Chronic social stress blunts core body temperature and molecular rhythms of *Cirbp* and *Rbm3* in mice lateral habenula

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Abstract (200 words)

Chronic social stress in mice causes behavioral and physiological changes resulting in perturbed rhythms of body temperature, activity and sleep-wake cycle. To further understand the link between mood disorders and temperature rhythmicity, we measured core body temperature (Tcore) in mice before and after exposure to the chronic social defeat stress (CSDS) paradigm. Our results revealed that Tcore amplitudes of stress resilient and susceptible mice are dampened during exposure to CSDS . However, after exposure to social stress, the temperature amplitude of resilient mice recovered faster than the susceptible mice. There were minimal changes in locomotor activity after stress exposure which correlates with regular rhythmic expression of *Prok2*, an output signal of the SCN (Suprachiasmatic nucleus). We also investigated the molecular changes associated with these dampened temperature rhythms, and determined the expression levels of thermosensitive genes *Cirbp*, *Rbm3* and *Hsf1*. Expression of *Rbm3* and *Cirbp* in the LHb (Lateral habenula) were blunted 1 day after CSDS. These molecular rhythms recovered 10 days later such that daytime expression was higher compared to nighttime, similar to stress-naive controls. *Hsf1* did not show robust rhythmic expression in the LHb.

Keywords

Core body temperature, chronic stress, mood disorder, blunted temperature rhythms, thermosensitive genes, circadian rhythms, Cirbp, Rbm3, Hsf1, Prok2.

Introduction

Daily cycles of light and temperature are the two primary environmental timing cues used by living systems to entrain their endogenous circadian rhythms to the solar day. In mammals, the suprachiasmatic nucleus (SCN), the principal biological clock, drives circadian rhythms of core body temperature (Tcore), which is maintained within a narrow range to allow optimal physiological functioning [1,2]. These rhythms can be entrained by external cues, predominantly light, but in the absence of these cues they exhibit endogenous free running characteristics. Tcore in homeothermic organisms are measured by internal and peripheral thermoreceptors. The preoptic area (POA) of the hypothalamus is the primary integrative site for thermoregulation [3]. The narrow range of Tcore rhythms is maintained by a complex feedback system consisting of heat-loss mechanisms that are activated when Tcore temperature rises, and heat-gain mechanisms which are activated when it falls [4-6]. It is hypothesized that daily Tcore rhythms are generated by SCN projections into hypothalamic regulatory centers and by modulation of thermogenic brown adipose tissue [7]. Moreover, body temperature is an intrinsic zeitgeber because changing the amplitude and frequency of temperature rhythms affects the rhythmic expression of key clock genes like *Bmal1* (Basic Helix-Loop-Helix ARNT Like 1) and *Per1* (Period 1) [8]. These changes are likely driven by changes in expression of thermo-sensitive genes such as *Rbm3* (RNA-binding Motif Protein 3), *Cirbp* (Cold-inducible RNA binding protein) and *Hsf1* (Heat Shock Factor 1) which are chaperones that modulate expression of a variety of target genes including the clock genes [6, 9-12].

Mood disorders and aberrations in Tcore rhythms are closely related. For example, patients often exhibit a shift or blunting of diurnal activity of cortisol and Tcore rhythms during depressive episodes [13, 14]. The relationship between circadian rhythms and depression is commonly attributed to molecular and pathophysiological changes in circuits connecting brain regions that regulate emotion, locomotor activity, sleep and temperature regulation [15]. Clinical studies indicate a relationship between desynchronization of Tcore rhythms and symptom severity, however, it is unclear whether the relationship is correlational or causal [14]. While the effects of stress on activity, sleep-wake cycles, and molecular rhythms have been investigated, mechanisms linking stress and Tcore rhythms need further exploration.

Stress-induced thermogenesis of brown adipose tissue resulting in emotional hyperthermia may be driven by the Lateral Habenula (LHb) and Ventral Tegmental Area (VTA) [16, 17] - two regions

that exhibit elevated pathophysiological firing in stress susceptible mice [18-21]. Pathophysiological changes in these regions may be responsible for stress induced changes in temperature rhythms. Moreover, changes in the daily rhythms of molecular processes in the SCN and LHb have been associated with the development of a variety of mood disorders [19, 22]. At the molecular level, *Rbm3* and *Cirbp* are expressed in response to lower temperatures while *Hsf1* expression is elevated in response to increased heat stress [23]. Deletion of *Rbm3* leads to a significant reduction in expression amplitudes of *Bmal1*,*Clock*, *Per1* and *Cry1* (Cryptochrome 1) [9]. Furthermore, patients affected by mood disorders exhibit a higher expression of *Rbm3* compared to healthy controls [24]. In the cell nucleus, *Cirbp* binds to the 3'-UTR of *Clock* mRNA and promotes its translocation to the cytoplasm, thereby enabling its translation [10-12]. *Hsf1* interacts with clock genes, such as *Bmal1,* in order to maintain synchronization of the cellular clock and clock-controlled adaptive responses to cellular stress [25]. Absence of *Hsf1* activity can lead to impaired hippocampal development and severe behavioral disruptions, such as depression and aggression [26]. We hypothesized that disrupted temperature rhythms in the mice model of stress and mood disorders cause perturbations in expression of thermosensitive genes which may lead to pathophysiological changes in the LHb and SCN. Further studies into changes in Tcore in a mouse model of stress and mood disorders will add valuable insight into molecular and physiological processes underlying these pathologies.

To understand the association between Tcore and mood disorders we used the chronic social defeat stress (CSDS) paradigm to investigate the effects of chronic stress on Tcore and expression of *Cirbp*, *Rbm3* and *Hsf1* (Fig. 1). Prior to stress all mice exhibited similar daily Tcore rhythms. During stress exposure both stress resilient and susceptible mice exhibited blunted Tcore rhythms. Tcore amplitudes were significantly blunted in susceptible, but not resilient mice, compared to controls during the first 5 days post-CSDS (recovery period). By days 6-10 of post-CSDS recovery, the average Tcore amplitude of susceptible mice had recovered as indicated by absence of differences between susceptible and control mice (Supplemental Table 2). Furthermore, control mice exhibit daily rhythmic expression of *Rbm3* and *Cirbp* in the SCN and LHb where transcript levels were higher in the day than the night in concordance with decreased core body temperature in the day compared to the night. In contrast, *Rbm3* and *Cirbp* expression is blunted post-CSDS in both resilient and susceptible mice 1 day after chronic stress. However, rhythmic expression returns to normal 10 days later.

