

# Evaluation of the Novel Orexin 2 Receptor Agonist ALKS 2680 on Measures of Arousal Circuit Activation in Rodents

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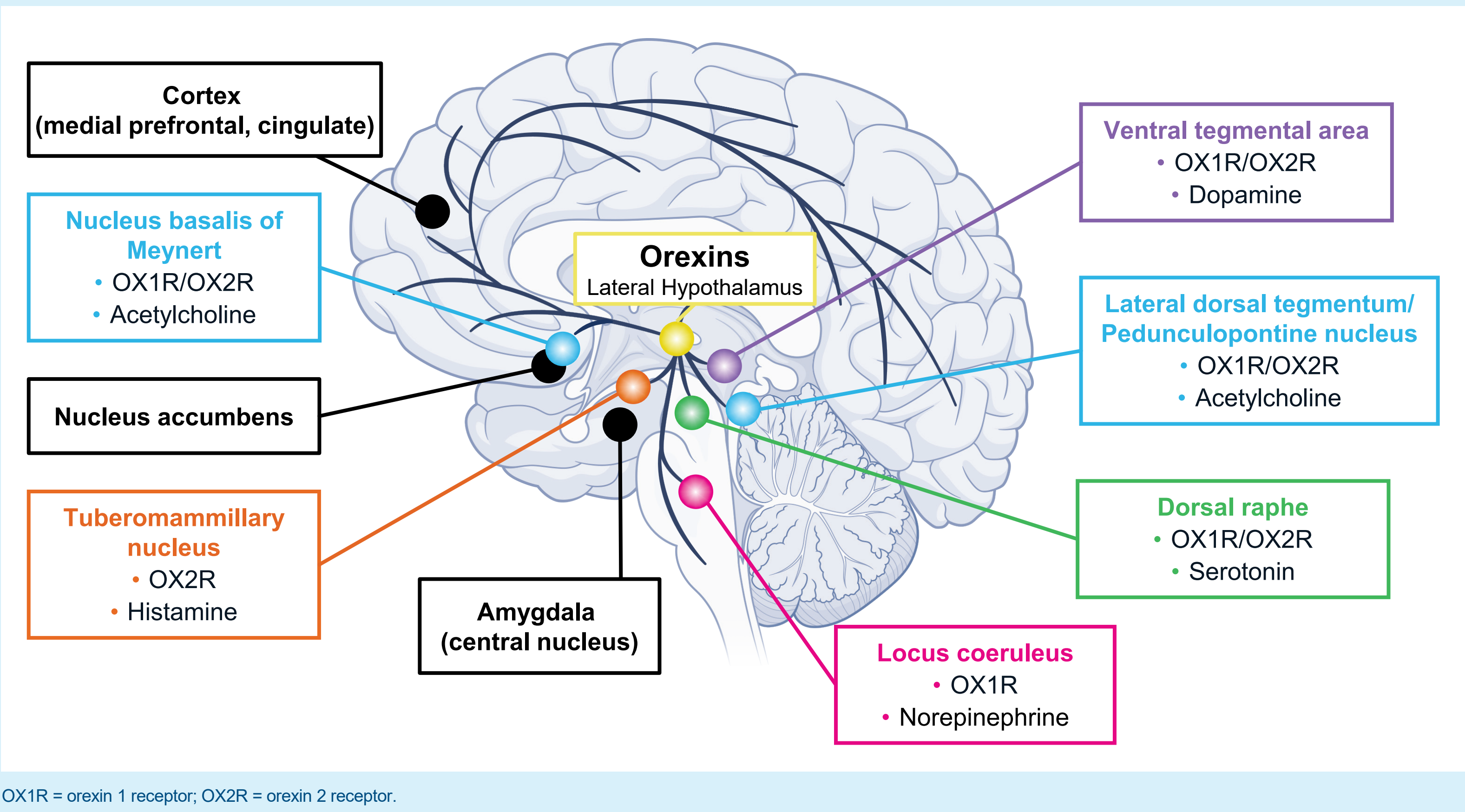
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## INTRODUCTION

