	
Weight	74.7 (20.38)		69.7 (10.7)	
Drinking Days/Week	2 (0.74)		2 (1)	
No. Drinks/Occasion	7 (2.31)		4.3 (2.3)	
BAC	Afternoon	Evening	Afternoon	Evening
Peak	0.0859 (0.012)	0.0789 (0.014)	.076 (0.014)	.080 (0.013)
No. of subjects peak at 0.1g/100ml	3	0	1	1

5.11.4 Summary of results for performance tasks

It was found that alcohol significantly impaired performance on all tasks. There were no significant time of day or block effects. The only interaction that was significant was the dose by block trend for performance on the auditory detection task under dual conditions. It was noted that errors were minimal on the auditory detection task under both single and dual conditions. However, one subject had extremely high error rates for the auditory detection task, single and dual conditions. While the reaction time scores of this subject were in the range of the other subjects their high error rates indicate that the reaction time data should be interpreted with some caution. Finally, the results of the high dose condition of study 1 were compared to the results of study 2, the alcohol condition, block 1. A number of

explanations were forwarded to explain the different findings between these two studies.

5.12 Biochemical measures

5.12.1 Melatonin

Table 5.10

Means and between subjects standard deviations for salivary melatonin levels (pg/ml) for each condition across blocks with the corresponding BAC (g/100ml).

		Block			
		0	30	120	210
Time of day		1200 hr	1330 hr	1530 hr	1700 hr
NAA	*M*	0.716	0.232	0.234	0.123
	SD	(± 0.873)	(± 0.405)	(± 0.432)	(± 0.278)
AA	*M*	0.945	1.524	0.283	0.135
	SD	(± 1.646)	(± 1.029)	(± 0.505)	(± 0.308)
BAC			0.064	0.065	0.043
		0	30	120	210
Time of day		1700 hr	1830 hr	2030 hr	2200 hr
NAE	*M*	0.105	0.169	0.514	2.010
	SD	(± 0.349)	(± 0.377)	(± 0.979)	(± 2.742)
AE	*M*	0.127	0.892	1.268	1.564
	SD	(± 0.421)	(± 1.113)	(± 2.368)	(± 2.556)
BAC			0.075	0.065	0.041

When the transport company delivered samples from subject 9 they appeared damaged. These data were considered invalid and were removed from the analysis (see Appendix N for their results). These data were removed from the melatonin analysis as levels deviated significantly from the other subjects.

A baseline measurement of melatonin upon arrival to the laboratory for each condition was sampled. In order to conduct a trend analysis the time intervals sampled have to be equal (Keppel, 1982). Given this, the '0' measurement was omitted from the analysis. A 2 x 2 repeated measures ANOVA was conducted on the baseline samples in order to detect any differences between baselines for each time of the day condition prior to alcohol ingestion. It was found that melatonin levels were significantly different for each time of day condition, $F(1,10) = 6.15$, $p = .03$, however, as expected, there was no significant difference prior to receiving alcohol within each time of the day condition or interaction (p values > .60).

There was a significant difference in melatonin levels for the alcohol and no alcohol conditions, $F(1,10) = 7.05$, $p = .02$, with the alcohol condition producing higher melatonin levels than the no alcohol condition. There was no effect of time of day on melatonin levels, $F(1,10) = 2.07$, $p = .18$. There was no significant linear trend for change in melatonin levels across blocks, $F(1,10) = 0.66$, $p = .44$. Similarly, the time of day by dose interaction was not significant, $F(1,10) = 0.16$, $p = .7$. The trend analysis showed that the linear dose and block interaction was significant, $F(1,10) = 12.55$, $p < .01$. It can be seen in Figure 5.11 that in the no alcohol condition melatonin increased across time. However, in the alcohol conditions, melatonin declined across time. Additionally, the linear interaction between time of day and block was also significant, $F(1,10) = 8.75$, $p = .01$. In the evening condition, melatonin increased across time, whereas in the afternoon condition, melatonin decreased across time (Figure 5.12). The three-way linear interaction was not significant, $F(1,10) = 0.15$, $p = .71$.

To explore the time of day by block interaction the analysis was separated into the two times of the day conditions. For the afternoon condition, there was a

significant effect of alcohol on melatonin, $F(1,10) = 8.92$, $p = .01$. Melatonin was significantly increased following alcohol ingestion in comparison to no alcohol. Melatonin levels showed significant linear and quadratic trends across test blocks, $F(1,10) = 36.60$, $p < .01$, $F(1,10) = 7.34$, $p = .02$. The interaction between dose and block was significant for linear and quadratic trends quadratic, $F(1,10) = 10.21$, $p = .01$, $F(1,10) = 18.28$, $p < .01$, interactions. It can be seen in Figure 5.13, that in the no alcohol condition, melatonin levels were stable across time whereas after alcohol ingestion subjects' melatonin levels decreased from 30 min post ingestion point to the melatonin levels of the no alcohol condition at 120 min and 210 min post ingestion.

Analyses of melatonin level for the evening conditions resulted in one statistically significant effect, all other contrasts had p values > 0.05. The dose by time linear interaction was significant, $F(1,10) = 5.69$, $p = .04$. While melatonin levels were higher in the alcohol condition the increase in melatonin levels across time in the no alcohol condition was steeper than the increase in melatonin across time after alcohol ingestion (Figure 5.14).

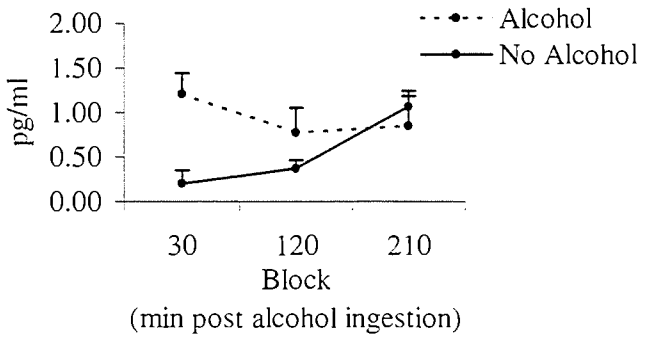


Figure 5.11. Mean melatonin levels across blocks for the alcohol and no alcohol conditions collapsed across time of day. Vertical bars depict standard error of means.

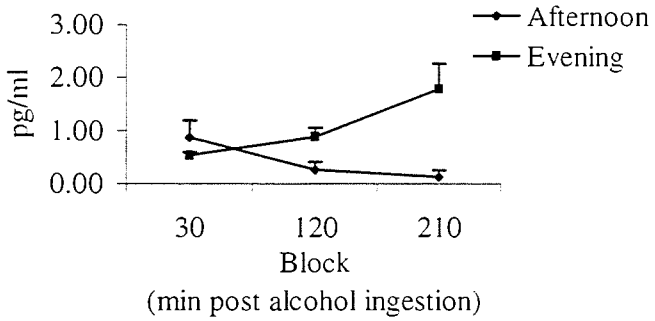


Figure 5.12. Mean melatonin levels across blocks for the afternoon and evening conditions collapsed across alcohol conditions. Vertical bars depict standard error of means.

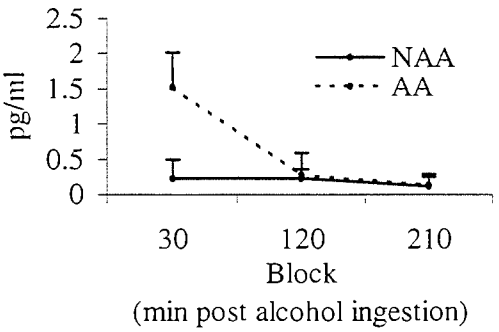


Figure 5.13. Mean melatonin levels under the afternoon alcohol and control conditions. Vertical bars depict standard error of the means.

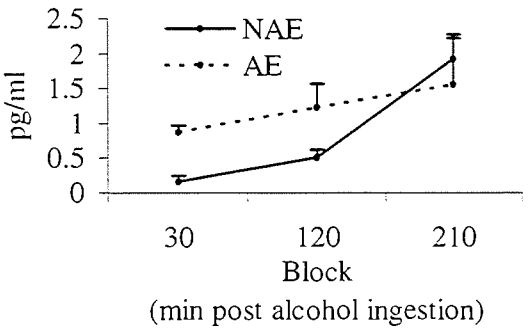


Figure 5.14. Mean melatonin levels under the evening alcohol and control conditions. Vertical bars depict standard error of the means.

5.12.2 Cortisol

Table 5.11

Means and between subjects standard deviations for salivary cortisol levels (ng/ml) for the four conditions across blocks with the corresponding BAC (g/100ml).

		Block			
		0	30	120	210
NAA	*M*	2.33	1.34	1.26	1.51
	SD	0.58	0.64	0.73	0.88
AA	*M*	3.56	1.77	1.13	1.85
	SD	(± 2.38)	(± 0.98)	(± 0.45)	(± 1.83)
BAC			0.064	0.065	0.043
NAE	*M*	1.27	0.60	0.26	0.53
	SD	(± 0.63)	(± 0.50)	(± 0.40)	(± 0.97)
AE	*M*	1.26	0.58	0.31	0.36
	SD	(± 0.72)	(± 0.76)	(± 0.41)	(± 0.51)
BAC			0.075	0.065	0.041

A baseline measurement of cortisol upon arrival to the laboratory for each condition was sampled. In order to conduct a trend analysis the time intervals sampled have to be equal (Keppel, 1982). Given this, the '0' measurement was omitted from the analysis. A 2 x 2 repeated measures ANOVA was conducted on the baseline samples in order to detect any differences between baselines for each time of the day condition prior to alcohol ingestion. It was found that cortisol levels were significantly different for each time of day condition, $F(1,10) = 18.52$, $p < .01$, however, as expected, there was no significant difference prior to receiving alcohol within each time of the day condition or interaction (p values > .05).

The cortisol data were analysed using 2 (dose) x 2 (time of day) x 3 (blocks) within subject's contrasts and trend analysis. There was no significant difference in

cortisol levels for the alcohol and no alcohol conditions, $F(1,11) = 0.25$, $p = .63$. There was, however, a significant time of day effect for cortisol levels, $F(1,11) = 44.89$, $p < .01$; cortisol levels were higher in the afternoon compared to the evening (see Figure 5.15). The cubic trend for change in cortisol levels across time was significant, $F(1,11) = 12.01$, $p < .01$. Cortisol levels appeared to decline across time and then increase at the 210 min block. Similar to the heart rate trend, a post hoc explanation for the increase at the 210 min block is that subjects may have been aroused by the anticipation of testing completion, particularly the evening condition (see Figure 5.15). The three-way quadratic interaction was significant, $F(1,11) = 12.01$, $p < .01$. This interaction indicated that while the trend in cortisol change across time under alcohol and no alcohol conditions was comparable, this was not the case in the afternoon conditions. In the afternoon, alcohol showed more of a quadratic trend across time. In comparison to the no alcohol condition cortisol levels were increased at the 30 min and 210 min block, and equal at the 120 min block (see Figure 5.17). Although, paired t-tests indicated that the increase in cortisol at 30 min and 210 min post alcohol ingestion was not statistically significant (p values > .20). None of the other interactions were significant (all p values > .10).

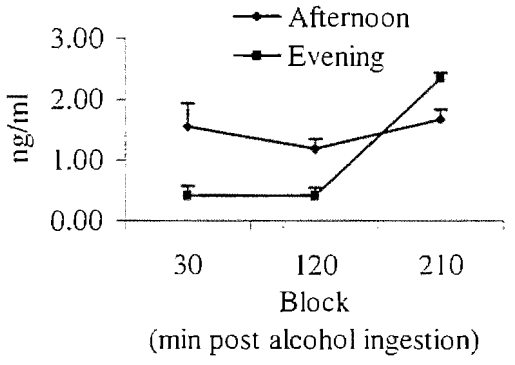


Figure 5.15. Cortisol levels across the test blocks for afternoon and evening conditions collapsed across alcohol conditions. Vertical bars depict standard error of means.

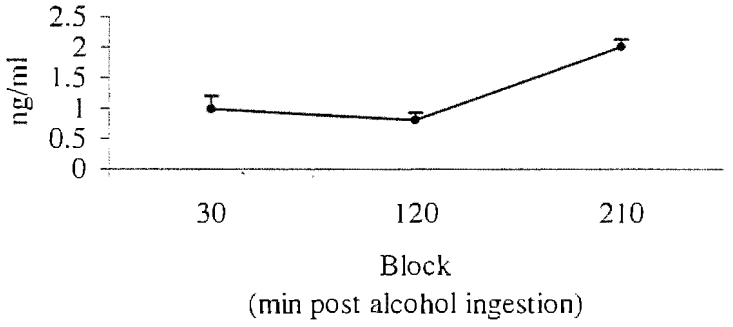


Figure 5.16. Cortisol levels across test blocks collapsed across alcohol and time of day conditions. Vertical bars depict standard error of means.

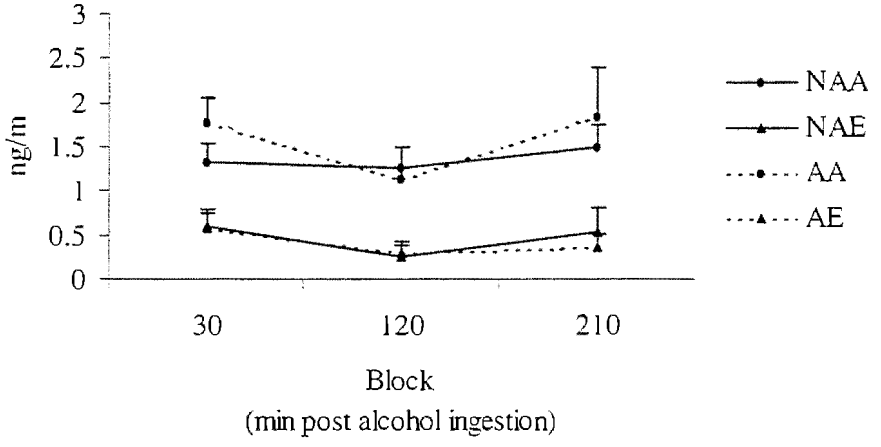


Figure 5.17. Cortisol levels across test blocks as a function of alcohol and time of day conditions. Vertical bars depict standard error of means.

5.12.3 Summary of biochemical results

Overall, there was a significant difference in melatonin levels for the alcohol and no alcohol conditions with the alcohol condition producing higher melatonin levels than the no alcohol condition. There was no effect of time of day on melatonin levels, although the interaction between time of day and block was significant.

For the cortisol results, there was no statistically significant difference between alcohol and no alcohol conditions. There was a significant difference between the two times of the day; as would be expected, cortisol levels were higher in the afternoon compared to the evening conditions. There were significant trends across blocks and the three-way interaction was significant.

5.13 Visual analogue scales

The variables that were reverse scored were recoded before data was analysed. A factor analysis was conducted on the VAS to reduce the variables to a smaller set of factors. Separate factor analyses were conducted on the data from the four conditions in order to extract the common variables (see Appendix Q). Three factors were extracted from the analyses. One was termed physical effects of alcohol, consisting of the variables nauseous, drunk, bored, and dizzy. Factor two, termed gregariousness, consisted of the variables talkative, happy, and social. Finally, the third factor, cognition, consisted of alertness and attention variables. The variables that did not consistently load onto the same factor across conditions were omitted. Mean scores for each of the VAS factors were calculated for each measurement period of the experimental conditions by averaging the scores obtained on items that comprised each of the factors.

Table 5.12

Means and between subjects standard deviations for the three factors for the four conditions across test blocks with the corresponding BAC (g/100ml).

Physical Effect					
		Blocks			
		0	30	120	210
NAA	M	1.31	1.35	1.19	1.19
	SD	(± 1.42)	(± 0.94)	(± 0.73)	(± 0.92)
AA	M	1.18	3.49	3.43	3.00
	SD	(± 1.28)	(± 1.77)	(± 2.41)	(± 1.88)
NAE	M	1.24	1.41	1.59	1.44
	SD	(± 1.16)	(± 1.21)	(± 1.28)	(± 1.21)
AE	M	0.58	3.32	3.09	2.12
	SD	(± 0.51)	(± 1.86)	(± 2.13)	(± 1.39)
Gregariousness					
		Blocks			
		0	30	120	210
NAA	M	7.31	7.57	8.21	8.28
	SD	(± 2.43)	(± 1.83)	(± 1.64)	(± 1.03)
AA	M	8.49	8.57	8.17	8.10
	SD	(± 1.22)	(± 1.57)	(± 1.76)	(± 1.93)
NAE	M	7.54	7.38	7.80	7.83
	SD	(± 2.22)	(± 2.35)	(± 2.36)	(± 2.20)
AE	M	7.79	8.43	7.86	7.50
	SD	(± 1.73)	(± 1.41)	(± 1.85)	(± 1.81)
Cognitive					
		Blocks			
		0	30	120	210
NAA	M	5.55	5.91	5.51	5.60
	SD	(± 1.20)	(± 1.24)	(± 0.77)	(± 1.06)
AA	M	5.19	5.16	5.39	6.35

	SD	(± 0.42)	(± 1.03)	(± 0.79)	(± 1.12)
NAE	M	4.98	5.11	5.23	5.68
	SD	(± 1.32)	(± 0.38)	(± 0.62)	(± 0.98)
AE	M	5.88	4.70	5.31	5.80
	SD	(± 1.48)	(± 0.94)	(± 1.08)	(± 1.15)
BAC	1300 hr		0.065	0.065	0.043
	1800 hr		0.075	0.065	0.041

Baseline measurements of subjective ratings using the VAS were made upon arrival to the laboratory for each condition. In order to conduct a trend analysis the time intervals sampled have to be equal (Keppel, 1982). Given this, the '0' measurement was omitted from the analysis. A 2 x 2 repeated measures ANOVA was conducted on the baseline subjective ratings in order to detect any differences between each time of the day condition prior to alcohol ingestion. No significant differences between conditions were noted for the physical (all p values > .10), cognitive (all p values > .09), or gregariousness factors (all p values > .10). Data from each of the factors derived from the VAS was analysed using orthogonal polynomial contrasts and trend analysis. No statistically significant effects were revealed for ratings on the gregariousness factor, (all p values > .10; see Appendix Q).

5.13.1 Dose

There was a significant difference between alcohol and no alcohol conditions on the physical factor; under alcohol conditions subjects had higher scores on the physical factor than under no alcohol conditions, $F(1,11) = 21.84$, $p < .01$. No statistically significant effects of alcohol were revealed on the cognitive factor, $F(1,11) = .09$, $p = .78$.

5.13.2 Time of day

No statistically significant effects of time of day were revealed on the physical or cognitive factors (all p values > 0.1).

5.13.3 Block

Significant changes across time were revealed for the physical factor and cognitive factors (see Figure 5.18). The linear trend for ratings on the physical factor were significant, $F(1,11) = 6.27$, $p = .03$. Ratings on the physical factor declined over time. Similarly, changes in ratings of cognitive factor across blocks were significant and showed a linear trend, $F(1,11) = 11.37$, $p < .01$. Ratings on the cognitive factor increased across time.

5.13.4 Interactions

None of the interactions were significant for any of the factors (see Appendix Q for analyses).

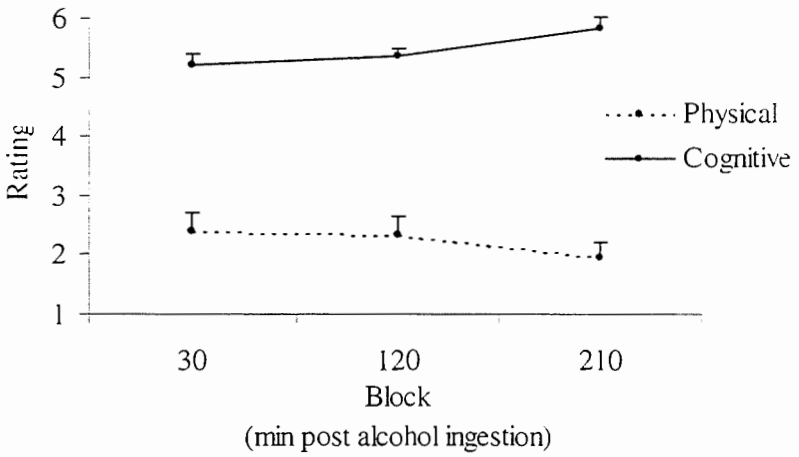


Figure 5.18. Subjective ratings of the physical and cognitive factors across blocks collapsed across alcohol and time of day condition. Vertical bars depict standard error of means.

5.13.5 Estimation of BAC

Data from the question asking subjects to estimate their BAC was not analysed as only five subjects gave responses for all blocks of the alcohol conditions on this question.

