Role of Layer 5 Pyramidal Neurons of the Medial Prefrontal Cortex in Sleep Homeostasis: Comparison in Young Adult and Aged Mice

Farahnaz Yazdanpanah Faragheh[1], Mattia Giovenzana[1], Shuzo Sakata[2], Derk-Jan Dijk[1], Julie Seibt[1]
[1]Surrey Sleep Research Centre, School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK
[2]Strathclyde Institute of Pharmacy and Biomedical Sciences, Glasgow, Scotland

INTRODUCTION

Sleep regulation was long thought to rely mainly on subcortical circuits. However, more recent work shows that the cortex, particularly layer 5 (L5) pyramidal neurons, plays a central role in sleep homeostasis. [Krone LB, et al., Nat Neurosci. 2021]. Several key questions related to this cortical regulation remain unresolved: how do these neurons regulate sleep pressure, how the nature of wakefulness shapes L5 neurons activity during subsequent sleep, and whether age-related sleep impairments arise from dysfunction in this specific neuronal population.

We hypothesise that Ca^{2+} dynamics in L5 pyramidal neurons directly reflect the build-up and dissipation of sleep pressure. We further predict that sleep deprivation involving higher cognitive load (e.g. novel object stimulation) elicits stronger L5 activation than low-load deprivation (e.g. gentle handling). Finally, we propose that these activity patterns are disrupted in aged mice, in line with increased sleep fragmentation and a reduced homeostatic rebound.

METHOD

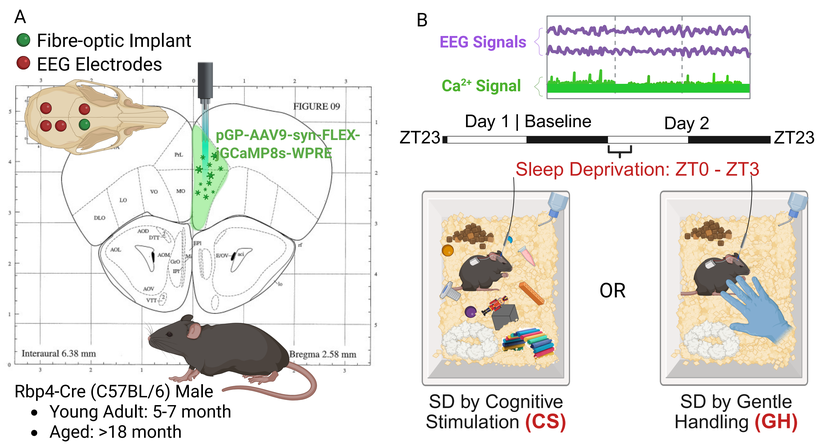
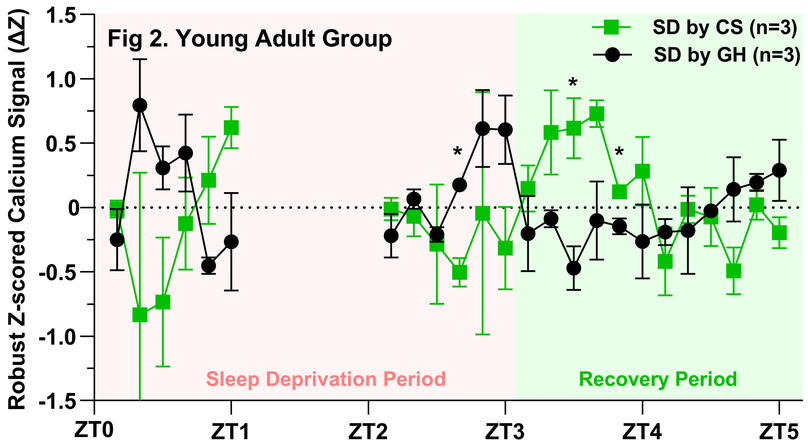
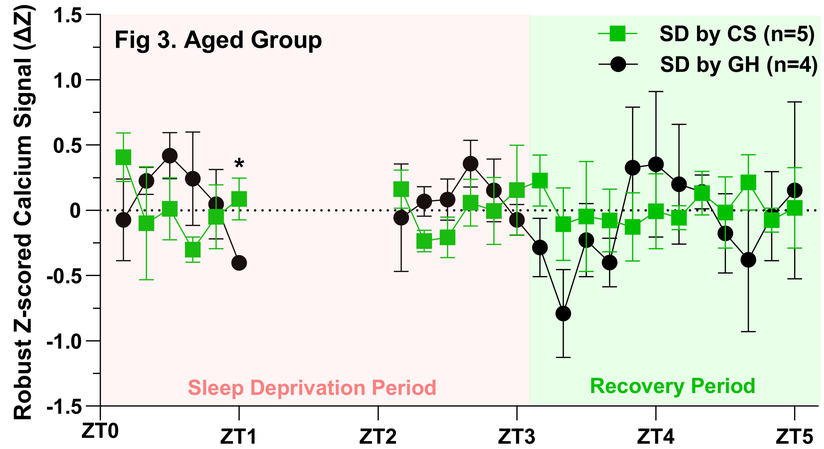
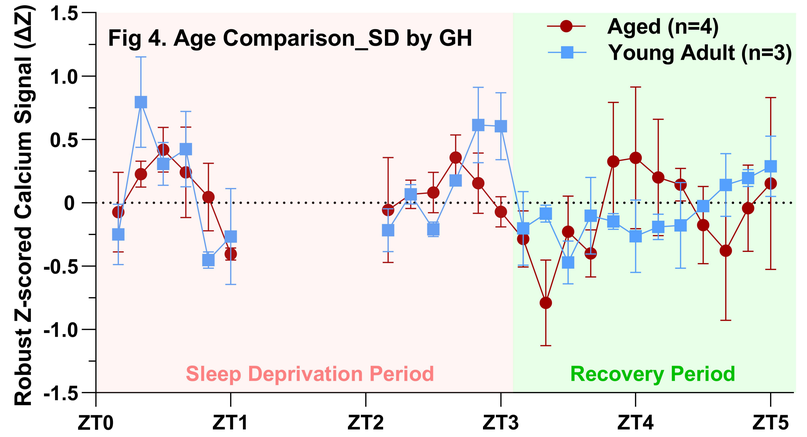


