

**The following Abstract is the verbatim abstract for the entire research proposal. The Research Plan, however, includes the specific aims and research protocols only as they pertain to Study 2. Study 1 and Study 3 are referred to in the other textual material, but these references are not pertinent to Study 2.**

## ABSTRACT

Studies of adults with alcohol dependence indicate a close relation between sleep disturbances and alcohol use and abuse. Few studies have examined these relations in young humans; fewer have examined the role of the circadian timing or sleep/wake homeostatic systems in alcohol utilization and metabolism; and none has attempted to determine associations of family history of alcoholism with sleep of offspring.

This project includes three studies aimed at assessing these associations. Study 1 provides the first attempt to examine sleep patterns, sleep architecture, and a marker of the circadian timing system (melatonin onset phase) in children ages 9 and 10, adolescents ages 15 and 16, and young adults ages 21 and 22 with (PH+) or without (PH-) parental history of alcohol abuse/dependence (N = 200). A subset of older participants have moderate to high prior experience with alcohol (etoh+). We will test hypotheses that sleep is more disturbed in (1) PH+ participants, (2) etoh+ participants, (3) and in older participants at an initial assessment. We also predict that participants with both PH+ and etoh+ will show most disruption. A 2.5-yr longitudinal component in a subgroup of 80 low alcohol-experience children and adolescents will test the hypotheses that (1) pre-existing sleep disruptions predict amount of alcohol use in the 2.5-year follow up and (2) alcohol use during follow-up predicts changes in sleep between the initial and the 2.5-year in-lab assessments.

In Study 2, effects of a moderate evening dose of alcohol on sleep, waking performance, and circadian phase will be studied in 64 adolescents (ages 15-16) and young adults (ages 21-22) with low alcohol exposure and PH+ or PH-. We will test the hypothesis that alcohol will alter sleep architecture and waking performance in predictable ways and that the effects will be blunted in PH+ offspring relative to PH- offspring. Additionally, we will assess whether alcohol-induced alterations in SWS are associated with slope of the decline in core body temperature following alcohol ingestion.

Study 3 uses the forced desynchrony paradigm, in which participants will live in the laboratory for nearly 2 weeks on a 20-hour day, to determine the independent and interactive contributions of the circadian timing system and sleep/wake homeostatic processes to the effects of moderate doses of alcohol on sleep, sleepiness, and waking performance. Study 3 involves a total of 24 FH- participants, ages 18 to 22.

## RESEARCH PLAN

### A. Specific Aims.

Associations between sleep and alcohol have been known for decades, and renewed interest in this area of investigation has been stimulated by recent findings and new scientific opportunities. The work of Gillin and colleagues showing that altered sleep architecture predicts relapse in adults with alcohol dependence, for example, points to the possibility that sleep disruption may predispose to alcohol use and dependence. Other important lines of investigation show acute effects of alcohol on sleep architecture, sleepiness, and performance and that alcohol and sleep loss act synergistically to impair alertness and performance. Yet many enigmas exist concerning how alcohol interacts with sleep and wakefulness as a function of the time at which it is taken—to wit, what is the role of the circadian timing system? Another yawning gap in the investigations of alcohol and sleep has been the role of adolescent development. Even though data are consistent and strong that many American teens drink and that adolescence is a time of maximal risk for alcohol use and abuse, no studies of human participants have examined prospectively the role of sleep disturbance in alcohol use of young humans, none has assessed the effects of alcohol on sleep and sleepiness in adolescent participants, and none has thoroughly investigated how the circadian timing system and sleep/wake homeostatic process together influence alcohol's effects in human participants of any age.

Investigations of alcohol use that take into account sleep and the circadian timing system have the potential to provide new models and modes of analysis in alcohol research. Family history studies, for example, show increased probability of alcohol abuse and dependence in youngsters with a positive family history, and some markers of risk have been identified. If sleep and/or circadian timing also differ as a function of family alcohol history, then new lines of investigation may emerge.

Thus, we approach this project with the goal of beginning to fill in gaps in (1) the description of sleep/wake and circadian timing in at-risk youngsters, (2) the effects of alcohol on sleep and circadian timing in adolescents and young adults, and (3) the relation of sleep/wake homeostasis and the circadian timing system to alcohol's effects. As we pursue these descriptive aims, we also plan to test a number of specific hypotheses. We propose three studies. Study 1 includes cross-sectional and longitudinal evaluations of sleep, circadian timing, and alcohol use in young people with and without parental history of alcohol abuse/dependence (PH+ or PH-) and with varying amounts of alcohol experience. Study 2 examines acute effects of moderate levels of alcohol taken in the evening. In Study 3, we use a specialized experimental protocol to dissect sleep/wake and circadian influences on alcohol's effects. These studies will address the following specific aims:

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- 5 To assess whether the acute sleep, sleepiness, circadian, and performance responses to alcohol differ in adolescents and young adults and whether these responses are blunted in those who are PH+. [Study 2.]
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## **B. Background and Significance.**

The rationale for this project derives from the convergence of several lines of investigation identifying a close link between sleep disturbance and alcohol use in adults, the association of family history with the development of alcohol abuse/dependence, the identification of adolescence as a period of risk for initiation and maintenance of alcohol use, and the dynamic interplay of alcohol use, insufficient sleep, and performance decrements. Furthermore, research technology now allows for close examination of the human circadian timing system as it interacts with the sleep/wake homeostatic process, providing an opportunity to examine these systems as they are implicated in the lines of investigation listed above.

The significance of the proposed work lies in the examination of adolescents to begin understanding the developmental pathways where alcohol use, family history of alcohol abuse/dependence, sleep, circadian timing, and performance converge. To date, we have little descriptive information of the phenomena in question. Furthermore, we have little information regarding key questions that immediately emerge when these broad issues are posed: is susceptibility to alcohol abuse (indexed by family history) a marker for sleep problems; is early exposure to alcohol at high levels a risk factor for sleep problems; do sleep problems elevate probability of increased alcohol use? Framing these developmental questions is the more general question of whether alcohol effects on sleep and performance in adolescents are similar to the effects in adults. We describe below the background and rationale for proposing three studies that address the basic questions enumerated here. These studies include longitudinal and cross-sectional methods, subject selection by alcohol use of individuals along with their family history, and protocols to examine sleep architecture, performance, circadian timing, and sleep/wake homeostasis. The intent of our approach is to provide the building blocks of fundamental knowledge that will provide a foundation for future research.

### Influence of Family History of Alcohol Abuse/dependence on Alcohol Use in Teens

Longitudinal studies demonstrate that children who are family-history positive (FHP) for alcohol abuse/dependence have increased risk of developing alcohol problems later in life (Grant, 1998; NIAAA, 1997). The exact nature of familial transmission remains unclear, but twin studies consistently support a genetically-based factor in the etiology of alcohol problems (McGue, 1995). Heritability estimates for men appear to be in the .5 to .6 range. Heritability estimates for women have been more difficult to identify, but significantly greater concordance in alcohol dependence has been observed among monozygotic (MZ) female twin pairs than dizygotic (DZ) pairs (McGue, 1997). In addition, FHP adolescents are more likely to display behaviors that increase risk for later alcohol problems, such as aggression and noncompliance, and these behaviors show greater concordance among MZ vs. DZ twin pairs (Rose et al., 1997). Studies of adoptees also support a biological component in the etiology of alcohol dependence (McGue, 1995). For example, adolescent alcohol use among adoptees is significantly related to their biological parents' drinking behavior (McGue et al., 1996). These findings have spurred investigation of genes that may influence predisposition to alcohol dependence. Genetic linkage studies have identified specific chromosomal regions that may contain genes influencing this predisposition (Long et al, 1998; Reich et al., 1998), but candidate genes from these regions have yet to be identified.

Biologically-based differences between family-history negative (FHN) and FHP populations could have a direct effect on alcohol consumption through the physiological response to alcohol. Schuckit and Smith (1996) examined the response to alcohol among young men who were FHP and found them less reactive to alcohol than men who were FHN. The men who reacted least were found to have the highest risk of developing alcohol dependence later in life. In a similar comparison study, a relatively smaller physiological response to alcohol on EEG was observed among men who were family history positive, and increased risk of developing alcohol dependence over the next 10 years was associated with smaller EEG responses (Volavka et al., 1996).

Many children of alcoholic parents do not go on to develop alcohol dependence, suggesting that expression of alcohol dependence among children who are FHP involves a complex relationship between genotype and environmental influences. Adoption studies (Sigvardsson et al., 1996) have supported a diathesis-stress model, which posits that disease expression in a biologically vulnerable organism is more likely under stressful environmental conditions. Biologically-based traits or abilities noted more frequently in children who are FHP,

such as emotional and/or behavioral dysregulation and impaired cognitive ability, may evoke responses from the environment that increase risk for early onset of drinking and later alcohol dependence. For example, increased family conflict and poor school performance may increase the child's negative affective response and association with a drug using peer group. Results from twin and adoption studies suggest that environmental factors appear to be more involved with the *initiation* of drinking than with the *maintenance* of drinking to problematic levels (Rose, 1998).

Sleep has not been assessed in these family history studies. We know that sleep is an important mediator of waking behavior. Children with sleep disorders may display poor behavioral regulation (cf., Guilleminault et al., 1981; Ali et al., 1993; 1994); children with behavior disorders may have sleep problems (e.g., Chervin et al., 1997; Picchietti et al., 1998); and experimental disruption of sleep can modify behavior (Fallone et al., 2000). If FHP children/adolescents also have disturbed sleep, then sleep disruption may not only serve as a marker of genotype and pose a risk for alcohol use in the "self-medication" model described below, but may also be a part of the conditions that predispose to alcohol use in such a diathesis-stress model. **In the cross-sectional component of Study 1, we will determine whether parental history of alcohol abuse/dependence is associated with poor sleep.**

#### Alcohol and Sleep Disruption in the Context of Alcohol Abuse/Dependence

Among the chief clinical complaints of adults with alcohol dependence is difficulty sleeping, which may be accompanied by daytime somnolence, disrupted circadian rhythms, and parasomnias (Gillin and Drummond, 2000). Highly fragmented sleep, indeed "polyphasic sleep" has been identified as a possible circadian rhythm disruption in severe alcohol dependent adults (Gillin and Drummond, 2000). Abnormal sleep patterns persist despite abstinence. After a few weeks of abstinence, sleep is characterized by prolonged sleep latency, reduced total sleep time, diminished SWS sleep, increased Stage 1 sleep, and elevated respiratory disturbances relative to normal controls (Ishibashi et al., 1987; Gillin et al., 1990a; Le Bon et al., 1997). Patients with a secondary diagnosis of major depression additionally have shorter REM latency and a higher percentage of REM sleep than patients without depression (Gillin et al., 1990b). Recovery of sleep quality is slow; even after 27 months of abstinence, REM sleep percentage remains elevated and REM latency is low (Drummond et al., 1998).

In general, the sleep complaints of adults with alcohol dependence focus on a difficulty initiating sleep, which has been hypothesized by some to lead to alcohol use in an attempt to achieve sedation leading to improved sleep (Vitiello, 1997). While resumption of alcohol use at 3 months after discharge from a treatment program is best predicted by measures of REM sleep—short REM latency, increased REM percentage and REM density (Clark et al., 1998, 1999; Gillin et al., 1994)—relapse beyond this duration is more strongly related to long sleep latency and poor sleep efficiency (Brower et al., 1998; Drummond et al., 1998). This latter finding is consistent with the speculation that self-medication with alcohol may be initiated in some patients for immediate relief of sleeplessness (Drummond et al., 1998). Furthermore, Roehrs and colleagues (1999) have shown that nonalcohol dependent insomniacs select alcohol as a hypnotic preferentially to placebo more often than normal sleepers, and an epidemiological study reported alcohol use as a sleep aid in 13.3% of 18- to 45-year-old adults (Johnson et al., 1998).

