Adolescent chronic sleep restriction promotes alcohol drinking in adulthood: evidence from epidemiological and preclinical data.

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Abstract

Study Objectives. Sleep disturbances during adolescence have been proposed as a contributing factor to the development of alcohol use disorder in adults. The purpose of this study was to test the longitudinal causal link between insufficient sleep and alcohol use by using epidemiological data and a controlled preclinical experiment.

Methods. The epidemiological dataset included 5497 participants of the Avon Longitudinal Study of Parents And Children cohort. Sleep and alcohol measures collected from interviews and questionnaires at 15 and 24 years of age were analyzed with multivariable linear regression and a cross-lagged autoregressive path model. Preclinical data were collected by sleep restricting Marchigian Sardinian alcohol preferring rats (n=40) from post-natal day 25 to 55 and measuring voluntary alcohol drinking concurrently and in adulthood. Polysomnography was used to validate the efficacy of the sleep restriction procedure. Behavioral tests were used to assess anxiety, risky behavior, and despair.

Results. After adjusting for covariates, we found a cross-sectional association between all sleep parameters and alcohol consumption at 15 years of age but not at 24 years. Alcohol consumption (Alcohol Use Disorder Identification Test for Consumption) at 24 years was predicted by insufficient sleep at 15 years whilst alcohol drinking at 15 years could not predict sleep problems at 24. Preclinically, adolescent chronic sleep restriction in rats escalated alcohol consumption during adolescence and led to higher alcohol drinking and increased propensity to risk-taking behavior in adulthood.

Conclusions. Our findings support the longitudinal and causal association between adolescent insufficient sleep and higher adult alcohol consumption.

Key words: insufficient sleep, ALSPAC, alcohol use disorders, msP rats, polysomnography, adolescence.

Introduction

Epidemiological research has shown that adolescents worldwide are chronically sleep deprived due to a combination of physiological, social, and environmental factors [1]. Increased use of technology at night [2], consumption of caffeine, academic and social demands combine with ontogenetic preference towards later sleep and wake up times and lead to reduced sleep duration and quality across adolescence [3–5]. Sleep fragmentation and sleep loss have been associated with emotional dysregulation [6, 7], increased psychosis [8, 9], and higher impulsive and risk-taking behaviors [10, 11], including higher substance abuse [12, 13]. Since adolescents are particularly prone to reward-seeking behavior and to the risk of developing substance use disorders and related psychiatric comorbidities [9, 14] it is particularly important to establish whether adolescent sleep restriction actively contributes to the development of such mental disorders.

The World Health Organization's global status report on alcohol and health (2018) and the American Survey on Drug Use and Health (2018) show that alcohol remains the substance most widely used by today's teenagers and represents the top risk factor for deaths and disability among the world's population aged 15-49 years [15, 16]. Although genetic predisposition is estimated to contribute to approximately 50–60% of the vulnerability to Alcohol Use Disorders (AUD) [17, 18], environmental factors can also play an important role, especially during adolescence that is characterized by delicate processes of maturation of brain regions important for decision making, risk assessment, and impulse control [9, 14]. The amount and quality of sleep experienced during adolescence is thought to affect not only adolescent behavior in the short term but to also result in long lasting changes in brain connectivity and functioning, which

in turn could lead to increased engagement in dangerous behaviors and alcohol drinking later in life, together with an increased risk of developing AUD and psychiatric comorbidities [9, 13].

Abundant cross-sectional studies show a bidirectional association between sleep and circadian disturbances and alcohol problems [19–21]. However, the studies showing a prospective association between adolescent sleep problems and alcohol use later in life are still limited. Longitudinal studies found that weekend sleep delays and problems falling asleep at 14-15 years predicted higher likelihood of alcohol use over a period of 1-2 years [22, 23], and that sleep difficulties (i.e. troubles falling or staying asleep) and fewer hours of sleep at 15 years could predict the odds of alcohol-related interpersonal problems and binge drinking 1 and 5 years later [24]. Different types of sleep disruption (restless sleep, sleep variability, insomnia) in late childhood and adolescence predicted a significant increase in different aspects of alcohol use, including age of first alcohol use and AUD onset during adolescence and young adulthood [25, 26]. In substance-naive 12 year old adolescents, shorter sleep and greater daytime sleepiness predicted the onset of alcohol use, heavy drinking, and alcohol-related consequences over a 4-year period [27]. These studies support the hypothesis that sleep impairment promotes alcohol use during adolescence, however most of them explored relatively short time intervals. Hence, here we assessed the cross-sectional and longitudinal associations between insufficient sleep and alcohol use over an interval of 9 years (at 15 and 24 years of age) in the Avon Longitudinal Study of Parents And Children (ALSPAC) cohort, a population-based, prospective birth cohort study of children born in 1991–1992 in the Bristol area (UK). Moreover, to better control for psychological and environmental factors that could contribute to the development of AUD and potentially confound the associations found, we tested the longitudinal causal link between adolescent insufficient sleep and voluntary alcohol consumption using a preclinical model of

chronic sleep restriction in a line of genetically selected alcohol preferring rats with innate high predisposition to drink voluntarily from an early age.

Methods

Epidemiological study

Participants

The Avon Longitudinal Study of Parents And Children (ALSPAC) is a population-based prospective cohort situated in South West England. Detailed information about the methods and procedures of ALSPAC study is available elsewhere [28–31]. Briefly, pregnant women resident in Avon, UK with expected dates of delivery between 1st April 1991 and 31st December 1992 were invited to take part in the study. 20,248 pregnancies have been identified as being eligible and the initial number of pregnancies enrolled was 14,541. Of the initial pregnancies, there was a total of 14,676 foetuses, resulting in 14,062 live births and 13,988 children who were alive at 1 year of age. When the oldest children were approximately 7 years of age, an attempt was made to bolster the initial sample with eligible cases who had failed to join the study originally. As a result, when considering variables collected from the age of seven onwards (and potentially abstracted from obstetric notes) there are data available for more than the 14,541 pregnancies mentioned above: The number of new pregnancies not in the initial sample (known as Phase I enrolment) that are currently represented in the released data and reflecting enrolment status at the age of 24 is 906, resulting in an additional 913 children being enrolled (456, 262 and 195 recruited during Phases II, III and IV respectively). The total sample size for analyses using any data collected after the age of seven is therefore 15,447 pregnancies, resulting in 15,658 foetuses. Of these 14,901 children were alive at 1 year of age. Of the original 14,541 initial pregnancies, 338 were from a woman who had already enrolled with a previous pregnancy, meaning 14,203 unique mothers were initially enrolled in the study. As a result of the additional phases of recruitment, a further 630 women who did not enroll originally have provided data since their

child was 7 years of age. This provides a total of 14,833 unique women (G0 mothers) enrolled in ALSPAC as of September 2021.

The study website contains details of all the data that is available through a fully searchable data dictionary and variable search tool (http://www.bristol.ac.uk/alspac/researchers/our-data/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. All participants have been followed up regularly with questionnaires on lifestyle behaviors and research clinics. Only data from participants who attended the teen focus clinic at 15 years were included in this study (n=5497). At the latter time point of our analyses, participants were 24-year-old.

Study data were collected and managed using REDCap electronic data capture tools hosted at the University of Bristol [32]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Exposure: Sleep

At 15 years, sleep parameters were derived from the following questions: "Assessment of how much sleep the participant gets" (3-point Likert type scale) for *sleep quantity*; "Length of time the participant sleeps on normal school night" for *hours of sleep per night*; "Problem participant has with sleepiness during daytime activities" for *daytime sleepiness*, and "Frequency participant feels that they get enough sleep" (5-point Likert type scale) for *sleep satisfaction* [33]. We created a *sleep insufficiency score* (min=0, max =3) at 15 years by summing up the minmax

scaled scores of the questions for sleep quantity, daytime sleepiness, and sleep satisfaction to summarize both the quantity and quality domains of sleep.

Sleep measures at 24 years were derived from the following questions [33]: “In past month, participant had problems getting to sleep or back to sleep”; “In past 7 nights, number of nights participant had sleep problems”; “In past week, length of time participant got to sleep on night with least sleep”; “In past 7 days, number of nights participant spent >=3 hours getting to sleep”; “In past 7 days, participant woke >2 hours early & couldn't get back to sleep”. We then constructed a total sleep insufficiency score at 24 years by summing up the minmax scaled score of the five questions.

