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The effect of reference choices on the spatio-temporal analysis of brain evoked potentials: The use of infinite reference

Dezhong Yao[a,b,*], Li Wang[b], Lars Arendt-Nielsen[b], Andrew C. N Chen[b]

[a]*School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, China*
[b]*Human Brain Mapping and Cortical Imaging Laboratory, The International Doctoral School in Biomedical Sciences and Engineering, Center for Sensory-Motor Interaction, Aalborg University, Fredrik Bajes Vej 7D3, Aalborg 9220, Denmark*

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Abstract

Reference is a very virtual issue in EEG and ERP. Understanding the difference of various references will make the applications more confident. In this work, somatosensory evoked potential (SEP) with stimulation on the right hand was studied. The SEP spatio-temporal analysis was conducted comparatively on six references, left mastoid (contralateral mastoid reference, CM), right mastoid (ipsilateral mastoid reference, IM), linked mastoids (LM), average reference (AR), vertex reference (Cz) and the infinity reference (IR) newly proposed in 2001. Among the six, CM is the one used in actual recordings, and the other five are obtained by off-line re-referencing. The comparison is conducted on four selected components (P30 ms, P40 ms, N90 ms and P230 ms) in both temporal and spatial aspects. The results show that references may have a distinct influence on the amplitudes of the scalp potentials, with relative error at some electrodes larger than 500%, and for some electrodes it may even change the polarity. Pair-wise multiple comparison (Tukey test) shows that the differences of peak values among various references are very significant ($P < 0.001$) between Cz and IR\CM\IM\LM, and significant ($P < 0.01$) between Cz and AR for component N90 ms; very significant ($P < 0.001$) between Cz and IR\CM\IM\LM\AR, significant between IM\LM and AR ($P < 0.01$), CM and AR ($P < 0.05$) for component P230 ms. The amplitude value order is CM/IM $\geqslant$ LM > IR > AR > Cz. The two-ways (the six references vs. the four Peaks) repeated measures ANOVA test shows the effect of different references depends on various components; there is a statistically significant interaction between reference and the peak ($P = < 0.001$). While for the spatial map of the potential amplitude, references will not affect the amplitude map shape if the color-bar is selected automatically, but if a fixed color-bar is chosen for data of various references, they may show some differences. These results mean a common reference is important for producing a comparable result between labs. As IR is theoretically a constant reference, we recommend it as the common choice in the future.

Keywords: Infinite reference; Contralateral and ipsilateral mastoid; Average; Cz reference; Amplitude; SEP

1. Introduction

The choice of an EEG reference is a critical issue for many studies [1–3]. In fact, both the evoked potential (EP) and the spontaneous potential (EEG) of neural activities are currently read in terms of components thought to reflect distinct neural generators [4–6]. Each component can be defined by characteristics such as polarity, scalp region, spectra, range of latencies and voltages. Moreover, for these characteristic values, a potential with neutral/zero reference is the desired objective data. It is well known that, in nature, only the potential difference between two points can be measured, so it is indispensable to set a reference in human scalp recordings [7], and we hope the neural electric field around the reference is a constant. The cephalic electrode, non-cephalic electrode, such as the vertex (Cz) [8,9], the tip of the nose [10,11], uni-Mastoid [12], Linked mastoids (LM) [13], neck ring [14], Average [15] reference, etc., each may yield some effects on the recordings [16], so different reference sites have been recommended for studies of different potentials [4,16,17].

The effect of a reference is due to that an activity near the reference electrode will affect measurements at all the other electrode sites and so do the temporal dynamic analysis and power

* Corresponding author. Tel./fax: +86 28 83206124.
E-mail address: dyao@uestc.edu.cn (D. Yao).

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spectra analysis of the EEG time series [18–20]. To solve this problem, great effort has been given to search for a relative non-active point on the body surface for a concrete EEG problem, and related arguments also existed for a long time [1,4,7,16–18]. However, there is no non-active point on the scalp surface whose potential can be considered to be zero [7] because the neural electric activities are distributed spatio-temporal dynamic processes. Recently, based on the technique improvement of high-density multi-channel recordings and computer computation resource, a new infinity reference (IR) was developed in 2001, which is a software method to approximately transform the scalp recordings with a scalp point as reference to recordings with a reference point at infinity [19,21]. As a point at infinity is far from all the possible neural electric sources, it brings a least effect on EEG recordings. Therefore, it provides an approximate zero reference, also a common reference for all cases in theory.

In a previous work, we have shown that a significant difference may be introduced by using different references [20] in power spectra analysis of EEG. In this paper, we will check the effect of various references on EP data. The contents include three main parts, part 1 shows the materials and methods, parts 2 and 3 show the effects of references on spatial and temporal aspects of a somatosensory evoked potential (SEP). For spatial aspects, we will show the amplitude maps shown with different color-bars; for temporal aspects, we will display the waveform changes and results of statistic tests and relative errors. And for clarity, the statistic test is focused on the selected four components P30 ($\sim$ 30 ms), P40 ($\sim$ 40 ms), N90 ($\sim$ 90 ms) and P230 ($\sim$ 230 ms).

2. Materials and methods

2.1. Sample and EEG recordings

Ten healthy right-handed adult volunteers (mean age: 27.7 years old, range 23–36 years old) were recruited from university staff and students. The study was approved by the Local Ethics Committee and in accordance with the Helsinki Declaration. Informed consent was obtained from each subject prior to the experiment. Experiments were performed in a quiet air-conditioned (20–21 °C) laboratory with soft natural light. Subjects were requested to sit in a comfortable chair and relax. The subjects kept their eyes open and fixed their gaze at a point on the wall during the experiment. The subjects were instructed to be alert to the sensation induced at the stimulation site, overly count the stimuli, and stay completely relaxed. The 124-channel electro-cap was mounted together with the auxiliary channels, including two electro-oculogram (EOGH and EOGV) channels and two mastoid reference channels (M1 and M2), thus totally 128 channels. Ag/AgCl electrodes (5 mm diameter) were carefully positioned on a nylon cap in accordance with the 10-5 extension of the International 10-10 electrode system [22], and they were attached (impedance $< 10\,\mathrm{k}\Omega$) to the scalp using electrode cream (EC2, Grass).

