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2 **Supplementary Information for**

3 **Laminar Specific fMRI Reveals Directed Interactions in Distributed Networks During** 4 **Language Processing**

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8 **This PDF file includes:**

9 Supplementary text

10 Figs. S1 to S5

11 Captions for Databases S1 to S2

12 References for SI reference citations

13 **Other supplementary materials for this manuscript include the following:**

14 Databases S1 to S2

15 Supporting Information Text

16 Methods and materials

17 Experimental design.

18 **Task paradigm.** Twenty-four native Dutch subjects (13 female, 18-30 years of age, 21 right handed) performed a single word
19 reading task which presented words, pseudo-words and false-font items as task conditions. Two subjects were ultimately
20 excluded from analysis owing to computer failure and signal dropout, leaving 22 data sets for analysis. Data for one subject
21 was unrecoverable following computer failure during image reconstruction. The second subject experienced signal dropout in
22 the left occipitotemporal sulcus and so was excluded from all analysis. Subjects had normal or corrected to normal vision and
23 were screened for reading impairment. Left handed participants were included because regions of interest were determined
24 through functional localization. Language function was observed to be left lateralized in all participants. Informed consent for
25 all experimental procedures was obtained in accordance with the procedures of ethical approval of the Donders Centre for
26 Cognitive Neuroimaging and the Erwin L. Hahn Institute.

27 Initially, the experiment was intended to use a 3×2 task design of Lexicality (words, pseudo-words, false-font items) \times Length
28 (short, long) with 120 items for each level, and where ‘short’ and ‘long’ designated the length of the words in terms of number
29 of syllables. The length manipulation was intended to vary the bottom-up signal contribution to the LOTS. It was determined
30 through piloting that the length manipulation was ineffective and was not analyzed as part of this study. The number of
31 participants was chosen on the basis of previous work using similar acquisition techniques (1). Our task thus included three
32 relevant conditions, two of which (words, pseudo-words) were conditions of interest for our analysis, and one of which (false-font
33 items) was used to localize the functional Region Of Interest (fROI) for analysis. Figure S5 visualizes mini-blocks from each
34 condition.

35 **Item creation.** Word items were selected from a list of high frequency, concrete Dutch nouns taken from the Celex database
36 (2). Words were selected to maximize frequency, minimize the standard deviation of word frequency, and minimize standard
37 deviation of these values for short and long items.

38 Pseudo-words were generated using Wuggy (3). Pseudo-word generation was constrained on the basis of phonemic
39 neighborhood density, consonant/vowel structure and the number of characters of the word items. The word and pseudo-word
40 stimuli, and the parameters used in Wuggy to generate the pseudo-word stimuli can be found in supplementary materials
41 (StimulusList.xlsx).

42 False-font items were created by rendering the word items in the false font. The false font (4) was designed to preserve the
43 low level features of familiar orthographically legal characters, but to be visually different from letter shapes. These items are
44 included in supplementary materials (FalseFontItems.pdf). Collapsing across the length manipulation, there were 240 items of
45 each stimulus type. Sample items can be seen in the main text.

46 **Stimulus presentation.** Items were presented during fMRI measurements taken over 12 runs. Runs were delimited by breaks in
47 data acquisition. Twenty items of each stimulus type were presented per run, with 60 items presented in total per run.

48 Individual stimuli were visually presented for 800ms in the center of the display. One item was presented per trial. Items
49 were rendered in white on a black background, as shown in the main text.

50 Presentation onset was jittered around the 3960 ms TR based on the design optimization calculations obtained using optseq
51 (5). A black screen was presented for the remaining 3160 ms of each trial. Stimuli were presented minimally with a 200 ms
52 delay from the trial onset and offset resulting in a minimum ISI of 400ms and a maximum ISI of 5920 ms. In practice, ISIs fell
53 between these two extremes. A fixation cross was presented for a full trial (3960 ms) at the beginning of each run and following
54 each mini-block.

55 Stimuli were presented in 5 item mini-blocks in which all 5 items were of the same condition type. Each mini-block was
56 followed by a fixation cross presented for the duration of one trial. On three pseudo-random occasions per run, a question mark
57 was presented that instructed the participant to indicate via button-box whether the previous mini-block contained existing
58 Dutch words. Button-box responses were not analyzed and were considered only to ensure participant compliance. Prior to the
59 experiment, subjects were briefed on the type of items they were to see and instructed to silently read the items on the screen.

60 The experiment was performed using Presentation® software (Version 16.1, Neurobehavioral Systems, Inc., Berkeley, CA,
61 www.neurobs.com). Two versions of the experiment were created with roughly half of the participants assigned to each version.
62 The versions differed in block and item order. The different experiment versions were intended to capture latent, unintended
63 effects inherent in presentation order or other version specific properties.

64 **Task design model.** The task design matrix included condition regressors, temporal and spatial dispersion derivatives, physiologic
65 regressors, motion regressors produced by using SPM version 12 (6), drift terms, frequency filters, outlier censors, and constant
66 terms modeling the mean signal per run. Outlier time points were determined using *3dToutcount* in AFNI (7). We considered
67 a voxel to be an outlier if the probability of the distance of its intensity value from the trend exceeded $p = 0.001$ as defined by
68 its location within a Gaussian probability distribution. Time points were excluded from analysis if 2% or more of the voxels at
69 that time point were categorized as outliers.

70 Stimulus onsets were modeled as instantaneous events with zero duration and convolved with the canonical hemodynamic
71 response function. Condition regressors were created separately for word, pseudo-word and false-font items; and for the long

72 and short items within each condition. In total six condition types were modeled, but only three were analyzed after collapsing
73 across length.

