**Cell Reports, Volume *16***

## Supplemental Information

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

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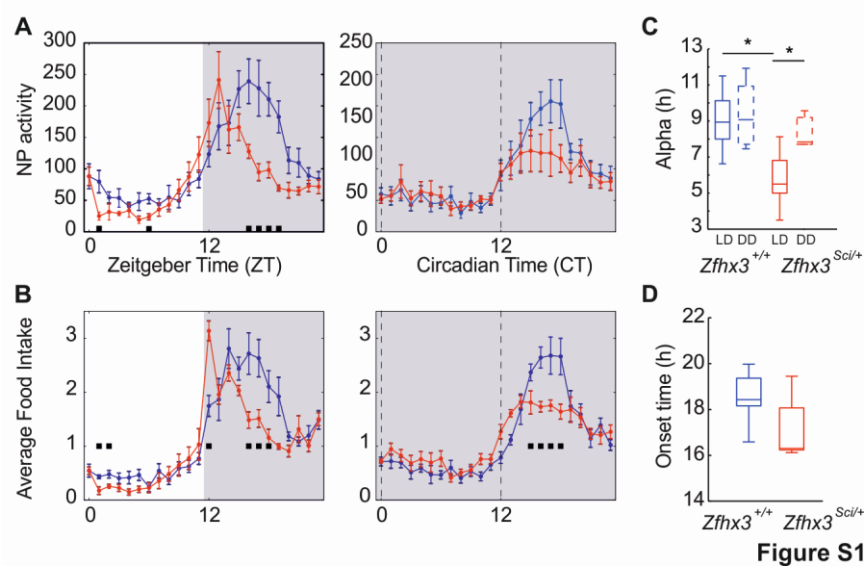


**Figure S1. Nose poking activity and food-intake in *Zfhx3*$^{Sci/+}$ mutant mice compared with wild-type *Zfhx3*$^{+/+}$ littermate controls, related to section "Light masking and food-seeking behavior in *Zfhx3*$^{Sci/+}$ mutants compared to littermate controls**

**"**. (**A**) Nose poking activity and (**B**) food intake over 24 hours are shown for mutants and wild-type animals in LD (left graph) and DD (right graph). Filled black squares within graphs represent statistically significant differences between *Zfhx3*$^{sci/+}$ and *Zfhx3*$^{+/+}$ mice. Data are presented as mean ± SEM. Box plot of (C) activity duration (Alpha) and (D) activity onset of mutants and wild-type for LD and DD conditions. Significant differences are indicated as follows: * $p<0.05$; ** $p<0.01$.