SEP was recorded with the unilateral left mastoid reference by using the 128-channel high density advanced neuro technology (ANT, The Netherlands) system. An example of raw data at channel PO4 (a left occipital channel) is shown in Fig. 4. Data were sampled at 2048 Hz through scalp electrodes and the band pass filters were set up at 0.05–100 Hz. The data before stimulus is 50 ms, and the stimulus interval is 524 ms. Electrical stimulation with 300 repeats was added to the little finger, the subjective pain level is 6. The details of electrical stimulus, pain level and pre-processing are shown in Appendix A. Finally, group average SEPs over artifact-free epochs of the 300 repeats are generated off-line.

Due to the individual differences of the latencies among the subjects, the four components P30 ($\sim$ 30 ms), P40 ($\sim$ 40 ms), N90 ($\sim$ 90 ms) and P230 ($\sim$ 230 ms) are selected subject by subject, the actual values of them are 35.10 ± 2.28(ms), 42.40 ± 4.91(ms), 90.20 ± 6.46(ms) and 227.00 ± 23.26(ms).

2.2. Re-references recordings

For checking the effect of different references, the EEG data are re-referenced off-line as below.

(1) *Contralateral mastoid* (*CM*) *reference recordings*: The original recording data are the left mastoid reference recordings, and as the stimulus is pain on the right hand, we noted it as CM recordings

$$V_{\mathrm{CM}} = V - TM_{\mathrm{left}}, \tag{1}$$

where the data matrix V_{CM} and V with size $n \times k$ represents scalp potential recordings at n electrodes with k samples, where T is a column vector with size $n \times 1$ and each of its elements being unity. V is the potential when it is referenced to a neutral point, M_{left} with size $1 \times k$ is the potential of the left mastoid point when referenced to a neural point and it was lost in the actual recordings V_{CM}.

(2) *LM reference recordings*:

Recordings with LM reference, noted as LMs, are [16]

$$\begin{aligned} V_{\mathrm{LM}} &= V - T(M_{\mathrm{left}} + M_{\mathrm{right}})/2 \\ &= (V - TM_{\mathrm{left}}) - T(M_{\mathrm{right}} - M_{\mathrm{left}})/2 \\ &= V_{\mathrm{CM}} - TV_{\mathrm{CM\text{-}right}}/2, \end{aligned} \tag{2}$$

where $V_{\mathrm{CM\text{-}right}}$ with size $1 \times k$ is the potential recorded at right mastoid with the left mastoid as reference, i.e., the recording in V_{CM} corresponding to the right mastoid electrode. M_{right} with size $1 \times k$ is the potential of the right mastoid point when referenced to a neural point.

(3) *Ipsilateral mastoid* (*IM*) *reference recordings*: Recordings with the right mastoid, noted as an IM reference as the stimulus is on the right hand, are

$$\begin{aligned} V_{\mathrm{IM}} &= V - TM_{\mathrm{right}} = (V - TM_{\mathrm{left}}) - T(M_{\mathrm{right}} - M_{\mathrm{left}}) \\ &= V_{\mathrm{CM}} - TV_{\mathrm{CM\text{-}right}} = V_{\mathrm{CM}} - (V_{\mathrm{CM}} - V_{\mathrm{LM}}) * 2 \\ &= 2V_{\mathrm{LM}} - V_{\mathrm{CM}}. \end{aligned} \tag{3}$$

This result means that V_{IM}, V_{LM} and V_{CM} are related to each other, where $V_{\mathrm{CM\text{-}right}}$ is the potential of the right mastoid point when referenced at the left mastoid.

(4) *Vertex Cz reference recordings*: Recordings with the vertex Cz electrode, noted as Cz reference (Cz), is

$$V_{Cz} = V - TM_{Cz} = (V - TM_{left}) - T(M_{Cz} - M_{left}) = V_{CM} - TV_{CM-Cz}, \quad (4)$$

where V_{CM-Cz} is the potential of the vertex Cz electrode when it was referenced at the left mastoid.

(5) *Average reference recordings*: Recordings referenced to the mean of all recording channels at each time point, noted as average reference (AR), are obtained by [16]

$$V_{AR} = V - T_{mean}(V) = V_{CM} - T_{mean}(V_{CM}), \quad (5)$$

where mean(∗) denotes the spatial average over all recording channels at each temporal sample point.

(6) *Infinity reference recordings*: Based on the theoretical electric relation between the scalp recordings with a specific reference and the neural electric sources, for a 'zero of potential' reference such as a reference at infinity (IR), we have

$$V = GS. \quad (6)$$

While for the CM recordings V_{CM}, we have

$$V_{CM} = G_{CM}S, \quad (7)$$

where G and G_{CM} are the transfer matrices determined by the head model, source configuration, electrode montage and reference, infinity and left mastoid, respectively. Based on Eqs. (6) and (7), we have [19]

$$V = GS = G((G_{CM})^{+}V_{CM}) = RV_{CM}, \quad (8)$$

where $(*)^{+}$ denotes the Moore–Penrose generalized inverse, and R is the transfer matrix. Based on Eqs. (6)–(8), we do not need to know the actual source S because what we really need are the transfer matrices G and G_{CM} which can be defined being the transfer matrix from the equivalent distributed source of the actual sources. Thus G and G_{CM} are determined by the head model, here a three-concentric sphere head model [23], configuration of the 124 electrodes [22] and the anatomical position of the equivalent distributed source, here a dipole layer above the cortical surface [24]. The details including the algorithm procedure are shown in the Appendix B and the original paper [19].

