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Dynamic spatiotemporal brain analyses using high performance electrical neuroimaging: Theoretical framework and validation

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HIGHLIGHTS

• Microsegmentation suite that differentiates transition states from stable ERP microstates.
• Differentiation of event-related brain microstates from changes in global field power.
• Integrated within- and between-subject bootstrapping procedures to assess solution robustness.
• Microstate algorithm to promote mapping both which and when brain regions is activated by a task.

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ABSTRACT

Background: Since Berger’s first EEG recordings in 1929, several techniques, initially developed for investigating periodic processes, have been applied to study non-periodic event-related brain state dynamics. New method: We provide a theoretical comparison of the two approaches and present a new suite of data-driven analytic tools for the specific identification of the brain microstates in high-density event-related brain potentials (ERPs). This suite includes four different analytic methods. We validated this approach through a series of theoretical simulations and an empirical investigation of a basic visual paradigm, the reversal checkerboard task. Results: Results indicate that the present suite of data-intensive analytic techniques, improves the spatiotemporal information one can garner about non-periodic brain microstates from high-density electrical neuroimaging data. Comparison with existing method(s): Compared to the existing methods (such as those based on k-clustering methods), the current micro-segmentation approach offers several advantages, including the data-driven (automatic) detection of non-periodic quasi-stable brain states. Conclusion: This suite of quantitative methods allows the automatic detection of event-related changes in the global pattern of brain activity, putatively reflecting changes in the underlying neural locus for information processing in the brain, and event-related changes in overall brain activation. In addition, within-subject and between-subject bootstrapping procedures provide a quantitative means of investigating how robust are the results of the micro-segmentation.

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1. Introduction

The rapid growth of large-scale, high-spatial resolution neuroimaging technology has advanced our understanding of the neural underpinnings of various complex cognitive and social processes. For instance, work in cognitive and social neuroscience has identified the neural correlates of information processing operations, ranging from basic perceptual processing (e.g., checkboard)
to more complex cognitive (e.g., object or face recognition, decision making, action understanding, embodied cognition) and social processing (e.g., pair bonding, love, empathy, cooperation). However, high-spatial resolution neuroimaging techniques, such as functional magnetic resonance imaging (fMRI), have been limited in terms of the temporal information they provide in studies of brain function. In addition, the cost of fMRI has placed constraints on the statistical power of most studies, which in turn has compromised the replicability of research findings (cf. Button et al., 2013; Cacioppo et al., 2013a).

A key theoretical objective in neuroscience and medicine is not only to specify what brain areas are recruited during a behavioral task, but also to specify when and in what specific combinations they are activated (e.g., Cacioppo et al., 2013b; Crits et al., 1995; Decety and Cacioppo, 2012; Ito et al., 2004; Ortigue et al., 2004, 2005; Ortigue and Bianchi-Demichel, 2008). By providing detailed information about the relationship between neuronal activity (i.e., post-synaptic dendritic potentials of a considerable number of neurons that are activated in pattern that yield a dipolar field) and the temporal resolution (millisecond by millisecond) of each component information processing operation required for behavioral performance, high-density electroencephalographic (EEG) recordings and averaged EEG (event-related potentials, ERPs) have provided a useful additional tool in investigations of brain function. Whereas fMRI analyses are performed in source space, EEG/ERP analyses are performed in sensor space, with high-density sensor recordings producing more detailed information about changes in brain activity measured across time and sensor space.

Since the first EEG study by German neurologist Hans Berger (1929), numerous techniques have been developed for investigating the brain state dynamics of periodic processes in the EEG, including standard waveform analyses, Fourier analysis, independent component analysis (ICA), principal component analysis (PCA), and k-means cluster analyses. Over the years, some have argued that measuring peaks and troughs was sufficient to the temporal processing of the brain, while others (e.g., Donchin and Heffley, 1978) argued, quite persuasively, that another approach, such as a statistical decomposition of the evoked brain states, was necessary. In the current work, we present a new method for identifying the underlying component structure of an ERP – specifically, we present a new method for identifying non-periodic brain state dynamics for the micro-segmentation and analysis of averaged high-density ERPs.

2. Non-periodic brain microstates

Over the past three decades, efforts have been made to complement the traditional analyses of ERP peaks and troughs at specific electrode positions with more comprehensive analyses of time-varying activity across the entire scalp. For instance, introduced in the 1980s by Dietrich Lehmann, the brain microstate approach (Lehmann and Skrandies, 1980) is a method to identify stable configurations of global electric brain activity (rather than signals collected from one electrode). Because this approach is extensively used and has been detailed previously in several review articles and scientific reports (Brunet et al., 2011; Decety and Cacioppo, 2012; Michel et al., 1999, 2001; Murray et al., 2008; Pascual-Marqui et al., 1995; Ortigue et al., 2004, 2005, 2009, 2010), here we provide only the essential details. With respect to ERP analyses, the brain microstate approach considers data in the spatial domain first, and then in the temporal domain, providing a display of the constantly changing spatial distribution of the brain activity. The goal of the brain microstate approach is to provide information about the brain activity associated with the sequence of discrete (and putatively non-periodic) information processing operations evoked by the presentation of a stimulus within the context or a particular experimental task, with exogenous ERP components sensitive to the characteristics of the stimulus and endogenous ERP components sensitive to the stimulus in the context of the task. This sequence of information processing is composed of a series of stable brain activities, called brain microstates, each of which is characterized by the performance of specific cognitive computations and a relatively stable spatial distribution of brain activity. For instance, after a visual presentation of a face, the sequence (also called syntax) of various evoked brain microstates is thought to reflect the different steps of face processing (cf. Pizzagalli et al., 2000, 2002; Lehmann et al., 2005).

The successive occurrence of brain microstates does not imply that their brain networks occur in a sequential way (Pascual-Marqui et al., 1995). The underlying mechanism by which the brain enters a microstate with a given brain network may be composed of any number of sequential or parallel physiological sub-processes. Investigators can address this issue in several ways. For instance, lesion studies permit tests of the role of and relationship (e.g., dependence) between temporally activated neural regions; fMRI can be used to investigate functional connectivity between regions of activation; and experimental studies in which microstate segmentation is applied to high-density EEG/ERP data can be used to test contrasting hypotheses (brain models) to explain the chronobiology of the observed microstates.

Common brain areas may sustain different microstates, and the same microstate may be observed in two different conditions (e.g., fear faces and sad faces). In the latter case, the intensity of the activation and/or the onset of the duration of this microstate, but not its configuration, may significantly vary between conditions. For instance, one microstate may occur earlier in one condition compared with another condition, which may provide valuable information regarding the temporal dynamics of these two conditions.

The notion underlying the brain microstate approach is that each microstate refers to a time-limited information processing operation. Consistent with this notion, a growing body of studies shows that the presence of different brain microstates is associated with distinct cognitive operations (Lehmann and Skrandies, 1980). As such, the global pattern of brain electrical activity identified as a microstate is characterized by its electrical maxima (positive and negative), the orientation of its maxima (anterior, posterior), the location of its maxima (left hemisphere, right hemisphere), and the onset and duration of the configuration (Lehmann and Skrandies, 1980, 1984). Each brain microstate may remain significantly stable for a certain amount of time (e.g., for tens to hundreds of milliseconds), and then changes into another brain microstate that remains stable again (e.g., Cacioppo et al., 2013a,b; Decety and Cacioppo, 2012; Ortigue et al., 2009, 2010). This approach suggests that the global pattern of brain electrical activity is modeled as being composed of a time sequence of decomposable brain microstates (Lehmann and Skrandies, 1980; Pascual-Marqui et al., 1995).

In the previous literature, these brain microstates have typically been identified using data clustering techniques (e.g., k-means cluster analysis) on the group-averaged ERPs of each experimental condition to identify the start, end, and nature of each brain microstate. Given the group averaged ERP data set consists of N discrete samples over n (e.g., 128 or 256) electrodes, the activity across the n electrodes at each discrete sample can be expressed as a topographic scalp potential map. In the classic approach to microstate segmentation, the N topographic maps are segmented by the k-means algorithm. The value of k defines the number of discrete microstates that will be identified; k can range from 1 to N, but in practice is usually limited to 1–20 for a time period of 500 ms post-stimulus onset. First, k timeframes (where timeframe refers to the electric potentials from all electrodes within a discrete range of time in the ERP) are selected at random. These k selected
timeframes serve as the initial template maps (where template map refers to the topographic scalp potential map that characterizes a given microstate) and are iteratively refined over the course of the algorithm.

Next, the pair-wise spatial correlation between the topographic map at each timeframe and the $k$ current template maps is computed. Each timeframe map is then said to be a “member” of the template map to which it most strongly correlates. Following the assignment of timeframes to $k$ groups, each of the $k$ template maps are re-defined as the arithmetic average of its respective set of member timeframes. This process of assigning timeframe membership and re-computing each template map is repeated until an iteration occurs in which no timeframe changes membership. The $k$ template maps that are computed in the final iteration of the algorithm are taken to be representative of the $k$ distinct stable microstates in the ERP. Because the quality of the clustering derived by the $k$-means algorithm is strongly dependent on the initial random selection of template map seeds, the whole $k$-means segmentation process is often repeated many hundreds of times and the result that maximizes some quality metric is taken as the best solution to the segmentation/clustering problem.

