Supporting Information

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SI Materials and Methods

Experimental Animals. All procedures and protocols were approved by the Institutional Animal Care and Use Committee at Yale University. Adult (24–42 g) WT and *Lmx1b*$^{f/f/p}$ littermate pairs of both sexes were housed in standard cages in a 12-h light/12-h dark regimen with food and water ad libitum. Genotyping was performed as previously described (1, 2). Generation (2) and breeding (1) of *Lmx1b*$^{f/f/p}$ mice have been described previously. Briefly, female *Lmx1b*$^{f/f}$ (WT) mice were mated with male *Lmx1b*$^{f/f}$ and *Lmx1b*$^{f/f/p}$ mice to produce progeny of these two genotypes in a 1:1 ratio.

EEG/EMG Data Acquisition and Analysis. EEG/EMG head mounts (8201; Pinnacle Technology, Inc.) and temperature/activity telemeters (G2 E-Mitter; Mini-Mitter, Inc.) were implanted as previously described (3). Animals were allowed to recover for at least 7 d before being studied. Animals were introduced to the recording chamber and allowed to acclimate as described below. Animals were fit with a small preamplifier (8202-SL; Pinnacle Technology, Inc.) attached directly to the implanted head mount. The head mount was attached to an amplifier (EX4-400 Quad Channel Differential Amplifier; Dagan Corp. or Model 440 Instrumentation Amplifier; Brownlee Precision Co.) by a tether and commutator (8204; Pinnacle Technology, Inc.). Data were digitized with an analog-to-digital converter (8206; Pinnacle Technology, Inc. or PCI-6221; National Instruments Corp.) and collected using software custom-written in Matlab (Mathworks, Inc.). EEG signals were amplified by 50,000 and band pass-filtered from 0.3 to 200 Hz. EMG signals were amplified by 50,000 and band pass-filtered from 10 to 1,000 Hz. Data were manually scored in 10-s epochs as W, NREM, or REM using software custom written in MatLab. Vigilance state was assigned using a standard approach (4) based on the EEG/EMG frequency characteristics as follows: W, low-amplitude high-frequency (7–13 Hz) EEG with high EMG power; NREM, high-amplitude low-frequency (0.5–4 Hz) EEG with moderate to low EMG power and lack of motor activity; and REM, moderate-amplitude moderate-frequency (4.5–8 Hz) EEG with minimal EMG power, except for brief bursts and minimal activity correlating with EMG bursts. Fast Fourier transform power spectra were created with Matlab for each 10-s epoch of data and used along with EEG and EMG characteristics to verify scoring.

Activity/Temperature/Gas Concentration Data Acquisition. Activity and body temperature were recorded via implanted telemeters. The recording chamber was positioned on top of a telemetry receiver (ER-4000 Energizer Receiver; Mini-Mitter, Inc.). Activity was registered when an animal changed location on top of the receiver. Temperature and activity data were relayed via the A-D converter to a desktop computer equipped with acquisition software custom written in MatLab. O_2 and CO_2 concentrations within the recording chamber were determined using CO_2 and O_2 gas analyzers (AEI Technologies) and recorded with the same custom software. Ambient temperature and relative humidity were recorded with temperature (BAT-12 microprobe thermometer; Physitemp Instruments, Inc.) and relative humidity (HIH-4602-A; Honeywell International, Inc.) sensors and the same software.

Twenty-Four–Hour Sleep Recordings. Animals were acclimated to the recording setup and EEG-tethered for at least 18 h before starting the 24-h data collection period. Animals were kept in a modified home cage for 24-h collections. Food and water were available ad libitum. The ambient temperature was maintained at 23, 30, or 33 °C. EEG, EMG, body temperature, and locomotor activity data were recorded as above. All experiments were videotaped (AF20 Webcam; Logitech) to aid in scoring of vigilance state.