(7) *Implementation of the re-referencing algorithms*: Based on the above formulae, the recordings with the other references IM, LM, AR, Cz are readily obtained from the actual recordings V_{CM}, by the above shown simple formulae with Matlab or any other program software, and the recordings with IR was obtained by a specific algorithm shown in Appendix B.

2.3. Data management and statistical analysis

The dependent measures of this study were focused on the amplitudes of the SEPs. The independent factors are (I) the references and (II) the peak stages. Thus, two-way repeated measures ANOVA was conducted. When significant overall effect was observed, the Tukey post hoc test was applied to examine the multiple comparisons of means. We adopted $p < 0.05$ or < 0.01 as statistical significant level, $p < 0.001$ as very significant level. Besides, in order to compare the effects induced by different references on the SEP response, the relative error (RE) was defined as the ratio of the standard deviation of the difference between two recordings corresponding to two references such as LM and IR to the standard deviation of one of the two recordings such as IR.

3. Results

3.1. Peak stages of the Finger SEPs

From the overlay of 124-channel somatosensory responses, four main stages of peak latencies were demonstrated from the grand average: 30, 40, 90 and 230 ms. Thus, the statistical analyses took into account of these peak stages. The x-axis and y-axis values of each peak for all the six references are the same for each subject. As shown in Fig. 1, the x-values of 30 and 40 ms were almost the same, but larger than those in 90 and 230 ms (pair-wise tests between P230 and P40, $p = 0.062$, between P230 and P30, $p = 0.067$). In contrast, the location profile of y-axis values varied greatly among the peak stages as seen in Fig. 1 (pair-wise test between P230 and P30\P40, $p = 0.004\backslash 0.01$; between N90 and P30, $p = 0.025$).

These results show us the activities transferred from postlateral to anterior of median line during these four peak stages after the stimulus elicited as shown in Fig. 1 based on the group means with standard-deviation bars of x-value and y-value (normalized in proportion). The effect of peak stages on the x-axis spatial positions of the four components is distinct only between the early two and the last peak.

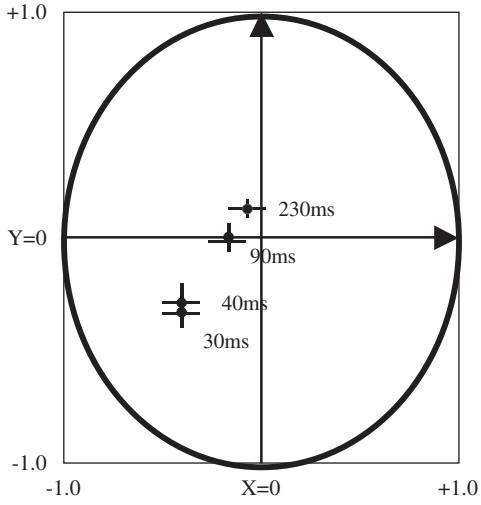

Fig. 1. The Spatial positions in relation to peak activations. The group means (dots) and standard-deviation (bars) of the focal maximal sites indicate that y-values differ between 30 ms/40 ms and 90 ms/230 ms, while x-values differ only between 30 ms/40 ms and 230 ms.

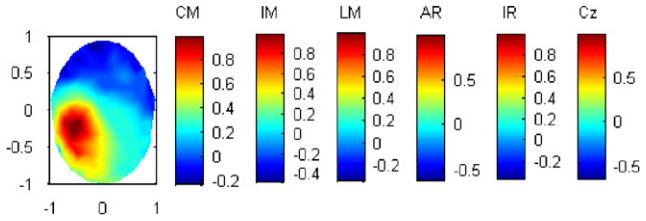

Fig. 2. Map of group averaged potential amplitude with automatically selected colorbars. The map (nose up) is the ERP at a selected time point (33 ms). The colorbars shown right side are correspond to different references, AR—average, IM—right mastoid (ipsilateral reference), CM—left mastoid (contralateral reference), LM—linked mastoids (bilateral reference), IR—infinity, Cz-vertex Cz electrode reference.

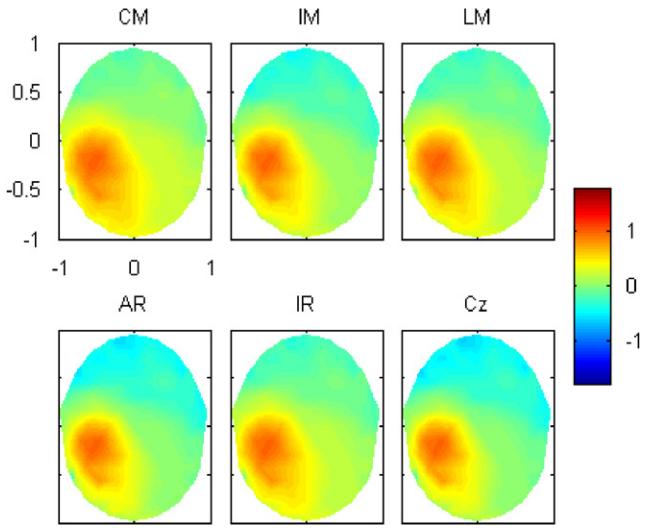

Fig. 3. Maps of the group averaged potential amplitudes with a unified color-bar. It is the potential at 33 ms after stimulation with six different references under a unified color-bar $(-1.831, +1.831)(\mu V)$.

3.2. Effects on the spatial amplitude maps

Fig. 2 shows the spatial amplitude map of the group average data at 33 ms (P30) with automatically selected color-bar by Matlab, the maps for the six references look similar (only shows one and omits the other three components P40, N90 and P230). The same shape map and different color-bars again confirm the fact that the effect of a reference choice is to add or subtract a constant value at all locations at each instant, like raising or lowering the water level in a landscape, without changing the surface [7].

Fig. 3 shows the spatial maps of the group averaged potential amplitude, where the absolute values of the maximum and minimum of the color-bars were being selected the maximum of the potential at 33 ms (P30) of the potentials (the figures of the other three components are omitted). The four sub-maps look similar, but distinct differences can be found, including the range of the active region and polarity.

