Concurrent multimodal estimation of cortical excitation and inhibition shows that EEG-based estimates are linked to the ratio of glutamate to GABA

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Abstract

Neural excitation/inhibition (E/I) balance is dynamically regulated on multiple timescales. Adaptive changes in E/I balance changes can support healthy development, learning, and cognition, while disordered E/I balance is implicated in neurodevelopmental disorders, neurodegenerative disorders, and states of impaired vigilance. There has been growing interest in inferring E/I balance from efficient and noninvasive measurements such as electroencephalography (EEG), and several algorithms have been proposed to estimate E/I balance from EEG recordings. Despite promising results, there has been a lack of validation studies testing the underlying neurochemical changes leading to increased or decreased EEG-based E/I. Here we assess E/I balance with concurrent EEG and magnetic resonance spectroscopy (MRS) in humans. We estimate a standard measure of E/I balance, glutamate concentration divided by GABA concentration, and assess the correspondence between each candidate EEG-based E/I algorithm to this MRS-based estimate. Due to the methodological interest in both between-subjects research (e.g., comparing disordered to healthy E/I) as well as within-subjects research (e.g., comparing pre-intervention to post-intervention E/I), we quantify the associations between EEG-based E/I and MRS-based E/I separately for between-subjects and within-subjects comparisons. We find that each EEG-based E/I algorithm shows reliable and positive associations with MRS-based E/I. While these associations are evident for between-subjects comparisons, they are quite weak for within-subjects comparisons. Candidate EEG-based E/I algorithms are thus likely to be reflecting, at least in part, relative concentrations of cortical glutamate and GABA, although poor signal quality appears to limit these methods when attempting to increase temporal precision and identify within-subjects variations.

Introduction

Brain functioning is largely regulated through a balance, or dynamically changing imbalance, of excitatory and inhibitory (E/I) activities (Hensch et al. 1998; Shew et al. 2011). Such dynamics are closely linked to a variety of phenomena including neural plasticity (Bavelier et al. 2010; Hensch et al. 1998; Vogels et al. 2011), brain development (Hensch 2004; Takesian and Hensch 2013) disordered functioning (Bruining et al. 2020; Geertjens et al. 2022; Nifterick et al. 2023), and cognition-related topics (Hellyer et al. 2016) such as vigilance (Yang et al. 2023) and decision-making (Amil and Verschure 2021). Unfortunately, E/I is extremely difficult to measure non-invasively (Ahmad et al. 2022; Craven et al. 2022), and continued refinement of E/I measurement is a core component of advancing these disparate fields. A variety of methods are used to assess E/I in humans. These include the use of electrophysiological signatures of E/I (e.g., using electroencephalography, EEG; Ahmad et al. (2022) and using magnetic resonance spectroscopy (MRS) to identify concentrations of excitatory and inhibitory neurotransmitters (e.g., Glutamate and GABA, respectively; Shibata et al. (2017). Each of these approaches holds great promise and yet faces serious limitations.

EEG-based approaches to quantifying E/I come from several theoretical and computational sources, including scale-free dynamics (Gao, Peterson, and Voytek 2017), criticality theory (Poil et al. 2012), and neural mass modeling (Yokoyama and Kitajo 2023), and have been used to investigate physiological underpinnings of phenomena ranging from vigilance (Yang et al. 2023) to Alzheimer's disease (Nifterick et al. 2023). Each of these has proposed mechanistic bases for the links between a scalp-based electrical signal and cortical excitation or inhibition, but empirical data tracking brain regions' neurochemistry to EEG measures has been largely lacking (Ahmad et al. 2022; Geertjens et al. 2022). The difficulty in establishing the biological bases of EEG-based E/I measures is offset, however, in their relative advantages: EEG is widely available, is inexpensive to implement, and provides data from multiple brain regions simultaneously.

In contrast, the use of MRS to study E/I presents a different set of trade-offs. Studies of E/I using MRS have been conducted to study brain plasticity, such as that involved in sleep-dependent consolidation of perceptual learning (Tamaki et al. 2020) or developmental differences in GABA and plasticity (Frank et al. 2022). While MRS-based E/I has the distinct benefit of having a clear biological basis (i.e., neurotransmitter concentrations such as Glutamate and GABA), it is limited to a single cortical location with fairly low temporal resolution, is limited to measurement inside a MRI scanner (thereby precluding, for example, measurement in certain populations or during certain behaviors), and is relatively expensive.

The field of E/I research using both EEG and MRS would benefit from linking the two domains. There have been multiple reports of associations between MRS-measured GABA concentrations with electrophysiological frequency bands (e.g., Balz et al. (2016); Edden et al. (2009)), but each of these studies measured

electrophysiology and MRS in separate sessions and were therefore limited to stable inter-individual correlations and were insensitive to functional changes that may occur on short timescales. Shorter timescales of change are necessary to understand in the context of many fields, including neuroplasticity, pharmacological interventions, and cognitive consequences of varying E/I.

There remains a clear need, then, for multimodal investigations of EEG-E/I in conjunction with MRS-E/I. Only by simultaneously measuring the two forms of E/I can both within-subject and between-subject variations in each of the two measures be assessed. It is possible that the two forms of E/I are empirically distinct from each other, for example, if EEG-E/I reflects rapidly-changing excitation and inhibition while MRS-E/I reflects E/I that only changes on a slower timescale, if EEG-E/I reflects specific neural subpopulations generating oscillatory signatures (Scheeringa and Fries 2019; Shafiei et al. 2023) while MRS-EI reflects the aggregate concentration of certain molecules in a large voxel, or if the relatively superficial neural populations generating scalp-measured electrophysiological changes display different dynamics than the relatively deep aggregate population contained within a MRS voxel. In contrast, it is possible that the two forms of E/I are indexing the same underlying biological processes, that is, the relative concentrations of the primary excitatory vs. inhibitory neurotransmitters in a large region of cortex. Here we address these questions by linking visual-area MRS and EEG recorded in participants who had completed a visual perceptual learning task.

