

This is a repository copy of Accounting for microsaccadic artifacts in the EEG using independent component analysis and beamforming.

White Rose Research Online URL for this paper: http://eprints.whiterose.ac.uk/96796/

Version: Accepted Version

Article:

Craddock, M, Martinovic, J and Müller, MM (2016) Accounting for microsaccadic artifacts in the EEG using independent component analysis and beamforming. Psychophysiology, 53 (4). pp. 553-565. ISSN 0048-5772

https://doi.org/10.1111/psyp.12593

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk https://eprints.whiterose.ac.uk/

Accounting for Microsaccadic Artefacts in the EEG using Independent Components

Analysis and Beamforming

Matt Craddock^{1,2}, Jasna Martinovic³, Matthias Müller¹

¹Institute of Psychology, University of Leipzig, Germany; ²School of Psychology, University of Leeds, UK;³School of Psychology, University of Aberdeen, UK

Running Head: ICA, beamforming, and artefactual gamma

Send correspondence to:

Matthias M. Müller

Institute of Psychology University of Leipzig Neumarkt 9-19 04109 Leipzig Germany Tel: +49 - 341 - 97 39 54 3

Fax: +49 - 341 - 97 35 96 9

Email: m.mueller@rz.uni-leipzig.de

Neuronal activity in the gamma-band range was long considered a marker of object representation. However, scalp-recorded EEG activity in this range is contaminated by a miniature saccade related muscle artefact. Independent Component Analysis has been proposed as a method of removal of such artefacts. Alternatively, beamforming, a source analysis method in which potential sources of activity across the whole brain are scanned independently through the use of adaptive spatial filters, offers a promising method of accounting for the artefact without relying on its explicit removal. We present here the application of ICA-based correction to a previously published dataset. Then, using beamforming, we examine the effect of ICA correction on the scalp-recorded EEG signal and the extent to which genuine activity is recoverable before and after ICA correction. We find that beamforming attributes much of the scalp-recorded gamma-band signal before correction to deep frontal sources, likely the eye muscles which generate the artefact related to each miniature saccade. Beamforming confirms that what is removed by ICA is predominantly this artefactual signal, and that what remains after correction plausibly originates in visual cortex. Thus, beamforming allows researchers to confirm whether their removal procedures successfully removed the artefact. Our results demonstrate that ICA-based correction brings about general improvements in signal-to-noise ratio suggesting it should be used along with, rather than be replaced by, beamforming.

Neuronal activity in the gamma-band range may play a critical role in visual perception (Martinovic & Busch, 2011). The electroencephalogram (EEG), with its high temporal resolution, provides a valuable method of studying macroscale neural oscillations. EEG has been used to examine gamma band activity during a range of tasks across a range of modalities. In particular, for many years, the role of induced gamma band activity (iGBA) – activity which is neither time- nor phase-locked to the onset of a stimulus – in visual object recognition was hotly debated (e.g. Busch, Herrmann, Müller, Lenz, & Gruber, 2006; Herrmann, Munk, & Engel, 2004; Martinovic, Gruber, & Müller, 2007; Müller, Gruber, & Keil, 2000; Tallon-Baudry & Bertrand, 1999). Across many studies, a peak in iGBA approximately 200-400 ms after stimulus onset was reliably observed when identifying or categorizing an object, leading many to conclude that iGBA was a marker of the activation of an object representation. However, Yuval-Greenberg, Tomer, Keren, Nelken, and Deouell (2008) convincingly demonstrated that induced gamma band activity in EEG data can be contaminated by muscle artefacts originating from miniature saccades. Thus, the role of miniature saccades needs to be assessed when studying iGBA using the EEG.

Miniature saccades occur even during fixation (for reviews, see Martinez-Conde, Macknik, Troncoso, & Hubel, 2009; Rolfs, 2009) and correlate with neural activity (Bosman, Womelsdorf, Desimone, & Fries, 2009; Dimigen, Valsecchi, Sommer, & Kliegl, 2009; Melloni, Schwiedrzik, Rodriguez, & Singer, 2009). Most tellingly, after the onset of a visual stimulus the rate of miniature saccades typically peaks in the same time window as the iGBA peak (Engbert & Kliegl, 2003; Yuval-Greenberg et al., 2008). Each miniature saccade produces a characteristic pattern of electrical, musclegenerated activity – the saccade spike potential (SSP). Electromyogenic signals such as these generate high-frequency, broadband noise which can easily be mistaken for genuine neural activity (Barlow, 1984; Coburn & Moreno, 1988; Goncharova, McFarland, Vaughan, & Wolpaw, 2003). The SSP artefact in particular poses a considerable problem. Unlike other artefacts such as blinks, muscle noise, and voltage-jumps, miniature saccades are too small – typically less than 1° of visual angle – to be easily detected using common artefact detection methods such as a simple inspection of the electroocculogram as recorded in the EEG. Furthermore, other artefacts are typically intermittent, and thus removal of artefact-contaminated trials is a practical solution. The ubiquity of miniature saccades would necessitate the rejection of a great many trials from analysis, with the consequence of a poor signal-to-noise ratio.

Two techniques which have recently been used to either correct or compensate for the SSP are Independent Component Analysis (ICA; Jung et al., 2000) and beamforming, a distributed source analysis method (Gross et al., 2001; Van Veen, van Drongelen, Yuchtman, & Suzuki, 1997). ICA is often used to isolate and remove the contribution of common artefacts such as blinks from the scalp recorded signal (Jung et al., 2000). Its key advantage over simple artefact rejection is that rather than completely removing artefact-contaminated data, full data is reconstructed with the artefactual contributions left out. However, ICA as normally applied does not adequately isolate the miniature saccade artefact. ICA can only find a limited number of sources – maximally, as many sources as electrodes – and better separates sources that contribute more variance to the EEG signal. Miniature saccades contribute a relatively small amount of the variance in the overall EEG signal. Hassler, Trujillo-Barreto, and Gruber (2011) construct virtual channels which consist only of miniature eye movement related activity. Thus, miniature eye movements contribute the majority of the variance to those channels, rendering the artefactual source more readily recoverable. Keren, Yuval-Greenberg, & Deouell (2010) instead re-epoch data to be time-locked to miniature saccades, detected by convolving the signal from ocular channels with a data-derived microsaccade filter. This ensures that ICA functions on data which has a relatively large artefactual component. Both methods thus improve the ability of ICA to maximally separate the contribution of the SSP to the signal from that of neural sources by increasing the amount of variance in the data which can be explained by the miniature saccade artefact.