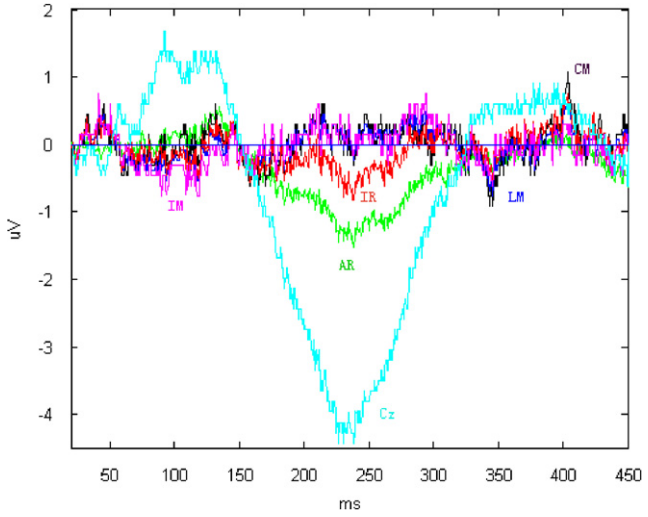

Fig. 4. Waveforms comparison of a selected channel of the group averaged data. The lateral axis is in ms, AR—average reference in green, IR—Infinity reference in red, CM—left Mastoid reference in black, LM—Linked mastoids in blue, IM—right mastoid in magenta, Cz–Cz electrode reference in cyan. In this map, LM and CM are almost overlapped. PO4 is an electrode in parietal region [22].

3.3. Relative error of potentials with different references

For the waveforms of group average data over the 10 subjects, the amplitude difference is distinct, and the polarity difference is also very distinct as shown in Fig. 4. The relative errors (REs) over all the 124 channels for a spatio-temporal window from 20 to 500 ms after stimulus is defined as the ratio of the standard deviation of the difference between CM\IM\LM\AR\Cz and IR to the standard deviation of the IR data are 40%, 41%, 35%, 59% and 219%, respectively, while REs between CM\IM\LM\Cz and AR are 110%, 113%, 108% and 211%, respectively. REs between IM\LM\Cz and CM are 33%, 16% and 209%, respectively. REs between LM\Cz and IM are 16% and 210%, respectively, and RE between LM and Cz is 212%.

If we check RE channel by channel, for example, a left occipital channel PO4, REs between CM\IM\LM\AR\Cz and IR are 109%, 111%, 96%, 160% and 596%, REs between CM\IM\LM\Cz and AR are 118%, 121%, 117% and 227%, respectively. The five curves of PO4 are shown in Fig. 4. Similarly, for a left temporal-frontal channel FC5, REs between CM\IM\LM\AR\Cz and IR are 52%, 53%, 46%, 76% and 285%, respectively; REs between CM\IM\LM\Cz and AR are 291%, 299%, 288% and 562%, respectively (figure omitted). Fig. 4 clearly shows that the widely used AR may cause polarity reverse, and different references may result in quite different waveforms, so again it shows the necessity of a unified reference in practice.

3.4. Difference of peak potentials with different references

The statistical results indicate very significant overall effects by peak stages on the peak potential amplitude ($F = 52.274$, $p < 0.001$). The difference in the mean values of potentials among the different references is very significantly

different ($F = 7.671$, $p < 0.001$). The effect of different references depends on what the peak (component) is. There is a statistically very significant interaction between reference and peak ($F = 15.361$, $p < 0.001$).

In order to reveal the detail of the effect of references on the values of the peak potential amplitude of the four components, the original data are shown in Fig. 5. Pairwise multiple comparisons (Tukey test) show that the differences of Peak values are very significant ($p < 0.001$) between Cz and IR\CM\IM\LM, and significant ($p < 0.01$) between Cz and AR for component N90 ms, very significant ($p < 0.001$) between Cz and IR\CM\IM\LM\AR, significant between IM\LM and AR ($p < 0.01$), CM and AR ($p < 0.05$) for component P230 ms, while all the other cases are not significant. The potential value order is CM/IM $\geqslant$ LM > IR > AR > Cz of the component P230, and such an order is the same as the case revealed in a study of spontaneous EEG power mapping except Cz which has not been checked in the previous EEG study [20]. Look at the values of the potential amplitudes (Fig. 5) and pay attention to the low signal/noise ratio shown in Fig. 4, we believe the non-significant effect on the amplitude of the references for the early components P30 and P40 is partly due to the noise effect.