Outcome: Alcohol consumption

Alcohol consumption at 15 years was calculated by multiplying the frequency at which the child reported to drink alcohol by the number of drinks consumed on a normal drinking day, as in [34]. The presence and severity of alcohol use disorder at 24 years was investigated using the Alcohol Use Disorder Identification Test for Consumption (AUDIT-C) [35, 36], obtained in a computer-assisted clinical interview.

Covariates

Based on previous literature of confounding factors associated with both exposure and outcome variables [37–40], biological sex at birth, mother’s AUDIT, mother’s occupational class, used as a proxy for maternal socioeconomic status, and presence of any psychiatric disorder at 15 years were included as covariates. Mother’s occupational class was derived at 32 weeks of gestation and used as a proxy for maternal socioeconomic status using the 1991 Office of Population,

Censuses and Surveys classification in UK (I-professional occupations, II-managerial and technical occupations, III skilled non-manual occupations, III skilled manual occupation, IV-partly skilled occupation, V-unskilled occupation) [41]. Mother's AUDIT-C was completed when the participant was 18 years old. The presence of psychiatric disorders was derived from the questionnaire Development and Well-Being Assessment (DAWBA) completed by the participant at 15 years of age, which assesses the likelihood of any psychiatric disorder (emotional, behavioural, and hyperactivity disorders) in one of six bands [42, 43].

Missing data

Attrition is a well-known problem in the ALSPAC dataset like other longitudinal studies and a complete-cases analysis could result in potentially biased findings [44]. Therefore, we imputed missing data. Table S1 describes the percentage of missing values for each variable. Univariate logistic regressions were used to evaluate the association between the independent variables and outcome (AUDIT-C at 24 years) data missingness. We observed that children from lower socioeconomic backgrounds, males, and individuals with mental health issues were more likely to skip follow-ups (Table S2), which indicates a relationship between the observed variables and data missingness. For this reason, exclusively including participants with complete data might potentially result in selection bias. To address this, we employed multivariable imputation by chained equations to impute missing data [45] using the 'futuremice' command in the 'mice' R package [46]. We imputed a total of 50 complete datasets (n = 5,497 all participants at the 15 years focus clinic) through 5 imputation iterations. The imputation matrix included all the variables in the regression models (biological sex at birth, alcohol consumption at 15 years, AUDIT-C at 24 years, mother's AUDIT, mother's social class, sleep measures at 15 and 24

years), and an additional set of auxiliary variables related to sleep issues, alcohol consumption, socioeconomic status, adverse childhood experiences, and adolescent psychopathology at 15 years, 24 years, and at multiple time points before 24 years. Including this extra set of auxiliary variables served a dual purpose: it increased the accuracy of the imputation by providing more information to estimate the missing data, and supported the assumption that data missingness was conditional on the factors we could observe and include in the imputation model (such as a participant's socioeconomic status or sex) rather than on the ones we couldn't include (Missing at Random, MAR, assumption) [47]

Statistical analysis

Continuous variables were described by mean and standard deviation (SD). Categorical data were expressed as percentages.

To determine whether reduced perceived sleep quantity and quality, daytime sleepiness, and hours of sleep were independent risk factors for alcohol use, we used multivariable linear regression with adjustment for the covariates (biological sex at birth, mother's AUDIT, social class, and the presence of any psychiatric disorder). Separate models were used to evaluate the association between sleep variables and alcohol consumption at 15 years, the association between sleep variables and AUDIT-C at 24 years, and the correlation between the sleep insufficiency score at 15 years and AUDIT-C score at 24 years (longitudinal model). The longitudinal model was also adjusted for alcohol consumption at 15 years to evaluate the long-term effect of insufficient sleep accounting for long course alcohol consumption thus including an auto-regressive component.

We examined model diagnostics using residual plots versus predicted plots and quantile–quantile plots. All model assumptions were met adequately.

We also conducted sensitivity analyses that were restricted to individuals who had data in both the exposure and outcome variables (complete cases analysis, Supplementary Tables 3 and 4). Results of analyses based on each imputed dataset were combined using Rubin's rules [48]. All analyses were conducted using R software, version 4.0.1 (R Core Team, 2020).

To further investigate the directionality of the association between alcohol use and sleep insufficiency we constructed a cross-lagged autoregressive path model. Sleep insufficiency at 15 years was modelled as a latent variable defined by the 3 self-report items of the sleep questionnaire described above, sleep insufficiency at 24 years was defined by 5 items of the sleep questionnaire at 24 years modelled as a latent variable. Alcohol problems at 15 and 24 years were modelled as latent variables defined by the minmax scaled number of drinks per week and the minmax scaled AUDIT-C score, respectively. Measurement invariance of the alcohol problems and sleep insufficiency constructs at the two time points were verified prior to analyzing the cross-lagged panel model. Alcohol problems were used as dependent variables, with the sleep insufficiency score as independent variable to evaluate whether sleep insufficiency predicted alcohol problems and vice versa for the reverse lagged association. Model paths were estimated using the unweighted least squares method with confidence interval bootstrapping while controlling for the three covariates that resulted as significant predictors of alcohol use in the longitudinal regression analysis (i.e., sex, maternal AUDIT-C and social class). The following predetermined parameters were chosen to evaluate adequate model fit: root mean square error of approximation below 0.08 and a comparative fit index and Tucker-Lewis Index of 0.95 or higher [49]. Significant pathways were established based on $\alpha = .05$. As for the

other analyses we applied the CLPM to the imputed datasets and to the original dataset as sensitivity analysis. The CLPM were estimated using the lavaan R package version 0.6-12 (R Project for Statistical Computing).

Preclinical study

Animals

Marchigian Sardinian alcohol preferring (msP) rats are characterized by high preference and high alcohol consumption levels, which make them a good model to study voluntary alcohol drinking and AUD since a young age [50–52]. Male rats were housed in pairs, under a 12-hour dark/light cycle, constant temperature with water and food *ad libitum*. All animal care and experimental procedures were carried out in accordance with the guidelines of the European Community Council Directive for Care and Use of Laboratory Animals (2010/63/EU), Decreto Legislativo Italiano 26 (2014), and authorized by the local Animal Care Committee and by the Italian Ministry of Health.

Chronic sleep restriction

At post-natal day (P) 25, rats were divided into two groups: the chronic sleep restricted (CSR) group and the yoke controls (YC). The CSR group (n=22) was allowed to sleep for 4 hours every day during the first 4 hours of the light period, and sleep restricted for the remaining 20 hours, for four consecutive days. Chronic sleep restriction was achieved by using sleep deprivation cages (AM Microsystem, Urbisaglia, Italy) equipped with a programmable rotating bar swiping the cage floor at 2-3 rpm and changing direction at random intervals between 20 sec to 2

minutes. To control for the stress induced by the bar rotation, the YC group (n=18) was subjected to bar rotation for 6 hours during the dark period (1 hour after lights off) and left undisturbed for the remaining 18 hours. On the fifth day, both groups were left undisturbed. This cycle of 4 days of sleep restriction plus 1 day of recovery was repeated 6 times, from P25 to P55.

Alcohol exposure and intake

Rats were exposed to a two-bottle choice (water and 10% alcohol solution) at P23 and P25 to determine baseline levels of alcohol preference and intake, on the recovery day throughout the 6 cycles of the CSR procedure (at P30, P35, P40, P45, P50, P55), and on alternating days between P90 and P96. Alcohol was available for 10 hours during the dark period, starting one hour after the light offset. The positions of the bottles were alternated. To measure alcohol and water intake, on the recovery day, a Plexiglas panel was inserted in the middle of the cage to temporarily separate the two rats. Measures of drinking and alcohol intake were recorded 30 minutes and 10 hours after bottle presentation.

EEG/EMG recordings and analysis

Polysomnographic recordings in nine rats, P38-50, were used to quantify the efficiency of the automated chronic sleep restriction during adolescence. Under isoflurane anesthesia (1.5-2% volume) 9 rats were implanted bilaterally with epidural screw electrodes over the frontal (AP: +2 mm, ML: ±2 mm) and parietal (AP: - 2mm, ML: ±3mm) cortices, and the cerebellum as reference and ground for chronic EEG recordings. Electrodes were fixed to the skull with dental

cement. Two stainless steel wires were inserted into neck muscles to record electromyogram (EMG).

