**Study Protocol**

**Official Title:** Delayed sleep phase and risk for adolescent substance use

**ClinicalTrials.gov ID (NCT number):** NCT03806296

**Protocol Date:** 08.04.2025

## Scientific Background

The acceleration of substance use (SU) that often begins in adolescence can culminate in substance use disorders (SUDs) and a host of other poor outcomes. Mounting evidence indicates that sleep and circadian rhythm disturbances are key contributors to adolescent SU. Delayed sleep phase (DSP), a phenotype characterized by later sleep timing, appears to confer particular risk for elevated SU, and ultimately, SUDs. However, the exact nature of this link and the mechanisms underlying it remain unclear. The extant literature is limited both conceptually (e.g., insufficient attention to neurobehavioral mechanisms) and methodologically (e.g., cross-sectional designs and self-report measures). Circadian misalignment resulting from a mismatch between late sleep timing and early school start times is a compelling potential contributor to elevated SU in adolescent DSP with plausible neurobehavioral mechanisms. We hypothesize that DSP-associated circadian misalignment decreases impulse control and increases reward sensitivity, thereby increasing SUD risk.

*NOTE: in the ClinicalTrials.gov protocol, the "Delayed Sleep Phase (DSP)" group is referred to as Late Sleep group, and the "normal phase group" is referred to as the Early/Mid Sleep group*

## Study Objectives

The proposed study will, for the first time, (1) comprehensively characterize the substance use disorder (SUD) risk profile associated with adolescent delayed sleep phase, and (2) probe whether SUD risk is diminished by altering sleep/circadian timing.

Aim 1: Laboratory Study—Compare DSP group to normal phase group on sleep/circadian factors, neurobehavioral markers, and SU. Compared to the normal phase group, the DSP group will exhibit: (H1a) later circadian phase, shorter sleep duration, and greater circadian misalignment; (H1b) greater behavioral and neural evidence of impulsivity and reward sensitivity; and (H1c) greater odds of cannabis and alcohol use.

Aim 2: Experimental Study—Probe the acute effects of a sleep phase-stabilizing manipulation on sleep and circadian rhythms, as well as neurobehavioral markers of SUD risk. Compared to the control group, the manipulation group will exhibit: (H2a) advances in circadian phase, increases in sleep duration, and reductions in circadian misalignment; and (H2b) reductions in behavioral and neural (fMRI) measures of increased impulsivity and reward sensitivity.

Exploratory Longitudinal Aim: Examine the prospective relationships between DSP characteristics and changes in SU via repeated bi-monthly assessments during a high-risk period for SU initiation/escalation.

## Study Design & Methods

The proposed study will, for the first time, (1) comprehensively characterize the SUD risk profile associated with adolescent DSP, and (2) probe whether SUD risk is diminished by altering sleep/circadian timing. The study will assess both established markers of SUD risk and putative neurobehavioral mechanisms (impulsivity and reward sensitivity). Specifically, we propose a comprehensive, multi-method approach to examining DSP's role in SUD risk, combining laboratory, experimental, and longitudinal studies.

We will recruit a sample of 150 eleventh and twelfth graders (16-19 y/o), divided between 100 DSP and 50 normal phase teens. We will focus on cannabis and alcohol use given their prevalent use in adolescents and evident links to DSP. This study combines Laboratory, Experimental, and Longitudinal protocols.

The Laboratory protocol (aka T1 or Baseline phase) compares a group of DSP adolescents to a group of normal phase adolescents on behavioral and neuroimaging (fMRI) tasks tapping impulsivity and reward sensitivity, as well as a circadian phase assessment. This protocol lasts 1 week, with a weeknight overnight visit at the end.

In the Experimental protocol (aka T2 or Post-Manipulation phase), we will probe whether stabilizing circadian phase in the DSP group by using sleep scheduling and chronotherapeutic approaches (e.g., bright light)

improves sleep and neurobehavioral function relevant to SUD risk. This protocol last 2 weeks, with a weeknight overnight visit at the end.

Finally, in the Longitudinal protocol we include repeated follow-up assessments (bi-monthly for the first 6 months; every 6 months afterwards) of sleep and SU to explore longitudinal associations during the high-risk transition to young adulthood.

**Eligibility Criteria**

General inclusion criteria:

• Age 16-19 years
• Currently in 11th or 12th grade and enrolled in a traditional high-school; or cyber school with synchronous classes (not home-schooled)

• Physically and psychiatrically healthy, as determined by instruments described below
• Provision of written informed consent and assent

Additional inclusion criterion for Experimental protocol
• Meets operational definition of delayed sleep phase (DSP; weekend bedtime ≥1 AM)

Exclusion criteria:

• Significant or unstable acute or chronic medical conditions. Examples of such conditions include, but are not limited to central nervous system disorders (e.g., head injury, seizure disorder, multiple sclerosis, tumor), cardiovascular or hemodynamically significant cardiac disease, liver disease (e.g., acute or chronic hepatitis, hepatic insufficiency), active peptic ulcer disease, inflammatory bowel disease, renal failure, arthritis, and diabetes and other endocrine disorders. Individuals with well-controlled health conditions that do not affect sleep or well- being (e.g., well-controlled thyroid disorders, asthma, or ulcer) will not be excluded. To evaluate these criteria, potential participants will complete a locally- developed Medical History Questionnaire. We will exclude women who are pregnant based on self-report during the screening process.