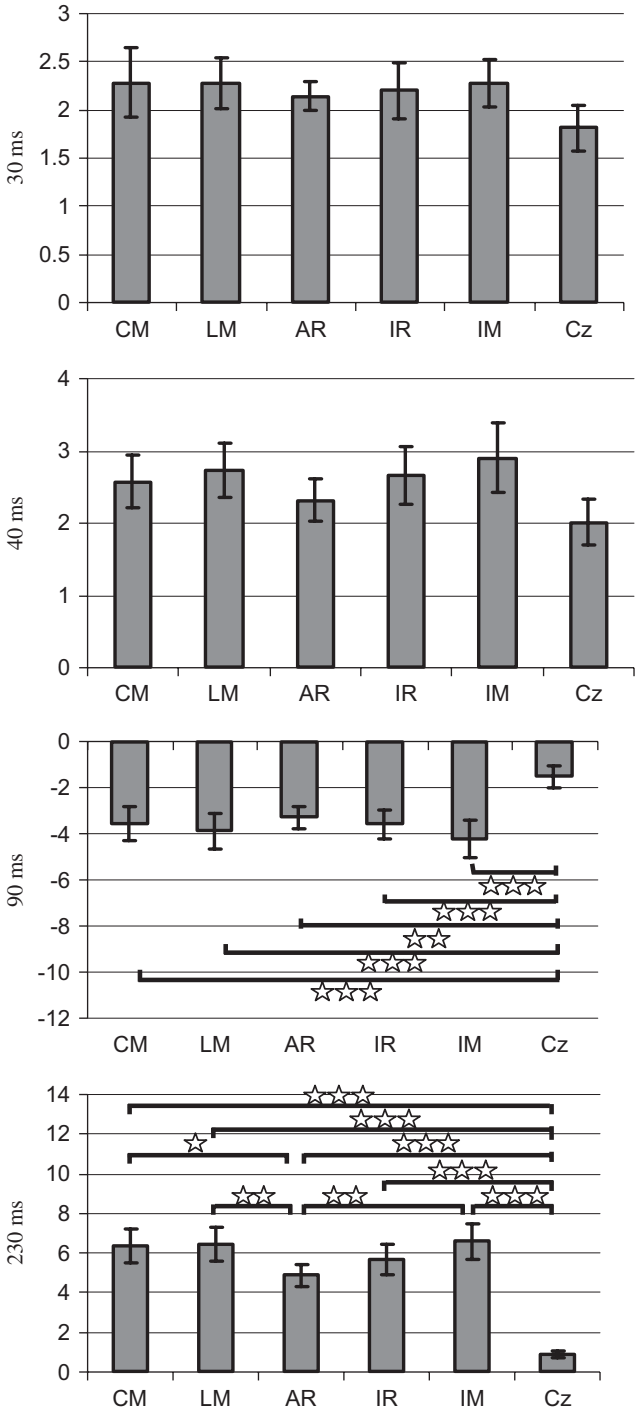

Fig. 5. The effect of references on peak amplitudes (μV) at peak stages (means and standard error of mean). The effect of references on peak amplitudes at 30, 40, 90, and 230 ms. Post hoc test significant differences as indicated by $*$ as $p < .05$, $**$ as $p < .01$, $***$ as $p < .001$.

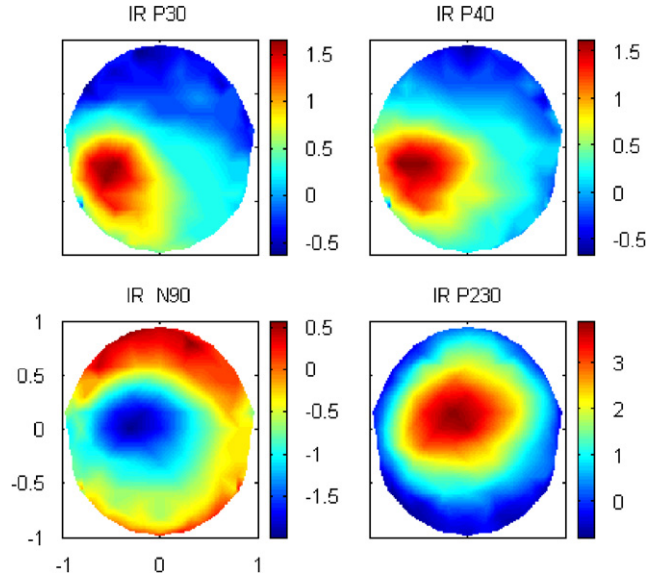

Fig. 6. The spatial maps of the four peaks of SEP with IR. The figures show the difference of the active centers at the four peak stages.

3.5. Reference effect on the peak potential difference

The pairwise multiple comparisons show that the differences of the potential amplitude values among the four peaks are very significant ($p < 0.001$) between P230 and N90\P40\P30, N90 and P40\P30 for CM, IM and LM. For AR, it is very significant ($p < 0.001$) between P230 and N90, N90 and P40\P30, and significant ($p < 0.05$) between P230 and P40\P30. For IR, the differences of amplitudes are very significant ($p < 0.001$) between P230 and N90/P30, N90 and P40/P30, significantly different ($p < 0.01$) between P230 and P40. For Cz, the amplitudes are very significantly different ($p < 0.001$) between N90 and P40/P30, and significantly different ($p < 0.05$) between P230 and N90 (Fig. 6). These results show that the significances of the amplitude differences between peaks are different for different references, i.e., the differences of the potential amplitude values among the four peaks between P230

and N90\P40\P30 are very significant ($p < 0.001$) for CM, IM and LM, and significant ($p < 0.05$) for AR and IR.

4. Discussion and conclusion

4.1. Spatial maps and ghost potential fields

Figs. 2 and 3 showed that if a fixed color-bar is chosen, the spatial amplitude maps corresponding to different references may have some distinct differences. In fact, Tomberg et al. [25] claimed that the choice of an "improper" reference, where AR was adopted, could introduce distortions in the map and that "ghost potential fields" may be created. In Fig. 3, there is also some minor differences that may be considered as "ghost" point. Geselowitz [7] had pointed out that 'ghost' potentials might appear with a change of reference under at least two circumstances. First, if map contours are sparse, some topographical features, such as local extrema, may be present in one map and not be present in the other. Another circumstance is if special significance is placed on the amplitude and sign of the potential, even when two maps have identical iso-potential contours, i.e., contours that pass through the same points on the scalp. For our case, the problem is that the similar information is displayed in different color range of a common color-bar, thus corresponding to a third case that may generate "ghost" potential.

In a word, the effect of reference on spatial map is not a crucial change, but a phenomenal change that we need to know in practice. And a unified reference will benefit the comparison between Labs.

4.2. Difference in potentials with different references

The relative errors between potentials (from 20 to 500 ms after stimulus) with different references are quite large as shown in Section 3.3. This phenomenon is due to the brain neural electrical activity actually being a spatio-temporal process. According to Figs. 1 and 6, the early stages (P30\P40) are more close to the left mastoid (CM), the late stages (N90\P230) are more close to the vertex (Cz) and the predominant neural activities (P230) of a SEP is in the sensory cortex which covers the vertex (Cz) region, so the REs between Cz and all the other references are distinctly larger than of any other cases. This fact means that we cannot chose Cz as the reference in a SEP study, and according to the REs between CM\IM\LM\AR\Cz and IR, LM seems to be the second choice following the ideal IR.

