Supplementary Materials for

Influence of Schizophrenia-Associated Gene *Egr3* on Sleep Behavior and Circadian Rhythms in Mice

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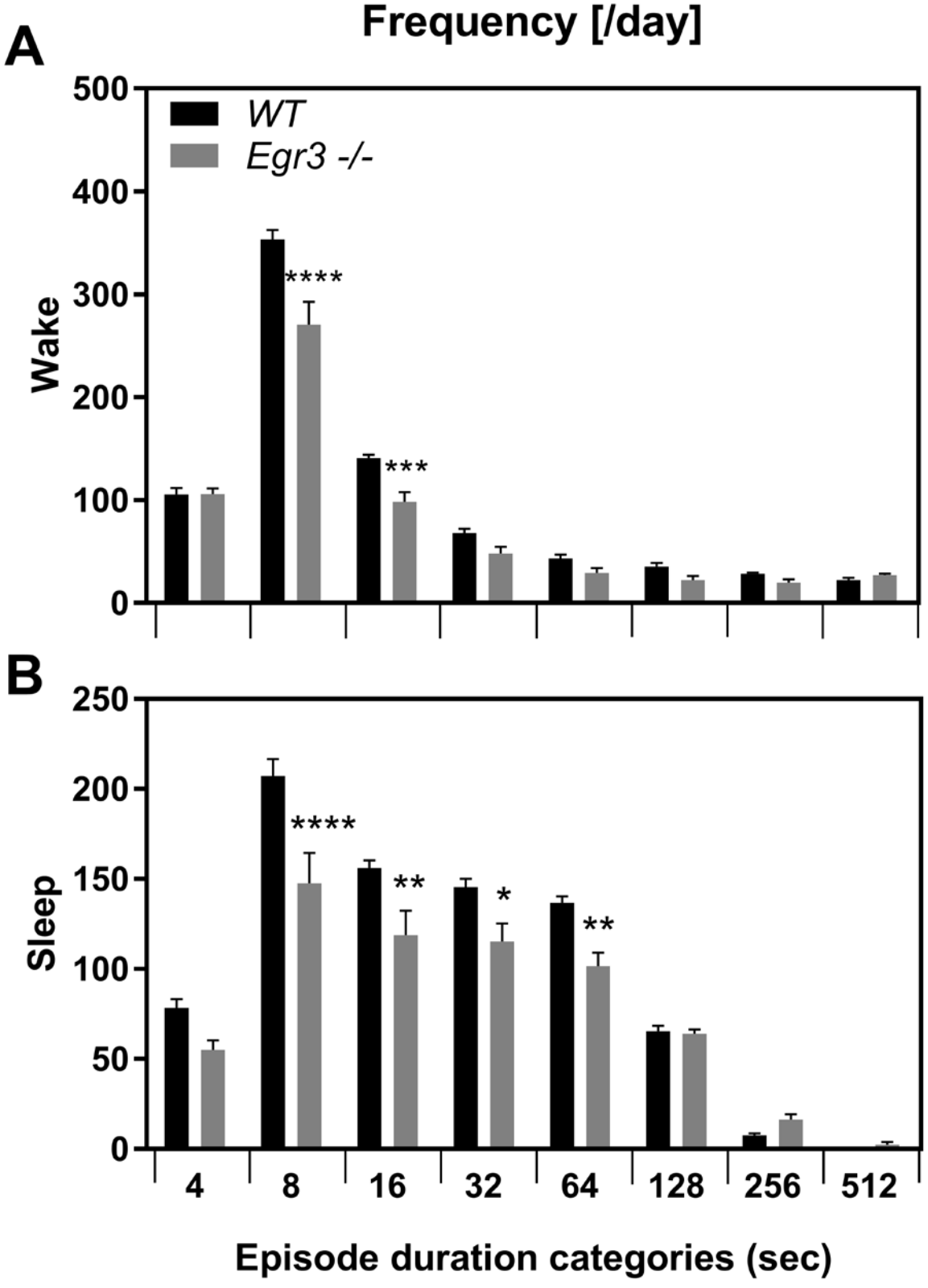
Materials and Methods

