

Variability of ICA decomposition may impact EEG signals when used to remove eyeblink artifacts

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Abstract

Despite the growing use of independent component analysis (ICA) algorithms for isolating and removing eyeblink-related activity from EEG data, we have limited understanding of how variability associated with ICA uncertainty may be influencing the reconstructed EEG signal after removing the eyeblink artifact components. To characterize the magnitude of this ICA uncertainty and to understand the extent to which it may influence findings within ERP and EEG investigations, ICA decompositions of EEG data from 32 college-aged young adults were repeated 30 times for three popular ICA algorithms. Following each decomposition, eyeblink components were identified and removed. The remaining components were back-projected, and the resulting clean EEG data were further used to analyze ERPs. Findings revealed that ICA uncertainty results in variation in P3 amplitude as well as variation across all EEG sampling points, but differs across ICA algorithms as a function of the spatial location of the EEG channel. This investigation highlights the potential of ICA uncertainty to introduce additional sources of variance when the data are back-projected without artifact components. Careful selection of ICA algorithms and parameters can reduce the extent to which ICA uncertainty may introduce an additional source of variance within ERP/EEG studies.

Descriptors: Independent component analysis, ICA, EEG, Artifacts, Eyeblinks, EEGLAB

Independent component analysis (ICA) is a powerful tool to isolate and remove nonneural artifacts such as eyeblinks from EEG signals (Stone, 2002), which in many respects may be superior to regression-based approaches for eyeblink correction (see Hoffmann & Falkenstein, 2008; Jung et al., 2000, for a comparison of ICA-based artifact removal relative to regression-based approaches). Conceptually, ICA assumes that individual sources of activity are unrelated and therefore statistically independent, rendering it possible to tease apart the underlying components (referred to as sources or components) that make up the EEG signal based upon maximizing their mutual independence (Stone, 2002). In this way, ICA appears ideally suited for separating artifactual activity such as eyeblinks from the underlying EEG signal given that they represent different (independent) physical processes. However, the implementation of this blind source separation technique results in the potential for variation in the separation of these underlying sources. That is, in order to maximize independence between sources, ICA approaches attempt to reduce higher-order statistical dependencies, which results in a multitude of potentially optimal solutions (Lee,

1998). Thus, previous investigations have observed that, given the same data, repeated ICA decompositions may return different solutions (Delorme & Makeig, 2004; Duann, Jung, Makeig, & Sejnowski, 2003; Duann et al., 2001; Esposito et al., 2002).

Within the context of removing artifactual activity from the EEG signal, inconsistent solutions for separating the artifact sources from the underlying EEG sources may induce additional variance within the reconstructed EEG signals when the artifact-related components are removed. Given the process by which blind source separation techniques work, variability in the source associated with the artifact may reflect the inclusion of aspects of nonartifactual sources. That is, when the EEG signals are reconstructed without the artifact component, there is the potential that real aspects of the signal may have unknowingly been removed. Conversely, it may also be that the variability in the source related to the artifact reflects misallocating artifact-related variance into nonartifact-related sources, meaning that aspects of the artifact may still be present when the EEG signal is reconstructed without the artifact component. In either case, if an ICA algorithm is highly variable in its solutions, then there is a potential that the process of reconstructing the EEG signal without the artifact-related components may mask or even induce statistical differences between conditions as a result of this uncertainty. However, the extent to which this variability in temporal ICA solutions ultimately influences the EEG signal when eyeblink-related activity is removed is not well established.

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The rise in popularity of ICA over the past decade coincides with the growing adoption of EEGLAB (Delorme & Makeig, 2004)—a MATLAB/octave-based graphical toolbox for data processing that includes a number of ICA algorithms that can easily be included within data processing workflows. Problematically, however, investigations that have utilized ICA approaches to remove eyeblink artifacts from EEG data rarely indicate the specific algorithm or settings utilized. The specific algorithms available (either by default or through EEGLAB extensions/plugins) also vary widely in their approach to blind source separation, which impacts on the extent to which variability across repeated decompositions might be observed. Although the EEGLAB documentation provides some guidance regarding preferential ICA algorithms and their limitations (Delorme & Makeig, 2004), a greater understanding of how this variability manifests across different ICA algorithms warrants further attention.

Within investigations that do report the ICA algorithm utilized, three methods appear to be particularly popular. Of these, SOBI (second-order blind identification; Belouchrani, Abed-Meraim, Cardoso, & Moulines, 1997) does not involve random partitioning of data and/or random assignments of weights and instead relies on cross-correlations to perform joint diagonalization in order to separate underlying sources. As eyeblinks should have a higher autocorrelation relative to other aspects of the EEG, the SOBI algorithm may be particularly well suited for the purpose of eyeblink removal. Further, such an approach also offers the benefit of absolute consistency across repeated decompositions. The recommended approach by Delorme and Makeig (2004), however, is the infomax algorithm (Bell & Sejnowski, 1995) or extended infomax algorithm (Lee, Girolami, & Sejnowski, 1999) given its ability to separate high-dimensional EEG data from artifacts such as eyeblinks and line noise as well as its ability to resolve dipolar components (Delorme, Palmer, Onton, Oostenveld, & Makeig, 2012). The infomax algorithm is based on minimizing redundancy between the outputs or, equivalently, maximizing the joint entropy of the components (Bell & Sejnowski, 1995; Jung et al., 2001). Another popular ICA algorithm available as an EEGLAB extension/plugin is the FastICA algorithm, which seeks to maximize non-Gaussianity of the resulting components through a fixed-point iteration scheme (Hyvärinen, 1999). The potential advantage of the FastICA algorithm is its ability to rapidly converge upon an optimal solution given the algorithmic approach (Hyvärinen & Oja, 2000). Accordingly, given the algorithmic differences, ICA uncertainty may differentially manifest as a result of the approach to separation of sources used within these algorithms (see Hyvärinen & Oja, 2000) for a more in-depth discussion of algorithmic differences).

Initial investigations of this ICA uncertainty have predominately focused on utilizing this variability in solutions to separate *signal* components from *noise* components (Artoni, Menicucci, Delorme, Makeig, & Micera, 2014; Groppe, Makeig, & Kutas, 2009; Harmeling, Meinecke, & Müller, 2004; Himberg, Hyvärinen, & Esposito, 2004; Meinecke, Ziehe, Kawanabe, & Müller, 2002). These investigations have attempted to utilize data resampling approaches to separate sources using different random subsets of the original data. By computing the independent components multiple times or under different conditions, those sources that represent signals should cluster together, whereas sources representing noise should not and can thus be discarded or recomputed. Using such an approach, Artoni and colleagues (2014) observed that the infomax algorithm produced a more reliable ICA decomposition than the FastICA algorithm across bootstrapped decompositions. The present literature reflects a focus on extracting source activations from

the EEG to get at the underlying component processes. For such uses, the data are transformed and ultimately analyzed in source space; thus, the focus is on identifying the many sources that reflect signals. However, as discussed above, ICA is also useful for isolating and removing sources of artifacts. In these instances, the focus is instead on identifying the few sources that reflect noise, so that the EEG data can be reconstructed without those sources. Therefore, a critical limitation of the present literature is that it provides little insight into the potential ramifications of this variability in ICA decompositions as it relates to alterations of the reconstructed EEG signal when artifact-related components are removed. Given the growing utilization of ICA for such a purpose, a greater understanding of the influence of this variability on the reconstructed EEG signal is necessary.

