Nighttime-specific gene expression changes in suprachiasmatic nucleus and habenula are associated with resiliency to chronic social stress

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Abstract

Mood disorder is bidirectionally associated with the dysregulation of circadian rhythms and sleep/wake cycle. However, the molecular mechanisms that link these processes remain largely unclear. The habenula (Hb) is a key brain region involved in mood regulation and depression, while the suprachiasmatic nucleus (SCN) is the body's central clock. We investigated the effects of chronic social defeat stress (CSDS) on gene expression in the Hb and SCN of stress-resilient and susceptible mice. Bulk RNA-seq analysis was conducted across four timepoints throughout the day, including the middle of the inactive (daytime) and active (nighttime) phases as well as during the transition periods. Differential expression analysis revealed a large number of differentially expressed genes and strong pathway activation in the resilient mice at ZT16 in both the Hb and SCN. This effect was timepoint and phenotype-specific. Our results reinforce the functional link between diurnal expression patterns and differential responses to stress. Specifically, we show potentially novel molecular mechanisms at night in the Hb and SCN that are associated with resilience to stress.

Keywords

Chronic stress, circadian rhythms, mood disorder, habenula, suprachiasmatic nucleus

1. Introduction

Major depressive disorder (MDD) is a highly heterogeneous disorder that affects mood, cognition, and basic physiological functioning (Maletic et al., 2007). Worldwide prevalence rates for year-long and lifetime occurrences of depressive disorders are 5.7% and 13%, respectively (Ferrari et al., 2013). At present half of the patients with MDD are resistant to classical treatments (Ionescu et al., 2015). There is growing evidence of the close connection between circadian rhythms and mood regulation (J. Z. Li et al., 2013). The majority of depressed patients show more severe symptoms in the morning which subside by evening (Joyce et al., 2005). Moreover, circadian processes are disrupted in some depressed patients, including diurnal hypersecretion of growth hormones, blunting of daily core body temperature and melatonin rhythms, and disruption of the sleep/wake cycle (Germain & Kupfer, 2008; Koenigsberg et al., 2004; Linkowski et al., 1985; Mendlewicz et al., 1985; Peeters et al., 2003; Walker et al., 2020).

Circadian rhythms are physiological, psychological and behavioural oscillations that follow an approximately 24-hour cycle. These internal rhythmic processes are maintained by multiple peripheral clocks throughout the body that are synchronised to a single central clock (Takahashi, 2017). In mammals, the suprachiasmatic nucleus (SCN), located in the hypothalamus, is the central timekeeper (Dibner et al., 2010). The SCN projects to other hypothalamic structures such as the paraventricular nucleus, subparaventricular zone, and dorsomedial hypothalamus (Germain & Kupfer, 2008; Vujovic et al., 2015). The SCN also projects to epithalamic structures associated with stress-coping such as the habenular complex, which is composed predominantly of lateral (LHb) and medial habenula (MHb) subregions (Baño-Otálora & Piggins, 2017; Vujovic et al., 2015). The direct and indirect projections of the SCN to mood-regulating areas of the brain are likely responsible for the diurnal modulation of pathological behaviours, such as anxious and depressive-like responses in mice (Landgraf et al., 2016; Vadnie et al., 2022).

The habenular complex (Hb) serves as a communication relay between the forebrain emotion-processing limbic system and the midbrain depression-associated aminergic centres (Proulx et al., 2014; Yang, Wang, et al., 2018). The Hb is known to be heavily involved in mediating stress responses (Baker et al., 2022; Gold & Kadriu, 2019) and has been associated with circadian modulation of depression (Young et al., 2022). Elevated firing in the LHb neurons projecting to the serotonergic dorsal raphae nucleus (DRN) is associated with social avoidance in chronically stressed mice (H. Liu et al., 2021). Passive coping, a behaviour mirroring apathy

and resignation in humans, has been associated with elevated activity in the reciprocal LHb-DRN projections (Coffey et al., 2020; L.-M. Yang et al., 2008; H. Zhang et al., 2018), medial prefrontal cortex projections to LHb (Warden et al., 2012) and LHb projections to the ventral tegmental area (VTA) (Cerniauskas et al., 2019; B. Li et al., 2011). Furthermore, the ameliorative effects of light therapy on depressive-like behaviour in mice are delivered through the retinal-LHb pathway terminating in the VTA and DRN (Huang et al., 2019). Likewise, ketamine rapidly blocks burst firing in the LHb and rescues depressive-like symptoms in rats and mice (Yang, Cui, et al., 2018). Pharmacological inhibition of LHb reverses treatment-resistant depressive-like symptoms in rats (Winter et al., 2011). Consistently, LHb lesions in rodents rescue depressive-like behaviour by increasing DRN serotonergic transmission (Yang et al., 2008). Developmental MHb lesion impairs voluntary exercise and induces despair-like and anhedonic phenotypes in mice, but without affecting the circadian period and sleep architecture (Hsu et al., 2014, 2016, 2017). MHb reacts to chronic stress in a molecularly-specific manner (Faron-Górecka et al., 2016). Studies in depressed humans suggest that both LHb and MHb exhibit increased metabolism and structural changes (Germann et al., 2021; Sartorius et al., 2010). Despite its importance, the effects of stress on molecular processes in the Hb remain underexplored.

Habenular clocks robustly modulate complex behaviours, such as anxiety-like responses to stressors (Basnakova et al., 2021). Daily variation in the firing of both LHb and MHb neurons is dependent on their functional molecular clock (Guilding et al., 2010; Sakhi, Belle, et al., 2014; Sakhi, Wegner, et al., 2014; Young et al., 2022). The LHb exhibits an independent endogenous molecular clock that is synchronised by the signals from the SCN (Baño-Otálora & Piggins, 2017; Hattar et al., 2006; Sakhi, Wegner, et al., 2014; C. Zhang et al., 2009). Core clock genes show rhythmic expression in the LHb *in vitro* and are in phase with the rhythms generated in the SCN (Baño-Otálora & Piggins, 2017; Z. Zhao et al., 2015). Moreover, the SCN innervates the LHb directly (Hu et al., 2020; Young et al., 2022; C. Zhang et al., 2009) and indirectly through the dorsomedial and lateral hypothalamic areas (Morin, 2013; Poller et al., 2013; Stamatakis et al., 2016). MHb sends input into the LHb (Kim & Chang, 2005). Hence, depression-associated circadian disruption might be attributed to the perturbations of circadian rhythmicity at the level of SCN, Hb or both.

Chronic social defeat stress (CSDS) is a robust and widely used chronic stress model that induces core symptoms of depression-like and PTSD-like behaviours in rodents (Krishnan et al., 2007; Petković & Chaudhury, 2022). The hallmark effect of CSDS is social avoidance as

measured by the social interaction (SI) test (Berton et al., 2006). Experimental mice exhibiting social avoidance are deemed stress-susceptible, while those that do not are as stress-resilient. These two subpopulations show different behavioural, physiological and molecular profiles (Chuang et al., 2010; Iñiguez et al., 2014; Krishnan et al., 2007; Schmidt et al., 2010). Such segregation allows investigations into underlying mechanisms that promote resilience to stress (Anacker et al., 2018; Cathomas et al., 2019). Chronic social stress in rodents disrupts body temperature, heart rate, locomotor activity rhythms and sleep patterns (Haniffa et al., 2023; Meerlo et al., 2002; Radwan et al., 2021a, 2021b). We have shown that LHb cells projecting to the DRN of stress-susceptible mice exhibit blunted diurnal activity while stress-resilient and stress-naïve mice exhibit robust daily rhythm in activity (H. Liu et al., 2021). This observation suggests that abnormal diurnal rhythmic activity in LHb cells projecting to the DRN may encode susceptibility to stress. However, the exact transcriptomic circadian profiles of the Hb and SCN in the stress-resilient and stress-susceptible mice have not yet been explored.

In this study, we investigated the effects of CSDS on the Hb and SCN transcriptome across the light/dark cycle. Mice were exposed to CSDS and sampled at four evenly spaced timepoints: ZT4 (mid-inactive phase), ZT10 (transition to active phase), ZT16 (mid-active phase), and ZT22 (transition to inactive phase). We hypothesised that the greatest changes would be observed in the stress-susceptible mice in the Hb, specifically during the light phase. We expected relatively few differences between the resilient and stress-naive mice, especially in the SCN. Contrary to our expectations, we found a strong gene upregulation and predicted activation of biological pathways at ZT16 (dark phase) in both the SCN and Hb of resilient mice. To our surprise, this transcriptomic signature was nighttime-specific and observed only in stress-resilient mice, whereas there were relatively few differences between the stress-susceptible and stress-naive groups across timepoints.

2. Material and Methods

2.1. Animals and Housing

Male C57BL/6J mice (8-10 weeks old; Jackson Laboratory) were housed in groups of 5 upon arrival for at least a week before the start of the chronic social defeat stress (CSDS) paradigm. CD1 male mice (retired breeders; Charles River Laboratories) were housed individually under standard housing conditions. All mice were housed with *ad libitum* access to food and water at 20 ± 1 °C and 50 ± 10% humidity under a 12-hr light/dark cycle (7:00 am / ZT0: lights on; 7:00

pm / ZT12: lights off). All experimental procedures were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals and approved by the New York University Abu Dhabi Animal Care and Use Committee (IACUC Protocol: 19-0004).

2.2. Chronic Social Defeat Stress

Chronic social defeat stress was performed as described previously (Golden et al., 2011). Experimental C57 mice were exposed to 10 minutes of physical stress followed by 24 hours of sensory stress in the cage of an unfamiliar CD1 aggressor mouse. CSDS was conducted daily over 15 days between ZT8 and ZT9. The control mice were housed in pairs in a separate room. All the mice were single-housed following the end of CSDS until extraction.

Social avoidance towards a novel non-aggressive CD1 mouse was measured in a two-stage social interaction (SI) test, as previously reported (Golden et al., 2011). The test was conducted between ZT7 and ZT10. TopScan video tracking system (CleverSys. Inc.) was used to automatically track mouse movement. Mouse phenotyping was based on the social interaction ratio (SI score), which was calculated as a ratio of time spent in the interaction zone with the social target present and social target absent, as described previously (Fig.S1A) (Golden et al., 2011). Experimental mice with SI scores below 100 were phenotyped as 'susceptible' and those with scores above 100 were phenotyped as 'resilient' (Fig.S2A). All of the values are reported as mean ±SEM. The data were analysed using one-way and two-way ANOVAs.

2.3. Tissue Sampling and Nuclei Isolation

Mice were anaesthetized using isoflurane and sacrificed 48-72 hours after the SI test by cervical dislocation. Sampling was done at four timepoints (ZT4, ZT10, ZT16, ZT22) across the light-dark cycle (Fig.1A-B). Extractions were carried out on a cold sterile surface, and the extracted brains were flash-frozen by immersion into -60 °C isopentane (Sigma Aldrich M3263) for 20 seconds. The brains were kept on dry ice at -80°C until further processing. The flash-frozen brain samples were then sliced on a Leica CM1950 cryostat (250 µm thickness) (Fig. S1B). The brain regions were isolated with reference to the Mouse Brain Atlas (Paxinos & Franklin, 2019). Region-specific punches were collected into separate 1.5ml EppendorfTM DNA LoBind tubes and snap-frozen in dry ice. For both the SCN and Hb, bilateral punches from within each biological replicate were combined (Fig. S1B).

2.4. RNA Isolation, Library Preparation and RNA Sequencing

RNA was isolated from the punches using the Qiagen RNeasy Micro Kit, as per the manufacturer's instructions. Isolated RNA was quantified and checked for quality on an Agilent Bioanalyzer instrument (Agilent Technologies). Samples with RIN values above 7 were used for the library preparation. All the libraries were prepared together using the NEBNext® Ultra™ II RNA Library Prep Kit (New England BioLabs Inc.) as per the manufacturer's protocol within one sitting. Sequencing was done on NovaSeq S1 flow cell (200 cycles) following Illumina's recommendations.

2.5. Differential expression and pathway enrichment analysis

Count preprocessing: Raw FASTQ sequenced reads were first assessed for quality using FastQC 1 (v0.11.5). The reads were then passed through Trimmomatic 2 (v0.36) for quality trimming and then processed with Fastp 3 to remove poly-G tails. Following the quality trimming, the reads were assessed again using FastQC. Post QC and QT, the reads were aligned to the mouse reference genome GRCm38.82 using HISAT2 4 with the default parameters The resulting SAM alignments were then converted to BAM format and coordinate sorted using SAMtools 5 v1.3.1. The sorted alignments were processed through Stringtie 7 v1.3.0 for transcriptome quantification. Finally, Qualimap 8 v2.2.2 was used to generate RNAseq-specific QC metrics per sample. All of the methods were executed using predefined YAML workflows using the BioSAILs 9 workflow management system.

Differential expression: Differential expression testing was performed in R package DESeq2 (v1.40.2) using multiple Wald tests (Love et al., 2014). To control for the false discovery rate, *p* values were adjusted using the Benjamini–Hochberg procedure. To obtain a better estimate of expression changes in low count genes, we performed log2-fold change (LFC) shrinkage using the *apeglm* method. We used an adjusted *p* value of 0.05 (5% FDR) and a log2-fold change (LFC) of 0.5 to identify differentially expressed genes (DEGs). Additional quality control was conducted to account for technical errors during sequencing. Samples within the same phenotype from ZT4 and ZT10 timepoints were computationally pooled together to provide an estimate of the 'daytime' expression, while ZT16 and ZT22 timepoint samples were pooled to provide an estimate of the 'nighttime' expression for each phenotype (Fig.1B). Pooling all the samples within the same phenotype provided an estimate of time-invariant 'global' expression corresponding to average expression throughout the day. Phenotypes were compared at each of

these levels. All the sample identities were assessed using previously published regional markers (Fig.1C).

Pathway analysis: Pathway enrichment analysis was conducted in the Ingenuity Pathway Analysis (IPA, Qiagen) on all genes that had passed the 5% FDR significance criterion. IPA was conducted only in those comparisons where the number of differentially expressed genes (DEGs) was higher than 20. Pathways were considered significant if –log(B-H *p*-value) was greater than 1.3. The analysis included only the pathways associated with the mouse nervous system, neurons, astrocytes, other tissues and unspecified cell types.

