scientific reports

OPEN

Chronic circadian disruption in adolescent mice impairs hippocampal memory disrupting gene expression oscillations

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Chronodisruption, the misalignment of internal biological systems with external environmental changes, negatively impacts health and cognition. A prevalent form, *social jet lag*, affects a large portion of adolescents and is associated with reduced academic performance, and impaired emotional regulation. Despite its prevalence, the mechanism by which adolescent chronodisruption results in cognitive deficits remains unexplored. Here, we induced chronic chronodisruption in adolescent mice through light/dark cycle manipulation. We performed a battery of established behavioral tests alongside an analysis of daily rhythms of expression of genes involved in relevant physiological functions. We report that adolescent circadian disruption induces impairments in short-term, social, and spatial memory without prompting anxiety-like behavior. Gene expression analyses revealed altered oscillation patterns of circadian clock genes in the hypothalamus and hippocampus. Furthermore, we identified alterations in daily patterns of genes involved in glucocorticoid and endocannabinoid transmissions as well as synaptic plasticity. This work emphasizes the importance of circadian homeostasis for cognitive function and hippocampal homeostasis. Thus, understanding the mechanism underlying adolescent chronodisruption is essential to better understand its long-term implications for cognitive function and overall health.

Keywords Adolescence, Behavior, Chronodisruption, Endocannabinoids, Gene expression, Memory impairments, Social jet lag

Chronodisruption describes a disturbance in the proper alignment of internal biological systems with external environmental cues, resulting in the critical loss of time order within physiological processes[1]. Under normal conditions, the endogenous circadian rhythms synchronize bodily functions with environmental factors like the day-night cycle, meal timing and social routines. However, modern society has significantly transformed human lifestyles resulting in a persistent desynchronization between the biological rhythms and daily activities[2].

This social-biological time discrepancy is referred to as *social jet lag* (SJL) and is operationalized as the difference in sleep midpoint between workdays and free days[3]. Epidemiological data estimates that 70% of the industrialized population experiences SJL[4] which has been linked to various pathological conditions[4,5]. Adolescents and young adults are particularly susceptible[6] with an average SJL of 2 h, 39 minutes[7] accompanied by impaired coping, reward function, impulse control, and emotional regulation[6,8–10] which are reflected in decreased academic performance[11]. In fact, the degree of SJL directly correlates with lower educational achievement[12]. Furthermore, SJL causes circadian misalignment[13] and dysregulation of the molecular circadian clock[14].

The mammalian molecular clock is an intrinsic timekeeping system synchronized by the suprachiasmatic nucleus (SCN) in the hypothalamus. It is driven by a network of proteins that maintain oscillatory transcriptional feedback loops[15]. The core positive limb is composed of the CLOCK: BMAL1 heterodimer, which initiates the transcription of multiple genes, including the *period* (*per*) and *cryptochrome* (*cry*) families[15]. The resulting PER and CRY proteins are the negative limb of the loop, inhibiting CLOCK: BMAL1 activity[15]. Hence, their degradation terminates the repression phase and restarts the transcription cycle. Additional regulators like NPAS2, DBP as well as nuclear receptors ROR and REV-ERBs, help to fine-tune the core circadian machinery[16]. The proper function of this machinery is crucial for regulating the timing of various biological processes throughout the day,

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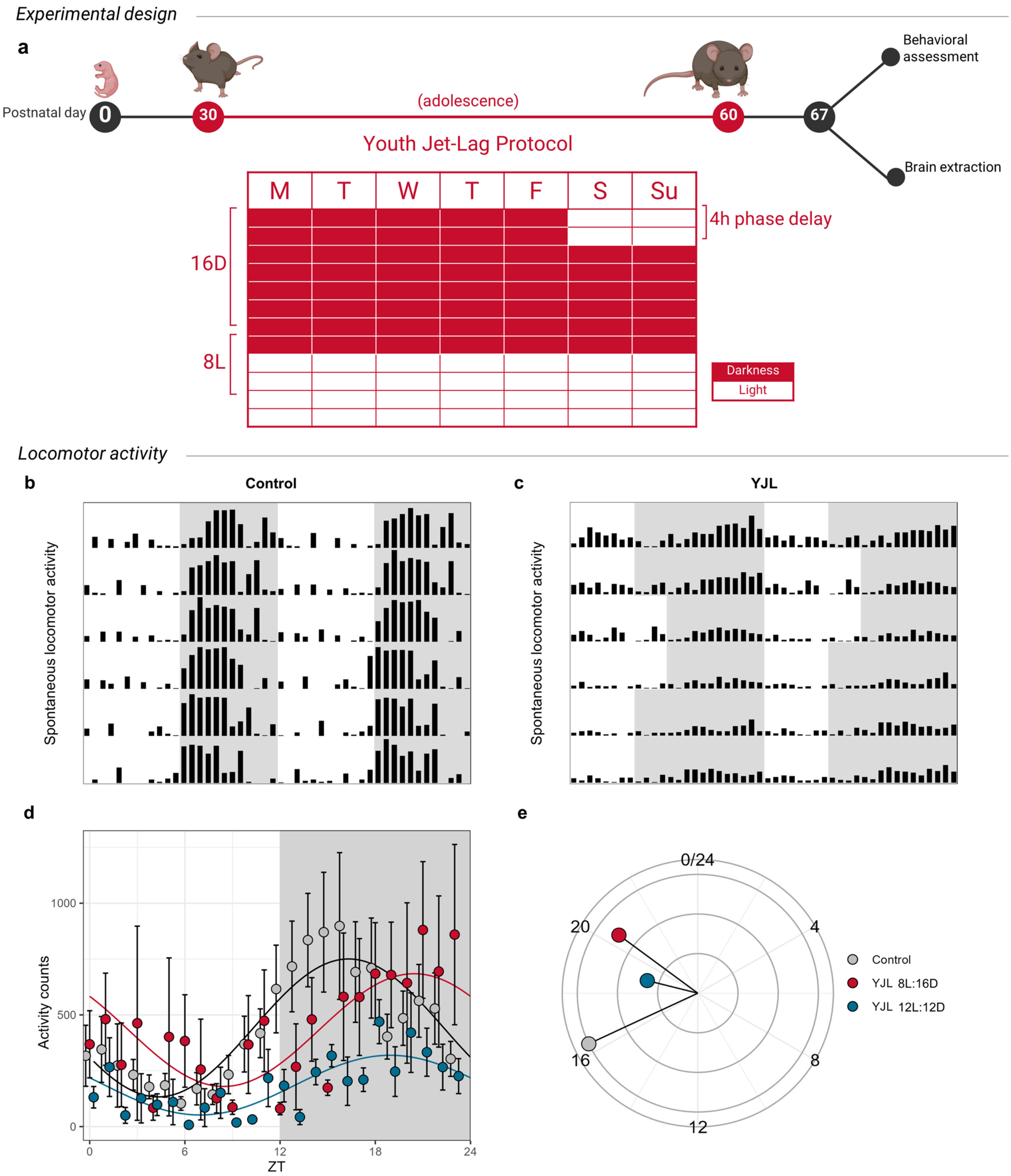


thus contributing to homeostatic control. While the daily patterns of expression of clock components have been well-characterized, the rhythmic patterns of clock outputs are less understood. It remains unclear if disruption of daily rhythms in clock genes directly translates into a loss of oscillation in output gene expression.

We do know, thanks to animal models, that the molecular clock within the central nervous system (CNS), interacts with critical modulatory systems like the endocannabinoid, dopaminergic, glutamatergic, and GABAergic systems[17–19] among others, to maintain bodily homeostatic conditions. Accordingly, and consistent with homeostatic failure as a basis of pathological conditions, clock malfunctioning leads to neurotransmission dysregulation. Nonetheless, whether this loss of homeostasis stems from disruptions of oscillations in clock-controlled genes and whether this is responsible for the pathological phenotypes remains to be investigated.

Consequently, our understanding of how chronodisruption, specifically SJL, ultimately translates into morbidity is limited. Several protocols have mimicked SJL in adult mice[20–22] reporting memory impairments[20]. This highlights the relevance of circadian rhythmicity for hippocampal homeostasis, regulating many forms

◀**Fig. 1**. Youth Jet Lag mice display distinct spontaneous locomotor activity patterns during the last week of the protocol. **a** Graphical representation of the experimental design. From PD30 to PD60 animals underwent a disruptive LD cycle with extended activity periods and phase delays. First, a 5-day pattern of 8 L:16D, followed by a 4-hour delay in the dark phase onset for 2 days, then returning to 8 L:16D. After 5 weeks, at PD60, animals had a one-week under standard LD conditions. Control mice were kept on a consistent 12 L:12D cycle throughout the study (PD30 to PD67). Subsequently, animals were used for behavioral experiments (PD67-77) or brain extractions (PD67-68). **b**, **c** Representative double-plotted actograms from control and YJL. The y-axis shows the monitored days and the x-axis the time of day expressed as ZT. Black bars symbolize spontaneous locomotor activity counts. The darkened areas of the plot represent the ambient illumination regime: grey for lights OFF, white for lights ON. **d** Spontaneous locomotor activity represented as sinusoidal waves representing the least-squares best fit trace for experimental groups; control (grey/black) and YJL activity during the last day of the 8 L:16D cycle (red) as well as during the last day of the 12 L:12D cycle (blue). Data are expressed as mean ($n=4$) and standard deviation. **e** Circlegram comparing acrophase and amplitude of spontaneous locomotor activity of the three groups assessed. The lengths of the lines represent the amplitude normalized to control group. The location of the color dots on the circle depicts the ZT of the acrophase. ZT0 represents the onset of the light phase.