- The orexin/hypocretin system acts as a key regulator of wakefulness through its connections to multiple downstream arousal neurotransmitter pathways<sup>1</sup> (Figure 1)
- Selectively targeting the orexin 2 receptor (OX2R) may provide novel treatment strategies for narcolepsy and related disorders
- ALKS 2680 is a highly potent, oral, and selective OX2R agonist being developed as a once-daily treatment for narcolepsy and idiopathic hypersomnia

FIGURE 1: Orexin-Mediated Brain Pathways Regulating Wakefulness



## OBJECTIVE

- To characterize network engagement and functional activation induced by ALKS 2680 in preclinical rodent models

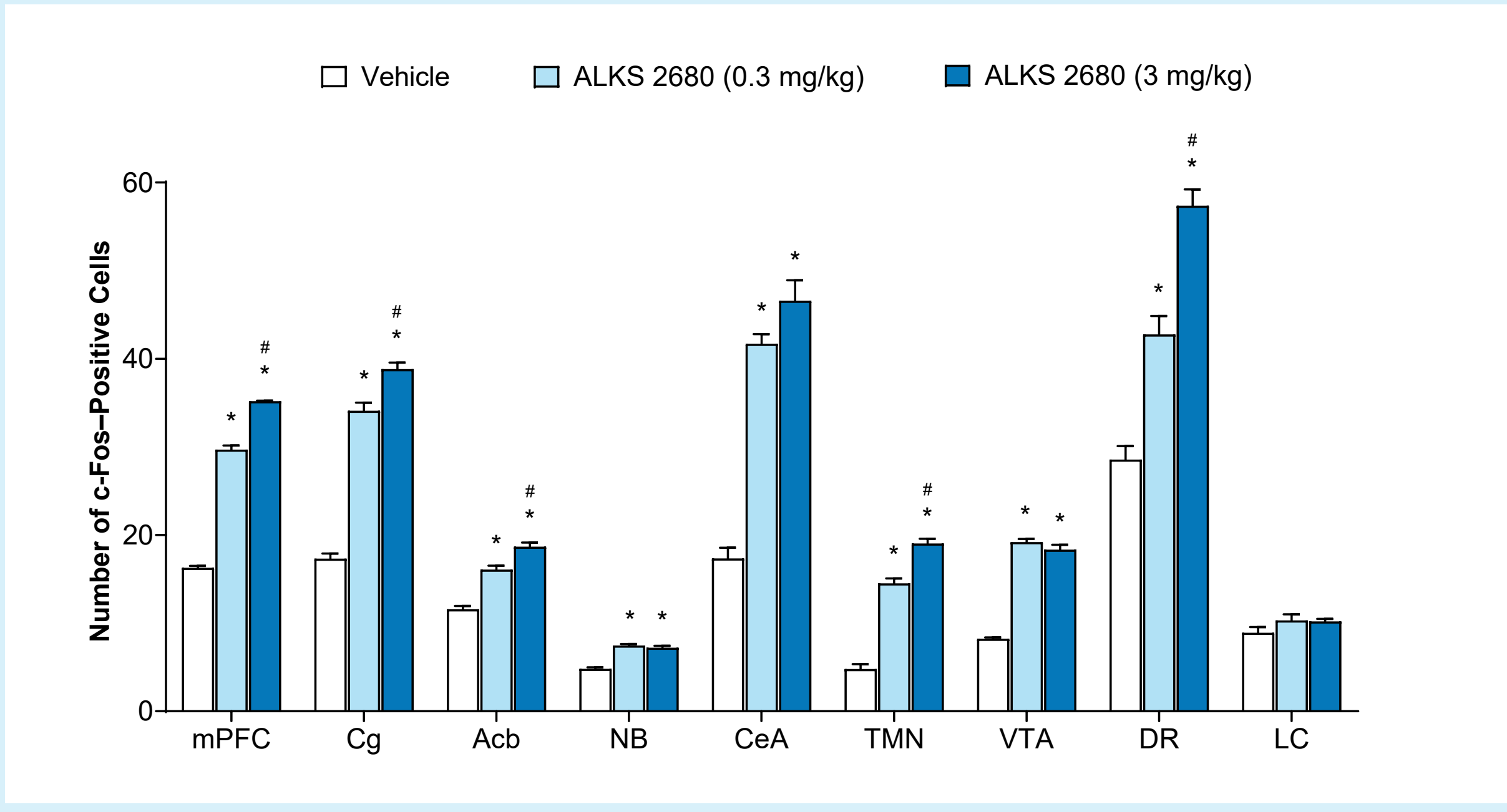
## METHODS

- c-Fos Quantification:** Male Sprague Dawley rats were orally administered vehicle, 0.3 mg/kg ALKS 2680, or 3 mg/kg ALKS 2680. Ninety minutes later, rats were processed for c-Fos immunohistochemistry. c-Fos-positive cells in brain areas of interest were counted using a custom macro in ImageJ software
- Ex Vivo Electrophysiology:** Whole-cell patch clamp recordings were performed in mouse acute brain tissue slices conserving the ventral tuberomammillary nucleus (TMN). Membrane potentials were recorded from electrophysiologically confirmed histaminergic neurons in response to bath application of increasing concentrations of ALKS 2680 or synthetic orexin B peptide in the presence of tetrodotoxin (1  $\mu$ M). Concentration-response curves were generated using GraphPad Prism 8.0; half-maximal effective concentrations were calculated from non-linear regression curve fitting
- Prefrontal Cortex Microdialysis:** Male Sprague Dawley rats were surgically implanted with guide cannula directed toward the prefrontal cortex. On experimental days, guides were replaced with probes continuously perfused with artificial cerebrospinal fluid. A total of 13 30-minute samples were collected: 3 baseline collections and 10 post-treatment collections. All samples were analyzed for neurotransmitter content by PsychoGenics using ultra-high pressure liquid chromatography with a tandem mass spectrometer
- In Vivo Electrophysiology:** Quantitative electroencephalography (EEG) and sleep-wake status were assessed in rats implanted with telemetry devices to collect data from frontal cortex, parietal cortex, and neck muscle electrodes. For each rat, a 2-hour baseline period was recorded, followed by a 6-hour post-dose period initiated immediately after dosing. Rats were orally administered vehicle, 1, 3, and 10 mg/kg ALKS 2680 during the inactive light phase when sleep pressure was highest. Power spectra in delta (1-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), beta (12-30 Hz), and gamma (30-100 Hz) frequency bands were computed from the frontal EEG. Sleep-wake states (wake, non-rapid eye movement sleep, and rapid eye movement sleep) were determined with manual sleep scoring. Statistical analysis was performed to compare the ALKS 2680 dose arms versus vehicle