5.13.6 Summary of results

For ease of comparison, a summary of the significant effects and interactions for all of the dependent variables studied are illustrated in Table 5.13. An `X' indicates a significant result.

Table 5.13

Significant main effects and interactions found for each dependent variable investigated in Study 2.

DV	Main Effects			Interactions			
	Dose	Tod	Block	Dose x Tod	Dose x Block	Tod x Block	3 way
Performance Tasks	X				X (DT – A. Det)		
Body temperature	X (Afternoon)		X				
Heart Rate	X		X	X			X
BAC	NA		X	NA	NA	X (Absorption)	
Melatonin	X		X		X	X	
Cortisol		X	X				X
VAS	X (Physical)		X (Physical & Cognitive)				

5.13.7 Graphical summary of primary findings

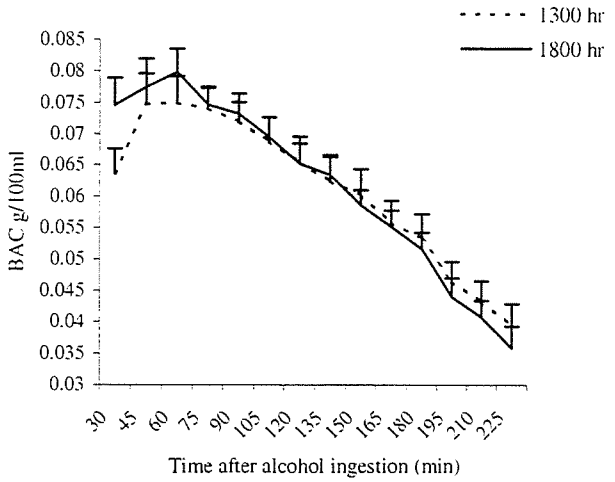


Figure 5.19. BACs across sampling time for afternoon and evening conditions.

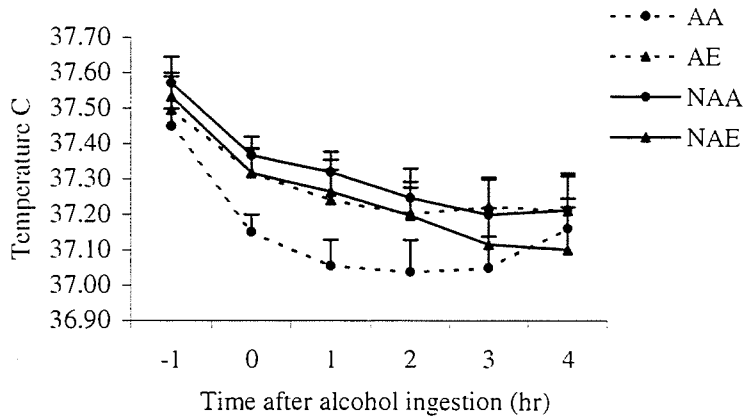


Figure 5.20. Body temperature trends across the test blocks for afternoon and evening alcohol and no alcohol conditions.

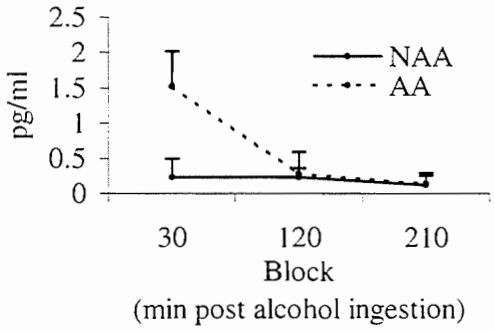


Figure 5.21. Melatonin trends across the test blocks for afternoon alcohol and no alcohol conditions.

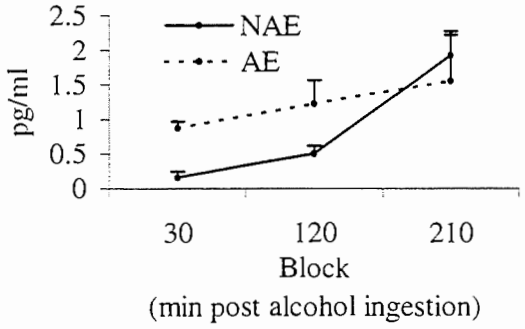


Figure 5.22. Melatonin trends across the test blocks for evening alcohol and no alcohol conditions.

Discussion

5.14.1 Alcohol effects

The general hypothesis that alcohol would influence performance, physiology, and biochemistry was supported. All of the dependent variables with the exception of cortisol revealed a significant change after alcohol ingestion. The body temperature of subjects who had consumed alcohol declined, although only when subjects consumed alcohol in the afternoon condition. In the evening condition, the body temperature of subjects did not decrease following alcohol ingestion. The body temperature of subjects receiving alcohol did not vary from that observed without alcohol until 2 hr post alcohol ingestion. At this time, body temperature in the control condition began to decline while the same subjects after consuming alcohol did not show a decline in core body temperature. These results support previous chronopharmacology of alcohol studies in animals that have found that the largest decreases in body temperature were found when animals were injected with alcohol during the dark phase (activity phase) of their diurnal cycle (Baird et al., 1998; Brick et al., 1984). Additionally, the results of the current study also support human studies, as alcohol appeared to have a more pronounced effect when it was consumed earlier in the day, 1300 hr compared to 1800 hr. Reinberg et al. (1975 cited in Reinberg 1992) reported that the 24 hr mean of oral temperature was found to be lowest compared to control temperature values when alcohol was consumed at 0700 hr compared to 1100 hr, 1900 hr, and 2300. Similarly, O'Boyle found that alcohol caused a significant decline in oral temperature at 0800 hr; in contrast, alcohol had no effect on temperature at 1600 hr. Additionally, as hypothesised, the heart rate of subjects significantly increased under alcohol conditions, supporting previous studies

that have found alcohol related heart rate increase (Bruce et al., 1999; Conrod et al., 1997; Sayette, 1993).

As would be expected, the performance of subjects was significantly poorer on all tasks after alcohol ingestion compared to when they did not receive alcohol. These results support the findings of previous studies that have found alcohol related impairment on dual tasks consisting of tracking and detection, such as Maylor et al. (1990), Miles et al. (1986), and Kerr et al. (1991). The self-reports from subjects also showed that the physical symptoms of alcohol were greater after drinking alcohol compared to when no alcohol was consumed. Other studies have also found similar results. For example, Yap et al. (1993) found that alcohol caused a significant decrease in perceived alertness, coordination, concentration, and attention. Likewise Lenné et al. (1999) found that subjective sleepiness was consistently higher under alcohol conditions compared to no alcohol conditions.

There was a significant effect of alcohol on melatonin. About the time of nocturnal melatonin onset (30 and 120 min blocks) an acute dose of alcohol appeared to increase melatonin. In the afternoon, at 30 min post alcohol ingestion, there was an increase in melatonin relative to the no alcohol condition melatonin levels. Following this, melatonin levels returned to levels of the no alcohol condition approximately two hours post alcohol ingestion. Comparatively, in the evening, alcohol also increased melatonin levels, although levels did not return to those of the no alcohol condition. This increase was sustained two hours post alcohol ingestion, however, the increase of melatonin in the no alcohol condition at 210 min was not produced to the same magnitude under alcohol conditions. Previous studies have demonstrated an alcohol related inhibition of melatonin in humans. The current study demonstrated this effect at the final block of testing (2230 hr) in the evening

condition. Röjdmark et al. (1993) demonstrated inhibition of melatonin by alcohol in humans. In comparison to the current study, repeated doses of alcohol were given and serum melatonin levels were sampled at two-hour intervals across the sleep phase. The greatest inhibition of melatonin was seen after alcohol when melatonin was around its peak (2400 hr – 0400 hr). The current study did not measure melatonin after 2230 hr, which is around the time of melatonin onset. However, the current study did show that melatonin levels after alcohol ingestion in the evening were below that of the no alcohol at the 210 min block, although this difference was not statistically significant. In summary, it appeared that alcohol increased melatonin levels prior to melatonin onset, that is, when melatonin levels were low.

In contrast, to the other dependent variables, alcohol did not affect cortisol levels. Reinberg (1992) also reported that alcohol did not have a significant effect on cortisol at 1100 hr or 1900 hr, however at 90 min post alcohol ingestion, a decrease in plasma cortisol was found at 0700 hr and an increase was found at 2300 hr. At these times baseline cortisol was at its highest and lowest levels, respectively. In contrast, Yap et al. (1993) found equal decreases in cortisol levels after a similar dose of alcohol at all four times of the day sampled, 0900 hr, 1500 hr, 2100 hr, and 0300 hr. Comparisons between studies showed varying results for the effect of alcohol on cortisol at different times of the day. Given the conflicting findings between these studies, further investigations are necessary that include varying doses of alcohol administered at more times with multiple sampling after alcohol ingestion in order to elucidate time of day variations in alcohol related changes in the cortisol response.

5.14.2 Time of day: Main effects and interactions

In the current study there were four dependent variables that had significant findings that involved time of day. The dependent variable that revealed a main effect of time of day was cortisol. Subjects had significantly higher cortisol levels in the afternoon compared to the evening irrespective of alcohol ingestion. These data support previous circadian rhythm research, such as, Yap et al. (1993) who found time of day effects for cortisol prior to the administration of alcohol. Yap found that the highest cortisol levels occurred at 0900 hr and the lowest levels at 2100 hr (the actual peak probably occurred earlier than 0900 hr however samples were not collected prior to 0900 hr).

No other main effects of time of day were found for body temperature, heart rate, performance, melatonin, or BAC. However, time of day did interact with both dose and block, independently, and as a 3-way interaction on a number of variables.

While no main effect of time of day was observed for subjects' BACs, a significant time of day by time post alcohol ingestion interaction was seen during alcohol absorption (measured as the first four readings up to 75 min post alcohol ingestion). On a review of the subject BAC experimental record forms it was noticed that subjects 10, 11, and 12, had eaten more food than the snack provided for the fasting periods. Subjects 10 and 11 had eaten more food prior to the afternoon session and subject 12 had eaten more food prior to the evening session. Inspection of their BAC curves revealed the classic flattening of the BAC curve caused by food in the digestive tract. Prior to the other experimental sessions, when these subjects conformed strictly to the fasting requirements, their BAC showed the same pattern of the other subjects (see Appendix O). Additionally, in two other cases, the first BAC reading in the afternoon condition appeared to be underestimated as the following

readings were similar to the 1800 hr reading. Thus, it is possible that the slower absorption seen in the afternoon could be influenced by these extraneous variables. It should be noted that while the meals (toasted sandwich for the 1300 hr condition and pasta for the 1800 hr condition) provided differed for the two times of alcohol administration conditions, assessment of the BAC curves after the meals were ingested revealed that the difference in food consumed did not impact differentially on the elimination rates of alcohol at the two times of day.

The significant interaction noted is consistent with the findings of other studies that have shown time of day variations in the pharmacokinetics of alcohol, although others have seen faster alcohol absorption earlier in day. For example, Lakatua et al. (1984) reported a faster alcohol absorption rate at 1000 hr compared to 2200 hr. Similarly, minimal elimination rates of alcohol disappearance have been reported to occur between 1200 hr and 2000 hr (Jones, 1974; Sturtevant et al., 1978). In the same way, Minors and Waterhouse (1980) demonstrated a significant circadian variation in the rate of alcohol decline, with the acrophase occurring at 0501 hr. Other studies have also reported time of day differences for mean BACs (Lenné et al., 1999; Yap et al., 1993). Lenné et al. (1999) found that peak BAC, estimated through breath analysis, was higher at 1200 hr and 1800 hr compared to 2300 hr. Similarly, Yap et al. (1993) found that peak BAC, measured using plasma, was highest at 0900 hr compared to 1500 hr, 1700 hr, and 0300 hr. There were no significant differences between the peak BACs at the other times of the day.

Additionally, a significant time of day by dose interaction was found for heart rate. Heart rate in the no alcohol condition was higher in the afternoon than in the evening (indicative of a time of day variation); however heart rate was the same after alcohol regardless of time of day. This pattern of results indicated that alcohol

increased heart rate more in the evening than in the afternoon. One explanation of this result is that given heart rate is lower in the evening; the sensitivity to alcohol's tachycardic effect may be heightened. This result supports the theory forwarded that maximum sensitivity to alcohol occurs when basal levels of the variables under study are at their lowest (Brick et al., 1984). In contrast, the only chronopharmacology of alcohol study to include heart rate as a measure (Yap et al. 1993) did not find a significant effect of alcohol on heart rate or a time of day variation in heart rate post alcohol ingestion. Similarly, the three-way interaction between dose, time of day, and block was significant. At both 1300 hr and 1800 hr alcohol ingestion lead to an increase in heart rate, although, heart rate was lower at 1800 hr than at 1300 hr when alcohol had not been previously ingested. At 1300 hr under both the no alcohol and alcohol condition heart rate declined from 30 min to 120 min and increased at 210 min, to be higher than the 30 min mean. However, at 1800 hr under the alcohol condition, heart rate declined across time, while the no alcohol condition had the same block pattern as the 1300 hr time of day.

A significant time of day by block interaction was noted for melatonin levels. In the evening condition, melatonin increased across measurement period, whereas in the afternoon condition, melatonin slightly decreased in the range of (0 – 1 pg/ml). This pattern is typical of the circadian rhythm of melatonin (Arendt, 1992). Average profiles indicate that daytime levels tend to be around <10 pg/ml[15]. The evening rise is initiated around 2100 hr, with maximum values occurring usually from 0100 hr - 0500 hr, declining to daytime levels by around 1000 hr (Arendt, 1992).

Time of day-mediated effects on performance was not found. Performance on the behavioural tasks was similar at both times of the day under alcohol and no

[15] Reports of daytime plasma levels can be a reflection of assay sensitivity.

alcohol conditions. Thus, there were no time of day effect on performance without alcohol and no differential impairment of performance by alcohol at 1300 hr. While this research was conducted under well controlled experimental conditions replication of the finding in study 1 was not achieved. The time of day variation in performance could have been a spurious result, as the effect was decreased with the additional training with alcohol. Alternatively, the effect may not have been seen in study 2 due to the change in computer mouse. The most likely explanation, however, is that alcohol related time of day differences in performance are not robust effects.

Similarly, findings from this study did not show time of day effects for the factors derived from the VAS. This supports the results found in study 1 that also failed to find time of day-mediated effects for the ratings made on the SSS. Comparatively, both Horne and Baumer (1991) and Horne and Gibbons (1991) found time of day and effects for sleepiness using the SSS. In study 1, it was suggested that methodological differences between study 1 and those of Horne and Baumer (1991) and Horne and Gibbons (1991) might account for the contrasting findings. In study 2 subjective states using a VAS were measured across the descending limb of the BAC curve, however time of day effects were not apparent. While it is possible that time of day effects are not apparent at these times, other studies have noted time of day effects for similar variables. For example, Lenné et al (1999) noted that ratings of inebriety were higher at 1200 hr and 1800 hr compared to 2300 hr, however Reinberg (1992) noted that subjective inebriety was highest at 2300 hr. Lenné et al (1999) also found significant time of day differences in ratings of sleepiness. Regardless of alcohol, subjects felt sleepier at 2300 hr than at 1200 hr and 1800 hr and at 1200 hr than at 1800 hr. Motivation ratings also showed time of day variability, regardless of alcohol, subjects felt more motivated at 1800 hr and

1200 hr than at 2300 hr. However, ratings of alertness did not vary between times of the day. Yap et al. (1993) found significant time of day differences for the ratings of alertness. Alcohol caused a significant decrease in alertness that was more pronounced at 1500 hr and 2100 hr. It appears that there are inconsistencies in the findings of time of day-mediated effects on subjective states. This could potentially be due to the different VAS used to measure these states. It is possible that these scales are not all measuring the same construct. Further, the times of the day and measurement points also differ between studies, which could also be contributing to the variability in findings.

5.14.3 Trends across time

All dependent variables, with the exception of those used in the performance tasks, showed significant changes across time regardless of alcohol or time of day. As hypothesised, BAC initially increased to a peak level at 60 min post alcohol ingestion. This peak was maintained for some 30 min, with decline in BAC over the remaining 225 min sampling period. This trend in BAC is usual of subjects who ingest an alcohol dosage to achieve a target BAC of 0.10 g/100ml (Reinberg, 1992; Yap et al., 1993). Core body temperature essentially decreased across time. In contrast, cortisol increased across time, and heart rate decreased from 30 min to 120 min and then increased at the 210 min block. A potential explanation for the increase in these measures at 210 min is that subjects may have became aroused due to an anticipation of the completion of testing sessions. Melatonin decreased from 30 min to 120 min and then increased at 210 min. The reports from subjects of the subjective effects also showed significant trends across time; ratings for the physical factor decreased across time and ratings on the cognitive factor increased across

time. These trends are consistent with previous reports. Lenné et al. (1999) found that subjects reported inebriety to be higher 40 min post alcohol ingestion compared to 20 min and 80 min post alcohol ingestion, and higher at 60 min than 20 min post alcohol ingestion.

These trends across time, regardless of alcohol, do not speak directly to the hypotheses forwarded. The interactions between dose and block are of more relevance. Dose by block interactions were found on performance (auditory detection, dual task condition) and melatonin.

It was hypothesised that under alcohol and no alcohol conditions performance on computerised tasks would show similar trends, that is, an improvement across blocks. While this was the case for three of the four performance tasks, there was a significant interaction between dose and block on RT for the auditory detection task under dual conditions. Performance improved across time under the alcohol condition and declined across time under the no alcohol condition. The impaired performance of subjects under the no alcohol condition was most likely due to boredom or fatigue resulting from the repeated testing procedure. In contrast, subjects who had consumed alcohol may have acquired an acute tolerance to the effects of alcohol, thus resulting in improved performance across time (Goldstein, 1983). Alternatively, the subjects could have tried harder to compensate for the impairing effects of alcohol. While it would be expected that both groups would experience boredom and/or fatigue, the subjects who ingested alcohol may have been less bored or fatigued due to the potentially arousing effect of alcohol.

Similarly, the interaction between dose and block was significant for melatonin levels; regardless of the time of day melatonin was collected. In the no alcohol condition melatonin increased across time, whereas, in the alcohol

conditions, melatonin decreased across time. Comparisons to other studies cannot be made, as it is believed that, at this time, no data on the effect of alcohol on daytime levels of melatonin have been published. It is also noted that when the analysis was restricted to each time of day condition, the dose by block interaction was significant under both time of day conditions.

5.14.4 Conclusions

In conclusion, alcohol led to a decrease in performance, and significantly affected all the physiological measures, with the exception of cortisol. Similarly, alcohol condition interacted with time since alcohol ingestion on the dependent variables of performance and melatonin. There were several findings involving the time of day alcohol was ingested. Cortisol was the only measure to show a main effect for time of day; afternoon cortisol levels were higher than evening cortisol levels. Likewise, heart rate, BAC, and melatonin were the only dependent variables to show interactions involving time of day.

In contrast to previous studies such as Jones (1974), Horne and Baumer (1991), and Horne and Gibbons (1991) this study has demonstrated that alcohol did not have time of day dependent effects on performance tasks or the subjective states as measured by the VAS. In contrast, time of day dependent alcohol effects were seen on the measures of heart rate, body temperature and melatonin. Additionally, the time of day by hours post ingestion interaction was significant for the absorption of alcohol. The time of day dependent effect of alcohol on heart rate showed that heart rate increased more when alcohol was consumed in the evening compared to the afternoon. In reference to body temperature, when alcohol was consumed during the active phase it had the effect of decreasing temperature, in contrast, alcohol

appeared to increase temperature when it was consumed in the evening, although this finding was not statistically significant. In the afternoon, melatonin levels increased after alcohol ingestion, relative to the no alcohol condition, at 2 hr post ingestion melatonin levels returned to those of the no alcohol condition. In contrast, in the evening, melatonin increased until 120 min then fell below that of the no alcohol condition at 210 min. In the evening melatonin levels of the no alcohol increased across time, which would be expected given the onset of melatonin.

Given the time of day dependent physiological responses to alcohol, there does appear to be support for a chronopharmacological explanation for the response of subjects to alcohol. According to chronopharmacological principles the time dependent efficiency of a drug may be explained either by temporal variations in the mode of action of this drug (i.e., chronesthesy or chronopharmacodynamics) or variations in its pharmacokinetics (i.e., chronokinetics) (Bruguerolle, 1992; Reinberg, 1990a). In this study time of day dependent effects were noted for the absorption of alcohol. It is possible that the time of day influence of alcohol on other measures, such as body temperature and melatonin could be due to differences in the pharmacokinetics of alcohol. However, it is also possible that the time related changes in alcohol's effects are linked to a temporal responsiveness of target tissues, chronesthesy, rather than alcohol's chronokinetics (Bruguerolle, 1992; Reinberg, 1990a).

