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Corresponding Author: Dr Peyman Adjamian, PhD

Corresponding Author's Institution: MRC

First Author: Peyman Adjamian

Order of Authors: Peyman Adjamian; Sian F Worthen; Arjan Hillebrand; Boris A Chizh; Anthony Hobson; Qasim Aziz; Paul L Furlong; Gareth R Barnes

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used in a MEG environment. We also show that in a reduced shielding environment the use of higher order synthetic gradiometry is sufficient to obtain signal-to-noise ratios that allow for accurate localisation of cortical sensory function.

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Effective electromagnetic noise cancellation with beamformers and synthetic gradiometry in shielded and partly-shielded environments

P. Adjamian[1], S. F. Worthen[2,3], A. Hillebrand[4], P.L.Furlong[2], B. A. Chizh[5], A. Hobson[6], Q. Aziz[3], G.R.Barnes[2]

[1] *MRC Institute of Hearing Research, University Park, Nottingham, UK.*

[2] *Wellcome Trust Laboratory for MEG Studies, Neurosciences, Aston University, Birmingham, UK.*

[3] *Barts and The London, Queen Mary College, University of London, UK.*

[4] *Department of Clinical Neurophysiology, Institute for Clinical and Experimental Neurosciences, VU University Medical Center,*

Amsterdam, The Netherlands.

[5] *Addenbrooke's Centre for Clinical Investigation, GlaxoSmithKline, Addenbrooke's Hospital, Cambridge, UK.*

[6] *Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, UK.*

Corresponding Author:

Dr Peyman Adjamian

MRC Institute of Hearing Research,

University Park,

Nottingham, UK

NG7 2RD

Tel: (0115) 922 3431 (ext. 262)

Fax: (0115) 951 8503

Email: peyman@ihr.mrc.ac.uk

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Abstract

The major challenge of MEG, the inverse problem, is to estimate the very weak primary neuronal currents from the measurements of extracranial magnetic fields. The non-uniqueness of this inverse solution is compounded by the fact that MEG signals contain large environmental and physiological noise that further complicates the problem. In this paper, we evaluate the effectiveness of magnetic noise cancellation by synthetic gradiometers and the beamformer analysis method of Synthetic Aperture Magnetometry (SAM) for source localisation in the presence of large stimulus-generated noise. We demonstrate that activation of primary somatosensory cortex can be accurately identified using SAM despite the presence of significant stimulus-related magnetic interference. This interference was generated by a contact heat evoked potential stimulator (CHEPS), recently developed for thermal pain research, but which to date has not been used in a MEG environment. We also show that in a reduced shielding environment the use of higher order synthetic gradiometry is sufficient to obtain signal-to-noise ratios that allow for accurate localisation of cortical sensory function.

Keyword: MEG, magnetic shielding, synthetic gradiometers, regularised and unregularised beamformers, SAM, ECD, CHEPS.

Introduction

Magnetoencephalography (MEG) is the non-invasive measurement of magnetic fields outside the head generated by the electrical activity of neurons within the brain. The aim of MEG is to increase the signal-to-noise ratio (SNR) of the recorded brain signals, and localise sources of these signals in the brain. This inverse problem is non-unique and ill-defined because there are many current configurations that could have produced the same magnetic fields. The cortical magnetic fields of interest are typically in the femtoTesla range which is approximately 100 million times smaller than the earth's magnetic field and about 1 million times smaller than typical urban environmental noise (Hamalainen et al., 1993; Vrba, 2002). In addition to environmental noise, the measured fields contain unwanted contributions from organs such as the heart, lungs and eyes, from muscle contractions, as well as from background brain activity arising from regions not being investigated. Therefore inverse methods must ideally provide noise cancellation as well as source localisation.

As the ultimate aim of MEG is to determine the spatial and temporal patterns of the current sources from regions of interest within the brain, sensors must be protected from various artefacts that contaminate these signals from nearby and distant sources. Typically, the first defence against environmental noise is passive magnetic shielding (Hamalainen et al., 1993). At the hardware level further noise reduction is achieved by using gradiometers to differentially sample the magnetic field around the head. This removes noise from distant sources where the magnetic interference is more or less uniform in space (Carelli and Leoni, 1986; Vrba 2002). First-order gradiometers for example consist of two coils wound in opposite directions; but the effectiveness of gradiometer coils is a function of the balance, or similarity between coils. Typically the effective balance of a coil can be synthetically

improved through the use of an array of reference channels. The reference channels are positioned far from the head such that they detect mainly environmental noise. Subsequently, a different linear combination of signals from the (noisy) reference channels is subtracted from each of the sensor outputs. The weights of the coefficients can either be fixed (after calibration) or determined adaptively to create a high-order synthetic gradiometer (Vrba and Robinson, 2001). In comparison to hardware gradiometry, synthetic gradiometers are a cost efficient option since one array of reference channels can be used for any number of sensor channels.

On the other hand, there are linear and non-linear mathematical techniques that provide not only noise cancellation, but also source localisation. These methods include signal space separation (Taulu et al., 2004), beamformers such as Synthetic Aperture Magnetometry (SAM) (Robinson and Vrba, 1999), Dynamic Imaging of Coherent Sources (Gross et al., 2001), Linearly Constrained Minimum Variance (Van Veen et al., 1997) or eigenspace beamformers (Sekihara et al., 1999), and multiple signal classification (MUSIC) (Mosher et al., 1992). A discussion of each of these methods is beyond the scope of this paper. Barnes and Hillebrand (2003) and Hillebrand et al. (2005) provide a detailed description of the SAM beamformer method and its applications. Put simply, the beamformer is a spatial filter that estimates source strength on a voxel by voxel basis and is used to build up an image of source activity throughout the brain. Noise cancellation is attained through this spatial filter. Similar to traditional frequency filters that select signals within a specified temporal range, the spatial filter selects signals only from specified spatial locations. All other signals, such as those arising from environmental sources, heart beat (electrocardiogram, ECG) or adjacent brain areas are minimised by the action of the spatial filter. For each voxel, the output of the spatial filter is a weighted sum of all the MEG sensors, and is called a virtual electrode with the

same millisecond time resolution as the original MEG signals. The virtual electrode timeseries can then be divided into active and passive epochs and power in pre-selected frequency bands can be calculated. The power difference between the two states can be calculated using all the epochs and then normalised by MEG sensor noise to obtain a statistical *pseudo-t* value (Vrba and Robinson, 2001). A 3-dimensional image of source power is obtained when this process is applied sequentially to each voxel in the brain.

The two studies presented in this paper demonstrate the effectiveness of noise cancellation using a combination of methods outlined above. Firstly, we demonstrate the robustness of SAM in the localisation of thermal pain-evoked potentials despite the presence of considerable stimulus-induced magnetic interference. Furthermore, by manipulating the regularisation parameter, we show how the magnetic interference is treated by the action of the beamformer. The cost of hardware magnetic shielding is significant, and even in well shielded rooms environmental noise (such as tramlines) can still be a significant barrier to recording. In this paper we degrade the quality of our shielded environment by opening the door of the room to look at the efficacy of other noise cancellation methods. In a second experiment, we demonstrate the accuracy of source localisation with dipole analysis and SAM with and without high order synthetic gradiometers on data collected in an 'open-door' environment.

Experiment 1

Methods

The Contact Heat-Evoked Potential Stimulator (CHEPS) (Medoc Ltd, Ramat-Yoshai, Israel) is a computerised thermal stimulator specifically designed to facilitate research investigating human sensory and nociceptive pathways. The present study was conducted to assess the suitability of this equipment in the MEG environment, as to date no study has combined the two. The CHEPS provides a rapid heating rate of 70° C/sec such that painful stimuli can be delivered from a skin temperature baseline of 32°C up to a maximum of 55°C in approximately 329 milliseconds. This is achieved using a special 27mm diameter probe that comprises two layers of stimulators, one external layer consisting of a very thin fast heating foil and one lower layer consisting of a Peltier element with heating and cooling capabilities. Although the probe has been designed for use in MR environment, and is suitable for EEG studies, the mechanism within the probe generates a significant magnetic field that produces a large artefact in the recorded MEG. This results in a shift in the range of the recorded MEG; activity in the absence of the stimulus is 950fT (peak to peak), compared to 41pT during stimulus presentation.

An example of the magnetic interference introduced by the CHEPS (coloured noise) in a single trial is shown by the black trace in Figure 1. The rise in temperature of the CHEPS is represented by the blue trace, showing temporal correspondence with the artefact in the MEG trace. The interference has a frequency peak of 2Hz and lasts for approximately 2 seconds. A similar level of noise was visible in all MEG channels across all trials. The grey trace illustrates the effect of applying a synthetic 3rd order gradiometer in reducing the level of the interference. Due to strength of the current produced by the CHEPS probe and the proximity of this to the MEG sensor array, the noise is not completely removed. Clearly, averaging the trials, therefore, would not remove the artefact from the traces and for this reason it is not possible to use Equivalent Current Dipole (ECD) modelling for localisation of the masked

electrophysiological evoked response. On the other hand, the beamformer spatial filter should act to minimise all magnetic sources bar those that arise from voxels near the target source location.

INSERT FIGURE 1 ABOUT HERE

To assess whether accurate source localisation can be achieved with the SAM beamformer method despite the presence of this large magnetic artefact, MEG data was collected following 30 rapid painful heat stimulations to the dorsum of the non-dominant hand. Informed written consent was obtained and the local ethics committee approved the experimental protocol.