4.3. Difference in peak potentials with different references

According to the results in the above sections, there is a very significant interaction between reference and peak (Section 3.4), in another word, the difference between the four peak potentials is different for different references (Section 3.5). The reason is due to the spatial maps of the four peaks being different as shown in Fig. 6. As the spatial maps are different, thus a reference will add or subtract different constants to the four components. Meanwhile, four of the six references are of specific spatial positions on the brain surface (CM-left mastoid, IM-right mastoid, LM-linked mastoids and Cz-vertex), the other two, AR and IR, are relatively independent to a concrete spatial positions on the brain surface, thus the differences among the four peak values may be different for different reference adopted, and such a fact finally may result in different significances of the differences between peak amplitudes as revealed in Section 3.5. Specifically, for this SEP example, due to the proximity of Cz to the position of the major activity of the late component at 230 ms, it certainly will result in the different significance when comparing with the other references. This fact again explains the effect of the reference on amplitude depends on various components.

4.4. General comments on the conventional references: IM\CM\LM\AR\Cz

One basic technique of EEG study is the reference, and it is an ongoing debate topic. Ideally, the potential recordings should represent a pure measure of activity near the recording site. The difficulty is that any potential is a relative measure that necessarily compares the recording site with another site (reference). If there is any un-neutral potential at the reference site, it will contribute equally to the resulting recordings of all channels, this is the case of the IM\CM-reference and other similar cases such as vertex (Cz), nose reference etc. Although it is sometimes argued that the mastoids (IM\CM) are relatively inactive, this has been persuasively shown to be false [26]. For the commonly recommended average reference (AR) [15], it assumes that the mean of all recording channels at each time point is approximately an inactive reference. However, this approximation is valid only with accurate spatial sampling of the whole scalp fields. Accurate sampling requires sufficient electrode density and full coverage of the head surface, otherwise, the reference effect will exist and bias the scalp recordings [16]. For the LM reference, if we consider it as a special case of the average reference (AR), it would be not a zero or a constant in a recording process in general.

In this work, for a SEP, our results confirm that different reference may result in different potential amplitude and even the polarity. It also may change the maps if the color-bar is not selected properly. While for temporal waveform, it may introduce significant change of the data including the waveform and polarity, thus may affect the physiological explanation of possible activities.

Also based on a detailed study [16], it was concluded that the choice of reference has substantial effects on analysis and interpretation, and recommended that the optimal choice of reference site depended on the study and the purpose of the analysis. Also, Hagemann et al [1] concluded that the choice of the EEG reference might be a critical issue for the study of anterior asymmetry in the alpha band, and they specially noted that reviews of the empirical literature should not treat the findings of different reference schemes as interchangeable.

4.5. Reference at infinity (IR)

Based on the technique improvement of multi-channel high-density recordings and modern computation technique, a better reference scheme seems possible as shown in our previous study [19,21] where a detailed simulation study of a new reference electrode standardization technique (REST) was given, which transfers a practical reference to IR, and the results showed that REST is very effective for the most important superficial cortical region and the standardization could be especially important in recovering the temporal waveform.

As the reference at infinity is far away from all the scalp electrodes thus providing a neutral reference [19–21], and the above results strongly argue a common accepted reference is very important for various applications, we recommend IR as the proper choice for further test.

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Appendix A. Electrical stimulus, pain level and data preprocessing

Electrical stimuli (0.2 ms square wave pulse, 300 trials, 1.9 Hz) were applied to the right little finger (Digit-5, D5) via a pin-electrode (diameter 1.24 mm). The electrode was fastened on a velcro-ring device, and the stimuli induced a clear and distinct pricking sensation at the fingertip. The stimulation reference electrode was placed at the base of the fifth metacarpophalangeal joints. A verbal rating scaling system (VRS) to assess the pain intensity was introduced to the subject prior to carrying out the experiment. The subject was familiarized with the VRS system and asked to report a number corresponding to the psychophysical feeling when the intensity of the stimulus varied.

The pain intensity was rated on a 0–10 verbal rating scale defined as follows: (0) no change, (1) barely intense, no pain, (2) intense, no pain, (3) fairly intense, but no pain, (4) slight pain (pain threshold), (5) mild pain, (6) moderate pain, (7) moderate–strong pain, (8) strong pain, (9) severe pain, and (10) unbearable pain. This study was aimed at the effects of different references, hence the activation of somatosensory cortex for D5 by noxious stimulation of moderate pain intensity was adopted to study the possible effect of reference on SEPs.

In order to define the painful intensity of the stimulation for each subject, a procedure for stimulation intensity verification was conducted before the experiment. The painful intensities for D5 were recorded 5 times by the method of ascending limit, and each mean value was used as the stimulation intensity for the little finger according to subjective rating of intensity for moderate pain (intensity—6). The painful intensities of stimulation over the group ($n = 10$, mean $\pm$ SD) were defined at 7.9 ± 3.0 mA by the subjects.

Somatosensory evoked potential (SEP) was recorded with the unilateral left mastoid reference and the vertical and horizontal eye movements (EOG) were recorded from a bipolar lead placed next to the orbit and they were used to monitor the EOG artifacts in EEG data. Fifty ms of pre-stimulus and 524 ms of post-stimulus were recorded as one epoch. Then these epochs were conducted to linear-detrend, artifact rejection and bad electrodes processing. The artifact rejection methods consisted of excluding the epochs with large amplitude (exceed ± 80 mV), DC bias, eye blinking, and slow eye movement coinciding with EOG. After rejection of the EOG contamination and non-specific artefacts, each set of EEG data was subjected to averaging for each subject. All electrodes were inspected, and bad electrodes were then interpolated from the neighborhood electrodes with the virtue values. Some of them, which could not be fixed, were removed. All these data processing procedures were carried out using the EEprobe program (ANT, The Netherlands). The four specific peaks of SEPs were extracted according to the compressed waveform of 124 channels.