Hypercapnic/Hypoxic Challenges. Animals were placed into the 350-cm^3 Plexiglas recording chamber equipped with bedding, food, and water and allowed to acclimate for 2 h on 3 d (consecutive) before the actual experimental trial. The gas within the chamber at baseline was RA (21% O_2, balance N_2) and was changed to hypercapnia (3, 5, 7, or 10% CO_2 with 21% O_2, balance N_2; 10 min) or hypoxia (5% O_2, balance N_2; 2 min). After a baseline recording period in RA for at least 60 min, animals were treated with four cycles of test gas alternating with RA. The gas was changed back to RA for 15–20 min between test gas exposures. Each test gas exposure was initiated after mice were noted to be asleep as determined by observation of the EEG/EMG recording, eye closure, and absence of motor activity. Flow rates were maintained at 710–760 mL/min with a flow meter (R-32004-10 or WU-32446-33; Cole–Parmer, Inc.). All compressed gas containers were obtained from Airgas East. To determine if the responses could be attributable to auditory, olfactory, or tactile (pressure) cues that might be associated with a gas change, control experiments were performed by changing the gas line from one delivering RA to another delivering RA.

Mechanical/Auditory Stimuli. Animals were placed into the recording chamber in RA. For mechanical stimulation, puffs of compressed air (2–18 psi, 100 ms, 1 cm from the animal's flank) were delivered through a small-diameter (0.039-in i.d.) polyethylene tube attached to a picospritzer and solenoid valve (General Valve). Auditory stimuli consisted of a mixture of 5-, 10-, 15-, and 20-kHz frequencies delivered over a range of intensities (45–90 dB) for 500 ms at a distance of 3 cm from the animal. Stimuli were generated and delivered with software custom written in Matlab.

1. Hodges MR, Wehner M, Aungst J, Smith JC, Richerson GB (2009) Transgenic mice lacking serotonin neurons have severe apnea and high mortality during development. *J Neurosci* 29:10341–10349.
2. Zhao ZQ, et al. (2006) Lmx1b is required for maintenance of central serotonergic neurons and mice lacking central serotonergic system exhibit normal locomotor activity. *J Neurosci* 26:12781–12788.
3. Hodges MR, et al. (2008) Defects in breathing and thermoregulation in mice with near-complete absence of central serotonin neurons. *J Neurosci* 28:2495–2505.
4. Franken P, Malafosse A, Tafti M (1998) Genetic variation in EEG activity during sleep in inbred mice. *Am J Physiol* 275:R1127–R1137.

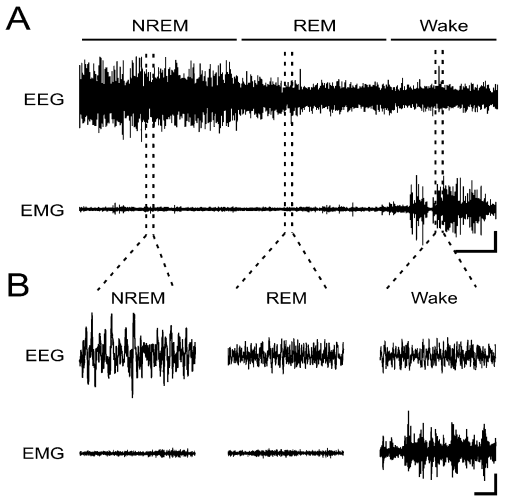

Fig. S1. Identification of normal vigilance states in *Lmx1b*$^{f/f/p}$ mice. (*A*) Continuous 5-min EEG (*Upper*) and EMG (*Lower*) traces depicting transition from NREM to REM to Wake as indicated. (Horizontal scale bar, 30 s; vertical scale bar, 5 μV.) (*B*) Ten-second segments of data from trace in *A*, as indicated by dashed lines. (Horizontal scale bar, 2 s; vertical scale bar, 5 μV.) In NREM, there is high-amplitude low-frequency EEG with low-amplitude EMG. In REM, there is low-amplitude moderate-frequency EEG with low-amplitude EMG. In Wake, there is low-amplitude high-frequency EEG with high-amplitude EMG.

