Poster W141

CNT-9982, an orexin receptor 2 agonist, enhances wakefulness in marmosets, and in the Wistar Kyoto rat model of major depressive disorder, normalizes the arousal state phenotype and alleviates behavioral despair

Sarah Wurts Black[1], Tod Steinfeld[1], Karl Gibson[2], Gregory R. Ott[3], Emiliangelo Ratti[2], Stephen J. Kanes[1], Mario Alberto Accardi[4], and Deborah S. Hartman[1]

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BACKGROUND

- OX2R agonists have been shown to enhance wakefulness in rodents, nonhuman primates, healthy volunteers, and patients with narcolepsy and IH[1-4]
- Based on an emerging understanding of orexin physiology, it is hypothesized that OX2R agonists may also modulate cognition, mood, and other neuropsychiatric and neurologic functions
  - Dysregulated orexin signaling in depression, including a blunted diurnal rhythm of OXA, or a reduction of OXA in patients with MDD who have attempted suicide,[5-7] has been reported in clinical studies
  - Reduced numbers of orexin neurons have been reported in Wistar Kyoto (WKY) rats, which recapitulate the hypothalamic-pituitary-adrenal axis dysregulation and behavioral symptoms seen in MDD[8]
- CNT-9982 is an orally available, brain-penetrating, highly potent (EC_{50} <100 picomolar) and selective (>5000-fold selective vs OX1R) OX2R agonist

OBJECTIVE

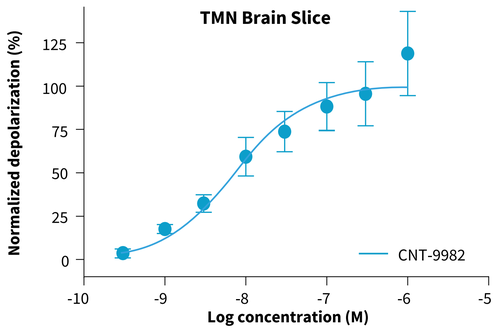
- To evaluate the effects of CNT-9982 on hypersomnolence and behavioral despair in the WKY rat model of MDD

METHODS

- **In Vitro Pharmacology.** i) Calcium mobilization (FLIPR) assays were performed using Chinese hamster ovary cells stably expressing human OX2R. Cells were loaded with a calcium-sensitive fluorescent dye, and agonist-evoked intracellular calcium responses were recorded following addition of test compound. ii) Electrophysiology was conducted in 300-µm coronal brain slices prepared from 3- to 4-week-old male C57BL/6J mice. Whole-cell current-clamp recordings were obtained from neurons in the ventral TMN at 30–32 °C in the presence of tetrodotoxin (1 µM). Histaminergic neurons were identified by their characteristic transient A-type outward current and hyperpolarization-activated inwardly rectifying current.
- **Animals and recordings.** Common marmosets (401–577 g, 4 males, 3 females in the colony at Evotec) and WKY and Wistar rats (8–11 males/genotype, 19–20 wks, purchased from Charles River for use at PsychoGenics) were surgically prepared with telemeters (HD-S02) for EEG and EMG monitoring. Arousal states were manually scored blind in 10 sec epochs using NeuroScore (Data Sciences Inc.). All stages of NREM sleep were combined into the single classification of NREM sleep to simplify analysis. Animals were recorded from home cages in LD12:12, with ad libitum water and environmental enrichment; marmosets were housed with a non-implanted marmoset of the opposite sex. Following EEG studies, rats were sacrificed at 45 wks of age; brains were extracted and processed for immunohistochemistry using anti-rabbit OXA antibody (ab6214, Abcam) at Neuroscience Associates. Bilateral OXA+ cell counts were performed across 3 anatomically matched sections per brain (Aiforia, AI for image analysis). A separate group of WKY rats (200–225 g) was evaluated for locomotor activity by beam breaks in the open field apparatus (Hamilton Kinder) followed by the forced swim test in a no-escape cylinder with water at ambient temperature 1 week later. Five-minute test windows occurred 1 h post dose during the rest phase. All animals were acclimated to handling, the test environment, and dosing procedures prior to experimentation. All experimental procedures were approved by the Italian Ministry of Health or the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.
- **Formulation, dosing, and efficacy study design.** CNT-9982 was formulated for acute oral dosing. Each dosing day, CNT-9982 was serially diluted and administered for EEG studies at the start of the dark period in a counterbalanced design, or, for the FST and open field studies, during the light period in a between-groups design. Data presented were the mean ± SEM. Significant differences from control (indicated by * on graphs) were determined by unpaired two-tailed t test (**Figures 3 and 4**) or Holm-Sidak's multiple comparisons test following analysis of variance (**Figures 5 and 6**).