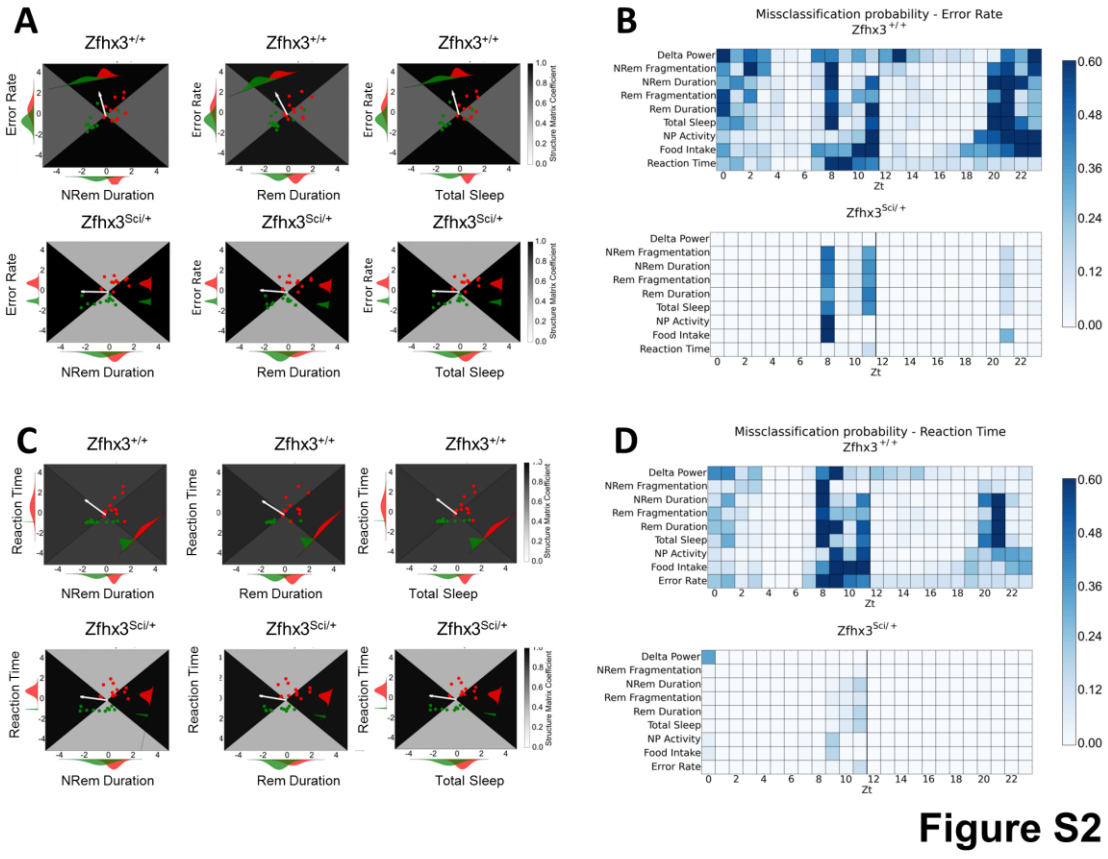


**Figure S2**

**Figure S2. Sleep is a poor discriminator between light and dark in *Zfhx3*$^{Sci/+}$ mutants, related to section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants"**. The error rates (**A,B**) and reaction times (**C,D**) of behavioral performance are plotted against total sleep and NREM and REM sleep. The data are presented as Z-scores of mean values for each zeitgeber time (ZT) across 24 hours. Red and green dots represent the values during the light and dark phases, respectively, while Gaussian plots (on the edge and within each graph) represent the distribution of the light and dark dataset projected over the Cartesian axis and orthogonal to the linear discriminant. White arrows within graphs mark the linear discriminant direction. The area between the bisectors represents the structure matrix coefficient corresponding to the behavioral variable (i.e., the error rate or reaction time), while the area outside the bisectors represents the coefficient relative to the sleep variable. The structure matrix coefficient is represented in grey-scale. See Table S3 and S4 for the numerical list of all structure matrix coefficients. Panels (**B**) and (**D**) are heat maps representing misclassifications of error rate and reaction time posterior probabilities. The probability (on the blue scale) of classifying an observation in light as dark and vice versa is shown, see table S2 for a list of classification probabilities.

**Table S1, related to figure S2 and section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants".**

| Genotype | Cognition | Sleep | Score |
|---|---|---|---|
| +/+ | Reaction Time | Delta Power | 0.958333 |
| +/+ | Reaction Time | Rem Time Course (min) | 0.833333 |
| +/+ | Reaction Time | NRem Time Course (min) | 0.916667 |
| +/+ | Reaction Time | Rem Fragmentation | 0.916667 |
| +/+ | Reaction Time | Sleep Time Course (min) | 0.916667 |
| +/+ | Reaction Time | NP Activity | 0.916667 |
| +/+ | Reaction Time | NRem Fragmentation | 0.958333 |
| +/+ | Reaction Time | Food Intake | 0.875 |
| +/+ | Reaction Time | Error Rate | 0.916667 |
| Sci/+ | Reaction Time | NP Activity | 1 |
| Sci/+ | Reaction Time | NRem Fragmentation | 1 |
| Sci/+ | Reaction Time | Error Rate | 1 |
| Sci/+ | Reaction Time | Rem Fragmentation | 1 |
| Sci/+ | Reaction Time | NRem Time Course (min) | 1 |
| Sci/+ | Reaction Time | Delta Power | 1 |
| Sci/+ | Reaction Time | Rem Time Course (min) | 1 |
| Sci/+ | Reaction Time | Food Intake | 1 |
| Sci/+ | Reaction Time | Sleep Time Course (min) | 1 |
| +/+ | Error Rate | Delta Power | 0.875 |
| +/+ | Error Rate | Rem Time Course (min) | 0.791667 |
| +/+ | Error Rate | NRem Time Course (min) | 0.791667 |
| +/+ | Error Rate | Rem Fragmentation | 0.916667 |
| +/+ | Error Rate | Sleep Time Course (min) | 0.833333 |
| +/+ | Error Rate | NP Activity | 0.791667 |
| +/+ | Error Rate | NRem Fragmentation | 0.833333 |
| +/+ | Error Rate | Food Intake | 0.833333 |
| +/+ | Error Rate | Error Rate | 0.708333 |
| Sci/+ | Error Rate | NP Activity | 0.958333 |
| Sci/+ | Error Rate | NRem Fragmentation | 1 |
| Sci/+ | Error Rate | Error Rate | 1 |
| Sci/+ | Error Rate | Rem Fragmentation | 1 |
| Sci/+ | Error Rate | NRem Time Course | 1 |

| | | | |
|---|---|---|---|
| | | (min) | |
| Sci/+ | Error Rate | Delta Power | 1 |
| Sci/+ | Error Rate | Rem Time Course (min) | 1 |
| Sci/+ | Error Rate | Food Intake | 0.958333 |
| Sci/+ | Error Rate | Sleep Time Course (min) | 1 |

**Table S1 LDA classification score, related to figure S2 and section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants".** Percentage of correct classification for each couple of variables.

**Table S2 (see excel file) Classification Post Probabilities, related to figure S2 and section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants".** Classification post probabilities form LDA classification. Each hour of the day is classified as "Dark" or "Light" if the estimated probability of belonging to the "Dark" or "Light" class is higher than 0.5.

