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Dynamic spatiotemporal brain analyses using high-performance electrical neuroimaging, Part II: A step-by-step tutorial

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HIGHLIGHTS
• Step-by-step tutorial for the objective identification of brain microsegmentation.
• Analytic procedures for forming a priori statistical contrasts of brain microstates.
• Complete microsegmentation of complex time-variant data.
• Guide to the high-performance microsegmentation suite, HPMS.
• Introduction of the Chicago Electrical Neuroimaging Analytics, CENA.

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ABSTRACT
Our recently published analytic toolbox (Cacioppo et al., 2014), running under MATLAB environment and Brainstorm, offered a theoretical framework and set of validation studies for the automatic detection of event-related changes in the global pattern and global field power of electrical brain activity. Here, we provide a step-by-step tutorial of this toolbox along with a detailed description of analytical plans (aka the Chicago Electrical Neuroimaging Analytics, CENA) for the statistical analysis of brain microstate configuration and global field power in within and between-subject designs. Available CENA functions include: (1) a difference wave function; (2) a high-performance microsegmentation suite (HPMS), which consists of three specific analytic tools: (i) a root mean square error (RMSE) metric for identifying stable states and transition states across discrete event-related brain microstates; (ii) a similarity metric based on cosine distance in n dimensional sensor space to determine whether template maps for successive brain microstates differ in configuration of brain activity, and (iii) global field power (GFP) metrics for identifying changes in the overall level of activation of the brain; (3) a bootstrapping function for assessing the extent to which the solutions identified in the HPMS are robust (reliable, generalizable) and for empirically deriving additional experimental hypotheses; and (4) step-by-step procedures for performing a priori contrasts for data analysis. CENA is freely available for brain data spatiotemporal analyses at https://hpenlaboratory.uchicago.edu/page/cena, with sample data, user tutorial videos, and documentation. © 2015 Elsevier B.V. All rights reserved.

1. Introduction

The development of large-scale, high-spatial resolution neuroimaging technologies has transformed human neuroscience. However, the costs and temporal limitations of metabolic neuroimaging have driven electrical neuroimaging toward new computational approaches to better quantify the neural dynamics of various complex cognitive and social processes. With the recent publication of our suite of analytic tools for the identification of stable evoked brain microstates in electrical neuroimaging (Cacioppo et al., 2014), our goal here is to provide users with guidelines and step-by-step instructions for implementing the basic functions of our analytic suite along with analytic procedures for forming and testing a priori statistical contrasts of these evoked brain microstates.

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 identify when and in what specific combinations...
they are activated (for review, see: Pizzagalli, 2007; for examples, see: Alexander et al., 2015; Berger, 1929; Cacioppo and Dorfman, 1987; Cacioppo et al., 2013, 2014; Decety and Cacioppo, 2012; Donchin and Heffley, 1978; Donders, 1969; Lehmann and Skrandies, 1980; Luck and Kappenman, 2012; Luria, 1966; Ortueto et al., 2004, 2005, 2010; Ortigue and Bianchi-Demicheli, 2008).

By providing detailed information about the brain microstates elicited by a stimulus, high-density electroencephalographic (EEG) recordings and averaged EEG (event-related potentials, ERPs) may provide a useful additional tool in investigations of brain function.

Over the years, several techniques have been developed to provide more comprehensive analyses of time-varying activity across the entire scalp (i.e., a multi-dimensional sensor space including all the electrodes) and to complement the traditional analyses of EEG and/or ERP peaks and troughs that were done on a single vector sensor-space, i.e., at specific electrode positions (e.g., Cacioppo and Dorfman, 1987; Donchin and Heffley, 1978; Lehmann and Skrandies, 1980; Luck and Kappenman, 2012). For instance, Dietrich Lehmann (e.g., Lehmann and Skrandies, 1980; Lehmann, 1987) introduced the brain microstate approach, which is a method for identifying stable configurations of global electric brain activity (rather than signals collected from one electrode). The identification of the distinct, evoked brain microstates elicited by a stimulus makes it possible to investigate robust changes in the configuration of activation in electrical neuroimaging data, where a configuration of activation is defined as a topographical map – the average evoked potentials at a given recording bin across n-dimensional sensor space where n the number of EEG recording channels. The goal of the brain microstate approach is to provide information about the brain activity associated with the sequence of discrete information processing operations evoked by the presentation (or anticipation) 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 (Lehmann, 1987), each of which is characterized by the performance of specific cognitive computations and a relatively stable spatial distribution of brain activity (Lehmann and Skrandies, 1980; Lehmann, 1987). Since the 1980s, the brain microstate approach has been applied successfully to several domains in cognitive neuroscience (e.g., language: Koenig and Lehmann, 1996, Ortueto et al., 2004; face perception: Thierry et al., 2007; Khanha et al., 2015, Michel et al., 1999, 2001, Oostenveld et al., 2011, Pascual-Marqui et al., 1994, 2014, for reviews), social neuroscience (e.g., affective attitude: Pizzagalli et al., 2002; pair bond: Cacioppo et al., 2013; desire: Ortueto and Bianchi-Demicheli, 2008), and psychiatry (e.g., schizophrenia, Koenig et al., 1999; Lehmann et al., 2005). Over the years, a few microstate-related freeware have been developed for academic purposes (e.g., the most popular is CARTOOL, Brunet et al., 2011; CENA, Cacioppo et al., 2014).

The notion underlying the brain microstate approach is that each microstate refers to a time-limited information processing operation (e.g., Lehmann, 1987; Lehmann and Michel, 2011; Koenig et al., 2002, 2011, 2014; Michel et al., 2009). 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, 1984). This approach suggests that the global pattern of brain electrical activity is modeled as being composed of a time sequence of decomposable brain microstates (Koenig et al., 2002, 2014; Lehmann and Skrandies, 1980; Pascual-Marqui et al., 1995). 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. The notion of identifying stable brain microstates based on the spatiotemporal information represents an important insight into the understanding of the chrononarchitecture of brain processes, but the utility and adoption of this brain microstate approach were limited in part by constraints in the quantitative methods used by investigators to identify and interpret brain microstates (cf. Cacioppo et al., 2014; Gartner et al., 2015; Koenig et al., 2002, 2011, 2014).

