

TITLE

The dynamic imprint of word learning on the dorsal language pathway

AUTHORS

María-Ángeles Palomar-García¹, Ana Sanjuán², Elisenda Bueichekú¹, Noelia Ventura-Campos¹, and César Ávila¹.

INSTITUTIONAL AFFILIATIONS

¹Neuropsychology and Functional Neuroimaging Group, Department of Basic Psychology, Clinical Psychology and Psychobiology. University Jaume I. 12071 Castellón. Spain.

²Computational Neuroscience Group, University Pompeu Fabra, Barcelona, Spain

AUTHOR RESPONSIBLE FOR CORRESPONDENCE

Name: César Ávila

Address:

Basic Psychology, Clinical Psychology and Psychobiology. Universitat Jaume I.

Avda. Sos Baynat, s/n. E-12071.

Castellón de la Plana, Spain.

E-mail: avila@psb.uji.es

Telephone +34964387662

Abstract

According to Hickok and Poeppel (2007), the acquisition of new vocabulary rests on the dorsal language pathway connecting auditory and motor areas. The present study tested this hypothesis longitudinally by measuring BOLD signal changes during a verbal repetition task and modulation of resting state functional connectivity (rs-FC) in the dorsal stream. Thirty-five healthy participants, divided into trained and control groups, completed fMRI sessions on days 1, 10, and 24. Between days 1 and 10, the trained group learned 84 new pseudowords associated with 84 native words. Task-related fMRI results showed a reduced activity in the IFG and STG while processing the learned vocabulary after training, returning to initial values two weeks later. Moreover, rs-fMRI analysis showed stronger rs-FC between the IFG and STG in the trained group than in the control group after learning, especially on day 24. These neural changes were more evident in participants with a larger vocabulary. Discussion focuses on the prominent role of the dorsal stream in vocabulary acquisition. Even when their meaning was known, newly learned words were again processed through the dorsal stream two weeks after learning, with the increase in rs-FC between auditory and motor areas being a relevant long-term imprint of vocabulary learning.

Keywords:

Brain plasticity; Intrinsic activity; Word learning; Dorsal stream; Longitudinal study.

1. Introduction

Vocabulary acquisition is especially relevant in native language development during infancy, but it is maintained at a lower rate throughout life. This process is also important for learners of a second language because they have to incorporate new words that are associated with words in their native language. Studies on the neural basis of vocabulary learning have been devoted to unveiling the keys to how these words are processed in the language network (Mestres-Missé et al. 2008; Davis et al. 2009; Hultén et al. 2010; Raboyeau et al. 2010). The aim of the present study was to longitudinally investigate the neural basis of vocabulary acquisition and the imprint this learning may leave on the language network at long-term during task performance and at rest.

The dual stream model of language processing hypothesizes the existence of two functionally distinct streams connecting posterior and anterior speech/language areas (Hickok and Poeppel 2007). The dorsal stream connects auditory areas in the superior temporal gyrus with motor areas in the premotor cortex and inferior frontal gyrus, and it is responsible for translating acoustic-based representations of speech signals into articulatory representations. The ventral stream involves different areas of the left and right superior, middle, and inferior temporal gyrus, and its function is related to processing the conceptual meaning of speech. According to the dual-route model, the dorsal stream is crucial for new vocabulary acquisition because it involves the generation of a sensory representation of the new word that codes the sequence of segments or syllables, and this representation guides motor articulatory sequences (Hickok and Poeppel 2007; Hickok 2012). However, this proposal does not specify how these acquired words are processed after learning their meaning and increasing their familiarity.

Auditory and motor areas communicate directly through the arcuate fasciculus (AF), a prominent white-matter tract proposed as participating in audiomotor processing in language

and music (Saur et al. 2008, 2010; Halwani et al. 2011). Saur et al. (Saur et al. 2008, 2010) reported that repetition of pseudowords compared to real words, activated the left anterior and posterior parts of the superior temporal region, along with frontal regions such as the left pars opercularis of the inferior frontal gyrus and the premotor areas (BA 44/6). In the same way, in the meta-analysis conducted by Davis and Gaskell (2009), phonological processing during unknown pseudoword processing was related to activation in the STG and opercular frontal areas, whereas processing of existing words activated more anterior, posterior and inferior regions of the lateral temporal lobe and inferior parietal regions, along with the pars orbitalis of the frontal lobe, areas related to lexical processing. Consistently with this framework, some studies have reported that activity in the inferior frontal and superior temporal cortex was related to new vocabulary acquisition (Raboyeau et al. 2004; Grönholm et al. 2005; Mestres- Missé et al. 2008; Hultén et al. 2009). Importantly, the enhancement of activity in these areas after training has been related to successful retrieval of this material 10 months later (Hultén et al. 2010).

Learning new vocabulary involves acquiring novel sensorimotor patterns that are likely to result in structural differences as well as functional brain changes. In keeping with this idea, a recent study by López-Barroso et al. (2013) combined tractography and fMRI to study whether the strength of anatomical and functional connectivity between auditory areas of the temporal lobe and Broca's regions was associated with new word learning ability (i.e. learning words of an artificial language through repetition). They found that microstructural properties and the strength of the functional connectivity between these regions in the left hemisphere correlated with word learning performance, which suggests that the human ability to learn new words relies on efficient and fast communication between these regions.

Brain connectivity can also be studied by means of resting-state functional magnetic resonance imaging (rs-fMRI), which allows us to measure the spontaneous activity of the

brain at rest, characterized by the co-activation of anatomically separate but functionally related brain regions. Previous studies have shown that the intensity of correlations within and between brain areas at rest has behavioral significance (Harmelech and Malach 2013; Guerra- Carrillo et al. 2014; Finn et al. 2015), and that the resting-state activity may reflect the repeated history of co-activation within or between brain regions, which may in turn be a predictor of individual differences while performing perceptual, intelligence, and memory tasks (Lewis et al. 2009; Baldassarre et al. 2012; Cole et al. 2012; Ventura-Campos et al. 2013; Bueichekú et al. 2015). Changes in rs-fMRI connectivity have also been reported in cross-sectional studies comparing effects of expertise, such as in experienced meditators (Taylor et al. 2013) and musicians (Palomar-García et al. 2016). Thus, functional connectivity at rest (rs-FC) may reflect the impact of brain plasticity on the brain after learning and be used as a complement to task-related fMRI in highlighting practice-related functional changes in the brain.

The aim of the present study was to use task-related and resting-state fMRI to investigate the changes in BOLD signal magnitude and functional connectivity (respectively) associated with word learning in three different time windows: at baseline, immediately after one week of learning, and two weeks after learning. We designed the experiment to ensure overlearning of all the new material, in order to obtain high and similar recall scores at both post-training time points of neural testing. A longer interval for the retest would imply lower recall and a less comparable situation. According to the dual route model, learning new vocabulary should be associated with increased participation of the dorsal stream including the left STG and left opercular IFG/premotor areas, as well as their audiomotor connectivity throughout the arcuate fasciculus. Consequently, training would be expected to not only affect these regions separately, but also to produce stable changes in the functional connectivity between the auditory and motor regions. In the present study, we used a verbal repetition task

and rs-fMRI at different time points to investigate the dynamic changes in neural activation due to learning the meaning and articulatory representation of new words. We hypothesized that: 1) auditory and premotor regions would be the main areas involved in pseudoword processing at baseline; 2) acquiring new words and their meanings would modify their processing in these areas; 3) this training would also impact on the functional connectivity between these areas during rest; and 4) these neural measures would be related to the measures of vocabulary size.