Methods

Ethics statement and animals: Animal protocols have been approved by the National Institute of Health Guide for Care and Use of Laboratory Animals (IACUC Protocol: 150005A2, 19-0004A1), as well as the NYUAD Animal Care and Use Committee. C57BL6/J mice of 5-7 weeks old were purchased from Jackson Laboratory (Bar Harbor, MS, USA). Single housed male CD1 retired breeder mice from Charles River laboratory (Wilmington, MS, USA) were used as resident aggressors for the CSDS paradigm. All experimental mice were maintained within standard housing conditions at a temperature of 21±2°C and humidity 50±10% controlled conditions with ad libitum access to food and water, as well as enrichment in the form of wood shavings. Mice were kept on a 12-to-12-hour light/dark cycle (light onset at 7am - ZT0, light offset at 7pm – ZT12).

Locomotor activity recording: Locomotor activity during baseline and recovery periods was measured using Actimetrics ClockLab wireless passive infrared (PIR) sensor system. The infrared sensor was placed on top of each cage, the data was collected using ClockLab Data Collection Software and analyzed with ClockLab Analysis Software (version 6.1.10).

Core body temperature recordings: Mice were anesthetized with a ketamine (100 mg kg−1) and xylaxine (10 mg kg−1) mixture. A wireless electronic telemetry pill (Anipill; BodyCap, Paris, France) was surgically implanted into the peritoneal cavity of all experimental mice. This surgical implantation was carried out by adopting and modifying a previous protocol [27]. The Anipill system was configured to collect temperature measurements every 15 minutes. Temperature recordings were set to begin immediately after pill activation and continued to collect and transmit data for the entire duration of the experiment. An open-source online software, Biodare2, was utilized to derive circadian parameters such as amplitude, phase, and period of temperature rhythms [28]. Fast Fourier Transform Non-Linear Least Squares (FFT NLLS) was employed to analyze average hourly data.

Chronic social defeat stress and behavioral analysis: For a period of 15 days, experimental C57BL/J6 mice were exposed to a territorial and aggressive CD1 mouse for 10 minutes of physical interaction. After each defeat session, C57BL/J6 mice were separated from the CD1 mice in the same cage using a perforated plexiglass divider that still enabled olfactory and visual sensory exposure. To prevent CD1 mice from becoming familiar with the experimental mice, C57BL/J6 mice were exposed to a novel aggressor each day. Control mice were kept in pairs in a similar cage set-up, with a plexiglas divider separating two control mice at all times. These mice were kept in the same room as experimental mice in order to keep ambient temperature constant.

To assess social aversion as a measure of depressive-like behavior, a white square plexiglass arena (dimensions 42 X 42 X 42.5cm) was used. The SI test, conducted under red light, consisted of two 2.5 minute sessions per mouse: one without a CD1 social target and with a CD1 social target. Data collected from TopScan was used to compare the time each mouse spent in each of the pre-defined zones. Mice were phenotyped based on the social interaction ratio (SI ratio) scores, which was calculated as described previously [29]. Experimental mice with scores < 100 were classified as 'susceptible' and those with scores ≥ 100 were classified as 'resilient'.

Brain sampling for molecular analysis: Sampling was started 24 hours following SI and completed within three days. Mice were anesthetized using 0.25ml isoflurane and then sacrificed by cervical dislocation followed by decapitation. Extractions were carried out on a cold sterile surface, and the extracted brains were directly immersed into cold isopentane (Sigma Aldrich M3263) and flash-frozen. Brains were sliced using the Leica CM1950 cryostat, using mouse brain atlas [30] as a reference to facilitate accurate identification of the chosen brain regions – the SCN and the LHb. Brain slices were visualized under a Leica stereoscope and tissue punches of the regions were collected into 1.5ml EppendorfTM DNA LoBind tubes and snap frozen using dry ice before being stored at -80℃ until RNA isolation.

RNA isolation and quantitative PCR : RNA was isolated using Qiagen RNeasy Micro Kit, as per manufacturer's instructions, and quantified using Nanodrop 8000 (Thermo Scientific). The

isolated RNA was converted to cDNA using the Thermoscientific Maxima H Minus First Strand cDNA Synthesis Kit as per manufacturer's protocol. The template cDNA was amplified using Qiagen Primers for the transcripts: Hsf1 (NM_008296), Rbm3 (NM_016809), Cirbp (NM_007705) and Prok2 (NM_015768) to quantify levels of mRNA in the SCN and LHb with GAPDH (NM_008804) as the housekeeping gene. Non-template controls were run for each primer pair. Thermofisher Applied Biosystems QuantStudio5 Real-Time PCR System was used for qPCR.

Statistical analysis: Prism 9 (Graphpad Software, La Jolla, CA, USA) was used for data plotting and statistical analyses. Statistical significance was determined using one-way and two-way ANOVAs, two-tailed independent *t*-tests and Tukey's HSD multiple comparison test (Supplemental table 1-2). All values are expressed as the mean ± standard error of the mean (SEM). The sample size (*n*) is noted in the specific figure legends for each experiment.