The literature on the acute effects of alcohol on performance and physiology is vast. In contrast, the data on the residual effects of alcohol or hangover on these measures is minimal and the few studies conducted have produced inconsistent results. Even less research has been conducted on the mechanisms of an alcohol hangover. Circadian rhythm disruption has been argued to be a salient factor in

explaining the mechanisms of a hangover (Baird et al., 1998). As the current study and other studies have shown, large acute doses of alcohol disrupt the circadian rhythm in a number of systems (sleep, thermoregulation). It is possible that alcohol hangover effects are due to sleep disturbances produced by alcohol rather than alcohol per se. These ideas will be explored in study 3, which examines the delayed effects of alcohol on body temperature and subjective states at the two administration times.

Chapter 6: Study 3 Delayed effects of alcohol at two times of the day on body temperature and subjective states

R-E-M-O-R-S-E!

Those dry martinis did the work for me: Last night at twelve I felt immense. Today I feel like thirty cents. My eyes blurred, my coppers hot, I'll try to eat, but I cannot. It's no time for mirth and laughter, the cold grey dawn of the morning after.

(George Ade, The Sultan of Sulu, 1903, cited in Wiese, Shlipak, & Browner, 2000, p.2).

6.1 Introduction

After alcohol ingestion, two actions occur in a chronological order. One is the initial drug effect followed by a compensatory response (Gauvin, Cheng, & Holloway, 1993). The symptoms of an alcohol hangover appear to peak at the time BACs approach zero and may continue for up to 24 hr (Gauvin et al., 1993). The symptoms of an alcohol hangover such as, headache, fatigue, thirst, and muscle aches are known, however, the mechanisms of the hangover are not very well understood (Swift & Davidson, 1998).

6.1.1 Conceptualisations of the alcohol hangover

According to Baird et al. (1998) and Gauvin et al. (1993) the literature on alcohol hangover conceptualises it as a toxic reaction to alcohol congeners or metabolites, physiological rebound from the initial drug effect, or a disruption to circadian rhythms. Evidence for each of these theories exists, although they may not be mutually exclusive in generating the hangover state (Baird et al., 1998; Gauvin et al., 1993).

6.1.1.1 Toxic reaction

Alcohol may directly contribute to a hangover via a number of processes (Swift & Davidson, 1998). Urinary output increases, as alcohol is a diuretic. Alcohol induces urine production by inhibiting the release of vasopressin from the pituitary gland (antidiuretic hormone). Further, the reduced levels of vasopressin prevent the kidneys from reabsorbing water and thereby increase urine production. Sweating, vomiting, and diarrhoea can also occur, adding to the fluid loss and electrolyte imbalances. Symptoms of dehydration include thirst, weakness, and dizziness, all of which can occur during hangover (Swift & Davidson, 1998). The abdominal pain, nausea and vomiting occasionally experienced after sufficient doses of alcohol can be attributed to the fact that alcohol is a gastric irritant. Alcohol directly irritates the stomach and intestines, causing inflammation of the stomach lining and delayed stomach emptying, especially when drinks with high alcohol content are consumed. Alcohol increases the production of gastric acid as well as pancreatic and intestinal secretion (Calder, 1997; Swift & Davidson, 1998). It has also been suggested (Calder, 1997; Swift & Davidson, 1998) that the accumulation of acetaldehyde (which is quite toxic even in small quantities) contributes to the hangover. Moreover, several alterations in the metabolic state of the liver and other organs in response to the presence of alcohol in the body can result in low blood sugar levels. The fatigue experienced during a hangover may result from low blood sugar level (Calder, 1997; Swift & Davidson, 1998). Finally, it is now believed that congeners can contribute to an alcoholic drink's intoxication effects and to the subsequent hangover. Drinks such as gin and vodka induce fewer hangovers than do beverages containing more congeners such as whiskey, brandy, and red wine (Swift & Davidson, 1998).

6.1.1.2 Rebound hyperthermia or circadian rhythm desynchrony

When the rectal temperature from animals following alcohol injection has been collected over an extended period of time, dose dependent hyperthermic disturbances are evident after the elimination of alcohol (Gallaher & Egner, 1987). This phenomenon has been termed by some as rebound hyperthermia (Gallaher & Egner, 1987). Rebound hyperthermia can be interpreted as a homeostatic phenomenon restricted to the temperature system or it could also be regarded as circadian rhythm disruption. Applying the homeostatic hypothesis to thermoregulation, it would be expected that acute alcohol induced hypothermia would initiate mechanisms to conserve or produce heat, reversing the acute drug effect and removal of the drug and the hypothermic influence would alter the balance of the temperature system to produce a hyperthermic state (Gallaher & Egner, 1987). An alternative theory to explain rebound hyperthermia is that alcohol produces circadian dysrhythmia with phase shifts/rhythm disruption, and the hyperthermia is not a response but rather an effect that is due to the absence of the normal circadian temperature trough (Holloway, Miller, King, & Bedingfield, 1993). For example, Sinclair and Gustafsson (1987) investigated behavioural changes in rats after a single injection of 2.5 g/kg alcohol or saline. At 20 - 24 hr after the injection, rats running wheel activity significantly increased. Further, 24 hr after alcohol there was an increase in body temperature (hyperthermia) and vocalization (measured as the number of audible vocalizations made during handling and temperature measurement) and a decrease in ambulation in a dark quiet open field. Each of these after effects was reduced or extinguished in rats with access to the running wheel, suggesting vigorous exercise may reduce hangover. It was proposed that the

increased running could be due to an alteration in circadian rhythms, increased sensitivity or reactivity to stimulation on the day after alcohol, or a compensatory reaction to the original drug effect (hypoactivity).

Similarly, Gallaher and Egner (1987) demonstrated rebound hyperthermia in rats. They observed a period of rebound hyperthermia beginning around the time of complete alcohol elimination and persisting for several days, after acute doses of alcohol ranging from 2 – 6 g/kg. The phenomenon generally occurred when baseline body temperatures were low. In addition to this, Gauvin et al. (1997) noted support for the circadian desynchrony theory of a hangover by observing that rats trained in a discrimination task, trained to discriminate hangover from normal homeostasis, completely generalized a phase advance of 8 hr to that hangover-training cue. Further evidence for the desynchrony theory of a hangover has been demonstrated by the persistent alterations found in both activity and body temperature rhythms lasting for at least 48 hr post injection, displaying a temporal pattern compatible with the time course of an alcohol hangover (Baird et al., 1998; Holloway et al., 1993).

Although alcohol can advance sleep onset the fatigue experienced during hangover could be the result of sleep disruption caused by alcohol (Swift & Davidson, 1998). In support of this claim, Roehrs, Yoon, and Roth (1991) found that a 0.80 g/kg dose of alcohol disturbed sleep in the second half of the night in healthy humans. Similarly, in their study of three human subjects who consumed alcohol before sleep, Mullin et al. (1933) found that rectal temperature dropped to a lower temperature than control during the first three hours then steadily increased for the last five hours of sleep intersecting and remaining significantly elevated above the mean control temperature for the final four and a half hours. Following the report of Mullin et al. (1933) there have been very few controlled investigations on the effect

of alcohol on temperature during the sleep phase, despite other human studies of the time dependent effects of alcohol (Reinberg, 1992; Yap et al., 1993). Eastman and Stewart (1994) investigated whether the results reported by Mullin et al. (1933) would generalize to women and other subjects. Three subjects, two women and one man, recorded their rectal temperature while carrying out their normal activities. On three nights the subjects drank alcohol (2 hr – 6 hr prior to sleep) and on other evenings (4 – 6 nights/subject) no alcohol was consumed. The type and amount of alcoholic beverage taken varied between and within subjects. In all three subjects, temperature was higher after alcohol consumption throughout the sleep phase. Although in subject 3, alcohol consumption resulted in a consistent decline in temperature prior to sleep onset and then showed an increase in temperature during sleep. Eastman and Stewart (1994, p. 142) concluded, "Alcohol consumption before sleep may change the rhythm waveform and create a masking factor that may obscure the phase oscillator controlling body temperature".

More recently, using a tightly controlled design, Danel et al. (2001) found that the core body temperature of subjects who had consumed alcohol was significantly higher at night. They used a repeated dosing method to maintain BACs at approximately 0.05 g/100ml throughout the session. Masking effects were controlled for as lights were controlled, subjects remained in bed, ambient temperature was maintained, and meals were also standardised. The mean lowest temperature was 0.36 °C higher in the alcohol session compared to the control session with seven of the nine subjects showing a hyperthermic effect of alcohol at night. Danel et al. (2001) suggested also that the effect of alcohol on core body temperature ultimately reduces the amplitude of the rhythm.

In summary, it has been shown that, in most cases, rebound hyperthermia is observed as alcohol is eliminated from the body. This is often described as a response by the body to counter hypothermia initially produced by alcohol. However, some researchers have challenged this theory, stating that the hyperthermia may not be a homeostatic rebound effect at all, but rather an effect that results from a dampening of the normal circadian temperature trough.

6.2 Aims and hypotheses

Study 2 aimed to investigate the effects of alcohol on performance, biochemical, and physiological variables, and subjectives states across 4 hr of the blood alcohol curve in an attempt to explore the role of circadian rhythms in time of day dependent alcohol-related changes. The current study, Study 3, was the second component of Study 2 and investigated delayed effects (5 hr – 14 hr post ingestion) of alcohol ingestion at two times of the day. The aim was to track changes in core body temperature throughout the dark phase to the following morning after alcohol ingestion. It was hypothesized that there would be significant differences in trends across time in core body temperature under alcohol and no alcohol conditions. Self-reports of subjective states at 0900 hr were also examined. It was hypothesized that ratings of subjective states would be significantly different after having consumed alcohol previously.

Method

6.3.1 Ethics approval

The James Cook University Human Ethics Committee approved the study (Ethics Approval H927).

6.4 Subjects

Given that this study is the second phase of the data collection of Study 2, the same subjects were studied.

6.5 Procedure

The procedure for the study followed on from the procedure of the acute effects of alcohol at two times of the day. Upon leaving the laboratory after the experimental session, subjects were told to stay at home, relax, and go to bed as normal. They were given a visual analogue scale to be completed at 0900 hr, before food, the following morning. While it would have been ideal to collect plasma samples across the sleep phase for determination of melatonin levels, this type of method was beyond the resources available.

Study 2 required subjects to log rectal temperature from one hour before they ingested alcohol until 0900 hr the following morning. This corresponded to 21 hr logging time for the afternoon conditions and 16 hr logging time for the evening conditions. This time frame allowed the measurement of rectal temperature prior to alcohol ingestion, across the blood alcohol concentration curve and during the sleep phase. The data of interest for this study was that collected 5 - 14 hr post ingestion. This time corresponded to 1830 hr – 0330 hr for the afternoon condition and 2330 hr – 0830 hr for the evening condition. The rectal temperature means and standard deviations for the four conditions across the 10 hr of logging are presented in Table 6.1.

Results

Core body temperature

Table 6.1

Means and between subjects standard deviations for rectal temperature (°C), 5 – 14 hr.

	Condition			
	AA	NAA	AE	NAE
M	37.06	37.04	36.81	36.69
SD	(± 0.44)	(± 0.55)	(± 0.38)	(± 0.41)

The results from the logging of core body temperature 5 - 14 hr post ingestion of alcohol are illustrated in Figure 6.1. Data were compressed into one-hour means (60 data points) and analysed using a 2 (dose) x 2 (time of day) x 10 (time after alcohol ingestion) repeated measures planned contrasts and trend analysis. Due to an incorrect logging procedure and data logger failure, the results of two subjects were excluded from the analysis. Missing values for other subjects were replaced by interpolation between nearest adjacent points.

There was a significant effect of time of day, $F(1,9) = 34.21$, $p < .01$. As expected, body temperature in the afternoon condition was higher than body temperature in the evening condition. However, there was no significant effect of alcohol, $F(1,9) = 0.76$, $p = .41$. There were significant linear and cubic trends across time, $F(1,9) = 72.46$, $p < .01$; $F(1,9) = 8.15$, $p = .02$. There was no significant interaction between time of day and dose, $F(1,9) = .44$, $p = .52$, nor were there any dose and hours post ingestion linear or quadratic trends (all p values >= .10). The interaction between time of day and hours post alcohol ingestion was significant showing linear, quadratic, and cubic interaction trends, respectively, $F(1,9) = 13.24$,

$p < .01$; $F(1,9) = 33.94$; $p = .01$; $F(1,9) = 5.12$, $p = .05$. In the afternoon, body temperature was stable from 5 - 10 hr post ingestion time and steadily declined until 14 hr post ingestion time. Under the evening conditions, body temperature declined from 5 - 10 hr post ingestion time and began to increase up until 14 hr post ingestion time. Contrasts restricted to temperature data collected in the afternoon conditions showed a significant linear dose by hours after alcohol ingestion interaction, $F(1,9) = 8.48$, $p = .02$. Under the no alcohol condition, body temperature declined across time, while under the alcohol condition body temperature also declined but not to the same extent as the control condition.

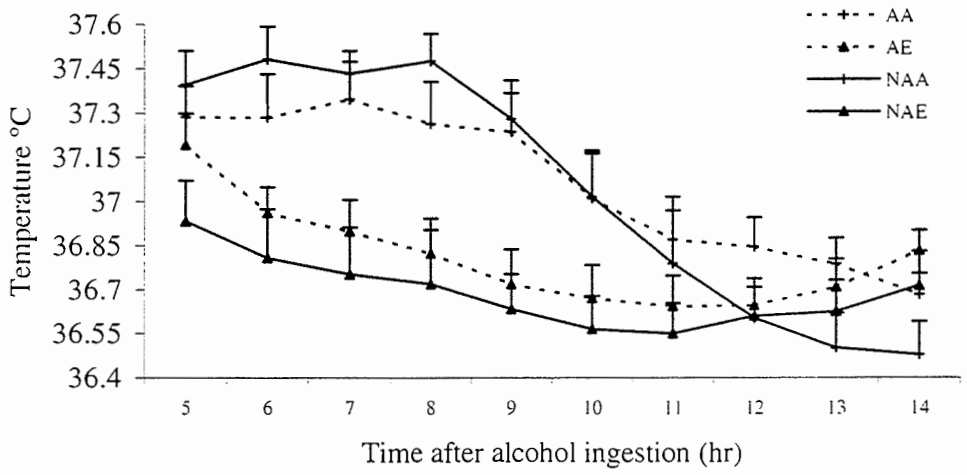


Figure 6.1. Mean core body temperature for the four conditions across time (5 hr – 14 hr). Vertical bars depict standard error of means.

Planned contrasts and trend analysis were conducted on the data from the solar time of 2330 hr to 0830 hr to determine whether previous alcohol ingestion affected core body temperature across the sleep phase (Figure 6.2). There was a statistically significant effect of alcohol on core body temperature, $F(1,9) = 6.46$, $p =$

.03. It was found that previous ingestion of alcohol significantly increased core body temperature across the sleep phase regardless of the time of day it was ingested (see Figure 6.3). The effect of time of day was not significant, $F(1,9) = 1.61$, $p = .24$. However, the effect of time was significant, showing linear and quadratic trends in body temperature change across time, $F(1,9) = 7.13$, $p = .03$; $F(1,9) = 31.27$, $p < .01$, respectively. None of the interactions were statistically significant (see Appendix Q).

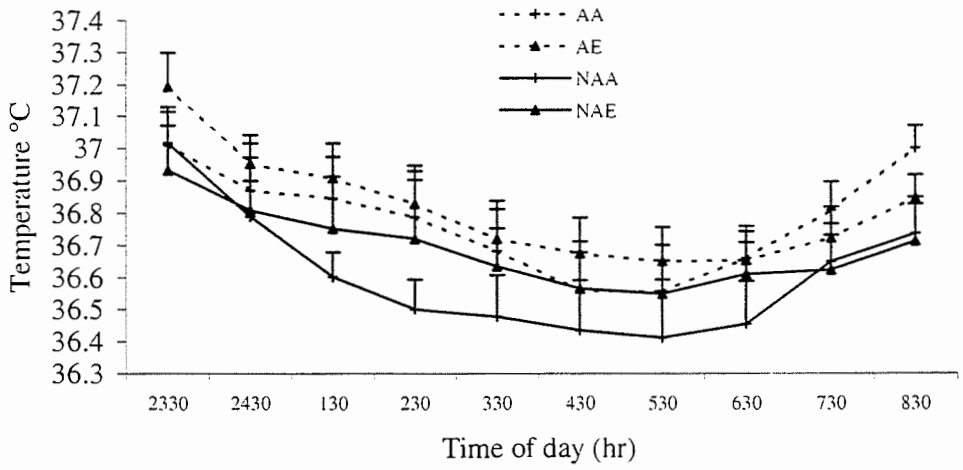


Figure 6.2. Mean core body temperature across the sleep phase for the four conditions with corresponding standard error.

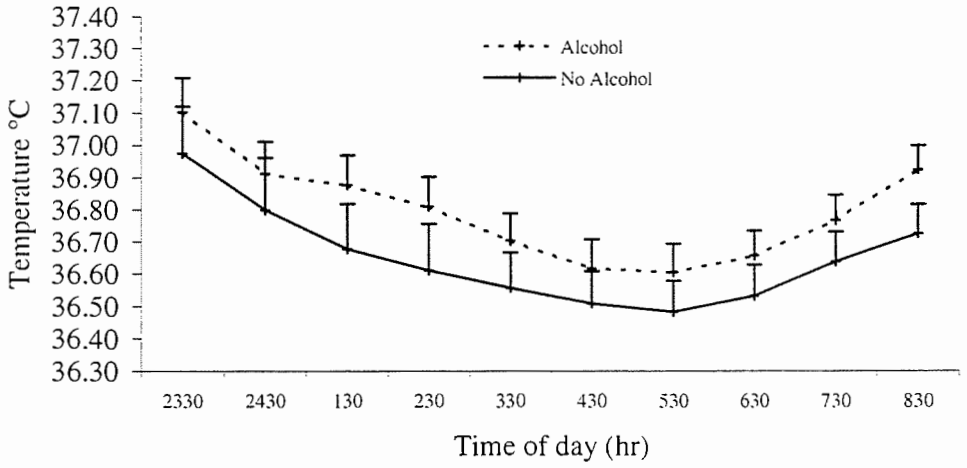


Figure 6.3. Mean core body temperature across the sleep phase for the alcohol and no alcohol conditions collapsed across time of day condition.

6.6.4 Visual Analogue Scale

Table 6.2

Means and between subjects standard deviations for the three factors derived from the VAS.

		Condition			
Factor		NAA	NAE	AA	AE
Physical	*M*	8.73	8.49	7.43	7.91
	SD	(± 1.05)	(± 1.33)	(± 1.76)	(± 1.35)
Gregariousness	*M*	7.66	7.72	8.14	7.50
	SD	(± 1.91)	(± 2.25)	(± 1.84)	(± 1.82)
Cognitive	*M*	5.61	5.35	5.57	5.41
	SD	(± 1.00)	(± 0.85)	(± 0.84)	(± 1.04)

ANOVAs, 2 (dose) x 2 (time of day), were conducted on each of factors derived from the VAS. There were only two significant findings from these analyses. The first was a significant effect of alcohol on ratings of the physical

factor $F(1,11) = 6.01$, $p = .03$. As would be expected, ratings were higher after previously consuming alcohol. There was no effect of alcohol on ratings of the cognitive or gregariousness factors, although there was an alcohol by time of day interaction on ratings of the gregariousness factor, $F(1,11) = 8.69$, $p = .01$. This effect is illustrated in Figure 6.4. The gregariousness factor consisted of the variables talkative, happy, and social. The decrease in ratings on the gregariousness factor at 0900 hr, after having consumed alcohol in the evening was greater in comparison to the afternoon alcohol condition. When no alcohol was consumed, scores for gregariousness, at 0900 hr, were slightly increased after attending an evening session compared to an afternoon session.

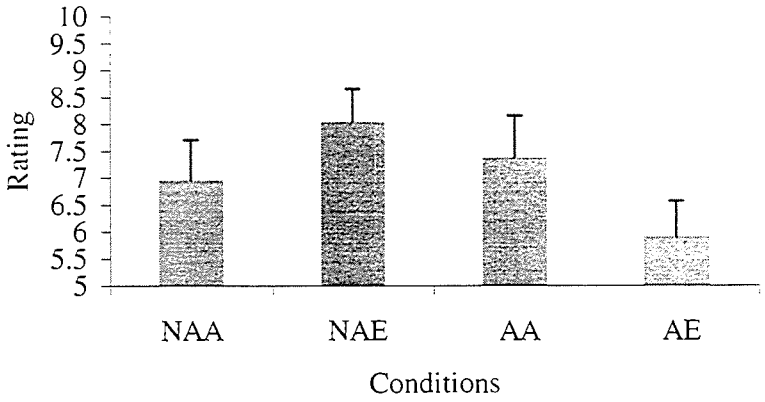


Figure 6.4. Mean scores for the gregariousness factor for each condition at 0900 hr. Vertical bars depict standard error of means.