No prospective study has examined sleep in individuals at risk for alcohol dependence. **Our longitudinal assessment in Study 1 will be the first to examine whether a sleep disturbance increases the probability of alcohol use** by looking at sleep in young humans who carry a family history of alcohol abuse/dependence but have not themselves begun to abuse alcohol. Furthermore, if poor sleep is associated with increased likelihood to initiate alcohol use, then we might expect young people with a familial predisposition for alcohol use who also have disrupted sleep to begin drinking sooner and to drink more than those who have little sleep disruption and genetic/familial risk. **The longitudinal component of Study 1 addresses this speculation as well.**

#### Acute Effects of Alcohol

Alcohol, Sleepiness, and Performance. Most of the data regarding the sleepiness-inducing or sedative effects of alcohol come from an extensive series of studies undertaken by the Detroit group of Roehrs and Roth. This elegant series of experiments used the well-characterized measure of sleep onset latency in the Multiple

Sleep Latency Test (MSLT, Carskadon et al., 1986) to identify the sedating effects of alcohol. When ingested at 0900 hours after 8 hours of sleep, alcohol induces a dose-dependent reduction in sleep onset latency on the MSLT (Roehrs et al., 1989a; Zwyghyuzen-Doorenbos et al., 1988) that persists across the day. This sedative effect of alcohol interacts with basal levels of sleepiness. Thus, for example, when time-in-bed is restricted to 4 hours the night before, sleep onset latencies following morning alcohol ingestion are further reduced relative to 8 hours time-in-bed (Roehrs et al., 1994). After four consecutive nights with 5 hours time-in-bed, a lower dose of alcohol (0.4 g/kg) produces levels of sleepiness similar to a higher dose (0.8 g/kg) following 8 hours time-in-bed (Zwyghyuzen-Doorenbos et al., 1988). Conversely, extending time-in-bed prior to alcohol administration mitigates the sedative effects of alcohol: after 10 or 11 hours time-in-bed, latencies on the MSLT following morning alcohol are longer than after 5 and 8 hours time-in-bed (Lumley et al., 1987), and they no longer differ from sleep latencies obtained with placebo after 7 to 8 nights of sleep extension (Roehrs et al., 1989b). A one-hour nap has also been used successfully to reduce sleepiness following alcohol ingestion relative to placebo no-nap levels (Roehrs et al., 1990, 1993).

The different effects of alcohol on sleepiness depending on the timing of alcohol ingestion present a puzzle that is of particular interest in this proposal. When alcohol is taken in the morning, MSLT scores are reduced for all tests, even though BAC is falling, and with no dose by time-of-day interactions. Importantly, several studies consistently showed residual sedation to alcohol at the 1600 test time on the MSLT (7 hours after alcohol ingestion), although blood alcohol concentrations in all participants at this time were zero (Zwyghyuzen-Doorenbos et al., 1988; Roehrs et al., 1989c; Roehrs et al., 1990).

In contrast, the few studies that have administered alcohol in the late afternoon (1600 hours) have found *reduced sedative effects* on evening MSLTs. Roehrs et al. (1992), for instance, found that evening latencies following 0.5 g/kg of alcohol administered at 1600 hours after 8 hours time-in-bed did not differ from placebo. The authors attributed these findings to the circadian decrease in sleepiness during the early evening hours. Interestingly, restricting time-in-bed the night before to 4 hours did not increase the sedative effect of alcohol in the evening (Roehrs et al., 1996). These findings suggest that other circadian factors, as yet unidentified, account for the reduced effects of alcohol in the evening hours. **Study 3 of the current proposal will add significant information to the consideration of this issue.**

As mentioned above, sleep loss and alcohol interact with regard to sleepiness. Similar results have been found with performance measures. In two field studies, Corfitsen (1982, 1996) found that simple reaction time was slowest among a group of self-assessed “very tired” alcohol-impaired drivers compared to “tired” and “rested” impaired drivers. In a laboratory study, Horne and Baumber (1991) found that simulated driving performance deteriorated to “dangerous” levels when even low doses of alcohol were ingested in the early afternoon. Roehrs et al. (1994) had participants ingest placebo or 0.6 g/kg of alcohol at 0900 hours following either 4 or 8 hours of sleep. Reaction times on a divided attention task were slowest and performance on a driving simulator (measured by lane deviations and “crashes”) deteriorated to the greatest extent in the 4 hour-alcohol condition. More recently, Arnedt et al. (in press) found that the combination of 20 hours of wakefulness and a BAC of 0.08 g% produced additive decrements in simulated driving performance relative to each condition alone. Taken together, these findings suggest that alcohol and sleepiness produce at least additive, and in some cases, synergistic decrements on commonly used measures of performance. **Study 3, with its assessment of sleep/wake homeostasis and alcohol administration, will provide additional data to assess this phenomenon.**

Alcohol and Sleep. A number of studies, beginning in the 1960s, have demonstrated a fairly consistent pattern of changes to sleep following the acute administration of alcohol. When ingested shortly before bedtime, up to 1 g/kg of alcohol reduces latency to sleep onset and to slow wave sleep (SWS, stages 3 and 4), decreases the percentages of REM, and increases the latency to REM sleep and the percentage of SWS (Gillin and Drummond, 1999; Yules et al., 1966, 1967; MacLean and Cairns, 1982; Roehrs et al., 1991; Williams et al., 1983). The alterations in REM sleep persist even when REM pressure is increased experimentally (Lobo and Tufik, 1997).

Of interest with regard to alcohol’s effects on sleep—particularly so in the context of adolescent development—is the disruption after alcohol of the relation between SWS and human growth hormone

production. Under normal circumstances, growth hormone (GH) production after childhood achieves a prominent peak each day in conjunction with SWS (Van Cauter, 2000). Thus, one might question whether the occurrence of increased SWS following alcohol consumption is associated with potentiation of GH release. In two studies that have examined GH following alcohol ingestion, however, alcohol markedly suppressed GH, even at a moderate dose (0.5 g/kg) (Prinz et al., 1980; Ekman et al., 1996). Methods of continuous blood sampling during sleep are not routine in our laboratory, so we do not plan to measure GH. We plan to use another method, however, to assess whether the alcohol-related change in SWS may be related to another physiological effect of alcohol, core temperature reduction. One of the most well-known physiological effects of a moderate dose of ethyl alcohol is the dilation of cutaneous blood vessels and consequent loss of core body temperature. Recent data in humans show a relation between the slope of the fall of core temperature before sleep onset and the initiation of sleep and amount of SWS (Murphy and Campbell, 1997), confirming earlier work (e.g., Dorsey et al., 1996). Although this association has not been confirmed in other experimental paradigms (Dijk and Czeisler, 1993), **the relationship will be testable in both Study 2 and Study 3.**

The magnitude of the changes in sleep architecture is largely dose-related. With moderate levels, a withdrawal effect may occur in the latter part of the night, as alcohol is metabolized and blood alcohol concentrations approach zero. As a result, sleep may be more shallow and disrupted during the second half of the night, with an increase in REM sleep and NREM Stage 1 (Gillin and Drummond, 2000; Roehrs et al., 1991; Rundell et al., 1972). Sleep disruption from alcohol may also be produced by gastric irritation, headache, or a full bladder (Gillin and Drummond, 2000).

As with alcohol's effects on sedation, the relation between effects on sleep and the time at which alcohol is presented is not straightforward. In particular, a moderate dose of alcohol ingested six hours before bedtime was shown to disrupt sleep overall and to reduce REM sleep during the night, even though breath alcohol concentrations were zero at bedtime (Landolt et al., 1996).

The effects of an evening dose of alcohol on sleep, sleepiness, and performance are of particular importance in the context of adolescence, because most adolescent drinking occurs in the evening hours (see Study 2 rationale for data from our group). Evening drinking may be followed by sleep or it may be followed by other activities, such as driving a car. In either case, we have no data to inform us about the acute effects of alcohol on these behaviors. **Study 2 of this proposal is the first to address the consequences of acute alcohol administration on the sleep of adolescents** and will assess whether the response of adolescents differs from that of young adults. Furthermore, **Study 2 examines the waking (sleepiness, performance, melatonin levels, rate of alcohol metabolism) response of adolescents and young adults to alcohol** consumed in the evening. In addition, **all these variables are examined in Study 2 with respect to how they are modified in those with a positive or negative parental history of alcohol abuse/dependence.**

Forced Desynchrony as a Means of Assessing Circadian and Sleep/Wake Homeostatic Processes. In order to examine the independent and interactive effects of the circadian timing system and sleep/wake homeostasis upon measures of interest, it is necessary to measure variables or systems at many times and many circadian phases. One way to accomplish such multiple measurement is to vary the length of time awake and asleep equalizing for time of day (cf., Åkerstedt et al., 1998); however, this type of approach has some difficulties in terms of creating a design that is orthogonal for both sleep/wake and time of day. An alternative experimental approach that has recently led to significant gains in our understanding of these systems is called Forced Desynchrony (FD) (Czeisler et al., 1990). The term "forced desynchrony" derives from concepts of circadian rhythms physiology and is best understood with a brief introduction to these concepts.

The internal mechanism or pacemaker that organizes daily biological processes in mammals has been localized to a small paired nucleus in the hypothalamus, the suprachiasmatic nucleus (Moore, 1997). This neuronal system has both intrinsic rhythmic properties and the capacity to entrain to rhythmic environmental stimuli. The principal entraining stimulus is the cycle of daylight and darkness. Recent findings have substantiated that the human circadian timing system—once thought to be insensitive to light signals—is quite exquisitely responsive to light (e.g., Czeisler 1995; Boivin and Czeisler, 1994; Boivin et al., 1998). One common metaphor for the circadian timing system is that of an oscillator whose oscillatory period is entrained

by an external time-giver, termed a *zeitgeber*. When we measure physiological variables around the clock in organisms living in natural conditions, the expressed period of the system is 24 hours, because the internal pacemaker is entrained to the natural 24-hour day length. When an organism is removed from natural conditions and placed into an environment with constant conditions—picture a hamster in a darkened cage with a running wheel and ad lib. food and water for weeks or months at a time—the internal oscillator is said to run free (*free run*) at its intrinsic period due to the absence of external control. Most organisms have a non-24-hour intrinsic period. In adult humans, the intrinsic period is thought to be approximately 24.18 hours (Czeisler et al., 1999); in adolescents, the intrinsic period may be slightly longer, approximately 24.33 hours (Carskadon et al., 1999a). These values of intrinsic period have been derived from humans living under FD conditions because it is difficult to study humans under constant conditions—picture a human alone in pitch darkness for weeks or months at a time.

The term *forced desynchrony* derives from the loss of synchrony between environmental and intrinsic oscillators that occurs when participants are studied while living on an imposed schedule outside the range of entrainment of the intrinsic circadian oscillator. Thus, physiological and behavioral patterns maintain their internally generated rhythmicity, but they desynchronize from the environmental cues and run free at the intrinsic oscillatory period. In order to accomplish such desynchrony, one could choose a very short (e.g., 20 hours) or a very long (e.g., 28 hours) cycle of rest-activity. It is important that the system run free from the environmental schedule so that scheduled sleep and waking events occur at varying phases of the internal circadian timing system; conversely, circadian phases occur at varying lengths of time since the offset of waking or the onset of sleep. Thus, by carefully measuring and tracking these parameters, we can assign to any variable the “circadian phase” and the “homeostatic time” at which it occurred and then assess contributions of these processes to measures of interest.

The anomalous findings described above regarding the sedative and sleep-related responses to alcohol beg the question of how alcohol interacts with the circadian timing system and the sleep/wake homeostatic process, which are difficult to separate in traditional experimental designs. **A major aim of Study 3 is to separate the effects of these processes using the forced desynchrony protocol, in which sleep and waking are scheduled on a non-24-hour day length.** Thus, we can examine how the effects of alcohol are modified by sleep/wake homeostasis and the circadian timing system. This information addresses not only the issue of adolescent drinking, but also concerns regarding alcohol use in shiftworkers and others with unusual timing of sleep.

### Sleep, Sleepiness, and Alcohol Use in Adolescents.

The associations between sleep pattern, sleepiness, and substance use in adolescents have been studied most extensively by Tynjala and colleagues (1997) in a Finnish sample of 11-, 13-, and 15-year-old students. These investigators reported a significant association among sleep habits, use of psychoactive substances (alcohol, tobacco, and coffee), and perceived sleepiness. Structural equation modeling identified that 20-25% of variance in perceived tiredness was explained by sleep habits and substance use (principally alcohol and smoking). Our survey data have also shown associations between sleep and alcohol (and tobacco) use in American adolescents who work more than 20 hours per week (Carskadon, 1990a): teens who worked more slept less, drank more, and smoked more.

Others have made eloquent statements regarding the need to understand the role of adolescence in the acquisition of alcohol abuse and dependence, largely in the context of animal models (Witt, 1994). We feel that the data summarized above—when combined with the growing recognition that the sleep of adolescents is insufficient and ill-timed—provide a compelling argument for beginning to take a closer look at the intersections and overlapping processes in adolescent humans. The strengths of our group stem from our longstanding involvement in the study of sleep, circadian rhythms, and behavior during adolescent development. Our focus on adolescent development and behavior has sensitized us to the high risk alcohol poses for adolescents who are chronically sleep deprived and have delayed circadian timing, and we are concerned that so little data are available to inform our understanding of these associations. Our group is poised to begin these studies: we have experience working with children, adolescents, and young adults; we have expertise in studying sleep, circadian timing, and behavior in these groups; we have taken steps in our

community to obtain cooperation for studying alcohol in adolescents; we have taken advantage of consultation with local experts in the study of alcohol; we have the motivation to focus our efforts, skills, and methods on these important issues. Too many questions remain to be answered, yet we hope that this proposal will provide a strong foundation to continue and expand this work.

