RESEARCH ARTICLE

# Differential sleep/wake response and sex differences following acute suvorexant, MK-1064 and zolpidem administration in the rTg4510 mouse model of tauopathy

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**Background and Purpose:** Transgenic mouse models of tauopathy display prominent sleep/wake disturbances which manifest primarily as a hyperarousal phenotype during the active phase, suggesting that tau pathology contributes to sleep/wake changes. However, no study has yet investigated the effect of sleep-promoting compounds in these models. Such information has implications for the use of hypnotics as potential therapeutic tools in tauopathy-related disorders.

**Experimental Approach:** This study examined polysomnographic recordings in 6-6.5-month-old male and female rTg4510 mice following acute administration of suvorexant (50 mg·kg$^{-1}$), MK-1064 (30 mg·kg$^{-1}$) or zolpidem (10 mg·kg$^{-1}$), administered at the commencement of the active phase.

**Key Results:** Suvorexant, a dual OX receptor antagonist, promoted REM sleep in rTg4510 mice, without affecting wake or NREM sleep. MK-1064, a selective OX$_2$ receptor antagonist, reduced wake and increased NREM and total sleep time. MK-1064 normalised the hyperarousal phenotype of male rTg4510 mice, whereas female rTg4510 mice exhibited a more transient response. Zolpidem, a GABA$_A$ receptor positive allosteric modulator, decreased wake and increased NREM sleep in both male and female rTg4510 mice. Of the three compounds, the OX$_2$ receptor antagonist MK-1064 promoted and normalised physiologically normal sleep, especially in male rTg4510 mice.

**Conclusions and Implications:** Our findings indicate that hyperphosphorylated tau accumulation and associated hyperarousal does not significantly alter the responses of tauopathy mouse models to hypnotics. However, the sex differences observed in the sleep/wake response of rTg4510 mice to MK-1064, but not suvorexant or

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zolpidem, raise questions about therapeutic implications for the use of $OX_2$ receptor antagonists in human neurodegenerative disorders.

## 1 | INTRODUCTION

A bidirectional relationship between sleep and neurological disease pathology is prominent in neurodegenerative disorders such as **Alzheimer's disease** (AD) and tau-variant frontotemporal dementia (FTD-tau), driven by amyloid-β (Aβ) and/or tau pathologies (Ju et al., 2014; Lim et al., 2014; Wang & Holtzman, 2020). Much of the preclinical research conducted thus far has focused on the effects of Aβ on sleep/wakefulness (e.g., Bero et al., 2011; Cirrito et al., 2005; Kang et al., 2009; Roh et al., 2012), although recently, attention has also turned to the involvement of tau (Holth et al., 2017; Holton et al., 2020; Koss et al., 2016; Zhu et al., 2018). Periods of extended wakefulness, or sleep deprivation, regulate the extracellular release of both Aβ (Bero et al., 2011; Cirrito et al., 2005, 2008; Tabuchi et al., 2015) and **tau** (Holth et al., 2019; Pooler et al., 2013; Wang et al., 2017; Wu et al., 2016; Yamada et al., 2014). In addition, the clearance of toxic metabolites such as Aβ and/or tau is enhanced during sleep (Xie et al., 2013). Combined with the proposed role that sleep plays in the consolidation of memory and learning (Diekelmann & Born, 2010), sleep therefore presents as a potentially viable therapeutic target in neurodegenerative models and/or disorders.

Studies investigating the bidirectional relationship between tauopathy mouse models and sleep are currently limited. The rTg4510 transgenic mouse model of tauopathy, which overexpresses human tau containing the P301L *MAPT* mutation (associated with FTD-tau in humans), exhibits a disrupted sleep/wake profile which manifests primarily as a hyperarousal phenotype particularly prominent during the active phase, without sleep compensation during the inactive (light) phase (Holton et al., 2020; Keenan et al., 2018). The rTg4510 sleep/wake phenotype mimics that of the tau transgenic PS19 mouse, which harbours the similar P301S *MAPT* mutation (Holth et al., 2017), albeit controlled by different promotors and with different transgene insertion points (Goodwin et al., 2019; Yoshiyama et al., 2007). Together these studies indicate that tau pathology alone is sufficient to drive sleep/wake changes (Holth et al., 2017; Holton et al., 2020). APP transgenic mice respond to dual **orexin receptor** (OX receptor) antagonists (DORAs; see Kang et al., 2009; Roh et al., 2014), including **lemborexant**, which dose-dependently increased sleep when administered during the inactive phase in an AD-like mouse model with both Aβ and tau pathologies (Beuckmann et al., 2021). However, no study has yet investigated how the hyperarousal phenotype of tau transgenic rTg4510 mice is affected by hypnotics, including more recent treatment modalities, such as OX receptor antagonists. Such information may promote and help design clinical trials on hypnotics as potential therapeutic approaches in tauopathy-related and other

neurodegenerative disorders characterised by sleep/wake disturbances.

The aim of the current study was therefore to assess the sleep/wake response of rTg4510 mice following acute administration of three different sleep-promoting compounds: the DORA **suvorexant** (Cox et al., 2010), the selective $OX_2$ receptor antagonist (2-SORA) **MK-1064** (Roecker et al., 2014) and the **$GABA_A$ receptor** positive allosteric modulator **zolpidem** (Arbilla et al., 1985). Suvorexant and zolpidem are marketed for the treatment of insomnia, whereas various 2-SORAs are in late stage clinical development (Hoyer et al., 2019). **Zolpidem** was selected as a standard, well-established hypnotic and a member of the "Z drugs" family, which increase total sleep time primarily by promoting NREM sleep, but suppress REM sleep in rodents (Hoyer et al., 2019). OX receptor antagonists are a relatively new addition to the hypnotic field (see Alexander, Christopoulos et al., 2021). DORAs such as suvorexant and almorexant robustly induce sleep in rodents (Betschart et al., 2013; Hoyer & Jacobson, 2013; Mang et al., 2012) and patients (reviewed in Clark et al., 2020; Jacobson et al., 2014), primarily by increasing REM sleep (Clark et al., 2020; Hoyer & Jacobson, 2013), although different DORAs may increase both REM and NREM sleep in preclinical species

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### What is already known

- Tau pathology contributes to sleep/wake disturbances.
- Mouse models of tauopathy display a prominent hyperarousal phenotype during the active phase.

### What does this study add

- GABAergic and orexinergic hypnotics promote sleep during the active phase in tau transgenic rTg4510 mice.
- rTg4510 mice exhibit sex differences in their response to $OX_2$ receptor selective antagonists.

### What is the clinical significance

- There may be potential implications for the use of $OX_2$ receptor selective antagonists in tauopathy-related disorders.

(see Gotter et al., 2014). Conversely, 2-SORAs increase sleep by modulating both NREM and REM sleep in a proportional, physiological-like manner (Betschart et al., 2013; Hoyer & Jacobson, 2013; Jacobson et al., 2017). Here, a single dose of suvorexant (50 mg·kg$^{-1}$), MK-1064 (30 mg·kg$^{-1}$) or zolpidem (10 mg·kg$^{-1}$) was administered to rTg4510 mice and wildtype (WT) littermates at the commencement of the active phase, when the rTg4510 hyperarousal phenotype is most prominent, and the subsequent sleep/wake profile relative to within-subject vehicle-treated controls was assessed.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Male and female rTg4510 transgenic mice and WT littermate controls were used as previously described (Ramsden et al., 2005; Santacruz et al., 2005). rTg4510 mice were generated in house by crossing Tg (tauP301L)4510 (FVB-$Fgf14^{Tg(tetO-MAPT*P301L)4510Kha}$; Jackson Lab Stock number 015815) mice with Tg (Camk2a-tTA)1Mmay (129SVeV background, Jackson Lab Stock number 016198) mice. Control animals were littermates, WT for both CaMKII-tTA and Tg (tauP301L) 4510. All mice were genotyped using a standardised tail DNA polymerase chain reaction (PCR) assay (Transnetyx Inc., Cordova, TN, USA). Mice used in this study were between 6 and 6.5 months of age during experimental sleep recordings, except for the study investigating MK-1064 blood and brain levels, performed in 11-month-old mice. All mice were group-housed (unless fighting occurred, which was rare) in standard, transparent open-top cages (29.5 × 16 × 13 cm), except during a 1-week acclimatisation period to open-topped, acrylic polysomnography (PSG) recording chambers (30.5 cm D × 30.5 cm H) and during PSG recordings, when they were single housed but could see, smell and hear cage mates. Sawdust, wooden chew blocks and tissue paper nesting material were provided throughout. Standard rodent food and water were available ad libitum. All experimentation was performed in accordance with the Prevention of Cruelty to Animals Act (2004), under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia (2013) and approved by The Florey Animal Ethics Committee (AEC number: 16-022). All efforts were made to minimise animal suffering. Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020).