74 **Acquisition.**

75 **Functional acquisition.** Near whole brain, submillimeter (0.943, 0.900 slice direction) resolution T2*-weighted GE-BOLD data
76 were acquired using a GRAPPA accelerated (acceleration factor 8×1) 3D-EPI acquisition protocol (8) with CAIPI shift $k_z =$
77 0 , $k_y = 4$ (9, 10); effective TE = 20ms, TR = 44ms, effective TR = 3960ms, BW = 1044Hz/Px, FoV = 215mm \times 215mm \times
78 215mm with 112 phase encode steps in the slice direction (100.8mm), $\alpha = 13^\circ$, partial Fourier factor=6/8 in both slice and
79 phase-encoding directions. The first phase encoding gradient was applied in the posterior to anterior direction. An axial slab
80 was collected in each subject and positioned to include the occipitotemporal sulcus. The 10cm slab was sufficient to allow
81 complete brain coverage in several subjects and near complete coverage in the remaining subjects. Data were acquired on a
82 Siemens Magnetom 7 Tesla scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel head coil (Nova Medical,
83 Wilmington, USA) at The Erwin L. Hahn Institute in Essen, Germany. Functional data consisted per subject of 12 3D-EPI
84 data sets of 77 volumes each, although some sessions were incomplete owing to time constraints or other difficulties. No session
85 contained fewer than 10 functional data sets.

86 **Anatomy acquisition.** Two anatomic images were acquired in each subject using the MP2RAGE (11) acquisition protocol (voxel
87 resolution = 0.75mm \times 0.75mm \times 0.75mm, TR = 6000ms, TE = 3.06ms, $T_{11} = 800$ ms, $T_{12} = 2700$ ms, $\alpha_1 = 4^\circ$, $\alpha_2 = 5^\circ$,
88 BW = 240Hz/Px, FoV = 240mm \times 240mm with 192 slices (144mm)) and a T1-weighted inversion recovery EPI (IR-EPI)
89 protocol based on the parameters used in the functional acquisition protocol. To create the T1 contrast, the following parameters
90 were modified from the functional acquisition: $\alpha = 90^\circ$, T1 = 800ms, TR = 200ms, TE = 20ms. Example images from each
91 type of anatomic acquisition can be seen in figure S1.

92 It was necessary to increase the number of phase encode steps in the slice direction from 112 to 160 and to expand the FoV
93 in the slice direction to ensure fully overlapping coverage with the functional data. The IR-EPI images were used for image
94 registration as they are known to provide high tissue contrast while preserving the geometric distortions of the functional
95 images (12). High accuracy, cross-modal registration is challenging, particularly with high resolution acquisitions known to
96 exaggerate geometric distortions. Performing co-registration taking the IR-EPI as the source image mitigated the challenges
97 caused by these distortions.

98 Field maps were also acquired in some subjects for potential use in distortion correction, though these were not used. The
99 IR-EPI provided sufficient contrast in the native functional space to facilitate high quality registration without the need for
100 distortion correction.

101 **Choice of acquisition method for laminar resolution imaging.** fMRI relies on the same neurovascular coupling mechanisms exploited
102 in standard BOLD imaging. In recent years there has been increased support for the concept that the neurovascular coupling
103 occurs at a sufficiently fine scale to make fMRI feasible (13). However, the requirement for submillimeter resolution has led to
104 considerable discussion as to the best MR-contrast for interrogating the hemodynamic response.

105 The standard gradient echo BOLD contrast is highly sensitive to functional activation, but is known to have a considerable
106 contribution from vessels downstream from the site of activation. The less commonly used spin-echo BOLD sequence only
107 acquires data from a subset of the contrast mechanisms that contribute to gradient echo BOLD, but is believed to have a
108 superior intrinsic spatial localization at high static magnetic field strengths (14–16). In addition, contrasts based on cerebral
109 blood flow and volume (CBF, CBV) should also be considered. It is technically far easier to test and compare these contrasts
110 in animal models, and historically such experiments largely preceded human fMRI (17–24). The conclusion drawn from these
111 was that CBV was consistently found to have the superior characteristics in terms of spatial resolution, and gradient echo
112 BOLD the poorest. Spin-echo BOLD and CBF are somewhere between these two extremes. This hierarchy may be explained
113 in terms of the current view that blood volume changes occur in the arterioles and capillaries (19, 25–27), and hence CBV
114 contrast should not be a downstream contrast as is BOLD.

115 The first laminar fMRI studies in humans are comparatively recent (28–30), and utilized gradient echo BOLD contrast.
116 Since then, the VASO technique for measuring CBV noninvasively (21) has been further developed for application for laminar
117 fMRI at high static magnetic field strengths (31–33), and a number of spin echo (34–36) and combined spin-echo and gradient
118 echo studies (37, 38) have been performed. Laminar CBF has to date not been published for human studies. Our rationale
119 for selecting gradient echo BOLD for the current study was based primarily on its exclusive ability to acquire high spatial
120 resolution data from large volumes within an acceptable acquisition time. The two main alternatives – CBV and spin-echo – are
121 currently techniques that are restricted in their volume coverage and suffer from comparatively long acquisition times (33, 36).

122 The whole-brain gPPI results we report suggest, however, that GE-BOLD may be capable of more refined spatial localization
123 than previously believed. As discussed in the main text, the ability of the gPPI to account for the task effects likely enhanced
124 our ability to localize signal variance unique to individual depth bins. Simulations from Markuerkiaga et al. (39) based on
125 reported depth dependent responses in visual cortex to identified a depth dependent peak to tail response ratio of at least 5:1
126 in all cortical depths at 7T, which would reduce the detectability of unique variance downstream from its source. The gPPI
127 results suggest that this ratio may be conservative, or perhaps influenced by task properties. Our reading experiment presented
128 stimuli at a high frequency relative to presentation rates discussed in Markuerkiaga et al. (39), which should have produced
129 relatively higher frequency task signal. The vasculature attributed to downstream BOLD effects consists of post capillary
130 vessels draining into larger vessels, whereby differences in the vessel length and flow velocity will act to reduce the coherence of

131 the signal leading the vasculature bed to act as a low-pass filter. High frequency task signal components would therefore be
132 expected to undergo greater attenuation than lower frequency components, and would experience a larger depth dependent
133 peak-to-tail response ratio. In light of our results, it seems clear that unique variance related to each bin was well localized,
134 and that signal contamination was isolated to the main task effects where it could be removed during the gPPI analysis.

