

Selective visual representation of letters and words in the left ventral occipito-temporal cortex with intracerebral recordings

Aliette Lochy^{a,b,c,1}, Corentin Jacques^{a,b,d,1}, Louis Maillard^{e,f}, Sophie Colnat-Coulbois^g, Bruno Rossion^{a,b,e,f,2}, and Jacques Jonas^{a,b,e,f}

^aPsychological Sciences Research Institute, UCLouvain, B-1348 Louvain-La-Neuve, Belgium; ^bInstitute of Neuroscience, UCLouvain, B-1348 Louvain-La-Neuve, Belgium; ^cCognitive Science and Assessment Institute, Education, Culture, Cognition, and Society Research Unit, Université du Luxembourg, L-4366 Esch-sur-Alzette, Luxembourg; ^dCenter for Developmental Psychiatry, Department of Neuroscience, KU Leuven, B-3000 Leuven, Belgium; ^eUniversité de Lorraine, CNRS, CRAN, F-54000 Nancy, France; ^fUniversité de Lorraine, CHRU-Nancy, Service de Neurologie, F-54000 Nancy, France; and ^gUniversité de Lorraine, CHRU-Nancy, Service de Neurochirurgie, F-54000 Nancy, France

Edited by Brian A. Wandell, Stanford University, Stanford, CA, and approved June 25, 2018 (received for review November 3, 2017)

We report a comprehensive cartography of selective responses to visual letters and words in the human ventral occipito-temporal cortex (VOTC) with direct neural recordings, clarifying key aspects of the neural basis of reading. Intracerebral recordings were performed in a large group of patients ($n = 37$) presented with visual words inserted periodically in rapid sequences of pseudofonts, non-words, or pseudowords, enabling classification of responses at three levels of word processing: letter, prelexical, and lexical. While letter-selective responses are found in much of the VOTC, with a higher proportion in left posterior regions, prelexical/lexical responses are confined to the middle and anterior sections of the left fusiform gyrus. This region overlaps with and extends more anteriorly than the visual word form area typically identified with functional magnetic resonance imaging. In this region, prelexical responses provide evidence for populations of neurons sensitive to the statistical regularity of letter combinations independently of lexical responses to familiar words. Despite extensive sampling in anterior ventral temporal regions, there is no hierarchical organization between prelexical and lexical responses in the left fusiform gyrus. Overall, distinct word processing levels depend on neural populations that are spatially intermingled rather than organized according to a strict postero-anterior hierarchy in the left VOTC.

word reading | lexical representation | intracerebral recordings | SEEG | fusiform gyrus

The ability to read relies on the rapid mapping of perceived visual letters and their combinations (i.e., visual word forms) to phonology and meaning. The central role of the left ventral occipito-temporal cortex (VOTC) in processing letter strings, initially suggested by pure alexia in lesion studies (1–3), is now widely accepted (4–6). However, within this region, the precise organization for processing letters and their combination remains largely unknown. Here, we report a comprehensive functional mapping of the VOTC for selective responses to visual letter strings and words with intracerebral recordings in a large population of individual human brains. Objective identification and quantification of VOTC activity during rapid discrimination of words from pseudofonts, nonwords, or pseudowords (Fig. 1) shed light on key issues regarding the neural basis of visual letter and word processing.

A first outstanding issue is whether there is a hierarchy of increasingly complex linguistic processes along the postero-anterior axis in VOTC (7). According to this view, bilateral early visual cortices, V1 to V4, or even MT (8), extract oriented bars, local letter features, and case-specific letter shapes (9), which are then recombined into increasingly abstract letter string representations up to the middle section of the left lateral fusiform gyrus [the visual word form area (VWFA)] (3, 7). This view is supported by functional magnetic resonance imaging (fMRI) evidence: in posterior VOTC, the fMRI signal does not differ for

various categories of wordlike stimuli (pseudofonts, consonant strings, pseudowords), while in the (more anterior) VWFA, the fMRI signal is larger for words and pseudowords than for less wordlike stimuli (10). However, opposite results have been reported, with larger fMRI activity for less wordlike stimuli in the VWFA (11, 12). Moreover, the location of the brain region showing greater selectivity for letter strings as opposed to non-letters varies from the VWFA site ($y \simeq -54$ in Talairach coordinates) to more anterior VOTC regions ($y \simeq -42$) (13, 14) (for a recent metaanalysis, see ref. 15), depending on the contrasted material (checkerboards, pseudofonts, or symbols), the task, as well as the stimulus presentation time.

In general, whether the functional organization of the VOTC obeys a hierarchy of increasingly complex representations from individual letters to words (10), and if so up to which region, has proved difficult to resolve not only due to methodological differences between studies but moreover because the fMRI signal-to-noise ratio (SNR) is not equivalent across the VOTC, with large magnetic susceptibility artifacts in its anterior portion (4). In this context, a complementary approach to increase understanding of the function of the VOTC in reading, which is not affected by such artifacts, is to record electrical field potentials in awake patients implanted with intracranial electrodes. While

Significance

The left ventral occipito-temporal cortex (VOTC) is a critical part of the reading circuitry. We made measurements with intracerebral electrodes in 37 participants to understand whether this region contains functionally separated brain loci for processing letters and words. Letter-selective responses are found in much of VOTC. Responses to word forms are absent in posterior VOTC but are present and intermingled with letter-specific responses in left anterior VOTC. The results are inconsistent with a hierarchical model in which posterior regions uniquely perform letter identification functions and increasingly anterior regions perform increasingly complex linguistic functions.

Author contributions: A.L., C.J., B.R., and J.J. designed research; A.L., C.J., S.C.-C., and J.J. performed research; A.L., C.J., and J.J. analyzed data; and A.L., C.J., L.M., B.R., and J.J. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Published under the PNAS license.

Data deposition: The raw electrophysiological data used for analyses in this paper, along with the xyz coordinates for the position of the electrodes, and the stimuli used have been deposited in the Dryad Digital Repository (doi:10.5061/dryad.6s39h64).

¹A.L. and C.J. contributed equally to this work.

²To whom correspondence should be addressed. Email: bruno.rossion@univ-lorraine.fr.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1718987115/-DCSupplemental.

Published online July 23, 2018.