3. Cluster analysis drawbacks

Several drawbacks to the cluster analysis approach to micro-segmentation have been identified. First, the user must specify the value of $k$ prior to analysis. However, the discrete computations performed by various brain regions need not map into cognitive operations in a one-to-one fashion, so the objective of performing this type of analysis is often to determine the value of $k$ itself. Moreover, the a priori specification of $k$ by an investigator also may introduce confirmatory bias at the expense of replicability or generalizability. There are a number of techniques that have been proposed in the literature for determining the “correct” value of $k$. The use of a cross-validation (CV) criterion, which is derived by dividing the global explained variance by the degrees of freedom, and the Krzanowski–Lai (KL) index has been advocated (Krzanowski and Lai, 1985; Brunet et al., 2011). By examining the values for these “quality” indices over a range of $k$ values, a point may be identifiable where only marginal improvement is achieved by identifying $k + 1$ clusters/template maps. However, identifying this inflection point in the KL and CV functions is non-trivial because it can be difficult to make a rigorous argument for the proposition that some value $k$ is “good enough”.

Second, the $k$-means algorithm mathematically identifies the spatial configurations that are sufficiently similar to belong to one of the user-specified number of clusters. These configurations are clustered regardless of where they occur across time. For this reason, the same spatial configuration can occur at different points in time, whereas the information processing operations evoked by a stimulus are thought to vary across time. When a given cluster is repeated, it is typically treated as reflecting a distinct information processing operation even though mathematically it represents a repeating microstate.

Third, in each iteration of the $k$-means algorithm, each individual timeframe (topographic map) is compared to the $k$ available template maps and is said to be a member of the template map group to which it most strongly correlates. This leads to a perhaps even more fundamental limitation of the $k$-means approach to segmentation. By definition, the $k$-means approach requires that every timeframe belong to some characteristic microstate. Stable states of brain activity may not always instantaneously change from one to the next. Rather, “transition periods” between pairs of stable states may occur. In transition periods the loci of brain activity migrates from one set of brain regions to another and results in an observed morphing of the topographic scalp potential maps that lie between the temporal windows in which two different stable microstates are observed.

This issue is depicted in illustrated in Fig. 1, where a stable microstate is observed from time 1–6, the transition from the first to the second stable microstates is observed from time 7–10, and the second stable microstate is observed from time 11–16 (the end of the recording period). The $k$-means approach necessitates that these “transition timeframes” be assigned membership to a template map group (microstate) as the mathematic of the $k$-means algorithm specifies that every timeframe (i.e., configuration) must belong to exactly one template map group. In the case illustrated in Fig. 1, micro-segmentation using the $k$-means algorithm would typically yield two stable microstates, and each of the topographic maps representing the brain activity during the transition period would be assigned to one of these microstates.

Note, however, that the transition timeframes should not belong to a stable microstate, as by definition they are not stable but instead they are part of a transition from one stable microstate to the next. The $k$-means approach therefore may be insufficient for identifying both stable and transition states. Combining transition timeframes with stable timeframes (i.e., microstates) degrades the quality of the template maps for each true stable microstate, as the averaging process used to compute their template maps includes in the calculation timeframes that resemble components of other (preceding and/or succeeding) microstates. The inclusion of transition states in microstates is particularly problematic when the onsets, durations, or offsets of the microstates are important to determine, or when source localization algorithms are used to investigate the underlying neural substrate for each microstate.

Finally, because the cluster analysis is performed on the overall ERP, no information is provided regarding how robust might be the micro-segmentation, and the $k$-means solution is assumed to accurately reflect the sequence of stimulus-evoked information processing operations shown by each individual. For these reasons, the basic $k$-means (as well as other clustering algorithm) approaches tell us nothing about how the number of microstates that are identified and the specific brain topographies associated with these micro-segments across time can vary across analysis runs and across participants.

The work that is presented here was initiated to address these issues by using a suite of quantitative methods for micro-segmentation. We begin with a definition of the suite of the quantitative methods used to identify non-periodic brain microstates and transition states. We describe the quantitative implementation of each method, and we evaluate this suite of quantitative methods for the identification of non-periodic brain microstates through a series of theoretical simulations and an experimental validation study using a basic visual paradigm, the reversal checkerboard task.

4. Data-driven suite of computing tools for micro-segmentation: material and methods

The present suite of high performance computing tools for micro-segmentation includes the following four quantitative methods: (1) a root mean square error (RMSE) metric for identifying transitions across discrete event-related brain states – that is, potential brain microstates; (2) a global field power (GFP) for identifying changes in the overall level of activation of the brain; (3) a similarity metric based on cosine distance to determine whether template maps for successive brain microstates differ in configuration of brain activity, global field power, or a combination

1 Krzanowski–Lai index is a criterion for determining the number of groups in a data set using sum-of-squares clustering.
of the two; and (4) a bootstrapping procedure for assessing the extent to which the solutions identified in the micro-segmentation are robust (reliable, generalizable) and for empirically deriving additional experimental hypotheses.

4.1. Root mean square error (RMSE) function

To identify stable brain microstates as well as transition states across these discrete brain microstates, we used a modified microstate segmentation algorithm that we derived from the gradual transition detection algorithm proposed by Volkmer et al. (2004) for identifying video scene changes. The algorithm incorporates a root mean square error (RMSE) metric and a Confidence Interval (CI) based on baseline data to identify potential stable and discrete brain microstates.

In gradual transition detection theory, Volkmer et al. (2004), studying video streams, observe that a gradual transition from one shot to another (such as a fade, wipe, or dissolve) can be automatically identified by locating instances of maximal distance between each video frame and a number of prior and subsequent frames. In the current adaptation of this theoretical approach, each discrete timeframe in an ERP (topographic potential map) is represented as a vector of $n$ electrode readings, and the RMSE is used to compute the distance between maps. As such, the proposed RMSE algorithm identifies the onset of stable states of brain activity by locating maxima in an inter-frame distance function applied to the $N$ discrete timeframes of the ERP.

RMSE is a frequently used measure of the differences between values predicted by a model or an estimator and the values actually observed. The differences between predicted and observed are termed residuals when the calculations are performed over the data sample that was used for the estimation, and are called prediction errors when computed out of sample. The RMSE serves to aggregate the magnitude of the errors in predictions for various times into a single measure of predictive power, or in our case, a quantification of the distance between two topographic maps. The RMSE function is defined as:

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \hat{x}_i)^2}{n}}$$  \hspace{1cm} (1)

where $n$ is the number of electrodes, $x_i$ is the voltage at electrode $i$ in the topographical map $x$ (the observed map at time $t$), and $\hat{x}_i$ is the voltage at electrode $i$ in the topographical map $\hat{x}$ (the observed map at time $t - L$). Thus, Eq. (1) provides the RMSE between the topographic maps of two timeframes, $x$ and $\hat{x}$, each comprised of $n$ discrete electrode values, indexed by i, which runs from 1 to $n$ (typically, 128 or 256 electrodes).

The proposed algorithm takes as input $n$ channels of ERPs, typically calculated across subjects and within conditions. The RMSE values over the specified baseline interval (e.g., within the last 400 ms of a jittered baseline) capture background noise levels and permit construction of a Confidence Interval (CI) around the mean RMSE value over the baseline interval. Because the RMSE over the baseline period is used as a measure of error variance in the RMSE function, the baseline from which these ERPs are calculated should be appropriately temporally jittered to avoid anticipatory responses to the stimulus onset.

In addition, a lag parameter, $L$, is provided to set the distance between topographical maps that are to be compared. $L$ is the minimum duration for a putative microstate, which means the time interval between topographical maps (i.e., map $x$ and map $\hat{x}$) that are to be compared is equal to the minimum duration for a putative microstate. In the case of exogenous (stimulus driven) ERP microstates this duration might be quite brief, whereas for endogenous ERP microstates this duration may be longer. The results of

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$^2$ The parameter, $L$, could be treated as a variable. As but one instance, $L_{\text{exogenous}}$ could be specified to represent the minimum duration specified for a microstate that occurs within 100 ms of the stimulus presentation (exogenous components), and $L_{\text{endogenous}}$ could be specified to represent the minimum duration specified for
an RMSE analysis is illustrated in Fig. 1, where the abscissa reflects arbitrary units of time, the ordinate reflects hypothetical RMSE values, \( L \) is specified to be 5 and the sampling period is 1. Point A reflects the onset of the transition period from hypothetical stable microstate #1 to microstate #2. Point B (first peak in the post-stimulus RMSE function that exceeds the specified CI (see Fig. 1) constitutes the start of the next putative event-related microstate, which extends either to the end of the recording epoch (as illustrated by Point C in Fig. 1) or until a significant decline and another significant rise in RMSE, as illustrated below. The timing of each microstate is peak-to-trough, inclusive. For instance, the peak to end of trough interval (Interval B to C in Fig. 1) represents a stable microstate.

The case depicted in Fig. 1 is simplified to illustrate the concepts of transition states and event-related brain microstates. In practice, local maxima/minima may represent noise rather than a true peak/trough. The RMSE algorithm in the micro-segmentation suite, therefore, defines a peak as a local maximum in the post-stimulus RMSE function that meets two conditions: (a) this local maximum exceeds the mean baseline (or, for all microstates following the first, exceeds the prior trough or the mean baseline, whichever is larger) by the CI (e.g., 2.575 \( \times \) SD for a 99% CI), and (b) it is followed by a decrease in RMSE that exceeds this CI. Thus, a local maximum in the post-stimulus RMSE that exceeds the CI but is followed by a small (i.e., less than the CI) decrease before RMSE rises again to reach a higher peak is disregarded as a peak. Conversely, troughs in the post-stimulus RMSE function are defined as a local minimum that is: (a) preceded by a decrease in RMSE from the prior peak that exceeds the CI (e.g., 2.575 \( \times \) SD for a 99% CI), and (b) is followed by an increase in the RMSE that exceeds this CI. For this reason, peak-to-trough intervals, inclusive, in the RMSE function represent discrete microstates.

