Functional characterisation of letter-specific responses in time, space and current polarity using magnetoencephalography

L. Gwilliams [a,b,*], G.A. Lewis [a], A. Marantz [a,b,c]

[a] Department of Psychology, New York University, United States
[b] NYUAD Institute, New York University Abu Dhabi, United Arab Emirates
[c] Department of Linguistics, New York University, United States

ABSTRACT

Recent neurophysiological evidence suggests that a hierarchical neural network of low-to-high level processing subserves written language comprehension. While a considerable amount of research has identified distinct regions and stages of processing, the relations between them and to this hierarchical model remain unclear. Magnetoencephalography (MEG) is a technique frequently employed in such investigations; however, no studies have sought to test whether the conventional method of reconstructing currents at the source of the magnetic field is best suited for such across-subject designs. The present study details the results of three MEG experiments addressing these issues. Neuronal populations supporting responses to low-level orthographic properties were housed posteriorly near the primary visual cortex. More anterior regions along the fusiform gyrus encoded higher-level processes and became active ~80 ms later. A functional localiser of these early letter-specific responses was developed for the production of functional regions of interest in future studies. Previously established response components were successfully grouped based on proximity to the localiser, which characterised location, latency and functional sensitivity. Unconventional anatomically constrained signed minimum norm estimates of MEG data were most sensitive to the primary experimental manipulation, suggesting that the conventional unsigned unconstrained method is sub-optimal for studying written word processing.

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Introduction

Primary responses to written words

Tarkiainen et al. (1999) was one of the first studies to successfully disassociate the neural dynamics of visual feature analysis and letter-specific recognition, in terms of both neuronal location and response timing. Employing magnetoencephalography (MEG), the authors identified two primary neural responses to written words, interpreted as an early linguistically insensitive response (Type One), and a later letter-specific response (Type Two — not to be confused with Type I & II type errors in the statistical sense). They interpret these responses as reflecting the role of the left inferior occipital–temporal cortex in supporting the processing of letter strings during visual word processing, and propose that the Type Two signals may act as the first port of call when filtering valid letter strings for further lexical processing.

A wealth of research utilising a wide range of methodologies has since been conducted on the neural processes underlying written language comprehension. Much of this research has focused on identifying distinct regions and stages of processing that are specifically responsive to linguistic content. Table 1 below outlines a number of such response components, which overlap to different extents in latency, spatial location and lexical sensitivity.

Recent investigation has suggested that rather than a single "lexically-sensitive region" of the brain, there exists a collection of hierarchical networks set along the occipital–temporal lobe and fusiform gyrus. The Local Combination Detector (LCD) model (Dehaene et al., 2005), for example, proposes graded sensitivity along the entire occipital–temporal cortex to abstract visual stimuli (i.e., written word forms) coded in a posterior-to-anterior progression. Under this account, posterior regions closest to the primary visual cortex are less selective and become active with less proximity to real words, while the most anterior portion of the fusiform overlapping with the Visual Word Form Area (VWFA) has greatest selectivity for high-level visual features.

Initial neuropsychological support for the LCD model was gathered from hierarchical neural detectors in macaque monkeys (Booth and Rolls, 1998; Rolls, 2000), finding neurons located anteriorly to support more abstract processing than populations located posteriorly. The

* Corresponding author at: 10 Washington Place, 6th Floor, New York, NY 10003, United States.
E-mail address: laura.gwilliams@nyu.edu (L. Gwilliams).

Table 1

Summary of response components and regions specifically associated with early written word processing. VWFA = "Visual Word Form Area"; LFA = "Letter Form Area"; WFA = "Word Form Area"; EEG = "Electroencephalography"; fMRI = "Functional Magnetic Resonance Imaging"; ECoG = "Electrocorticography".

Response/region	Latency	Location(s)	Sensitivity	Method(s)	Representative studies
Type One	~100 ms	V1; occipital cortex	Luminosity, visual complexity; non-linguistic properties	MEG	Tarkiainen et al. (1999); Helenius et al. (1999)
Type Two	~140 ms	Temporal-occipital junction	Symbol strings vs. Letter strings, legibility of letter strings	MEG	Tarkiainen et al. (1999); Helenius et al. (1999)
M130	~130 ms	Occipital lobe	Orthographic/surface properties (e.g., bigram freq., orthographic affix freq.)	MEG	Solomyak and Marantz (2009, 2010); Lewis et al. (2011)
M170	~170 ms	Occipital–temporal cortex and fusiform gyrus	Morphological properties (e.g., lemma freq., morphological affix freq., transition probability)	MEG	Solomyak and Marantz (2009, 2010); Lewis et al. (2011); Zweig and Pylkkänen (2009); Fruchter et al. (2013)
VWFA	~180 ms	Fusiform gyrus; Talairach co-ordinates (x = −43, y = −54, z = −12)	High level features such letter shapes; real words vs. consonant strings	EEG & fMRI	Cohen et al. (2000, 2002); Dehaene et al. (2001, 2002)
LFA	~160 ms	Occipital–temporal cortex; MNI co-ordinates (−40, −78, −18)	Consonants vs. false fonts	ECoG, MEG & fMRI	Thesen et al. (2012)
WFA	~225 ms	Posterior fusiform gyrus; MNI co-ordinates (−46, −52, −20)	Real words vs. consonants	ECoG, MEG & fMRI	Thesen et al. (2012)

generalisation of these results to humans was directly tested later by Vinckier et al. (2007). Using Functional Magnetic Resonance Imaging (fMRI), they compared responses along the occipitotemporal cortex to visually presented items differing systematically in their similarity to valid words: 1) false fonts; 2) strings of infrequent letters; 3) infrequent bigrams; 4) infrequent quadrigrams; 5) frequent quadrigrams; and 6) real words. Consistent with the LCD model, they found gradient sensitivity to real words in a posterior-to-anterior progression. The authors characterised the specialisation of responses along the "visual word form system" as supporting evidence for a graded sensitivity anterior from the occipital lobe towards the defined location of the VWFA.

Recent work by Lewis et al. (2011) examined the influence of linguistic variables of varying "abstractness" on an MEG component (putatively overlapping with the VWFA) associated with morphological detection (the MEG M170; Solomyak and Marantz, 2009, 2010). Restricted ROI analyses of posterior and anterior portions of the M170 yielded distinct sensitivities to linguistic variables: the anterior ROI showed an effect of transition probability from stem to suffix of apparently morphologically complex words, whereas the posterior portion was only sensitive to surface (high-level n-gram) frequency. Transition probability effects were associated with activation of abstract morphemic representations, and surface frequency effects with activation of concrete n-gram representations. The sources set more anterior upon the fusiform gyrus therefore appeared to code more abstract representations. This finding is in corroboration to the LCD model as well as Vinckier et al.'s (2007) results, and suggests that even within a single evoked response component, defined by the timing of a peak response over MEG or Electroencephalography (EEG) sensors, not all sources may display the same sensitivity.

Later work by Thesen et al. (2012) employed fMRI and time-sensitive methodologies including MEG and intracranial EEG recordings to identify distinct temporal and spatial attributes of a "letter-form area" (LFA) and a "word-form area". Findings included letter-selective responses peaking 160 ms post-stimulus onset, around 60 ms earlier than activation of the VWFA (Cohen et al., 2000, 2002). The authors propose a feed-forward structure of responses, whereby the system assesses information for word-likeliness at different stages of processing, and subsequently carries it forward through the system. This implies that access to the visual word form (VWF: Warrington and Shallice, 1980) is first fed by identification of valid letters, and then valid word-shapes, suggesting that the first stage of visual word recognition is not VWF access but rather identification of valid letters in posterior regions.

Taken together, these findings strongly suggest that the visual system is composed of neuronal populations that support graded low-to-

high level processing in a posterior-to-anterior arrangement. As such, established response components and regions can be placed along this graded visual processing system, both to index the complexity of processing they support and to assess their similarity to other response components.

Methods of source estimation

As illustrated, investigation into the neurophysiological underpinnings of visual word processing has focused on categorising stages of processing based on information such as timing, location, and sensitivity of particular neural responses. An additional dimension that has been used in methodologies that measure electrical current directly, either from the cortical surface (Electrocorticography: ECoG) or through the skull (EEG), is the polarity of the electrical current (in this case, relative to the reference electrode). For example, the N400 component is defined by negative current potential, and the P600 by a positive current potential. Although polarity relative to a reference electrode is arbitrary, activity with opposing polarities reflect distinct response components. Directionality is therefore an important dimension for characterising responses in these methodologies.

MEG does not measure the electrical current in the brain directly, but rather the magnetic field generated around the brain from which location, amplitude and direction of neuronal currents can be estimated. In many methods of source localisation, including the MNE software used in this paper, source estimation is achieved by placing a current dipole at each source to be estimated, spread across the brain's volume or across the cortical surface. Six co-ordinates are required to characterise a dipole vector within this MRI co-ordinate space: three to position the origin and three to position the vector tip. The method of deriving a single-value current estimate depends on the orientation constraint applied. "Free orientation" applies no constraint on the dipoles' orientation, and amplitude is estimated from the vector's norm (length). Consequently, amplitude always has a positive value, and is referred to as "unsigned". In "fixed orientation" the dipole is cortically constrained by being projected onto the axis normal to the cortical surface. The current estimate is then calculated from the norm of the projected vector - assigned a positive value when the dipole is directed out of the cortical mass, and negative when oriented into the cortical mass. As cortically constrained estimates can realise either positive or negative values, they are referred to as "signed". The vast majority of MEG studies have adopted the convention of using free orientation in source reconstruction, thus removing directional information.