Data was recorded in shielded environment using a whole head CTF 275-channel scanner with 1st order hardware gradiometer coil configuration (VSM Medtech, Canada) at a sampling rate of 1200Hz. The procedure was performed on eight participants for whom pain tolerance threshold was obtained. This was at an average level of 48.1 ± 1.5^{o}C across participants, and perceived to be strong pain; equivalent to a visual analogue scale (VAS) of 7 (on a 0 to 10 scale from no sensation to unbearable pain). Each of the 30 trials lasted 7 seconds with pre-stimulus time of 2 seconds and randomised inter-stimulus intervals of between 8 to 12 seconds. In addition to this 'contact' condition, the procedure was repeated a second time for one of the participants to create a 'blocked' condition. In this, the thermal sensation was blocked by placing a heat-resistant cloth between the CHEPS probe and the participant's hand. As artefacts can influence source localisation, the implementation of this

condition provided comparative data to certify that stimulus-related source localisation was not simply due to the stimulus-generated artefacts. Co-registration of the MEG data with the participants' anatomical MRI was performed using a modification of the surface-matching approach described in Adjamian et al. (2004). Instead of using a bite-bar to stabilize the subject's head during head digitization with the Polhemus, a reference coil is attached to the subjects head when headshape points are collected. This reduces the influence of head movement during digitization.

Dual state SAM analysis comparing active and passive states was performed on both datasets to identify peak conditions in the primary (SI) and secondary (SII) cortices, regions known to be activated in pain studies (see Peyron et al., 2000; Derbyshire, 2003 for reviews). Pre- and post-stimulus time windows of half a second, one second, and two second length were compared for the frequency bands of 13-20 and 20-30Hz, corresponding to the beta rhythm exhibited by the sensorimotor cortices (Hari and Salmelin, 1997; Lounasmaa et al., 1996) and in which changes have been reported in response to pain (Chang et al., 2002; Huber et al, 2006; Lalo et al., 2007). For each of the eight participants, the SAM comparison showing activation within the primary somatosensory cortex was then converted into MNI space using a method of normalisation (Friston, et al, 1994) to enable comparisons across participants and with previously reported source reconstructions following stimulation of the hand. For the participant who was also involved in the 'blocked' condition, those voxels in the SAM images that showed activation of somatosensory cortex, time-frequency spectrograms were produced using Morlet wavelet analysis. A bootstrapping analysis was then applied to obtain statistically significant levels (Graimann et al., 2002).

To enable us to determine the accuracy of the thermal stimulus-related cortical activation, data were compared to that obtained for each participant following electrical stimulation of the dorsum of the same hand, a procedure for which reliable cortical locations are produced and which are in the absence of any artefact. MEG data was recorded following 100 painful electrical stimulations to the dorsum of the non-dominant hand at a sampling rate of 1200Hz. A constant-current high-voltage stimulator (DS7, Digitimer, Ltd., Welwyn Garden City, Herts, UK) was externally triggered (DSN, Digitimer Ltd.) to deliver 100 square-wave pulses of 200μS duration at a rate of 0.2Hz. The level of stimulation, determined by the participants' pain threshold, was an average of 27 ± 19.8mA across participants, and equivalent to a VAS score of 7 (on a 0 to 10 scale). Each trial was 3 seconds duration, which included 1 second pre-trigger data. SAM analysis was performed on the non-filtered data using a time window (passive vs active time windows) of 0.5 seconds before and after stimulus onset, in the same beta frequency bands as before (13-20Hz and 20-30Hz). Again, the SAM comparison showing activation within the primary somatosensory cortex was subsequently converted into MNI space (Friston et al, 1994)

Results

In the 'contact' condition, the peak activation was in the contralateral SI region in response to thermal somatic pain in all time-windows and for both frequency bands despite the presence of significant magnetic artefact in the raw data. These activations were spatially similar to those seen in response to the electrical stimulus. Table 1 shows the MNI coordinates of the normalised peak activation for each participant in the 20-30Hz frequency band and time window of two seconds pre- and post-stimulus onset for the thermal and five hundred milliseconds pre- and post-stimulus onset for the electrical stimulations. The similarity of the

locations is illustrated in Figure 2. For the 'blocked' thermal condition, no focal activation was found in either SI or SII (as illustrated in Figure 3).

INSERT TABLE 1 ABOUT HERE

INSERT FIGURE 2 ABOUT HERE

Time-frequency analysis of spectral power in right SI was performed on individual data for the 'contact' and 'blocked' thermal datasets to determine the temporal dynamics of cortical activation. The results of this analysis are shown in Figure 3. On the left, activation of right sensory cortex during the contact condition is shown. The bootstrap time-frequency analysis for the beamformer spatial filter output at that location (or virtual electrode) shows a significant increase in power ($p<0.05$) in the beta band between two and three seconds post-stimulus. The 'blocked' stimulus condition SAM image is shown on the top right, and the bootstrap time-frequency analysis for the voxel that was activated in the 'contact' condition highlights the absence of any stimulus-related cortical activity in that region.

INSERT FIGURE 3 HERE

In order to assess the noise reduction quality of the beamformer we reconstructed the spatial filter at the same location and frequency band (13-20Hz) using a heavily regularised version

of the algorithm. In the case of the CTF software, this corresponds to adding an identity matrix of sufficient magnitude to the data covariance matrix such that the information in the latter is washed out. We determined this value empirically (typically mu=5000) by observing at which point the correlation between the original beamformer weights and the regularized weights asymtoted. This effectively turns the beamformer weights into dipolar lead fields (i.e. equivalent to looking at the signal projected through a dipole at the same location (Hillebrand and Barnes 2003). Figure 4 shows un-regularised (top) and fully regularised (bottom) SAM images and VEs. For the regularised beamformer, the noise generated by the CHEPS is projected into the spatial filter, contaminating the signals of interest and is revealed by an un-physiologically large source amplitude. Across participants the beamformer gives a significant reduction in the environmental noise contamination at this location, with a ration of 7.5 : 1 for the regularised : unregularised beamformer output. Table 2 lists the rms values for these beamformer data for all 8 participants.

INSERT TABLE 2 ABOUT HERE

INSERT FIGURE 4 ABOUT HERE

Experiment 2

Methods

In this experiment we set out to demonstrate the accuracy of functional localisation with SAM and ECD in an 'open-door' environment, with and without 3rd order synthetic gradiometers. The MEG scanner at the Wellcome Trust Laboratory for MEG Studies at Aston University is equipped with 275 1st order hardware gradiometers, placed in a shielded room that comprises two layers of μ-metal and one layer of Al. To fulfil the aims of this study, MEG data was collected twice on the same single participant, once with the door of the shielded room closed and subsequently with the door of the shielded room open. Again informed written consent was obtained and the local ethics committee approved the experimental protocols.

MEG data was recorded following 100 painful electrical stimulations to the dorsum of the non-dominant hand at a sampling rate of 1200Hz. A constant-current high-voltage stimulator (DS7, Digitimer, Ltd., Welwyn Garden City, Herts, UK) was externally triggered (DSN, Digitimer Ltd.) to deliver 100 square-wave pulses of 200μS duration at a rate of 0.2Hz. The level of stimulation, determined by the participant's pain threshold, was 23mA and equivalent to a VAS score of 7 (on a 0 to 10 scale). Each trial was 3 seconds duration, which included 1 second pre-trigger data.

The collected data was analysed with either 3rd or no synthetic gradiometer settings, resulting in four datasets: "open-door No Synthetic Gradiometer"; "open-door 3rd Order Synthetic Gradiometer"; "closed-door 3rd Order Synthetic Gradiometer" and "closed-door No Synthetic Gradiometer". The datasets were filtered between 0.5 and 40Hz and the 100 trials averaged. ECD analysis was performed using a time window of 25 to 125ms, corresponding to the temporal dynamics of the primary component of the somatosensory evoked response (see figure 5) followed by Monte Carlo analysis to obtain 95% confidence intervals (Singh et al.,

1997). SAM analysis was performed on the non-filtered data using time windows (passive vs active time windows) of the first 1 and 0.5 seconds before and after stimulus onset, respectively, in the frequency bands 13-20Hz and 20-30Hz. ECD and SAM data were transformed to MNI space (Friston et al., 1994). To obtain a measure of the effect of total shielding on noise attenuation at the sensor level, we recorded two sets of noise data with no subject in the scanner, first with the MSR door open and then repeated with the MSR door closed. 150 trials of 2 second duration were collected using a sampling rate of 1200Hz, 3rd order synthetic gradiometers and comb filter at 50 Hz for the removal of the powerline.

Results

Figure 5 shows the averaged sensory evoked fields (SEF) with the maximum peak occurring at 70ms post-stimulus onset for all four datasets. The dipolar magnetic field pattern for this peak of the evoked response can be clearly seen in the topographic maps.

The early averaged SEF appears unaffected by the MSR door, as illustrated by the 1st and 3rd traces and by their respective topographic maps where the dipolar pattern is indicative of right somatosensory cortex activation. For the open-door recording, the only visible difference was the increased noise present with the synthetic gradiometer removed (2nd trace). This increase in environmental noise is also demonstrated by the less focal dipolar magnetic field pattern in the corresponding topographic map for the peak of the evoked response at 70ms. However, it is clear from the overlay of the channels in figure 5 that the later sensory component (P300) is less ambiguous when complete magnetic shielding is applied.

