Trace Amine-Associated Receptor 1 Agonists as Narcolepsy Therapeutics

Supplementary Information

SUPPLEMENTAL METHODS AND MATERIALS

Experimental Protocol 1: Efficacy of TAAR1 full and partial agonists in WT and Taar1 KO mice

Surgical procedures. Under isoflurane anesthesia, a sterile telemetry device (G2 E-Mitter; Phillips Respironics, Bend OR) was surgically implanted in the peritoneal cavity and a prefabricated EEG/EMG headmount (8201-C; Pinnacle Technologies, Lawrence KS) was affixed to the skull with 4 stainless steel screws positioned approximately 1.5 mm lateral to the sagittal suture, +2.0 mm and -4.0 mm from bregma. Two stainless steel braided EMG wires were inserted into the trapezius muscle and sutured in place. The headmount was secured with conductive silver epoxy and dental acrylic. Analgesia was managed with pre-surgical line block (bupivacaine 0.25%, 0.8 mL/kg), followed by buprenorphine (0.1 mg/kg, s.c.) upon emergence from anesthesia. Ketoprofen (5 mg/kg, s.c., q.d.) was administered for 3 d post-surgery.

EEG/EMG, T_b and LMA recording and analysis. Mice were allowed 3 weeks to recover post-surgery and had at least 4 days of adaptation to tethers, handling and dosing procedures prior to data collection. Mice were studied in their home cages with extended vertical Plexiglas sides that permitted the animal to be tethered for EEG/EMG recordings. Cages were kept inside ventilated, light-tight sound-attenuated chambers in a 12:12h light-dark cycle. Room temperature (22±2^{o}C), humidity (50±20% relative humidity), and lighting conditions (LD12:12) were monitored continuously via computer. Animals were inspected daily in accordance with AAALAC and SRI guidelines and body weights were taken weekly during cage changes.

EEG/EMG data were continuously recorded using iox2 (EMKA Technologies, France). Mice were connected to flexible cables attached to swivel commutators (Pinnacle Technologies) mounted above the cage's center, allowing unrestricted movement across the entire cage. Electrophysiological signals were amplified with Grass Model 15 amplifiers; EEG signals were high- and low-pass filtered at 0.3 Hz and 300 Hz, respectively, and EMG signals were high- and low-pass filtered at 3 Hz and 6 KHz, respectively. Amplified electrophysiological signals were sampled at 500 Hz. Locomotor activity (LMA) and core body temperature (T_b) were continuously recorded from the implanted E-Mitters at 1-min intervals via inductive telemetry using Vitalview (MiniMitter/Philips Respironics). LMA and T_b and EEG/EMG data were collected simultaneously. LMA and T_b data were analyzed in Clocklab (Actimetrics; Evanston IL).

Experimental Protocol 2: TAAR1 full and partial agonists in mouse models of narcolepsy

Animals. DTA mice were the double transgenic offspring of *orexin/tTA* mice (C57BL/6-Tg(orexin/tTA)G5/Yamanaka), which express the tetracycline transactivator exclusively in Hcrt neurons (1) and mice that express diphtheria toxin A fragment in the absence of dietary doxycycline (B6.Cg-Tg(tetO-DTA)1Gfi/J). Both parental strains were obtained from Akihiro Yamanaka (Nagoya University, Japan) and were from a C57BL/6J genetic background. Data were collected from two cohorts of animals: the first cohort contained 10 Atax and 6 DTA mice, and the second cohort contained 9 DTA mice. Two of 10 Atax mice were excluded from analysis because of poor EEG signal quality. Seven of 15 DTA mice were removed from study because either the mouse (*n*=2) or implant (*n*=2) did not survive, the EMG or EEG signal was poor quality (*n*=2), or the mouse experienced premature Dox(-) due to experimenter error. All mice of the

desired sex and genotype that were available for surgery were used (i.e., no randomization of subjects was required). Atax mice were aged 14±0.9 weeks (35±0.8 g) and DTA mice were aged 12±0.9 weeks (30±0.9 g and maintained on Dox) at the time of surgery.

Surgical procedures. Mice were anesthetized with isoflurane (breathing rate and tactile responsiveness were continually monitored) and sterile telemetry transmitters (F20-EET, Data Sciences Inc., St Paul, MN) were placed i.p. along the midline. Biopotential leads were routed subcutaneously to the head, and EMG leads were positioned bilaterally through the nuchal muscles. Cranial holes were drilled 1 mm anterior to bregma and 1 mm lateral to midline and, contralaterally, 2 mm posterior to bregma and 2 mm lateral to midline. EEG leads were placed subcranially over the dura and were attached to the skull with cyanoacrylate and dental acrylic. Analgesia was managed with pre-surgical line block (bupivacaine 0.25%, 0.8 mL/kg) and ketoprofen (5 mg/kg, s.c.) followed by buprenorphine (0.1 mg/kg, s.c.) upon emergence from anesthesia. Ketoprofen (5 mg/kg, s.c., q.d.) continued for 3 d post-surgery.