135 **Image registration.** High quality registration is critical to laminar fMRI. Given the complications inherent in the registration of
136 submillimeter data, different combinations of tools were necessary to achieve accurate registrations in different participants.
137 The criteria for success were constant, however, across all participants and of an entirely anatomical basis. Alignment quality
138 was determined by visual inspection of brain edges and the left occipitotemporal sulcus. The registration procedure is described
139 in this section.

140 **Motion correction.** Image realignment was performed using *spm_realign* from SPM 12 (6), with parameter values set to achieve
141 the highest quality registration. During this step, a mean functional image was computed to be used as the base image in
142 cross-modal registration.

143 **Skull removal.** Skull removal was performed on functional and anatomic data prior to cross-modal image registration. Different
144 skull removal procedures were used depending on image modality. The FreeSurfer (40) watershed function was applied to the
145 IR-EPI data sets, sometimes following a first pass B1 bias field correction (discussed in *B1 Correction*). Nearly all processed
146 brains required manual intervention to remove voxels containing unwanted skull or tissue, or to reintroduce voxels removed in
147 error. Skull removal was performed on all MP2RAGE images in the same manner.

148 Skull removal was performed on the mean functional images produced during realignment. In this procedure, we manually
149 edited the result of AFNI's *3dAutomask* program. *3dAutomask* is typically used to remove the skull in images with poor tissue
150 contrast, such as with T2*-weighted images. Parameters for this program were optimized on a per subject basis, and all results
151 were manually edited to ensure that only voxels containing skull were removed. We found these results to be adequate on
152 the basis of visual inspection following manual intervention, where 'adequate' describes results which did not contain residual
153 skull or exclude voxels containing brain-matter. *3dAutomask* parameters were iteratively optimized until a result was obtained
154 which reasonably limited the necessary manual intervention.

155 **B1 correction.** B1 correction on the IR-EPI data was unsuccessful on 5 data sets using the standard tools available in the
156 FreeSurfer suite, resulting in failed skull removal and inaccurate segmentations. We were able reduce B1 inhomogeneity by
157 applying an additional B1 correction before applying FreeSurfer tools. Our approach was to calculate a first-pass transform
158 for the mean functional and IR-EPI images (with the skull) and apply the transformation to the mean functional image. We
159 then utilized the B1 bias captured in the mean functional image to correct the IR-EPI anatomic images. Following the initial
160 coregistration, the mean functional image was smoothed and voxel-wise intensity scaled between $0.3v$ and $0.9v$ of its intensity
161 value v to prevent extreme values from unduly influencing bias correction. The IR-EPI was then divided by the scaled image,
162 and the result was taken as the corrected image. The corrected image could then be coregistered to the original mean functional
163 image and used as the input dataset for the standard FreeSurfer processing pipeline. We observed a marked improvement in
164 both the coregistration results and the results of the FreeSurfer segmentation and surface generation after performing this
165 correction (figure S2). Computation time was drastically reduced as well, in some cases up to 15 hours.

166 **Coregistration.** Within-subject coregistration was performed using the skull-removed mean functional image and the skull-
167 removed IR-EPI image. Using this image set mitigated registration error owing to image distortion typically observed in
168 EPI acquisitions. High quality coregistration was crucial to the laminar analysis featured in this experiment, as the accurate
169 definition of tissue boundaries in functional space follows only from a highly accurate coregistration of the structural and
170 functional images. Note also that the transformation computed in this step was applied to the structural image to avoid
171 introducing interpolation errors.

172 Several coregistration tools were used to calculate optimal image alignment. For a given subject, multiple transforms were
173 calculated and visually inspected. The best alignment as determined by visual inspection was taken for further analysis.
174 Volume coregistration was performed using FreeSurfer's robust, outlier insensitive registration cost function as implemented in
175 *mri_robust_register*. If the resulting transformation resulted in poor registration, we then used the NMI cost function
176 implemented in *mri_robust_register* and finally the NMI cost function implemented in AFNI's *3dAllineate*. If necessary,
177 manual improvements were applied to the best transformations generated by these tools. Registration quality was assessed by
178 visual inspection of alignment along the left occipitotemporal sulcus and brain edges.

179 In 11 subjects, failure to reconstruct surfaces from the IR-EPI image made it necessary to perform surface reconstruction
180 on MP2RAGE data, and therefore to bring the MP2RAGE surfaces into register with the functional data.. Following the
181 initial coregistration described above for the IR-EPI images, the IR-EPI images were generally in good alignment with the
182 task data and could serve as the source image for this purpose. In this case, MP2RAGE surfaces were aligned to the IR-EPI
183 volumes using FreeSurfer's boundary based registration program. If the IR-EPI volume was not in good alignment with the
184 functional data, an initial alignment between the MP2RAGE and functional data was first computed using the tools described
185 in the previous paragraph before performing the boundary based registration. The boundary based registration procedure is
186 described below in a dedicated section.

187 **Normalization for group analysis.** Functional data for each subject were mapped into MNI space for use in the whole-brain gPPI
188 group analysis. The skull-removed MP2RAGE image was first brought into alignment with the skull-removed mean functional
189 image. After inspecting the quality of the registration, the functionally aligned MP2RAGE images were aligned to the MNI128
190 template available in standard FreeSurfer installations. This transformation was concatenated with that obtained from the
191 inverted matrix from the initial coregistration and applied to the motion corrected functional data. The result of this spatial
192 normalization was MNI mapped functional data for each subject.

193 **Tissue segmentation and surface generation.** Tissue segmentation was performed in FreeSurfer using the skull-removed IR-EPI
194 image. Failures to properly reconstruct subject surfaces were addressed by inserting control points, applying additional
195 normalization as described in a previous section, or disabling the correction of defects in surface topology if they did not
196 occur in experiment critical regions. The IR-EPI images commonly included artifacts in noncritical locations that would
197 result in discontinuities in the surface and unsuccessful surface generation. As these defects did not often occur near LOTS,
198 it was possible to generate accurate surfaces even after bypassing correction. If surface reconstruction failed following these
199 interventions, the MP2RAGE dataset was used in place of the IR-EPI, and additional registration steps were applied (discussed
200 below).

201 **Boundary based registration.** Surfaces reconstructed from the IR-EPI image did not require additional alignment to the functional
202 data beyond resampling the FreeSurfer generated surfaces from “conformed space” to functional space. “Conformed space,”
203 native to FreeSurfer, is a 1mm isotropic 256^3 grid in the RAS coordinate system.