Gene ontology: We visualised the associated DEGs and the top 5 IPA pathways from the resilient-to-control comparisons at ZT16 for both the SCN and Hb using gene-concept networks. Gene-concept networks represent the relative coordinates of the enriched pathways based on the number and differential expression of the associated DEGs. Therefore, it is possible to identify overlapping DEGs that have the largest contribution to the enrichment of the top 5 activated pathways.

Detection of rhythmic genes: Log-normalised count values were used to determine the rhythmic expression profile of core clock genes across all phenotypes, using the JTK method in the R-implemented version of the ECHO (v.4.0) tool (De los Santos et al., 2020; Hughes et al., 2010).

3. Results

3.1. Behavioural Results

Following CSDS, mice were classified as stress-resilient or stress-susceptible (Fig. S2A-C) (Golden et al., 2011). One-way ANOVA revealed significant variation in SI scores ($F(2,120) = 63.28$, $p < 0.001$). Tukey's post hoc comparison revealed significant differences between controls and susceptible mice ($p < 0.001$, 95% C.I. = [65.4, 106.8]), and resilient and susceptible mice ($p < 0.001$, 95% C.I. = [58.92, 94.9]) (Fig. S2B). Stress-naïve and resilient mice did not differ in SI scores ($p = 0.72$, 95% C.I. = [-8.6, 26.9]). Resilient and control mice exhibited similar locomotor patterns, while susceptible mice exhibited a more constrained movement range (Fig.S2D-E).

3.2. Transcriptomic Analysis

3.2.1. Differential Expression Analysis

We observed the highest number of DEGs between resilient and control mice at all levels of comparison, both in the SCN and Hb. The majority of these DEGs were upregulated in the resilient phenotype. In the SCN (Fig. 2A-B), at global and daytime comparisons only a few genes were differentially expressed between any of the phenotypes. Unexpectedly, ZT4 and ZT10 comparisons did not reveal substantial phenotypic differences. However, at nighttime, we found 338 DEGs in resilient mice relative to controls, out of which 269 were upregulated (Fig.2A,D). The nighttime differences in the SCN stemmed largely from phenotypic differences at ZT16 where we found 1058 DEGs out of which 549 were upregulated (Fig.2B,E). At ZT22, we observed 34 DEGs in resilient relative to susceptible mice. However, other phenotypic comparisons at ZT22 did not reveal any major differential expression patterns. These results suggest that the largest changes in stress-induced gene expression observed in the SCN occur during the nighttime at ZT16.

In the Hb (Fig.4A-B) expression patterns were similar to those observed in the SCN. Global comparisons in the Hb revealed 68 upregulated DEGs in the resilient mice relative to controls (Fig.4A,C). Gene expression patterns did not robustly differ between any of the three phenotypes during the daytime. Specifically, neither ZT4 nor ZT10 yielded any discernible differences in gene expression patterns (Fig. 4B). However, nighttime comparison between the phenotypes revealed 55 upregulated DEGs in resilient mice relative to controls (Fig.4A,D). Susceptible mice did not show significant differential expression patterns relative to either the resilient or control mice. We found that the large differences in gene expression profiles observed during global and nighttime analysis are the result of the large gene upregulation at ZT16 in stress-resilient compared to control and susceptible mice (Fig.4A-B). Specifically, 143 DEGs were identified in the resilient mice relative to controls at ZT16, out of which 129 were upregulated (Fig.4B,E). Consistent with the observed similarity between susceptible and control transcriptional signatures, we observed 78 upregulated DEGs in resilient mice compared to stress-susceptible mice, but not between the susceptible and control mice (Fig.4B). No robust differences were observed at ZT22 between any of the phenotypes (Fig.4B). These results suggest that the stress-induced differences in Hb gene expression patterns, both at global and day or night analysis, are driven by changes at the ZT16 timepoint.

Taken together, these results suggest that resilient mice show robust transcriptomic differences relative to control mice in both the Hb and SCN. The observed differences were largest at ZT16 in both brain regions, with SCN showing larger transcriptional response than the Hb.

3.2.2. Pathway Enrichment Analysis

Pathway enrichment analysis in the SCN was performed at nighttime and at ZT16 in the resilient-to-control comparisons (Fig.3). Specifically, at nighttime, cAMP-response element binding protein (CREB) signalling (z = 5), S100 family signalling (z = 4.6), G-protein coupled receptor (GPCR) signalling (z = 3.9), synaptogenesis (z = 3.8), cAMP-mediated signalling (z = 3.5), SNARE signalling pathway (z = 2.8), and calcium signalling (z = 2.1) were all activated and enriched in the SCN of resilient mice relative to the controls. At ZT16, the enriched pathways included an expanded list of those observed from the nighttime comparison (Fig.3A-B). These pathways can be divided into two broad functional groups: signalling and plasticity-related pathways. Serotonin receptor (z = 3.9), neurovascular coupling (z = 3.8), oxytocin (z = 3.3), calcium (z = 3.1), ephrin receptor (z = 3.1), opioid (z = 2.6), α-adrenergic receptor (z = 2.5), oestrogen receptor (z = 2.5), and glutamate receptor (z = 1.9) signalling pathways were all enriched in the resilient mice relative to controls at ZT16 (Fig.3A). Broad signalling pathways, such as CREB (z = 6.3), GPCR (z = 5.2), S100 family (z = 5.2) and cAMP-mediated signalling (z = 3.8) were also predicted to be strongly activated. Several plasticity-related pathways were also enriched at this timepoint including synaptogenesis (z = 5.1), SNARE (z = 3.5), reelin (z = 3.4), CCK/Gastrin-mediated signalling (z = 2.7), ERBB (z = 2.7), synaptic long-term depression (z = 2.5), neuregulin (z = 2.3), and synaptic long-term potentiation (z = 1.9) signalling pathways (Fig.3B). Furthermore, at ZT16, circadian rhythm signalling pathway was significantly enriched in the resilient mice without clear activation pattern. Both signalling and plasticity-related pathways in the resilient mice were enriched by common DEGs. Some of these genes include: *Adora2a*, *Adra2c*, *Chrm 1-5*, *Cacng3*, *Camk4*, *Creb3l1*, *Gucy1a1, Grm 5* and *8*, *Hrh2, Htr* family, *Kcnj 4-5*, *Ngfr*, *Oprd1*, *Prkcg*, and *Rasd2* (Fig.3C).

In the Hb, pathway enrichment was observed in global, nighttime and ZT16 comparisons between resilient and control mice (Fig. 5). At global comparison level, opioid (z = 2.7), RAC (z = 2.2), and calcium (z = 2.1) signalling pathways were significantly enriched (Fig.5A). At nighttime, the enriched pathways in resilient mice included calcium signaling (z = 2.4), opioid signaling (z = 2.2), and RAC signaling (z = 2). Unlike the SCN, relatively few pathways were highly enriched and activated in the resilient mice at ZT16 timepoint (Fig.5A-B). At ZT16,

synaptogenesis (z = 3.3), calcium (z = 3.3), cAMP-mediated (z = 2.8), dopamine feedback in cAMP (z = 2.6), opioid (z = 2.5), synaptic long-term potentiation (z = 2.4), and granzyme A signalling (z = 2.2) were all significantly enriched in the resilient mice relative to controls (Fig.5A-B). Only oxidative phosphorylation was predicted to be inhibited (z = - 2.4). At all three levels of comparison, the circadian rhythm signaling pathway was significantly enriched in resilient mice, but no prediction could be made regarding its activation. Several genes drive the activation of these pathways in the Hb of resilient mice. These genes include: *Adcy1*, *Adcy9*, *Cacna1h*, *Cacna2d1*, *Camk2b*, *Camkk1, Gad1*, *Grin2a*, *Grin2b*, *Prkce* and *Ppp3ca* (Fig.5C). Our results suggest a strong association between chronic stress resilience and enrichment of signalling and plasticity-related pathways in the SCN and Hb, specifically at ZT16.

3.2.3. Rhythmicity of core clock genes in SCN and Hb

JTK analysis revealed the core clock genes (*Per1*, *Per2*, *Per3*, *Cry1*, and *Arntl*) to be rhythmic in the SCN (p_{adj} <0.05) of control mice (Fig.6A-B). Resilient and susceptible mice exhibited similar rhythmic profiles of the core clock genes to control mice, with the exception of *Arntl* and *Per1*. *Arntl* was arrhythmic in both stress-resilient and stress-susceptible mice, while *Per1* was arrhythmic only in the SCN of stress-susceptible mice. Similarly, to the SCN, these core clock genes were rhythmic in all three phenotypes in Hb, with the exception of *Per1, Per3,* and *Cry1* which were arrhythmic in the Hb of control mice (Fig.6A-B).

4. Discussion

Resiliency and Homeostatic Response

The close relationship between circadian rhythms and mood disorders warranted a systemic investigation of transcriptomic changes in the principal clock (SCN) and brain regions that modulate mood, such as the Hb (Basnakova et al., 2021; Salaberry et al., 2019; Young et al., 2022). We found that chronic stress elicited a robust change in the gene expression in the SCN, and to a lesser degree in the Hb (Fig.2 & 4). This transcriptomic signature was specific to resilient mice and was observed exclusively at ZT16. In contrast, stress-susceptible and control mice did not differ transcriptomically. Large differential upregulation in resilient mice was associated with enrichment and predicted activation of signalling and plasticity-related pathways (Fig.3 & 5). Resiliency is an active homeostatic process and involves transcriptional changes in a diffuse network of interconnected brain regions (Franklin et al., 2012; Lorsch et

al., 2019). The current understanding is that chronic stress imposes an adaptive challenge that requires behavioural flexibility and physiological homeostatic buffering (Pfau & Russo, 2015). The inability to elicit such a response leads to failure in stress-coping and strongly contributes to associated stress-induced pathologies (Chang et al., 2015; Wu et al., 2013). Resilient mice do not show behavioural deficits, likely because of large transcriptomic changes. Early studies found that resilient mice exhibited large and unique upregulation of neurotrophic factors and stress-responsive genes in the prefrontal cortex (Krishnan et al., 2007). Moreover, other studies found strong gene upregulation in resilient mice relative to control and susceptible mice in midbrain regions such as the nucleus accumbens and ventral tegmental area (Bagot et al., 2014, 2016; Golden et al., 2013; Isingrini et al., 2016). Our findings that large transcriptomic changes occur in resilient mice at a specific time at night (ZT16) add a significant temporal dimension to the molecular mechanisms that drive resilience to stress. Our data, along with previously published physiological and circuit studies, suggest that temporal patterns of regional activity may play a crucial role in driving a particular phenotype. For example, stress-susceptible mice exhibit elevated firing in the LHb during the day relative to stress-resilient and stress-naïve mice, suggesting a diurnal disruption at the level of LHb (H. Liu et al., 2021). Specifically, increased burst firing in the LHb is caused by signals from other stress-response regions (H. Liu et al., 2021; Zheng et al., 2022). Supporting previous findings, our data suggests a tight association between large transcriptional upregulation and successful stress-coping mechanisms.

SCN transcriptomic profile

We observed significant upregulation of the adenosine A2a receptor gene (*Adora2a)* in the SCN of resilient mice at ZT16. Adenosine is known to regulate *Per1* and *Per2* expression via A1/A2A receptor activation which is hypothesised to integrate both the light and sleep signalling to track homeostatic sleep pressure and regulate sleep architecture - both of which were previously found to be differentially impacted in resilient and susceptible mice (Jagannath et al., 2021; Radwan et al., 2021a, 2021b). Upregulation of *Adora2a* in the SCN of resilient mice at ZT16 may be a subcomponent of adaptive homeostatic response that maintains consolidated sleep. Interestingly, the upregulation of A2A receptors in the forebrain is associated with depressive-like behaviour and decreased synaptic plasticity following UCMS (Calker et al., 2019). Resilient mice displayed strong upregulation of *Ntrk1* and *Ngfr* in the SCN at ZT16. NGF binds to Ntrk1 and Ngfr (Bathina & Das, 2015; Miranda et al., 2019). Moreover, NGF levels are significantly lower in depressed patients (Tseng et al., 2015) while increasing NGF levels has antidepressant properties (Mondal & Fatima, 2019; Overstreet et al., 2010). Furthermore, BDNF which has

functional roles in synaptic plasticity, stress response and antidepressant action in several midbrain regions also binds to Ngfr (Chaudhury et al., 2015; Koo et al., 2019). Ngfr is specifically synthesised in cholinergic neurons which strongly modulate SCN neuronal excitability and modulate circadian rhythmicity at night (Bina & Rusak, 1996b, 1996a; C. Liu & Gillette, 1996). Interestingly, besides observing strong upregulation of *Ngfr* in resilient mice, we observed high upregulation of cholinergic muscarinic receptors *Chrm 1-5.* These receptors play a role in fear and emotional responsiveness (Navarria et al., 2015; Santini et al., 2012). Cholinergic signalling in the SCN can induce phase shifts in locomotor rhythms and activity (Hut & Van der Zee, 2011). However, it is unclear at present how increased muscarinic receptor expression at ZT16 in the SCN drives resilience. A recent study demonstrated that the stress-associated neuropeptide corticotropin-releasing hormone (CRH), which is upregulated in the stress-resilient mice in our study, increases cholinergic interneuron firing that leads to potentiation of dopamine transmission via M5 activation (Julia C. Lemos et al., 2019; Maksymetz et al., 2019). Our observation that CRH is upregulated in resilient mice adds to the recent and novel findings that CRH-expressing neurons are present in the SCN (Y. Wang et al., 2021). It was already known that rhythmic expression of glucocorticoids is regulated by AVP neurons in the SCN that activate CRH-expressing neurons in the PVN resulting in ACTH production in the pituitary glands and consequent release of glucocorticoids (Jones et al., 2021). The functional role of increased CRH expression in the SCN at ZT16 in resilient mice has yet to be elucidated. The resilient phenotype has also been associated with increased cAMP and GPCR-mediated signalling (Bagot et al., 2016; J. Yang et al., 2020). We found that these pathways have been consistently activated in the SCN of resilient mice. Overall, only a few studies have examined stress effects on SCN physiology and those predominantly focused on the expression of select clock genes (Landgraf et al., 2016; Ota et al., 2018, 2021; Tahara et al., 2015). Our observations highlight potentially new molecular mechanisms in the SCN that promote resilience to stress.