Once the set of potential microstates has been specified, the representative template map for each is calculated by taking the average of the timeframes (i.e., topographic maps) that are identified as being members of a given microstate. In the illustration in Fig. 1, this would mean averaging the maps for times 1–6 to derive the template map for the first hypothetical microstate and averaging across the maps for times 11–16 to derive the template map for the second microstate. Perusal of the configurations of brain activity associated with each microstate and transition state in Fig. 1 illustrates that the exclusion of the topographic maps from the transition state in the template maps for each microstate should improve the identification of the onset and offsets of stable brain microstates and, therefore, improve performance of source localization algorithms used to investigate the underlying neural correlates for these microstates.

In summary, the RMSE analysis decomposes the ERP into a baseline state, transition states, and discrete event-related microstates. The RMSE micro-segmentation does not require the a priori specification of the number of event-related microstates, and it produces timing information regarding the onset and duration of each microstate. As such, the RMSE algorithm improves hypothesis testing over k-cluster analyses by eliminating a confirmatory bias and increasing the ways in which empirical evidence can disconfirm an investigator’s a priori hypotheses. The RMSE analysis is only the first step in a series of analyses required for micro-segmentation, however. To illustrate why this is the case and the additional analyses that have been implemented to identify distinct event-related microstates, we simulated a set of different ERP outcomes (see Section 5.1, below). We describe these simulations after defining the remaining components of the micro-segmentation suite.

### 4.2. Global field power function

Global field power (GFP) is equivalent to the standard deviation of the electrode voltages for a given timeframe (topographic map). To identify changes in the overall level of activation of the brain, we use the GFP function defined as follows:

\[
\text{GFP} = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n}}
\]

where \( x_i \) is the voltage at electrode \( i \) in the map \( x \), \( \bar{x} \) is the average voltage of all electrodes of the map \( x \) and \( n \) is the number of electrodes in map \( x \). As was done for the RMSE values over the specified baseline interval, a CI is calculated for the GFP values around the mean GFP value over the same specified baseline interval. Meaningful changes in GFP levels are then determined in the same way as for RMSE.

### 4.3. n-dimensional cosine similarity metric

To determine whether the microstates identified in the RMSE differ in the configuration of brain activity, the global field power, or a combination of the two, we employ a multi-dimensional cosine similarity metric based on the cosine distance between template maps for successive event-related microstates. Each topographic map can be represented as a vector in \( n \)-dimensional sensor space where \( n \) represents the number of electrodes, and the length of the vector within a given dimension representing the ERP amplitude recorded at the given electrode site in the topographic map.

Consider the simplified illustration in Fig. 2 where each topographic map consists of the measurements at 2 electrode sites, Electrode 1 and Electrode 2. For illustrative purposes, only a limited range of positive values for Electrode 1 and Electrode 2 are depicted; Fig. 2 therefore illustrates the upper right quadrant of the 2-dimensional vector space for the brain activity recorded over Electrodes 1 and 2. Each of the topographic maps (i.e., \( t_1 \), \( t_2 \), \( t_3 \), and \( t_4 \)) in this hypothetical microstate can be depicted as a vector within this two-dimensional sensor space, and the template map for the microstate (\( T \)) is the mean of these vectors.

In vector space, two topographic or template maps that represent the exact same configuration of brain activity will be perfectly co-linear (regardless of GFP or magnitude) and therefore the angle formed by the two vectors will be 0°. Conversely, two vectors that represent exactly opposite configurations of brain activity (i.e. the positive values in one map are negative in the other and vice versa), again regardless of magnitude will point in opposite directions and therefore form an angle of 180°. Taking the cosine of the angle formed by two vectors means that perfectly similar map vectors yield a value of 1 whereas perfectly dissimilar map vectors yield a value of −1.

We use a modified cosine distance as the metric to quantify the difference between any given topographic map and/or template maps (i.e., vectors in \( n \)-dimensional sensor space). For any given pair of such vectors \( A \) and \( B \), we define similarity as:

\[
\text{Similarity} \ (A, B) = 1 - \cos(\theta) = 1 - \frac{A \cdot B}{||A|| \cdot ||B||}
\]

where \( \theta \) is the angle formed by vectors \( A \) and \( B \), and \( ||.|| \) indicates magnitude. The similarity values produced by this formula range from 0 indicating exactly equal configurations of brain activity (regardless of GFP), to 2 indicating exactly opposite configuration, with in-between values indicating intermediate similarity or
dissimilarity. The standard deviation for the metric specified in Eq. (3) for a given microstate is specified as:

\[ \text{SD} = \sqrt{\frac{\sum_{i=1}^{m} \text{Similarity} (t_i, T)^2}{m}} \]  

where \( t_i \) is the vector representation of each topographical map in \( n \)-dimensional sensor space, \( T \) is the mean of the constituent topographical maps within a given microstate, and \( m \) represents the number of topographical maps that were averaged to generate the template map \( T \) for the microstate.

To determine whether the microstates identified in the RMSE differ in the configuration of brain activity a confidence interval around each template map \( T \) is constructed within the \( n \)-dimensional sensor space based on the differences in the angles between each template map vector’s constituent topographic maps, \( t_i \). We specify a 95% confidence interval as

\[ \text{CI} = 1.96 \times \sqrt{\frac{\sum_{i=1}^{m} \text{Similarity} (t_i, T)^2}{m}} \]  

In Fig. 2, the 95% confidence interval around template map \( T \) for the constituent topographic maps \( t_1, t_2, t_3, \) and \( t_4 \) is illustrated by the dashed lines around \( T \). The obtained value for CI specifies the cosine distance (representing the angles around \( T \)) within which a subsequent configuration of brain activity across the \( n \)-dimensional sensor space would be evaluated as equivalent to the microstate represented by template \( T \). Specifically, the template map, \( T' \), for the successive event-related microstate would be compared to the preceding microstate, \( T \), by calculating the cosine distance between \( T' \) and \( T \), as specified by Eq. (3). If this value falls outside the CI around \( T \), then the microstate \( T' \) would be interpreted (with 95% confidence) as representing a significantly different configuration of brain activity – that is, a distinct microstate whether or not GFP also changed between the two microstates. In this way, the \( n \)-dimensional cosine distance metric makes it possible to determine whether template maps for successive brain microstates differ in configuration of brain activity, global field power, or a combination of the two.

To summarize thus far, a template map \( T \) is determined for each microstate identified by the RMSE algorithm by averaging across the constituent topographic maps. The template map for a given microstate is equivalent to the mean of the topographic maps and can be represented in this \( n \)-dimensional sensor space. In addition, each of the constituent topographic maps for a given template map can also be depicted in this \( n \)-dimensional sensor space. The standard deviation of the similarity between each topographic map and their associated template map provides a measure of the variability in the configuration within a given microstate in \( n \)-dimensional vector space. This measure of variability is useful for two reasons. First, a confidence interval can be specified for each microstate based on the similarity of the topographic map vectors and the template map vector, and the angle between \( T \) (the mean \( n \)-dimensional vector for a microstate) and \( T' \) (the mean vector for the \( T + 1 \)st microstate) can be used to evaluate statistically the likelihood that this succeeding microstate identified by RMSE represents the same or a different configuration of brain activity across the \( n \)-dimensional sensor space, independent of GFP. Thus, with the exception of the first event-related microstate, the cosine similarity metric makes it possible to evaluate quantitatively which of the succeeding microstates identified by the RMSE algorithm change in the configuration of the activity from the preceding event-related microstate across the sensor space (putatively reflecting a change in neural locus of these scalp potentials) and which represents a change in magnitude but not in the configuration of brain activity (putatively reflecting a change in the overall activation of a given neural locus rather than a change in the neural locus of these scalp potentials). This is possible because the cosine distance is a measure of difference in orientation of two or more vectors (i.e. template or topographic maps) and does not consider their magnitude.

Second, the baseline state in fMRI has been found to represent a default mode of brain activity, but this default baseline is determined separately from the temporally jittered pre-stimulus baselines used in event-related electrical (or functional) neuroimaging. The standard deviation metric provides a means of determining the extent to which any baseline state represents primarily noise or some underlying time-locked microstate. The topographic maps observed during the baseline can be treated as vectors \( \mathbf{t}^0 \), and the template map for the baseline can be treated as vector \( T^0 \). If the baseline is appropriately jittered (i.e., if the individual topographic maps during baseline are not time-locked to a stimulus), then the scalp potential maps should represent random fluctuations around zero, reflecting random error. If this is the case, then the standard deviation of the vector angles formed between the constituent topographical maps \( \langle \mathbf{t}^0 \rangle \) and the baseline template map \( T^0 \) should be large. To determine the standard deviation that would be expected by chance in an \( n \)-dimensional sensor space, we performed a series of Monte Carlo simulations, each consisting of 10,000 iterations in which a value for each sensor was randomly generated, and the mean \( T \) and standard deviation was determined using Eq. (4). Monte Carlo simulations were performed for two \( n \)-dimensional sensor spaces (\( n \): 64 or 128 sensor space...
topographic maps) × three different numbers of topographical maps \((m: 3\) topographic maps, reflecting a short microstate; 30 topographic maps, reflecting a long microstate; and 300 topographic maps, reflecting a baseline state), for a total of six simulations of 10,000 iterations. The results are illustrated in Fig. 3.

