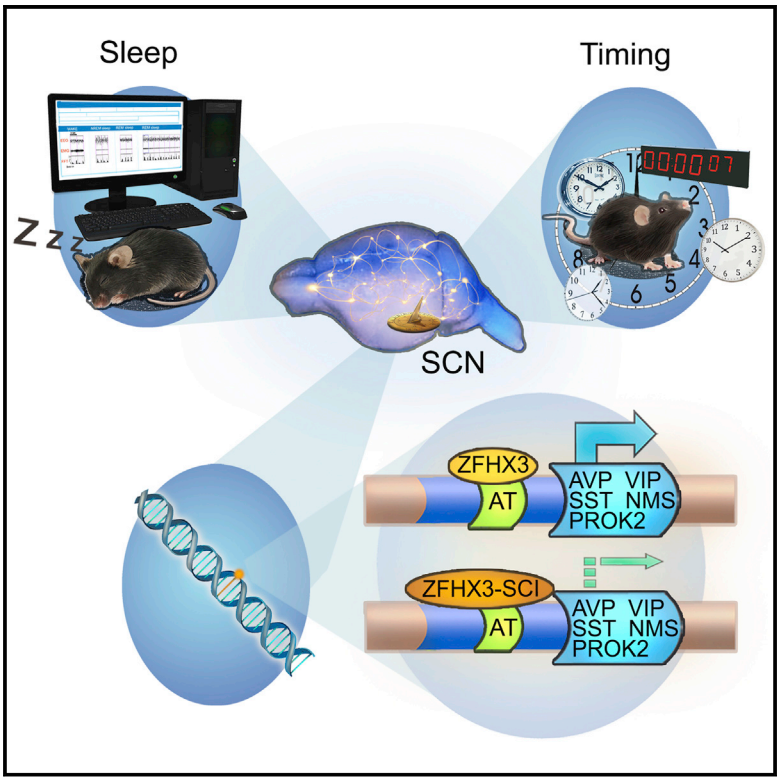
Report

Cell Reports

The *Zfhx3*-Mediated Axis Regulates Sleep and Interval Timing in Mice

Graphical Abstract



Authors

Edoardo Balzani, Glenda Lassi, Silvia Maggi, ..., Michelle Simon, Patrick M. Nolan, Valter Tucci

Correspondence

valter.tucci@iit.it

In Brief

Balzani et al. report that the transcription factor *Zfhx3*, which is primarily expressed in the suprachiasmatic nucleus of the hypothalamus and which regulates circadian rhythms, modulates sleep homeostatic functions and short-interval behavioral responses in mice.

Highlights

- The *Zfhx3*$^{Sci/+}$ mutation leads to a defect in sleep homeostasis
- This mutation accelerates biological timers across timescales
- The *Zfhx3*$^{Sci/+}$-dependent gene network contains a significant number of sleep targets
- Sleep and the circadian clock are predictors of behavioral performance in mice

Balzani et al., 2016, Cell Reports *16*, 615–621
July 19, 2016
http://dx.doi.org/10.1016/j.celrep.2016.06.017

Cell Reports
Report

The *Zfhx3*-Mediated Axis Regulates Sleep and Interval Timing in Mice

Edoardo Balzani,[1,3] Glenda Lassi,[1,3] Silvia Maggi,[1,3] Siddharth Sethi,[2] Michael J. Parsons,[2] Michelle Simon,[2] Patrick M. Nolan,[2] and Valter Tucci[1,*]
[1]Department of Neuroscience and Brain Technologies, Istituto Italiano di Tecnologia, via Morego, 30, 16163 Genova, Italy
[2]MRC Harwell, Harwell Science and Innovation Campus, Oxfordshire OX11 0RD, UK
[3]Co-first author
*Correspondence: valter.tucci@iit.it
http://dx.doi.org/10.1016/j.celrep.2016.06.017