INSERT FIGURE 5 ABOUT HERE

Source localisation with Monte Carlo analysis showed that the open-door environment affected the dipole localisation with and without 3rd order synthetic gradiometry. The results are shown in Figure 6 (top row), where the dipoles for each dataset are marked by the green circles. SAM analysis in the 20-30Hz beta band is also shown (bottom row). In each case the SEF is localised to the right somatosensory cortex, although there is a small change in location when compared with that from the door closed condition, 3rd order dataset (Table 3).

The Monte Carlo volumes in each condition were equally in the order of 0.01cm^3 indicating that, surprisingly, the extra noise did not influence confidence interval. Localisation coordinates in MNI (Montreal Neurological Institute) space are given in Table 3 for the dipole and the SAM results, as well as the Monte-Carlo (MC) error volumes for the dipole model solutions. For the closed-door data, when 3rd order synthetic gradiometers are applied there is a small shift in localisation with SAM. This shift in localisation is greater for the ECD analysis, showing the influence of gradiometer settings on the accuracy of localisation with the dipole model. Interestingly, 3rd order gradiometer localisation with both techniques is affected by the opening of the door, but again larger differences were observed with ECD compared to SAM.

INSERT FIGURE 6 ABOUT HERE

INSERT TABLE 3 ABOUT HERE

Discussion

We have shown that the beamformer analysis technique of SAM can accomplish two main objectives of neuroimaging with MEG despite the presence of significant magnetic interference; firstly increasing the SNR of measured brain activity through noise reduction of the spatial filter, and secondly reliably localising activity within the brain. We demonstrated that MEG data treatment and functional localisation is possible with SAM even in the presence of large electromagnetic artefacts. It was shown that this localisation with SAM is robust in situations where traditional dipole approaches cannot succeed as they require careful noise elimination. We also demonstrated that it is possible to collect MEG data and localise function in an environment that is not completely shielded by having the door of the MSR open. While the magnetic artefact introduced by the CHEPS can partly be removed by applying a high-pass filter, much of it still remains in the filtered data. Due to the temporal dynamics of the cortical response coinciding with this artefact, other MEG analysis techniques, such as ECD, could not be successfully applied. In such circumstances, the beamformer method provides a reliable alternative.

We were concerned by the relatively late (~2.5s) appearance of the beta peak in the contact condition (Figure 3, left panel) as it is delayed compared to that reported previously in the literature (see Apkarian et al, (2005) or Garcia-Larrea et al, (2003) for reviews). We

hypothesise that the delay is due to the nature of this novel contact heat stimulator targeting selective nociceptive fibres. For example, evoked potentials induced by brief (16ms) laser stimulation have a temporal range of 70-400ms (see Garcia-Larrea et al, 2003 for a review) and a corresponding induced beta rebound at around 500-700ms after stimulus offset. Contrast these findings with evoked potentials generated by the CHEPS and other similar heat stimulators (600ms stimulus duration) which are slower and longer in duration (500-1000ms) (Le Pera et al, 2002). Consequently, the sensation may therefore be more comparable to that experienced in tonic thermal pain, for which delayed increases in beta power have also been reported (Huber et al, 2006). Finally, there is some evidence to suggest that a power increase in the beta frequency bandwidth may be indicative of selective activation of the A-delta nociceptive fibres only (Raij et al, 2004). This is a potential area for further investigation, but is outside the remit of this study.

Similarly, we selectively chose to investigate activation within the beta rhythm only. It is well documented that (pain-related) cortical activity occurs across a wide frequency range; for example magnetic evoked fields to electrical stimulation have been localised using SAM in the 50-200Hz frequency range (Hirata et al, 2002) and the importance of slower alpha and theta frequencies have been implicated in chronic pain populations (Stern et al, 2006). Although we did not find such responses in the primary somatosensory cortex (Figure 3), now we have shown the ability of beamformer techniques to remove unwanted stimulus-related artefacts, there is the potential to complete more thorough investigations of pain processing using MEG.

The average SEF showed slightly different morphology for data collected in open MSR compared to that collected in closed-door MSR (figure 5). Specifically, whilst the latencies of

the first two peaks were the same, the amplitude of these components was reduced in the absence of full magnetic shielding. Furthermore, the different shielding and gradiometer conditions had less of an influence on source localisation using SAM, compared to using an ECD solution (Table 3). In general, for simple paradigms where only one source exists the source of cortical activity localised by dipole and SAM should agree. Note that our MEG scanner is equipped with first order hardware gradiometers consisting of two oppositely wound coils, which cancel a significant amount of more distant noise. Therefore the results can not be generalised to data collected with systems equipped with magnetometers. The localisation results (Table 3) with and without complete shielding indicate that this can be achieved without higher order synthetic gradiometry and magnetic shielding. Table 3 is further indicative of localisation reliability with SAM as opposed to ECD procedures, particularly in open-door environments. However, it must be noted that our experimental paradigm was a relatively simple one involving low-level sensory processes with strong and focal responses. The averaged SEF traces (Figure 5) show that the later component of the evoked response can only be seen when high-order gradiometers and/or magnetic shielding were applied (1^{st}, 3^{rd} and 4^{th} overlays). This late response is equivalent to the P300 potential (Sutton et al., 1965), which is thought to reflect cognitive processes (Polich & Kok, 1995). Whilst it is outside the remit of this study to investigate this later activation in more detail, one might expect that by using SAM, it would be possible to localise the source of this activity, regardless of the application or absence of high-order gradiometers or magnetic shielding.

As for the correspondence between the hand area of SI, our dipole and SAM locations, both with and without MSR are consistent with previously reported data from stimulation to the dorsum of the hand. For example, Bingel, et al. (2004) reported SI locations of 35.1 -32.6

58.9; Inui et al. (2003), showed SI locations at 36 -36 48, and we report an average of 37.6 -15.8 59.3 for SAM and 37.1 -16.4 61.3 for the dipole (ignoring the source localized to the wrong hemisphere). We acknowledge, however, that these locations are somewhat anterior in location to those reported in previous studies, and suggest that this may reflect activation of the motor cortex due to the nature of the stimulus and the consequent twitching of the fingers.

In conclusion, we have shown that beamformers can be very useful in the presence of large magnetic interference and in poorly shielded environments, offering robust spatial resolution. Costs permitting, heavy shielding and effective hardware noise cancellation are clearly desirable. Despite incurring such costs one could still arrive at a situation where environmental noise is extremely pernicious (for example in cities with tram-lines) or if indeed the noise is created by the patient themselves (eg pacemakers, cochlear implants). In such cases, or where the budget available is limited, we have been able to show that software noise cancellation through beamformer spatial filtering solutions are highly effective.

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Figure 1. Example of the magnetic artefact in a single MEG channel over the right central region produced by the CHEPS mechanism before (black trace) and after (grey trace) application of 3rd order synthetic gradiometer. The CHEPS trace (in blue) shows the temperature of the probe over the time-course of one trial rising from 32^{o}C to 48^{o}C at 70^{o}C/s in approximately 230ms, sustained at 48^{o}C for 500ms, then returning back to 32^{o}C at a rate of 40^{o}C/s. The vertical line indicates the onset of the stimulus. Note the duration of the artefact. Also note the attenuation of signal amplitude due to the 3rd order gradiometer (black versus grey trace).

Figure 2. Peak SI beamformer localisation for the CHEPS (red) and electrical (blue) stimulation. Shown is a top view (left panel), right view (middle panel), and back view (right

panel) of the MNI template brain. Each mark represents a different participant, such that there are 8 red and 8 blue marks. Activations are observed in the 20-30Hz frequency band and for the two second pre-and post-stimulus onset for thermal and 500ms pre- and post-stimulus onset for electrical time-windows, respectively. Note the concordance in S1 peak location for the two different stimulus modalities, which is further illustrated by the average MNI locations shown in Table 1. These locations are consistent with previously published data following painful stimulation to the dorsum of the hand (Derbyshire et al, 1997).

Figure 3. Examples of beamformer localisation of the CHEPS stimulus for 'contact' (left) and 'blocked' (right) conditions. The top panel shows SAM volumetric images of event-related power change in the 13-20Hz band. The red colour indicates increase in source power. For the contact condition, focal activation of the right SI is shown (both images are thresholded to half the maximum intensity). This is absent from the blocked condition (top right). The bootstrap time-frequency analysis (bottom row) shows induced stimulus related increase in power (15-20Hz) for the contact condition appearing after 2 seconds, which is absent from the blocked condition. Note that the stimulus related artefact appears in frequencies < 5Hz in both time-frequency plots which were not removed as the beamformer weights were created in the 13-20Hz band in this instance.

Figure 4. The effect of the beamformer regularisation parameter. Both images are thresholded to half the maximum intensity. On the top is the un-regularised SAM image showing the heat-evoked sensory cortex activation as increase in power relative to baseline (t=2.7) in the 13-20Hz band (shown in red). The VE trace shows the spatial filter output and time-course of this activity. Note that the image is 'sharp' and stimulus-related artefact has been removed. On the bottom is the regularised SAM image (μ=5000) again showing relative

power increase in the 13-20Hz band. The image now contains projected noise, as indicated by the widespread activity and un-realistic relative power values (t=527). The VE trace shows that the regularization destroys the action of the spatial filter and that the stimulus-related artefact becomes visible in the source space and the VE timecourse. Note that the SAM volume now appears much less focal.