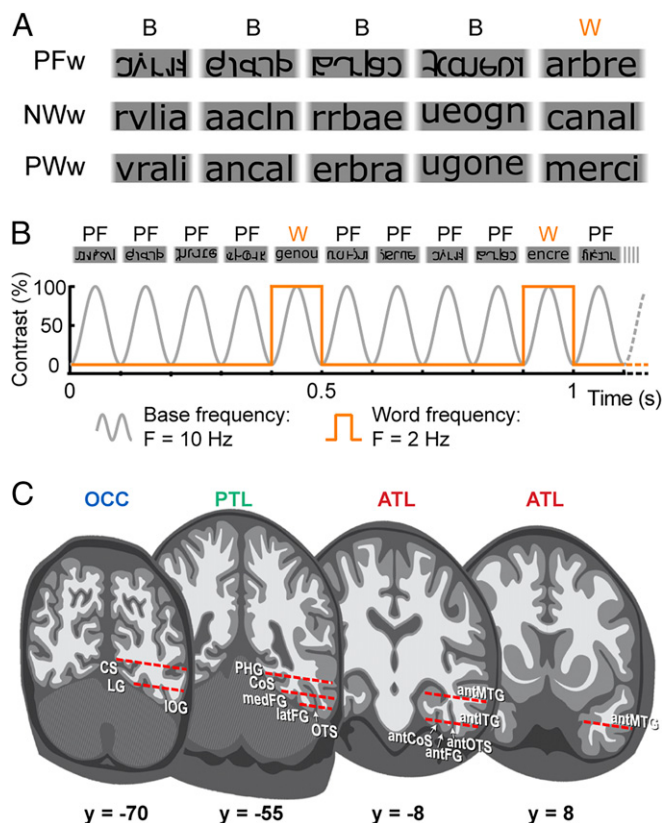


Fig. 1. Fast periodic visual stimulation (FPVS) and SEEG methods. (A) Experimental conditions and example stimuli. Words (w) are inserted within three different types of base stimuli (B), that is, in three conditions: words in pseudofonts (PFw), in nonwords (NWw), and in pseudowords (PWw). (B) FPVS paradigm. Sequences of PW, NW, and PF are presented at a rate of 10 Hz through sinusoidal contrast modulation with words inserted every fifth item, at 2 Hz, that is, 10 Hz/5 (the PFw condition is represented here). (C) Schematic coronal representation of the typical trajectories of depth electrodes implanted in the VOTC. Electrodes consist of 8–15 contiguous recording contacts (red rectangles) spread along the electrode length, along the medio-lateral axis. Acronyms: ant, anterior; ATL, anterior temporal lobe; CoS, collateral sulcus; CS, calcarine sulcus; FG, fusiform gyrus; IOG, inferior occipital gyrus; ITG, inferior temporal gyrus; lat, lateral; LG, lingual gyrus; med, medial; MTG, middle temporal gyrus; OCC, occipital lobe; OTS, occipito-temporal sulcus; PHG, parahippocampal gyrus; PTL, posterior temporal lobe.

fMRI measurements typically reflect a general elevation of neural activity only partly due to stimulus-locked responses, intracranial recordings may isolate stimulus-locked responses (16). A seminal study using subdural grids of electrodes [electrocorticography (ECoG)] reported that various letter strings (words, pseudowords, or nonwords) generate an equivalent intracranial response (N200) over the posterior fusiform gyrus, while selectivity to words interpreted in terms of semantic processing was found only in more anterior sections of the VOTC (17). More recently, letter-selective responses (consonant strings > pseudofonts) were found in a more posterior region of the left fusiform gyrus than word-selective responses (words > consonant strings) (18). These observations support a hierarchy of word form processing in the VOTC. However, these recent recordings were performed in targeted VOTC subregions, such that a comprehensive mapping and quantification across the whole VOTC for selective responses to letter strings and words aiming at thoroughly evaluating its hierarchical organization, is currently lacking. Moreover, in these studies, the use of ECoG, which is restricted to the gyral surface, may have underestimated responses arising from sulci. The success of a comprehensive mapping for letter and word processing may thus be enhanced by

evenly measuring responses from both gyri and sulci with depth intracerebral electrodes, and by a relatively large recording sample (see ref. 19).

A second outstanding issue regarding the neural basis of letter and word processing is whether the VOTC, in particular the VWFA, is sensitive to whole-word form (i.e., lexical) representations of written strings or is only tuned to prelexical factors characterizing letter strings (e.g., orthographic structure, such as frequently co-occurring letters in a given orthography). This issue is usually operationalized by testing for differential representation of visual real words (lexical items) and pseudowords (orthographically and phonologically plausible sequences of letters) in the VWFA. Here again, previous studies yielded conflicting findings. On the one hand, most fMRI studies did not report differential responses to words vs. pseudowords in this region (10, 20, 21), supporting a prelexical representation level in the VWFA (3, 7, 22). On the other hand, larger responses have been found in this region for pseudowords vs. words (23–26), and for nonfrequent vs. frequent words (27); these observations being interpreted in terms of neural sensitivity to whole-word forms, thus lexical representation. fMR-adaptation paradigms have also shown that the decrease of the response due to pseudoword repetition depends on the number of repeated letters, with a single letter identity change in words resulting in full release from adaptation (24, 25), hence supporting whole-word form coding. Finally, recent intracranial electrophysiology findings suggest that words and pseudowords recruit identical populations of neurons in the VWFA but at different timescales (28). However, the stimuli compared in that study differed only by prelexical factors (number of common letters or bigram frequency). Moreover, since depth recording in three individual brains was limited to the left VWFA only, whether differential responses to words and pseudowords extend to other VOTC regions remains unknown.

Here, we clarify these issues by providing a comprehensive functional mapping of the VOTC for selective responses to visual letter strings at the level of letter recognition, prelexical, or lexical processing. We report data of 37 patients (60 hemispheres) implanted in the VOTC with multiple intracerebral electrodes, each comprising 8–15 recording contacts (2,172 recording contacts in the gray matter) during stereoencephalography (SEEG) (Fig. 1C). In our paradigm, validated in scalp EEG studies (29, 30), visual words appear periodically among different stimuli presented at 10 Hz and varying in wordlikeness (Fig. 1*A* and *B*): (*i*) pseudofonts (PF; [Movie S1](#)), (*ii*) letter strings that form orthographically implausible words [nonwords (NW; [Movie S2](#))], or (*iii*) letter strings that form orthographically plausible words [pseudowords (PW; [Movie S3](#))].

We classified each intracerebral recording contact based on the pattern of present (+) or absent (−) significant responses in these three conditions. Three response patterns have a clear theoretical interpretation, reflecting increasingly complex linguistic processing levels. First, contacts with a significant response only to words among PF but not among NW or PW (+PFw, −NWw, −PWw) reflect a letter-selective neural response. Second, discrimination of words from PF and NW but not from PW (+PFw, +NWw, −PWw) reflects a prelexical level of processing. Finally, discrimination of words in all three conditions (+PFw, +NWw, +PWw) reflects a lexical processing level. We focus on these three response patterns and refer to these responses as “word discrimination responses” in the manuscript. Importantly, wordlike stimuli (PF, NW, PW) are presented at a rapid rate of 10 Hz in different 70-s streams, with words inserted every five items (i.e., every 500 ms or 2 Hz; Fig. 1 *A* and *B*; [Movies S1–S3](#)). In these conditions, briefly presented words (100-ms stimulus onset asynchrony) are forward- and backward-masked by wordlike stimuli, and word discrimination neural responses can be objectively identified and quantified in the intracerebral EEG spectrum at 2 Hz and harmonics (see refs. 19 and 31 for this frequency-tagging approach in intracerebral SEEG mapping). Moreover, the rapid presentation rates and low attentional-demand tasks reduce the involvement of higher level (semantic) processes (10).