Hb Transcriptomic Profile

Adcy1 and *Adcy9* were upregulated in the Hb of resilient mice relative to controls at ZT16. Adenylyl cyclases play a pivotal role in cAMP-mediated and the ERK½-CREB signaling cascade and are known to modulate neuronal physiology (Ha & Redmond, 2008; D. Lee, 2015; H. Wang et al., 2004). Overexpression of *Adcy1* in the forebrain prevented BDNF downregulation in stressed mice, thereby promoting stress resiliency (M. Yang et al., 2020). Likewise, *Adcy1* forebrain overexpression induces greater long-term potentiation (LTP) (H.

Wang et al., 2004). In the Hb, we observed ZT16-specific upregulation of other plasticity-modulating genes, such as *Grin2a, Grin2b*, *Cacna1h*, *Camk2b*, and *Ppp3ca* which correlate with large predicted activation of synaptogenesis and LTP signaling pathways. Previous reports have shown that resilient mice exhibit greater LTP relative to susceptible mice in the hippocampus associated with higher expression levels of *Grin2b* and *Camk2* (C.-W. Lee et al., 2021). This upregulation of plasticity-related genes may be a part of a larger homeostatic response that is associated with stress resiliency (Christoffel et al., 2011; J. Wang et al., 2018). We also observed upregulation of *Gad1, Gabra5, Kcnq3* and *Npy1r* genes. Chronic mild stress was found to impair GABA release and uptake in the medial prefrontal cortex and is associated with depressive-like behaviour (Ma et al., 2016). In the LHb, in line with our findings, sub-chronic mild stress accumulation decreases long-term depression (LTD) of GABAergic neurons (Lalive et al., 2022). Furthermore, male mice with *Kcnq3* mutation that inhibits GABA binding exhibit reduced self-grooming, behaviour indicative of apathy (Chen et al., 2023; Petković & Chaudhury, 2022). Neuropeptide Y action through its Y1 receptor has been associated with stress resilience and anxiolysis in several brain regions, including the Hb (Reichmann & Holzer, 2016; Sweis et al., 2013). In LHb, *Npy1r*-expressing neurons play a critical role in chronic stress adaptation by driving palatable food intake and reducing anxiety-like behaviour (Ip et al., 2019).

Clock Genes

Since our analysis revealed a strong effect of stress on the SCN and Hb at a specific timepoint, we examined the expression rhythms of clock genes in both of these nuclei. Chronic stress induces arrhythmicity of *Arntl* in the SCN of both resilient and susceptible mice. Despite the previous observation that specific knockdown of *Arntl* leads to behavioural despair, anxiety and helplessness in the chronic mild stress model (CMS), our observation suggests that its arrhythmicity in the SCN does not directly impact mood regulation (Landgraf et al., 2016). Another study found that CMS in rats induces perturbation of *Per2* in the LHb, without affecting the SCN rhythms (Christiansen et al., 2016). In our study, *Per1*, *Per3*, and *Cry1* were arrhythmic in the Hb of stress-naive mice, but not resilient and susceptible mice. Although unexpected, these results can be attributed to the subdivisional differences at the level of MHb and LHb (H. Zhao & Rusak, 2005). LHb activation, as measured by *c-Fos* immunoreactivity, is larger at nighttime in both nighttime-stressed and stress-naive rats, suggesting circadian modulation of stress response (Chastrette et al., 1991). Importantly, this effect was specific to LHb and was not observed in the MHb (Chastrette et al., 1991). Furthermore, clock gene expression in the MHb has yet to be systematically investigated, though it has been reported previously in hamsters and

rats (Shieh, 2003; Yamamoto et al., 2001). Despite both the LHb and MHb showing diurnal variation in firing (Sakhi, Belle, et al., 2014; Sakhi, Wegner, et al., 2014), the LHb displays more evident rhythms of higher amplitude than the MHb (Baño-Otálora & Piggins, 2017; Guilding et al., 2010; H. Zhao & Rusak, 2005). However, varying methodological approaches and experimental settings are the likely cause behind inconsistent study conclusions (Young et al., 2022). For example, a study using *in situ* hybridisation reported more prominent *Per1* and *Per2* rhythms in the MHb rather than the LHb (Olejniczak et al., 2021). It has been suggested that the most parsimonious conclusion from these studies would be that clock gene expression in the LHb is overall at a low level and that it might be localised to the medial part of the LHb that is bordering the lateral part of the MHb (Young et al., 2022). In the present study we did not differentiate between the two subregions and reduced spatial resolution might have contributed to the observed arrhythmicity of clock genes in stress-naive mice.

Conclusion and Future Directions

The SCN and Hb are heterogeneous regions with many functionally and molecularly distinct subregions (Guilding & Piggins, 2007; Wallace et al., 2020). Nonetheless, to the best of our knowledge, no previous study has systematically examined the Hb or SCN bulk transcriptome in the context of murine chronic stress models across multiple timepoints throughout the circadian cycle. We are the first to show that the largest transcriptomic changes in the SCN and Hb of resilient mice occur at night. This highlights the importance of sampling over a 24-hour cycle to accurately determine dynamic changes in gene expression following stress exposure. Most studies never consider diurnal changes in expression profiles. Our time-dependent transcriptomic analysis suggests novel potential molecular mechanisms for driving resiliency and susceptibility to stress that would not be evident in samples collected within a constrained daytime period. Such findings would further support time-dependent treatment administration that might enhance its efficacy. So far, transcriptomic studies have examined only specific subpopulations of the Hb in the context of functional experiments designed to probe specific behavioural regulation (Cerniauskas et al., 2019; Cui et al., 2018; Weil et al., 2022). Likewise, SCN studies were limited to investigating a limited predetermined list of genes (Landgraf et al., 2016). Given the distinct functions and projection patterns of these subregions, the next steps should involve establishing a relationship between specific depression endophenotypes and expression patterns of specific subpopulations, possibly through the utilisation of single-cell RNA-seq. Ultimately, the 'key to solving the mystery of depression' might still lie in genetics (Nestler et al., 2002).

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Declaration of competing interest

None.

References

Anacker, C., Luna, V. M., Stevens, G. S., Millette, A., Shores, R., Jimenez, J. C., Chen, B., & Hen, R. (2018). Hippocampal neurogenesis confers stress resilience by inhibiting the ventral dentate gyrus. *Nature*, *559*(7712), 98–102. https://doi.org/10.1038/s41586-018-0262-4

Bagot, R. C., Cates, H. M., Purushothaman, I., Lorsch, Z. S., Walker, D. M., Wang, J., Huang, X., Schlüter, O. M., Maze, I., Peña, C. J., Heller, E. A., Issler, O., Wang, M., Song, W., Stein, Jason. L., Liu, X., Doyle, M. A., Scobie, K. N., Sun, H. S., … Nestler, E. J. (2016). Circuit-wide Transcriptional Profiling Reveals Brain Region-Specific Gene Networks Regulating Depression Susceptibility. *Neuron*, *90*(5), 969–983. https://doi.org/10.1016/j.neuron.2016.04.015

Bagot, R. C., Labonté, B., Peña, C. J., & Nestler, E. J. (2014). Epigenetic signaling in psychiatric disorders: Stress and depression. *Dialogues in Clinical Neuroscience*, *16*(3), 281–295.

Baker, P. M., Mathis, V., Lecourtier, L., Simmons, S. C., Nugent, F. S., Hill, S., & Mizumori, S. J. Y. (2022). Lateral Habenula Beyond Avoidance: Roles in Stress, Memory, and Decision-Making With Implications for Psychiatric Disorders. *Frontiers in Systems Neuroscience*, *16*, 826475. https://doi.org/10.3389/fnsys.2022.826475

Baño-Otálora, B., & Piggins, H. D. (2017). Contributions of the lateral habenula to circadian timekeeping. *Pharmacology Biochemistry and Behavior*, *162*, 46–54. https://doi.org/10.1016/j.pbb.2017.06.007

Basnakova, A., Cheng, R.-K., Chia, J. S. M., D'Agostino, G., Suryadi, Tan, G. J. H., Langley, S. R., & Jesuthasan, S. (2021). The habenula clock influences response to a stressor. *Neurobiology of Stress*, *15*, 100403. https://doi.org/10.1016/j.ynstr.2021.100403

Bathina, S., & Das, U. N. (2015). Brain-derived neurotrophic factor and its clinical implications. *Archives of Medical Science*, *6*, 1164–1178. https://doi.org/10.5114/aoms.2015.56342

Berton, O., McClung, C. A., DiLeone, R. J., Krishnan, V., Renthal, W., Russo, S. J., Graham, D., Tsankova, N. M., Bolanos, C. A., Rios, M., Monteggia, L. M., Self, D. W., & Nestler, E. J. (2006). Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. *Science*, *311*(5762), 864–868. https://doi.org/10.1126/science.1120972

Bina, K. G., & Rusak, B. (1996a). Muscarinic receptors mediate carbachol-induced phase shifts of circadian activity rhythms in Syrian hamsters. *Brain Research*, *743*(1), 202–211. https://doi.org/10.1016/S0006-8993(96)01043-8

Bina, K. G., & Rusak, B. (1996b). Nerve growth factor phase shifts circadian activity rhythms in Syrian hamsters. *Neuroscience Letters*, *206*(2), 97–100. https://doi.org/10.1016/S0304-3940(96)12432-0

Calker, D., Biber, K., Domschke, K., & Serchov, T. (2019). The role of adenosine receptors in mood and anxiety disorders. *Journal of Neurochemistry*, *151*(1), 11–27. https://doi.org/10.1111/jnc.14841

Cathomas, F., Murrough, J. W., Nestler, E. J., Han, M.-H., & Russo, S. J. (2019). Neurobiology of Resilience: Interface Between Mind and Body. *Biological Psychiatry*, *86*(6), 410–420. https://doi.org/10.1016/j.biopsych.2019.04.011

Cerniauskas, I., Winterer, J., de Jong, J. W., Lukacsovich, D., Yang, H., Khan, F., Peck, J. R., Obayashi, S. K., Lilascharoen, V., Lim, B. K., Földy, C., & Lammel, S. (2019). Chronic Stress Induces Activity, Synaptic, and Transcriptional Remodeling of the Lateral Habenula Associated with Deficits in Motivated Behaviors. *Neuron*, *104*(5), 899-915.e8. https://doi.org/10.1016/j.neuron.2019.09.005

Chang, L. J., Reddan, M., Ashar, Y. K., Eisenbarth, H., & Wager, T. D. (2015). The challenges of forecasting resilience. *Behavioral and Brain Sciences*, *38*, e98. https://doi.org/10.1017/S0140525X14001496

Chastrette, N., Pfaff, D. W., & Gibbs, R. B. (1991). Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus. *Brain Research*, *563*(1–2), 339–344. https://doi.org/10.1016/0006-8993(91)91559-J

Chaudhury, D., Liu, H., & Han, M.-H. (2015). Neuronal correlates of depression. *Cellular and Molecular Life Sciences*, *72*(24), 4825–4848. https://doi.org/10.1007/s00018-015-2044-6

Chen, K. J., Yoshimura, R., Edmundo, C. A., Truong, T. M., Civelli, O., Alachkar, A., & Abbott, G. W. (2023). Behavioral and neuro-functional consequences of eliminating the KCNQ3 GABA binding site in mice. *Frontiers in Molecular Neuroscience*, *16*. https://www.frontiersin.org/articles/10.3389/fnmol.2023.1192628

Christiansen, S., Bouzinova, E., Fahrenkrug, J., & Wiborg, O. (2016). Altered Expression Pattern of Clock Genes in a Rat Model of Depression. *International Journal of Neuropsychopharmacology*, *19*(11), pyw061. https://doi.org/10.1093/ijnp/pyw061

Christoffel, D. J., Golden, S. A., & Russo, S. J. (2011). Structural and synaptic plasticity in stress-related disorders. *Reviews in the Neurosciences*, *22*(5), 535–549. https://doi.org/10.1515/RNS.2011.044

Chuang, J.-C., Cui, H., Mason, B. L., Mahgoub, M., Bookout, A. L., Yu, H. G., Perello, M., Elmquist, J. K., Repa, J. J., Zigman, J. M., & Lutter, M. (2010). Chronic social defeat stress disrupts regulation of lipid synthesis. *Journal of Lipid Research*, *51*(6), 1344–1353. https://doi.org/10.1194/jlr.M002196

Coffey, K. R., Marx, R. G., Vo, E. K., Nair, S. G., & Neumaier, J. F. (2020). Chemogenetic inhibition of lateral habenula projections to the dorsal raphe nucleus reduces passive coping and perseverative reward seeking in rats. *Neuropsychopharmacology*, *45*(7), 1115–1124. https://doi.org/10.1038/s41386-020-0616-0

Cui, Y., Yang, Y., Ni, Z., Dong, Y., Cai, G., Foncelle, A., Ma, S., Sang, K., Tang, S., Li, Y., Shen, Y., Berry, H., Wu, S., & Hu, H. (2018). Astroglial Kir4.1 in the lateral habenula drives neuronal bursts in depression. *Nature*, *554*(7692), 323–327. https://doi.org/10.1038/nature25752

De los Santos, H., Collins, E. J., Mann, C., Sagan, A. W., Jankowski, M. S., Bennett, K. P., & Hurley, J. M. (2020). ECHO: An application for detection and analysis of oscillators identifies metabolic regulation on genome-wide circadian output. *Bioinformatics*, *36*(3), 773–781. https://doi.org/10.1093/bioinformatics/btz617

Dibner, C., Schibler, U., & Albrecht, U. (2010). The Mammalian Circadian Timing System: Organization and Coordination of Central and Peripheral Clocks. *Annual Review of Physiology*, *72*(1), 517–549. https://doi.org/10.1146/annurev-physiol-021909-135821

Faron-Górecka, A., Kuśmider, M., Kolasa, M., Żurawek, D., Szafran-Pilch, K., Gruca, P., Pabian, P., Solich, J., Papp, M., & Dziedzicka-Wasylewska, M. (2016). Chronic mild stress alters the somatostatin receptors in the rat brain. *Psychopharmacology*, *233*(2), 255–266. https://doi.org/10.1007/s00213-015-4103-y

