WUN progress report

We have made considerable progress in the last year thanks to the funding from this award.

Characterize the activity of limbic neuron projections in muscle-atonia regulating regions.

We have previously demonstrated a striking increase in dopamine release in the nucleus accumbens during both REM sleep and cataplexy (this work was published last year: Toth et al., 2023). Furthermore, we showed that increasing dopamine release in this region was sufficient to promote entrances into both REM sleep and cataplexy. We have begun to characterize the downstream projections by which the nucleus accumbens may regulate these states. Preliminary data suggests that neurons in the nucleus accumbens that project to the ventrolateral periaqueductal gray (vlPAG), a midbrain region known to gate REM sleep, increase their activity prior to transitions into REM sleep, suggesting a role for this pathway in regulating REM sleep transitions (Figure 1). Unfortunately, these studies were stalled due to animal and reagent availability.

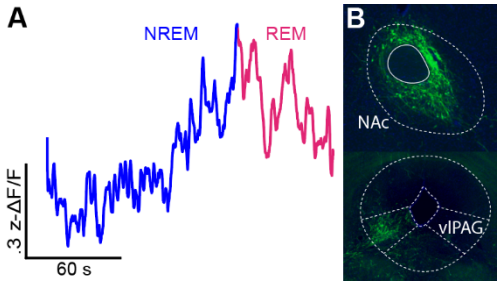

Figure 1. (A) Representative *in vivo* Ca^{2+} recording of limbic-brainstem projections during a NREM-to-REM transition. (B) NAc→vlPAG projecting neurons.

Determine the endogenous release and functional role of norepinephrine in sleep and cataplexy.

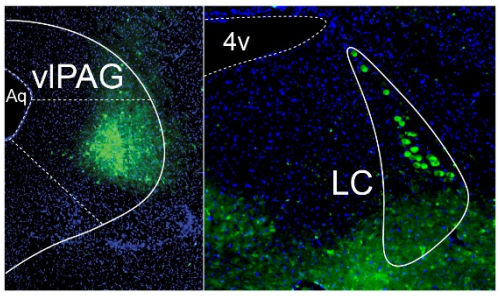

Figure 2. Injection of a retrograde virus expressing GFP in the vlPAG (left). Retrogradely labelled cells in the LC (right).

In our preliminary studies above, when mapping projections to the vlPAG, in addition to seeing projections from the nucleus accumbens, we found that norepinephrine neurons in the locus coeruleus (LC-NE) also project to the vlPAG (Figure 2). LC-NE has been implicated in sustained attention and maintenance of muscle tone during periods of arousal. LC neurons fire maximally during wake and minimally during cataplexy and REM sleep, suggesting that LC-NE release may suppress the activity of muscle atonia-generating brainstem regions to enable normal muscle tone during waking. Accordingly, NE receptor pharmacology increases muscle tone and effectively reduces cataplexy frequency in narcoleptic mice.

We began working to characterize the contributions of the LC-NE system in REM sleep and cataplexy, with a specific focus on LC projections to the vlPAG. To evaluate NE release dynamics during sleep, GRAB-NE was expressed in the vlPAG-LPT of mice fitted with an EEG headcap (Figure 3A). Similar to previous characterizations, we found that NE release in the vlPAG was highest during wakefulness and lowest during REM sleep. During NREM sleep, NE release remained elevated and fluctuated at an infraslow timescale (Figure 3B, C). Optogenetic stimulation of LC-NE projections to the vlPAG did not have an effect on NREM sleep, but strongly suppressed REM sleep (Figure 3D-F). When recording NE release in OX KO mice during episodes of cataplexy, we found that release was abruptly suppressed at the onset of cataplexy, returning to baseline only after the mice transitioned back to wakefulness (Figure 4A-C). Finally, when stimulating NE release in the vlPAG, we found that similar to REM sleep, this suppressed cataplexy frequency (Figure 4D-F). These experiments suggest a need for decreased NE release in the vlPAG to permit entrance into both REM sleep and cataplexy.

Collectively, these studies reveal an important mechanism by which both limbic and NE circuits regulate the occurrence of REM sleep phenomenon. We will continue working to elucidate the roles of these circuits in both healthy sleep and narcolepsy, with the goal of publishing independent studies in peer-reviewed journals. We thank the WUN foundation for their support of this work.

