Bachelor’s degree in Human Biology

Final degree project

Pompeu Fabra University

Spontaneous Neuronal Activity is correlated to Statistical Learning performance.

Computation of ALFF and fALFF indices on resting-state fMRI.

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Abstract

Statistical learning (SL) is a mechanism that enables us to detect and learn probabilistic regularities and patterns from the environment. Previous studies have explored the role of SL in resting-state functional connectivity, but none of them has focused on spontaneous neuronal activity (SNA) and whether it can predict performance at a word segmentation task. Here we compute the functional segregation indices, ALFF and fALFF, on resting-state functional MRI (rs-fMRI) data and correlate them to statistical learning performance after listening to an artificial language stream. Our results show that there is a significant negative correlation between fALFF index and SL performance after a 4-minute exposure at bilateral temporo-occipital junction. This region seems to play a role in auditory attention and speech perception and, according to our results, is relevant for statistical learning when SNA is taken into account.

Keywords: statistical learning, spontaneous neuronal activity, amplitude of low frequency fluctuation, temporo-occipital junction

Tutor authorization

I, Miguel Burgaleta Díaz hereby authorize the presentation of this manuscript as the Final degree project of the student Sandra Sanahuja Irene for the degree in Human Biology of Pompeu Fabra University.

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Introduction

We live in an environment that is packed with statistical regularities and patterns and in order to survive it is necessary to learn and predict them. Interestingly, humans show a remarkable sensitivity to patterned regularities as a result of a cognitive mechanism called **statistical learning** (SL) (Oropella, 2019). This ability allows us to extract the statistical regularities from the world and learn about the environment that surrounds us. Evidence shows that statistical learning is present throughout the lifespan and that it is not only a human ability, but it is also manifested in other animals such as primates (Miyashita, 1988) or domestic chicks (Santolin et al., 2016). This ability has been demonstrated in different domains (temporal and spatial), modalities (auditory, visual, tactile) and a broad spectrum of stimuli such as syllables (Saffran et al., 1996), shapes (Fiser and Aslin, 2001), spatial locations (Mayr, 1996), melodies (Creel et al., 2004) or non-linguistic sounds (Gebhart et al., 2009).

Statistical learning has been studied mostly in the linguistic domain, especially in the auditory modality, where it has been proposed as a partial solution to the word segmentation problem. Unlike written text, in the speech there are not clear boundaries between words and SL allows to discretize the words that make up a continuous speech stream via complex computations (Sengupta et al., 2019; Oropella, 2019). The simplest computation is the estimation of **transitional probabilities** (TP)[1] between syllable sequences (Pelucchi et al., 2009), meaning the computation of the probability of two syllables being pronounced together on a speech. The transitional probabilities are usually higher for syllables within a word than in the boundaries between words. For instance, in the sentences *the baby is there*, *this baby cries a lot* and *look at that baby laughing*, the probability of the syllable *by* following the syllable *ba* is 1.0 (*ba* predicts *by*) but it is only 0.33 for the syllables *the* and *ba*, *this* and *ba* or *that* and *ba*. Thus, we can say that the words *theba*, *thisba* and *thatba* are far less probable than the word *baby*.

The computation of transitional probabilities is available from ages as young as 8 months, as was demonstrated by Saffran, Aslin and Newport (Saffran et al., 1996). Their study was one of the first studies to investigate statistical learning. They showed that babies are able to use the transitional probabilities between syllables to extract word-like units that form a continuous speech. They created an artificial speech, without the prosodic clues typical of the natural speech, made up by four nonsense trisyllabic words presented an equal amount of times and following each other with an equal probability.

[1] The transitional probabilities are described as Probability (Y|X) = Frecuency (XY) / Frequency (X)

Saffran et al. exposed 8-month-old babies to this artificial stream for 2 minutes and observed if the infants were able to distinguish the words from part-words (consisting of the last syllable of a word and the two first of the next word). Due to the design of the experiment, the probability of three syllables forming a part-word is 0.33, while the probability of forming a word is 1.00. The results showed that the infants were able to discriminate the words from the part-words, thus they had to be using the statistical regularities of the speech to learn the possible words (Saffran et al., 1996).