### 2.2 | Materials

The DORA suvorexant (Suvo; AdooQ Bioscience, Redfern, Australia), 2-SORA MK-1064 (Merck and Co., Inc, Kenilworth, USA) and the GABA$_A$ positive allosteric modulator zolpidem (Zolp; Sapphire Bioscience, Redfern, Australia) were used. Drugs were prepared fresh as suspensions on the day of administration. The vehicle for suvorexant

and zolpidem was 0.5% methylcellulose (MC; Sigma-Aldrich, North Ryde, Australia) in water. The vehicle for MK-1064 was 20% D-α-tocopherol polyethylene glycol 1000 succinate (TPGS; Sigma-Aldrich, North Ryde, Australia) in water. All drugs were administered by oral gavage in a volume of 10 ml·kg$^{-1}$.

### 2.3 | Experimental study design

All mice were initially housed on a 12-h light/dark cycle (lights on at 0700 h) before being moved into a new housing area with their respective experimental light cycle (lights on at 0300 h) 10 days before EEG/EMG surgery. The PSG experimental protocol involved three consecutive 23 h PSG recordings, commencing at the start of the active phase (Zeitgeber time (ZT)12). On Day 1, mice were administered water (10 ml·kg$^{-1}$, p.o.) to habituate to oral gavage. On Day 2, they received vehicle (MC or TPGS, 10 ml·kg$^{-1}$, p.o.). On Day 3, 50 mg·kg$^{-1}$ suvorexant, 30 mg·kg$^{-1}$ MK-1064 or 10 mg·kg$^{-1}$ zolpidem were administered p.o. The doses for suvorexant (Betschart et al., 2013; Hoyer et al., 2013), MK-1064 (Gotter et al., 2016; Roecker et al., 2014) and zolpidem (Fox et al., 2013; Steiner et al., 2011) were selected based on literature and preliminary data in-house, and for the OX receptor antagonists, as providing efficacious induction of sleep and receptor occupancy of the OX receptors of ≥90%. Administration of compounds was performed within a time window of 5–15 min before the start of the recordings. Experimental group sizes were as follows: Suvorexant experiment: WT ($n = 18$; 7 males, 11 females); rTg4510 ($n = 13$; 7 males, 6 females). MK-1064 experiment: WT ($n = 15$; 8 males, 7 females); rTg4510 ($n = 15$; 8 males, 7 females). Zolpidem experiment: WT ($n = 16$; 7 males, 9 females); rTg4510 ($n = 13$; 7 males, 6 females). Mice were allocated to treatment groups pseudo-randomly to ensure an appropriate number of genotype and sex in each treatment and randomly assigned to a drug treatment sequence with some mice receiving one, two or all three active compounds, with their own respective water and vehicle recordings, with a minimum 1-week wash-out period between experiments. Experimenters were blinded to the genotype of the mice during in-life experimentation and blinded to all experimental conditions (treatment, sex and genotype) for PSG scoring and analysis of compound levels in the plasma and brain.

### 2.4 | Plasma and brain levels of MK-1064

To assess blood and brain levels of MK-1064, a separate group of mice ($n = 17$) received MK-1064 once daily, as described above, for six consecutive days. On the sixth day, mice were deeply anaesthetised (lethal overdose of sodium pentobarbitone) 1 or 4 h after MK-1064 administration, followed by rapid collection of hemi-brains and blood (via cardiac puncture) which was immediately centrifuged at 10,621 RCF for 2 min at 4°C for the collection of plasma. Plasma and brain samples were snap frozen on dry ice and stored at −80°C until analysis for levels of MK-1064 as previously described (Gotter et al., 2016).

## 2.5 | Polysomnographic (PSG) recordings and analysis

### 2.5.1 | Surgery

Mice were anaesthetised with isoflurane (5% for induction, 2–3% for maintenance) in oxygen and placed in a stereotaxic frame. Meloxicam (1–3 mg·kg$^{-1}$, Ilium, Cenvet, Kings Park, Australia) and 500 μl of warm saline were administered subcutaneously; 50 μl of 1% lignocaine HCl (Pfizer, Clifford Hallam Healthcare, Keysborough, Australia) was administered near the incision site, then the skull was exposed and EEG/EMG preformed head mounts (Catalogue number: #8201-SS, Pinnacle Technology Inc., Lawrence, KS, USA) were surgically implanted. EEG1 and EEG2 electrodes (screws inserted into the head mount) were positioned over the parietal and frontal lobe, respectively. Reference and ground electrodes were positioned at equivalent regions, respectively, and a non-penetrating anchor screw imbedded into the skull over the motor cortex. Two EMG wires attached to the head mount were inserted into the neck musculature and sutured (7-0 Prolene, Ethicon, Clifford Hallam Healthcare, Keysborough, Australia) into place. Dental cement was positioned under and around the head mount using an 18G drawing-up needle and allowed to set. The mouse was removed from the stereotaxic frame and placed into a warm recovery box (30°C) until it was moving freely, then returned to its original cage. Mice were carefully monitored once daily for 7–10 days post-surgery and then at least twice per week thereafter.

### 2.5.2 | Recordings

Mice were individually housed in transparent, cylindrical sleep recording chambers (29 cm diameter × 30 cm height) for 1 week of recovery and acclimatisation. Head mounts were plugged into the pre-amplifier 24 h before recordings began. Sirenia Acquisition version 1.8.0 software (Pinnacle Technology Inc.) was used for EEG/EMG acquisition. Three channels were recorded: EEG1, EEG2 and EMG. The sampling rate was 200 Hz; 100-Hz EEG and EMG low pass filters were applied. Recordings began at 1500 h (ZT12) and finished at 1400 h (ZT11). Following EEG/EMG recordings all mice were returned to their original cages.

### 2.5.3 | Analysis

Sirenia recording files were imported into Somnivore™ (Allocca et al., 2019) in 4-s epochs for vigilance state analysis. Each recording was scored by first training the Somnivore machine learning algorithm with 101 epochs of wake, NREM and REM, each. The entire hypnogram was then populated using Somnivore's auto-score function. Contextual scoring rules included a minimum bout length setting of 8 s (two contiguous epochs) for both wake and NREM, and 12 s (three contiguous epochs) for REM. A further 50 epochs of each state were then trained, and the auto-score function was run again. The entire recording was then manually checked and corrected as required. Sleep latencies were defined as the time from the start of the recording to the first occurrence of 50 consecutive epochs (200 s) of sleep, 50 consecutive epochs (200 s) of NREM sleep, or three consecutive epochs (12 s) of REM sleep. Vigilance state-specific normalised EEG power spectral data were obtained using Somnivore (Allocca et al., 2019) for the first 3 h post-administration for wake and NREM and for the first 6 h for REM (due to the relative scarcity of REM sleep during the active phase in rTg4510 mice). For power spectral analysis, all transition epochs were excluded and signals from EEG1 and EEG2 electrodes were averaged. Frequencies in the 0- to 100-Hz range were analysed.

## 2.6 | Data and statistical analysis

Statistical analyses were performed using GraphPad Prism (version 9.2.0) or R (version 3.6.1)/RStudio (version 1.2.5001). Time spent in each vigilance state across the 23 h recording and vigilance state-specific normalised EEG power were analysed by a repeated measures linear mixed effects model in R, using the *lmer* function. Data for each compound were analysed within genotype and sex, versus the respective vehicle. Post hoc analyses were performed using the *emmeans* function, with Tukey's multiple comparisons test. REM as a percentage of total sleep (% REM / TST), average bout numbers, bout durations, sleep latencies and time spent in each vigilance state per phase were analysed by Student's paired *t* test between compound and respective vehicle. Percentage change from vehicle data was analysed by two-way ANOVA with Sidak's multiple comparisons test within genotype. Post-hoc tests were run only if F achieved P<0.05 and there was no significant variance inhomogeneity. Each individual group was also analysed by one-sample *t* test versus baseline/vehicle (0%). For all analyses, $P \leq 0.05$ was considered to be statistically significant. Data and statistical analysis complied with the recommendations of the *British Journal of Pharmacology* on experimental design and analysis in pharmacology (Curtis et al., 2018).

## 2.7 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/22 (Alexander, Christopoulos, et al., 2021; Alexander, Mathie, et al., 2021).