Experimental group	*p* value	*r* square	Average	Acrophase	Amplitude	Pairwise models *p* value
Control	1.22E-09	0.357	440.4	16.3	308.8	0.0004
YJL 8 L:16D	0.001	0.136	433.7	20.5	252.4	
YJL 12 L:12D	4.88E-06	0.231	186.0	18.9	133.6	

Table 1. Kronos' rhythmic analysis output of locomotor activity.

of neuroplasticity[23] and supporting memory formation, retrieval, and persistence[24,25]. Nevertheless, preclinical research of adolescent chronodisruption and its impact on cognition is largely neglected despite the higher prevalence of SJL in young humans. Investigating how developmental disruption of circadian rhythms impacts CNS homeostasis is essential to understand of the pathophysiology of SJL. This study is, to our knowledge, the first attempt to investigate chronodisruption in adolescent mice and its downstream effects on the circadian expression of neurotransmission systems. Our goal was to develop a novel model of chronodisruption in adolescent mice that recapitulates SJL symptomatology. Additionally, we aimed to determine whether alterations in natural rhythms of gene expression outside of the central pacemaker are preserved, and if disrupted, to explore their relationship with the observed pathological phenotype. For this, we first characterized the model at a behavioral level. Then, we assessed physiological chronodisruption via gene expression analyses throughout the 24 h cycle. This approach allowed the study of daily rhythms in circadian clock genes as well as external targets such as components from the endocannabinoid, glucocorticoid, glutamatergic and GABAergic systems. Overall, our research shows that disruption of adolescent circadian rhythms induces memory impairments and a loss of circadian homeostasis in the hypothalamus and hippocampus.

Results

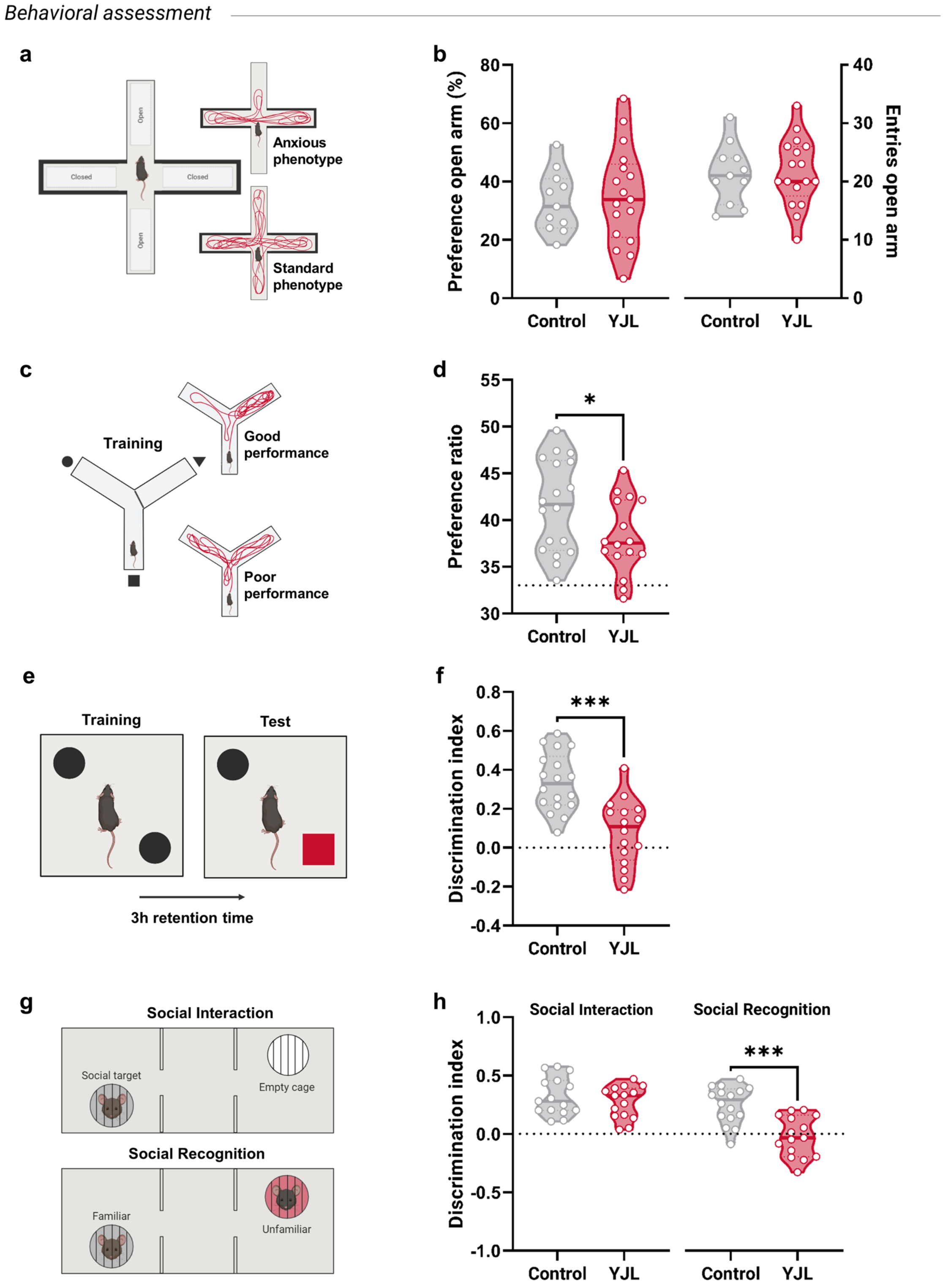
Aberrant light cycles in adolescent mice induce alterations in daily locomotor activity rhythms

Spontaneous locomotor activity of YJL and controls was recorded to assess daily rhythms (Fig. 1). Sinusoidal curves were generated with Kronos (Fig. 1d). The Generalized Linear Model (GLM) results provided estimated coefficients for the sine and cosine and were found to be significant across all groups (Table 1) with strong evidence of periodicity and varying degrees of goodness-of-fit, as evidenced by the R-squared values ranging from 0.136 to 0.357 across groups. The acrophase and amplitude for each group is reported in Table 1 and graphically represented as a circlegram in Fig. 1e. Furthermore, when assessing differential rhythmicity, the p-value for the pairwise models indicated that the rhythmic patterns vary significantly across experimental conditions ($p<.001$). Specifically, the ANOVAs indicated significant differences for 'control vs. YJL 12:12', $F(1, 186)=52.75$, $p<.0001$, and 'YJL 8:16 and YJL 12:12', $F(1, 186)=24.29$, $p<.0001$. However, no differences were found for 'control and YJL 8:16' (Supplementary Information, Table S2).

Chronodisruptive protocol mimicking social jet lag during adolescence induces impairments in spatial, short-term memory, and social recognition

Elevated plus maze

In the Elevated Plus Maze (EPM), reduced preference for the open arms was taken as a measure of anxiety-like behavior. Results indicated that the means of time spent in open arms were similar: for controls and YJL (Fig. 2a, b). Indeed, no significant differences were found between groups for either the percentage of preference or the number of entries in open arms (Preference for open arms: $t(26)=0.36$, $p=.719$, $r=.005$; Entries in open arms: $t(26)=0.15$, $p=.885$, $r=.0008$). The two-way ANOVA showed no significant main effects of sex nor interaction sex vs. experimental group, $F(1, 24)=0.82$, $p=.374$ (data not shown).



Reference memory

The reference memory test was used to evaluate spatial memory indicated by an increased preference for the novel arm over the familiar arms. In this case, both groups showed a preference for the novel arm (Fig. 2c, d). However, the unpaired *t*-test revealed significant differences between groups, $t(32) = 2.18$, $p = .037$, $r = .129$, indicating a decreased performance of the YJL group. The two-way ANOVA revealed no significant interaction sex vs. experimental group, $F(1, 29) = 0.01$, $p = .920$. Generally, the mean preference of females (41.62%) was higher than the males' (38.64%) regardless of the experimental group (data not shown). However, this was marginally not significant for sex, $F(1, 29) = 4.13$, $p = .051$.

◀**Fig. 2**. Youth Jet Lag mice display impairments in spatial, short-term, and working social memory but no anxiety-like behavior or social avoidance. **a** Graphical representation of the EPM apparatus. **b** Percentage of preference (control: *n* = 11; x̄ =33.08% ±10.54, YJL: *n* = 17; x̄=35.15% ±16.77) and number of entries in the open arm of the EPM (control: *n* = 11; x̄=21.27 ±5.16, YJL: *n* = 17; x̄=21.59 ±5.81) **c** Schematic representation of the Reference memory test. **d** Percentage of preference for the novel arm of the Y-Maze in the reference memory test (control: *n* = 18; x̄=41.54% ±4.94, YJL: *n* = 16; x̄=38.17% ±3.97). Dotted line at y = 33 represents pure chance. **e** Visual representation of the NOR experimental paradigm. **f** Discrimination index during the NOR test (control: *n* = 18; x̄=0.336 ±0.15, YJL: *n* = 16; x̄=0.079 ±0.17). Dotted line at y = 0 represents no preference for either object. **g** Graphical representation of the three-chamber social interaction and recognition test. **h** Discrimination indexes during the three-chamber social interaction (control: *n* = 15; x̄=0.323 ±0.16, YJL: *n* = 15; x̄=0.273 ±0.14) and social recognition test (control: *n* = 16; x̄=0.250 ±0.16, YJL: *n* = 15; x̄=−0.021 ±0.17). Dotted line at y = 0 represents no preference between interaction cups. Statistical significance was calculated by Student's *t*-tests. **p* <.05; ***p* <.01; ****p* <.001. Data are expressed in violin plots as medians, individual values and 25 and 75% percentiles for control (grey) and YJL (red) group.