Several **functional magnetic resonance imaging** (fMRI) studies have shown that a wide neuronal network is activated while using statistical learning to segment words from a continuous speech. This network includes several language production and perception related areas, such as Broca's territory (inferior frontal gyrus, middle frontal gyrus and premotor areas) or Wernicke's territory (posterior superior temporal gyrus) (Cunillera et al., 2009; López-Barroso et al., 2013; McNealy et al., 2006), but also areas that activate in all statistical learning tasks (not only language-related SL tasks) such as the hippocampus, medial temporal lobe, basal ganglia, thalamus or the memory system (Frost et al., 2015).

These studies have provided information about the neural activity throughout specific tasks, but task-evoked brain activity does not take into account the role of the dynamic interactions between and within brain networks that take place in resting state. Hence, as important as it is to determine the brain areas that are active in the course of a task, it is to determine the intrinsic activity because, while task-related activity only accounts for a minimal part of all the energy consumption of the brain, the brain metabolism during resting state represents almost a 20% of the total body energy use (Raichle, 2006).

Functional MRI during rest (**resting state fMRI** or rs-fMRI) allows to calculate the blood-oxygen level-dependent (BOLD) large-amplitude spontaneous low-frequency (<0.1 Hz) fluctuations that are temporally correlated across functionally related areas, which are referred to as **functional connectivity** (FC)[2] (Biswal et al., 2010). Resting state functional connectivity (rsFC) reflects the intrinsic functional organization of the brain and it has been suggested to be an expression of the network behaviour underlying high level cognitive function. There are multiple methods to analyse rs-fMRI data to determine neuronal networks, but they all can be placed in two groups: functional integration methods and functional segregation methods. **Functional integration methods** focus on the functional connectivity between different brain areas, and only a few studies have

[2] Functional connectivity is defined as the temporal dependency of neuronal activation patterns of anatomically separated brain regions (van den Heuvel et al., 2010)

related functional connectivity and language acquisition (Yang et al., 2015) or statistical learning (Sengupta et al., 2019). These investigations suggest that language acquisition induces changes in the functional connectivity of brain areas related to language comprehension and production, memory and attention. Specifically, Sengupta et al. (2019) found out that listening to an artificial language changes the resting-state connectivity between the Dorsal Attention Network and the Default Mode Network, thus suggesting that attentional and working memory processes are involved in statistical learning mechanisms.

On the other hand, **functional segregation methods** focus on the local function of specific brain regions, relying on the analysis of rs-fMRI activity instead of the analysis of rs-fMRI connectivity (Lv et al., 2018). Crucially, to date the relationship between functional segregation indices and statistical learning performance has not been investigated. In this research we will tackle this issue by studying the potential association between statistical word learning and the **Amplitude of Low Frequency Fluctuations** (ALFF). This index, developed by Yu-Feng et al. (2007), measures the total power of the BOLD signal within the low-frequency range (0.01-0.08 Hz), which is proportional to regional neuronal activity (Lv et al., 2018) and has been suggested to correlate with the rate of regional glucose metabolism (Nugent et al., 2015). The **Fractional Amplitude of Low Frequency Fluctuations** (fALFF) method is a variant that measures the ratio of the power at the low frequency range (0.01-0.08 Hz) to that of the total power in the entire detectable frequency range (0-0.25 Hz) (Zou et al., 2008). Thus, these analyses measure the regional spontaneous brain activity at rest.

Project hypothesis

The aim of this project is to test, for the first time, whether there is a correlation between the performance on the statistical learning task and the ALFF and fALFF indices. We hypothesize that, should this relationship be significant, it would be observed in brain areas previously related with SL tasks, such as inferior frontal gyrus, left temporal cortex, medial temporal lobe, basal ganglia, left superior parietal lobule or right posterior cingulum (Frost et al., 2015; Sengupta et al., 2019)

Objectives

- Compute the correlation between the number of correct answers in a SL task and the selected functional segregation indices.
- Understand the concepts of ALFF and fALFF and what are the differences between them and functional connectivity.

- Learn how to use the Data Processing Assistant for Resting-State fMRI (DPARSF) to preprocess rs-fMRI data and compute the rs-fMRI indices.
- Implement ALFF and fALFF on the analysis of rs-fMRI data in statistical learning studies.