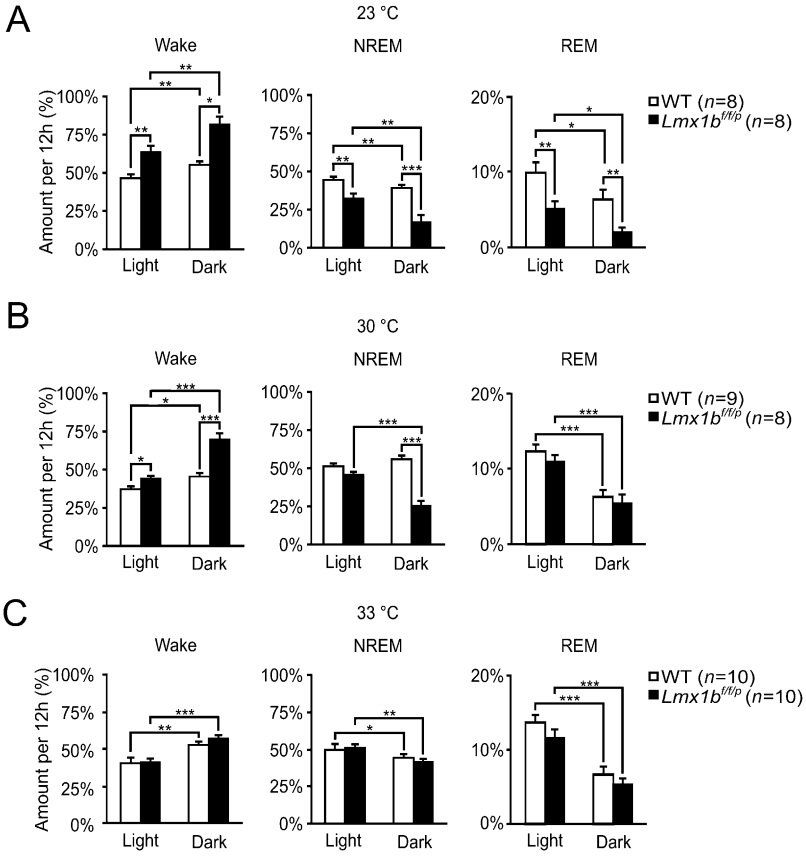

Fig. S2. Percentage of time spent in each vigilance state in light vs. dark at 23 °C (*A*), 30 °C (*B*), and 33 °C (*C*). The increased proportion of time *Lmx1b*$^{f/f/p}$ mice spent awake and the decreased proportion of time spent in NREM at 23 and 30 °C is more pronounced in the dark. At 23 °C, *Lmx1b*$^{f/f/p}$ mice also spent significantly less time in REM. This difference was not appreciated at 30 °C. Bar graphs depict percentage of 12-h light and 12-h dark periods spent in W, NREM, and REM, respectively, for WT (white) and *Lmx1b*$^{f/f/p}$ (black) mice. *$P < 0.05$; **$P < 0.005$; ***$P < 0.0001$.