In the Monte Carlo simulations, we found that with respect to the number of topographic maps that go into a template map, in the limit the SD for the cosine similarity metric approaches 1. This is expected because if the topographic maps \((t_i)\) are evenly distributed around the sensor space, the average distance from the template map to each the topographic maps would be 1 and the standard deviation would be 1. Smaller numbers of topographic maps imply greater sampling error, which means each (small) sample of topographic maps will appear less random than they are, and the histograms of standard deviations will be more distant from 1 than when the number of topographic maps is large. Note, too, that the distribution of sample outcomes is more precise the greater the number of topographic maps that go into the calculation (see left versus right columns in Fig. 3) and for the number of sensors \((64\) vs. \(128)\) that go into each topographic map (see top vs. bottom rows in Fig. 3). The difference between the latter is small as would be expected given the size of these sensor spaces.

The SDs that are observed in these Monte Carlo simulations should be much larger than the SD for a discrete event-related microstate, which is defined in terms of a stable series of similar topographic maps. Because the confidence interval for a baseline that represents random noise (e.g., appropriately jittered baselines in ERP studies) would be so large as to include true event-related microstates, the cosine similarity metric is not applicable to determine whether the template map for the first microstate falls within the CI for the preceding baseline. However, when the baseline represents primarily background noise levels rather than a specific, time locked microstate, the first event-related microstate identified by RMSE by definition represents a unique microstate, and this is the case regardless of GFP.

False positives in the identification of discrete microstates are a possibility, especially as the time series for topographic maps increases. For this reason, the default for RMSE analyses is a 99% CI. However, neither the RMSE function nor the cosine similarity metric is suited to detect such false positives. For this and other issues in micro-segmentation, we turn to bootstrapping using high performance computing.

### 4.4. Between-subjects and within-subjects bootstrapping

Typically, one assumes that the series of event-related microstates evoked across trials or across participants is homogeneous. This assumption may not be justified, however. We therefore implemented a bootstrapping procedure to identify heterogeneities in the timing or number of microstates as well as their representative template maps across analysis trials, runs, or participants. Specifically, high performance computing permits the application of a bootstrapping-segmentation process in which the microstate segmentation routine is run on a large number of bootstrapped ERPs. This bootstrapping procedure can be performed either within-subjects or across groups of subjects. In the case of within-subject bootstrapping, at each iteration a unique ERP is “bootstrapped” by a process of random selection from the
available trials in a given subject’s EEG recording for a given condition, with the selected trials then averaged to generate an ERP for that subject and condition. In between-subjects bootstrapping, a pre-processing step must be performed in which each subject’s EEG recordings for a given condition are reduced to a within-subject ERP by averaging as depicted in Fig. 4. The rest of the between-subjects bootstrapping procedure is the same as the within-subjects procedure but instead of performing a random selection from the set of one subject’s available trials, the bootstrapped ERP is generated by selecting from the set of all subjects ERPs for the given condition. In either case, a random sample of \( r \) (without replacement) of the available \( N \) possibilities is used to generate the bootstrapped ERP.

Following each bootstrap ERP generation phase, the resulting ERP (either within- or between-subjects) is subjected to the microstate segmentation routine. These steps are repeated a large number of times (on the order of thousands to quadrillions). The total number of unique bootstrapped ERPs (i.e., possible unique combinations samples of size \( r \) from a population of size \( N \)) is given by (N choose \( r \))

\[
\binom{N}{r} = \frac{N!}{r!(N-r)!}
\]

For instance, if \( N=50 \) participants in a study and \( r=30 \) participants in each bootstrapped ERP, the total number of unique bootstrapped ERPs that can be calculated across these 50 participants is \( 50!/ (30! * 20!) = 47,129,212,246,893 \). Bootstrapping can be performed on a subset of perhaps several thousand of these more than 47 trillion combinations or, using high performance computing, the entire population of bootstrapped ERPs could be generated and analyzed. The results from each run are aggregated to determine the distribution of solutions and the robustness of the solution derived when performing the analysis on all \( N \) participants (i.e., the grand average solution). A unimodal, leptokurtic distribution of solutions for a given microstate cantered on the grand average solution increases the confidence in the overall solution, whereas a multimodal, platykurtic distribution of solutions for a microstate signals that the microstate lacks robustness (e.g., significant unidentified sources of variance or moderator variables are operating). The replicability of a microstate and the performance of source localization algorithms should be superior for robust than nonrobust microstates.

5. Validation

5.1. Simulated data

All simulated ERPs were generated on a 128 electrode set (sensor space) with a sampling rate of 1000Hz with “stimulus onset” specified as time 0 and the simulated ERP ranging from \(-30 \)ms to \(+69 \)ms (i.e., 100 one-millisecond bins). The baseline onset for RMSE

\[ \text{The latter strategy has advantages such as using computational tools to empirically generate new hypotheses about possible sources of variance (e.g., an individual difference) in the brain’s microstates.} \]
analyses is the onset of the simulated time series plus L, the specified lag between the topographical map \( x \) (the observed map at time \( t \)), and the topographical map \( \hat{x} \) (see Eq. (1)). As a result, the baseline onset in these simulations is \(-24 \text{ ms}\). The 128 electrodes were partitioned into two non-overlapping sub-sets corresponding to anterior and posterior electrode locations, henceforth referred to as the anterior or posterior electrode set. The RMSE, GFP, and cosine similarity metric between successive microstates are used to disambiguate the processes underlying the ERP. To inspect the assumption of homogeneity and to identify false positives, bootstrapping was performed 1000 times, and the results of the micro-segmentation across these bootstraps were quantified. Inspection of the results of the bootstrapping (e.g., the percent of bootstrapped runs that produced peaks or valleys within ±5% and ±10% time window around the mean lag) was used to provide a quantitative means of identifying robust micro-segmentation solutions and assist in distinguishing robust microstates from false positives.

5.1.1. Simulation set 1: same configuration and same power

We begin with the case in which the global electrical brain activity (i.e., configuration) and the GFP are the same across the ERP. This case represents the null hypothesis, that is, the absence of an event-related microstate. These ERPs were generated by: (a) setting electrical potential at sensors in the anterior and posterior electrode sets to 0 \( \mu \text{V} \), respectively, across all timeframes in the simulated recording (i.e., no signal; see Fig. 5, Panel A), and (b) adding a value in the range ±10 \( \mu \text{V} \) from a uniformly distributed pseudorandom set at each timeframe across the entire simulated recording and for each sensor to introduce random noise (Fig. 5, Panel B). Fifty such ERPs were generated, and the grand mean was calculated (Fig. 5, Panel C).

The RMSE algorithm outlined above was applied to the maps from the 128 sensors across time, and a 99% CI was calculated based on the brain activity during baseline. The RMSE analysis shows no evidence for an event-related microstate (Fig. 6, Panel A), and the bootstrapping results confirmed the absence of any replicable microstate solutions (Fig. 6, Panel B). Analyses of the GFP function produced similar results. Thus, the algorithm accurately specified that there was no change from baseline in the configuration of activation or in the overall level of brain activation.

5.1.2. Simulation set 2: different configurations and the same power

We next examined a simulated case in which the stimulus evokes changes in global configuration of electrical brain activity but not in the GFP. The specific case illustrated in Fig. 7 represents two event-related changes in the neural locus of the ERP, leading to
two distinct event-related microstates. The simulated ERP at each of the 128 sensors was generated as specified in Fig. 7. The template maps in each panel in Fig. 7 depict time-time-averaged electrical activity over the specified temporal window.

The RMSE algorithm was applied to the grand mean across all subjects, and a 99% CI was calculated based on the brain activity during baseline (Fig. 8, Panel A). The RMSE analysis shows a stable baseline state followed by the transition to the first stable event-related microstate from 17 to 30 ms, and transition to a second stable microstate from 38 to 69 ms (i.e., the end of the recording trial).

The cosine similarity metric provides an important test of whether the 128-dimensional vector representing the template map for the $n$th microstate falls within or outside the confidence interval of the vector representing the template map for the $n$th microstate. The 95% CI for the vector for microstate 1 was ±.0004, and the cosine distance between the vectors for microstates 1 and 2 was 2.0, which means the second microstate fell outside the 95% confidence interval surrounding the vector for the first microstate, indicating (accurately) that the second microstate represented a sufficiently different configuration to be designated a separate microstate – indeed, the configuration of activity in microstate 2 was precisely the opposite of that found in microstate 1.

The between-subjects bootstrapping results for the RMSE analysis indicated a two microstate solution in 99.8% of the runs and a homogeneity across participants in the onsets and offsets for the two microstates identified in the overall analysis. For instance, the distributions for the onset of microstate 1 (Fig. 8, Panel B, $\Sigma_1$), offset for microstate 1 (Fig. 8, Panel B, $\Sigma_2$), and onset for microstate 2 (Fig. 8, Panel B, $\Sigma_3$; the offset for microstate 2 was the end of the recording interval in all cases) indicate robust microsegmentation results across subjects. These results correspond well to the underlying signal embedded in noise in this simulation – an initial microstate that extended from 14 to 30 ms and a second distinct microstate that extended from 38 to 69 ms, plus random noise unique to each subject. Finally, analyses of the GFP function (not shown) indicated that there was an increase in GFP during the first transition period. In the transition between state1 and state2 there was a large drop in GFP followed immediately by another large rise. During each of the microstates, the GFP was high and stable with small blips due to noise.

