**Supplementary Materials**

# Materials and Methods

## Model Development

In this study, we have refined our model to incorporate the pivotal role of the GCN2-eIF2α-ATF4 pathway (GCN2, general control nonderepressible 2; eIF2α, eukaryotic translation initiation factor 2α; ATF4, activating transcription factor 4) within the integrated stress response (ISR) as an internal mechanism in the suprachiasmatic nucleus (SCN). This pathway is essential for the resilience of the central mammalian clock located in the SCN. Our model also highlights the interconnected reliance of this master clock’s response to metabolic stress signals on feedback from the hypothalamic-pituitary-adrenal (HPA) axis. This relationship is framed within the SCN topology-HPA axis model established in our previous work [1, 2]. Distinctively, the model differently represents the SCN as a heterogeneous collection of GCN2-eIF2α-ATF4 pathway-mediated, damped neuronal oscillators and further incorporates the hypothetic, indirect effect of glucocorticoids (CORT), which is output from the HPA axis, on upregulating the neurotransmitter expression within SCN neurons (**Figure 1**).

### The ISR pathway (GCN2-eIF2α-ATF4 signaling cascade)-mediated intra-neuronal and inter-neuronal coupling in the SCN

Based on the mechanism elucidated by Pathak and Cao et al. [3], where the ISR pathway modulates the circadian characteristics of SCN clock by regulating the transcription of clock gene (*Per2*), we integrated the GCN2-eIF2α-ATF4 pathway with the autoregulatory clock dynamics into each single neuron in the SCN. Upon the GCN2 is activated () (**Equation 1**), eIF2α is phosphorylated () (**Equation 2**) to initiate the ISR and promote the translation of transcriptional modulators such as ATF4 (**Equation 3**), which then enhances the transcription of *Per/Cry* mRNA (**Equation 9**) by binding to the *Per2* promoter region and modulate the clock genes and proteins dynamics. The deactivation/ dephosphorylation or selective degradation of these proteins are described using negative multipliers of protein concentrations and corresponding rate constants.

*A single cell (cell i) in the SCN:*

(1)

(2)

(3)

Similar to our prior works [1, 2], the SCN in this study is represented as a single compartment that consists of a population of neurons with heterogeneity, where the component SCN neurons release neurotransmitters (*V*) (**Equation 4**) for both self-coupling at the cellular level and inter-neuronal communication at the tissue level. The secretion of neurotransmitters to the extracellular medium are assumed to be induced upon the activity of PER/CRY proteins (**Equation 4**) and their functions as inter-cellular coupling signals are distance-dependent, implying that the adjacent neurons of the neurotransmitter-releasing neuron are more affected (**Equation 5-7**). Specifically, the entry coupling signals to each cell (both the same neurons and affected/ coupled neighbors) (*Q*) is proportional to the intra-SCN inter-neuronal coupling strength (*K*) and the strength of coupling signal (*F*) (**Equation 5**), which is calculated by the average concentration (local mean field) of neurotransmitters (**Equation 6**) released by all cells within the threshold distance () (**Equation 7**).

*Intra-cellular and inter-cellular coupling mechanisms of the SCN:*

(4)

(5)

(6)

(7)

Furthermore, experimental evidence suggests that in the SCN, activated GCN2 phosphorylates eIF2α in a rhythmic pattern [3], the driving force of the oscillation in GCN2 activity, however, is not fully understood. Given the significances of neurotransmitters involved coupling mechanism and of GCN2-eIF2α-ATF4 pathway to the SCN clock’s robustness/ plasticity, we thus hypothesized that the GCN2-eIF2α-ATF4 signaling cascade, which activates the transcription of *Per/Cry*, and accordingly the rhythm in each cell are triggered by the entry neurotransmitter effects (the first term in **Equation 1**).