2. Materials and methods

2.1. - Participants

Thirty-nine Spanish speakers participated in the study. Four participants were excluded from the analyses due to excessive head movements (more than 2 mm of translation or 2 degrees of rotation) during one of the three fMRI acquisitions. The final sample consisted of thirty-five Spanish speakers. Eighteen participants formed the trained group (9 women; mean age 19.9 years old, standard deviation (SD); ± 1.16) and 17 were included in the control group (12 women; mean age $20 \pm .87$ years old). All participants were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield 1971). Intellectual level was evaluated with the Vocabulary subtest of the WAIS-III (trained group: mean 12.1 ± 1.4 ; control group: mean 11.5 ± 1.5) and the block design subtests (trained group: mean 11.4 ± 3.03 ; control group: mean 11 ± 2.8). There were no significant between-group differences in age or on general intellectual functioning measures ($p > 0.10$). None of them had suffered any neurological or psychiatric disorders, and they had no history of head injury with loss of consciousness. Written informed consent was obtained from all participants, and they received monetary compensation for their participation. This research was approved by the Ethics Committee of the University Jaume I.

2.2. - Experiment overview

To obtain a longitudinal perspective, the experiment consisted of three identical MRI scan sessions. Session 1 (S1) on day 1 was held before learning; Session 2 (S2) was held on day 10, after six 1-hour learning sessions in the trained group and after a no-training period in the control group; and Session 3 (S3) took place on day 24, after a two-week period with no-training. After S2 and S3, all the participants of the trained group performed cued-recall retention tests (see **Fig. 1** for the experimental overview).

2.3. - Stimuli

One-hundred and sixty-eight Spanish words were selected from the Snodgrass and Vanderwart picture database (Snodgrass and Vanderwart 1980). Pseudowords were created by recombining the syllables of the Spanish words and the pronunciation was adjusted to conform to Spanish phonology. To make sure that the pseudowords were not similar to existing words in Spanish, Catalan or English, we created a questionnaire to assess similarity and administered it to 10 native Spanish speakers. Any pseudoword that was similar to an existing word was replaced and evaluated again. In the final sample, there were no differences in syllable length between words and pseudowords.

After the pseudowords had been created, they were divided into two sets of 42 stimuli and combined with words to obtain two sets of 84 word-pseudoword pairs (see Supplementary Table I). One set of pseudowords was used for training and the other for a control conditions (i.e. untrained pseudowords) in the fMRI task. The pseudoword sets were

counter-balanced across trained participants (i.e. nine participants were trained with one set and nine with the other set). The two sets were matched on word length in terms of number of phonemes and syllables. The Spanish words in the two sets, which were the same ones associated with the different pseudowords in each group, were used for the learning condition, and another pool of 42 words was used as a control condition for the fMRI task (i.e. untrained words). The Spanish words were matched in terms of word frequency, age of acquisition, and semantic category (half were manufactured objects and the other half were natural). All 336 stimuli were recorded digitally by a female-speaker.

2.4. - Learning phase (only the trained group)

Participants were taught 84 new pseudowords associated with the corresponding Spanish words during six 1-hour sessions held between the first and second fMRI sessions.

Vocabulary learning was implemented in a computer using a self-paced method presenting pseudoword-word pairs, which was programmed with the Inquisit software (Neurobehavioral Systems, Inc., Albany, CA, USA). The training program included the auditory and visual presentation of 84 pairs, including 12 different blocks of 7 pseudoword- word pairs that were repeated twice consecutively. Each pseudoword-word pairs lasted for approximately 500 ms. At the end of each training block, there was a cued-recall test with feedback. In other words, a Spanish word was presented and the participant had to recall the associated pseudoword. After the participant's response, the correct response was presented. At the end of each training session and also after the scanning session in the second and third fMRI sessions, participants performed a cued-recall test to evaluate their progression (see Supplementary **Fig. 1**).

2.5. - Experimental task and procedure

The same fMRI protocol was used for the three fMRI sessions. They consisted of a resting state in which participants were instructed to simply rest with their eyes closed and not sleep or think about anything in particular. This period was followed by the overt verbal- repetition task with their eyes closed. During the task, participants listened to words and pseudowords, and they were instructed to repeat them aloud.

The stimuli were presented according to a block design paradigm with 4 conditions: 1) trained pseudowords: pseudowords associated with Spanish words during training; 2) untrained pseudowords: pseudowords not associated with Spanish words; 3) trained words: Spanish words associated with pseudowords during training; and 4) untrained words: Spanish words not associated during training. Note that the pseudowords used in the control condition differed not only in their lack of a semantic content, but also in familiarity. This was done intentionally in order to simulate vocabulary-learning conditions, which means learning new articulatory representations and meanings after a repeated use, a process that does not occur with unknown words.

For each of the conditions, there were 7 blocks with 6 stimuli per block, or a total of 42 stimuli per condition. From our two sets of 84 word-pseudoword pairs, we used one set of 42 pseudowords for the control conditions (i.e. untrained pseudowords), and the other set for the training condition (i.e. trained pseudowords). The Spanish words associated with the pseudowords during training were used for the trained conditions, and the other pool of 42 words was used for the untrained conditions. To avoid lexical conflict across conditions, the two components of a pseudoword-word pair were never presented together in the same task. For example, within the training conditions, the 42 pseudowords presented in condition 1 (i.e. trained pseudowords) never belonged to the same pair as the 42 words presented in condition

3 (i.e. trained words). The presentation of all conditions was counterbalanced. Participants had to repeat the stimuli aloud and exactly as they heard them.

The task was programmed using the Presentation software (Neurobehavioral System, Inc., Albany, CA). Auditory stimuli were presented through fMRI compatible headphones (VisuaStim, Resonance Technology, Inc., Northridge, CA, USA). A compatible microphone, with a noise cancellation system, was also used to record participants' verbal responses. Each auditory stimulus lasted for approximately 600 ms, with an inter-trial interval (ITI) of 3 seconds. The total duration of each block was, therefore, 18 seconds.

Before scanning, participants were carefully trained outside the scanner to ensure that they fully understood the task and to avoid head movements as much as possible. The pseudowords and words that appeared during the practice task were not used later during the fMRI task. Pseudowords were described as spoken words comparable to comprehensible words, but with no meaning, that the subjects had not heard before.

2.6. - fMRI parameters

The same fMRI protocol was used for all three scanning sessions. Images were acquired on a 1.5-T Siemens Avanto (Erlangen, Germany). Participants were placed in a supine position in the fMRI scanner and fixation cushions were used to reduce motion artifacts. FOV positioning and slice orientation were set during the first session, and they were then automatically aligned in the following runs using the Siemens AutoAlign feature. A 3D structural MRI was acquired for each subject, using a T₁-weighted magnetization-prepared rapid gradient-echo sequence (time repetition/time echo (TR/TE) = 2200/3.8 ms, matrix=256 x 256 x 160, voxel size = 1 x 1 x 1 mm). For the rs-fMRI, 270 volumes were recorded using a gradient-echo T₂*-weighted echo-planar imaging sequence (TR/TE= 2000/48 ms, matrix = 64 x 64, voxel size = 3.5 x 3.5 mm, flip angle = 90°, slice thickness = 4 mm, slice gap = 0.8 mm, 24 interleaved axial slices). A gradient

–echo T2*-weighted echo-planar imaging sequence was used to obtain 359 volumes for the fMRI task (TR /TE = 2500/50 ms, matrix = 64 x 64, voxel size = 3.5 x 3.5 mm, flip angle = 90°, slice thickness = 3.6 mm, slice gap = 0.4 mm, 29 interleaved axial slices). All the scanner acquisitions were aligned to the plane that intersected the anterior and posterior commissures (AC-PC), and they covered the whole brain.