After 7 days of recovery, rats were transferred to the sleep restriction chambers, and after 24 hours of habituation, connected to the acquisition board (Open Ephys) by a recording head stage SPI cable (Intan Technologies) and a commutator. Polysomnographic recording was carried out continuously for 7 days and consisted of 24 hours of undisturbed baseline, 4 consecutive days of bar rotation (either 20 hours for the CSR group or 6 hours per day for the YC group), and 2 days of undisturbed recovery. EEG and EMG signals were filtered (EEG: high-pass filter at 0.1 Hz; low-pass filter at 100 Hz; EMG: high-pass filter at 10 Hz; low-pass filter at 70 Hz). All signals were sampled at 1000 Hz resolution and down sampled at 512Hz for analysis. Waking, NREM sleep, and REM sleep were manually scored off-line (SleepSign, Kissei COMTEC, Matsumoto, Japan) in 4-s epochs according to standard criteria.

Behavioural tests

The first testing session started about 36 hours after the last ethanol exposure cycle (P57), whereas the second session started after 5 weeks of recovery (> P105). All tests were performed during the dark phase.

Open field test. Rats were habituated to the test room for 10 minutes and then placed at the edge of a white Plexiglas arena (43.4 x 43.4 x 30.3 cm) and allowed to move freely for 20 mins while being recorded (Med Associates, St Albans, VT, USA).

Light dark box. A Plexiglas box (40 x 40 x 40cm) was divided into two compartments connected by a sliding door (10 cm × 10 cm). The light chamber was brightly illuminated by a lamp.

Animals were allowed to habituate to the test room for 5 mins and then placed in the centre of the light chamber facing the wall of the arena to explore the two chambers. The test lasted 5 mins.

Novelty suppressed feeding test. After 24hrs of food deprivation, each animal was placed in a novel Plexiglas arena (L 76.5 cm x 76.5 cm x h 40 cm) with a brightly illuminated center (~1000Lux) containing a previously weighed food pellet. The animals were allowed free exploration and access to the food pellet for 5 minutes: the latency to their first bite and the amount of food consumed were measured. Rats that did not consume any food were automatically assigned a latency of 300 sec. After the 5 minutes, rats were returned to their home cage and allowed free access to another food pellet for 5 minutes to compare feeding behavior in a familiar environment.

Forced swim test. Rats were placed in a cylindrical container (50cm height and 20cm in diameter) filled with water (25±2ºC) to a depth of 30cm for 6mins. Only activities in the final 4mins were considered for analysis. Behaviors were manually scored by two researchers blind to the animal's condition. Immobility was considered as when the limbs and shrunk were immobile only to allow for floating.

Statistical analysis

Statistical tests used are specified in each figure legend. Bivariate correlations between behavioral tests data were evaluated using non-parametric Spearman's test. The significance level alpha was set to 0.05. Analysis was performed with GraphPad Prism 9.5.1 and Python 3.9.

Results

In the first part of this study, we carried out cross-sectional and longitudinal analyses to investigate the link between sleep insufficiency and development of alcohol use disorder in a human population study, across a period of 9 years. Information on the population, sleep, and alcohol variables in the imputed and complete dataset is summarized in Table 1. All models were adjusted for the presence of psychiatric disorders, maternal AUDIT-C and social class, and biological sex at birth.

Cross-sectional effect of sleep deprivation on alcohol consumption at 15 and 24 years of age.

All distinct sleep variables as well as the multidimensional score of sleep insufficiency were associated with alcohol consumption at 15 years of age so that shorter and low-quality sleep were associated with higher alcohol consumption (Table 2). For sensitivity analyses, the same models were tested in the complete cases dataset in which we confirmed the association between the insufficient sleep score and alcohol consumption (Table S3). Conversely, we did not find any association between sleep and AUDIT-C score at 24 years (Table 2).

Longitudinal effect of sleep deprivation on alcohol consumption.

We then tested whether insufficient sleep at 15 years predicted patterns of alcohol consumption at 24 years using a linear model that included an autoregressive term for alcohol consumption at 15 years. We found that higher alcohol consumption at 24 years was associated with fewer hours of sleep during weekdays, lower sleep quantity and satisfaction, a higher daytime sleepiness and a higher insufficient sleep score at 15 years (Table 2, Supplementary Table 4).

To assess the directionality of the association, we used an autoregressive cross-lagged path model and found that sleep insufficiency at 15 years had a significant effect on AUDIT-C at 24 (Sleep T1 → Alcohol T2, Table 3). Conversely, the estimated number of alcoholic drinks per week at 15 years of age did not predict sleep insufficiency later in life at F24 (Alcohol T1 → Sleep T2) (Figure 1).

In summary, these results support the hypothesis of a unidirectional causal relation between sleep insufficiency in mid-adolescence and alcohol use disorder in emerging adulthood.

Chronic sleep restriction escalates alcohol drinking during adolescence and adulthood in msP rats.

To further test such causal link, we sleep restricted adolescent msP rats and measured alcohol drinking and other behavioral parameters in adulthood. We first quantified the efficacy of our automated sleep restriction protocol (Figure 2A) in a cohort of msP rats using polysomnographic recordings. During the chronic sleep restriction (CSR), we observed a reduction in total time spent in NREM and REM sleep on average of 53% and 47%, respectively, and an increase of 43% in total wake duration relative to 24-hour of undisturbed baseline (Figure 2B). During the recovery day, an increase in total sleep time was found, mostly accounted for by increased REM sleep (+70% relative to baseline). During the 4-hour sleep opportunity, total time spent in NREM sleep was not significantly increased relative to the same period in baseline, but NREM sleep episodes were more consolidated (lasted longer and were fewer, Figure S1 A and B). Time spent in REM sleep increased by 180% (Figure 2 C NREM and REM), an effect due to both a higher number and length of REM sleep bouts (Figure S1 C and D). In controls, there was no significant effect on the time spent in NREM and REM sleep relative to the 24-hour baseline (Figure 2D).

To determine the effect of adolescent CSR on voluntary alcohol consumption, another cohort of msP rats was subjected to either CSR or control manipulation from post-natal day (P) 25 to P55 (see Material and Methods and Figure 3 A and B). Both groups showed a gradual increase in alcohol consumption when exposed to an intermittent 2-bottle choice paradigm during the recovery day. However, rats that underwent CSR showed a faster escalation in alcohol drinking relative to controls, reaching higher levels of alcohol intake at earlier times (Figure 3 C and D). When rats were tested again for alcohol intake after 5 weeks, increased alcohol consumption persisted in the CSR group relative to controls at 10 hours (Figure 3 G and H). Water consumption remained similar between groups across the entire experiment (Figure 3 E, F, I, J).

Sleep restriction and alcohol drinking promote short- and long-lasting risky behavior.

To identify whether CSR and alcohol drinking were associated with short and/or long-lasting behavioral changes, we performed two batteries of tests (T1 and T2, Figure 3A).

In the open field (OF) test, while the controls showed a significant decline in time spent moving in the arena and in the distance traveled from late adolescence to young adulthood, rats previously exposed to CSR maintained higher, adolescent-like values of travelling when tested later in life (Figure 4A, left). Conversely, the two groups spent a similar amount of time in the center of the arena relative to the edges (Figure 4A, right). A subgroup of rats underwent the light/dark box test, where CSR rats showed a tendency to spend more time in the lit box and to enter the lit chamber relative more rapidly than controls (Figure 4B).

In the novelty suppressed feeding (NSF) test, we found that the amount of food consumed was unchanged between controls and CSR, both in the arena and in the home cage, even though a decrease in food consumption in the home cage was observed with age in both groups

(adolescent vs adult, Figure 4C). However, CSR rats showed reduced latency to approach and eat food in the unfamiliar arena, whereas in the home cage no significant difference was detected (Figure 4D). The difference in latency was maintained when animals were tested again after 5 weeks of recovery. To rule out the possibility that the observed decreased latency was due to an increased appetite in response to the CSR procedure, we measured food consumption during recovery as well as during the NSF test: no difference was found between the groups (Figure 4 C and E). Moreover, 6 cycles of CSR affected neither growth rate nor body weight throughout the experiment (Figure 4F). No differences were found between groups in the forced swim test (FST, Figure 4G).

In summary, rats exposed to adolescent CSR showed increased locomotion in the OF test and decreased latency to approach and eat food in an unfamiliar environment relative to yoked controls. No alterations in despair levels were highlighted.