RESULTS

Figure 1: CNT-9982 activated histaminergic neurons in the TMN, a brain region mediating wake promotion

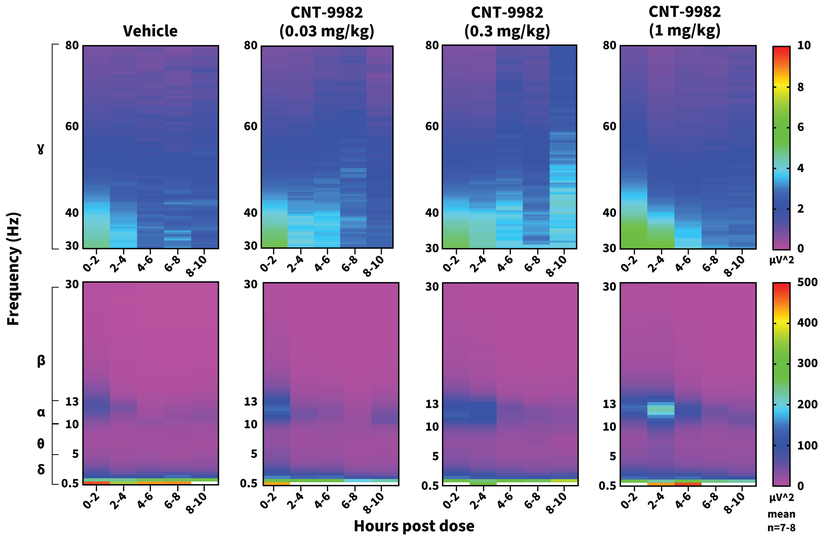


Membrane depolarization potency

Agonist	EC_{50} (nM)
CNT-9982	7.3
OXA	31.7

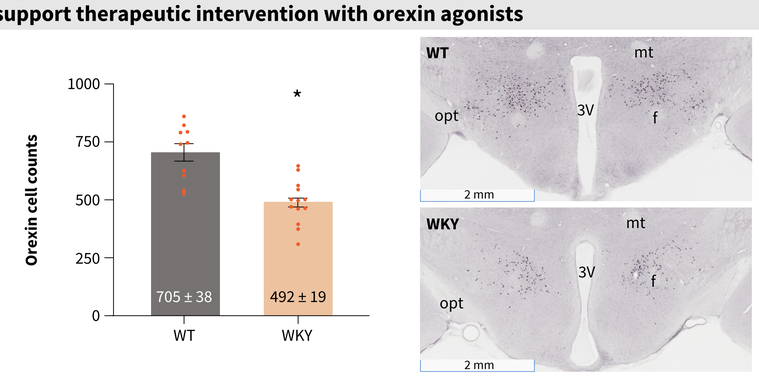
Membrane depolarization of histaminergic neurons in *ex vivo* mouse TMN brain slices by CNT-9982. Concentration-response data are expressed as mean ± SEM of normalized depolarization values (% maximum); each point reflects 3–6 neurons.