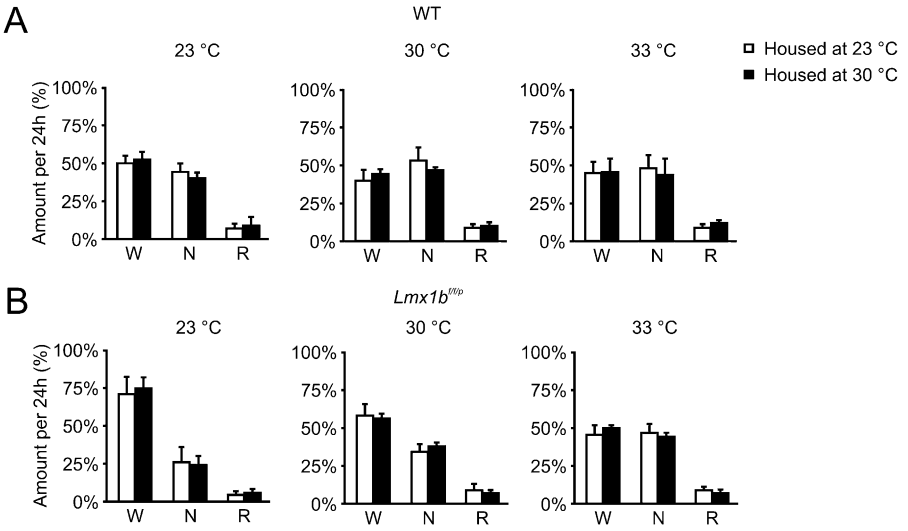

Fig. S3. Preexperimental housing temperature had no effect on sleep/wake architecture in WT or *Lmx1b*$^{f/f/p}$ mice at any given recording temperature. Bar graphs depict percentage of 24-h recording period spent in W, NREM (N), and REM (R), respectively, for WT (*A*) and *Lmx1b*$^{f/f/p}$ (*B*) mice when housed at 23 °C (white) or 30 °C (black) for 10 d and then recorded at 23 °C (*Left*), 30 °C (*Middle*), or 33 °C (*Right*).