Several issues have been noted regarding the current implementation of the microsegmentation algorithms (e.g., k-cluster analysis) that has been used in the published literature on brain microstates. For instance, programs that implement k-means algorithms for microstate segmentation (e.g., CARTOOL’s microstate segmentation is based on cluster analysis using either a modified k-means cluster analysis or an atomize and agglomerate hierarchical cluster analysis, Brunet et al., 2011) request that users specify the range of clusters prior to analysis (see CARTOOL’s snapshot in supplementary Fig. S1). The specification of the range of clusters an investigator expects to find in the data could introduce a confirmatory bias, potentially to the detriment of experimental replicability when other investigators expect a different range of clusters.

Second, Murray et al. (2008) wrote a popular tutorial on microstate analysis in which they addressed the clustering techniques (k-cluster, hierarchical clustering) that are used in CARTOOL. Murray et al. (2008, pp. 259–260) then discussed how users might identify the optimal number of template maps. When describing the various criteria (e.g., the Krzanowski-Lai [KL] criterion) for doing so in CARTOOL, they state that: “its highest value should in principle indicate the optimal clustering. In practice, however, the KL will nearly all the time peak for three segments due to the very nature of the data we analyze . . . Though this peak at three segments can theoretically be of some interest, we advise considering the subsequent highest peak as the one indicating the optimal number of template maps, though additional peaks may also ultimately be of interest if they lead to statistically significant results” p. 260). This method may permit investigators to engage in a research practice now known as p-hacking, which has become a concern in scientific practice because it can undermine the replicability of the results in a field (Head et al., 2015). Replicability is a pre-requisite for validity. One of our goals in developing CENA was to improve the replicability of results in the field by determining the number of microstates based on the data rather than based on the discretion of an investigator.

Recently, advances have been made in the conceptualization and detection of “transition periods” between stable brain microstates (e.g., Cacioppo et al., 2014; Gartner et al., 2015). The Marchov chain analysis (e.g., Brodebeck et al., 2012; Gartner et al., 2015; Koenig et al., 2002), for instance, uses a probabilistic approach to defining microstates and transition states. The transition states are not equivalent to those described in CENA, however (Cacioppo et al., 2014). CENA uses the baseline to create a model of the noise level in the EEG. This information is used in the initial pass through the EEG data using a root mean square error (RMSE) metric to identify candidates for stable evoked brain microstates and intervening transition states. The final microstate structure is determined by analyzing these candidate microstates using a

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1 Because the brain microstate approach is extensively used and has been detailed previously in several review articles and scientific reports (for example, see: Brunet et al., 2011; Cacioppo et al., 2014; Gartner et al., 2015; Koenig et al., 2002; Lehmann and Michel, 2011; Michel et al., 1999, 2001, 2009; Murray et al., 2008; Pascual-Marqui et al., 1995), we do not describe it in detail here.

2 Here and throughout this paper, we refer to issues that exist in common scientific practices, for instance, given the current implementation of CARTOOL, not to what may be a mathematical possibility or requirement.
cosine metric in $n$-dimensional vector space, where $n$ represents the number of EEG recording channels (described below).\footnote{At the theoretical limit, a baseline that represents pure noise will approach a mean vector length of zero and no angle. In empirical practice, this means that a baseline that reasonably modes noise should have a short template (mean) vector for the baseline than for microstates, and it will have high variance in terms of the angle of the constituent topographical vectors that represent the baseline (for details and illustrative simulations, see Cacioppo et al., 2014).} It may be an empirical question as to whether a Markov chain analysis or CENA best models changes in brain activity but they are distinct mathematical approaches that produce distinct depictions of the underlying event-related brain activity.

The accurate identification of brain microstates (and “transition states”) can be seen as advantageous in (re)discovering information “lost” in traditional approaches to ERP analyses as well as in reinforcing the existence of common ERP components and common ERP topographies, such as N170 or P100. For instance, in Cacioppo et al., 2014, we report an empirical study combining the visual checkerboard task with CENA in which we found some brain microstates to coincide with peaks or troughs in the visual ERP over the occipital regions, and others to be detectable in the absence of a peak or trough. By dramatically improving the spatio-temporal information provided by noninvasive electrical neuroimaging, we believe CENA promises to improve the information available in EEG/ERP studies of the human brain, and contribute to replicable research findings in neuroimaging through statistically well-powered studies, data aggregation across shared datasets, and high-performance computational approaches to identifying potential sources of heterogeneity within high density EEG/ERP datasets.

The CENA plugin (Cacioppo et al., 2014) Matlab Toolbox for Brainstorm (Tadel et al., 2011) allows the objective detection of non-periodic event-related changes in the global pattern of electrical brain activity and the identification of transition states as well as stable states as unique and separate entities.\footnote{The current version of CENA focuses on time domain data. Analyzes of unfiltered frequency domain data will be implemented in a future version of CENA. In theory, the analysis would proceed unchanged if the trial-level data were frequency-filtered prior to averaging and microsegmentation using CENA, but the interpretation of brain activity (both configurations and field power) would be limited to activation within the frequency range of the filtered data.} The CENA toolbox includes a high-performance microsegmentation suite (HPMS), which consists of three specific tools: (1) an RMSE metric for identifying stable states and transition states across discrete event-related (or evoked) brain microstates; (2) a global field power (GFP; Lehmann and Skrandies, 1980) metric; and (3) a similarity metric based on cosine distance between stable microstates in $n$ dimensional sensor space to determine whether template maps for successive brain microstates differ in configuration and/or GFP of brain activity. Although a cosine metric has been part of vector mathematics for many years, the manner in which it is used in CENA – for instance, to determine which putative microstates identified by the RMSE reflect differences in global configurations of activity, and which reflect differences in global field power – is unique. Moreover, our cosine metric operates on the configuration, in contrast to the global dissimilarity measure that is used in other programs (e.g., CARTOOL) as “a measure of topographic differences of scalp potential maps” (see Eq. (2) in Brunet et al., 2011). Theoretical and empirical rationales for the HPMS can be found in Cacioppo et al. (2014).