EEG/EMG, T_b and activity recording and analysis. Mice were permitted at least 3 weeks post-surgical recovery and at least 1 week adaptation to running wheels, handling and dosing procedures prior to data collection. Throughout the study, mice were housed individually in home cages with access to food, water, nestlets and running wheels *ad libitum*. Room temperature (22±2^{o}C), humidity (50±20% relative humidity), and lighting conditions (LD12:12) were monitored continuously via computer. Animals were inspected daily in accordance with AAALAC and SRI guidelines and body weights were taken weekly during cage changes.

EEG and EMG were sampled at 250 Hz. Digital videos were recorded at 10 frames per second, 4CIF de-interlacing resolution.

Data analysis and statistics. Data were manually scored in 10-s epochs by experts (≥ 96% inter-rater reliability) using Neuroscore 2.1 (Data Sciences Inc., St. Paul, MN). Each scorer was blind to treatment condition and scored all records across conditions for their assigned animals. All statistical tests were performed using SigmaPlot 12.5 (Systat Software Inc., San Jose, CA) and from single measurements per animal per condition. Sample sizes per mouse model (n=8) were based on the number of animals estimated to provide sufficient power to detect the smallest expected scientifically relevant difference between group means (30%) at $P \leq 0.05$ with a power of 0.8. The power calculations were computed using G*Power v. 3.1.5 (2).

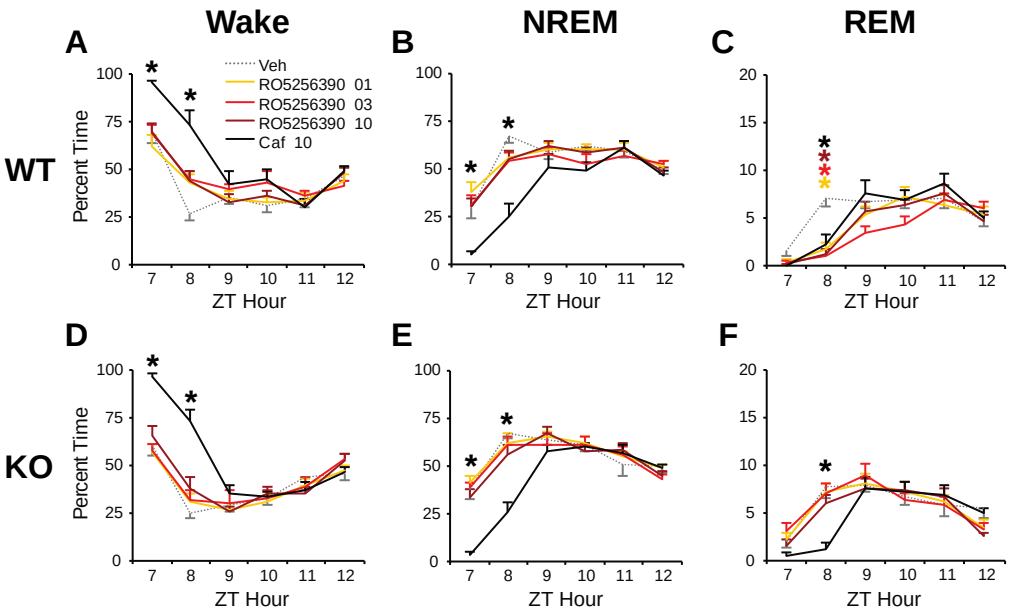

Figure S1. Hourly amounts for the effects of RO5256390 (1-10 mg/kg, warm colors), caffeine (Caf, 10 mg/kg, black) or vehicle (Veh, gray) on all 3 states in WT mice (A-C) and *Taar1* KO mice (D-F) after treatment at zeitgeber time (ZT) 6. Data are presented as mean + s.e.m. from *n*=13 WT and 11 *Taar1* KO mice. Two-way mixed-factor ANOVA and *post hoc* Bonferroni *t* tests: * *P*<0.05 vs Veh; + *P*<0.05 vs WT.

SUPPLEMENTAL REFERENCES

1. Tabuchi S, Tsunematsu T, Black SW, Tominaga M, Maruyama M, Takagi K, et al. (2014): Conditional ablation of orexin/hypocretin neurons: a new mouse model for the study of narcolepsy and orexin system function. J Neurosci 34:6495-6509.
2. Faul F, Erdfelder E, Lang AG, Buchner A (2007): G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39:175-191.