2.7. - Behavioral analysis

Behavioral data were processed with the IBM SPSS Statistics software. Regarding the learning measure, an ANOVA was conducted for the trained group using the 8 cued-recall scores (i.e. measures obtained after the six learning sessions and after S2 and S3) as the within-subjects factor.

We also registered accuracy of responses during the repetition task. These data were analyzed for the trained group using a repeated measures 2 x 2 x 3 ANOVA including Type of words (Pseudoword vs Word), Training (Trained vs Untrained); and Sessions (Session 1, Session 2 vs Session 3) as within-subjects factors.

2.8. - fMRI analysis

2.8.1. - Repetition task analysis

2.8.1.1. - Preprocessing

Image processing and statistical analyses were carried out using SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK). The preprocessing of the functional data involved realignment to correct for possible head movements during acquisition, where the images

from each time point were introduced as sessions. Sessions were first realigned with each other by aligning the first scan from each session with the first scan of the first session, and then they were registered to the mean image. Excessive head motions were found in 4 participants (more than 2 mm of translation or 2 degrees of rotation), and they were excluded from further analysis. After realignment, the fMRI time series was normalized into a standard space (Montreal Neurological Institute, MNI coordinates) using SPM8's EPI template (voxel size 3 mm³). Finally, spatial smoothing was conducted by applying an isotropic 8-mm Gaussian Kernel full-width at a half maximum (FWHM).

2.8.1.2.- First level analysis

Statistical analyses were performed in the context of the General Linear Model (Friston et al. 1995) for each participant and for each time point. In the first level analysis, the conditions of interest corresponding to the pseudowords and words were modeled. The BOLD signal was estimated by convolving the stimulus onset with the canonical hemodynamic response function. Six motion realignment parameters were included to explain signal variations due to head motion, that is, as covariates of non-interest. A high-pass filter (128s) was applied to the functional data to eliminate low-frequency components. Then, contrast images were obtained to directly compare our conditions of interest: 1) pseudowords (i.e. trained and untrained) > words (i.e. trained and untrained); 2) untrained pseudowords > untrained words; and 3) trained pseudowords > untrained words. The contrast images resulting from the first level analyses were then used in the second level analyses.

2.8.1.3.- Localizer

A one-sample t-test was run to assess differences between pseudowords and words before learning with the contrast pseudowords (i.e. trained and untrained) > words (i.e. trained and untrained) in both groups. To avoid false positives in the fMRI analyses (Woo et al. 2014), the statistical criterion was set at $p < 0.05$, Family-Wise Error (FWE) cluster-corrected

for multiple comparisons (voxel-level uncorrected threshold of $P < 0.001$; cluster size appears in each result). We used this analysis as a localizer of STG and IFG activity during pseudoword processing. Using the WFU Pickatlas software (Maldjian et al. 2003), the local maxima in each region of interest was used to draw a sphere with an 8 mm radius. We used these STG and IFG regions of interest (ROIs) as seeds for further task and rs-fMRI analyses.

2.8.1.4.-Task-related analysis: ROI and Whole-brain analysis

The mean BOLD signal for the two ROIs was extracted in two different contrasts in the three sessions: a) untrained pseudowords > untrained words; and b) trained pseudowords > untrained words. These data were analyzed using two separate (i.e. one for each ROI) repeated-measures 2 x 3 ANOVAs, with the training condition (untrained pseudowords and trained pseudowords) and sessions (S1, S2 and S3) as within-subjects factors. The IBM SPSS program was employed for this purpose. The values obtained from this analysis allowed us to longitudinally assess the BOLD signal changes in the auditory-motor regions in the three fMRI sessions. This analysis was performed only with the trained group because the control group does not have a trained condition.

Additionally, we performed Pearson's correlation analysis of the two ROIs mean BOLD signal and the behavioral measures (Vocabulary and Block design subtests of the WAIS-III) in the three fMRI sessions. The multiple-comparisons between-sessions analysis threshold was set to $p < 0.05$, applying a Holmes-Bonferroni correction to counteract the problem of multiple comparisons.

At an informative level, an additional whole-brain one-sample t-test was conducted with the trained group in each session with the trained pseudowords relative to the untrained words contrast, in order to determinate other complementary changes in the brain in sessions 2 and 3.

2.9. - Resting-State analysis

2.9.1 - Preprocessing

We used the Data Processing Assistant for Resting-State Toolbox (DPARSF, <http://rfmri.org/DPARSF>) (Chao-Gan and Yu-Feng 2010) to carry out resting-state MRI data processing. Preprocessing included the following steps: 1) slice-timing correction for interleaved acquisitions (the 23rd slice was used as the reference point); 2) head motion correction; 3) T1 co-register to functional image; 4) new segmentation to Dartel; 5) removal of spurious variance through linear regression: six parameters from the head motion correction, the global mean signal, the white matter signal and the cerebrospinal fluid signal; 6) removal of the linear trend in the time series; 7) spatial normalization to the Montreal Neurological Institute (MNI) (3mm³); 8) spatial smoothing with 4 mm FWHM Gaussian Kernel; and 9) band-pass temporal 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).

2.9.2 - Seed-Based rs-FC Analysis

We tested the relationship between the two ROIs obtained in the localizer. To test whether possible rs-FC differences would be specific to the left hemisphere, we also calculated the rs-FC value for the homologous area in the right hemisphere. The mean time course of all voxels in each seed region was used to calculate pairwise linear correlations (Pearson's correlation). Individuals' *r* values were normalized to *z* values using Fisher's *z* transformation. In order to analyze the changes in rs-FC in S1, S2 and S3 between groups, we performed mixed-design ANOVAs on the *z*-values, with session (S1, S2 and S3) and laterality (left vs. right) as within-subjects factor, and group (Experimental vs. Control) as between-subjects factor, on the *z* value, using the IBM SPSS statistics software.

We also performed Pearson's correlation analysis with the z-values obtained for the rs-FC between the seeds regions with Vocabulary and Block design subtests from the WAIS-III in the three fMRI sessions. The multiple-comparisons between sessions analysis threshold was set to $P < 0.05$, applying a Holmes-Bonferroni correction to control the problem of multiple comparisons.

3. - Results

3.1. - Behavioral data: learning

The training protocol was successful, as revealed by the analysis of the cued-recall test scores over time (see **Fig. S1**). There was a significant increase in the scores in the learning phase [$F(6,108) = 168.38, p < 0.001$], especially from day 1 to day 4, reaching an asymptotic level. Immediately after the learning phase, in session 2, mean accuracy was very high, 98.5% (SD=2.3), and it only dropped slightly two weeks later, in session 3, 94.2% (SD=6.2). Therefore, training had short- and long-term effects on vocabulary knowledge.

With regard to the accuracy values during the verbal repetition task (see Supplementary Table II), the $2 \times 2 \times 3$ ANOVA analysis yielded a significant three-way Words, Training and Sessions interaction ($F_{2,34} = 46.52, p < 0.001$). This result indicated that the training had different effects on accuracy depending on the type of words. Post-hoc analyses revealed that accuracy in S2 ($F_{1,17} = 62.20; P < 0.001$) and S3 ($F_{1,17} = 26.01; p < 0.001$) was higher than in S1 for the trained pseudowords.