## 3 | RESULTS

### 3.1 | Hyperarousal in rTg4510 mice

To allow comparison of the effects produced by the three treatment modalities in rTg4510 and WT mice, most figures depict the data for

each compound side by side. Figure 1 compares the 23-h post administration PSG data obtained with suvorexant, MK-1064, zolpidem and their respective vehicles in male and female rTg4510 and WT mice, whereas Figure 2 depicts the cumulative total sleep time (TST) measured over 12 h for the same mice. It is evident that rTg4510 mice show a hyperarousal phenotype in all cohorts, although there are variations, since each control group was specific within each treatment modality. Hence, the hyperarousal appears less pronounced in some female than male rTg4510 mice (Figure 1a, e,i). As seen in Figure 2, cumulated differences in TST at 12 h were markedly lower in some female rTg4510 (Figure 2b,d,f) than in male rTg4510 mice (Figure 2a,e) when compared with WT mice, although for male rTg4510 mice this difference is less pronounced in the MK-1064 group (Figure 2c). Despite the hyperarousal which takes place during the active phase, the rTg4510 mice showed no sleep compensation during the following inactive period, whether in controls or drug-treated males or females (Figure 1). This is particularly evident when comparing wake (Figure 1a,e,i), NREM (Figure 1b,f,j) or TST (Figure 1d,h,l) in both males and females. During the inactive period, the respective PSG traces of rTg4510 and WT mice are virtually superimposable whether in control or drug-treated groups, in contrast to what is observed in the cumulative TST traces during the active phase (Figure 2).

## 3.2 | Suvorexant

The sleep/wake architecture of rTg4510 and WT mice was significantly altered by suvorexant (Figures 1, 3, 4, 5, 6, and S1 and Table S1), whereby REM sleep was enhanced in the absence of effects on NREM sleep. Suvorexant did not affect wake (Figure 1a), NREM (Figure 1b) or TST (Figure 1d) in rTg4510 or WT mice. Male and female WT mice spent significantly more time in REM sleep following suvorexant administration for up to 4 h (Figure 1c). Similar effects were observed with male rTg4510 mice, whereas female rTg4510 mice exhibited increased REM sleep only during the first hour post treatment (Figure 1c). Suvorexant had minimal effects on the cumulated TST during the 12 h following application, since only REM sleep was impacted in all treatment groups, and REM makes a small contribution to TST in general (Figure 2a,b). Indeed, when
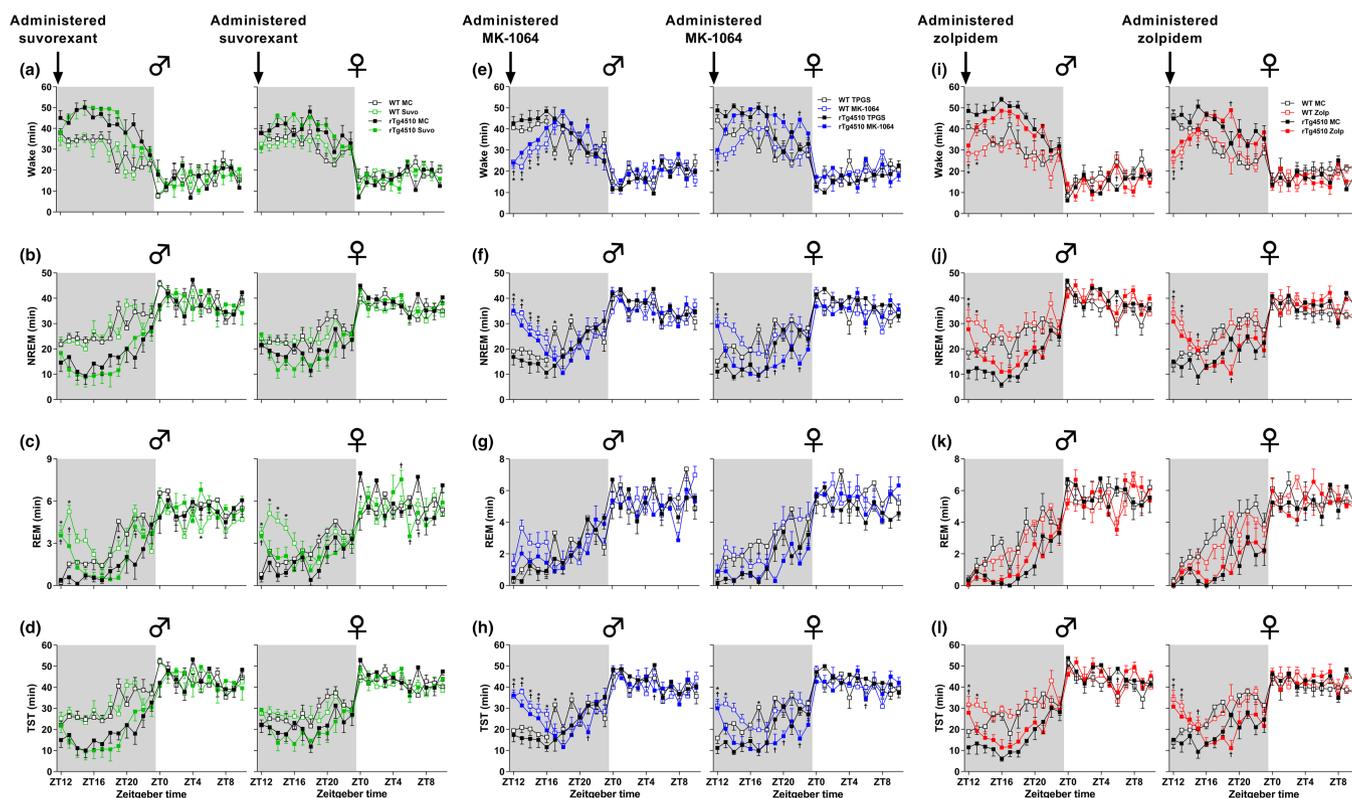


**FIGURE 1** Sleep/wake profiles following acute suvorexant, MK-1064 or zolpidem administration in rTg4510 and WT mice. (a–d) Time spent in wake (a), NREM (b), REM (c) and total sleep time (TST) (d) vigilance states per hour across a 23-h recording period in rTg4510 and WT mice administered 50 mg·kg$^{-1}$ suvorexant (Suvo) or methylcellulose (MC) vehicle. Males: $n = 7$ per group; females: WT MC $n =11$, WT Suvo $n = 11$, rTg4510 MC $n = 6$, rTg4510 Suvo $n = 6$. (e–h) Time spent in wake (e), NREM (f), REM (g) and TST (h) vigilance states per hour across a 23-h recording in rTg4510 and WT mice administered 30 mg·kg$^{-1}$ MK-1064 or TPGS vehicle. Males: $n = 8$ per group; females: $n = 7$ per group. (i–l) Time spent in wake (i), NREM (j), REM (k) and TST (l) vigilance states per hour across a 23-h recording in rTg4510 and WT mice administered 10 mg·kg$^{-1}$ zolpidem (Zolp) or MC vehicle. Males: $n = 7$ per group; females: WT MC $n = 9$, WT Zolp $n = 9$, rTg4510 MC $n = 6$, rTg4510 Zolp $n = 6$. Data are means ± SEM. *$P \leq 0.05$ versus WT vehicle, †$P \leq 0.05$ versus rTg4510 vehicle, by repeated measures linear mixed effects model with Tukey's multiple comparisons post hoc test. Dark shading represents the active phase
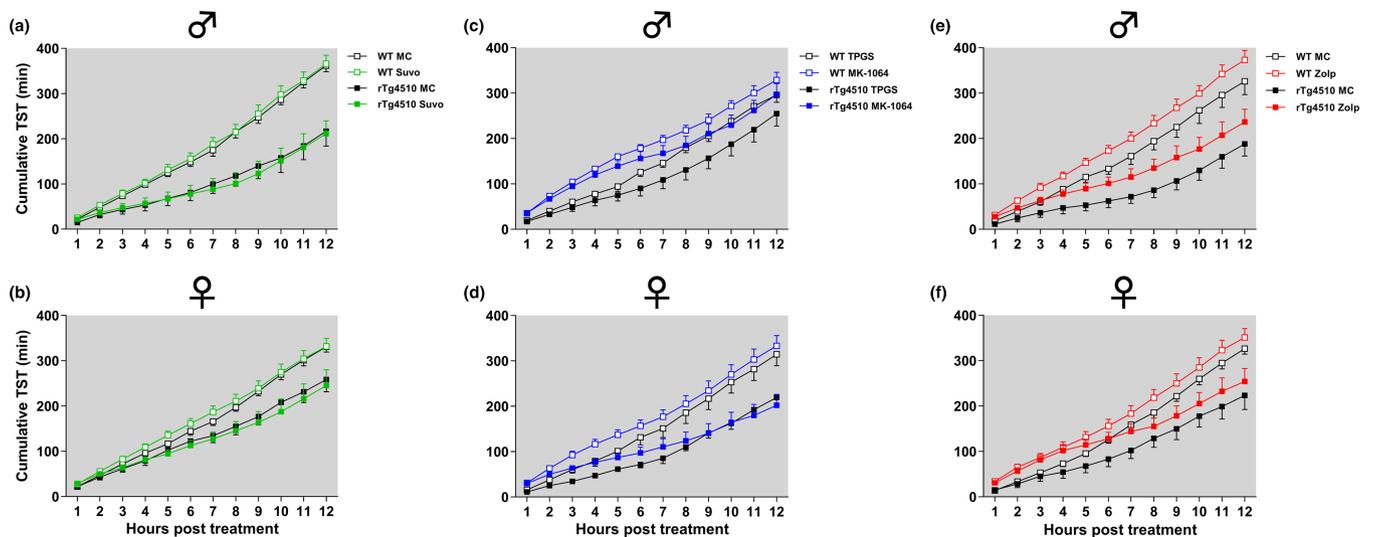
**FIGURE 2** Cumulative total sleep time (TST) following acute suvorexant, MK-1064, zolpidem or vehicle administration in rTg4510 and WT mice. (a–f) Cumulative TST (minutes) in rTg4510 and WT mice administered vehicle or suvorexant (a, b), MK-1064 (c, d) or zolpidem (e, f) for up to 12 h post administration. Data are means ± SEM. Suvorexant, males: $n = 7$ per group; females: WT MC $n = 11$, WT Suvo $n = 11$, rTg4510 MC $n = 6$, rTg4510 Suvo $n = 6$. MK-1064, males: $n = 8$ per group; females: $n = 7$ per group. Zolpidem, males: $n = 7$ per group; females: WT MC $n = 9$, WT Zolp $n = 9$, rTg4510 MC $n = 6$, rTg4510 Zolp $n = 6$. Dark shading represents the active phase