Figure 2: In healthy nonhuman primates, CNT-9982 activated the brain at low doses



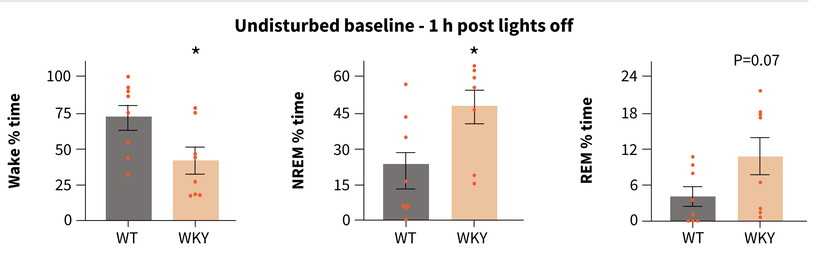
- CNT-9982 increased EEG spectral power during wakefulness in the marmoset brain in a dose- and time-related manner

Figure 3: In the WKY rat model of MDD, decreased numbers of orexin neurons support therapeutic intervention with orexin agonists



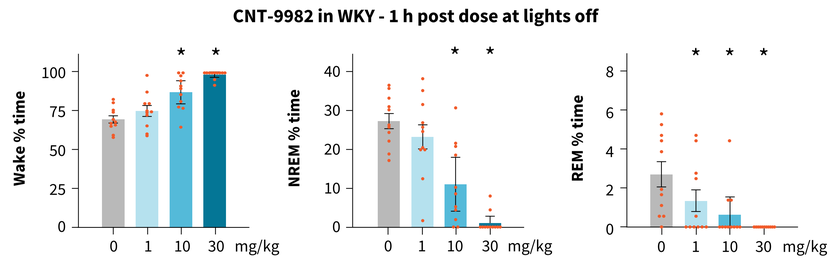
- WKY rats had 30.3% fewer OX+ neurons than WT Wistar rats

Figure 4: WKY rats showed a phenotype of hypersomnolence consistent with reduced orexin tone



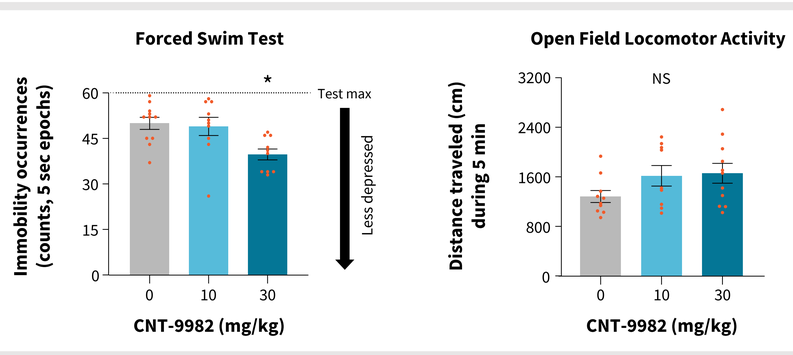
- WKY rats spent less time awake and more time asleep than WT Wistar rats during the first hour of the active phase in undisturbed baseline recordings
- WKY rats showed a phenotype of reduced brain activity on the EEG power spectrum (**Supplement 1**)

Figure 5: CNT-9982 normalized the hypersomnolent phenotype of WKY rats



- CNT-9982 increased time awake and decreased time in NREM and REM sleep during the active phase in a dose-related manner
- CNT-9982 increased activity in the gamma band during wakefulness (**Supplement 1**)

Figure 6: CNT-9982 reduced behavioral despair after an acute dose in the WKY rat model of MDD



- After an acute oral dose of CNT-9982 (30 mg/kg), WKY rats spent less time immobile in the Forced Swim Test of behavioral despair
- CNT-9982 did not change the activity level of the same WKY rats as measured in the Open Field Test
  - WKY rats in the EEG study showed a phenotype of reduced motor activity that was normalized to WT levels after CNT-9982 without changing core body temperature (**Supplement 2**)
- The improvement of the symptom of depression (behavioral despair) in WKY rats was not confounded by a non-specific increase in motor activity

CONCLUSIONS

- Immediate antidepressive effects were observed after a single dose of CNT-9982 at 30 mg/kg, within the wake-promoting dose range
- WKY rats showed a phenotype of hypersomnolence consistent with reduced orexin tone
  - WKY rats had 30.3% fewer numbers of OX+ neurons than WT Wistar rats
  - NREM and REM sleep were increased, and wakefulness was decreased, in WKY rats during the first hour of the active phase compared with WT Wistar rats
- CNT-9982 normalized the hypersomnolent phenotype of WKY rats compared with WT Wistar rats with a dose-related increase in wakefulness and decrease in NREM and REM sleep