Cortically-constrained, signed estimates model the physiology of the cortical mass, thus making it the most anatomically motivated method

given the hypothesised source of the MEG signal in pyramidal cells of the cortex, which are oriented perpendicular to the cortical surface (Hämäläinen et al., 1993). Furthermore, current flowing from the base of a pyramidal cell to the pial surface is likely to support distinct processes from neuronal populations whose current flows from the surface into the cortical mass; however, the exact differences between polarities of the current are poorly understood (da Silva, 2010). Assuming that switches in polarity mirror distinct responses, unsigned free orientation loses one dimension of discrimination. As a consequence, neuronal populations whose current shifts from one polarity to another may be incorrectly characterised as displaying just one rather than two separate responses. Furthermore, the timing of distinguished response peaks may be misidentified due to erroneous averaging (see Fruchter and Marantz, 2015).

One complication of using fixed orientation, where sources are fit perpendicular to the surface, is caused by the convolution of the cortex. Fixed orientation results in the localisation of the reconstructed current as outgoing activity on one side of a sulcus and ingoing on the other side. One of the localisations and directionalities represents the "true" source of activity, while the other represents a reconstruction "bleed". Averaging positive and negative sources together would lead to a shallower estimate of source amplitude, and thus reduce sensitivity to experimental manipulations. Care must therefore be taken not to cancel out activity by selecting both positive and negative sources when averaging over a ROI; for example, anatomical regions often encapsulate sources from both sides of a cortical fold, which would include both positive and negative data estimates if utilising a cortically constrained method of signing data. Instead, a region needs to be selected in such as way that the inclusion of opposite polarity is minimised.

Despite extensive investigation into the different methods of generating source estimates of EEG and MEG data, there is little study into the consequences of using signed versus unsigned estimates in terms of sensitivity to experimental manipulations across subjects. Comparisons have instead focused on the spatial accuracy of locating a source in data simulations when using free, fixed and loose dipole constraining parameters, often in a single subject. Such work has advocated the advantages of incorporating anatomical constraints on source orientations to avoid over-estimating the spatial extent of activity (Chang et al., 2013; Dale et al., 2000; Hauk, 2004; Lin et al., 2006; Liu et al., 1998, 2002). To our knowledge, only one study has considered the implications of activity polarity relative to experimental sensitivity (Fruchter and Marantz, 2015, Appendix B), although comparing the functional differences between methods was not the main purpose of the experiment. The current study therefore focused on the relative experimental sensitivity of two methods of source reconstruction (free unsigned, fixed signed) rather than spatial accuracy. Based on this comparison we determined whether the sign of MEG data is a useful dimension to retain, and characterised the directionality of Type Two responses relative to the cortical surface. The dataset employed a FreeSurfer average brain to localise responses, and did not combine MEG data with individual subject structural MRIs — information that is not always available to researchers. It must therefore be stressed that present results aimed to compare experimental sensitivity of signed and unsigned estimates and *not* accuracy in localisation. If, in spite of this inherently imperfect estimation, using cortically constrained methods proved to be a superior method, it would suggest that the sign is an important aspect of the signal to retain.

Letter-specific functional ROI

The use of functionally defined regions of interest (fROIs) is frequent with methodologies such as fMRI, but is not common practice with MEG. This technique involves running an orthogonal experimental task known to robustly evoke a functionally specific region of the brain, with subsequent analysis of the same region for a critical manipulation of interest in a separate experiment (see Poldrack, 2007 for a

discussion of fROIs in fMRI). This "localiser", which is typically run on the same sample of participants during the same experimental session, allows for a motivated method of selecting a particular region of the brain to analyse.

Importantly for the present purposes, if the fROI is identified using threshold-based cluster tests (explained in detail below), it avoids the complication of polarity striping that comes along with using fixed orientation. This is because the fROI will consist of a uni-directional set of sources, which will match the response of interest *if* the processes underlying the localiser and the critical experiment are the same. Furthermore, it has advantages over anatomically defined regions in avoiding analysing larger areas than necessary (a problem when correcting for multiple comparisons across space, and when averaging activity within a given ROI), and is not constrained by borders between parcellated regions. Thus, if the localiser truly identifies the same underlying response, statistical power should be increased, and the analysis will be less susceptible to Type I errors.

Given the statistical and theoretical advantages of utilising a localiser of functionally specific activity, we assessed whether fROIs of early letter-specific responses could be created by results of a reduced version of the Tarkiainen paradigm. To achieve this, we replicated a study known to evoke both lower- and higher-level lexical processing - Solomyak and Marantz (2010: henceforth "S&M") involving a visual lexical decision task of mono-morphemic and bi-morphemic words. The original study's main finding was that activity around 170 ms in the fusiform gyrus (M170) was modulated by transition probability from stem to suffix of the morphologically complex words, as well as by morphological but not orthographic affix frequency. The M170 response component appeared to index high-level processes sensitive to sub-lexical (i.e., morphological) structure. Earlier and more posterior processing at the M130 was sensitive only to surface properties of the stimulus, whereby activity was modulated by orthographic affix frequency.

Testing the utility of Tarkiainen et al.'s paradigm as a localiser importantly allowed us to assess whether cluster-based fROIs can overcome the issue of activity cancellation of positive and negative sources when using cortically constrained estimates. Further, if lexical variables from the S&M replication modulate activation of the region(s) shown to display Type Two responses in the abridged Tarkiainen design, it would suggest that the Type Two response can be used to localise the lower and higher level lexical processing as identified in S&M. For the present purposes, this would enable a functional comparison between neuronal populations underlying the Type Two component and other established responses and regions in the literature.

Aims

The principle aim was to establish the location, timing, and functionality of the Type Two response relative to associated regions (e.g., VWFA, letter-form area, word-form area) and responses (e.g., M130, M170) defined in the literature, as well as the posterior-to-anterior progression of higher-level processes more broadly. The original Tarkiainen et al. study employed multiple dipole modelling analysis, which does not provide information regarding the spatial coverage of response-specific activation in a region. To fully ascertain the spatio-temporal extent of Type Two activity, we ran an English-adaptation of Tarkiainen et al.'s (1999) study while recording neural responses with MEG. We then conducted a distributed source analysis to locate brain regions most sensitive to our critical manipulations.

The second aim was to develop a localiser of early letter-specific activity for use as a fROI in future experiments. To do so, we identified a manageable abridgement of the Tarkiainen paradigm to be administered as an orthogonal task within the same recording session as a replication of the S&M study — consisting of a visual lexical decision task of mono-morphemic and bi-morphemic words. We examined the influence of the lexical variables from S&M on activity in the regions

identified by the localiser to determine whether the Type Two response taps into the same neuronal populations as the M130 or M170 components. Determining the functional sensitivity of these regions would allow us to: 1) make stronger connections with other MEG responses, following aim one above; 2) position the regions supporting the Type Two response relative to the hierarchical LCD model; and 3) determine whether underlying neural populations are shared between components, allowing the Type Two responses to be used as a localiser of letter-specific activity more broadly construed.

In aiming to characterise the Type Two response, the final goal of this study was to ascertain the direction of the neural current at the source of the recorded magnetic field with respect to the cortical surface. This purpose is built upon the assumption that a shift in polarity indexes a distinct neural response, and that retaining the sign of activation avoids loss of evoked responses due to averaging. Ultimately, we assessed whether using signed estimates allows for greater ease in discriminating neural responses in comparison to unsigned source estimates. To avoid the issue of averaging over positive and negative estimates, all analyses employed a threshold-based permutation cluster test to identify spatial regions that elicit activation of the same polarity.

To address these questions, we conducted three MEG experiments: 1) a full Tarkiainen et al. (1999) replication, 2) an abridged localiser, and 3) an S&M replication.

Method

Participants

Participants in all three experiments were right-handed native English speakers with normal or corrected-to-normal vision and were recruited from the NYU Abu Dhabi community. Written informed consent was provided by all participants prior to data collection. The Tarkiainen replication experiment included 16 participants (6 females, mean age = 23.8, SD = 4.5, median = 22, range = 18–31). The abridged localiser and S&M replication experiment included 24 participants (17 females, mean age = 21.9, SD = 6.18, median = 20, range = 19–50).

Materials

Tarkiainen replication

Stimuli were an English adaptation of materials originally developed by Tarkiainen et al. (1999). There were 950 items in total, consisting of four different categories of stimulus: i) pure Gaussian noise; ii) single element: one letter (total of 25 letters, without the letter "O") or one geometrical symbol (a triangle, diamond or square); iii) two-element: two-letter English syllables (25 different syllables) or two-element symbol strings (four possible combinations of a triangle, diamond, square or circle): and iv) four-element: four-letter English words (50 different words) or four-element symbol strings (four combinations of

triangle, diamond, square or circle). All four-letter words two-syllable common English nouns (e.g., *SODA, PONY, ATOM*). Letters, syllables and words were embedded in four different levels of Gaussian noise, increasing from 1, 8, 16, to 24. The Gaussian noise was zero centred, and the variance of the noise was set to 0.0234, 0.0938, 0.375 and 1.5, respectively. Symbols of all lengths were always presented in the lowest noise, and served as controls for the low-noise letters, syllables and words. Items in noise levels 1 and 8 were clearly visible, whereas noise level 16 made identification more difficult, and items at noise level 24 were extremely difficult to identify. Fig. 1 shows examples of these stimuli.