Ferrari, A. J., Charlson, F. J., Norman, R. E., Flaxman, A. D., Patten, S. B., Vos, T., & Whiteford, H. A. (2013). The Epidemiological Modelling of Major Depressive Disorder: Application for the Global Burden of Disease Study 2010. *PLoS ONE*, *8*(7), e69637. https://doi.org/10.1371/journal.pone.0069637

Franklin, T. B., Saab, B. J., & Mansuy, I. M. (2012). Neural Mechanisms of Stress Resilience and Vulnerability. *Neuron*, *75*(5), 747–761. https://doi.org/10.1016/j.neuron.2012.08.016

Germain, A., & Kupfer, D. J. (2008). Circadian rhythm disturbances in depression. *Human Psychopharmacology: Clinical and Experimental*, *23*(7), 571–585. https://doi.org/10.1002/hup.964

Germann, J., Mameli, M., Elias, G. J. B., Loh, A., Taha, A., Gouveia, F. V., Boutet, A., & Lozano, A. M. (2021). Deep Brain Stimulation of the Habenula: Systematic Review of the Literature and Clinical Trial Registries. *Frontiers in Psychiatry*, *12*, 730931. https://doi.org/10.3389/fpsyt.2021.730931

Gold, P. W., & Kadriu, B. (2019). A Major Role for the Lateral Habenula in Depressive Illness: Physiologic and Molecular Mechanisms. *Frontiers in Psychiatry*, *10*, 320. https://doi.org/10.3389/fpsyt.2019.00320

Golden, S. A., Christoffel, D. J., Heshmati, M., Hodes, G. E., Magida, J., Davis, K., Cahill, M. E., Dias, C., Ribeiro, E., Ables, J. L., Kennedy, P. J., Robison, A. J., Gonzalez-Maeso, J., Neve, R. L., Turecki, G., Ghose, S., Tamminga, C. A., & Russo, S. J. (2013). Epigenetic regulation of RAC1 induces synaptic remodeling in stress disorders and depression. *Nature Medicine*, *19*(3), 337–344. https://doi.org/10.1038/nm.3090

Golden, S. A., Covington, H. E., Berton, O., & Russo, S. J. (2011). A standardized protocol for repeated social defeat stress in mice. *Nature Protocols*, *6*(8), 1183–1191. https://doi.org/10.1038/nprot.2011.361

Guilding, C., Hughes, A. T. L., & Piggins, H. D. (2010). Circadian oscillators in the epithalamus. *Neuroscience*, *169*(4), 1630–1639. https://doi.org/10.1016/j.neuroscience.2010.06.015

Guilding, C., & Piggins, H. D. (2007). Challenging the omnipotence of the suprachiasmatic timekeeper: Are circadian oscillators present throughout the mammalian brain?: Circadian oscillators in mammalian brain. *European Journal of Neuroscience*, *25*(11), 3195–3216. https://doi.org/10.1111/j.1460-9568.2007.05581.x

Ha, S., & Redmond, L. (2008). ERK mediates activity dependent neuronal complexity via sustained activity and CREB-mediated signaling. *Developmental Neurobiology*, *68*(14), 1565–1579. https://doi.org/10.1002/dneu.20682

Haniffa, S., Narain, P., Hughes, M. A., Petković, A., Šušić, M., Mlambo, V., & Chaudhury, D. (2023). Chronic social stress blunts core body temperature and molecular rhythms of Rbm3 and Cirbp in mouse lateral habenula. *Open Biology*, *13*(7), 220380. https://doi.org/10.1098/rsob.220380

Hattar, S., Kumar, M., Park, A., Tong, P., Tung, J., Yau, K.-W., & Berson, D. M. (2006). Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *The Journal of Comparative Neurology*, *497*(3), 326–349. https://doi.org/10.1002/cne.20970

Hsu, Y.-W. A., Gile, J. J., Perez, J. G., Morton, G., Ben-Hamo, M., Turner, E. E., & De La Iglesia, H. O. (2017). The Dorsal Medial Habenula Minimally Impacts Circadian Regulation of Locomotor Activity and Sleep. *Journal of Biological Rhythms*, *32*(5), 444–455. https://doi.org/10.1177/0748730417730169

Hsu, Y.-W. A., Morton, G., Guy, E. G., Wang, S. D., & Turner, E. E. (2016). Dorsal Medial Habenula Regulation of Mood-Related Behaviors and Primary Reinforcement by Tachykinin-Expressing Habenula Neurons. *ENeuro*, *3*(3). https://doi.org/10.1523/ENEURO.0109-16.2016

Hsu, Y.-W. A., Wang, S. D., Wang, S., Morton, G., Zariwala, H. A., De La Iglesia, H. O., & Turner, E. E. (2014). Role of the Dorsal Medial Habenula in the Regulation of Voluntary Activity, Motor Function, Hedonic State, and Primary Reinforcement. *The Journal of Neuroscience*, *34*(34), 11366–11384. https://doi.org/10.1523/JNEUROSCI.1861-14.2014

Hu, H., Cui, Y., & Yang, Y. (2020). Circuits and functions of the lateral habenula in health and in disease. *Nature Reviews Neuroscience*, *21*(5), 277–295. https://doi.org/10.1038/s41583-020-0292-4

Huang, L., Xi, Y., Peng, Y., Yang, Y., Huang, X., Fu, Y., Tao, Q., Xiao, J., Yuan, T., An, K., Zhao, H., Pu, M., Xu, F., Xue, T., Luo, M., So, K.-F., & Ren, C. (2019). A Visual Circuit Related to Habenula Underlies the Antidepressive Effects of Light Therapy. *Neuron*, *102*(1), 128-142.e8. https://doi.org/10.1016/j.neuron.2019.01.037

Hughes, M. E., Hogenesch, J. B., & Kornacker, K. (2010). JTK_CYCLE: An Efficient Nonparametric Algorithm for Detecting Rhythmic Components in Genome-Scale Data Sets. *Journal of Biological Rhythms*, *25*(5), 372–380. https://doi.org/10.1177/0748730410379711

Hut, R. A., & Van der Zee, E. A. (2011). The cholinergic system, circadian rhythmicity, and time memory. *The Cholinergic System and Brain Function*, *221*(2), 466–480. https://doi.org/10.1016/j.bbr.2010.11.039

Iñiguez, S. D., Riggs, L. M., Nieto, S. J., Dayrit, G., Zamora, N. N., Shawhan, K. L., Cruz, B., & Warren, B. L. (2014). Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. *Stress*, *17*(3), 247–255. https://doi.org/10.3109/10253890.2014.910650

Ionescu, D. F., Rosenbaum, J. F., & Alpert, J. E. (2015). Pharmacological approaches to the challenge of treatment-resistant depression. *Dialogues in Clinical Neuroscience*, *17*(2), 16.

Ip, C. K., Zhang, L., Farzi, A., Qi, Y., Clarke, I., Reed, F., Shi, Y.-C., Enriquez, R., Dayas, C., Graham, B., Begg, D., Brüning, J. C., Lee, N. J., Hernandez-Sanchez, D., Gopalasingam, G., Koller, J., Tasan, R., Sperk, G., & Herzog, H. (2019). Amygdala NPY Circuits Promote the Development of Accelerated Obesity under Chronic Stress Conditions. *Cell Metabolism*, *30*(1), 111-128.e6. https://doi.org/10.1016/j.cmet.2019.04.001

Isingrini, E., Perret, L., Rainer, Q., Amilhon, B., Guma, E., Tanti, A., Martin, G., Robinson, J., Moquin, L., Marti, F., Mechawar, N., Williams, S., Gratton, A., & Giros, B. (2016). Resilience to chronic stress is mediated by noradrenergic regulation of dopamine neurons. *Nature Neuroscience*, *19*(4), 560–563. https://doi.org/10.1038/nn.4245

Jagannath, A., Varga, N., Dallmann, R., Rando, G., Gosselin, P., Ebrahimjee, F., Taylor, L., Mosneagu, D., Stefaniak, J., Walsh, S., Palumaa, T., Di Pretoro, S., Sanghani, H., Wakaf, Z., Churchill, G. C., Galione, A., Peirson, S. N., Boison, D., Brown, S. A., … Vasudevan, S. R. (2021). Adenosine integrates light and sleep signalling for the regulation of circadian timing in mice. *Nature Communications*, *12*(1), 2113. https://doi.org/10.1038/s41467-021-22179-z

Jones, J. R., Chaturvedi, S., Granados-Fuentes, D., & Herzog, E. D. (2021). Circadian neurons in the paraventricular nucleus entrain and sustain daily rhythms in glucocorticoids. *Nature Communications*, *12*(1), 5763. https://doi.org/10.1038/s41467-021-25959-9

Joyce, P. R., Porter, R. J., Mulder, R. T., Luty, S. E., McKENZIE, J. M., Miller, A. L., & Kennedy, M. A. (2005). Reversed diurnal variation in depression: Associations with a differential antidepressant response, tryptophan:large neutral amino acid ratio and serotonin transporter polymorphisms. *Psychological Medicine*, *35*(4), 511–517. https://doi.org/10.1017/S0033291704003861

Julia C. Lemos, Jung Hoon Shin, & Veronica A. Alvarez. (2019). Striatal Cholinergic Interneurons Are a Novel Target of Corticotropin Releasing Factor. *The Journal of Neuroscience*, *39*(29), 5647. https://doi.org/10.1523/JNEUROSCI.0479-19.2019

Kim, U., & Chang, S.-Y. (2005). Dendritic morphology, local circuitry, and intrinsic electrophysiology of neurons in the rat medial and lateral habenular nuclei of the epithalamus. *Journal of Comparative Neurology*, *483*(2), 236–250. https://doi.org/10.1002/cne.20410

Koenigsberg, H. W., Teicher, M. H., Mitropoulou, V., Navalta, C., New, A. S., Trestman, R., & Siever, L. J. (2004). 24-h Monitoring of plasma norepinephrine, MHPG, cortisol, growth hormone and prolactin in depression. *Journal of Psychiatric Research*, *38*(5), 503–511. https://doi.org/10.1016/j.jpsychires.2004.03.006

Koo, J. W., Chaudhury, D., Han, M.-H., & Nestler, E. J. (2019). Role of Mesolimbic Brain-Derived Neurotrophic Factor in Depression. *Biological Psychiatry*, *86*(10), 738–748. https://doi.org/10.1016/j.biopsych.2019.05.020

Krishnan, V., Han, M.-H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., LaPlant, Q., Graham, A., Lutter, M., Lagace, D. C., Ghose, S., Reister, R., Tannous, P., Green, T. A., Neve, R. L., Chakravarty, S., Kumar, A., Eisch, A. J., Self, D. W., … Nestler, E. J. (2007). Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018

Lalive, A. L., Nuno-Perez, A., Tchenio, A., & Mameli, M. (2022). Mild stress accumulation limits GABAergic synaptic plasticity in the lateral habenula. *European Journal of Neuroscience*, *55*(2), 377–387. https://doi.org/10.1111/ejn.15581

Landgraf, D., Long, J. E., Proulx, C. D., Barandas, R., Malinow, R., & Welsh, D. K. (2016). Genetic Disruption of Circadian Rhythms in the Suprachiasmatic Nucleus Causes Helplessness, Behavioral Despair, and Anxiety-like Behavior in Mice. *Biological Psychiatry*, *80*(11), 827–835. https://doi.org/10.1016/j.biopsych.2016.03.1050

Lee, C.-W., Fang, Y.-P., Chu, M.-C., Chung, Y.-J., Chi, H., Tang, C.-W., So, E. C., Lin, H.-C., & Lin, H.-C. (2021). Differential mechanisms of synaptic plasticity for susceptibility and resilience to chronic social defeat stress in male mice. *Biochemical and Biophysical Research Communications*, *562*, 112–118. https://doi.org/10.1016/j.bbrc.2021.05.064

Lee, D. (2015). Global and local missions of cAMP signaling in neural plasticity, learning, and memory. *Frontiers in Pharmacology*, *6*. https://www.frontiersin.org/articles/10.3389/fphar.2015.00161

Li, B., Piriz, J., Mirrione, M., Chung, C., Proulx, C. D., Schulz, D., Henn, F., & Malinow, R. (2011). Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature*, *470*(7335), 535–539. https://doi.org/10.1038/nature09742

Li, J. Z., Bunney, B. G., Meng, F., Hagenauer, M. H., Walsh, D. M., Vawter, M. P., Evans, S. J., Choudary, P. V., Cartagena, P., Barchas, J. D., Schatzberg, A. F., Jones, E. G., Myers, R. M., Watson, S. J., Akil, H., & Bunney, W. E. (2013). Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proceedings of the National Academy of Sciences*, *110*(24), 9950–9955. https://doi.org/10.1073/pnas.1305814110

Linkowski, P., Mendlewicz, J., Leclercq, R., Brasseur, M., Hubain, P., Golstein, J., Copinschi, G., & Cauter, E. V. (1985). The 24-Hour Profile of Adrenocorticotropin and Cortisol in Major Depressive Illness*. *The Journal of Clinical Endocrinology & Metabolism*, *61*(3), 429–438. https://doi.org/10.1210/jcem-61-3-429

Liu, C., & Gillette, M. U. (1996). Cholinergic regulation of the suprachiasmatic nucleus circadian rhythm via a muscarinic mechanism at night. *The Journal of Neuroscience*, *16*(2), 744.