### C. Preliminary Studies.

A great deal of Carskadon's previous work has been devoted to the study of sleep patterns in children and adolescents. It is important to understand, therefore, that the fundamental tools of sleep research and chronobiology are very familiar to the investigator and, more important, that she is unique in having worked with these techniques in children and adolescents. Co-investigators on and consultants to this project bring a rich background in alcohol use and abuse (Monti), laboratory administration of alcohol (Roehrs), and developmental psychopathology and family studies (Seifer). Therefore, the following summary of preliminary research includes data from studies that provide the direct theoretical background for the application, indicate familiarity and previous experience working with young people using complex experimental protocols, and show our capability to recruit and maintain suitable research subjects.

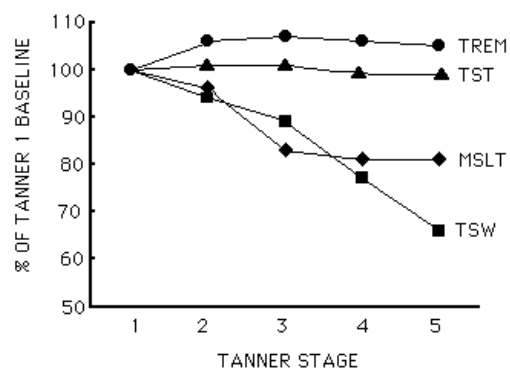
Our understanding of the development of sleep patterns in adolescents has advanced considerably in the last 25 years. Although the full characterization of teen sleep regulation remains to be accomplished, our previous research provides a basis from which to begin examining the effects of alcohol.

In the mid-1970s we became aware that sleep patterns change fundamentally at the transition to adolescence: the reported timing of sleep begins to delay in early adolescence. Associated with the delay of sleep is a decline in the amount of sleep obtained and an increase in the discrepancy between school nights and weekend nights. Although nonschool night "oversleeping" was acknowledged as recovery from insufficient sleep during the school week, it was generally assumed that the sleep requirement declines with age (the older you are, the less sleep you need).

A longitudinal study begun in 1976 attempted to examine this axiom (Carskadon, 1979; 1982; Carskadon et al., 1980; 1983). This remarkably successful research approach produced a wealth of information regarding sleep and sleepiness in children and adolescents. Boys and girls enrolled in this research project at ages 10, 11, or 12 and came to the lab for a 72-hour assessment each year for 5 or 6 years. During the 72-hour sessions, testing included nocturnal sleep recordings, measurement of physiological sleepiness using the MSLT and introspective sleepiness, as well as a variety of performance tests. Assessment of puberty was performed using Tanner staging of secondary sexual characteristics (Tanner, 1962).

Our hypothesis was that the reduced sleep need in older children would manifest as less sleep within a 10-hour nocturnal window. This hypothesis was not confirmed: regardless of age or developmental stage, the children all slept about 9 1/4 hours. Figure 1 summarizes the major findings of this longitudinal study, showing relationships among sleep, physiological sleepiness, and Tanner stages, with data normalized to the values at Tanner stage 1. (Age-related trends were similar.) Although the total amount of sleep (TST) at night remained constant across the developmental stages, slow wave sleep (SWS) declined significantly. Physiological sleepiness (MSLT) also showed a clear relationship to developmental progression, with greater physiological sleepiness in older teens. These findings were consistent for boys and girls. Because average total sleep time at night was nearly identical for subjects at each developmental stage, changes in SWS and daytime sleepiness were not due to a change in nocturnal sleep amount. One conclusion, therefore, was that the need for sleep does not change across adolescent development.

Figure 1





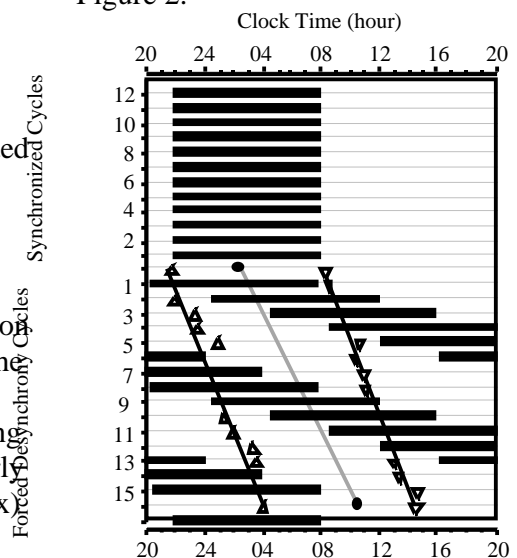
These data indicate clearly that important developmental changes in the sleep/wake system occur during the adolescent years, and they form the core of our knowledge base on the development of the sleep process. One of the most important aspects of this data set was the importance of holding constant the scheduled sleep opportunity across the longitudinal assessments, a procedure we will maintain in the current project with our “stabilization” sleep schedules. Previous work in adolescents, either cross-sectional (Williams et al., 1974) or longitudinal (Karacan et al., 1975), had identified a stable amount of SWS across adolescent development. In both studies, however, scheduled sleep was permitted to vary according to the participants “usual schedule,” which in every case was to sleep less with increasing age. Thus, two factors resulted in a more constant value for SWS with adolescent development: first, when data are reported as a percentage of total sleep time, a constant amount of SWS (or even reduced amount of SWS) will be expressed as a larger percentage of the whole; second, reducing sleep leads to increased pressure for SWS and larger amounts of SWS. [This is one of the fundamental tenets of the homeostatic sleep/wake process.]

Although we have shown that psychosocial factors, such as academic obligations, social opportunities, employment, and school start time, play a role in setting the timing for and in limiting sleep of adolescents, reports of a teen sleep delay from surveys conducted by us and by others in other countries (Andrade et al., 1993; Ishihara et al., 1990; Bearpark and Michie, 1987; Strauch and Meier, 1988) suggested that intrinsic developmental changes may also play a role. This realization led to our initial attempt to assess circadian phase preference in adolescents. Our study provided the first evidence that the circadian timing system likely undergoes significant developmental change during adolescent development, supporting involvement of a biological factor associated with puberty in the adolescent phase preference delay (Carskadon et al., 1993).

Our subsequent studies have attempted to confirm a developmentally-mediated phase delay in adolescents using more precise measures. When examined in the laboratory, for example, one of the best ways to identify time in the intrinsic biological clock in humans is to examine melatonin secretion (Lewy and Sack, 1989). Melatonin is a hormone that is regulated by the circadian timing system and produced by the pineal gland. Melatonin secretion occurs during nocturnal hours in both day-active species, like humans, and in night-active species. Melatonin can be measured from saliva samples collected in dim lighting conditions. Under strictly controlled conditions, we found a significant correlation between pubertal development and circadian timing: more mature adolescents had a later timing of the termination of melatonin secretion (Carskadon et al., 1997). One stream of our ongoing research on adolescent sleep examines mechanisms and attempts to determine more precisely how the circadian timing system is linked to the sleep/wake system during adolescent development. One of the important tools used in this research will be brought to bear on questions examined in Study 3 of the current proposal. Because of the complexity of the experimental design and underlying concepts, we present a somewhat more elaborated description of our ongoing project using the forced desynchrony (FD) protocol.

We have used FD in our laboratory over the last five summers, with participants as young as 9 years old. In general, we enroll participants in the FD protocols only if they have significant experience sleeping away from home and have successfully completed another of our protocols involving at least one overnight stay in the laboratory. This procedure maximizes the chances for successful completion of the study. We have used the 28-hour version of the protocol, as shown in Figure 2. Participants are placed on an Optimized sleep schedule with a fixed light-dark (LD) phase (lights on = 0800; lights off = 2200) while living at home. The in-lab part of the study begins with a single overnight on this schedule followed by a procedure called “constant routine,” which “unmasks” the underlying physiological signals used to characterize circadian phase, particularly core body temperature. The 28-hour schedule with lights on (15 lux) for 16.33 hours and lights dimmed (<1 lux) for 11.67 hours is maintained over the course of 12, 28-hour cycles (14 external days).

Figure 2. Forced Desynchrony Protocol

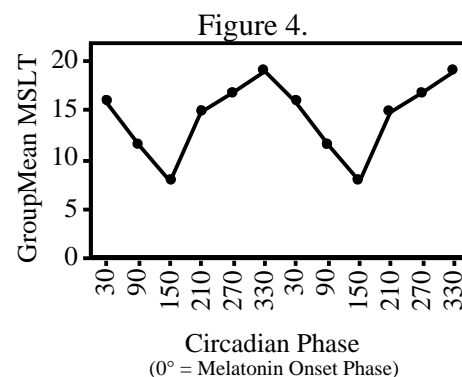
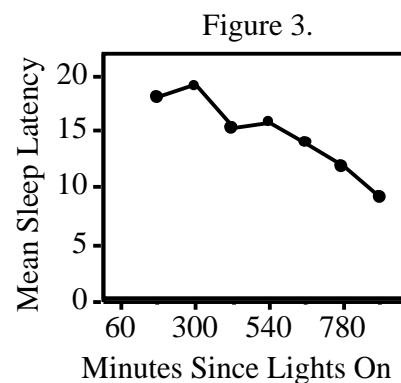


Note that the in-lab study also ends with a constant routine. These bookend constant routine studies were initially considered critical to obtain accurate measures of circadian period, providing the most accurate phase assessments at the beginning and end of the period of free running rhythm. Our experience with FD shows that the phase assessments from salivary melatonin collected during waking portions of the FD provide a very robust measure of period and that the constant routine will not be needed for Study 3 of this proposal.

We present here data from five boys and 5 girls (ages 10.9 to 15.2 year; mean = 13.7 year). Saliva samples (2 ml) were collected during all waking episodes at 30-minute or 1-hour intervals for determination of melatonin levels. The times of melatonin onset (up triangles) and offset (down triangles) were computed by interpolation from values rising above or falling below, respectively, a 4 pg/ml threshold. Rectal temperature was sampled only on the constant routines and bracketing nights. Minimum temperature phase (dark circles) was determined using the method of Brown and Czeisler (1992). Intrinsic circadian period for each participant was estimated using each of these three phase markers. Figure 2 illustrates these data in one 13.9-year-old girl. The average estimate of intrinsic circadian period computed with core temperature was  $24.30 \pm .20$  hours; for melatonin onset was  $24.33 \pm .21$  hours; for melatonin offset was  $24.35 \pm .21$  hours. Correlation coefficients indicated strong coherence among the estimates of period, ranging from  $r = .962$  to  $r = .995$ .

In addition to providing an excellent measure of the intrinsic circadian period, FD provides opportunities to explore circadian timing system and the sleep/wake homeostatic processes. Thus, one can assess for any measure collected across the FD, the relative contributions of circadian phase and of length of time awake (or asleep, for sleep variables) to the expression of the measure. We illustrate this process below using data from the MSLT; keep in mind, however, that the same general scheme can be applied to a variety of measures taken when participants are awake (cf., Johnson et al., 1992; Dijk et al., 1992; Boivin et al., 1997; Wyatt et al., 1999) or asleep (cf., Dijk and Czeisler, 1995; Dijk et al., 1996).