ICA-based methods show successful attenuation of the SSP artefact, revealing an underlying GBA increase in response to familiar relative to unfamiliar objects (Hassler et al., 2011; Keren et al., 2010). The corrected GBA response appears to have narrower frequency extent, longer temporal extent, and lower amplitude than the typical broadband peak, and it does not follow the rate of miniature saccades (Hassler et al., 2011). However, ICA is not the only approach that allows characterisation of artefact-free iGBA; another option is beamforming (Hipp and Siegel, 2013).

Whereas ICA is used to isolate and remove artefactual signals, beamforming is a source analysis technique in which the contribution of potential dipole locations within the brain to the measured signal is estimated independently of other locations using spatial filters. Thus, estimates of source activity in posterior regions should be relatively uncontaminated by the miniature saccade artefact, which should mostly be present in frontal areas, close to the eyes. This approach has several advantages over the ICA-based methods. First, it does not require the explicit detection, isolation, and removal of artefact related signals. Second, since it estimates activity in source space without explicit removal of artefactual signal, it provides a less ambiguous answer to the question of the origin and morphology of gamma-band activity, which remains somewhat ambiguous after ICA-based artefact correction.

Hipp and Siegel (2013) found that beamforming effectively attenuated both the miniature saccade artefact and other muscle noise, allowing the recovery of an ongoing gamma band signal in visual cortex which was largely unobservable in sensor space. However, Hipp and Siegel (2013) employed a task using a high-contrast, continuously moving stimulus. Previous demonstrations of the efficacy of ICA-based correction (Hassler et al., 2011; Keren et al., 2010) and many of the previous studies of object recognition which were likely confounded by miniature saccade artefacts (e.g. Busch et al., 2006; Martinovic et al., 2007; Tallon-Baudry & Bertrand, 1999) used stationary objects. Although the pattern of miniature saccades produced in Hipp and Siegel's study was comparable to that typically observed in object recognition experiments (Yuval-Greenberg et al, 2008), it is unclear whether the underlying sources of gamma band activity are comparable. Thus, it is possible the underlying sources in object recognition tasks may be more or less readily recoverable using beamforming.

We recently published an article examining object-related gamma band activity after correction for the miniature saccade artefact using ICA (Craddock, Martinovic, & Müller, 2015). Here we report the application of the beamforming approach to the same data, and compare how beamforming and ICA approaches function in a typical object recognition experiment. In the first part of the article, we will demonstrate how the ICA approach attenuates the saccade spike artefact and allows recovery of an ongoing gamma band signal. In the second part of the article, we re-analyze the data in the frequency domain using beamforming, comparing the data before and after correction with ICA in order to localize artefact sources and determine which sources are removed by ICA.

Method

Note that sections regarding the participants, stimuli, and design of the experiment are an edited reproduction from Craddock, Martinovic, and Müller (2015), where a complete version is available.

Participants

Fifteen participants (ages 19 - 32, mean = 24 years) were recruited from the participant database of the University of Leipzig EEG-Laboratory. 13 were right-handed, 2 left-handed. 3 were male, 12 female. Individual written informed consent was obtained. The study conformed to the Code of Ethics of the World Medical Association and followed the guidelines for electrophysiological recordings of the local ethics committee of the University of Leipzig. Participants either received class credit or a small honorarium for participating.

Stimuli and Apparatus

Four-hundred and eighty stimuli were created using 240 greyscale photographs taken from a commercial image database (Hemera Photo Objects). Half of these photographs depicted natural objects (e.g. animals, fruit) and half showed man-made objects (e.g. furniture, tools). Each photograph showed only a single object against a neutral grey background. Two-hundred and forty non-object noise textures were created by randomising the phase of the Fourier transformation of each object image. Thus, each object image had a matching noise texture with the same amplitude spectrum and spatial frequency content. The objects were also spatially filtered to produce high and low spatial frequency versions to address hypotheses related to coarse-to-fine visual processing (Craddock et al., 2015). Here, we focus only on the contrast between objects and non-objects (see Figure 1). The mean (global luminance) and standard deviation (RMS contrast) of every image was adjusted to match the mean global luminance and RMS contrast of the full set of object images.

The stimuli were presented centrally on a 17" cathode ray tube screen (refresh rate 85 Hz) at a screen resolution of 1024×768 pixels, and seen from a viewing distance of 80 cm. Stimulus size was 400×400 pixels. At this viewing distance, the stimuli subtended approximately 10 degrees of visual angle in each direction. Presentation and timing of the stimuli was controlled using the Cogent toolbox for Matlab (Cogent, <u>www.vislab.ucl.ac.uk/Cogent/</u>; The Mathworks, Inc, Natick, Massachusetts). Participants' task was to discriminate living from non-living objects, and objects in general from non-object noise textures. Participants responded via button press.