Discussion

6.7.1 Delayed effects of alcohol on body temperature

The study presented data on the delayed effects of alcohol acutely administered at two times of the day, 1300 hr and 1800 hr, in humans. Alcohol

significantly impacted on core body temperature and self-reports of the physical effects of alcohol and gregariousness ratings 14 hr post alcohol ingestion. It should be pointed out that the measurement period, 5 hr - 14 hr post alcohol ingestion, corresponded to a solar time period of 1830 hr – 0330 hr for the afternoon condition and 2330 hr – 0830 hr for the evening condition. In the overall analysis, conducted as a function of hours post alcohol ingestion, the body temperature of subjects under alcohol conditions was slightly higher than under no alcohol conditions, although this was not statistically significant. There were significant trends in temperature change across time. Body temperature declined across time with the steepest decline occurring from 9 hr to 12 hr. The only significant interaction was between time of day and time post ingestion. In the afternoon condition, body temperature was stable from 5 hr – 9 hr and then generally declined across time with the steepest decline occurring from 9 hr to 12 hr. In contrast, the body temperature of subjects in the evening condition declined from 5 hr – 10 hr post ingestion and then began to steadily increase until 14 hr post ingestion. These differences would be expected given that these data were collected at different solar times.

When the contrasts were restricted to afternoon body temperature data, there was a significant dose by time after alcohol ingestion interaction. Having previously drunk alcohol in the afternoon subjects' body temperature was lower than that when no alcohol was consumed until approximately 9 hr after drinking, which corresponded to a solar time of 2230 hr. At this time, body temperature was equal in the no alcohol and alcohol conditions. After 10 hr post ingestion, body temperature of subjects in no alcohol condition began to decline. While the temperature of subjects who had previously consumed alcohol also declined, the decline was not as steep. This trend supports the results found by Danel et al. (2001). In their study

subjects had body temperatures that were lower than the control condition initially (daytime 1240 hr – 1400 hr), which then increased to be higher than the control during the night (0300 hr – 0820 hr. This trend was also found in the early study by Mullin et al. (1933), and the more recent human studies by Eastman and Stewart (1994). In the same way, the body temperature of subjects after an acute dose of alcohol in the afternoon provided support for the results of other researchers who have observed the rebound effect on temperature in animals (Baird et al., 1998; Gallaher & Egner, 1987; Holloway et al., 1993). Comparable to these studies, the rebound effect observed in the afternoon condition coincided with the normal decline in body temperature, which typically starts around 2330 hr. While the body temperature of subjects, not under the influence of alcohol declined, the body temperature of subjects who had had alcohol did not decline. The body temperature of subjects who consumed alcohol in the evening condition also did not decline to no alcohol levels throughout the sleep phase. This lack of decline in body temperature was apparent when temperature values were normally at their lowest. Previous alcohol ingestion significantly increased core body temperature across the sleep phase (2330 hr – 0830 hr) compared to the control condition. Baird et al. (1998) found similar results in rats injected with alcohol at four times of the day, stating "the entire period of apparent hyperthermia rebound, suggested to be a marker of ethanol hangover states, was contained within a time of day at which temperature values are normally at their lowest" (p. 311). Baird et al. (1998) proposed that the apparent hyperthermia could be interpreted as either a phase advance or phase delay in the circadian rhythm of ethanol treated animals. Like Baird et al. (1998) the results of the current study support the circadian desynchrony hypothesis given that the body temperature of subjects under the influence of alcohol increased when the body

temperature of subjects in the no alcohol condition was at its lowest. While some would argue that this could be due to homeostatic mechanisms, that is a physiological response to counter the decrease in temperature initially induced by alcohol, the fact that increased temperature values occurred throughout the sleep phase regardless of time of alcohol ingestion (1300 hr or 1800 hr) suggests a possible circadian effect rather than a homeostatic phenomenon, although a homeostasis explanation cannot be ruled out entirely.

Subjects in this experiment were not maintained under a constant routine, and masking effects such as ambient temperature, food intake, and activity were not completely controlled. It is possible that variations in subjects sleep and activity cycles could have influenced the results of this study. Given this, the findings of this study should be interpreted with some caution. Other the studies such as those by Mullin et al. (1933) and Eastman and Stewart (1994) did not control for masking effects, however, the study Danel et al. (2001) used a tightly controlled procedure. The results of the present study and these previous studies consistently show the same trends.

6.7.2 Delayed effects of alcohol on subjective states

Two measures on the subjective ratings made by subjects using the VAS showed a significant effect due to alcohol. Subjects felt less social the morning after evening alcohol ingestion and subjects rated the physical symptoms of alcohol more highly after having consumed alcohol previously. These results are in line with that of previous hangover studies that have found that subjects feel less aroused (Finnigan et al., 1998) have poorer mood (Collins, 1980), and greater fatigue, anxiety, and sleepiness (Collins & Chiles, 1980). However, it should be noted that at 0900 hr, it

was 14 hr since alcohol ingestion for the evening condition and 18 hr since alcohol ingestion for the afternoon group. It would therefore be expected that any residual effects of alcohol would be stronger for the evening group compared to the afternoon alcohol condition due to fewer hours since alcohol ingestion. Additionally, it is also possible that the evening group could have had less sleep although not all subjects completed the question in regards to sleep onset and wake time to conclusively determine this claim.

6.7.3 Conclusion

In summary, evidence for the circadian desynchrony theory of a hangover has been demonstrated by the alteration found in the core body temperature rhythms of subjects lasting for at least 14 hr post alcohol consumption. Alcohol significantly impacted on the body temperature and ratings of subjective states 14 hr post alcohol ingestion. Regardless of alcohol ingestion time, after drinking alcohol the body temperature of individuals remained higher than that of the no alcohol condition from 2330 hr – 0830 hr. This trend supports the findings of Danel et al. (2001), Eastman and Stewart (1994), and Mullin et al. (1933), and the animal studies that have found disruption to the temperature rhythm after extended periods of time (Baird et al., 1998; Gallaher & Egner, 1987; Holloway et al., 1993). Thus, it is possible that alcohol hangover effects could be due to circadian rhythm disturbances potentially caused by alcohol. However, it is acknowledged that physiological rebound from the initial alcohol effect could also be contributing to the effects of alcohol on body temperature.

Chapter 7: General discussion

7.1 Discussion of Study 1

In Study 1, the interaction between dose of alcohol and time of day of alcohol administration was statistically significant for performance on the pursuit rotor task under single and dual task conditions. Under the low dose of alcohol, time off target was similar whether alcohol was administered at 1300 hr or 1800 hr. However, it was found that when a high dose of alcohol was ingested in the afternoon, there was a significant increase in time off target for the pursuit rotor task under single and dual task conditions, compared to when alcohol was administered in the evening condition. This result supports the research of Jones (1974), Horne and Baumer (1991), Horne and Gibbons (1991), and Lenné et al. (1999) who found that alcohol related performance impairment was increased after consuming alcohol earlier in the day compared to later in the day. It should be noted, however, that this time of day effect was seen only in one task and only under dual task testing conditions. It is noted that with additional training after the consumption of alcohol the time of day variation was limited to performance on the pursuit rotor task under dual conditions only.

While BACs after the low and high doses of alcohol differed significantly, there were no time of day differences between BACs measured for the low or high doses of alcohol. This finding was consistent with other studies that have reported no significant circadian phase differences in human BAC (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence et al., 1983; Reinberg, 1992;), alcohol metabolism enzymes such as alcohol dehydrogenase in rats (Brick et al., 1984), time to reach peak BAC, AUC, or elimination rates (Yap et al., 1993). It should be noted, however, that other studies have found variations in the pharmacokinetics of alcohol

at different times of the day (Jones, 1974; Lakatua, et al., 1984; Minors & Waterhouse, 1980; Sturtevant et al., 1978). It was not possible to comment on pharmacokinetics from this study as BACs were only monitored for the duration of the performance testing (i.e., 15 min). Additionally, while subjects were asked to fast prior to arrival at the laboratory, it was noted throughout the experiment that subjects' had consumed differing types and amounts of food three hours prior to fasting. Consequently, subjects' stomach contents differed across sessions, which could have had an influence on alcohol pharmacokinetics, consequently influencing the BACs of individuals and potentially their performance on tasks (Millar et al., 1992).

Subjects' scores on the SSS revealed no statistically significant effects. Sleepiness was rated as similar pre and post alcohol consumption, and at both doses of alcohol, and at both times of the day alcohol was consumed. Similarly, there were no significant interactions. These results are in contrast to previous studies that have used the SSS and other types of subjective rating scales (Horne & Baumer, 1991; Horne & Gibbons, 1991). While the times of the day alcohol was administered were comparable to other studies, the times after alcohol ingestion that the SSS was administered were not comparable. In the current study sleepiness was measured prior to task performance, that is, at peak BAC. Changes in the levels of sleepiness due to alcohol, dose of alcohol, and time of day may not have been detected, as levels of sleepiness may need to be measured over a longer period of time. Similarly, given sleepiness levels were measured at peak BAC, alcohol may have been having more of a stimulatory effect rather than a depressant effect on subjects. Previous studies have measured subjective states, for example, sleepiness, after lengthy performance testing and over much longer periods after alcohol ingestion.

Thus, these differences in procedure between study 1 and other chronopharmacology studies examining sleepiness could explain the lack of significant findings on the SSS.

Findings from this study have implications for other experimental research. Experimental psychologists should be aware that performance can be influenced by the time of the day testing is conducted. This being the case, experimental manipulations should be conducted at the same time of day to avoid variability due to of time testing.

7.2 Discussion of Study 2

Study 2 aimed to further investigate the time of day variation in alcohol related performance impairment found in Study 1. This study included an alcohol free condition, a more extended period of measurement, up to 4 hr post alcohol ingestion, and other dependent variables such as body temperature, heart rate, melatonin, and cortisol. The study demonstrated that alcohol had a significant main effect on all measures with the exception of cortisol. There was a significant effect of alcohol on performance, core body temperature, heart rate, melatonin and the physical symptoms factor of the VAS. Time of day effects were noted, as a main effect for cortisol, and as part of an interaction with dose and/or block, for heart rate, melatonin, and BAC. With the exception of performance, all dependent variables displayed significant trends across the measurement period. In contrast to study 1 and other studies (Jones, 1974; Horne & Baumer, 1991; Horne & Gibbons, 1991; Lenné et al., 1999; Reinberg, 1992) study 2 demonstrated that alcohol did not have time of day dependent effects on performance tasks or subjective states as measured by the VAS used in this study. Although the findings of study 2 are consistent with

Yap et al. (1993) who reported that performance on a divided attention task and digit symbol coding after alcohol ingestion was not dependent on the time of day it was ingested.

Possible explanations for the difference between the findings of study 1 and study 2 are offered. First, it is probable that the significant time of day by dose effect found in Study 1 could have been a spurious result, as the effect was decreased with the additional training with alcohol. Alternatively, the effect may not have been seen in study 2 due to a change in the measurement equipment. Additionally, subjects in study 1 had more trials under alcohol conditions (4 compared to 2), suggesting that subjects in study 1 may have developed behavioural tolerance (Goldstein, 1983). Although subjects in study 2 undertook a repeated testing schedule whereby three blocks of testing for each experimental session were required. Moreover, there were also differences in the gender proportions and drinking histories of subjects between study 1 and study 2 which could also be potential explanations for the divergent results. Given that the studies were conducted under well controlled conditions, the most likely explanation for the lack of replication is that alcohol related time of day variations in performance are not robust effects which can be easily over-ridden by other factors.

The time of day by dose interaction for heart rate showed that heart rate for the no alcohol condition was higher in the afternoon condition compared to the evening condition (indicative of the time of day variation). However, regardless of time of day, heart rate levels were the same under both alcohol conditions. This indicated that heart rate increased more in the evening compared to the afternoon. It is noted that this effect could be due to the difference in baseline heart rate. Sayette (1993) stated that the law of initial values asserts that physiological responses occur

within a certain range of values. Therefore, when initial values approach these limits, the physiological reactivity can be restricted. Therefore, it is possible that because baseline heart rate was higher in the afternoon the response to alcohol was restricted in comparison to the response made in the evening when baseline levels were lower.

Only one other chronopharmacology of alcohol study has included heart rate as a measure (Yap et al., 1993) and they did not find time of day variations for heart rate prior to or after alcohol administration. However, the methodology between the current study and Yap et al. (1993) differed in a number of ways. Firstly, Yap et al. (1993) did not use a no alcohol control condition, and took one sample prior to drinking, and at 60 min and 120 min post drinking at the four times of day conditions. The current study measured heart rate over seven minutes at 30, 120, and 210 min post alcohol ingestion and at these same times under no alcohol conditions.

Alcohol appeared to increase melatonin levels prior to 2230 hr, relative to no alcohol conditions. At 2230 hr melatonin levels after alcohol appeared to reduce the rise in melatonin that was seen in the no alcohol condition, although this difference between alcohol and no alcohol conditions at the 210 min block under evening conditions was not statistically significant. The primary argument forwarded by previous researchers for nocturnal alcohol-inhibited melatonin secretion is that there is a down regulation of the pineal beta-receptors in response to increased noradrenergic transmission caused by alcohol administration (Moss et al., 1986; Röjdmark et al., 1993; Schmitz, Sepandj, Pichler, & Rudas, 1996). Given pineal melatonin synthesis is regulated by adrenergic neurotransmission, reduced melatonin secretion may be a reflection of decreased sympathetic outflow (Moss et al., 1986). The data from this study supports this contention in that alcohol had an opposing

effect (to the baseline trend of melatonin) on the melatonin levels of those individuals who had received alcohol after melatonin onset. Further support for this argument, an opposing effect on melatonin levels by alcohol, was shown on daytime levels of melatonin (Figure 5.11). When alcohol is given during the day, alcohol has the effect of increasing melatonin (daytime levels of melatonin are very low as they are suppressed by light). However, in the current study this study melatonin was used as a dependent variable to investigate the role of circadian rhythms in time of day mediated alcohol effects and not designed to explore the mechanisms for alcohol's effect on melatonin. In light of this, future research may benefit from exploring this "opposing effect" as a possible mechanism for the variation in melatonin levels at different alcohol administration times.

While no interactions involving time of day were noted for temperature the fact that alcohol had a significant effect on temperature in the afternoon and not in the evening suggested that alcohol had a more pronounced effect in the afternoon compared to the evening. The body temperature of subjects who had consumed alcohol declined, although only when subjects consumed alcohol in the afternoon condition. These results support previous chronopharmacology of alcohol studies in animals that have found that the largest decreases in body temperature were found when animals were injected with alcohol during the dark phase (activity phase) of their diurnal cycle (Baird et al., 1998; Brick et al., 1984). Additionally, the results of the current study also support human studies, as alcohol appeared to have a more pronounced effect when it was consumed earlier in the day, 1300 hr compared to 1800 hr. Reinberg et al. (1975 cited in Reinberg 1992) reported that the 24 hr mean of oral temperature was found to be lowest compared to control temperature values when alcohol was consumed at 0700 hr compared to 1100 hr, 1900 hr, and 2300.

Similarly, O'Boyle found that alcohol caused a significant decline in oral temperature at 0800 hr; in contrast, alcohol had no effect on temperature at 1600 hr.

In study 2, a time of day influence on the absorption of alcohol was noted. Previous research has shown significant circadian variations for the pharmacokinetics of alcohol (Jones, 1974; Lakatua et al., 1984; Lerné et al., 1999; Minors & Waterhouse, 1980; Sturtevant et al., 1978; Yap et al., 1993). However other studies have not reported significant circadian phase differences in human BAC (Horne & Baumer, 1991; Horne & Gibbons, 1991; Lawrence et al., 1983; Reinberg, 1992), alcohol metabolism enzymes such as alcohol dehydrogenase in rats (Brick et al., 1984), or time to reach peak BAC, AUC, and elimination rates (Yap et al., 1993).

According to chronopharmacological principles the time dependent efficiency of a drug can be explained either by chronesthesy or chronokinetics (Reinberg, 1990a). In light of the time of day variation found for BAC absorption it is possible that this variation could account for time related disruption to body temperature, heart rate, and melatonin. Although it seems unlikely as the more pronounced effect (greater decline in body temperature) was noted in the afternoon when alcohol absorption was shown to be slower. Therefore, it is probable that the time related changes in alcohol's effects are linked to a temporal responsiveness of target tissues, chronesthesy, in addition to alcohol's chronokinetics.

In summary, while no time of day differences in behavioural measures or subjective states were noted, the most noteworthy finding was that alcohol had a more pronounced effect, indicated by a decline in temperature, after alcohol administration at 1300 hr. This finding has important implications for public health and workplace safety.

7.3 Discussion of Study 3

Study 3 presented data on the delayed effects of alcohol (5 hr – 14 hr) on body temperature and subjective following alcohol administration at 1300 hr and 1800 hr. Circadian rhythm disruption has been argued to be a salient factor in explaining the mechanisms of a hangover (Baird et al., 1998). Along with other studies, study 2 found that alcohol disrupted body temperature after both alcohol administration times. It has been proposed that alcohol hangover effects could be due to sleep disturbances resulting from the effects of alcohol on circadian rhythms rather than a toxic reaction to alcohol.

Alcohol had statistically significant effects on the core body temperature rhythm and ratings of subjective states up to 14 hr post alcohol ingestion. Previous ingestion of alcohol at 1300 hr lowered subjects' body temperature compared to the no alcohol condition from 5 hr – 9 hr after alcohol consumption corresponding to a solar time of (1830 hr – 2230 hr). After this time the body temperature of subjects who had not drunk alcohol began to decline while the body temperature of subjects who had consumed alcohol did not decline to the same extent. After drinking alcohol in the evening, the body temperature of individuals remained slightly higher than the no alcohol condition following the same trend.

The body temperature data from study 3 is consistent with the results of other researchers who have observed the rebound episode in animals (Baird et al., 1998; Gallaher & Egner, 1987; Holloway et al., 1993). These studies have shown that when rectal temperature following alcohol injection is collected over an extended period of time hyperthermic disturbances are evident after the elimination of alcohol (Gallaher & Egner, 1987). Baird et al. (1998) proposed that the apparent hyperthermia seen in animal subjects could be interpreted as either a phase advance

or phase delay in the circadian rhythm of ethanol treated animals. While some would argue that these findings could be due to homeostatic mechanisms, the fact that the increased body temperature values occurred throughout the sleep phase regardless of time of alcohol ingestion (1300 hr or 1800 hr) could be suggestive of a circadian disruption as subjects who consumed alcohol did not experience the normal circadian temperature decline throughout the sleep phase. Research on the effect of alcohol on body temperature during the sleep phase, using human subjects, has shown that alcohol consumption prior to sleep significantly increases core body temperature, relative to baseline levels (Danel et al., 2001; Eastman & Stewart, 1994; Mullin et al., 1933). The design and procedures used in these studies differed considerably, for example doses of alcohol, control over subjects' sleep/ wake and activity levels, and food intake; however, it has been consistently shown that alcohol consumption has an effect on core body temperature.

The temperature data provided the most compelling evidence of a hangover effect. Only the gregariousness subscale on the VAS showed a significant change in the morning after evening alcohol ingestion. Subjects felt less sociable at 0900 hr following evening ingestion of alcohol. This result is in line with that of previous hangover studies that have found that subjects feel less aroused (Finnigan et al., 1998) have poorer mood (Collins, 1980), and greater fatigue, anxiety, and sleepiness (Collins & Chiles, 1980). Nevertheless, it should be noted that at 0900 hr, any residual effects of alcohol would be stronger for the evening group compared to the afternoon alcohol condition due to fewer hours since alcohol ingestion.

In summary, evidence for the desynchrony theory of a hangover has been demonstrated by the disruption to body temperature lasting for at least 14 hr post injection. Regardless of time of alcohol ingestion, drinking alcohol dampened the

temperature decline of individuals throughout the sleep phase. This trend supports the findings of Mullin et al. (1933), Eastman and Stewart (1994), Danel et al. (2001), and animal studies (Baird et al., 1998; Gallaher & Egner, 1987; Holloway et al., 1993) who have found disruption to the temperature rhythm after the administration of alcohol. Alcohol hangover effects could be due to circadian rhythm disturbances caused by alcohol. Alcohol been shown to disturb sleep patterns; for example, variable reductions in sleep latency and intermittent wakefulness, and effects on total sleep time (Buysse, 1991). Whether this effect is in part due to disruption to the circadian rhythms of melatonin secretion and core body temperature is unknown at this time. Nevertheless, one of the reasonable consequences of disturbance to the core body temperature is disruption to sleep. However, it is acknowledged that physiological rebound from the initial alcohol effect could also be contributing to the effects of alcohol on body temperature. Regardless of the mechanisms for this effect, evidence that alcohol's effects extend beyond its metabolism can provide important information for workplace safety, particularly those jobs that involve high risk.