Abridged localiser

A subset of the original stimuli was used for the localiser: the four-element (four letter words and length-matched symbols) and one-element items (single letter and single symbol), in the lowest and highest noise levels. This resulted in a subset of 300 trials (50 trials × 6 conditions).

S&M replication

The S&M replication experiment was a lexical decision task of 530 items, including 265 English words that served as critical stimuli. These words were split into a series of five conditions, with 53 items per condition. 1) *Truly Complex Free* items contained a stem morpheme whose orthography is unchanged when combined with a suffix (e.g., "mile" is orthographically identical when appearing in "mileage"). Furthermore, the meaning of the stem when combined with a suffix was the same as its meaning in isolation — mileage means the number of miles. 2) *Truly Complex Bound* items contained a stem morpheme that changes its orthography when combined with a suffix (e.g., "social" loses the final 'L' to become "sociable"). But again, the meaning of the stem is maintained in the complex form. 3) *Pseudo-Complex* items such as "trolley" contained a stem morpheme whose use in isolation has an unrelated meaning to that of the complex word (e.g., "troll" is semantically unrelated to "trolley"). 4) *Unique Stem* items such as "excursion" did not contain a stem morpheme that appears in isolation (i.e., there is no such word as "excurse"). However, their form and meaning matched that of productively formed words with the same suffix — for example, "excursion" means "the event of going on a trip" in the same way that "explosion" means "the event of blowing up". 5) *Pseudo-Unique Stem* items such as "winter" also did not contain an attested stem morpheme, but their form and meaning did not match productively formed words with the same suffix — "winter" does not mean someone who does something as an occupation, in the way that "baker" means someone who bakes.

The collection of items varied in their values on a range of lexical variables known to modulate early letter-specific activity, presented in Table 2 below.
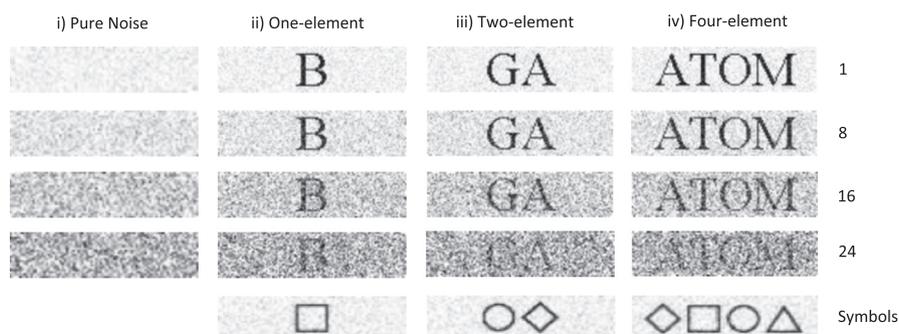

Fig. 1. Full set of stimuli used in the experiment. All are English adaptations of those used by Tarkiainen et al. (1999).

Table 2
Lexical statistics of S&M replication experiment. LM = Log of the Mean; OLF = Orthographic Log Frequency; MLF = Morphological Log Frequency; LF = Log Frequency; TP = Transition Probability.

Condition	Bigram LM		Affix OLF		Affix MLF		Surface LF		Lemma LF		Log TP	
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD
Truly Complex Free *Mileage*	3.63	0.14	5.51	0.59	4.68	0.39	1.8	0.55	3.08	0.56	1.28	0.7
Truly Complex Bound *Sociable*	3.53	0.13	5.07	0.4	4.45	0.37	1.75	0.55	2.49	0.72	0.75	0.66
Pseudo-Complex *Trolley*	3.54	0.12	5.16	0.56	4.1	0.83	1.65	0.63	2.78	0.97	1.29	0.71
Unique Stem *Excursion*	3.59	0.15	5.16	0.46	4.13	0.65	1.69	0.72	NA	NA	NA	NA
Pseudo-Unique Stem *Winter*	2.54	0.15	5.31	0.57	4.22	0.84	1.61	0.58	NA	NA	NA	NA

Procedure

All participants' head shapes were digitised using a hand-held FastSCAN laser scanner (Polhemus, VT, USA) to allow for co-registration during data preprocessing. Five points on each participant's head were also digitised: just anterior of the left and right auditory canal, and three points on the forehead. Marker coils were later placed at the same five positions to localise each participant's skull relative to the sensors. These marker measurements were recorded just before and after the experiment in order to track the degree of movement during the recording.

At the beginning of each of the three experiments, participants completed a practice session outside of the machine to ensure full understanding of the task prior to entering the magnetically shielded room. The Tarkiainen replication was recorded in one session, while the S&M replication and localiser were recorded together in a separate session.

MEG data were recorded continuously using a 208 channel axial gradiometer system (Kanazawa Institute of Technology, Kanazawa, Japan), with a sampling rate of 1000 Hz and applying an online low-pass filter of 200 Hz.

Stimuli in all three experiments were displayed using Presentation software. Stimuli were projected onto a screen 85 cm away from the individual's face.

Tarkiainen replication

Stimuli were organised into four blocks, so that all of the items within a single block contained the same stimuli type; i.e., all of the single-element items were within a single block, and all of the four-element items were displayed in a separate block. There were 8 possible block orders, and two participants were allocated to each block-order. The order of stimulus presentation was randomised within conditions/blocks but was the same for all subjects. It was ensured that the same word did not appear within 10 trials of presentation to avoid repetition effects.

Each item was presented in a centrally placed rectangular patch (~12.7 × 5 cm), and was displayed on the screen for 60 ms with a 2 s inter-stimulus interval. Participants were instructed to focus on the images and to verbally report the item if a question mark appeared. The prevalence of these question-mark trials was 5% (40 out of 950 trials) and served to aid concentration. There were no question-mark trials during the pure noise block. As this block was 20% shorter, it was easier to maintain concentration. The whole experiment lasted around 40 min.

Abridged localiser

Stimuli were presented using the same parameters as the full paradigm. Unlike the full Tarkiainen replication, stimulus order was fully randomised within and between six blocks of presentation so that each block contained a mixture of the six types of stimuli; this was done in order to make the task more engaging. Each block lasted around

60 s, and participants were asked not to blink during stimulus presentation to minimise artefacts in the MEG recording. No overt task was employed due to the brevity of the paradigm; participants were simply asked to pay attention to the items as they appeared on-screen. The localiser took around 6 min.

S&M replication

Each trial began with a fixation cross ("+") for 400 ms, followed by the critical item for 2 s. Participants were asked to indicate whether the item was a word or not by pressing one of two buttons with their left hand. No feedback was provided. Order of stimulus presentation was fully randomised, and each participant received a unique randomisation. The experiment was split into 4 blocks and lasted around 20 min.

Analysis

Data from all three experiments underwent the same preprocessing steps. The continuous MEG data were first noise-reduced by utilising eight magnetometer reference channels located away from the participant's head, using the Continuously Adjusted Least Squares Method (CALM; Adachi et al., 2001), with MEG160 software (Yokohawa Electric Corporation and Eagle Technology Corporation, Tokyo, Japan). The noise-reduced MEG data was imported into MNE-Python (see Gramfort et al., 2014), low pass filtered at 40 Hz, and epoched from 200 ms pre-stimulus onset to 800 ms post-stimulus onset. In order to clean the data, we automatically rejected all trials whose amplitude exceeded a $+/- 2000$ femtotesla threshold; additional artefact rejection was performed through manual inspection of the data, removing noisy trials that were contaminated with movement artefacts or extraneous noise. These clean epochs were averaged across conditions to produce an evoked signal at each MEG sensor.

To reconstruct the location of MEG sensors relative to the individuals' heads, the neuromagnetic data were co-registered with the FreeSurfer average brain (CorTechs Labs Inc., Lajolla, CA). This involved scaling and orienting the average brain to the participant's head-shape. The digitised scan was imported, the digital fiducial points were aligned to the coil markers' position, and the average brain was expanded or reduced to fit the size of the digital scan. Next, an ico-4 source space was created, consisting of 2562 vertices per hemisphere, each corresponding to a potential electrical source. At each vertex, activity was computed for the forward solution with the Boundary Element Model (BEM) method, which provides an estimate of each MEG sensor's magnetic field. For each subject, the inverse solution was computed from the forward solution and the grand average activity across all trials.

Two different orientation parameters were employed in the inverse solution and applied to the data: 1) signed fixed orientation, which defines the direction of the current normal to the cortex by projecting dipoles perpendicular to the cortical surface and estimating activity from the magnitude of the current dipole normal to the cortex; and 2)

unsigned free orientation, which allows the fitted dipole at each potential electrical source to orient in any direction. Estimates are calculated from the magnitude (absolute length) of the current dipole fitted at the source. The inverse solution was applied to each condition average employing an SNR value of 3, which produced a conversion into noise-normalised Dynamic Statistical Parameter Map (dSPM) units (see Dale et al., 2000).