**Fig. 6.** RMSE Microsegmentation results for Monte Carlo simulations depicting random noise. Panel A: Results of the RMSE microsegmentation routine applied to the grandmean of all 50 simulated individual’s ERPs. No unique microstates are detected. Panel B: Summary of the results of 1000 bootstrap RMSE analyses to identify the onsets and offsets of the microstates. In each application of the bootstrap routine, 30 out of the available 50 simulated individual ERPs were selected at random, averaged together, and the resulting ERP subjected to the RMSE segmentation algorithm. Temporal locations of peaks/valleys are accumulated over the 1000 iterations and normalized by the number of iterations to compute the percentage of bootstrap runs in which a peak/valley is identified at a specific sampling bin. Results indicate the identification of microstate onsets and offsets at levels that would be expected by chance.
5.1.3. Simulation set 3: same configuration and the different power

In our third series of simulations, we examined the case in which the stimulus evokes a global configuration of electrical brain activity (i.e., a single microstate) that was expressed across the microstate at two distinct levels of GFP. The specific case illustrated in Fig. 9 represents one event-related change in the neural locus of the ERP that extended from 14 to 69 ms, during which time there was an increase in the amount of brain activation (i.e., GFP). The simulated ERP at each of the 128 sensors was generated as specified in Fig. 9.

The RMSE algorithm was applied to the grand mean across all subjects, and a 99% CI was calculated based on the brain activity during baseline (Fig. 10). The initial RMSE analysis shows a stable baseline state followed by the transition to a stable event-related microstate from 17 to 30 ms and a second from 36 to 69 ms (i.e., the end of the recording trial). Importantly, the cosine similarity metric indicated that the second microstate did not differ from the first. Specifically, the 95% CI for the template vector for microstate 1 was ±.0004, and the cosine distance between the template vectors for microstates 1 and 2 was ±.00001, which means the second microstate fell within the 95% CI surrounding the vector for the first microstate, indicating (accurately) that the second microstate represented the same microstate (Fig. 10, Panel A).

The between-subjects bootstrapping results for the RMSE analysis, which are summarized in the middle and bottom of Fig. 10, indicated a two microstate solution in 100% of the 1000 runs. The RMSE final overall RMSE analysis indicated there was a single microstate from 17 to 69 ms. The distribution for the onset of microstate 1 (Fig. 10, Panel B, Σ1) indicates that the onset of the microstate identified in the overall analysis was homogeneous across subjects. The offset of this microstate was the end of the recording interval in all cases, as well. These results again correspond well to the underlying signal embedded in noise in this simulation – an initial microstate that extended from 14 to 69 ms that included an increase in GFP from 31 to 37 ms that was
not associated with a change in the global configuration of brain activity.

5.1.4. Simulation set 4: different configurations and different power

The final case we examined is when the stimulus evokes two changes in the configuration of global electrical brain activity and changes in global field power. This case represents event-related changes in the neural locus of the ERP, leading to two distinct event-related microstates, each of which is also associated with a different GFP. The simulated ERP at each of the 128 sensors was generated as specified in Fig. 11. The template maps in each panel in Fig. 11 depict time-time-averaged electrical activity over the specified temporal window.

The RMSE analysis, summarized in Fig. 12, shows a stable baseline state followed by a transition to a stable event-related microstate from 17 to 30 ms, followed by a brief transition to a second stable microstate that extended from 38 to 69 ms (i.e., the
end of the recording trial). The cosine similarity analysis indicated that the 95% CI for the vector for microstate 1 was ±0.004, and the cosine distance between the vectors for microstates 1 and 2 was 2.0; as in Simulation Set #2 (above), the second microstate represented a separate microstate, with the configuration of activity in microstate 2 precisely the opposite of that found in microstate 1 even though the GFP differed for these microstates (see Fig. 11, Panel A).

The between-subjects bootstrapping results for the RMSE analysis, which are summarized in Fig. 12 (Panels B and C), indicated a two microstate solution in 99.4% runs. The distributions for the onset of microstate 1 (Fig. 12, Panel B, Σ1), offset for microstate 1 (Fig. 12, Panel B, Σ2), and onset for microstate 2 (Fig. 12, Panel B, Σ3; the offset for microstate 2 was the end of the recording interval in all cases) indicate that the onsets and offsets for the two microstates identified in the overall analysis are homogeneous across participants (see Fig. 12, Panel C, for summary statistics).

In sum, the RMSE algorithm permits the identification of event-related microstates. Note that the RMSE approach does not require that the number of microstates to be provided a priori, and it is able to identify “transition states” thereby excluding transition timeframes from sets of stable timeframes (the microstates themselves). In addition, the construction and application of a CI for global field power permits the detection of event-related changes in the overall amount of brain activity, and the n-dimensional cosine similarity metric makes it possible to test whether the vector representing the template map for the n + 1st microstate falls within or outside the confidence interval for the vector representing the template map for the nth microstate, providing information about whether or not a putative microstate reflects a change in the overall configuration of brain activity from the preceding configuration (i.e., a new microstate). As illustrated in these simulations, this suite of analytic tools accurately differentiates among discrete microstates and changes in GFP.

5.2. Experimental data

To further investigate the performance of this suite of computing tools for micro-segmentation, we conducted an empirical study using a basic visual paradigm, the reversal checkerboard task, in which the pattern reverses every 500 ms. The checkerboard task is common because there is considerable inter-subject reliability in
Fig. 10. RMSE microsegmentation results for Monte Carlo simulations depicting random noise and one post-stimulus microstate with variable global field power. Panel A: Results of the RMSE microsegmentation routine applied to the grandmean of all 50 simulated individual’s ERPs. One stable Microstate is identified with onset at 17 ms post-stimulus and persists until the end of the ERP at 69 ms. In the initial phase of the RMSE segmentation procedure a second microstate is identified with time window 36 ms to 69 ms post-stimulus but in the final result it is merged with the first microstate as it fell within the confidence interval of cosine distance of the first microstate. Panel B: Summary of the results of 1000 bootstrap RMSE analyses to identify the onset and offset of the microstate. In each application of the bootstrap routine, 30 out of the available 50 simulated individual ERPs were selected at random, averaged together, and the resulting ERP subjected to the RMSE segmentation algorithm. Temporal locations of peaks/valleys are accumulated over the 1000 iterations and normalized by the number of iterations to compute the percentage of bootstrap runs in which a peak/valley is identified at a specific sampling bin. Panel C: Summary of the distribution of peaks found in bootstrap analysis within ±5 ms time window around the microstate onset identified in the overall microsegmentation analysis of the RMSE curve. The time-weighted mean of peaks in the overall analysis is 17.0 ms. Bootstrap results indicate a microstate onset in 89.3% of the runs within the ±5 ms time window. No additional solutions were found for the ±10% window. Note: Microstate 1 was maintained throughout the recording interval, so the offset of microstate 1 is specified as the end of the recording interval, which in this simulation is 69 ms.

terms of the visual ERP that it elicits. Specifically, a negative peak appears at a latency of about 70–95 ms, a larger amplitude positive peak appears at about 100–120 ms, a more variable negative peak appears around 140–160 ms, and a later, smoother positive peak around 200 ms (Lehmann and Skrandies, 1980; Luck and Kappenman, 2012; Regan, 2009; Sutter, 2010; Slotnick et al., 1999; Di Russo et al., 2003, 2005; Emmerson-Hanover et al., 1994). As in the simulation studies, the RMSE, GFP, and cosine distance between successive microstates are used to disambiguate the processes underlying the ERP. As outlined above, these three analytic tools do not provide a test of how robust are the results. For instance, individual differences in stimulus-evoked information processing and false positives are not discernible using these three analyses alone. To investigate the extent to which the microstates are reliable and generalizable across the sample, we performed between-subjects bootstrapping.

5.2.1. Experimental design and participants

Participants were 22 volunteers (8 females) with a mean age of 23.18 (SD = 3.92) years. All were right-handed (Edinburgh Handedness Inventory; Oldfield, 1971), and had normal or corrected to-normal visual acuity. None had any prior or current neurological
or psychiatric impairment, as ascertained by a detailed anamnesis. Prior to participation, volunteers provided written informed consent that had been approved by the Institutional Review Board of the University of Chicago.

The experimental design was a 2 (Task instructions: passive viewing vs active visual search) × 2 (Counterbalanced Order) between-subjects factorial design. We focus here on the data from the passive viewing condition because this replicates the instructional condition in the checkerboard reversal task (Schneider et al., 1993). In this condition, participants were instructed to passively view the center of a reversing checkerboard.

5.2.2. Procedure

Checkerboards had a spatial frequency of 1 cycle/deg, covered 5.4 x 5.57° of visual angle and were reversed every 500 ms (duration confirmed by photocell measurements; E-prime Psychology Software Tools Inc., Pittsburgh, USA). A red cross of 1° of visual angle was placed in the top center of the monitor and the participants were instructed to fixate this cross throughout visual stimulation. Stimuli were displayed in black and white on a monitor screen, with refresh rate of 60 Hz. Visual stimuli were presented on a PC computer using EGI-E-prime Psychology Software Tools Inc., Pittsburgh, USA under Windows XP, which provides control of display durations and accurate recordings of reaction times. Participants were comfortably seated 100 cm away from a PC computer screen in which stimuli were presented centrally. The task consisted of 250 checkerboard reversals.