Novel object recognition

The Novel Object Recognition test (NOR) evaluated recognition memory indicated by increased preference for the novel object compared to the familiar one. During this test, the average total time of object exploration during the test was 53.99 s (*SEM* = 2.32) and 53.29 s (*SEM* = 2.97) for the controls and the YJL group, respectively (Fig. 2e, f), with no significant differences, *t*(34) = 0.19, *p* =.851, *r* =.001 (data not shown). In addition, we found that the control mean discrimination index ($M_{control}$ = 0.336, *SEM* = 0.04) indicated a higher preference towards the novel object. Meanwhile, the mean discrimination index of YJL (M_{YJL} = 0.08, *SEM* = 0.04) indicated no preference between objects. The unpaired two tailed *t*-test yielded significant differences between groups, *t*(32) = 4.70, *p* <.0001, *r* =.408. The two-way ANOVA revealed no significant main sex effect, *F*(1, 31) = 0.51, *p* =.480, nor interaction between sex and experimental group, *F*(1, 31) = 0.09, *p* =.770 (data not shown).

Social interaction and recognition

During the social interaction test, time spent in the room containing another animal compared to the empty compartment was taken as a sociability indicator. The average discrimination indexes for control ($M_{control}$ = 0.32, *SEM* = 0.04) and YJL (M_{YJL} = 0.27, *SEM* = 0.04) indicated that both groups showed preference for the stimulus mouse over the empty cup (Fig. 2g, h). Additionally, the *t*-test revealed no significant statistical differences in sociability between groups, *t*(28) = 0.92, *p* =.366, *r* =.029. Social recognition was represented by an increased time exploring the novel mouse compared to the familiar one (Fig. 2h, right). The average control discrimination index remained higher than 0 ($M_{control}$ = 0.25, *SEM* = 0.04) whereas the mean for YJL plummeted (M_{YJL} = −0.020, *SEM* = 0.044), indicating no preference between stimulus mice. The *t*-test revealed significant differences between experimental groups, *t*(29) = 4.55, *p* <.0001, *r* =.414. The two-way ANOVA yielded no significant main effects of sex, *F*(1, 27) = 0.47, *p* =.501, nor interaction between variables, *F*(1, 27) = 0. 001, *p* =.97, (data not shown).

Adolescent chronodisruption induces alterations in daily expression of the molecular clock and genes involved in neurotransmission in the hypothalamus and memory processing in the hippocampus

Regarding circadian clock genes, we found area-dependent significant oscillations (Fig. 3 and Supplementary Information, Table S3). In the hypothalamus (Fig. 3a-j), the control group showed significant oscillation of *arntl1*, *dbp*, *npas2*, and *per2*, whereas for the YJL group, significant oscillation was reported for *arntl1*, *dbp*, and *npas2*, but not *per2*. Although *npas2* was reported to follow a circadian pattern of expression in both groups, the pairwise model indicated that the patterns were significantly different between controls and YJL (*p* =.004). Particularly, we observed an acrophase advancement of 5.6 h. Meanwhile, in the hippocampus (Fig. 3k-t) controls exhibited significant oscillation of *arntl1*, *cry1*, *cry2*, *dbp*, *nr1d2*, *per1* and *per2*, whereas the YJL group showed oscillation in *arntl1*, *cry1*, *dbp*, *nr1d2* and *per2*, but not *cry2* nor *per1*.

Regarding targets outside the molecular clock, Kronos identified significant oscillations in several genes in the hypothalamus (Supplementary Information, Table S4) and the hippocampus (Supplementary Information, Table S5). In the hypothalamus, control animals showed a significant oscillation for: *avp*, *crh*, *crhr1*, *crhr2*, *napepld*, *pparα*, *slc17a6*, and *th* (Fig. 4a-h). Alternatively, in YJL, significant oscillations were reported for *pparα*, and *slc17a6*, but not for *avp*, *crh*, *crhr1*, *crhr2*, *napepld* nor *th*. While in the hippocampus of controls, we found significant oscillation for *crhr2*, *drd2*, *gabra1*, *gsk3b*, *maoa*, *mgll*, *npy*, *ntrk2*, *slc17a8* and *slc1a2*, *but not napepld*, (Fig. 5a-j). In the YJL, however, significant oscillation was only reported for *gsk3b* and *napepld*, *but not for crhr2*, *drd2*, *gabra1*, *maoa*, *mgll*, *npy*, *ntrk2*, *slc17a8* and *slc1a2*. Importantly, the pairwise model indicated that although *gsk3b* did oscillate in a circadian manner in the YJL, its sinusoidal curve was significantly different to that of the control (*p* =.04). Particularly, we observed an acrophase advancement of 5.5 h in the YJL group (Fig. 5d).

Discussion

The present study provides novel insights into the consequences of chronic adolescent chronodisruption on cognitive function and gene expression rhythms in the CNS. We developed a novel adolescent mouse model of circadian disruption that induces impairments in short-term and spatial memory as well as in social recognition without affecting other cognitive domains like sociability or exploratory and anxiety-like behaviors. Chronodisruption was evidenced by alterations in daily activity rhythms under the disruptive LD cycle, and region-specific changes in clock gene expression. Specifically, in YJL animals, the oscillatory profile of *per2*

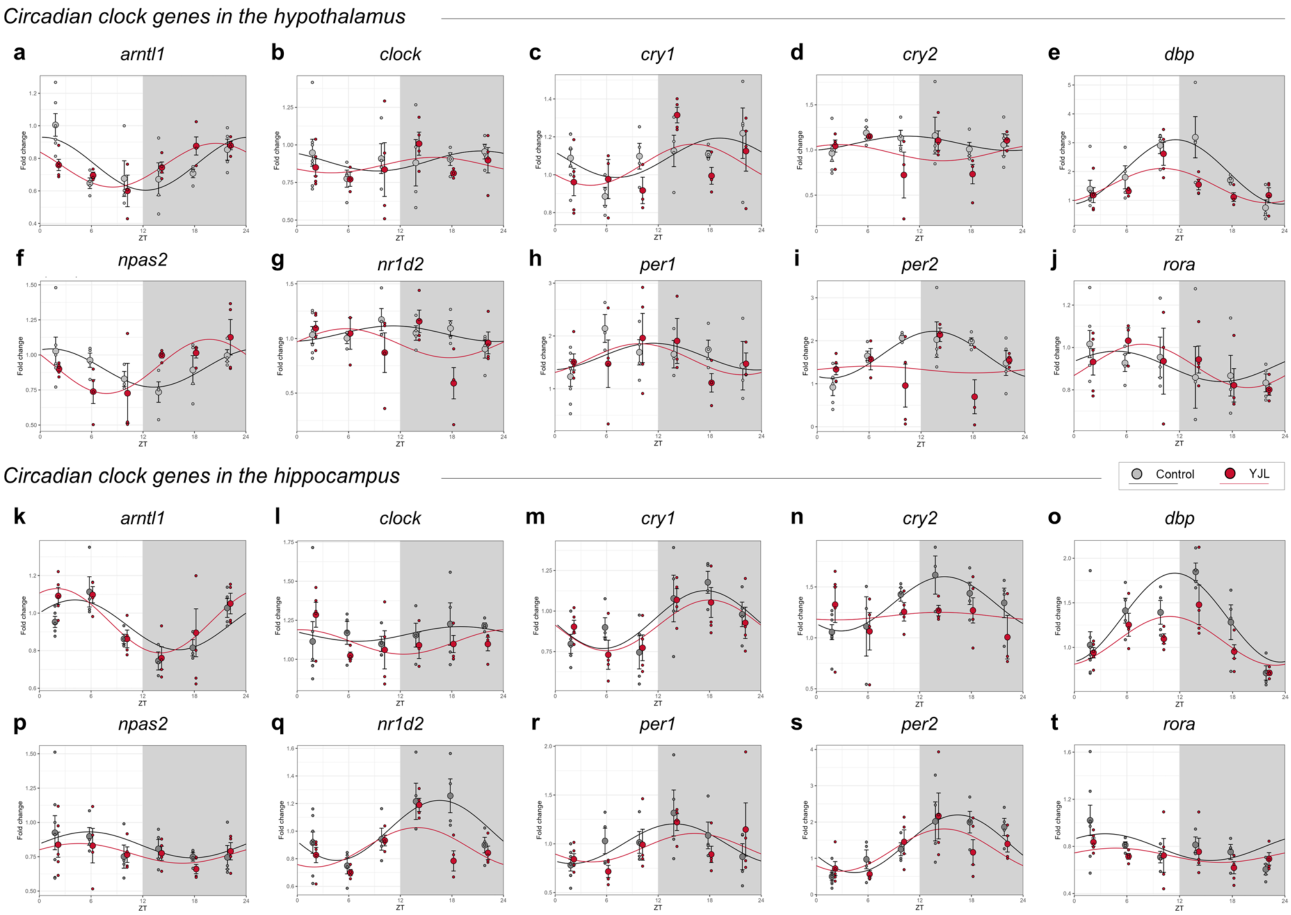


Fig. 3. Youth Jet Lag mice display circadian disruption of circadian clock genes in the hypothalamus and hippocampus. Rhythmic analysis output on clock genes mRNA levels in the hypothalamus and hippocampus. Sinusoidal waves fold change of mRNA expression fold change of hypothalamus **a***artl1*, **b***clock*, **c***cry1*, **d***cry2*, **e***dbp*, **f***npas2*, **g***nr1d2***h***per1*, **i***per2* and **j***rora*. Sinusoidal waves fold change of mRNA expression fold change of hypothalamus **a***artl1*, **b***clock*, **c***cry1*, **d***cry2*, **e***dbp*, **f***npas2*, **g***nr1d2***h***per1*, **i***per2* and **j***rora*. Data is normalized to the median sample of the control group at ZT2. Sinusoid curves represent the least-squares best fit trace for both experimental groups; control (grey/black) and YJL (red). Data are expressed as mean, individual values ($n = 3–5$) and error bars represent standard deviation.

and *npas2* was altered in the hypothalamus while the hippocampus exhibited a loss of oscillations of *cry2* and *per1*. Furthermore, we describe for the first time the daily expression rhythms of genes including *crhr1*, *crhr2* and *napepld* in the hypothalamus, and *drd2*, *gabra1*, *gsk3b*, *maoa*, *mgll*, *npy*, *ntrk2*, *slc17a8*, *slc1a2*, in the hippocampus. Importantly, the YJL group displayed a region-dependent loss of oscillation in the expression of genes related to regulation of the hypothalamic-pituitary-adrenal (HPA) axis, the extended endocannabinoid system, as well as neurotransmitter uptake and synaptic plasticity processes.