CENA also provides an additional set of analytic tools to support a priori inferential statistical comparisons between conditions. For instance, CENA includes a bootstrapping procedure for assessing the extent to which the solutions identified in the microsegmentation are robust (reliable, generalizable) and for empirically deriving additional experimental hypotheses. In addition, CENA includes procedures for performing a priori contrasts for data analysis. The aim here is to describe the functions and analyses in the CENA toolbox, all of which are available under a common interface running as a plugin in Brainstorm (Tadel et al., 2011) under MATLAB environment. By extending existing electrical neuroimaging tools, the CENA toolbox can be helpful to both researchers and clinicians who aim to automatically decompose brain processes and identify neural biomarkers and event-related changes in the global pattern and global field power of electrical brain activity in an objective and robust way.

2. Methods and results

2.1. Basic functions

CENA utilizes various standard functions in Brainstorm, such as the averaging function and Brainstorm head models. Additionally, CENA adds functionality to Brainstorm in three general domains: (a) Operations to create ERP waveforms configurations, (b) Operations on ERP waveforms configurations, and (c) Operations to provide statistical contrasts between ERP waveform configurations. These three operations are described below.

2.2. Operations to create ERP waveform configurations

Brainstorm (Tadel et al., 2011) offers a variety of tools for preprocessing EEG data and creating ERP waveforms, including an “Average file” function. CENA add to this set the difference wave function that operates on and creates ERP difference waveform configurations as described next.

2.2.1. Difference wave function

This CENA function offers users the possibility to create a difference waveform configuration between two $n$-dimensional ERPs by subtracting the ERP waveform elicited by one condition (e.g., ERP_A) from the ERP waveform elicited by another condition (ERP_B). The output of this difference waveform function is computed as ERP_A – ERP_B, which results in a $T \times n$ matrix with $T$ as the number of timeframes and $n$ as the number of electrodes. When processing two ERPs via the Brainstorm routine window at the bottom of the Brainstorm interface, ERP_A will be the ERP at the top of the list and ERP_B will be the ERP second in the list. The CENA function constructs a “difference waveform” that putatively represents physiological processes that are different between two conditions.

Accordingly, this function could be used, as in any other standard difference wave functions, to isolate a component of interest (e.g., N400) for two different conditions (e.g., Luck, 2014; Luck and Kappenman, 2012). However, the limitation of any difference waveform functions is that physiological processes are “usually not additive, that is, do not occur such that the physiological processes in one condition equal those processes in the other conditions plus or minus one other processes” (Picton et al., 2000). As a consequence, a difference waveform does not necessarily show which of the original waveforms contained the additional component. The interpretation of a difference waveform is, thus, not straightforward (Picton et al., 2000). Therefore, we rather recommend using the present difference waveform function as a first step toward the identification of differential stable microstates between two conditions that are better understood through the high performance microsegmentation of each condition, respectively. See Sections 2.1.2 and 2.2 for further details.

2.3. Operations on ERP waveform configurations

As outlined in Cacioppo et al. (2014), CENA toolbox provides a high-performance microsegmentation suite (HPMS) that can be
applied to different configurations of ERP waveforms. A description of HPMS toolbox is described next. For more details about the algorithms behind HPMS, see Cacioppo et al. (2014).

2.3.1. A high-performance microsegmentation suite (HPMS)

2.3.1.1. Root mean square error (RMSE) analysis. The first HPMS step uses a root mean square error (RMSE) analysis that decomposes the n-dimensional ERP waveform based on noise levels detected during the baseline period into two types of event-related brain states: (i) discrete stable microstates, and (ii) transition states between these microstates transitions are not immediate (Cacioppo et al., 2014). Compared to previous microstate programs using k-mean clustering (e.g., CARTOOL, Brunet et al., 2011), CENA does not require the a priori specification of the number or the range of the expected number of event-related brain microstates, as it uses RMSE and it produces timing information regarding the onset and duration of each stable event-related microstate as well as transition states. With the RMSE algorithm, CENA 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.

In each iteration of the k-means algorithm, each individual time-frame (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 when applied to the microstate approach. By definition, the k-means approach applied to some microstate programs requires that every time-frame belong to some characteristic microstate. Stable states of brain activity may not always instantaneously change from one state to the next (for examples in resting state or spontaneous EEG, see Brodbeck et al., 2012; Gartner et al., 2015; Koenig et al., 2002; for examples in ERP, see Cacioppo et al., 2014). Rather, as described in Cacioppo et al., 2014, “transition periods” between pairs of stable evoked brain microstates may occur. Based on the RMSE metric, these “transition periods” are not another type of brain microstate, but they rather represent a transition between two brain microstates. Accordingly, a “transition state” does not represent a cognitive function or an underlying neural mechanism in the same way as does a stable microstate. In transition periods the loci of brain activity migrates from one brain region (or distinct 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 Fig. 1, where RMSE is depicted on the ordinate and the (hypothetical) topographical maps of brain activity across time (in milliseconds) is depicted on abscissa. In Fig. 1, a stable microstate is observed from time 1 to 6, the transition from the first to the second stable microstate is observed from time 7 to 10, and the second stable microstate is observed from time 11 to 16 (the end of the recording period). The k-means approach as implemented in CARTOOL results in any such “transition timeframes” being 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, microsegmentation using the k-means algorithm would typically yield two stable microstates (e.g., one from 1 to 8 and another one from 9 to 16), and each of the topographic maps representing the brain activity during the transition period would be assigned to one of these microstates (e.g., see Brunet et al., 2011, pp. 5–6).

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 (preceeding 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.