Results

In order to localise the Type One and Type Two responses in our data, we ran spatio-temporal permutation cluster tests over the time-windows (80–130 ms, 130–180 ms) and regions (occipital and temporal lobes) reported in Tarkiainen et al.'s (1999) results using the *Eelbrain* module in Python (https://pythonhosted.org/eelbrain/). This established the location of sources sensitive to the experimental manipulation, and when these sensitivities arose. All cluster-based permutation tests reported here followed the procedures detailed in Maris and Oostenveld (2007).

In order to maximise statistical power and account for extraneous variation, we coded conditions as binary variables within a regression analysis. Combining regression with spatio-temporal permutation cluster tests involved three stages (summarised in Fig. 2). First, the source estimates of each trial within a selected region were used in turn as the dependent measure of an ordinary least squares regression. The data were in the form of N number of 3-dimensional matrices with the shape: space (number of vertices in the tested ROI) × time (number of milliseconds within the window interest) × item (number of trials). The design matrix of this regression model included variables of interest (e.g., noise level, string type and stimulus type), a random intercept, and nuisance variables such as number of elapsed trials. This was applied to each subject's data separately, resulting in a beta coefficient for each vertex in the source space, for each millisecond within the selected time window, for each variable of interest, for each subject. Second, a one-sample *t*-test was performed on the distribution of beta values across subjects for each variable separately, again at each vertex and time-point, in order to test if their value was significantly different from zero. This resulted in a matrix of *t*-values, with a dimension for each vertex and time-point. Third, all *t*-values exceeding a $p < .05$ threshold were clustered based on spatio-temporal adjacency. As only *t*-values with the same polarity are clustered together, clusters of different underlying polarity are identified as separate regions. If a cluster consisted of a minimum of 10 vertices and lasted for at least 20 ms, the *t*-values within this cluster were summed, resulting in a cluster-level statistic, for comparison with test statistics of 10,000 random permutations of the data. Each permutation involved shuffling predictor values at random, and re-computing the cluster statistic of the permuted data to form a distribution of cluster-level *t*-values. If the original critical test statistic fell at or below the 2.5th percentile, or beyond the 97.5th percentile of this distribution, the cluster was considered significant at a corrected level of $p < .05$. This *p*-value was then corrected for multiple comparisons over time and space following Maris and Oostenveld (2007). This procedure was followed identically for both fixed and free source estimates.
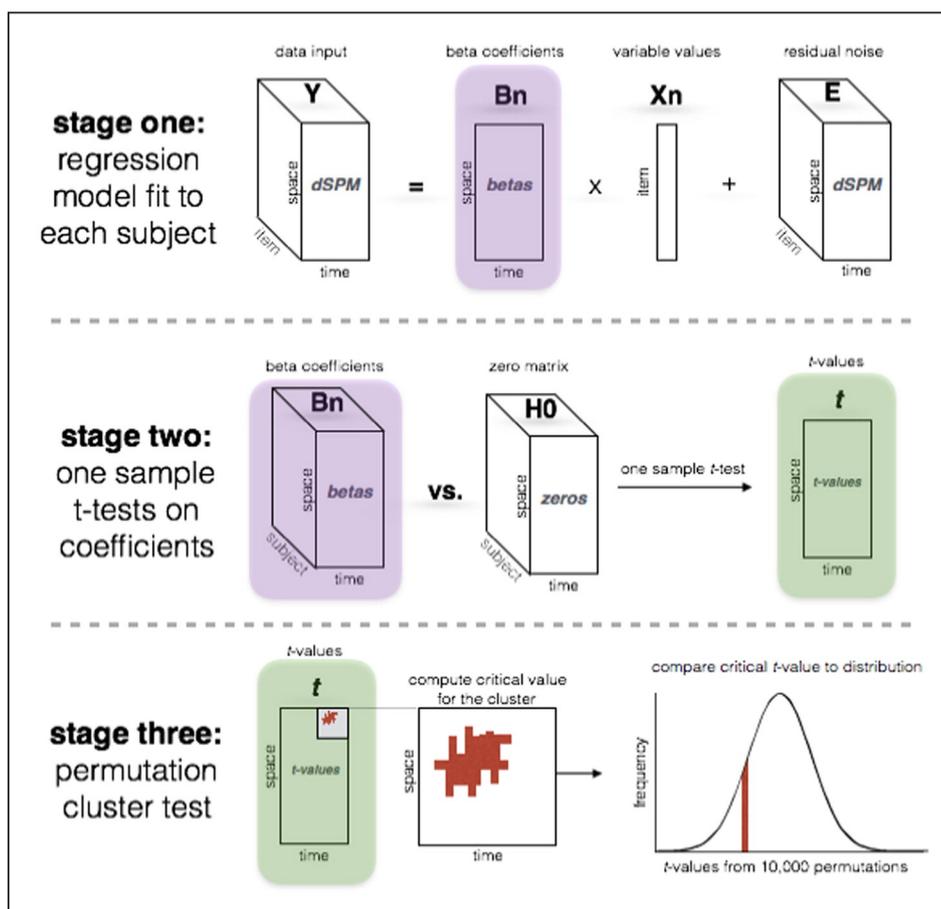
Fig. 2. Stages of cluster test based on mixed effects regression. n = place holder for each variable of interest. Depiction illustrates computations for a single variable — the same procedure is carried out to form clusters for each variable. H0 = null hypothesis.

Type One response: Distributed source analysis

Tarkiainen et al. (1999) localised the Type One response bilaterally in the occipital lobe, with peak latency of dipole activity between 95 and 115 ms post stimulus onset. All stimulus items displayed a correlation between activation and noise, whereby more noise elicited higher amplitude of activity, and there was no fall-off in activation between level 8 noise and level 24 noise. Activity also positively correlated with stimulus length, whereby longer items elicited more activation.

To capture this response, we included Noise Level and Stimulus Type as variables of interest. Noise Level was coded as follows: 1: 0, 8: 1, 16: 2, 24: 3; Stimulus Type was coded as follows: blank: 0, letter: 1, syllable: 2, word: 3. A nuisance variable, the number of elapsed trials, was also included to account for variance due to fatigue, but will not be reported here. The regression analysis was run over the 50 ms time-window of 80–130 ms, and vertices were restricted to the occipital and temporal lobes bilaterally (see Fig. 3A), merged into one region per hemisphere. This region included the FreeSurfer *aparc* parcellation labels: lateral-occipital, cuneus, lingual, pericalcarine, fusiform, middle-temporal and inferior-temporal (available for download at http://surfer.nmr.mgh.harvard.edu).

Analyses on both free and fixed estimates identified the Type One response (see Table 3 and Supplementary figures S2 & S3). For Noise Level, the signed fixed data yielded three significant clusters in each hemisphere (p's < .05); for Stimulus Type, two left lateralised clusters and three right lateralised clusters were identified (p's < .012). The free unsigned estimates identified one significant cluster in each hemisphere for Noise Level, both p < .01; one cluster was identified for Stimulus Type in the left hemisphere (p < .014), while two non-significant clusters were identified in the right hemisphere (p > .2). Note that more clusters are found for signed estimates because sources with opposing polarity are not identified as adjacent neighbours and therefore form separate clusters.
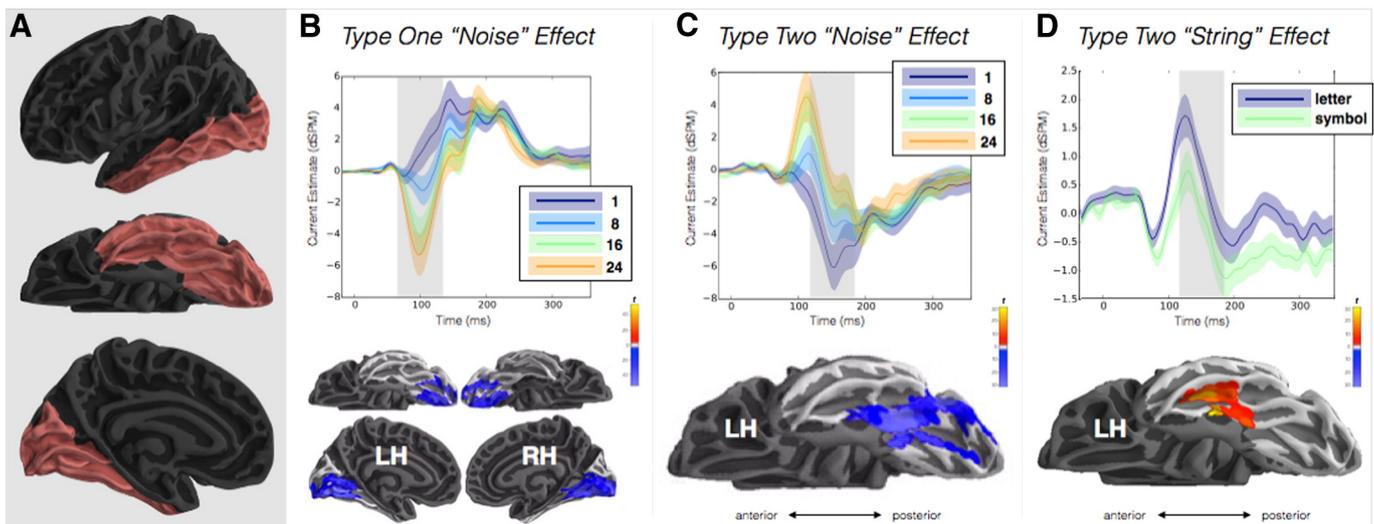
Type Two response: distributed source analysis

The Type Two response reported by Tarkiainen et al. (1999) was characterised by a fall-off in activity at the highest noise level (24) eliciting greater activity for the lower noise letter strings than the strings with the highest level of noise. It was also reported that greater activity was observed for letter strings over length-matched symbol strings. The peak latency of the fitted dipoles was between 140 and 170 ms post stimulus onset, and was localised to the left-hemisphere occipital–temporal junction.