3.2. - Functional localizer

A one-sample t-test comparing pseudowords to words at baseline in both groups was used as a localizer of brain areas involved in pseudoword processing. This analysis yielded a large cluster of activation from the left anterior and posterior parts of the superior temporal gyrus to the left pars opercularis/anterior insula of the left inferior frontal gyrus ($P < 0.05$ Family Wise Error (FWE) cluster-corrected, voxel-wise threshold of $p < 0.001$ with a cluster size of 446 voxels, see **Fig. 2A.1** and Supplementary Table III). From the local maxima of the cluster, we extracted two main regions of interest (ROIs) that localized the activity during pseudoword processing: 1) auditory ROI in the left superior temporal gyrus (STG) centered on $x = -63$, $y = -16$, $z = 1$; and 2) motor ROI in the left pars opercularis centered on $x = -60$, $y = 14$, $z = 19$. These ROIs were subsequently used for the task-related and resting-state fMRI analyses.

3.3. - Task-related results

3.3.1. - ROI analyses

We investigated the activity of the STG and IFG ROIs obtained in two different contrasts for each session: trained pseudowords $>$ untrained words and untrained pseudowords $>$ untrained words. The ANOVA included two within-subjects factors: Session (1, 2 or 3) and Training (untrained vs. trained pseudowords). The analysis of the IFG ROI revealed a marginally significant Training \times Session interaction ($F_{2,34} = 2.77$, $P = 0.07$; **Fig. 3A**). Follow-up post hoc tests revealed that the existence of a significant quadratic relationship showing a reduction in the IFG activity during Session 2, compared to Sessions 1 and 3, for the trained pseudowords ($F_{1,17} = 4.96$; $P < 0.05$, see **Fig. 3A**).

The analysis of the STG ROI showed a significant main effect of the training condition ($F_{1,17} = 7.95$; $P = 0.01$; **Fig. 3B**), indicating that average signal values for the untrained

pseudowords were higher than for the trained pseudowords (see **Fig. 3B**). Although showing a similar trend, the Training x Session interaction did not reach significance.

Finally, Pearson's correlation analyses showed a significant linear relationship between the mean BOLD signal in the STG (trained pseudowords vs. untrained words) with the Vocabulary score in the session 3 ($r_{(18)} = 0.64$; $P < 0.01$, see **Fig. 3C**). Correlations were not significant for the Block Design subtest.

3.3.2. - Whole-brain analyses

We conducted whole-brain analysis with the trained group using the trained pseudowords > untrained words contrast. Results in session 1 were similar to those presented in the localizer section. In session 2, and after the new pseudowords were trained, we found significant activations in the left IPL/Angular gyrus, the left premotor cortex and the right cerebellum ($P < 0.05$ FWE cluster-corrected, voxel-wise threshold of $P < 0.001$, with a cluster size of 57 voxels, see **Fig. 2A.2** and Supplementary Table III). Session 3 showed activation in the left IPL, the left premotor cortex, the left pars opercularis of the left IFG, the bilateral SMA/ACC, and the right cerebellum ($P < 0.05$ FWE cluster-corrected, voxel-wise threshold of $p < 0.001$, with a cluster size of 181 voxels, see **Fig. 2A.2** and Supplementary Table III). In session 3, using a lower threshold ($P < 0.005$), we also found activation in the left STG corresponding to the area reported in the localizer section.

The opposite contrast, untrained words > trained pseudowords also produced relevant results. In session 1, activations were focused on the precuneus/posterior cingulate cortex (PCC), the bilateral fusiform/parahippocampal gyrus, the bilateral posterior middle temporal gyrus/posterior inferior temporal gyrus, the bilateral pars orbitalis of the IFG, and the bilateral IPL/angular gyrus ($p < 0.05$ FWE cluster-corrected, voxel-wise threshold of $p < 0.001$, with a cluster size of 83 voxels, see **Fig. 2B** and Supplementary Table III). Importantly, this contrast

did not yield significant activations in session 2. Finally, the bilateral angular gyrus/posterior middle temporal gyrus again showed greater activation on words than on pseudowords in session 3 ($P < 0.05$ FWE cluster-corrected, voxel-wise threshold of $p < 0.001$, with a cluster size of 59 voxels, see **Fig. 2B** and Supplementary **Table III**).

3.4. - rs-FC results

Four ROIs were used for the rs-FC analysis (left IFG, left STG and their right-hemispheric homologues, see **Fig. 4A**). This analysis yielded a significant three-way interaction among Laterality, Session, and Group ($F_{(2, 64)} = 3.06$, $P = 0.05$; see **Fig. 4B**). This interaction indicated that trained participants increased their functional connectivity more than the control group between the seeds in the left hemisphere after training. Follow-up post hoc tests revealed that this effect was significant when comparing session 3 to session 1 ($F_{1,33} = 4.67$; $P < 0.05$), but not in other comparisons between sessions ($P > 0.10$).

Finally, there was a positive correlation in session 3 between the left STG-IFG rs-FC in session 3 and the Vocabulary score ($r_{(18)} = 0.68$; $P = 0.002$, see **Fig. 4C**). We found no significant correlations with the Block design subtest.

4. - Discussion

In the present study, we investigated the impact of learning a new vocabulary on the pattern of activation during the task and communication at rest in the STG (auditory) and IFG (motor) regions. These regions were selected due to their involvement in the pseudoword processing required to transform pseudowords into words. The longitudinal perspective adopted to investigate brain changes associated with learning a new vocabulary revealed two relevant results. First, while processing the new vocabulary, there was a reduction in brain activity after training in the IFG and STG that returned to baseline two weeks later. Second, there were stable changes in rs-FC between the STG and IFG after training that were still observable two weeks after. Therefore, the temporal pattern of changes reflects an acute change in local pseudoword processing, in contrast to more stable changes in rs-FC measures, even in the absence of the task. These long-lasting effects on the intrinsic activity of the brain could act as an imprint of vocabulary learning in the brain. Next, these results are discussed in detail.

4.1. - Training effects on brain activity

We focus on the task related fMRI results obtained from the comparison of the untrained pseudowords and words. The baseline results coincided with previous studies showing that the left IFG and STG were involved in pseudoword processing, whereas the angular gyrus, the medial and inferior temporal lobe, and the pars orbitalis of the inferior frontal gyrus were more active for words than pseudowords (Saur et al. 2008, 2010; Davis et al. 2009; Rodríguez-Fornells et al. 2009). In addition, activation in the STG and IFG was replicated in sessions 2 and 3 when comparing untrained pseudowords to untrained native words (see Fig. 2, untrained group), thus showing the consistency of the effect. Therefore, these areas were likely to be strongly involved in pseudoword processing during the one-week training carried out between sessions 1 and 2. Both the activity in these areas and the direct auditory-motor interactions

between these areas have been proposed to be highly relevant in the acquisition of new vocabulary (López-Barroso et al. 2013, 2015). Theoretical views reported that auditory-motor interactions involve generating a new sensory representation of the new word and keeping this auditory representation in an active state [i.e. phonological short-term memory (Hickok and Poeppel 2004; Buchsbaum et al. 2005; Jacquemot and Scott 2006)]. At the same time, this sensory representation can then be used to guide the production of motor articulatory sequences (Hickok and Poeppel 2004, 2007; Saur et al. 2008).