A strict hierarchical view of letter string representation in the VOTC predicts a posterior-to-anterior hierarchy of responsive contacts following the three levels of word discrimination tested. Moreover, if the VWFA responds only to statistical mappings of recurring letters and not to whole-word forms (3, 10), words should not be discriminated from PW in this region but rather in the ventral anterior temporal lobe, extensively sampled here. However, if the VWFA is tuned to whole-word forms (25), contacts reflecting lexical discrimination should be found in this region, with a potential intraregion hierarchical organization (i.e., prelexical posterior to lexical anterior contacts).

Results

Despite the brief recording time (two to six sequences of 70 s per condition), high SNR word discrimination responses were identified in the VOTC exactly at the word presentation frequency (2 Hz) and its harmonics following fast Fourier transform (FFT) (Fig. 2A) of SEEG data. Significant word discrimination responses were determined based on a combination of the first four harmonics (i.e., 2, 4, 6, and 8 Hz; Fig. 2B) and a z-score transform ($z > 3.1$, $P < 0.001$; Fig. 2C).

Broad Distribution of Word Discrimination Responses Along the Left VOTC. Across the VOTC, we found 212 significant word discrimination contacts in 27 participants, that is, 9.8% of all recorded contacts. Note that word discrimination contacts can be letter selective (+PF, -NW, -PW), prelexical (+PF, +NW, -PW), or lexical (+PF, +NW, +PW). Every word discrimination contact was labeled according to the individual anatomy (Table 1 and Fig. 3A), using a topographic parcellation of the VOTC (*SI Appendix, Fig. S1*; see ref. 32). Word discrimination contacts were distributed broadly across the VOTC with two prominent features: (i) these contacts were much more numerous in the left hemisphere; and (ii) their density was highest around the left fusiform region (Fig. 3A and *SI Appendix, Fig. S1*). In addition, anterior temporal lobe (ATL) word discrimination contacts were mostly restricted to its posterior part, close to the junction with the posterior temporal lobe (PTL) (Fig. 3A).

Overall, the proportion of word discrimination contacts relative to all recorded contacts (i.e., responsive and unresponsive) was much higher in the left than in the right hemisphere (left: 169/1,326, 12.7%; right: 43/846, 5.1%; two-tailed permutation test, $p < 0.0001$). To estimate and visualize the prominence of word discrimination contacts at a more local level over the group of participants, we computed maps of the proportion of word discrimination contacts relative to all recorded contacts (Fig. 3B). In the left hemisphere, the proportion of word discrimination contacts was significantly above zero ($P < 0.01$) in the ventro-medial occipital cortex (VMO) and all along the left fusiform gyrus and adjacent sulci (from the posterior fusiform gyrus close to the occipital lobe to the anterior fusiform gyrus). We found a similar pattern of results when considering the local proportions of individual brains showing word discrimination contacts (*SI Appendix, Fig. S2*). In the right hemisphere, the proportion of contacts was also significantly above zero along the fusiform gyrus with a maximum in the posterior lateral fusiform gyrus (latFG) (Fig. 3B). Nevertheless, the proportion of word discrimination contacts was significantly higher in the left hemisphere in part of the VMO and along the fusiform gyrus (Fig. 3C).

Spatial Dissociation Between Letter and Word Processing Levels. Fig. 4 illustrates the classification of letter-selective (+PFw, -NWw, -PWw), prelexical (+PFw, +NWw, -PWw), and lexical (+PFw, +NWw, +PWw) word discrimination contacts with the data of a few individual participants (*SI Appendix, Fig. S3*). The respective number of contacts at each level of discrimination is shown in Table 2. The other classes of response patterns, that is, significant responses in at least one condition but without an unambiguous a priori theoretical interpretation (e.g., -PFw, +NWw, +PWw, exhibiting a discrimination response for words presented among the most but not the least wordlike conditions; *SI Appendix, Table S1*), were not considered in the main analysis.

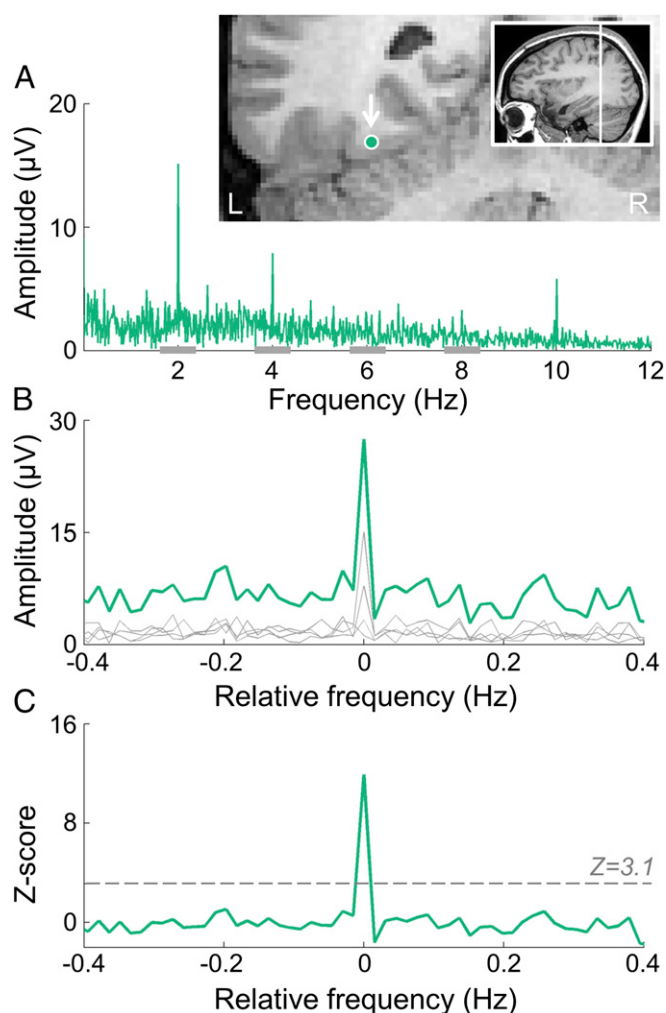


Fig. 2. Objective and high-SNR intracerebral word discrimination responses recorded in the VOTC. (A) SEEG frequency-domain responses recorded at an individual recording contact in the PFW condition. The displayed contact was the 10th best of this type, with an average response amplitude of 21 μ V, while the best contact had a response amplitude of 86.8 μ V. The anatomical location of the contact (in the left latFG, white arrow) is shown in a coronal MRI slice. Word discrimination responses are observed at the exact word stimulation frequency and harmonics (mainly at 2 and 4 Hz). (B) Significant word discrimination responses were determined by first segmenting the FFT spectrum into four segments centered at the frequency of word stimulation and its harmonics up to 8 Hz (i.e., 2, 4, 6, and 8 Hz). Individual FFT segments are shown in gray (see horizontal gray bars on the X axis in A, representing the length of each FFT segment). The four segments, containing both the signal and the surrounding noise, were then summed (green line). The 0 mark corresponds to the word stimulation frequency. (C) Z-score transformation of the summed FFT spectrum for statistical purpose. The Z score at the word stimulation frequency exceeds 3.1 ($P < 0.001$), indicating that this contact shows a significant word discrimination response.