7.4 Limitations and future research

Very little research on the chronesthesy and chronokinetics of alcohol has been conducted on humans. The main reasons for the lack of human research in this area are practical and ethical considerations. For example, while it is possible to keep rats under constant conditions and have knowledge of their drug and behavioural learning history, it is impossible to completely control for all of these variables in humans. Further, using humans to collect this type of data is laborious and expensive and often places many inconveniences on subjects. In the experiments of this thesis, subjects were not maintained under a constant routine, and masking

effects such as ambient temperature, food intake, and activity were not completely controlled. It is possible that variations in subjects sleep and activity cycles could have influenced the results of this study. While a constant routine would have been ideal to conduct these experiments, particularly for the measurement of melatonin and core body temperature, the imposed cost and special laboratory conditions were not justifiable for the other variables and research questions of this thesis. While the studies by Mullin et al. (1933) and Eastman and Stewart (1994) did not control for masking effects, the study Danel et al. (2001) used a tightly controlled procedure. The results of the all of these studies consistently show the same trends, thus it is contended that the findings of this study are sound and that the ecological validity of this study adds strength to more laboratory based studies.

Related to this, while time of day variations in the alcohol response were noted for body temperature and melatonin levels, the circadian parameters, mesor, acrophase, amplitude, and period were not evaluated, as one complete circadian cycle for these measures was not collected. Thus, whether alcohol would differentially alter these parameters, as a function of time of day could not be determined from this thesis. It was thought that asking subjects to log rectal temperature for a period of 24 hr, for four sessions, could have hindered recruitment. In hindsight, subjects who were involved in this research probably would have continued to log their temperature, as they were interested in the research and the information they would receive about their physiology. Similarly, melatonin samples were collected at five time points, none of which were throughout the sleep phase, although some of the collections were made as melatonin levels were increasing. Future research should examine the chronergy of alcohol, that is, its effect on the biological rhythm, by combining information from chronokinetics and chronesthesy to interpret the

influence of alcohol as a whole. One way this could be achieved is by obtaining rectal temperature data and multiple samples of melatonin for one circadian cycle in order to analyse circadian parameters for alcohol/time of day differences.

Similarly, due to practical requirements, only two times of the day were chosen as alcohol administration times. Time of day variations in the response to alcohol may not have been detected (e.g. cortisol, performance impairment, subjective states) because the study did not measure during the windows in which the effects occur. Future research should include more alcohol administration times, for example a very early morning time and a very late evening time, to explore the dependent measures using the same methodology. It is possible that increased temperature values throughout the sleep phase are a homeostatic phenomenon. However, if the same pattern were found at other times of the day then further support would be found for the notion of a circadian disruption throughout the sleep phase. Similarly, earlier sampling times would further elucidate whether alcohol does indeed increase melatonin levels when alcohol is given during the day or early evening and if melatonin levels are inhibited once nocturnal melatonin onset occurs.

A number of variables are known to influence the pharmacokinetics of alcohol particularly when the route of administration is oral. For example, although subjects were provided with snacks and a meal during the course of experiments, prior dietary intakes could have influenced the pharmacokinetics of alcohol. Moreover, the TBW formula does not directly factor body fat into the equation. When calculating the TBW of a person, their TBW could be over or under estimated, thus affecting the dose of alcohol required to reach a target BAC. This explanation could account for the variation seen in BACs obtained in the experiments. Measurement error of the breathalyser may also have had an influence on the BAC

results. If the chronokinetics of alcohol are to be clearly elucidated, future research should measure BAC using plasma samples.

To date, chronopharmacology studies of alcohol that have used physiological measures rather than performance measures have not included a separate control condition to assess the effects of alcohol, as the primary interest of most of these studies was to assess the circadian variation of alcohol responses rather than the effect of alcohol per se. Most chronopharmacology of alcohol studies have included baseline measurements of variables prior to alcohol ingestion, potentially to reduce the time burden on subjects involved in the research. The only study (Lenné et al., 1999) that has included a separate control condition has investigated alcohol and time of day changes in performance rather than physiological measures and thus baseline measures would not be appropriate given the potential confounding effects of practice. The current study included both baseline measures for the variables of temperature, melatonin, cortisol, and VAS and a separate no alcohol condition for all measures. The no alcohol condition did not require subjects to drink juice; rather this condition was included to collect data on the variables of interest to the study when subjects were engaged in their usual daily activities. Thus, a strength of this research is the fact that baselines and a separate no alcohol condition were incorporated providing a study with more ecological validity. Juxtaposing this strength, however, not providing subjects with juice in the no alcohol condition makes assessment of pharmacological differences problematic, as the effects of alcohol could be argued to be the result of juice rather than alcohol. Although, a post-hoc pilot study of the effect of orange juice was undertaken using two subjects. Data showed that ingestion of orange juice did not significantly impact on body temperature in both subjects (see Appendix P). While the pilot study of the effect of juice on core body

temperature showed no difference between juice and no juice conditions, differences were not explored for performance measures. The amount juice that would have been required to be consumed for study 2 would not have significantly impacted on performance. Nevertheless, future research should be undertaken to explore differences between placebo, control (beverage), control (no beverage) and an alcohol condition to identify possible differences between conditions.

7.5 Implications of the research

An expanding area of chronopharmacology is the interaction between alcohol and circadian rhythms. Very little research has been conducted on the interaction between alcohol and circadian rhythms, particularly using human subjects. This thesis has focused on humans' acute and delayed physiological and biochemical responses to alcohol intoxication at different times of the day. Interesting findings from this thesis that add to the alcohol and circadian rhythm literature include findings on the effect of alcohol on melatonin levels. At present very little data exist on the effects of alcohol on melatonin levels in humans. Additionally, this thesis has presented findings on the effects of drinking alcohol at two times of the day on rectal temperature for 14 hr post alcohol ingestion inclusive of the sleep phase for human subjects. One of the major findings of this thesis was that drinking alcohol at 1300 hr has the acute effect of decreasing subjects' body temperature, relative to a no alcohol condition, yet this trend was not seen after drinking alcohol at 1800 hr; that is body temperature did not decline, relative to the no alcohol condition. Moreover, it was found that regardless of the administration time of alcohol, that is, 1300 hr or 1800 hr, the delayed effect of alcohol on a subjects' body temperature is a disruption during the sleep phase (2330 hr – 0830 hr). This research showed that alcohol

appears to dampen the circadian temperature trough compared to a no alcohol condition.

Given the relationship between body temperature, melatonin, and sleep, this finding alludes to the existing evidence that alcohol disrupts sleep. This result supported previous animal and human studies that have found that alcohol ingestion resulted in an increase in body temperature when body temperature in a no alcohol condition was low. These data have not been previously collected after a single dose at different alcohol administration times. In conclusion, a clearer understanding of alcohol's effects on the circadian rhythms has important consequences for the investigation of physiology and behaviour and the effects drugs have on these processes. Researchers conducting behavioural, physiological, and pharmacological studies need to consider the time of day measures are examined as effects could be influenced by the time of day the studies are performed. Likewise, the findings of this study have implications for public health and workplace safety.

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Glossary

The terms in this glossary have been defined using the following sources.

Brugerolle (1994)
Giles & Kapur (1991)
Julien. (1998)
Reinberg (1990a)
Touitou & Haus (1992)

Acrophase: a measure of the time of the peak of an oscillation.

Amplitude: maximum minus minimum value, one half of the peak to trough difference.

Biological clock: Mechanism within an organism that is capable of generating repeated cycles (oscillations, rhythms), whose period is relatively insensitive to temperature, and which can be synchronised by environmental stimuli

Chronergy: The rhythmic change of the response of the organism to a drug according to its chronokinetics and it chronesthesy (see below).

Chronesthesy: Rhythmic changes in the susceptibility or sensitivity of a target biosystem to a chemical agent. Potentially caused by temporal changes in receptors of target cells, membrane permeability etc.

Chronokinetics: Study of the temporal changes in absorption, distribution, metabolism, and elimination of a drug.

Chronotherapy: Delivery of medical treatment taking into consideration chronopathalogy, chronokinetics, and chronesthesy to enhance the desired pharmacological effect and reduce side effects of the drug.

Circadian: The term used to describe rhythms with an about 24 hr cycle length (20 – 28 hr).

Circadian typology: The recognition of variations between individuals in their circadian rhythms. Morning (M-) and Evening (E-) types are generally regarded as the extremes of a continuum on which the intermediate, Neither (N-), types represent the largest category. This difference occurs in all physical and psychological cycles.

Consinor Analysis: Statistical method involving cosine curve fitting for detecting and quantifying time series data to describe biological rhythm parameters. The parameters derived are mesor, amplitude, and acrophase.

Central Nervous System (CNS): The brain and spinal cord.

Desynchronisation: loss of synchronisation between two or more rhythms so that they show independent periods.

Diurnal: active in the day time

Dose-response relationship: Relation between drug doses and the response elicited at each dose level.

Drug: Chemical substance used for its effect on bodily process.

Drug administration: Procedures through which a drug enters the body (oral, injection).

Endogenous rhythm: A rhythm that is Presumably a genetically fixed biological rhythm, persisting when outside time cues are not present.

Entrainment: synchronisation of a rhythm by a repetitive signal (e.g. the recurring light-dark cycle).

Errors on reaction time task: Errors were defined as a false alarm (making a response when one was not required) or a miss (not making a response when one was required). Errors made by subjects were minimal.

Free-run: natural self sustained rhythm that exists in the absence of a synchronising signal. When human is free running their cycle appears to be longer than 24 hours.

Homeostatic hypothesis of medical treatment: This hypothesis forwards that the body's functions are in a constant steady state throughout the day and night. This directs treatment towards maintaining or restoring the steady state (i.e., ensuring a steady state of the drug is in the bloodstream).

Hypothalamus: Structure located at the base of the brain, above the pituitary gland. The control site of temperature regulation and secretion of hormones.

Lighting regime: The light-dark cycle (LD), or (LL), constant light, or (DD) constant dark conditions used in chronobiologic studies.

Masking of a rhythm: alteration of the usual shape and/or parameters of a rhythm due to random or non-random environmental stimuli, persisting for the duration of the stimulus only (without persistent alterations of endogenous components).

MESOR: (Midline Estimating Statistic of Rhythm): The value midway between the highest and lowest values of the cosine function best fitting to the data.

Nocturnal: active in the night-time.

Oscillation: a cycle.

Oscillator: Something that changes regularly or cyclically. For example, oscillator neurons, which generate regular breathing or locomotory rhythms.

Pacemaker: driving oscillation, a rhythm that controls other rhythms, e.g. the SCN in humans.

Period (τ): the length of a cycle; designated by the greek letter tau; the duration of time required for a rhythm to complete one cycle.

Pharmacodynamic: Study of the interactions of a drug and the receptors responsible for the action of alcohol.

Pharmacokinetics: Study of the factors that influence the absorption, distribution, metabolism, and excretion of a drug.

Pharmacology: Branch of science that deals with the study of drugs and their actions on living systems.

Placebo: Pharmacologically inert substance that may elicit a significant reaction largely because of the mental set of the subject or the physical setting in which the drug is taken,

Phase (ϕ): (of a rhythm) the instantaneous state of an oscillation with a period, represented by the value of the variable and all its time derivatives; phase specifies the relationship between an event and something else (e.g. time according to a clock) and requires specification of phase reference points.

Phase advance: an earlier timing of an acrophase or peak time, with respect to clock hour from normal or with reference to a set of environmental cues.

Phase delay: A delayed timing of an acrophase or peak time, with respect to clock hour from normal or with reference to a set of environmental cues.

Phase response curve: plot of phase shifts in response to pulses plotted versus time the pulse was given.

Phase shift ($\Delta\phi$): a single displacement of an oscillation along the time axis.

Photoperiod: light-time, length of light time; daylength.

Photoperiodic: phenomenon that is responsive to daylength (or night length).

Rebound hyperthermia: When the rectal temperature of animals following alcohol injection, is collected over an extended period of time, dose dependent hyperthermic disturbances are evident after the elimination of alcohol. This phenomena can be explained as a response by the body to counter hypothermia initially produced by alcohol. However, the hyperthermia may not be a homeostatic rebound effect at all, but rather an effect that results from a dampening of the normal temperature trough.

SCN: suprachiasmatic nucleus; region of the hypothalamus thought to generate circadian rhythm information that is conveyed neurally to the pineal gland in some species; bilateral.

Scotoperiod: nightlength; length of the dark time.

Side effect: Drug induced effect that accompanies the primary drug effect for which the drug is administered.

Synchronisation: State of a system when two or more variables exhibit periodicity with the same frequency.

Time series: A series of measurements obtained as a function of time.

Tolerance: Tolerance refers to a reduction in the physiological response generally because of prolonged frequent use.

Total Body Water (TBW): Total Body Water (TBW) refers to the sum of the volumes of the individual components of body water. The distribution volume of alcohol is affected by the body composition and that relates to factors such as age, weight, and gender.

Toxic effect: Drug induced effect harmful to any organ or system. Drug toxicity includes both the relatively minor side effects that invariably accompany drug administration and the more serious manifestations that occur in a small percentage of subjects who take a drug.

Trough: The lowest point in a series of measurements obtained as a function of time.

Zeitgeber: a synchroniser, entraining agent, time giver, time signal, time cue, e.g. dawn or dusk.

Appendices

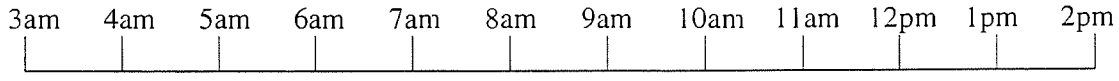
Appendix A: Circadian Continuum Scale (Lehtonen & Graham, 1998)

You are probably very well aware of what time of day you feel at your best, and what time of day you prefer to engage in various activities. However, because of work, study, family or other commitments we often have to plan our activities at times which may not suit our particular *best time' of day.

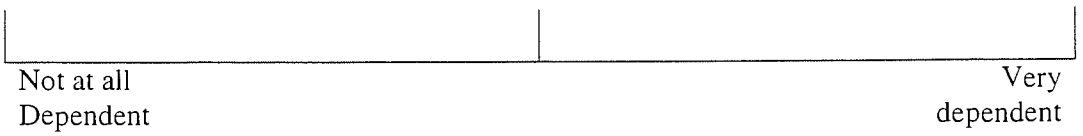
This questionnaire aims to find out what your 'actual' preferred times for certain activities are. When you answer this questionnaire, assume that you are free from work, study, family and all other commitments, and are free to choose your preferred time of activities simply on the basis of your `best time' of day.

Name: _____ Age: ____ Gender: M/ F

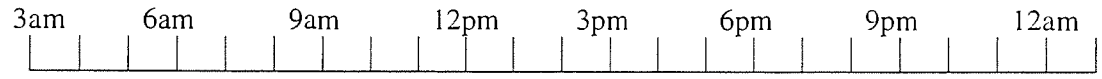
Q1. Assuming you are totally free of commitments, what time of day would you wake up?



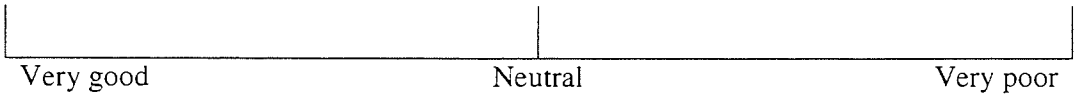
Q2. If you wanted to wake up one morning at 7am, how dependent would you be on an alarm clock?



Q3. Assuming you are totally free of commitments, at what time do you think you are at your mental 'peak'?

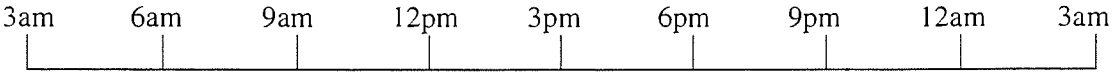


Q4. How is your general mood upon waking in the morning?

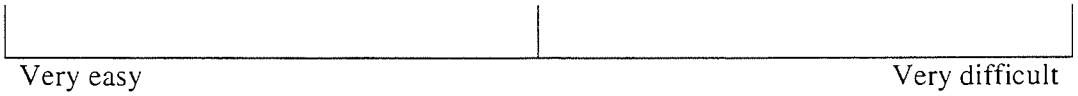


Q5. If you awoke at Sam, how badly would you **WANT** to go back to sleep again?

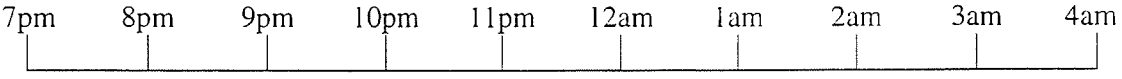
Q6. At what time of the day do you usually feel you have the most energy?



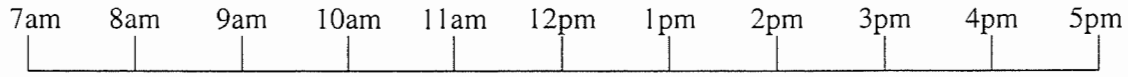
Q7. If you woke at 6am, how **EASY** would you find it to fall back to sleep again?



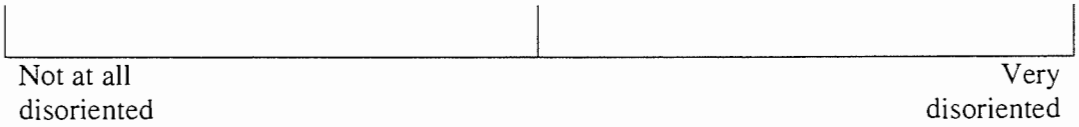
Q 8. Assuming you are totally free of commitments, what time would you normally go to bed?



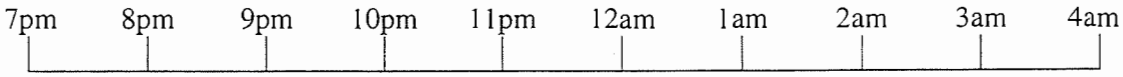
Q9. At which time on the following time scale do you feel it is easiest to get yourself motivated about something?



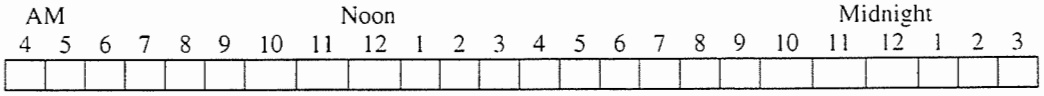
Q10. If you awoke at 6am, how disoriented would you feel?



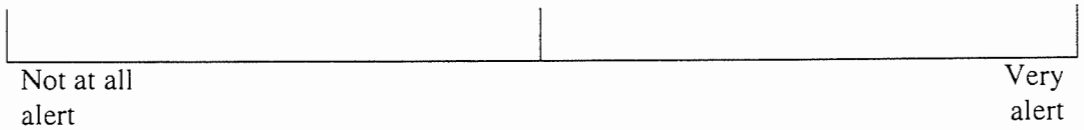
Q11. At what time in the evening do you start to feel sleepy?



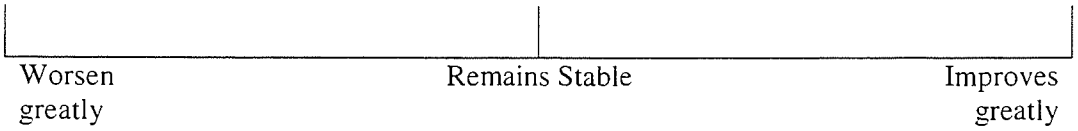
Q12. You have decided to take on 2 hours of part-time work for several weeks. Assume that you are totally free of any other commitments, and you are free to choose your own working hours. You enjoy the work and get paid a bonus for performance. Which 2 consecutive hours of the day would you choose in order to be at your most productive?



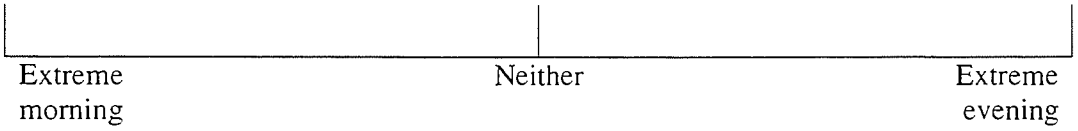
Q13. Place a mark which you feel best describes your experience of waking



Q14. After waking, does your general mood normally improve through the course of the day or worsen through the course of the day?