The aim of the present investigation was to determine the extent to which this ICA uncertainty may influence the reconstructed EEG signal within a real data set when eyeblink-related artifacts are removed. To best characterize this ICA uncertainty, a data set containing EEG from 32 participants was submitted to temporal ICA decomposition and subsequent postprocessing 30 separate times for each of the three ICA algorithms. Although there is a wide assortment of potential other ICA algorithms that could be investigated, these ICA algorithms represent three popular algorithms that are available for use with EEGLAB either in the default distribution or through a plugin. Thus, these algorithms can be implemented as turnkey solutions; not requiring specialized hardware, additional software, or alterations to aspects of the EEGLAB code, rendering them particularly likely to be used by the general psychophysiological investigator. Further, these three algorithms can more broadly be construed as representing the key methodological approaches currently employed to blind source separation, with SOBI providing insight into the stability of lower-order statistical approaches, FastICA providing insight into approaches utilizing approximation methods to model higher-order statistics, and infomax to provide insight into higher-order statistical approaches. In contrast to existing studies investigating ICA uncertainty that used different subsets of the same data, within the present investigation ICA decomposition utilized the full data set for each decomposition. As identical data were submitted each time, any variation in the final result must then be the result of the ICA decomposition. Although it is impossible in this context to determine what the true optimal solution is, this approach allows for characterizing the potential variation in ICA solutions. Further, conducting 30 repetitions of each decomposition provides a perspective on the most likely range of solutions given assumptions for regression toward the mean on any individual decomposition of the data.

In this context, the P3 ERP—an aspect of the EEG signal time-locked to the presentation of a stimulus—offers a prime test case for how this variability might impact empirical findings. Stimulus-locked ERPs such as the P3 are widely utilized within the psychophysiological community (Polich, 2007). Although traditionally investigated within the EEG space, prior investigations have used source separation techniques such as PCA (Kamp, Murphy, & Donchin, 2013) and ICA (Debener, Makeig, Delorme, & Engel, 2005; Makeig et al., 2002) to investigate the P3 in source space. Remaining in source space could conceptually allow for characterizing the extent to which these ICA algorithms may be misallocating variance between the P3 and the eyeblink across repeated decompositions. However, source separation techniques generally produce multiple ICA components that relate to the P3 (Debener et al., 2005; Makeig et al., 2002), consistent with the premise that the P3 ERP results from multiple neural generators (Polich, 2007).

Given the well-characterized understanding of the P3 ERP and the increasing use of ICA as a means to remove artifacts and then return to EEG space, the present investigation focused on the extent to which the P3 was influenced by removal of the eyeblink artifact component. In EEG space, the P3 is characterized by topographic maxima surrounding parietal electrode sites, yet has a broad topographical distribution (Polich, 2007). As the eyeblink manifests in a frontal topographic distribution (Delorme & Makeig, 2004), ICA uncertainty would be hypothesized to modulate the amplitude of the P3 within frontal electrodes. The extent to which the variability in ICA solutions might more broadly influence other aspects of the EEG signal was further assessed by investigating the overall level of variability within each electrode across all sampling points. These two approaches were used to address the question of how algorithm choice relates to ICA-induced variability and, given the findings, can variability resulting from using a specific ICA algorithm be reduced (see following sections.) These questions are answered and discussed separately with a final overall conclusion provided in Overall Discussion.

How Does Algorithm Choice Relate to ICA-Induced Variability?

Method

Thirty-two college-aged young adults (11 female; 19.3 ± 0.9 years) from Michigan State University participated in this investigation. All participants provided written informed consent in accordance with the Institutional Review Board at Michigan State University and reported being free of any neurological disorder, psychological condition, previous history of head trauma, cardiovascular disease, and physical disabilities, and indicated normal or corrected-to-normal vision.

Procedure. EEG activity was recorded in response to a well-established perceptually challenging three-stimulus oddball task that required responding with a right-hand thumb press only when an infrequent target stimulus occurred, while ignoring all other stimuli (Pontifex, Parks, Henning, & Kamijo, 2015). All stimuli were presented focally on a computer monitor at a distance of 1 m for 100 ms, with a 1,000-ms response window and a 1,700-ms intertrial interval using PsychoPy, 1.76 (Peirce, 2009). Three blocks of 175 trials were presented resulting in a total of 63 target stimuli, for a total task duration of approximately 9 min. EEG activity was recorded from 64 electrode sites arranged in an extended montage based on the International 10-10 system (Chatrian, Lettich, & Nelson, 1985) using a Neuroscan Quik-cap (Compumedics, Inc., Charlotte, NC). Recordings were referenced to averaged mastoids (M1, M2), with AFz serving as the ground electrode, and impedances were less than 10 k Ω . A bipolar recording configuration was used to monitor electrooculographic (EOG) activity with an electrode placed above and below the orbit of the left eye. The continuous data were digitized at a sampling rate of 1000 Hz and amplified with a factor of 500 in a frequency range from DC to 70 Hz. The EEG data were then imported into EEGLAB (Delorme & Makeig, 2004) and prepared for temporal ICA decomposition. All data processing was conducted using an Apple iMac with a 3.5 GHz Intel Core i7 processor and 32 GB RAM. Data more than 2 s prior to the first event marker and 2 s after the final event marker were removed to restrict computation of ICA components to task-related activity. The continuous data were then filtered using a 0.05 Hz high-pass 2nd order Butterworth

IIR filter to remove slow drifts (Mognon, Jovicich, Bruzzone, & Buiatti, 2011), and the mastoid electrodes were removed prior to ICA decomposition.

To characterize the variability in ICA solutions, the data were processed by repeating 30 independent decompositions for each algorithm (SOBI, infomax, and FastICA). Example code for the implementation of each approach is provided in Figure 1. The SOBI and FastICA algorithms were run using the default EEGLAB settings. The infomax algorithm was run using the binary implementation with the extended option using the default settings. Per the default settings within EEGLAB, the infomax algorithm was initialized using the identity matrix, while the FastICA algorithm was initialized using a random matrix. For each of the ICA algorithms, no PCA reduction in components was used such that the number of components extracted was the same as the number of channels in the EEG data. Following each ICA computation, the eyeblink artifact components were identified and removed. Identification of the eyeblink artifact components was performed using the *icablinkmetrics* function (Pontifex, Miskovic, & Laszlo, 2016). Specifically, a time domain approach was used to identify the components that exhibited statistically significant correlation and overlap (assessed using convolution) with the eyeblink activity, as well as produced a statistically significant reduction in the eyeblink artifact present within the EEG (Pontifex et al., 2016). The reduction in the eyeblink artifact present within the EEG was quantified by computing the convolution between the rectified mean EEG activity across all electrodes during the occurrence of an eyeblink and the rectified mean EEG activity across all electrodes during this same period following removal of each ICA component (Pontifex et al., 2016). Secondary verification was performed using the *EyeCatch* function (Bigdely-Shamlo, Kreutz-Delgado, Kothe, & Makeig, 2013), which uses spatial information to distinguish the eyeblink-related components based upon the correlation between the scalp map projection for each ICA component and a database of over 3,000 templates. All blink-related components identified by the *icablinkmetrics* function were also identified by the *EyeCatch* function. This procedure resulted in each participant's data being recomputed and processed 90 separate times (three algorithms $\times$ 30 repeats of the data processing for each participant). Because of the deterministic nature of the SOBI procedure (Belouchrani, Abed-Meraim, Cardoso, & Moulines, 1997), the repetitions for this approach should be irrelevant—with all repetitions returning identical solutions. Thus, the inclusion of this algorithm also provides a validation check of the methodology described below.