Type Two sources were identified with two spatio-temporal cluster tests. The first was run only on responses to letter strings (not symbol strings or blank stimuli), and only on noise levels 8 and 24. This is because, unlike the Type One response, the Type Two response of Tarkiainen was not characterised by a linear relationship between noise level and amplitude. Rather, there was a drop-off in activation at the highest noise level (24) when the letters became illegible. To capture this relationship we selected just the level of noise that was found to elicit the largest response (8) and the smallest response (24). The design matrix included Noise Level and Stimulus Type as predictors. Noise Level was coded as follows: 24: 0, 8: 1; Stimulus Type was coded as above. Note that the coding scheme for Type One Noise assumes higher activity for noisier strings, whereas Type Two Noise assumes higher activity for clearer strings — this should be noted when interpreting the sign of reported t-values. We restricted time-points to a 50 ms window between 130 and 180 ms, and vertices to the same temporal-occipital region utilised in the Type One analysis. A summary of these results is presented in Table 4; Noise Level responses are presented in Fig. 6; String Type responses are displayed in Supplementary figures S2 & S3.

For the signed fixed data, three significant clusters were sensitive to the Noise Level variable (8 > 24): p's < .05. Clusters formed for the Stimulus Type variable were not significant (p's > .1). When testing unsigned free data, no clusters were formed either for the Noise Level variable or Stimulus Type.

The second cluster test was run just on responses to letter and symbol strings at the lowest noise level. The design matrix included String Type and Stimulus Type as predictors. String Type was coded as follows: symbols: 0, letters: 1; Stimulus Type was coded as follows: one element: 0, two elements: 1, four elements: 2. Spatial and temporal restrictions were the same as the first Type Two analysis above. For the signed fixed data, one cluster survived corrections for multiple comparisons both for the String Type variable p = .046 and the Stimulus Type variable (p = .037). When using unsigned free source estimates, one significant cluster was formed for String Type (p = .014), and no clusters were formed for Stimulus Type.

Fig. 3. Summary of distributed source analysis using signed fixed estimates. (A) Location of spatial vertices used in the analysis. (B) Activation averaged over the most significant cluster of the Type One "Noise" response. Brains presented in ventral (above) and medial (below) view. (C) Activation averaged over the most significant cluster of the Type Two "Noise" response. Brain presented in ventral view. (D) Activation averaged over the most significant cluster of the Type Two "Symbol" response. Clusters on the brains represent t-values at each estimated source, averaged over the shaded time window. Grey shadowing indicates the time during which the cluster was significant — cluster location illustrates position when averaged over this significant time-window. LH = left hemisphere, RH = right hemisphere.

Table 3
Summary statistics: results of the spatio-temporal regression analysis of the Type One Noise Level variable, for both fixed and free source estimates. Cluster size is measured in number of vertices. The green highlighted row corresponds to the most significant "fixed" cluster.

Variable	Data	Cluster size	Hemisphere	Cluster start (ms)	Cluster stop (ms)	Cluster t-statistic	Corrected p-value
Noise level (24 > 8)	Fixed	77	Left	80	130	−2035.61	<.0001
		52	Left	80	130	−853.324	0.0185
		20	Left	80	130	−455.428	0.0504
		20	Left	80	130	386.917	0.0634
		76	Right	80	130	−1978.87	0.0001
		57	Right	80	130	1583.32	0.0019
		48	Right	80	130	−984.855	0.0133
		15	Right	80	130	363.749	0.0703
		23	Right	80	130	315.11	0.0902
	Free	224	Left	80	130	3303.5	0.0003
		209	Right	80	115	2569.49	0.0048

Linking responses to M100, M130 and M170

The next aim was to directly link the responses found in source space to the MEG response components M100, M130 and M170, which are defined based on peaks in sensor data. Using the fixed orientation signed data and results from the full dataset, we projected activation in each cluster back into sensor space to determine which peak (and therefore, component) it corresponded to. Note that this is only possible for data created with fixed orientation, as polarity of the magnetic field cannot be reconstructed using unsigned current estimates. The location of the cluster was used to mask the grand average source estimate, setting all vertices outside of the cluster to zero. The forward solution, which is a matrix that characterises the mapping from source space to sensor space, was then applied to the masked grand average, translating current estimates at each source to femtotesla amplitude at each sensor. Examining the sensor-level responses of each cluster allows us to directly infer to which component it corresponds. Fig. 4 illustrates the results of the cluster projection for a single representative subject. For cluster projection averaged over all subjects, see Supplementary figure S1.

From inspecting the grand average response, three peaks were identified, with a latency of 85 ms, 149 ms and 172 ms (marked with vertical dashed lines in the top panel of Fig. 4B). These timings fall within the range of what has previously been found for the peak latency of the M100, M130 and M170, respectively (Solomyak and Marantz, 2009,

2010; Simon et al., 2012; Lewis et al., 2011; Fruchter and Marantz, 2015). Each of the three projected clusters was found to correspond to one of these evoked peaks, consistent both in terms of the sensor topography and the timing of increased sensor amplitude. These results therefore link the Type One response to the M100 component, the Type Two Noise Level response to the M130 component, and the M170 response to the Type Two String Type component.

*Functional localiser*

Designing the abridged paradigm

We next ran an exploratory analysis to identify stimuli from the Tarkiainen replication that would yield significant effects of the Type Two response. These stimuli would be used in the abridged Tarkiainen adaptation for the purpose of identifying an fROI for use in future studies. We found that using just the four-element (four letter words and length-matched symbols) and one-element items (single letter and single symbol), in the lowest and highest noise levels achieved this goal. This resulted in a subset of 300 trials (50 trials × 6 conditions), which would take a participant around 6 min to complete.

The posterior Type Two-Noise response was identified based on responses to the one and four element letter string items (recall that the noise level for all symbol strings was 1). A regression analysis of Noise Level created a beta value for each vertex and each millisecond from

Table 4
Summary statistics: Results of the spatio-temporal regression analysis of the two Type Two variables, for both fixed and free source estimates. Cluster size is measured in number of vertices. The green highlighted rows correspond to the most significant "fixed" clusters for each variable.

Variable	Data	Cluster size	Hemisphere	Cluster start (ms)	Cluster stop (ms)	Cluster t-statistic	Corrected p-value
Noise level (8 > 24)	Fixed	58	Left	130	180	−967.281	0.002
		21	Left	130	180	283.296	0.0384
		16	Left	130	175	267.156	0.0449
		19	Left	130	170	244.938	0.0591
		12	Left	130	175	167.875	0.1485
	Free				*No clusters*		
String type	Fixed	23	Left	130	180	315.442	0.0463
		15	Left	140	180	−157.317	0.2101
	Free	124	Left	130	180	1378.6	0.014
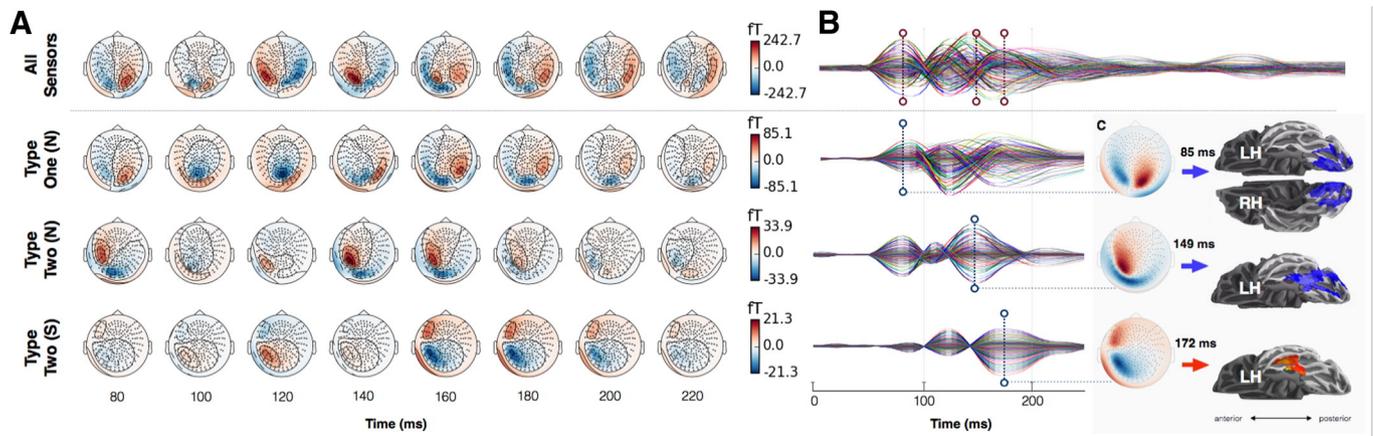
Fig. 4. Projecting clusters identified in source-space back into sensor-space: data for one representative subject. (A) Sensor topographies averaged over 20 ms bins. Top panel: Average response across all trials for all sensors. Lower three panels: Evoked responses across sensors that contributed to the significant cluster of each response. N = "Noise Level Response", S = "String Type Response". (B) Butterfly plots for the grand average (top panel) and each projected cluster (lower three panels). Dashed vertical lines indicate peaks of the M100, M130 and M170 respectively. (C) Topography at the sensor peak for each of the three responses, and their corresponding clusters in source space. All brains presented in ventral position. LH = left hemisphere, RH = right hemisphere.

stimulus onset to 500 ms after onset. The same threshold-based spatio-temporal cluster tests reported above were performed from 130 to 180 ms on the same temporal-occipital region analysed in the full paradigm. This resulted in one significant cluster of 64 vertices in the lateral-occipital lobe for the entirety of our time-window of interest ($p < .001$). An identical regression was run over the same time-window and spatial extent on the String Type variable, comparing low-noise letter strings to low-noise symbol strings. This identified the anterior Type Two String Type response: one significant cluster of 19 vertices in the inferior temporal lobe from 145 to 180 ms ($p < .001$).