Appendix B. Infinity reference recordings

The infinity reference (IR) is based on the fact that the use of scalp potentials to determine the neural electrical activities or their equivalent sources does not depend on the reference [7], so the equivalent distributed sources of the unknown underneath neural sources may be approximately reconstructed from scalp EEG recordings with a scalp point or average as reference [19]. Then the potentials referenced at infinity are approximately reconstructed from the equivalent distributed sources.

The primary simulation studies with assumed neural sources in a three-concentric sphere head model included effects of electrode number, volume conductor model and noise effects. The results showed that IR is very effective for the most important superficial cortical region and the standardization could be especially important in recovering the temporal waveform and frequency domain power information of EEG recordings [19]. Recently, this technique has been checked for a realistic head model with boundary element method, and the results further confirmed its effectiveness in practice [21].

About the principle, according to the electromagnetic theory, for a ‘zero of potential’ reference, we have

$$V = GS, \tag{6}$$

where G is the transfer matrix determined by the head model, source configuration, electrode montage and reference. While for CM (the left mastoid) recordings V_{CM}, we have

$$V_{\mathrm{CM}} = G_{\mathrm{CM}}S, \tag{7}$$

where the transfer matrix G was changed to G_{CM}. Now based on the equations (6) and (7), we see that the source (S) is the

same, and this fact just means that the reference will not affect the use of noiseless scalp potentials to solve the localization of neural active sources [7,18]. Based on Eqs. (6) and (7), we have

$$V = GS = G((G_{CM})^{+}V_{CM}) = RV_{CM}, \quad (8)$$

where $(*)^{+}$ notes the Moore–Penrose generalized inverse, i.e. a minimum norm solution, and R is our final correction matrix which is determined by the transfer matrix G_{CM} and G, and so it is determined by the head model, electrode montage and the primary reference. Based on Eqs. (6)–(8), we do not need to know the actual source S because what we really need are the transfer matrices G and G_{CM}. Hauk [27] recently pointed out that in the absence of reliable a priori information about the generating sources, the maximum-likelihood approach, the minimum norm approach, and the resolution optimization approach all yield the minimum norm pseudoinverse (MNP), and strongly suggests that the classical minimum norm solution is a valuable method whenever no reliable a priori information about source generators is available. For our reference-correction problem, we don't know anything of the generators in general, thus a simple and meaningful MN inversion is utilized here.

As potential produced by any sources can be equivalently produced by a source distribution which encloses the actual source inside the distribution [24,28], we may assume an equivalent source distribution on the cortical surface that encloses all the possible neural electric sources inside, then the matrix G and G_{CM} are surely determined by the head model, electrode montage and the spatial geometric information of the assumed equivalent source distribution model [19,24]. In this work, the head model is assumed to be the usual three-concentric-sphere model, the radii of the three concentric spheres are 0.87 (inner radius of the skull), 0.92 (outer radius of the skull) and 1.0 (radius of the head), and the conductivities are 1.0 (brain and scalp) and 0.0125 (skull). The forward theory of the three-concentric-sphere model can be found in literature (Rush and Driscoll, 1969). The assumed equivalent source distribution model is assumed to be a discrete equivalent dipole layer source on a closed surface formed by a spherical cap surface with radius $r = 0.869$ and a transverse plane at $z = -0.076$. A discrete approximation of the closed surface was further assumed to be formed of 2600 radial dipoles on the spherical cap surface and 400 radial dipoles on the transverse plane, so the total number of the equivalent sources was $2600+400=3000$ [19]. The electrode montage is the 10-5 extension of the International 10-10 electrode system [22] with 124 effective electrodes involved in the calculation (the EOGH and EOGV are excluded).

Due to the limited spherical cap electrode array on the scalp surface, the theoretically closed dipole layer [24,28] could not be perfectly reconstructed, the equivalence between the inverted dipole layer and the neural sources inside the layer is approximate and the approximation is different for different dipole locations and orientations, so the efficiency of IR also is different for different dipole locations and orientations. For the same three-concentric sphere head model, equivalent source model and a similar electrode montage, the effectiveness of IR has been evaluated in detail in literature [19], and the results show that REST may be very effective for the most important superficial cortical region [19].

In summary, IR was realized by the following procedures:

(1) The electrode montage is given and the scalp recordings V_{CM} are got in actual experiments . A head model such as the three-concentric sphere head model as noted above is assumed and an equivalent source model such as the discrete dipole layer source model noted above is assumed too.
(2) Based on the above electrode montage, head model and equivalent source model, calculate the transform matrix G in Eq. (6) and matrix G_{CM} in Eq. (7) by EEG forward formula [23,24].
(3) Calculate the general inverse G_{CM}^{+} of the matrix G_{CM} by singular value decomposition (SVD) and calculate the standardization matrix R in Eq. (8) from G_{CM} and G_{CM}^{+}.
(4) Calculate the final reconstructed EEG recording V according to Eq. (8) from the known recording V_{CM}.

The program was developed under Matlab 6.1 and it may be run on Windows 9x/NT/2000 systems.

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Dezhong Yao Professor and the Dean of the School of Life Science and Technology University of Electronic Science and Technology of China, Chengdu, China. Dr. Yao received his Ph.D. in 1991 from the Chengdu University of Technology, China, his Master's degree in 1988 from the University of Zheijing, Hangzhou, China, his Bachelor's degree in 1985 from the Southwest Univeristy, Chongqing, China. In 1991 he was a faculty member of UESTC, and professor since 1995, UESTC. He was a visting scholar from July 1997 to August 1998 in the University of Illinois at Chicago, USA and from January 1999 to June 1999 in the Beijing Lab of Cognitive Science, China. He was a visiting Professor from November 2000 to May 2001 in the McMaster University, Canada and from November 2003 to February 2004 in Aalborg University, Denmark. Since November 2001, he is the Dean of the School of Life Science and Technology, UESTC.
He is a member of the International Advisory Board of Physics in Medicine and Biology, 2005.
His areas of interest include Fractional Brain mapping (EEG/ERP/fMRI), Biomedical signal/image processing.