To assess the variability related to the stimulus-locked P3 ERP component following removal of the eyeblink component from the continuous EEG, epochs were created for correct trials from -100 to $1,000$ ms around the stimulus, baseline-corrected using the prestimulus (-100 to 0 ms) period, and filtered using a 30 Hz low-pass IIR filter (Pontifex et al., 2015). To ensure consistency across repeated ICA decompositions, no epochs were rejected. Following computation of the mean epoch, the amplitude of the P3 ERP component was evaluated as the difference between the mean prestimulus baseline and the largest positive-going peak within a 300–700 ms latency window. The variability of P3 amplitude resulting from removal of the eyeblink component was quantified at each electrode using the interquartile range (IQR) across the 30 repeated decompositions for each electrode site. This approach provides insight into the effect of different ICA implementations on the range of possible P3 amplitudes observed within each single subject across repeated decompositions.

```

clear; clc; % Clear memory and the command window
eeglab; % Start eeglab: Requires Matlab Signal Processing Toolbox & Statistics Toolbox

% Determine what algorithm to run
% ['sobi' | 'infomax' | 'infomax_studywise' | 'fastica' | 'fastica_iter' | 'fastica_symm' | 'fastica_symm_tanh' | 'fastica_symm_tanh_identity']
algorithmselection = 'infomax_studywise';

% Save study wise random state prior to beginning any data processing
if ~(exist('studywiserandomstate.mat', 'file') > 0)
    studywiserandomstate = rng; save('studywiserandomstate.mat', 'studywiserandomstate')
end

% Read in a continuous EEG dataset with any bad channels/segments removed
EEG = pop_loadset('filename', 'RawEEG.set', 'filepath', '/Studies/');

% Remove additional data points 2 seconds outside of triggers
winPadding = 2;
winStart = (EEG.event(1).latency - (EEG.srate * ((1000/EEG.srate) * winPadding)));
winStop = (EEG.event(end).latency + (EEG.srate * ((1000/EEG.srate) * winPadding)));
if (winStart < 1); winStart = 1; end; if (winStop > EEG.pnts); winStop = EEG.pnts; end;
EEG = pop_select( EEG, 'point', [winStart, winStop]); EEG = eeg_checkset(EEG);

% HighPass Filter the data to remove slow drift
EEG = pop_basicfilter(EEG, 1:size(EEG.chanlocs,2), 'Filter', 'highpass', 'Design', 'butter', 'Cutoff', 0.05, 'Order', 2, 'Boundary', 87);

% Compute ICA
switch algorithmselection
case 'sobi'
    EEG = pop_runica(EEG, 'icatype', 'sobi');
case 'infomax'
    EEG = pop_runica(EEG, 'icatype', 'binica', 'options', {'extended', 1});
case 'infomax_studywise'
    temp = load('studywiserandomstate.mat'); studywiserandomstate = temp.studywiserandomstate;
    rng(studywiserandomstate);
    % Note that this requires EEGLAB v13.5.4b or newer
    EEG = pop_runica(EEG, 'icatype', 'runica', 'options', {'extended', 1, 'block', floor(sqrt(EEG.pnts/3)), 'anneal', 0.98, 'reset_randomseed', 'off'});
case 'fastica'
    EEG = pop_runica(EEG, 'icatype', 'fastica');
case 'fastica_iter'
    EEG = pop_runica(EEG, 'icatype', 'fastica', 'options', {'maxNumIterations', 3000});
case 'fastica_symm'
    EEG = pop_runica(EEG, 'icatype', 'fastica', 'options', {'approach', 'symm'});
case 'fastica_symm_tanh'
    EEG = pop_runica(EEG, 'icatype', 'fastica', 'options', {'approach', 'symm', 'g', 'tanh'});
case 'fastica_symm_tanh_identity'
    EEG = pop_runica(EEG, 'icatype', 'fastica', 'options', {'approach', 'symm', 'g', 'tanh', 'initGuess', eye(size(EEG.chanlocs,2))});
end
EEG = eeg_checkset(EEG);

% Find Eye Blink Component(s) - Requires:
% icablinkmetrics EEGLAB Extension - https://scn.ucsd.edu/wiki/EEGLAB_Extensions
% EyeCatch is included within the Measure Projection Toolbox - https://scn.ucsd.edu/wiki/MPT
contaminated_channel = find(strcmp({EEG.chanlocs.labels}, 'VEOG')); % Channel where eye blink is present
EEG.icaquant = icablinkmetrics(EEG, 'ArtifactChannel', EEG.data(contaminated_channel,:), 'VisualizeData', 'True');
eyeDetector = pr.eyecatch; EEG.icaquant.eyelC = eyeDetector.detectFromEEG(EEG);
EEG.icaquant.eyeblickcomponent = intersect(EEG.icaquant.identifiedcomponents, EEG.icaquant.eyelC);

% Remove Eye Blink Component(s)
EEG = pop_subcomp(EEG, EEG.icaquant.eyeblickcomponent, 0);

```

Figure 1. Example MATLAB EEGLAB syntax for each of the ICA algorithms and approaches examined.

To determine the extent to which the variability might more broadly relate to other aspects of the EEG, the overall level of variability resulting from the ICA computation was assessed across all sampling points of the EEG. Following ICA computation, the continuous EEG data were rectified and centered across each electrode to reduce the influence of any voltage shifts following removal of the eyeblink component. The IQR of the 30 repeated decompositions was then computed for each data point within the continuous EEG

data following removal of the eyeblink components. The overall level of variability was subsequently quantified using the mean IQR within each electrode. This approach provides insight into the effect of different ICA implementations on the range of potential variability across all points of the EEG, not just those points time-locked to specific aspects of the EEG signal.

To characterize the efficacy of the ICA algorithms in removing eyeblink-related artifacts, triggers were inserted in the raw EEG

Table 1. Median Solution and Variability (Mean $\pm$ 1 SD) Within Each Region for Each of the ICA Algorithms

	Infomax			FastICA				Parallel approach with tanh contrast with identity matrix
	SOBI	Default	Studywise random state	Default	Increased iterations	Parallel approach	Parallel approach with tanh contrast	
Median solution for P3 amplitude (μV)								
Frontal	4.1 ± 3.9	4.4 ± 5.0	4.4 ± 5.4	5.2 ± 5.1	5.1 ± 5.2	5.4 ± 5.6	4.5 ± 5.0	4.5 ± 5.4
Central	9.3 ± 4.6	9.7 ± 5.0	9.7 ± 5.4	10.0 ± 4.9	9.9 ± 5.0	10.2 ± 5.1	9.7 ± 5.0	9.7 ± 5.4
Occipital	11.0 ± 4.7	11.4 ± 4.6	11.4 ± 5.1	11.4 ± 4.4	11.4 ± 4.5	11.5 ± 4.5	11.4 ± 4.5	11.4 ± 5.1
Interquartile range of P3 amplitude (μV)								
Frontal	0.0	0.08 ± 0.10	0.0	0.91 ± 0.88	0.73 ± 0.52	0.19 ± 0.26	0.12 ± 0.09	0.0
Central	0.0	0.04 ± 0.04	0.0	0.57 ± 0.65	0.47 ± 0.39	0.10 ± 0.14	0.06 ± 0.06	0.0
Occipital	0.0	0.02 ± 0.02	0.0	0.50 ± 0.69	0.43 ± 0.49	0.08 ± 0.15	0.03 ± 0.03	0.0
Interquartile range of overall EEG (μV)								
Frontal	0.0	0.22 ± 0.14	0.0	2.96 ± 1.61	2.93 ± 1.62	0.51 ± 0.56	0.37 ± 0.24	0.0
Central	0.0	0.09 ± 0.06	0.0	1.63 ± 1.10	1.56 ± 1.04	0.26 ± 0.42	0.15 ± 0.11	0.0
Occipital	0.0	0.06 ± 0.04	0.0	1.48 ± 1.40	1.47 ± 1.55	0.23 ± 0.54	0.09 ± 0.06	0.0
Median solution for percent reduction of eyeblink amplitude (%)								
Frontal	81.9 ± 19.0	94.1 ± 4.9	92.3 ± 10.9	87.0 ± 9.7	87.0 ± 9.7	86.9 ± 16.4	92.9 ± 8.1	92.1 ± 11.6
Central	75.0 ± 20.2	86.4 ± 13.5	85.2 ± 19.0	74.7 ± 19.0	74.6 ± 19.2	75.7 ± 29.3	83.1 ± 21.7	84.3 ± 20.2
Occipital	50.7 ± 36.6	65.3 ± 37.3	65.2 ± 44.2	46.1 ± 41.1	48.5 ± 39.4	53.7 ± 42.1	58.1 ± 47.3	61.7 ± 48.4
Interquartile range of percent reduction of eyeblink amplitude (%)								
Frontal	0.0	0.6 ± 0.6	0.0	8.1 ± 8.4	7.5 ± 7.3	1.4 ± 2.3	0.8 ± 0.8	0.0
Central	0.0	1.2 ± 1.2	0.0	16.4 ± 17.6	15.5 ± 16.9	3.3 ± 6.6	1.8 ± 1.6	0.0
Occipital	0.0	2.7 ± 3.1	0.0	32.9 ± 25.7	32.6 ± 28.6	8.1 ± 19.4	5.0 ± 6.5	0.0

corresponding to the apex of the eyeblink as identified by cross-correlations of 0.98 or higher between a canonical eyeblink waveform and the activity recorded from the vertical EOG (VEOG) electrode using the eyeblinklatencies function of the icablinkmetrics2.0 toolbox. Epochs were then created from -200 to 200 ms around each eyeblink and baseline-corrected using the first data point in each epoch. The amplitude of the eyeblink artifact was evaluated as the rectified mean amplitude within a -50 to 50 ms window. The same measurement was also conducted on the unaltered original EEG data to allow for computation of the percent reduction in the eyeblink artifact. The variability of eyeblink artifact removal was quantified using the IQR of the percent reduction in the eyeblink artifact across the 30 repeated decompositions for each electrode. This approach provides insight into the effect of different ICA implementations on the range of possible reductions in the eyeblink artifact observed within each single subject across repeated decompositions.