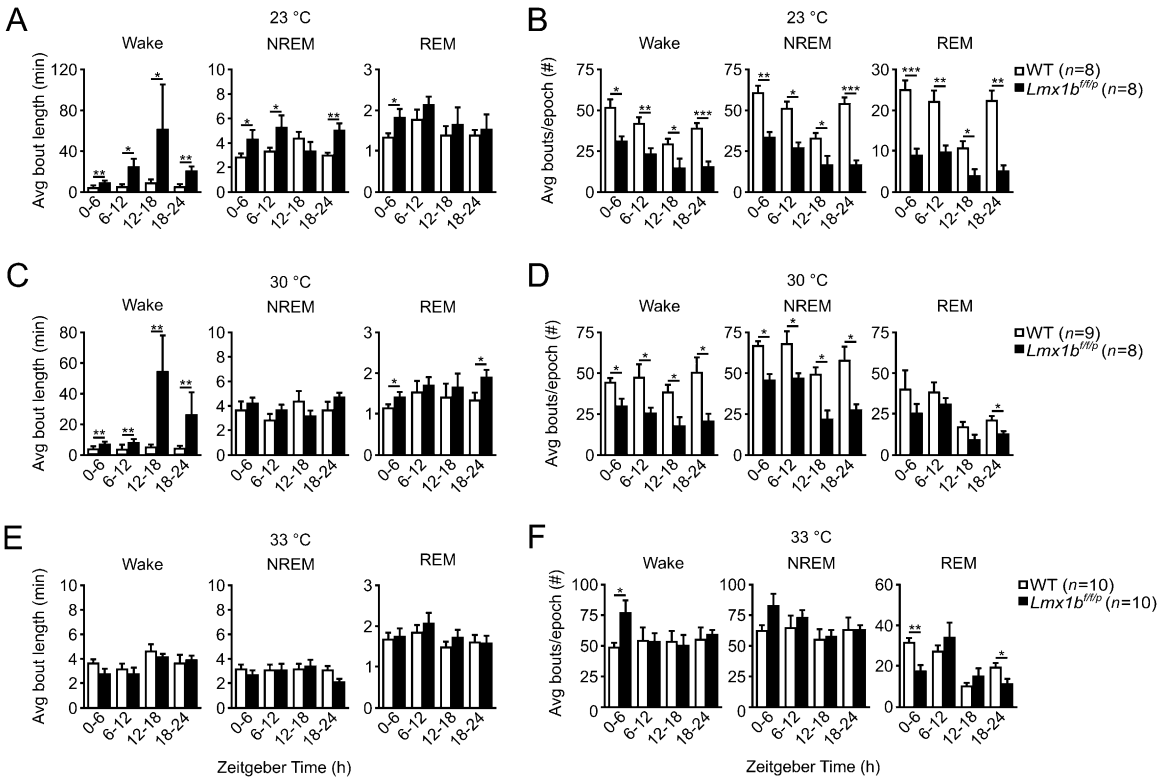

Fig. S4. *Lmx1b*$^{f/f/p}$ mice had longer wakefulness bouts, and thus fewer vigilance state transitions, during the dark period at 23 and 30 °C but not at 33 °C. The average length of each bout (*A*, *C*, and *E*) and the total number of bouts (*B*, *D*, and *F*) of Wake, NREM, and REM for WT (white) and *Lmx1b*$^{f/f/p}$ (black) mice are shown for each 6-h epoch throughout the 24-h recording period at 23 °C (*Top*), 30 °C (*Middle*), and 33 °C (*Bottom*). *$P < 0.05$; **$P < 0.005$; ***$P < 0.0001$.

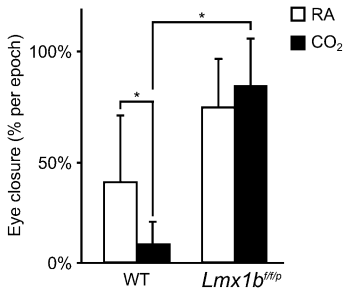

Fig. S5. *Lmx1b*$^{f/f/p}$ mice spent more time with their eyes closed during hypercapnia compared with WT mice. Shown is the percentage of time spent with eyes closed for WT (*Left*) and *Lmx1b*$^{f/f/p}$ (*Right*) mice in RA (white) and 7% CO_2 (black). WT mice spent a significantly reduced proportion of time with their eyes closed in hypercapnia compared with RA. In contrast, *Lmx1b*$^{f/f/p}$ mice spent a similar proportion of time with their eyes closed in hypercapnia compared with RA. There was no significant difference in the percentage of time with eyes closed in RA for either genotype (n = 6 for each genotype). *P < 0.05.

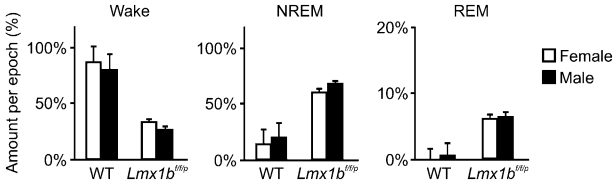

Fig. S6. There was no sex difference in the percentage of time spent in each vigilance state during the hypercapnic challenge. Bar graphs depict the percentage of hypercapnic challenge spent in Wake, NREM, and REM, respectively, for female (white; n = 4 WT, 5 *Lmx1b*$^{f/f/p}$) and male (black; n = 5 WT, 5 *Lmx1b*$^{f/f/p}$) WT and *Lmx1b*$^{f/f/p}$ mice as indicated.

Table S1. Vigilance state distribution differs in male but not female mice

	W		NREM		REM	
Sex	WT (% ± SEM)	*Lmx1b*$^{f/f/p}$ (% ± SEM)	WT (% ± SEM)	*Lmx1b*$^{f/f/p}$ (% ± SEM)	WT (% ± SEM)	*Lmx1b*$^{f/f/p}$ (% ± SEM)
Male	39.1 ± 3.4	57.6 ± 3.5*	52.5 ± 3.8	33.5 ± 2.2*,**	8.5 ± 1.0	8.9 ± 1.7
Female	43.9 ± 5.0	50.2 ± 1.1	48.8 ± 4.5	41.6 ± 1.3	7.3 ± 1.7	8.2 ± 1.4

Female *Lmx1b*$^{f/f/p}$ mice spent slightly more time awake and less time in NREM sleep than WT mice, but these differences were not statistically significant (P = 0.13 and P = 0.09, respectively). Female *Lmx1b*$^{f/f/p}$ mice also spent slightly less time awake (P = 0.05) and more time in NREM sleep (P = 0.015) compared with their male counterparts. Data are from animals housed at 23 °C and recorded at 30 °C [n = 4 for female WT and male and female *Lmx1b*$^{f/f/p}$, n = 5 for male WT; amount per 24 h (% ± SEM)]. *P < 0.05 between genotypes; **P < 0.05 between sexes.