204 Surfaces reconstructed from MP2RAGE images underwent an additional registration step using *bbregister*, FreeSurfer’s
205 boundary based registration (BBR) tool. The goal of this procedure was to produce surfaces in register with the functional data.
206 As the IR-EPI and MP2RAGE data sets were generally well aligned from the coregistration procedure described previously,
207 the main purpose of the BBR was to find a solution accommodating the distortions affecting surface placement along the fROI.
208 Using a boundary based cost function, IR-EPI images that were unable to be used for surface generation were aligned with the
209 boundaries generated from the MP2RAGE images. The inverse of this transformation was then applied to the surfaces to align
210 the boundaries to the IR-EPI image. If the alignment generated by the boundary based registration procedure was found to
211 be inaccurate, simple solutions to improve accuracy involved optimizing the registration for the fROI through a weighting
212 mask, manual intervention, or improving the alignment of the two images prior to the boundary based registration. Failing a
213 simple solution, we also computed a nonlinear boundary based registration (41). In this approach, the registration algorithm
214 recursively divided and aligned surface segments to increase registration accuracy.

215 The importance of highly accurate image alignment in laminar resolution imaging cannot be overstated. In the present
216 work, registration inaccuracies in excess of 1mm had the potential to displace entire bins, leading to meaningless results. Great
217 care was taken to ensure accurate registrations and alignment of the surfaces with the functional images. As in the other
218 registration procedures, registration quality was assessed only through visual inspection of key anatomy.

219 **Equivolume contouring.** The gray matter volume of each subject was partitioned into equivolume bins using the OpenFmri
220 (<https://github.com/TimVanMourik/OpenFmriAnalysis>) implementation of the equivolume contouring approach described in Waehnert
221 et al. (42). The equivolume method increases the likelihood that the histological profile of each bin is consistent throughout
222 the given region.

223 We partitioned the gray matter volumes into 3 bins: the smallest number of bins which allowed for the dissociation of the
224 deep, middle and superficial contributions to the overall BOLD signal. For the purpose of the spatial GLM, it was necessary
225 to include two additional non-cortical bins representing white matter and CSF volumes respectively. The inclusion of these
226 additional bins was due to partial volume effects caused by voxels extending outside the cortical strip. Voxels observed within
227 these boundaries were assigned a value representing the fractional volume observed within a particular set of boundaries.

228 The ultimate output of this procedure was a 4D dataset whose first 3 dimensions represented spatial coordinates and whose
229 4th dimension represented the different bins. Incrementing over the 4th dimension indices gave the fractional volume of each
230 voxel found in that particular bin. This volume is referred to as the layer-volume distribution.

231 In our partition scheme, the volume subsuming the six histological layers was partitioned into three bins. We argue that the
232 histologically coarse bins were sufficient to dissociate top-down and bottom-up signal contributions. At the mesoarchitectural
233 level of lfMRI, it is not practical to measure individual histological layers. One common approach to this challenge has
234 been to consider a simplified model of layer interactions which merges supragranular (layers I,II,III), granular (layer IV) and
235 infragranular (layers V,VI) histological layers into three logical layers based on shared connection tendencies (1, 28, 37, 43, 44).
236 This model is based in large part on patterns of laminar connectivity discovered in Rockland & Pandya (45) when exploring the
237 link between anatomy and functional hierarchy, and schematized in the Felleman and Van Essen hierarchy (46). The simplified
238 laminar model has proven valuable when constrained by functional data, and has informed efforts in lfMRI.

239 **Physiologic noise removal.** Cardiac and respiration data were collected concurrently with the functional data using a pulse
240 oximeter and pneumatic belt. Physiologic regressor estimation up to the 6th (cardiac) and 8th (respiration) order was performed
241 using a modified version of the PhysIO toolbox of the TAPAS suite (47). These minor modifications were necessary to account
242 for unique log file formats produced by the equipment at the scan site. Regressors were then included in the design as nuisance
243 regressors. Regressor quality was assessed with a partial *F*-test.

244 **Non-laminar analysis.** The task design was fitted using the generalized least squares regression implemented in the OpenFmri
245 analysis suite. First level T -statistics were calculated in MATLAB version R2014B (The Mathworks Inc.) for condition and
246 contrast effects in each subject individually. Several versions of the first level analysis were performed to calculate parameter
247 fits in both native space and MNI space, and with different levels of spatial smoothing applied. This was necessary owing to
248 requirements of the different analyses reported in this work.

249 Native space data with were spatially smoothed with a 1mm and 4mm Gaussian kernel. Data smoothed with the 4mm
250 kernel were mapped to MNI space and used in the whole-brain gPPI analysis. Data smoothed with the 1mm kernel were
251 analyzed in the first level GLM used to identify the fROIs. Following fROI identification, the fROIs identified in each subject
252 were resampled to native resolution and used as an inclusive mask of the original resolution, native space functional data. The
253 voxels included in this mask then underwent depth-dependent signal extraction.

254 **Procedure to define functional region of interest.** Anatomically, the region of interest was located proximal to the fundus of
255 the occipitotemporal sulcus. It was functionally defined as a cluster of voxels which responded to visually presented words and
256 pseudo-words, but preferentially to pseudo-words. In addition, this region is known to express reduced BOLD amplitude to
257 false-font items compared to items composed of orthographically legal characters (48).

258 The region was defined in each subject through a series of masking operations implemented with AFNI's voxel-wise dataset
259 calculator *3dcalc*. These operations were performed on the t -statistics from the 1mm smoothed, native space analysis. First,
260 voxels were removed if they did not reach threshold in both the word and pseudo-word conditions. This was defined as a
261 t -statistic where $1 \leq t \leq 3$. T was initially set to $t = 2$ and was increased or reduced if the number of surviving voxels fell
262 outside of the desired range (see below). We then removed all voxels with a larger t -statistic for the false-font condition than
263 for either the word or pseudo-word conditions. Finally, voxels were excluded if the difference between t -statistics of words
264 and pseudo-words was larger than the original activation threshold. Clusters were considered for inclusion if they were 1)
265 located within the extent of the occipitotemporal sulcus, if 2) cluster size was between 100 and 400 voxels, if 3) 30-50%
266 of the voxels responded preferentially to the word condition over the pseudo-word condition, and if 4) the total response was
267 comparable between the word and pseudo-word preferred voxels when considering the proportion of voxels preferring each
268 condition. These criteria were selected to isolate a functional region which is known to respond preferentially to both words
269 and pseudo-words compared to false-font items, prefer pseudo-words to words, and contain a mixture of individual voxels which
270 prefer each condition. The fROI selection procedure was biased by design toward pseudo-word activation because stronger
271 pseudo-word activation is a functional feature of the region (48).