Statistical analysis. In order to remain robust to the potential for outliers resulting from the ICA computations, the median was used as a measure of central tendency and the IQR was used as a measure of variability. To quantify how the variability resulting from ICA computations manifests across the scalp, three regions of interest were defined by taking the mean IQR across electrodes in the following regions: frontal (FP1/Z/2, AF3/4, F7/5/3/1/Z/2/4/6/8), central (FT7/8, FC5/3/1/Z/2/4/6, T7/8, C5/3/1/Z/2/4/6, TP7/8, CP5/3/1/Z/2/4/6), and occipital (P7/5/3/1/Z/2/4/6/8, PO7/5/3/Z/4/6/8, O1/Z/2). To facilitate direct comparison of the variability observed across each of the ICA algorithms, the median and IQR of P3 amplitude, the overall EEG, and the percent reduction in the eyeblink artifact are provided in Table 1.

Results

Across all participants, ICA computations were based on 147.9 ± 12.7 data points for each ICA weight (data points/channels²).

The mean number of eyeblink artifacts present within the data across participants was 107.7 ± 74.1 (min: 36, max: 326). Across the 30 repetitions of each ICA algorithm for each participant, the mean computation time for each algorithm was 0.7 ± 0.1 min for the SOBI algorithm, 9.1 ± 2.2 min for the infomax algorithm, and 3.7 ± 1.6 min for the FastICA algorithm.

Central tendency. *Median solution for P3 amplitude.* The median P3 amplitude revealed attenuation of P3 amplitude across the scalp in response to the SOBI algorithm (8.1 ± 4.4 μ V), relative to the infomax (8.5 ± 4.9 μ V) and FastICA (8.9 ± 4.8 μ V) algorithms (see Figure 2).

Median solution for percent reduction of the eyeblink. The median percent reduction in eyeblink artifact indicated a greater reduction in the amplitude of the eyeblink artifact for the infomax algorithm ($81.9 \pm 17.2\%$), relative to the FastICA ($69.2 \pm 20.6\%$) and SOBI ($69.2 \pm 22.9\%$) algorithms (see Figure 2).

Variability. *IQR of P3 amplitude.* The IQR of P3 amplitude was such that the FastICA algorithm exhibited greater variability of P3 amplitude (0.66 ± 0.68 μ V) than the infomax (0.05 ± 0.05 μ V) and SOBI algorithms (0.0 ± 0.0 μ V; see Figure (3 and 4), and 5). Across all algorithms, greater variability of P3 amplitude was observed for frontal electrode sites (0.50 ± 0.45 μ V), relative to central (0.30 ± 0.33 μ V) and occipital electrode sites (0.26 ± 0.35 μ V; see Figure 4 and 5).

IQR of the overall EEG. The IQR of the overall EEG similarly reflected greater variability for the FastICA algorithm (2.02 ± 1.3 μ V), relative to the infomax (0.12 ± 0.08 μ V) and SOBI algorithms (0.0 ± 0.0 μ V; see Figure 4). Across all algorithms, greater variability in the overall EEG was observed for frontal electrode sites (1.60 ± 0.83 μ V), relative to central (0.86 ± 0.56 μ V) and occipital electrode sites (0.77 ± 0.71 μ V).

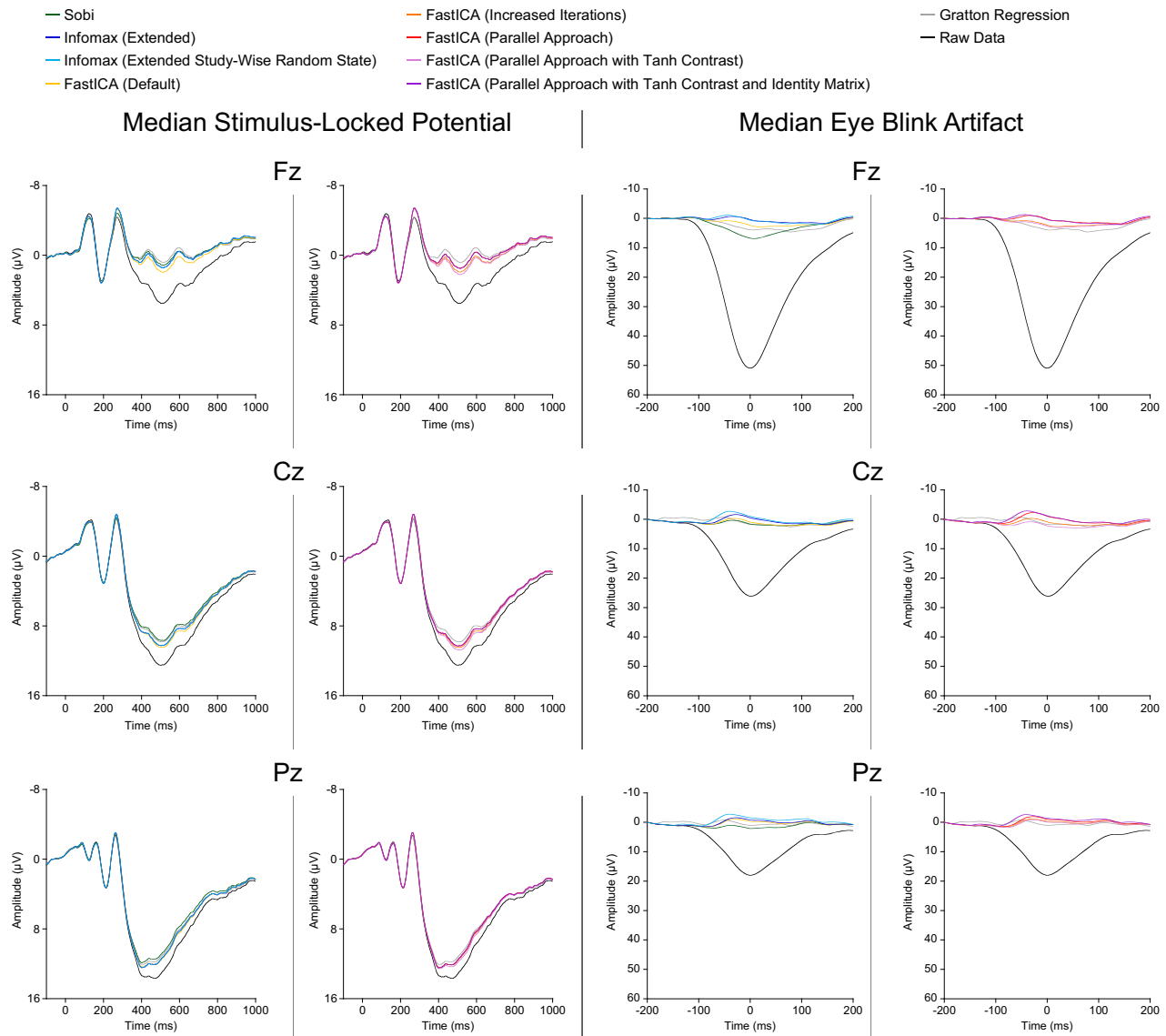


Figure 2. Illustration of the grand-averaged stimulus-locked potential and eyeblink artifact for the median solutions for three popular ICA algorithms and from utilizing different algorithm parameters. For comparison, these grand averages are plotted alongside the grand average of the raw uncorrected data, and data corrected using the Gratton regression-based approach (Gratton, Coles, & Donchin, 1983).