## RESULTS

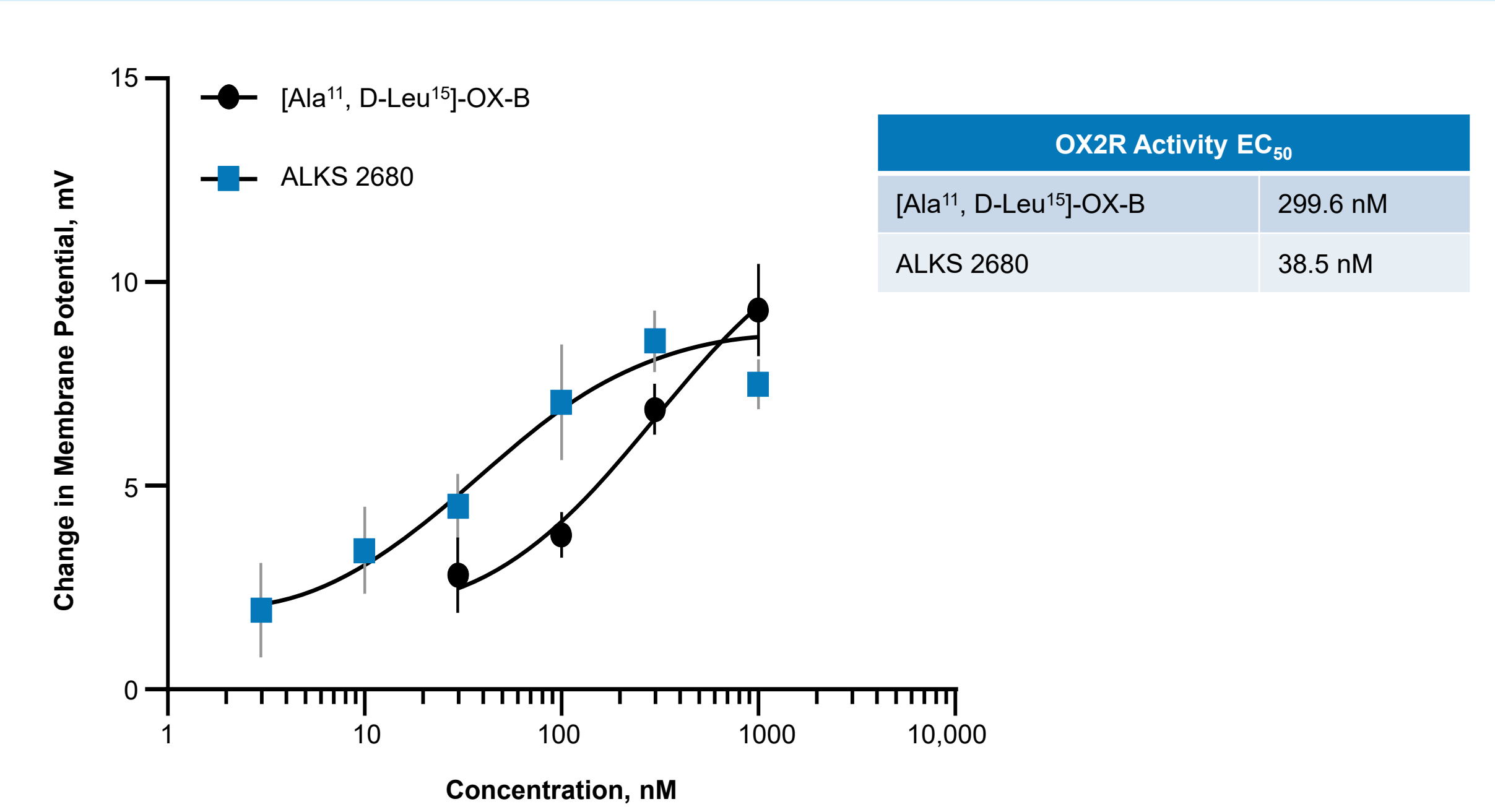
- ALKS 2680 dose-dependently increased neuronal activity in wakefulness-associated neurocircuitry, including OX2R-expressing brain regions (Figure 2)

FIGURE 2: Effects of Acute Oral Administration of ALKS 2680 on Neuronal c-Fos Expression



- ALKS 2680 induced robust, concentration-dependent increases in neuronal excitability of histamine neurons in mouse TMN (Figure 3)

