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ZONA INCERTA LHX6 NEURONS ARE MOST ACTIVE DURING NREM AND REM SLEEP AND AFTER PROLONGED WAKING

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Introduction: Emerging data shows that GABA neurons in the zona incerta (ZI) play a prominent role in regulating sleep. Transfer of the orexin gene into ZI neurons blocks cataplexy in narcoleptic orexin-knockout mice (Liu et al., JNeurosci, 2011) and vGAT GABA neurons in the ZI anticipate onset of NREM (Blanco-Centurion et al., SLEEP, 2021). To identify the subtype of GABA neurons regulating sleep, we examined the activity of Lhx6 neurons. This study tests the hypothesis that Lhx6 neurons show peak calcium fluorescence during sleep, and that the fluorescence is further increased after prolonged waking.
Methods: In Lhx6-cre mice (mice=4; all females; 4-5 mo), rAAV-DIO-GCaMP6s was delivered stereotaxically to the ZI (isofluorane anesthesia) and a GRIN lens, along with EEG and EMG electrodes were implanted. 21d later a miniscope (INSCOPIX) was attached, and after 3d of adaptation, sleep and fluorescence in individual Lhx6 neurons were recorded for 4h (baseline). On another day, the mice were kept awake for 6h (gentle handling; 9a-3p) and fluorescence in Lhx6 neurons was recorded for 2h during recovery sleep. The imaged data from the two recording periods (baseline and recovery sleep) was combined into a single data file and the change in fluorescence was determined against the mean image frame (F0). Previously, we and others found that the GCamP calcium fluorescence is a direct measure of action potentials and serves as a marker of activity.
Results: 97 neurons were automatically extracted (PCA-ICA analysis; blinded without knowledge of sleep state). In 66 neurons (68%) the average fluorescence was significantly higher during REM, NREM or both, compared to waking (Mixed Model ANOVA; SPSS25; $P<0.01$). In this population the fluorescence was significantly higher during recovery sleep compared to baseline ($P<0.001$) indicating increased activity of the sleep-active neurons during recovery sleep. With ensuing sleep, the increase in fluorescence gradually returned to baseline levels, attesting to the fluorescence as a marker of homeostatic sleep pressure. In 14 neurons (14%), fluorescence was highest in waking as compared to the other states during baseline, and in these neurons, fluorescence did not increase after sleep loss. Interestingly, six neurons (6%) were most active in waking and NREM but silent in REM (REM-off).
Conclusion: This is the first study to measure fluorescence in individual neurons after sleep loss. We find that the fluorescence in two-thirds of ZI Lhx6 neurons is tightly linked to sleep, and that the average fluorescence is further increased after prolonged waking. Microendoscopy is superior to indirect measures such as c-FOS or photometry in gauging sleep pressure in individual neurons.
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BRAIN BACTERIAL PEPTIDOGLYCAN IS REGION-SPECIFIC AND CHANGES AFTER ISCHEMIC STROKE

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Introduction: Sleep disorders and ischemic stroke (IS) are large health burdens. Almost half the USA population reports disturbed sleep and 795,000 Americans suffer a stroke annually. Despite dysregulated sleep being a stroke risk factor that can exacerbate injury and prolong recovery, sleep deprivation immediately preceding experimental stroke is neuroprotective. Bacteria and microbial products associate with (patho-)physiologies, including sleep phenotypes and IS. Peptidoglycans (PGs) are bacterial cell wall components found in diseased and healthy adults, and in developing and sleep-deprived brains. Although they influence atherosclerosis, an IS risk factor, PGs have not been characterized in post-stroke brain.
Methods: Aged (63 weeks) male wildtype mice (n=5) underwent permanent left middle cerebral artery occlusion, a model that mimics atherosclerotic IS, the most common type in humans. Surgeries began at Zeitgeber time (ZT) 12, lasted 30-40 minutes, and were followed by a 2-2.5-hour recovery period before sacrifice at ZT15. Brain stem (BS), somatosensory and prefrontal cortices (Sctx, PFC) were dissected, homogenized in phosphate buffered saline, and centrifuged. A standardized murine peptidoglycan ELISA (MyBioSource) was used for PG/MP quantification in resultant supernates. Brain areas in the left (L) IS, and right (R) control, hemispheres were compared by two-way ANOVA and Tukey's HSD tests.
Results: Mean PG values are expressed as ng/mg tissue wet weight ± SEM. Post-IS PG in the injured L Sctx (4.28±0.48) was lower ($F(2,21)=49.29$, $p<0.05$) than the uninjured R Sctx (5.66±0.29; $p=0.048$). There were no hemispheric PG differences in either BS or PFC. L hemispheric PG was greater in BS (7.59±0.40) versus Sctx ($p=0.0008$) and PFC (3.82±0.21, $p=0.0002$). R hemispheric PG was also greater in BS (8.26±0.53) versus Sctx ($p=0.005$) and PFC (3.56±0.56, $p=0.0006$) and in Sctx versus PFC ($p=0.02$).
Conclusion: This study confirms our parallel study, presented in a separate abstract herein (English et al), that PG regulation is unique within brain areas. Additionally, PG values in uninjured Sctx and in BS were similar between studies. Finally, current results suggest that reduced cerebral blood flow induced by IS reduces PG in the affected brain area. Further, PG may have a role in sleep deprivation-related IS injury and recovery.
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MATERNAL SLEEP QUALITY ACROSS PREGNANCY PREDICTS NEWBORN NEURODEVELOPMENT

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Introduction: The prenatal period is characterized by immense fetal neuronal growth. Such rapid growth can increase fetal susceptibility to prenatal environmental insults (Barker, 1998). A promising prenatal process that may alter fetal development is maternal prenatal sleep quality. Poor prenatal sleep quality is a public health concern affecting approximately 78% of pregnant individuals (Lucena et al., 2018). In rodents, maternal sleep deprivation across gestation predicts offspring hippocampal neurogenesis, with pups exposed to sleep deprivation early and late in pregnancy exhibiting more anxiety and depression-like behaviors (Peng et al., 2015). In humans, poor sleep quality in other developmental stages predicts hippocampi and amygdalae changes (Marshall & Born, 2007; Saghir et al., 2018). However, the relation between prenatal sleep quality and offspring brain