

Characterization of sleep architecture in male and female mice with kynurenine 3-monooxygenase deficiency

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BACKGROUND

Individuals with psychotic disorders, such as schizophrenia (SZ) and bipolar disorder (BPD), frequently have sleep disturbances.

Kynurenine 3-monooxygenase (KMO), a pivotal enzyme of the kynurenine pathway (KP) of tryptophan catabolism, converts kynurenine to the metabolite 3-hydroxykynurenine.

Reduced *Kmo* gene expression and KMO enzyme activity have been found in postmortem brain tissue of patients with SZ concurrent with increased levels of kynurenic acid (KYNA), a neuroactive metabolite of the KP that inhibits glutamatergic and cholinergic neurotransmission.

Recent studies demonstrate that acute increase in endogenous KYNA increases wakefulness and adversely impacts rapid eye movement (REM) sleep (Pocivavsek et al. 2017 *Sleep*).

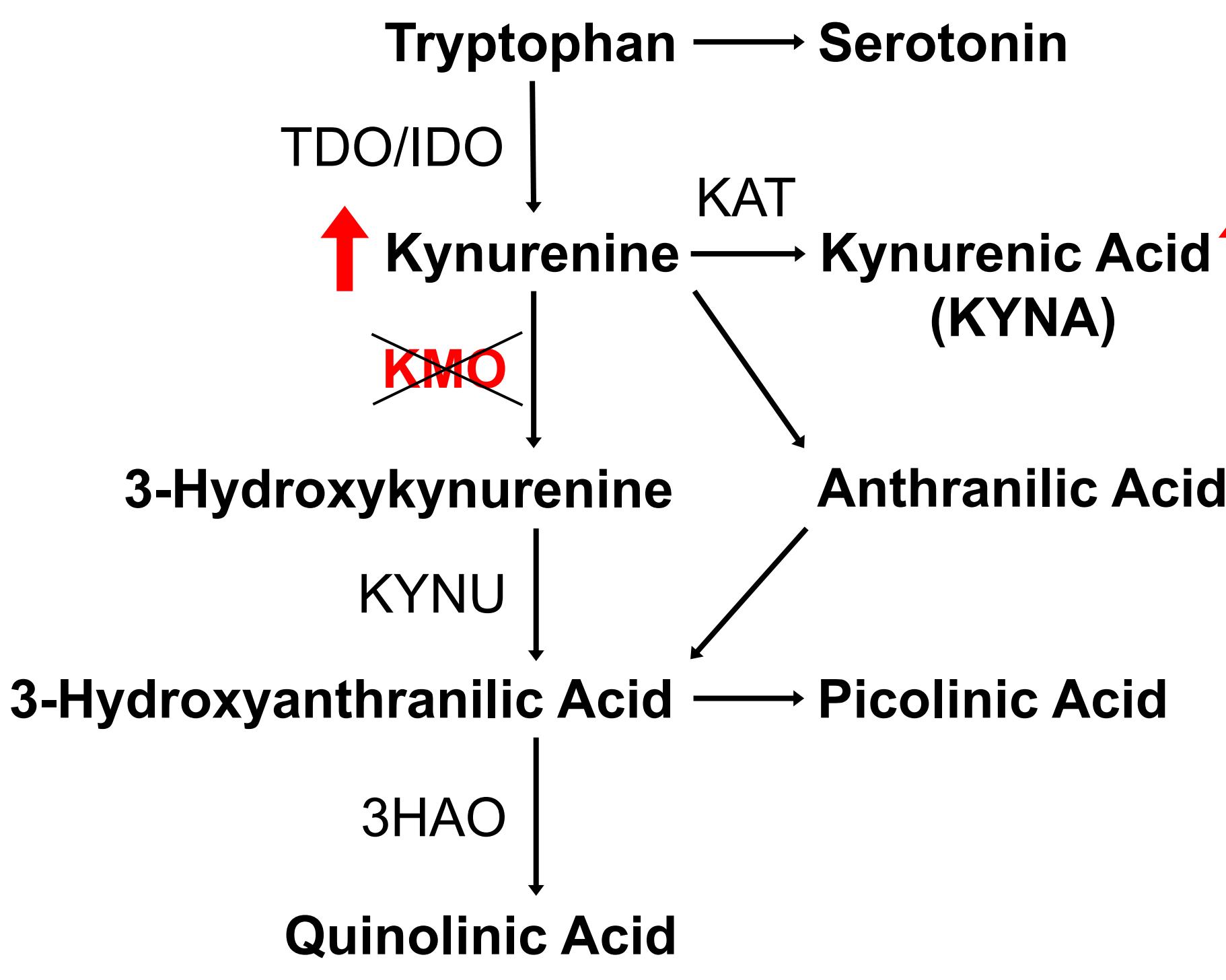


Figure 1. Schematic of the kynurenine pathway (KP).