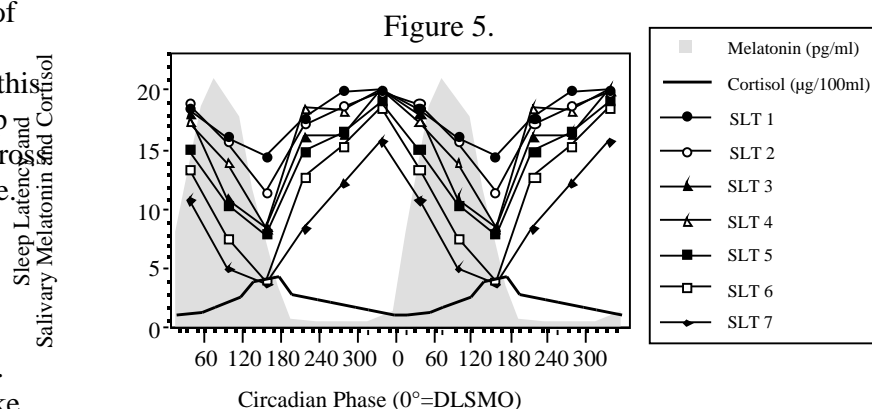
We were the first to measure sleep propensity using MSLT in FD and thus had the opportunity to examine sleep propensity at all circadian phases but also at different times since sleep offset. Sleep latency tests (SLT) occurred at 2-hr intervals beginning 150 minutes after "lights on" in each FD cycle. Intrinsic period was estimated for each subject by regression across melatonin onset phases as described above. Each SLT sample was assigned a value for phase based on the individual's computed intrinsic circadian period and a value for time awake based on the interval since scheduled "lights on" time signifying the end of a sleep episode. The contribution of the sleep/wake homeostatic system to the level of sleep propensity was estimated from data averaged into 2-hr bins of time since "lights on;" the circadian contribution was derived from data in  $60^\circ$  bins (melatonin onset =  $0^\circ$ ). MANOVA analysis identified significant main effects of time since lights on ( $F_{6,54} = 38.9$ ;  $p < .001$ ), circadian phase ( $F_{5,45} = 44.6$ ;  $p < .001$ ) and a significant interaction ( $F_{30,270} = 1.87$ ;  $p < .005$ ). The homeostatic effect is depicted in Figure 3, showing the decrease in sleep latency as a function of time awake independent from effects of circadian phase. In other words, regardless of internal "clock" time, the longer the waking interval, the greater is sleep propensity. The independent influence of the circadian timing system on this sleep latency is illustrated with a double plot in Figure 4, showing that sleep propensity is strongly related to circadian phase (regardless of how long one has been awake), with greatest propensity toward the end of the circadian nighttime and lowest propensity toward the beginning of the circadian nighttime. [Although this pattern seems counterintuitive, the timing of these circadian and homeostatic effects sets up opposing forces that serve to maintain waking alertness across the day and consolidated sleep across the night (Borbely et al., 1989; Edgar et al., 1993; Dijk and Czeisler, 1995).] The interaction of these processes is such that the longer one has been



awake, the greater is the contribution of the circadian timing system to level of sleep propensity. Figure 5 illustrates this interaction, depicting the average sleep latency for each of the 7 tests given across a cycle as a function of circadian phase. The melatonin and cortisol secretory patterns are also shown for reference. Thus, if awake only 2.5 hours (SLT1), latency to sleep onset is high and minimally affected by circadian phase.

On the other hand, after 16 hours awake

(SLT7), the average sleep latency is reduced and the greatest effect is apparent at the circadian phase occurring toward the end of melatonin secretion. We shall apply this experimental approach in Study 3 to examine how the timing of alcohol administration at various circadian phases and lengths of time into the waking day (or time before sleep onset) affect sleep, sleepiness, and performance.



#### D. Research Design and Methods.

This proposal includes three studies. Protocol considerations specific to each study and specific analysis plans are presented following this general description of procedures. The time-line for the studies is included in the Budget Justification.

##### A. Participants

1. Recruitment. Before enrolling in any study, participants generally come to the laboratory for a tour of the facilities and an explanation and demonstration of procedures. Such tours are useful for recruitment and to familiarize participants with the laboratory and personnel so that their expectations about the study and their assent to take part can be well informed. Participants are recruited from a number of sources. Adult participants are recruited from local colleges and universities. Newspaper advertisements are used for recruiting all age groups. This project requires recruitment of a sample that includes young people who have a parent with a history of alcohol abuse/dependence. In order to assure that we have adequate access to such individuals, we will recruit using the facilities of a number of alcohol dependence treatment centers. We have spoken to the directors of two treatment centers in the Providence area who have indicated willingness to assist with recruitment: the VA Hospital has approximately 600 alcohol abuse/dependent outpatients in treatment each year; the Providence Center sees 317 patients annually in residential treatment for substance abuse/dependence and about 60 patients per month in outpatient treatment, most of whom are alcohol dependent. We note that a co-investigator on this project, Dr. Seifer, has successfully used a similar recruitment strategy for 15 years to recruit children of parents with identified psychopathology.

All research participants are paid. For this project, although young people are our primary study participants, parents are paid to compensate them for their time for the Family History Screening Interview. Our aim for compensation is to provide a tangible incentive to take part in and complete the research, without providing a monetary reward so large as to be coercive to certain potential subjects. We feel that the inducements described in the budget justification section of this proposal achieve our goals and are appropriate reimbursements for the time and effort of our participants. Fees are not offered for “drinking alcohol” but for the time and effort involved in participating in the research.

2. Inclusion and Exclusion Criteria. Our goal is not to obtain a group of “supernormal” youngsters and young adults, but to find participants who are healthy, within a normal range on parameters of interest, and meet specific inclusion and exclusion criteria. The three studies proposed below have slightly varying criteria. Study 1 requires children, adolescents, and young adults with and without parental history of alcohol abuse/dependence, as well as adolescents and young adults who have very little or fairly substantial personal experience with alcohol. We shall not constrain sleep schedules for recruiting Study 1 participants, since self-

selected sleep patterns are dependent variables of interest. Study 2 also needs adolescents and young adults with and without parental history of alcohol abuse/dependence, all with moderate personal experience with alcohol, and with additional constraints on sleep/wake schedules that would affect the variables of interest. For Study 3, we will limit the sample to parental history negative, moderate alcohol experience young adults, and we will apply specific sleep/wake schedule constraints.

Determination of fitness for inclusion in an individual study is made with a four-tier process that includes telephone interviews of participant and parent, completion of paper and pencil questionnaires, personal interview, and medical evaluation. The forms, questionnaires, interviews, and medical assessments that provide both demographic and inclusion/exclusion information are described below; first, we highlight the criteria.

Parental History (PH) of Alcohol Abuse/dependence: participants are classified as PH+ if one or both biological parents report a life-time history consistent with DSM-IV criteria for alcohol abuse/dependence; participants are classified as PH- if neither parent reports such a history. In the event that one parent is unavailable for interview (e.g., death or divorce), a negative parental history can be affirmed if the interviewed parent is negative and has a high degree of confidence and good recent knowledge of the absent parent. For PH+, a positive self-report of the available parent will suffice, and information about the absent parent (positive or negative) is accepted if there is a high degree of confidence and good recent knowledge. In the PH+ missing-parent situation, we may not be able to assign a *degree* of genetic loading when knowledge is not confident or current; however, we do not exclude a participant on this basis.

Personal Alcohol Experience. For Study 1, participants are classified as low alcohol (Etoh-) or high alcohol (Etoh+) experience based upon the criteria outlined below. We are not studying young children (ages 9 or 10) with Etoh+, because we do not feel we could locate enough qualified candidates. For example, data from the Monitoring the Future epidemiological survey of Johnston et al. (2000) indicate that even by 8<sup>th</sup> grade fewer than half of youngsters have ever used alcohol. On the other hand, because 70% to 80% of high school students (Johnston et al., 2000) and even more young adults have some lifetime experience with alcohol, we decided not to include a “zero experience” group, since the pool of potential participants would be quite small. Thus, for purposes of Study 1, participants are classified as Etoh- if they report no prior experience with alcohol, experience with alcohol only in small quantities for religious observance, or <1 standard drink per month on average, drinking 2 drinks on a given occasion, and never getting drunk, as confirmed by the DUSI-R and the 1-month Time-Line Follow Back interview (see below). This set of criteria is comparable to the “low-intensity” definition of Beck and Treiman (1996). Participants are classified as Etoh+ if they report past history of alcohol use (>10 lifetime experiences for adolescents and >20 experiences for young adults) and current alcohol use frequency 1 time per week, averaging 5 to 20 drinks per week, as confirmed by the DUSI-R and 1-month Time-Line Follow Back interview. Unlike the Beck and Treiman (1996) definition of high-intensity alcohol users, we do not require that Etoh+ participants report getting drunk each month. Participants are excluded if they meet DSM criteria for alcohol abuse or dependence. For Study 2 and 3 participants, moderate alcohol use criteria include current drinking of 1 time per month, averaging 2 drinks per occasion, but not exceeding an average of 10 drinks per week. These criteria are similar to those used to define moderate drinking by Beck and Treiman (1996).

General Medical/Behavioral Exclusions include personal or family (first degree) history of narcolepsy, sleep apnea syndromes, restless legs syndrome, psychotic disorder, or genetically transmitted neurologic disorder; personal history of or diagnosis with epilepsy or other neurologic disorder (including fetal alcohol syndrome), metabolic disorders, chronic medical conditions (e.g., cancer, diabetes, kidney disease, active asthma) or infectious illness; current illness, fever, symptoms of respiratory infection or allergy at the time of in-lab assessments; current use of prescribed psychoactive agents or other drugs that may affect the sleep/wake cycle or daytime sleepiness/alertness or the circadian timing system (except alcohol, as required for inclusion); physical handicap that interferes with testing (e.g., blind, deaf); mental retardation or pervasive developmental disorder; expressed suicidal intent or attempts; history of shift work or travel beyond 2 time zones in the 3 months before in-lab assessments. Participants must have sufficient knowledge of the English language to complete study requirements.

Additional Exclusions for Study 2 and Study 3 Participants. The following additional exclusion criteria are required for participants in Studies 2 and 3 who will be receiving alcohol: scores on Center for Epidemiological Studies Depression Scale falling outside normal limits; morningness/eveningness (M/E) score falling 2 standard deviations from mean values of subjects in the same grade (age) and of the same sex, from our laboratory norms or published norms; body weight is 20% above or 20% below normal values adjusted for age, sex, and height; self-reported sleep schedules that vary by greater than 3 hours across a week or that indicate chronic insufficient sleep accompanied by overt signs of excessive sleepiness (e.g., falling asleep inappropriately); use of drugs or medications that contraindicate alcohol; pregnancy; current sleep problem or sleep disorder as noted in self or parental report; for example, excessive daytime sleepiness, enuresis, sleepwalking, night terrors, sleep apnea syndromes, insomnia, narcolepsy; history of mononucleosis, hepatitis, or other disorder specifically affecting liver function; abnormal liver function values. Participants with known sensitivity to alcohol will be excluded; particularly in the case of volunteers of Asian descent, they will be excluded if their alcohol experience is too low to assess this factor. All participants in Studies 2 and 3 must report some prior experience with alcohol in at least moderate amounts. Parents of adolescents in Study 2 must also acknowledge prior alcohol experience in the participant. Participants are excluded if average alcohol use exceeds 10 standard drinks per week.

## **B. Methodology: Procedures**

### **1. Pre-Study Assessments: Screening and Demographic Information.**

a. Telephone Screening Questionnaire. Each candidate is given an explanation of the study and fairly extensive interview over the telephone that focuses on the exclusion factors. The telephone screening is completed by parents and children.

b. Sleep Habits Survey (Appendix). All candidates complete the Sleep Habits Survey. This form is used to collect demographic and screening information from volunteers for the present study. In general, the questions cover the volunteer's usual sleep schedule, morningness/eveningness (Smith et al., 1989), sense of well-being (Rutter et al., 1976), life satisfaction (Diener et al., 1985) depressed mood (Kandel et al., 1982), and pubertal development (Carskadon and Acebo, 1993). Adults complete the abridged Sleep Questionnaire and Assessment of Wakefulness (Douglass et al., 1994), which covers current sleep patterns and sleep disorders symptoms.

c. Sleep, Medical, Educational, and Family History Form (Appendix). This form is a data-gathering tool used routinely for studies in our laboratory. One parent (usually mother) completes this form for the child prior to entry into the study. Young adults complete an adult version of this form.

d. Menstrual Status Form (Appendix). All menarchal girls and young women complete the menstrual status form, either with the help of their mothers or as an interview with a female research assistant.

e. Child Behavior Checklist (CBCL)- Parent Version (Appendix). For young participants, Child Behavior Checklist (CBCL), a widely-used global behavioral rating scale, assesses current behavior problems for all subjects in the study. Parent versions of the CBCL have well-established reliability and validity for youngsters up to 16 years (Achenbach et al., 1983). CBCL scales measure multiple dimensions of behavior, including inattentiveness, hyperactivity, aggressiveness, and delinquent conduct. Parents (chiefly mothers) complete the CBCL before initial laboratory evaluations.

f. Youth Self Report (YSR) Form (Appendix). The YSR is a self-report form standardized on adolescents to evaluate the adolescent's emotional/behavioral problems (Achenbach et al., 1991). Three measures are derived from this scale: Internalizing (withdrawn, somatic, anxious/depressed behaviors), Externalizing (conduct problems, aggressive behaviors), and Total Behavior Problems.

g. Revised Symptom Checklist-90 (SCL-90-R) (Appendix). The SCL-90-R is a pencil and paper self-report symptom inventory that takes about 15 minutes to complete and is given to adult participants to provide a measure of current distress with the Global Severity Index (Derogatis, 1975; 1994).

h. Center for Epidemiological Studies Depression Scale (CES-D) (Appendix). The CES-D is a general assessment for depressive mood that has been normed for adolescents and adults (Radloff et al., 1977). This form is used to provide a basal, demographic value for depressed mood.