Methods

Participants

40 participants took part in this study. The mean age of the participants was 22.70 ± 4.01 and out of the 40 subjects 32 were females and 8 were males. All of them were right-handed and none of them reported any neurological or psychiatric disorder nor hearing or language disability. Participants were native speakers of Catalan and/or Spanish. Written consent was obtained from each participant and they were paid for their participation in the study. One participant had to be excluded because the fMRI scan presented artifacts. Thus, this study was performed with 39 participants in total (Sengupta et al., 2019).

Stimuli and procedure

The study is composed by two parts: a behavioural test and a resting-state functional magnetic resonance image acquisition. The order of behavioural testing and MRI acquisitions were compensated across participants. Before the experiment, participants were told that they would listen to an "alien language" and that they would be tested on the words that they were able to learn (Sengupta et al., 2019).

All participants listened to artificial language audio streams in the behavioural test, which were composed by four tri-syllabic words pseudo-randomly concatenated (consecutive repetition of the same word was not allowed). The transitional probabilities of this artificial language were 1 within words and 0.33 between words (Sengupta et al., 2019).

All the syllables used in the artificial languages were different and were created according to the principles used by previous statistical learning studies (Aslin et al., 1998; Saffran et al., 1996). The words were synthetized with the speech synthesizer software MBROLA (Dutoit et al., 1996) based on the concatenation of diphones at 16 kHz from the Spanish male database (es2) and they were 696 ms long. None of the artificial words or part-words were Spanish or Catalan words (Sengupta et al., 2019).

Behavioral testing

The design of the behavioural test allowed to estimate statistical learning. Participants sat in a comfortable armchair in a sound-attenuated room while they listened to two counterbalanced artificial language streams. One of the streams lasted 2 minutes and the other was 4 minutes long, without breaks or pauses. After listening to each stream, subjects were asked to perform a two-alternative forced choice test. The two different speech lengths were used to check if there was an observable improvement with more exposition to the artificial language. Participants had to choose which one of the two options presented was a word of the artificial language by pressing a button (Sengupta et al., 2019).

MRI acquisition

Participants were instructed to rest with their eyes closed and not to think in anything and not to sleep. The acquisition of the images was made in a GE 1.5 T scanner using a gradient-echo T2*-weighted echoplanar imaging sequence in the axial plane (TR, 2000 ms; 50 ms; matrix 64x64; voxel size 3.75x3.75 mm; flip angle, 90º; slice thickness, 4 mm; FOV = 240) and 120 volumes. Additionally, a 142-slice, 3D, spoiled gradient-recalled acquisition sequence was obtained in the sagittal plane (TR = 1.33 ms, TE = 3.3 ms, inversion time = 600 ms, flip angle = 10º, FOV 260, matrix size 256x256, in-plane resolution 0.98 mm^2, slice thickness 1.2 mm) (Sengupta et al., 2019) (Fig. 2a).

Image preprocessing

All rs-fMRI data was preprocessed using the Data Processing Assistant for Resting-State fMRI or DPARSF (Chao-Gan and Yu-Feng 2010, http://rfmri.org/DPARSF) (Fig. 1), which is based in some functions in Statistical Parametric Mapping (SPM) (http://www.fil.ion.ucl.ac.uk/spm) and Resting-State fMRI Data Analysis Toolkit (REST) (Song et al., 2011, http://www.restfmri.net). The process of preprocessing included (a) the removal of the first four time-points to ensure signal stabilization (leaving a total of 116 volumes for final analysis), (b) slice timing correction, (c) head motion correction of the functional volumes of each subject using a six-parameter (rigid-body) linear transformation with a two-pass procedure, (d) spatial normalization to the MNI standard space (Fig. 2b and 2c), (e) spatial smoothing with an isotropic Gaussian kernel of 4mm full-width at half-maximum (FWHM), (f) temporally bandpass filtering (0.01-0.08 Hz) to reduce the effect of low-frequency drift and high-frequency noise (Biswal et al., 1995; Lowe et al., 1998). Further preprocessing steps included the removal of sources of spurious variance through linear regression such as six parameters from rigid body correction of head motion, global mean signal and white matter and CSF signals (Sengupta et al., 2019).