Materials and Methods

Experimental Design and Statistical Analysis

We re-analyzed data from Study 1 of Tamaki et al. (2020), with 18 subjects each sleeping for between 60 and 90 minutes after completing a perceptual learning task. EEG (polysomnography; PSG) and MRS were recorded throughout the sleep. To assess the extent to which candidate EEG-based measures of E/I were reflecting the changes in neurotransmitter concentrations measured by MRS-based E/I, we first estimated a single E/I value using each method for each 5-minute period of the recorded data. We then used cross-validation to estimate out-of-sample variance explained when predicting MRS-based E/I using EEG-based E/I ($dRsq_{oos}$; see below). We separated these estimates into between-subjects and within-subjects analyses; while much previous work has focused on the relatively stable differences between individuals' levels of excitation and inhibition (Balz et al. 2016; Edden et al. 2009), within-subjects variation in E/I are central to many research domains such as vigilance (Yang et al. 2023) and learning-related neuroplasticity (Bavelier et al. 2010; Tamaki et al. 2020) because these topics are inherently concerned with intra-individual changes. Separating these results helps identify both the overall associations between MRS-E/I and EEG-E/I as well as their relative isomorphism in the context of different applications.

For within-subjects and between-subjects analyses we used two statistical approaches in conjunction. In the first, in order to best estimate the effect size (i.e., correlation coefficient) between E/I measures, we fit a Bayesian multivariate linear mixed-effects model (BMVLMEM) using **brms** in R (Bürkner 2017). Using this approach we specified each of the candidate E/I measures as variables to predict (i.e., "response" variables). Each response variable was fit with an overall intercept, a random-effects by-participant intercept, and a fixed-effect autoregressive term with an order of one to account for non-independence of temporally proximal measurements. Within the multivariate model the correlations across the response variables of the by-participant intercepts were estimated (in brms "Wilkensen" notation the formula for each E/I measure was `response ~ 1 + ar(time, order = 1) + (1 | subjectR | subjectID)`). In this way we estimated simultaneously the between-subjects effects, in the form of the correlations between different response variables' by-participant intercepts, as well as within-subjects effects, in the form of the residual correlations remaining after accounting for the autoregressive and by-subject terms. We report these two correlations, both between-subjects and within-subjects, as our best estimate of the links between each measure. In addition, this model included an estimation of the remainder of the correlation matrix (e.g., between all EEG-E/I measures as well as estimates of Glx and GABA+); we report these correlations to determine the extent to which our EEG measures are isomorphic and are potentially more reflective of Glx or GABA+ variations (see Figure S1).

As tests of reliability, and in consideration of the possibility of overfitting our data, we augmented our BMVLMEM with a maximum-likelihood cross-validation approach. This approach involves iteratively and randomly partitioning the data into "training" and "test" sets, fitting a maximum-likelihood regression model predicting MRS-E/I to the training set, and assessing the R-squared (i.e., proportional reduction in sum of squared error) on the test set. Between-subjects and within-subjects analyses were necessarily implemented somewhat differently. Between-subjects cross-validation involved first calculating the subject-level means for each value, and then using simple random partitioning of this aggregated dataset into 80%/20% subsets, with data being fit with a bivariate robust linear model to 80% of the data and out-of-sample delta R-squared ($dRsq_{oos}$) assessed on the remaining 20%. In contrast, within-subjects cross-validation involved randomly selecting a single datapoint from each participant to keep as a test set, fitting a linear model including a participant covariate (i.e., to control for participant-level means in MRS-E/I) to the remaining training set, and then assessing the proportional change in out-of-sample squared error ($dRsq_{oos}$) when compared to a model with only the participant covariate as a predictor. To control for between-subjects variations in EEG-based E/I measures, for each of these within-subjects cross-validation models the EEG-E/I measure was first normalized within participants (i.e., Yeo-Johnson transformed to minimize univariate skew, and then z-scored). As will be seen, fairly large between-subjects effects are evident, which limits the amount of remaining variance left to be explained by within-subjects effects. We report the median $dRsq_{oos}$ of 500 resampled models for each EEG-E/I measure.

MRS-based E/I

MRS-based E/I is typically implemented by identifying a brain region of interest (e.g., early visual areas) and placing a single voxel in that region. Using magnetic resonance, the spectroscopic signal is then measured in that voxel over the course of several minutes and the relative concentrations of excitatory and inhibitory neurotransmitters are estimated (Shibata et al. 2017). These concentrations can then be examined separately or combined into a single measure of E/I. Using such methods it would be considered high spatial resolution to have a single voxel that is 15mm per side and a high temporal resolution to have one measurement every 2 minutes. The relative trade-offs of MRS measures of E/I are clear, then: While MRS-E/I is a relatively direct measure of actual molecules of interest within the brain, this measurement technique is resource-intensive, spatially and temporally imprecise, and the measured spectrum is itself a noisy measure. Indeed, the estimation of neurotransmitter concentrations has no consensus standard and results from a single MR sequence may vary depending on the estimation algorithm (e.g., inter-algorithm correlations between GABA concentration estimates ranging from below .1 to above .8; Craven et al. (2022).