Figure 5. Average SEF and topographic map (view from above, nose at the top, left ear on the left, right ear on the right) of the magnetic fields due to the electrical stimulation of the subject's left wrist. On the top, averaged SEF for MEG data recorded with the door of the shielded room open. 3rd order synthetic gradiometery is also applied. The corresponding topography at a latency of 70ms shows clear dipolar activity above the right central channels, indicative of activity within the right somatosensory cortex. On the second row, the same recording is shown with no synthetic gradiometry applied. Note that the averaged trial contains more random noise from the environment and the topographic map of the magnetic dipole also displays more noise. On the third row, data is shown where the door of the shielded room was closed and a 3rd order synthetic gradiometer applied. There is no obvious difference in noise between this trial and the early components of the averaged SEF when the door is open, as evident also from the magnetic field pattern at 70ms. On the bottom row, averaged data is shown with the MSR door closed but no synthetic gradiometer applied.

Figure 6. Localisation of sensory cortex from electrical stimulation of the left wrist for the 4 conditions using ECD analysis (top row) and SAM (13-20Hz) (bottom row). The source locations are represented by a green circle. For the SAM images the increase in source power is shown in red and is also indicated by the pseudo-t values (green label in the images).

*** Revision Notes**

My colleagues and I would like to thank the reviewers for their insightful comments. We have now made substantial changes to the manuscript which takes into consideration the comments by both reviewers. We provide comprehensive replies to each comment raised by the reviewers. Our comments are written in red colour font below.

Reviewers' comments:

Reviewer #1: The authors clearly demonstrated the methodological effectiveness of magnetic noise cancellation by synthetic gradiometers and beamformer method of SAM under two conditions; first, under the interference of electromagnetic noise generated by a heat stimulator, and secondly, in the absence of hardware shielding. These methods will be highly useful especially in clinical situations where the optimal recording condition is often difficult to be kept due to the artifact from the metals or electrical devices implanted within patient's bodies. In addition, MEG recordings without a magnetically shielded room will expand the application of MEG. Therefore, with respect to methodological significance, this paper is potentially sufficient to be published. However, there are several major and many minor points to be improved.

[Major points]
#1. Page 10, Paragraph 2
MEG data were recorded from only one subject. This journal does not necessarily require many subjects, because methodology is main focus. However, only one subject is too small. We can not evaluate the reproducibility or stability of the present method. Otherwise, the authors should carefully discuss much more based on the evidence from simulation or phantom data.
We have now included data from additional participants to indicate the stability and reliability of our results. These are shown in Tables 1 and 2 and in a new Figure 2, and discussed in corresponding sections of the manuscript.

#2. Page 11, Paragraph 2
Why did not the authors investigate other frequency bands than 13-20 and 20-30 Hz?
The authors should indicate the evidence (some references) that the CHEPS or other heat stimuli evoke the responses in the 13-20Hz and 20-30Hz.
We should have made this clearer. In the Methods section of experiment 1 on page 8 we have added: ("…for the frequency bands of 13-20 and 20-30Hz, corresponding to the beta rhythm exhibited by the sensorimotor cortices (Hari and Salmelin, 1997; Lounasmaa et al., 1996)"); we now also report that these beta frequencies are also involved in pain processing and included additional references (Lalo et al, 2007; Huber et al, 2006; Chang et al 2002), inclusive of which are studies which have shown beta activation in response to thermal stimulation (Huber et al, 2006). Figure 3 (bottom row) supports our a priori choice for the frequency bands.

#3. Page 12, Paragraph 2; Figure 3
Time frequency analysis shows the beta activity from 2 to 3 seconds after the heat stimulus onset. Although the heat stimulus persists 7 seconds, why does the beta activity occurs only for such a limited period?
This is a good point. We were also curious. However the images indicate the activity is not artefact of the stimulus but physiological response associated with the sensation. Although each trial analysed was 7 seconds in duration this comprised baseline and post-stimulus recovery

periods as well as the stimulus delivery. The thermal stimulus used in the study lasted for a period of approximately 1130 milliseconds, comprising time to maximum (230ms), maintenance at maximum for 500ms, and decrease back to baseline (400ms) periods. Although the increase in beta power is delayed compared to that reported previously, we hypothesise that it is the same physiological mechanism which is just delayed due to the nature of the stimuli. We have added the following paragraph in the discussion. "We were concerned by the relatively late (~2.5s) appearance of the beta peak in the contact condition (Figure 3. Left panel) as it is delayed compared to that reported previously in the literature (see Apkarian et al, (2005) or Garcia-Larrea et al, (2003) for reviews). We hypothesise that the delay is due to the nature of this novel contact heat stimulator targeting selective nociceptive fibres. For example, evoked potentials induced by brief (16ms) laser stimulation have a temporal range of 70-400ms (see Garcia-Larrea et al, 2003 for a review) and a corresponding induced beta rebound at around 500-700ms after stimulus offset. Compare this to those evoked potentials generated by this contact and other similar heat stimulators (600ms stimulus duration) which are slower and longer in duration (500-1000ms) (Le Pera et al, 2002). Consequently, the sensation may therefore be more comparable to that experienced in tonic thermal pain, for which delayed increases in beta power have also been reported (Huber et al, 2006). Finally, there is some evidence to suggest that a power increase in the beta frequency bandwidth may be indicative of selective activation of the A-delta nociceptive fibres only (Raij et al, 2004). This is a potential area for further investigation, but is outside the remit of this study."

#4. Page 14, Paragraph 2
SAM analysis was performed in the 13-20Hz and 20-30Hz to investigate the evoked responses to electrical stimulation to the hand. However, an SEF paper using SAM reported robust response in the 50-200Hz to electrical stimulation to the median nerve stimulation (ref. 1). The authors should indicate the evidence why it is sufficient to analyze only 13-20 and 20-30Hz bands.
We have now included a paragraph to discuss this, referring to the evidence in the 50-200 Hz which the reviewer kindly brought to our attention (see also response above). "Similarly, we selectively chose to investigate activation within the beta rhythm only. It is well documented that (pain-related) cortical activity occurs across a wide frequency range; for example magnetic evoked fields to electrical stimulation have been generated using SAM in the 50-200Hz frequency range (Hirata et al, 2002) and the importance of slower alpha and theta frequencies have been implicated in chronic pain populations (Stern et al, 2006). Now we have shown the ability of beamformer techniques to remove unwanted stimulus-related artefacts, there is now the potential to complete more thorough investigations of pain processing using MEG"

#5. Page 18, Paragraph 1
The authors compared P3 response only with respect to MSR and synthetic gradiometer. Can SAM analysis delineate this P3 response?
This is a very good question. We analysed the SAM virtual electrode traces to look for evidence of the P300 response but found none. We think this may be due to the increased spatial selectivity of SAM (which tends to suppress activity in neighbouring regions). We have not analysed this as it was not within the remit of this study. We have inserted the following sentence in the discussion on page 17: "Whilst it is outside the remit of this study to investigate this later activation in more detail, one might expect that by using SAM, it would be possible to localise

the source of this activity, regardless of the application or absence of high-order gradiometers or magnetic shielding."

[Minor points]
#1. Page 11, Paragraph 3
The authors stated that bootstrapping analysis provides statistically significant levels. Why did not you indicate statistical significance levels to indicate stimulus-evoked response?
We apologise. The significance level was 0.05. We have now inserted this in the manuscript.

#2. Page 11, Paragraph 3
What does 'a widespread increase in power' mean for the 'blocked' condition neurophysiologically?
The 'widespread increase in power' in this context was in reference to the absence of localized activity in the somatosensory cortex similar to that in the unblocked condition. It was basically a thresholding error. On reflection the phrase was not used appropriately and we have removed this on page 9 and replaced with: "For the 'blocked' thermal condition, no focal activation was found to either SI or SII (as illustrated in Figure 3)."
We have also revised the image so that both SAM images are thresholded to the same extent, now showing the absence of activity in the blocked condition on the right.

#3. figure legend of Figure 1
The meaning of symbols; m, w, S and P should be explained.
This figure has now been removed from the paper along with the relevant text in agreement with the second reviewer's recommendation.

#4. Figure 2
Location of the MEG sensor that the data in Figure 2 were recorded should be indicated.
The time-series represent MEG activity recorded over the right central region (taken from channel MRC16). However, all channels recorded the same artefact. We now say this in the legend for figure1: "The time-series represents recording from a channel over the right parietal region." This is now figure 1.

#5. figure legend of Figure 2
In the second sentence, '70oC/s' should be corrected.
This has now been corrected to read 70^{o}C/s. This is now figure 1.

#6. Figure 3
The authors should indicate the meaning of the number as indicated in green color in the left SAM image. This number is not indicated in the right SAM image.
We have now removed the label from the SAM images.

#7. figure legend of Figure 3
What does 'middle figures' indicate?
These words have now been removed.

#8. Figure 4
VE of unregularised SAM (the right upper graph) does not seem to have any apparent responses to electrical stimulation. The authors should clearly state if any responses exists.
Figure 4 is showing the VE following thermal and not electrical stimulation. The inclusion of this figure is to demonstrate the removal of the artefact by the implementation of an unregularised beamformer, although we acknowledge that there is no clear evoked response following thermal stimulation.