EEG Recording and Analysis

Continuous EEG was recorded from 64 locations using active Ag-AgCl electrodes (BioSemi Active-Two amplifier system; Biosemi, Amsterdam, The Netherlands) placed in an elastic cap. This system uses two active electrodes positioned in close proximity to the electrode POz of the international 10– 20 system (Jasper, 1958) in place of the "ground" electrodes in other EEG amplifiers: Common Mode Sense (CMS) acts as a recording reference and Driven Right Leg (DRL) serves as ground (Metting van Rijn, Peper, & Grimbergen, 1990, 1991). Four electroocculograms (EOG) – above and below the right eye and outer canthi of each eye – were recorded in order to exclude trials with blinks and large eye movements, and for use in the detection and removal of miniature eye movements. EEG signal was sampled at a rate of 512 Hz and segmented into epochs starting 1 sec prior and lasting 1 sec after stimulus onset. EEG data processing was performed using the EEGLAB (Delorme & Makeig, 2004) and FieldTrip toolboxes (Oostenveld, Fries, Maris, & Schoffelen, 2011) combined with in-house procedures running under the Matlab (The Mathworks, Inc, Natick, Massachusetts) environment. The Fully Automated Statistical Thresholding for EEG Artifact Rejection (FASTER) plug-in for EEGLAB was used for artefact rejection and interpolation of globally and locally artefact contaminated channels (Nolan, Whelan, & Reilly, 2010).

Miniature saccade detection and correction method. We performed corrections using the microDetect plug-in for EEGLAB, which was created in-house

(<u>https://github.com/craddm/microDetect</u>). For each participant we created a radial EOG (the mean of the four EOG channels re-referenced to the posterior parietal electrode Pz; rEOG) using the data from

all conditions, concatenating epochs lasting from 500 ms before stimulus onset until 700 ms after stimulus onset. We convolved the rEOG with the saccadic-potential filter supplied by Keren et al. (2010). The saccadic-potential filter is a matched filter with an impulse response that matches the average normalized saccade-related potential of the five subjects from Keren et al. (2010). As an alternative to using the matched filter, a 30-80 Hz band-pass Butterworth filter may be applied to the rEOG. Both procedures pass features with temporal and spectral profiles comparable to miniature eye movements, although all detection steps are conducted in the temporal domain. Subsequently, local peaks greater than three times the standard deviation of the filtered rEOG were marked as potential miniature saccades.

After detection, we used Independent Component Analysis (ICA) to identify and remove artefactual activity. We used the Extended Infomax algorithm implemented in the EEGLAB toolbox. ICA decomposes statistically independent inputs or signal generators from a signal which comprises a linear mixture of those inputs. Individual components can then be removed or examined in isolation. Keren et al. (2010) suggest performing ICA on epochs centred on the event of interest rather than whole epochs or continuous data; the more that the data submitted to ICA focuses on miniature saccades, the better the separation of ocular from neural ICA components. Using the markers derived from the filtered-rEOG, we extracted peri-saccadic epochs from the full EEG dataset lasting 100 ms before and after the onset of each miniature saccade. Epochs were formed only around potential miniature saccades falling within a window from 500 ms before stimulus onset until 700 ms after stimulus onset.

The mean-centred, peri-saccade epochs with data from all 64 scalp channels and the four EOG channels were then submitted to the ICA algorithm. To compensate for the reduction in rank due to average referencing, a principal component analysis (PCA) was applied to remove a single from the data before conducting the ICA. Failure to account for this reduction in rank sometimes causes ICA to return components that contain high-frequency noise, which, if not removed, introduce that noise to the back-projected scalp-level data when other components are removed. Typically these components also capture some genuine brain activity, and thus their removal is undesirable. After decomposition, the resulting components were inspected for those resembling the three primary types of components identified by Keren et al. (2010): the average saccade, the vertical saccade, and the horizontal saccade. These components were identified by means of their topography and time course. Figure 2 shows an example of the time course and topography of a typical miniature saccade related ICA component. In addition, the time course of these components usually correlates highly with that of the rEOG or individual EOG channels, which can be checked using the microDetect plug-in. The weights of the ICA decomposition of the peri-saccade epochs were copied to the full dataset, and components that reflected miniature saccades were rejected.

Time-frequency analysis. Time-frequency representations for both sensor and source space were obtained using sliding-window FFT methods implemented in the FieldTrip toolbox (Oostenveld et al., 2011). We applied bandstop 2nd order Butterworth filters from 83-87 Hz and from 48-52 Hz to remove activity relating to the (85 Hz) refresh rate of the monitor and 50 Hz power line noise, respectively. The linear trend was also removed. Total power (both evoked activity and activity neither time- nor phase-locked to stimulus onset) was estimated by performing time-frequency transformations on each trial and then averaging across trials. High-frequency power (30 to 110 Hz in 4 Hz steps) was estimated using multitapers (Mitra & Pesaran, 1999), with a fixed time window of 250 ms and 5 orthogonal Slepian tapers, yielding a frequency smoothing of approximately 12 Hz. Activity was normalized by dividing by the mean of a baseline window from 400 to 100 ms before stimulus onset, yielding a measure of percentage change relative to baseline activity. Electrodes for the analysis of GBA were selected by averaging the data across all conditions and choosing the region of maximal gamma band activity in parietal and occipital areas in the uncorrected data.

Beamforming method. An adaptive linear spatial filtering method (beamforming) was used to identify the sources responsible for producing the gamma-band signal recorded from the scalp. Beamforming allows the estimation of activity at any given location in the brain. We created a grid of source locations at 10 mm intervals, yielding 1960 possible dipoles within the brain. For each of these locations, the beamformer creates three orthogonal filters representing the three spatial dimensions. The first step is to create a leadfield. The leadfield is a matrix containing the solution to the forward problem for each dipole location and orientation, describing the projection of a unit amplitude signal from the dipole to each sensor on the scalp. The filters for each location are then formed from a combination of the forward model and an estimate of the co-variance between sensors. In the absence of individual structural magnetic resonance imaging scans, we created a standard leadfield using a standard boundary element model (Oostenveld, Stegeman, Praamstra, & van Oosterom, 2003) derived from a standard structural scan ("colin27"); both provided in the Fieldtrip toolbox, Oostenveld et al., 2011). We also used standard electrode positions corresponding to 10-20 space. The source locations and standard electrode positions were mapped to Montreal Neurological Institute (MNI) space. Since we were interested in localizing a signal with a maximum in a known frequency range and time window, we performed beamforming using Dynamic Imaging of Coherent Sources (DICS; Gross et al., 2001), which localizes sources in the frequency domain. With this method, the cross-spectral density matrix is used to assess co-variance between sensors.