Liu, H., Rastogi, A., Narain, P., Xu, Q., Sabanovic, M., Alhammadi, A. D., Guo, L., Cao, J.-L., Zhang, H., Aqel, H., Mlambo, V., Rezgui, R., Radwan, B., & Chaudhury, D. (2021). Blunted diurnal firing in lateral habenula projections to dorsal raphe nucleus and delayed photoentrainment in stress-susceptible mice. *PLOS Biology*, *19*(3), e3000709. https://doi.org/10.1371/journal.pbio.3000709

Lorsch, Z. S., Hamilton, P. J., Ramakrishnan, A., Parise, E. M., Salery, M., Wright, W. J., Lepack, A. E., Mews, P., Issler, O., McKenzie, A., Zhou, X., Parise, L. F., Pirpinias, S. T., Ortiz Torres, I., Kronman, H. G., Montgomery, S. E., Loh, Y.-H. E., Labonté, B., Conkey, A., … Nestler, E. J. (2019). Stress resilience is promoted by a Zfp189-driven transcriptional network in prefrontal cortex. *Nature Neuroscience*, *22*(9), 1413–1423. https://doi.org/10.1038/s41593-019-0462-8

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for

RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Ma, K., Xu, A., Cui, S., Sun, M.-R., Xue, Y.-C., & Wang, J.-H. (2016). Impaired GABA synthesis, uptake and release are associated with depression-like behaviors induced by chronic mild stress. *Translational Psychiatry*, *6*(10), e910–e910. https://doi.org/10.1038/tp.2016.181

Maksymetz, J., Joffe, M. E., Moran, S. P., Stansley, B. J., Li, B., Temple, K., Engers, D. W., Lawrence, J. J., Lindsley, C. W., & Conn, P. J. (2019). M1 Muscarinic Receptors Modulate Fear-Related Inputs to the Prefrontal Cortex: Implications for Novel Treatments of Posttraumatic Stress Disorder. *Biological Psychiatry*, *85*(12), 989–1000. https://doi.org/10.1016/j.biopsych.2019.02.020

Maletic, V., Robinson, M., Oakes, T., Iyengar, S., Ball, S. G., & Russell, J. (2007). Neurobiology of depression: An integrated view of key findings. *Neurobiology of Depression*, 11.

Meerlo, P., Sgoifo, A., & Turek, F. W. (2002). The Effects of Social Defeat and Other Stressors on the Expression of Circadian Rhythms. *Stress*, *5*(1), 15–22. https://doi.org/10.1080/102538902900012323

Mendlewicz, J., Linkowski, P., Kerkhofs, M., Desmedt, D., Golstein, J., Copinschi, G., & Van Cauter, E. (1985). Diurnal Hypersecretion of Growth Hormone in Depression*. *The Journal of Clinical Endocrinology & Metabolism*, *60*(3), 505–512. https://doi.org/10.1210/jcem-60-3-505

Miranda, M., Morici, J. F., Zanoni, M. B., & Bekinschtein, P. (2019). Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. *Frontiers in Cellular Neuroscience*, *13*, 363. https://doi.org/10.3389/fncel.2019.00363

Mondal, A. C., & Fatima, M. (2019). Direct and indirect evidences of BDNF and NGF as key modulators in depression: Role of antidepressants treatment. *International Journal of Neuroscience*, *129*(3), 283–296. https://doi.org/10.1080/00207454.2018.1527328

Morin, L. P. (2013). Neuroanatomy of the extended circadian rhythm system. *Experimental Neurology*, *243*, 4–20. https://doi.org/10.1016/j.expneurol.2012.06.026

Navarria, A., Wohleb, E. S., Voleti, B., Ota, K. T., Dutheil, S., Lepack, A. E., Dwyer, J. M., Fuchikami, M., Becker, A., Drago, F., & Duman, R. S. (2015). Rapid antidepressant actions of scopolamine: Role of medial prefrontal cortex and M1-subtype muscarinic acetylcholine receptors. *Neurobiology of Disease*, *82*, 254–261. https://doi.org/10.1016/j.nbd.2015.06.012

Nestler, E. J., Barrot, M., DiLeone, R. J., Eisch, A. J., Gold, S. J., & Monteggia, L. M. (2002). Neurobiology of Depression. *Neuron*, *34*(1), 13–25. https://doi.org/10.1016/S0896-6273(02)00653-0

Olejniczak, I., Ripperger, J. A., Sandrelli, F., Schnell, A., Mansencal-Strittmatter, L., Wendrich, K., Hui, K. Y., Brenna, A., Ben Fredj, N., & Albrecht, U. (2021). Light affects behavioral despair involving the clock gene Period 1. *PLOS Genetics*, *17*(7), e1009625. https://doi.org/10.1371/journal.pgen.1009625

Ota, S. M., Kong, X., Hut, R., Suchecki, D., & Meerlo, P. (2021). The impact of stress and stress hormones on endogenous clocks and circadian rhythms. *Frontiers in Neuroendocrinology*, *63*, 100931. https://doi.org/10.1016/j.yfrne.2021.100931

Ota, S. M., Suchecki, D., & Meerlo, P. (2018). Chronic social defeat stress suppresses locomotor activity but does not affect the free-running circadian period of the activity rhythm in mice. *Neurobiology of Sleep and Circadian Rhythms*, *5*, 1–7. https://doi.org/10.1016/j.nbscr.2018.03.002

Overstreet, D. H., Fredericks, K., Knapp, D., Breese, G., & McMichael, J. (2010). Nerve growth factor (NGF) has novel antidepressant-like properties in rats. *Pharmacology Biochemistry and Behavior*, *94*(4), 553–560. https://doi.org/10.1016/j.pbb.2009.11.010

Paxinos, G., & Franklin, K. B. (2019). *Paxinos and Franklin's the mouse brain in stereotaxic coordinates*. Academic press.

Peeters, F., Nicholson, N. A., & Berkhof, J. (2003). Cortisol Responses to Daily Events in Major Depressive Disorder. *Psychosomatic Medicine*, *65*(5), 836–841. https://doi.org/10.1097/01.PSY.0000088594.17747.2E

Petković, A., & Chaudhury, D. (2022). Encore: Behavioural animal models of stress, depression and mood disorders. *Frontiers in Behavioral Neuroscience*, *16*, 931964.

Pfau, M. L., & Russo, S. J. (2015). Peripheral and central mechanisms of stress resilience. *Neurobiology of Stress*, *1*, 66–79. https://doi.org/10.1016/j.ynstr.2014.09.004

Poller, W. C., Madai, V. I., Bernard, R., Laube, G., & Veh, R. W. (2013). A glutamatergic projection from the lateral hypothalamus targets VTA-projecting neurons in the lateral habenula of the rat. *Brain Research*, *1507*, 45–60. https://doi.org/10.1016/j.brainres.2013.01.029

Proulx, C. D., Hikosaka, O., & Malinow, R. (2014). Reward processing by the lateral habenula in normal and depressive behaviors. *Nature Neuroscience*, *17*(9), 1146–1152. https://doi.org/10.1038/nn.3779

Radwan, B., Jansen, G., & Chaudhury, D. (2021a). Abnormal Sleep Signals Vulnerability to Chronic Social Defeat Stress. *Frontiers in Neuroscience*, *14*, 610655. https://doi.org/10.3389/fnins.2020.610655

Radwan, B., Jansen, G., & Chaudhury, D. (2021b). Sleep-wake dynamics pre- and post-exposure to chronic social stress. *IScience*, *24*(10), 103204. https://doi.org/10.1016/j.isci.2021.103204

Reichmann, F., & Holzer, P. (2016). Neuropeptide Y: A stressful review. *Advances in NPY Research*, *55*, 99–109. https://doi.org/10.1016/j.npep.2015.09.008

Sakhi, K., Belle, M. D. C., Gossan, N., Delagrange, P., & Piggins, H. D. (2014). Daily variation in the electrophysiological activity of mouse medial habenula neurones. *The Journal of Physiology*, *592*(4), 587–603. https://doi.org/10.1113/jphysiol.2013.263319

Sakhi, K., Wegner, S., Belle, M. D. C., Howarth, M., Delagrange, P., Brown, T. M., & Piggins, H. D. (2014). Intrinsic and extrinsic cues regulate the daily profile of mouse lateral habenula neuronal activity. *The Journal of Physiology*, *592*(22), 5025–5045. https://doi.org/10.1113/jphysiol.2014.280065

Salaberry, N. L., Hamm, H., Felder-Schmittbuhl, M.-P., & Mendoza, J. (2019). A suprachiasmatic-independent circadian clock(s) in the habenula is affected by Per gene mutations and housing light conditions in mice. *Brain Structure and Function*, *224*(1), 19–31. https://doi.org/10.1007/s00429-018-1756-4

Santini, E., Sepulveda-Orengo, M., & Porter, J. T. (2012). Muscarinic Receptors Modulate the Intrinsic Excitability of Infralimbic Neurons and Consolidation of Fear Extinction. *Neuropsychopharmacology*, *37*(9), 2047–2056. https://doi.org/10.1038/npp.2012.52

Sartorius, A., Kiening, K. L., Kirsch, P., von Gall, C. C., Haberkorn, U., Unterberg, A. W., Henn, F. A., & Meyer-Lindenberg, A. (2010). Remission of Major Depression Under Deep Brain Stimulation of the Lateral Habenula in a Therapy-Refractory Patient. *Biological Psychiatry*, *67*(2), e9–e11. https://doi.org/10.1016/j.biopsych.2009.08.027

Schmidt, M. V., Scharf, S. H., Sterlemann, V., Ganea, K., Liebl, C., Holsboer, F., & Müller, M. B. (2010). High susceptibility to chronic social stress is associated with a depression-like phenotype. *Psychoneuroendocrinology*, *35*(5), 635–643. https://doi.org/10.1016/j.psyneuen.2009.10.002

Shieh, K.-R. (2003). Distribution of the rhythm-related genes rPERIOD1, rPERIOD2, and rCLOCK, in the rat brain. *Neuroscience*, *118*(3), 831–843. https://doi.org/10.1016/S0306-4522(03)00004-6

Stamatakis, A. M., Van Swieten, M., Basiri, M. L., Blair, G. A., Kantak, P., & Stuber, G. D. (2016). Lateral Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral Habenula Regulate Feeding and Reward. *The Journal of Neuroscience*, *36*(2), 302–311. https://doi.org/10.1523/JNEUROSCI.1202-15.2016

Sweis, B. M., Veverka, K. K., Dhillon, E. S., Urban, J. H., & Lucas, L. R. (2013). Individual differences in the effects of chronic stress on memory: Behavioral and neurochemical correlates of resiliency. *Neuroscience*, *246*, 142–159. https://doi.org/10.1016/j.neuroscience.2013.04.052

Tahara, Y., Shiraishi, T., Kikuchi, Y., Haraguchi, A., Kuriki, D., Sasaki, H., Motohashi, H., Sakai, T., & Shibata, S. (2015). Entrainment of the mouse circadian clock by sub-acute physical and psychological stress. *Scientific Reports*, *5*(1), 11417. https://doi.org/10.1038/srep11417

Takahashi, J. S. (2017). Transcriptional architecture of the mammalian circadian clock. *Nature Reviews Genetics*, *18*(3), 164–179. https://doi.org/10.1038/nrg.2016.150

Tseng, P.-T., Lin, P.-Y., Tu, K.-Y., Cheng, Y.-S., Wu, C.-K., & Chen, Y.-W. (2015). Significantly lower nerve growth factor levels in patients with major depressive disorder than in healthy subjects: A meta-analysis and systematic review. *Neuropsychiatric Disease and Treatment*, 925. https://doi.org/10.2147/NDT.S81432

Vadnie, C. A., Petersen, K. A., Eberhardt, L. A., Hildebrand, M. A., Cerwensky, A. J., Zhang, H., Burns, J. N., Becker-Krail, D. D., DePoy, L. M., Logan, R. W., & McClung, C. A. (2022). The Suprachiasmatic Nucleus Regulates Anxiety-Like Behavior in Mice. *Frontiers in Neuroscience*, *15*, 765850. https://doi.org/10.3389/fnins.2021.765850

Vujovic, N., Gooley, J. J., Jhou, T. C., & Saper, C. B. (2015). Projections from the subparaventricular zone define four channels of output from the circadian timing system: Projections from the SPZ. *Journal of Comparative Neurology*, *523*(18), 2714–2737. https://doi.org/10.1002/cne.23812

Walker, W. H., Walton, J. C., DeVries, A. C., & Nelson, R. J. (2020). Circadian rhythm disruption and mental health. *Translational Psychiatry*, *10*(1), 28. https://doi.org/10.1038/s41398-020-0694-0

Wallace, M. L., Huang, K. W., Hochbaum, D., Hyun, M., Radeljic, G., & Sabatini, B. L. (2020). Anatomical and single-cell transcriptional profiling of the murine habenular complex. *ELife*, *9*, e51271. https://doi.org/10.7554/eLife.51271

Wang, H., Ferguson, G. D., Pineda, V. V., Cundiff, P. E., & Storm, D. R. (2004). Overexpression of type-1 adenylyl cyclase in mouse forebrain enhances recognition memory and LTP. *Nature Neuroscience*, *7*(6), 635–642. https://doi.org/10.1038/nn1248

Wang, J., Hodes, G. E., Zhang, H., Zhang, S., Zhao, W., Golden, S. A., Bi, W., Menard, C., Kana, V., Leboeuf, M., Xie, M., Bregman, D., Pfau, M. L., Flanigan, M. E., Esteban-Fernández, A., Yemul, S., Sharma, A., Ho, L., Dixon, R., … Pasinetti, G. M. (2018). Epigenetic modulation of inflammation and synaptic plasticity promotes resilience against stress in mice. *Nature Communications*, *9*(1), 477. https://doi.org/10.1038/s41467-017-02794-5

Wang, Y., Hu, P., Shan, Q., Huang, C., Huang, Z., Chen, P., Li, A., Gong, H., & Zhou, J.-N. (2021). Single-cell morphological characterization of CRH neurons throughout the whole mouse brain. *BMC Biology*, *19*(1), 47. https://doi.org/10.1186/s12915-021-00973-x

Warden, M. R., Selimbeyoglu, A., Mirzabekov, J. J., Lo, M., Thompson, K. R., Kim, S.-Y., Adhikari, A., Tye, K. M., Frank, L. M., & Deisseroth, K. (2012). A prefrontal cortex–brainstem neuronal projection that controls response to behavioural challenge. *Nature*, *492*(7429), 428–432. https://doi.org/10.1038/nature11617

Weil, T., Daly, K. M., Yarur Castillo, H., Thomsen, M. B., Wang, H., Mercau, M. E., Hattar, S., Tejeda, H., & Fernandez, D. C. (2022). Daily changes in light influence mood via inhibitory networks within the thalamic perihabenular nucleus. *Science Advances*, *8*(23), eabn3567. https://doi.org/10.1126/sciadv.abn3567