Nuisance regression

Micro-head movements between time points can induce artifactual inter-individual and inter-group variability in rs-fMRI measures (Power et al. 2012a, Power et al., 2012b; Satterthwaite et al. 2012; Van Dijk et al. 2012). Recent work has reported that higher-order models demonstrate benefits in removing head-motion effects (Satterthwaite et al. 2013; Yan et al., 2013) so, in this project, Friston 24-parameter model (Friston et al., 1996) was used to regress out head motion effects from the realigned data. Head motion was also controlled at the group-level by taking mean framewise displacement as a covariate (Satterthwaite et al. 2013; Yan et al., 2013). White matter and cerebrospinal fluid signals were regressed out to reduce cardiac and respiratory effects. Linear and quadratic trends were also included as regressors because BOLD signal demonstrates low-frequency drifts (Yan et al., 2016)

rs-fMRI indices: ALFF and fALFF calculation

The ALFF images were computed by extracting power spectra via a Fast Fourier Transformation of the time series for each voxel and calculating the sum of amplitudes in the low-frequency bands (0.01 – 0.08 Hz). The ALFF measure at each voxel represents the averages square root of the power across 0.01 - 0.08 Hz. Normalization is made by dividing the ALFF of each voxel by the individual mean within-brain ALFF value (Fig. 3a). To compute the fALFF images, a ratio of the power at the low-frequency range to that of the entire frequency range (0 – 0.25 Hz) was performed (Fig 3b). Finally, both ALFF and fALFF images were spatial smoothed with an isotropic Gaussian kernel of 8 mm of full-width at half-maximum (FWHM) (Bu et al., 2019; Zou et al., 2008).

Statistical analysis

Pearson's R statistics for the association between SL performance and ALFF/ fALFF were computed voxelwise while controlling for potential effects of age and gender. Analyses were carried out via permutation-based inference (Nichols and Holmes, 2002), implemented using the randomise tool of the FSL package (FMRIB, Oxford, UK; http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise). The Threshold-Free Cluster Enhancement algorithm (Smith and Nichols, 2009) was applied to detect clusterwise statistical signal while avoiding the setting of arbitrary cluster-forming thresholds. Resulting statistical maps were thresholded at $p < 0.05$ corrected for multiple comparisons (family-wise error rate below 5%).

Results

Our results reveal a significant negative correlation ($p < 0.05$) between fALFF and statistical learning performance after 4 min of exposure to an artificial language significant in right and left temporo-occipital junctions (Fig. 4). This correlation was not found between fALFF and performance after 2 min of exposure.

Pearson's R test did not show significant correlation between ALFF and SL performance at either 2 or 4 min of artificial language exposure.

Discussion

To our knowledge, this is the first study to investigate the potential relationship between statistical word learning and the resting-state fMRI indices Amplitude of Low Frequency Fluctuations (ALFF) and fractional Amplitude of Low Frequency Fluctuations (fALFF). Many studies have demonstrated that the Low Frequency Fluctuations (LFFs) of BOLD rs-fMRI signal are closely related to the spontaneous neuronal activities (SNA) (Goldman et al., 2002; Logothetis et al., 2001; Lu et al., 2007; Mantini et al., 2007). and that they are related to the rate of regional glucose consumption, making ALFF more sensitive to changes in regional brain activity than other methods of rs-fMRI analysis.

Although functional connectivity analysis can provide us with more holistic information of a set of brain regions within a network, it does not tell whether there is a change in the spontaneous brain activity or where this change is located. The advantage of ALFF and fALFF methods also lies in the simplicity of the analysis without any underlying hypothesis, enabling the exploration of spontaneous brain activity on a whole-brain scale without the need of defining an *a priori* seed region, thus allowing to avoid the bias that may be produced (Lv et al., 2018).

Our results show a significant negative correlation between fALFF at bilateral temporo-occipital junctions (TOJ) and performance in a SL task. fALFF selectively suppresses artifacts from nonspecific brain areas while enhancing signals from cortical regions associated with brain activity, thus improving the sensitivity and specificity in detecting spontaneous activity when compared with ALFF (Zou et al., 2008). Interestingly, we did not observe any significant correlation in regions typically found to activate in statistical learning tasks (inferior frontal gyrus, left temporal cortex, medial temporal lobe, basal ganglia, left superior parietal lobule, right posterior cingulum; Frost et al., 2015; Sengupta et al., 2019).