272 Further considerations were made with respect to the importance of cluster contiguity. Given the high spatial resolution of
273 our data, it was possible to distinguish populations of active voxels spanning the CSF boundary bridging the occipitotemporal
274 sulcus. Following the removal of the voxels located in CSF in some subjects, formerly contiguous clusters became distinguishable.
275 We determined that the most reasonable approach was to include formerly contiguous voxels in the laminar analysis. Given that
276 this region is often functionally defined and generally identified near the fundus of the occipitotemporal sulcus, partial volume
277 effects have almost certainly influenced fMRI measurements at standard resolutions. The decision to exclude populations
278 of voxels stranded on either side of the chasm would have proven arbitrary in that non-laminar studies investigating this
279 region typically lack the resolution to distinguish fusiform and inferior temporal populations. We concluded that allowing for
280 discontinuities in the left OTS fROI more faithfully adhered to the literature definition of the region than an ad hoc justification
281 for voxel removal. Native space functional ROIs for all participants can be seen in figures S3 and S4.

282 **Depth dependent signal extraction.** The fROI produced through the procedure described above was used to mask the layer-
283 volume distribution. This resulted in a layer-volume distribution specific to the fROI. By treating this distribution as a design
284 matrix such that rows were voxels and columns were bins, it was possible to regress it against the signal observed in the fROI
285 for each time point in the experiment (49, 50).

286 Fitting the voxel-volume distribution to each time point in the experiment yielded the relative contribution of each bin
287 to the overall signal at each time point, thereby representing a depth dependent time-series for each depth bin. These were
288 treated similarly to voxel time-courses and used to fit the task design.

289 The task design model was then fitted to the extracted depth dependent time courses. Percent signal change was calculated
290 as a division between β -weights assigned to each condition and the average weight assigned to the constant terms. The percent
291 signal change values were then analyzed at the group level in an ANOVA and subsequent two-tailed, paired t -test comparing
292 the responses to words and pseudo-words. T -statistics and ANOVA results were determined to be significant at $p = 0.01$.
293 These analyses were implemented in MATLAB.

294 **Statistical analysis.**

295 **Intraregional gPPI.** The gPPI analysis is a generalized version of the PPI analysis. In the generalized form, the analysis is designed
296 to span the full experiment space (51). In gPPI analysis, the first level model is extended by including the time-course of a
297 seed region in addition to interaction terms of the seed region with each task condition regressor.

298 As the goal of this analysis is to observe the effect of the interaction between the task and the neuronal response of a seed
299 region, a deconvolution is typically applied to the seed time-course before computing the interaction term. Given the problems
300 associated with deconvolution (52) and the novel nature of this work, we omitted deconvolution from our gPPI analysis. The
301 depth dependent hemodynamic response function (HRF) is not well understood. In the absence of this knowledge, the task

302 regressors used in this experiment were created using the canonical HRF. Deconvolution based on the canonical HRF would
303 have therefore exacerbated errors in modeling introduced by the initial convolution. Furthermore, the design of this study
304 sequentially presented five items of each condition type, essentially in 20 second blocks. O'Reilly et al. (52) have shown that
305 omitting deconvolution is not expected to greatly affect the outcome in block designs such as that used in the present study. We
306 therefore considered it more prudent to omit rather than include deconvolution in this analysis. There is no known precedent
307 for laminar specific gPPI. The gPPI design was created by adding seed region time-courses and interaction terms to the original
308 design. Interaction terms were calculated as the product of the detrended depth dependent time-series and binary condition
309 vectors (1 when a condition response was expected, 0 when it was not) derived from the task regressors. A time point was
310 included in the interaction term if the task regressor diverged from 0 by 0.0001.

311 Different models were created for each inter-regional analysis to assess the interaction between two depth- bins while
312 alternating seed/target assignment. We did not model the third remaining bin. Six models were created in total, each containing
313 six interaction terms (each of the six conditions multiplied by the seed-region time-course), the seed-region itself, and the
314 full design as discussed previously. Group effects were assessed using AFNI's *3dANOVA3*. Paired two-tailed *t*-statistics were
315 computed on the word and pseudo-word condition contrast. Results were deemed significant at $p = 0.01$.

316 **Bin to whole brain gPPI.** In a separate gPPI analysis, we modeled the task-dependent effect of the deep and middle bins on the
317 whole brain. The superficial bin was excluded from the whole brain gPPI for several reasons. The LOTS is hypothesized to
318 connect to left temporal cortex through either primarily bottom-up or top-down configurations (48). To distinguish among these
319 and thus explore the ability of fMRI to distinguish between top-down from bottom-up network arrangements, it was necessary
320 only to include the predicted top-down and bottom-up bins associated with word reading. Voxels within the superficial bin are
321 also susceptible to partial volume artifacts due to vessels on the pial surface, which could possibly affect the analysis. The
322 inclusion of the superficial bin would have required the gPPI model to include ten additional regression terms which would
323 have been collinear with the experimentally interesting deep and middle bin terms, and so this was not considered further in
324 the interests of a parsimonious data analysis.

325 The analysis was performed on the MNI normalized data with a 4mm Gaussian smoothing kernel applied. Alignment quality
326 was assessed partially on the basis of alignment accuracy of the middle temporal gyrus. Individual subject *t*-statistic maps were
327 used in conjunction with subject anatomy and the MNI template used for normalization to determine the alignment quality of
328 task critical regions. Inaccuracies in subject registrations were addressed with a manually created, secondary transformation
329 containing small translations intended to improve task critical region alignment without introducing large global inaccuracies.
330 As the experimental question related to the depth dependent connectivity to regions that respond to the word/pseudo-word
331 contrast, the use of the non-laminar first level maps to facilitate alignment was independent of the gPPI analysis. Group results
332 were assessed using AFNI's *3dANOVA3*, as in the previous section.