administered at the beginning of the active phase, suvorexant did not significantly alter % change from vehicle in wake or NREM states in male or female WT or rTg4510 mice (Figure 3a–d). Percent change in REM sleep time was increased particularly in male mice up to 6 h after dosing, but had mostly returned to baseline by 12 h (Figure 3e,f). Sleep and NREM latencies were either unaffected or even tended to increase following suvorexant (Figure 4a,g), whereas REM sleep latency decreased in all groups (Figure 4d), in contrast to MK-1064 or zolpidem.

Suvorexant increased the number of wake bouts in female, but not male mice, compared with the respective vehicle treatment across both the first half (Figure 5a) and full active phase (Figure S5A). Suvorexant similarly increased the number of NREM bouts in female, but not male mice, compared with their respective vehicle treatment across the first half (Figure 5b) and full active phase (Figure S5D). During the first 6 h following suvorexant administration, the number of REM bouts was significantly increased in WT mice (Figure 5c). The majority of male and female rTg4510 mice also exhibited an increase in REM bout number following suvorexant administration compared with vehicle during this period; however, this did not reach overall statistical significance (Figure 5c). Similar to both wake and NREM bout numbers, only female mice exhibited increased numbers of REM bouts across the full active phase (Figure S5G).

Wake bout duration was unaffected by suvorexant treatment in the first 6 h post administration (Figure 5d). During the first half of the active phase, suvorexant decreased NREM bout duration in male and female rTg4510 mice, but only in female WT mice (Figure 5e). During the first 6 h following suvorexant administration, REM bout duration was significantly increased in male and female rTg4510 mice (Figure 5f). The majority of male and female WT mice also

exhibited an increase in REM bout duration following suvorexant administration compared with vehicle during this period; however, this did not reach overall statistical significance in all groups (Figure 5f).

Given the previously reported differences between DORAs, 2-SORAs and Z-drugs like zolpidem on the relative proportions of REM sleep, REM was assessed as a proportion of total sleep across the active phase (Figure 6). In agreement with previous observations in WT mice, all suvorexant-treated groups in the present study showed an increase in % REM / TST compared with vehicle (Figure 6a), in contrast to that seen with MK-1064 or zolpidem. EEG power was largely unaffected by suvorexant treatment (Figure S2 and Table S2), except for a shift in the theta peak during REM sleep in male rTg4510 mice (Figure S2D). In summary, suvorexant affected predominantly REM sleep in both WT and rTg4510 mice but had a minimal influence on wake and NREM states. EEG power in transgenic and WT mice showed some differences, which remained largely unaffected by suvorexant.

## 3.3 | MK-1064

The sleep/wake architecture of rTg4510 and WT mice was significantly altered by MK-1064 (Figures 1, 3, 4, 5, and S1 and Table S3). MK-1064 predominantly affected the wake and NREM vigilance states. Male rTg4510 and WT mice spent significantly less time awake following MK-1064 administration during the first half of the active phase (Figure 1e). In contrast, female rTg4510 and WT mice exhibited a marked decrease in wake time only during the first hour post MK-1064 administration (Figure 1e). Male rTg4510 and WT mice
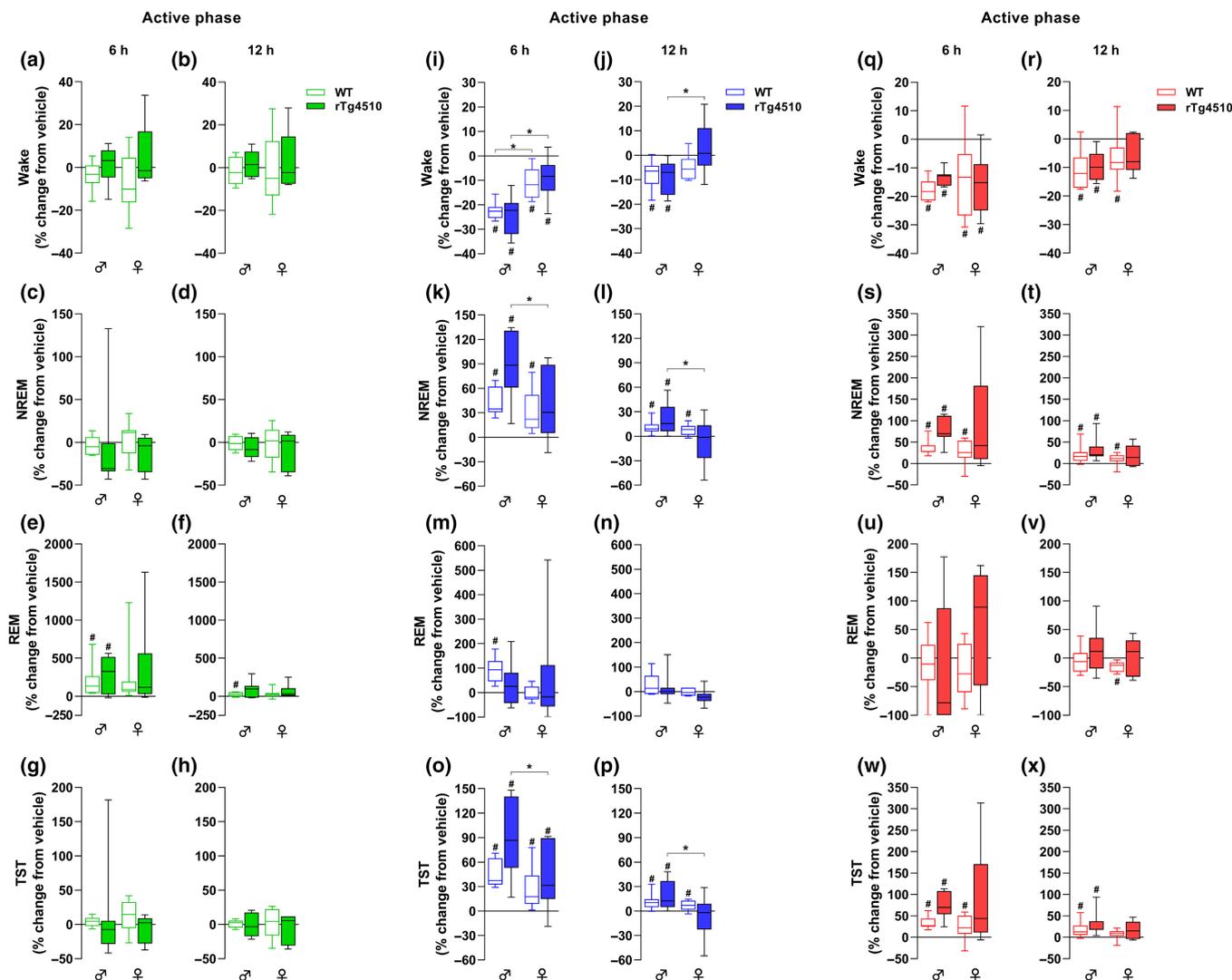
**FIGURE 3**    Sleep/wake profile changes from vehicle (within subject) during the active phase following acute suvorexant, MK-1064 or zolpidem administration in rTg4510 and WT mice. (a–h) Percentage change from vehicle for wake (a, b), NREM (c, d), REM (e, f) and total sleep time (TST) (g, h) vigilance states during 6 or 12 h of the active phase in rTg4510 and WT mice administered suvorexant. Males: $n = 7$ per group; females: WT MC $n = 11$, WT Suvo $n = 11$, rTg4510 MC $n = 6$, rTg4510 Suvo $n = 6$. (i–p) Percentage change from vehicle for wake (i, j), NREM (k, l), REM (m, n) and TST (o, p) vigilance states during 6 or 12 h of the active phase in rTg4510 and WT mice administered MK-1064. Males: $n = 8$ per group; females: $n = 7$ per group. (q–x) Percentage change from vehicle for wake (q, r), NREM (s, t), REM (u, v) and TST (w, x) vigilance states during 6 or 12 h of the active phase in rTg4510 and WT mice administered zolpidem. Males: $n = 7$ per group; females: WT MC $n = 9$, WT Zolp $n = 9$, rTg4510 MC $n = 6$, rTg4510 Zolp $n = 6$. *$P \leq 0.05$ within genotype, by two-way ANOVA, #$P \leq 0.05$ versus baseline/vehicle (0%), by one-sample $t$ test