5.2.3. EEG data collection

Continuous surface electroencephalogram (EEG) was recorded from 128 AgAgCl carbon-fiber coated electrodes using an Electric Geodesic Sensor Net (GSN300; Electrical Geodesic, Inc., OR;
Fig. 12. RMSE microsegmentation results for Monte Carlo simulations depicting random noise and two distinct post-stimulus microstates that differ also in global field power. Panel A: Results of the RMSE microsegmentation routine applied to the grandmean of all 50 simulated individual’s ERPs. Microstate 1 is identified in the time window ranging from 17 ms to 30 ms post-stimulus and Microstate 2 in the time window ranging from 38 ms post-stimulus and persists until the end of the ERP at 69 ms. Panel B: Summary of the results of 1000 bootstrap RMSE analyses to identify the onsets and offsets of the microstates. In each application of the bootstrap routine, 30 out of the available 50 simulated individual ERPs were selected at random, averaged together, and the resulting ERP subjected to the RMSE segmentation algorithm. Temporal locations of peaks/valleys are accumulated over the 1000 iterations and normalized by the number of iterations to compute the percentage of bootstrap runs in which a peak/valley is identified at a specific sampling bin. Panel C: Summary of the distribution of peaks/valleys found in bootstrap analysis within a ±5% time window around the microstate onset (or offset) identified in the overall microsegmentation analysis of the RMSE curve. The onset of microstate 1 is depicted in the left panel. The time-weighted mean of peaks in the overall analysis is 17.0 ms. Bootstrap results indicate a microstate onset in 96.8% of the runs within the ±5% time window. No additional solutions were found for the ±10% window. The offset of microstate 1 is depicted in the middle panel. The time-weighted mean of valleys in the overall analysis is 29.6 ms. Bootstrap results indicate a microstate offset in 100% of the runs within the ±5% time window. The onset of microstate 2 is depicted in the right panel. The time-weighted mean of peaks in the overall analysis is 38.0 ms. Bootstrap results indicate a microstate onset in 100% of the runs within the ±5% time window. No additional solutions were found for the ±10% window. Note: Microstate 2 was maintained throughout the recording interval, so the offset of microstate 2 is specified as the end of the recording interval, which in this simulation is 69 ms.

http://www.egi.com/), where EEG electrodes are arrayed in a regular distribution across the head surface and the inter-sensor distance is approximately 3 cm. The EEG was digitized at 250 Hz (corresponding to a sample period of 4 ms), band-width of 0.01–200 Hz, with the vertex electrode (Cz) serving as an on-line recording reference. Impedances were kept below 100 kΩ. Data logging were via NetStation 4.5. Data were collected in two sessions with brief intervening rest periods for the participant. As in previous visual-evoked potential (VEP) studies (e.g., Luck, 2014, pp. 244–245; Nunez and Srinivasan, 2006; Ortigue et al., 2004; Tunik et al., 2008), the data were band pass filtered between 1 and 30 Hz with a roll-off slope of 12 dB/Octave.

5.2.4. ERP data pre-processing
Electrophysiological data were first pre-processed at the individual level. All trials were visually inspected for oculomotor (saccades and blinks), muscles, and other artifacts. Channels with corrupted signals were interpolated. Surviving epochs of EEG were
averaged for each participant to calculate the ERP. The ERP is illustrated in Fig. 13 for the O1 and O2 recording sites. The ERP morphology observed over the O1 and O2 sensor sites is similar to that observed previously, with a negative peak around 96 ms, a larger positive peak around 128 ms, a second negative peak around 180 ms, and a smoother positive peak around 240 ms.

5.2.5. ERP data reduction and analyses

The RMSE and the GFP micro-segmentation algorithms were next applied to the high-density ERP grand average recorded across the scalp. The resulting RMSE and GFP functions and CIs for each are depicted in Fig. 14. The lag, L, was specified as 8 ms, a 99% CI was used to construct thresholds for the RMSE and GFP analyses, and a 95% CI was used for cosine metric analyses. The RMSE algorithm identified: (a) a stable baseline configuration from the start of the baseline (−152 ms) to stimulus onset, (b) the first discrete event-related microstate from 92 to 100 ms, (c) the second microstate from 116 to 132 ms, (d) the third microstate from 144 to 164 ms, (e) the fourth microstate from 180 to 208 ms, and (f) a fifth microstate from 224 to 436 ms.

A 128-dimensional cosine similarity metric analysis was performed next to determine whether each successive microstate represented a significant change from the preceding microstate in the overall configuration of electrical activity across the sensor space. The cosine distance between each contiguous pair of microstates fell outside the 95% CI for the earlier of the two microstates, indicating five discrete event-related microstates. Specifically, the cosine distance between microstates 1 and 2 was 1.82, which fell well outside the 95% CI for microstate 1 of ±0.01. Similarly, the 95% CI and cosine distance between each of the succeeding microstates was (i.e., microstates 2 and 3, microstates 3 and 4, microstates 4 and 5) fell outside the 95% CI of the earlier of the two microstates (cosine distances = 0.114, 1.76, and 1.24, respectively; CIs = ±0.003, ±0.113, and ±0.449, respectively).

The between-subjects bootstrapping results for the RMSE analysis are summarized in Fig. 14 (Panels B and C). The analyses, which are summarized in Fig. 14, indicated more robust micro-segmentation for early than late microstates, as would be expected. Specifically, in the first 2 microstates the bootstrapping indicated 98–100% homogeneity whereas in the last 2 microstates the bootstrapping indicated homogeneity had dropped to 50–60%. Interestingly, the bootstrapping also indicated that five microstates were identified in only 26.8% of the runs. Although this was the modal solution, four microstates were identified in 20.3% of the runs, six microstates were identified in 23.1% of the runs, and seven microstates were identified in 14.4% of the runs. (The remaining 15.4% of the runs identified various numbers of microstates ranging from two to ten.) Together, these results suggest that all participants may not be showing the same microstate structure during the reverse checkerboard task, and specifically that any such individual differences in the neural responses to this task are especially likely to be emerging after the second microstate (i.e., after 132 ms).

Inspection of the GFP function and CI (Fig. 15) indicates three distinct epochs during which time GFP changed. GFP increased from basal levels beginning at 48 ms post-stimulus, peaking at 96 ms, falling to a trough at 108 ms, increasing to a second peak at 128 ms, falling to a trough at 188 ms, rising to a third (but lower) peak at 236 ms where it remained fairly stable through the rest of the recording period.

Between-subjects bootstrapping was then performed to investigate how robust were these changes in GFP across subjects. The GFP analysis was performed on the same bootstrapped ERPs used in the RMSE analyses. The results are displayed in Panel B of Fig. 15, and the summary statistics are provided in Panel C and the caption of Fig. 15. The results paralleled those for RMSE, with the overall analysis showing reasonably robust results with increasing variability during the latter segments of the post-stimulus period.

5.2.6. Illustrative inverse solution

Although the current quantitative suite of tools focuses on the identification of discrete event-related brain microstates, it should improve performance of source localization algorithms (see Discussion). To illustrate the potential value of such data, we used a distributed linear inverse solution to estimate the sources in the brain that gave rise to each of the microstates in the checkerboard task. The inverse matrices applied here for illustrative purposes were based on a low-resolution brain electromagnetic tomography (LORETA) model of the unknown current density in the brain.
Fig. 14. RMSE Microsegmentation results for the Checkerboard Task. Panel A: Results of the RMSE microsegmentation routine applied to the grandmean of all 22 individual’s ERPs. Microstate 1 is identified in the time window ranging from 92 ms to 100 ms post-stimulus, Microstate 2 in the time window ranging from 116 ms to 132 ms post-stimulus, Microstate 3 in the time window ranging from 144 ms to 164 ms post-stimulus, Microstate 4 in the time window ranging from 180 ms to 208 ms post-stimulus, and Microstate 5 from 224 ms post-stimulus and persists until the end of the ERP at 436 ms. Panel B: Summary of the results of 1000 bootstrap RMSE analyses to identify the onsets and offsets of the microstates. In each application of the bootstrap routine, 11 out of the available 22 individual ERPs were selected at random, averaged together, and the resulting ERP subjected to the RMSE segmentation algorithm. Temporal locations of peaks/valleys are accumulated over the 1000 iterations and normalized by the number of iterations to compute the percentage of bootstrap runs in which a peak/valley is identified at a specific sampling bin. Panel C: Summary of the distribution of...
Since LORETA belongs to the class of distributed inverse sources, it is capable of dealing with multiple simultaneously active sources of a priori unknown location.

The applied version of LORETA was used with a lead field (solution space) calculated on a realistic head model using SMAC (Spinelli et al., 2000) on an average brain model provided by the Montreal Neurological Institute (MNI). Our head model included 3005 solution points, selected from a 6 mm × 6 mm × 6 mm grid equally distributed within the gray matter. Source estimations were rendered on the MNI/McGill average standard brain supplied by Cartool. As an output, this approach provides current density measures (in μA/mm²) at each solution point. The results of our micro-segmentation defined five event-related microstates (and corresponding template maps) during which intracranial sources were estimated with the distributed source inverse solution (LORETA). Although LORETA provides one current source density maximum for each microstate, it may also, as a distributed inverse solution, detect additional simultaneously active sources at other solution points. These distributed activations may be more or less intense across microstates. (For this determination, refer to the GPF function for each microstate in Fig. 15.)

Source estimations for the event-related microstates are depicted in Fig. 16. LORETA distributed source inverse estimation of the active intracranial generators of event-related microstate 1 (92–100 ms), and LORETA revealed a bilateral activation of the occipital cortex with a current source density maximum located in the primary visual cortex (Talairach coordinates, −82, 13; BA17). The second event-related microstate (116–132 ms) was characterized by neural loci just anterior to the prior microstate with a current source density maximum again located in the secondary visual cortex, V2 (Talairach coordinates, −3, −81, 18; BA17). The third microstate (144–164 ms) was associated with a bilateral activation in the parahippocampal gyrus (Talairach coordinates, −11, −15; BA28), a region that has been found in fMRI research to be activated by the checkerboard task (Rajimehr et al., 2011). In addition, visual inspection of the other brain activation sites found for microstate 3 revealed activation in the anterior cingulate area, which is known to be involved in attention. LORETA estimation of the active intracranial generators for event-related microstate 4 (180–208 ms) showed bilateral activation in the parietal lobe/precuneus (Talairach coordinates, −15, −76, 47; BA7), a brain region known to be involved in visuospatial processing (Cavanna and Tröuble, 2006). Finally, event-related microstate 5 (224–436 ms) was characterized by activation of the dorsal anterior cingulate (Talairach coordinates, −3, 26, −7; BA32), an area involved in attention, anticipation, and conflict monitoring (Weissman et al., 2005).