i. Drug Use Screening Inventory (DUSI-R) for Youth (Appendix) or Adults as appropriate is used to characterize substance use over the past year and to provide substance use comorbidity information in our initial screening assessments of participants (Tarter and Hegedus, 1991).

j. Time-Line Follow Back Interview (Appendix). The Time-Line Follow Back Interview has been used routinely in adult participants of alcohol intervention studies to measure substance use (including alcohol) over intervals of 1, 3, and 6 months (the latter after “practice” with the technique). Monti and colleagues (in press) have also successfully used the 1-month version of this measure in adolescents as young as 13 years. We administer the 1-month TLFB interviews for screening and to classify parents and children in initial evaluations and in the 6-month follow-up assessments of Study 1.

k. Family History Screening Interview (Appendix). A modified version of the Family History Screen of Weissman et al. (2000) is administered by a trained clinician to the participant’s genetic mother and father and to step-parents living in the home. This instrument has shown high validity and good reliability for identifying major depression, anxiety disorders, substance dependence (including alcohol), and suicide attempts in a screening format. The modification is to include self-report and to add the spouse and the child participant as key family members. When used for this project, the interviewer conducts the structured interview, and when clinically relevant, the interviewer follows up with relevant modules from the Structured Clinical Interview for DSM-IV [SCID (First, Spitzer, Gibbon, and Williams, 1995)] for our key inclusion/exclusion criteria, including psychosis, full DSM alcohol abuse/dependence factors, and DSM depression criteria.

l. Physical Examination and Tanner Staging. The final assessment of each participant includes a brief physical examination and, for child and adolescent participants, Tanner staging of pubertal development (Tanner, 1962) performed by our consulting physician, who has attained high reliability in Tanner staging. Tanner staging yields two variables for each child: for boys, stage of genital development and pubic hair growth; for girls, stage of breast development and pubic hair growth. When the two variables differ, the pubic hair growth score is used, since measurement reliability is highest with this variable (Tanner, 1962). Tanner staging will not be performed in young adults, all of whom are assumed to be Tanner stage 5.

## **2. Field Monitoring Procedures (*data are scored by individuals blind to participant group status*).**

a. Sleep/Wake Diary (Appendix). Participants complete a sleep/wake diary throughout all at-home portions of the studies. These diaries document bedtimes, rising times, amount of sleep, and so forth. Data gathered with the diary are compared to and verified via actigraphy.

b. Actigraphy. The actigraph is a device the size of a digital wrist watch (1.75” x 1.3” x .38” weighing 2 oz.) worn on the nondominant wrist (Mini-motionlogger AMI, Ardsley, NY, USA). The accelerometer sensor interfaces with solid-state memory, initialized by a software program and requiring no further manipulation. The actigraph is initialized for 1-minute epochs, Zero-Crossing mode, with filter setting 18. This enables continuous monitoring for a period longer than a week. After the actigraph is initialized, it is given to the participant with a demonstration of how to wear and care for it. Participants wear the device throughout at-home and in-lab portions of each study. When the participant returns to the lab, the data are downloaded, printed, and compared to the diary information. Discrepancies are identified and the actigraph record annotated for periods when the device is off or where the actigraph record is not congruent with behavior (e.g., napping during a car ride). The data are analyzed to estimate sleep/wake using algorithms validated by our laboratory (Sadeh et al., 1994). Comparisons are made with the participant’s sleep diary to confirm bedtime and rising times and compliance with the study schedules. Actigraph variables are described in Acebo et al., 1999 in the Appendix.

c. Schedules. Depending upon the goals of the individual study, participants are asked to keep their usual, self-selected schedule or are placed on a strict schedule for sleeping and waking. We use three types of sleep schedules. Actigraphy and sleep diaries are used throughout; morning phone calls to the laboratory answering machine to report sleep times are required on the Self-Selected schedule; morning and evening phone calls to the laboratory answering machine to report sleep times are required during the Stabilization and Optimization schedules.

- **Self-Selected**: Participants on the Self-Selected portion of a study are asked to maintain their usual schedule, to sleep at home (no sleepovers) without visitors, and to avoid “all-nighters.” Otherwise, we do not apply restrictions. The Self-Selected schedule permits us to assess sleep patterns of participants under relatively “naturalistic” conditions.
- **Stabilized (Stabilization)**: As a lead-in to the in-lab portions of Studies 1 and 2, it is important to fix participants’ at-home sleep schedules in order to (1) minimize sleep restriction, (2) stabilize the circadian timing system, and (3) permit meaningful comparisons of in-lab parameters across participants and over time. Many sleep parameters, such as sleep latency, amount of slow wave sleep, REM sleep latency, and so forth are very responsive to prior sleep history and/or to circadian phase (cf., Carskadon and Dement, 2000). The stabilization procedure allows us to tailor the schedule for individuals, while fixing the sleep pattern for 10 to 12 nights provides adequate consistency prior to the study so that sleep parameters are not affected. The Stabilization schedule for each participant is determined by data from the week of self-selected actigraphy in conjunction with the requirements of the participant’s activities. Thus, bedtime and rising time are determined by participants’ required activities (such as school starting time), and the schedule includes a minimum of 10 hours per night time in bed for participants ages 14 or younger, 9 hours per night time in bed for participants ages 16 to 20, and 8.5 hours per night time in bed for participants ages 21 and older. Participants are placed on a schedule providing more sleep if their sleep habits questionnaire or self-selected actigraphy record indicates a pattern of more sleep than this minimum. These minimum amounts are intended to provide a schedule that is relatively consistent within age groups and provides adequate sleep for all participants (though not necessarily ‘optimal sleep’). On Stabilization nights, participants are required to sleep at home (no sleepovers) without visitors, in bed with lights out and without other activities (e.g., television, radio) during the scheduled sleeping hours. Participants are asked to limit caffeine to a maximum of one drink not taken within 12 hours of scheduled sleep time and to eliminate all other substance and medication use throughout the Stabilization procedure.
- **Optimized (Optimization)**: The optimized schedule is used for participants enrolled in Study 3. Optimization is intended to (1) provide recovery from prior sleep debt, (2) stabilize participants to the same circadian phase, and (3) permit in-lab study procedures to be scheduled coincidentally. For Optimization, all participants are given an identical 10-hour time in bed sleep schedule (2200 to 0800), which they maintain for 12 to 14 nights prior to their in-lab study. Furthermore, on Optimization nights, participants are required to sleep at home (no sleepovers) without visitors, in bed with lights out and without other activities (e.g., television, radio) during the scheduled sleeping hours, and wearing eye shades provided by the lab throughout the hours designated for sleeping. Participants are asked to eliminate all caffeine and other substance and medication use throughout the Optimization procedure.

d. **Dim Light Salivary Melatonin Onset (DLSMO)**. This measure is described in detail below, and noted here, as we are collecting this phase marker at the end of actigraphy collection on the Self-Selected schedule. Participants come to the lab on one evening on which 10 saliva samples are collected at 30-minute intervals, the times of which are determined based upon the actigraph bedtimes on the preceding 5 nights. The last scheduled saliva time is set at the nearest quarter of an hour after the mean reported bedtime. Thus, a subject with a mean reported bedtime of 2320 has the first sample collected at 1900 repeated each half hour until 2330. See below for specific information on saliva collection procedures and computation of melatonin onset phase.

### **3. Laboratory Procedures (data are scored by individuals blind to participant group membership status).**

a. **General Considerations**. Several studies proposed in this application require young people to undergo complex, demanding, and lengthy laboratory evaluations, under conditions that we know are inherently difficult, for example, sleep deprivation. Based on our previous research, we are confident in the capacity of children, adolescents, and young adults to undergo the procedures and even to enjoy the experience. We are also sensitive to the personal resources a participant requires to succeed and the supports that the laboratory must provide to enhance the likelihood for success. Our considerations recognize not only our regard for the well-being of our study participants, but also our concerns for the overall success of the research endeavor. The effort and expense involved in creating this research environment are substantial, and we intend to maximize the

payoff of the investment. In order to achieve this goal, we provide experimental protocols that subjects are willing to volunteer for and that they are able to complete.

Our strategy to accomplish these goals relies in part on making the laboratory procedures relatively noninvasive. Human sleep and circadian rhythms research requires continuous or frequent measures of the parameters of interest, which usually include rectal temperature and blood constituents. Such studies have been largely limited to adult subjects, who are able to tolerate more invasive procedures. We have succeeded in obtaining core temperature data from children and adolescents of the ages proposed in this, and we also have had success with salivary measures rather than blood measures. Furthermore, procedures for gathering sleep data using measures from skin and scalp surface are well tolerated by young people. By acquiring these minimally invasive measures in the context of the state-of-the-art experimental paradigms and using well controlled laboratory conditions, we are confident that we obtain accurate estimates of circadian and sleep parameters. Furthermore, we are confident of ability to recruit young people capable of succeeding.

b. Polysomnography. All three studies involve continuous polysomnographic recordings during in-lab scheduled sleep episodes. Continuous monitoring also takes place during all multiple sleep latency tests or performance testing. Polysomnography involves continuous monitoring of a number of physiological variables, all of which are assessed with noninvasive techniques. We routinely record referential (contralateral mastoid or ear lobe) electroencephalogram (EEG) from C3, C4, O1, and O2 electrodes measured according to the standard 10-20 system of electrode placements (Jasper, 1958) applied to the scalp with collodion-soaked gauze pads; referential electro-oculogram (EOG) from surface electrodes taped at the left and right outer canthi; chin electromyogram (EMG) from electrodes taped over the mentalis/submentalis; electrocardiogram (ECG) from electrodes taped on right shoulder and left side [modified lead II]. Thermocouples, leg EMGs, and oximetry are used only on initial in-lab nights to screen for occult sleep disorders. For daytime recordings, participants may not wear the chin EMG or EKG electrodes, and recordings are continuous only during testing.

Polysomnographic recordings are made using Grass Model 8 polygraphs and amplifiers and digitized (100 samples per sec.) on Astromed/Grass Braintree systems. Recordings are coincidentally recorded to paper at a paper speed of 10 mm/sec. For EEG and EOG channels, the low frequency cut-off is 0.3 cps, high frequency 30 cps, and the calibration level is 50  $\mu$ V/cm. These are standard techniques (Bornstein, 1982; Carskadon and Rechtschaffen, 1989; Rechtschaffen and Kales, 1968).

Nocturnal polysomnograms are visually scored in 30-second epochs according to standard criteria (Rechtschaffen and Kales, 1968). Our technologists have inter- and intrarater reliability assessed semiannually to a level of  $>.90$ . Epoch data are entered into a computer program (Somnibus, Behavioral Cybernetics, Cambridge, MA) for calculation of parametric descriptions of each night—that is, amount of total sleep and sleep stages, latency to sleep onset and to various sleep stages, and so forth—along with a sleep histogram. Transient ( 15 seconds) arousals are evaluated according to the criteria of Carskadon et al. (1982) and tabulated for NREM and REM sleep. Apneas are scored during any stage of sleep when airflow stops for 10 seconds (Guilleminault et al., 1978). Hypopneas are scored when the excursion of the pens monitoring airflow and effort drops to  $<50\%$  of the preceding level for 10 seconds, and when the return to basal excursion is accompanied by any signs of arousal in the recording. Leg movements are coded according to the methods of Coleman et al. (1982). In addition, for the analysis of sleep stages during the FD protocol of Study 3, the Somnibus file is transformed into a file providing a minute-by-minute time-linked data set. This data set is then linked to circadian time (after determination of intrinsic circadian period), and the sleep data can be evaluated for analyses examining circadian and sleep/wake homeostatic hypotheses (see analysis section of Study 3). The records collected during performance tests are analyzed for unintentional sleep episodes (microsleeps) according to laboratory procedures that code as microsleep those sleep episodes  $\geq 3$   $<15$  seconds.

c. Multiple Sleep Latency Test (MSLT). The MSLT is an operationally-defined measure of daytime sleep tendency or sleep propensity (Carskadon and Dement, 1982; Carskadon et al., 1986). EEG and EOG are continuously recorded and monitored; on the first day of MSLT testing for each participant, chin EMG is also recorded. For each test, the participant is put to bed in an individual bedroom and given the instructions to: "lie quietly, keep your eyes closed, and try to fall asleep." The light level is  $<1$  lux. Study 1 participants are allowed to sleep for up to 20 minutes in order to examine sleep stage structure, particularly REM sleep onsets.