We then explored possible correlations between behavioral parameters and found that, in adult rats, alcohol consumption positively correlated with OF ambulatory time and distance (Figure 5A and B), and with time spent in the center of the OF arena (Figure 5C). CSR rats also showed a negative correlation between the amount of alcohol consumed at 30 minutes and the latency to approach food in the NSF test, so that rats with the highest levels of alcohol consumption in the first 30 minutes were also the fastest to approach the food in the arena. By contrast, controls showed an opposite trend (Figure 5D). A significant negative correlation was found between ambulatory time in the OF test and time spent immobile in the FST (Figure 5E). Finally, we found a longitudinal correlation between adolescent alcohol consumption and adult performance in the NSF test: the average amount of alcohol consumed during the 2nd, 3rd, and 4th cycle of

treatment during adolescence negatively correlated with the latency of approaching food in the arena in adulthood (Figure 5F and G).

Discussion

The present study tested the hypothesis that insufficient sleep during adolescence promotes alcohol drinking in young adulthood by using both epidemiological and preclinical data. The epidemiological data were derived from a well-characterized cohort in which self-reports of sleep patterns and alcohol consumption were obtained from questionnaires taken 9 years apart. We found a cross sectional association between all parameters of sleep and alcohol consumption at 15 years of age, with 1.7 more drinks per week for every unitary increase in the sleep insufficiency score. Moreover, the autoregressive cross-lagged path model showed that AUDIT-C at 24 years was predicted by insufficient sleep at 15 years, highlighting a prospective developmental effect of mid adolescent insufficient sleep on young adult alcohol consumption. These findings confirm and extends on the results of other cross-sectional [19, 20, 53, 21] and longitudinal reports [22–24, 26, 27, 54–57] suggesting the reproducibility and validity of such associations in different population studies.

However, we failed to detect any concurrent association between sleep and AUDIT-C score at age 24 years, and we found that alcohol drinking at 15 years could not predict sleep problems at 24. These findings are in contrast with other studies reporting a bidirectional association between insomnia and alcohol use in adolescents and adults [22, 58–61] but see [62], and are unlikely explained by the high percentage of missing data for the sleep insufficiency score at 24 years (Supplementary Table1) [63].

The long-lasting effect of sleep insufficiency on alcohol related behavior raises the question of what the reason for such a link could be. Significant shortcomings intrinsic to clinical studies make it difficult to answer this question. For instance, pre-existent mental health problems might

be the cause for both the sleep problems and the development of AUD [64]. However, even when psychiatric disorders are accounted for as covariates, here and in other studies, the longitudinal association between sleep parameters and alcohol use persists [54, 55]. Other factors, such as peer influence and engagement in social activity, which might affect sleep schedules and promote alcohol drinking without a causal relationship between the two, may also play a role. Moreover, in our ALSPAC population, the sleep and alcohol measurements were self-reported and might have been affected by recall bias and social desirability bias.

Therefore, to better control for different factors that might synergistically interact to contribute to alcohol use, and that are particularly difficult to account for with human subjects, we developed a preclinical rat model of adolescent sleep restriction and alcohol drinking.

Previous experiments using rodents showed that sleep restriction is cross sectionally associated with increased alcohol consumption [65–67] and reduced sensitivity to the motor-impairing effects of alcohol in adult rodents [68], but only few experiments have focused on sleep manipulations during adolescence and looked at the effects on alcohol drinking later on. Atrooz and colleagues disturbed sleep for 6-8 hours a day, for fourteen days between childhood and early adolescence (post-natal day, P, 19-31) in Sprague Dawley rats and found increased voluntary consumption of alcohol one week after the end of the manipulation [69]. However, in prairie voles, reduced REM sleep and fragmented NREM sleep between P14 and P21 were not associated with differences in voluntary baseline alcohol drinking in adult individuals but led to higher alcohol intake immediately following foot shock [70]. Here, we found that if exposed to CSR between P25-P55, genetically selected alcohol preferring msP rats escalated alcohol consumption and led to higher alcohol drinking later in life. Our experimental paradigm explored a longer temporal interval and controlled for the stress induced by the sleep restriction procedure,

by having the control group exposed to the bar rotation experienced by the CSR group. We used polysomnography to validate the sleep restriction procedure in adolescent rats. Moreover, our sleep restriction protocol preserved a sleep opportunity window during the first hours of the light period, when rats are naturally more propense to be asleep and sleep pressure is higher [71]. This helped exclude the effect of a circadian shift in sleep, which is likely occurring with other sleep deprivation procedures and can have additional effects on alcohol drinking behavior [72].

Notably, msP rats [50] are believed to drink alcohol, at least in part, as an anxiety relieving mechanism [52, 73, 74]. Indeed, control rats showed a trend for a positive correlation between alcohol consumption and latency to approach food in an unfamiliar environment, the latter being considered a sign of anxiety. However, other typical markers of anxiety, such as the time spent in the center of the open field arena or the time spent in the light box, did not differ from controls. If anything, relative to controls, CSR rats showed a tendency towards a less anxious-like behavior in the open field test and in the NSF test. At first glance, these results could appear counterintuitive because existent reports showed that repeated adolescent alcohol binge drinking [75] and sleep loss [76] can increase anxiety and depressive symptoms in early and mid-adulthood. On the other hand, it is also known that disrupted sleep is associated with global risky decision making in adolescents, including illicit substance use, sexual risk-taking, and road/transport-related safety [10]. Hence, it is likely that the CSR-induced increased alcohol consumption levels observed were due to a heightened propensity to engage in risky behaviors or in impulsive actions rather than to increased levels of anxiety.

In addition to heightened impulsivity and changes in emotional responses, altered regulation of reward processes [77, 78] and changes in preference for caloric beverages [79–82] may be also linked to sleep loss and increase alcohol consumption. While here we did not measure overall

caloric intake, we attempted to control for this confounding factor by giving the rats *ab libitum* access to food throughout the experiment. 24-hour measurements of food consumption during the recovery period did not reveal a significant difference in overall food intake (g) between CSR and controls. Moreover, in our experimental paradigm, alcohol exposure occurred during the CSR recovery day, when theoretically, caloric intake should go down and be similar to control conditions [81]. Finally, increased alcohol intake persisted for many weeks of recovery after the sleep restriction procedure, when potential differences in energy expenditure should have ceased.

In conclusion, our findings favor the hypothesis that insufficient sleep represents a contributing factor for future AUD associated with increased propensity to risk-taking and impulsive behavior. Future experiments should explore the biological mechanisms linking sleep loss to the development of this complex neuropsychopathology and psychiatric comorbidity.

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Figure legends

Figure 1. Cross-lagged panel model evaluating the cross-sectional and longitudinal association between sleep and alcohol. Sleep parameters at 24 years consisted of item 1: “In past month, YP had problems getting to sleep or back to sleep”; item 2: “In past 7 nights, number of nights YP had sleep problems”; item 3: “In past week, length of time getting to sleep on night with least sleep”; item 4: “In past 7 days, number of nights spent >=3 hours getting to sleep”; item 5 “In past 7 days, YP woke >2 hours early & couldn't get back to sleep”. Significant paths and relative estimates are in bold.

Figure 2. Automatic sleep restriction. A) 7 days of EEG/EMG recordings, gray segments indicate 12 hours of lights on, black segments indicate 12 hours of lights off, red segments indicate activation of bar rotation (20 hours in the CSR group, 6 hours in the YC group). **B)** Time spent in each physiological state, expressed as % of a 24-hour baseline, for the CSR group. Repeated measure ANOVA was performed on raw data (minutes), for NREM sleep $F_{day}(1.789, 7.155)=13.11$ $p=0.0045$; for REM sleep, $F_{day}(2.23,10.25)=39.35$ $p<0.0001$; for wake $F_{day}(1.57, 7.21)=18.29$ $p=0.002$. Dunnett’s multiple comparisons post hoc tests against baseline. **C)** Time spent in NREM and REM sleep as % of 24-hour baseline during the 4 hours of sleep opportunity (4hr S opp) and the 20 hours of bar rotation (20hr bar ON). LME model was performed on raw data (minutes), for NREM sleep $F_{day}(1.99, 19.86)=20.50$ $p<0.0001$; for REM sleep $F_{day}(5,23)=39.35$ $p<0.0001$. Dunnett’s multiple comparisons post hoc tests against baseline. **D)** Time spent in each physiological state, expressed as % of a 24-hour baseline, for the YC group. CSR = chronic sleep restriction; YC = yoked controls; Ctrl = control; rec = recovery. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