Our primary results use the originally-published MRS data (Tamaki et al. (2020); Study 1), wherein a MEGA-PRESS sequence was used (1.25s TR; 2.2 × 2.2 × 2.2 cm voxel, placed on midline in early visual cortices; 3 Tesla Siemens scanner). We made two modifications to the data reported in the previous publication, namely, partial volume correction (PVC) to account for relative proportions of grey matter (i.e., to mitigate the influence of grey matter proportion on associations with EEG measures) and separation into 5-minute sections of time instead of 10-minute time sections (mean per participant = 13.1, sd = 3.6). As with the originally-published work, we used LCModel software (Provencher 1993) to identify Glx and GABA+ concentrations normalized to NAA. We then estimate MRS-E/I ratio by dividing Glx by GABA+. We chose to continue using LCModel to estimate Glx and GABA+, rather than other candidate softwares, for several reasons: To maintain congruence with the original findings, because reliable results in the original paper indicate that E/I from LCModel was reflecting true underlying cortical changes, and because LCModel has been found to provide the most “typical” estimates of neurotransmitter concentrations (i.e., highest correlation of GABA estimates with the median of estimates from other algorithms; Craven et al. (2022).

EEG-based measures of E/I

We assessed four approaches to inferring E/I ratio from EEG (i.e., EEG-E/I), with three of these approaches being separated into three canonical frequency bands, leading to 10 EEG-E/I measures total to test associations with MRS-E/I. The first, overall amplitude, is not a measure of E/I *per se*, but associations between MRS-E/I within specific frequency bands have been observed previously (e.g., theta and low beta; Tamaki et al. (2020)). Following amplitude, we assessed EEG-E/I derived from scale-free dynamics reflected by the 1/f power slope (Gao, Peterson, and Voytek 2017), an extension of this scale-free perspective that derives from criticality theory

(Bruining et al. 2020), and a set of estimates utilizing a neural mass model of excitatory and inhibatory dynamics (Yokoyama and Kitajo 2023).

For details of processing steps such as MR artifact rejection see Methods in Tamaki et al. (2020). In all analyses we report EEG measures estimated from the closest 7 electrodes to the MRS voxel (i.e., most posterior; Oz, O1, O2, PO3, PO4, & PO7; we replicated the overall pattern of our results when examining only Oz, O1, & O2). We applied each of the following analysis approaches to each 5-minute measurement period separately.

Frequency band amplitude

As one of the most basic measures identifiable within EEG signals, we included simple amplitude (i.e., square root of power) measures from several frequency bands. We used finite impulse response (FIR) bandpass filtering to isolate theta (5-8Hz), alpha (8-12Hz), and low beta (12-20Hz) bands, estimated the amplitude in 5-second windows (see section *EEG-E/I from criticality theory*), and then calculated the trimmed mean amplitude for each 5-minute measurement period (i.e., after removing the 5% lowest and 5% highest amplitude windows, calculating the arithmetic mean of the amplitudes within the remaining windows). We would not expect this approach to produce diverging results from, for example, simply averaging the amplitude across the entire 5-minute period.

While there have been some indications of links between frequency bands and E/I balance (Balz et al. 2016; Duncan, Wiebking, and Northoff 2014; Porjesz et al. 2002), we include amplitude here primarily as "a baseline" approach. That is, as an algorithmically simple derivation from resting-state EEG, we consider amplitudes to be valuable measures to assess overall data reliability (e.g., see section *Across-channel correlations*), test the divergence of EEG-E/I measures from amplitudes (see section *Associations among EEG measures*), as well as providing parsimonious reflections of MRS measures if the relevant correlations were to be found.

EEG-E/I from 1/f slope ("1/f")

Another approach derives from the widespread observation that neural activity, in temporal, spatial, and frequency domains, displays scale-free (power-law, or "1/f" when in the frequency domain) dynamics (Hahn et al. 2017; He 2014; Linkenkaer-Hansen et al. 2001). Computational derivations of the slopes of these 1/f distributions have been linked to cortical E/I balance (Gao, Peterson, and Voytek 2017; Lendner et al. 2020). As with previous work (Gao, Peterson, and Voytek 2017; Yokoyama and Kitajo 2023) we estimated the 1/f slope coefficient between 30Hz and 50Hz (bandpass filtered) for each window of interest; we implemented this approach with Python scripts available in conjunction with Yokoyama and Kitajo (2023). The following approach, based in criticality theory, is an elaboration of these principles of scale-free dynamics.

EEG-E/I from criticality theory ("fE/I")

A third approach is grounded in the computational biology framework of criticality, which characterizes the nature of many biological systems as existing at the cusp of state transitions (Bak 1999; Márton et al. 2014; Poil et al. 2012). One application of this theoretical approach is to neural criticality, which proposes that neural activity

exists approximately at a balance between excitation and inhibition in order to maximize the flexibility of macro-scale neural computations (Avramiea et al. 2022; Cocchi et al. 2017; Shew et al. 2011). To estimate E/I EEG, we use an algorithm designed to index excitation/inhibition ratio (Juarez Martinez et al. 2019; Ahmad et al. 2022), which is based in criticality theory and has been validated in computational, pharmacological (Bruining et al. 2020), and animal models (Kat et al. 2024). We applied this approach to the same three bandpass-filtered frequency bands we considered when examining simple amplitude measures. In brief, this algorithm first determines the degree of *criticality* (here, linked to the exponent of Detrended Fluctuation Analysis (DFA) Long-Range Temporal Correlations (LRTC)). This uses links between the window size of an analyzed signal and the variability in its cumulative detrended amplitude profile. Second, this degree of criticality is linked to the signal amplitude; in inhibitory-dominant regimes these two values should be positively correlated, while in excitation-dominant regimes they should be anticorrelated (Bruining et al. 2020). Using MATLAB code published in conjunction with Bruining et al. (2020), fE/I values were estimated for each of the above-defined frequency bands and then within- and between-subject correlations with MRS-E/I were estimated. As in the publication establishing the method, estimation of fE/I used a window size of 5 seconds with an inter-window overlap of .8 (Bruining et al. 2020); we also tested the effects of increasing the window sizes to 15 seconds and found minimal changes, and here report only 5-second window sizes. For each 5-minute measurement period we also estimated the DFA coefficient and excluded measurement periods with DFA below .6, as recommended by the developers of this approach (Bruining et al. 2020; Diachenko et al. 2024).