With the HPMS, CENA users can automatically identify stable microstates (in the example depicted in Fig. 1: one from 1 to 6; and one from 11 to 16) and transition states (from 7 to 10, as depicted in Fig. 1). CENA toolbox currently allows users to perform two types of HPMS: the HPMS for one condition (HPMS single) or a HPMS to compare two or more conditions (HPMS multiple). Menu options of the HPMS function (either HPMS single or HPMS multiple) allow users to select two different levels (either a 95% or 99%) of confidence interval (CI) for: (i) thresholding RMSE peaks and valleys, and (ii) performing a cosine metric analysis to determine whether time-adjacent microstates differed in configuration (described below). In addition, the menu options allow users to specify the duration of their baseline (e.g., period prior a stimulus onset) and to tune the size of the RMSE lag for the HPMS at a minimum duration that is appropriate to their study. We recommend that the baseline to be time-jittered, variable in length, and corrected to ensure the best possible model of noise. (Time-jittering the baseline is typical in fMRI research and is done to ensure the baseline is a reasonable model of the background noise level for the signal of interest).6

Because most ERP research (and most microstate analyses) focuses on post-stimulus event-related brain states, post-stimulus brain microstates (evoked brain microstates) are the primary focus of the present version of CENA. However, if the experimenter were interested in pre-stimulus states, a straightforward modification of the experimental design would be sufficient to permit investigation of these prestimulus (e.g., anticipatory) microstates. A typical trial structure is: (i) jittered, variable-length baseline, (ii) stimulus onset, and (iii) post-stimulus period during which evoked microstates are identified and investigated. If one were interested in the event-related anticipatory microstates and wished to use CENA, the trial structure could be modified as follows: (i) jittered, variable-length baseline, (ii) a fixed-interval pre-stimulus period that makes it possible for the subject to anticipate the stimulus onset (and during which evoked anticipatory microstates can be identified and investigated), (iii) stimulus onset, and (iv) post-stimulus period (during which evoked microstates can be identified and investigated).

As described in Cacioppo et al. (2014), the 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 evoked brain microstate, which means the time interval between topographical maps (i.e., map x and map x+L) that are to be compared. In the case of exogenous (stimulus driven; Cacioppo et al., 2000) ERP microstates this duration might be quite brief, whereas for

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6 A 95% CI is recommended in between-subjects contrasts, while a 99% CI is recommended in within-subjects contrasts.

6 It is conceivable that microstates exist during baselines. For any such microstates to be detectable, however, they would need to be quite large or they would need to be time-locked to permit an improvement in the signal to noise ratio with averaging. The requirement for such baseline microstates to be time-locked across trials and subjects when the baselines are time-jittered and variable in duration is unlikely to be met. Nevertheless, CENA makes it possible to measure the adequacy of a baseline as a model for noise (see Footnote 1).
endogenous ERP microstates this duration may be longer. Because a brain microstate must have a minimum duration of a few consecutive time points to be meaningful of a functional brain processing (Ortigue et al., 2004; Pascual-Marqui et al., 1995), we recommend an L lag (the minimum duration for a putative microstate) of approximately 8 ms (for basic visual tasks, such as a checkerboard) and at least 12 ms for more complex cognitive task.

2.3.1.2 Cosine similarity metric. To confirm whether the microstates identified in the RMSE differ in the configuration of brain activity, we employ a multi-dimensional cosine similarity metric based on the cosine distance between template maps for successive evoked brain microstates (Cacioppo et al., 2014). Although the cosine similarity metric resolves ambiguities left by the RMSE analysis, the RMSE analysis is a necessary first step to identify candidate brain microstate based on the ERP configuration across n-dimensional sensory space.

Specifically, the RMSE analysis identifies significant changes in the stable event-related pattern of EEG activation across the n-dimensional sensor space. However, there are two reasons such a change in the RMSE function may occur (Cacioppo et al., 2014): (1) a different stable evoked brain microstate was elicited, typically interpreted as meaning that one or more of the cortical sources underlying the prior event-related microstate had changed; or (2) the same stable evoked brain microstate was maintained but GFP increased (or decreased), typically interpreted as meaning that the level of activation of the set of cortical sources underlying the event-related microstate had increased (or decreased). Once the putative stable microstates have been identified by the RMSE, each topographical map within a microstate can be expressed within an n-dimensional (e.g., 128-dimensional) vector space, the template (i.e., mean) map for the microstate can be expressed in this microstate, a confidence interval region can be determined around this template map in 128-dimensional space (see Cacioppo et al., 2014, for details). If the succeeding evoked brain microstate identified by RMSE is the result of a change in the location of the underlying neural sources of the n-dimensional event-related waveform, the cosine metric between the template map for an event-related microstate and the template map for the succeeding microstate should differ. This is because different configurations of activity produce different vector angles in n-dimensional vector space. However, if the succeeding evoked brain microstate identified by RMSE is the result of a change in the level of neural activation (i.e., GFP) rather than a change in source location, then the representation of these microstates in n-dimensional vector space differ in the length of the vector but not in the angle of the vector (Cacioppo et al., 2014). Therefore, the RMSE is followed by an analysis based on a cosine similarity metric (for details, see Cacioppo et al., 2014).

The results of a HPMS single provide two types of outputs: One output with “preliminary” results (provided users select the option “yes” to the question “plot preliminary results” (supplementary Fig. S2), and one output with final results (Fig. 2). If the user is interested in changes in GFP, then outputting the preliminary results should be selected. If one has no interest in GFP, then there is no need to output the preliminary results. The preliminary output includes information about microstates before they are merged using a multi-dimensional cosine similarity metric based on cosine-distance function that determines whether template maps for successive brain microstates differ in configuration of brain activity. The final output includes, on the other hand, results after the merging of the brain microstates. A comparison of these outputs permits one to identify which microstates identified by the RMSE analysis were subsequently determined by the analysis based on the cosine metric as the same microstate but at a different GFP. Changes in GFP levels within the same microstate are provided in the GFP outputs for the microstates in the preliminary results that were merged in the final results.

Both the preliminary (Fig. S2) and final outputs (Fig. 2) are organized similarly. They both display two figures and five tables. Although the figures provide a visual representation of the HPMS results of the RMSE analysis routine and the GFP analysis routine applied to the Grand Mean ERPs across conditions (or for the mean ERPs for within a condition), the tables provide numerical values.