The ERP over the occipital sites showed a negative peak at 96 ms, a positive peak at 128 ms, a negative peak at approximately 180 ms, and a positive peak at 240 ms (see Fig. 13). The current micro-segmentation suite, which operates on configurations of activation rather than on morphological peaks or troughs at specific sensor regions, identified five event-related microstates during the reversible checkerboard task (see Fig. 14). The early negative peak over occipital regions found in the prior literature (and replicated here) was identified in the present analysis as the first event-related microstate (and an increase in GPF) that extended from 92 to 100 ms. The second microstate identified in this study was associated with a stable configuration of brain activity from 116 to 132 ms and corresponded to the first positive component (the P1) reported in the prior literature and observed in the current study. The third event-related microstate was associated with a stable configuration from 144 to 164 ms and was not evident as a morphological peak or trough in the ERP recorded over the occipital regions.

The fourth event-related microstate was associated with a stable configuration from 180 to 208 ms and corresponded to the second negative component in the literature and observed here. Finally, a less robust fifth microstate was suggested based on a stable configuration from 224 to 436 ms that corresponded to the smaller, second positive peak observed over the occipital regions around 240 ms.

In sum, the estimated neural loci of microstates 1 and 2 were maximal in regions of the visual cortex, and the loci of microstates 4 and 5 reflected anterior regions involved in visual attention, as would be expected based on prior research. For instance, in a study using fMRI and EEG, Di Russo et al. (2002) reported that the checkerboard task activated brain regions similar to those identified here (Di Russo et al., 2002). Interestingly, the results for microstate 3 suggested that the parahippocampal region and anterior cingulate, higher order components of the ventral visual pathway and attentional system, respectively, were briefly activated, consistent with prior brain imaging work showing the activation of the parahippocampal region to checkerboard stimulation (e.g., Rajimehr et al., 2011). This microstate would not have been identified had the analyses been limited to morphological features of the ERP over the occipital regions. Together, these data illustrate the potential value of the micro-segmentation analysis suite and effective source estimation programs to investigate not only which regions are activated by a task but also when these areas are activated.

6. General discussion

Microstates are conceptualized as a time-limited information processing operation in the brain. The micro-segmentation suite developed in this paper is designed to identify quasi-stable non-periodic event-related microstates of the brain based on changes in the pattern of global electrical activity as measured by high-density EEG. An RMSE metric is first applied to high-density ERP data to identify the transitions across discrete event-related brain states, and the GPF time series is analyzed to identify changes in the overall level of activation of the brain. To determine whether
the microstates identified by the RMSE metric differ in the configuration of brain activity, the GFP, or a combination of the two, an n-dimensional cosine distance metric is used to determine whether the template map for a putative microstate differs from the template map from the preceding microstate. Finally, a bootstrapping procedure is used to assess the extent to which the solutions identified in the micro-segmentation are robust (reliable, generalizable).

The transition between microstates need not be all or none, but rather may be incremental. For this reason, the micro-segmentation analysis suite has been developed to improve the specification of the configuration, number, timing, and duration of event-related microstates by distinguishing among microstates, transition states, and changes in GFP. The resulting parameters each reflect unique information about brain function, and each can be subjected to statistical analysis to determine the effects of various within-subjects and between-subjects factors to investigate information processing in the normal, waking human brain. Moreover, the current suite of analytic tools improves hypothesis testing over prior micro-segmentation techniques by eliminating the confirmatory bias that results from an investigator specifying a priori how many event-related microstates should be observed, and by increasing the ways in which empirical evidence can disconfirm an investigator’s a priori hypotheses, improve replicability, and promote empirically grounded hypothesis generation.

The results of the simulation studies confirmed that the micro-segmentation analysis suite correctly identified stable periods and changes in the overall pattern of brain activity independent of GFP. In each simulation, we produced an event-related signal embedded in considerable random noise at the level of the individual subject, with 50 unique subject-level ERPs produced by varying the random noise in which the signal was embedded. The RMSE and GFP algorithms were applied to the overall ERP, and our multidimensional cosine distance metric was used to determine when a putative succeeding microstate was in fact part of the prior microstate. Finally, bootstrapping was used to assess how robust (e.g., repeatable) was the identification of each microstate. The nature of the simulated event-related signal varied across simulations, but in each case this high performance micro-segmentation suite accurately captured the underlying signal in the simulation. For instance, if the location of activation across the scalp changed but the overall activity did not change, the micro-segmentation suite correctly identified this as a new microstate. On the other hand, if the location of activation did not change but the overall activity did, the micro-segmentation suite correctly identified this as a change in activity (power) but not a change in microstate.

An empirical validation study was performed using the well-studied visual checkerboard task. We replicated the standard visual ERP and demonstrated that the presence or absence of a morphological peak (or trough) in an ERP over a region of interest is separable from the presence or absence of a microstate. Specifically, as illustrated in the third microstate in the checkerboard task, an event-related microstate can occur in the absence of an ERP peak (or trough) over a specific region of interest (e.g., the occipital cortices in the case of the visual checkerboard task). This result, putatively attributable to the distal neural locus and dipole orientation of this microstate, illustrates an important advantage of analyzing changes in the configuration of activity over the entire high-density sensor space. On the other hand, the first, second, fourth, and fifth microstates we identified in the reversible checkerboard task illustrate the co-occurrence of event-related microstates and ERP peaks and troughs over a region of interest. In addition, the current analysis provides information about the onset, offset, and duration of these microstates; and the results of the bootstrapping step provide information about the robustness of these parameters. For instance, the bootstrapping results showed very high agreement across subjects in onsets and offsets for the first two microstates and more modest agreement across subjects for the last three microstates.

Such a result could reflect increased temporal jitter across subjects for the later microstates, but the bootstrapping results also showed that although the modal, median, and mean number of microstates detected was 5.5, and 5.37, respectively, 20.3% of the runs produced a four microstate solution and another 23.1% produced a six microstate solution. These results suggest that there may be individual differences in the measured patterns of electrical activity evoked by the checkerboard task – especially following the second microstate. For instance, the microstate structure for these latter states may differ if some participants devote more cognitive resources to anticipating the next checkerboard reversal than others. Although whether the individual differences found in these latter microstates reflect differences in the neural processes evoked by the checkerboard task, differences in the cognitive operations evoked by the task, and/or anatomical differences in dipole orientations is beyond the scope of the present paper, but these results illustrate the potential theoretical value of testing rather than assuming homogeneity in microstate structures (or information processing operations) across all subjects.

The current analytic approach makes it possible to investigate possible neural organizations underlying baseline states even in the absence of a clear morphological peak or trough. These empirical findings, together with the results of the simulations, indicate that the present analytic approach increases the spatiotemporal information that one may glean from high-density ERP data.

Finally, we focused here on the microstates because this has been the focus previously in the literature. However, one of the interesting observations to emerge from our analytic approach is that there are transition states between the quasi-stable microstates of the brain. These transition states may be of considerable interest, as well, as they putatively represent the transfer of information between microstates. The transition states, therefore, may provide information about the nature and timing of this information transfer through the brain. Second, according to continuous flow conceptions of human information processing dating back more than three decades (e.g., Ericksen and Schultz, 1979), information extracted early in the processing of a stimulus is consistent with a range of possible responses, and each of these responses receives initial activation. As information continues to accumulate, activation continues to accumulate in response channels that remain viable. A given response is evoked when the activation of its channel exceeds criterion. Importantly, continuous flow models of information processing reject the notion that information proceeds in a step-by-step fashion in which the computations performed at any given step (or microstate) are completed before any information is passed onto the next step (or microstate). Instead, information processing is depicted as proceeding through a series of computations in a semi-continuous fashion. It is conceivable that the transition states provide a means of investigating the effects of experimental conditions on this information flow. However, transition states are also affected by latency jitter so it would be

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3 The cosine standard deviation metric is used to determine whether the Template map (e.g., baseline) reflects an underlying neural signal or noise.

4 If the cartoon of microstates and transition states depicted in Fig. 1 were to represent the average ERP within a condition for a single (simulated) subject, one can imagine stacking a set of such cartoons while jittering the microstate onset latency across (simulated) subjects. This would show that individual differences in the onset latency of the same microstate across subjects manifest as a longer transition state than actually shown by any individual (simulated) subject. Note, temporal jitter can also operate across trials within an individual. Because temporal jitter is cumulative, it tends to be greater for later microstates than for early microstates. Therefore, transition states tend to be longer for later than earlier microstates, all else constant.
Fig. 15. GFP analysis for the Checkerboard Task. Panel A: Results of the GFP analysis routine applied to the grandmean of all 22 simulated individual’s ERPs. The table below the plot in Panel A indicates the temporal location of peaks/valleys identified by the GFP analysis routine. Panel B: Summary of the results of 1000 bootstrap GFP analyses to identify the locations of peaks/valleys. In each application of the bootstrap routine, 11 out of the available 22 simulated individual ERPs were selected at random, averaged together, and the resulting ERP subjected to the GFP segmentation algorithm. Temporal locations of peaks/valleys are accumulated over the 1000 iterations and normalized by the number of iterations to compute the percentage of bootstrap runs in which a peak/valley is identified at a specific sampling bin. Panel C: Summary of the distribution of peaks/valleys found in bootstrap analysis within ±5% (red) and ±10% (blue) time windows around the locations of a peak/valley identified in the analysis of the overall GFP curve. Top Left Panel: GFP valley at $t=48$ ms. The time-weighted mean of valleys in both the ±5% and ±10% windows is 49.67 ms. Bootstrap results indicate a valley in 30.1% of the runs within both the ±5% and ±10% time windows. Top Middle Panel: GFP peak at $t=96$ ms. The time-weighted mean of peaks in the ±5% and ±10% time windows are 95.67 ms and 95.68 ms, respectively. Bootstrap results indicate a peak in GFP in 99.7% and 99.8% of the runs within the ±5% and ±10% windows, respectively. Top Right Panel: GFP valley at $t=108$ ms. The time-weighted mean of valleys in the ±5% and ±10% time windows are 106.93 ms and 106.92 ms, respectively. Bootstrap results indicate a valley in GFP in 99.7% and 99.8% of the runs within the ±5% and ±10% windows, respectively. Bottom Left Panel: GFP peak at $t=128$ ms. The time-weighted mean of peaks in the ±5% and ±10% time windows are 110.98 ms and 111.09 ms, respectively. Bootstrap results indicate a peak in GFP in 99.7% and 99.8% of the runs within the ±5% and ±10% windows, respectively.
important to attend to this source of variance for the analysis of transition states to be interpretable.