Hickok and Poeppel (2007) also proposed that, as the word becomes familiar, the nature of the sensory-motor interaction might change. After training, when participants have processed these stimuli hundreds of times, our results showed that activity in the IFG decreased to a word-like level, indicating that the need for these areas was reduced when these pseudowords became similar to words. STG showed the same pattern but the effect was not significant. In addition, these results were associated with other changes in the brain showing the involvement of the dorsal pathway: the contrast between trained pseudowords > untrained words activated the inferior parietal lobe, the premotor cortex, and the cerebellum. The inferior parietal lobe is a key area in successful second language learning, as demonstrated in different anatomic and functional studies (Hickok and Poeppel 2004; Buchsbaum et al. 2005; Jacquemot and Scott 2006; Baldassarre et al. 2012; López-Barroso et al. 2015). The premotor cortex and the cerebellum are relevant in articulating learned pseudowords (Price 2012). These results are important because these areas do not participate in initial stages during of pseudoword processing, but they do appear when this learning is established.

Even after the intensive training, the lack of activity in the STG and IFG was short-lived: after two-weeks without any exposure to these stimuli, the effect disappeared, and the acquired words were again processed through the dorsal route (i.e. the activity returned to initial values). These changes occurred even though the participants remembered the semantic meaning of the word with 94% accuracy. This could reflect a temporary semantic priming effect that dissipates

after two weeks without training. When compared to native words, the processing of trained pseudowords also required the participation of the premotor cortex, the cerebellum, and the inferior parietal lobe, with broader activations. However, a new area involved in language control also participated: the SMA/ACC. This area is activated when language control is needed, especially when participants must use the second language (Garbin et al. 2010; Abutalebi et al. 2013; Abutalebi and Green 2016). Overall, results suggest that these pseudowords may be returning to brain activity levels in the dorsal stream similar to those of untrained pseudowords, but the language control network is recruited to implement the processing of meaning and phonology.

Beyond the main interest of the study, our design also allowed us to investigate the role of the ventral language stream in this task. In fact, the word > pseudoword contrast in session 1 produced a significant activation in the left posterior system middle temporal gyrus. As expected, words generated more semantic processing in the ventral stream than non-words. This activation disappeared in Session 2, demonstrating that trained pseudowords generated a similar semantic processing to words immediately after training. Importantly, the activation in the middle temporal gyrus reappeared again in Session 3, demonstrating again that trained pseudowords produced lower semantic processing two weeks after learning. This effect should be investigated in future studies.

4.2. - Relationship between Auditory and Motor regions at rest

Whereas the increased in task-related BOLD activation was ephemeral, increased rs-FC measures seemed to maintain a hallmark of learning two weeks later. Our results were consistent with Hickok and Poeppel's idea (2007) that the dorsal stream is crucial for new vocabulary processing. Moreover, the rs-FC values between the left STG and the left IFG (pars opercularis) at baseline were positive and strong, indicating that the relationship between the

two areas was chronically established. As expected, we found a greater increase in rs-FC between the left STG and the left IFG (pars opercularis) in the trained group after learning (significant in session 3), compared to controls. These changes in rs-FC may reflect intrinsic and stable modifications related to vocabulary learning. This result agrees with previous studies showing that training in specific functions increased FC between local and distant brain areas involved in learning a new task (Lewis et al. 2009; Baldassarre et al. 2012; Ventura-Campos et al. 2013). Therefore spontaneous activity patterns in the rs-fMRI may reflect a priori biases and the individual's propensities as a result of experience (Harmelech and Malach 2013). They also coincide with previous studies showing the relevance of the arcuate fasciculus, the white matter pathway that connects the IFG and STG, in pseudoword processing and learning. Saur et al. (Saur et al. 2008, 2010) demonstrated that the dorsal language pathway connecting two areas that resemble the IFG and STG seeds employed in the present study was associated with repetition of pseudowords, compared to repetition of real words. In addition, Lopez-Barroso et al. (López-Barroso et al. 2013, 2015) showed that individual differences in vocabulary acquisition correlated with the strength of the anatomical and functional connectivity of the direct connections between Broca's area and auditory brain areas in the left hemisphere (i.e. arcuate fasciculus). The correlation obtained was restricted to the left long segment of the arcuate fasciculus connecting auditory regions in the auditory STG to Broca's area. The present study has tested these associations longitudinally, showing that acquiring new vocabulary increased rs-FC, not only after learning, but also two weeks later.

4.3. - The role of pretraining vocabulary

The changes in BOLD magnitude and rs-FC found in this study were associated with individual differences in vocabulary size. Specifically, in session 3, participants in the trained group with higher scores on the Vocabulary subtest of the WAIS showed: 1) stronger rs-FC between IFG and STG; and 2) higher response in STG while processing the learned material. Previous studies have shown that individual differences in vocabulary acquisition were positively associated with phonological memory in children (Santos and Bepi-Lopes 2012; Newbury et al. 2015) and adults (Papagno and Vallar 1995). These results were also consistent with a study in 20-month-old children showing that pseudoword familiarization indexed by evoked potentials (N200-400 repetition effect) was observed only in children with larger productive vocabularies (Torkildsen et al. 2009). These studies demonstrated that vocabulary acquisition was more associated with phonological than semantic language processing, as observed in entities such as specific language disorders where both cognitive processes (i.e. phonological memory and vocabulary) are simultaneously underdeveloped (Gray 2006; Moav-Scheff et al. 2015; Jackson et al. 2016).

Our study has served to localize the neural basis of pseudoword processing in the left STG and IFG, as well as their connectivity. Consistent with our results, DTI studies revealed that Vocabulary scores and phonological processing were related to a stronger degree of lateralization of the arcuate fasciculus in the left hemisphere (Lebel and Beaulieu 2009). Thus, individual differences in vocabulary size contribute to determining vocabulary acquisition mediated by the left STG and IFG.

4.4. - Conclusion

We have provided evidence that learning a new vocabulary is associated with fluctuations in BOLD activity and increased coupling between auditory and motor regions. Task-related results allow us to show the dynamics of the left STG and IFG regions while processing the learned material in the short-term, whereas the rs-FC between the two areas seemed to act as a long-term neural signature of this learning. Furthermore, these processes were stronger in individuals with higher Vocabulary scores. Overall, this pattern of results might help to understand second-language vocabulary learning in the classroom setting. Our results showed that learned vocabulary tends to be processed in the brain through the dorsal stream, even when we know the meaning of the acquired words, after a short period (i.e. two weeks) without using them. This result highlights the need to continuously use the acquired vocabulary in order to treat them as words, especially in beginners.

Acknowledgments

This work was funded by County Council Department of Education, Culture and Sports of the Valencian Community of Spain (APOSTD/2012068), the Ministry of Economy and Competitiveness of Spain (CSD2007-0.0012, PSI2013-47504-R and FPDI2013-17045) and the Universitat Jaume I (P1-1B2012-38).