Results and discussion

We found that daily rhythms in Tcore are affected by chronic social stress. Prior to stress, all mice exhibited lowest Tcore in the daytime when mice are least active and highest at nighttime where they are most active (Fig. 2a(i,ii)). However, during CSDS both resilient and susceptible mice generally exhibited higher Tcore in the daytime and lower Tcore in the nighttime compared to controls (Fig. 2b(i,ii)). Daytime Tcore of resilient mice returns to control levels immediately after CSDS, while susceptible mice continue to exhibit elevated Tcore for a longer period during the recovery phase (Fig. 2c(i,ii)). Analysis of Tcore rhythms was performed by measuring Tcore amplitude during baseline, CSDS and recovery (Fig. 2d). During CSDS, both resilient and susceptible mice exhibited significantly lower Tcore amplitudes compared to controls. However, during the first 5 days of recovery period, Tcore amplitudes were significantly blunted in susceptible, but not resilient mice, compared to controls. In contrast there were no significant differences in Tcore amplitude between control and susceptible from day 6 to 10-days of the recovery period. These observations align with clinical findings that patients with depression exhibit blunted diurnal rhythms where nighttime temperatures are abnormally high (when people are usually least active), while day time temperatures are largely unaffected [14]. Previous studies have shown aberrations in physiological functioning in susceptible mice [18, 31-33]. It is likely that homeostatic factors that maintain Tcore in resilient mice buffer against stress-induced

processes that drive elevated daytime Tcore and greater blunting of Tcore amplitudes in susceptible mice. Our study correlates with previous observations that more severe stress induces significantly greater blunting of temperature rhythms [34]. We had previously shown that susceptible and resilient mice displayed reduced overall sleep homeostasis [35]. Moreover, susceptible mice exhibited significant deficient NREM recovery sleep responses and increased NREM sleep fragmentation following CSDS [35-36]. Since sleep and Tcore are closely related it may be that chronic stress- causes pathophysiological changes in mechanisms that regulate both the sleep architecture and Tcore regulation .

Next we investigated whether expression of the thermosensitive genes *Rbm3*, *Cirbp* and *Hsf1* correlates with the observed changes in temperature profiles immediately after CSDS and 10-days of recovery. *Rbm3* and *Cirbp* are both expressed in response to lower temperatures, oscillate diurnally and have been linked to circadian function [6, 37, 38]. The expression of *Rbm3* and *Cirbp* in the LHb of control mice is significantly higher in the day (ZT11), when Tcore is lower, compared to the night (ZT20), when Tcore is higher (Fig. 3a(i),b(i)). In contrast, immediately after CSDS, there was no significant difference between day and night expression of *Rbm3* and *Cirbp* in the LHb of susceptible and resilient mice. However, after 10 days of recovery, the diurnal differences in expression of *Rbm3* and *Cirbp* (Fig. 3a(ii),b(ii)) in the LHb of both susceptible and resilient mice are similar to control mice suggesting recovery of daily rhythmic expression following the recovery of temperature rhythms.These results correlate with the previous findings that mild hypothermia results in peak expression of *Cirbp* and *Rbm3* which decreases significantly following deep hypothermia [11]. In turn, hyperthermia causes substantial decreases in expression of these genes in cultured mammalian cells [11].

Chronic mild stress affects rhythmic expression of clock genes in various brain regions including the LHb [39]. We speculate that this may be due to stress-induced changes in *Rbm3* and *Cirbp* expression in the LHb. Following 10 days of post-CSDS recovery, both susceptible and resilient mice show robust daily rhythms where daytime expression was significantly greater than night (Fig 3a(ii),b(ii)). Our observations of the effect of stress on temperature and molecular rhythms are in close agreement with previous findings where repeated 5-day social defeat stress resulted in decreased amplitude of heart rate and body temperature which persisted for at least 10-days after the final confrontation [40]. *Rbm3* regulates local synaptic translation and neuronal activity, and knockdown of *Rbm3* at specific times of the day increases firing of cultured hippocampal cells

[38]. Changes in clock gene expression induced by sleep deprivation is hypothesized to be regulated by increased daytime *Cirbp* expression when mice are asleep and Tcore is lower, and decreased nighttime expression when mice are active and Tcore is higher [41]. When mice are asleep, the increased expression of *Cirbp* attenuates expression of wake-inducing/sustaining genes, such as *Homer.* Sleep deprivation leads to prolonged attenuation of *Cirbp* expression and, consequently, increased expression of wake-inducing genes [41].

Since we did not observe any effect of stress on locomotor rhythms, we wondered whether this lack of effect was associated with expression patterns of genes that regulate daily cycles of locomotion. Prokineticin 2 (*Prok2)* is one such clock-controlled gene whose product is an important neuropeptide output signal of the SCN that regulates locomotor rhythms [42]. Our data shows that *Prok2* undergoes robust rhythmic expression in the SCN (Supplemental Fig. 4). This implies that stress exposure does not affect rhythmic expression in the SCN as both resilient and susceptible mice express higher levels of *Prok2* during daytime relative to the nighttime, as previously reported [43] (Supplemental Fig. 4). Moreover, the rhythmic expression of *Prok2* in susceptible and resilient mice is comparable to control mice, with the locomotor data showing all phenotypes exhibit similar locomotor rhythms before and after stress exposure (Supplemental Fig. 2).

A relatively stable expression of *Prok2,* in the SCN in stress-exposed mice supports the hypothesis that the SCN is buffered against environmental stressors, enabling normal entrainment to light . Moreover, clock gene expression following CMS was shown to be stable in the SCN but not in other regions involved in mood regulation [39]. Previous, light pulse experiments on stress-exposed and control groups revealed phase delay of the body temperature rhythm without any changes in the circadian pacemaker functions [44]. The findings on locomotor activity and *Prok2* expression from our study closely match previous studies [39,44] and indicate that pacemaker function remains unaffected by different forms of chronic stress (Supplemental Fig.3).

Hsf1 did not show robust rhythms in either LHb or SCN. Studies have shown that exposure to decreased temperature (cold shock) does not generate a significant expression of *Hsf1*, consistent with the findings from this study [45]. Additionally, the lack of significant changes within the LHb indicate that there may be region specific differences in rhythmic expression following exposure to chronic stress (Supplemental Fig. 5).

Conclusion

Taken together, this study provides further evidence for stress-induced changes in temperature regulation and differential expression of thermo-sensitive genes. Importantly, it identifies some physiological differences in daily temperature cycles between susceptible and resilient mice. These represent crucial steps into a more systematic understanding of the temperature imbalances experienced by patients diagnosed with mood disorders. The preliminary findings of this study highlight the interplay between temperature dysregulation and thermo-responsive gene expression in the LHb. One limitation of this study is the lack of multiple sampling timepoints across the day-night cycle that would assess rhythmic gene expression with better resolution. Future studies are warranted to include more timepoints, investigate expression of other clock genes and their rhythmicity in response to stress. Some genes may appear relatively unaffected at the mRNA levels but the protein levels may be altered. Future experiments to investigate the changes in protein expression would enable an additional level of insight into the interplay of stress and core body temperature regulation.