Saccade-locked activity. For an analysis of saccade-locked activity, the data were epoched to the onset of saccades as detected by the algorithm above. We calculated the cross-spectral density matrix for the frequency range $64 \text{ Hz} \pm 12 \text{ Hz}$ using a multitaper FFT (5 tapers) across a time window from 150 ms before to 150 ms after saccade-onset. Note that an initial saccade-locked time-frequency analysis showed that the high frequency component of the saccade occupied this time window using these parameters. To counteract rank-deficiency introduced by average referencing, we used a regularization parameter (lambda) of 10% of the mean of the diagonal of the cross-spectral matrix. The beamformer created a single common set of spatial filters based on the uncorrected data, which were subsequently used to estimate activity in both the uncorrected and corrected data. Thus, any changes observed cannot be due to changes in the spatial filter introduced by the correction.

Stimulus-locked activity. For the analysis of stimulus-locked (i.e. object-related) activity, we calculated the cross-spectral density matrix for the frequency range 64 Hz \pm 12 Hz using a multitaper FFT and averaged across the time window from 200-400 ms after stimulus onset (i.e. the peak time range of the microsaccadic spike artefact). We combined the data from all conditions prior to ICA-based correction for each participant. Again, the beamformer created a single common set of spatial filters which were subsequently applied to both uncorrected and corrected data across all conditions. To counteract rank-deficiency introduced by average referencing, we used a regularization parameter (lambda) of 10% of the mean of the diagonal of the cross-spectral matrix. We applied the common

spatial filters to data from the Object and Non-object conditions in order to estimate source-localized activity in the specified time window and frequency range. In addition, we obtained time-resolved estimates of sources at specific locations identified during the course of the analysis by passing each individual trial through the common spatial filters derived in the first step of the beamforming analysis.

Beamforming statistical analysis. We contrasted the DICS estimates of source space activity at each location across different conditions over the whole brain. To control for multiple comparisons, we computed the maximum statistic under the null hypothesis using 5000 permutations. In brief, this generates a reference null distribution by randomly shuffling each individual datapoint across conditions and spatial locations and computing the t-statistic at each grid location. For each of 5000 iterations, the largest negative and largest positive t-values are stored, yielding a bimodal distribution of the expected negative and positive t-values under the null hypothesis of no difference between conditions. The 2.5th and 97.5th percentile of this reference distribution are then used as thresholds for significance at p = .05. We compared saccade-locked activity before and after correction directly, and also combined the Object and Non-object conditions and compared the sources of gamma band activity before and after correction in the critical 200-400 ms window. Finally, we also conducted separate comparisons of the Object and Non-object conditions on the data before and after correction using ICA. For visualization purposes, we overlaid beamforming results on the standard structural MRI. Anatomical locations were determined in MNI space using the Automated Anatomical Labeling atlas (AAL; Tzourio-Mazoyer et al., 2002). Note that some sources fall in locations which are not labelled using this atlas; these are indicated with the term "outside parcellation".

Results

Detection and Correction of Miniature Saccades from EEG data

We first examined the rate of miniature saccades as detected from the EEG. Figure 3A shows the detection rate for objects and non-objects before correction. Note the clear peak between 200-400 ms for objects, which is preceded by a reduction in saccade rate immediately after stimulus onset. This

pattern is consistent with that observed in previous studies during the typical paradigms used in gamma band studies of object recognition (Hassler et al., 2011; Keren et al., 2010; Yuval-Greenberg et al., 2008). After correction, we redetected miniature saccades from the corrected dataset using the threshold from the uncorrected dataset. Figure 3B shows a clear reduction in the rate of miniature saccades for both objects and non-objects.

We tested the effects of the removal procedure on the rate of miniature saccades in the critical 200-400 ms time window using repeated measures ANOVA with the factors Object (Object/Non-object) and Correction (before/after). Where necessary, post-hoc t-tests were conducted with Bonferroni-Holm correction for multiple comparisons. We report here only results involving the Correction factor. There was a significant reduction in the number of miniature saccades detected after correction [F(1,14) =18.93, p < .001, η_{E}^{2} = .32]. There was also a significant Object × Correction interaction [F(1,14) = 22.93, p < .001, η_{E}^{2} = .18] (see Figure 4). This interaction was driven by the much larger decrease in detected saccades for objects. After correction, the rate of saccades for objects was not significantly different from the saccade rate for non-objects before correction (p = .4). All other comparisons were significant (ps < .001). Note that the post-correction rate is very low, and the remaining significant difference between objects and non-objects is likely due to zero-bounding.

The Effect of Correction on Total Gamma Band Activity in Sensor Space

Gamma-band activity locked around the miniature saccades detected by the algorithm showed the expected pattern: a large centro-parietal peak, with additional areas of high activity around frontal and eye electrodes (see Figure 5). After correction, this peak was largely removed.

Figure 6 shows gamma band activity in response to objects before correction averaged across a frequency range of 40-90 Hz and a time window of 200-400 ms after stimulus onset as a topography, a ³/₄ frontal view, and as a time-frequency representation. Before correction, the expected patterns can clearly be seen: activity spans many parietal and occipital electrodes, and a clear peak at frontal eye electrodes is visible on the ³/₄ view. The time-course of this activity shows the expected broadband peak in amplitude from 200-400 ms on object trials, which is absent on non-object trials.

After correction, clear reductions in activity at frontal areas and over the parieto-occipital area are visible. The time-course after correction in Figure 6 shows that the clear peak in tGBA from 200-400 ms in response to objects is considerably reduced, while activity on non-object trials is largely unchanged. This suggests that artefact was successfully attenuated. Note that the post-correction topography indicates that the remaining tGBA forms bilateral clusters at parieto-occipital sites, which remain partly within the original cluster selected for analysis. Thus, the residual tGBA apparent in the figure may mostly reflect activity at these two clusters.