References

- Abutalebi J, Della Rosa PA, Ding G, Weekes B, Costa A, Green DW. 2013. Language proficiency modulates the engagement of cognitive control areas in multilinguals. *Cortex*. 49:905-911.
- Abutalebi J, Green DW. 2016. Neuroimaging of language control in bilinguals: neural adaptation and reserve. *Biling Lang Cogn*. 1-10.
- Baldassarre A, Lewis CM, Committeri G, Snyder AZ, Romani GL, Corbetta M. 2012. Individual variability in functional connectivity predicts performance of a perceptual task. *Proc Natl Acad Sci U S A*. 109:3516-3521.
- Buchsbaum BR, Olsen RK, Koch P, Berman KF. 2005. Human dorsal and ventral auditory streams subserve rehearsal-based and echoic processes during verbal working memory. *Neuron*. 48:687-697.
- Bueichekú E, Ventura-Campos N, Palomar-García M-Á, Miró-Padilla A, Parcet M-A, Barrós-Loscertales A, Ávila C. 2015. Functional connectivity between superior parietal lobule and primary visual cortex «at rest» predicts visual search efficiency. *Brain Connect*. 5:517-526.
- Chao-Gan Y, Yu-Feng Z. 2010. DPARSF: A MATLAB toolbox for «pipeline» data analysis of resting-state fMRI. *Front Syst Neurosci*. 4:13.
- Cole MW, Yarkoni T, Repovs G, Anticevic A, Braver TS. 2012. Global connectivity of prefrontal cortex predicts cognitive control and intelligence. *J Neurosci*. 32:8988-8999.
- Davis MH, Di Betta AM, Macdonald MJE, Gaskell MG. 2009. Learning and consolidation of novel spoken words. *J Cogn Neurosci*. 21:803-820.
- Finn ES, Shen X, Scheinost D, Rosenberg MD, Huang J, Chun MM, Papademetris X, Todd Constable R. 2015. Functional connectome fingerprinting: identifying individuals based on patterns of brain connectivity. *Nat Neurosci*. 18:1664-1671.
- Friston KJ, Holmes AP, Worsley KJ, Poline J-P, Frith CD, Frackowiak RSJ. 1995. Statistical parametric maps in functional imaging : a General Linear Approach. *Hum Brain Mapp*. 2:189-210.
- Garbin G, Sanjuan A, Forn C, Bustamante JC, Rodriguez-Pujadas A, Belloch V, Hernandez M, Costa A, Ávila C. 2010. Bridging language and attention: Brain basis of the impact of bilingualism on cognitive control. *Neuroimage*. 53:1272-1278.
- Gray S. 2006. The relationship between phonological memory, receptive vocabulary, and fast mapping in young children with specific language impairment. *J Speech Lang Hear Res*. 49:955-969.
- Grönholm P, Rinne JO, Vorobyev V, Laine M. 2005. Naming of newly learned objects: A PET activation study. *Cogn Brain Res*. 25:359-371.
- Guerra-Carrillo B, Mackey AP, Bunge SA. 2014. Resting-State fMRI: a window into human brain plasticity. *Neuroscientist*. 20:522-533.
- Halwani GF, Loui P, Rüber T, Schlaug G. 2011. Effects of practice and experience on the

- arcuate fasciculus: comparing singers, instrumentalists, and non-musicians. *Front Psychol.* 2:1-9.
- Harmelech T, Malach R. 2013. Neurocognitive biases and the patterns of spontaneous correlations in the human cortex. *Trends Cogn Sci.* 17:606-615.
- Hickok G. 2012. The cortical organization of speech processing: feedback control and predictive coding the context of a dual-stream model. *J Commun Disord.* 45:393-402.
- Hickok G, Poeppel D. 2004. Dorsal and ventral streams: a framework for understanding aspects of the functional anatomy of language. *Cognition.* 92:67-99.
- Hickok G, Poeppel D. 2007. The cortical organization of speech processing. *Nat Rev Neurosci.* 8:393-402.
- Hultén A, Laaksonen H, Vihla M, Laine M, Salmelin R. 2010. Modulation of brain activity after learning predicts long-term memory for words. *J Neurosci.* 30:15160-15164.
- Hultén A, Vihla M, Laine M, Salmelin R. 2009. Accessing newly learned names and meanings in the native language. *Hum Brain Mapp.* 30:976-989.
- Jackson E, Leitao S, Claessen M. 2016. The relationship between phonological short-term memory, receptive vocabulary, and fast mapping in children with specific language impairment. *Int J Lang Commun Disord.* 51:61-73.
- Jacquemot C, Scott SK. 2006. What is the relationship between phonological short-term memory and speech processing? *Trends Cogn Sci.* 10:480-486.
- Lebel C, Beaulieu C. 2009. Lateralization of the arcuate fasciculus from childhood to adulthood and its relation to cognitive abilities in children. *Hum Brain Mapp.* 30:3563-3573.
- Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M. 2009. Learning sculpts the spontaneous activity of the resting human brain. *Proc Natl Acad Sci.* 106:17558-17563.
- López-Barroso D, Catani M, Ripollés P, Dell'Acqua F, Rodríguez-Fornells A, de Diego-Balaguer R. 2013. Word learning is mediated by the left arcuate fasciculus. *Proc Natl Acad Sci.* 110:13168-13173.
- López-Barroso D, Ripollés P, Marco-Pallarés J, Mohammadi B, Münte TF, Bachoud-Lévi A-C, Rodríguez-Fornells A, de Diego-Balaguer R. 2015. Multiple brain networks underpinning word learning from fluent speech revealed by independent component analysis. *Neuroimage.* 110:182-193.
- Maldjian JA, Laurienti PJ, Kraft RA, Burdette JH. 2003. An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage.* 19:1233-1239.
- Mestres-Missé A, Càmarà E, Rodríguez-Fornells A, Rotte M, Münte TF. 2008. Functional neuroanatomy of meaning acquisition from context. *J Cogn Neurosci.* 20:2153-2166.
- Moav-Scheff R, Yifat R, Banai K. 2015. Phonological memory and word learning deficits in children with specific language impairment: A role for perceptual context? *Res Dev Disabil.* 45-46:384-399.
- Newbury J, Klee T, Stokes S., Moran C. 2015. Exploring expressive vocabulary variability in

