## SUPPLEMENTARY INFORMATION

### PSG, "score_method"

Recordings were scored in 10-s epochs for sleep/wake states utilizing automated methods in Somnivore (Somnivore, Ltd., Pty; Allocca et al., 2019). Each scoring period was provided to Somnivore (ver. 1.1.4.0) in EDF format from which 100-200 10-sec epochs of each state, Wake, Non-Rapid Eye Movement (NREM) sleep, and Rapid Eye Movement (REM) sleep were selected by an expert human scorer as training data. The selected data were used to train a classifier and applied in a supervised machine learning model to automatically score each state for the duration of each recording. Following this initial scoring, the accuracy of the autoscored data was assessed visually. If the automated scoring was determined by the expert human scorer not to generalize well, an additional 1-21 misidentified epochs were provided to the training dataset and automatic scoring of the recording was repeated. Manual review and correction of the autoscored dataset was then performed when the following occurred: 1) single epochs of REM sleep and 2) REM sleep directly following wake.

### Animal, "experimental_procedures"

**a. Surgical Procedures**: Male (N = 7; age: 10.4 ± 0.5 weeks) and female (N = 7; age: 9.9 ± 0.3 weeks) C57BL6/J mice were implanted with telemetric devices (F20-EET; DSI, St-Paul, MN, USA) for recording 1 electroencephalogram (EEG) channel and 1 electromyogram (EMG) channel, subcutaneous body temperature ($T_{sc}$) and activity. Mice were anesthetized with isoflurane (induction: 3–5% isoflurane in oxygen delivered at 1 L/min; maintenance: 1–2% isoflurane in oxygen delivered at 1 L/min). Telemeters were placed in a blunt-dissected subcutaneous (SC) pocket located on the left dorsum and biopotential leads were routed to the head. EMG leads were placed in the right nuchal muscle. Cranial holes were drilled through the skull at -2.0 mm AP from bregma and 2.0 mm ML and on the midline at −1 mm AP from lambda. The EEG leads were inserted into these holes and affixed to the skull with dental acrylic. The incision was closed with absorbable suture. Analgesia was managed with meloxicam (5 mg/kg, SC) and buprenorphine (0.05 mg/kg, SC) prior to emergence from anesthesia and for the first day post-surgery. Meloxicam (5 mg/kg, SC) was continued for 2 d post-surgery. A minimum of two weeks of post-surgical recovery were allowed before experimental protocols were initiated.

After surgery, mice were housed individually in home cages with access to food, water, and nestlets *ad libitum*. Room temperature (22±2°C), humidity (50±20% relative humidity), and lighting conditions (LD12:12, where Zeitgeber time (ZT) 0=lights on and ZT12=lights off) were monitored continuously. Animals were inspected daily in accordance with AAALAC and SRI guidelines. All experimental procedures were approved by the Institutional Animal Care and Use Committee at SRI International.

**b. Experimental Design:** Figure 1 depicts the dosing schedule used in each arm of this crossover study. Each mouse received two treatments in a counterbalanced manner; 16 days elapsed between the final treatment in the first arm of the study and the 1st treatment in the second study arm:

Treatment 1: Bidaily SC injections of escalating doses of morphine sulfate (Day 1: 5 mg/kg, Day 2: 10 mg/kg, Day 3: 20 mg/kg, Day 4: 40 mg/kg and Day 5: 80 mg/kg) over 5 days followed by 7 days without injections.
Treatment 2: Bidaily SC injections of saline over 5 days followed by 7 days without injections.

The experimental cohorts in this study were equally sized. In this treatment paradigm, one experimental cohort received Treatment 1 in the first study arm, then Treatment 2 in the second study arm following the washout period, while the other experimental cohort received the treatments in the opposite order with Treatment 2 occuring first, then Treatment 1 following washout. For each treatment, mice received bidaily SC injections at ZT1 and ZT13 of constant volume (10 ml/kg; Figure 1). Mice received a habituation injection of saline (volume: 10 ml/kg) 2 days before initiating each arm.

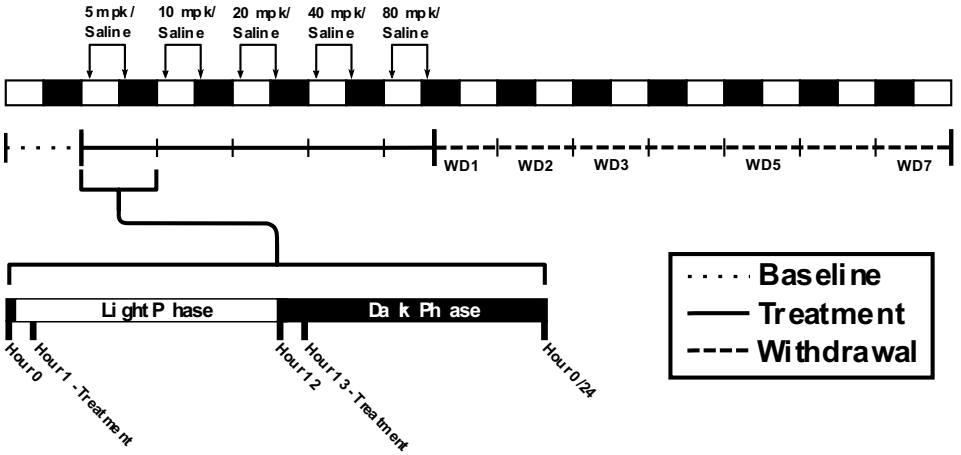


**Figure 1.** Schematic illustrating the experimental design and dosing schedule used in this study. After a 24-h baseline EEG/EMG recording, experimental treatments were initiated at hour ZT1 on the following day and continued for the next 5 days. Bidaily subcutaneous (s.c.) dosings occurred at ZT1 and ZT13 on each day during the 5-day treatment phase, with the morphine dose doubling every day. The withdrawal phase lasted 7 days, commencing at the final dose. An EEG recording was initiated at ZT13 on WD1 and continued for 71 h through WD3. Separate 24-h recordings were initiated at ZT12 on WD5 and WD7. Abbreviations: WD, Withdrawal day.

**c. Duration of data collection:**

All mice underwent a 24-h baseline (BL) recording prior to each study arm in which EEG, EMG, $T_{sc}$, signal strength, and gross motor activity were recorded via telemetry using Ponemah (DSI, St-Paul, MN, USA). EEG and EMG were sampled at 500 Hz. $T_{sc}$ and signal strength were recorded at 10 Hz, while gross motor activity was recorded at 1 Hz. After the last morphine dose at ZT13 on Day 5, EEG, EMG, $T_{sc}$, signal strength, and gross motor activity were continuously recorded for 71 hours (withdrawal days (WD) 1-3) and for 24 hours on WD5 and WD7.

**References**

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