RESEARCH AIM

We presently investigate the impact of genetic disruption of *Kmo* (using mice with chronically elevated KYNA because of a deficiency in *Kmo*; Giorgini et al. 2013 *J Biol Chem*) on sleep-wake behavior and architecture in mice.

METHODS

Animals

Sleep-wake phenotype was characterized during the light and dark phases in adult mice, age 3-6 months. Male and female mice were divided into groups based on their genotype: C57BL/6J wild-type (WT), knock-out (*Kmo*^{-/-}) (KO), heterozygous (*Kmo*^{+/-}) (HET), and wild-type offspring from heterozygous parents (*Kmo*^{+/-}) (WK). Animals were kept on a 12/12 h light-dark cycle, where lights on corresponded to zeitgeber time (ZT) 0 and lights off to ZT 12. Animals received *ad libitum* access to food and water for the duration of the experiment.

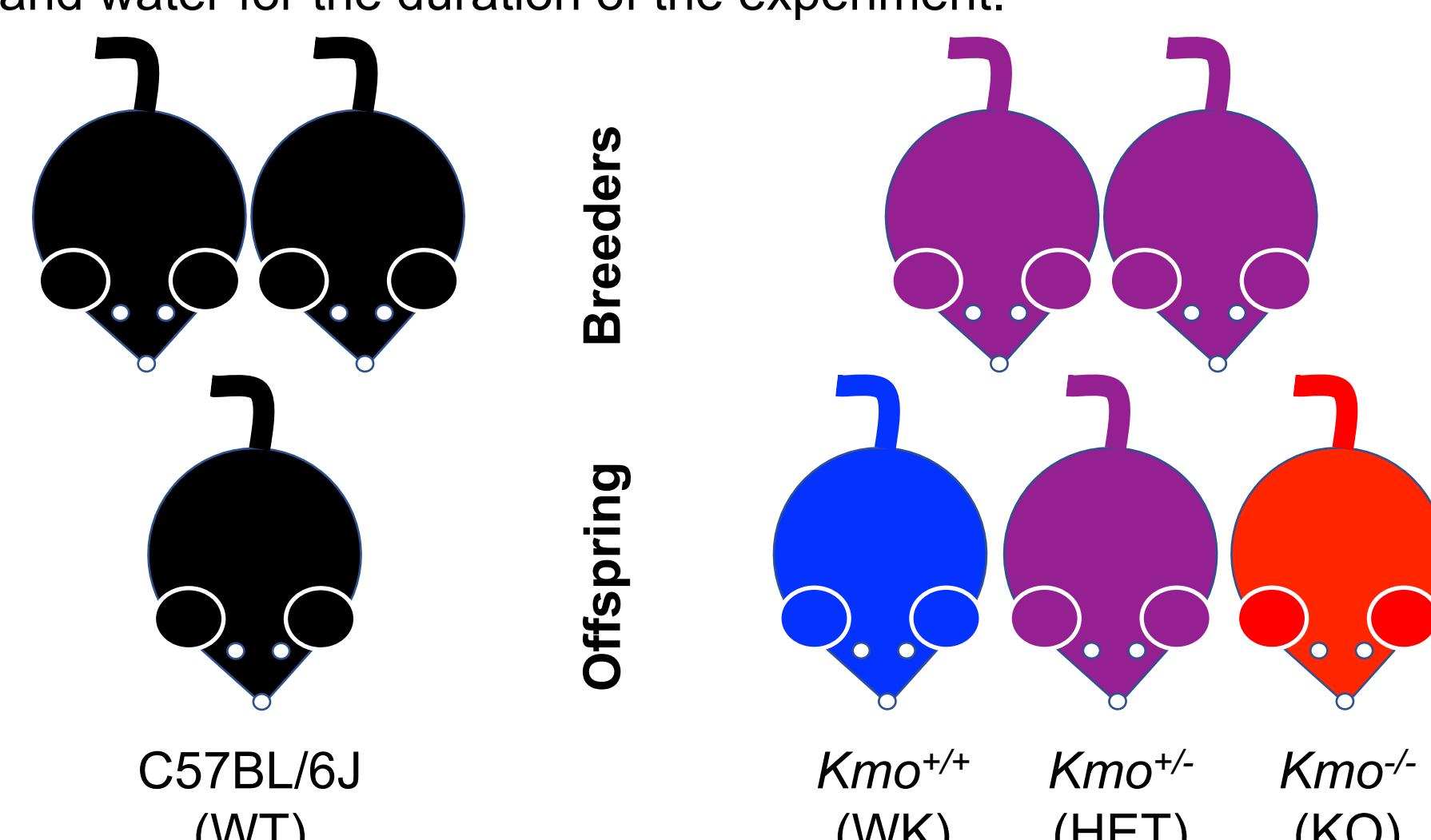


Figure 2. Representation of breeders' and offspring's genotype.

Surgery

Under isoflurane anesthesia, animals were placed in a Stoelting stereotaxic frame and implanted with telemetry transmitters with EEG/EMG electrodes (PhysioTel HD-X02; Data Science International). Briefly, the telemetry transmitter was intraperitoneally implanted through a dorsal incision of the abdominal region. An incision at the midline of the head was made to secure EEG leads to two surgical screws implanted into 0.5 mm burr holes at 1.9 mm anterior/+1.0 mm lateral and 3.4 mm posterior/-1.8 mm lateral relative to bregma and secured with dental cement. Two EMG leads were inserted directly into the dorsal cervical neck muscle at approximately 1.0 mm apart and sutured into place. The skin on top of the head was sutured and animals were allowed to recover post-operatively for ≥ 14 days before the start of experimentation.