This led to the exclusion of 16.2% of the contacts. Although interpretation of these responses is not straightforward, these contacts may reflect subtle lexical and prelexical processes (*Discussion*). Therefore, we performed the same analyses as presented here, including these contacts, yielding virtually identical findings (*SI Appendix, Fig. S4*).

The spatial organization of the three types of word discrimination contacts across all individual brains is displayed in Fig. 5A. Letter-selective contacts were the most frequent and were widely distributed across all individually defined anatomical VOTC regions [VMO, inferior occipital gyrus (IOG), medial fusiform

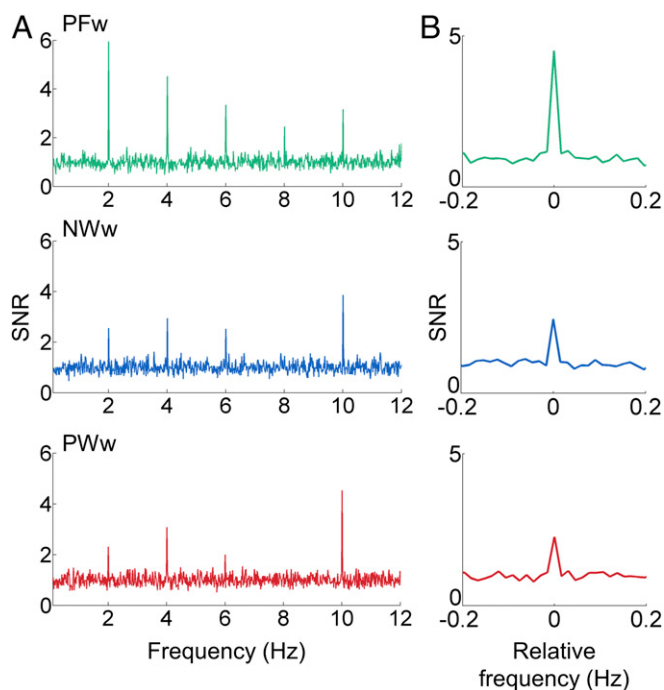


Fig. 7. SEEG lexical responses over the left hemisphere. (A) SNR frequency spectra recorded over the 18 lexical contacts in the left hemisphere for the PFw (top row), NWw (middle row), and PWw (bottom row) conditions. Raw FFT spectra were first averaged across the 18 lexical contacts separately for each condition and then transformed to SNR (as the ratio of the amplitude at the frequency bin of interest to the averaged amplitudes at neighboring bins). (B) Data from A replotted as summed-harmonic segmented FFT spectra (summed FFT spectra were averaged across the 18 lexical contacts separately for each condition and then transformed to SNR).

cortical hierarchy of reading. During reading acquisition, letters are first learned in isolation, that is, as single symbols representing specific phonemes, leading to an early left hemispheric lateralization in their cortical representation (30). Letters may be combined into syllables, morphemes, and words, and new combinations of letters may be encountered throughout the lifetime as new words. The independent representation of letters from words is supported by neuropsychological case studies of pure alexia, where patients cannot read words “at a glance” but can still recognize and name letters one by one (33, 34).

Another finding of the current study is that letter-selective responses were found up to the middle and even anterior FG, with a progressive decrease in proportion, not magnitude, of responses along the posterior-anterior axis (Fig. 6). This suggests that, while letters are selectively coded in the left posterior VOTC, this process extends in a distributed network of regions and overlaps with higher-level processes in anterior VOTC. This is consistent with the greater selectivity for words vs. pseudofonts even in anterior ventrotemporal regions in fMRI (e.g., refs. 10 and 13). Such larger responses to words (vs. pseudofonts) in these anterior regions have been interpreted as showing higher selectivity for increasingly large segments of letter strings (7, 10), or as reflecting phonological or semantic feedback (32, 35). Here, our data reveal letter selectivity even in anterior regions of the VOTC: on the very same contacts, words may not be distinguished from other alphabetic strings.

Identification of the Visual Word Form Area in Intracerebral Recordings. In our cartography of the VOTC, the left midFG showed the largest letter-selective response in amplitude across all significant contacts (*SI Appendix, Table S2*) and, most importantly, showed significant responses to words among other alphabetic strings (nonwords or pseudowords). This region corresponds relatively well to the VWFA as found in fMRI. While

previous intracranial studies did not identify this region (17), or targeted directly and solely the midFG (28), it emerges here from our sampling of the whole VOTC with direct intracerebral recordings. While the range of coordinates of the region found here overlaps with the VWFA, the average coordinate is slightly anterior ($y \approx -40$) to the VWFA as typically defined in fMRI (e.g., $y \approx -54$ in ref. 3), with individual electrode contacts up to $y = -25$ here (Fig. 6B). As presented in the Introduction, this difference may be due to difficulties in recording the anterior section of the VOTC in fMRI due to large magnetic susceptibility artifacts (4). This observation further illustrates the additive value of human brain mapping with intracerebral recordings, in particular with a large sample of participants, a wide spatial coverage, and objective quantification of significant responses as afforded by EEG frequency tagging (31).