correspondingly also spent significantly more time in NREM sleep following MK-1064 administration (Figure 1f). In contrast to males, female WT and rTg4510 mice exhibited a marked increase in NREM sleep only during 1–2 h post MK-1064 administration (Figure 1f). No statistically significant changes in REM sleep were observed in any treatment group, although there was a tendency for an increase (Figure 1g) at least in male WT mice (Figure 3m). The relative effect on TST was near identical to that for NREM sleep (Figure 1h).

Figure 2 illustrates the cumulative effects of MK-1064 on TST over time. The effects are particularly pronounced during 6–8 h post-treatment in all groups, although they are much more limited in female WT and rTg4510 mice. In essence, female rTg4510 mice tend to show

a transient "normalisation" (defined as non-different from vehicle-treated WT mice) of TST during the first 4 h whereas male MK-1064-treated rTg4510 mice show a TST profile which is superimposable to that of male WT non-treated mice from 8 h onward. Accordingly, % change in wake versus vehicle 6 h following MK-1064 administration (Figure 3i,j) was decreased in all groups compared with their respective vehicle, an effect which persisted particularly in male mice to 12 h (Figure 3j). In contrast, female rTg4510 mice were the only group to show a small increase in wake duration, of 17 min, over the 12-h period ($t_{(6)} = 0.811$, $P > 0.05$, Figure 3j). MK-1064 significantly increased NREM sleep time versus vehicle at 6 and 12 h after dosing in WT mice of both sexes and in male rTg4510 mice, but not in
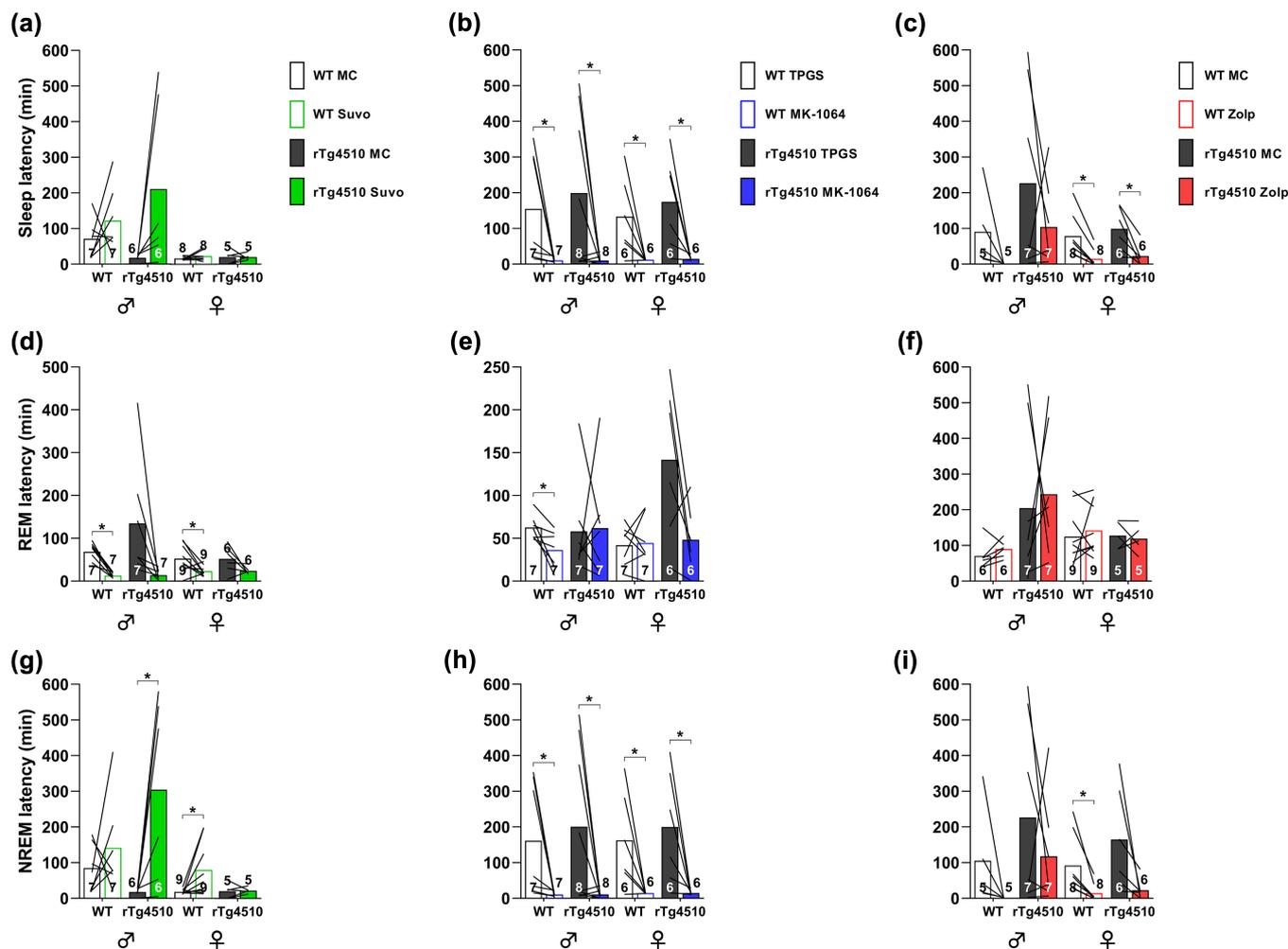
**FIGURE 4** Effects of acute suvorexant, MK-1064 or zolpidem administration on sleep latencies in rTg4510 and WT mice. (a–c) Sleep latency following acute suvorexant (a), MK-1064 (b) or zolpidem (c) administration in rTg4510 and WT mice. (d–f) REM sleep latency following acute suvorexant (d), MK-1064 (e) or zolpidem (f) administration in rTg4510 and WT mice. (g–i) NREM sleep latency following acute suvorexant (g), MK-1064 (h) or zolpidem (i) administration in rTg4510 and WT mice. Bars represent means and lines individual animals. $n = 5$–9 per group, per sex. * $P \leq 0.05$ versus vehicle, by Student's paired $t$ test

female rTg4510 mice (Figure 3k,l). This was statistically greater in male rTg4510 mice compared with female rTg4510 mice (Figure 3k,l), although no difference between male and female WT mice was observed for this parameter. A similar pattern of response was observed for % change in TST as for NREM sleep (Figure 3o,p). By contrast, MK-1064 did not significantly affect average REM sleep in any treatment group (Figure 3m,n), with the possible exception of male WT mice at 6 h.

In agreement with the above, MK-1064 reduced latency to sleep and NREM sleep in all groups (Figure 4b,h), whereas latency to REM sleep was minimally impacted (Figure 4e). MK-1064 administration significantly increased both wake (Figure 5g) and NREM (Figure 5h) bouts in male rTg4510 mice, but had no effect in other groups (Figure 5g,h). REM bouts were significantly increased in only male WT mice across the first half of the active phase (Figure 5i), full active phase and the entire recording (Figure S5P,R). Wake bout duration was not significantly affected by MK-1064 administration (Figures 5j

and S6J–L), in part due to large variation for this parameter. Across the first 6 h following MK-1064 administration, NREM bout duration was significantly increased in male mice of both genotypes, but not in female mice of either genotype (Figure 5k). Male rTg4510 also exhibited an increase in REM bout duration across the first half of the active phase (Figure 5l) and the entire recording (Figure S6R), compared with vehicle. This effect was absent in female rTg4510 mice (Figures 5l and S6R). In contrast to suvorexant, MK-1064 had minimal direct effects on REM (Figures 1, 3, 4, 5, and S1), although the lack of change in the proportion of % REM / TST (Figure 6) indicates that since TST sleep is increased, REM sleep was enhanced in a physiological manner. MK-1064 influenced EEG power (Figure S3 and Table S4) mainly by increasing delta power during NREM sleep in all four groups, except for WT female mice (which trended towards increased delta power; Figure S3C,I,J).