9. Figure 4
The authors should indicate the parameter of the longitudinal axis of the VE graphs.
We have now inserted the correct scale for this graph.

#10. Figure 5
Stimulus onset seems to be identical with the peak latency of the P1 component. Is this correct?
No it is not. We thank the reviewer for bringing this to our attention. We have now corrected this figure to reflect the stimulus onset time accurately, which is approximately 70ms before the evoked response.

#11. Tables
Captions of the tables should be briefly described and placed below the tables instead of placing in the figure legends section.
This has now been applied as advised.

[References]
1) Hirata M, et al. Frequency-dependent spatial distribution of human somatosensory evoked neuromagnetic fields. Neurosc Lett 2002; 318: 73-76.

Reviewer #2:

This is an interesting paper that demonstrates the effect of 1) Higher-order gradiometry 2) Room shielding and 3) Spatial-filtering (beamformers) on the SNR of evoked responses and source localization accuracy.

While there is much of merit in this paper, the aims and findings are poorly presented and need substantial re-working. Here are my specific queries/recommendations

1) Is open-door the same as unshielded? Presumably some shielding effect still occurs from the room, even if the door is open. If the results in this paper do not generalize to fully unshielded environments, then some of the key claims of the Authors are unfounded. Also the work presented here does not look at the stability of the ERFs or localization accuracy in the presence of, say, moving metal objects in the local environment - presumably some MEG labs will be better suited to open-door working the others.
The reviewer is correct. Open-door environment does not equate to unshielded in terms of the amount of noise that is present at the sensor level. We have now replaced ‘unshielded’ and

‘NoMSR’ to ‘open-door’ in the manuscript. We have also stated in the discussion that this paper deals with partly shielded rooms which might pave the way for similar future studies in completely open and unshielded environments.

2) In the abstract the Authors' claim that by using higher-order gradiometers accurate source localizations can be obtained. In fact the Authors' data show that the P300 can only be properly recovered using the MSR and that without the MSR both the dipole and SAM localizations are driven deeper than might be predicted for the hand area of S1.
In the previous submission the data were recorded from the same subject but on different days inside and outside the MSR. We were worried that coregistration errors could have led to some of the above discrepancies. We repeated this experiment collecting both open and closed door runs back to back. As for the correspondence between the hand area of S1, our dipole and SAM locations, both with and without MSR are consistent with previously reported data from stimulation to the dorsum of the hand. For example, Bingel, et al. (2004) reported S1 locations of 35.1 -32.6 58.9; Inui et al, showed S1 locations at 36 -36 48, and we report and average of 37.6 -15.8 59.3 for SAM and 37.1 -16.4 61.3 for the dipole. We acknowledge, however, that these locations are somewhat anterior in location to those reported in previous studies, and suggest that this may reflect activation of the motor cortex due to the nature of the stimulus and the consequent twitching of the fingers. We have now added the following in the Discussion:
“As for the correspondence between the hand area of SI, our dipole and SAM locations, both with and without MSR are consistent with previously reported data from stimulation to the dorsum of the hand. For example, Bingel, et al. (2004) reported SI locations of 35.1 -32.6 58.9; Inui et al. (2003), showed SI locations at 36 -36 48, and we report an average of 37.6 -15.8 59.3 for SAM and 37.1 -16.4 61.3 for the dipole. We acknowledge, however, that these locations are somewhat anterior in location to those reported in previous studies, and suggest that this may reflect activation of the motor cortex due to the nature of the stimulus and the consequent twitching of the fingers.”

As to the P300 the reviewer is correct, it was certainly clearer in the MSR and we have mentioned this in the Discussion on page 17.

3) In general the introduction is too long and recapitulates methodology that is easily accessible in recent publications, including the Authors' own recent review articles. Figure 1 is also superfluous as versions of it are shown in other publications.
We agree with the reviewer and now substantially edited and rewritten the introduction which is now brief and covers the aspects that are directly relevant to the study methodology. We have also removed figure 1.

4) In the introduction the Authors claim that performing MEG in unshielded environments is "highly desirable". I wonder if this is really the case. Surely for the majority of MEG applications the best possible SNR is desirable in order to properly categorize brain function, including what might be weaker components such as the P300? The Authors data seem to be showing that combining the MSR with higher-order gradiometry and beamformer localization is the best way to proceed as it gives the best SNR and localization accuracy. It also allows experiments to be performed in the presence of large stimulus artifacts. The latter seems to be the strongest part of this paper and should be the key focus of the manuscript.

We agree. We have now toned down the introduction and refocused the paper accordingly.

5) Page 10, second paragraph (after Figure 2). It is not clear to me how the Authors define "accuracy" of source localization with SAM in this paper. Some consistency of localization is demonstrated (although not using the MSR appears to drive the activation inappropriately deep) but accuracy is not really assessed.
To explore this point further we have now included comparable results on the same participants following painful electrical stimulation, a stimulus modality that is well-documented in the literature and which yields reproducible source localisations. The beamformer data following thermal stimulation are spatially coincident to those following electrical stimulation and therefore it is on this basis that we refer to accuracy of SAM in successful artefact removal and source localisation. Furthermore, in experiment two, the results show same area of activation with both SAM and dipoles.

6) Page 11, end of 1st para. What modification of the surface-matching approach is used?
The surface-matching algorithm is exactly the same where a set of headshape points is collected with the help of the Polhemus isotrack. This is then matched to an extracted headshape from the MRI. However, we no longer used a bite-bar to keep the head motionless. We clarify this in the text by adding the following sentence on page 7: “Instead of using a bite-bar to stabilize the subject’s head during head digitization with the Polhemus, a reference coil is attached to the subjects head when headshape points are collected. This reduces the influence of head movement during digitization.”

7) Page 12 (Results). Does a Beta power-increase 2.5 seconds after the onset of stimulation make sense? This seems quite late. How does this compare to previous sensory/pain activation studies?
Please refer to our reply to Reviewer 1, Major Point 3

8) Page 12 (2nd para) In the "blocked" condition, what is the Authors' intepretation of the widespread bilateral Beta power increase?
Please see response to Reviewer 1, minor points 2.

9) Page 12. Last line. Can the Authors give some sense of what a Mu=5000 actually means?
We have now described what we mean by this in the text in page 10. (it is effectively the factor required to wash out the information in the data covariance matrix- making the SAM algorithm into a dipole fit).

10) Page 14. 2nd Para. Why was the participant's data transformed to the MNI space? This makes no sense in the context of a single-subject study.
Using the CTF software (VSMMedtech), the results are displayed such that they are in arbitrary coordinates and do not relate to any other coordinate that a reader maybe familiar with. Conversion to MNI provides standard space for comparison allowing us to refer to other literature on the localization of the hand area.

11) Page 15, 2nd para. The fact that the P300 is more apparent when the MSR is used seems to be a strong argument for using shielding!

Yes, this is reasonable and we have now toned down this claim. We should stress however that the P300 was not the purpose of this study, and that the main goal was to show that recording of a basic physiological response is feasible.

12) Page 15, end of Page. It seems clear that the SAM localization is driven inappropriately deep when no MSR is used. Can the Authors' please comment on this?
Yes, we have addressed this in our response to comment 2.

13) Table 1: Why MNI co-ordinates?
See reply to number 10.

14) Table 1: Confidence volumes should be listed in the table for the ECDs.
This has now been done.

16) Page 16 and Table II. I am genuinely confused as to what the distances are in Table II. For example what are the dipole shifts relative to? More explanation is needed!
This table has now been removed. The new Table 2 shows the ratio of regularised and unregularised beamformer values

17) Page 17. The first paragraph and Figure 7 contribute little and can be omitted.
We agree with the reviewer about this and have now removed the figure.

18) Page 19, end of the 2nd paragraph. It is not necessary to use regularization to smooth data for group analysis - surely post-hoc smoothing could be used?
We have now removed this section.

19) Figure 3. The spectrograms shown in Figure 3 do not look thresholded. Was bootstrapping really used?
We can confirm that bootstrapping was indeed used. The significance level was set 0.05 which explain some sparse bursts of activity. We have now inserted the significance level to clarify this.

20) Figure 3. Looking at the spectrograms, there appears to be a clear evoked response for the blocked condition. Can the Authors comment on this?
Yes, we suspect that this activity is in fact stimulus-related artifact, which as shown in figure 1, occurs in low frequencies and lasts for approximately the same duration (0 to 1.5 seconds). For this analysis, the beamformer weights were created in the 13-20Hz and thus the noise was not rejected by the beamformer. Some level of this artifact can also be seen in the spectrogram for the unblocked condition on the left. We comment on this in the caption for figure 3 where we have added the following:
"Note stimulus related artefact appears in frequencies < 5Hz in both time-frequency plots which were not removed as the beamformer weights were created in the 13-20Hz band in this instance."

21) Figure 4. Are the traces shown on the same amplitude scale? Amplitude scales should be shown.

The amplitude scale which is the same for both graphs is now shown in the revised figure.

22) Figure 6. On the ECD plots, confidence eliipsoids should be shown as these could give further information about localization robustness for the various conditions.
We have done this, one surprising finding was how small these ellipsoids were and we comment on this in the Results section of experiment 2.