Figure 3. Experimental design and alcohol and water consumption. A) Experimental design for adolescent chronic sleep restriction (CSR) and yoked control (YC) groups. Each white rectangle represents 1 day of 6-hour bar rotation (Ctrl); each red rectangle represents 1 day of 20-hour bar rotation (CSR); yellow-gray rectangles represent recovery days during which rats could drink water and ethanol. CSR = chronic sleep restriction; YC = yoked controls; Ctrl = control; T1 and T2 indicate batteries of behavioral tests. **B)** sleep restriction chamber and 2-bottle choice paradigm: rats could choose between drinking water or a solution of 10% alcohol. **C)** alcohol intake across the 6 cycles of sleep restriction, measured 30 minutes after presenting the 2 bottles (water and 10% ethanol). LME model $F_{group}(1,38)=16.24$ $p=0.0003$. Post hoc Šídák's multiple comparisons test, cycle 3 adjusted $p=0.0003$, cycle 4 $p=0.0027$. **D**) alcohol intake across the 6 cycles of sleep restriction, measured 10 hours after presenting the 2 bottles (water and 10% ethanol). LME model $F_{group}(1,36)=15.59$ $p=0.0004$. Post hoc Šídák's multiple comparisons test, adjusted p value for cycle 2 $p=0.0326$, cycle 3 $p=0.0155$, and cycle 4 $p=0.0078$. **E)** water intake across the 6 cycles of sleep restriction, measured 30 minutes after presenting the bottles. LME model $F_{group}(1,38)=0.55$, $p=0.46$. **F)** water intake across the 6 cycles of sleep restriction, measured 10 hours after presenting the bottles. LME model $F_{group}(1,38)=1.95$, $p=0.17$. **G)** average ethanol consumption, measured 30 minutes after bottle presentation, over 7 days of intermittent (1 day on, 1 day off) 2-bottle choice in adulthood, Mann Whitney test $p=0.46$. **H)** average ethanol consumption, measured 10 hours after bottle presentation, over 7 days of intermittent (1 day on, 1 day off) 2-bottle choice in adulthood, Mann Whitney test $p=0.03$. **I)** and **J)** average water consumption, measured 30 minutes (G) and 10 hours (H) after bottle presentation, over 7 days of intermittent (1 day on, 1 day off) 2-bottle choice in adulthood, Mann

Whitney test p=0.91 and p=0.77. CSR group in red (N=22), YC group in blue (N=18). ns= not significant, * p<0.05, ** p<0.01, *** p<0.001.

Figure 4. Behavioral tests. A) Open field test. *Left*, open field test shows a decline in total distance traveled from adolescence to adulthood in the YC group (N=18) but not in the CSR group (N=22). Two-way ANOVA found a main effect of age $F_{age}(1,37)=6.976$ p=0.012, and an interaction between age and group $F_{group\ x\ age}(1,37)=4.35$ p=0.043, which were confirmed by Šídák's multiple comparisons post hoc tests, adolescent p=0.43, adult p=0.049, YC adolescent vs adult p=0.0053, CSR adolescent vs adult p=0.9. *Right*, time spent in the center of the open field. Two-way ANOVA found only an effect of age $F_{age}(1,37)=17.30$ p=0.0002, confirmed by Šídák's multiple comparisons post hoc tests: YC p=0.057, CSR p=0.0015. **B)** Light-dark box. We found a trend towards shorter latency to enter the light box and longer time spent in the light box in CSR (N=9) relative to YC (N=10). p value for Mann-Whitney test displayed. **C)** Novelty Suppressed Feeding test. *Left*, food consumption in the arena, two-way repeated measure (rm) ANOVA $F_{group}(1,37)=3.16$ p=0.083, $F_{age}(1,37)=3.04$ p=0.089, $F_{age\ x\ group}(1,37)=0.36$ p=0.54. *Right*, food consumption in home-cage, two-way rmANOVA $F_{age}(1,37)=56.37$ p<0.0001, $F_{group}(1,37)=0.2$ p=0.6, $F_{age\ x\ group}(1,37)=0.55$ p=0.46, Šídák's post hoc test for YC and CSR p<0.0001. **D)** Novelty Suppressed Feeding test. *Left*, latency to eat in the arena, two-way rmANOVA $F_{group}(1,37)=13.35$ p=0.0008, $F_{age}(1,37)=0.01$ p=0.9, $F_{age\ x\ group}(1,37)=0.05$ p=0.8. Šídák's multiple comparisons post hoc tests, adolescent p=0.047, adult p=0.018. Right, latency to eat in the home cage, two-way rmANOVA $F_{group}(1,37)=0.66$ p=0.42, $F_{age}(1,37)=5.74$ p=0.022, $F_{age\ x\ group}(1,37)=0.22$ p=0.64. **E)** food consumption over a period of 24 hour, two-way rmANOVA $F_{group}(1,13)=0.18$ p=0.67, $F_{age}(1,13)=165,9$ p<0.0001, $F_{age\ x\ group}(1,13)=3.5$ p=0.083,

Šídák's post hoc test for YC (N=8) and CSR (N=7) $p<0.0001$. **F)** Measures of body weight during adolescence (left) and young adulthood (right), CSR N=22, YC N=18, Mann-Whitney $p=0.22$. **G)** Forced swim test. Right, time spent immobile, Mann-Whitney $p=0.43$. Left, latency to immobility Mann-Whitney $p=0.48$.

YC= yoke controls; CSR= chronic sleep restriction; NSFT= novelty suppressed feeding test; ns= not significant, * $p<0.05$, ** $p<0.01$, ****$p<0.0001$.

Figure 5. Significant correlations between behavioral measurements. A and **B)** Positive correlation between alcohol consumption and ambulatory time and distance in adult rats (N total=39; *left* time $\rho=0.42$ $p=0.008$; *right* distance $\rho=0.37$ $p=0.022$). **C)** positive correlation between alcohol consumption and % of time spent in the center of the open field (OF) arena (N=39; $\rho=0.45$ $p=0.004$). **D)** Correlation between the amount of alcohol consumed at 30 minutes and the latency to approach food in the arena in CSR adult rats (N=18; $\rho=-0.59$, $p=0.01$) and YC (N=16; $\rho=0.49$ $p=0.054$). **E)** Negative correlation between ambulatory time in the OF test and time spent immobile in the forced swim test (FST) (N=23; $\rho=-0.45$ $p=0.029$). **F** and **G)** Longitudinal correlation between adolescent alcohol consumption and adult performance in the novelty suppressed feeding test (NSFT) (N= 34; $\rho=-0.45$ $p=0.008$ at 30 minutes *left*, $\rho=-0.44$ $p=0.01$ at 10 hours *right*). ρ= Spearman's coefficient. For correlations with the latencies at the NSFT, rats that did not engage in food consumption during the test were removed from the analysis. YC=yoke controls; CSR = chronic sleep restriction.

Table 1. Sleep and alcohol variables used in the original dataset and in the 50 imputed datasets.

	Original dataset	Imputed datasets
Participants (Male %)	5497 (47.1)	5497 (47.1)
Sleep insufficiency score at 15 years, mean score (SD)	1.19 (0.54)	1.12 (0.54)
Hours of sleep per night at 15 years	8.26 (0.94)	8.24 (0.94)
Sleep insufficiency score at 24 years, mean score (SD)	2.88 (0.84)	2.12 (1.0)
Alcoholic drinks per week at 15years, mean n (SD)	3.33 (12.35)	3.11 (12.47)
AUDIT-C at 24 years, mean score (SD)	5.19 (2.49)	5.25 (2.46)
Maternal AUDIT, mean score (SD)	8.07 (3.01)	7.77 (3.24)
Social class, mean score (SD)		
I	339 (7.65)	391 (7.11)
II	1602 (36.15)	1911 (34.76)
III (non-manual)	1807 (40.78)	2271.5 (41.32)
III (manual)	287 (6.47)	376.5 (6.84)
IV	344 (7.76)	468.5 (8.52)
V	52 (1.17)	77.0 (1.40)
Psychiatric disorders, mean score (SD)	2.19 (0.83)	2.20 (0.84)

SD=standard deviation

Table 2. **Cross-sectional and longitudinal associations between sleep parameters and alcohol consumption measures**