It should be considered that while circadian disruption is often associated with metabolic changes which could indirectly impact behavior and cognition, here, we detected no differences in body weight changes between groups (Figure S1).

Overall, the present work offers a novel chronodisruption model that recapitulates cognitive deficits observed in humans. First, LD cycle disruption significantly altered daily locomotor activity rhythms in YJL mice. Control mice exhibited an acrophase consistent with previous reports for the C57BL/6 strain[26,27]. However, the YJL group presented a delay in the acrophase during the 8 L:16D cycle, a common observation in short photoperiods[28]and indicative of adaptation to the aberrant LD cycle. Additionally, YJL mice showed differential activity patterns relative to the type of cycle they were being exposed to, i.e. 8 L:16D or 12 L:12D with 4 h delay of the onset of light phase. These alterations suggest an entrainment to the disruptive LD cycle and point to a potential dysregulation in the timing of circadian processes. Indeed, our molecular analyses of well-reported oscillating clock genes in the hypothalamus, the integrating region of circadian signals, confirm a desynchronization of the circadian machinery evidenced by the loss of daily fluctuations in *per2* and the advancement of *npas2* acrophase. Collectively, these findings evidence that our model induces chronodisruption.

Regarding behavioral assessment, YJL animals displayed impairments in hippocampal-mediated memory, such as short-term and spatial memory as well as social recognition. This is in accordance with previous studies that have linked circadian disruption to worsened memory performance[29–31]. In this sense, exposure

Oscillating genes in the hypothalamus

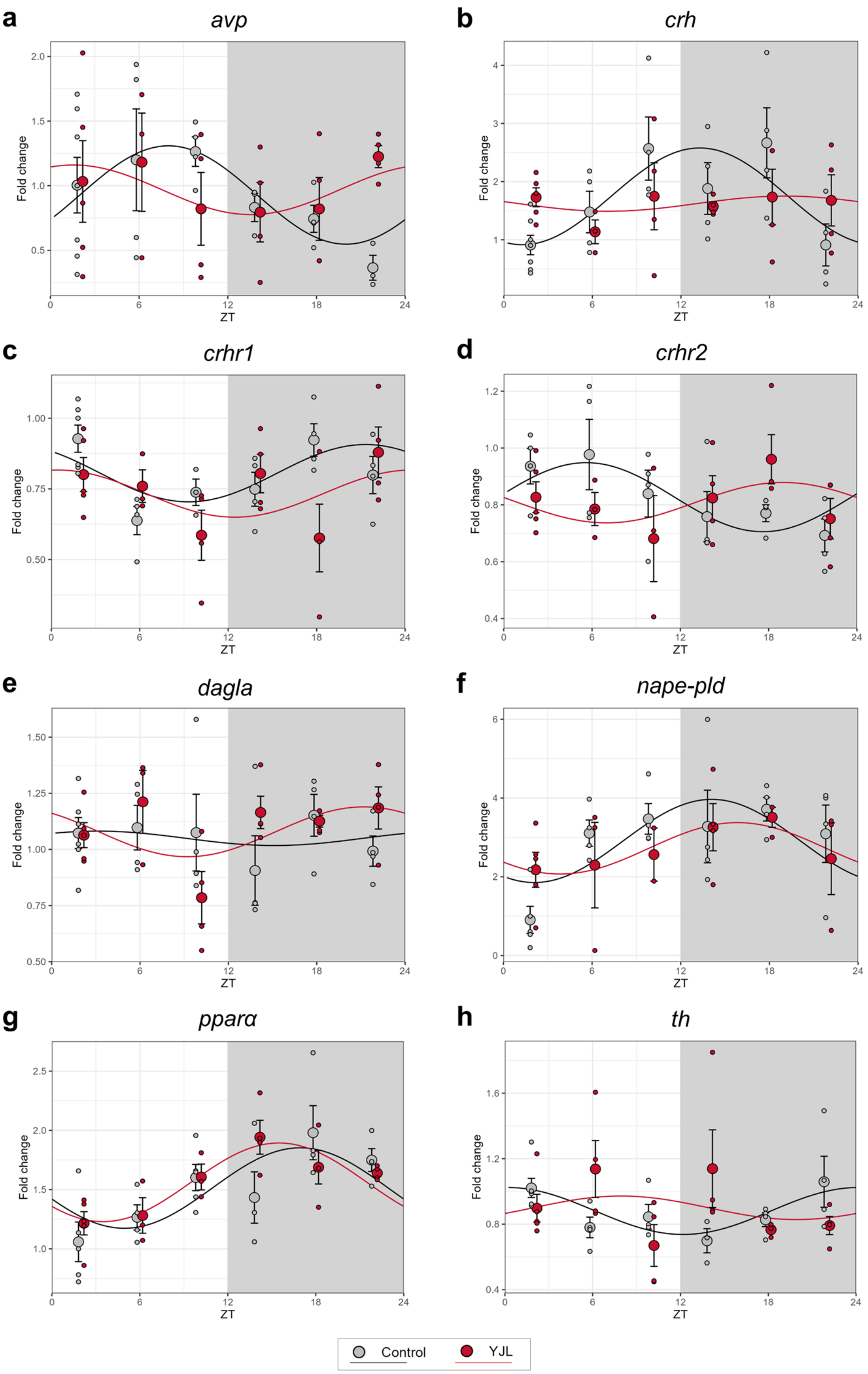


Fig. 4. Youth Jet Lag mice display circadian disruption of naturally oscillating genes in the hypothalamus. Rhythmic analysis output on a selection of genes with circadian oscillation levels of mRNA in the hypothalamus. Sinusoidal waves fold change of mRNA expression fold change of **a***avp*, **b***crh*, **c***crhr1*, **d***crhr2*, **e***dagla*, **f***nape-pld*, **g***pparα* and **h***th*. Data is normalized to the median sample of the control group at ZT2. Sinusoid curves represent the least-squares best fit trace for both experimental groups; control (grey) and YJL (red). Data are expressed as mean, individual values ($n = 3–5$) and error bars represent standard deviation.