Editorial comments:

In the Reference list all Journals' titles should be abbreviated. Articles' pages are in the wrong format, i.e. 691-698 should be 691-8.
Please refer to Authors' guidelines.

All page numbers and journal abbreviations are now corrected.

*** Title page-incl. type of article and authors' name and affiliation**

Effective electromagnetic noise cancellation with beamformers and synthetic gradiometry in shielded and partly-shielded environments

P. Adjamian[1], S. F. Worthen[2,3], A. Hillebrand[4], P.L.Furlong[2], B. A. Chizh[5], A. Hobson[6], Q. Aziz[3], G.R.Barnes[2]

[1] *MRC Institute of Hearing Research, University Park, Nottingham, UK.*

[2] *Wellcome Trust Laboratory for MEG Studies, Neurosciences, Aston University, Birmingham, UK.*

[3] *Barts and The London, Queen Mary College, University of London, UK.*

[4] *Department of Clinical Neurophysiology, Institute for Clinical and Experimental Neurosciences, VU University Medical Center, Amsterdam, The Netherlands.*

[5] *Addenbrooke's Centre for Clinical Investigation, GlaxoSmithKline, Addenbrooke's Hospital, Cambridge, UK.*

[6] *Immuno-Inflammation CEDD, GlaxoSmithKline Medicines Research Centre, Stevenage, Hertfordshire, UK.*

Corresponding Author:

Dr Peyman Adjamian

MRC Institute of Hearing Research,

University Park,

Nottingham, UK

NG7 2RD

Tel: (0115) 922 3431 (ext. 262)

Fax: (0115) 951 8503

Email: peyman@ihr.mrc.ac.uk

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Figure

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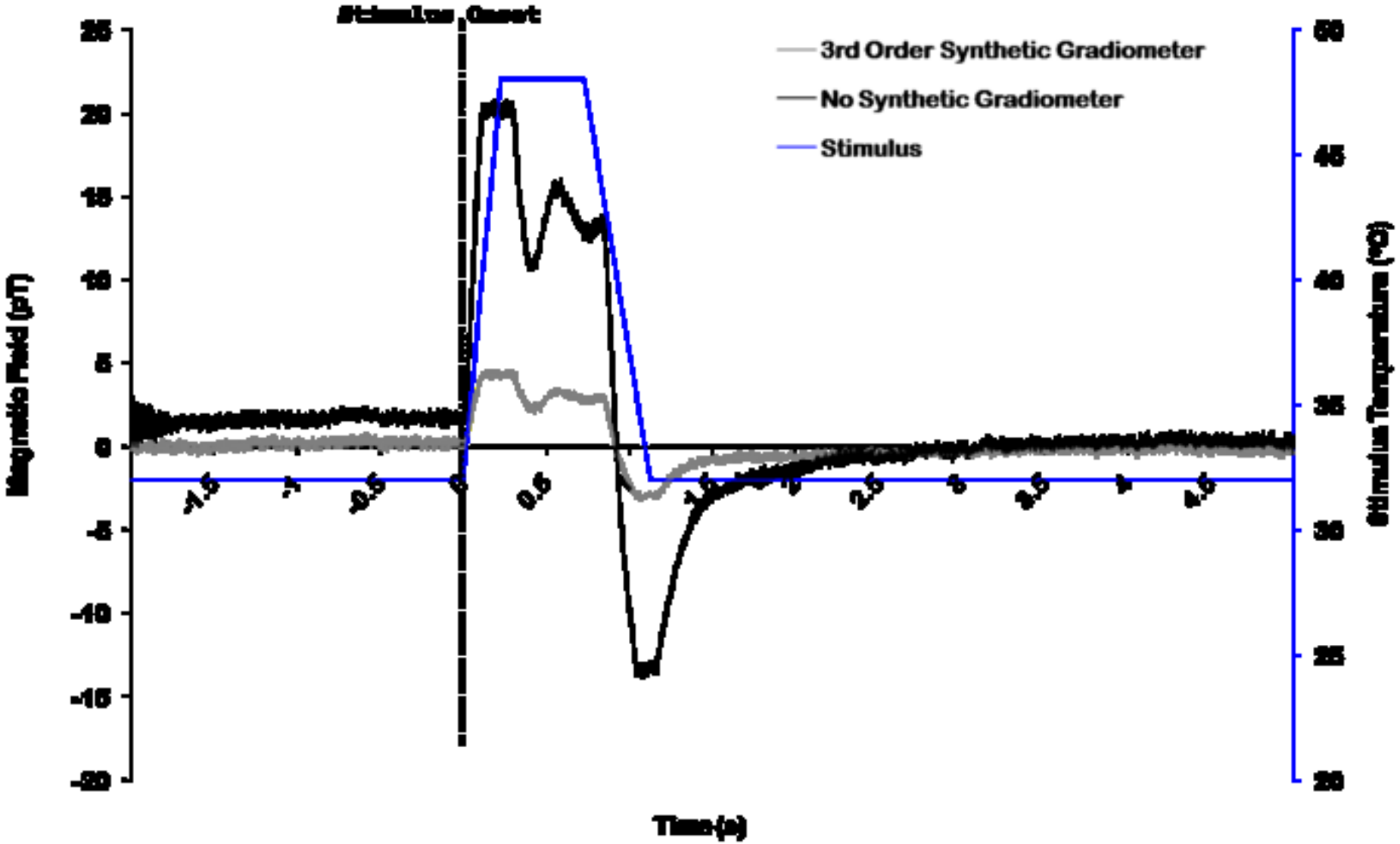

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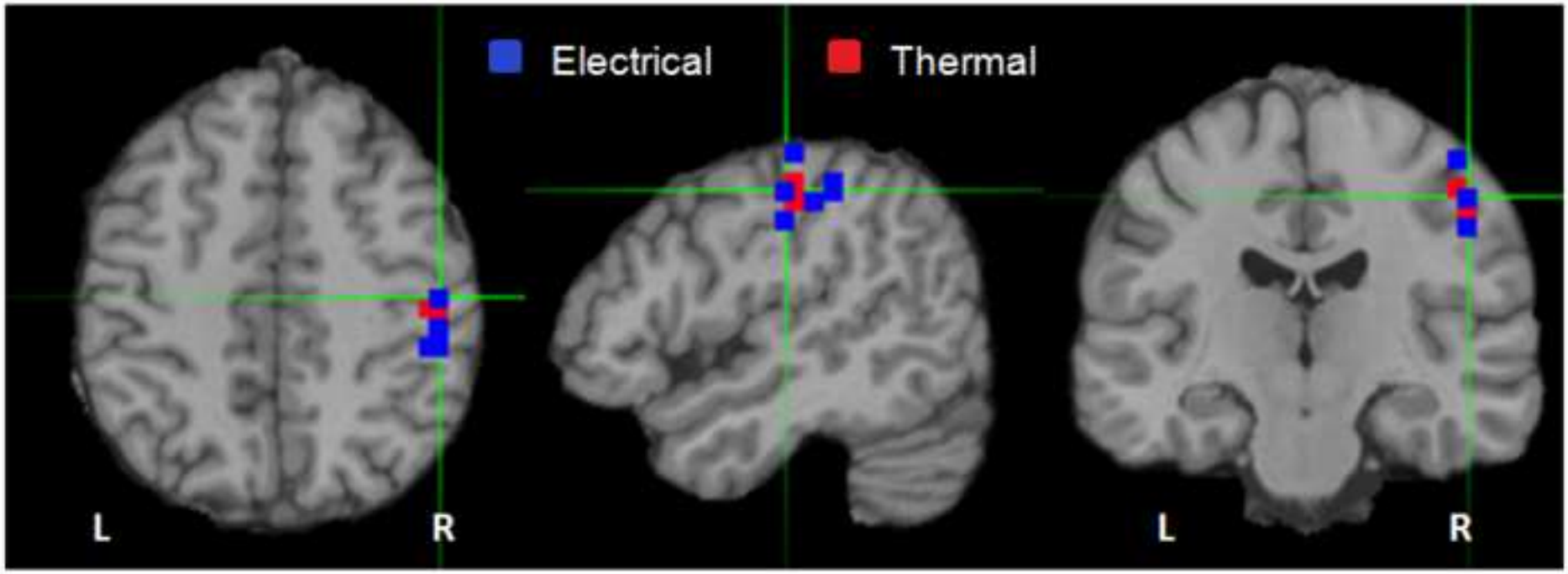