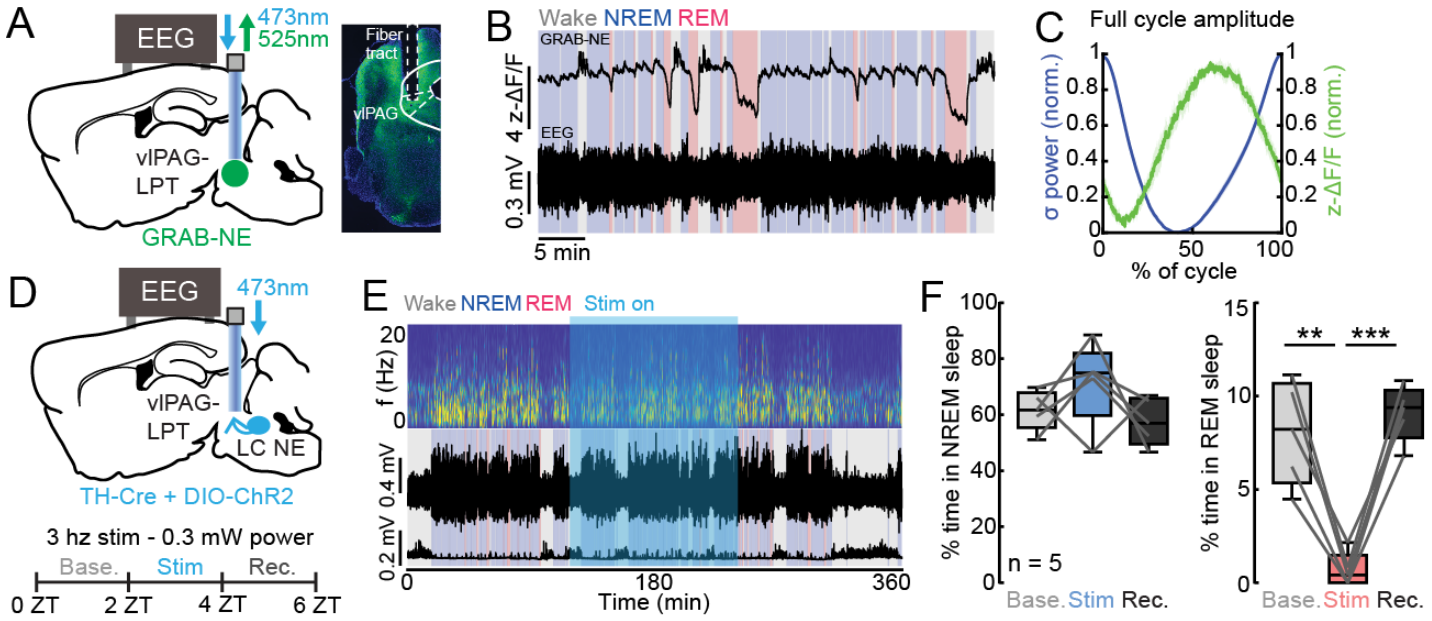

Figure 3. (A) Viral strategy to record NE release in the vlPAG during sleep (left). Representative histology of GRAB-NE expression in the vlPAG (right). (B) Representative recording of NE release during sleep. (C) NE release in the vlPAG is antiphase to infraslow sigma power. (D) Strategy to tonically stimulate LC-vlPAG projections during sleep. (E) Representative 6h recording during which stimulation occurred during hours 2-4. (F) While tonic stimulation had little effect on NREM sleep, REM sleep was strongly suppressed during this period.

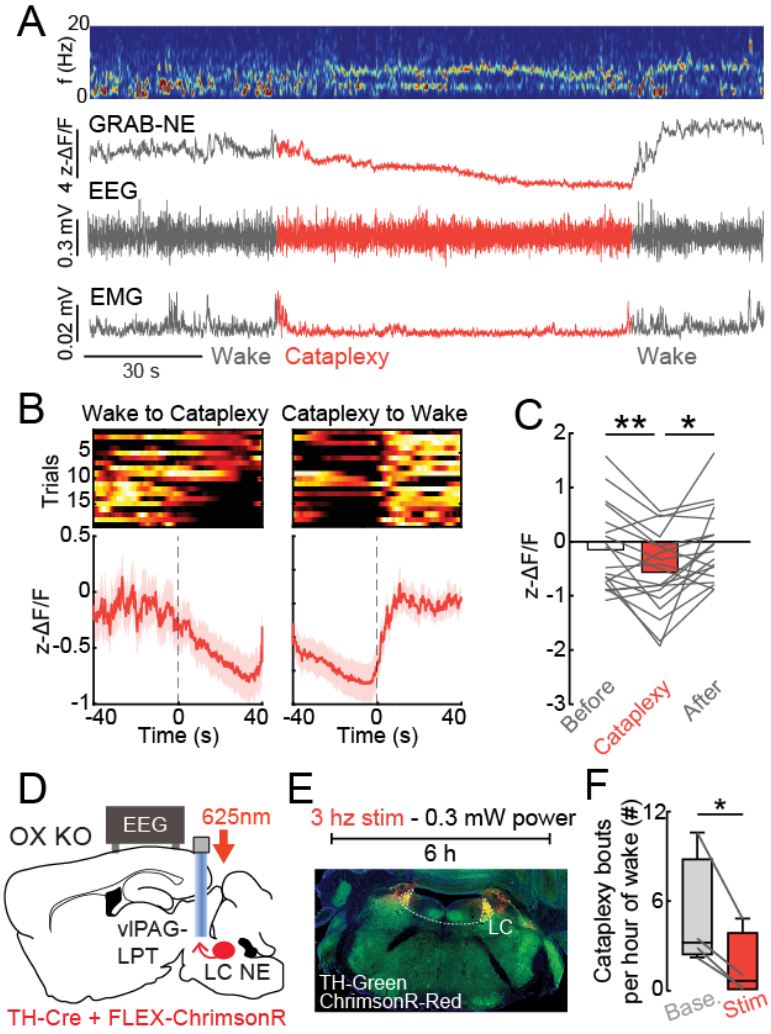

Figure 4. (A) Representative spectrogram, GRAB-NE trace, EEG, and EMG during a wake to cataplexy transition. (B) Trial averaged response of NE release at the onset and offset of cataplexy. (C) NE release was significantly suppressed during cataplexy. (D) Viral strategy to stimulate NE release in the vlPAG in OX KO mice. (E) Representative histology of ChrimsonR expression in the LC. (F) Stimulation of LC-vlPAG projections significantly suppressed cataplexy frequency.