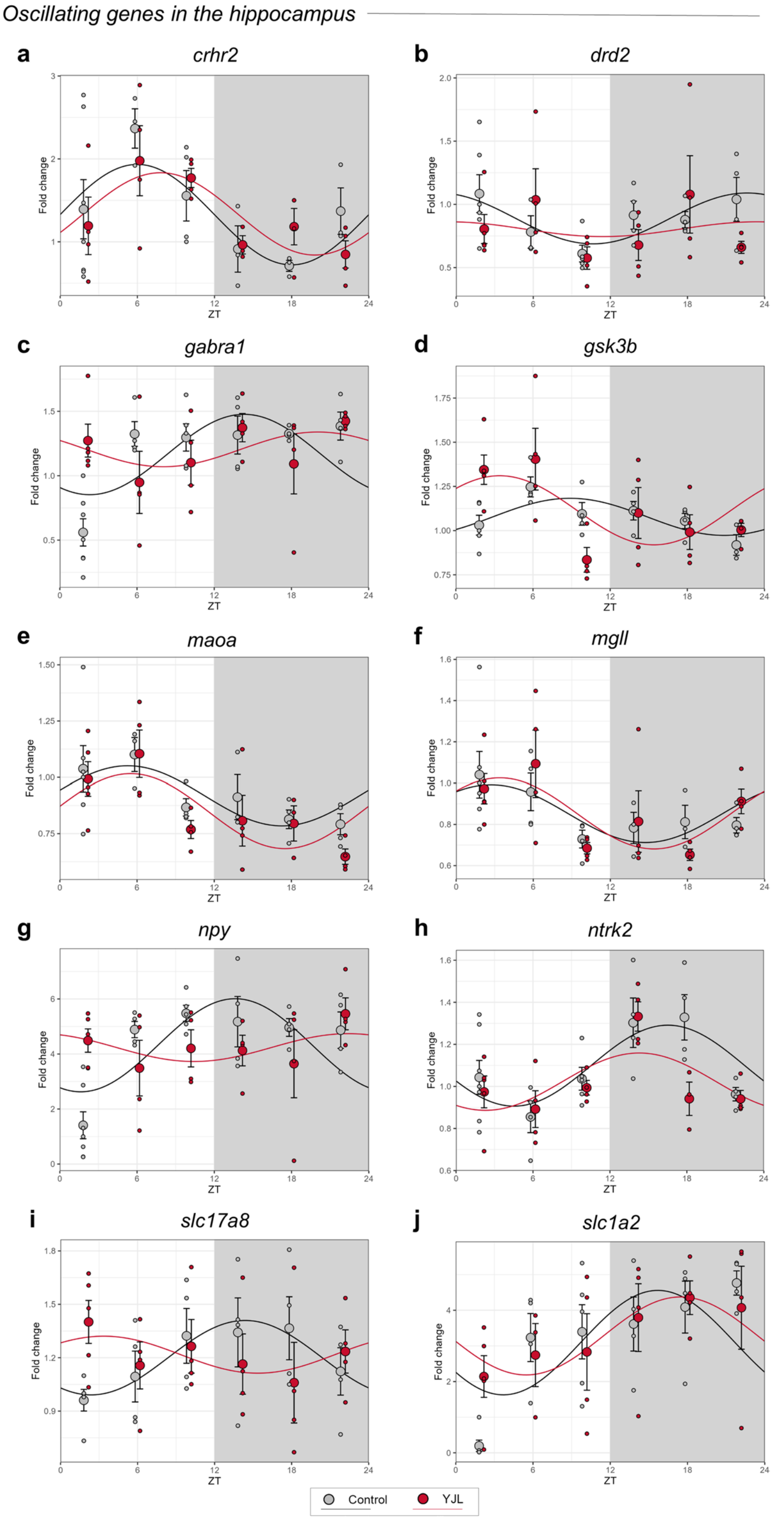


Fig. 5. Youth Jet Lag mice display circadian disruption of naturally oscillating genes in the hippocampus. Rhythmic analysis output on a selection of genes with circadian oscillation levels of mRNA in the hypothalamus. Sinusoidal waves fold change of mRNA expression fold change of **a***crhr2*, **b***drd2*, **c***gabra1*, **d***gsk3b*, **e***maoa*, **f***mgll*, **g***npy*, **h***ntrk2*, **i***slc17a8* and **j***slc1a2*. Data are normalized to the median sample of the control group at ZT2. Sinusoid curves represent the least-squares best fit trace for both experimental groups; control (grey) and YJL (red). Data are expressed as mean, individual values ($n = 3–5$) and error bars represent standard deviation.

to an aberrant LD cycle during adulthood has been shown to impair NOR without affecting EPM[32] in mice. Furthermore, a LD cycle of 12 L:9D worsens spatial memory performance in the Morris water maze[33]. In aged mice, chronic phase advances impair spatial working memory[34]. Regarding social memory, to the best of our knowledge, no research has addressed the effects of chronic chronodisruption in mice. Though, it is known that acute exposure to a bright light negatively affects social recognition via oxytocin neurons in the supraoptic nucleus[35]. However, in most of this existing research, both chronodisruption and behavioral testing occur during adulthood, rather than developmental periods.

One recent study investigated early-life chronodisruption (from birth until weaning) induced by a chronic advancement of the light phase every other day[36]. Behavioral tests were conducted during adulthood and reported impairments in spatial and working memory, which aligns with our observations. Additionally, another study has applied a similar approach to our YJL model aiming to observe the effects of chronodisruption[37] in which mice underwent a protocol with an aberrant LD cycle during adolescence, comparable to our YJL paradigm. Authors reported impairments in the NOR and active avoidance test. Such findings are in accordance with the present work and further provide evidence of the association between adolescent chronodisruption and memory impairments. Hence, our work is the first one to report social recognition and spatial memory deficits following chronodisruption during adolescence. This approach is particularly relevant since, during this period, humans exhibit greater susceptibility to circadian disruption due to intrinsically delayed sleep-wake phases.

Although both sexes were included as a variable, this was never found to interact with our experimental manipulation. Unfortunately, contextualizing this finding within existing literature is challenging since most studies on LD-induced chronodisruption are performed exclusively on male mice[20,22,30]. However, and despite known sex differences in circadian biology[38]generally, circadian disruption seems to affect male and female rodents similarly[36,39].

In the context of anxiety-like behavior, exposure to drastically altered LD cycles is considered a stressful experience for the animals[40] and chronic stress has been linked to memory impairments[41]. Since YJL animals display no changes in anxiety-like behavior in the EPM at basal conditions, we infer that memory impairments are unlikely due to stress derived from the aberrant LD cycle, probably due because in our procedure changes of LD cycle were relatively mild. In addition, social withdrawal is a hallmark of chronic stress[42]. Since we report no social avoidance, the YJL phenotype might be substantially different to that of anxiety-like behavior arising from a stress-inducing protocol. Hence, the explanation of the worsened memory performance seems to be contingent to chronodisruption during adolescence.

At the molecular level, behavioral alterations in YJL were accompanied by molecular evidence of chronodisruption within the hypothalamus, such as alterations in the oscillation of *per2* and *npas2*. Additionally, we provide the first characterization of the oscillatory profile of *crhr1* and *crhr2* within the hypothalamus. In this area, glucocorticoid release follows a strict circadian organization evidenced by daily fluctuations of *crh* mRNA and blood plasma glucocorticoid levels[43]. In this context, our finding reinforces the control of circadian clock genes in glucocorticoid transmission. Additionally, we observe a loss of oscillation of *crh*, *crhr1* and *crhr2* in YJL which can be directly linked to molecular clock alterations. Previously it has been observed that the function of *per2* in the hypothalamus is tightly related to the control of corticosterone secretion[44]. Therefore, it is possible that the disruption of daily rhythms of *crh*, *crhr1* and *crhr2* in YJL is associated with the disruption of hypothalamic *per2* oscillations. Notably, changes in HPA components did not reflect absolute differences in total expression levels, as indicated by AUC results (Figure S2), but rather a disruption in the timing of their rhythmic expression, which might serve as an explanation for the lack of differences during the EPM between YJL and control. Furthermore, both *npas2* and *per2* are key regulators of glutamatergic transmission[45]. Although we report a significant overall downregulation in the expression of VGLUT3 (*slc17a8*) in YJL animals (Figure S2), no significant differences in rhythmic expression were observed in other glutamatergic genes, suggesting limited impact on hypothalamic excitatory tone.

In the hippocampus, a region essential for memory processing, we found circadian oscillation in the expression of core clock genes in basal conditions (*arntl1*, *cry1*, *cry2*, *dbp*, *nr1d2*, *per1* and *per2)*, consistent with prior studies[46]. Furthermore, we describe, for the first time, daily rhythm of expression in genes critical for synaptic function (*crhr2*, *drd2*, *gabra1*, *gsk3b*, *maoa*, *mgll*, *npy*, *ntrk2*, *slc17a8*, *slc1a2)*, as well as *napepld* in YJL mice, which emphasizes the relevance of circadian control of hippocampal functioning and synaptic plasticity[25].

As expected, adolescent chronodisruption induced a loss in daily expression patterns of clock genes, such as *cry2* and *per1*, as well as those genes whose rhythms of expression have been first characterized by the present study. Interestingly, the alterations in the molecular clock can be linked to the observed memory impairments. Although to date, no role of *cry2* in memory function has been established, it has been observed that reduction of hippocampal *per1* mRNA levels using siRNA prior to training impairs learning, while its overexpression prevents age-related memory deficits[47]. Moreover, PER1-knockout animals perform significantly worse in hippocampal-dependent long-term spatial learning tasks[48]. Furthermore, prolonged activation of glucocorticoid receptors has been linked to loss of *per1* oscillation in the hippocampus resulting in loss of circadian variation in LTP and impaired memory[49]. This observation fits well with our results, in which a loss of hypothalamic *per2* oscillation is accompanied by a dysregulation of *crh*, *crhr1* and *crhr2* gene expression which, in turn, might lead to the disruption of *per1* daily rhythms in the hippocampus potentially contributing to memory impairments. This effect might occur through the regulation of CREB-mediated transcription since absence of PER1 disrupts both CREB-dependent gene expression and long-term hippocampal memory formation[50,51].

Interestingly, the hippocampus is not directly innervated by the SCN. Instead, circadian signals are relayed via the medial septum, through GABAergic signaling, and into the hippocampus via cholinergic networks[52]. This septo-hippocampal interaction is critical in memory formation[53] and therefore, it has been implicated as a potential mechanism of memory impairments by chronodisruption[54]. In this sense a disruptive LD phase shift induces memory impairments by a septal influence of hippocampal excitability[54]. This is consistent with our findings since *per1* and *per2* are known to influence membrane excitability in hippocampal CA1 neurons.

Regarding gene targets outside of the molecular clock, we observed a loss in the rhythmic expression of *ntrk2* –a well-established mediator of long-term potentiation (LTP)[55] and memory formation– following YJL. This is line with previous studies describing altered normal fluctuation of BDNF/TrkB levels in response to exposure to an aberrant LD cycle and associated with memory impairments[56]. Furthermore, chronodisruption-induced memory impairments are accompanied by a decreased amplitude of LTP[57]. In fact, though less described, LTP magnitude and characteristics vary between the light and dark phase[25,58]. Additionally, neurotrophic factors like BDNF and TrkB play an important role as modulators of hippocampal dendritic spine morphology, which is essential for both synaptic plasticity and memory processing[59]. Studies in rodents have revealed circadian oscillations in spine density of the CA1 and DG subregions[60]. Notably, these daily rhythms in morphology are affected by the LD cycle and light pulses[61,62]. This implies that circadian rhythms regulate light-induced rapid remodeling of hippocampal neurons. Indeed, aberrant LD cycles like constant light impact diurnal differences in spine morphology and spatial working memory[63]. Since in our model we observe alterations in daily rhythms of TrkB, it is likely that some interferences on LTP and synaptic plasticity are contributing to the YJL phenotype.