Cross-sectional association between sleep parameters and alcohol consumption at 15 years of age.			
Parameter	**Estimate**	**95% CI**[1]	**p-value**
Sleep insufficiency score	1.7	0.84, 2.6	<0.001
Hours of sleep per night	-0.92	-1.5, -0.38	0.001
Daytime sleepiness	1.6	0.71, 2.5	<0.001
Sleep quantity *	0.99	0.16, 1.8	0.020
Sleep satisfaction *	0.65	0.09, 1.2	0.023
Cross-sectional association between sleep insufficiency score and AUDIT-C score at 24 years of age.			
Parameter	**Estimate**	**95% CI**[1]	**p-value**
Sleep insufficiency score	0.01	-0.11, 0.13	0.9
Longitudinal association between sleep parameters at 15 years and AUDIT-C at 24 years			
Parameter	**Estimate**	**95% CI**[1]	**p-value**
Hours of sleep per night	-0.11	-0.20, -0.03	0.009
Sleep insufficiency score	0.35	0.21, 0.5	<0.001
Daytime sleepiness	0.16	0.05, 0.26	0.003
Sleep quantity *	0.25	0.09, 0.40	0.002
Sleep satisfaction *	0.24	0.15, 0.34	<0.001

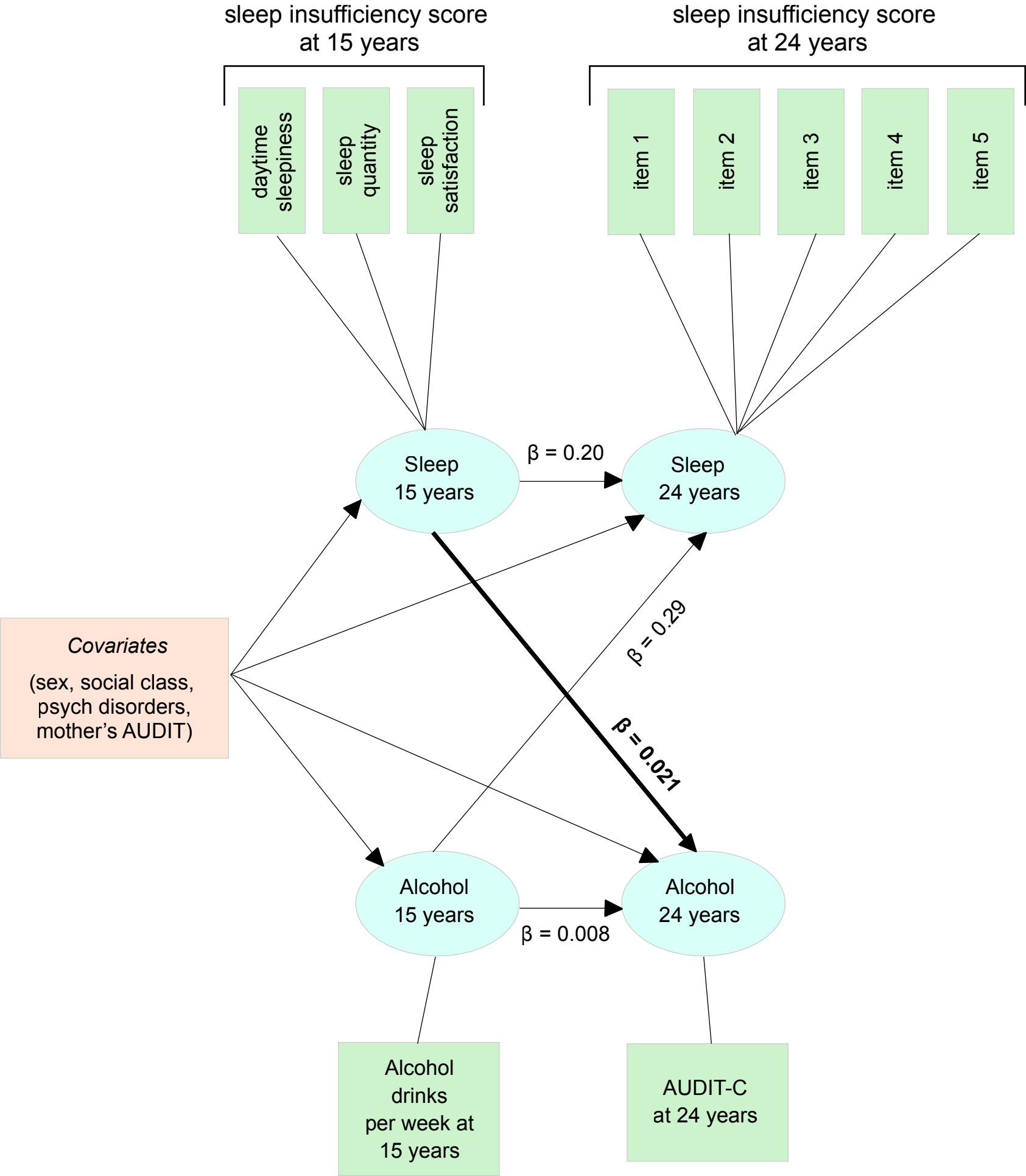
[1]CI = Confidence Interval

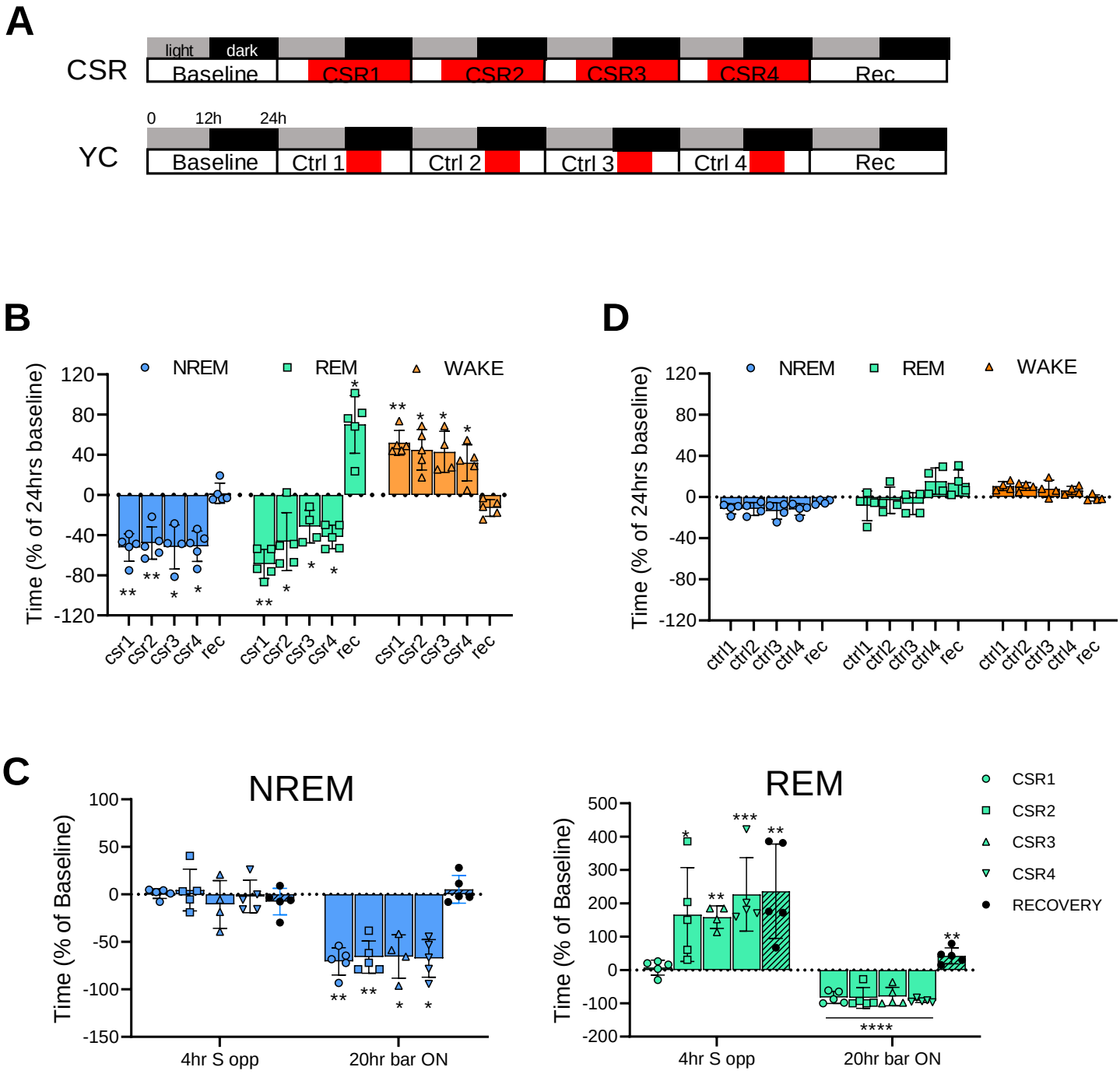
* Responses are on an inverse scale, with higher scores indicating less sleep or low satisfaction