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7 The parameter, L, could be treated as a variable. As but one instance, Lendogenous could be specified to represent the minimum duration specified for a microstate that occurs within 100 ms of the stimulus presentation (exogenous components), and Lendogenous could be specified to represent the minimum duration specified for a microstate that occurs more than 100 ms after the stimulus (i.e., endogenous components).
for the HPMS results. The first table provides quantitative information about the stable microstates. This table includes information about the duration (timing in milliseconds, ms) of each stable brain microstates (start and end) and the mean global field power (Avg GFP), the GFP maximum amplitude (in microvolts, µV) and the GFP standard deviation for each stable brain microstate. The timing of each stable microstate is peak-to-trough, inclusive (Cacioppo et al., 2014). For instance, as described in Cacioppo et al. (2014), 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 evoked brain microstates. In practice, local maxima/minima may represent noise rather than a true peak/trough. The RMSE algorithm in our microsegmentation 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*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*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 (Cacioppo et al., 2014).

2.3.1.3. Global field power. The second table in the output displays the time (in ms) and the amplitude (in microvolts, µV) of GFP peaks and valleys. Users interested in changes in magnitude (rather than changes in topography only) will find this GFP information useful.

Initially introduced by Lehmann and Skrandies (1980), the 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:

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

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 (Cacioppo et al., 2014).

The last three tables provide quantitative information about the cosine distance between template maps, the standard deviation of cosine distances of topographic maps (“topomaps” i.e., the average evoked potentials at a given recording bin across \(n\)-dimensional sensor space where \(n\) the number of EEG recording channels; Cacioppo et al., 2014) in each template map, and a membership identification code for the template maps. 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 space.

\[\text{\footnotesize{Note:}}\] The template map for a given microstate is equivalent to the mean of the topographic maps. A template map is determined for each microstate identified by the RMSE algorithm by averaging across the constituent topographic maps. In addition, 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 inn-dimensional vector space (see Cacioppo et al., 2014 for details).
vector space. This measure of variability is useful for two reasons. First, a confidence interval (CI) 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 \(T+1\) 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 evoked brain microstate, the cosine similarity metric makes it possible to evaluate quantitatively which of the succeeding microstates identified by the RMSE algorithm represent changes 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 (Cacioppo et al., 2014).

Finally, the HPMS function allows users to export each template maps and estimate their brain source using Brainstorm tools and head models. For instance, as we did in a recent CENA study of the Stroop task (Cacioppo et al., 2015), one can use the Brainstorm forward model that is calculated with a symmetric Boundary Element Model (Gramfort et al., 2010; Kybic et al., 2005) generated with OpenMEEG on the cortical surface of a template MINI brain (colin27 atlas) with a 1 mm resolution (Collins et al., 1998; Tzourio-Mazoyer et al., 2002). Cortical source estimations (in picoamperes-meters; pAm) can then be (i) estimated with a constrained inverse model of EEG sources using the standard weighted minimum-norm current estimate (wMNE; Baillet et al., 2001) and (ii) mapped to a distributed model consisting of 15,002 elementary current dipoles, as implemented in Brainstorm (Tadel et al., 2011). The source activity at each cortical location is standardized using the z-score transformation with respect to the average and standard deviation of the source activity during baseline period. Then, statistical analyses (e.g., t-test) can be done to statistically validate whether (or not) the observed qualitative modulations of brain activations are significantly different between two microstates. While CENA offers statistical comparison between conditions at the level of the brain microstates, it does not offer statistical comparison for the brain sources. Because CENA is implemented in Brainstorm (Tadel et al., 2011), estimates of the inverse solutions and source analysis should be performed using Brainstorm statistical analyses functions. As stated on the Brainstorm website, however: “Brainstorm does not offer a very large variety of statistical tests. However, it is easy to export your source results and process them with a specialized program” (e.g., Statistical Parametric Mapping, SPM).

When inverse solutions are applied to ERPs derived from studies that have been largely investigated and replicated in lesion and fMRI/MEG studies, or when ample evidence exists about the neural substrates (e.g., reversal checkerboard, Stroop, Posner paradigm), inverse solutions can be interpreted with more confidence (i.e., in a confirmatory way) than they would otherwise to suggest when these substrates might be active. On the other hand, when the inferences drawn from the inverse solutions concern a phenomenon that has not been extensively studied using lesions and fMRI/MEG, these solutions serve the purpose of hypothesis generation – not hypothesis testing.

2.3.1.4. Bootstrap function. The final function of the CENA toolbox is between-subjects and within-subjects bootstrapping procedures. Typically, one assumes that the series of brain 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. This data-intensive analytic approach, made possible by the use of high-performance computing, promises to dramatically improve the spatio-temporal information provided by noninvasive electrical neuroimaging. Indeed, this data-intensive 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 (see Cacioppo et al., 2014 for details). 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 microsegmentation 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 \(r\)):

\[
\binom{N}{r} = \frac{N!}{r!(N-r)!}
\] (2)

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 \(50!/(30!\cdot20!) \approx 47,129,212,246,893\). Bootstrap 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.\(^9\)

The results from each bootstrap 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 centered 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 brain source localization algorithms on each one of the stable microstates should be superior for robust than nonrobust microstates. Although there are specific analytic opportunities made possible by doing analyses on all possible combinations of bootstrapped ERPs, runs of 10,000 or even 1000 can be useful in identifying metrics from the HPMS (including the identification of a “discrete” microstate) that lack robustness.

\(^9\) 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.
2.4. Operations to provide statistical contrasts between ERP waveform configurations

The tools in HPMS within CENA permit the identification of a number of metrics in event-related electrical neuroimaging. The remaining tools in CENA are designed to support contrasts between conditions in evoked brain microstates, as represented in ERP waveform configurations. Fig. 1 illustrates the mapping between stable and transition brain microstates and ERP waveform configurations, and the distinctions between topographical maps and template maps, and Fig. 3 illustrates the CENA plugin editor as implemented in Brainstorm.