KEY FINDINGS

REM sleep architecture is altered in male *Kmo*^{+/-} (HET) mice

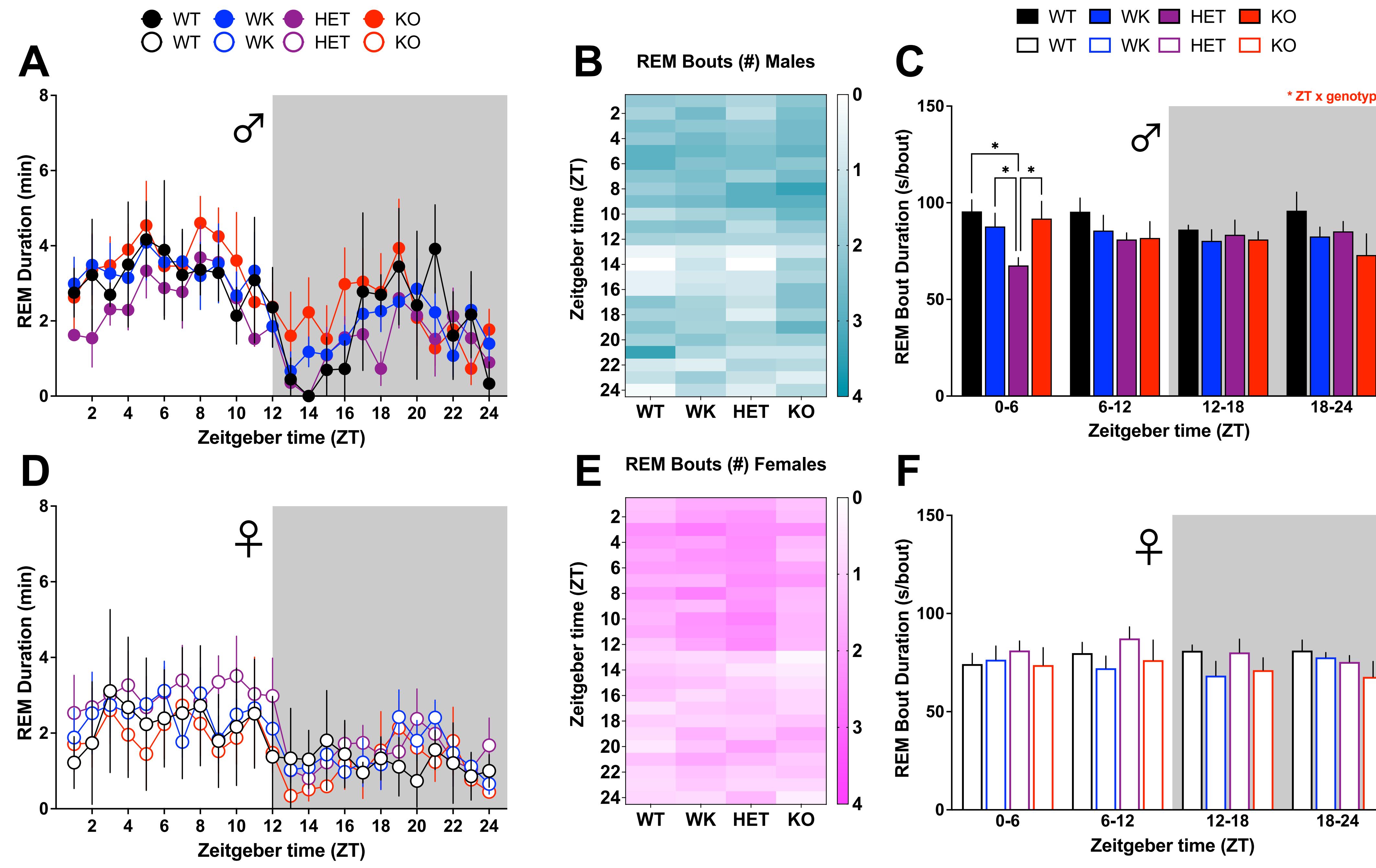


Figure 3. Altered REM architecture in *Kmo* HET males during the first half of the light phase. (A) REM duration in males. (B) REM bout number in males. (C) REM bout duration in males. (D) REM duration in females. (E) REM bout number in females. (F) REM bout duration in females. Data are mean ± SEM and were analyzed by RM two-way ANOVA with Fisher's LSD post hoc test: *p<0.05; N = 3-8 per group.

Architecture of slow wave, non-REM (NREM) is altered in *Kmo*^{+/-} female (WK) mice from *Kmo*^{+/-} parents