Abbreviations

Core body temperature (Tcore), Suprachiasmatic nucleus (SCN), Preoptic area (POA), Brown adipose tissue (BAT), Bmal1 ((Basic Helix-Loop-Helix ARNT Like 1), Per1 (Period 1), RNA-binding Motif Protein 3 (Rbm3) , Cold inducible RNA binding protein (Cirbp), Heat Shock Factor 1 (Hsf1) , LHb (Lateral Habenula), VTA (Ventral Tegmental Area), chronic social defeat stress (CSDS) , Passive Infrared (PIR), Fast Fourier Transform Non-Linear Least Squares (FFT NLLS).

Author contributions

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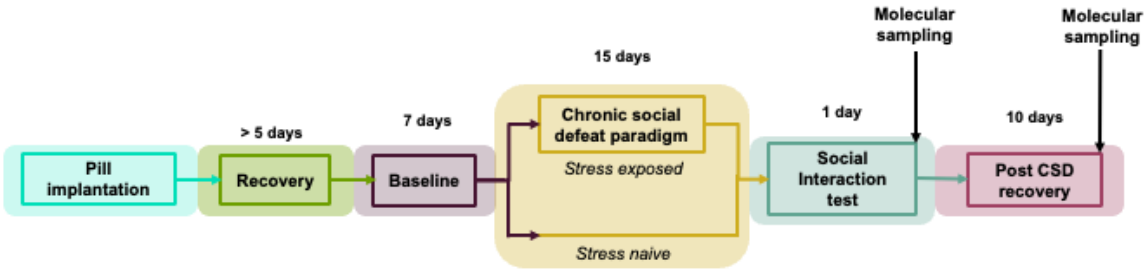
Figure 1



General procedure and timeline for investigating the association between stress, diurnal temperature rhythms and changes in expression of thermosensitive genes.