EEG-E/I from neural mass model ("NMM-E/I")

A fourth approach comes from a body of work instantiating neural mass models, that is, computational models of small-scale neural circuitry such as cortical columns; such models can be scaled up and can simulate complex dynamics and characterize dynamic and nonlinear features of neural activity (Cooray, Rosch, and Friston 2023; Jansen and Rit 1995). Yokoyama and Kitajo (2023) extended earlier neural mass models (Jansen and Rit 1995), which had been developed for purposes such as modeling epileptic dynamics, with a key insight: such models can include estimates of the relative activity of circuits' excitatory and inhibitory neural populations. Following this, and so they could compare to the 1/f approach described above, Yokoyama and Kitajo (2023) showed that the E/I ratio derived from such models better captured the fluctuating activity of humans' electrophysiological activity during sleep. This approach to estimating E/I ratio is quite new, however, and to our knowledge has not been widely assessed. We estimated this NMM-E/I using Python code available in conjunction with Yokoyama and Kitajo (2023) for each 5-minute measurement period, and for each of the canonical frequency bands described above (ie., theta, alpha, & low Beta). We also tested the NMM-E/I on a single wider set of frequencies, more similar to the approach implemented in Yokoyama and Kitajo (2023), on bandpass-filtered data between 5Hz and 20Hz (i.e., the lower and upper limits of the three frequency bands considered in the above analyses). The

resulting NMM-E/I values did not improve correpondence to MRS-E/I relative to the values estimated from distinct canonical frequency bands.

EEG-E/I reliability assessment

We implemented a cross-channel correlation approach to estimate a heuristic reliability of our EEG-based measures. Due to basic principles of volume conduction (Burle et al. 2015), as well as our intention to measure relatively large-scale variations in E/I, we expected highly homogeneous estimates of E/I across our measured channels. Because each of our EEG-E/I methods involved first estimating E/I for each channel we were able to, within each method, estimate the cross-channel correlations. We tested these correlations within subjects by finding the pairwise cross-channel partial rank correlations in estimated E/I values, partialling out participant-level intercepts. We then report the median cross-channel partial rank correlation. While we do not have a strict reliability criterion for these correlation measures, we believe that the relative cross-channel correlations should provide a general sense of the computational stability of the measures (see Figure S2).

Between-subjects variability relative to within-subjects variability

In light of differences in between-subjects relative to within-subjects correspondences between EEG-based and MRS-based measures of E/I, we sought to quantify the relative between-subjects and within-subjects variances for each measure. To determine between-subjects variances we first found each participant's median value for each measure, then calculated the variances of these values. To determine within-subjects variances we first calculated each participant's variance for each measure, then found the median of these variances. The ratio of between-subjects variance to within-subjects variances is reported in Figure S3.

Results

We first assessed data quality and excluded 5-minute windows in which at least one E/I measure demonstrated high noise relative to signal. MRS data quality was quantified using the ratio of the uncertainty of molecules' estimated concentration relative to the corresponding estimate; as in the originally published work, a 20% level of this "Cramer-Rao lower bound" was used for MRS exclusion. We also assessed EEG quality using the detrended fluctuation analysis (DFA) exponent coefficient criterion which Bruining et al. (2020) describe in conjunction with the fE/I method; this involves estimating the DFA coefficient and excluding windows in which the coefficient is less than .6. While the original sample included 18 participants' datasets, three participants had only three or fewer remaining MRS values and were excluded from further analysis. This left 15 participants data, with a mean number of 5-minute samples of 13.1.

Between-subjects associations between EEG measures and MRS-based E/I

Inter-individual differences in certain measures of EEG-E/I were correlated with MRS-E/I (see Figure 1). Each of the candidate algorithms produced E/I estimates with more than 5% of predictive variance explained in at least one frequency band (i.e., $dRsq_{oos} > .05$); we consider each of the links with positive $dRsq_{oos}$ values to be reliable. The criticality-based fE/I approach derived from alpha oscillatory activity was most closely linked to MRS-E/I across participants, followed by 1/f slope, then NMM-E/I derived from low beta activity, and then fE/I derived from theta band. All other measures, including overall amplitudes, were not reliably predictive of inter-individual differences in MRS-E/I