2.4.1. Considerations prior analyses

Before performing any analyses (including those described above), topographical maps for the Grand Mean ERP waveforms should be inspected for artifacts or bad channels in the recordings. The Grand Mean is used because it generally represents the best estimate of integrity of the ERP recordings across time and it avoids any confounding bias in editing based on expected differences between conditions. Verify that the same number of trials for a given subject contributes to each cell of the within-subjects design, and that the same mean number of trials was used to create any ERP waveforms that are to be compared, including between-subjects.

The analysis of the Grand Mean ERP waveform (or the waveform for any single condition) is simple in the sense that it simply represents the dynamics of brain microstates in response to one type of stimulus (or condition) in one group of subjects (see Cacioppo et al., 2014). An example of CENA procedure for a one-factor (one condition) from a reversal Checkerboard task (see Cacioppo et al., 2014 for details) can be found in Fig. 3. In this example, a HPMS single was applied to the grand mean from 22 individual’s ERPs (Fig. 3A and B). Results revealed five stable microstates (Fig. 3C–E). As shown in Fig. 3D, Microstate 1 was 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. Results from the bootstrapping are illustrated in Fig. 3F. A 2D layout for each microstate is illustrated in Fig. 3G, along with the estimated brain source localization for each microstate (Fig. 3H).

In the next section, we describe the tools and procedures users can use to form, test, and interpret a priori statistical contrasts between experimental conditions in the context of a two-factor mixed model with A (a1, a2) × B (b1, b2), in which A serves as a between-subjects factor and B serves as a within-subjects...
factor (see Fig. 4). The analyses are based on orthogonal\(^\text{10}\) contrasts comparing pairs of event-related ERP waveforms. For a factor with two levels, this is simply the contrast between the ERP waveforms between these two levels; for a factor with three levels, this means specifying a priori two orthogonal contrasts (e.g., level 1 vs. level 2; level 3 vs. mean of level 1 and level 2); and so forth. Accordingly, procedures for a one-factor between-subjects design can be reduced to those described in Section 2.4.2, the procedures for a one-factor within-subjects design reduced to those described in Section 2.4.3; and the procedures for factorial designs involving more than two factors are straightforward extrapolations of the procedures described in Sections 2.4.2–2.4.4.

2.4.2. Main effect test for Factor A. The main effect for Factor A involves the following steps

1. Using the Average function in Brainstorm, average the a1b1 topographical maps (i.e., \(n\)-dimensional ERP waveform) and a1b2 topographical maps to create the topographical maps for Mn,a1.
2. Average the a2b1 and a2b2 topographical maps to create the topographical maps for Mn,a2.
3. Using the CENA Difference Waveform function, difference the Mn,a1 and Mn,a2 topographical maps to create the topographical maps for the Main Effect for A. That is, difference the \(n\)-dimensional waveform created in Step-2 from the corresponding waveform created in Step-1 above.
4. Average the Mn_a1 and Mn_a2 topographical maps to create the topographical maps for the Grand Mean. That is, average the ERPs created in Steps-1 and -2.
5. Perform the HPMS on the topographical maps created in Step-1 to create the microsegmentation (and template maps) for Mn_a1.
6. Perform the HPMS on the topographical maps created in Step-2 to create the microsegmentation (and template maps) for Mn,a2.
7. Perform the HPMS on the Grand Mean ERP created in Step-4 to create the microsegmentation for the periods of time in which the brain microstates did not differ as a function of Factor A.
8. Perform the HPMS on the topographical maps created in Step-3 to identify the periods of time in which the ERP waveform did and did not differ significantly as a function of Factor A.

(8a) For the time period(s) in which Step-8 shows no significant differences in ERP waveform as a function of Factor A, refer to the results from Step-7 to characterize the evoked brain microstates across Factor A. That is, for the time period(s) that the ERP waveform did not differ as a function of Factor A, identify the microstate structure based on the results of Step-7 (i.e., Grand Mean) and perform brain source localization on each of these microstate(s).

(8b) For the time period(s) in which Step-8 shows significant differences in ERP waveform as a function of Factor A, refer to the results from Step-5 and Step-6 to characterize the distinct evoked brain microstates within each level of Factor A. That is, for the time period(s) that the ERP waveform did differ as a function of Factor A, identify the microstate structure separately for each level of Factor A (i.e., Step-5 and Step-6 above) and perform brain source localization on each of these microstate(s).

2.4.3. Main effect test for Factor B. The main effect for Factor B involves the following steps

1. Using the Average function in Brainstorm, average the a1b1 topographical maps (i.e., \(n\)-dimensional ERP waveform) and a2b1 topographical maps to create the topographical maps for Mn,b1.
2. Average the a1b2 and a2b2 topographical maps to create the topographical maps for Mn,b2.
3. Using the CENA Difference Waveform function, difference the Mn,b1 and Mn,b2 topographical maps to create the topographical maps for the Main Effect for B. That is, difference the \(n\)-dimensional waveform created in Step-2 from the corresponding waveform created in Step-1 above.
4. Average the Mn,b1 and Mn,b2 topographical maps to create the topographical maps for the Grand Mean. That is, average the ERPs created in Steps-1 and -2.
5. Perform the HPMS on the topographical maps created in Step-1 to create the microsegmentation (and template maps) for Mn,b1.
6. Perform the HPMS on the topographical maps created in Step-2 to create the microsegmentation (and template maps) for Mn,b2.
7. Perform the HPMS on the Grand Mean ERP created in Step-4 to create the microsegmentation for the periods of time in which the brain microstates did not differ as a function of Factor B.
8. Perform the HPMS on the topographical maps created in Step-3 to identify the periods of time in which the ERP waveform did and did not differ significantly as a function of Factor B.

(8a) For the time period(s) in which Step-8 shows no significant differences in ERP waveform as a function of Factor B, refer to the results from Step-7 to characterize the evoked brain microstates across Factor B. That is, for the time period(s) that the ERP waveform did not differ as a function of Factor B, identify the microstate structure based on the results of Step-7 (i.e., Grand Mean) and perform brain source localization on each of these microstate(s).