IQR of the percent reduction of the eyeblink. The IQR of the percent reduction in eyeblink artifact observed greater variability for the FastICA algorithm ($19.13 \pm 15.78\%$), relative to the infomax ($1.51 \pm 1.51\%$) and SOBI algorithms ($0.0 \pm 0.0\%$; see Figure 4 and 5). Across all algorithms, greater variability of the percent reduction of the eyeblink artifact was observed for occipital electrode sites ($17.81 \pm 13.70\%$), relative to frontal ($4.34 \pm 4.29\%$) and central ($8.81 \pm 9.16\%$) electrode sites.

Discussion

The aim of the present investigation was to determine the extent to which ICA uncertainty may influence the underlying EEG signal within a real data set when the eyeblink-related artifact is removed. This uncertainty was characterized using EEG data from 32 participants, decomposed and postprocessed to create stimulus-locked ERP averages 30 separate times for three popular ICA algorithms. The FastICA algorithm was associated with the greatest variability

in P3 amplitude, with over half a microvolt separating the first and third quartiles of possible outcomes. This variability in potential P3 amplitudes for the FastICA algorithm was over 10 times greater than the variability observed when the infomax algorithm was utilized, which exhibited less than 100 µV difference between the smallest and largest quartiles of possible outcomes. Further, a key finding from this investigation was that such variability resulting from both the FastICA and infomax algorithms was not restricted to the P3 ERP component. Rather this variability was more generally observed across all EEG sampling points, with the FastICA algorithm varying by 2 µV, while the infomax algorithm varied by just over 100 µV.

The present investigation also quantified the extent to which these ICA algorithms were effective at removing the eyeblink-related artifact from the EEG. Although it is important to note that the use of real data precludes the ability to know the “true” uncontaminated EEG, conceptually, as the eyeblink does not occur time-locked to a particular aspect of the EEG, signal averaging across

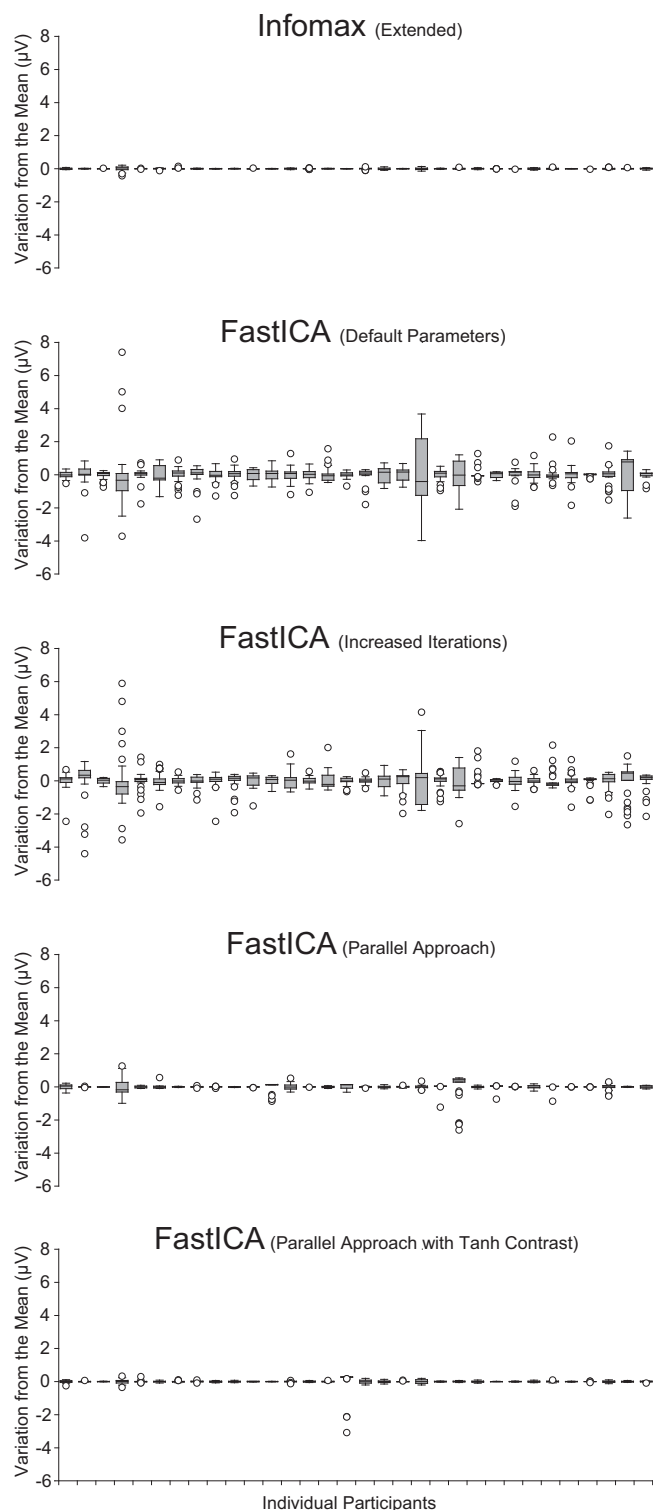


Figure 3. Illustration of the distribution of global P3 amplitude across each of the 30 repeated decompositions—relative to the mean of the decomposition for each participant—for the infomax ICA algorithm and in response to utilizing different algorithm parameters.

epochs surrounding the eyeblink should eliminate most EEG activity unrelated to the eyeblink. Thus, as evident in Figure 5, there is virtually no activity in this time window following removal of the eyeblink component. Utilizing such an approach, findings from this

investigation indicate that the infomax algorithm was superior to the FastICA and SOBI algorithms in reducing the eyeblink artifact. Further, the FastICA algorithm was observed to exhibit the greatest variability in the reduction of the eyeblink artifact, varying by approximately 19%; while the infomax algorithm exhibited only 1.5% variation in the reduction of the eyeblink artifact.

Collectively, then, the particular strengths and weaknesses of the specific ICA algorithms are apparent. The SOBI algorithm, while invariant in its solutions and relatively quick, was not able to reduce the eyeblink artifact to the same extent as the infomax algorithm. The FastICA algorithm, while quick, was observed to exhibit a high degree of variability in potential solutions that was of sufficient magnitude to influence experimental findings. The infomax algorithm resulted in the greatest reduction in the eyeblink artifact, yet was computationally slow, taking over three times as long as the FastICA algorithm and nine times as long as the SOBI algorithm. Moreover, uncertainty in the infomax algorithm did produce a small amount of variability in potential solutions; however, within the context of traditional EEG/ERP experiments, it is questionable if 1\10 µV of variability would influence experimental findings.

Can Variability Resulting from Using the FastICA Algorithm Be Reduced?

The high degree of variability observed across repeated decompositions when using the FastICA algorithm was surprising given its popularity to date. Accordingly, in an attempt to understand the nature of this variability and the extent to which it may be reduced, we investigated several potential causes of this ICA uncertainty. Our first hypothesis was that this variability in solutions may result from an insufficient number of iterations being allowed. That is, within the present investigation the default settings were used based upon the premise that if an investigator does not specifically mention alterations of algorithmic settings in a manuscript, then the default parameters must have been used. Within the FastICA algorithm, the default parameters may have limited the number of search iterations for each component, and the algorithm may have suffered from a convergence problem (i.e., failing to center on the optimal solution). Increasing the number of iterations may therefore allow the FastICA algorithm to iterate through a greater number of potential solutions for each component, increasing the time necessary for computation but allowing a more optimal solution to be obtained. Finding such an optimal solution should ultimately reduce the variability across repeated decompositions. Another potential source of the increased variation across repeated decomposition observed for the FastICA algorithm may be the default approach of sequentially computing the solution for each ICA component (Delorme, Sejnowski, & Makeig, 2007). When components are estimated sequentially, the order in which components are found can drastically alter the subsequent solutions for other components (Ollila, 2010). Thus, it may be that the greater variability observed for the FastICA algorithm comes from resolving components in different orders across repeated decompositions. Estimating the components in parallel, in a manner more similar to that of infomax, may result in more consistent ICA solutions across repeated decompositions. Further, Glass and colleagues (2004) observed that the utilization of the default cubic contrast function may misallocate variance when multiple sources are similar, resulting in greater variability in ICA solutions across repeated decompositions. Utilization of the tanh contrast function in the FastICA algorithm, alternatively, was observed to result in greater similarity