Figure 2

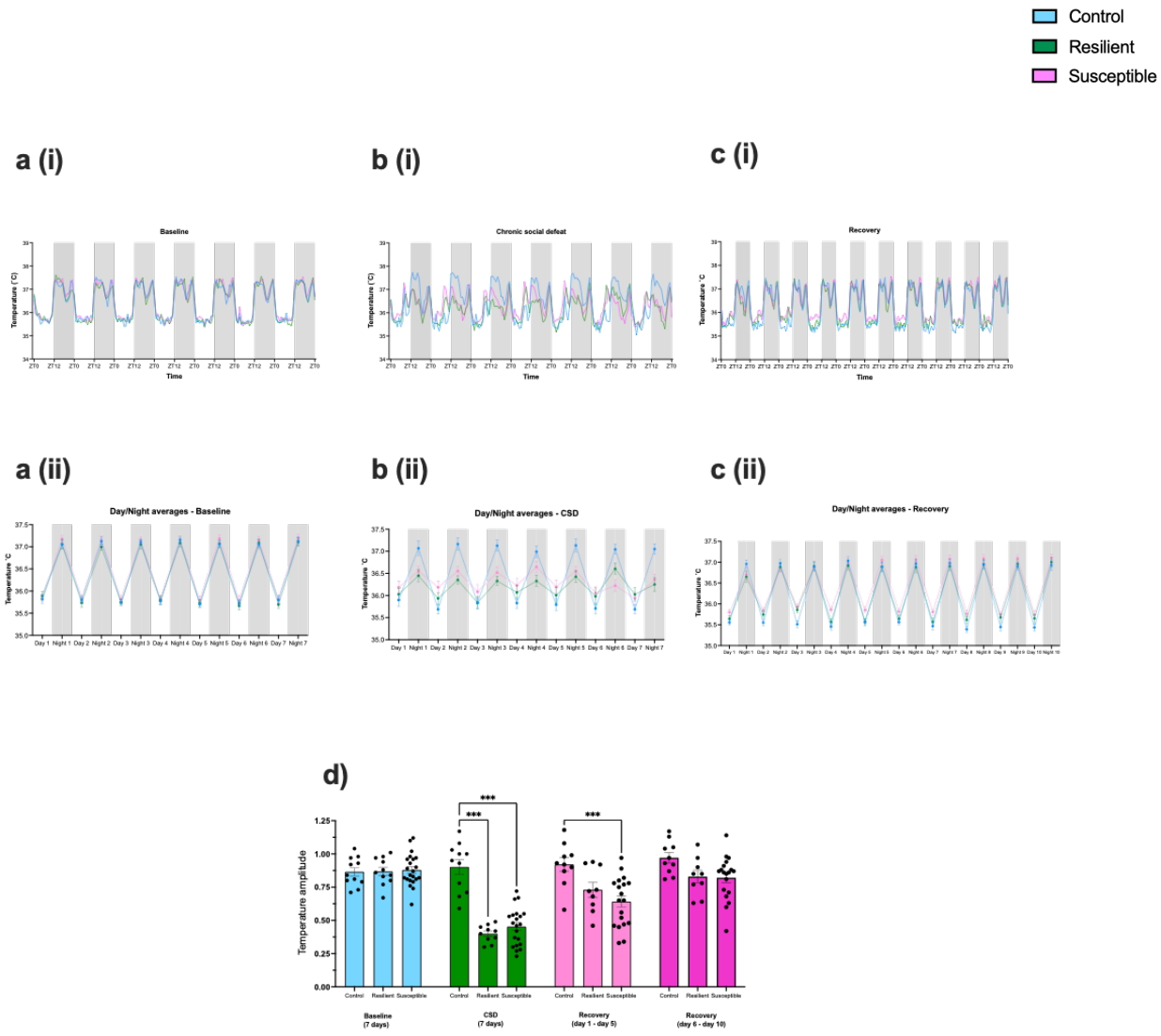


Figure 2. Average core body temperature rhythms during baseline, CSDS and recovery. a(i) No baseline differences in Tcore rhythms between control, resilient and susceptible mice. Control, resilient and susceptible mice show comparable Tcore rhythms prior to CSDS in 12H:12H light/dark conditions. **b(i)** CSDS blunts the temperature amplitude of resilient and susceptible mice compared to controls (N=11). **c(i)** The Tcore of susceptible mice remains blunted longer than resilient mice during the recovery period. **a-c (ii)** Average day and night values (averaging 12h day and night separately for each phenotype) of Tcore during baseline, CSDS and recovery were calculated for each phenotype. **Baseline:** Average day and night temperatures did not significantly differ between phenotypes. **CSDS:** Susceptible and resilient mice exhibit significantly lower Tcore most nights compared to controls. A two-way ANOVA revealed significant main effect of phase ($F_{13,462} = 21.90$, $p < .001$), significant main effect of phenotype ($F_{2,462} = 11.36$, $p < .001$), and significant interaction between the two factors ($F_{26,462} = 4.738$, $p < .001$). In most nights, resilient and susceptible mice exhibited significantly lower average Tcore compared to controls (Supplemental Table 1). **Recovery:** Daytime Tcore of susceptible mice was significantly higher relative to control mice for most days following CSDS. Based on a two-way ANOVA, main effects of phase ($F_{19,660} = 148.7$, $p < .001$) and phenotype ($F_{2,660} = 20.08$, $p < .001$) were significant. Daytime Tcore averages of susceptible mice during recovery were higher on most days relative to controls (Supplemental Table 2). **(d)** Average Tcore amplitudes during baseline, CSDS and recovery were calculated for each phenotype. **Baseline**: There was no difference in baseline Tcore amplitude between the phenotypes. **CSDS**: Tcore amplitude was significantly blunted in susceptible and resilient mice during CSDS relative to controls (Supplemental Table 2). **Recovery**: Both susceptible and resilient mice exhibited significantly blunted amplitude relative to the controls during the first 5 days of recovery. Susceptible, but not resilient, mice continued exhibiting blunted amplitude from day 6 to day 10 of recovery . (a,d) Baseline: control ($N = 11$), resilient ($N = 11$), and susceptible mice ($N = 23$); (b,e) CSDS: control ($N = 11$), resilient ($N = 10$), and susceptible mice ($N = 21$) and (c,f) Recovery post-CSDS in control ($N = 10$), resilient ($N = 9$), and susceptible mice ($N = 19$). *** indicate $p < .001$.