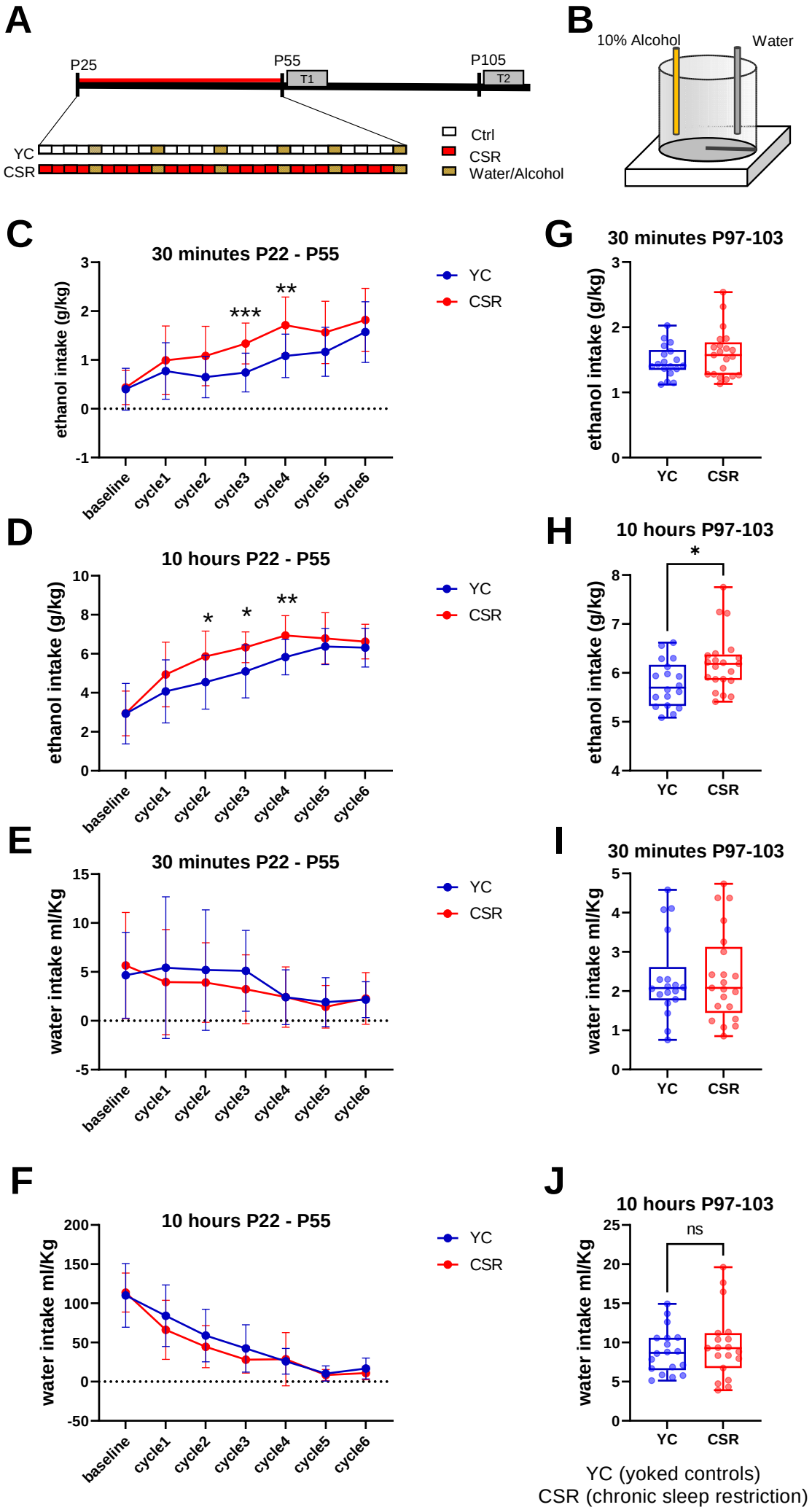
Table 3. Evaluation of autoregressive, concurrent, and cross-lagged correlations between insufficient sleep score and alcohol use.

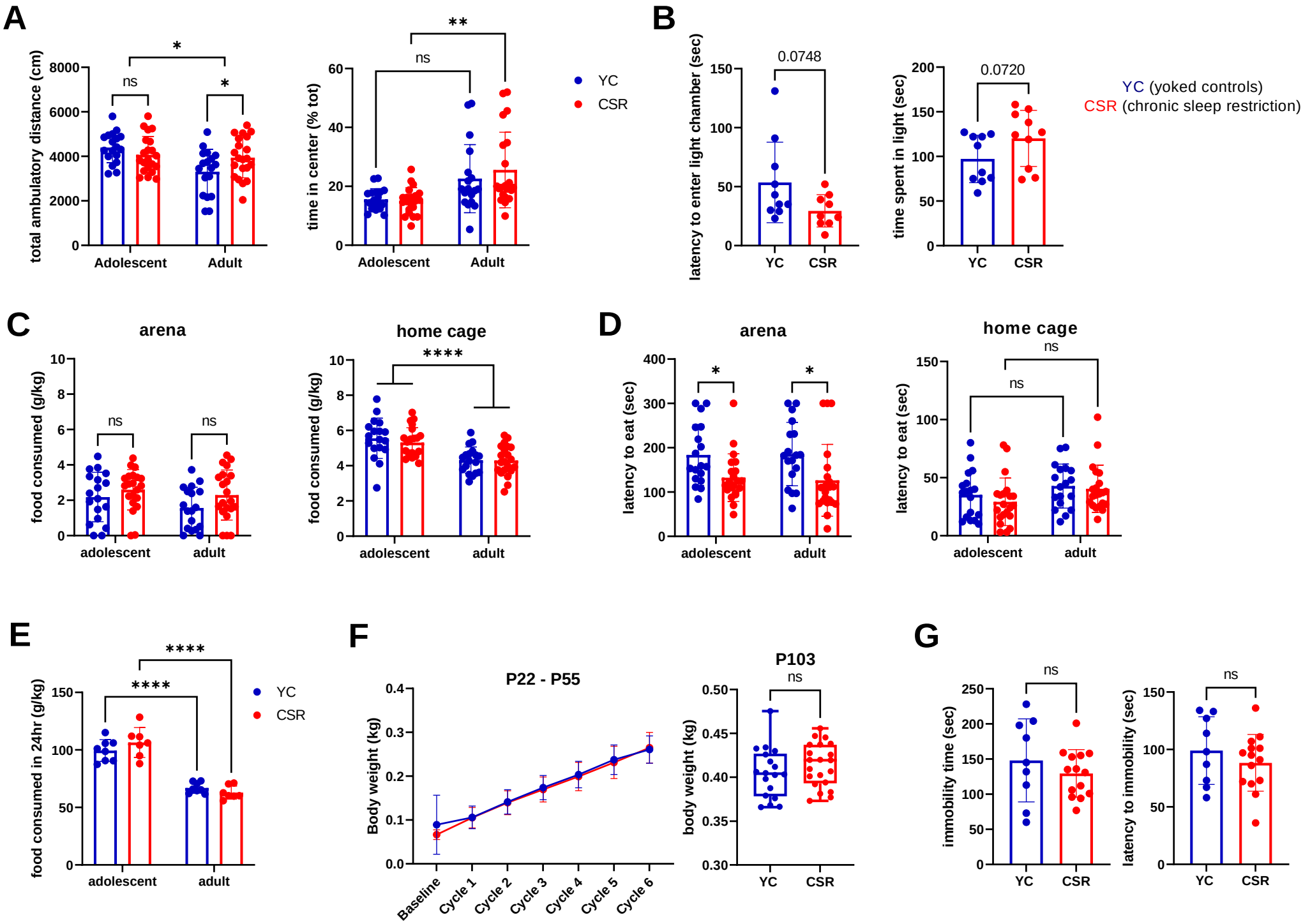
Autoregressive correlation	**Estimate**	**95% CI**[1]	**p-value**
Sleep T1 → Sleep T2	0.20	-0.31 – 0.72	0.44
Alcohol T1 → Alcohol T2	0.01	-0.095 - 0.11	0.88
Cross lagged correlation	**Estimate**	**95% CI**[1]	**p-value**
Sleep T1 → Alcohol T2	**0.021**	**0.012 - 0.03**	**<0.001**
Alcohol T1 → Sleep T2	0.29	-1.92 – 2.49	0.805
Concurrent correlation	**Estimate**	**95% CI**[1]	**p-value**
Sleep T1 → Alcohol T1	**0.0037**	**0.001 - 0.006**	**0.039**
Sleep T2 → Alcohol T2	-0.001	-0.004 - 0.003	0.892