(8b) For the time period(s) in which Step-8 shows significant differences in ERP waveform as a function of Factor B, refer to the results from Step-5 and Step-6 to characterize the distinct evoked brain microstates within each level of Factor B. That is, for the time period(s) that the ERP waveform did differ as a function of Factor B, identify the microstate structure separately for each level of Factor B (i.e., Step-5 and Step-6 above) and perform brain source localization on each of these microstate(s).

\(^{10}\) The orthogonality that is mentioned is related to design, not variance (e.g., PCA, etc.)
2.4.4. A × B interaction test

When possible, produce simple main effect difference topographical maps within-subjects rather than between-subjects to minimize the error in these difference maps. In this example, Factor A is a between-subjects factor and Factor B is a within-subjects factor, so the simple main effect tests would be calculated within each level of A.

(1) Using the CENA Difference Waveform function, difference the a1b1 and a1b2 topographical maps to create the topographical maps for the simple main effect for a1. That is, difference the ERPs created in Step-2 from the waveform created in Step-1 above.

(2) Difference the a2b1 and a2b2 topographical maps to create the topographical maps for the simple main effect for a2.

(3) Difference the topographical maps for the simple main effect for a1 and the topographical maps for the simple main effect for a2 to create the topographical maps for the A × B interaction (i.e., the difference of the differences). That is, difference the n-dimensional waveform created in Step-2 from the corresponding waveform created in Step-1 above.

(4) Average the Mn·a1 and Mn·a2 topographical maps to create the topographical maps for Grand Mean. (This waveform should already be completed if the steps in Sections 2.4.2 and 2.4.3 have been completed.)

(5) Perform the HPMS on the topographical maps created in Step-1 to create the microsegmentation (and template maps) for the simple main effect for a1.

(6) Perform the HPMS on the topographical maps created in Step-2 to create the microsegmentation (and template maps) for the simple main effect for a2.

(7) Perform the HPMS on the topographical maps created in Step-3 to create the microsegmentation (i.e., epochs of significant difference) for the simple main effects for a1 and a2. The output of this step specifies the periods of time during which Factors A and B interacted significantly (at an alpha-level determined by the CI used – typically a 99% CI, producing an alpha-level of .01) to produce the observed brain microstates.

(8) Perform the HPMS on the Grand Mean ERP created in Step-4 to create the microsegmentation for the periods of time in which Factors A and B did not interact to produce the brain microstates.

(8a) For the epochs in which the results of Step-7 show no significant differences, refer to the results of Step-8 to characterize the evoked brain microstates. If main effects were also absent for this epoch, then source localization should be performed on the observed microstate(s) during this epoch in the Grand Mean. If the main effect for Factor A and/or Factor B is significant for this epoch, then refer to the results above to characterize the evoked brain microstate(s) observed during this epoch.

(8b) For the epochs in which the results of Step-7 show significant differences in the waveforms, refer to the results of Steps-5 and -6 to characterize the distinct evoked brain microstates as a function of Factors A and B. For such an epoch, source localization should be performed on the observed microstate(s) during this epoch separately for the microstates identified and in Steps-5 and -6. HPMS analyses and source localization within each cell (e.g., a1b1, a1b2, a2b1, and a2b2) may also be performed as a means of breaking down the interaction to all possible pairwise comparisons.

3. Example

Let’s take a one-factor (Stimulus Type) within-subjects design with two conditions (i.e., color words versus non-words – viz., the letter string XXXXXX) as an example. In this example, 70 subjects (mean age = 23.59, SD = 5.62 years) performed a classic Stroop interference task in which they were instructed to gaze at the center of the screen and indicate, as quickly and as accurately as possible, the color of the ink of each centrally presented string of letters while their brain activity was continuously recorded from a 128 Electric Geodesic Sensor Net (Electrical Geodesic Inc., Oregon; http://www. egi.com/; Fig. 5A). As described in Cacioppo et al. (2015), behavioral analyses confirmed a classic Stroop interference effect ($p < .001$; partial eta squared = .23), with the color of color words identified more slowly ($M = 736.03$; $SD = 141.14$) than the color of nonwords ($M = 651.42$; $SD = 129.89$). The CENA results extended these behavioral results, by unraveling the spatio-temporal brain dynamic evoked in response to each condition. A detailed description of the steps we took to obtain those results is presented next.

3.1. Select CENA plugin

Once we preprocessed our EEG/ERP data at the individual level, excluded EEG epochs containing eye blinks or other transient muscular and/or electric noise, we created an ERP file in response to each experimental condition for each one of our 70 subjects and interpolated each ERP to correct bad channels. Then, we created a group-averaged ERP for each condition (see Fig. 5B for color words condition (top panel) and for the XXX condition (bottom panel). After verifying the average of these two group-averaged ERP was deprived of any noise, we selected CENA plugin as implemented in the latest version of Brainstorm.

3.2. Perform HPMS

a. First, we performed an HPMS single on the difference waveform (Fig. 5C) of your two experimental conditions to identify the number of microstates (each represented as a template map, defined as the mean of the topographical maps that constitute a microstate) when the two conditions differ from one another. To do so:

(1) Drag and drop the ERP of each condition into the Brainstorm “File to process” window;

(2) Click on run and Click on CENA to have access to the CENA functions;

(3) Select the CENA Difference wave function and Click run.

The difference is computed as InputA – InputB. When feeding two ERPs into the Brainstorm file to process window InputA will be the ERP at the top of the list and InputB will be the ERP second in the list. As explained above, this step allows users to create the topographical maps for the Main Effect of the Factor (here, Stimulus Type).