Otherwise, we use standard procedures for the experimental version of the MSLT (Carskadon et al., 1986), in which tests are ended at 3 consecutive epochs of sleep (including stage 1) or the first epoch of stage 2; otherwise, tests continue for 20 minutes. The primary outcome measure is latency from lights out to the first sign of sleep (>15 sec stage 1 sleep). Slow eye movements (SEMs) are also coded.

d. Forced Desynchrony (FD). Study 3 uses a 20-hour FD to evaluate how circadian and sleep/wake homeostatic processes interact with alcohol administration to affect sleep, melatonin, performance, mood, and sleepiness. We selected a 20-hour imposed schedule rather than 28-hours for several reasons. First, humans experience a free run in either circumstance (cf., Wyatt et al., 1999). Second, the 20-hour protocol, with its quicker cycling of wake and sleep, provides a greater number of measures over a shorter length of time and thus is significantly more economical. The major drawback of the 20-hour versus 28-hour day length is that the sleep/wake homeostatic system can be assessed only over a relatively short span of time awake (or asleep). Given the nature of our questions, the proposed 13h 20m days and 6h 40m nights will be adequate.

In order to perform FD, participants undergo the Optimized schedule described previously for 12 to 14 cycles before coming to the laboratory. The laboratory conditions during the FD require low level lighting to permit accurate collection of melatonin levels and to avoid affecting the circadian system by inducing “relative coordination” to the light/dark schedule. We keep light levels at 15 lux during waking phases and <1 lux during sleeping phases. To assess circadian phase for individuals at any instant during the FD, we need to obtain an estimate of intrinsic period for each participant; hence, we measure melatonin levels in saliva samples obtained in all waking phases, giving phase markers permitting period assessment by linear regression through multiple cycles. The schedule for FD procedures is outlined in the methods section of Study 3.

e. Core Body Temperature. Core body temperature is measured on two nights in Study 2 and throughout the FD protocol in Study 3. The measure is obtained by placing a thermistor probe (disposable esophageal/rectal flexible thermistor probe, Mini-Mitter, Inc., Sunriver, Oregon) 10 cm within the rectum. Each probe is prepared before use by marking the correct insertion depth with an easily located tape flag. Participants are given a lesson in probe insertion and take a kit home for practicing before attempting to insert the probe in the lab. All participants must report successful insertion before returning to the lab. For recording, the thermistor is attached to a Mini-Logger 2000 data logger (Mini-Mitter, Inc., Sunriver, Oregon), a small, battery-operated computer designed to acquire, store and download temperature to a host computer. Temperature is collected in 1-min intervals and can be viewed either continuously or intermittently on the host computer throughout data collection. In the lab, the thermistor probe is inserted by the participant in the privacy of the bathroom. To minimize logger movement, the case is pinned to the side of the bed or attached by a plastic clip to the participant’s clothing. At the end of the data collection interval, temperature data are downloaded on a PC and extraneous data points (e.g., temperature deviations of > 0.4°C in a 5 min. period related to probe slips, bathroom trips) are edited out. Editing is done conservatively, and temperature deviations not clearly associated with probe slips and/or bathroom trips are brought to a consensus meeting for review and discussion, while blind to condition and group membership.

f. Salivary Melatonin and Cortisol. Saliva samples (2 ml) are collected using the salivette system (Sarstedt, Newton, NC, USA), in which a participant chews on a plain (not citric impregnated) cotton cylinder, which is then placed in a test tube and centrifuged for two minutes. If participants consistently require more than one cylinder to produce adequate saliva, they chew parafilm to stimulate saliva and deposit it directly into a tube. Samples are provided in dim (<40 lux) light while seated for at least 5 minutes. Participants rinse their mouths and brush their teeth (without toothpaste) before samples that occur following drinks, meals, or snacks. Saliva is frozen (-20° C) within 4 hours. Frozen samples are taken to the Miriam Hospital Lipid Laboratory (Linda Bausserman, Ph.D.) for radioimmunoassay using the Alpco (Windham, NH) assay kits.

Phase of melatonin secretion onset has been used as a circadian phase marker for a number of years (Lewy and Sack, 1989), and the salivary determination of the marker is achieving increasing support (e.g., Voultsios et al., 1997). We have had outstanding success using this marker in our adolescent studies. Our melatonin phase determinations are made using a threshold method in which 4 pg/ml is used as the threshold value. According to Deacon and Arendt, salivary melatonin is approximately 40% of the plasma value (Deacon et al., 1994); hence, the 4 pg/ml level is comparable to Lewy’s 10 pg/ml threshold (Lewy and Sack, 1989). We determine the

time of melatonin onset and offset phases by linear interpolation from the samples bracketing the threshold, provided that the melatonin level continues to rise (onset) or fall (offset) in the subsequent sample. This method works well for adolescents in whom salivary melatonin levels consistently rise above 4 pg/ml during nocturnal hours. We are concerned that lower melatonin levels in adults may render this method of calculating phase invalid or that it may impair our ability to compare phase position across age groups. Therefore, we plan to utilize Method 3 of Voultsios and colleagues to estimate melatonin onset phase for these comparisons (Voultsios et al., 1997), which computes melatonin onset phase relative to individual levels.

Cortisol levels are also examined from morning saliva samples in Studies 1 and 2 and throughout FD in Study 3. In protocols that unmask circadian rhythms, cortisol generally begins to rise a bit before the falling limb of the melatonin cycle (see Figure 5 taken from adolescents examined during a constant routine unmasking), and the circadian phase with high levels of cortisol is generally coincident with the greatest circadian propensity for REM sleep (cf., Moore-Ede et al., 1983). Because of this usual temporal relationship, cortisol levels will also be examined to explain the presence of REM on the morning MSLTs. Furthermore, cortisol levels are compared across groups in Study 1 and examined with specific reference to alcohol ingestion in Studies 2 and 3.

g. Introspective Measures. Throughout all phases of the in-lab protocols, participants complete a simple set of visual analog measures derived from the work of Monk and colleagues to assess alertness and mood at 30-minute intervals (Monk, 1989). The scales are completed by marking on a 100 mm line how “you feel right now.” The scales range from “very little” to “very much” with the following items: How alert do you feel? How sad? How tense? How much of an effort is it to do anything? How happy? How weary? How calm? How sleepy? Overall, how do you feel? The Stanford Sleepiness Scale (SSS), a 7-point Likert scale, is also included on this form (Hoddes et al., 1972a; 1972b). The scales are administered in tandem, take about 1 minute to complete, and provide an introspective estimate of the level of sleepiness.

h. Neurobehavioral Assessment Battery. We use the Neurobehavioral Assessment Battery (NAB) of Dinges to measure a set of parameters at 2-hour intervals during the in-lab studies (Dinges et al., 1997). The NAB includes a number of additional introspective measures (the SSS, the 9-point Karolinska Sleepiness Scale (Gilberg et al., 1994), and the activation-deactivation checklist (ADACL, (Thayer, 1978)), probed word memory task, a 10-minute simple reaction time test, a 90-second digit symbol substitution task, and a series of brief time estimation trials. The testing battery lasts approximately 25 minutes. Light levels are minimally (1 to 2 lux) affected by the computer screen during the testing battery. Participants are paced through the testing battery from the control room so that the tests and associated forms are completed at the same protocol time by every participant. Participants are monitored continuously with EEG and EOG to document any microsleep episodes. If the volunteer stops performing or falls asleep for 30 seconds, the research assistant (using the intercom system) reminds the volunteer to continue working. The data are evaluated for signs of drowsiness (slow eye movements) and microsleep episodes, tabulated by 30-second epochs.

i. Divided Attention Task. Divided attention tasks are commonly used to assess performance deficits arising from sedation or intoxication. We use a computerized divided attention task requiring continuous tracking of a central stimulus with simultaneous attention and response to peripheral or central stimuli (Roehrs et al., 1989). Dependent measures derived from this 15- or 30-minute task include tracking time, central reaction time, and peripheral reaction time. Signs of sleep are monitored as on the NAB.

j. Driving Simulation. Our laboratory is in the process of completing validation tests of a driving simulation program [York Driving Simulator (YDS; York Computer Technologies, Kingston, Ontario, Canada)] that combines over-learned skills with a cognitive task. The driving simulation/cognitive test is 30 minutes in length. Signs of sleep are monitored as on the NAB. The computer screen for the test adds a minimal intensity of light (~2.5 lux) to the room light levels. The driving program presents a forward view from the driver’s seat of a straight two-lane highway, with lane markings and signs and signals appropriate for the road environment. A digital speedometer appears at the bottom of the screen, and simple line drawings (as opposed to video simulation) portray objects and induce a sense of apparent motion by using real-time perspective generation. The participant is instructed to stay in the right hand lane, to obey all speed signs, and to avoid the simulated obstacles. The dependent measures for the driving task include: tracking (deviation in feet of the vehicle’s

center from the center of the right-hand lane), tracking variability, speed deviation and variability, and “off-road” incidents.

The simultaneous cognitive component is a working memory task adapted from similar tasks (e.g., Drummond et al., 1999), and appears on the computer screen at eye-level while participants are driving. Two types of tasks are presented—a “count” and a “computation” task—which are alternated in 1-minute blocks for 4 blocks, followed by a 1-minute “rest” block. Dependent measures for the cognitive task include: number of correct, incorrect and omitted responses, and the speed of reaction time.

k. Alcohol Administration Protocol (Study 2 and Study 3). Alcohol beverages are administered with participant and assigned research assistant blind to condition. A research assistant uninvolved with the laboratory protocol prepares and delivers the beverages to participants and acquires breathalyzer readings. A balanced placebo design to control for participant’s expectancy of alcohol effects was considered but ruled out primarily because it is a fully between-subjects design. Under the circumstances of our project, the expense of the fully between subjects approach is prohibitive. We are reassured about our choice, however, because the variables of interest in this study—introspective, physiological, and performance—are minimally affected by expectancy (Marlatt and Rohsenow, 1980). Our beverage composition and administration protocol follow a number of other guidelines suggested for studies using the balanced placebo design (Rohsenow and Marlatt, 1981). Participants are screened upon arrival at the laboratory via self-report and breath analysis using an AlcoSensor IV hand-held breath analyzer (Intoximeters, Inc, St. Louis, MO) to ensure compliance with the pre-laboratory protocol regarding alcohol consumption. Participants are routinely weighed at the start of in-lab sessions. In Study 2, no food or beverage other than water is consumed within 4 hours of alcohol/placebo administration. In Study 3, small meals are taken at 2-hour intervals, so that stomach contents are relatively similar at each time of alcohol or placebo administration.

The “alcohol” beverage consists of 80 proof alcohol acquired as prescribed beverage alcohol through the hospital pharmacy mixed with chilled tonic (Schweppes) in a 1:4 ratio with a wedge of fresh lime run around the rim. The “placebo” beverage is a similar chilled tonic with lime drink having three drops of vodka floated on the surface. For each participant, the volume of liquid is equivalent for both conditions and distributed equally between three glasses. Drinks are consumed by each participant in his/her room under video observation, without social interaction except to encourage participants to pace their drinking and to consume the drinks within 30 minutes (one glass every 10 minutes). Participants provide a breath sample for analysis of alcohol concentration after allowing 30 minutes for absorption (Rohsenow and Marlatt, 1981), and they are not informed of their alcohol concentration value until debriefing.

Based on recent findings with maritime cadets of similar ages to our young adult participants (Howland et al., 2000), the alcohol is administered in a 0.6 g/kg dose for adult men and, consistent with Friel et al. (1999), 0.55g/kg for adult females (i.e., a male:female dosing ratio of 1.1:1). We know of no information regarding administration of alcohol to adolescents, and thus, we plan to use the same doses for our teenaged participants. If, however, we find differences in the metabolism of alcohol as a function of age, we will adjust the dose accordingly. We anticipate this dose of alcohol to produce blood alcohol concentrations in the range of 0.045 to 0.05 g% for all participants. Except in very large participants consuming more than 50 or 60 g of alcohol, we anticipate zero BAC levels to be achieved within an interval of no more than 6 hours from ingestion.

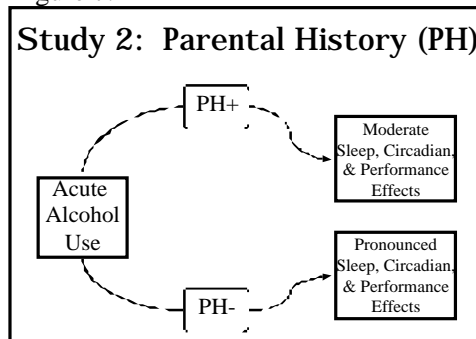
Participants are informed prior to enrolling for either Study 2 or Study 3 that they will receive either a low or moderate dose of alcohol during the course of the experiment. Although this procedure is somewhat misleading (given that the “low” dose is physiologically null), we feel that it is useful to control for expectancies that may occur. Although our primary sleep variables are not susceptible to expectancies, certain secondary variables may be susceptible. Furthermore, we shall implement two additional procedures to minimize the likelihood of alcohol detection, based on recommendations from our consultant, Dr. Timothy Roehrs, and based upon Rohsenow and Marlatt (1981): (1) taste acuity is reduced by having participants rinse their mouths with mouthwash immediately before ingesting the beverages, and (2) proprioceptive cues are controlled by ensuring that participants remain seated throughout the alcohol procedures and by providing a distraction after they consume the beverages. Participants in both the placebo and alcohol conditions provide

breath samples for analysis of alcohol content at 30-minute intervals; no participant is provided feedback during the studies about breathalyzer results.