### The clock gene dynamics in the SCN neurons

The intrinsic dynamics of the clock genes and proteins network is modeled using the same gene regulatory network [4, 5] as our previous works [1, 2, 6, 7], which consists of interacted positive and negative transcriptional translational feedback loops. The positive branch is constituted by a sequence of indirect activation of *Bmal1* transcription by nuclear PER/CRY protein (**Equation 12**), translation of *Bmal1* mRNA (**Equation 13**), nuclear translocation of cytoplasmic BMAL1 protein (**Equation 14**), and heterodimerization to CLOCK/BMAL1 complex (**Equation 15**). In contrast, the inhibition of CLOCK/BMAL1-induced *Per/Cry* transcription by the nuclear PER/CRY (**Equation 9**) upon the translation to cytoplasmic PER/CRY protein (**Equation 10**) and subsequent translocation to the nucleus (**Equation 11**) forms the negative branch.

The light entraining effect on the SCN oscillators is retained as additive terms that describe the independent photic-induced *Per/Cry* transcription from the CLOCK/BMAL1-activated transcription [8] (**Equation 9**), with the 12L/12D light/dark cycle is modeled by a step function in the *in silico* experiments (**Equation 8**).

(8)

*A single cell (cell i) in the SCN:*

(9)

(10)

(11)

(12)

(13)

(14)

(15)

### The HPA axis mediated ISR stressor effect on central circadian clock

The exact mechanism through which the GCN2-eIF2α-ATF4 pathways within SCN neurons are activated by ISR signals and the SCN clock’s robustness is affected by unpredictable stress perturbations remains unclear. However, since the HPA axis is a major neuroendocrine system to regulate the stress response and glucocorticoids, known as the primary effectors of the HPA axis, are believed to enable the entrainment of neurotransmitter expression in the SCN [9], one hypothesis could be that stresses affect the SCN clock through an indirect extra-SCN pathway, which in this study is assumed to be the glucocorticoids-mediated stress-transducing feedback of the HPA axis on SCN neurotransmitters. In other words, our model assumed that the neurotransmitters convey both intra-SCN coupling and extra-SCN stress information.

The stress responses of the HPA axis are initiated by the release of corticotropin releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus, which then induces the production and secretion of adrenocorticotropic hormone (ACTH) by the anterior pituitary gland, followed by the output of stress hormones, glucocorticoids (CORT), from the adrenal gland (**Equation 16-18**). The stress activities of glucocorticoids, in turn, activate the expression of neurotransmitters (*V*) (the first term in **Equation 4**), transducing the stress effect to the SCN, which consequently stimulates the activation of GCN2-eIF2α-ATF4 pathway in each cell (**Equation 1**). Parameter in **Equation 4** denotes the strength of the CORT-dependent feedback loop to the V activities in SCN neurons.

*HPA axis:*

(16)

(17)

(18)

### The circadian rhythm of HPA axis and glucocorticoid receptor dynamics

Built upon our previous models [1, 2, 7], the neurotransmitter *V* released from the SCN downregulates the secretion of CRH [10] (**Equation 16**), driving the circadian rhythm of the HPA axis. Besides, a closed CORT-dependent negative feedback loop that inhibits the production of CRH and ACTH is reserved, which is essential to maintain the homeostasis and terminate the stress response of the HPA axis. Specifically, the secreted CORT binds to its receptor () (**Equation 20**) in the hypothalamus and anterior pituitary gland. Then, the formed cytoplasmic glucocorticoid-receptor complexes () (**Equation 21**) translocate to the nucleus () (**Equation 22**) and exert the CORT’s negative regulation on the activities of CRH (**Equation 16**) and ACTH (**Equation 17**), as well as the receptor gene’s transcription () (**Equation 19**).

*Glucocorticoid receptor dynamics in the HPA axis:*

(19)

(20)

(21)

(22)

## Methods

### Quantification of clock resistance to photic perturbation

A “jet lag” protocol is believed to be a useful and quantifiable evaluation tool of the SCN clock’s capability to have its unremitting oscillation resistant to an unexpected large phase shift in the external light/dark (LD) cycle, in which the SCN’s robustness against this photic perturbation is assessed by the speed of resynchronization of SCN neurons upon exposure to the sudden advance or delay of the LD cycle [11]. In this study, a 6-h advance of the 12h/12h LD cycle was introduced on the 21st day for both entrained WT and GCN2 KO individuals. An individual that has a less robust SCN clock is expected to complete the re-entrainment of the ensemble of/ the resynchronization of component neuronal oscillators faster (i.e., in fewer days).