Results support OX2R activation as a potential MOA for rapid-onset treatment to improve mood symptoms and alleviate hypersomnolence in neurologic and psychiatric disorders

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ABBREVIATIONS

AI, artificial intelligence; EC50, half maximal effective concentration; EEG, electroencephalogram; EMG, electromyogram; FLIPR, fluorescent imaging plate reader; FST, forced swim test; IH, idiopathic hypersomnia; MDD, major depressive disorder; MOA, mechanism of action; NREM, non-rapid-eye-movement; NS, not significant; OX+, orexin; OXA, orexin A; OX1R, orexin receptor 1; OX2R, orexin receptor 2; OXA, orexin-A; REM, rapid-eye-movement; SEM, standard error of the mean; TMN, tuberomammillary nucleus; WKY, Wistar Kyoto; WT, wild type

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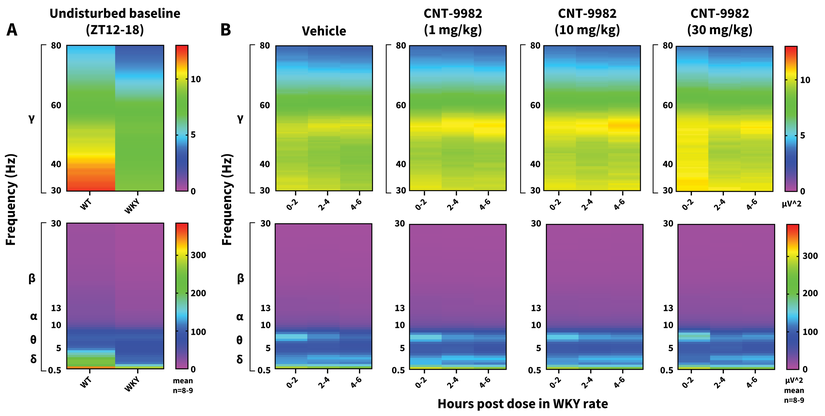
SUPPLEMENTAL

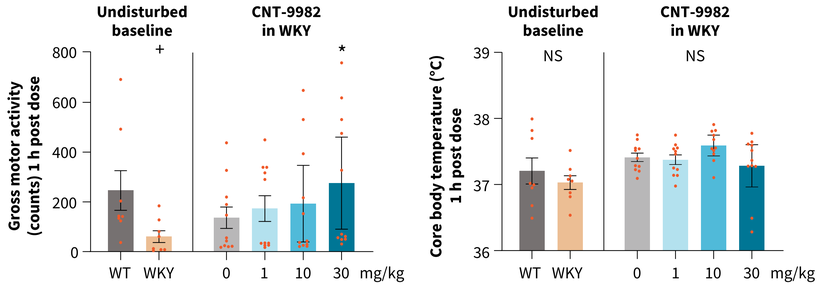
Supplement 1: Changes in EEG spectral power during wakefulness in WKY rats following oral CNT-9982



Comparison of EEG spectral power between WT Wistar and WKY rats (A), and changes in EEG spectral power during wakefulness in WKY rats following oral CNT-9982 (B).

- WKY rats showed a phenotype of reduced brain activity on the EEG power spectrogram
- CNT-9982 increased activity in the gamma band during wakefulness

Supplement 2: Normalization of gross motor activity and core body temperature between WKY and WT Wistar rats following a single dose of CNT-9982



Baseline n=8/genotype, 1 h test window began at the start of the active phase. CNT-9982 was dosed at the start of active phase in 11 male WKY rats. *P<0.05 vs 0 mg/kg, +P<0.05 vs WT.

- CNT-9982 mildly increased gross motor activity in WKY rats to the level observed in WT Wistar rats in the first hour post dose (blue bars, left panel)
- Core body temperature did not differ between WKY and WT Wistar rats, nor did CNT-9982 change it
- Hyperlocomotion was not observed after CNT-9982 in WKY rats