Then:

(1) Clear the “File to process” Brainstorm window. Drag and drop the difference waveform into the “File to process” Brainstorm window;

(2) Click on run and Click on CENA to have access to the CENA functions;

(3) Click on CENA-HPMS single. Enter timing information about the duration of our baseline, RMSE lag duration, CI for thresholding RMSE peaks and valleys, and CI for merging time-adjacent microstates> Check “yes” to the option “Export template maps”, and specify that we wanted to plot the preliminary results;

(4) Click on run.
b. Then, we performed another HPMS single on the average waveform of the two experimental conditions to identify the number of microstates when the two conditions do not differ from one another (Fig. 5D). To do so:

1. Drag and drop the ERP of each condition into the Brainstorm “File to process” window;
2. Click on run and Click on average > Average files > Click run;
3. Clear list in the “File to process” Brainstorm window and Drag and drop the average waveform into the “File to process” Brainstorm window;
4. Click on run and Click on CENA to have access to CENA functions;
5. Click on CENA-HPMS single. Enter timing information about the duration of our baseline, RMSE lag duration, CI for thresholding RMSE peaks and valleys, and CI for merging time-adjacent microstates;
6. Check «yes» to the option «Export template maps», and specify that you want to plot the preliminary results;
7. Click on run.

c. Finally, we performed an HPMS multiple with each experimental condition to identify the number of microstates evoked in response to each experimental condition (see Fig. 5E for the color words condition and Fig. 5H for the non-words, XXXXX, condition). To do so:

1. Drag and drop the ERP of each condition into the Brainstorm “File to process” window and Click on run;
2. Click on CENA to have access to the CENA functions;
3. Click on CENA-HPMS multiple. Enter timing information about the duration of our baseline, RMSE lag duration, CI for thresholding RMSE peaks and valleys, and CI for merging time-adjacent microstates;
4. Check «yes» to the option «Export template maps», and specify that you want to plot the preliminary results;
5. Click on run.

In the present Stroop task, the parameters were as follows: An L lag of 12 ms, a baseline period from -284 ms\(^{11}\) pre-stimulus to

\(^{11}\) The 284 ms value is automatically generated in Brainstorm by subtracting the lag duration (here, 12 ms) from the entire baseline (here, 300 ms minus one time point corresponding to stimulus onset). In this study, the EEG was digitized at 250 Hz (corresponding to a sample bin of 4 ms per time point), bandwidth at 0.01–200 Hz, with the vertex electrode (C2) serving as an on-line recording reference; and impedances were kept below 100 k\(\Omega\).
48 ms post-stimulus, and a 99% CI to detect significant rises or falls in the RMSE function, and a 95% CI for the cosine metric analysis to determine whether the n + 1st microstate differed significantly in configuration from the nth microstate. Fig. 5F displays results about microstate onsets and offsets obtained from the HPMS of the difference between color words condition minus the non-word condition, while Fig. 5G shows results about the microstate onsets and offsets obtained from the HPMS of the grand mean of the two conditions. Finally, as illustrated in Figs. 5I and 6, a graphical representation of the CENA complete temporal brain dynamics obtained from HPMS performed in two experimental conditions was performed in order to report final results.

4. General discussion and conclusion

The recent advances in neuroimaging technology and computational neuroscience are offering new and more rigorous ways to identify automatically stable brain microstates. The Chicago Electro-Neuroimaging Analytics (CENA) benefits from such advances (Cacioppo et al., 2014). Unlike previous methods of microsegmentation that require a priori specification of the range of stable microstates in an ERP, CENA offers an automatic (data-driven) identification of brain microstates (Cacioppo et al., 2014). Moreover, CENA 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. Together, the computational procedures in CENA increase the likelihood that the identification of stable brain microstates and transition brain microstates are likely to be robust, replicable, and generalizable (Cacioppo and Cacioppo, 2013). Finally, the bootstrapping feature in CENA allows an objective identification of the most frequent stable microstates in a sample of subjects or across trials within subjects (Cacioppo et al., 2014). Within-subject and between-subject bootstrapping procedures provide an additional, powerful, and objective means of investigating how robust are the results of the microsegmentation.

Furthermore, CENA provides users with a data-intensive analytic suite of tools that parses the ERP into three types of states: a baseline state, stable microstates, and non-stable transitions between these states. This feature is a real advantage in the identification of the entire spatio-temporal dynamic of a time-variant data set, as it allows users to differentiate stable states from transition states. Combining transition timeframes (which by definition are not stable but instead are part of a transition from one stable microstate to the next; Cacioppo et al., 2014) 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 (Cacioppo et al., 2014). 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. By allowing specific differentiation between transition and stable states, CENA is an analytical suite of tools that solve the issue of incomplete, sporadic, and unreliable temporal state detection.

Finally, by analyzing time-varying activity in a multidimensional sensor space (across the entire scalp) rather than in a single vector space (at specific electrode positions), CENA makes it possible to investigate possible neural organizations underlying baseline states even in the absence of a clear morphological peak or trough (Cacioppo et al., 2014). Because it differentiates stable brain microstates from transitions between states, the new
quantitative procedure for identifying evoked brain microstates introduced here may serve as a better basis for source localization algorithms used to investigate the underlying neural correlates for these microstates. This, in turn, may lead to the identification of more defined biomarkers for various neuropsychiatric and neurologic diseases.

Conflict of interest

The authors have a patent pending on CENA (State identification in data with a temporal dimension; Cacioppo, S., Cacioppo, J. T., Weiss, R. M., & Runesha, H. B.; U. S. Patent pending. The University of Chicago) but the authors specified in the patent that the use of CENA for academic research in electrical neuroimaging is to be free to users.

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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.neuropsychologia.2015.09.004.

References

Glossary

CENA: Chicago Electrical Neuroimaging Analytics
CI: Confidence interval
EEG: Electroencephalogram

Electrical Neuroimaging: Corresponds to the use of different approaches, such as high-density EEG and algorithms integrating brain source localization, to directly (or indirectly) image the spatiotemporal electrical dynamics, structure, and function of the brain.

ERP: Event-related potentials
Evoked brain microstates: Post-stimulus brain microstates elicited in anticipation of or in response to a stimulus
GFP: Global field power
Microstates: Temporal segments of quasi-stable brain states

Template Map: Mean of the topographic maps. A template map is determined for each candidate brain microstate identified by the RMSE metric by averaging across the constituent topographic maps, and for each final microstate identified by the cosince metric by averaging across the constituent topographic maps.

Topographic Map (or TopoMap): Map of the evoked potentials at a given recording bin (time point) across n-dimensional sensor space where n the number of EEG recording channels
wMNE: weighted minimum-norm current estimate