We statistically compared total gamma band activity before and after correction using repeated measures ANOVA with the factors Object (object or non-object) and Correction (before or after). We averaged across the frequency range 40-90 Hz, the electrode cluster indicated in Figure 6, and the time window 200-400 ms. Greenhouse-Geisser corrections were performed and post-hoc ttests with Bonferroni-Holm correction where necessary. Surprisingly, the main effect of Correction was not significant [F(1,14) = 2.16, p = .2, $\eta_g^2 = .02$]. However, there was a significant Object × Correction interaction [F(1,14) = 22.38, p < .001, $\eta_g^2 = .06$], which indicated that there was a significant decrease in tGBA after correction for objects (p < .001), with, if anything, a marginal increase for non-objects (p = .04).

Source-space Effects of ICA Correction

A direct comparison of the uncorrected and corrected saccade-locked data found that ICA removed signal from frontal areas, directly behind and above the eyes, but also significantly increased signal in multiple areas in the brain (see Fig. 8A). In the critical 200-400 ms time window, when the post-stimulus peak in miniature saccades occurs, there is a significant difference in a fronto-central source with a centre of gravity behind the eyes (Fig. 8B). Thus, the predominant effect of correction in this window is to reduce the artefact around frontal sources, with little smaller, non-significant effects on other areas.

Source-space Analyses of Uncorrected and Corrected Object-related Activity

Before correction for the miniature eye movement artefact, a clear difference in relative power between Objects and Non-Objects is observable (see Figure 9A). A substantial frontally generated gamma band signal, which spans a wide frontal brain area and has a centre of gravity behind the eyes, clearly indicates the presence of a miniature eye movement related artefact, consistent with the higher rate of such eye movements in the Object condition. Regions in which this signal is significant include the right frontal inferior orbital cortex and the right temporal pole (Fig 9B). The local maximum observed at MNI co-ordinates [4 1 -32] fell outside the areas covered by the AAL atlas, but is directly behind the eyes. Since the miniature saccade artefact is driven by the eye muscles, this localization is consistent with the expected location of the artefact source. Notably, however, the maximum tstatistic is located in right occipital middle and temporal middle cortices (Fig 9C, D), suggesting that non-artefactual sources may be recoverable through beamforming before ICA-based correction.

After correction for the miniature eye movement artefact, the frontal difference between Objects and Non-objects is no longer clear, suggesting that any remaining differences are well matched across conditions. Notably, the remaining maximum in relative power is in right occipital middle and occipital inferior cortices (Fig 10A), overlapping with an area in which a significant difference between conditions was observed before correction (Fig 9). Activity in this area remains significant after correction for multiple comparisons (Fig 10B). However, the maximum t-statistic is located in the left and right precuneus and cingulum, see Figs 10C and 10D.

We examined the time-courses of high-frequency activity in these regions before and after correction by applying the beamformer's spatial filters to the single trial time-domain data at the local maximum behind the eyes before and after ICA correction (MNI: -4 1 -32; Fig. 11). Before ICA based correction, a clear broadband spike in gamma band activity is visible from 200-400 ms on object conditions only (Fig. 11A). After correction, this broadband spike is no longer apparent, clearly demonstrating both that ICA removes activity from this source, and that the beamformer accurately localizes it (Fig. 11B).

We also looked at activity at the local maximum in the precuneus [MNI: -4 -49 38; see Fig. 12]. Activity before correction shows a notable peak in the approximate time window of the microsaccade artefact. In comparison to the more typical artefact-like signal seen in Fig. 11A, this

peak appears to have maxima at the upper and lower ends of its frequency range (Fig. 12A). After correction, this peak is largely removed, suggesting that if it is not the miniature saccade artefact per se, it is a similar artefact removed during ICA-based cleaning (Fig. 12B).

At the local maximum in right middle occipital cortex [MNI: 46 -93 0; Fig. 13] before ICAbased correction, there is little indication of a broadband, temporally confined spike in gamma band activity, although there is noticeably higher activity for Objects relative to Non-objects across all conditions (Fig 13A). After correction, the gamma band signal increases in relative magnitude, though appears to occupy largely the same frequency and temporal range (Fig 13B).

Discussion

We examined two methods of accounting for the contribution of microsaccadic artefacts to the scalprecorded EEG signal: Independent Component Analysis and beamforming. Both methods involve spatial filtering of the EEG data, but are employed to two different ends. ICA is used to remove microsaccade artefacts and allow subsequent analysis of gamma-band activity in sensor space, while beamforming moves the data into source space, and should minimize the contribution of the artefact to activity in areas distant from the eyes. We showed, first, how ICA successfully removes signals which are related to microsaccades. Before correction with ICA, our data showed the typical artefactual microsaccade-generated gamma-band signal, and the detection algorithm also found the characteristic pattern of microsaccades in the EEG data. After correction with ICA, object-related gamma band activity is still visible at the scalp level, leaving a bilateral gamma band signal over parieto-occipital regions. The overlap of the gamma band topography after correction with the topography before correction leaves open the possibility that some of the remaining gamma band activity is residual activity from the microsaccades. However, our beamforming analyses strongly suggest that the remaining activity results from genuine cortical rather than artefactual sources.

First, we found that a large source of gamma band activity before correction is located behind the eyes, consistent with generation by microsaccades. A time-frequency analysis of activity at this deep frontal source confirmed that it showed the expected pattern of activity generated by a miniature saccade artefact. Specifically, a clear broadband burst between 40-80 Hz was visible on Object trials from 200-400 ms, but was not present on Non-object trials. A direct comparison of the uncorrected and corrected saccade-locked data in source space showed that ICA removes a frontal component of the data and increases the power estimated by the beamformer at a variety of sites throughout the brain. Comparison of the uncorrected and corrected sources of GBA in the 200-400 ms range clearly demonstrated that the net result of ICA correction is the removal of the deep frontal source (i.e. the eye muscles) with limited overall impact on other sources. This strongly supports the use of ICA for the removal of miniature saccade artefacts, and clearly shows that beamforming attributes scalprecorded artefactual gamma-band to deep frontal areas, away from posterior cortical sources.