333 The parameters of the spatial AutoCorrelation Function (ACF) representing the smoothness of the data were computed
334 using AFNI's *3dFWHMx* on the residual time-series of first level analysis. The ACF parameters were used by the AFNI
335 program *3dClustSim* to compute the likelihood of random clusters given the ACF parameters 0.5815, 2.9134 and 8.3001, in a
336 volume of the dimensions $63 \times 82 \times 55$ with 2mm isotropic voxels. The dimensions of the volume used for permutation testing
337 were determined with a group level functional data mask. Clusters were deemed significant at $p_{\text{uncorr}} = 0.001$, $\alpha = 0.05$.

338 The task-independent connectivity from the deep and middle bins was assessed using the deep and middle bin time-courses
339 included in the gPPI model. The final results were visualized using the rendering plugin in AFNI. In addition to subjects
340 2 and 19, subject 4 was excluded from this analysis. We were unable to successfully bring subject 4 into MNI space. Large
341 inaccuracies in the registration resulted in the exclusion of this subject from the whole brain analysis. This subject was included
342 in all analyses performed in native space.

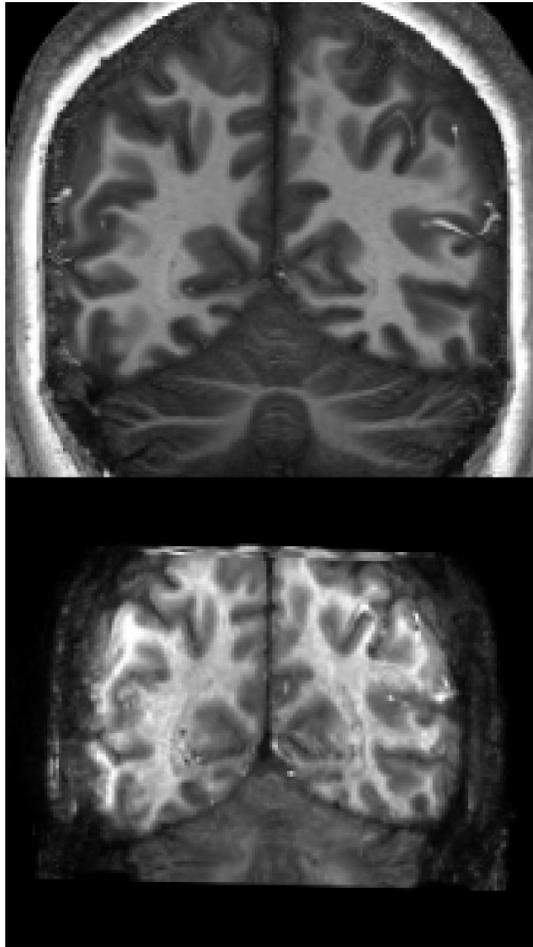


Fig. S1. Different anatomy acquisitions used in this experiment. MP2RAGE (top), IR-EPI (bottom). The left hemisphere is shown on the right side of the images.

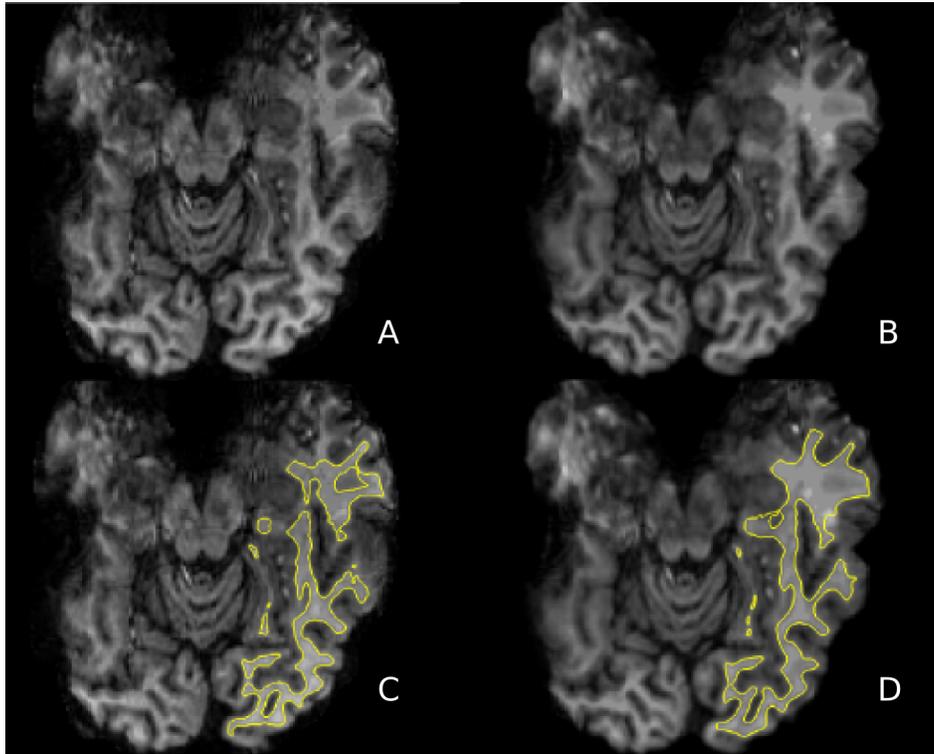


Fig. S2. Comparison of FreeSurfer white matter surface generation on the left hemisphere before and after supplemental B1 correction. (A) uncorrected; (B) corrected; (C) surfaces generated from A; (D) surfaces generated from B. The left hemisphere is shown on the right side of the image.