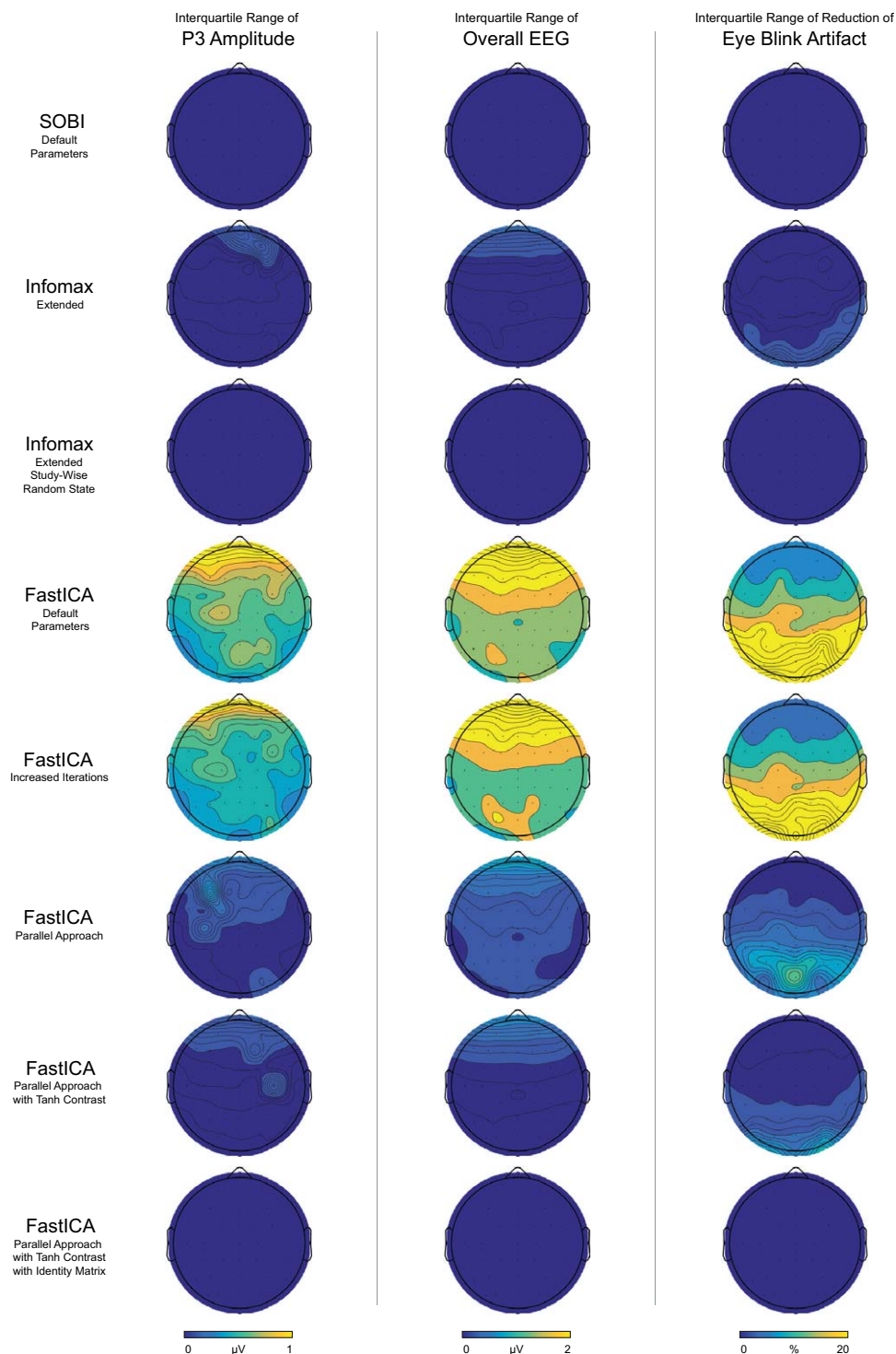


Figure 4. Illustration of the topographic distribution in variability for P3 amplitude, the overall EEG, and the reduction in the eyeblink artifact across all participants as a function of three popular ICA algorithms and from utilizing different algorithm parameters.

of the EEG data to the infomax algorithm following removal of the eyeblink component (Glass et al., 2004). Collectively then, the default FastICA parameters may not be ideally tuned to the characteristics of EEG data, which may have contributed to the manifestation of the increased variability across repeated decompositions (Artoni et al., 2014). Accordingly, we specifically tested the extent to which variability induced by the FastICA algorithm following removal of the eyeblink component might be reduced by increasing

the number of iterations, using a parallel approach, and using the tanh contrast function in association with the parallel approach.

Method

Procedure. Replicating the methodological approach used above, the data were processed by repeating 30 independent decompositions of the FastICA algorithm. To ensure that the variability was

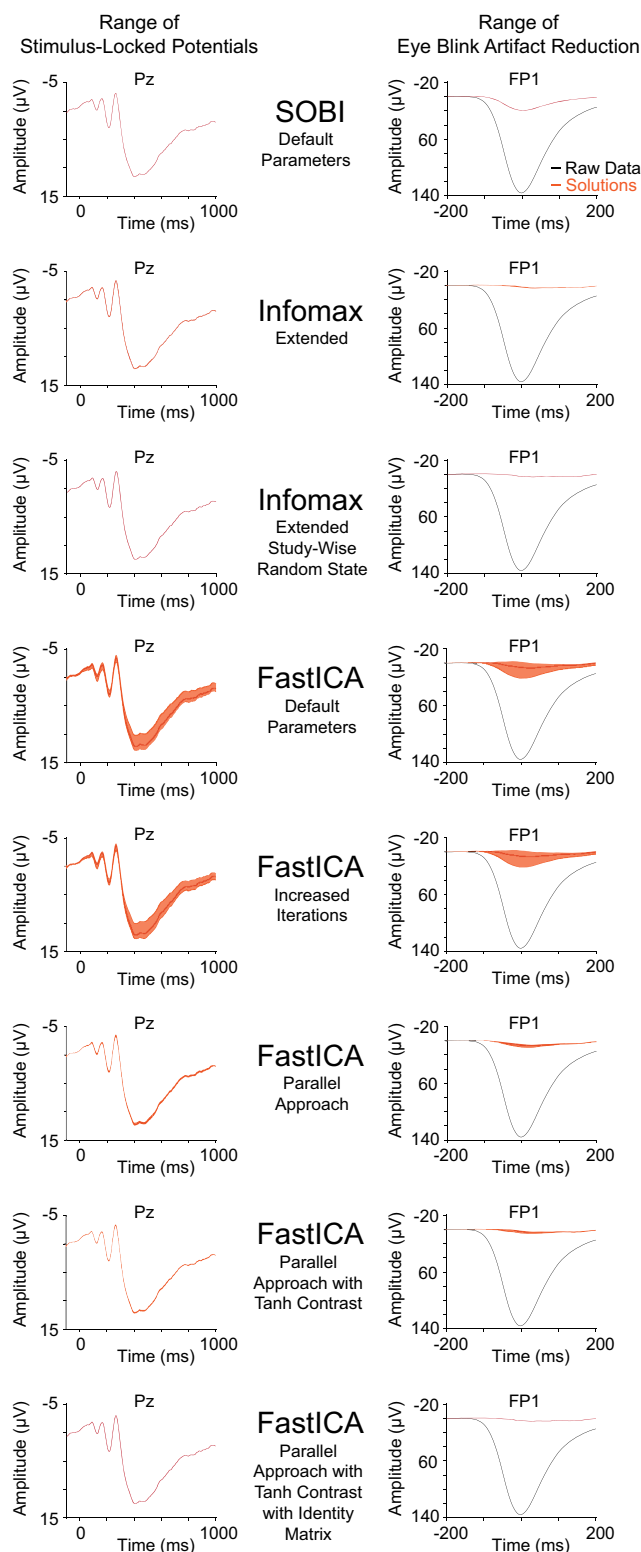


Figure 5. Illustration of the variability for the stimulus-locked average and eyeblink artifact average waveforms across all participants as a function of three popular ICA algorithms and from utilizing different algorithm parameters.

not increased as a result of failing to converge upon an optimal solution, this process was repeated by increasing the maximum number of iterations from 1,000 to 3,000. This process was further repeated using the parallel approach with a stability parameter

enabled, and again changing the contrast function to tanh using the parallel approach with a stability parameter enabled. Exemplar MATLAB EEGLAB code is provided in Figure 1. Following each ICA computation, the eyeblink artifact component was identified

using the `icblinkmetrics` function (Pontifex et al., 2016), verified using the `EyeCatch` function (Bigdely-Shamlo et al., 2013), and removed. All postprocessing and variability quantification approaches following removal of the eyeblink artifact component were identical to those used above.