• Past or current bipolar disorder or psychotic disorders, which will be evaluated using the Mini-International Neuropsychiatric Interview for Children and Adolescents (MINI KID).

• Past or current substance use disorders other than alcohol use disorder or cannabis use disorder. We will not exclude participants for subsyndromal symptoms in this domain. The MINI-KID will be used for diagnostic assessment of substance use disorders. We will also assess the likely presence of physiological dependence on alcohol or cannabis via the withdrawal subscales from the Customary Drinking and Drug Use Record (CDDR), and participants that indicate that they would not be able to refrain from use during lab visits will be excluded.

• Past month recreational drug use other than cannabis and nicotine. Cannabis and non- daily nicotine/tobacco use will be permitted, given their high degree of co-use with alcohol. Excluding any nicotine use would also limit generalizability: ~38% of 12th graders report past-month tobacco use (YBRS, 2015), and prevalence is higher in those with regular AU (NSDUH, 2015). Therefore, we will to exclude daily nicotine users. Excluding daily nicotine users will markedly reduce confounding effects on sleep and reward function, and non-daily users should not suffer from withdrawal while abstaining during laboratory procedures. Self- reported drug use will be assessed using the Time Line Follow Back method. In addition, a Breathalyzer, salivary cotinine and urine drug screen will be administered prior to the start of the circadian phase assessment visits to the Sleep and Behavioral Neuroscience Center (SBNC).

• Current syndromal sleep disorders other than insomnia and delayed sleep phase disorder, including narcolepsy, restless legs syndrome, obstructive sleep apnea, and current night shift work (i.e., any work occurring between the hours of midnight and 6:00 a.m.). We will not exclude for subsyndromal symptoms or disorders in these domains. We will not exclude for insomnia and delayed sleep phase disorder because of their conceptual overlap and high co-occurrence with delayed sleep phase. Sleep disorders will be diagnosed

according to criteria in the DSM-5 and the International Classification of Sleep Disorders, 3rd Edition, 2014. These disorders will be evaluated using clinical interview and the Structured Interview for Sleep Disorders.

• Medications that interfere with sleep and/or reward function. (e.g., hypnotics, benzodiazepines, anxiolytics, antipsychotics, decongestants and sedating antihistamines, beta blockers, corticosteroids). In order to evaluate medications, participants will complete a listing of current medications, including prescription and over the counter medications, “natural” preparations, and nutritional supplements. Participants must be free of psychotropic medications for a minimum of 2 weeks. We will not exclude participants who are taking antidepressants. We will also not exclude participants who are taking stimulants prescribed for ADHD. However, such participants must be willing and able to discontinue stimulants for 24 hours prior to study visits.

• Conditions that would interfere with the MRI procedures. These include the presence of implanted medical devices such as cardiac pacemaker, aneurysm clip, ear implant, IUD, shrapnel, neurostimulators or other metal devices (e.g. dental braces); and fear of closed spaces.

**Statistical Considerations**

We will calculate actigraphy and EMA summary measures, examine SU distributions, and perform preliminary analyses. For *a priori* hypotheses, all models will be adjusted for potential confounding factors (e.g., sex). P-values and effect sizes with 95% confidence intervals will be used to evaluate statistical and clinical significance, respectively. After model-fitting, diagnostics will be used to evaluate model assumptions and fit. Transformations or non-parametric methods will be used if normality assumptions are violated. Analyses for the experimental study will adhere to an intent-to-treat principle and include all randomized subjects as assigned.

Aim1:
The DSP group will exhibit (H1a) later circadian phase, shorter sleep duration, and greater circadian misalignment and (H1b) greater behavioral and neural evidence of impulsivity and reward sensitivity: We will use a regression model to test the effect of group on each a priori outcome, controlling for relevant confounders. H1c: The DSP group will exhibit greater odds of cannabis and alcohol use. We will use a generalized linear model to test whether the relative risk of cannabis use and/or alcohol use in the past month differs by group, controlling for relevant confounders.

Aim 2:
The manipulation group will exhibit (H2a) advances in circadian phase, increases in sleep duration, and reductions in circadian misalignment; and (H2b) reductions in behavioral and neural (fMRI) measures of increased impulsivity and reward sensitivity. Mixed effect models will be used to test the effect of group on changes in each *a priori* outcome, controlling for relevant confounders.