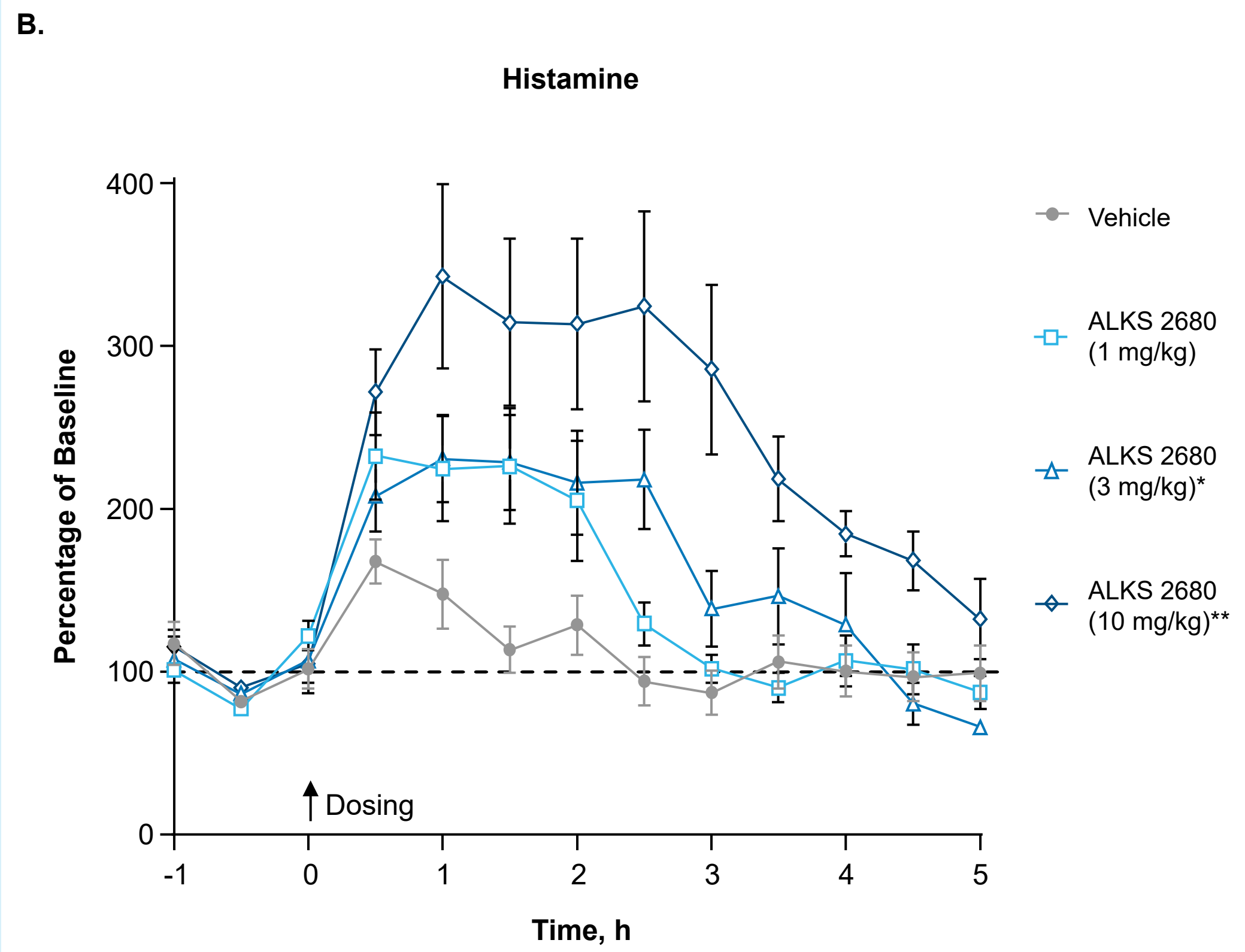
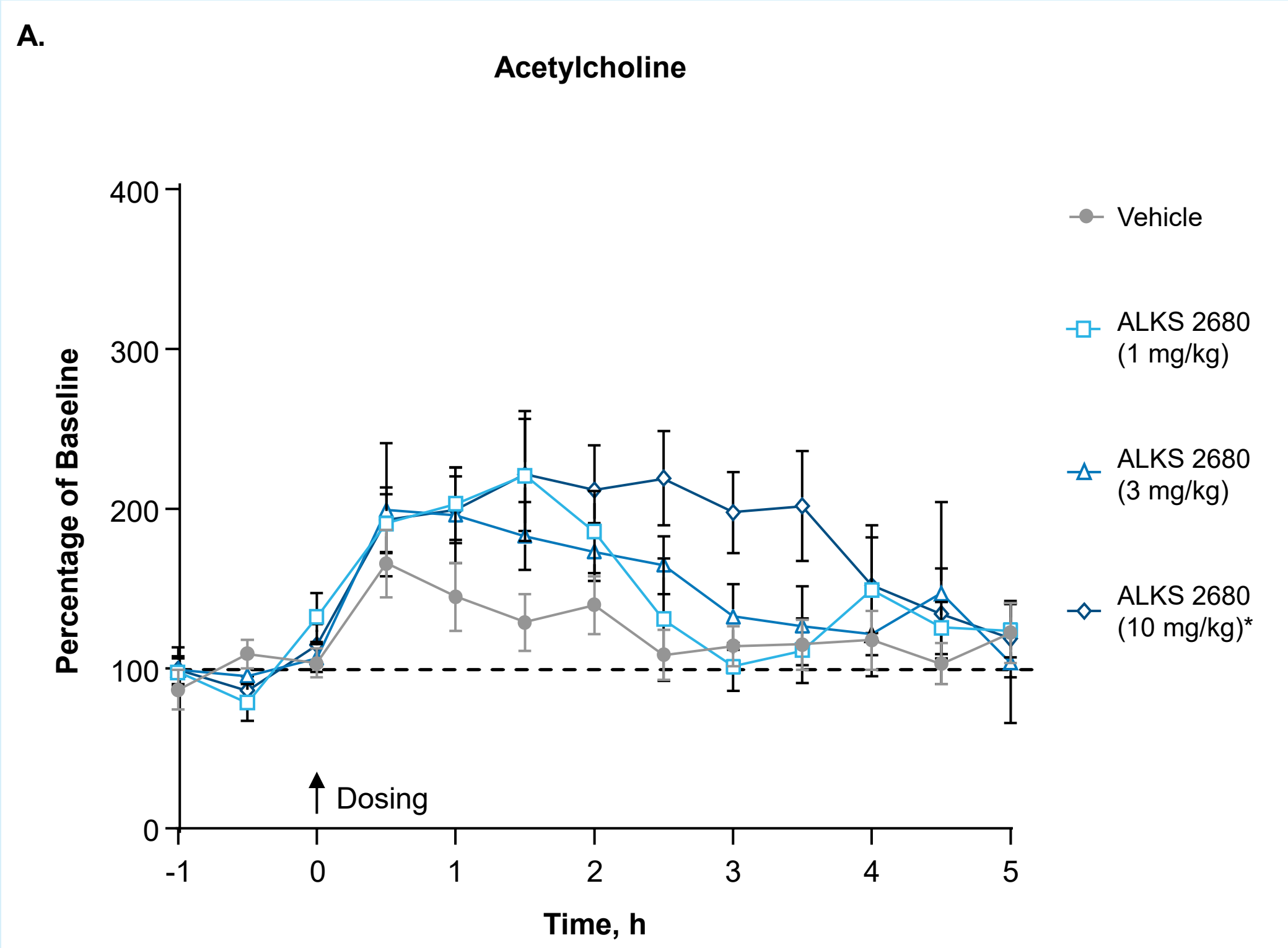
FIGURE 3: Effects of ALKS 2680 or Synthetic Orexin B on Histamine Neuron Membrane Potential Depolarization in Acute Mouse Brain Slices Conserving the TMN