Second, an additional source in right middle occipital gyrus was also significantly active before correction. This occipital source survived correction using ICA. However, after correction, the maximally significant difference between Object and Non-object trials was located in the cingulate/precuneus, which was not clearly visible before correction. This strongly suggests that the artefact may have impaired the ability of beamforming to estimate activity from some areas of the brain. Specifically, the precuneus is located under the area of the scalp where the projection of the artefact is maximal in sensor space (see Fig. 5). Time-frequency analyses of activity at posterior sources showed that only the signals in the precuneus followed the typical artefactual pattern before correction. No broadband peak was visible in the critical 200-400 ms time window at the occipital source. Indeed, both sources showed overall higher activity after correction, despite the lack of a statistically significant effect of correction in these areas.

Activity in the precuneus was also observed in an MEG study of gamma band activity using a similar object recognition paradigm (Friese, Supp, Hipp, Engel, & Gruber, 2012). MEG is relatively resistant to microsaccade artefacts, and thus it seems plausible that this source represents a genuine cortical source of gamma-band activity. It is therefore encouraging that our beamforming analysis finds it active here. Comparisons between the post-correction gamma-band activity at the scalp level and activity at specific sources is difficult because of the spectral specificity of the DICS filters. Nevertheless, the activity at the scalp level (Fig.6) resembles the source-localized occipito-temporal activity in frequency-range and temporal extent (see Fig. 13). Thus, it seems highly likely that activity

visible at the scalp level after ICA correction also reflects a genuine cortical source of gamma-band activity.

Hipp and Siegel (2013) reported that ICA based correction had little effect on posterior gamma-band sources. One possible explanation for the discrepancy between their results and our results is that ICA is necessarily subjective. It is possible that our removal procedures and choices systematically differed. For example, either the present authors or Hipp and Siegel may have chosen components for removal that contained more or less genuine signal, or more or less artefactual signal. Notably, the changes which are observed appear to be largely changes of scale, with common features observable in the plots of activity both before and after correction. This strongly suggests that ICA correction brought about general improvements in signal-to-noise ratio in the gamma-band, allowing better recovery of source activities through the beamformer.

In conclusion, we confirmed that ICA and beamforming both successfully identify microsaccade artefacts in scalp-recorded EEG. Beamforming successfully localizes a major source of scalp-recorded gamma-band activity behind the eyes, without any requirement for explicit detection of miniature eye movements. It allows recovery of some genuine activity without the need for explicit correction for the artefact, and thus may be particularly suitable in cases where, for example, no simultaneous eye-tracking was performed, or the automatic, algorithmic approaches to detection and correction (Hassler et al., 2011; Keren et al., 2010) are unsuccessful. Furthermore, beamforming clearly shows that when ICA is applied, the majority of the signal removed most likely originates from the eye muscles behind the eyes, leaving genuine activity largely unaltered. If anything, ICA improved the results from the beamforming analysis, suggesting that a combination of both approaches may yield a clearer picture of underlying, genuine cortical activity than either approach alone. Finally, the activity visible after ICA correction at the scalp level most likely reflects genuine cortical activity, and is reflected in the beamforming results. Thus, ICA correction alone may be sufficient to examine gamma-band activity using the EEG.

Practical Guidelines

As noted above, our microDetect plug-in for EEGlab implements many of the steps required for the detection and correction of miniature saccade artefacts (<u>https://github.com/craddm/microDetect</u>).

- Use individual eye channels, not bipolarized channels. A typical four-electrode set-up as used here is advised. The topographical signatures of the miniature eye movement artefact may differ slightly according to the arrangement of the eye electrodes.
- 2) Ensure that good quality signal is recorded from the eye channels. Miniature saccades share characteristics with high-frequency noise, and thus its presence may increase false-positive detections. If one channel is nevertheless particularly noisy, it may be better to remove it and retain only the good quality eye channels.
- 3) Account for rank-deficiency before performing ICA. Referencing and other typical procedures such as interpolation may reduce the rank of the data. Remove rather than interpolate bad channels, and if necessary interpolate them after ICA has been performed. Perform PCA before running ICA to reduce number of dimensions in the data to the rank. Alternatively, remove
- 4) To identify appropriate miniature saccade related components, examine the topography, timecourse and time-frequency representation of the components. Assess the correlation of the components with the rEOG or other eye channels, as is possible with the microDetect plug-in.
- 5) Even if planning to use beamforming, ICA correction may yield significant improvements in the estimates of source activity. In addition, the algorithmic detection of miniature saccades provides a strong indication of whether the patterns of iGBA in the data may be artefactual in origin.