Following our observations, other components that might be contributing to the YJL phenotype include: (1) dopamine receptor D2R which is required for hippocampal-dependent memory and plasticity[64](2) GluA1 and GluA2 receptors determinant contributors to memory retrieval[65](3) DAGLα, NAPE-PLD, PPARα, and MAGL, key components of the endocannabinoid system which plays a key role in memory due to its regulation of synaptic plasticity processes such as LTP[66]or 5) GSK3B, which is required for memory reconsolidation[67]. In fact, circadian rhythmicity of GSK3B activation regulates synaptic plasticity in the hippocampus[68] and proteasomal degradation of CRY2 which might be related to our observation of altered *cry2* expression.

A key strength of this work lies in its focus on analyzing variations in gene expression, particularly for targets outside the circadian clock machinery. This approach is crucial since most studies describe either a complete absence or a general reduction/increase in protein and/or mRNA levels. In contrast, our study reveals a loss of circadian oscillation, without significant changes in overall expression (Figure S2). This finding underscores the limitations of single-time point analysis, since changes in overall expression levels of oscillating molecules could simply reflect a shift in the peak of expression rather than a true up- or down- regulation. Hence, our work emphasizes the importance of incorporating the temporal dimension into molecular analyses. However, it is important to consider changes in mRNA expression patterns do not necessarily reflect changes in protein abundance or function. Assessing these parameters by incorporating proteomic analyses or functional assays could contribute to fully understand the biological impact of these transcriptional factors.

Despite its relevance, experimental approaches incorporating circadian variables remain uncommon, particularly in behavioral neuroscience. This scarcity is due to intrinsic limitations in the methodological techniques since factoring in the variable time greatly complicates experimental design. One limitation is the relatively small sample size per timepoint, inherent to the time-series design. This constraint, combined with natural biological variability, contributes to increased variability in gene expression measurements, which might hinder the accurate detection or modeling of circadian rhythms. In this line, most genes that were rhythmic in the YJL group also showed rhythmicity in controls, suggesting thet chronodisruption altered existing rhythms rather than inducing new ones. *Nape-pld* in the hippocampus was an exception, but due to high variability, this result should be interpreted with caution.

Our current approach stands out as innovative, as it has not only allowed us to assess the loss of gene expression rhythmicity in the context of YJL, but also to identify genes whose circadian expression was previously ignored. Therefore, to effectively build on these findings and keep combining chronobiology with behavioral neurobiology, it is essential to develop and implement new methods or resources that overcome these experimental limitations.

In conclusion, our study provides valuable insights into the interplay between circadian rhythms, memory, and daily fluctuations in gene expression. We show that the YJL model effectively induces chronodisruption, evidenced by altered daily rhythms in locomotor activity and oscillatory patterns in molecular clock genes. We conclude that chronic disruption of circadian rhythms during adolescence induces deficits in short-term, spatial, and social working memory as well as alterations in daily expression of genes associated with endocannabinoid system, glucocorticoid release, and synaptic plasticity within the hypothalamus and hippocampus. This work underscores the relevance of circadian dynamics into hippocampal function and homeostasis and highlights adolescence as a vulnerable period.

Methods

Animals

Male and female C57BL/6 mice at postnatal day 30 (PD30) were purchased from Charles River (Lyon, France) and delivered to our animal facility (UBIOMEX, PRBB). Mice were grouped-housed at a stable temperature (22 °C ± 2) and humidity (55% ± 10%), with food and water *ad libitum*. Animal weights were recorded daily (Supplementary Information, Figure S1). All tests were conducted during the dark phase in standard dim light conditions (15–25) lux from PD67 to PD74.

Animal care and experimental protocols were approved by the Barcelona Biomedical Research Park – Universitat Pompeu Fabra Ethical Committee for Animal Research (CEEA-PRBB-UPF). In all the studies, animals were treated in compliance with the European Communities Council Directive (86/609/EEC) and the ARRIVE guidelines for the care and use of laboratory animals.

Youth jet lag paradigm

We developed a novel chronic circadian disruption protocol contemplating a short photoperiod combined with a phase delay. The onset of the light phase was labeled as ZT0. The protocol consisted of two different light/dark (LD) conditions. First, an 8 L:16D cycle for the first five days (lights ON at ZT0 and OFF at ZT8) shown to induce changes in circadian phasing[28]. Then, during the sixth and seventh day we applied a 4 h shift on the onset of the light phase (12 L:12D, lights OFF at ZT14). On the eighth day, the cycle returned to 8 L:16D. Animals subjected to said protocol constituted the experimental group: "youth jet lag" (YJL). The protocol was implemented during the adolescent-like period, although the exact duration of adolescence in mice is still a subject of debate. It is widely accepted that this phase begins around the onset of puberty at approximately PD30 and extends until sexual maturity is reached at PD60[69–71]. Notably, some circadian parameters can help to delimit this developmental window, including a characteristic delay of the circadian phase that emerges at the onset of puberty, peaks at PD35-45 and stabilizes at around PD60[72,73] when the adulthood begins. Hence, when PD60 was reached, all animals spent one week in standard inverted LD conditions (lights ON at ZT0, lights OFF at ZT12) prior to further testing. Control mice were housed under a standard inverted LD cycle throughout the entire procedure.

Locomotor activity

The evaluation of daily rhythms of locomotor activity allowed us to evidence the effects of the aberrant light dark cycle directly in the behavior of the animals. For this purpose, the spontaneous locomotor activity of the animals was recorded during the last week of the YJL paradigm (PD53-PD60) to avoid social isolation stress, which can introduce stress-related confounds, and when the most pronounced disruptions were expected. Alterations in locomotor activity were evaluated by monitoring the locomotion of a small cohort ($n = 10$) from PD53 to PD60 (LE8825, LE8816; Panlab s.l.u., Barcelona, Spain). The cohort included animals from both control (3 males and 2 females) and YJL (3 females and 2 males) groups. Since the recordings were performed at PD53, the experimental group was undergoing the last week of the YJL procedure and hence, were housed under two different light schedules. Meanwhile, the recording of the control group occurred under standard LD conditions. For the statistical analysis with Kronos, data of different days had to be collapsed. For YJL, two distinct days were considered due to the differences in light schedules. For this, we selected the last days of the two different light cycles of PD55 (8 L:16D) and PD57 (12 L:12D) to evaluate the effects of the 4 h light phase onset delay. For control mice, we considered the activity of PD57.

Elevated plus maze

The EPM (LE-842, Panlab s.l.u., Barcelona, Spain) was used to evaluate anxiety-like behavior, following the methodology of previous studies[74]. Briefly, the apparatus featured a black cross-shaped design with two enclosed arms and two open arms that stood 30 cm above the floor. Mice were placed in the intersection of the arms and allowed to explore the maze for 5 min. Animals' movement in the maze was assessed by an automated tracking software: Smart (Panlab s.l.u., Barcelona, Spain). The number of entries in each arm was considered and the percentage of time spent in the open arms was calculated with Eq. 1.

$$Preference\ for\ open\ arms\ (\%) = \frac{(time\ in\ open\ arms)}{(time\ in\ open\ arms\ +\ time\ in\ closed\ arms)} \times 100 \tag{1}$$

Reference memory test

The reference memory test was performed as previously described[75] to evaluate spatial reference memory. Briefly, we employed a Y-maze with three arms separated by 120º angles each featuring one visual cue. The experiment began with a five-minute training session in which entrance to one of the arms was blocked. After a 1-hour inter-trial interval, we performed the test session. The entrance was unblocked, and mice were allowed to freely explore all arms for 5 min. The Smart Software tracked the time spent in each of the arms. The preference ratio was then calculated using Eq. 2.

$$Preference\ for\ novel\ arm\ (\%) = \frac{time\ in\ novel\ arm}{time\ in\ all\ arms} \times 100 \tag{2}$$

Novel object recognition

The NOR test was performed to evaluate short-term declarative memory as described in Garcia-Baos et al., (2023)[75]. The experiment comprised habituation, training, and test sessions all 5 min long. During habituation, mice were introduced to the empty arena. In the training session, mice explored the box containing two identical objects (familiar objects). Following a retention time of 3 h, the test session was conducted, replacing one of the familiar objects with a novel one. Both test sessions were video recorded (Logitech C270) and analyzed using the software BORIS[76] by a double blinded observer. The discrimination index was computed using Eq. 3.

$$Discrimination\ index = \frac{time\ novel\ object - time\ familiar\ object}{time\ novel\ object + time\ in\ familiar\ object} \tag{3}$$

Social interaction and recognition test

The social interaction and social recognition tests were performed as previously described[77] to assess sociability and social memory. The test utilizes a three-chamber arena, two wired cups and unfamiliar juvenile mice (PD30) for social interaction. It is divided into 3 ten-minute sessions. During habituation, subject mice explored the maze where the lateral chambers contained an empty wire cup each. During sociability, an unfamiliar juvenile mouse was enclosed in one of the cups and the other remained empty. Lastly, in the social novelty session, a second unfamiliar mouse was enclosed in the second wire cup, which had remained empty in the previous session. The Smart software was used to track the animal and assess the time spent in the areas surrounding the cups. The discrimination index was calculated with Eq. 4.

$$Discrimination\ index = \frac{time\ cup\ A - time\ cup\ B}{time\ cup\ A + time\ cup\ B} \tag{4}$$

Time-point tissue collection

Brain sample collection was performed to obtain different measurements of gene expression throughout the 24 h cycle to study circadian rhythmicity[78]. For this, all animals were housed in a standard LD cycle (12 L:12D) from PD60 to PD67. At PD67, mice were sacrificed by cervical dislocation at 6 different time-points: ZT2, ZT6, ZT10, ZT14; ZT18 and ZT22. The hippocampus and hypothalamus regions were isolated (See Supplementary Information), extracted and stored at −80 C for further analysis.