Longitudinal Study Methods. Female WT and *Egr3*−/− littermate mice were generated from matings of heterozygous *Egr3*+/- parents that had been back-crossed to a C57BL/6NTac background for greater than 20 generations and are therefore considered congenic. Mice (n = 6-8 per genotype; 4 months of age at the start of the experiment) were acclimated to single housing in the colony room for 9 days prior to sleep and circadian assessment with the *PiezoSleep* Mouse Behavior Tracking System for **9 weeks** (3 weeks under an entrained 14:10 LD cycle, followed by 3 weeks each of DD then LL; constant conditions separated by 14 days of LD reentrainment, which were not analyzed). Estrous was not monitored during this ~3-month observation window so that the animals could remain undisturbed from direct human contact and remain naïve to overt time cues while they were free-running. Given the long duration of each component of the study, any influence of the estrous cycle on sleep-circadian behavior is likely to have averaged out (Prendergast et al., 2014; Smarr et al., 2017). The piezo apparatus consisted of four individual transparent polycarbonate compartments. The floor of each compartment was fitted with a polyvinylidine diflouride (PVDF) square film (17.8 X 17.8 cm, 110 mm thick), which functioned as a pressure sensor (Measurement Specialties, Inc., Hampton, NY, USA). The PVDF films were connected to a recording station and coupled to an input differential amplifier. Pressure signals were generated and classified via a non-invasive high-throughput classifier across each 2s epoch of the recordings as either motions consistent with wake activity, or the relative inactivity associated with sleep. Under this classification scheme, sleep was characterized by small periodic pressure measurements displaying regular amplitudes, typical of the respiration of a quiescent mouse (Donohue et al., 2008). Wake was scored when there was an absence of the sleep signal and when higher amplitude, irregular-spiking pressure movements associated with behavioral activity were present (assessed by SleepStats, Signal Solutions LLC, Lexington, KY). Circadian analyses were conducted with a "percent wake" variable, which was measured by calculating the percentage of 2s bins scored as "wake" (0-100%) within each minute of the recording. Here, the minute-by-minute "percent-wake" variable was used as a proxy measure for locomotor activity. All circadian analyses based on this percent-wake variable were quantified using ClockLab software (Actimetrics, Wilmette, IL), including NPCRA and assessments of circadian robustness or amplitude, phase angle of entrainment (based on estimated onsets and offsets), free-running rhythm (*tau*), and the duration of the active phase (*alpha*). For phase marker quantification in ClockLab, daily onsets were calculated using a template-matching algorithm that searched for a timepoint at the intersection of a 10-h period of relative immobility followed by a 12-h period of consolidated waking behavior that exceeded the 20th percentile level for the percent-wake measure. Daily offsets were determined using a reciprocal template-matching strategy.


A
Frequency [/day]
WT
Egr3 -/-
Wake
500
400
300
200
100
0
****
***
B
Sleep
250
200
150
100
50
0
****
**
*
**
4
8
16
32
64
128
256
512
Episode duration categories (sec)

Supplementary Figure 1. Histogram of wake-sleep bout duration. The frequency per day (averaged over a 7-day period) of individually defined bouts of **Wake** and **Sleep** with different episode durations was analyzed and assigned to one of eight bins of logarithmically increasing size (**4**-7, **8**-15, **16**-31, **32**-63, **64**-127, **128**-255, **256**-511, and > **512** sec). Each vertical bar represents the mean (± SEM) number of bouts per bin over a 24-hour period (averaged across 7 days of recording). (**A**) *Egr3*-/- mice exhibited fewer bouts of wake than WT littermates (repeated measures, two-way ANOVA, genotype x wake duration category interaction, $F(7, 84) = 11.24$, $p < 0.0001$; main effect of genotype, $F(1, 12) = 15.58$, $p = 0.0019$; main effect of wake duration category, $F(7, 84) = 529.0$, $p < 0.0001$). Post-hoc analyses revealed that the *Egr3*-/- mice has fewer short bouts of wake (8 – 16 sec). This tendency to sustain waking activity for longer periods of time is consistent with other arousal phenotypes noted in *Egr3*-/- mice during the study (e.g., increased overall wake, expanded *alpha)* (**B**) *Egr3*-/- mice also exhibited fewer bouts of sleep than WT littermates (repeated measures, two-way ANOVA, genotype x sleep duration category interaction, $F(7, 84) = 8.176$, $p < 0.0001$; main effect of genotype, $F(1, 12) = 15.49$, $p = 0.0020$; main effect of sleep duration category, $F(7, 84) = 233.1$, $p < 0.0001$). Post-hoc analyses revealed that, in addition to showing fewer short bouts of sleep (8 – 16 sec), *Egr3*-/- mice also displayed fewer medium bouts of sleep (32 – 64 sec), than their WT littermates. These data suggest that both wake and sleep are more consolidated in *Egr3*-/- mice and may help to explain the time-series measured increase in circadian amplitude of their daily activity rhythm. Bins where frequency differences are observed between genotypes are marked with an asterisk in the figure; statistical significance was determined via post-hoc, with Sidak's multiple comparisons test, * $p < 0.05$, ** $p< 0.01$, *** $p<0.001$, **** $p<0.0001$.

References

Donohue KD, Medonza DC, Crane ER, O'Hara BF (2008) Assessment of a non-invasive high-throughput classifier for behaviours associated with sleep and wake in mice. Biomed Eng Online 7:14.

Prendergast BJ, Onishi KG, Zucker I (2014) Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci Biobehav Rev 40:1-5.

Smarr BL, Grant AD, Zucker I, Prendergast BJ, Kriegsfeld LJ (2017) Sex differences in variability across timescales in BALB/c mice. Biol Sex Differ 8:7.