Since the effects of MK-1064 were of shorter duration in female rTg4510 mice (Figures 1, 2, and 3), we determined the brain and
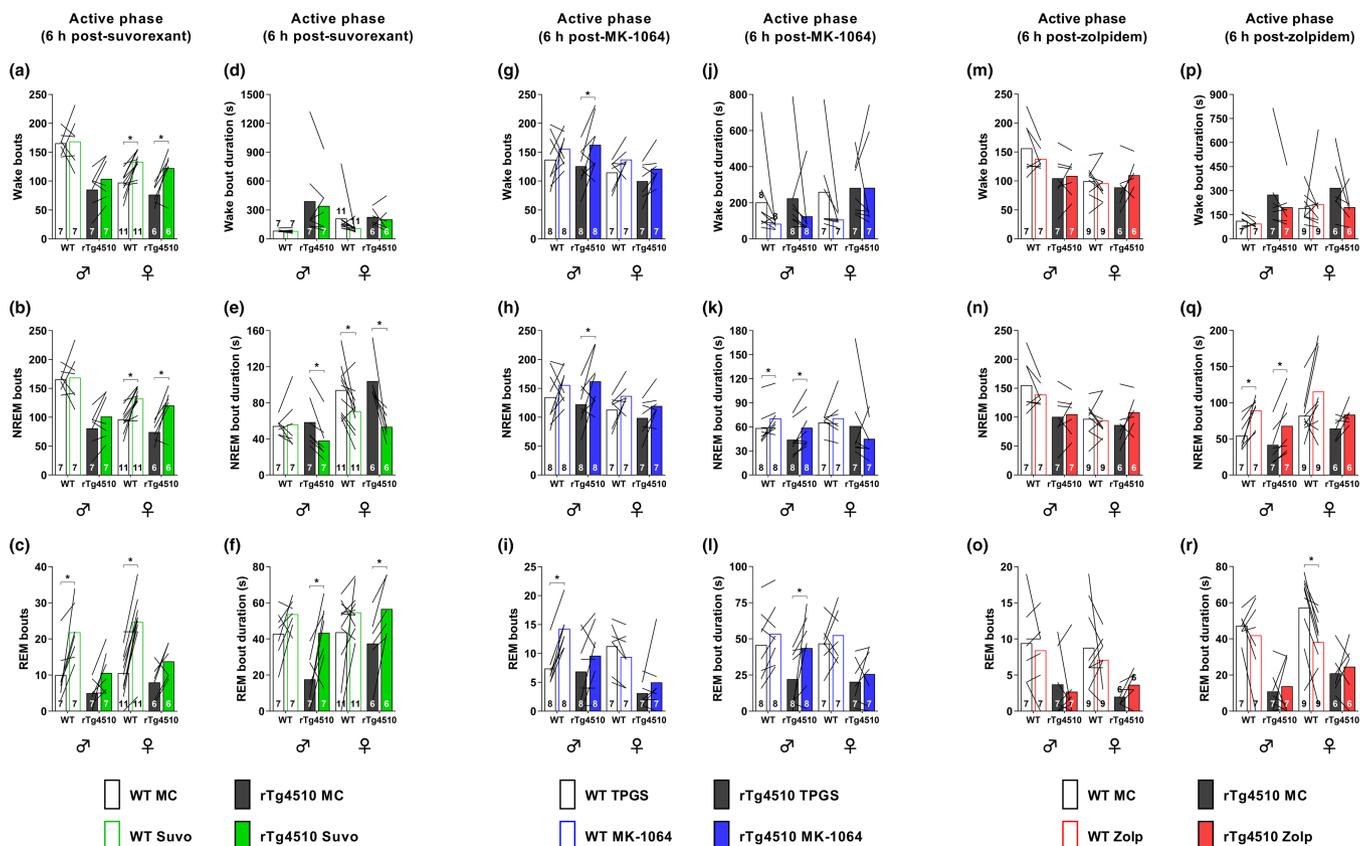
**FIGURE 5** Effects of acute suvorexant, MK-1064 or zolpidem administration on vigilance state bouts and bout duration in rTg4510 and WT mice during the active phase for the 6 h post administration. (a–c) Total number of wake (a), NREM (b) and REM (c) bouts in rTg4510 and WT mice administered 50 mg·kg$^{-1}$ suvorexant (Suvo) or methylcellulose (MC) vehicle. (d–f) Mean wake (d), NREM (e) and REM (f) bout duration in rTg4510 and WT mice administered 50 mg·kg$^{-1}$ Suvo or MC vehicle. $n = 6$–11 per group, per sex. (g–i) Total number of wake (g), NREM (h) and REM (i) bouts in rTg4510 and WT mice administered 30 mg·kg$^{-1}$ MK-1064 or TPGS vehicle. (j–l), Mean wake (j), NREM (k) and REM (l) bout duration in rTg4510 and WT mice administered 30 mg·kg$^{-1}$ MK-1064 or TPGS vehicle. $n = 7$–8 per group, per sex. (m–o) Total number of wake (m), NREM (n) and REM (o) bouts in rTg4510 and WT mice administered 10 mg·kg$^{-1}$ zolpidem (Zolp) or MC vehicle. (p–r) Mean wake (p), NREM (q) and REM (r) bout duration in rTg4510 and WT mice administered 10 mg·kg$^{-1}$ zolpidem (Zolp) or MC vehicle. $n = 6$–9 per group, per sex. Bars represent means and lines individual animals. *$P \leq 0.05$ versus vehicle, by Student's paired $t$ test
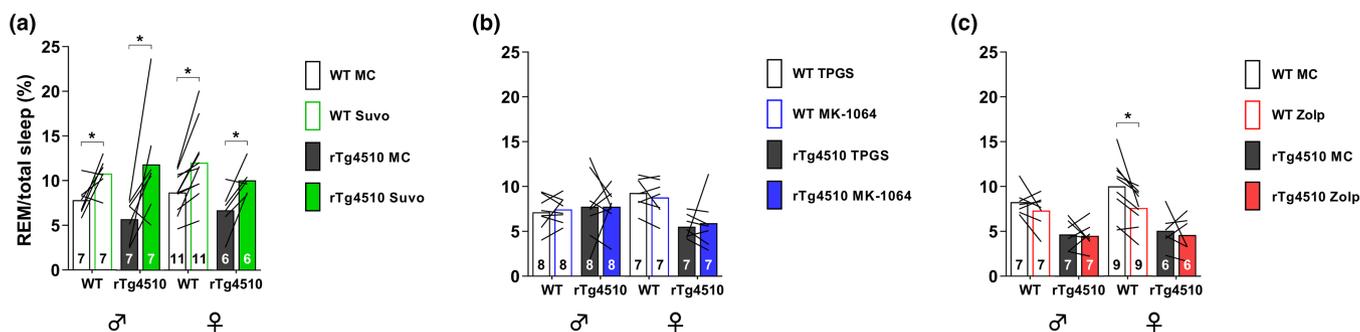


**FIGURE 6** Effects of acute suvorexant, MK-1064 or zolpidem administration on REM sleep as a proportion of total sleep in rTg4510 and WT mice. (a–c) REM sleep as a percentage of total sleep during the active phase following acute suvorexant (a), MK-1064 (b) or zolpidem (c) administration in rTg4510 and WT mice. Bars represent means and lines individual animals, $n = 6$–11 per genotype, per sex. *$P \leq 0.05$ versus vehicle, by Student's paired $t$ test

plasma levels in a satellite group of mice. Mean ± SD plasma levels of MK-1064 determined 1 and 4 h post administration were 10.4 ± 2.0 and 8.46 ± 1.1 μM and brain levels 1.3 ± 0.28 and 0.98 ± 0.15 μM, respectively. There was no apparent influence of sex or genotype (Figure S7), thus no evidence to suggest that female rTg4510 mice metabolised MK-1064 faster than rTg4510 males, which could have explained the sex differences reported above.

Altogether, MK-1064 had marked effects on the sleep wake cycle in WT and rTg4510 mice, although the effects in females were shorter-lived, particularly in rTg4510 females. In contrast to suvorexant which affected REM sleep, MK-1064 had major effects on wake, NREM and TST, whereas REM sleep remained proportional to TST. Figure 2 suggests that at least in male rTg4510 mice, the sleep wake cycle was "normalised" to a large extent when compared to WT-vehicle treated mice, whereas in female rTg4510 mice, this effect was of shorter duration.