Results of localiser

To assess the suitability of the reduced stimulus set as a localiser of early lexical processing, we ran the abridged Tarkiainen paradigm during the same recording session as the replication of the S&M visual lexical decision task. The S&M stimuli included nonwords and an equal number of mono-morphemic and bi-morphemic words falling along continua of linguistic variables tested in the original S&M study, including transition probability from stem to suffix, and orthographic and morphological affix frequency. Also included were bigram frequency and lemma frequency, which were found to be significant determiners of M130 and M170 activity in later studies (Lewis et al., 2011; Simon et al., 2012). We later tested the modulation of activity in localised regions as a function of these continuous lexical variables.

Data analysis procedures were identical to those used with the full dataset: Type Two sources were identified by running regression-based spatio-temporal cluster tests on a 130–180 ms time-window in the temporal-occipital region. Tests on fixed signed data revealed clusters located very close to, and with the same current polarity as, those found in the full dataset: an effect of Noise ($p = .01$), and of String Type 200–250 ms ($p = .009$). Unsigned free data did not identify any clusters where responses to noise level 8 were greater than noise level 24, and no clusters were formed for String Type. Only Type One clusters were identified with free unsigned estimates.

Using signed fixed estimates, the next step was to extract regions found to be sensitive to the Tarkiainen localiser experiment, and to test the same regions' sensitivity to lexical variables in the lexical decision task. Note that the localiser experiment contained an orthogonal set of stimuli, which insured that the technique was statistically sound and not "double dipping". To do this, we ran temporal cluster tests on the S&M data from 80 to 180 ms (chosen to encompass potential M130 and M170 effects) in each Tarkiainen-identified region, assessing the significance of the five continuous variables. The regression included all items with meaningful values on a given variable (i.e., not listed as "NA" in Table 2).

Activity in the posterior Noise Level region significantly correlated with orthographic affix frequency ($p = 0.008$) from 80 to 100 ms; morphological affix frequency ($p = 0.03$) from 80 to 100 ms and log transform of mean bigram frequency ($p = 0.01$) from 100 to 130 ms. No clusters surpassed the threshold for either of the other two variables. The anterior String Type region correlated with lemma frequency from 135 to 170 ms ($p = .001$) and log transition probability from 130 to 170 ms ($p = .008$). No other variables surpassed the cluster-forming threshold.

As functional localisers are usually applied at the subject rather than group level, it was also attempted to apply the localiser within each subject. Because the implementation of regression-based spatio-temporal permutation cluster tests requires a distribution of beta values over subjects ("Stage Two" illustrated in Fig. 2), we first tried localising within-subject responses with t-tests, which can be performed at the level of a single subject. High and low noise was compared for the Type Two-Noise response, and letter and symbol strings for the Type Two-Symbol response. However, this method yielded no clusters for any of the individual subjects.

As a second implementation, within-subject responses were localised by clustering beta values obtained from "Stage One" of the mixed effects regression procedure. To do this, we applied the full regression design matrix to the within-subject data, to create a map of beta values over the cortical surface. These beta coefficients were transformed into t-values. Clusters were formed if at least ten spatio-temporally adjacent samples surpassed a $+2$ threshold for the Type Two String Type response, or -2 threshold for the Type Two Noise Level response, (directionality was based on the polarity observed in the full data analysis). Vertices within the cluster with the highest average t-value were extracted and considered that subject's localised sensitivity. A temporal cluster test was then run at the group level over the averaged values within these localised vertices (time windows and variables identical to those described above), to determine if these sources were significantly modulated by the lexical variables in the S&M replication. The variable that correlated most significantly with localised activity was log transition probability ($p = .06$) between 160 and 175 ms; however, this method was clearly less sensitive than applying the localiser at the group level. It therefore appears that there is insufficient signal in the abridged paradigm to apply it as a localiser within a single subject.

Finally, we wanted to test whether running the abridged paradigm was more beneficial than simply extracting the cluster location from

the full dataset. When running a temporal regression test on the S&M data (as detailed above), but averaging activity over the two Type Two clusters found in the full dataset rather than the localiser, no clusters were formed for the lexical variables of interest. This suggests that applying a localiser at the group level aids the identification of functionally specialised regions.

Discussion

The primary goal of the present study was to compare the relative localisation and timing of the letter-specific Type Two response (Tarkiainen et al., 1999) with other established MEG responses (e.g., M130, M170). We achieved this by conducting a replication study of Tarkiainen et al.'s original MEG experiment, combining distributed source analysis, spatio-temporal cluster tests and the projection of responses back into sensor space. The second goal was to assess the functionality of these responses. For this purpose, we designed an abridged version of the Tarkiainen experiment that may be used to localise letter-specific responses in future studies. We assessed sensitivity of these fROIs by testing the influence of continuous variables from the S&M replication on neural activation of the fROIs; in doing so, we further linked the fROIs' sensitivity to other reported components in the literature. Our final goal was to spell out differences between cortically constrained source estimates and unconstrained methods of MEG source reconstruction and to characterise the Type Two responses in terms of current polarity.

Type One

The Type One response was localised bi-laterally in the occipital lobe between 80 and 130 ms post stimulus onset, beginning in V1/BA 17 and extending up to V3/BA 19. This response encapsulates the primary visual evoked response at around 100 ms, eliciting the field pattern over sensors typically associated with the M100. Consistent with Tarkiainen et al., signal amplitude of the Type One response increased systematically as a function of noise (greater Gaussian noise correlated with greater amplitude of activity), and increasing stimulus length, suggesting that the neural populations underlying this response are sensitive to the visual complexity of a given stimulus.

Type Two

The sensitivity to both String Type (Symbol Strings vs. Letter Strings) and Noise level (8 > 24) were originally reported by Tarkiainen et al. to originate at the same place and time. However, we found two main effects for the Type Two response, each localising to different patches of cortex at different time-points, realising opposing polarity of the current with fixed orientation (see Fig. 3). The main effect of Noise (8 > 24) (henceforth the "Type Two-Noise" response) was found at the junction between the occipital cortex and temporal lobe, as was the original location reported by Tarkiainen et al. The main effect of String Type (henceforth "Type Two-Letter" response), however, was localised at the anterior-most portion of the fusiform gyrus, more anterior than the Type Two-Noise response. The posterior Noise response had negative polarity, whereas the anterior Letter response had positive polarity.

When projecting the two Type Two clusters back into sensor space (Fig. 4), the observed switch in cluster polarity was also present at the sensor level (i.e., the magnetic field over sensors shifted direction). Furthermore, each response was found to have a distinct spatial and temporal profile that corresponded to peak responses of the grand average, and was distinct from the other projected clusters. The Type One response corresponded to the M100 peak; the Type Two-Noise response corresponded to the M130 peak, and the Type Two-Letter response corresponded to the M170 peak. Interestingly then, our results do not support the previous links that have been made between the M130 and the earlier Type One response (Lewis et al., 2011). Instead, they suggest that the Type One response is driven by much lower-level properties of the stimulus. Further, they suggest that the Type Two response as identified by Tarkiainen et al. should be analysed as two functionally distinct components.