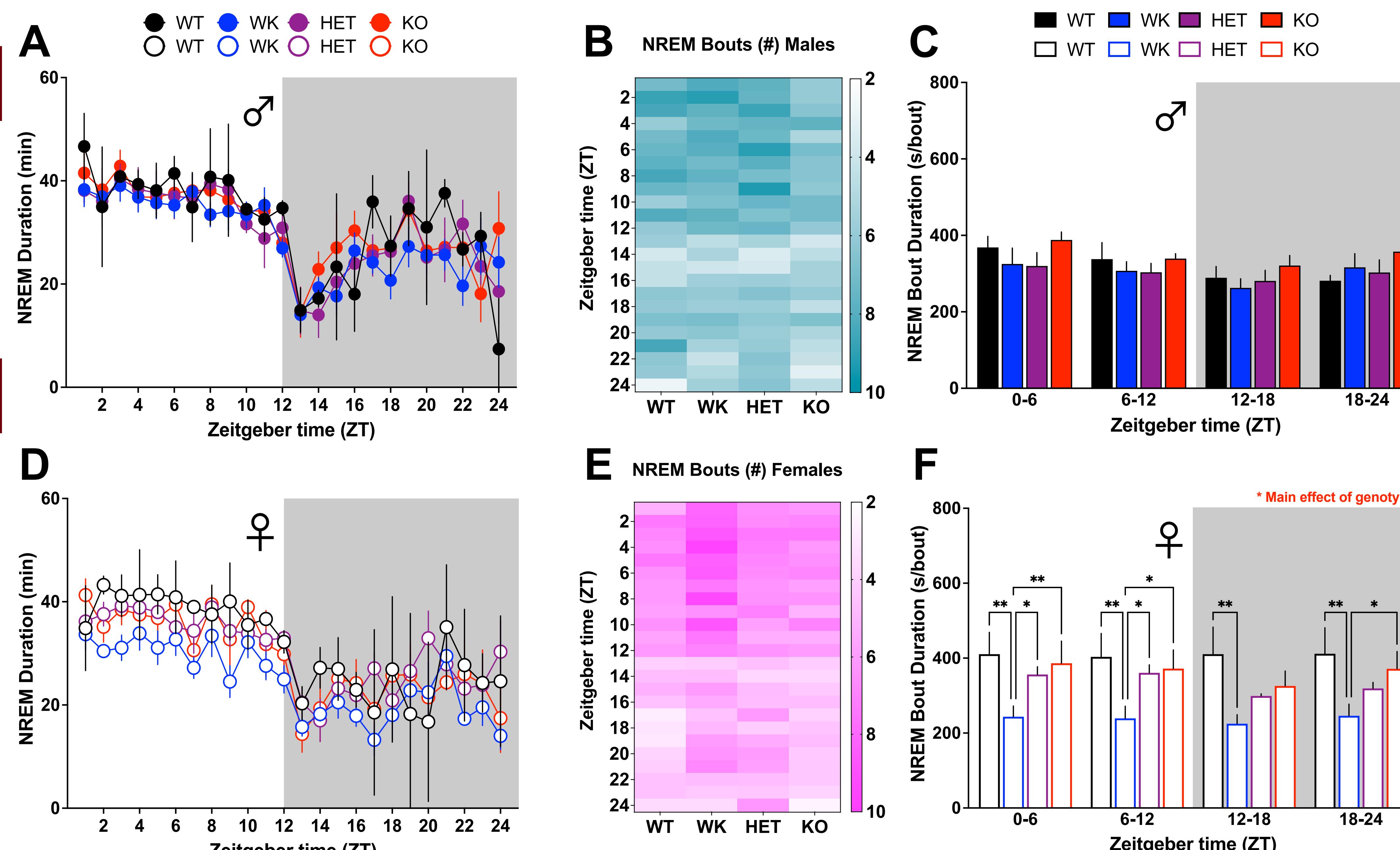


Figure 4. NREM bout duration is reduced in *Kmo* WK females throughout both light and dark phases. (A) NREM duration in males. (B) NREM bout number in males. (C) NREM bout duration in males. (D) NREM duration in females. (E) NREM bout number in females. (F) NREM bout duration in females. Data are mean ± SEM and were analyzed by RM two-way ANOVA with Fisher's LSD post hoc test: *p<0.05, **p<0.01; N = 3-8 per group.

Spectral power during sleep indicates a peak in theta during REM and delta during NREM; no differences among *Kmo* genotypes in male mice