## 3.4 | Zolpidem

The sleep/wake architecture of rTg4510 and WT mice was significantly altered by zolpidem (Figures 1, 3, 4, 5, 6, and S1 and Table S5), which predominantly influenced wake and NREM vigilance states. Male and female WT and rTg4510 mice spent significantly less time awake following zolpidem administration (Figure 1i), although the extent and duration varied between the different treatment groups. Male and female WT and rTg4510 mice also spent significantly more time in NREM sleep (Figure 1j) although as for wake, the effect size and duration were group dependent. No significant changes in REM sleep were observed at any individual time point (Figure 1k), keeping in mind that the level of REM sleep at this ZT time is typically very low. The relative effect on TST observed in the different groups was nearly identical to that for NREM sleep (Figure 1l). The cumulated TST appeared to normalise rTg4510 TST to that of WT-vehicle in males for up to 4 h and in females for up to 7 h post treatment (Figure 2e,f). Beyond these time points, the TST curves in both female and male rTg4510 mice are well below those of the respective WT mice in contrast to what was observed following MK-1064 treatment in male rTg4510 mice (Figure 2c).

Following zolpidem administration, % change in wake time during the first half of the active phase was significantly decreased in all groups, as was the case by 12 h, except for female rTg4510 mice which tended towards reduced wakefulness ($P = 0.08$; Figure 3q,r), although no sex differences within genotype were detected. Percent change in NREM sleep was increased in all groups except for female rTg4510 mice (Figure 3s,t), although, as for wake, sex differences within genotype were not significantly different. Percent change in REM sleep time was not significantly affected by zolpidem across the active phase, with the exception of female WT mice which exhibited a significant decrease by 12 h (Figure 3u,v). An increase in TST appeared more prominent in male compared with female mice (Figure 3w,x). Most mice administered zolpidem exhibited a significant decrease in wake and increase in

NREM and TST across the entire 23 h recording (Figure S1AA,AD, AJ).

Zolpidem reduced latency to sleep and NREM sleep in female mice only (Figure 4c,i), although the trend was present in all groups. There was no significant effect on REM sleep latency (Figure 4f). Wake bouts were largely unaffected by zolpidem administration (Figures 5m and S5S–U), except for a small decrease in total wake bouts across the entire 23 h recording in male WT mice (Figure S5U). The number of NREM bouts was similarly unaffected (Figure 5n), except for a significant decrease in male WT mice across the inactive phase and entire recording (Figure S5W,X). REM bouts were also not significantly altered (Figure 5o), except for an increase in REM bout numbers in female WT mice during the inactive phase (Figure S5Z). Wake bout duration was not affected by zolpidem administration (Figures 5p and S6S–U). NREM bout duration was significantly increased in male mice of both genotypes, but not in female mice of either genotype (Figure 5q). REM bout duration was significantly decreased in female WT mice only, during the active phase (Figure 5r) and the entire recording (Figure S6AA).

Zolpidem exhibited small, inconsistent effects on EEG power (Figure S4 and Table S6). It decreased EEG power only in male WT mice and increased delta power during NREM sleep in female WT mice (female rTg4510 mice tended towards increased power; Figure S4). Zolpidem also sporadically reduced EEG power across frequencies during REM sleep in female WT mice, although this was predominantly outside of the theta frequency range (Figure S4D).

Zolpidem significantly decreased % REM / TST only in female WT mice (Figure 6c). Altogether, zolpidem reduced wake and increased NREM and TST across the four treatment groups with slight variations in effect size and duration. There may be a trend towards a reduction in REM sleep but given the low REM baseline during the active phase, significant reductions may be difficult to detect. In contrast to MK-1064, zolpidem did not "normalise" sleep in the rTg4510 mice to a comparable extent.

## 4 | DISCUSSION

Mutant tau overexpression contributes to sleep/wake disruptions in tauopathy mouse models, characterised by a pronounced hyperarousal phenotype during the active phase without compensation during the inactive phase (Holth et al., 2017; Holton et al., 2020; Koss et al., 2016; Zhu et al., 2018). Whether this phenotype influences sleep/wake responses to different hypnotic classes had not previously been investigated. The present study therefore examined the modulation of sleep/wake in rTg4510 and WT mice following acute administration of suvorexant, MK-1064 or zolpidem at the commencement of the active phase, when the hyperarousal phenotype is most prominent in rTg4510 mice (Holton et al., 2020). Suvorexant promoted REM sleep in both male and female WT and rTg4510 mice, without overly affecting wake or NREM sleep, as previously reported in non-transgenic C57BL/6 mice (Betschart et al., 2013; Hoyer et al., 2013). On the other hand, MK-1064 effectively attenuated the hyperarousal

phenotype of male rTg4510 mice by decreasing wake and increasing NREM sleep and TST, in alignment with previous preclinical studies using 2-SORAs (Gotter et al., 2016; Roecker et al., 2014). Male mice exhibited a pronounced and sustained response to MK-1064. By contrast, although female rTg4510 mice displayed a marked early response to MK-1064, this effect declined very rapidly. Zolpidem, on the other hand, decreased wake and increased NREM sleep and TST similarly in both male and female rTg4510 mice, with a slightly more prominent response in male mice.

Suvorexant increased REM sleep for several hours post-dose in WT and rTg4510 mice of both sexes, without significantly affecting wake and NREM sleep, although it is possible that a higher dose of suvorexant may have influenced these vigilance states. The promotion of REM sleep in the present study parallels that observed by Betschart et al. (2013), in which 50 mg·kg$^{-1}$ suvorexant administration at the beginning of the active phase significantly increased REM sleep for up to 5 h post-dose, compared with vehicle, in C57BL/6 mice, with minimal effects on NREM sleep. These data indicate that the selective promotion of REM sleep by suvorexant is not impaired in the rTg4510 tauopathy mouse model. In terms of REM sleep architecture, REM bout number and duration were typically higher in all mice following suvorexant treatment compared with vehicle, however, suvorexant increased REM sleep in WT mice predominantly by increasing the number of REM bouts, without significantly altering REM bout duration. Conversely, the promotion of REM sleep in rTg4510 was driven primarily by an increase in REM bout duration, but not total REM bouts. These data suggest that hyperphosphorylated tau accumulation and the sleep/wake phenotype of rTg4510 mice affects the response to suvorexant, but only to a limited extent.

MK-1064 decreased wake time and increased NREM sleep time markedly for up to several hours post-dose in WT and rTg4510 mice, consistent with previous observations in C57BL/6 mice (Gotter et al., 2016; Roecker et al., 2014) although female and especially female rTg4510 mice were less responsive. MK-1064 normalised the amount of wake, NREM and TST in the active phase to that of vehicle-treated WT levels in male rTg4510 mice. By contrast, female rTg4510 mice exhibited an initially marked, but then very rapidly decreasing response to the hypnotic effects of MK-1064. Unlike suvorexant, which antagonises both the $OX_1$ receptor and $OX_2$ receptor, MK-1064 is $OX_2$ receptor selective and the presence of the $OX_2$ receptor is essential for the sleep promoting effects of 2-SORAs and DORAs (Gotter et al., 2016; Mang et al., 2012). The diminished response following MK-1064, but not suvorexant administration in female rTg4510 mice suggests potential differences in $OX_2$ receptor function and/or expression. Interestingly, female rTg4510 mice, like WT and rTg4510 males, responded to MK-1064 with an increase in delta power during NREM sleep. This suggests that $OX_2$ receptor signalling may remain relatively intact in cortico-thalamic circuits in rTg4510 females but may be disrupted in wake promoting regions such as the monoaminergic nuclei. Indeed, we have previously demonstrated that male rTg4510 mice exhibit altered $OX_1$ receptor mRNA expression in the locus coeruleus (LC) but without changes in $OX_2$ receptor expression (Keenan et al., 2021), which aligns with the robust

response to the $OX_2$ receptor selective antagonist MK-1064 in male rTg4510 mice. Female rTg4510 mice, however, were not examined in that study and investigations into $OX_2$ receptor expression and/or function in female rTg4510 mice are thus warranted. Nevertheless, the initial, albeit transient, hypnotic response in female rTg4510 mice suggests that $OX_2$ receptor function is still at least partially intact. Although not particularly evident in the present study, we have previously observed an exacerbated hyperarousal phenotype in female rTg4510 mice compared with males (Keenan et al., 2018, 2019). This led us to hypothesise that the blunted response to MK-1064 may be attributable to an inability to overcome this heightened hyperarousal drive. To further investigate this concept, we also assessed the sleep/wake response of rTg4510 mice following acute administration of the $GABA_A$ receptor positive allosteric modulator zolpidem.

