# Glutamate Concentration is Directly Correlated with Alpha Power Attenuation from Eyes Closed to Eyes Open in Human Occipital Lobe

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**Target Audience**: Researchers interested in neurochemical correlates of classic electroencephalography signals in humans

**Purpose**: Electroencephalography, or EEG, has been a critical tool of neuroscientists for nearly a century. An EEG signal can be transformed into its component frequencies, and one of the most well studied frequency bands is alpha (8-12 Hz). Alpha power is strongest during wakeful rest with eyes closed, however this signal is significantly attenuated upon eye opening. The neurochemical contributions to this phenomenon are not clearly understood. Pyramidal glutamatergic neurons in layer IV of the occipital cortex (OCC) are known to fire at approximately the alpha frequency at rest and this steady rate of OCC neuronal firing is regulated by γ-aminobutyric acid (GABA) interneurons. During visual activation, this firing becomes asynchronous. Since EEG records net electrical activity of thousands of firing neurons, it is likely that excitation and resultant desynchronization of glutamatergic pyramidal neurons during visual activation cause the observed alpha power attenuation. Therefore, we hypothesize that more glutamatergic neurons would result in higher excitability of the OCC and therefore more alpha attenuation. Additionally, GABAergic interneurons would exhibit an inverse relationship with alpha attenuation. To test these hypotheses, we applied sequential measurements of glutamate-glutamine (Glx) and GABA using J-coupled edited MRS and assessed correlations with alpha power attenuation in the OCC.

**Methods:** Informed consent was obtained from healthy volunteers (n=15; age=23±3 yrs, sex=M), after which subjects underwent an EEG and MRS experiment. ***EEG*** *Experiment:* Subjects were fitted with an electrode cap connected to array of 128 Ag-AgCl electrodes in a standard, preconfigured montage. Electroogulogram (EOG) activity was recorded from electrodes placed below the left eye and on the outer canthus of the left and right eye to remove blinking artifacts. Subjects were asked to rest quietly in a darkened room for two minutes with eyes open followed by two minutes with eyes closed. Order of eyes open/eyes closed was counter-balanced across subjects. EEG and EOG activities were digitized with a sampling rate of 256 Hz. During data collection recordings were referenced to single central electrode. *Analysis:* Recordings were referenced to a common average reference of all 128 electrodes. Blink artifacts were removed using an ocular artifact correction algorithm. Data analysis was restricted to an occipital region of interest containing 14 electrodes in order to better compare EEG measures to MRS measures (***Figure 1a).*** Power calculations in the alpha bandwidth (8-12 Hz) were done using a consecutive non-overlapping 1-second Hanning window. Data were normalized for each subject by dividing power in the alpha bandwidth by power in all other bandwidths (0.01-60 Hz). Alpha attenuation was calculated by subtracting normalized alpha power during eyes open from normalized alpha power during eyes closed. ***MRS****: Experiment*: Single voxel spectroscopy was performed on a 3T Philips Achieva scanner using a J-difference edited MEGA-PRESS[4] sequence to concurrently measure GABA and Glutamate+glutamine (Glx). 384 averages of the spectra were acquired using TR/TE=2000/73. Spectroscopy voxel placement (25x30x22 mm) is illustrated (***Figure 1b***) *Analysis:* Each spectral line was frequency and phase corrected using Creatine (Cr) peak as reference. LCModel[5] was used to detect the ratio of Glx to Cr, ([Glx]/[Cr]) as well as the ratio of GABA to Cr ([GABA]/[Cr]). Robust quality of each spectra was ensured by using a Cramer-Rao lower bound ≤ 15%. Correlation of [Glx]/[Cr] and [GABA]/[Cr] with alpha power attenuation was assessed using Pearson's correlation analysis.

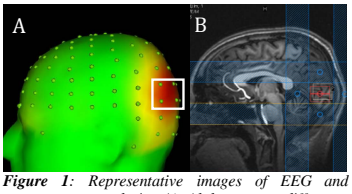


***Figure 1****: Representative images of EEG and spectroscopy analysis. A) Alpha power difference between eyes open and eyes closed projected onto a scalp with warmer colors representing greater alpha attenuation. Green points represent electrodes and the white box represents the approximate region of interest used for calculations. B) Spectroscopy voxel placement and in the occipital lobe*

**Results:** Subjects showed the predicted attenuation in alpha power from eyes closed to eyes open (***Figure 2***). As predicted, a significant positive relationship was found between the [Glx]/[Cr] and alpha attenuation (r=0.50, p<0.03, ***Figure 3a***). The relationship between [GABA]/[Cr] and alpha attenuation was not significant, however a negative trend was seen (r=0.27 p=0.18, ***Figure 3b***).

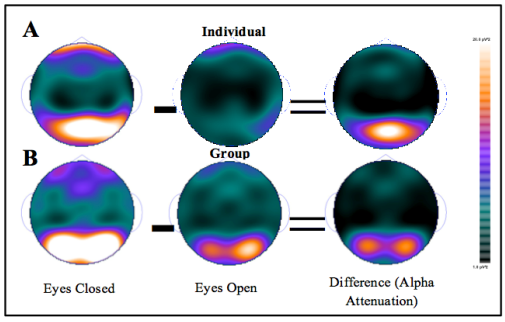


***Figure 2****: 2-dimensional topographic plots of electrical activity during rest. A) Representative images from one subject, B) Average activity for all subjects (n=14). The difference in power between eyes closed and eyes open is the alpha attenuation value. Scale range is 1 to 20 pV²*

**Discussion:** These results demonstrate that the difference in alpha power between eyes closed and eyes open during rest may provide an index of cortical excitability or glutamatergic neuron density in the OCC. While this result does not conclusively prove that the attenuation seen in alpha power between eyes open and eyes closed is due to the desynchronization of pyramidal glutamatergic neurons, it does add support to the model. Several factors need to be considered while interpreting this study: First, the measured peak, Glx, is a combination of signals from glutamate and glutamine and contribution of non-neuronal physiological processes cannot be eliminated. Secondly, detection of GABA using J-difference editing uses long TEs. However, Glx has a short transverse relaxation rate (T2) and is better detected at short TE (~ 25 ms). This could be addressed by performing additional MRS scans at short TE or using multi-TE MRS sequences. Finally, one subject showed an increase in alpha power during eyes open (***Figure 3***). While unexpected, this is not physiologically impossible and may warrant further study. Future work will determine if an individual's concentration of glutamate in OCC is correlated with visual activation measured with EEG, or performance in a visual behavioral task.

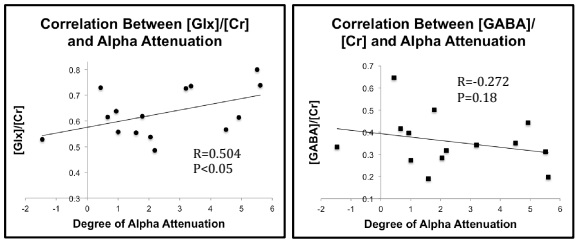


***Figure 3:*** *There is a significant positive relationship between the occipital concentration of glutamate ([Glx]/[Cr]) and alpha attenuation (r=0.504, p=0.028), however there is no significant relationship between [GABA]/[Cr] and alpha attenuation (r=-0.272, p=0.18)*

**Conclusion**: This work demonstrates that alpha power attenuation is more strongly related to glutamatergic concentration than GABAergic concentration in the OCC. These data support the hypothesis that the attenuation in alpha power seen in EEG research is due to the activation and desynchronization of glutamatergic neurons.

**References:**

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