Concentration-response curves for ALKS 2680 and synthetic orexin B peptide [Ala<sup>11</sup>, D-Leu<sup>19</sup>]-OX-B recorded from n = 2-6 cells from a total of 8 mice. Data expressed as mean  $\pm$  SEM. EC<sub>50</sub> values calculated from non-linear regression fitting.  
Ala = alanine; EC<sub>50</sub> = half-maximal effective concentration; Leu = leucine; OX-B = orexin B; OX2R = orexin 2 receptor; SEM = standard error of the mean; TMN = tuberomammillary nucleus.

- ALKS 2680 increased acetylcholine and histamine in rat prefrontal cortex (Figure 4)

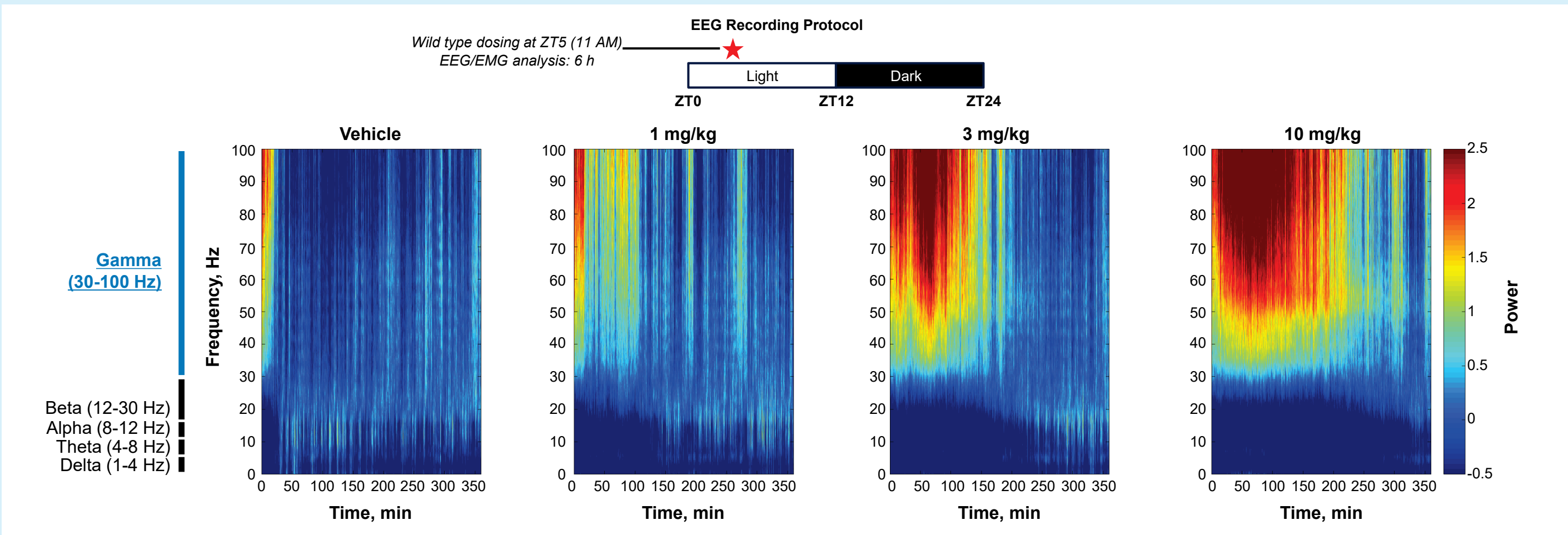
FIGURE 4: Percentage Change From Baseline Values in Prefrontal Cortex Neurotransmitters Following Acute Oral Administration of ALKS 2680



\*P < 0.05, \*\*P < 0.01 (n = 10).  
Time course of effects of vehicle or ALKS 2680 (1, 3, 10 mg/kg) administration (n = 8-10 per group) on (A) extracellular acetylcholine or (B) histamine in prefrontal cortex of freely moving rats. Values are presented as mean  $\pm$  SEM of the percentage of pre-dosing baseline (time points -1 to 0). Administration of vehicle or ALKS 2680 is indicated by the arrow. Asterisk (\*) represents statistical significance from vehicle as determined by a heterogeneous mixed model with repeated measures. SEM = standard error of the mean.

- ALKS 2680 dose-dependently increased high-frequency power and decreased low-frequency power correlating with cortical activation in rats during period of high sleep pressure (Figure 5)