References

- Barlow, J. S. (1984). EMG artifact minimization during clinical EEG recordings by special analog filtering. Electroencephalography and Clinical Neurophysiology, 58(2), 161–174. http://doi.org/10.1016/0013-4694(84)90030-0
- Bosman, C. A., Womelsdorf, T., Desimone, R., & Fries, P. (2009). A Microsaccadic Rhythm Modulates Gamma-Band Synchronization and Behavior. The Journal of Neuroscience, 29(30), 9471–9480. http://doi.org/10.1523/JNEUROSCI.1193-09.2009
- Busch, N. A., Herrmann, C. S., Müller, M. M., Lenz, D., & Gruber, T. (2006). A cross-laboratory study of event-related gamma activity in a standard object recognition paradigm. NeuroImage, 33(4), 1169–1177. http://doi.org/10.1016/j.neuroimage.2006.07.034
- Coburn, K. L., & Moreno, M. A. (1988). Facts and artifacts in brain electrical activity mapping. Brain Topography, 1(1), 37–45. http://doi.org/10.1007/BF01129338
- Craddock, M., Martinovic, J., & Müller, M. M. (2015). Early and late effects of objecthood and spatial frequency on event-related potentials and gamma band activity. BMC Neuroscience, 16(1), 6. http://doi.org/10.1186/s12868-015-0144-8
- Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. Journal of Neuroscience Methods, 134(1), 9–21. http://doi.org/10.1016/j.jneumeth.2003.10.009
- Dimigen, O., Valsecchi, M., Sommer, W., & Kliegl, R. (2009). Human Microsaccade-Related Visual Brain Responses. The Journal of Neuroscience, 29(39), 12321–12331. http://doi.org/10.1523/JNEUROSCI.0911-09.2009
- Engbert, R., & Kliegl, R. (2003). Microsaccades uncover the orientation of covert attention. Vision Research, 43(9), 1035–1045. http://doi.org/10.1016/S0042-6989(03)00084-1
- Friese, U., Supp, G. G., Hipp, J. F., Engel, A. K., & Gruber, T. (2012). Oscillatory MEG gamma band activity dissociates perceptual and conceptual aspects of visual object processing: A combined repetition/conceptual priming study. NeuroImage, 59(1), 861–871. http://doi.org/10.1016/j.neuroimage.2011.07.073

- Goncharova, I. I., McFarland, D. J., Vaughan, T. M., & Wolpaw, J. R. (2003). EMG contamination of EEG: spectral and topographical characteristics. Clinical Neurophysiology, 114(9), 1580– 1593. http://doi.org/10.1016/S1388-2457(03)00093-2
- Gross, J., Kujala, J., Hämäläinen, M., Timmermann, L., Schnitzler, A., & Salmelin, R. (2001).
 Dynamic imaging of coherent sources: Studying neural interactions in the human brain.
 Proceedings of the National Academy of Sciences, 98(2), 694–699.
 http://doi.org/10.1073/pnas.98.2.694
- Hassler, U., Barreto, N. T., & Gruber, T. (2011). Induced gamma band responses in human EEG after the control of miniature saccadic artifacts. NeuroImage, 57(54), 1411–1421. http://doi.org/10.1016/j.neuroimage.2011.05.062
- Herrmann, C. S., Munk, M. H. J., & Engel, A. K. (2004). Cognitive functions of gamma-band activity: memory match and utilization. Trends in Cognitive Sciences, 8(8), 347–355. http://doi.org/10.1016/j.tics.2004.06.006
- Hipp, J. F., & Siegel, M. (2013). Dissociating neuronal gamma-band activity from cranial and ocular muscle activity in EEG. Frontiers in Human Neuroscience, 7, 338. http://doi.org/10.3389/fnhum.2013.00338
- Jasper, H. (1958). The ten twenty electrode system of the international federation. Electroencephalography and Clinical Neurophysiology, 10, 371–375.
- Jung, T.-P., Makeig, S., Humphries, C., Lee, T.-W., Mckeown, M. J., Iragui, V., & Sejnowski, T. J. (2000). Removing electroencephalographic artifacts by blind source separation. Psychophysiology, 37(2), 163–178. http://doi.org/10.1111/1469-8986.3720163
- Keren, A. S., Yuval-Greenberg, S., & Deouell, L. Y. (2010). Saccadic spike potentials in gamma-band EEG: Characterization, detection and suppression. NeuroImage, 49(3), 2248–2263. http://doi.org/10.1016/j.neuroimage.2009.10.057
- Martinez-Conde, S., Macknik, S. L., Troncoso, X. G., & Hubel, D. H. (2009). Microsaccades: a neurophysiological analysis. Trends in Neurosciences, 32(9), 463–475. http://doi.org/16/j.tins.2009.05.006

- Martinovic, J., & Busch, N. A. (2011). High frequency oscillations as a correlate of visual perception. International Journal of Psychophysiology, 79(1), 32–38. http://doi.org/10.1016/j.ijpsycho.2010.07.004
- Martinovic, J., Gruber, T., & Müller, M. M. (2007). Induced Gamma Band Responses Predict Recognition Delays during Object Identification. Journal of Cognitive Neuroscience, 19(6), 921–934. http://doi.org/10.1162/jocn.2007.19.6.921
- Melloni, L., Schwiedrzik, C. M., Rodriguez, E., & Singer, W. (2009). (Micro)Saccades, corollary activity and cortical oscillations. Trends in Cognitive Sciences, 13(6), 239–245. http://doi.org/16/j.tics.2009.03.007
- Metting van Rijn, A., Peper, A., & Grimbergen, C. (1990). High-quality recording of bioelectric events. Medical and Biological Engineering and Computing, 28(5), 389–397. http://doi.org/10.1007/BF02441961
- Metting van Rijn, A., Peper, A., & Grimbergen, C. (1991). High-quality recording of bioelectric events. Medical and Biological Engineering and Computing, 29(4), 433–440. http://doi.org/10.1007/BF02441666
- Mitra, P. P., & Pesaran, B. (1999). Analysis of Dynamic Brain Imaging Data. Biophysical Journal, 76(2), 691–708. http://doi.org/10.1016/S0006-3495(99)77236-X
- Müller, M. M., Gruber, T., & Keil, A. (2000). Modulation of induced gamma band activity in the human EEG by attention and visual information processing. International Journal of Psychophysiology, 38(3), 283–299. http://doi.org/16/S0167-8760(00)00171-9
- Nolan, H., Whelan, R., & Reilly, R. B. (2010). FASTER: Fully Automated Statistical Thresholding for EEG artifact Rejection. Journal of Neuroscience Methods, 192(1), 152–162. http://doi.org/10.1016/j.jneumeth.2010.07.015
- Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Intell. Neuroscience, 2011, 1:1–1:9. http://doi.org/10.1155/2011/156869