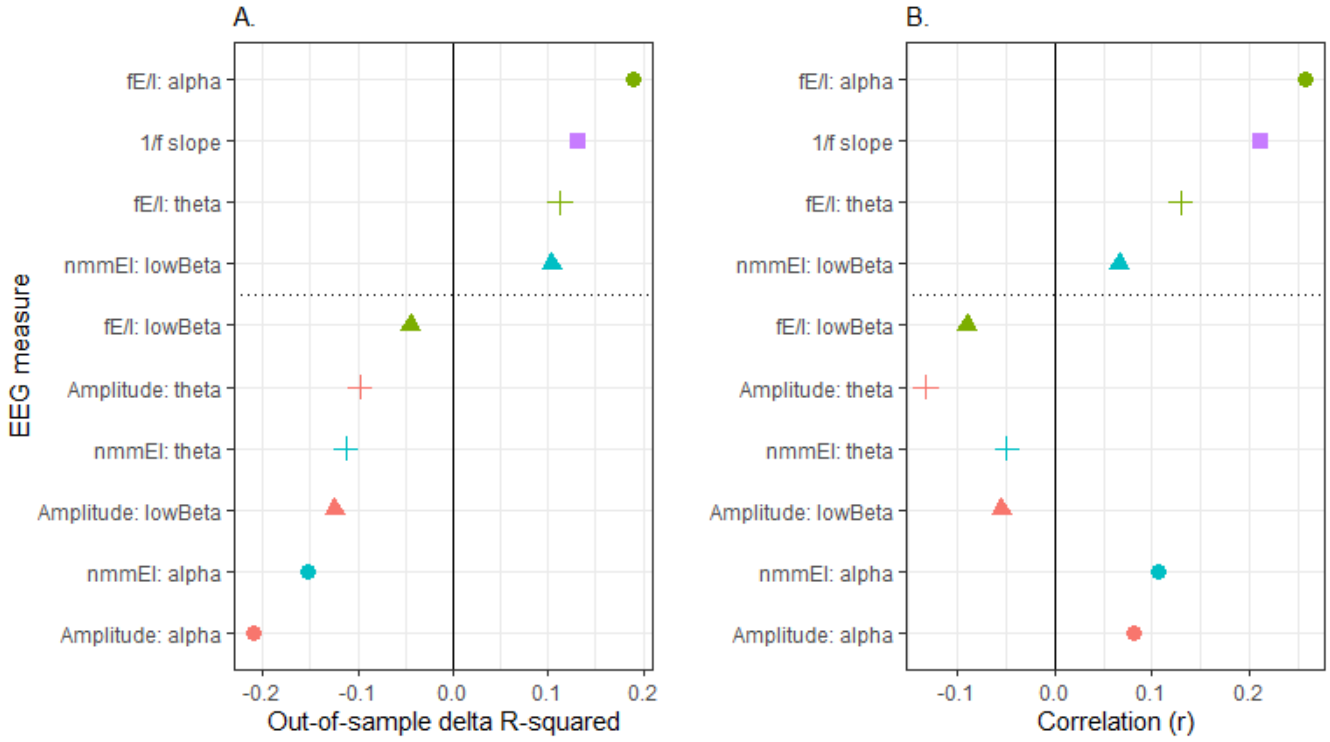

Figure 1. Between-subject associations between EEG-E/I measures and MRS-E/I. (A) Out-of-sample [cross-validated predictive] proportion of variance explained ($dRsq_{oos}$). (B) Correlation of by-participant random intercepts from the Bayesian multivariate mixed-effects model. Each panel is sorted by descending $dRsq_{oos}$. Dashed line separates variables explaining reliable out-of-sample variance with those that do not; we consider all associations above the dashed line to be reliable.

Within-subjects associations between EEG measures and MRS-based E/I

After controlling for participant-level effects, several approaches to EEG-E/I were reliably associated with MRS-E/I (i.e., $dRsq_{oos} > 0$; see Figure 2). While this wide range of EEG-E/I approaches providing within-subjects out-of-sample predictiveness for MRS-E/I is promising, a closer investigation of the effects tempers this promise somewhat. First, although out-of-sample variance explained was positive, it was quite small, with only simple

amplitude measures providing more than 1% of predictive value. Second, of the EEG measures with positive $dRsq_{oos}$ values, only theta amplitude had a positive correlation with MRS-E/I (r = 0.082). These results are in striking contrast to the between-subjects findings, where a relatively large amount of variance was explained with positive relations between EEG-E/I and MRS-E/I measures.

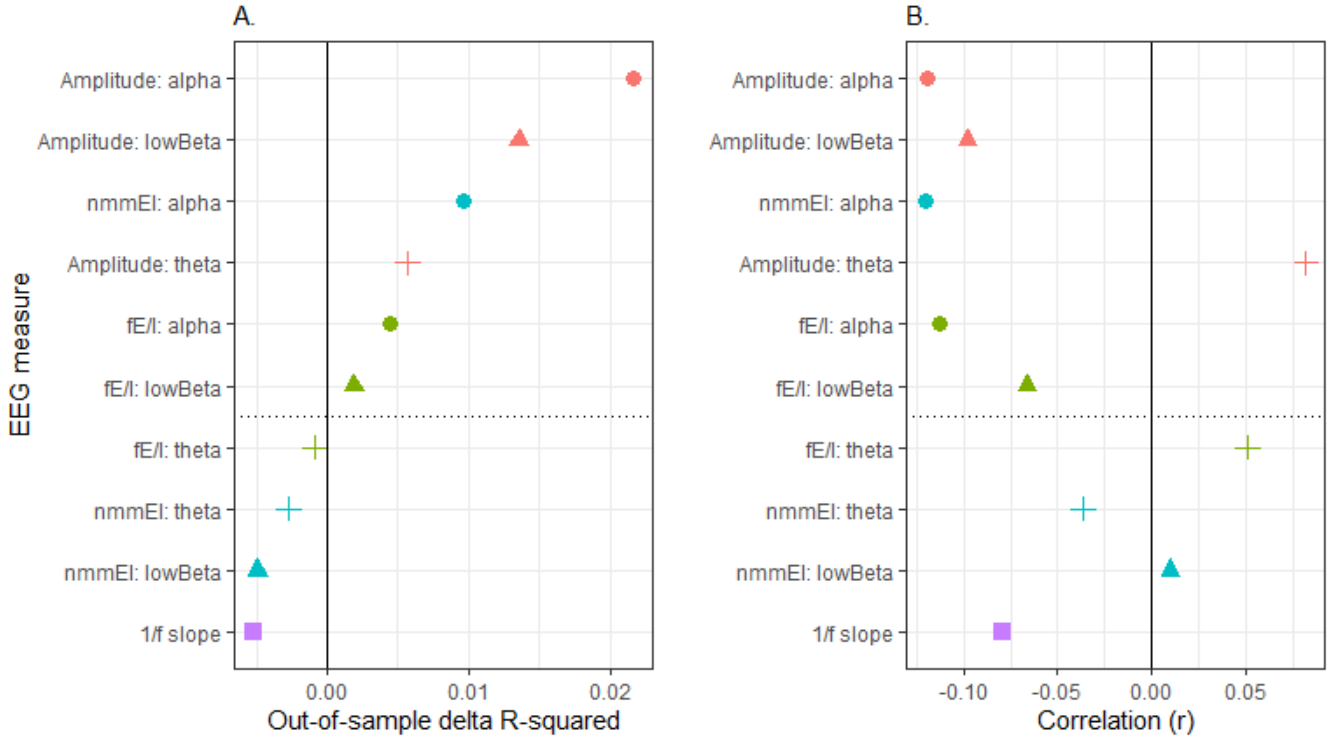

Figure 2. Within-subject associations between EEG-E/I measures and MRS-E/I. (A) Out-of-sample [cross-validated predictive] proportion of variance explained ($dRsq_{oos}$). (B) Correlation of by-participant random intercepts from the Bayesian multivariate mixed-effects model. Each panel is sorted by descending $dRsq_{oos}$. Dashed line indicates whether variables explained out-of-sample variance or did not; we consider all associations above the dashed line to be reliable.