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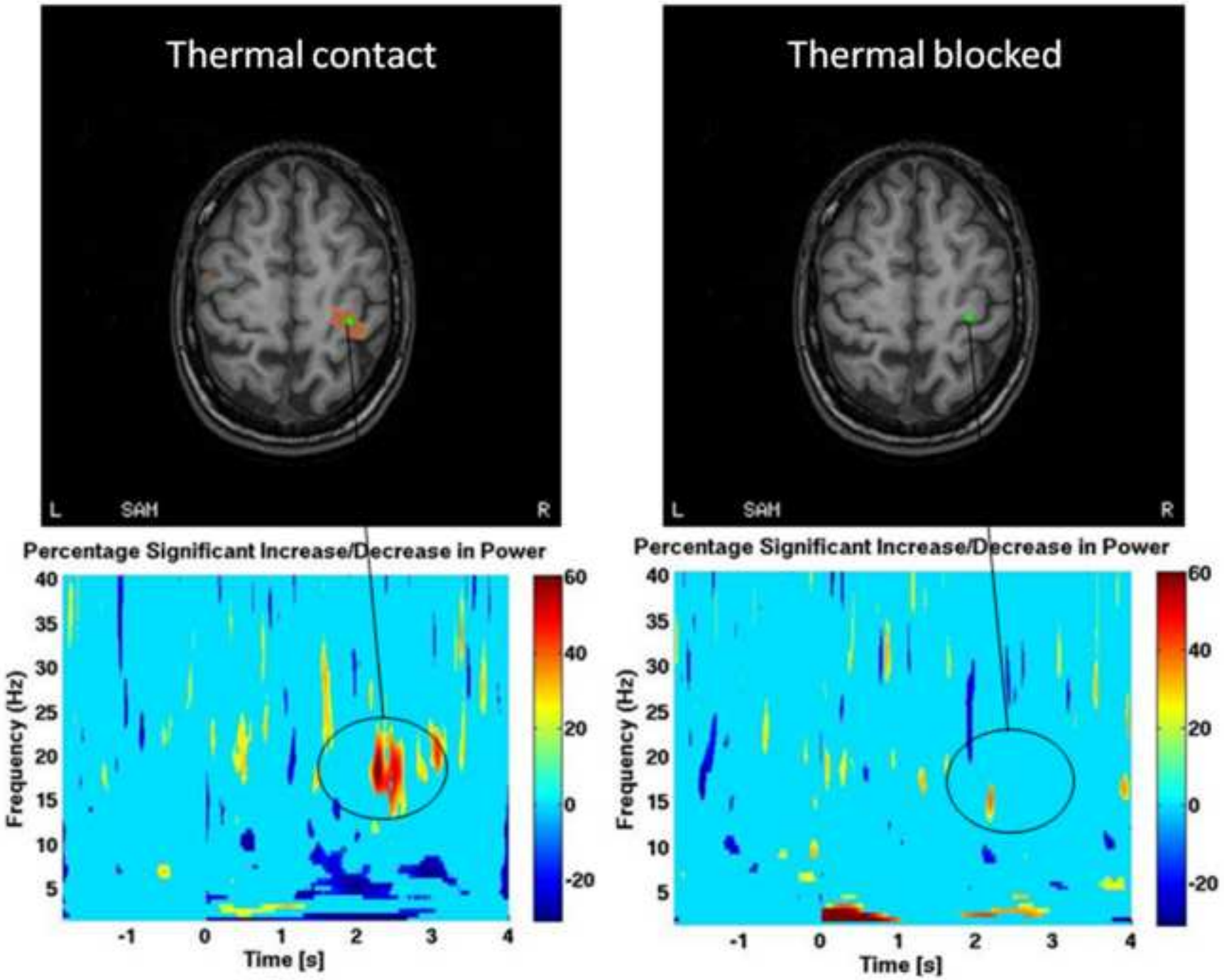

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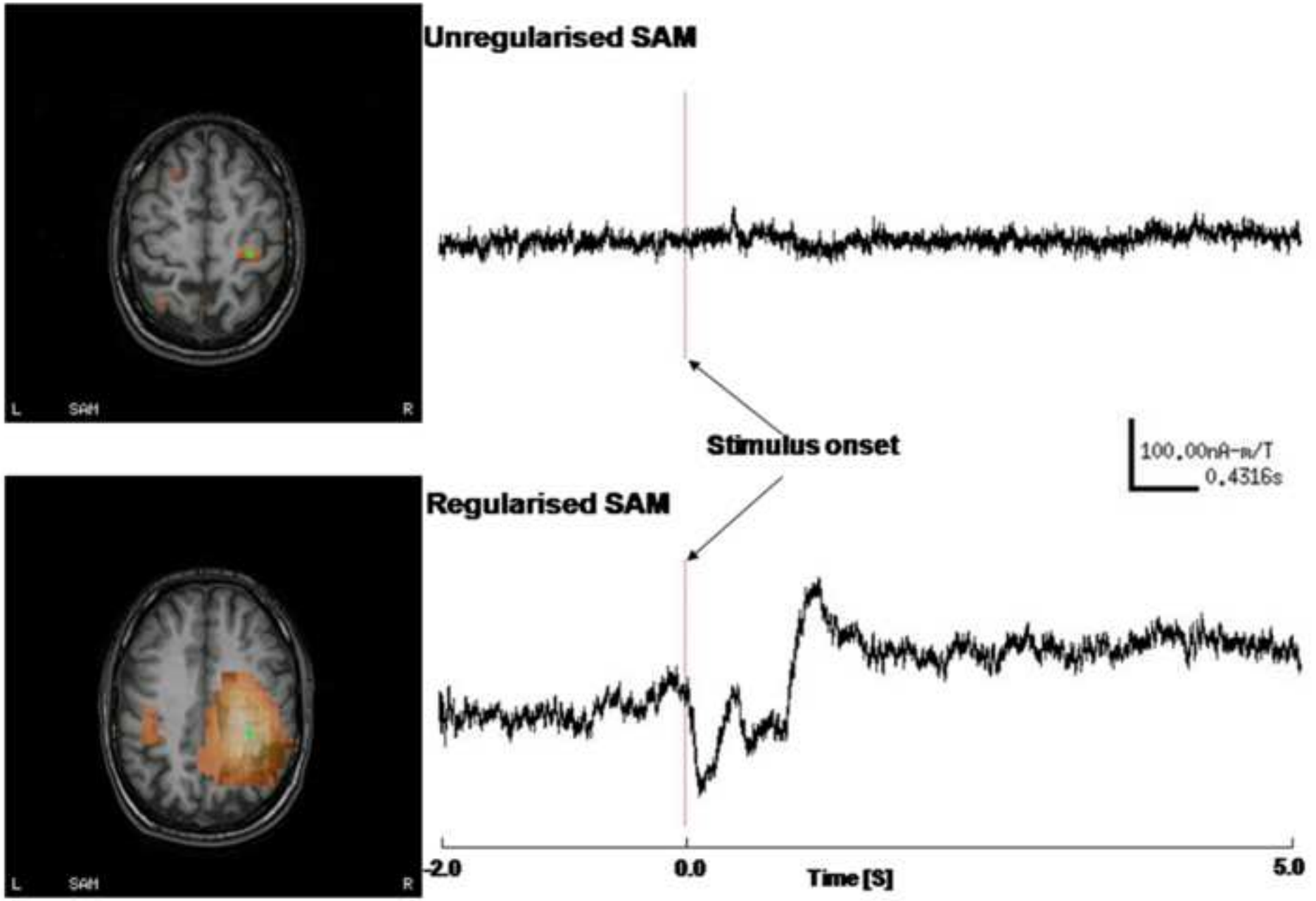

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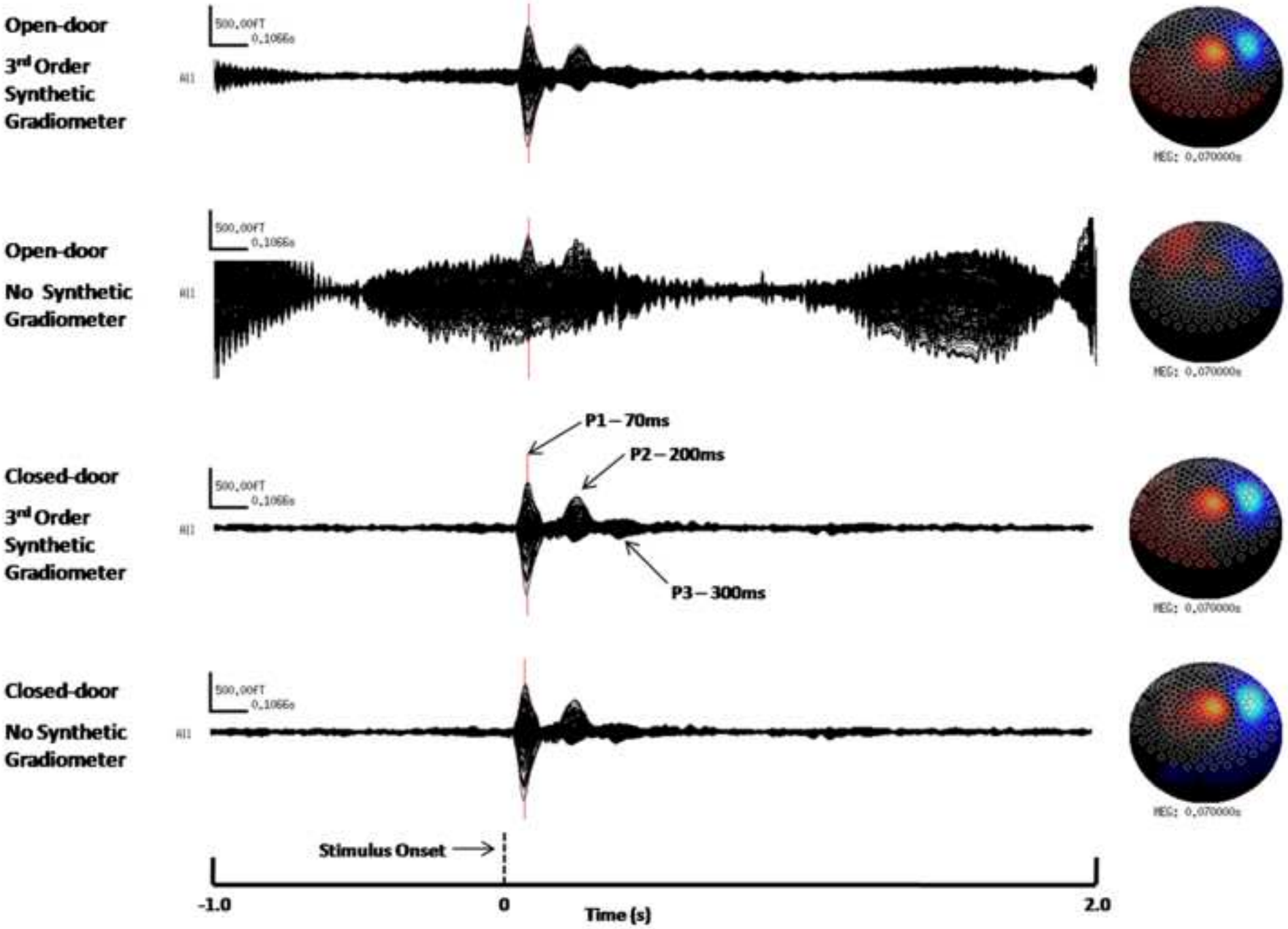