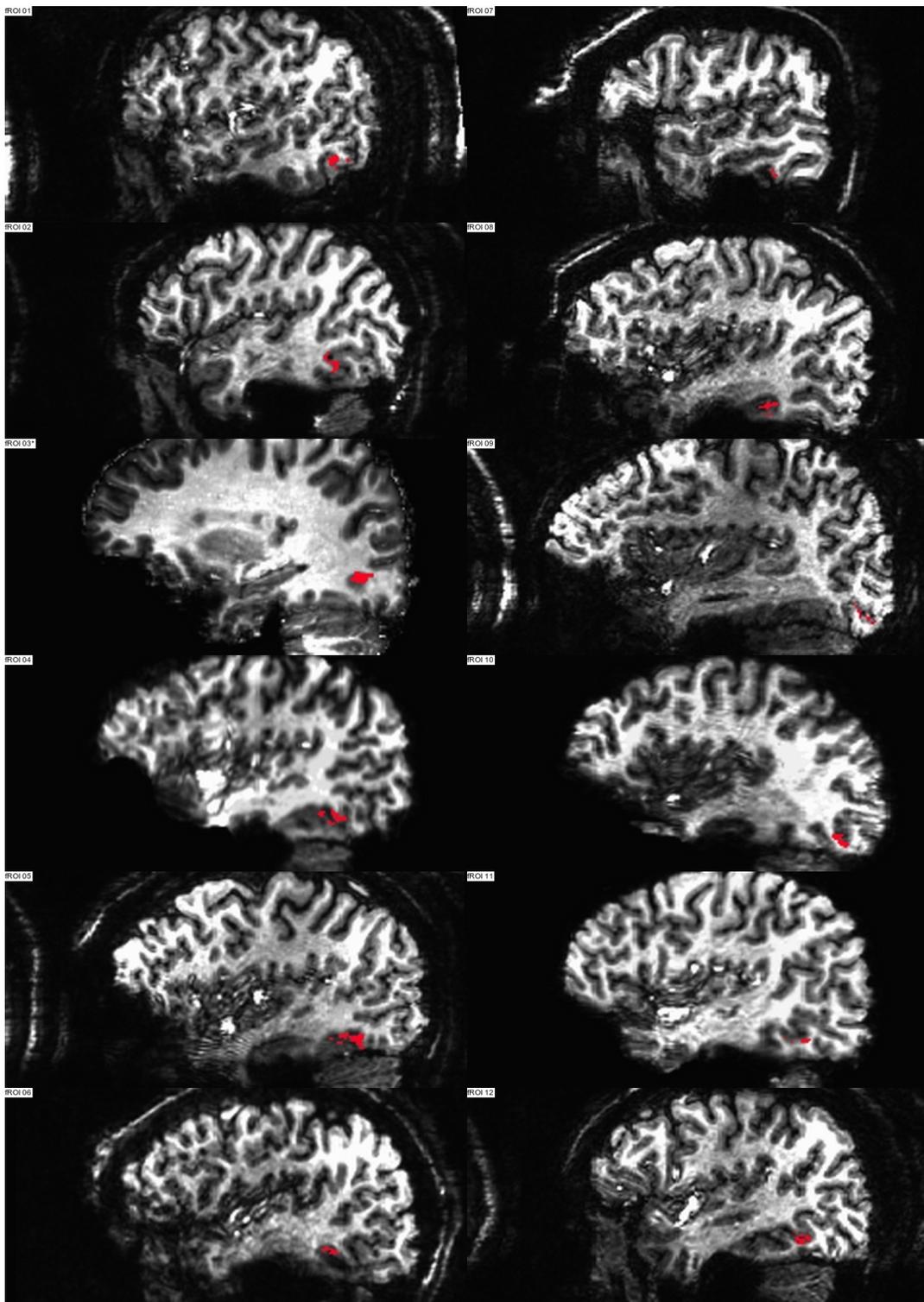


Fig. S3. Native space fROIs 1-12
*Subject 03 excluded from whole-brain gPPI analysis