6.1. Implications for source localization estimation

The accurate specification of event-related microstates should improve estimates of source localization for several reasons. First, prior micro-segmentation procedures have characterized the entire event-related potential as a series of distinct microstates, whereas the mathematical approach used here distinguishes between transition states and discrete event-related microstates. Estimates of source localization should benefit from the distinction between stable microstates and transitions between successive microstates. An ancillary benefit of this analysis is that onsets and offsets are provided for each event-related microstate and for each transition state.

Second, quantification of these event-related microstates are based on brain activity measured across the scalp rather than at a single point on the scalp. For instance, the identification of the third microstate in our reversible checkerboard task made it possible to identify potential anterior sources for a brain state that was not apparent in the ERPs recorded over occipital regions.

Third, by averaging across the stable brain maps recorded during a given microstate, the template maps provide more reliable estimates of the true configuration of brain activity that was elicited by the task. By improving the input data for source localization estimates, the output should also be better. The standard deviation of the cosine metric for a baseline state or a microstate provides additional information about the internal consistency of the template maps (configurations of activity across the sensor space). The additive nature of temporal jitter implies that the standard deviation of this cosine metric is likely to increase as one moves from early to later microstates. The standard deviation of the cosine metric, which can be thought of as an internal consistency measure, may prove useful in determining whether a source localization algorithm is likely to produce a valid estimate.

Fourth, GFP and the configuration of brain activity recorded across the scalp are not synonymous, and within-subjects and between-subjects factors can have different effects on each. When the configuration of brain activity is unitized by dividing by the corresponding GFP across time, it becomes difficult to discern what the resulting metric means in terms of underlying neurobiology. This is because by combining information about the global pattern of brain activity and the global field power, it becomes difficult to know whether an observed change is attributable to a change in brain configuration (suggesting a change in the neural locus), a change in GFP (suggesting an change in the level of activation of the brain, possibly from the same neural locus), or a combination of the two. In the present micro-segmentation analysis, GFP and the RMSE functions are treated as distinct metrics, with the former indexing the level of brain activation and the latter indexing the global pattern of brain activity apparent in high-density EEG. As illustrated in the simulation studies and found in the empirical study, these metrics, and the cosine distance between the template maps for

at $t = 128$ ms. The time-weighted mean of peaks in both the $\pm 5\%$ and $\pm 10\%$ windows is 127.14 ms. Bootstrap results indicate a peak in 100% of the runs within both the $\pm 5\%$ and $\pm 10\%$ time windows. Bottom Middle Panel: GFP valley at $t = 188$ ms. The time-weighted mean of valleys in the $\pm 5\%$ and $\pm 10\%$ time windows are 186.87 ms and 189.43 ms, respectively. Bootstrap results indicate a valley in GFP in 54.9% and 70.6% of the runs within the $\pm 5\%$ and $\pm 10\%$ windows, respectively. Bottom Right Panel: GFP peak at $t = 236$ ms. The time-weighted mean of peaks in the $\pm 5\%$ and $\pm 10\%$ time windows are 235.9 ms and 234.46 ms, respectively. Bootstrap results indicate a peak in GFP in 76.5% and 84.2% of the runs within the $\pm 5\%$ and $\pm 10\%$ windows, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
success microstates, distinguish between a change in neural locus and a change in activation at a given locus.

Finally, the incorporation of a bootstrapping procedure makes it possible to empirically test the assumption that the microstates are homogeneous within or across participants. Greater confidence can be placed in the results of source localization estimates when bootstrapping supports the homogeneity assumption. Although it is still important to validate of any such source localization estimates using, for instance, fMRI, improvements in the inputs to source localization algorithms should produce more accurate, reliable, and generalizable estimates. Bootstrapping procedures may also prove useful for investigating the circumstances (e.g., runs or participants) that are associated with a robust outcome, which not only should lead to better source localization estimates but to empirically testable hypotheses about the processes or moderating variables responsible for the initially observed heterogeneity.

Note that if there is a local stability but changes elsewhere, then the change in overall configuration across the n-dimensional sensor space would emerge as a new microstate. If, for instance, information is processed at two distinct neural loci at a given point in time (say, microstate 1) and there is a transition of the information to two different neural loci for information processing, then this would be reflected as a new microstate (microstate 2) as long as there was a corresponding change in the electrical activity across the n-dimensional sensor space. However, if the information being processed at the two distinct neural loci during microstate 1 is continues to be processed by one of these two loci but the second locus is replaced by a new neural locus whose activity manifests as a change in the n-dimensional sensor space, this would also appear as a new microstate (microstate 2'). That is, the analyses performed in this paper would not be sufficient to investigate local stability. However, it is a simple matter to adapt the analyses described in this paper to do so; this adapted procedure would also provide a means of distinguishing between microstate 2 and microstate 2'.

Typically, one would have a theoretical rationale for investigating the local stability of a particular sensor space. If this were the case, then the analyses described in this paper: (a) would be performed for the entire n-dimensional sensor space, and (b) would be repeated for the constrained sensor space for which the possibility of local stability were of theoretical interest. For instance, one might first perform the analysis of a visual ERP paper over 128-sensor space and then limit the analysis to a reduced sensor-space covering the visual cortices to investigate local stability within that sensor region. Local stability within a particular sensor space would be evidenced as the absence of any microstates (or the maintenance of a given microstate – such as the continuous activation of a single region of the visual cortex). If one had no theoretical rationale for investigating local stability, exploratory data analyses could nevertheless be performed to investigate the possibility of local stabilities. To do so, one would again begin with the analyses for the entire n-dimensional sensor space. One could then repeat the analyses for each constrained sensor space in which one wanted to investigate local stability. Given the exploratory nature of the latter approach, one would typically want to replicate (cross-validate) the analyses in an independent sample.

7. Conclusion

Analyses that are designed to investigate periodic brain processes (e.g., independent or principal component analysis; K-means cluster analysis) have been applied to study non-periodic event-related component processes. Analytic techniques that rely on the full time series have two limitations not encountered by the present approach to identifying non-periodic brain dynamics: (a) the characterization early components (segments) may be influenced by what happens in later segments of the time series, and (b) there is no statistical evaluation of the segmentation. The former means that what is measured and gleaned in tasks limited to early components – and the theories and predictions they produce – may not be replicated by or generalize to studies that are thought to build on but extend these early operations to examine more complex (and later) information processing operations. Without a statistical evaluation of the segmentation, it is unclear whether the results are robust. Whether or not the present micro-segmentation analysis suite proves more informative in studies of information processing in the brain than these time series analyses is an empirical question, but it is encouraging that statistical evaluation of the segmentation is possible and the identification of later microstates cannot influence the identification of earlier microstates.

There are several additional advantages of the current micro-segmentation approach over prior methods for identifying microstates such as those based on k-clustering methods. First, this micro-segmentation approach does not require the a priori estimation of the number of cluster maps that could best explain the entire time period of interest after a stimulus onset. This feature allows for data-driven microstate automatic detection rather than for any potential ‘microstate cherry picking.’ As noted by Brunet et al.’s (2011) “Changing the number of clusters might change the results at this level by proposing more or less map differences across time or between conditions” (p. 7). Second, the current approach does not require the hand-picking of time periods of interest to perform second-level statistical analyses (e.g., fitting procedure in Cartool). Third, the current approach uses a statistical approach to identify the optimal number of cluster maps rather than a cross-validation criterion derived by dividing the global explained variance by the degrees of freedom, which depends on the number of electrodes). Finally, the bootstrapping feature in the current approach permits identification of the most frequent stable microstates (and transition states) in a sample of subjects or across trials within subjects.

In sum, theoretical simulations and empirical data were presented for a new method for identifying brain state dynamics based on the micro-segmentation and analysis of high-density event-related brain potentials. The current approach applies a suite of quantitative methods to distinguish between event-related changes in the global pattern of brain activity, putatively reflecting changes in the underlying neural locus for information processing in the brain, and event-related changes in overall brain activation. In addition, within-subject and between-subject bootstrapping procedures provide a quantitative means of investigating how robust are the results of the micro-segmentation. Tests performed on synthetic data and on real ERP measurements showed that the proposed suite of data-intensive analytic techniques, made possible by the use of high performance computing, provides new and unique spatiotemporal information about event-related (non-periodic) microstates.

Conflict of interest

The authors declare having no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jneumeth.2014.09.009.

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