- two-year-olds: the role of working memory. *J Speech, Lang Hear Res.* 58:1761-1772.
- Oldfield R. 1971. The assessment and analysis of handedness: the Edinburgh Inventory. *Neuropsychologia.* 9:97-113.
- Palomar-García M-Á, Zatorre RJ, Ventura-Campos N, Bueichekú E, Ávila C. 2016. Modulation of Functional Connectivity in Auditory–Motor Networks in Musicians Compared with Nonmusicians. *Cereb Cortex.* bhw120.
- Papagno C, Vallar G. 1995. Verbal short-term memory and vocabulary learning in polyglots. *Q J Exp Psychol A.* 48:98-107.
- Price CJ. 2012. A review and synthesis of the first 20 years of PET and fMRI studies of heard speech, spoken language and reading. *Neuroimage.* 62:816-847.
- Raboyeau G, Marcotte K, Adrover-Roig D, Ansaldo A. 2010. Brain activation and lexical learning: the impact of learning phase and word type. *Neuroimage.* 49:2850-2861.
- Raboyeau G, Marie N, Balduyck S, Gros H, Démonet J-F, Cardebat D. 2004. Lexical learning of the English language: A PET study in healthy French subjects. *Neuroimage.* 22:1808-1818.
- Rodríguez-Fornells A, Cunillera T, Mestres-Missé A, de Diego-Balaguer R. 2009. Neurophysiological mechanisms involved in language learning in adults. *Philos Trans R Soc.* 364:3711-3735.
- Santos MT, Befi-Lopes DM. 2012. Vocabulary, phonological awareness and rapid naming: contributions for spelling and written production. *J Soc Bras Fonoaudiol.* 24:269-275.
- Saur D, Kreher BW, Schnell S, Kümmerer D, Kellmeyer P, Vry M, Umarova R, Musso M, Glauche V, Abel S, Huber W, Rijntjes M, Hennig J, Weiller C. 2008. Ventral and dorsal pathways for language. *PNAS.* 105:18035-18040.
- Saur D, Schelter B, Schnell S, Kratochvil D, Küpper H, Kellmeyer P, Kümmerer D, Klöppel S, Glauche V, Lange R, Mader W, Feess D, Timmer J, Weiller C. 2010. Combining functional and anatomical connectivity reveals brain networks for auditory language comprehension. *Neuroimage.* 49:3187-3197.
- Snodgrass JG, Vanderwart M. 1980. A standardized set of 260 pictures: norms for name agreement, image agreement, familiarity, and visual complexity. *J Exp Psychol Learn Mem.* 6:174-215.
- Taylor VA, Daneault V, Grant J, Scavone G, Breton E, Roffe-Vidal S, Courtemanche J, Lavarenne AS, Marrelec G, Benali H, Beauregard M. 2013. Impact of meditation training on the default mode network during a restful state. *Soc Cogn Affect Neurosci.* 8:4-14.
- Torkildsen J von K, Friis Hansen H, Svangstu JM, Smith L, Simonsen HG, Moen I, Lindgren M. 2009. Brain dynamics of word familiarization in 20-month-olds: effects of productive vocabulary size. *Brain Lang.* 108:73-88.
- Ventura-Campos N, Sanjuán A, González J, Palomar-García M-Á, Rodríguez-Pujadas A, Sebastián-Galles N, Deco G, Ávila C. 2013. Spontaneous brain activity predicts learning ability of foreign sounds. *J Neurosci.* 33:9295-9305.
- Woo C-W, Krishnan A, Wager TD. 2014. Cluster-extent based thresholding in fMRI analyses: pitfalls and recommendations. *Neuroimage.* 91:412-419.

Figure 1. Overview of the experiment

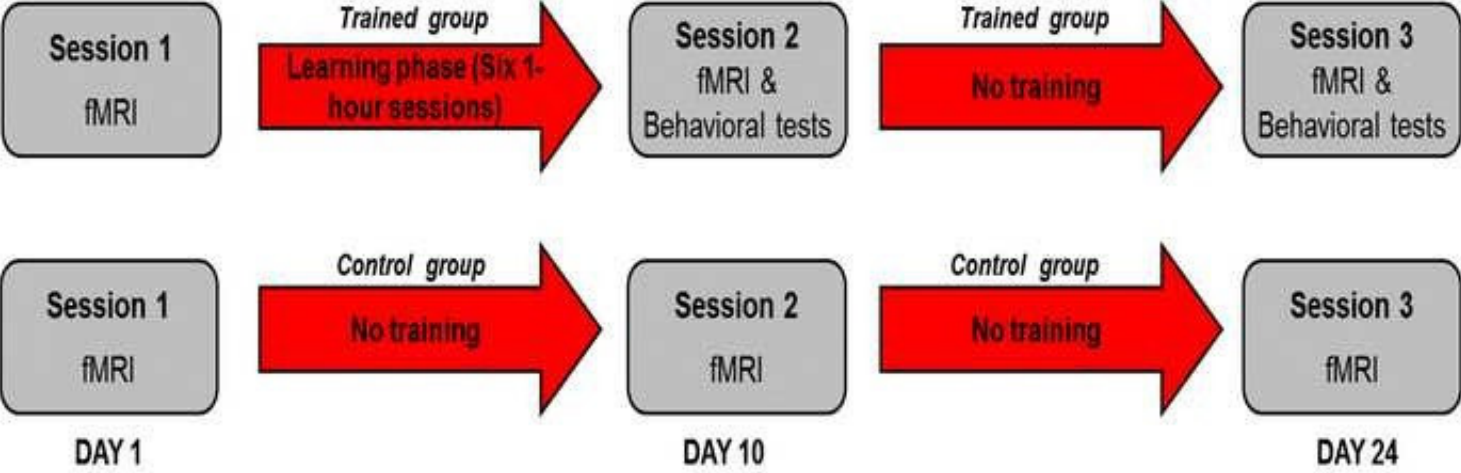


Figure 2. Brain activation maps illustrating: A) the effect of training on pseudowords compared to untrained words. (1) Session 1/Localizer (blue), session 3 (red), and the common regions (violet); (2) Session 2 (green), session 3 (red), and the common regions (yellow). B) The untrained words compared to trained pseudowords activations. Session 1 (blue), session 3 (red), and the common regions (violet).

FOOTNOTES: Color bar represents t-values. Results were $p < 0.05$ FWE cluster-corrected. L=Left. R=Right. Coordinates are in the MNI space.