TOJ has been demonstrated to be involved in different cognitive abilities, including language and visual perception and even though its role is not consistently evident across word segmentation studies, this area has been reported to be active in several investigations regarding statistical learning (e.g. Sandoval et al., 2017; Karuza et al., 2013). Plante et al. (2015) suggested that this area has a function in auditory attention; as a matter of fact, activity in TOJ has been posited to rise as the stimulus diverges from familiar material and more listener resources are required, meaning that activation will be greater when listening to part-words than words of the artificial language (Clark and Wagner.,2003).

Hickok and Poeppel (2000) also claimed that temporo-occipital junctions and its surroundings are involved in the ventral pathway of speech perception, which is important for interfacing sound-based representations of speech.

In addition, Ardilla et al. (2015) demonstrated that the temporo-occipital junction participates in a language system that is related to complex language processing and understanding and that it is structurally and functionally connected with the inferior frontal gyrus (Broca's territory), premotor cortex (involved in speech perception) and inferior temporal gyrus (involved in speech perception and auditory attention) among other language-related areas.

Therefore, and in light of previous evidence, here we suggest that temporo-occipital junction is a highly connected area that plays a role in speech perception and auditory attention, and that it seems to be relevant for statistical learning when its spontaneous activity is taken into consideration. Future studies should further investigate the specific role of low frequency fluctuations in SL-related brain function.

Conclusion

In this project we studied for the first time the relationship between fALFF index and statistical learning performance. The results showed that there is a negative significant correlation between fALFF and the number of correct answers in the behavioural test in the temporo-occipital junction, bilaterally.

This study presents a new approach to predict performance in cognitive tasks and also to study statistical learning not from the functional connectivity, but from the spontaneous neuronal activity perspective.