Q15. Do you consider yourself to be a `morning' type, an `evening type' or neither?



Thank you very much for your time and cooperation.

Comments

If you have any comments that you would like to make (either regarding the questions or any difficulty you may have had answering any of them) please feel free to comment below.

Appendix B: Composite Scale

Subject	Study 1		Study 2	
	CS	CCS	CS	CSS
1	39	106.7	33	126.6
2	25	92.3		121
3	29	108.9	40	100.1
4		98.5		99.6
5		113.8		103.4
6		85	35	127.7
7		84.3	38	127
8	33	133.5		107.3
9		116		108.7
10	25	135.9	34	100.9
11		104	38	91.7
12		126.2	37	120.1
Mean	30.2	108.8	36.4	111.2
St Dev	5.9	17.3	2.5	12.7

CCS Criteria

Greater than 137.7	E
80.1 – 137.7	N
Less than 80.1	M

CS Criteria

22 and less	E
23-43	I
44 and above	M

DIRECTIONS. Please *check* the response for *each* item that best describes *you*.

1. Considering only your own "feeling best" rhythm, at what time would you get up if you were entirely free to plan your day?

   5:00–6:30 a.m. _____
   6:30–7:45 a.m. _____
   7:45–9:45 a.m. _____
   9:45–11:00 a.m. _____
   11:00 a.m.–12:00 (noon) _____

2. Considering you only "feeling best" rhythm, at what time would you go to bed if you were entirely free to plan your evening?

   8:00–9:00 p.m. _____
   9:00–10:15 p.m. _____
   10:15 p.m.–12:30 a.m. _____
   12:30–1:45 a.m. _____
   1:45–3:00 a.m. _____

3. Assuming normal circumstance, how easy do you find getting up in the morning? (Check one.)

   Not at all easy _____
   Slightly easy _____
   Fairly easy _____
   Very easy _____

4. How alert do you feel during the first half hour after having awakened in the morning? (Check one.)

   Not at all alert _____
   Slightly alert _____
   Fairly alert _____
   Very alert _____

5. During the first half hour after having awakened in the morning, how tired do you feel? (Check one.)

   Very tired _____
   Fairly tired _____
   Fairly refreshed _____
   Very refreshed _____

6. You have decided to engage in some physical exercise. A friend suggests that you do this one hour twice a week and the best time for him is 7:00–8:00 a.m. Bearing in mind nothing else but your own "feeling best" rhythm, how do you think you would perform?

   Would be in good form _____
   Would be in reasonable form _____
   Would find it difficult _____
   Would find it very difficult _____

7. At what time in the evening do you feel tired and, as a result, in need of sleep?

   8:00–9:00 p.m. _____
   9:00–10:15 p.m. _____

10:15 p.m.–12:30 a.m. _____
12:30–1:45 a.m. _____
1:45–3:00 a.m. _____

8. You wish to be at your peak performance for a test which you know is going to be mentally exhausting and lasting for two hours. You are entirely free to plan your day, and considering only your own "feeling best" rhythm, which ONE of the four testing times would you choose?

8:00–10:00 a.m. _____
11:00 a.m.–1:00 p.m. _____
3:00–5:00 p.m. _____
7:00–9:00 p.m. _____

9. One hears about "morning" and "evening" types of people. Which ONE of these types do you consider yourself to be?

Definitely a morning type _____
More a morning than an evening type _____
More an evening than a morning type _____
Definitely an evening type _____

10. When would you prefer to rise (provided you have a full day's work—8 hours) if you were totally free to arrange your time?

Before 6:30 a.m. _____
6:30–7:30 a.m. _____
7:30–8:30 a.m. _____
8:30 a.m. or later _____

11. If you always had to rise at 6:00 a.m., what do you think it would be like?

Very difficult and unpleasant _____
Rather difficult and unpleasant _____
A little unpleasant but no great problem _____
Easy and not unpleasant _____

12. How long a time does it usually take before you "recover your senses" in the morning after rising from a night's sleep?

0–10 minutes _____
11–20 minutes _____
21–40 minutes _____
More than 40 minutes _____

13. Please indicate to what extent you are a morning or evening *active* individual.

Pronounced morning active (morning alert and evening tired) _____
To some extent, morning active _____
To some extent, evening active _____
Pronounced evening active (morning tired and evening alert) _____

Appendix C: Screening Instrument

SCREENING INSTRUMENT

Participant Name_____

Address_____

_____

Telephone_____

Age_____

Please indicate if you have used any of the drugs/substances listed below in the last month. If so, how often and when was the last time you used the drug/substance. Your responses are entirely confidential.

- **Stimulants** (includes cocaine & amphetamines, e.g. XTC & speed)
  Y **N** _____
- **Depressants** (calming, sleep inducing, anxiety-reducing, e.g. barbituates, benzodiazepines such as valium, muscle relaxants, sleeping tablets)
  Y **N** _____
- **Hallucinogens** (Marijuana, LSD, Mushrooms)
  Y **N** _____
- **Opiates** (methadone, heroin, codeine) **Y** **N** _____
- **Inhalants** (e.g. glue, petrol) **Y** **N** _____

The following questions refer to treatments you may be receiving.

Are you receiving treatment from a doctor for any medical condition? **Y** **N**

Are you currently undergoing, or have you recently undergone, any therapy with a psychologist, psychiatrist or other counselor for any emotional or psychological problems? **Y** **N**

Have you ever sought treatment for alcohol problems? **Y** **N**

Are you on any prescription medication, or receiving any medicines regularly? This includes: pain killers, antihistamines, antidepressants, antibiotics, sleeping pills, major tranquilizers, insulin, ventolin? **Y** **N**

The following questions refer to your typical pattern of alcohol consumption for an average week.

- During a typical week on how many days would you drink alcohol?_____days
- On how many occasions per day would you drink (e.g. would you normally drink after work, at lunch, at night)_____
- How many drinks would you consume on each occasion? (e.g. cans, pots, glasses, bottles, nips etc.)

  _____
- When did you have your last drink?

  _____
- How much did you drink at that time?

  _____
- Have you ever drunk 5 - 7 standard alcoholic drinks within 1 - 2 hours?

  Y **N**

What was the effect on you (e.g. were you ill) _____

The following questions refer to your caffeine and cigarette intake.

Are you a cigarette smoker? **Y** **N**

If yes, how many cigarettes would you smoke per day? _____

Could you refrain from smoking without withdrawal for approximately 3 hours?

Y **N**

Do you drink coffee/tea? **Y** **N**

How many cups of coffee or tea do you drink on the average day? _____

Could you refrain from drinking coffee/tea without withdrawal for approximately 3 hours? **Y** **N**

(For females) To the best of your knowledge, are you pregnant, or attempting to fall pregnant? **Y** **N**

Appendix D: Preparation Instructions

Preparation Instructions

- Please do not drink alcohol for 24 hours prior to the experiment.
- Please do not take any other drug (does not include oral contraceptives) for 3 days prior to the experiment.
- Please do not drink coffee or smoke cigarettes for 1 hour prior to the experiment appointment times.
- Do have a light meal 2-3 hours prior to your experiment appointment times. Do not have any snacks or drinks (except water) between this light meal and your appointment time. Food will be provided after the testing session.
- On the day of the experiment you are not to drive or ride a vehicle to or from the university. Transportation will be provided.
- You will be given an amount of alcohol in the form of vodka and orange juice, which you will consume in a certain period of time. A number of physiological and behavioural measures will be taken throughout the testing sessions.
- You have the right to withdraw from the experiment at any time. If you do withdraw and have already consumed alcohol you must stay within the laboratory facilities until your breath alcohol concentration level has returned to zero. Further, you are instructed not to drive a motor vehicle until the following morning after the experiment.
- The estimated time required per testing session is 8 - 10 hours. Approximately two hours is required for the testing session, the remainder being leisure/recuperation time waiting for your breath alcohol reading to return to zero.

Please contact me if you require further information.

Madonna Devaney
James Cook University (Ph: 40 421359)

Appendix E: Consent Form Study 1

Consent Form

Charting the interface between Alcohol, Time of Day, Dosage, and Performance

Information

1) I _____ age _____ years agree to participate in the study conducted at James Cook University, Cairns.
2) This study examines the effects of alcohol consumption (at a medium or a high dose) on peoples' ability to perform a number of tasks.
3) The test sessions will be conducted on the same day of the week for the next 4 weeks. The practice of tasks will take approximately 30-60 minutes. The alcohol session will last for 5 - 10 hours depending on the dosage consumed. This time includes drinking the alcoholic beverage, participation in the testing sessions, and waiting for my BAC to return to zero.
4) During the test session I will be in the psychology experimental laboratory or the waiting room. At all times the primary investigator or a research assistant will supervise me.

Requirements

5) I agree to refrain from drinking alcoholic beverages for 24 hours prior to the testing session. Given that the experiment involves controlled doses of alcohol, on arrival at the laboratory a breath alcohol test will be taken before beginning the session. If the test is positive, the session will be cancelled and a new session time allocated.
6) I agree to refrain from the consumption of other drugs for three days prior to the experiment. Additionally, I agree to advise the investigator of any drugs consumed within the last three days, being aware that all information given is strictly confidential.
7) I agree to refrain from eating 2 hours prior to the testing session: and I understand that food will be given after the testing session.
8) I understand that I will be asked to consume alcohol that may result in a blood alcohol concentration 0.10%. This is equivalent to the blood alcohol level

achieved after drinking approximately 7-8 standard drinks (a standard drink = 1 glass of wine, a nip of scotch, or a pot of beer) over 2 hours.

9) I understand that this alcohol dose is likely to cause signs of intoxication. As such:

10) I agree, that upon commencement of the test session, I will not leave the grounds of James Cook University until released by the investigator. I further understand that the investigator will not release me until my blood alcohol level has returned to zero (0.00 g%).

11) I agree that it is compulsory for me to be driven to, and home from, the testing session at James Cook University Cairns.

12) I will be paid $100 on the completion of all four experimental sessions.

13) I understand that my participation in this research is completely voluntary and I may withdraw from the research at any time. However, I understand that if I withdraw and have already consumed alcohol that I must stay within the laboratory facilities until my breath alcohol concentration level has returned to zero.

14) I understand that only people involved with the research at James Cook University, Cairns will have access to the all information and data provided by me. I understand that all information will be treated with confidentiality and individuals will not be personally identifiable in any reports of this research.

15) Please circle Yes (Y) or No (N) for the following questions.

a. Have you used prescription/nonprescription drugs in the last month? Y N
b. Are you undergoing medical or psychiatric/psychological treatment? Y N
c. Have you previously sought treatment for alcohol problems? Y N
d. Can you consume, without becoming ill, 5-7 standard drinks in 1-2 hrs? Y N
e. Can you refrain from cigarette smoking for 3 hours without withdrawal? Y N

f. Can you refrain from caffeine for 3 hours without withdrawal? Y N

g. For females, do you have knowledge of being pregnant or are you attempting to fall pregnant? Y N

Appendix F: BAC data of pilot study

		Block		
Target BAC	Subject	1	2	3
0.02 g/100ml	1	0.008	0.008	0.006
	2	0.022	0.023	0.020
	3	0.014	0.014	0.015
	4	0.025	0.025	0.025
	5	0.014	0.016	0.017
	6	0.015	0.019	0.019
	7	0.019	0.016	0.013
	M	0.017	0.017	0.016
	SD	0.006	0.006	0.006
0.05 g/100ml	8	0.040	0.041	0.043
	9	0.040	0.042	0.044
	10	0.054	0.047	0.054
	11	0.047	0.049	0.050
	12	0.035	0.044	0.045
	M	0.043	0.045	0.047
	SD	0.007	0.003	0.005
0.10 g/100ml	13	0.077	0.078	0.078
	14	0.089	0.103	0.101
	15	0.078	0.087	0.100
	16	0.067	0.081	0.079
	M	0.078	0.087	0.090
	SD	0.009	0.011	0.013

Appendix G: Charting BAC Form

Name:		Subject No:	
Gender	**Weight (kg)**	**Height (cm)**	**Age (yr)**
Strength of Vodka	**Time (min)**		
Total Body Water	**Grams of ethanol**		
-2.1000	0.0000		
0.02	**0.05**	**0.08**	**0.10**

Misc:

Level required 0.03 Amount needed

Charting of alcohol consumption:

Time started:

Time finished:

Time started exp't:

Time finished exp't:

BrAC on arrival:

Time last ate:

Food comsumed

Time started drinking:

Time finished drinking:

Level required:

BrAC:		BrAC:	
Level	Time	Level	Time

Appendix H: SSS

Stanford Sleepiness Scale

Rate your alertness using the following scale

DEGREE OF SLEEPINESS	SCALE RATING
Feeling active, vital, alert, or wide awake	1
Functioning at high levels, but not at peak; able to concentrate	2
Awake, but relaxed; responsive but not fully alert	3
Somewhat foggy, let down	4
Foggy; losing interest in remaining awake; slowed down	5
Sleepy, woozy, fighting sleep, prefer to lie down	6
No longer fighting sleep, sleep onset soon; having dream-like thoughts	7
Asleep	X

Appendix I: Task instructions

Task Instructions

Instructions for these tasks are: -

(1) **Auditory Detection Task**: - A tone will be presented at random intervals. As soon as you hear the tone press the space bar key on the computer keyboard. The aim is have a fast reaction time to the presented tone.

(2) **Pursuit rotor task**: - Presented on the screen is a star. When the trial begins, an animated mouse within a target circle will appear on the right-hand side of the star. Using the computer mouse, you are required to keep the animated mouse within the target circle while the circle moves around the star. Please be aware that at the start of a new trial the animated mouse (or cursor) will appear inside the target circle at the right-hand side of the star. It is not necessary to move the cursor to this position.

(3) **Dual Task**: - This task combines both the auditory and tracking tasks. The previous instructions for these tasks apply. However, the primary aim is to give equal attention to both tasks so performance will not be comprised on either task. You are required to keep the animated mouse on the target as it moves around the star while at the same time responding quickly to the tone upon its presentation.

Appendix J: Waiver of release

Waiver

Following my consumption of alcohol as part of this study I participated in at James Cook University, Cairns, I have been shown my breathalyser reading and note that it is above 0.0%. I have been advised that I should not leave the University before my breathalyser has returned to 0.0% but have chosen to do so of my own free will. I have also been advised that I should under no circumstances drive a motor vehicle until I can be sure that my blood alcohol level is below the legal limit for driving in Queensland (0.05%).

Despite this I have left James Cook University against advice. I anticipate no adverse consequences arising from my participation in the study. I therefore release James Cook University or any employee, member or representative thereof, from all or any claim that I may have arising out of my participation in the study.

NAME _____

SIGNED _____

WITNESSED BY _____

DATE _____/_____/2000

Appendix K: Consent Form Study 2

CONSENT FORM

Charting the interface between Alcohol, Time of Day, Circadian Rhythms, and Performance

1) I _____ age _____ years agree to participate in the study conducted at James Cook University, Cairns.

2) The study explores the effects of drinking alcohol on my physiology and the ability to perform a number of tasks.

3) On arrival, 1 hour prior to alcohol ingestion, a breath alcohol test will be taken before beginning the session. If the test is positive, the session will be cancelled and a new session time allocated. If the test is negative the session will continue.

4) **Procedural Information:**

1. One hour before the consumption of alcohol a number of measurements will be taken. I will be weighed and my height measured to calculate the dosage of alcohol needed to reach the required blood alcohol concentration. Saliva samples will be taken before alcohol administration and at 4 points after alcohol ingestion. This is done to extract measures of cortisol (an arousal indicator), melatonin (sleep indicator), and immunoglobulin A (peripheral immune indicator). I will also be required to indicate my mood state throughout the session by indicating how I feel on a questionnaire designed to measure mood. Measurement of core body temperature, through the use of a rectal temperature thermometer connected to an electronic data logger, will begin 1 hour before alcohol administration and continue until I awake the next morning (approximately 17 hours of logging required). I have been given full written and verbal instructions of how to use the temperature logging equipment. My heart rate will also be measured before alcohol and after administration. However, I understand that this measurement will not give me any medical information about the condition of my heart. I have

been shown equipment, probes, datalogger, test tubes, heart rate monitor and electrodes, and the breathalyser, that will be used to measure these variables.

2. During the test session I will be in the psychology experimental laboratory or the waiting room. At all times the primary investigator or a research assistant will supervise me. I will be given the allocated dose of alcohol to be consumed over 30 minutes. I was specifically instructed to pace drinking equally over the allotted 30 minutes. I did not observe preparation of the beverages. During alcohol ingestion, breath alcohol analysis will be conducted twice (10 min, 20 min).
3. Thirty minutes after the completion of drinking, saliva samples will be collected (melatonin and cortisol), heart rate monitored, mood scales filled out, and testing on the computerised tasks undertaken (short term memory task, tracking, auditory detection, and a dual task (tracking and auditory detection)). I will be breathalysed every 15 minutes during the testing session.
4. The same procedure will be undertaken at 2 hr and 3.5 hr after the completion of drinking.
5. I will also give saliva samples (melatonin and cortisol) at 0900 hr the following day along with ratings made on the VAS. I will not have to return to the university the next day to produce saliva samples. I will be given a kit (test tubes and VAS) by the researcher to use at home. The researcher will collect my saliva samples and mood scales from me.
6. I have asked the primary researcher questions about the procedure and measurements and I have received satisfactory answers.

5) I agree to refrain from drinking alcoholic beverages for 24 hours prior to the testing session.

6) I agree to refrain from the consumption of other drugs for three days prior to the experiment.

7) I will be asked to consume a dosage of alcohol that may result in a blood alcohol concentration 0.10%. This is equivalent to the blood alcohol level achieved after drinking approximately 7-8 standard drinks (a standard drink = 1 glass of wine, a nip

of scotch, or a pot of beer) over 2 hours. This dose of alcohol is likely to cause signs of intoxication.

8) After commencement of the test session, I will not leave James Cook University until released by the investigator. The investigator will not release me until my blood alcohol level is zero.

9) I agree that it is compulsory for me to be driven to and home from the testing session at James Cook University Cairns.

10) I agree to refrain from eating 2 hours prior to the testing session. Food will be given after the testing session. I will have the light meal provided by the researcher 2-3 hours before arrival to the session.

11) I will be given $100 on completion of the fourth testing. I may also expect to gain information from the study that may be useful in understanding the effects of alcohol on the rhythms of my body and performance after drinking alcohol.

12) My participation in this research is completely voluntary and I may withdraw from the research at any time. However, I agree that if I withdraw and have already consumed alcohol than I must stay within the laboratory facilities until my breath alcohol concentration level has returned to zero.

13) Only people involved with the research at James Cook University, Cairns will have access to the data. All information will be treated with confidentiality and individuals will not be personally identifiable in any reports of this research.

14) I acknowledge that I have read the above statement that explains the nature and the objectives of the investigation to my satisfaction. Before signing this document I have been given the opportunity to ask questions relating to any possible risks I might encounter as a result of participating in this research, and I have received satisfactory answers.

15) In light of the foregoing I hereby release James Cook University, Cairns or any employee, member or representative thereof, from all or any claim that I may have arising out of my participation in this study. I understand that this document in no way limits my rights of law from any damage that might arise from negligence on the part of the investigators.

16) Please circle Yes (Y) or No (N) for the following questions.

a. Have you used prescription/nonprescription drugs in the last month? Y N
b. Are you undergoing medical or psychiatric/psychological treatment? Y N
c. Have you previously sought treatment for alcohol problems? Y N
d. Can you consume, without becoming ill, 5-7 standard drinks in 1-2 hrs? Y N
e. Can you refrain from cigarette smoking for 3 hours without withdrawal? Y N
f. Can you refrain from caffeine for 3 hours without withdrawal? Y N
g. For females, do you have knowledge of being pregnant or are you attempting to fall pregnant? Y N

Name of Participant _____

Signed _____Date_____

I certify that I have explained the study to the participant. I have given adequate and truthful answers to all questions raised by the participant. Thus, I consider that he/she understands what the experiment involves.