Discussion

We have seen that EEG could be used to estimate E/I in a manner that is compatible with, but not wholly overlapping with, variations in E/I estimated from MRS. This mechanistic basis in the ratio between glutamate and GABA was true for several distinct approaches to EEG-E/I. In particular, EEG-E/I between-subjects was associated with MRS-E/I between-subjects, to the extent that they are comparable to (or even larger than) the correlations between MRS neurotransmitter concentrations found when comparing different estimation algorithms (Craven et al. 2022). In particular, the "functional E/I" algorithm derived from criticality theory was most closely associated with between-subjects differences in MRS-E/I; this reliance on temporal variations in alpha power

corroborates the work of the developers of the fE/I algorithm (Bruining et al. 2020; Diachenko et al. 2024). Beyond this criticality-based EEG-E/I measure, each of the other EEG-E/I approaches were also positively associated with between-subjects variations in MRS-E/I. These results showed broad support for each of the approaches, which were themselves not highly correlated (see Figure 3). Future work may benefit from combining these approaches to best characterize cortical E/I. The relative contribution of estimates from separate frequencies is of particular interest; the generative mechanisms underlying variations in theta, alpha, and beta rhythms provides the eletrophysiologist with a rich and multidimensional signal from which to infer shifts in cortical states. Indeed, independent frequency bands have been previously associated with glutamate and/or GABA levels (Porjesz et al. 2002; Tamaki et al. 2020), and a broad spectrum of frequencies showed links to MRS-E/I when analyzed with different algorithms in the current work (i.e., theta and alpha analyzed with fE/I; low beta analyzed with a neural mass model; gamma when calculating a 1/f slope). We believe that combining amplitude and E/I estimates from several frequency bands may provide additional precision, but we are hesitant to attempt such a combinatorial search within our limited dataset.

In contrast to between-subjects patterns of E/I balance, within-subjects associations between MRS-E/I and EEG-E/I were much weaker; indeed, only simple theta amplitude was reliably and positively associated with within-subjects variations in MRS-E/I. The reasons for the weakness of these associations cannot be known with certainty, but it is likely to arise partly to a decrease in signal strength and an increase in noise when compared to between-subject comparisons. That is, E/I is likely to vary a small amount within the course of 60-90 minutes of sleep, when compared to inter-individual differences in E/I, thereby limiting the magnitude of the variations we were able to observe. At the same time, estimating EEG-E/I as well as MRS-E/I on windows of only 5 minutes is likely to lead to relatively noisy estimates; while shorter measurement windows measuring GABA with MRS have been used in the past (Frank et al. 2022), longer windows (e.g., 10 minutes) are more standard. Unfortunately our available data is limited enough that estimating such within-subjects associations between EEG-E/I and MRS-E/I appears to be largely overwhelmed by the reduced signal-to-noise ratio; the MR context of these estimates may also have itself reduced data quality in estimating EEG-based E/I.

There may be alternative sources of divergences between EEG-based and MRS-based E/I ratios. On one hand, the neural mechanisms giving rise to each of our measured signals may be distinct; while MRS measured bulk concentrations of molecules in a relatively deep and spatially precise portion of posterior cortex, our EEG measurements were reflecting temporal variations in the activity of relatively superficial and widespread cortical neurons. Similarly, the causal determinants of oscillatory activities, and their variations over time, are not yet known with temporal or molecular precision; it may be the case that cell types, neuromodulators, or long-range connections are influencing EEG signals which would not be reflected in the measurement of Glx and GABA+ in a single voxel within posterior cortices (Ahmad et al. 2022).

Our results also reinforce the fact that the phenomena we are considering may be varying over time on fundamentally different timescales. One of the most notable functions of variations in excitation and inhibition are their changes over developmental time, that is, years or even decades (Hensch 2004; Takesian and Hensch 2013), which in contrast, cognition-related modulation of E/I would necessarily operate on sub-minute timescales (Hellyer et al. 2016). The methods we considered provide clear tendencies toward longer or shorter timescales, with MRS-based measures needing a minimum of five minutes, NMM-based measures providing estimates which are nominally relevant on the sub-second level, and the other methods falling somewhere in between. It may be possible, then, that these measures simply do not reflect underlying cortical changes that operate on similar timescales, leading to a lack of evident relations between them.

Robust links between EEG-E/I and MRS-E/I at a between-subjects level provides validation for EEG-based measures to be used in certain areas of research for which they have developed, namely, in investigating special populations such as individuals with ASD (Bruining et al. 2020), Alzheimer's disease (Nifterick et al. 2023), or monogenetic neurodevelopmental disorders (Geertjens et al. 2022). Our results indicate that the between-person differences in E/I revealed by contrasting these groups with healthy controls are likely to have common mechanistic bases whether they are measured using EEG or MRS. In contrast, for research which relies heavily on both within-person and between-person contrasts, such as the study of neuroplasticity (Shibata et al. 2017) or vigilance (Yang et al. 2023), EEG-E/I measures may not allow the assumption of mechanistic isomorphism.