## Study 2. Effects of Evening Ingestion of Alcohol on Sleep, Circadian Phase, and Performance as a Function of Parental History of Alcohol Abuse/dependence.

**Rationale.** As summarized in the background section of the proposal, the effects of acute alcohol administration on the sleep of nonalcoholic adults are fairly consistent: reduced latency to sleep onset, increased SWS, reduced REM sleep, increased REM latency. Nothing is known about effects of alcohol on sleep in adolescents, nor in adolescents or young adults with parental history of alcohol abuse/dependence. The issue of parental history will frame all assessments in Study 2, based upon findings that individuals with positive family history of alcoholism show blunted response to physiological and some psychological effects of alcohol (Schuckit and Smith, 1996; Volavka et al., 1996). Figure 7 depicts the model that frames this major component of Study 2.

Figure 7.



Many other issues, however, are addressed in this study. For example, the effect of evening alcohol administration on the circadian timing system, as marked by melatonin secretion, is unknown. This effect may be particularly important in adolescents because of their inherent tendency to phase delay. Knowledge regarding alcohol metabolism and performance effects of an evening alcohol dose is also important in the adolescent group. Our limited data indicate that adolescents use alcohol most frequently in the evening. We track weekly substance use of high school students in one of our studies; these data indicate that over 90% of reported alcohol use occurs in the evening. Roehrs and colleagues (1992) showed that evening alcohol is less sedating than morning alcohol in young adults. We do not know whether adolescents also have this “protection” or how long it may persist after alcohol ingestion. Because we *do* know that many young people drink alcohol in the evening and then stay awake beyond their usual bedtime and perhaps operate motor vehicles under this combination of circumstances, we feel it is important to assess performance capacity after evening alcohol, taking into account BAC, melatonin level, and time of night. These parameters have not been assessed concurrently in adults or adolescents, nor has parental history of alcohol abuse/dependence been taken into account. This study, therefore, has the opportunity to break significant new ground in understanding the actions of alcohol and the risks posed for evening alcohol use in young people.

Age Group	Moderate Alcohol Experience	
	PH+	PH-
Ages 15 and 16	n = 16	n = 16
Ages 21 and 22	n = 16	n = 16

**Participants.** Participants will be adolescents ages 15 and 16 and young adults ages 21 and 22 with and without a positive parental history of alcohol abuse/dependence (8 males and 8 females in each age group). All participants will be screened as described in the general methods section and will report moderate alcohol use. Participants in Study 1 are not eligible for Study 2; siblings of Study 1 participants may take part in Study 2. We plan to perform the first one or two sessions of Study 2 with participants in the age 21-22 year group in order to establish the experimental procedures. We do not intend to complete all the older subjects before enrolling teens, however, as we do not wish to introduce cohort effects that might interfere with interpretation of the results.

**Methods.** After screening and study orientation, participants undergo 10 to 12 cycles on the sleep Stabilization schedule prior to in-lab sessions and remain on that schedule throughout the study, except as indicated below. Every participant spends 5 nights in the lab, three consecutive nights for Adaptation, Placebo-Sleep, and Alcohol-Sleep conditions (single-blind) and 2 nonconsecutive nights for Placebo-Wake and Alcohol-Wake conditions. The Placebo-Wake and Alcohol-Wake conditions occur in counterbalanced order (double-blind), and they are separated from the Alcohol-Sleep night and each other by 5 to 7 nights on the Stabilization schedule at home. In order to minimize expectation effects, participants are told that they will receive a drink with alcohol, either low or moderate amount, on the last 4 nights of the study (see general methods section for alcohol administration protocol). Participants leave the lab during days between the in-lab sessions, with instructions to avoid caffeine within 12 hours of scheduled sleep time and to abstain entirely from alcohol and other drugs or medications. Urine is collected for toxicology, and breath alcohol concentration levels (BAC) are obtained upon arrival each evening.

The in-lab sessions begin with an Adaptation night on which participants arrive in the lab approximately 6 hours before scheduled bedtime. At this time, schedule compliance is confirmed by careful review of actigraphy and sleep diary records, a meal is provided (sandwich, fruit, small serving of chips or pretzels, cookie, and drink), and orientation for the laboratory session occurs. Throughout laboratory stays, light levels are held at 40 lux to avoid light suppression of melatonin secretion. Participants are prepared for polysomnography and core temperature recording as described under general methods. Saliva samples are collected at 30-minute intervals beginning 5 hours before scheduled bedtime for determination of melatonin onset phase. The in-lab sleep schedule on Adaptation night is delayed by 30 minutes (both bedtime and rising time) so that a saliva sample can be obtained 30 minutes after usual bedtime in order to limit data loss that can occur if melatonin onset lags usual bedtime. [In our experience, melatonin onset phase leads usual bedtime by about 60 minutes, but a small percentage of individuals may have a negative phase angle of melatonin secretion, that is, melatonin onset delayed until after usual sleep onset.] This strategy should not affect sleep on the Adaptation night; if anything, delaying sleep by 30 minutes may improve sleep by decreasing sleep latency (often prolonged on the first night in the lab). Sleep is monitored throughout the Adaptation night, and these data are used only to document that minimal sleep restriction (which could affect sleep on the subsequent night) has occurred. Although rectal probes will be in place on the Adaptation night and connected to the recording device, these data will not be used. Probes are worn for adaptation purposes only on this first night.

Bedtime and risetime on the second and third nights are kept consistent with the participant's Stabilization schedule. The second and third in-lab nights include the methods described above, except no saliva samples are collected, and an alcohol-containing drink is given starting 90 minutes before scheduled bedtime to be consumed by 60 minutes before scheduled bedtime. The Placebo version of the alcohol beverage (as described in the methods section) is given on the second night and the Alcohol version on the third night in a single-blind condition. This order is maintained to avoid the possibility that effects of alcohol on sleep on one night affect sleep on the subsequent night. Decades of previous sleep research studies indicate that the second night of in-lab sleep is an adequate baseline for such comparisons. BAC is measured 60, 30, and 5 minutes before lights out as described in the general methods section (blind to participants and all but one staff member). Polysomnography and core temperature are monitored continuously beginning 90 minutes before scheduled bedtime. The specific dependent variables derived from the Sleep nights in Study 2 are: Sleep Onset Latency, REM Sleep Latency, REM Sleep Percent, Slow Wave Sleep Percent, and Slope of Core Body Temperature decline from alcohol/placebo ingestion time to lights out and from sleep onset through the first 3 hours of sleep. Performance test practice is provided during free time before lights out on the first three in-lab nights to overcome practice effects before the 4<sup>th</sup> in-lab night.

The second phase of Study 2 includes two nonconsecutive nights on which waking effects of evening alcohol ingestion are compared. As on other in-lab nights, participants arrive approximately 6 hours before Stabilization bedtime, are checked for schedule and substance compliance, are given a meal, hooked up for monitoring, and undergo a number of tests at scheduled intervals. Participants remain in bed in dim light (40 lux) with the bed at a 70° angle for performance testing and a 45° angle for MSLT testing (a procedure we have used with success in the past). The testing schedule is individualized for participants relative to their

Stabilization sleep schedules. Thus, for example, a participant with scheduled bedtime at 2300 drinks at 2130-2200 and goes to bed at 0300, five hours after ingesting the drink. Participants are permitted to sleep 60 minutes past their Stabilization rising times to mitigate the sleep restriction occurring on this night. They are required to return to the Stabilization schedule on the following night. Saliva samples are collected at 30 minute intervals along with visual analog measures of sleepiness and mood, as well as breath ethanol concentration (after alcohol/placebo ingestion). Beginning 2.5 hours before alcohol/placebo ingestion, participants are given an MSLT, repeated at 90-minute intervals until lights out. The Neurobehavioral Assessment Battery and Divided Attention Task are given 180 minutes before and 30, 120, and 210 minutes after alcohol/placebo ingestion. Driving Simulation is tested at 90 minutes before and 60, 150, and 240 minutes after alcohol/placebo ingestion.

The following specific variables will be assessed: BAC over time; melatonin area under the curve from samples collected before lights out; MSLT sleep latencies for each test; NAB variables that include PVT lapses, PVT fastest 10% reaction time, number of words recalled on probed memory recall, correct attempts on digit symbol substitution; DAT variables that include tracking time, missed stimuli, and reaction time for stimuli; Driving Simulation variables that include number of crashes, lane deviation, and speed variation.

Analysis. As indicated in the rationale above, Study 2 contains hypothesis-driven and descriptive elements. *All hypotheses implicate Parental History* as indicated in Figure 7; that is, responses to alcohol administration will be mitigated in PH+ participants. We have no specific hypotheses concerning age or sex; however, these variables will be assessed in all analyses and in descriptions of the findings. When no sex or age effects are found, these factors will not be included in subsequent analyses.

Our initial hypotheses concern the data collected on the Sleep nights (Nights 2 and 3). We predict that alcohol will alter sleep versus placebo in a manner similar to that seen in previous studies: decrease sleep onset latency, increase SWS, decrease REM sleep, increase REM latency, decrease REM sleep percent. This hypothesis will be tested with multivariate analysis of variance of Night 2 to Night 3 change scores, including PH group and age as between subject factors. Our hypothesis will be supported by main effects of condition, i.e., change scores significantly different from zero; our overarching hypothesis regarding PH+/PH- will be supported by a significant effect of parental history group. Our subhypothesis concerning age effects will be supported by a significant effect of age group. We also will test the hypotheses that the slope of core temperature decline is steeper following alcohol administration and that temperature slope is associated with the alcohol-related increase in SWS. These hypotheses will be tested by a similar multivariate approach to assess differences in core temperature slopes and by correlation of the temperature slope variables with SWS to determine association between these variables. Our hypothesis will be supported by a significant correlation showing that steepness of core temperature decline is correlated with increased SWS.

For the waking data collected on Nights 4 and 5, we will need to analyze for time of test, as well as for differences between conditions (change scores). In general, we hypothesize that sleepiness will increase and performance will decrease following alcohol administration and that these effects will be greatest in the hours after Stabilization bedtime (even though BAC will likely be falling or may even be zero before the last test). To test these hypotheses, we will perform repeated measures analysis of variance with time of test as a within subject factor and parental history as a between subjects factor. Our hypothesis will be supported in variables showing a significant effect of condition (change scores differ from zero) and time of test. With regard to the hypothesis concerning PH, we will find confirmation with a significant main effect of parental history.

Sample Size Determination. With a sample size of 32 for each parental history group and 32 for each age group and an alpha of .05, we will have ample power (.94) to detect large effect size differences and moderate power (.58) to detect medium effect size differences.

## **E. Human Subjects.**

### **1. Characteristics of the Subject Population.**

The proposed research will include a total sample of 288 participants, including children (ages 9-10 years), adolescents (ages 15-16 years) and adults (ages 18-22 years). We will include equal numbers of male and female participants with (PH+) and without (PH-) parental history of alcohol abuse/dependence. The research will be conducted in three studies: Study 1 will include 40 children with low alcohol experience (20 PH+, 20 PH-), 80 adolescents, half with low alcohol experience (20 PH+, 20 PH-), and half with high alcohol experience (20 PH+, 20 PH-), and 80 adults, half with low (20 PH+, 20 PH-), and half with high (20 PH+, 20 PH-) alcohol experience; Study 2 will comprise 32 adolescent and 32 adult participants (16 PH+, and 16 PH- in each group); and Study 3 will include 24 normal adult participants without a family history of alcohol abuse/dependence, and with minimal alcohol experience. Attrition will be most likely at the follow-up assessment in Study 1, which we expect to arise primarily from loss of interest, inability to locate participants, or inability to meet the study schedule. Participants in all three studies will be generally medically and psychologically healthy, and additionally for Study 2 and Study 3, they will maintain relatively regular sleep schedules, have no liver-related illness, and females will not be pregnant. The most recent edition of the Chronicle of Higher Education provided the following ethnic distribution in the state of Rhode Island: 0.5% American Indian, 2.2% Asian, 4.8% Black, 92.5% Caucasian, and 6.2% Hispanic. In reviewing recruitment numbers for an ongoing NIH-project, our numbers were similar to the state numbers: 89% of the sample is Caucasian, 2-3% Asian, 6% Black, and 4-5% Hispanic. We therefore expect a representative sample for Study 1, although Study 2 and Study 3 may have restricted numbers of Asian volunteers. For these studies, Asian candidates will be carefully screened for difficulties metabolizing alcohol, because of a high preponderance (more than 50%) of Asians with an allele of the ALDH2 gene (National Institute on Alcohol Abuse and Dependence, 2000). All participants in the proposed studies must have sufficient knowledge of English to complete the requirements of the studies.