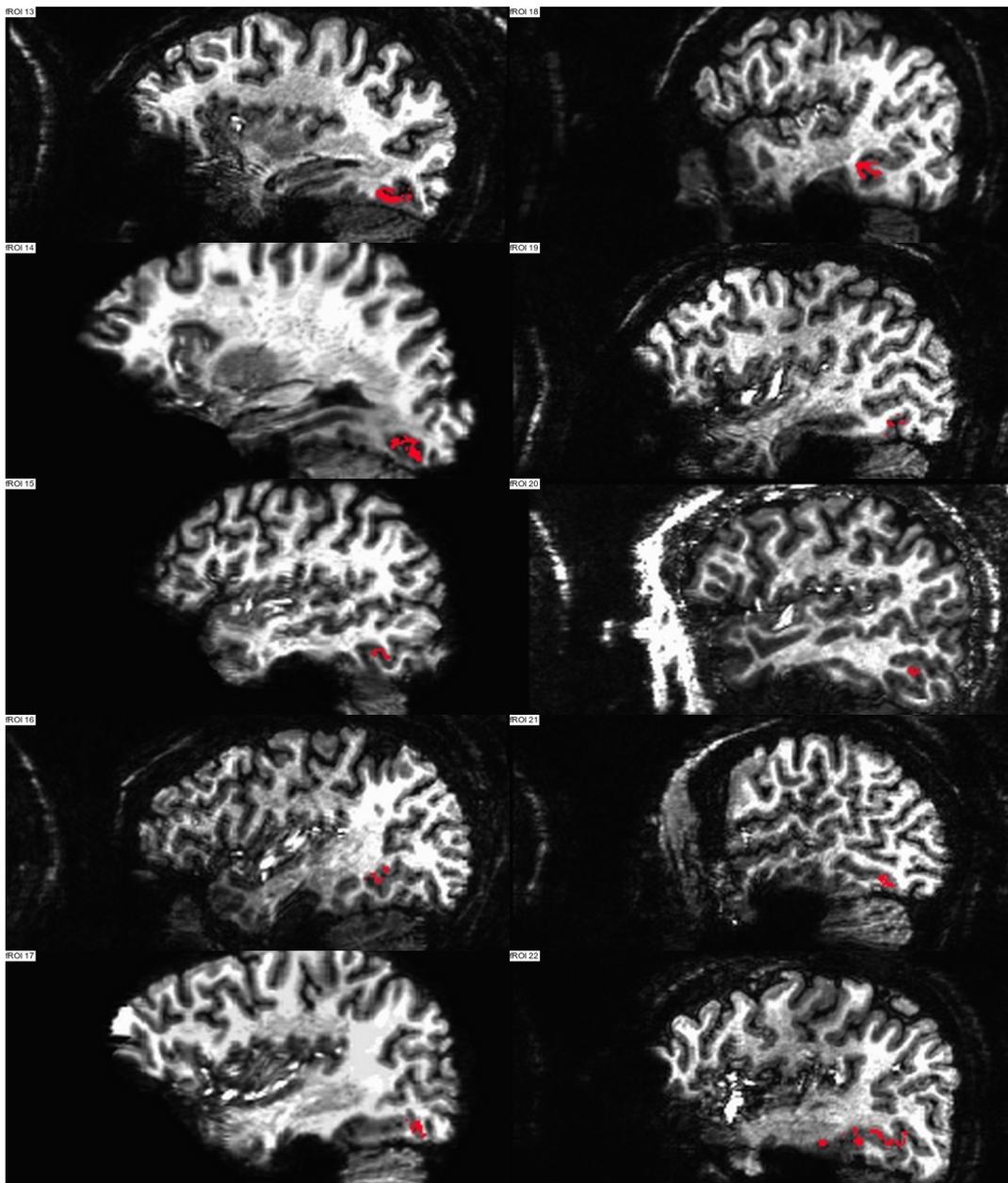


Fig. S4. Native pace fROIs 13-22

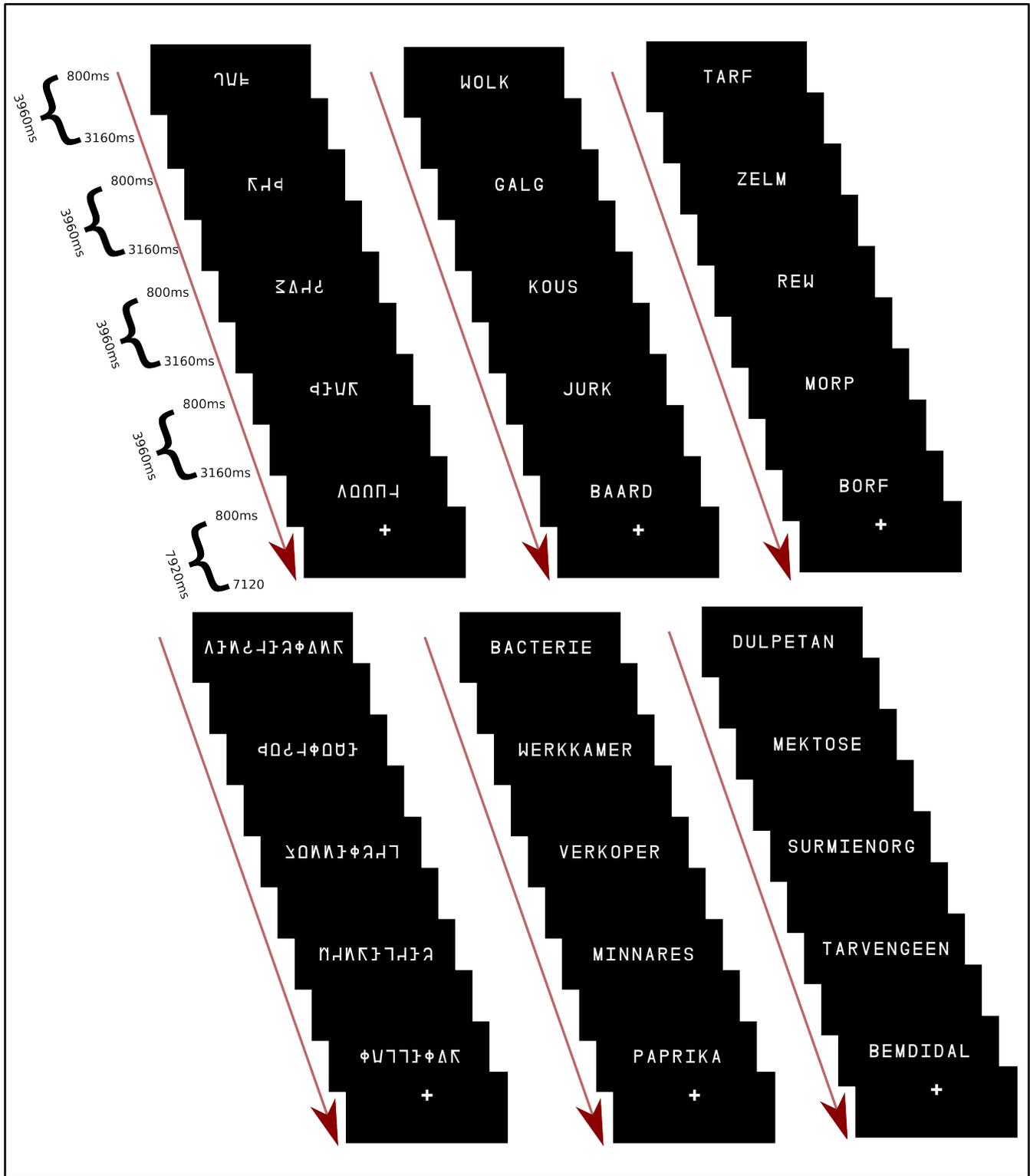


Fig. S5. Sample stimuli showing mini-blocks from each condition. Stimuli were presented in 5 trial mini-blocks with the items from the same condition presented in the trials within a block. The duration of each trial was 3960 ms. Each item was presented for 800 ms within the 3960 ms according to parameters obtained using optseq2. A black screen was presented for the remaining 3160 ms in each trial. A fixation was presented for the duration of one trial (3960 ms) at the beginning of each run and following each mini-block. From left to right: false fonts, words, pseudo-words. One syllable items are shown in the top portion and three syllable items are shown on the bottom.

343 **Additional data table S1 (StimulusList.xlsx)**

344 word and pseudo-word items

345 **Additional data table S2 (FalseFontItems.pdf)**

346 false-font items

347 **References**

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