Statistical analysis. The analytical strategy mirrored that detailed above, with the median and interquartile range of P3 amplitude, the overall EEG, and the percent reduction in the eyeblink artifact provided in Table 1.

Results

The default FastICA algorithm exhibited a mean computation time of 3.7 ± 1.6 min. Increasing the possible iterations of the FastICA algorithm resulted in a mean computation time of 6.8 ± 3.5 min. The mean computation time was 1.2 ± 0.7 min for the FastICA algorithm using the parallel approach and 1.0 ± 0.2 min for the FastICA parallel approach using the tanh contrast.

Central tendency. Median solution for P3 amplitude. The median P3 amplitude was observed to be attenuated for the FastICA parallel approach using tanh at frontal/central electrode sites (mean: 7.1 ± 5.0 μ V), relative to other variants of the FastICA algorithm (mean: 7.6 ± 5.1 μ V; see Figure 2).

Median solution for percent reduction of the eyeblink. The median percent reduction in eyeblink artifact was observed to be greater for the FastICA parallel approach using tanh ($78.0 \pm 21.7\%$), relative to other variants of the FastICA algorithm (mean: $70.5 \pm 19.2\%$; see Figure 2).

Variability. IQR of P3 amplitude. The IQR of P3 amplitude was observed to be greater for the default FastICA algorithm and FastICA with increased iterations (mean: 0.60 ± 0.54 μ V), relative to the FastICA parallel approach and the FastICA parallel approach using tanh (mean: 0.10 ± 0.11 μ V; see Figure (3 and 4), and 5).

IQR of the overall EEG. The IQR of the overall EEG similarly reflected greater variability for the default FastICA algorithm and FastICA with increased iterations (mean: 2.00 ± 1.31 μ V), relative to the FastICA parallel approach and the FastICA parallel approach using tanh (mean: 0.27 ± 0.31 μ V; see Figure 4).

IQR of the percent reduction of the eyeblink. The IQR of the percent reduction in eyeblink artifact observed greater variability for the default FastICA algorithm and FastICA with increased iterations (mean: $18.83 \pm 16.20\%$), relative to the FastICA parallel approach and the FastICA parallel approach using tanh (mean: $3.38 \pm 5.91\%$; see Figure 4 and 5).

Discussion

In contrast to our initial hypothesis that the variability observed across repeated decompositions when using the FastICA was the result of a failure of the algorithm to converge upon an optimal solution, the findings reported herein suggest that a major contributor to this variability is the sequential approach for determining each component. That is, the parallel implementations of the FastICA algorithm resulted in an approximately 80% reduction in the variability observed for P3 amplitude, the overall EEG, and the percent reduction in the eyeblink artifact relative to the default implementation of FastICA. Thus, resolving components in parallel

appears to result in more consistent ICA solutions across decompositions relative to the default implementation of FastICA. Although not drastically different from the default cubic contrast function, utilization of the tanh contrast function of the parallel FastICA algorithm, as suggested by Glass and colleagues (2004), did result in a small reduction in variability for P3 amplitude, the overall EEG, and reduction of the eyeblink with the additional benefit of a faster computation time. However, the tanh contrast function was associated with a slightly smaller P3 amplitude across frontal and central electrodes.

Can Variability Be Reduced by Controlling the Random Parameters?

Although the variability was minimized by utilizing the FastICA algorithm with the parallel approach and tanh contrast function, perfectly consistent results were not observed across repeated decompositions. One potential source of this remaining variability in the FastICA algorithm is the default utilization of an initial guess of the ICA weights by way of a randomly generated matrix. Thus, on each decomposition iteration, the FastICA algorithm generates a different initial starting point for computing the ICA weights. Such a source of variability may also be involved in the variability observed from utilization of the infomax algorithm, as upon initialization of the algorithm the data points used for training are randomly reordered. Thus, on each decomposition iteration, the infomax algorithm may converge upon a different local optima solution resulting in the observed variability. Accordingly, we specifically tested the extent to which these random parameters contributed to the observed variability across repeated decompositions.

Method

Procedure. Replicating the methodological approach used above, the data were processed by repeating 30 independent decompositions. To determine how the variability related to the infomax algorithm may result from differences in the random reordering of training samples across repeated decompositions, the MATLAB implementation of infomax (`runica`) was utilized with the parameter to reset the random seed turned off (an option available in EEGLAB version 13.5.4b or newer), allowing for initializing the algorithm using a studywise random state. As such a parameter was not available through the binary implementation of infomax (`binica`), all other parameters of the MATLAB implementation of infomax (`runica`) were set to be equivalent to those used in the binary implementation of infomax (`binica`) with the block size heuristic drawn from MNE-Python (Gramfort et al., 2013) to most closely approximate the heuristic used in the binary implementation (see Figure 1). To determine how the variability related to the FastICA algorithm may result from differences in the random allocation of the initialization matrix, the FastICA algorithm using the parallel approach with tanh contrast was initialized using the identity matrix as the initial guess for each repeated decomposition. Exemplar MATLAB EEGLAB code is provided in Figure 1. All postprocessing and variability quantification approaches following removal of the eyeblink artifact component were identical to those used above.

Statistical analysis. The analytical strategy mirrored that detailed above, with the median and IQR range of P3 amplitude, the overall EEG, and the percent reduction in the eyeblink artifact provided in Table 1.

Results

The infomax algorithm with a studywise random state (run using the MATLAB implementation of infomax) exhibited a mean computation time of 14.2 ± 4.4 min, whereas the FastICA algorithm using the parallel approach with tanh contrast and initialized with the identity matrix exhibited a mean computation time of 1.2 ± 0.5 min.

Central tendency. *Median solution for P3 amplitude.* The median P3 amplitude revealed no systematic differences between the infomax algorithm (8.5 ± 4.9 μ V) and the infomax algorithm with a studywise random state (8.5 ± 5.3 μ V). Similarly, no systematic differences in the median P3 amplitude were observed between the FastICA algorithm using the parallel approach with tanh contrast initialized with a random matrix (8.6 ± 4.8 μ V) and that initialized with the identity matrix (8.5 ± 5.3 μ V).

Median solution for percent reduction of the eyeblink. The median percent reduction in eyeblink artifact revealed no systematic differences between the infomax algorithm ($81.9 \pm 18.6\%$) and the infomax algorithm with a studywise random state ($80.9 \pm 24.7\%$). Similarly, no systematic differences in the median percent reduction in eyeblink artifact were observed between the FastICA algorithm using the parallel approach with tanh contrast initialized with a random matrix ($78.0 \pm 25.7\%$) and that initialized with the identity matrix ($79.4 \pm 26.7\%$).

Variability. *IQR of P3 amplitude.* Neither the infomax algorithm with a studywise random state nor the FastICA algorithm using the parallel approach with tanh contrast and initialized with the identity matrix exhibited any variability in P3 amplitude (0.0 ± 0.0 μ V).

IQR of the overall EEG. Neither the infomax algorithm with a studywise random state nor the FastICA algorithm using the parallel approach with tanh contrast and initialized with the identity matrix exhibited any variability in the overall EEG (0.0 ± 0.0 μ V).