Figure

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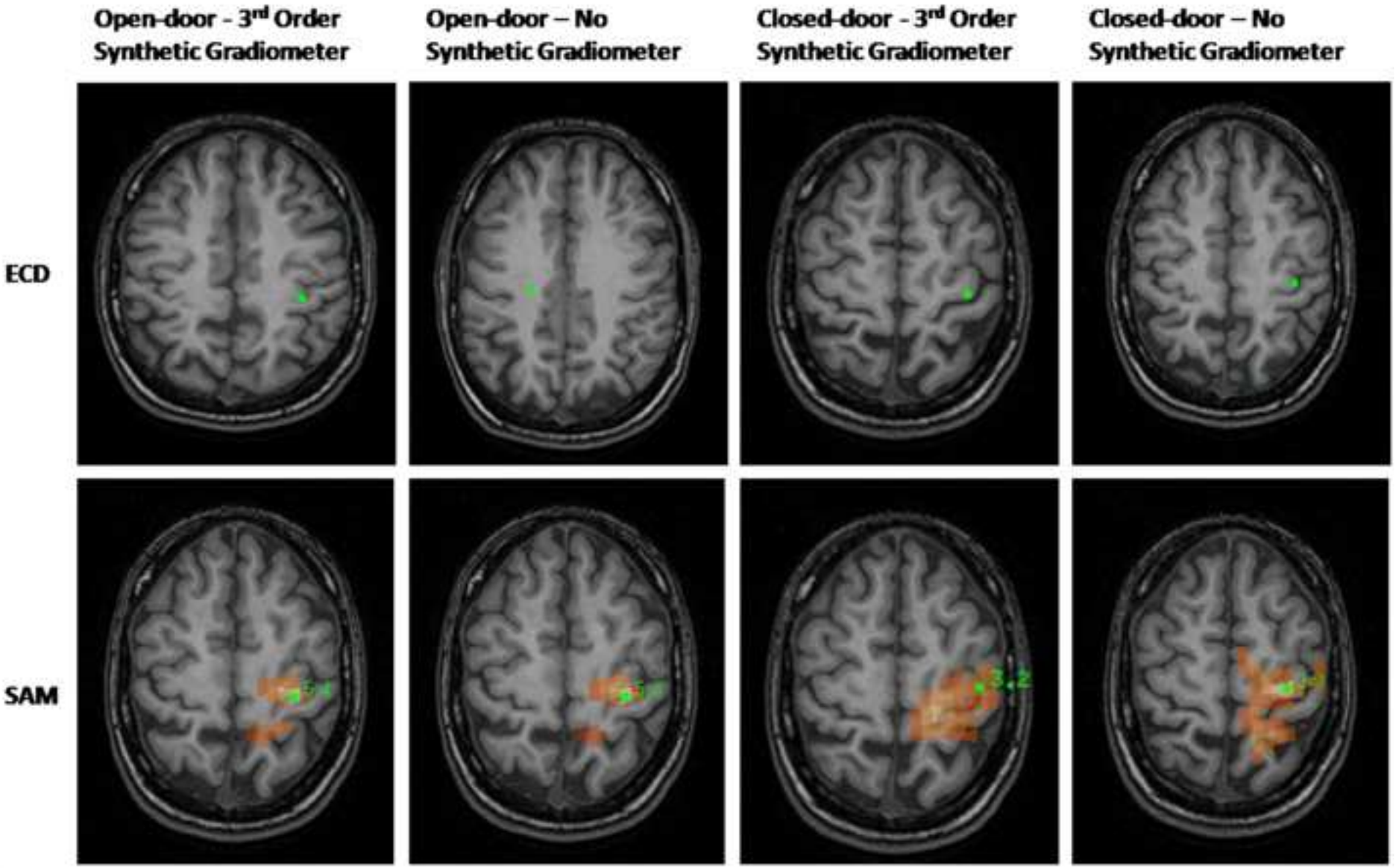

Table

Participant	*Thermal MNI Co-ordinates*			*Electrical MNI Co-ordinates*		
	X	*Y*	*Z*	*X*	*Y*	*Z*
1	47.7	-18.1	47.7	48.2	-21.1	60
2	51.2	-21.1	45	51.2	-18.1	48
3	56.7	-24.5	37	51.2	-33.1	48
4	44.7	-20.9	50.6	51.2	-33.1	51
5	41.7	-35.9	43.1	51.2	-18.1	39
6	47.7	-17.7	60	51.2	-27.1	45
7	41.7	-32.7	48.4	48.2	-33.1	48
8	41.7	-21	47.9	48.2	-27.1	48
Group Average	**41.2**	**-23.9**	**47.4**	**50.0**	**26.3**	**48.3**

Table 1 – Peak SI activations from the SAM analysis (20-30 Hz) are shown using MNI coordinates for the 8 participants for the thermal (left column) and electrical (right column) stimulation. The locations are depicted in a standardised brain in Figure 2.

Table

Participant	*Regularised Beamformer (Root Mean Square) [(nAm)^2]*	*Unregularised Beamformer (Root Mean Square) [(nAm)^2]*	*Ratio Regularised : Unregularised*
1	21.96	7.89	2.78 : 1
2	131.11	13.83	9.48 : 1
3	66.70	9.34	7.14 : 1
4	82.32	8.68	9.48 : 1
5	57.22	6.79	8.42 : 1
6	83.11	7.22	11.51 : 1
7	48.72	6.81	7.15 : 2
8	25.74	6.09	4.22 : 1
Group Average	**64.61**	**8.33**	**7.52 : 1**

Table 2 – Power (rms) of the regularised and unregularised beamformer in the 0 to 100 Hz band using the entire length of each trial (-2 to 5 seconds). The higher values of the regularised (mu=5000) beamformer indicate the noise projected into the spatial filter which creates unphysiologically large MEG signal. For each participant, the ratio of the regularised/unregularised beamformer output is shown in the column on the right. Note that on average there is approximately an 8-fold reduction of projected noise for the unregularised beamformer.

Table

	MC DipoleFit (x, y, z)		SAM Peak (x, y, z) (mm)
	Co-ordinates (x, y, z) (mm)	Monte-Carlo Error Volume (mm^3)	
Open, 3rd order synthetic Gradiometers	34.1 -17.1 62.0	10.604	33.1 -18.1 60.0
Open, No synthetic Gradiometers	-13.1 -8.0 49.0	1.106	36.1 -18.1 57.0
Closed, 3rd order synthetic Gradiometers	42.2 -15.1 56.0	0.514	38.2 -19.0 57.0
Closed, No synthetic Gradiometers	35.1 -17.1 66.0	2.579	33.1 -18.1 63.0

Table 3 – Dipole and SAM peak locations for electrical pain stimulation under different noise levels. The Monte Carlo volumes indicate that the best fit results are obtained when both shielding and highest-order gradiometers are applied emphasising their combined effect on noise reduction. The dipole technique performs worst in terms of reconstructed location in open-door environment and no synthetic gradiometry (2nd row, and 2nd column in figure 2). In such situations the beamformer clearly performs better.

*** List of four potential Reviewers (with full address, email, phone, fax)**

Suggested Reviewers

1-Professor Andrew T. Smith
Department of Psychology
Royal Holloway, University of London
Egham
Surrey
TW20 OEX
ENGLAND

Email: a.t.smith@rhul.ac.uk
Phone: 01784 443717

2-Dr Sylvain Baillet
Centre MEG-EEG - LENA UPR640
Hôpital Pitié Salpétrière
47 Bd de l'hôpital
75651 Paris CEDEX 13
Tel: 0142161161
Fax: 0145862537
sylvain.baillet@chups.jussieu.fr

3-Professor Mayasuki Hirata
Department of Neurosurgery,
Osaka University Graduate School of Medicine,
Osaka, Japan

Fax: +81-6-6879-3659.
mhirata@nsurg.med.osaka-u.ac.jp

4-Dr Matthew Brookes
Room MR17 Sir Peter Mansfield Magnetic Resonance Centre
University Park
NG7 2RD
Tel: 0115 846 6003
Fax: 0115 951 5166
matthew.brookes@nottingham.ac.uk