Gene expression analysis

RNA isolation and RT-PCR

Total RNA isolation was performed via the TRIZOL method (Supplementary Information). Reverse transcription was performed by High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol, as previously described.

OpenArray™ technology

Custom array plates were designed and obtained from Thermo Fisher Scientific Inc. (Supplementary Information, Table S1). To perform the OpenArray™ analyses, 2.5 µl of cDNA sample was mixed with 2.5 µl TaqMan OpenArray™ Real-Time Master Mix (Thermo Fisher #4462159) and loaded into a single well of a 384-well plate[79]. Custom OpenArray™ plates were then automatically loaded using the AccuFill System (AccuFill System User Guide, PN4456986) and run in QuantStudio 12 K. Data were analyzed using ExpressionSuite Software v1.3 (Thermo Fisher Scientific, 2018–2020). The amplification was normalized to the geometric mean of selected reference endogenous genes *actb*, *gapdh*, *hprt1* and *b2m*. Fold-change values were calculated using the ΔΔCt method using the median value of the ZT2 control as reference sample[78].

Statistical analysis

We assessed normality and homoscedasticity across all data sets using the Shapiro-Wilk and Spearman's test. Parametric tests were employed when assumptions of normal distribution and equal variance were met. For single-factor, two-group analyses, we used two-tailed unpaired Student's *t*-tests. Alternatively, for between-group designs involving two variables, we employed a two-way ANOVA with Bonferroni post-hoc corrections. The present study includes male and female mice in all experiments and considers 'sex' as a variable whenever the number of animals was sufficient. When analyses revealed no statistically significant differences based on sex, data were pooled. Statistical significance was set at $p<.05$.

Circadian rhythms were analyzed using the computational tool Kronos[78,80]. This software evaluates circadian rhythms within biological datasets by decomposing the time variable into sine and cosine components and using a GLM to assess rhythmicity. Sinusoid curves can then be predicted from each outcome variable. The proportion of variance explained by each individual predicted curve with the corresponding *p*-value, along with the acrophase and amplitude is calculated. Additionally, Kronos allows for pairwise comparisons between experimental groups for statistical evaluation of differential rhythmicity.

Lastly, to compare overall gene expression between experimental groups throughout the cycle, we calculated the area under the curve (AUC) and used the total area and standard error to perform unpaired Student's *t*-tests or with Welch's correction each gene.

Data were statistically analyzed, and violin plots were generated using GraphPad Prism 8.0. Actograms were created using Microsoft Excel for Microsoft Office 365. Sinusoidal curves and circlegrams were generated using R package Kronos.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon request.

Received: 21 February 2025; Accepted: 16 July 2025

References

1. Erren, T. C. & Reiter, R. J. Defining chronodisruption. *J. Pineal Res.* **46**, 245–247 (2009).
2. Pilorz, V., Helfrich-Förster, C. & Oster, H. The role of the circadian clock system in physiology. *Pflügers Archiv - Eur. J. Physiol.* **470**, 227–239 (2018).
3. Wittmann, M., Dinich, J., Merrow, M. & Roenneberg, T. Social jetlag: misalignment of biological and social time. *Chronobiol Int.* **23**, 497–509 (2006).
4. Caliandro, R., Streng, A. A., van Kerkhof, L. W. M., van der Horst, G. T. J. & Chaves, I. Social Jetlag and Related Risks for Human Health: *Timely Rev. Nutr.* **13** (2021).
5. Beauvalet, J. C. et al. Social jetlag in health and behavioral research: a systematic review. *ChronoPhysiol. Ther.* **7**, 19–31 (2017).
6. Van der Vinne, V. et al. Timing of examinations affects school performance differently in early and late chronotypes. *J. Biol. Rhythms.* **30**, 53–60 (2015).

7. Roenneberg, T., Allebrandt, K. V., Merrow, M. & Vetter, C. Social jetlag and obesity. *Curr. Biol.* **22**, 939–943 (2012).
8. Claudatos, S., Baker, F. C. & Hasler, B. P. Relevance of sleep and circadian rhythms to adolescent substance use. *Curr. Addict. Rep.* **6**, 504–513 (2019).
9. de Souza, C. M. & Hidalgo, M. P. L. Midpoint of sleep on school days is associated with depression among adolescents. *Chronobiol Int.* **31**, 199–205 (2014).
10. Mathew, G. M., Li, X., Hale, L. & Chang, A. M. Sleep duration and social jetlag are independently associated with anxious symptoms in adolescents. *Chronobiol Int.* **36**, 461–469 (2019).
11. Haraszti, R. Á., Ella, K., Gyöngyösi, N., Roenneberg, T. & Káldi, K. Social jetlag negatively correlates with academic performance in undergraduates. *Chronobiol Int.* **31**, 603–612 (2014).
12. Smarr, B. L. & Schirmer, A. E. 3.4 Million real-world learning management system logins reveal the majority of students experience social jet lag correlated with decreased performance. *Sci. Rep.* **8**, 4793 (2018).
13. Baron, K. G. & Reid, K. J. Circadian misalignment and health. *Int. Rev. Psychiatry*. **26**, 139–154 (2014).
14. Takahashi, M. et al. Chronotype and social jetlag influence human circadian clock gene expression. *Sci. Rep.* **8**, 10152 (2018).
15. Partch, C. L., Green, C. B. & Takahashi, J. S. Molecular architecture of the mammalian circadian clock. *Trends Cell. Biol.* **24**, 90 (2013).
16. Guillaumond, F., Dardente, H., Giguère, V. & Cermakian, N. Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. *J. Biol. Rhythms*. **20**, 391–403 (2005).
17. Vaughn, L. K. et al. Endocannabinoid signalling: has it got rhythm? *Br. J. Pharmacol.* **160**, 530–543 (2010).
18. Kim, J. et al. Implications of circadian rhythm in dopamine and mood regulation. *450 Mol. Cells*. **40**, 450–456 (2017).
19. Chi-Castañeda, D. & Ortega, A. Circadian regulation of glutamate transporters. *Front Endocrinol. (Lausanne)* **9** (2018).
20. Haraguchi, A. et al. Use of a social jetlag-mimicking mouse model to determine the effects of a two-day delayed light- and/or feeding-shift on central and peripheral clock rhythms plus cognitive functioning. *Chronobiol Int.* **38**, 426–442 (2021).
21. Liu, K. et al. Social jetlag and damage to male reproductive system: epidemiological observation in European and Chinese populations and biochemical analyses in mice. *SSRN Electron. J.* https://doi.org/10.2139/SSRN.3482809 (2019).
22. Oneda, S., Cao, S., Haraguchi, A., Sasaki, H. & Shibata, S. Wheel-running facilitates phase advances in locomotor and peripheral circadian rhythm in social jet lag model mice. *Front Physiol* **13** (2022).
23. Snider, K. H., Sullivan, K. A. & Obrietan, K. Circadian Regulation of Hippocampal-Dependent Memory: Circuits, Synapses, and Molecular Mechanisms. *Neural Plast.* **2018** (2018).
24. Hasegawa, S. et al. Hippocampal clock regulates memory retrieval via dopamine and PKA-induced GluA1 phosphorylation. *Nat. Commun.* **10**, 5766 (2019).
25. Eckel-Mahan, K. Circadian oscillations within the Hippocampus support memory formation and persistence. *Front Mol. Neurosci* **5** (2012).
26. Dial, M. B. et al. Effects of time-restricted exercise on activity rhythms and exercise-induced adaptations in the heart. *Sci. Rep.* **14**, 146 (2024).
27. Schroeder, A. M. et al. Voluntary scheduled exercise alters diurnal rhythms of behaviour, physiology and gene expression in wild-type and vasoactive intestinal peptide-deficient mice. *J. Physiol.* **590**, 6213–6226 (2012).
28. Weinert, D., Freyberg, S., Touitou, Y., Djeridane, Y. & Waterhouse, J. M. The phasing of circadian rhythms in mice kept under normal or short photoperiods. *Physiol. Behav.* **84**, 791–798 (2005).
29. Loh, D. H. et al. Rapid changes in the light/dark cycle disrupt memory of conditioned fear in mice. *PLoS One*. **5**, 1–12 (2010).
30. Fujioka, A. et al. Effects of a constant light environment on hippocampal neurogenesis and memory in mice. *Neurosci. Lett.* **488**, 41–44 (2011).
31. Colwell, C. S. How a disrupted clock May cause a decline in learning and memory. *Circadian Med.* 235–248. https://doi.org/10.1002/9781118467831.ch16 (2015).
32. LeGates, T. A. et al. Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature* **491**, 594–598 (2012).
33. Deibel, S. H. et al. Impaired Morris water task retention following T21 light dark cycle exposure is not due to reduced hippocampal c-FOS expression. *Front. Behav. Neurosci.* **16**, 1025388 (2022).
34. Liu, J. A. et al. Chronic phase advances reduce recognition memory and increases vascular cognitive dementia-like impairments in aged mice. *Sci. Rep.* **14**, 1–10 (2024).
35. Huang, Y., Liao, P., Yu, J., Shih-Kuo & Chen (eds), (Alen). Light disrupts social memory via a retina-to-supraoptic nucleus circuit. *EMBO Rep* 24, e56839 (2023).
36. Ameen, R. W., Warshawski, A., Fu, L. & Antle, M. C. Early life circadian rhythm disruption in mice alters brain and behavior in adulthood. *Sci Rep* **12** (2022).
37. Bonilla, P., Shanks, A., Nerella, Y. & Porcu, A. Effects of chronic light cycle disruption during adolescence on circadian clock, neuronal activity rhythms, and behavior in mice. *Front. Neurosci.* **18** (2024).
38. Dib, R., Gervais, N. J. & Mongrain, V. A review of the current state of knowledge on sex differences in sleep and circadian phenotypes in rodents. *Neurobiol. Sleep. Circadian Rhythms.* **11**, 100068 (2021).
39. Tam, S. K. E. et al. Dim light in the evening causes coordinated realignment of circadian rhythms, sleep, and short-term memory. *Proc. Natl. Acad. Sci.* **118**, e2101591118 (2021).
40. Sakellaris, P. C., Peterson, A., Goodwin, A., Winget, C. M. & Vernikos-Danellis, J. Response of mice to repeated photoperiod shifts: susceptibility to stress and barbiturates. *Proc. Soc. Exp. Biol. Med.* **149**, 677–680 (1975).
41. Conrad, C. D. A critical review of chronic stress effects on Spatial learning and memory. *Prog Neuropsychopharmacol. Biol. Psychiatry*. **34**, 742–755 (2010).
42. Tran, I. & Gellner, A. K. Long-term effects of chronic stress models in adult mice. *J. Neural Transm.* **130**, 1133–1151 (2023).
43. Kwak, S. P., Morano, M. I., Young, E. A., Watson, S. J. & Akil, H. Diurnal CRH mRNA rhythm in the hypothalamus: decreased expression in the evening is not dependent on endogenous glucocorticoids. *Neuroendocrinology* **57**, 96–105 (2008).
44. Russell, A. L. et al. Knockout of the circadian gene, Per2, disrupts corticosterone secretion and results in depressive-like behaviors and deficits in startle responses. *BMC Neurosci.* **22**, 5 (2021).
45. Beaulé, C., Swanstrom, A., Leone, M. J. & Herzog, E. D. Circadian modulation of gene expression, but not glutamate uptake, in mouse and rat cortical astrocytes. *PLoS One*. **4**, 1–8 (2009).
46. Dębski, K. et al. The circadian dynamics of the hippocampal transcriptome and proteome is altered in experimental Temporal lobe epilepsy. *Sci Adv* **6** (2020).
47. Kwapis, J. L. et al. Epigenetic regulation of the circadian gene Per1 contributes to age-related changes in hippocampal memory. *Nat. Commun.* **9**, 3323 (2018).
48. Jilg, A. et al. Temporal dynamics of mouse hippocampal clock gene expression support memory processing. *Hippocampus* **20**, 377–388 (2010).
49. Birnie, M. T. et al. Circadian regulation of hippocampal function is disrupted with corticosteroid treatment. *Proc. Natl. Acad. Sci. U S A*. **120**, e2211996120 (2023).
50. Rawashdeh, O. et al. PERIOD1 coordinates hippocampal rhythms and memory processing with daytime. *Hippocampus* **24**, 712–723 (2014).