Signed (investigator) _____Date_____

Appendix L: Visual Analogue Scale

Uncoordinated	_____	Coordinated
Alert	_____	Not Alert
Not Sleepy	_____	Sleepy
Talkative	_____	Not Talkative
Not Fatigued	_____	Fatigued
Happy	_____	Not happy
Social	_____	Not social
Problems attending	_____	No problems attending
Not dizzy	_____	Dizzy

Not Drunk _____ Drunk

Comfortable _____ Not comfortable

Bored _____ Not bored

Nauseous _____ Not nauseous

Estimated BAC: _____%

Appendix M: Logging temperature instructions

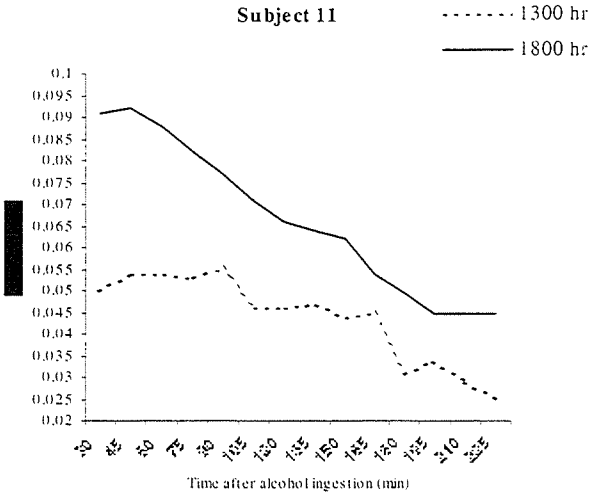
Logging Temperature Instructions

1. Remove probe from packaging. Connect the plug of the probe into the data logger. You should hear a click when the probe is correctly connected.
2. Slowly insert the other end of the probe into the rectum. It is suggested that 10-15cm of the probe be inserted to ensure the probe does not work itself free. Do not use any lubricant, as the probe may be more likely to work itself free.
3. The probe needs to be removed when showering and when performing bowel motions. Please reinsert the probe as soon as possible after these events. When removing and re-inserting the probe you can clean it with a dry tissue.
4. The logger can be kept in a pocket or underwear. Try to ensure the cord does not protrude loosely from the body as it may catch on objects.
5. When logging is complete you should remove the probe and disconnect it from the data logger. The probe is to be discarded.

Appendix N: Subject 9 Melatonin Data

Condition		Melatonin level pg/ml
911		0.73
912		6.25
913		15.12
914		27.56
915		4.70
921		0.00
922	No sample	0.00
923		0.00
924		0.00
925		1.30
931		27.36
932		7.69
933		18.40
934		8.80
935	Missed sample	
941		0.00
942		25.37
943		5.65
944		9.64
945		2.48

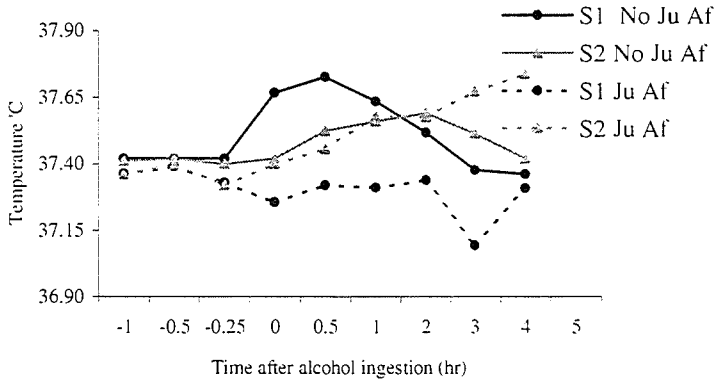
Appendix O: BAC curves for three subjects




Subject 12
1300 hr
1800 hr
0.1
0.095
0.09
0.085
0.08
0.075
0.07
0.065
0.06
0.055
0.05
0.045
0.04
0.035
0.03
0.025
0.02
Time after alcohol ingestion (min)

Appendix P: Effect of orange juice on body temperature of two subjects

Subject	Juice Admin	Juice cond	Time after alcohol ingestion: -1	-0.5	-0.25	0	0.5	1	2	3	4
			Solar time of day								
			12-1230	1230-1300	1300-1315	1315-1330	1330-1400	1400-1430	1430-1530	1530-1630	1630-1730
1	1300hr	No Juice	37.42	37.42	37.42	37.67	37.73	37.64	37.52	37.38	37.36
2			37.41	37.42	37.40	37.42	37.52	37.56	37.59	37.52	37.42
1		Juice	37.37	37.39	37.33	37.26	37.32	37.31	37.34	37.10	37.31
2			37.36	37.40	37.32	37.40	37.46	37.58	37.58	37.67	37.74
			1700-1730	1730-1800	1800-1815	1815-1830	1830-1900	1900-1930	1930-2030	2030-2130	2130-2230
1	1800hr	No Juice		38.03	37.90	37.79	37.83	37.84	37.84	37.31	37.15
2				37.60	37.38	37.33	37.32	37.25	37.24	37.30	37.01
1		Juice		37.56	37.49	37.37	37.39	37.65	37.22	37.07	36.88
2				37.18	37.16	37.16	37.16	37.16	37.07	36.97	36.91



Appendix Q: Statistical analyses for all studies

Study 1: BAC Study 1
Tests of Within-Subjects Contrasts

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	2.836E-02	73.211	.000
Error(DOSE)	Linear			11	3.874E-04		
TOD		Linear		1	1.174E-06	.002	.966
Error(TOD)		Linear		11	6.273E-04		
TIME			Linear	1	2.407E-04	6.370	.028
			Quadratic	1	3.335E-05	1.137	.309
Error(TIME)			Linear	11	3.778E-05		
			Quadratic	11	2.934E-05		
DOSE * TOD	Linear	Linear		1	7.951E-05	.156	.700
Error(DOSE* TOD)	Linear	Linear		11	5.094E-04		
DOSE * TIME	Linear		Linear	1	3.267E-05	1.457	.253
			Quadratic	1	1.168E-05	.970	.346
Error(DOSE* TIME)	Linear		Linear	11	2.242E-05		
			Quadratic	11	1.204E-05		
TOD * TIME		Linear	Linear	1	4.167E-08	.002	.963
			Quadratic	1	2.689E-05	3.038	.109
Error(TOD*TIME)		Linear	Linear	11	1.888E-05		
			Quadratic	11	8.851E-06		
DOSE * TOD * TIME	Linear	Linear	Linear	1	1.550E-04	7.027	.023
			Quadratic	1	5.556E-06	1.420	.258
Error(DOSE* TOD*TIME)	Linear	Linear	Linear	11	2.206E-05		
			Quadratic	11	3.912E-06		

Study 1: Performance tasks
Tests of Within-Subjects Contrasts

Source	Measure	DOSE	TOD	df	Mean Square	F	Sig.
DOSE	PR	Linear		1	1.997E-02	2.997	.111
	DTPR	Linear		1	7.386E-02	1.798	.207
	Aud	Linear		1	6007.411	16.561	.002
	DTAud	Linear		1	5943.900	8.494	.014
Error(DOSE)	PR	Linear		11	6.663E-03		
	DTPR	Linear		11	4.108E-02		
	Aud	Linear		11	362.754		
	DTAud	Linear		11	699.812		
TOD	PR		Linear	1	5.434E-03	.222	.647
	DTPR		Linear	1	5.262E-02	1.996	.185
	Aud		Linear	1	115.402	.127	.729
	DTAud		Linear	1	.710	.001	.978
Error(TOD)	PR		Linear	11	2.451E-02		
	DTPR		Linear	11	2.636E-02		
	Aud		Linear	11	911.171		
	DTAud		Linear	11	891.780		
DOSE * TOD	PR	Linear	Linear	1	.148	15.223	.002
	DTPR	Linear	Linear	1	.429	9.724	.010
	Aud	Linear	Linear	1	588.267	.878	.369

	DTAud	Linear	Linear	1	6635.518	4.777	.051
Error(DOSE*TOD)	PR	Linear	Linear	11	9.751E-03		
	DTPR	Linear	Linear	11	4.412E-02		
	Aud	Linear	Linear	11	669.940		
	DTAud	Linear	Linear	11	1388.913		

Study 1: Error

Source	DOSE	TOD	df	Mean Square	F	Sig.
DOSE	Linear		1	1.752E-03	2.235	.163
Error(DOSE)	Linear		11	7.839E-04		
TOD		Linear	1	4.688E-04	1.170	.303
Error(TOD)		Linear	11	4.006E-04		
DOSE * TOD	Linear	Linear	1	1.875E-05	.117	.738
Error(DOSE*TOD)	Linear	Linear	11	1.597E-04		

Study 1: SSS

Tests of Within-Subjects Contrasts

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	2.344	3.947	.072
Error(DOSE)	Linear			11	.594		
TOD		Linear		1	1.042E-02	.018	.896
Error(TOD)		Linear		11	.579		
TIME			Linear	1	3.760	2.830	.121
Error(TIME)			Linear	11	1.329		
DOSE * TOD	Linear	Linear		1	9.375E-02	.142	.714
Error(DOSE*TOD)	Linear	Linear		11	.662		
DOSE * TIME	Linear		Linear	1	1.760	3.449	.090
Error(DOSE*TIME)	Linear		Linear	11	.510		
TOD * TIME		Linear	Linear	1	9.375E-02	.371	.555
Error(TOD*TIME)		Linear	Linear	11	.253		
DOSE * TOD * TIME	Linear	Linear	Linear	1	.510	1.669	.223
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	11	.306		

Study 1: Contrasts for performance (additional experimental sessions)

Tests of Within-Subjects Contrasts

Source	Measure	Measure	DOSE	TOD	df	Mean Square	F	Sig.
DOSE	PR	TR	Linear		1	4.266E-03	.201	.663
	DTPR	DTTR	Linear		1	9.712E-02	6.896	.024
	Aud	TONE	Linear		1	4423.539	5.554	.038
	DTAud	DTTO	Linear		1	18378.859	11.869	.005
Error(DOSE)	PR	TR	Linear		11	2.125E-02		
	DTPR	DTTR	Linear		11	1.408E-02		
	Aud	TONE	Linear		11	796.448		
	DTAud	DTTO	Linear		11	1548.495		
TOD	PR	TR		Linear	1	3.790E-03	.405	.538
	DTPR	DTTR		Linear	1	2.373E-02	2.885	.118
	Aud	TONE		Linear	1	1.301	.002	.964
	DTAud	DTTO		Linear	1	63.450	.117	.739
Error(TOD)	PR	TR		Linear	11	9.369E-03		
	DTPR	DTTR		Linear	11	8.226E-03		
	Aud	TONE		Linear	11	626.049		

	DTAud	DTTO		Linear	11	542.632		
DOSE * TOD	PR	TR	Linear	Linear	1	6.696E-03	1.698	.219
	DTPR	DTTR	Linear	Linear	1	3.536E-02	6.230	.030
	Aud	TONE	Linear	Linear	1	178.206	.290	.601
	DTAud	DTTO	Linear	Linear	1	.114	.000	.991
Error(DOSE*TOD)	PR	TR	Linear	Linear	11	3.942E-03		
	DTPR	DTTR	Linear	Linear	11	5.676E-03		
	Aud	TONE	Linear	Linear	11	615.357		
	DTAud	DTTO	Linear	Linear	11	799.143		

Study1: Error for the DT auditory detection (additional experimental sessions)

Source	DOSE	TOD	df	Mean Square	F	Sig.
DOSE	Linear		1	9.187	2.320	.156
Error(DOSE)	Linear		11	3.960		
TOD		Linear	1	2.083E-02	.021	.886
Error(TOD)		Linear	11	.975		
DOSE * TOD	Linear	Linear	1	4.687	2.448	.146
Error(DOSE*TOD)	Linear	Linear	11	1.915		

Study 2: BAC

Source	TOD	TIME	df	Mean Square	F	Sig.
TOD	Linear		1	4.046E-05	.034	.858
Error(TOD)	Linear		11	1.207E-03		
TIME		Linear	1	4.733E-02	227.236	.000
		Quadratic	1	4.175E-03	32.879	.000
		Cubic	1	7.942E-04	23.466	.001
Error(TIME)		Linear	11	2.083E-04		
		Quadratic	11	1.270E-04		
		Cubic	11	3.384E-05		
TOD * TIME	Linear	Linear	1	7.430E-04	6.649	.026
		Quadratic	1	1.027E-04	3.524	.087
		Cubic	1	6.436E-05	4.216	.065
Error(TOD*TIME)	Linear	Linear	11	1.117E-04		
		Quadratic	11	2.915E-05		
		Cubic	11	1.527E-05		

Study 2: Temperature analysis acute effects afternoon conditions
Tests of Within-Subjects Contrasts

Source	DOSE	TIME	df	Mean Square	F	Sig.
DOSE	Linear		1	.853	6.390	.032
Error(DOSE)	Linear		9	.134		
TIME		Linear	1	1.221	27.582	.001
		Quadratic	1	.755	48.884	.000
		Cubic	1	2.725E-02	2.388	.157
Error(TIME)		Linear	9	4.426E-02		
		Quadratic	9	1.545E-02		
		Cubic	9	1.142E-02		
DOSE * TIME	Linear	Linear	1	2.551E-02	.290	.603
		Quadratic	1	.115	5.721	.040
		Cubic	1	2.984E-03	.220	.650
Error(DOSE*TIME)	Linear	Linear	9	8.788E-02		
		Quadratic	9	2.007E-02		

	Cubic	9	1.356E-02

Study 2: Temperature analysis acute effects evening conditions
Tests of Within-Subjects Contrasts

Source	DOSE	TIME	df	Mean Square	F	Sig.
DOSE	Linear		1	2.274E-02	.130	.726
Error(DOSE)	Linear		9	.174		
TIME		Linear	1	1.501	10.498	.010
		Quadratic	1	.264	14.175	.004
		Cubic	1	3.106E-02	4.921	.054
Error(TIME)		Linear	9	.143		
		Quadratic	9	1.865E-02		
		Cubic	9	6.311E-03		
DOSE * TIME	Linear	Linear	1	8.408E-02	1.685	.226
		Quadratic	1	6.038E-03	.311	.590
		Cubic	1	4.430E-04	.055	.819
Error(DOSE*TIME)	Linear	Linear	9	4.988E-02		
		Quadratic	9	1.939E-02		
		Cubic	9	7.989E-03		

Study 2: Heart rate
Tests of Within-Subjects Contrasts

Source	DOSE	TOD	BLOCK	TIME	df	Mean Square	F	Sig.
DOSE	Linear				1	22709.131	58.797	.000
Error(DOSE)	Linear				11	386.229		
TOD		Linear			1	847.502	2.270	.160
Error(TOD)		Linear			11	373.404		
BLOCK			Linear		1	895.810	8.673	.013
			Quadratic		1	3896.625	32.071	.000
Error(BLOCK)			Linear		11	103.290		
			Quadratic		11	121.502		
TIME				Linear	1	383.470	15.559	.002
				Quadratic	1	204.160	.859	.374
				Cubic	1	181.359	7.886	.017
Error(TIME)				Linear	11	24.646		
				Quadratic	11	237.552		
				Cubic	11	22.998		
DOSE * TOD	Linear	Linear			1	1342.819	6.836	.024
Error(DOSE*TOD)	Linear	Linear			11	196.438		
DOSE * BLOCK	Linear		Linear		1	230.330	2.310	.157
			Quadratic		1	201.095	1.388	.264
Error(DOSE*BLOCK)	Linear		Linear		11	99.722		
			Quadratic		11	144.836		
TOD * BLOCK		Linear	Linear		1	124.973	2.054	.180
			Quadratic		1	10.484	.119	.736
Error(TOD*BLOCK)		Linear	Linear		11	60.845		
			Quadratic		11	87.918		
DOSE * TOD * BLOCK	Linear	Linear	Linear		1	1205.830	13.189	.004
			Quadratic		1	157.855	1.625	.229

Error(DOSE*TOD*BLOCK)	Linear	Linear	Linear		11	91.425		
			Quadratic		11	97.122		
DOSE * TIME	Linear			Linear	1	21.748	.331	.576
				Quadratic	1	144.430	2.977	.112
				Cubic	1	58.413	2.283	.159
Error(DOSE*TIME)	Linear			Linear	11	65.638		
				Quadratic	11	48.511		
				Cubic	11	25.582		
TOD * TIME		Linear		Linear	1	45.300	1.695	.220
				Quadratic	1	31.184	1.130	.311
				Cubic	1	16.743	.361	.560
Error(TOD*TIME)		Linear		Linear	11	26.732		
				Quadratic	11	27.595		
				Cubic	11	46.363		
DOSE * TOD * TIME	Linear	Linear		Linear	1	23.800	1.451	.254
				Quadratic	1	5.592	.059	.812
				Cubic	1	28.273	.577	.463
Error(DOSE*TOD*TIME)	Linear	Linear		Linear	11	16.406		
				Quadratic	11	94.715		
				Cubic	11	48.991		
BLOCK * TIME			Linear	Linear	1	.726	.027	.871
				Quadratic	1	7.368	.372	.554
				Cubic	1	1.734	.289	.602
			Quadratic	Linear	1	12.151	.539	.478
				Quadratic	1	.663	.020	.890
				Cubic	1	.146	.010	.922
Error(BLOCK*TIME)			Linear	Linear	11	26.462		
				Quadratic	11	19.821		
				Cubic	11	5.999		
			Quadratic	Linear	11	22.530		
				Quadratic	11	33.288		
				Cubic	11	14.745		
DOSE * BLOCK * TIME	Linear		Linear	Linear	1	7.555	1.221	.293
				Quadratic	1	13.605	.458	.513
				Cubic	1	29.586	2.054	.180
			Quadratic	Linear	1	20.216	.455	.514
				Quadratic	1	2.470E-03	.000	.991
				Cubic	1	7.895	.386	.547
Error(DOSE*BLOCK*TIME)	Linear		Linear	Linear	11	6.190		
				Quadratic	11	29.704		
				Cubic	11	14.405		
			Quadratic	Linear	11	44.455		
				Quadratic	11	18.041		
				Cubic	11	20.435		
TOD * BLOCK * TIME		Linear	Linear	Linear	1	3.662	.251	.626
				Quadratic	1	2.518	.072	.793
				Cubic	1	22.445	1.294	.279
			Quadratic	Linear	1	18.723	1.341	.271
				Quadratic	1	1.066	.107	.749
				Cubic	1	4.601	.337	.573
Error(TOD*BLO		Linear	Linear	Linear	11	14.607		

CK*TIME)								
				Quadratic	11	34.779		
				Cubic	11	17.346		
			Quadratic	Linear	11	13.962		
				Quadratic	11	9.941		
				Cubic	11	13.653		
DOSE * TOD * BLOCK * TIME	Linear	Linear	Linear	Linear	1	14.538	.738	.409
				Quadratic	1	66.870	7.007	.023
				Cubic	1	.342	.048	.831
			Quadratic	Linear	1	12.450	.240	.634
				Quadratic	1	1.619	.188	.673
				Cubic	1	9.367	.695	.422
Error(DOSE*TOD*BLOCK*TIME)	Linear	Linear	Linear	Linear	11	19.695		
				Quadratic	11	9.543		
				Cubic	11	7.180		
			Quadratic	Linear	11	51.889		
				Quadratic	11	8.626		
				Cubic	11	13.473		

Study 2: Heart rate excluding 210m block

Tests of Within-Subjects Contrasts

Source	DOSE	TOD	BLOCK	TIME	df	Mean Square	F	Sig.
DOSE	Linear				1	18150.642	51.013	.000
Error(DOSE)	Linear				11	355.807		
TOD		Linear			1	297.406	1.078	.321
Error(TOD)		Linear			11	275.828		
BLOCK			Linear		1	1528.405	13.271	.004
Error(BLOCK)			Linear		11	115.173		
TIME				Linear	1	237.425	10.437	.008
				Quadratic	1	163.550	.818	.385
				Cubic	1	109.156	5.760	.035
Error(TIME)				Linear	11	22.748		
				Quadratic	11	199.948		
				Cubic	11	18.950		
DOSE * TOD	Linear	Linear			1	2591.783	16.549	.002
Error(DOSE* TOD)	Linear	Linear			11	156.612		
DOSE * BLOCK	Linear		Linear		1	22.021	.159	.698
Error(DOSE* BLOCK)	Linear		Linear		11	138.637		
TOD * BLOCK		Linear	Linear		1	7.759	.073	.791
Error(TOD* BLOCK)		Linear	Linear		11	105.730		
DOSE * TOD * BLOCK	Linear	Linear	Linear		1	42.013	.669	.431
Error(DOSE* TOD*BLOCK)	Linear	Linear	Linear		11	62.815		
DOSE * TIME	Linear			Linear	1	15.086	.242	.632
				Quadratic	1	63.723	1.664	.224
				Cubic	1	66.405	2.856	.119
Error(DOSE* TIME)	Linear			Linear	11	62.261		
				Quadratic	11	38.300		
				Cubic	11	23.247		
TOD * TIME		Linear		Linear	1	59.312	3.115	.105
				Quadratic	1	31.934	1.268	.284
				Cubic	1	25.914	.456	.513