Figures

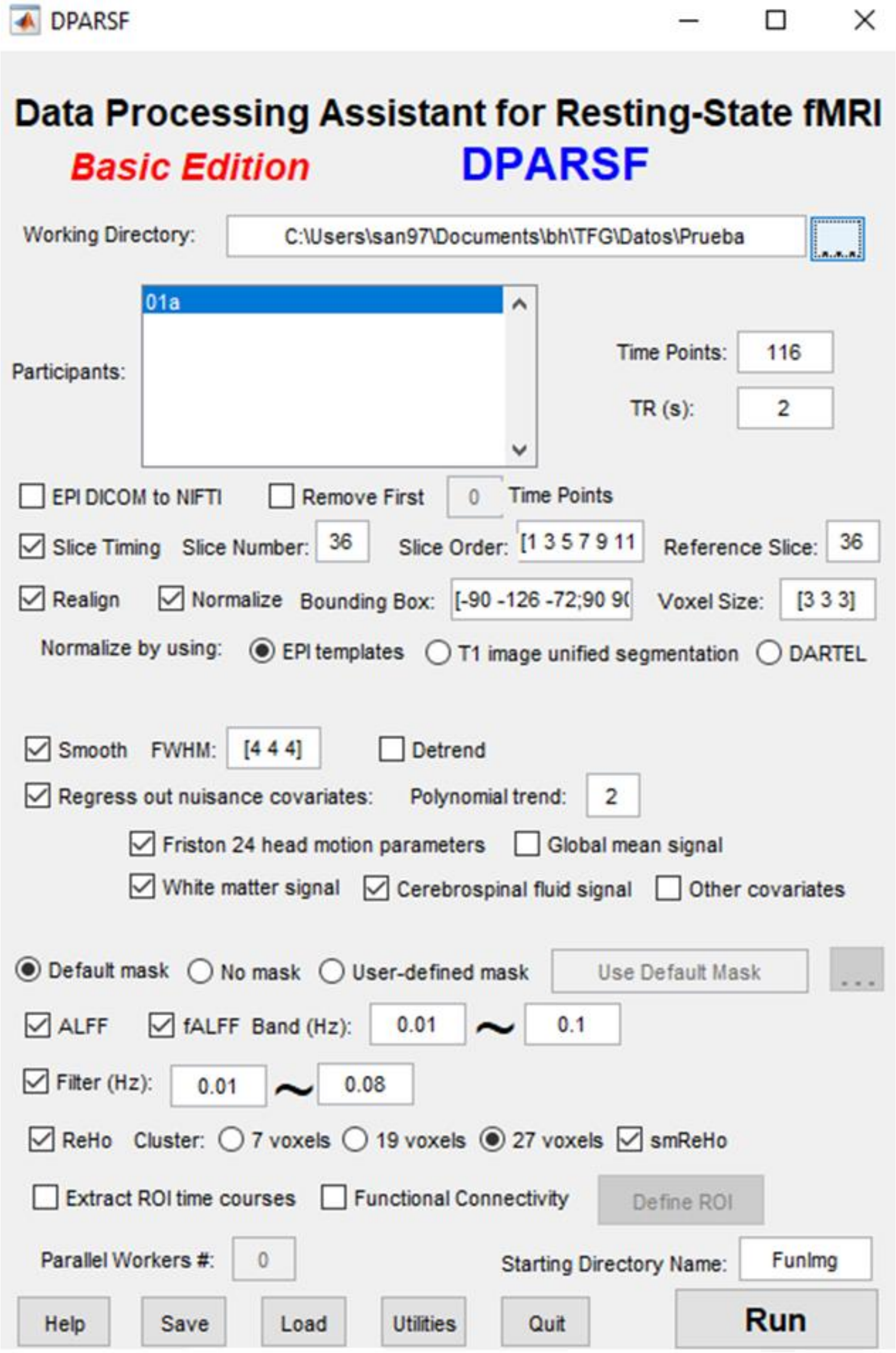

Fig 1. Settings of DPARSF used to analyse rs-fMRI images.