### Assessment of clock entrainment behavior

The phase response curve (PRC) and its area under curve (AUC) were used to evaluate SCN clock’s differential responses to photic and non-photic (stress) entrainers. To produce the photic stimulated PRC, a 3 h-lasting light pulse with 0.5\*nominal light intensity was imposed to the system in constant darkness (DD) every hour and corresponding maximum ensemble phase shifts of SCN neuronal oscillators were recorded. For the non-photic stimulated PRC, a 3 h-lasting stress pulse with 5\*nominal stress intensity was introduced to the same system in the DD environment at same time intervals, and corresponding data of the representative output were collected.

### Sensitivity analysis of model response to parameters

A local sensitivity analysis approach [12] was utilized to evaluate the impact of perturbations in parameters that are associated with the newly introduced ISR (GCN2-eIF2α-ATF4) pathway and its mediated intra-SCN coupling mechanism on the completion time of the jetlag-induced re-entrainment of the SCN clock. The investigated parameters were varied by ±20%, ±50%, and ±100%, and one at a time with other parameters fixed. As the relative sensitivity indices () for a varied parameter (), relative jetlag-induced ensemble re-entrainment rate (completion time) was determined as the ratio of the relative change of the state variable (), jetlag-induced ensemble re-entrainment rate, to the relative change of the parameter value (**Equation 23**) [13-15], where represents different variation strategies employed to the parameter and the reference/ baseline values for computing the relative changes are corresponding nominal values. Larger values of over different parameter variations ( for the same ) reflect a more influential parameter that significantly affects the jetlag-induced resynchronization speed of SCN neuronal oscillators, or said, the robustness of SCN clock.

(23)

### *In silico* implementation of individual variability

By hypothesizing that the stress effect on the resistance of circadian timing system, represented by CORT amplitude, is related to the functioning of intra-SCN ISR pathway, the strength of extra-SCN tissue feedback, and the perceived stress level of the system, three respectively representative parameters, , , and , were sampled using Sobol algorithm to capture the individualization in these physiological properties. The resulting virtual population was further screened with the criterion that simulated CORT oscillations peak within a ±2 h window of the standard CORT peaking time (ZT12), to ensure the investigated individuals have (similar) homeostatic CORT rhythms.