Error(TOD* TIME)		Linear		Linear	11	19.039		
				Quadratic	11	25.182		
				Cubic	11	56.777		
DOSE * TOD * TIME	Linear	Linear		Linear	1	1.120	.065	.803
				Quadratic	1	6.377	.093	.767
				Cubic	1	14.067	.299	.596
Error(DOSE* TOD*TIME)	Linear	Linear		Linear	11	17.103		
				Quadratic	11	68.934		
				Cubic	11	47.087		
BLOCK * TIME			Linear	Linear	1	11.866	.332	.576
				Quadratic	1	4.252	.115	.741
				Cubic	1	.980	.088	.773
Error(BLOCK* TIME)			Linear	Linear	11	35.775		
				Quadratic	11	37.037		
				Cubic	11	11.174		
DOSE * BLOCK * TIME	Linear		Linear	Linear	1	27.754	.761	.402
				Quadratic	1	3.562	.112	.744
				Cubic	1	26.554	.860	.374
Error(DOSE* BLOCK*TIME)	Linear		Linear	Linear	11	36.469		
				Quadratic	11	31.669		
				Cubic	11	30.881		
TOD * BLOCK * TIME		Linear	Linear	Linear	1	7.787	.496	.496
				Quadratic	1	1.018E-02	.001	.982
				Cubic	1	17.862	1.254	.287
Error(TOD* BLOCK*TIME)		Linear	Linear	Linear	11	15.703		
				Quadratic	11	18.104		
				Cubic	11	14.239		
DOSE * TOD * BLOCK * TIME	Linear	Linear	Linear	Linear	1	1.321	.022	.885
				Quadratic	1	8.920	1.286	.281
				Cubic	1	8.662	.779	.396
Error(DOSE* TOD*BLOCK* TIME)	Linear	Linear	Linear	Linear	11	60.177		
				Quadratic	11	6.938		
				Cubic	11	11.112		

Study 2: Performance tasks
Tests of Within-Subjects Contrasts

Source	Measure	DOSE	TOD	BLOCK	df	Mean Square	F	Sig.
DOSE	DT Aud	Linear			1	57581.272	20.059	.001
	Aud	Linear			1	17364.695	9.899	.009
	DT PR	Linear			1	7.468	14.154	.003
	PR	Linear			1	7.916	12.738	.004
Error(DOSE)	DT Aud	Linear			11	2870.619		
	Aud	Linear			11	1754.129		
	DT PR	Linear			11	.528		
	PR	Linear			11	.621		
TOD	DT Aud		Linear		1	117 928	.103	.754
	Aud		Linear		1	4004.019	2.295	.158

	DT PR		Linear		1	.316	1.133	.310
	PR		Linear		1	8.400E-02	.201	.662
Error(TOD)	DT Aud		Linear		11	1146.298		
	Aud		Linear		11	1744.505		
	DT PR		Linear		11	.279		
	PR		Linear		11	.417		
BLOCK	DTAud			Linear	1	223.124	.123	.733
				Quadratic	1	459.953	.523	.485
	Aud			Linear	1	3933.179	2.440	.147
				Quadratic	1	.250	.000	.983
	DTPR			Linear	1	1.043	2.900	.117
				Quadratic	1	.115	.574	.465
	PR			Linear	1	.693	1.662	.224
				Quadratic	1	.204	1.955	.190
Error(BLOCK)	DTAud			Linear	11	1821.300		
				Quadratic	11	880.271		
	Aud			Linear	11	1612.007		
				Quadratic	11	513.920		
	DTPR			Linear	11	.360		
				Quadratic	11	.201		
	PR			Linear	11	.417		
				Quadratic	11	.104		
DOSE * TOD	DT Aud	Linear	Linear		1	2567.681	2.151	.170
	Aud	Linear	Linear		1	392.795	.606	.453
	DT PR	Linear	Linear		1	3.630E-03	.029	.869
	PR	Linear	Linear		1	.346	1.562	.237
Error(DOSE*TOD)	DT Aud	Linear	Linear		11	1193.599		
	Aud	Linear	Linear		11	648.454		
	DT PR	Linear	Linear		11	.127		
	PR	Linear	Linear		11	.222		
DOSE * BLOCK	DTAud	Linear		Linear	1	17568.871	8.746	.013
				Quadratic	1	154 364	.191	.670
	Aud	Linear		Linear	1	1256.690	1.823	.204
				Quadratic	1	49.006	.060	.812
	DTPR	Linear		Linear	1	1.430	3.193	.102
				Quadratic	1	1.925E-02	.282	.606
	PR	Linear		Linear	1	1.278	2.430	.147
				Quadratic	1	1.167E-02	.105	.752
Error(DOSE*BLOCK)	DTAud	Linear		Linear	11	2008.759		
				Quadratic	11	806.344		
	Aud	Linear		Linear	11	689.346		
				Quadratic	11	821.191		
	DTPR	Linear		Linear	11	.448		
				Quadratic	11	6.834E-02		
	PR	Linear		Linear	11	.526		
				Quadratic	11	.111		
TOD * BLOCK	DTAud		Linear	Linear	1	503.930	.196	.666
				Quadratic	1	106.741	.281	.607
	Aud		Linear	Linear	1	5.217	.003	.955
				Quadratic	1	405.489	.536	.479
	DTPR		Linear	Linear	1	3.735E-02	.161	.696
				Quadratic	1	1.021E-02	.070	.796
	PR		Linear	Linear	1	6.356E-02	.963	.348
				Quadratic	1	3.417E-03	.068	.799

Error(TOD* BLOCK)	DTAud		Linear	Linear	11	2569.497		
				Quadratic	11	380.239		
	Aud		Linear	Linear	11	1568.137		
				Quadratic	11	756.492		
	DTPR		Linear	Linear	11	.232		
				Quadratic	11	.145		
	PR		Linear	Linear	11	6.600E-02		
				Quadratic	11	5.045E-02		
DOSE * TOD * BLOCK	DTAud	Linear	Linear	Linear	1	58.201	.047	.833
				Quadratic	1	445.587	.513	.489
	Aud	Linear	Linear	Linear	1	1071.568	2.769	.124
				Quadratic	1	338.675	.488	.500
	DTPR	Linear	Linear	Linear	1	3.015E-02	.577	.464
				Quadratic	1	6.496E-03	.068	.800
	PR	Linear	Linear	Linear	1	.133	.687	.425
				Quadratic	1	5.816E-02	.567	.467
Error(DOSE*TOD*BLOCK)	DTAud	Linear	Linear	Linear	11	1242.302		
				Quadratic	11	868.005		
	Aud	Linear	Linear	Linear	11	386.981		
				Quadratic	11	694.614		
	DTPR	Linear	Linear	Linear	11	5.226E-02		
				Quadratic	11	9.603E-02		
	PR	Linear	Linear	Linear	11	.194		
				Quadratic	11	.103		

Study 2: Melatonin

Tests of Within-Subjects Contrasts

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	5.343	7.052	.024
Error(DOSE)	Linear			10	.758		
TOD		Linear		1	12.918	2.073	.181
Error(TOD)		Linear		10	6.233		
TIME			Linear	1	1.242	.657	.436
			Quadratic	1	1.882	18.534	.002
Error(TIME)			Linear	10	1.889		
			Quadratic	10	.102		
DOSE * TOD	Linear	Linear		1	7.842E-02	.155	.702
Error(DOSE* TOD)	Linear	Linear		10	.505		
DOSE * TIME	Linear		Linear	1	7.614	12.552	.005
			Quadratic	1	5.846E-03	.033	.860
Error(DOSE* TIME)	Linear		Linear	10	.607		
			Quadratic	10	.178		
TOD * TIME		Linear	Linear	1	21.429	8.754	.014
			Quadratic	1	2.183E-03	.010	.923
Error(TOD*TIME)		Linear	Linear	10	2.448		
			Quadratic	10	.222		
DOSE * TOD * TIME	Linear	Linear	Linear	1	5.876E-02	.150	.707
			Quadratic	1	2.424	6.259	.031
Error(DOSE* TOD*TIME)	Linear	Linear	Linear	10	.392		

	Quadratic	10	.387

Study 2: Melatonin afternoon condition
Tests of Within-Subjects Contrasts

Source	DOSE	TIME	df	Mean Square	F	Sig.
DOSE	Linear		1	3.358	8.918	.014
Error(DOSE)	Linear		10	.377		
TIME		Linear	1	6.177	36.608	.000
		Quadratic	1	.878	7.349	.022
Error(TIME)		Linear	10	.169		
		Quadratic	10	.119		
DOSE * TIME	Linear	Linear	1	4.505	10.206	.010
		Quadratic	1	1.334	18.279	.002
Error(DOSE*TIME)	Linear	Linear	10	.441		
		Quadratic	10	7.299E-02		

Study 2: Melatonin evening condition
Tests of Within-Subjects Contrasts

Source	DOSE	TIME	Type III Sum of Squares	df	Mean Square	F	Sig.
DOSE	Linear		2.063	1	2.063	2.327	.158
Error(DOSE)	Linear		8.866	10	.887		
TIME		Linear	16.495	1	16.495	3.957	.075
		Quadratic	1.006	1	1.006	4.922	.051
Error(TIME)		Linear	41.683	10	4.168		
		Quadratic	2.044	10	.204		
DOSE * TIME	Linear	Linear	3.167	1	3.167	5.690	.038
		Quadratic	1.096	1	1.096	2.228	.166
Error(DOSE*TIME)	Linear	Linear	5.567	10	.557		
		Quadratic	4.919	10	.492		

Study 2: Cortisol
Tests of Within-Subjects Contrasts

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	.247	.251	.626
Error(DOSE)	Linear			11	.983		
TOD		Linear		1	38.728	44.890	.000
Error(TOD)		Linear		11	.863		
TIME			Linear	1	2.371E-03	.003	.960
			Quadratic	1	3.459	12.012	.005
Error(TIME)			Linear	11	.882		
			Quadratic	11	.288		
DOSE * TOD	Linear	Linear		1	.625	.813	.386
Error(DOSE*TOD)	Linear	Linear		11	.768		
DOSE * TIME	Linear		Linear	1	8.703E-02	.075	.790
			Quadratic	1	.280	1.559	.238
Error(DOSE*TIME)	Linear		Linear	11	1.164		
			Quadratic	11	.180		

TOD * TIME		Linear	Linear	1	.441	.442	.520
			Quadratic	1	.299	.965	.347
Error(TOD*TIME)		Linear	Linear	11	.996		
			Quadratic	11	.310		
DOSE * TOD * TIME	Linear	Linear	Linear	1	5.301E-03	.008	.932
			Quadratic	1	.886	5.356	.041
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	11	.687		
			Quadratic	11	.165		

Study 2: Rotated component matrixes for VAS for the four experimental conditions

Rotated Component Matrix

	Component		
	1	2	3
ALERT11	.437	.829	
TALK11	.834	.328	
HAP11	.892	.378	
SOC11	.922		
COM11	.734	.585	
NAU11			.931
BOR11	-.885		
CO11	.377	.844	
SLEEP11		-.841	
FAT11	-.702	-.639	
ATT11	.806		-.300
DIZ11	-.942		
DRU11			.708

Rotated Component Matrix

	Component			
	1	2	3	4
ALERT32	.752			
TALK32	.355	.877		
HAP32		.965		
SOC32		.968		
COM32			.513	-.400
NAU32			.945	
BOR32			.970	
CO32	.871			
SLEEP32	-.822			
FAT32	-.796	-.403		
ATT32	.907			
DIZ32				.913
DRU32				.897

Rotated Component Matrix

	Component		
	1	2	3

ALERT21	.371	-.635	.605
TALK21	.849		
HAP21	.955		
SOC21	.846		
COM21	.874		-.383
NAU21		.822	-.347
BOR21	-.505	.678	.338
CO21	.650		.443
SLEEP21	-.475	-.485	-.383
FAT21	-.365		.691
ATT21	.804		
DIZ21		.564	-.476
DRU21		.715	.453

Rotated Component Matrix

	Component		
	1	2	3
ALERT42	.611	.633	
TALK42	.945		
HAP42	.953		
SOC42	.824		
COM42	.398	.737	-.364
NAU42			.914
BOR42	-.676		.578
CO42		.872	
SLEEP42	-.773	-.523	
FAT42	-.758	-.339	.471
ATT42	.838		-.410
DIZ42		-.606	.642
DRU42	.334	-.748	.406

Study 2: VAS

Tests of Within-Subjects Contrasts

Physical Factor

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	105.593	21.836	.001
Error(DOSE)	Linear			11	4.836		
TOD		Linear		1	.464	.158	.698
Error(TOD)		Linear		11	2.925		
TIME			Linear	1	4.950	6.271	.029
			Quadratic	1	.830	1.049	.328
Error(TIME)			Linear	11	.789		
			Quadratic	11	.791		
DOSE * TOD	Linear	Linear		1	4.344	1.636	.227
Error(DOSE*TOD)	Linear	Linear		11	2.655		
DOSE * TIME	Linear		Linear	1	3.674	3.945	.073
			Quadratic	1	.439	.356	.563
Error(DOSE*TIME)	Linear		Linear	11	.931		

			Quadratic	11	1.233		
TOD * TIME		Linear	Linear	1	.435	.646	.439
			Quadratic	1	.373	.475	.505
Error(TOD*TIME)		Linear	Linear	11	.673		
			Quadratic	11	.785		
DOSE * TOD * TIME	Linear	Linear	Linear	1	1.260	1.464	.252
			Quadratic	1	4.835E-03	.007	.937
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	11	.861		
			Quadratic	11	.736		

Tests of Within-Subjects Contrasts
Cognitive

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	.120	.093	.766
Error(DOSE)	Linear			11	1.285		
TOD		Linear		1	4.393	2.862	.119
Error(TOD)		Linear		11	1.535		
TIME			Linear	1	9.773	11.376	.006
			Quadratic	1	1.005	4.051	.069
Error(TIME)			Linear	11	.859		
			Quadratic	11	.248		
DOSE * TOD	Linear	Linear		1	8.867E-03	.010	.921
Error(DOSE*TOD)	Linear	Linear		11	.853		
DOSE * TIME	Linear		Linear	1	6.166	4.227	.064
			Quadratic	1	2.223E-02	.110	.747
Error(DOSE*TIME)	Linear		Linear	11	1.459		
			Quadratic	11	.202		
TOD * TIME		Linear	Linear	1	.915	1.549	.239
			Quadratic	1	.531	.838	.380
Error(TOD*TIME)		Linear	Linear	11	.591		
			Quadratic	11	.634		
DOSE * TOD * TIME	Linear	Linear	Linear	1	1.385	3.450	.090
			Quadratic	1	.251	.687	.425
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	11	.401		
			Quadratic	11	.366		

Tests of Within-Subjects Contrasts
Gregariousness

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	3.816	.645	.439
Error(DOSE)	Linear			11	5.919		
TOD		Linear		1	6.208	1.645	.226
Error(TOD)		Linear		11	3.774		
TIME			Linear	1	1.238E-02	.012	.916
			Quadratic	1	.766	2.879	.118
Error(TIME)			Linear	11	1.068		
			Quadratic	11	.266		
DOSE * TOD	Linear	Linear		1	.148	.038	.850
Error(DOSE*T	Linear	Linear		11	3.930		

OD)							
DOSE * TIME	Linear		Linear	1	8.730	6.261	.029
			Quadratic	1	.225	.284	.605
Error(DOSE*TIME)	Linear		Linear	11	1.394		
			Quadratic	11	.791		
TOD * TIME		Linear	Linear	1	1.159	.851	.376
			Quadratic	1	.397	.738	.409
Error(TOD*TIME)		Linear	Linear	11	1.363		
			Quadratic	11	.538		
DOSE * TOD * TIME	Linear	Linear	Linear	1	.174	.198	.665
			Quadratic	1	.142	.216	.651
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	11	.881		
			Quadratic	11	.656		

Study 3: Temperature
Tests of Within-Subjects Contrasts

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	.459	.763	.405
Error(DOSE)	Linear			9	.601		
TOD		Linear		1	9.199	34.206	.000
Error(TOD)		Linear		9	.269		
TIME			Linear	1	15.904	72.464	.000
			Quadratic	1	.103	.807	.392
			Cubic	1	.746	8.146	.019
Error(TIME)			Linear	9	.219		
			Quadratic	9	.127		
			Cubic	9	9.157E-02		
DOSE * TOD	Linear	Linear		1	.263	.439	.524
Error(DOSE*TOD)	Linear	Linear		9	.599		
DOSE * TIME	Linear		Linear	1	.318	3.084	.113
			Quadratic	1	.109	1.894	.202
			Cubic	1	7.191E-02	2.252	.168
Error(DOSE*TIME)	Linear		Linear	9	.103		
			Quadratic	9	5.774E-02		
			Cubic	9	3.193E-02		
TOD * TIME		Linear	Linear	1	4.017	13.242	.005
			Quadratic	1	2.372	33.936	.000
			Cubic	1	.354	5.122	.050
Error(TOD*TIME)		Linear	Linear	9	.303		
			Quadratic	9	6.990E-02		
			Cubic	9	6.914E-02		
DOSE * TOD * TIME	Linear	Linear	Linear	1	.932	5.536	.043
			Quadratic	1	7.872E-06	.000	.995
			Cubic	1	5.339E-02	.916	.363
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	9	.168		
			Quadratic	9	.178		
			Cubic	9	5.827E-02		

Study 3: Temperature afternoon conditions

Tests of Within-Subjects Contrasts

Source	DOSE	TIME	df	Mean Square	F	Sig.
DOSE	Linear		1	1.356E-02	.013	.912
Error(DOSE)	Linear		9	1.054		
TIME		Linear	1	17.953	60.408	.000
		Quadratic	1	.744	7.735	.021
		Cubic	1	1.064	12.649	.006
Error(TIME)		Linear	9	.297		
		Quadratic	9	9.620E-02		
		Cubic	9	8.412E-02		
DOSE * TIME	Linear	Linear	1	1.170	8.477	.017
		Quadratic	1	5.376E-02	.321	.585
		Cubic	1	.125	2.955	.120
Error(DOSE*TIME)	Linear	Linear	9	.138		
		Quadratic	9	.167		
		Cubic	9	4.216E-02		

Study 3: Sleep phase (2330 hr – 0830 hr) core body temperature

Tests of Within-Subjects Contrasts

Source	DOSE	TOD	TIME	df	Mean Square	F	Sig.
DOSE	Linear			1	2.185	6.461	.032
Error(DOSE)	Linear			9	.338		
TOD		Linear		1	.363	1.607	.237
Error(TOD)		Linear		9	.226		
TIME			Linear	1	2.432	7.132	.026
			Quadratic	1	5.891	31.267	.000
			Cubic	1	.146	2.388	.157
Error(TIME)			Linear	9	.341		
			Quadratic	9	.188		
			Cubic	9	6.117E-02		
DOSE * TOD	Linear	Linear		1	5.415E-02	.247	.631
Error(DOSE*TOD)	Linear	Linear		9	.219		
DOSE * TIME	Linear		Linear	1	5.721E-04	.002	.967
			Quadratic	1	9.304E-04	.008	.932
			Cubic	1	7.581E-02	2.028	.188
Error(DOSE*TIME)	Linear		Linear	9	.316		
			Quadratic	9	.123		
			Cubic	9	3.739E-02		
TOD * TIME		Linear	Linear	1	.141	1.363	.273
			Quadratic	1	.316	6.650	.030
			Cubic	1	1.066E-02	.159	.700
Error(TOD*TIME)		Linear	Linear	9	.103		
			Quadratic	9	4.758E-02		
			Cubic	9	6.714E-02		
DOSE * TOD * TIME	Linear	Linear	Linear	1	.140	.654	.439
			Quadratic	1	9.800E-02	2.898	.123
			Cubic	1	9.083E-02	2.681	.136
Error(DOSE*TOD*TIME)	Linear	Linear	Linear	9	.214		
			Quadratic	9	3.382E-02		

		Cubic	9	3.388E-02	

Study 3: VAS

Source	Measure	df	Mean Square	F	Sig.
Dose	Physical	1	18.533E-03	.038	849
	Social	1	8.953	2.676	.130
	Cognitive	1	19.075E-03	.034	.857
Error (Dose)	Physical	11	.225	075	.789
	Social	11	3.346	.135	.721
	Cognitive	11	.266	096	.763
TOD	Physical	1	3.203E-02		
	Social	1	.420		
	Cognitive	1	4.441 E-02		
Error (TOD)	Physical	11	.424		
	Social	11	3.122		
	Cognitive	11	.463		
Dose * TOD	Physical	1	3.172	1.109	.315
	Social	1	19.853	8.685	.013
	Cognitive	1	1.222	1.283	.281
Error (Dose * TOD)	Physical	11	2.862		
	Social	11	2.286		
	Cognitive	11	.953		