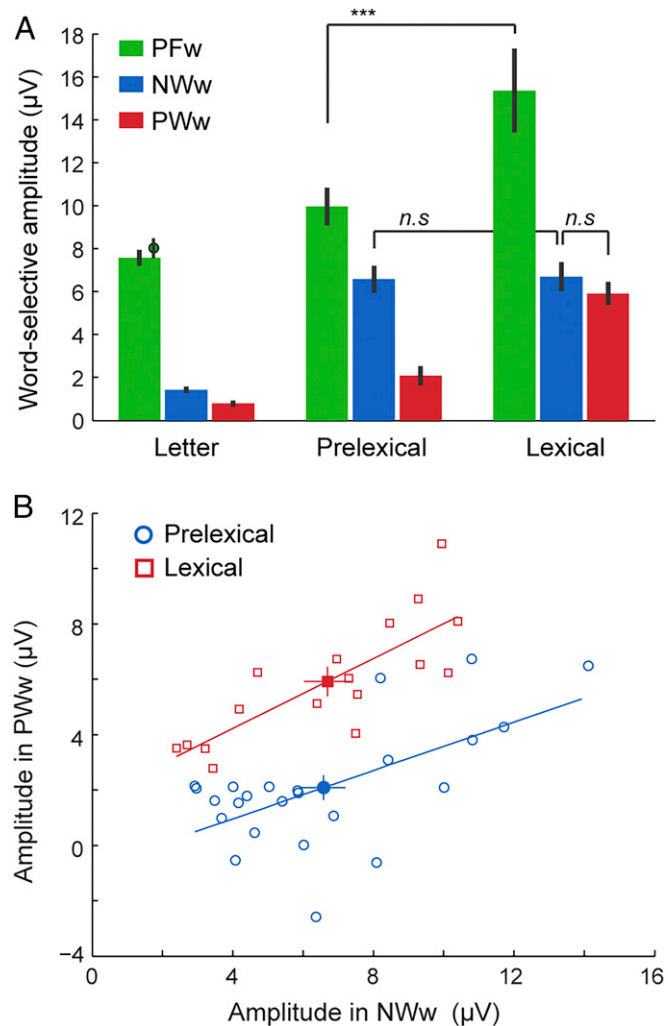


Fig. 8. Amplitude quantification across conditions and processing levels. (A) Response amplitudes were quantified as the average of the amplitudes across recording contacts, separately for each processing level (letter, prelexical, and lexical) and each condition (PFw, NWw, and PWw). The additional data point over the left-most column represents the averaged response amplitude over contacts located in the same anatomical regions as the prelexical and lexical contacts (latFG, medFG, antFG, antOTS, and ant-CoS). (B) Relationship between the amplitude in the PWw and the NWw conditions, separately for the prelexical and the lexical recording contacts. Each white-filled point is a single contact, and the mean across contacts is shown as filled markers. Lines represent the least-square linear fit computed separately for prelexical and lexical contacts. Error bars are the SEM.

An Objective Lexical Representation in the Left midFG. First, we found strong evidence for neuronal populations in the left midFG sensitive to familiar, known words, supporting visual lexical representations of written words. Whether the VWFA, and the VOTC in general, hold visual lexical representations is the matter of a long-standing debate in the scientific reading community. On the one hand, the VWFA is thought to compute abstract but prelexical representations (3, 7); on the other hand, it is thought to be tuned for whole-word forms and to discriminate words differing only by one letter (24, 25, 28). The present observations provide original and direct evidence supporting the latter view. Second, despite a wide sampling of the VOTC, including the most anterior ventral temporal regions, significant lexical responses were found almost exclusively in the left midFG (i.e., including the location of the VWFA), highlighting the fundamental role of this local region in reading. Note that this conclusion also holds even when lowering the statistic threshold (*SI Appendix, Fig. S5*) and when including response patterns that do not obey our strict criterion of significance for the three contrasts (*SI Appendix, Fig. S4*). Indeed, a number of additional contacts show responses to words only when presented among letter strings but not among pseudofonts (*SI Appendix, Table S1*). These contacts could potentially reflect more subtle processes, for example, selectivity to word forms only in the context of letters, that is, when a baseline level of activity is sufficiently high.

Both the large number of highly variable word and pseudoword stimuli used in the present study, and the strict control of stimuli in which pseudowords shared the same letters as words in a rearranged order (*Materials and Methods* and ref. 29) rule out an account of lexical responses in the midFG in terms of mere physical differences between words and pseudowords. This is supported by the lack of lexical responses in posterior visual regions sampled in this study. Moreover, although words and pseudowords differed in bigram frequency (*Materials and Methods*), and this factor can modulate VWFA activity in fMRI (36), lexical responses in this paradigm are unlikely to be related to this, for several reasons. First, in scalp EEG, the robust selective response over the left occipito-temporal cortex for words inserted in pseudowords is not reduced when bigram frequency is equated (29). Moreover, directly contrasting pseudowords differing significantly in bigram frequency does not lead to any EEG activity (29). Obviously, this does not completely rule out putative effects of bigram frequency inside the brain that would not be captured on the scalp. In this context, however, there is evidence that lexical contacts in the present intracerebral study do not show a modulation of the response according to bigram frequency. Specifically, while the difference in bigram frequency with words is much higher for NW ($7,387 \pm 3,291$) than PW ($2,979 \pm 4,014$), NWw responses are not significantly larger than PWw responses in lexical contacts (Fig. 8A). Finally, a complementary analysis (*SI Appendix, Fig. S6*) shows that the modulation of bigram frequency in different PWw sequences does not correlate with the response amplitude in these sequences.

Given the high stimulus presentation rate (10 Hz) as well as the forward and backward masking provided by the stimuli in the sequence (Fig. 1), we would like to argue that word selectivity effects found here likely reflect neural coding for visual word forms rather than integration of an abstract visual form with higher-order factors stemming from putative feedback from anterior language areas (32, 37). Supporting this view, we found no evidence of representations of letters and words anteriorly to the midFG in the left VOTC, contrary to intracranial findings with a slower stimulus presentation rate, revealing late semantic effects (17). Future studies could examine this issue further by defining the timing of lexical effects in the VOTC. This investigation is possible with the approach used here (e.g., see ref. 38) but requires inserting more stimuli between word items, reducing SNR as word repetition decreases in a given sequence length.

Interestingly, the contacts classified as lexical discriminate words from nonwords and pseudowords with a similar range of response amplitudes (Fig. 8). This result argues against additive processing

stages (i.e., prelexical then lexical) at different time points, or parametrically modulated sensitivity to orthographic structure. Indeed, both of these modes of processing would have generated larger responses to words in the NWw than in the PWw condition in lexical contacts. Rather, in these left midFG populations of neurons classified as lexical, whole-word recognition is relatively independent of the letter strings' plausibility context.

Since responses to words among nonwords (NWw) are not larger in lexical than prelexical contacts, these contacts do not differ only quantitatively, that is, by a generally larger response in lexical contacts. However, the larger response to words among pseudofonts on these lexical contacts suggests that neuronal populations tuned to lexical representations could be less activated by nonletters. These representations could therefore reflect a recognition pattern within an orthographic memory system (26) or a storage of whole-word forms, that is, a lexicon (24, 25, 39). Such a unique visual coding of individual words, allowing fast recognition processes, is necessary for fluent reading. This is consistent with neuropsychological studies showing that lesions in the left midFG cause deficits in word reading, leading to slow and effortful letter-by-letter reading (2, 34, 40, 41).

Nonhierarchical Spatial Prelexical and Lexical Representations. Finally, a crucial finding of our intracerebral recording study is that different levels of word processing appear intermingled in the same region of the midFG: besides the lexical responses identified, some contacts responded to words among orthographically implausible nonwords (i.e., prelexical response) and not to words among pseudowords, without a clear spatial hierarchy between the two types of contacts (Fig. 5). This finding—which also holds when lowering the statistic threshold (*SI Appendix, Fig. S5*) and when including contacts that do not conform to the three strict combinations of conditions to define the three processing levels (*SI Appendix, Fig. S4*)—does not support the proposal of strict successive stages for the coding of letters, bigrams, and quadrigrams at specific positions along the y axis [$y = -64$, -56 , and -48 respectively; LCD model (7)]. Indeed, according to this view, lexical contacts should have been located, on average, more anteriorly than prelexical contacts. Also, many contacts with the largest responses in the NWw condition did not discriminate words among pseudowords, ruling out a simple explanation in terms of magnitude of response (Fig. 8). Rather, responses to words at a prelexical level only may be considered as indirect evidence for responses to pseudowords: populations of neurons that are sensitive to the plausibility or statistical regularity of letter combinations rather than to letter strings previously encountered, that is, whole-word forms.