Li Wang Research Assistant of Human Brain Mapping and Cortical Imaging Laboratory, Ph.D. candidate in Biomedical Engineer of the International Doctoral School in Biomedical Sciences and Engineering, Center for Sensory-Motor Interaction, Aalborg University, Denmark. He received his M.S.c. degree in Acoustics in 2002 from the Aalborg University, Denmark. He accepted Postgraduate degree in advanced education in Industrial Automation, Automation Research Center of the Northeastern University, Shenyang, PR China from 1997 to 1999. He obtained College Diploma in the Industrial Enterprise Electric Automation, Industrial Technology Academy of the Harbin University of Science and Technology, Harbin, PR China in 1991. His work focuses on cortical plasticity and pain study.

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Lars Arendt-Nielsen Professor, Dr. Med. Sci., Ph.D. Center for Sensory–Motor Interaction, Aalborg University, Denmark. Prof. Lars Arendt-Nielsen accepted Dr. Sci. in Medicine, The Medical Faculty, Aarhus University in 1994; Ph.D. in Biomedical Sciences, Faculty of Science and Technology, Aalborg University in 1987; Postgraduate student, University College, London 1983–1984; M.Sc. in Biomedical Sciences, Aalborg University in 1983; and B.Sc. in Biomedical Sciences, Aarhus University in 1980.

2005–2011 Councillor, International Association for the Study of Pain.
2004– Board member, University Board, Aalborg University.
2001–Board member, Department of Health Science and Technology, Aalborg University.
2001–Director, Center for Biomedical Sciences. A National Research Program on Pain and Brain.
2000–2005 Member of the Danish Research Education Council.
2000–2005 Member of the Danish Technical Research Science Council.
1999– Head of the International Doctoral School of Technology and Science, Aalborg University.
1997–Director of the International Doctorate School in Biomedical Science and Engineering, Center for Sensory-Motor Interaction, Aalborg University.
1993–1999 Head and principal investigator, Danish Cancer Pain Research Center.
1993–Principal investigator, Center for Sensory–Motor Interaction.
1993–Professor in Biomedical Sciences, Aalborg University, Department for Health Science and Technology, former Department Medical Informatics and Image Analysis.

1992–1999 Member of the Board, The Open University, Aalborg.
1992–1993 Senior Research Fellow, Aalborg University.
1990–1991 Senior Research Fellow, The Danish Cancer Society.
1988–1989 Associate professor, Aalborg University.
1987–1988 Assistant professor, Aalborg University.
1987–Research fellow, The Danish Cancer Society.
1984–1987 Ph.D. student, Aalborg University.
1983–1984 Research fellow, Department of Clinical Neurophysiology, The National Hospital for Nervous Diseases, London.
1980–1981 Research assistant, Department of Physiology, Aarhus University.

Publications:
He has published approximately 455 publications and he is first author of approximately 86 of them. Most papers concentrate on human pain research (experimental and clinical) and on motor control.
Full list: Articles — Abstracts and Proceedings

Research activities:
The research activities have been concentrated on biomedical sciences within neuroscience. The specific research areas have been on pain and on motor control. The main focus is on human experimental pain research—basic and clinical applications. A substantial network of international collaborations with 15 different countries has been established with universities, hospitals, and the biomedical/pharmaceutical industry. Satellite laboratories have been established in six different countries.

Lectures:
Has participated in approximately 140 international conferences with related published abstracts/proceedings and he has given several lectures at Danish and foreign universities and hospitals. Has given 102 invited lectures at international meetings/workshops/seminars. Furthermore, he has hosted the six Ph.D. courses "The Neurobiology of The Pain System" and "Sensory physiological testing methods".

Teachings:
Has 17 years of university teaching and he has achieved experience by teaching nurses, physiotherapists and he has held specialising-courses for medical doctors within neurology, orthopaedics, anaesthesiology, oncology and general practice. Has acted as Ph.D. supervisor for 15 students (14 graduated).

Appointments:
Editorial board member of "Journal of Electrophysiological Kinesiology", "European Journal of Pain, "Journal of Clinical Pain" and reviewer for "Clinical Neurophysiology", "Brain", "Journal of Applied Physiology", "Muscle and Nerve", "Pain", "Journal of Neurophysiology", "Journal of Psychophysiology", "Acta Anaesthesiologica Scandinavica", "The Journal of Rheumatology", "Progress in Neuro-Psychopharmacology & Biological Psychiatry", "Anesthesiology", "Medical Engineering & Physics", Experimental Brain Research", "Brain Research", "Journal of Neuroscience Methods", "Journal of Musculoskeletal Pain", "European Journal of Pain", "Pain", "Journal of Clinical Pain", "European Journal of Applied Physiology", Anesthesia & Analgesia, and "The Wellcome Trust". Guest Editor, Clinical Journal of Pain, Special Issue on Musculoskeletal Pain—new directions and perspectives, 2001. Guest Editor, European Journal of Pain 2002. Editor on "Smerter—en lærebog", FADL's Forlag, Denmark, Danish Educational Book on Pain, 2002. Section Editor, Encyclopedic Reference of Pain, Springer Verlag, 2002. Guest Editor, European Journal of Pain Special Issue on Gender and Pain, 2004.
He has been in organizing/scientific committee for six international congresses. EEC expert reviewer Biomed I, EEC expert advisory group on Biomedical Sciences, Head of campaign "Year of the Brain 1997—Pain".

GCP experience:
Has been principal investigator for seven drug screening GCP studies and one large multicenter study. Collaborations on GCP studies have been performed with GSK, Pfizer, BSP Pharma, ASTRA, Pierre Fabre, Allergan, and Coloplast.

Others:
Chairman of the Local Organising Committee of the 25th Annual SASP (Scandinavian Association for the Study of Pain) Meeting, 4–7 April 2002, Aalborg, Denmark (500 delegates).