IQR of the percent reduction of the eyeblink. Neither the infomax algorithm with a studywise random state nor the FastICA algorithm using the parallel approach with tanh contrast and initialized with the identity matrix exhibited any variability in the percent reduction in eyeblink artifact ($0.0 \pm 0.0\%$).

Discussion

Consistent with our hypothesis that controlling the random parameters of the ICA algorithms would reduce the variability, specifying the random state of the infomax algorithm and initializing the FastICA algorithm using the parallel approach with tanh contrast with the identity matrix both eliminated the variability across repeated decompositions. An important distinction, however, is that although fixing the random seed allows for reproducible results, this approach merely hides the stochastic nature of the source separation problem. That is, there may be many local optima that an ICA algorithm can converge upon, with this approach representing a point estimate from the distribution of potential results that may be equally valid or even superior to the fixed solution in separating the artifact from the signal. Conversely, however, the use of a studywise random state is effectively no different than the standard approach to utilizing the infomax algorithm. That is, within a typical workflow, an investigator only performs ICA decomposition a

single time with the random state reinitialized for each participant. Thus, in such an instance, the resultant decomposition again only reflects a point estimate from the distribution of potential results. The use of a studywise random state still allows for the random ordering of training samples for the infomax algorithm, but simply standardizes the random state across all participants to allow for reproducible findings.

Overall Discussion

Within the context of this investigation, the default implementation of the FastICA algorithm appears suboptimal for use with EEG, as altering the algorithmic approach and contrast functions enhanced its ability to separate the eyeblink artifact components from the background EEG as well as enhanced its computational speed. Although there may be particular context when the default FastICA parameters are ideal, based upon the findings from this investigation and others, it would seem that removing eyeblink artifacts from EEG data are not such a case. Indeed, within the statistics and machine-learning communities, the FastICA algorithm has long been viewed as a poor signal separation algorithm (Bach & Jordan, 2002; Learned-Miller & John, 2003; Matteson & Tsay, 2016). However, by altering the FastICA parameters to utilize a parallel approach with the tanh contrast function and an initial guess of the identity matrix, the results that are obtained are consistent and reproducible. When the context of the data processing does not necessitate a high degree of computational speed, it would appear that the extended infomax algorithm offers a preferable approach for ICA decomposition for the purpose of eyeblink artifact removal given that the infomax algorithm was more effective in reducing the eyeblink artifact than any other algorithm. Further, the utilization of a studywise random seed allowed for obtaining consistent and reproducible ICA decompositions. Although within the context of the present investigation we are unable to specifically speak to the mechanism underlying the superior performance of the infomax algorithm, such a finding likely reflects the nature of the computational approach. The SOBI algorithm relies on lower-order statistical approaches that provide optimal stability, but appear unable to fully differentiate the activity related to the eyeblink artifact from other activity within the EEG signal. FastICA provides greater computational speed at the inherent cost of accuracy given the use of estimates within the fixed-point iteration approach, while the infomax algorithm is better able to parse the signals present within the EEG as a result of the higher-order statistical approach at the cost of computational speed. Indeed, previous findings have observed that the infomax algorithm is better able to recover dipolar sources, which are compatible with cortical generators observed within the EEG signal (Delorme et al., 2012). Although speculative, it seems reasonable to assume that if the infomax algorithm is better suited for finding cortical EEG sources, it would also be better in retaining cortical activity when noncortical sources are removed.

As the present investigation specifically addressed the variability of the infomax and FastICA algorithms within EEGLAB, the generalizability of the findings to ICA implementations in other software packages is as of yet unknown. Conceptually, the variability of the ICA solutions should be very similar; however, seemingly minor differences in the way that the algorithms are implemented have the potential to modify the consistency in ICA solutions across repeated decompositions. Indeed, a key difference that could impact the ability to obtain both an optimal and reproducible ICA solution is the way in which the infomax algorithm

implementation approaches setting of the random state. If the implementation does not allow access to specifying the random seed—as is the case in the current binary implementation of the infomax algorithm in EEGLAB (binica)—the ability to obtain reproducible results is hindered. Alternatively, if the implementation relies upon a fixed seed, the algorithm would obtain consistent and reproducible results at the potential cost of the algorithm converging upon a local-optimal solution—impacting the ability of the algorithm to obtain the true optimal solution (if such a solution even exists within real EEG data). Depending upon the implementation of these algorithms, the default preferences for using a random relative to a fixed seed may differ. The benefit of open source data processing platforms, however, is that such differences between software implementations can more easily be detected and modified (as in the case of updates to the runica function in EEGLAB version 13.5.4b or newer) to allow the investigator to specify the parameters appropriate for their needs. Further, as the integration between MATLAB and other software packages, such as Python, continues to be enhanced, it will be increasingly possible to utilize newer ICA algorithms or superior ICA implementations made available in other programming languages.

It is also necessary to note that the efficacy of the ICA decomposition is dependent upon adequate preprocessing of the data. As low-frequency aspects of the EEG signal typically account for large proportions of variance, the application of a high-pass filter can improve the ICA decomposition by removing slow drifts and DC components (Winkler, Debener, Müller, & Tangermann, 2015; Zakeri, Asseondi, Bagshaw, & Arvanitis, 2014). Similarly, including electrode channels located near the eye can improve the ICA decomposition for the purposes of artifact removal, as the electrodes provide greater information for the ICA algorithm to separate the artifact from the background EEG (Zakeri et al., 2014).

Collectively, the present investigation illustrates how three popular ICA algorithms vary in the consistency of their solutions across repeated decompositions using real EEG data. While the present investigation used an automated eyeblink component selection approach that could have impacted the resultant variability, the benefit of such an approach is the consistent application of the same standards for component identification across repeated decompositions, relying on convergence between two very different methodologies of eyeblink component selection (i.e., icablinkmetrics—relying on time domain information, and EyeCatch—relying on spatial information). Any variation then ultimately occurs as a result of the ICA decomposition, with the data following each decomposition representing one possible outcome. The external relevance of these findings is that, when ICA approaches

are utilized to reconstruct data without a particular artifact, it is important to understand the potential for hidden variance to be induced. Although the measures of central tendency indicated minimal differences in P3 amplitude across the three ICA approaches (as illustrated in Figure 2), these findings should be interpreted as reflective of a lack of differences in the midpoint of the solutions across repeated decompositions across the three ICA approaches. Whereas the interquartile range reflects the magnitude of the variation in the reconstructed data observed within each single subject across repeated decompositions, thus the P3 ERP component on any particular decomposition would be expected to fall somewhere within the IQR (as illustrated in Figure 3).

Although this investigation used only data recorded in response to a visual cognitive task, speculatively such variability should be observed regardless of the context surrounding the recording of the EEG signal so long as eyeblink artifacts occur. Such speculation is drawn from the consistency between those findings specifically focused on the P3 ERP component and those findings assessing the variability across all sampling points of the EEG. Thus, although this investigation utilized the P3 ERP component as a test case, these findings should similarly generalize to other ERP components. An important caveat to the present findings is that they do not speak to the accuracy of the reconstructed EEG signal following removal of the eyeblink artifact. That is, as real EEG data were used, we are unable to know what the “ground truth” activity was during the eyeblink activity. Thus, these ICA algorithms may differ not only with respect to the consistency of their solutions, but the accuracy of the solutions as well. Further, research is thus necessary to investigate the accuracy of these ICA algorithmic approaches. However, the present findings speak to the need for investigators developing new ICA-based approaches to be attentive not only to the accuracy of their approach but to the potential uncertainty inherent within their approach. As Artoni and colleagues (2014) have demonstrated, such variability can be of use within signal space for differentiating more stable sources that likely reflect signal from those more variable sources that likely reflect noise. However, within the context of reconstructing the EEG signal when artifact components are removed, these findings highlight the ramifications of ICA uncertainty as it has the potential to introduce additional sources of variance within EEG/ERP measures. Given the growing interest in the repeatability of psychophysiological findings, investigators should be particularly aware of the relative strengths and limitations of the algorithm and parameters utilized for ICA decomposition as it relates to the potential to introduce such additional sources of variance within their data.

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