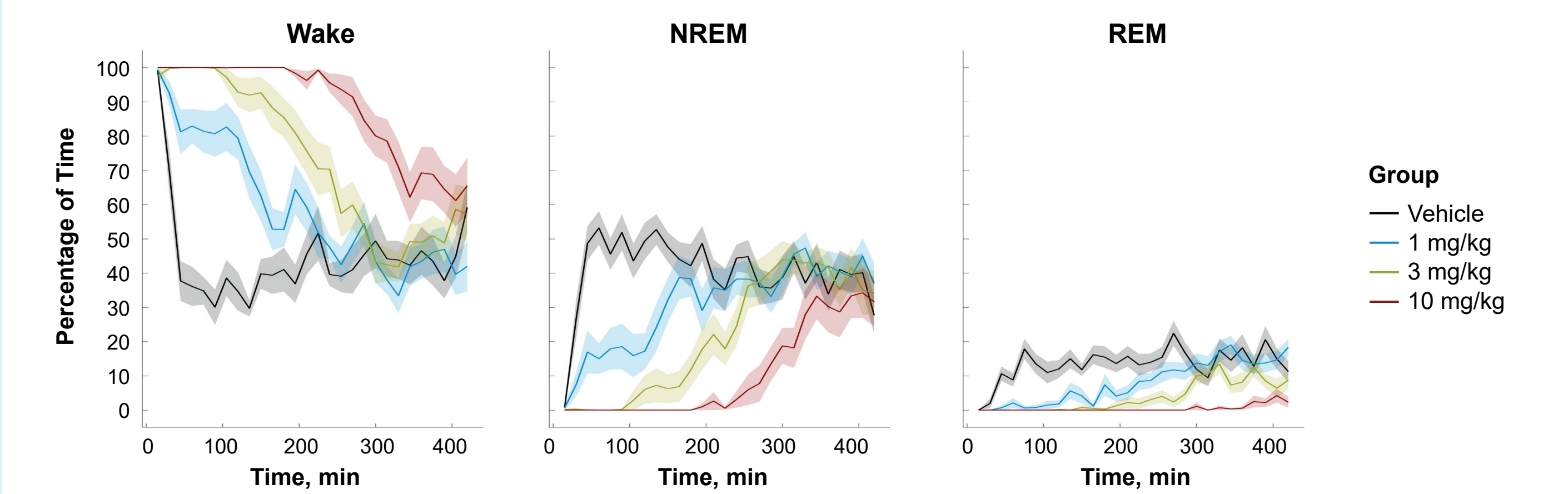
FIGURE 5: Baseline Normalized EEG Spectra Following Acute Oral Administration of Vehicle or ALKS 2680



Time course of effects of vehicle or ALKS 2680 (1, 3, 10 mg/kg) administration (n = 14 per group, 4-arm Latin square crossover) on baseline normalized EEG spectra in rats. Warm colors (yellow to red) indicate an increase in power relative to pre-dosing baseline. Cool colors (blue to green) indicate a reduction in power relative to pre-dosing baseline. Inset provides an overview of recording protocol on experimental days. EEG = electroencephalography; EMG = electromyography; Hz = hertz; ZT = zeitgeber time.

- ALKS 2680 dose-dependently increased wakefulness and suppressed sleep in wild-type rats (Figure 6)

FIGURE 6: Effects of Acute Oral Administration of ALKS 2680 on Percentage of Time for Awake, NREM, and REM Sleep Stages



Time course of effects of vehicle or ALKS 2680 (1, 3, 10 mg/kg) administration (n = 14 per group, 4-arm Latin square crossover) on percentage of time for awake, NREM, and REM sleep stages in rats. Data are presented in 15-minute bins organized by sleep-wake state and are expressed as mean values and SEM at each time point. NREM = non-rapid eye movement; REM = rapid eye movement; SEM = standard error of the mean.

## CONCLUSIONS

- ALKS 2680 induces a pattern of neuronal activation that includes OX2R-expressing neurons in brain regions associated with wakefulness and vigilance
- Lack of activation at the locus coeruleus confirms the selectivity of ALKS 2680 for the OX2R
- ALKS 2680 increases excitability in a neuronal population associated with wake promotion and stabilization with a 7.8-fold increase in potency compared to synthetic orexin B peptide
- ALKS 2680 dose-dependently elevated neurotransmitters, acetylcholine and histamine, associated with increased arousal and vigilance in the prefrontal cortex
- Selective activation of OX2R by ALKS 2680 promotes cortical activation and wakefulness during periods of high sleep pressure in wild-type rats
- These preclinical data support further investigation of ALKS 2680 for the treatment of narcolepsy and idiopathic hypersomnia, including cognition- and wakefulness-related symptoms

### Reference

1. Jászberényi M, et al. *Biomedicines*. 2024;12(2):448.

### Acknowledgments

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### Disclosures

JB, LAT, and BR are employees and shareholders of Alkermes. AMP and CBP were employees of Alkermes during this study.