**Table S3, related to figure S2 and section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants"**

| Structure Matrix | | |
|---|---|---|
| **Predictors** | **+/+** | **Sci/+** |
| Error Rate | -0.805883 | -0.661087 |
| Delta Power | 0.43916 | 0.060493 |
| Error Rate | -0.625255 | -0.869062 |
| Reaction Time | -0.999902 | -0.899102 |
| Error Rate | -0.821917 | -0.799303 |
| Food Intake | 0.921304 | 0.126105 |
| Error Rate | -0.603221 | -0.789809 |
| NP Activity | 0.999752 | 0.212532 |
| Error Rate | 0.572109 | -0.997646 |
| NRem Fragmentation | 0.913856 | -0.148462 |
| Error Rate | 0.576577 | -0.995094 |
| Rem Fragmentation | 0.964197 | -0.330813 |
| Error Rate | 0.644586 | -0.999314 |
| NRem Time Course (min) | 0.978866 | -0.317905 |
| Error Rate | 0.641139 | -0.986749 |
| Rem Time Course (min) | 0.921822 | -0.332129 |
| Error Rate | 0.631573 | -0.998176 |
| Sleep Time Course (min) | 0.977305 | -0.324256 |

**Table S4, related to figure S2 and section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants"**

| Structure Matrix | | |
|---|---|---|
| **Predictors** | **+/+** | **Sci/+** |
| Reaction Time | -0.887205 | -0.727621 |
| Delta Power | 0.302325 | 0.064357 |
| Reaction Time | -0.999902 | -0.899102 |
| Error Rate | -0.625255 | -0.869062 |
| Reaction Time | -0.906951 | -0.901492 |
| Food Intake | 0.635709 | 0.137476 |
| Reaction Time | -0.760803 | -0.971491 |
| NP Activity | 0.788475 | 0.252687 |
| Reaction Time | -0.680946 | -0.900162 |
| NRem Fragmentation | -0.680161 | -0.12948 |
| Reaction Time | 0.775524 | -0.939467 |
| Rem Fragmentation | 0.810967 | -0.301885 |
| Reaction Time | -0.808214 | -0.954912 |

| | | |
|---|---|---|
| NRem Time Course (min) | -0.767482 | -0.29363 |
| Reaction Time | -0.851974 | -0.930689 |
| Rem Time Course (min) | -0.765986 | -0.302793 |
| Reaction Time | -0.807977 | -0.949996 |
| Sleep Time Course (min) | -0.781817 | -0.298294 |

**Table S3,S4 Canonical Structure Matrix, related to figure S2 and section "Sleep is a poor predictor of circadian times in *Zfhx3*$^{Sci/+}$ mutants".** The canonical structure matrix reveals the correlations between each variable and the linear discriminant function. It is a measure of how much a variable is related to the discriminant function.

**Supplemental Experimental Procedures**

***Linear discriminant analysis procedure, related to section "Sleep is a poor predictor of circadian times in Zfhx3Sci/+ mutants".***

To understand how sleep and behavioral measures allow differentiating light and dark phases over 24 hours, we performed a linear discriminant analysis (LDA) of reaction times and error rates versus sleep parameters for each hour during the 24 hour cycle (Figure S2). To avoid differences among units, we computed z-scores to normalize the raw data. Then, an optimal linear discriminator between light and dark values was extracted for each pair of variables. The contribution of each variable to the classification was quantified by computing structure matrix coefficients (SMCs). Classification score for each couple of measures we tested are reported in Table S1. All numerical information for the posterior probabilities are listed in Table S2 and represented in Figure S2B,D in blue-scale. SMCs are reported in Tables S3 and S4 and are represented in Figure S2A,C by gray-scale areas. Light and dark values across sleep (x axis) and behavior (y axis) prompted the fit of Gaussian distributions along the orthogonal plane derived from the LDA.

***Integration of sleep and behavioral data across circadian times, "Sleep is a poor predictor of circadian times in Zfhx3*$^{Sci/+}$ *mutants"***

For each LDA, we identified the best linear separator between the light and dark datasets. We then fitted Gaussian distributions to the projection of the data along the direction orthogonal to the discriminant line (white arrow in Figure S2A,C) derived from the LDA line. Interestingly, we found that in wild-type animals, both sleep and behavioral measures significantly discriminated between light and dark phases, whereas in mutant animals, the discriminative power between variables was unbalanced. Indeed, the structure matrix coefficients, which express measures of discriminative power for each variable, showed that *Zfhx3*$^{+/+}$mice exhibited similar coefficients among all paired variables (Figure S2 and Table S3,4). Remarkably, in *Zfhx3*$^{Sci/+}$ mice, the SMCs for sleep measures (Figure S2A,C and Table S3,4) were very low (gray areas), while for behavioral measures, they were very high (black areas). Furthermore, when we examined the probability of being misclassified among the light and dark phases for each set of paired variables and for each time of the day, we observed a higher probability of misclassifying observations during the light-dark transitions in wild-type animals,

suggesting that the distribution of the datasets throughout the 24 hour cycle was homogenous in wild-type mice. Overall, these results suggest that both behavior and sleep are good predictors of light/resting and dark/active performance in *Zfhx3*$^{+/+}$ wild-type mice, and both variables equally discriminate throughout the 24 hour cycle.