Figure 3

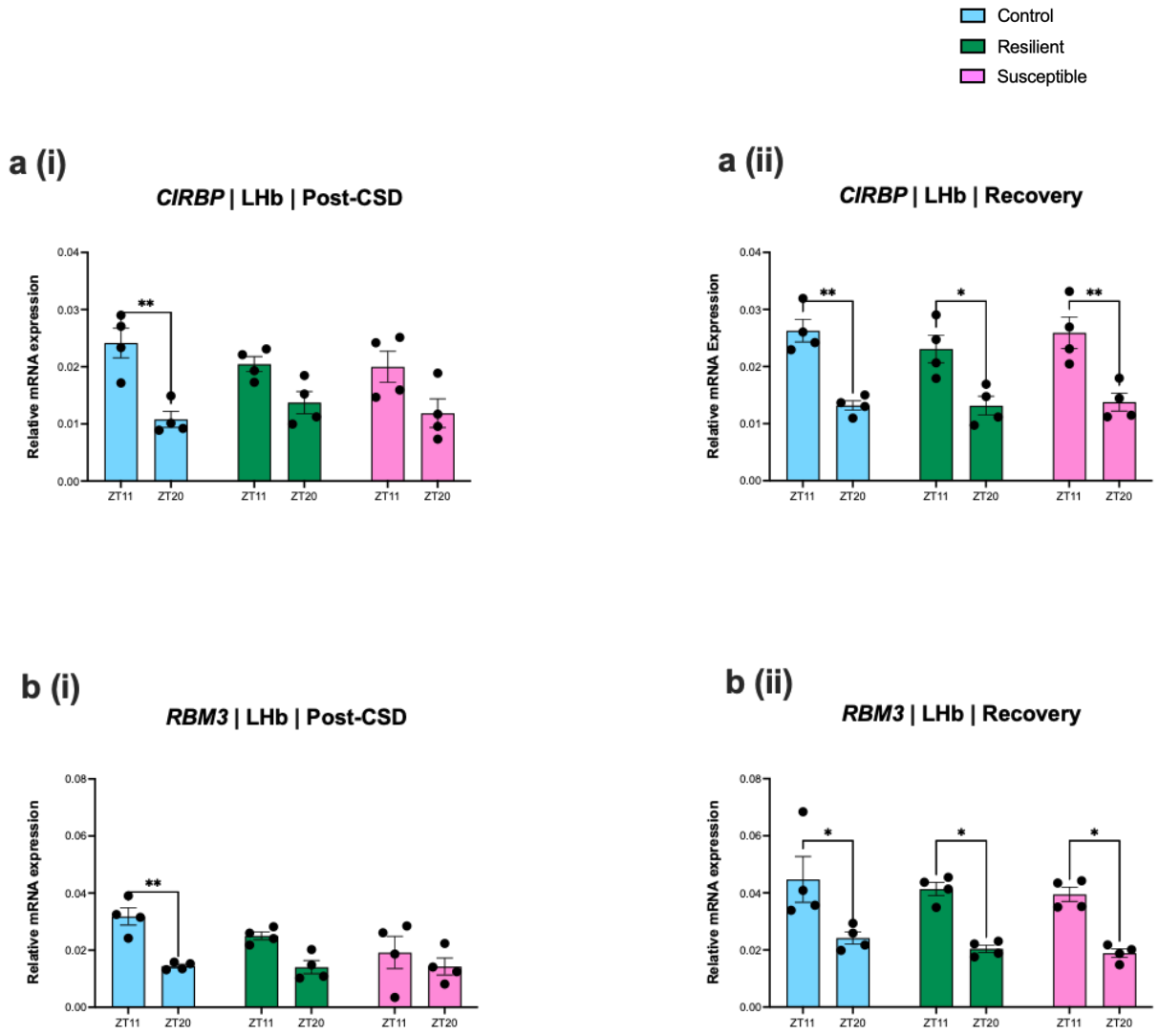
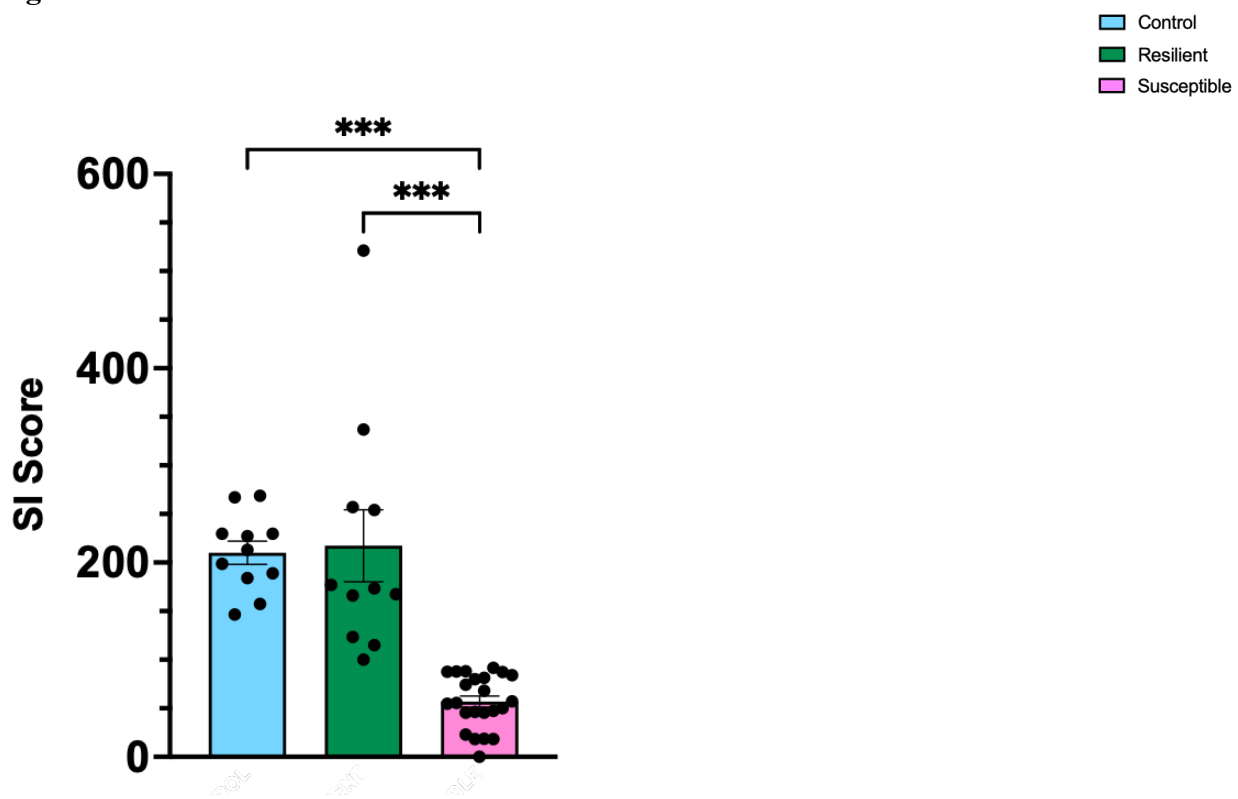


Figure 3. Diurnal rhythmic expression of *Cirbp* and *RBM3* in the LHb is blunted immediately after CSDS. a(i) & b(i) Control, but not resilient and susceptible mice, display significantly larger mRNA expression of *Cirbp* (Tukey's HSD, $p < .01$, 95% C.I. = [0.00, 0.02] and *RBM3* ($p < .01$, 95% C.I. = [0.00, 0.03] in the LHb at ZT11 compared to ZT20 post-CSD. **a(ii) & b(ii)** The molecular rhythms of *Cirbp* and *RBM3* in resilient [*Cirbp:* $p = .02$, 95% C.I. = [0.00, 0.02]; *RBM3:* $p = .01$, 95% C.I. = [0.00, 0.04]] and susceptible [*Cirbp:* $p < .01$, 95% C.I. = [0.00, 0.03]; *RBM3*: $p = .01$, 95% C.I. = [0.00, 0.04]] mice return 10 days after recovery and are at similar levels comparable to control [*Cirbp:* $p < .01$, 95% C.I. = [0.00, 0.02]; *RBM3:* $p = .01$, 95% C.I. = [0.00, 0.04]]. Error bars represent mean ± SEM.* and ** indicate $p < .05$ and $p < .01$, respectively.