51. Rawashdeh, O., Jilg, A., Maronde, E., Fahrenkrug, J. & Stehle, J. H. Period1 gates the circadian modulation of memory-relevant signaling in mouse hippocampus by regulating the nuclear shuttling of the CREB kinase pP90RSK. *J. Neurochem*. **138**, 731–745 (2016).
52. Müller, C. & Remy, S. Septo–hippocampal interaction. *Cell. Tissue Res.* **373**, 565–575 (2018).
53. Khakpai, F., Nasehi, M., Haeri-Rohani, A., Eidi, A. & Zarrindast, M. R. Septo-hippocampo-septal loop and memory formation. *Basic. Clin. Neurosci.* **4**, 5–23 (2013).
54. Ruby, N. F. et al. Spatial memory and long-term object recognition are impaired by circadian arrhythmia and restored by the GABAA antagonist Pentylenetetrazole. *PLoS One*. **8**, e72433 (2013).
55. Minichiello, L. TrkB signalling pathways in LTP and learning. *Nat. Rev. Neurosci.* **10**, 850–860 (2009).
56. Asadian, N. et al. Chronic light deprivation induces different effects on Spatial and fear memory and hippocampal BDNF/TRKB expression during light and dark phases of rat diurnal rhythm. *Behav. Brain. Res.* **418**, 113638 (2022).
57. Li, S. et al. Influence of circadian disorder on structures and functions of neurons in hippocampus of mice. *Biol. Rhythm Res.* **48**, 639–645 (2017).
58. Chaudhury, D., Wang, L. M. & Colwell, C. S. Circadian regulation of hippocampal Long-Term potentiation. *J. Biol. Rhythms*. **20**, 225–236 (2005).
59. McEwen, B. S., Nasca, C. & Gray, J. D. Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. *Neuropsychopharmacology* **41**, 3–23 (2016).
60. Ikeno, T., Weil, Z. M. & Nelson, R. J. Photoperiod affects the diurnal rhythm of hippocampal neuronal morphology of Siberian hamsters. *Chronobiol Int.* **30**, 1089–1100 (2013).
61. Ikeno, T., Weil, Z. M. & Nelson, R. J. Timing of light pulses and photoperiod on the diurnal rhythm of hippocampal neuronal morphology of Siberian hamsters. *Neuroscience* **270**, 69–75 (2014).
62. Verma, V., Kumari, R. & Singaravel, M. Chronic altered light–dark cycle differentially affects hippocampal CA1 and DG neuronal arborization in diurnal and nocturnal rodents. *Chronobiol Int.* **39**, 665–677 (2022).
63. Schröder, J. K. et al. Effects of the Light/Dark Phase and Constant Light on Spatial Working Memory and Spine Plasticity in the Mouse Hippocampus. *Cells* **12** (2023).
64. Espadas, I. et al. Dopamine D2R is required for Hippocampal-dependent memory and plasticity at the CA3-CA1 synapse. *Cereb. Cortex*. **31**, 2187–2204 (2020).
65. Pereyra, M. & Medina, J. H. AMPA receptors: A key piece in the puzzle of memory retrieval. *Front Hum. Neurosci* **15** (2021).
66. Marsicano, G. & Lafenêtre, P. Roles of the endocannabinoid system in learning and memory. *Curr. Top. Behav. Neurosci.* **1**, 201–230 (2009).
67. Kimura, T. et al. GSK-3beta is required for memory reconsolidation in adult brain. *PLoS One*. **3**, e3540 (2008).
68. Besing, R. C. et al. GSK3 activity regulates rhythms in hippocampal clock gene expression and synaptic plasticity. *Hippocampus* **27**, 890–898 (2017).
69. Bell, M. R. Comparing postnatal development of gonadal hormones and associated social behaviors in rats, mice, and humans. *Endocrinology* **159**, 2596–2613 (2018).
70. Martini, M. & Valverde, O. A single episode of maternal deprivation impairs the motivation for cocaine in adolescent mice. *Psychopharmacol. (Berl)*. **219**, 149–158 (2012).
71. Spear, L. P. & Brake, S. C. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. *Dev. Psychobiol.* **16**, 83–109 (1983).
72. Hagenauer, M. H. & Lee, T. M. The neuroendocrine control of the circadian system: adolescent chronotype. *Front. Neuroendocrinol.* **33**, 211 (2012).
73. Hagenauer, M. H., Perryman, J. I., Lee, T. M. & Carskadon, M. A. Adolescent changes in the homeostatic and circadian regulation of sleep. *Dev. Neurosci.* **31**, 276 (2009).
74. Martín-Sánchez, A. et al. Comorbidity between alzheimer's disease and major depression: a behavioural and transcriptomic characterization study in mice. *Alzheimers Res. Ther.* **13**, 73 (2021).
75. Garcia-Baos, A. et al. The role of PPAR-γ in memory deficits induced by prenatal and lactation alcohol exposure in mice. *Mol. Psychiatry*. **28**, 3373–3383 (2023).
76. Friard, O. & Gamba, M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* **7**, 1325–1330 (2016).
77. Portero-Tresserra, M. et al. Maternal separation increases alcohol-drinking behaviour and reduces endocannabinoid levels in the mouse striatum and prefrontal cortex. *Eur. Neuropsychopharmacol.* **28**, 499–512 (2018).
78. Berbegal-Sáez, P. et al. Lack of Bmal1 leads to changes in rhythmicity and impairs motivation towards natural stimuli. *Open. Biol.* **14**, 240051 (2024).
79. Abraham, N. A., Campbell, A. C., Hirst, W. D. & Nezich, C. L. Optimization of small-scale sample Preparation for high-throughput openarray analysis. *J. Biol. Methods*. **8**, e143 (2021).
80. Bastiaanssen, T. F. S. et al. Kronos: A computational tool to facilitate biological rhythmicity analysis. *BioRxiv* https://doi.org/10.1101/2023.04.21.537503 (2023).

Acknowledgements

The authors would like to thank Dr. Thomaz Bastiaanssen and colleagues at the APC Microbiome for their guidance in the fundamentals of the R programming for Kronos analyses and to Dr. Alba García-Baos for her assistance in carrying out the experiments.

Author contributions

IGL and OV were responsible for the study conceptualization. IGL and PBS performed the experiments. IGL and OV analyzed and interpreted data. OV was responsible for supervision, project administration and funding acquisition. IGL and OV wrote the original draft. All authors critically reviewed and approved its content.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-12237-7.

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