Both the spatial organization of prelexical and lexical contacts and the absence of additivity of responses in lexical contacts strongly suggest that prelexical processes and lexical processes are functionally separated (Fig. 8B) rather than constituting ordered additive, spatially hierarchical, stages in visual word recognition. Hence, within the midFG region, distinct neuronal populations compute different levels of representation of words: a letter-selective representation coding for abstract letters; a coarse word representation sensitive to visual characteristics such as plausibility of letter combinations; and a precise, fine-grained representation where neurons have become tuned to previously encountered words. This finding was afforded by our approach of intracerebral recordings where each electrode contact records electrophysiological responses from a limited neuronal population, compared with commonly used fMRI analyses where spatial smoothing and region-of-interest approach average responses originating potentially from different neuronal populations.

In summary, our large-scale intracerebral recording study with fast periodic visual stimulation clarifies the neural basis of reading by revealing (i) a spatial dissociation in the left VOTC between a posterior IOG/FG letter-selective representation, and a representation for words, at prelexical and lexical levels, confined to the middle and anterior section of the left fusiform gyrus; (ii) true lexical responses, that is, fine-tuning to real words, in a region extending more anteriorly than the visual word form area typically

identified with fMRI; (iii) prelexical responses suggesting sensitivity to the statistical regularity of letter combinations only; and (iv) a lack of spatial organization between qualitatively distinct prelexical and lexical responses in the left fusiform gyrus.

Materials and Methods

Participants. The study included 37 native French-speaker participants (20 females; mean age, 33 ± 8.4 y; 35 right-handed) undergoing clinical intracerebral evaluation with depth electrodes (SEEG) (42) for refractory partial epilepsy, studied in the Epilepsy Unit of the University Hospital of Nancy between September 2013 and June 2016. Participants with at least one intracerebral electrode implanted in the VOTC were included in the study (Fig. 1C). They gave written consent to participate to the study, which was approved by the Ethics Committee of the University Hospital of Nancy.

Intracerebral Electrode Implantation and Recording. Intracerebral electrodes were stereotactically implanted within the participants' brains for clinical purposes, that is, to delineate their seizure onset zones (43). Each 0.8-mm diameter intracerebral electrode contains 8–15 independent recording contacts of 2 mm in length separated by 1.5 mm from edge to edge (for details about the electrode implantation procedure, see ref. 44). Intracerebral EEG was sampled at a 512 Hz and referenced to either a midline prefrontal scalp electrode (FPz, in 32 participants) or an intracerebral contact in the white matter (in five participants).

Fast Periodic Visual Stimulation Paradigm. The paradigm was previously validated in a scalp EEG study (29).

Stimuli. Stimuli were words, pseudowords, nonwords, and pseudofonts (30 of each type), all composed of five elements (letters or pseudofonts) (Fig. 1A). French words were selected from the Lexique 3.55 database (45) with the following criteria: they were frequent common nouns (84.99 per million) in singular form, with limited orthographic neighbors (average, 1.9; range from 0 to 4), no foreign language origin, and no accents. PW and NW were built on an item-by-item basis by rearranging the letters of the words [e.g., from the word "avril" ("April") were built the PW "vrali" and the NW "rvlia"]. PF items were also built on an item-by-item basis: letters from words were vertically flipped, segmented, and segments were rearranged into five pseudoletters with the same overall size as the original word. Each word thus had a corresponding PW, NW, and PF containing the exact same amount of black-on-white contrast, so that all conditions were similar in terms of lower-level visual properties. Bigram frequencies were calculated with Wordgen (46) and are reported as summated type bigram frequencies (from the French CELEX database). As in Lochy et al. (29), bigram frequencies differed between words (mean $\pm$ SD: 12,038 $\pm$ 3,715) and nonwords [4,650 $\pm$ 2,803; $t_{(29)} = 10.665$, $P < 0.0001$] as well as between words and pseudowords [9,059 $\pm$ 4,293; $t_{(29)} = 4.485$, $P < 0.0001$]. This was done intentionally to respect natural differences in bigram frequencies. Importantly, however, differences in bigram frequency alone do not contribute to selective responses to words recorded in this paradigm since (i) these responses are also found for words among pseudowords equated in bigram frequency and (ii) no response emerges when two sets of PWs differing in bigram frequency are contrasted [experiment 2; Lochy et al. (29)]. Stimuli were presented in Verdana font, with the size ranging from 4.8 to 7.7 (width) and 1.15 to 2 (height) degrees of visual angle.

Procedure. Participants viewed continuous sequences of visual stimuli (PW, NW, or PF) presented periodically at a rate of 10 Hz through sinusoidal contrast modulation (from 0 to 100% in 50 ms, then back to 0% in 50 ms) with words inserted as every fifth item, so that the word presentation frequency was 2 Hz (10 Hz/5) (Fig. 1B and Movies S1–S3). A sequence started by a fixation cross displayed for 2–5 s, followed by 70 s of visual stimulation: 66 s of stimulation at full contrast flanked by 2 s of fade-in and fade-out, wherein contrast gradually increased or decreased, respectively. Randomly selected words were inserted in three different sequences of base stimuli (PF, NW, or PW) resulting in three conditions (Fig. 1A): words embedded in PW (PWw; Movie S3), in NW (NWw; Movie S2), and in PF (PFw; Movie S1). Each condition was repeated two times, resulting in six sequences for a total of ~10 min of testing time, including short breaks. The experiment was repeated a second time for 13 participants and a third time for 3 participants, depending on their availability. Participants were not informed about the periodicity of the words and were unaware of the objectives of the study. No participant had seizures in the 2 h preceding the recordings. During the sequences, participants were instructed to fixate a small blue cross (15 pix) presented continuously at the center of the stimuli, and to detect and respond to (by key press) brief (200-ms) nonperiodic color changes (blue to red, six times per sequence) of this fixation cross.

Intracerebral EEG Analysis. Intracerebral EEG analysis largely followed a procedure of a recently reported group study on the neural basis of selective face perception (31).

Frequency domain processing. Segments of SEEG corresponding to stimulation sequences were extracted, starting 2 s after the onset of the sequence (i.e., after the fade-in period) until ~68 s (before stimulus fade-out) so as to contain an integer number of 2-Hz cycles (~66 s). No artifact rejection was performed because intracerebral artifacts (mainly epileptic spikes, but also electro-oculographic and electro-myographic activity, since we used a prefrontal scalp electrode as reference electrode for most of the participants) are more broadly distributed across the frequency spectrum than the frequencies of interest (i.e., 2 Hz, 10 Hz, and their respective harmonics). Sequences were averaged in the time domain separately for each condition and each participant, and amplitude spectrum was computed for each contact using FFT.