WT and rTg4510 mice exhibited a relatively similar decrease in wake and increase in NREM sleep/TST following zolpidem administration, with a slightly larger effect size in male mice. REM sleep across the active phase, however, was decreased only in female WT mice, suggesting a sex difference in responses to zolpidem, which may be related to sex differences in metabolism of this drug, as is noted in rats and humans (Peer et al., 2016; Yoon et al., 2021). Reduction in REM sleep in rTg4510 mice may not have been evident due to the low levels of REM in these animals. Zolpidem promotes sleep via a global suppression of CNS activity, through modulation of $GABA_A$ receptors, without affecting OX receptors. Ablation of dense OX receptor expressing LC or tuberomammillary nucleus (TMN) neurons in rats attenuated almorexant-mediated promotion of REM sleep but had no influence on the efficacy of zolpidem in promoting sleep (Schwartz et al., 2016), indicating zolpidem does not act via these regions.

It has been reported that female sex hormones can influence sleep by promoting wakefulness and decreasing NREM sleep in C57BL/6 mice, an effect which is abolished by ovariectomisation (Paul et al., 2006). This supports the finding of the present study that male mice respond more prominently to hypnotic compounds but does not explain the transient response of female rTg4510 mice to MK-1064, but not zolpidem or suvorexant. Assessment of the levels of MK-1064 in the brains and plasma of male and female rTg4510 and WT mice showed they were comparable across groups and did not decrease significantly between 1 and 4 h post administration, confirming that gross sex differences in drug metabolism cannot explain the rapidly declining effect of MK-1064 on sleep/wake in female rTg4510 mice, whilst keeping in mind that 1 h post dose, the MK-1064 effect size was comparable in male and female rTg4510 mice. Furthermore, the approximate equipotency of suvorexant and zolpidem in all four groups of mice rules out major differences in metabolism for female rTg4510 mice, per se. Hence, there are clearly inherent differences in female rTg4510 mice that contribute to the diminished response to MK-1064. The exact mechanisms involved remain to be elucidated but may relate to previously reported sex differences in rTg4510 mice, in which female mice have more advanced tau pathology than males, by the same age (Yue et al., 2011), which may influence the orexin system. Overall, such differences reinforce

the importance of investigating both sexes in rodent preclinical studies, as previously recommended (Docherty et al., 2019).

REM as a proportion of total sleep during the active phase was assessed following acute suvorexant, MK-1064 or zolpidem administration. The DORA suvorexant increased sleep predominantly by disproportionately promoting REM sleep as previously reported in rodents (Betschart et al., 2013; Hoyer et al., 2013) and humans (Clark et al., 2020), whilst the 2-SORA MK-1064 produced a balanced increase of both NREM and REM sleep in WT and rTg4510 mice. It should, however, be noted that increases in both NREM and REM sleep have also been reported in response to other DORAs in preclinical species (Gotter et al., 2014). Zolpidem, however, preferentially promoted NREM sleep and suppressed REM sleep albeit only in female WT mice. REM as proportion of total sleep remained quite stable in rTg4510 mice, which is most probably due to the limited time that rTg4510 mice spend in REM sleep during the active phase, as a result of their hyperarousal phenotype. These data further reinforce the concept that $OX_2$ receptor selective antagonists promote a more physiological type of sleep (Betschart et al., 2013; Hoyer et al., 2019; Hoyer & Jacobson, 2013; Jacobson et al., 2017), even in a mouse model of neurodegenerative tauopathy. Furthermore, although zolpidem increased NREM sleep in both male and female rTg4510 mice without overly suppressing REM sleep, it is not an ideal candidate for tauopathy-related and other neurodegenerative disorders. Prominent disadvantages of benzodiazepines, or Z-drugs such as zolpidem, include adverse drug–drug interactions, cognition impairment, negative effects on balance leading to falls, and overall, a general inhibition of brain function (Kales et al., 2015; Winrow & Renger, 2014); hence contraindicating them as first choice hypnotics in neurodegenerative disorders. In contrast, OX receptor antagonists do not appear to impair cognition in humans and animals (Morairty et al., 2014; Neylan et al., 2020), and indeed can improve memory in animal models of insomnia and amyloidogenesis (Gamble et al., 2020; Zhou et al., 2020). A 2-SORA may therefore be the most suitable therapeutic principle to restore physiological sleep in neurodegenerative disorders (Hoyer et al., 2019). However, based on the present data, investigations into potential sex effects regarding the response to MK-1064 in tauopathy-related disorders in a clinical setting are warranted. On the other hand, the 2-SORA seltorexant was previously assessed in major depressive disorder and no sex effects were observed (Recourt et al., 2019), although this does not rule out possible tauopathy-driven alterations in the efficacy of 2-SORAs.

Finally, it should be noted that the rTg4510 is an insertion–deletion (INDEL) model, in that inserted transgenes disrupted endogenous genes, including deletion of fibroblast growth factor 14 (Gamache et al., 2019; Goodwin et al., 2019). The effect of these deletions on the hyperarousal phenotype of rTg4510 or their acute responses to hypnotics is unknown, although non-INDEL tau transgenic mice also show hyperarousal (e.g., Holth et al., 2017). However, Holton et al. (2020) did not observe differences in PSG between rTg4510-WT mice, rTg4510 mice treated with doxycycline from 13 weeks of age and the parental rTg4510 strain that carries the tau transgene (Tg4510-tTA), although the latter two are INDELs.

Furthermore, the effect of suppressing the tau transgene on hyperarousal is age-dependent. Whereas suppression of the tau transgene with doxycycline from 13 weeks of age prevented hyperarousal (Holton et al., 2020, i.e., irrespective of the INDELs, which are not influenced by doxycycline), when doxycycline was given to 18-week-old rTg4510 mice, hyperarousal was not reduced (Keenan et al., 2018), perhaps due to progressive invasion of tau pathology into the ascending arousal circuitry (Keenan et al., 2021). That transgene suppression did not rescue the hyperarousal phenotype after a certain age, yet these mice retain the ability to respond to hypnotic compounds, is interesting and potentially of translational value; this warrants further investigation in non-INDEL tau transgenic models.

In conclusion, we have assessed the sleep/wake response of rTg4510 mice following acute administration of sleep-promoting compounds during the active phase, when the hyperarousal phenotype is most evident. The DORA suvorexant promoted REM sleep in WT and rTg4510 mice, but had no effect on wake and NREM sleep states at the dose selected. By contrast, the $OX_2$ receptor selective antagonist MK-1064 effectively attenuated the hyperarousal phenotype by decreasing wake time and increasing NREM and TST particularly in male rTg4510 mice, whereas it showed limited efficacy in female rTg4510 mice, as the response effect size decreased rapidly during the few hours post administration. MK-1064 "normalised" the sleep phenotype in male rTg4510 mice across the entire active phase, whereas the effect was only transient in female rTg4510 mice. These differences cannot be explained by sex-related differences in metabolism, since male and female WT and rTg4510 mice show comparable brain or plasma levels, with very minimal differences between 1 or 4 h post administration. Finally, zolpidem promoted NREM sleep and decreased wake in both male and female rTg4510 mice, but in contrast to MK-1064, zolpidem did not normalise the sleep phenotype to WT levels, where both male and female rTg4510 mice treated with zolpidem are clearly separated from their respective controls in terms of TST following administration. These data reveal sex differences in the response of rTg4510 mice to MK-1064, highlighting potential alterations in $OX_2$ receptor function and/or expression in females in response to tau overexpression. The proportionally similar increase in NREM and REM sleep following MK-1064, but not suvorexant or zolpidem, reinforces the notion that $OX_2$ receptor selective antagonists promote a more physiological-like sleep architecture in both WT mice and a tau transgenic mouse model of neurodegenerative tauopathy. In conclusion, our data indicate that rTg4510 mice can indeed respond positively to sleep-promoting therapeutics despite a prominent and persistent hyperarousal phenotype during the active phase. Nevertheless, the noted sex differences suggest further investigations into sex-specific effects of hyperphosphorylated tau on $OX_2$ receptor pharmacology are warranted.

## AUTHOR CONTRIBUTIONS

RJK contributed to the experimental design and conduct, data acquisition and analysis, prepared all figures, and wrote the first draft of the manuscript. HD contributed to the experimental design and conduct, and data acquisition and analysis. LCD contributed to the experimental design and conduct, and data acquisition. JC contributed to data analysis. GA created and supplied the software program used for EEG sleep analysis. LHJ and DH contributed to the experimental design and manuscript writing. All authors contributed to manuscript revision and proofreading.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for Design and Analysis, and Animal Experimentation, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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