Functional ROI

Functionally localised ROIs have a number of advantages over anatomically defined parcellations or ROIs based on peaks in grand-average sensor or source data. In particular, fROIs are not constrained by borders between regions, and do not require analysing larger regions than necessary – a complication when correcting for multiple comparisons in cluster-based analyses. Provided that the localiser and critical experiment tap into the same neural sensitivities, a fROI should yield the least variation between the location of the effect and the region
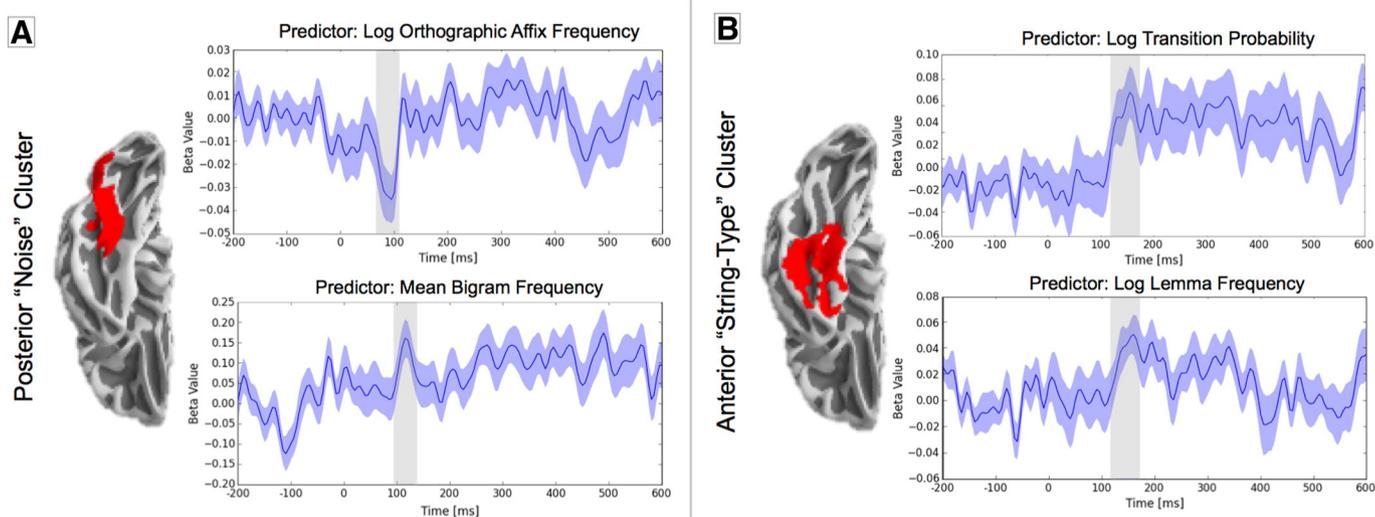
Fig. 5. Beta values of the regression analysis between (A) the posterior localiser and log orthographic affix frequency and mean bigram frequency; (B) the anterior localiser and log transition probability and log lemma frequency. Beta coefficients are averaged over the spatial extent of the cluster. Grey shading indicates when the beta values were significantly different from zero.

being tested, thus providing the greatest level of statistical power. Functional ROIs also remove the experimenter's role in selecting a region based on visual inspection of the data, and do not employ arbitrary parameters to localise regions. Further, fROIs are particularly pertinent for cortically constrained source estimates: as the localiser is based on a threshold-based cluster analysis, it identifies a region of homogenous polarity, and thus fully overcomes the issue of activity cancellation - the principal motivation against using signed source estimates with MEG data.

In testing just a subset of the experimental materials from Tarkiainen et al. (1999), we found a very robust effect for both Type Two-Noise and –Letter responses when using just the four-element block (four letter words, noise levels 1 and 24 and length-matched symbols), and one-element block (one letter, noise levels 1 and 24 and one symbol) which took participants only ~6 min to complete. The location and timing of each Type Two response corresponded with the full dataset, supporting that the same neural sensitivities can be localised even when using one quarter of the stimulus materials. This set is therefore an ideal candidate for a localiser paradigm.

Tests of the posterior Type Two-Noise response revealed significant modulation of activity by orthographic and morphological affix frequency between 80 and 100 ms, and log mean bigram frequency between 100 and 130 ms. Numerous studies have associated both of these variables with the M130 component (Lewis et al., 2011; Simon et al., 2012; Solomyak and Marantz, 2009, 2010), suggesting that the posterior localiser identified spatio-temporal regions associated with lower-level orthographic processing.

Analysis of the more anterior Type Two-Letter response showed that log lemma frequency and log transition probability significantly modulated activity from 130 to 170 ms (morphological affix frequency was not a significant determiner). Both of these variables have previously been associated with the M170 response (Lewis et al., 2011; Simon et al., 2012), suggesting that regions supporting the anterior Type Two-Letter response are shared with the M170, which is responsible for more abstract lexical processing. Regression coefficients of the continuous variables are presented in Fig. 5.

This functional disassociation between posterior and anterior responses corresponds to recent results employing cortically constrained MEG data from a lexical decision task (Chen et al., 2015). The authors found that activity in posterior portions of the fusiform correlated with bigram frequency ~ 100 ms post onset, while anterior regions correlated with word surface frequency ~ 160 ms, thus strongly corroborating the present Results. Lewis et al. (2011) also reported similar results using unconstrained signed estimates of MEG data, finding that only posterior portions of the anterior M170 ROI displayed surface frequency effects. This was interpreted as indexing a "high-ngram" effect, and the activation of more concrete representations.

Our findings therefore support that the abridged Tarkiainen paradigm can be used to successfully localise early posterior orthographic processing, as well as later anterior sub-lexical processing. More specifically, finding activity in these regions to significantly correlate with variables shown to modulate the M130 and M170 is strong functional evidence that the Type Two-Noise and -Letter responses are analogous to those MEG components, in line with the clusters' peaks in the sensor data reported above.

The stimuli used in the localiser of the present study are available to download, either on github (https://github.com/LauraGwilliams/TarkiainenLocaliser.git) or by contacting the first author.

Graded lexical sensitivity of Type Two

Each linguistic variable included in the localiser analysis is associated with a certain level of processing complexity and has been linked to a specific response component in previous literature. Orthographic affix frequency and bigram frequency are linked to surface orthographic properties entailing relatively low-level processing and access to concrete representations. Both variables modulated activity in the Type

Two-Noise region, which is in line with the LCD model prediction that posterior cortical regions subserve lower level processes. The posterior sensitivities of this region are consistent with the "letter-form" area (Thesen et al., 2012) and the M130 component (Solomyak and Marantz, 2010), suggesting that the responses and the localiser share underlying neural mechanisms.

Variables such as lemma frequency and transition probability are linked to more abstract processing involving the connection between input and stored word forms. The LCD model accordingly positions neurons tuned to higher-level processes along anterior portions of the fusiform gyrus, in agreement with our results. The sensitivity of the Type Two-Letter response to these variables, in addition to its anterior location, are consistent with equating the Type Two-Letter response with the M170 as identified by S&M, with the VWFA (Cohen et al., 2000) and with the "word-form" areas (Thesen et al., 2012) that appear to involve higher-level processing.

The posterior-to-anterior progression of abstract processing is thus supported by our results, whereby lower-level sensitivities such as that to letter frequency arise posteriorly, and higher order variables such as lemma frequency appear to be encoded more anteriorly. Our results are in full corroboration with the LCD model as tested by Vinckier et al. (2007), and offer striking similarities in localisation and functionality between the two Type Two responses and the "letter-form" and "word-form" regions identified by Thesen et al. (2012). Together our findings support the hypothesis that words are first processed through the orthographic properties of letter strings, followed by the processing of word forms and sub-lexical structure.
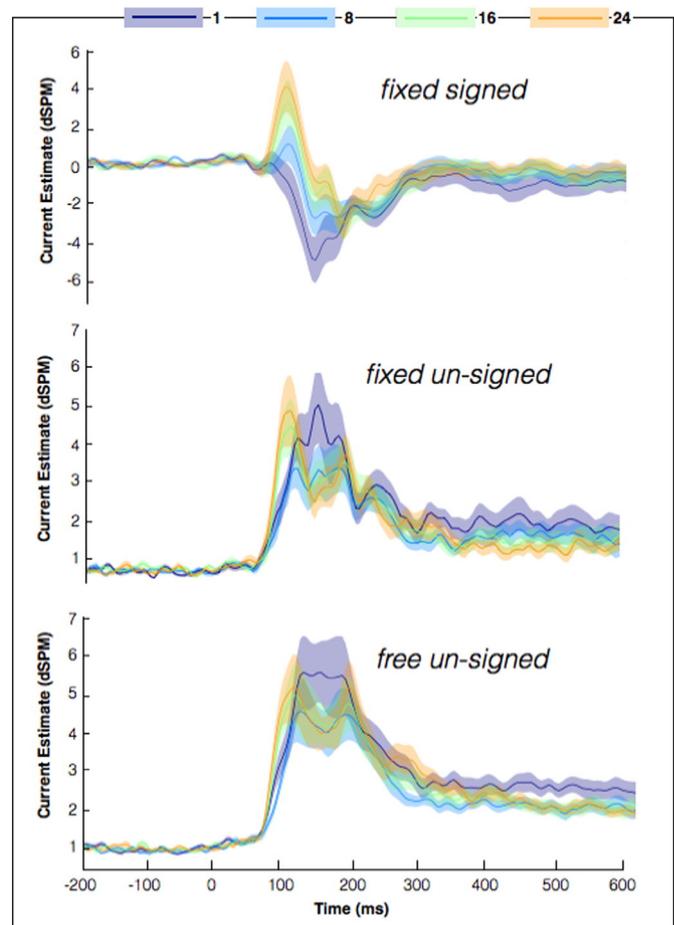

Fig. 6. Timecourse of activation averaged over the Type Two-Noise cluster found in the full dataset (shown in Fig. 3C), for three methods of source estimation. Values 1, 8, 16 and 24 correspond to noise level of the stimulus.

Comparing source estimate constraints

The final issue to discuss is the implication of applying different methods of source reconstruction. We directly assessed whether, in comparison to signed source estimates, conventional unsigned source estimates of MEG data would reduce the ability to discriminate between spatio-temporally neighbouring responses or "lose" evoked peaks by taking absolute strength of activation.