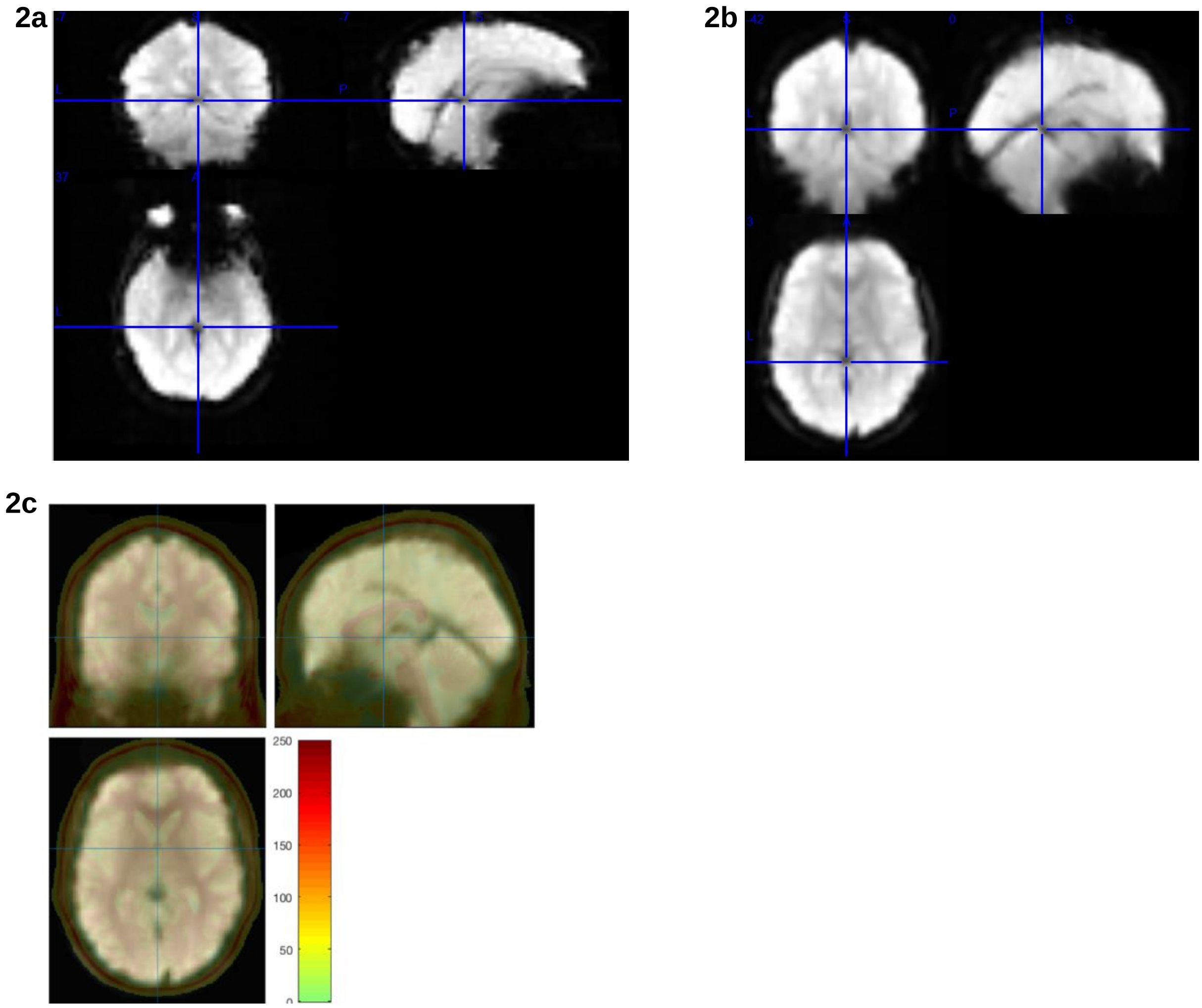

Fig 2. Representation of preprocessing steps. (a) rs-fMRI image without preprocessing. (b) rs-fMRI image after normalization to the MNI space. (c) Normalization quality control.

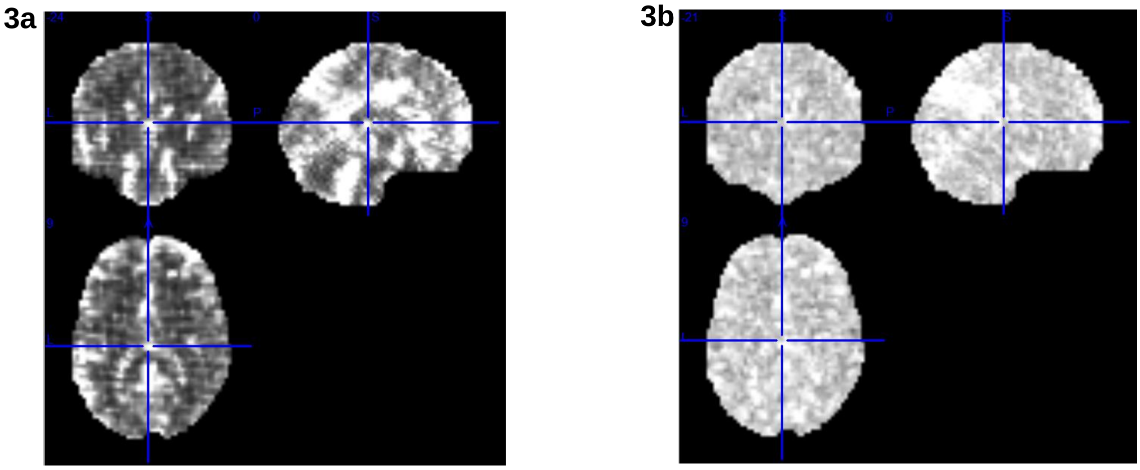

Fig 3. Computation of rs-fMRI indices. (a) ALFF. (b) fALFF.

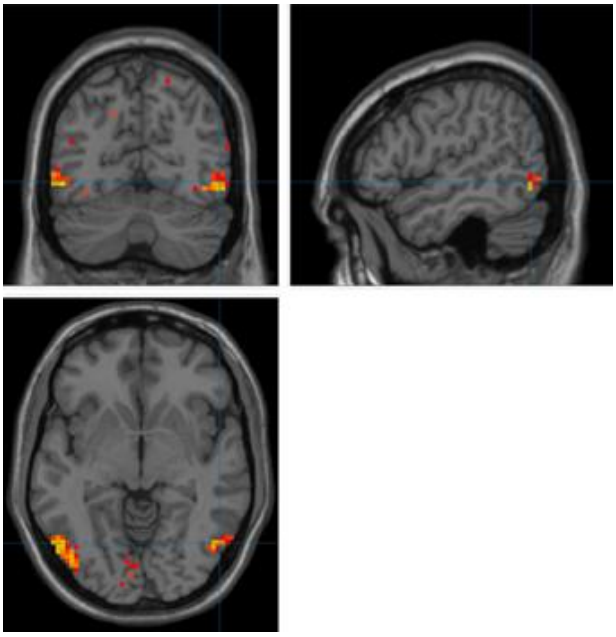

Fig. 4. fALFF at bilateral temporo-occipital junction shows a significant negative correlation with SL performance after 4 min of exposure to an artificial language.

Conflict of interest

I declare that I have no conflict of interest.

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