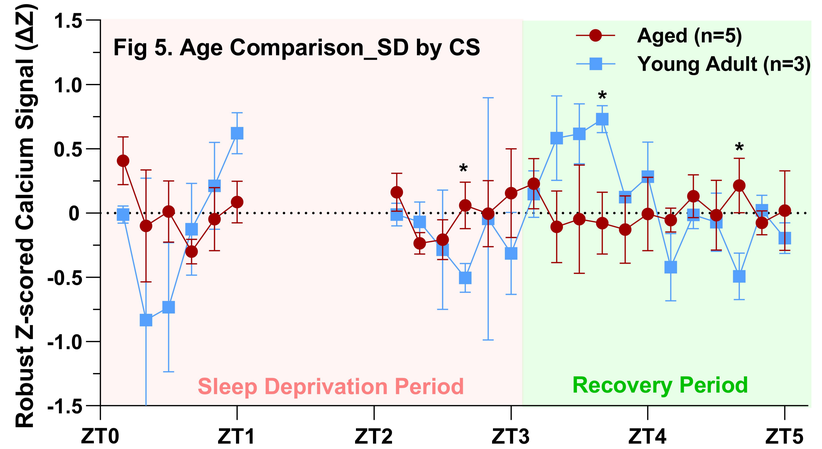
Fig.1. (A) Unilateral virus injection site in the mPFC; Locations of screws for two-channel DSI EEG telemetry, and fiber-optic cannula on skull; (B) L5 Neuronal calcium dynamics were tracked across a 24-hour baseline (Day 1) and through a 3-hour sleep deprivation (SD) by either gentle handling (low cognitive load) or novel object exposure (high cognitive load) (Day 2). Subsequent recovery sleep on Day 2 was monitored to quantify the age-dependant homeostatic rebound in relation to prior L5 activity.

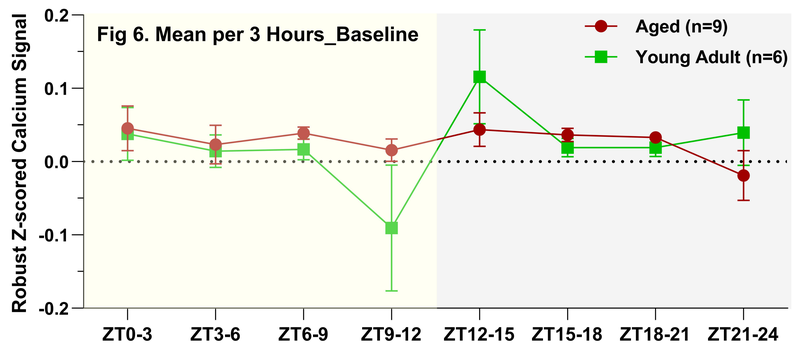
PRELIMINARY RESULTS











Preliminary results indicate that mPFC L5 pyramidal neurons activity during SD and recovery depends on both age and wakefulness content.
In young mice, Ca^{2+} activity shows opposite dynamics during SD and recovery depending on SD type. High cognitive load (SD by CS) induces a marked surge in L5 activity during the first hour of recovery, significantly greater than after low-load SD (GH) (**Fig 2**). In aged mice, Ca^{2+} responses are blunted, remaining near baseline regardless of SD method (**Fig 3**). Age comparisons show similar activity under GH (**Fig 4**), but distinct dynamics with CS, particularly during recovery (**Fig 5**).
Baseline data suggest age-related divergence across the 24 h light/dark (LD) cycle: young mice show clear mPFC activity fluctuations at the LD transition, whereas aged mice remain relatively flat (**Fig 6**). Ongoing data collection will increase sample sizes, and future EEG integration will link these calcium dynamics to specific sleep–wake states.

Data are presented as mean robust Z-score ± SEM; x-axis indicates Zeitgeber time (ZT).

ACKNOWLEDGEMENT

This project is funded by the Doctoral College (grant number TC9036), University of Surrey, UK

CONCLUSION

By focusing on mPFC L5 pyramidal neurons' role in sleep homeostasis, this work fills a key gap in understanding cortical contributions to age-related sleep dysfunction and provides a mechanistic foundation for targeted interventions aimed at ameliorating age-related sleep problems.