Real-Time Measurement of Lactate, Glucose and Glutamate Concentration Change During Wake and Sleep

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Abstract

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Introduction

• Biosensors are an emerging technology that can be used to rapidly measure analyte changes as quickly as one sample per second during periods of physiological transitions such as those seen during sleep and wake.1,2,3
• Extracellular lactate concentrations in the brain have been reported to be lower during NREM sleep as compared to wake and REM.4,5
• Glucose has been reported to be elevated during the NREM period.3,5
• In concordance with its role as the primary excitatory neurotransmitter, extracellular glutamate increases with wake and during REM sleep.1,2
• For this study we used EEG and EMG recording in conjunction with high-resolution electrochemical biosensors to record lactate, glucose and glutamate within the mouse cortex throughout multiple, uninterrupted sleep/wake cycles.
• This data demonstrates multiple, simultaneous biosensor measures as recorded in the mouse and marks the first high-resolution study of three neurometabolic substances across multiple sleep and wake periods.

Methods

• Twelve young (age 11-13 wks) wild-type C57Bl mice were housed under a 12 hour light / 12 hour dark cycle with food and water available ad lib.
• All mice were surgically implanted with bilateral cannulas for biosensor insertion in the prefrontal cortex (AP +1.7 to +1.9 ML +/- 1.5 DV -0.5) along with cross-cortical electromyograph (EMG) recording electrodes. Electromyograph (EMG) recording electrodes were inserted bilaterally in the nuchal muscles.
• Each mouse was implanted with either one lactate and one glucose biosensor (n=6) or one lactate and one glutamate biosensor (n=6) at the beginning of the lights off period. Simultaneous EEG and biosensor readings were recorded over a 24 hour period.
• Following the baseline recording period, mice in the lactate/glucose groups were sleep deprived for six hours at the beginning of the lights on period using a slowly rotating bar within their home cage. To prevent ascorilation to the sleep deprivation stimulus, the direction of bar rotation was randomly reversed every 30-90 seconds.
• All biosensors were constructed with a sensing cavity coated with a selective layer designed to react with the analyte of interest as well as interference layers to reject confounding electroactive substances. Following implantation, all biosensors were post-calibrated by stepwise addition of their specific analyte of interest at regular intervals (Figure 1a). Amperometric changes in individual biosensor readings were used for measurement of concentration changes in vivo. Specificity of biosensor analyte detection was further validated by simultaneous implantation of a working sensor and a null sensor (constructed in an identical fashion but lacking the sensing enzyme) concurrently within the same animal and recording over a 12 hour period (Figure 1b).

Results

• Individual results from two mice are shown below. Lactate and glutamate tend to track with sleep and wake (Figure 2a) while glucose concentration has pronounced dips during waking periods and peaks during NREM sleep onset. (Figure 2b).

Figure 2: Multiple sleep/wake cycles recorded using simultaneous EEG and (a) lactate/glutamate biosensors or (b) lactate/glucose biosensors plotted during the lighter period. Episode scored as wake are marked in red, NREM sleep epochs are colored blue and REM sleep epochs are indicated in green. Concentration change for each analyte is indicated on the y-axis. The lower graph (c,d) correspond to time periods on the upper graphs indicated by the solid box. In all expanded graphs, lactate concentration change is plotted in a similar manner to the large-scale graphs with colors indicating sleep/wake state and the secondary analyte (c) glutamate or (d) glucose plotted as an overlay in green or orange, respectively.

• Analysis of all sleep and wake episodes greater than 15 minutes show clear and significant changes in lactate, glutamate and glucose (Figure 3). Lactate sensor readings from both cohorts were not significantly different from each other during wake (two-way ANOVA, P<0.05) and sleep/peripartum periods and were combined.

Figure 3: Average per-minute concentration change for lactate (blue), glucose (orange), nM) and glutamate (green, null) during all (a) wake or (b) sleep episodes greater than 15 minutes in length. The bars represent SEM. "n"=P<0.05 as compared to the baseline value for that analyte using Tukey's.

• Lactate concentration changes by five standard deviations over baseline within one minute following wake or sleep transitions. Elevations in lactate concentration correlated nearly perfectly with time awake for every waking episode greater than five minutes in length while declines in lactate corresponded with time asleep (Figure 4).

Figure 4: Correlational analysis between wake episode length and increased concentrations in lactate (blue), glucose (orange) and glutamate (green) or (a) wake or (b) sleep periods greater than five minutes in length.

• Lactate concentration increased rapidly during sleep deprivation onset and remained elevated throughout the entire six hours of enforced sleep deprivation (Figure 5).

Figure 5: Average lactate concentration change in five animals during 15 minute baseline (open bar), six hours of enforced sleep deprivation (solid bar) and 20 minutes of recovery sleep (hashed bar). The dark blue line indicates the mean change in lactate concentration and the light blue lines indicate S.E.M.

Conclusions

• Changes in physiological state affect extracellular concentrations of lactate, glucose and glutamate.
• Lactate rises during wake periods and declines during NREM sleep.
• Glucose exhibits a transient decrease during waking and a transient surge during NREM sleep onset.
• Lactate and glucose changes stabilize within 20 minutes following wake or sleep onset.
• Lactate concentration can be used to detect sleep/wake transitions in the absence of EEG data.

References


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