While our results indicate that several recently developed algorithms for measuring E/I using EEG are indeed reflecting variations in the cortical ratio of glutamate to GABA, several limitations our present in our findings. The size of our dataset does not allow for precise estimates of effect sizes nor compelling rejection of candidate EEG-E/I measures, and the study of sleeping humans may lead to idiosyncratic results when contrasted to wakefulness; each of these limitations motivates future comparisons using concurrent multimodal imaging in awake humans. Further, with larger sample sizes, it would be possible to identify several additional useful aspects of links between EEG-E/I and neurotransmitter concentrations, such as functional nonlinearities or optimal combinations of EEG-E/I measures.

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Supplemental Material

Associations among EEG measures

In addition to our primary questions regarding links between EEG-E/I measures and MRS-based E/I, we additionally explored two additional questions. First, we assessed the extent to which different EEG-based measures of E/I were themselves collinear; second, we assessed the associations between each EEG-E/I measure was associated with either Glx or GABA+, as opposed to these molecules' ratio in the form of MRS-E/I. To assess these relations we extracted the by-participant random intercepts and the residual correlations from the Bayesian multivariate linear mixed-effects model (see Figure S1 see also Figures 1b, 2b).

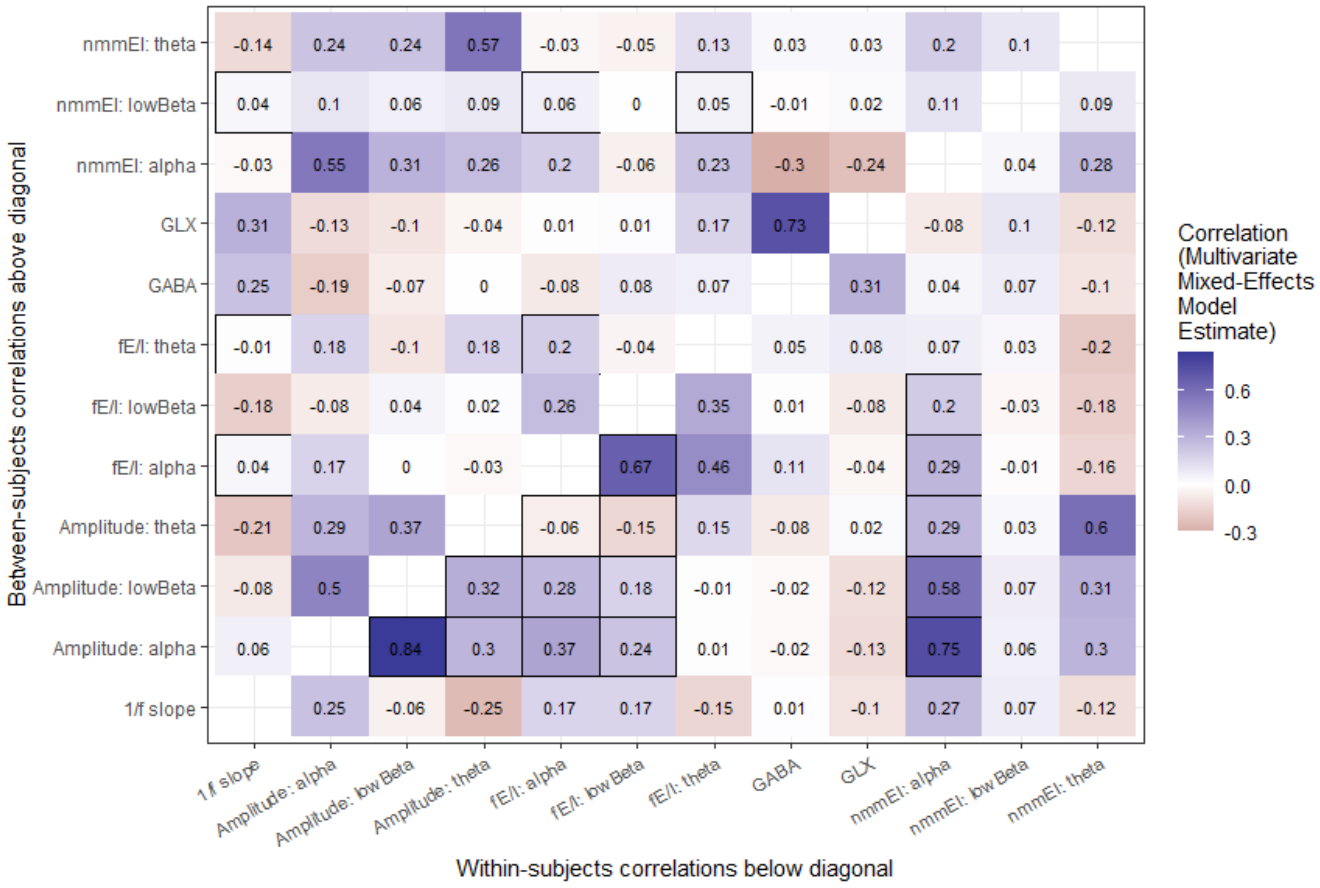

Figure S1. Correlations among measures apart from MRS-E/I (i.e., EEG measures as well as separated Glx and GABA+ concentration estimates). Above the diagonal are between-subjects correlations (see Figure 1b); below the diagonal are within-subjects correlations (see Figure 2b). Cells of EEG measures with reliable out-of-sample predictiveness of MRS-E/I are outlined. All estimates are drawn from a single multivariate mixed-effects model.