Winter, C., Vollmayr, B., Djodari-Irani, A., Klein, J., & Sartorius, A. (2011). Pharmacological inhibition of the lateral habenula improves depressive-like behavior in an animal model of treatment resistant depression. *Behavioural Brain Research*, *216*(1), 463–465. https://doi.org/10.1016/j.bbr.2010.07.034

Wu, G., Feder, A., Cohen, H., Kim, J. J., Calderon, S., Charney, D. S., & Mathé, A. A. (2013). Understanding resilience. *Frontiers in Behavioral Neuroscience*, *7*. https://doi.org/10.3389/fnbeh.2013.00010

Yamamoto, S., Shigeyoshi, Y., Ishida, Y., Fukuyama, T., Yamaguchi, S., Yagita, K., Moriya, T., Shibata, S., Takashima, N., & Okamura, H. (2001). Expression of the Per1 gene in the hamster: Brain atlas and circadian characteristics in the suprachiasmatic nucleus. *Journal of Comparative Neurology*, *430*(4), 518–532. https://doi.org/10.1002/1096-9861(20010219)430:4<518::AID-CNE1047>3.0.CO;2-H

Yang, J., Sun, J., Lu, Y., An, T., Lu, W., & Wang, J.-H. (2020). Revision to psychopharmacology mRNA and microRNA profiles are associated with stress susceptibility and resilience induced by psychological stress in the prefrontal cortex. *Psychopharmacology*. https://doi.org/10.1007/s00213-020-05593-x

Yang, L.-M., Hu, B., Xia, Y.-H., Zhang, B.-L., & Zhao, H. (2008). Lateral habenula lesions improve the behavioral response in depressed rats via increasing the serotonin level in dorsal raphe nucleus. *Behavioural Brain Research*, *188*(1), 84–90. https://doi.org/10.1016/j.bbr.2007.10.022

Yang, M., Ding, Q., Zhang, M., Moon, C., & Wang, H. (2020). Forebrain overexpression of type 1 adenylyl cyclase promotes molecular stability and behavioral resilience to physical stress. *Neurobiology of Stress*, *13*, 100237. https://doi.org/10.1016/j.ynstr.2020.100237

Yang, Y., Cui, Y., Sang, K., Dong, Y., Ni, Z., Ma, S., & Hu, H. (2018). Ketamine blocks bursting in the lateral habenula to rapidly relieve depression. *Nature*, *554*(7692), 317–322. https://doi.org/10.1038/nature25509

Yang, Y., Wang, H., Hu, J., & Hu, H. (2018). Lateral habenula in the pathophysiology of depression. *Current Opinion in Neurobiology*, *48*, 90–96. https://doi.org/10.1016/j.conb.2017.10.024

Young, C. J., Lyons, D., & Piggins, H. D. (2022). Circadian Influences on the Habenula and Their Potential Contribution to Neuropsychiatric Disorders. *Frontiers in Behavioral Neuroscience*, *15*, 815700. https://doi.org/10.3389/fnbeh.2021.815700

Zhang, C., Truong, K. K., & Zhou, Q.-Y. (2009). Efferent Projections of Prokineticin 2 Expressing Neurons in the Mouse Suprachiasmatic Nucleus. *PLoS ONE*, *4*(9), e7151. https://doi.org/10.1371/journal.pone.0007151

Zhang, H., Li, K., Chen, H.-S., Gao, S.-Q., Xia, Z.-X., Zhang, J.-T., Wang, F., & Chen, J.-G. (2018). Dorsal raphe projection inhibits the excitatory inputs on lateral habenula and alleviates depressive behaviors in rats. *Brain Structure and Function*, *223*(5), 2243–2258. https://doi.org/10.1007/s00429-018-1623-3

Zhao, H., & Rusak, B. (2005). Circadian firing-rate rhythms and light responses of rat habenular nucleus neurons in vivo and in vitro. *Neuroscience*, *132*(2), 519–528. https://doi.org/10.1016/j.neuroscience.2005.01.012

Zhao, Z., Xu, H., Liu, Y., Mu, L., Xiao, J., & Zhao, H. (2015). Diurnal Expression of the Per2 Gene and Protein in the Lateral Habenular Nucleus. *International Journal of Molecular Sciences*, *16*(8), 16740–16749. https://doi.org/10.3390/ijms160816740

Zheng, Z., Guo, C., Li, M., Yang, L., Liu, P., Zhang, X., Liu, Y., Guo, X., Cao, S., Dong, Y., Zhang, C., Chen, M., Xu, J., Hu, H., & Cui, Y. (2022). Hypothalamus-habenula potentiation encodes chronic stress experience and drives depression onset. *Neuron*, S0896627322000472. https://doi.org/10.1016/j.neuron.2022.01.011

Figure 1

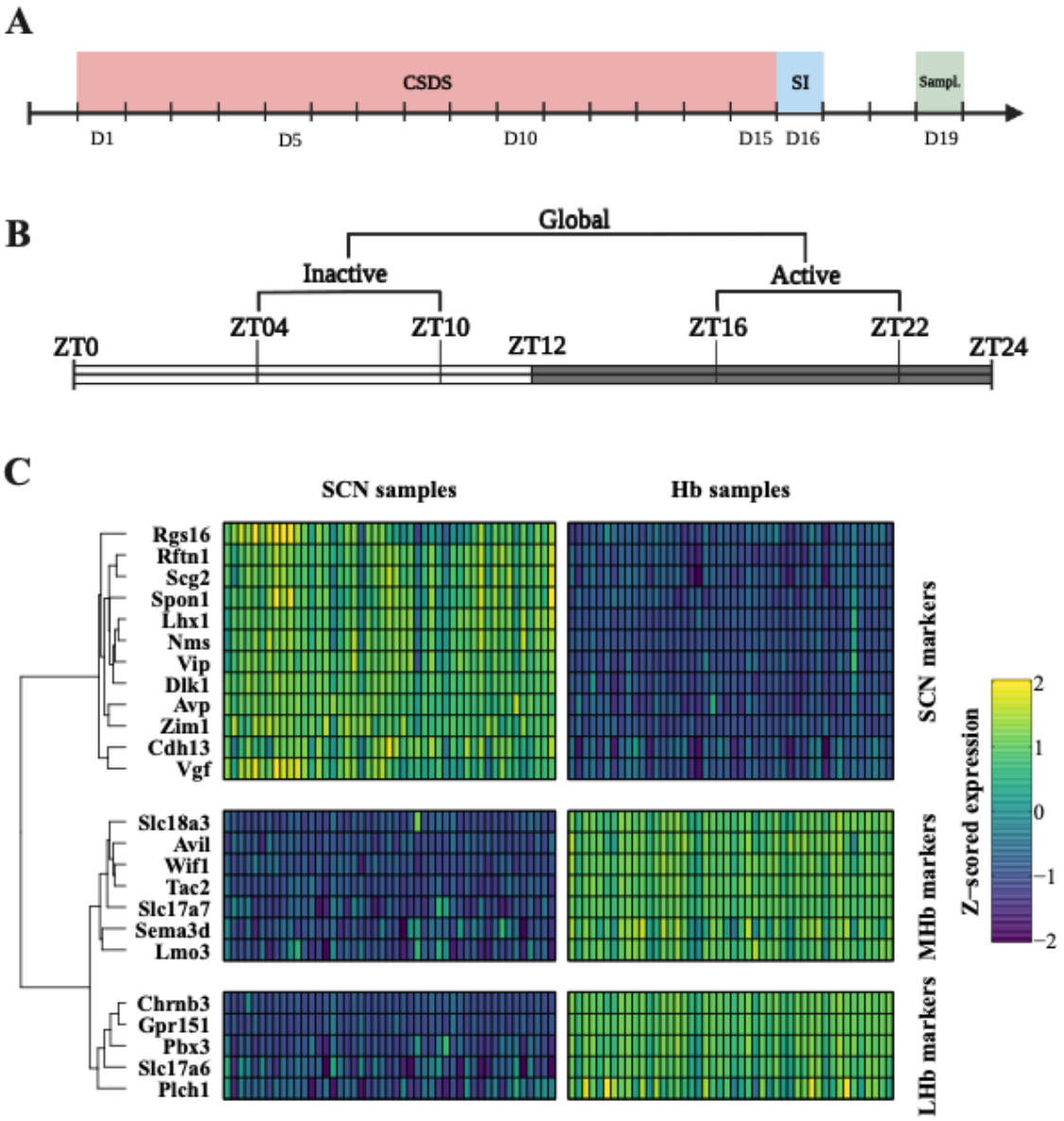


Figure 1. Experimental design and quality control. (A) Timeline for investigating the effect of stress on temporal changes in gene expression in stress-naïve and stress-exposed animals. **(B)** Sampling timepoints and emerging comparison levels from pooling the sampling timepoints. **(C)** Heatmap of canonical marker genes in the SCN and Hb. Normalized gene expression (variance stabilizing log2-transformed counts) across samples for reported marker genes specific to SCN, LHb and MHb (Wallace et al., 2020; Wen et al., 2020).

Figure 2

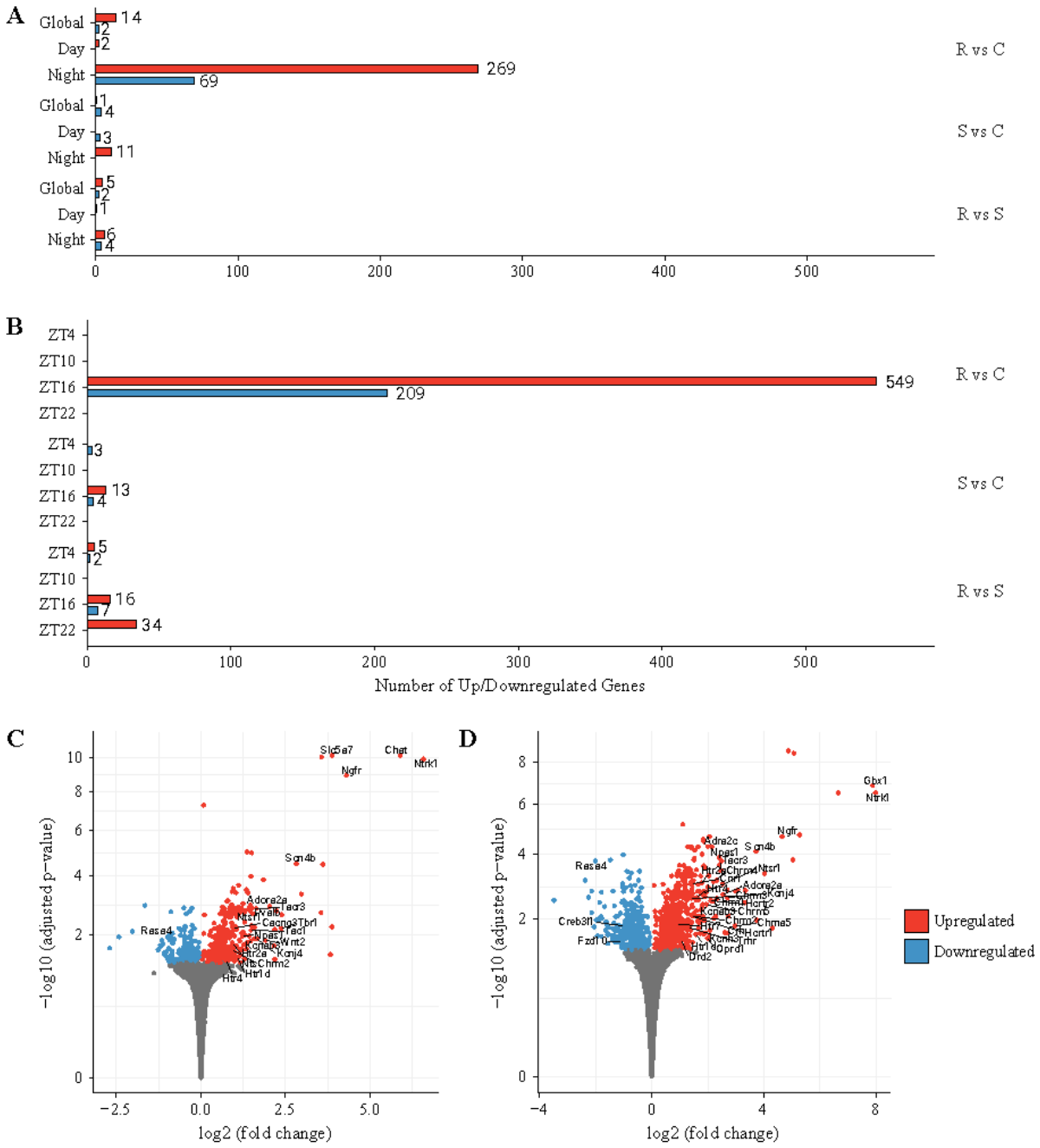


Figure 2. Differentially expressed genes (DEGs) at different time point levels across phenotypes in the SCN. (A) Number of DEGs at pooled timepoints. At the global comparison level, there were 16, 5 and 7 DEGs (FDR < 0.05 and |log2FC| > 0.5) in resilient-to-control, susceptible-to-control and resilient-to-susceptible comparisons, respectively. Daytime comparison revealed 2, 3 and 1 DEGs for resilient-to-control, susceptible-to-control and susceptible-to-resilient comparisons, respectively. Night time comparison revealed 338, 11 and 10 DEGs for

resilient-to-control, susceptible-to-control and susceptible-to-resilient comparisons, respectively. **(B)** Number of DEGs at specific timepoints. At ZT4, there were 0, 3 and 7 DEGs for resilient-to-control, susceptible-to-control and resilient-to-susceptible comparisons. At ZT10, there were no DEGs for any phenotypic comparison. At ZT16, there were 758, 17 and 23 DEGs for resilient-to-control, susceptible-to-control and resilient-to-susceptible comparisons, respectively. At ZT22, there were 0, 0 and 34 DEGs for resilient-to-control, susceptible-to-control and resilient-to-susceptible, respectively. **(C)** Nighttime comparison. Volcano plot showing the DEGs in resilient mice relative to controls at nighttime comparison level in SCN. **(D)** ZT16 comparison. Volcano plot showing the significant differentially expressed genes in resilient mice relative to controls at ZT16 in SCN. Red and blue points represent significantly up-regulated and down-regulated genes, respectively.