Word discrimination responses. Word discrimination responses significantly above noise level at the word stimulation frequency (2 Hz) and its harmonics were determined in each condition as follows: (i) the FFT spectrum was cut into segments centered at the word response frequency and the four first harmonics, that is, 2, 4, 6, and 8 Hz (10 Hz corresponds to the base visual stimulation frequency and was not included in the analyses; no word discrimination responses were found above 10 Hz), and surrounded by 25 neighboring bins on each side (Fig. 2A); (ii) the amplitude values of these four FFT segments were summed (Fig. 2B); (iii) the summed FFT spectrum was transformed into a Z score (Fig. 2C). Z scores were computed as the difference between the amplitude at the word frequency bin and the mean amplitude of 48 surrounding bins (25 bins on each side, excluding the 2 bins directly adjacent to the bin of interest, i.e., 48 bins) divided by the SD of amplitudes in the corresponding 48 surrounding bins. A contact was considered as showing a word discrimination response in a given condition if the Z score at the frequency bin of word stimulation exceeded 3.1 (i.e., $P < 0.001$, one-tailed: signal > noise).

Levels of word discrimination contacts. Based on the pattern of word discrimination responses across the three conditions (i.e., significant or not), we labeled each contact according to three hierarchical levels of word discrimination: (i) contacts showing a significant word discrimination response only when presented among pseudofonts, that is, not in the NWw and PWw conditions, were defined as letter selective (+PFw, –NWw, and –PWw); (ii) contacts showing significant responses in the PFw and NWw conditions but not in the PWw condition were defined as prelexical (+PFw, +NWw, and –PWw); and (iii) contacts showing significant responses in the three conditions (PFw, NWw, and PWw) were defined as lexical (+PFw, +NWw, and +PWw). These three types of contacts are referred to as word discrimination contacts. Contacts that displayed a significant response in at least one condition but that did not conform to any of these three combinations of conditions (e.g., exhibiting a significant response in PWw and NWw conditions but not in more basic PFw condition) were excluded from the main analyses (SI Appendix, Fig. S4 and Table S1). Note that the three word processing levels result from combining an increasing number of significant conditions (1–3), resulting in a stricter statistical criterion (thus a different likelihood of false positives) across the three levels. Because these conditions are not statistically independent, one cannot solve this issue by simply adapting the statistical threshold according to the number of tests performed. This issue is not present when including all contacts responding to at least one condition and labeling these contacts according to a single test (SI Appendix, Supplemental Analysis and Fig. S4).

Quantification of response amplitude. Baseline-corrected amplitudes were computed as the difference between the amplitude at each frequency bin and the average of 48 surrounding bins (i.e., 50 bins, excluding the 2 bins directly adjacent to the bin of interest, i.e., 48 bins). Word discrimination responses were quantified as the sum of the baseline-subtracted amplitudes at the word frequency from the first until the fourth harmonic (2 Hz until 8 Hz) (38), and averaged for each condition and contact type. We removed a single outlier lexical contact in which the response amplitude in each condition was more than 3 SDs away from the mean. SNR spectra were also calculated as the ratio between the amplitude at each frequency bin and the average of the corresponding 48 surrounding bins for display purposes and comparison across studies.

Contact Localization in the Individual Anatomy. The exact position of each contact in the individual anatomy was determined by fusing the post-operative CT scan with a T1-weighted MRI. Contacts inside the gray matter were anatomically labeled in the individual anatomy using the same topographic VOTC parcellation as in ref. 32 (SI Appendix, Fig. S1), based on anatomical landmarks. Major VOTC sulci (collateral sulcus and occipito-temporal sulcus) served as medio-lateral divisions. Postero-anterior divisions were the anterior tip of the parieto-occipital sulcus for the border between occipital and temporal lobes, and the posterior tip of the hippocampus for the border between PTL and ATL.

Group Visualization and Proportion Analyses in MNI Space. In a separate analysis, anatomical MRIs were spatially normalized to determine Talairach and MNI coordinates of intracerebral contacts. MNI coordinates of the intracerebral contacts were used to perform group analyses and visualization. Using MNI transformed coordinates, we computed the local proportion of word discrimination intracerebral contacts across the VOTC. Local proportion of contacts was computed in volumes (i.e., “voxels”) of size $15 \times 15 \times 100$ mm (for the X, left–right; Y, posterior–anterior; and Z, inferior–superior dimensions, respectively) by steps of $3 \times 3 \times 100$ mm over the whole VOTC. A large voxel size in the Z dimension was used as a way to collapse across contacts along the inferior–superior dimension.

For each voxel, we extracted the following information across all participants in our sample: (i) the number of recorded contacts located within the voxel; (ii) the number of contacts showing a significant response for each level of word discrimination; (iii) the number of participants having at least one contact recorded in the voxel; and (iv) the number of participants having at least one contact showing a significant word discrimination response. From these values, for each voxel and each level of word discrimination, we computed the proportion of significant contacts/participants over recorded contacts/participants (proportions are crucial here since sampling differs across regions). To ensure reliability and reproducibility, we only considered voxels in which at least two participants showed significant responses. Then, for each voxel, we determined whether the

proportion of significant contacts was significantly above zero using a bootstrap procedure in the following way: (i) sampling contacts from the voxel (the same number as the number of recorded contacts in the voxel) with replacement; (ii) determining the proportion of significant contacts for this bootstrap sample and storing this value; (iii) repeating steps i and ii 5,000 times to generate a distribution of bootstrap proportions and to estimate the *P* value as the fraction of bootstrap proportions equal to zero. In addition, we also statistically compared proportions of word discrimination contacts across corresponding voxels of the left vs. right hemisphere using permutation tests with 20,000 permutations.

We also visualized the variations in the proportions of contacts in each word processing level as a function of the posterior–anterior axis (Y dimension) in the left hemisphere. Proportions were computed along the Y dimension (MNI coordinates) using a running average procedure (in segments of 15 mm by steps of 3 mm) by collapsing contacts across the X (lateral–medial) and Z (inferior–superior) dimensions. The resulting proportion profiles were normalized between 0 and 1.

ACKNOWLEDGMENTS. We thank the participants for their involvement in the study, and Talia Retter for careful editing of a previous version of the manuscript. This work was supported by European Research Council Grant facessvpe 284025 and Belgian Science Policy Office Grant PAI/33.