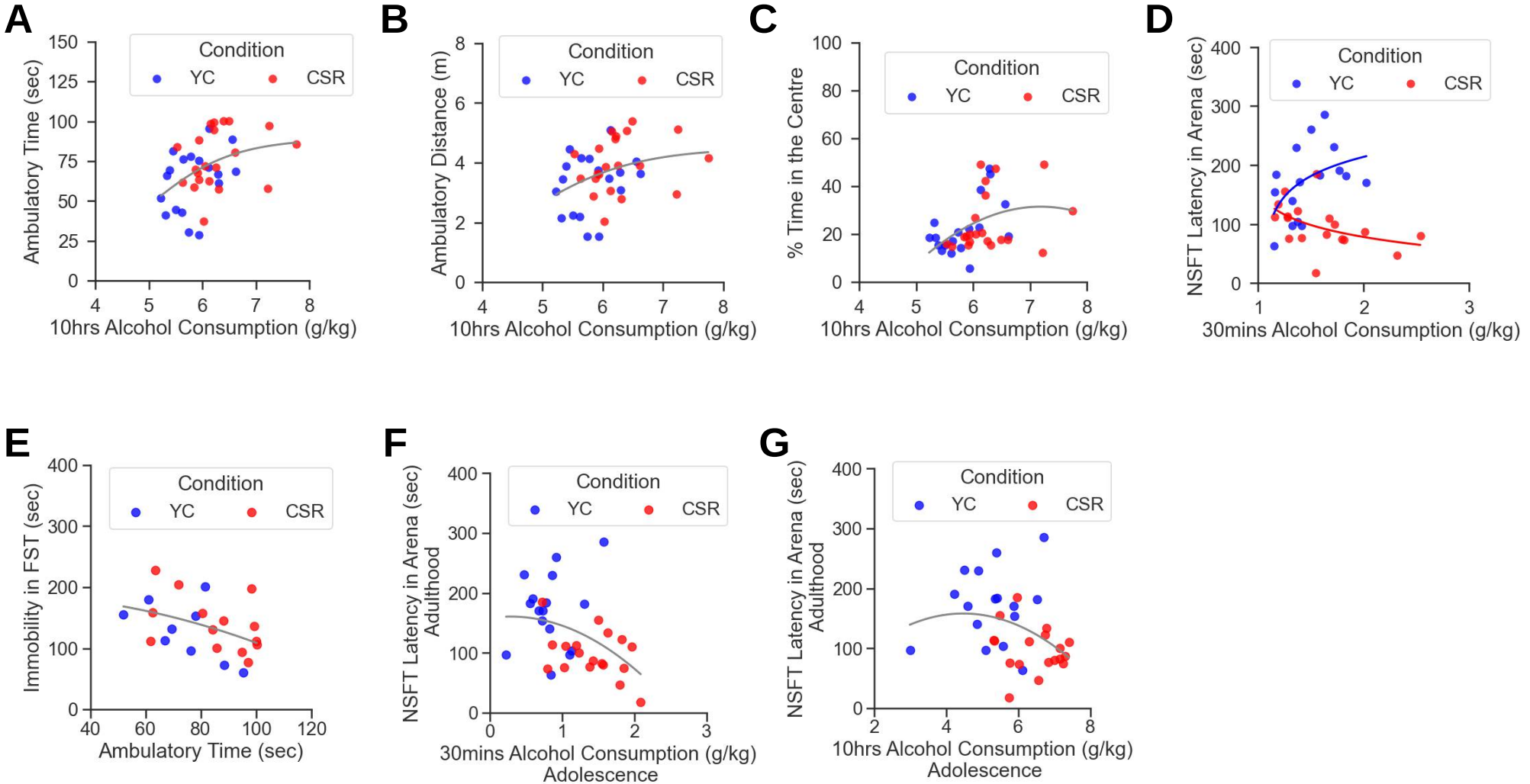
Sleep T1=insufficient sleep score at 15 years of age. Sleep T2=insufficient sleep score at 24 years of age. Alcohol T1= alcohol consumption at 15 years of age. Alcohol T2=AUDIT-C score at 24 years of age. [1] CI = Confidence Interval.


sleep insufficiency score at 15 years
sleep insufficiency score at 24 years
daytime sleepiness
sleep quantity
sleep satisfaction
item 1
item 2
item 3
item 4
item 5
Sleep 15 years
β = 0.20
Sleep 24 years
β = 0.29
β = 0.021
Covariates
(sex, social class, psych disorders, mother's AUDIT)
Alcohol 15 years
β = 0.008
Alcohol 24 years
Alcohol drinks per week at 15 years
AUDIT-C at 24 years


A
CSR
light
dark
Baseline
CSR1
CSR2
CSR3
CSR4
Rec
YC
0
12h
24h
Baseline
Ctrl 1
Ctrl 2
Ctrl 3
Ctrl 4
Rec
B
NREM
REM
WAKE
Time (% of 24hrs baseline)
120
80
40
0
-40
-80
-120
csr1
csr2
csr3
csr4
rec
D
ctrl1
ctrl2
ctrl3
ctrl4
rec
C
NREM
Time (% of Baseline)
100
50
0
-50
-100
-150
4hr S opp
20hr bar ON
REM
500
400
300
200
100
0
-100
-200
CSR1
CSR2
CSR3
CSR4
RECOVERY


A
P25
P55
P105
T1
T2
YC
CSR
Ctrl
CSR
Water/Alcohol
B
10% Alcohol
Water
C
30 minutes P22 - P55
ethanol intake (g/kg)
baseline
cycle1
cycle2
cycle3
cycle4
cycle5
cycle6
YC
CSR
D
10 hours P22 - P55
ethanol intake (g/kg)
E
30 minutes P22 - P55
water intake ml/Kg
F
10 hours P22 - P55
water intake ml/Kg
G
30 minutes P97-103
ethanol intake (g/kg)
H
10 hours P97-103
ethanol intake (g/kg)
I
30 minutes P97-103
water intake ml/Kg
J
10 hours P97-103
water intake ml/Kg
ns
YC (yoked controls)
CSR (chronic sleep restriction)


A
total ambulatory distance (cm)
8000
6000
4000
2000
0
ns
*
*
Adolescent
Adult
time in center (% tot)
60
40
20
0
ns
**
Adolescent
Adult
YC
CSR
B
latency to enter light chamber (sec)
150
100
50
0
0.0748
YC
CSR
time spent in light (sec)
200
150
100
50
0
0.0720
YC
CSR
YC (yoked controls)
CSR (chronic sleep restriction)
C
arena
food consumed (g/kg)
10
8
6
4
2
0
ns
ns
adolescent
adult
home cage
food consumed (g/kg)
10
8
6
4
2
0
****
adolescent
adult
D
arena
latency to eat (sec)
400
300
200
100
0
*
*
adolescent
adult
home cage
latency to eat (sec)
150
100
50
0
ns
ns
adolescent
adult
E
food consumed in 24hr (g/kg)
150
100
50
0
****
****
adolescent
adult
YC
CSR
F
P22 - P55
Body weight (kg)
0.4
0.3
0.2
0.1
0.0
Baseline
Cycle 1
Cycle 2
Cycle 3
Cycle 4
Cycle 5
Cycle 6
P103
ns
body weight (kg)
0.50
0.45
0.40
0.35
0.30
YC
CSR
G
immobility time (sec)
250
200
150
100
50
0
ns
YC
CSR
latency to immobility (sec)
150
100
50
0
ns
YC
CSR


A
Condition
YC
CSR
Ambulatory Time (sec)
10hrs Alcohol Consumption (g/kg)
B
Condition
YC
CSR
Ambulatory Distance (m)
10hrs Alcohol Consumption (g/kg)
C
Condition
YC
CSR
% Time in the Centre
10hrs Alcohol Consumption (g/kg)
D
Condition
YC
CSR
NSFT Latency in Arena (sec)
30mins Alcohol Consumption (g/kg)
E
Condition
YC
CSR
Immobility in FST (sec)
Ambulatory Time (sec)
F
Condition
YC
CSR
NSFT Latency in Arena (sec)
Adulthood
30mins Alcohol Consumption (g/kg)
Adolescence
G
Condition
YC
CSR
NSFT Latency in Arena (sec)
Adulthood
10hrs Alcohol Consumption (g/kg)
Adolescence