Figure 3

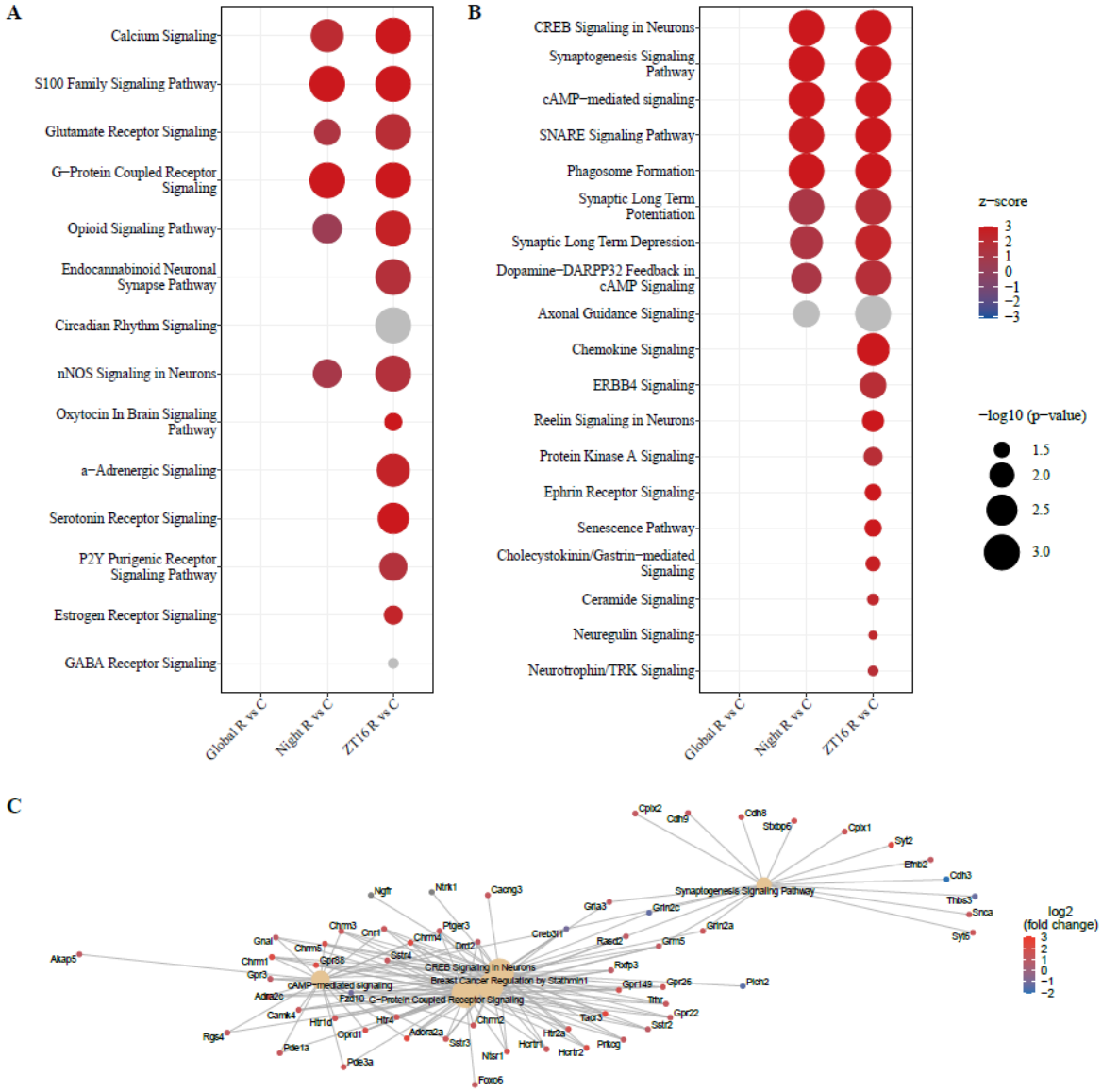


Figure 3. Enriched pathways in the SCN of resilient mice relative to controls. The activation pattern of enriched biological pathways in the SCN. All lists are sorted by adjusted p-value. Red denotes activation ($z > 0$), blue denotes inhibition ($z < 0$), white denotes no net effect ($z = 0$), and grey denotes unpredicted activity. **(A)** Enriched signaling pathways. **(B)** Enriched plasticity-associated pathways. **(C)** Gene-concept networks of the most enriched IPA pathways at ZT16 between resilient and control phenotypes in the SCN.

Figure 4

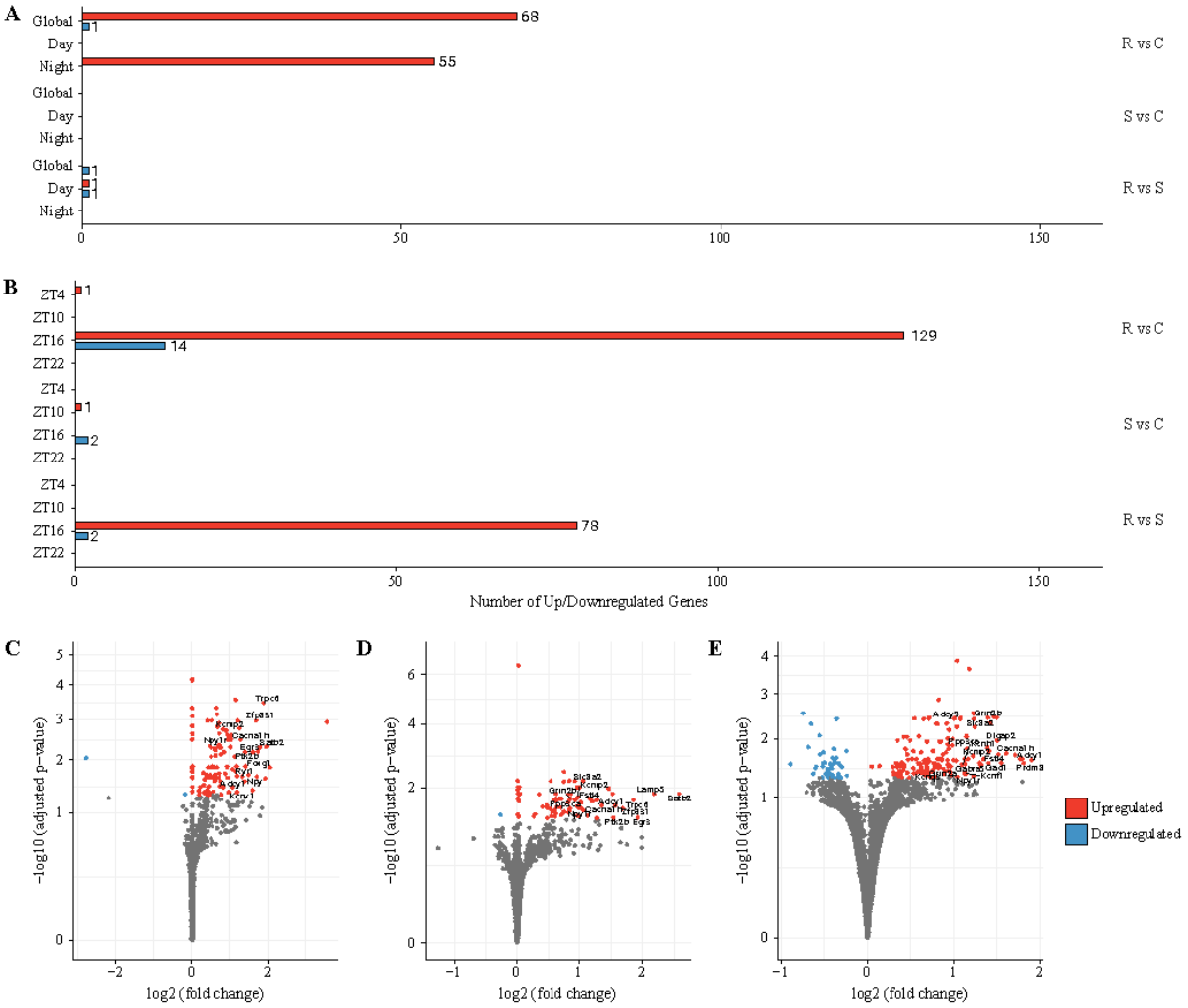


Figure 4. Differentially expressed genes (DEGs) at different time point levels across phenotypes in the Hb. (A) Number of DEGs at pooled timepoints. At the global comparison level, there were 69, 0 and 1 DEGs for resilient-to-control, susceptible-to-control and resilient-to-susceptible comparisons, respectively. At the daytime comparison level, there were 0, 0 and 2 DEGs for resilient-to-control, susceptible-to-control and susceptible-to-resilient comparisons, respectively. At the nighttime comparison level, there were 55, 0 and 0 DEGs for resilient-to-control, susceptible-to-control and susceptible-to-resilient, comparisons, respectively. **(B)** Number of DEGs at specific sampled timepoints. At ZT4, there were 1, 0 and 0 DEGs for resilient-to-control, susceptible-to-control and resilient-to-susceptible comparisons. At ZT10, there were no DEGs for resilient-to-control and resilient-to-susceptible comparisons, and only 1 DEG in susceptible-to-control comparison. At ZT16, there were 143, 2 and 80 DEGs for resilient-to-control, susceptible-to-control and resilient-to-susceptible comparisons, respectively. At ZT22, there were no DEGs in any of the phenotypic comparisons. **(C)** Global comparison. Volcano plot showing the DEGs in resilient mice relative to controls at global comparison level in Hb. DEGs were labeled based on |log2FC| > 1.0 and biological relevance. **(D)** Nighttime comparison. Volcano plot showing the DEGs in resilient mice relative to controls at nighttime comparison level in Hb. **(E)** ZT16 comparison. Volcano plot showing the DEGs in resilient mice relative to controls

at ZT16 timepoint in Hb. Red and blue points represent significantly upregulated and downregulated genes, respectively.

Figure 5

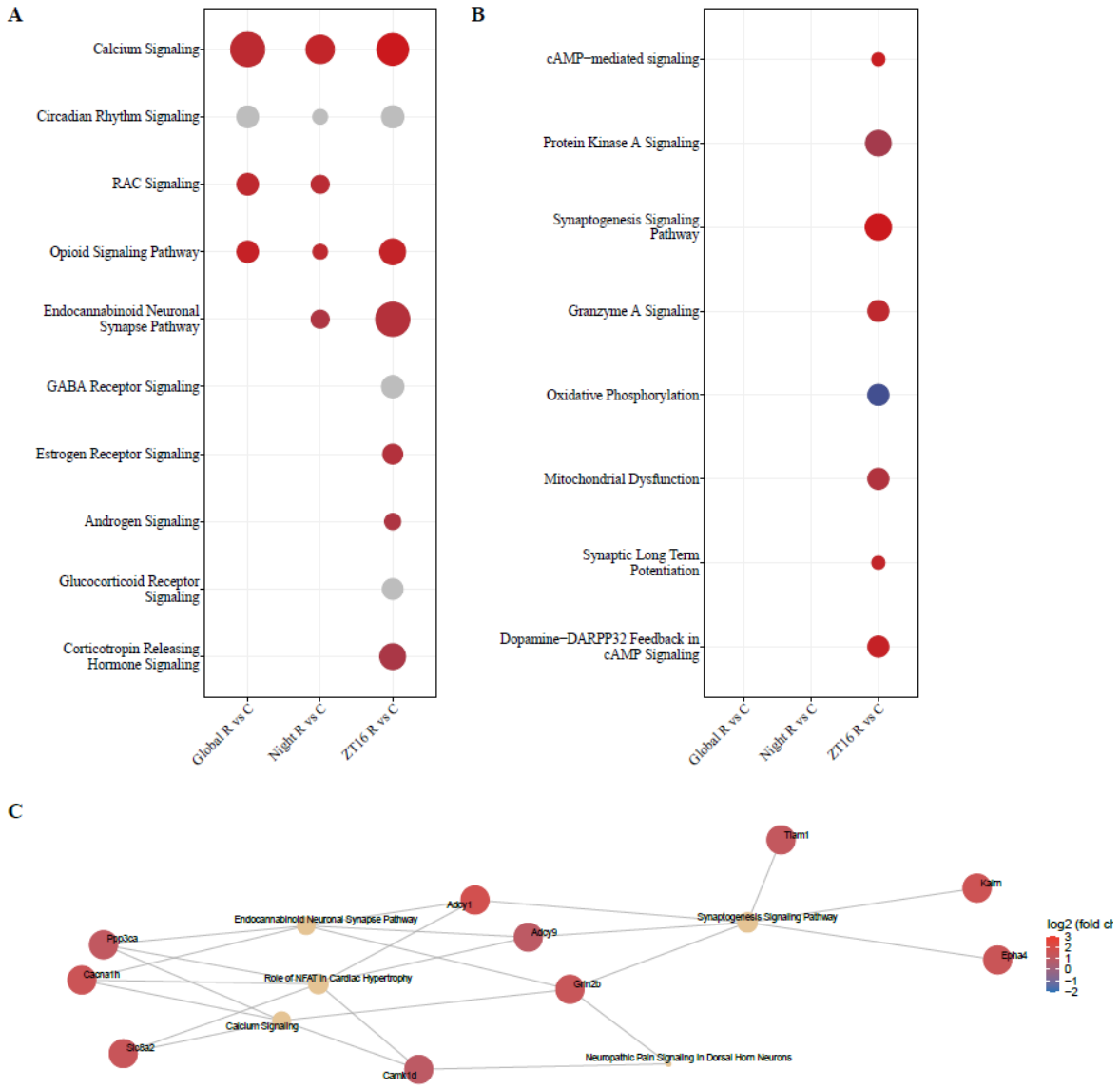


Figure 5. Enriched pathways in the Hb of resilient mice relative to controls. The activation pattern of enriched biological pathways in the Hb. All lists are sorted by adjusted p-value. Red denotes activation ($z > 0$), blue denotes inhibition ($z < 0$), white denotes no net effect ($z = 0$), and grey denotes unpredicted activity. **(A)** Enriched signaling pathways. **(B)** Enriched plasticity-associated pathways. **(C)** Gene-concept networks of the most enriched IPA pathways at ZT16 between resilient and control phenotypes in the Hb.