- Dejerine J (1892) Contribution à l'étude anatomo-pathologique et clinique des différentes variétés de cécité verbale. *Mémoires la Société Biol* 4:61–90.
- Damasio AR, Damasio H (1983) The anatomic basis of pure alexia. *Neurology* 33:1573–1583.
- Cohen L, et al. (2002) Language-specific tuning of visual cortex? Functional properties of the visual word form area. *Brain* 125:1054–1069.
- Wandell BA (2011) The neurobiological basis of seeing words. *Ann N Y Acad Sci* 1224:63–80.
- Taylor JSH, Rastle K, Davis MH (2013) Can cognitive models explain brain activation during word and pseudoword reading? A meta-analysis of 36 neuroimaging studies. *Psychol Bull* 139:766–791.
- Shuster S, Havelka S, Richlan F, Luderndorfer P, Hutzler F (2015) Eyes on words: A fixation-related fMRI study of the left occipito-temporal cortex during self-paced silent reading of words and pseudowords. *Sci Rep* 5:12686.
- Dehaene S, Cohen L, Sigman M, Vinckier F (2005) The neural code for written words: A proposal. *Trends Cogn Sci* 9:335–341.
- Rauschecker AM, et al. (2011) Visual feature-tolerance in the reading network. *Neuron* 71:941–953.
- Szwed M, et al. (2011) Specialization for written words over objects in the visual cortex. *Neuroimage* 56:330–344.
- Vinckier F, et al. (2007) Hierarchical coding of letter strings in the ventral stream: Dissecting the inner organization of the visual word-form system. *Neuron* 55:143–156.
- Vogel AC, Petersen SE, Schlaggar BL (2012) The left occipitotemporal cortex does not show preferential activity for words. *Cereb Cortex* 22:2715–2732.
- Tagamets M-A, Novick JM, Chalmers ML, Friedman RB (2000) A parametric approach to orthographic processing in the brain: An fMRI study. *J Cogn Neurosci* 12:281–297.
- Olulade OA, Flowers DL, Napoliello EM, Eden GF (2013) Developmental differences for word processing in the ventral stream. *Brain Lang* 125:134–145.
- Turkeltaub PE, Gareau L, Flowers DL, Zeffiro TA, Eden GF (2003) Development of neural mechanisms for reading. *Nat Neurosci* 6:767–773.
- Martin A, Schurz M, Kronbichler M, Richlan F (2015) Reading in the brain of children and adults: A meta-analysis of 40 functional magnetic resonance imaging studies. *Hum Brain Mapp* 36:1963–1981.
- Hermes D, Nguyen M, Winawer J (2017) Neuronal synchrony and the relation between the blood-oxygen-level dependent response and the local field potential. *PLoS Biol* 15:e2001461.
- Nobre AC, Allison T, McCarthy G (1994) Word recognition in the human inferior temporal lobe. *Nature* 372:260–263.
- Thesen T, et al. (2012) Sequential then interactive processing of letters and words in the left fusiform gyrus. *Nat Commun* 3:1284.
- Rossion B, Jacques C, Jonas J (February 26, 2018) Mapping face categorization in the human ventral occipitotemporal cortex with direct neural intracranial recordings. *Ann N Y Acad Sci*, 10.1111/nyas.13596.
- Gaillard R, et al. (2006) Direct intracranial, fMRI, and lesion evidence for the causal role of left inferior temporal cortex in reading. *Neuron* 50:191–204.
- McCandliss BD, Cohen L, Dehaene S (2003) The visual word form area: Expertise for reading in the fusiform gyrus. *Trends Cogn Sci* 7:293–299.
- Dehaene S, Cohen L (2011) The unique role of the visual word form area in reading. *Trends Cogn Sci* 15:254–262.
- Bruno JL, Zumberge A, Manis FR, Lu Z-L, Goldman JG (2008) Sensitivity to orthographic familiarity in the occipito-temporal region. *Neuroimage* 39:1988–2001.
- Glezer LS, Jiang X, Riesenhuber M (2009) Evidence for highly selective neuronal tuning to whole words in the “visual word form area.” *Neuron* 62:199–204.
- Glezer LS, Kim J, Rule J, Jiang X, Riesenhuber M (2015) Adding words to the brain's visual dictionary: Novel word learning selectively sharpens orthographic representations in the VWFA. *J Neurosci* 35:4965–4972.
- Baech A, Kravitz D, Baker C, Op de Beeck HP (2015) Influence of lexical status and orthographic similarity on the multi-voxel response of the visual word form area. *Neuroimage* 111:321–328.
- Kronbichler M, et al. (2004) The visual word form area and the frequency with which words are encountered: Evidence from a parametric fMRI study. *Neuroimage* 21:946–953.
- Hirshorn EA, et al. (2016) Decoding and disrupting left midfusiform gyrus activity during word reading. *Proc Natl Acad Sci USA* 113:8162–8167.
- Lochy A, Van Belle G, Rossion B (2015) A robust index of lexical representation in the left occipito-temporal cortex as evidenced by EEG responses to fast periodic visual stimulation. *Neuropsychologia* 66:18–31.
- Lochy A, Van Reybroeck M, Rossion B (2016) Left cortical specialization for visual letter strings predicts rudimentary knowledge of letter-sound association in preschoolers. *Proc Natl Acad Sci USA* 113:8544–8549.
- Jonas J, et al. (2016) A face-selective ventral occipito-temporal map of the human brain with intracerebral potentials. *Proc Natl Acad Sci USA* 113:E4088–E4097.
- Price CJ, Devlin JT (2011) The interactive account of ventral occipitotemporal contributions to reading. *Trends Cogn Sci* 15:246–253.
- Habekost T, Petersen A, Behrmann M, Starrfelt R (2014) From word superiority to word inferiority: Visual processing of letters and words in pure alexia. *Cognit Neuropsychol* 31:413–436.
- Leff AP, et al. (2001) The functional anatomy of single-word reading in patients with hemianopic and pure alexia. *Brain* 124:510–521.
- Kherif F, Josse G, Price CJ (2011) Automatic top-down processing explains common left occipito-temporal responses to visual words and objects. *Cereb Cortex* 21:103–114.
- Binder JR, Medler DA, Westbury CF, Liebenthal E, Buchanan L (2006) Tuning of the human left fusiform gyrus to sublexical orthographic structure. *Neuroimage* 33:739–748.
- Price CJ, Devlin JT (2003) The myth of the visual word form area. *Neuroimage* 19:473–481.
- Retter TL, Rossion B (2016) Uncovering the neural magnitude and spatio-temporal dynamics of natural image categorization in a fast visual stream. *Neuropsychologia* 91:9–28.
- Wimmer H, Luderndorfer P, Richlan F, Kronbichler M (2016) Visual experience shapes orthographic representations in the visual word form area. *Psychol Sci* 27:1240–1248.
- Beverford DQ, Ratcliffe NR, Rhodes CH, Reeves AG (1997) Pure alexia: Clinical-pathologic evidence for a lateralized visual language association cortex. *Clin Neuropathol* 16:328–331.
- Seghier ML, et al. (2012) Reading without the left ventral occipito-temporal cortex. *Neuropsychologia* 50:3621–3635.
- Talairach J, Bancaud J (1973) Stereotaxic approach to epilepsy. Methodology of anatomo-functional stereotaxic investigations. *Prog Neurol Surg* 5:297–354.
- Salado AL, et al. (2018) sEEG is a safe procedure for a comprehensive anatomic exploration of the insula: A retrospective study of 108 procedures representing 254 transopercular insular electrodes. *Oper Neurosurg (Hagerstown)* 14:1–8.
- Jonas J, et al. (2015) Beyond the core face-processing network: Intracerebral stimulation of a face-selective area in the right anterior fusiform gyrus elicits transient prosopagnosia. *Cortex* 72:140–155.
- New B, Pallier C, Ferrand L, Matos R (2001) Une base de données lexicales du français contemporain sur internet: Lexique™. *Année Psychol* 101:447–462.
- Duyck W, Desmet T, Verbeke LPC, Brysbaert M (2004) WordGen: A tool for word selection and nonword generation in Dutch, English, German, and French. *Behav Res Methods Instrum Comput* 36:488–499.