The present study consistently found unsigned free orientation to be less sensitive to experimental manipulations than signed fixed estimates. For the full Tarkiainen dataset, analyses on free unsigned data did not form any clusters above the $p < .05$ threshold for the Type Two-Noise response, and for the abridged paradigm, free unsigned data failed to uncover both the Type Two-Noise response and the Type Two-Letter response. Analyses using fixed signed data straightforwardly identified all of these responses, even in the absence of structural MRIs for participants – the presence of which should only serve to improve accuracy further.

Fig. 6 presents the source estimates of the Type Two-Noise response when reconstructing the source of activity using three methods: 1) signed data fixed normal to the cortical surface, whereby negative activity corresponds to current flowing into the cortical mass, and positive to current flowing out of the cortical mass; 2) unsigned fixed data, taking the norm of the dipole fitted perpendicular to each vertex; and 3) unsigned free data, which does not retain the direction of the source and allows the dipole to freely orient in any direction. Recall that free

unsigned data did not elicit a reliable Type Two-Noise response in the full dataset. All sources are from the same spatial cluster identified with the signed fixed estimates, shown in Fig. 3C.

As can be seen in Fig. 6, the separation between peaks of activity is much less clear for the unsigned estimates, as compared to the signed methodology. Because there is temporal overlap between one response and the next in this region, and because the two responses have opposing polarity, averaging these values distorts the actual relationship between activation and the given experimental conditions. This erroneous averaging across polarity is therefore likely to explain why Type Two-Noise responses were not identified using unsigned estimates. For the timecourse of activation found for the Type One response and Type Two String Type response with both free unsigned and fixed signed estimates, refer to Supplementary materials (Figures S2 & S2).

In the study conducted by Fruchter and Marantz (2015, Appendix B), the authors analysed their data employing the two methods of source reconstruction discussed above. They found that when comparing source estimation methods, unsigned data greatly reduced activation peaks. This dampening of evoked responses can also be observed when comparing the unsigned MEG and signed ECoG measurements in Thesen et al.'s (2012) study. Figs. 4h and i from their paper represent the local field potential recorded from the cortical surface directly, and the MEG source reconstruction from the same region. There we can see very clear polarity shifts in the ECoG data, allowing for discrimination between response components in this dataset; however, in the unsigned free orientation MEG data, it is unclear whether the "bumps" of
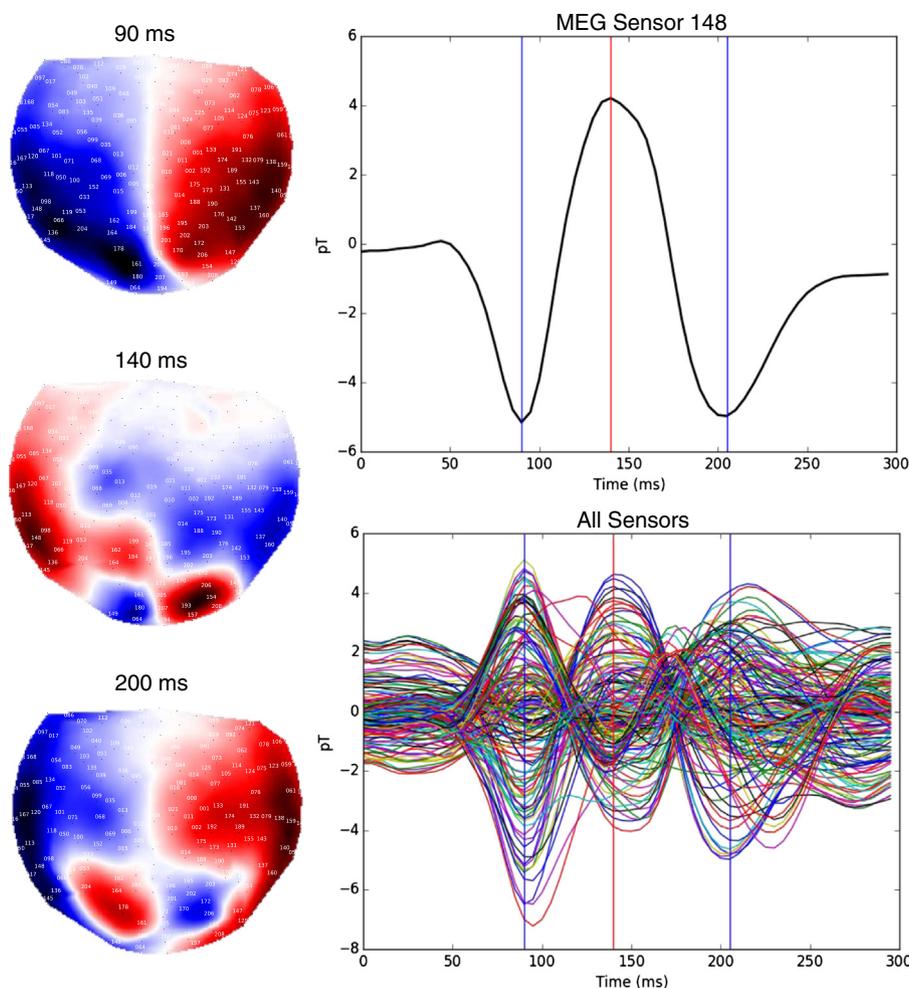

Fig. 7. Polarity of magnetic field at a single sensor (above) and across all sensors (below) averaged over subjects and items. Topographic plots show the polarity of the magnetic field at each of the three peaks in the sensor data.

activity reflect separate responses. One level of discrimination is therefore lost when removing this polarity information, as compared to the method that records the electric potentials generating the MEG signal.

Such shifts in polarity are not negligible and are clearly observed at the sensor level: A single channel will shift between a positive and negative magnetic field depending upon the orientation of the dipole at the source. Fig. 7 below illustrates the strength of the magnetic field at a left lateralised sensor averaged across all subjects and items. In line with the timing we observed for the source analysis of the Type Two responses, a field reversal is also apparent, indicating that the current dipole(s) in that region flip direction at different time-points.

This field reversal was also observable when projecting the activity of clusters into sensor space for a single subject. The direction of the dipole for the Type Two responses was clearly oriented differently (Fig. 4C), corresponding to the polarity differences also observed at the source level. Even without structural MRIs for individual subjects, the polarity of the clusters found with fixed orientation lined up with the sensor data, such that the field pattern over sensors corresponded to polarity of sources. This is consistent with the polarity difference between the M130 and M170 when using a free signed methodology (assigning polarity based on whether the dipole is oriented up or down with respect to the head) observed in the original Solomyak and Marantz (2010) analysis and in follow-up studies (Lewis et al., 2011; Simon et al., 2012).

This is only a limited investigation into the question of current reconstruction. Further study should aim to ascertain whether all negative response components reflect functionally different computations from all positive components, or if the directionality of the current is an arbitrary dimension of discrimination (similar to polarity in EEG). The present study illustrates that polarity of the reconstructed sources is an important element of MEG data, and can be used to disassociate functionally discrete (in this case, Type Two) neighbouring responses. Furthermore, using signed estimates appears to be more experimentally robust when analysing a reduced dataset, as well as a more sensitive method, especially when current dipoles rapidly switch direction. Finding evidence, both at the sensor and source level, that the neural generators underlying the identified responses are alternating in polarity highlights the importance of current dipole directionality for MEG data.

Summary

Using distributed source analysis of MEG data, we localised the Type Two response of Tarkiainen et al.'s (1999) study for comparison with other established response components and regions of lexical-specific activity. The Type Two response localised to two different regions with different preferences: 1) preference for visible over obscured letter strings in the lateral-occipital lobe with negative activity with respect to the cortical surface; 2) preference for letter over symbol strings in the anterior fusiform gyrus with positive activity. When testing the lexical sensitivities of these regions as part of an abridged paradigm, functional responses were shared between the posterior Type Two-Noise response, the M130 and "letter-form" area, and between the Type Two-Letter response, the M170 and "word-form" area. These results suggest that each case evokes the same underlying processes, and crucially that a subset of the stimuli materials is sufficient to localise these response components with notable accuracy.

In order to address the issue of source reconstruction with MEG data, we applied two methods to the current dataset and compared the results to the findings of Tarkiainen et al. (1999). In the presence of rapidly alternating polarity, utilising cortically constrained estimates was the most sensitive approach, ensuring the preservation of evoked response components. By contrast, cortically unconstrained unsigned estimates were susceptible to the loss of discrimination between activation peaks. In this regard, our findings directly indicate that, arbitrary or not, retaining the sign of MEG data can allow for greater sensitivity to experimental manipulations and an additional level of discrimination.

Bringing our results together, we are able to characterise two localisers of letter-sensitive responses for future studies in time, space and current directionality with respect to the cortical surface. We propose that the posterior Type Two sensitivity to visible letter strings can be used to localise orthographic processing, and the anterior Type Two sensitivity to letter strings over symbol strings can localise higher-level processing of sub-lexical structure, such as morphological composition. Our results promote the use of cortically constrained signed estimates of MEG data, in unison with functional ROIs when investigating letter-specific neurophysiological responses in future studies.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2016.02.057.

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