# 2 Supplementary Tables

Table S1. Nominal values of model parameters and their sources. (\*) denotes the estimated parameters in this study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| # | Parameter | Value | Unit | Compartment | Description/ References |
| 1 |  | 3 |  | SCN and coupling mechanism | Activation rate of GCN2 [16] |
| 2 |  | 10 |  | Total GCN2 concentration (\*) |
| 3 |  | 4 |  | Inactivation rate of GCN2 [16] |
| 4 |  | 0.25 |  | Phosphorylation rate of eIF2α [16] |
| 5 |  | 15 |  | Total eIF2α concentration [16] |
| 6 |  | 10 |  | Dephosphorylation rate of eIF2α [16] |
| 7 |  | 3 |  | Production rate of ATF4 (\*) |
| 8 |  | 3 |  | Degradation rate of ATF4 (\*) |
| 9 |  | 1.3 | 1 | Intra-SCN coupling strength (\*) |
| 10 |  | 0.5 | 1 | Threshold distance within the SCN population [2] |
| 11 |  | 1 |  | Synthesis rate of neurotransmitters [16] |
| 12 |  | 4 |  | Degradation rate of neurotransmitters [16] |
| 13 |  | 0 |  | HPA axis-to-SCN feedback strength (\*) |
| 14 |  | 0.0325 | 1 | Light intensity [17] |
| 15 |  | 9 |  | Maximal rate of *Per/Cry* transcription [5] |
| 16 |  | 1 |  | Michaelis-Menten constant of *Per/Cry* transcription [5] |
| 17 |  | 0.56 |  | Inhibition constant of *Per/Cry* transcription [5] |
| 18 |  | 1 |  | Strength of ATF4 activation effect [16] |
| 19 |  | 2 |  | Hill coefficient of activation effect of ATF4 [16] |
| 20 |  | 3 |  | Hill coefficient of inhibition of *Per/Cry* transcription [16] |
| 21 |  | 0.18 |  | Degradation rate of *Per/Cry* mRNA [16] |
| 22 |  | 1 |  | Light sensitivity of the SCN [16] |
| 23 |  | 0.33 |  | Complex formation rate of cytoplasmatic PER/CRY [5] |
| 24 |  | 2 |  | Number of PER/CRY complex forming subunits [5] |
| 25 |  | 0.1 |  | Degradation rate of cytoplasmatic PER/CRY complex [16] |
| 26 |  | 0.36 |  | Nuclear import rate of the PER/CRY complex [16] |
| 27 |  | 0.02 |  | Nuclear export rate of PER/CRY complex [5] |
| 28 |  | 0.18 |  | Degradation rate of the nuclear PER/CRY complex [16] |
| 29 |  | 1 |  | Maximal rate of *Bmal1* transcription [16] |
| 30 |  | 2.16 |  | Michaelis-Menten constant of *Bmal1* transcription [5] |
| 31 |  | 3 |  | Hill coefficient of activation of *Bmal1* transcription [5] |
| 32 |  | 1.1 |  | Degradation rate of *Bmal1* mRNA [16] |
| 33 |  | 0.24 |  | Translation rate of BMAL1 [5] |
| 34 |  | 0.09 |  | Degradation rate of cytoplasmatic BMAL1 [16] |
| 35 |  | 0.45 |  | Nuclear import rate of BMAL1 [5] |
| 36 |  | 0.06 |  | Nuclear export rate of BMAL1 [5] |
| 37 |  | 0.18 |  | Degradation rate of nuclear BMAL1 [16] |
| 38 |  | 0.09 |  | Activation rate of nuclear CLOCK/BMAL1 complex [5] |
| 39 |  | 0.003 |  | Deactivation rate of nuclear CLOCK/BMAL1 complex [5] |
| 40 |  | 0.13 |  | Degradation rate of nuclear CLOCK/BMAL1 complex [16] |
| 41 |  | 0.6 | 1 | HPA axis and glucocorticoid receptor dynamics | Perceived stress level of the system to a standard stress event (\*) |
| 42 |  | 0.38 |  | Zero-order synthesis rate constant of CRH [18] |
| 43 |  | 6.54 |  | Hypothalamic negative feedback [7] |
| 44 |  | 0.35 |  | First-order rate constant for CRH degradation [7] |
| 45 |  | 4.39 |  | Michaelis-Menten constant for CRH degradation [7] |
| 46 |  | 3 |  | Neurotransmitter coupling strength of the HPA (\*) |
| 47 |  | 0.46 |  | First-order rate constant for synthesis of ACTH [7] |
| 48 |  | 1.63 |  | Pituitary negative feedback [7] |
| 49 |  | 1 |  | First-order rate constant for degradation of ACTH [7] |
| 50 |  | 0.85 |  | Michaelis-Menten constant for ACTH degradation [7] |
| 51 |  | 0.73 |  | Feedforward adrenal sensitivity [7] |
| 52 |  | 0.72 |  | First-order rate constant for CORT degradation [7] |
| 53 |  | 0.18 |  | Michaelis-Menten constant for CORT degradation [7] |
| 54 |  | 540.7 |  | Baseline value of free cytosolic CORT receptor [19] |
| 55 |  | 25.8 |  | Baseline value of CORT receptor mRNA [19] |
| 56 |  | 2.9 |  | Zero-order rate constant for synthesis of CORT receptor mRNA [19] |
| 57 |  | 26.2 |  | CORT concentration at which CORT receptor mRNA synthesis drops to its half [19] |
| 58 |  | 1.2 |  | First-order rate constant for degradation of CORT receptor [19] |
| 59 |  | 0.49 |  | CORT receptor recycle fraction from nucleus to cytoplasm [19] |
| 60 |  | 0.57 |  | Rate of CORT receptor recycling from nucleus to cytoplasm [19] |
| 61 |  | 0.003 |  | Second-order rate constant for CORT-receptor binding [19] |
| 62 |  | 0.06 |  | First-order rate constant for degradation of CORT receptor [19] |
| 63 |  | 0.11 |  | First-order rate constant for degradation of CORT receptor mRNA [19] |
| 64 |  | 0.63 |  | Rate of CORT receptor translocation from cytoplasm to nucleus [20] |

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