- Oostenveld, R., Stegeman, D. F., Praamstra, P., & van Oosterom, A. (2003). Brain symmetry and topographic analysis of lateralized event-related potentials. Clinical Neurophysiology, 114(7), 1194–1202. http://doi.org/10.1016/S1388-2457(03)00059-2
- Rolfs, M. (2009). Microsaccades: Small steps on a long way. Vision Research, 49(20), 2415–2441. http://doi.org/16/j.visres.2009.08.010
- Tallon-Baudry, C., & Bertrand, O. (1999). Oscillatory gamma activity in humans and its role in object representation. Trends in Cognitive Sciences, 3(4), 151–162. http://doi.org/10.1016/S1364-6613(99)01299-1
- Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., ... Joliot, M. (2002). Automated Anatomical Labeling of Activations in SPM Using a Macroscopic
 Anatomical Parcellation of the MNI MRI Single-Subject Brain. NeuroImage, 15(1), 273–289. http://doi.org/10.1006/nimg.2001.0978
- Van Veen, B. D., van Drongelen, W., Yuchtman, M., & Suzuki, A. (1997). Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. IEEE Transactions on Biomedical Engineering, 44(9), 867–880. http://doi.org/10.1109/10.623056
- Yuval-Greenberg, S., Tomer, O., Keren, A. S., Nelken, I., & Deouell, L. Y. (2008). Transient Induced Gamma-Band Response in EEG as a Manifestation of Miniature Saccades. Neuron, 58(3), 429–441. http://doi.org/10.1016/j.neuron.2008.03.027

Author Notes

The authors would like to thank Renate Zahn and Karolin Meiß for their assistance conducting the recordings. This work was funded by the Deutsche Forschungsgemeinschaft (German Research Foundation; DFG), grant number MU 972/16-1.

Fig. 1 Example stimuli. Upper row shows objects, lower row shows non-objects.

Fig. 2 Time course and topography of a miniature saccade IC component. (A) the event related potential (ERP) of the IC, (B) Topographical distribution of the IC, (C) ERP image showing the single-trial activity of the IC component.

Fig. 3 Mean saccade rate (averaged across subjects) as detected from the EEG eye channels (bin width = 20 ms). Upper panels (A) show saccade rate before correction; lower panels (B) depicts saccade rate after correction. Left column shows saccades on Object trials; right column shows saccades on Non-object trials.

Fig. 4 Mean miniature saccade rate as detected from the EOG channels in the 200-400 ms time window before and after correction with ICA. Left panel depicts mean saccade rate before correction for Objects (red) and Non-objects (blue). Right panel shows the same after correction. Error bars are bootstrapped 95% confidence intervals

Fig. 5 Topography of saccade locked gamma band activity averaged from 40-90 Hz and from -100 ms before to 150 ms after saccade onset. Left before correction; right after correction. Colours show percent change from baseline.

Fig. 6 Total gamma band activity before and after correction. Upper row shows a topography and 3⁄4 frontal view of activity on Object trials averaged across all three spatial frequency conditions, 40-90 Hz and over the 200-400 ms time window after stimulus onset before (left) and after correction (right). The black oval shows the cluster of electrodes selected for further analysis. The lower row shows time-frequency plots of total gamma band activity from the selected electrode cluster on object and non-object trials before and after correction

Fig. 7 tGBA before and after correction at the parietal/parieto-occipital cluster indicated on

Figure 6. Left panel shows activity before correction for objects (red) and non-objects (blue), while the right panel shows activity after correction. Error bars show bootstrapped 95% confidence intervals

Fig. 8 Source space differences between artefact-contaminated and artefact-corrected activity.

Group t-statistics are overlaid on axial slices of the standard brain. Panel A) saccade-locked activity. Panel B) activity at 200-400 ms after stimulus-onset. Colours represent the t-statistic; red indicates higher activity before correction, blue indicates higher activity after correction. Saturated colours are regions significant at p = .05 corrected for multiple comparisons.

Fig. 9 Source space differences in gamma band activity between Object and Non-object trials before correction for miniature eye movement artefacts. Group t-statistics are overlaid on the standard brain. Upper panels show orthogonal slices centred on the maximum relative power difference between conditions (MNI co-ordinates [4 1 -32]; Outside parcellation). Panel A) Relative differences in power between Objects and Non-objects. Panel B) t-statistic thresholded at p = .05corrected for multiple comparisons. Lower panels show orthogonal slices centred on the maximum tstatistic (MNI co-ordinates [46 -69 8; Right middle occipital gyrus/middle temporal gyrus]. Panel C) unthresholded t-statistic and D) thresholded t-statistic at p = .05 corrected for multiple comparisons

Fig. 10 Source space differences in gamma band activity between Object and Non-object trials after correction for miniature eye movement artefacts. Upper panels, show orthogonal slices centred on the maximum relative power difference between conditions (MNI co-ordinates [46 -93 0]; Right middle occipital gyrus/inferior occipital gyrus). Panel A) Relative differences in power between Object Present and Object Absent conditions. Panel B) t-statistic thresholded at p = .05 corrected for multiple comparisons. Lower panels show orthogonal slices centred on the maximum t-statistic (MNI co-ordinates [-4 -49 38]; Left posterior cingulate/precuneus). Panel C) unthresholded t-statistic and Panel D) thresholded t-statistic at p = .05 corrected for multiple comparisons.

Fig. 11 Grand average time-frequency plots of the peri-ocular source [MNI -4 1 -32]. Panel A

shows activity before correction. Panel B shows activity after correction. In each panel, the left column shows the activity on Object trials, while the right column shows activity on non-object trials. Scale is percent change in signal. Time zero indicates stimulus onset.

Fig. 12 Grand average time-frequency plots of activity at a source in the precuneus/cingulate

[**MNI -4 -49 38**]. Panel A shows activity before correction; Panel B shows activity after correction. Columns show Object and Non-object trials. Scale is percent change in signal. Time zero indicates stimulus onset.

Fig. 13 Grand average time-frequency plots of activity at a source in right middle

occipitotemporal cortex [MNI 46 -93 0]. Panel A shows activity before correction; Panel B shows activity after correction. Columns show Object and Non-object trials. Scale is percent change in signal.