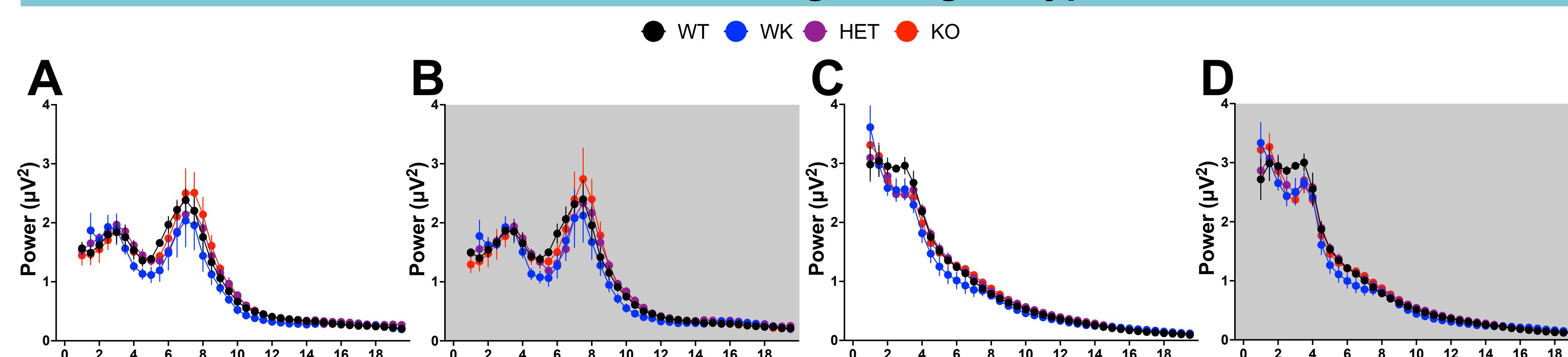
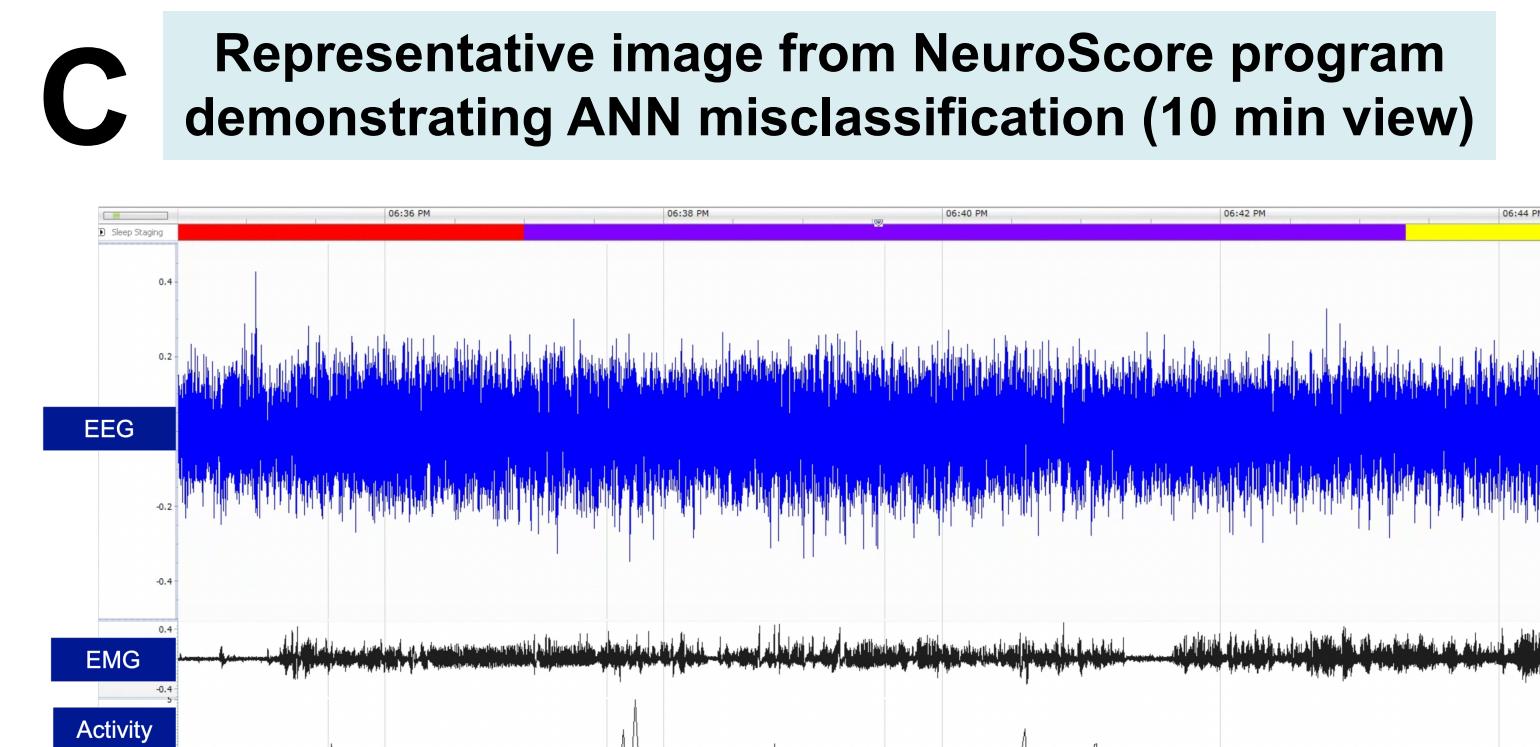
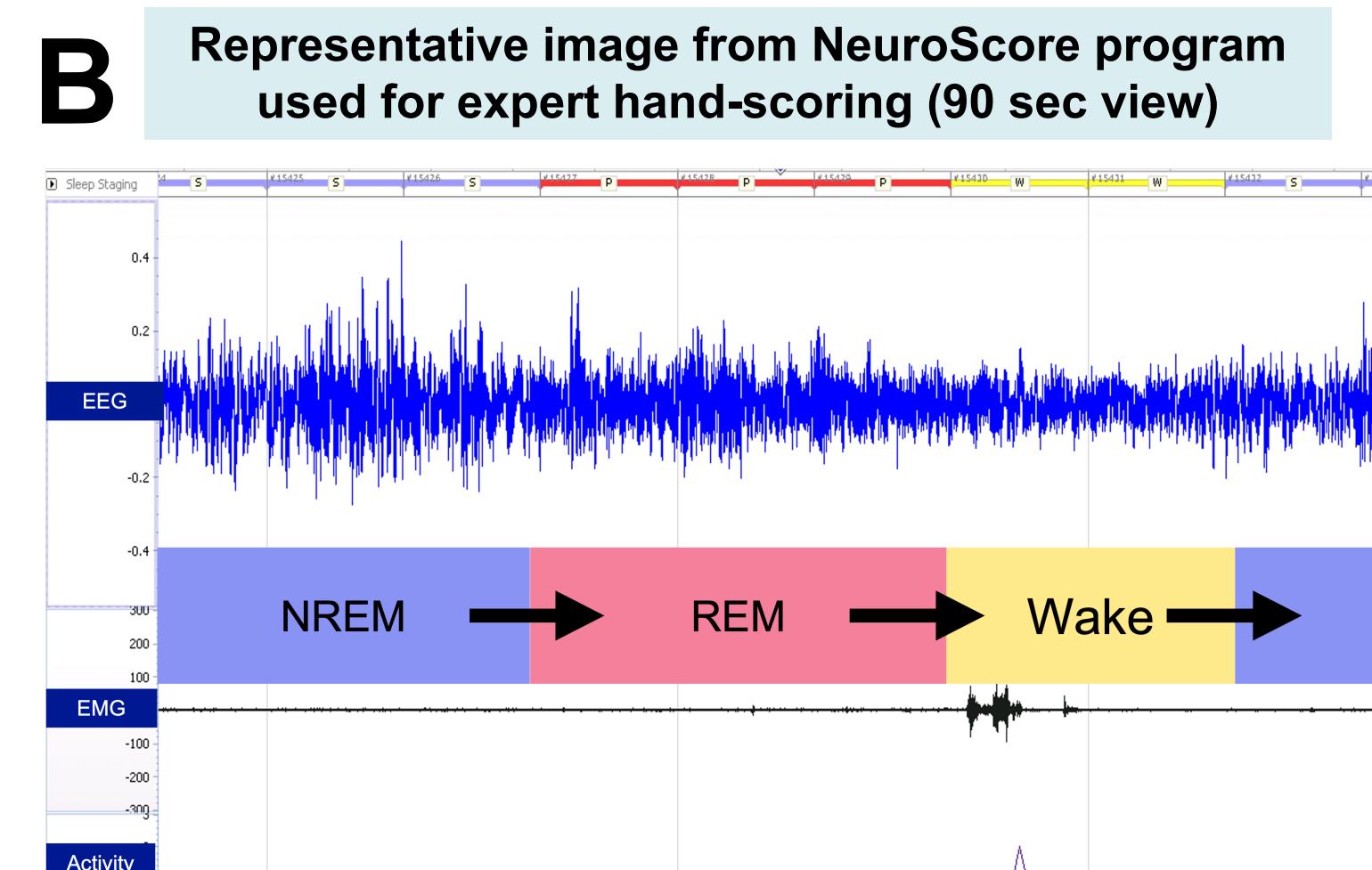
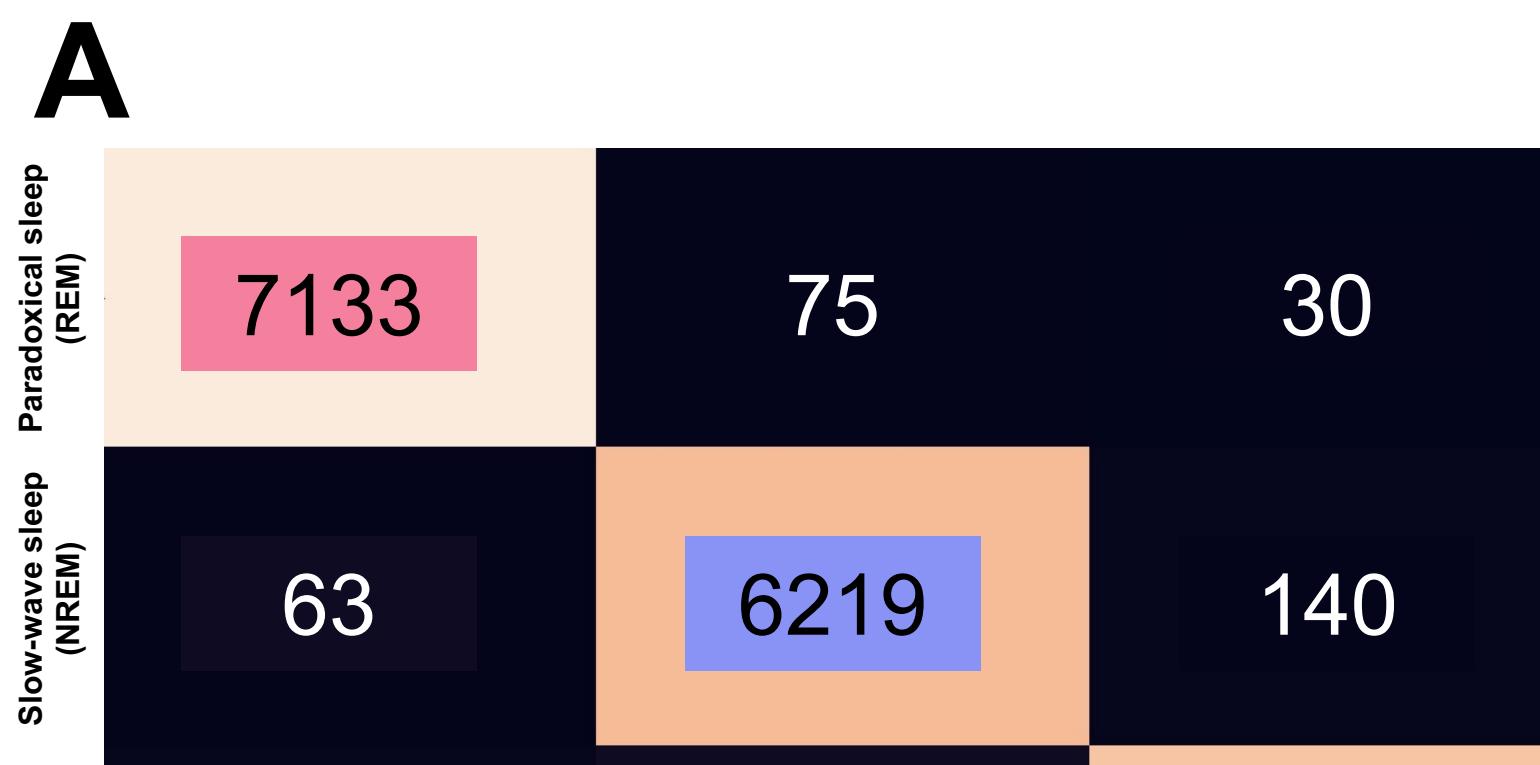


Figure 5. Spectral power data in males. (A) REM spectrum in the light phase. (B) REM spectrum in the dark phase. (C) NREM spectrum in the light phase. (D) NREM spectrum in the dark phase. N = 3-8 per group.

Artificial neural network (ANN) methodology performs classification tasks regulated by supervised predictive machine learning.

Confusion matrix demonstrates testing accuracy for each machine learning model based on performance of the classifiers for paradoxical sleep (REM), slow-wave sleep (NREM) and wake.



CONCLUSIONS

Kmo genotype in adult mice impacts REM and NREM sleep architecture in a sex-specific manner.

- In males, REM bout duration is decreased in *Kmo*^{+/-} (HET)
- In females, NREM bout duration is decreased in wild-type offspring (WK) from *Kmo*^{+/-} parents

Parental *Kmo* genotype contributes to the sleep phenotype of offspring.

Although ANN produces 96% accuracies in vigilance state classification, it may make misclassifications, and we are working to further improve the network.

For more research on genetically modified *Kmo* mice please refer to:

- Poster 73 (CMCC lower level, Sarisha Menon) for relative cage activity and temperature data
- Poster 82 (CMCC lower level, Morgan Lambert) for parental behavior, breast milk metabolites, and offspring cognitive performance

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