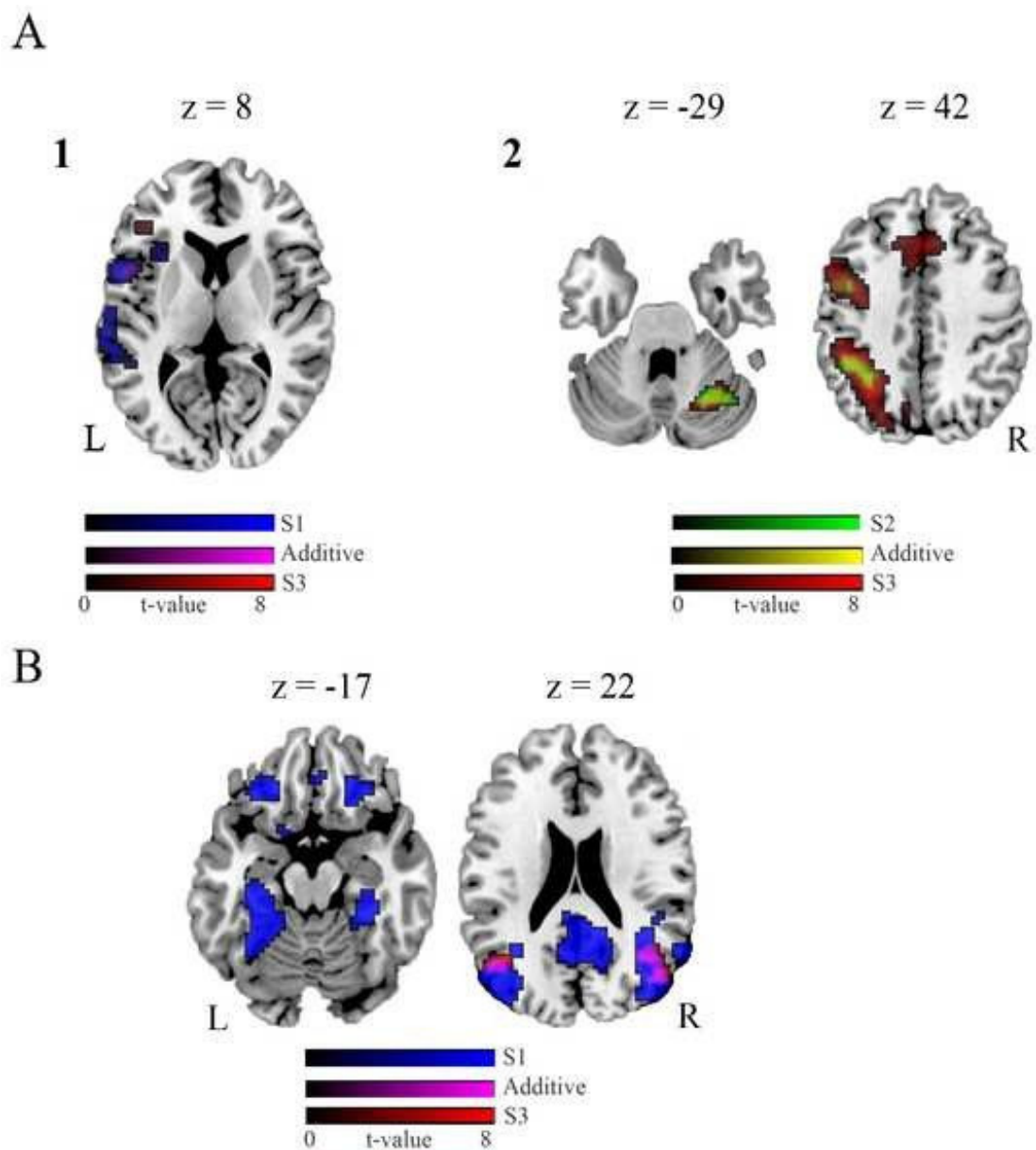


Figure 3. Task-related results. Mean BOLD signal and standard error mean bars in the IFG

(A) and STG (B) for two conditions: 1) untrained pseudowords vs. untrained words (green lines); 2) trained pseudowords vs. untrained words (yellow lines) in the three fMRI sessions.

(C) The scatter plot shows that in session 3 the mean BOLD signal for the left STG correlated significantly with the vocabulary score.

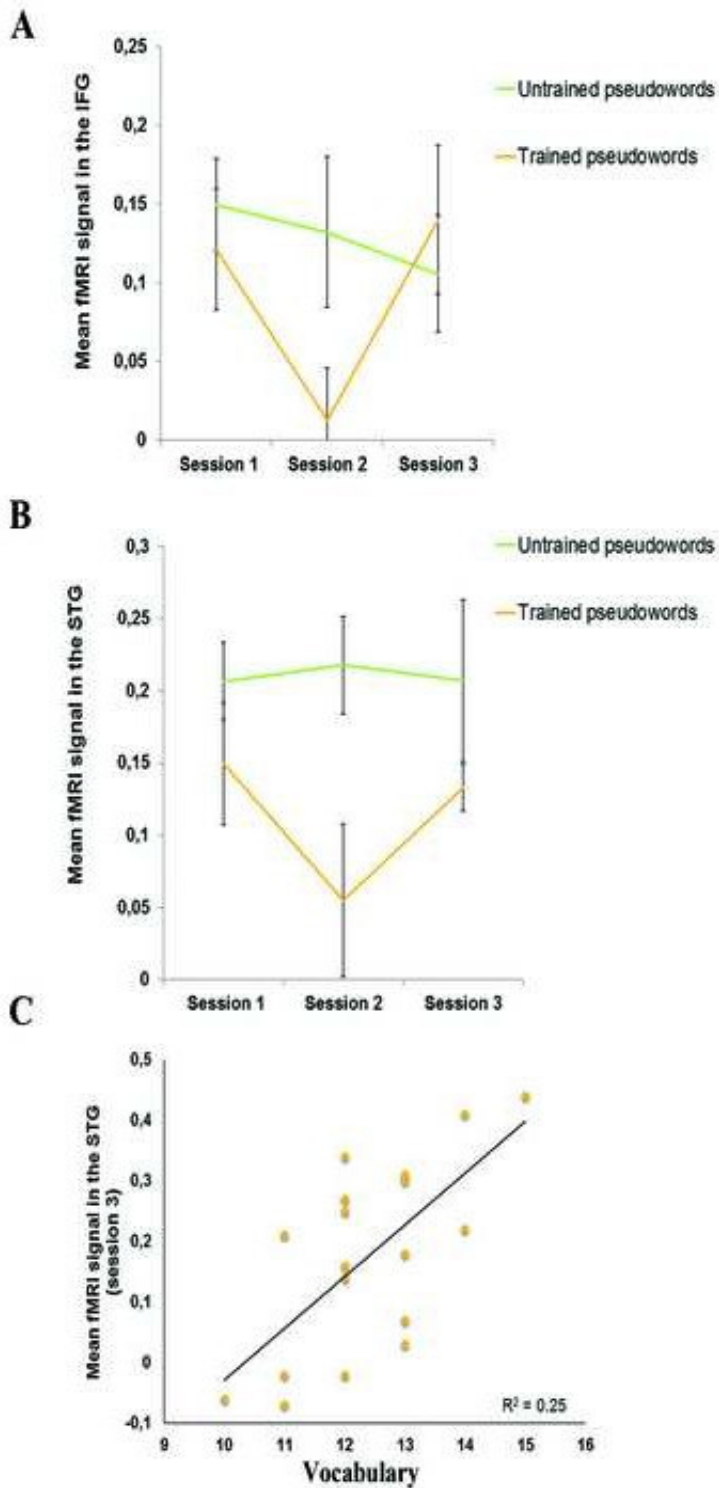
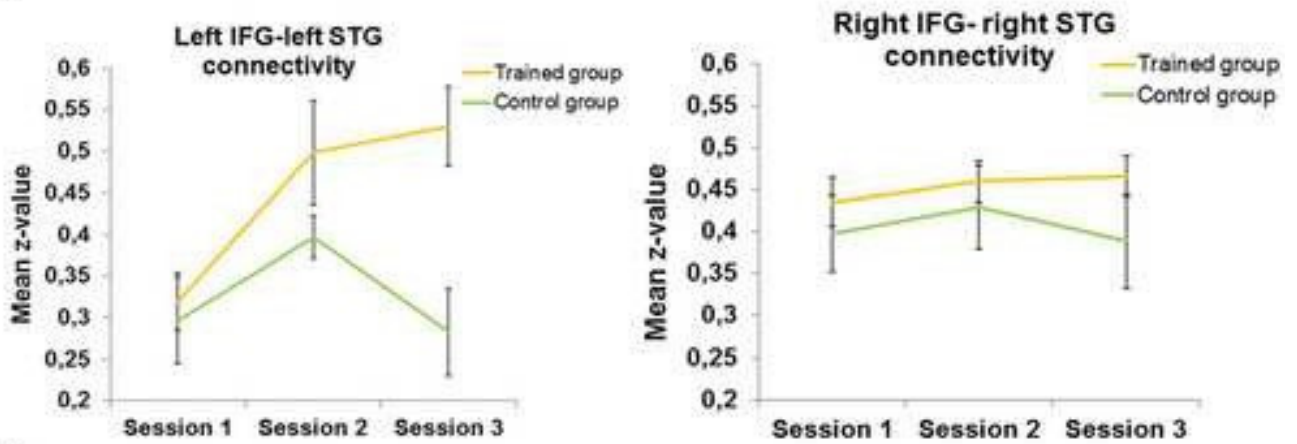


Figure 4. Rs-FC results. (A) Illustration of the location of the 4 seed ROIs in the left hemisphere (left IFG and left STG) and the right hemisphere (right IFG and the right STG). (B) The graph shows the comparison of functional connectivity at rest (mean z-value) and standard error mean bars. FC between the left IFG and left STG was higher in the trained group than in the control group when comparing the session 3 to session 1, $P < 0.05$. (C) The scatter plot shows that in session 3 the connectivity between the left IFG and the left STG (z-value) correlated significantly with the vocabulary score

A



B



C