SUMMARY

An AT motif-dependent axis, modulated by the transcription factor *Zfhx3*, influences the circadian clock in mice. In particular, gain of function of *Zfhx3* significantly shortens circadian rhythms and alters the transcriptional activity of an important class of neuropeptides that controls intercellular signaling in the suprachiasmatic nucleus (SCN) of the hypothalamus. The ZFHX3/AT axis revealed an important, largely cell-nonautonomous control of the circadian clock. Here, by studying the recently identified circadian mouse mutant *Zfhx3*$^{Sci/+}$, we identify significant effects on sleep homeostasis, a phenomenon that is outside the canonical circadian clock system and that is modulated by the activity of those neuropeptides at a circuit level. We show that the *Zfhx3*$^{Sci/+}$ mutation accelerates the circadian clock at both the hourly scale (i.e., advancing circadian rhythms) and the seconds-to-minutes scale (i.e., anticipating behavioral responses) in mice. The in vivo results are accompanied by a significant presence of sleep targets among protein-protein interactions of the *Zfhx3*$^{Sci/+}$-dependent network.

INTRODUCTION

The intrinsic circadian clock significantly influences the performance of multiple biological processes. Research on circadian biology has predominantly investigated the classical transcriptional-translational feedback loop (TTFL), in which core clock genes are cell autonomously regulated via the E-box DNA motif. Recently, a new circadian axis was identified (Parsons et al., 2015). Using a circadian-driven forward genetics approach in mice, we discovered a point mutation in exon 9 of the zinc finger homeobox 3 (*Zfhx3*) gene, a transcription factor that is highly expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus. The mutation, which results in a G→T transversion, causes shortening of the circadian period in short circuit (*Sci*) mice. This experimental model revealed an important consensus AT motif that functions as a clock-regulated transcriptional axis. Interestingly, in searching for transcriptional changes in the SCN caused by the *Zfhx3*$^{Sci/+}$ mutation, we identified a significant functional module of closely connected genes. In particular, the *Zfhx3*$^{Sci/+}$ mutation downregulates important neuropeptides, such as vasoactive intestinal peptide (Vip), its receptor (Vipr2), and the neuropeptide receptor prokineticin receptor 2 (Prokr2). These peptidergic systems are known to play fundamental roles in sleep-wake regulation (Hu et al., 2011; Piggins and Cutler, 2003) and in circadian cell-cell signaling (Maywood et al., 2006). The interplay between the circadian clock and sleep processes over 24 hr influences many behavioral/cognitive traits, such as timing at the scale of seconds to minutes (Lassi et al., 2012; Agostino et al., 2011). The ability to perceive and produce short-interval behavioral responses is known as "interval timing," and it is a primary property of many cognitive functions (e.g., attention and decision making). For example, in conditioning behaviors, interval timing permits the association between a stimulus and a reward (or a punishment): in risk assessment, the association between a choice and the payoff and, in feeding opportunities, the association between two meals. Both the circadian clock (hourly scale) and interval timing (seconds-to-minutes scale) are basic mechanisms for anticipatory behaviors involved in many daily life behaviors.

Here, we studied sleep in *Zfhx3*$^{Sci/+}$ mice and littermate controls and discovered that they exhibit significant alterations in sleep homeostasis. We also investigated the function of short-interval timing in working-for-food cognitive tasks and discovered that, as for the circadian clock, the interval timing mechanism also runs faster in *Zfhx3*$^{Sci/+}$ mice. The exploration of gene enrichment ontologies revealed a significant presence of sleep terms among *Zfhx3*$^{Sci/+}$ SCN targets compared with wild-type SCNs.

RESULTS

The *Zfhx3*$^{Sci/+}$-Dependent Gene Network Includes a Significant Presence of Sleep Targets and Cell Response Signaling Pathways

Specific analyses of the RNA sequencing data (reported in Parsons et al., 2015) in the SCN of *Zfhx3*$^{Sci/+}$ and wild-type control mice allowed us to investigate whether the mutation affected specific pathways associated with sleep. A densely connected subnetwork (module 1; Parsons et al., 2015) displayed a significant