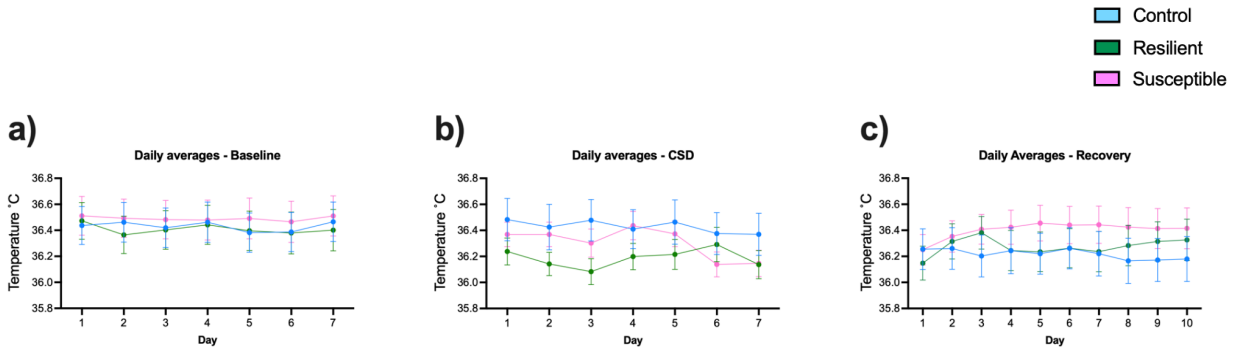
Supplementary figures

Figure 1



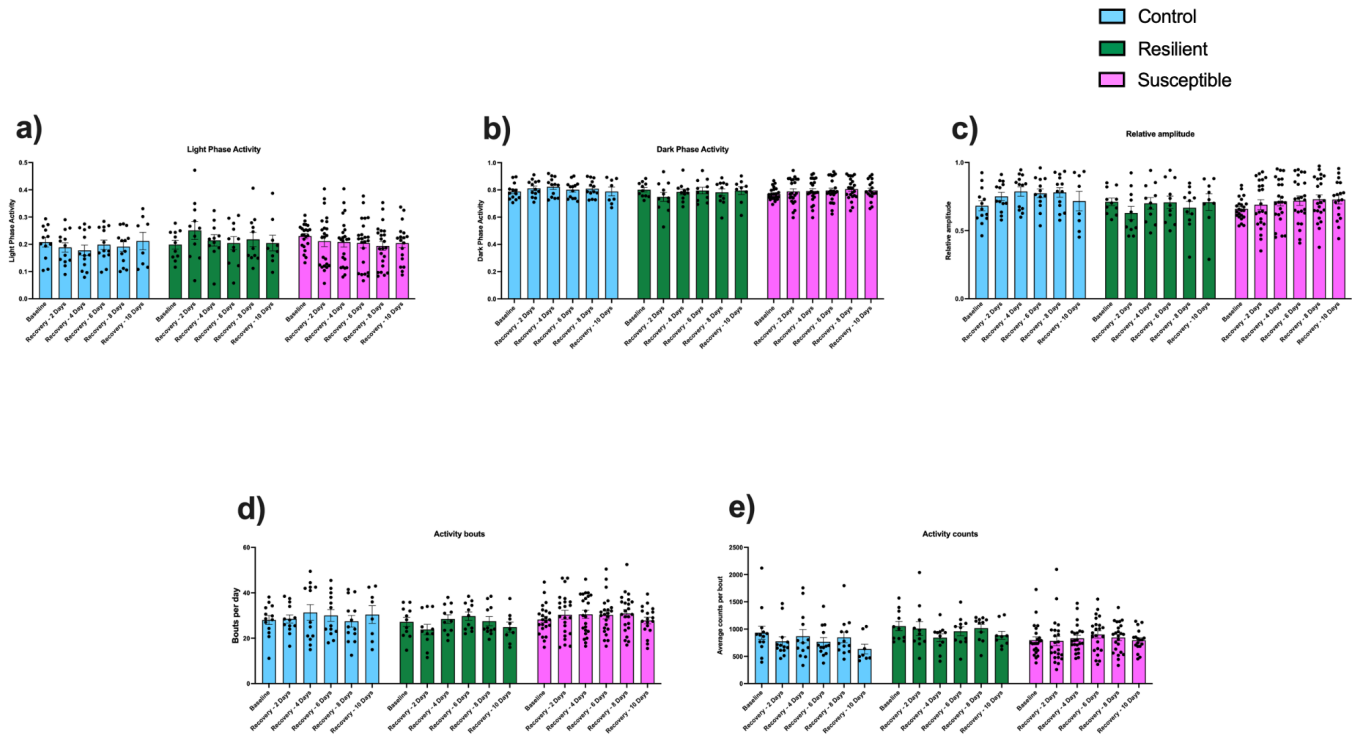
Social interaction (SI) scores for the control, resilient and susceptible mice following CSDS. Experimental mice were exposed to the CSDS paradigm for 15 consecutive days. Following the final day of defeat, all mice were subjected to the SI test and the obtained scores were used to phenotype the mice as either stress susceptible or stress resilient. A one-way ANOVA revealed a significant difference in social interaction between control/ resilient and susceptible mice ($F_{2,42}$ = 31.63, p = .02). There was a significant difference in SI values between control and susceptible (Tukey's HSD, p < .001, 95% C.I. = [94.21, 212.0]) as well as resilient and susceptible mice (p < .001, 95% C.I. = [101.5, 219.3]). A total of N = 23 mice with SI <100 were identified to be stress susceptible. Control mice (N = 11) and stress resilient mice (N = 11) with SI >100 were selected for post-defeat molecular and recovery analysis. Tukey's HSD test was used to compare the differences across phenotypes and error bars represent mean ± SEM. *** indicate p < .001.

Figure 2



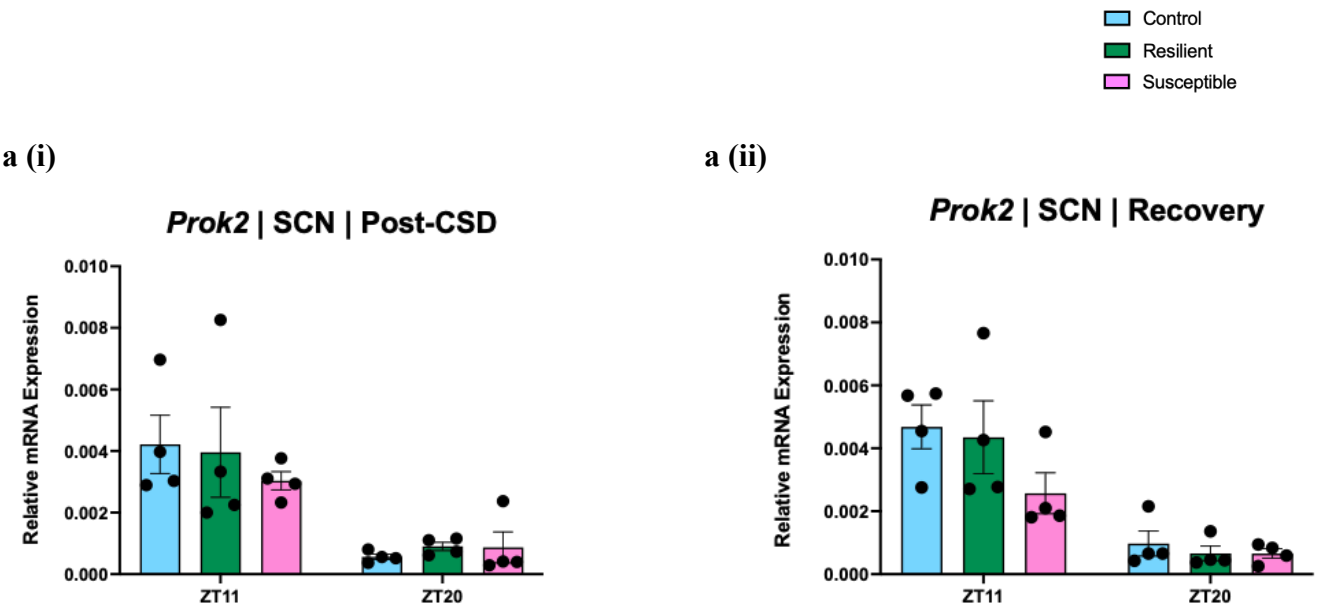
Daily temperatures averages: **(a)** The daily average temperature for each day was calculated for 7 days of baseline, **(b)** last 7 days of CSDS and **(c)** 10 days of post-CSDS recovery. Temperature was collected every hour. The error bars represent Mean ± SEM.