### **2. Sources of Research Material.**

All data obtained will be used for the purposes of the research projects only. Sources of data include telephone screening, various forms and questionnaires (see appendix), clinical interviews with parents; rest-activity data from wrist actigraphy; sleep-wake diary information, and data and specimens collected in the laboratory, including: polysomnographic data, mood and sleepiness ratings, performance testing, core body temperature, breath ethanol measures, blood samples (for liver function and pregnancy screening), urine samples (for toxicology screening), and saliva samples (for melatonin and cortisol levels). All data are coded with an ID number specific to each participant.

### **3. Recruitment of Participants and Consent Procedures.**

Participants will be recruited from a variety of sources. Some of the children and adults will be recruited from local schools with whom we have built working relationships through other NIH-funded projects. Adult participants will be recruited from local colleges and universities. Newspaper advertisements are used to recruit candidates of all ages. Advertisements and informational brochures will emphasize the sleep and circadian rhythms aspects of the studies. In no instance will the projects be "hyped" as studies providing alcohol as the primary focus. [Any material used for advertising or recruiting will be sent to the IRB for review and approval before it is used.] Since we require a large number of participants from families who have at least one parent with alcohol abuse/dependence, we will access many participants from alcohol dependent treatment centers in the state of Rhode Island. Two specific sites we have identified are the Providence Center Residential Treatment Program and Outpatient program and the Veteran's Affairs Hospital of Providence. Before enrolling

in any study, participants come to the laboratory for an orientation session, consisting of a tour of the facilities and explanation of the procedures involved in the study. Because of the rigorous nature of these studies, we encourage participants and their parents (if appropriate) to ask questions to ensure that they are clear about the procedures involved. In addition, we ensure that a senior investigator involved in the project speaks with each family about the specifics of the study, answers any questions, and obtains signed informed consent of the child, adolescent and parents or adult participants.

#### 4. Potential Risks.

Three experimental protocols are described in this application: All studies involve interviews and screening measures, diary and actigraphy recording, overnight sleep/sleepiness assessment, and salivary melatonin assessment. Studies 2 and 3 involve the administration of ethyl alcohol and measurement of core body temperature. Study 3 involves a forced desynchrony protocol.

Let us first examine the potential risks of the interviews and screening measures, diary and actigraphy recording, overnight sleep/sleepiness assessment, and salivary melatonin assessment included in all studies. The risks to participants participating in these aspects of the studies are minimal. Interviews and screening measures include questions about alcohol and drug use. The confidentiality of child and adult responses to these questions will be ensured with a federal certificate of confidentiality. The sleep and other physiological recordings pose no vital risks to volunteers. We have used these procedures with many children and adolescents with no lasting effects, even when electrodes and other sensors are kept in place for several days and nights. An occasional volunteer experiences a slight rash from the electrode/tape used in the sleep recordings. These effects are minimized by careful electrode application and removal procedures, and the rash dissipates within several days. Risks of drawing blood for blood tests include fainting, pain, bruising or hematoma at the site, and rarely, infection.

As to the administration of ethyl alcohol in studies 2 and 3, alcohol is contraindicated during pregnancy and in combination with certain medications (e.g., sedatives, hypnotics, anticonvulsants, anti-anxiety agents, anti-depressants, anti-psychotics, antihistamines). Study volunteers will be carefully screened and excluded from participation if medical condition or medication regimen contraindicate alcohol consumption. Female volunteers will be required to have a negative pregnancy test (assessed from a screening blood sample) and to assure appropriate methods of birth control in order to participate in the study. In addition, participants in studies 2 and 3 must have previous experience with alcohol, but must not have a history of alcohol problems. The levels of ethanol to be ingested by participants in these studies are low to moderate and are not associated with adverse physical symptoms in adults. Nausea and vomiting, for example, are not commonly reported until levels at 4 to 6 times the maximum dose in this study (Glod, 1998). Previous experience with alcohol consumption, relatively small doses, and the controlled, non-social dosing conditions during the studies should minimize the risk that study participation could lead to an increase in recreational use of alcohol. Debriefing of study volunteers should further minimize this risk.

[Consumption of alcohol prior to age 21 is prohibited by state law. The Attorney General for the state of Rhode Island has provided the PI with a waiver of prosecution for participants who are under the legal drinking age when they are exposed to alcohol in laboratory protocols. In addition, the Ethics Committee for Lifespan (corporate parent to Bradley Hospital), has approved the dosing of under-age study participants in the manner described here. Supporting documents are in the Appendix.]

During laboratory assessment in Study 2 and Study 3, each participant's core body temperature is continuously recorded using a rectal thermometer. Rectal temperature measurement has been used in many studies of circadian rhythms in adult humans and is a safe, comfortable procedure. We have used this measure in children as young as 9 years old with no problems for the children.

Study 3 involves a forced desynchrony protocol that requires participants to stay in the laboratory for 10 consecutive days (which they experience as about 12 cycles, because their sleep/wake cycle is scheduled for 20-hour days). Participants are scheduled to be awake for 13 hours 20 minutes and to sleep for 6 hours 40 minutes. Thus, the shorter scheduled wake time compensates for the shorter sleep period. Because the circadian pacemaker continues to oscillate with a period closer to 24 hours, the scheduled sleeping times pass into and out



of linkage with the appropriate internal sleep phase. Therefore, on certain of the “nights” the participants may experience some difficulty sleeping. In-lab sleep schedules will generally coincide with a normal diurnal schedule for the last day of study participation, thereby easing the transition from the laboratory. We do not anticipate that any participant will accrue a sleep deficit that cannot be repaid with several nights of normal sleep at home following the study. Participants will be living apart from society for 10 consecutive days, confined to the laboratory area, isolated from time cues of the external world, and unable to communicate directly with family and friends. On the other hand, participants are able to interact regularly with laboratory staff members and occasionally with one another. The waking hours involve a structured schedule that keeps participants mentally active and minimizes "down time". Therefore, we anticipate the risk for psychological distress related to the forced desynchrony protocol to be minimal. We have used the forced desynchrony paradigm in our lab over the last five summers, with participants as young as 9 years old. In general, we enroll participants in the FD protocols only if they have significant experience sleeping away from home and have successfully completed another of our protocols involving at least one overnight stay in the laboratory.

##### 5. Procedures for Minimizing Risk.

Several mechanisms will be employed to maintain confidentiality and encourage honest reporting throughout the screening, assessment, and follow-up procedures. First, participants and their family members are protected through careful treatment of all data files, which are accessible only to authorized members of the laboratory staff and are unavailable to parents, school authorities, or other parties, without written permission of the child and parents. All records receive a code, so that subject identification cannot be made from the files. Publication of findings or academic presentations will maintain participant anonymity. In addition, a federal certificate of confidentiality will be obtained to protect disclosure of illegal substance use by parent and/or participants from use in potential prosecution. Finally, we will inform parents through our informed consent process that we plan to collect information regarding alcohol and illicit drug use from our child and adolescent participants, and that this information will not be shared with the parent without the child's consent. Parents must agree to this confidentiality policy in order for their children to participate in the study. Clinical personnel will review screening and assessment information and will follow-up with participants and parents when serious clinical conditions are revealed, such as imminent risk to harm self or others. As for the risk of local skin irritation with electrode attachment, we have been quite successful in minimizing this risk by exercising great care in electrode application and removal. We avoid abrasive conductive gel, and we use hypoallergenic tape. Furthermore, during consecutive days of laboratory assessment, electrodes are repositioned as necessary to avoid tender placements. Risks from obtaining blood samples will be minimized by having licensed professionals draw the blood.

Risks related to ethyl alcohol administration are mitigated by several factors:

- Volunteers will be excluded from participation in the alcohol administration studies if medical condition contraindicates alcohol consumption (e.g., pregnancy, gastritis, history of epilepsy), if they have a history of alcohol-related problems, or if they have no experience with alcohol. In order for adolescents to be considered for participation in study 2, parental report must confirm adolescent alcohol experience and adolescents must indicate that they have consumed up to two "standard drinks" (i.e., 3 ounces of liquor or 2, 4-ounce glasses of wine or 1, 12-ounce bottle of beer) without adverse effects.
- All dosing procedures will be conducted pursuant to the NIAAA *Recommended Council Guidelines on Ethyl Alcohol Administration in Human Experimentation*.
- Dosing will only occur during laboratory assessment and will be followed by a night's sleep. Participants will be monitored continuously for adverse reaction during the dosing and absorption phases and during the night after dosing. A physician will be on-call in case of emergency. Breath alcohol concentration levels (BAC) will be assessed prior to the participant leaving the laboratory to ensure 0.00 g% BAC.
- Participants consume the alcohol in a nonsocial, laboratory setting, an unlikely scenario for subsequent alcohol ingestion to occur. We feel that this setting is protective—because participants are continuously

monitored—and provides a sufficient barrier to subsequent alcohol use outside of the laboratory environment. Furthermore, participant debriefing will include information about legal issues related to drinking and will emphasize the physical, behavioral, social, and emotional consequences.

- Participants are informed at the start of the study that their safety requires that they remain in the lab until alcohol levels are not detectable and that they must agree to this in writing.
- Participants are not permitted to drive themselves to the laboratory when alcohol is being administered in any dose.
- Staff are instructed that participants be asked to remain until they see the study director or medical consultant.
- If an adult participant insists on leaving before alcohol is at undetectable levels, a staff member accompanies the participant (usually by taxi) until the participant is in a safe environment (i.e., home).
- In the case of a minor child, the participant is not permitted to leave until a parent or guardian arrives to transport him/her home.

The risk of developing a problem with alcohol as a result of participating in study 2 or 3 are unknown; however, we intend to provide motivational interviewing about alcohol abuse and addiction in our debriefing meetings with these participants to mitigate this potential risk.

The rectal thermometer sensor used to measure body temperature is small, pliable, flexible, and harmless. The sensor is inserted approximately 4 inches into the rectum and secured in place with tape. The subject is asked to check the probe by feel at intervals to determine that it remains in place. If the participant is unable to insert the probe him/herself, a member of the staff is available to assist with the procedure, although all participants are given probes before the study begins and must report success inserting the probe before coming to the lab.

With regard to the Forced Desynchrony protocol, we are very comfortable studying children and adolescents in a longer (20 day/night) protocol and we are utterly confident of our ability to perform the precise, controlled measurements this study requires while providing a supportive environment for our young adult participants. Research assistants provide support in managing protocol elements and clinical staff monitor the psychological well-being of study participants. Finally, the consulting medical staff will follow closely any participants who show a sensitivity to these procedures and take steps as necessary to treat any skin irritation or difficulties that may arise.

## 6. Risk/Benefit.

While there are some risks associated with partaking in these studies, we believe that the preponderance of evidence pointing to substantial substance use in adolescence coupled with the growing recognition that the sleep of adolescents is insufficient and ill-timed provide a compelling argument for beginning to take a closer look at the intersections and overlapping of these processes. The strengths of our group stem from our longstanding involvement in the study of sleep, circadian rhythms, and behavior during adolescent development. Our focus on adolescent development and behavior has sensitized us to the high risk alcohol poses for adolescents who are chronically sleep deprived and have delayed circadian timing, and we are concerned that so little data are available to inform our understanding of these associations. Although the protocols we propose are not simple and may, for some, involve a degree of stress relative to usual activities, our lab has demonstrated that these modern techniques are eminently workable in children, adolescents, and young adults, particularly when applied in the context of a supportive environment. Our group is poised to begin these studies: we have experience working with children, adolescents, and young adults; we have expertise in studying sleep, circadian timing, and behavior in these groups; we have taken steps in our community to obtain cooperation for studying alcohol in adolescents; we have taken advantage of consultation with local experts in the study of alcohol; we have the motivation to focus our efforts, skills, and methods on these important issues. Too many questions remain to be answered, yet we hope that this proposal will provide a strong foundation to continue and expand this work.

Participants will benefit from the debriefing and motivational interview session at the conclusion of the study where they will receive information about alcohol abuse and addiction and support for choosing to use alcohol wisely.

## **F. Vertebrate Animals. N/A**

## **G. Literature Cited.**

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#### **H. Consortium/Contractual Arrangements. N/A**

**I. Consultants.** See letters on following pages.