Across-channel correlations

One way to assess the reliability of EEG-based measures, which were each estimated separately for each channel, was to examine the correlations between the same measure across the posterior channels we analyzed. Due to our interest in relatively macro-scale changes in E/I, as well as the basic properties of volume conduction (Burle et al. 2015), we expected that our EEG-based E/I estimates would have highly positive correlations with one another; this would indicate that the underlying algorithms are computationally stable. For most measures we do observe the expected large positive correlations between channels, although the NMM-based estimates are much more weakly inter-correlated (See Figure S2). Broadly, then, we see that measures of amplitude, fE/I, and 1/f slope all demonstrate expected cross-channel correlations indicating reliability, while the low correlations of NMM measures may be due to true underlying ability to measure independent measures from nearby electrodes, or may be due to computational instability of the NMM approach; further investigation of this topic may be of interest for future research.

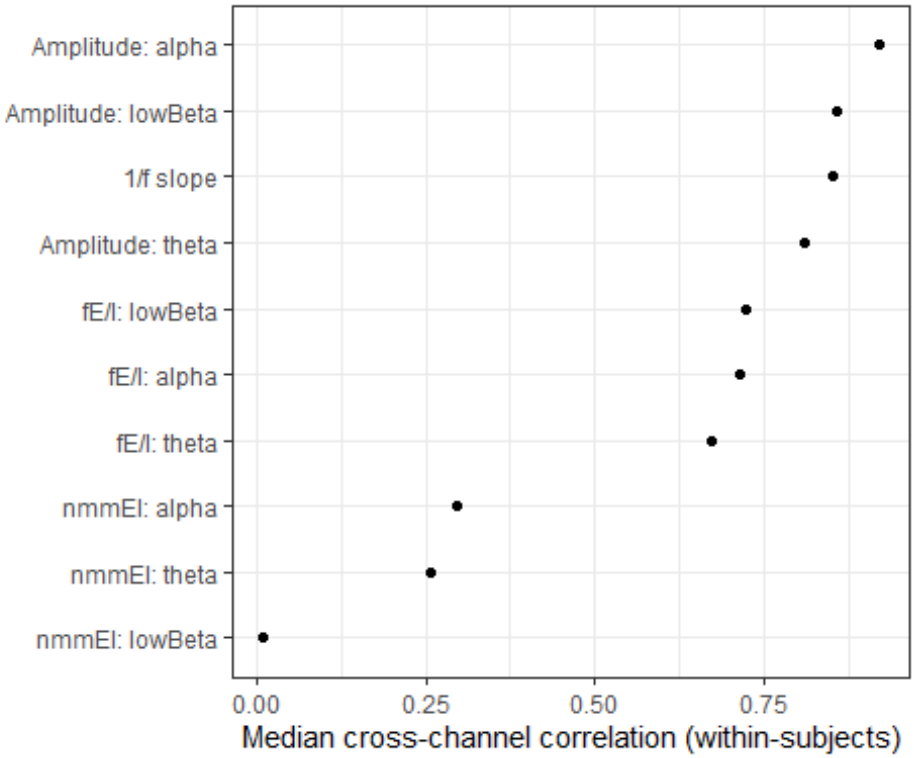

Figure S2. Median rank correlations between analyzed posterior channels' estimates of EEG-E/I. Most estimates were highly correlated across channels, although NMM-based estimates had relatively low cross-channel reliability.

Ratio of between-subjects to within-subjects variance

As a second exploration into the consistency of within-subjects estimates, which may influence the reliability of within-subjects E/I measures, we examined the ratio of between-subjects variance to within-subjects variance.

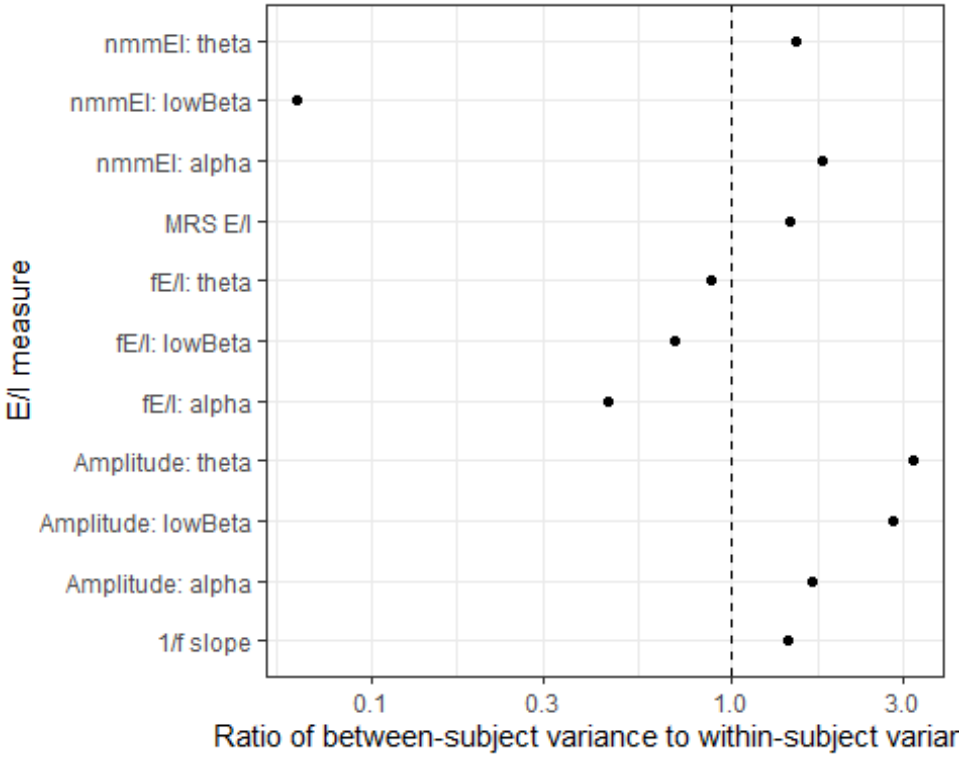

Figure S3. Ratios of between-subjects variances to within-subjects variances. Most measure had less than a 3:1 ratio of these variances, with MRS-based E/I having between-subjects variances approximately 1.45 times as large as within-subjects variances.