Figure 3



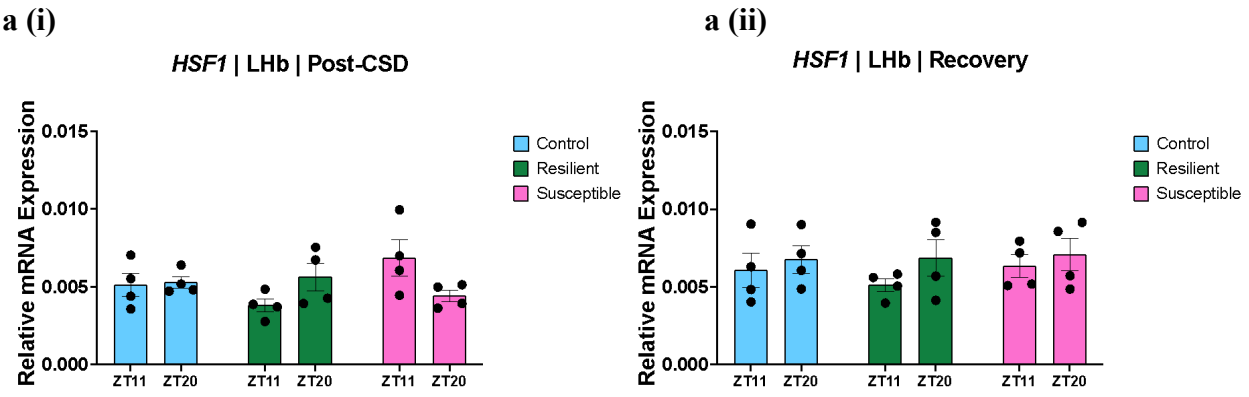
Chronic social stress has no effect on locomotor activity rhythms on resilient and susceptible mice following CSDS. **(a & b)** Light phase and dark phase activity shows no significant difference between phenotypes. **(c)** Relative activity amplitude showed no significant difference when comparing across phenotypes and conditions. **(d)** No significant difference in activity bouts. **(e)** No significant difference in activity counts. Activity parameters were calculated using ClockLab Software. The error bars represent Mean ± SEM.

Figure 4



Expression analysis of *Prok2*: a(i, ii). There is no significant difference/blunting in resilient and susceptible as compared to controls for Prok2 expression both post-CSD and recovery.

Figure 5



Expression analysis of *HSF1* : a(i,ii) *Hsf1* mRNA levels does not show robust rhythmic expression in the LHb after CSDS or post recovery.

Table 1. Tukey's HSD multiple comparison tests for day and night average Tcore values during CSDS.

Tukey's HSD Multiple Comparison Tests	Mean Diff.	95% CI		Adjusted *p* value
		LL	*UL*	
Night 1				
Control vs. Resilient	0.62	0.17	1.07	.004
Control vs. Susceptible	0.52	0.07	0.96	.02
Day 2				
Control vs. Susceptible	-0.50	-0.95	-0.05	.03
Night 2				
Control vs. Resilient	0.81	0.36	1.26	< .001
Control vs. Susceptible	0.61	0.16	1.06	.004
Night 3				
Control vs. Resilient	0.80	0.35	1.25	< .001
Control vs. Susceptible	0.61	0.16	1.06	.004
Night 4				
Control vs. Resilient	0.66	0.22	1.11	.002
Night 5				
Control vs. Resilient	0.71	0.26	1.16	< .001
Control vs. Susceptible	0.58	0.14	1.03	.006
Night 6				
Control vs. Resilient	0.44	-0.01	0.89	.06
Control vs. Susceptible	0.82	0.37	1.27	< .001
Night 7				
Control vs. Resilient	0.80	0.35	1.25	< .001
Control vs. Susceptible	0.69	0.24	1.14	< .001

Note: Only significant ($p \leq .05$) and comparisons approaching significance ($p \leq .08$) are reported. All the values, except for *p*, have been rounded to two decimals.

Table 2. Tukey's HSD multiple comparison tests for Tcore amplitude values.

Tukey's HSD Multiple Comparison Tests	Mean Diff.	95% CI		Adjusted *p* value
		LL	*UL*	
CSD				
Control vs. Resilient	0.5	0.29	0.71	< .001
Control vs. Susceptible	0.45	0.27	0.63	< .001
Recovery (Day 1 – Day 5)				
Control vs. Resilient	0.19	-0.03	0.41	.17
Control vs. Susceptible	0.28	0.09	0.47	< .001
Recovery (Day 6 – Day 10)				
Control vs. Resilient	0.14	-0.08	0.36	.61
Control vs. Susceptible	0.15	-0.04	0.34	.26

Note: All the values, except for *p*, have been rounded to two decimals.