Figure 6

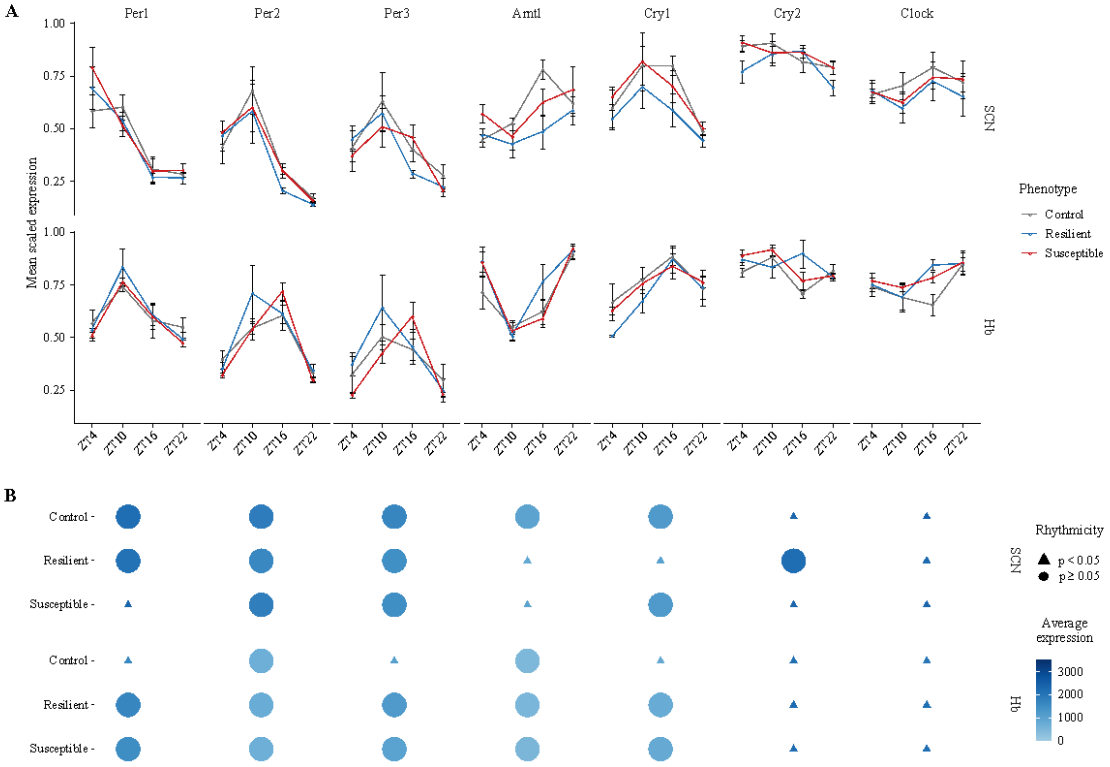
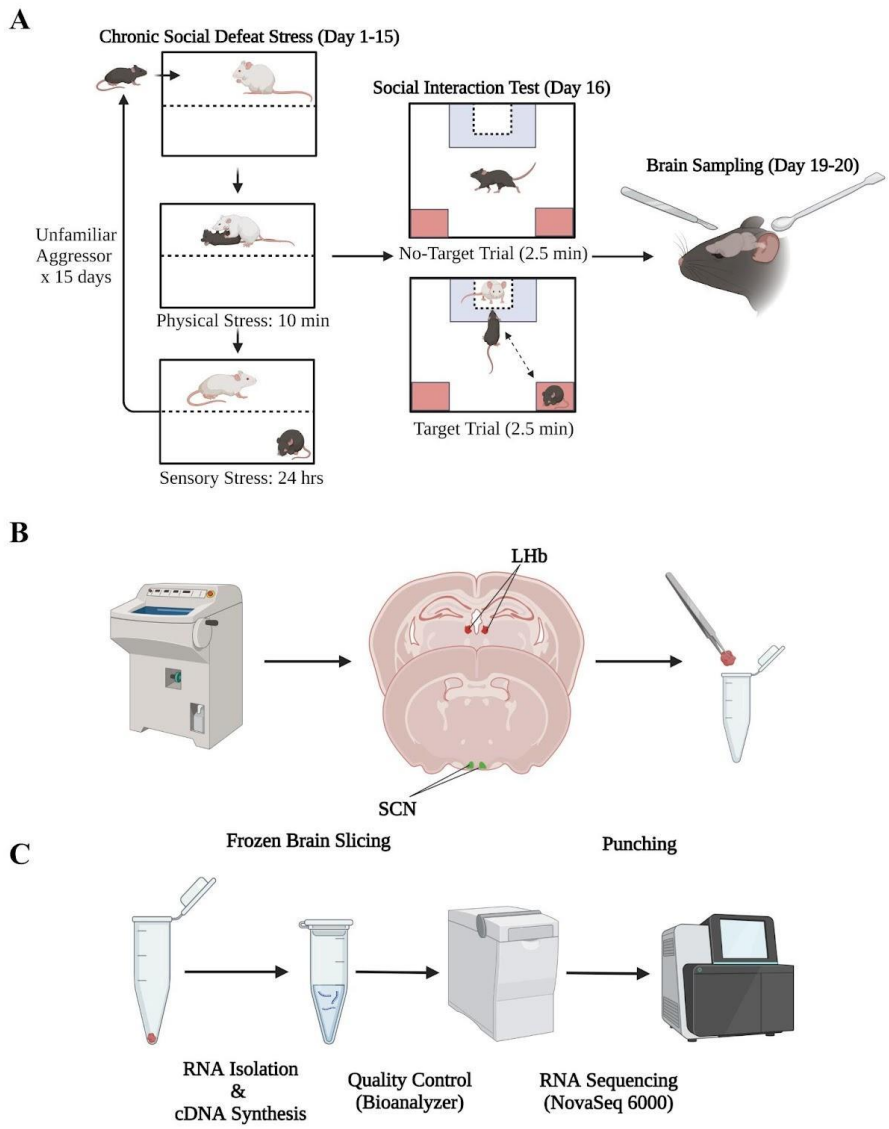


Figure 6. Clock gene rhythmicity. (A) Temporal expression of core clock genes in the SCN and Hb of control, resilient and susceptible mice. All the clock genes show previously reported temporal expression in the SCN of control mice. **(B)** Dotplot showing rhythmic expression of clock genes in SCN and Hb. **SCN**: *Per1* and *Cry1* are arhythmic in susceptible and resilient mice respectively. *Arntl* is arrhythmic in both resilient and susceptible mice. **Hb**: *Per1*, *Per3* and *Cry1* are arrhythmic in the control mice, while being rhythmic in both the resilient and susceptible mice.

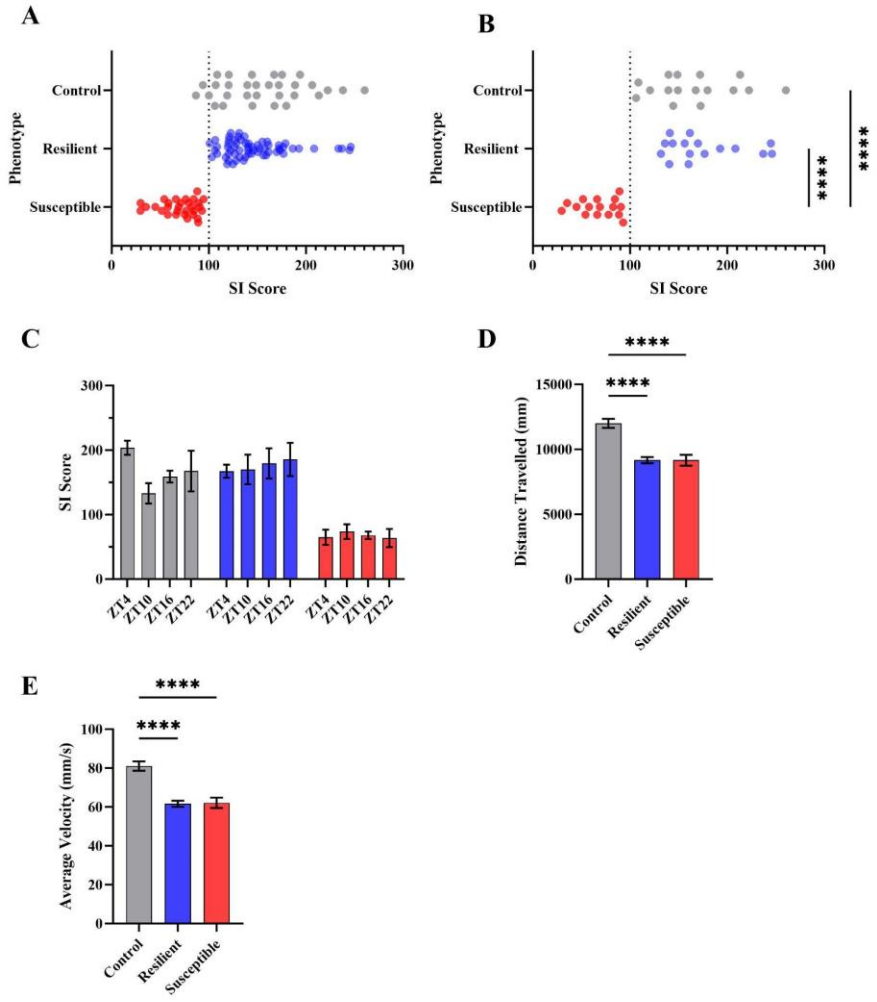
Supplemental Figures

Supplemental figure 1



Supplemental figure 1. **Graphical summary of the methodology. (A)** Behavioural experimental methods and timeline. **(B)** Experimental methods for slicing and tissue punching. **(C)** Experimental methods for RNA isolation, quality control and sequencing.

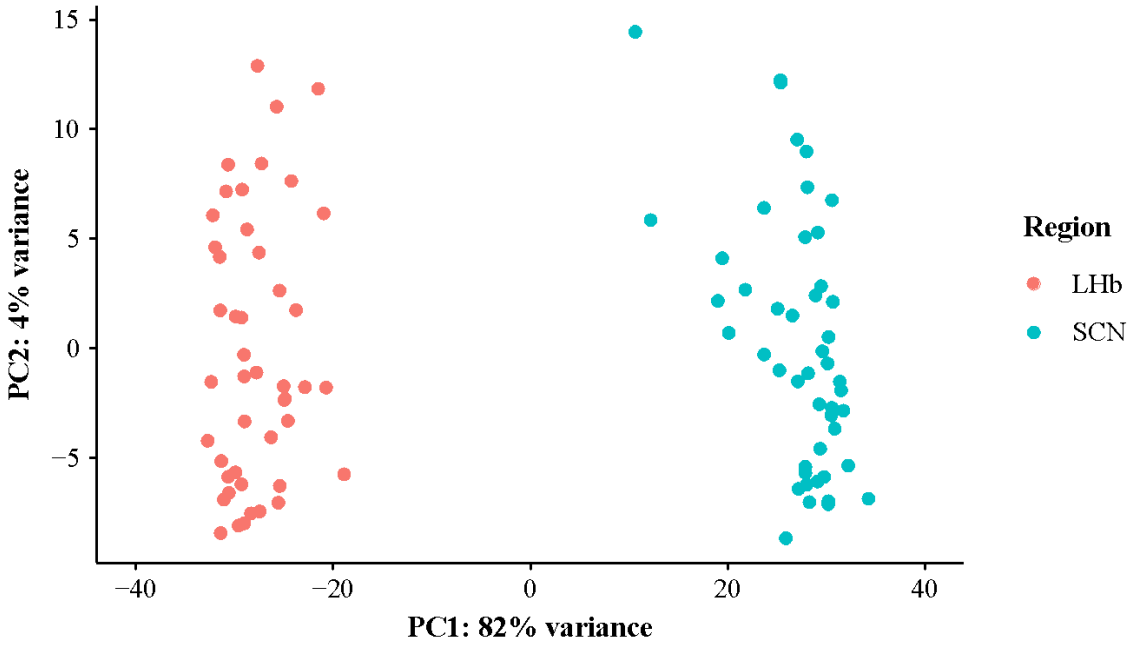
Supplemental figure 2



Supplemental Figure 2. **Additional behavioural measures. (A)** One-way ANOVA revealed significant variation in SI scores ($F(2,120) = 63.3$, $p < .001$). Tukey's post hoc comparison revealed significant differences between controls and susceptible mice ($p < .001$, 95% C.I. = [65.4, 106.8]), and resilient and susceptible mice ($p < .001$, 95% C.I. = [58.9, 94.9]). Stress-naïve and resilient mice did not differ in SI scores ($p = .44$, 95% C.I. = [-8.6, 26.9]). **(B)** One-way ANOVA revealed significant variation in SI scores of sequenced mice ($F(2,45) = 45.3$, $p < .001$). Tukey's post hoc comparison revealed significant differences between sequenced control and susceptible mice ($p < .001$, 95% C.I. = [67.8, 128.6]), and resilient and susceptible mice ($p < .001$, 95% C.I. = [77.9, 138.4]). Stress-naïve and resilient mice did not differ in SI scores ($p = .72$, 95% C.I. = [-40.2, 20.6]). **(C)** Within phenotype, sample groups did not differ between timepoints in the SI scores. **(D)** One-way ANOVA for total distance travelled was significant ($F(2,120) = 23.3$, $p < .001$). The observed differences were significant between control and susceptible mice ($p < .001$, 95% C.I. =

[1624, 4059]), and control and resilient mice ($p < .001$, 95% C.I. = [1796, 3889]) (Fig.1C). **(E)** One-way ANOVA for average velocity travelled was significant ($F(2,120) = 24.2$, $p < .001$). There were significant differences in average velocity between control and susceptible mice ($p < .001$, 95% C.I. = [10.8, 27]), and control and resilient mice ($p < .001$, 95% C.I. = [12.5, 26.4]). All values and bar plots are reported as mean±SEM. Significance levels: ** $p < .01$, *** $p < .001$.

Supplemental figure 3



Supplemental Figure 3. PCA plots showing clear separation between SCN and Hb samples. First principal component represents the regional identity of the samples and explains 82% of data variability.