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Why do we need to use a zero reference? Reference influences on the ERPs of audiovisual effects

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Abstract

Using ERPs in the audiovisual stimulus, the current study is the first to investigate the influence of the reference on experimental effects (between two conditions). Three references, the average reference (AR), the mean mastoid (MM), and a new infinity zero reference (IR), were comparatively investigated via ERPs, statistical parametric scalp mappings (SPSM), and LORETA. Specifically, for the N1 (170–190 ms), the SPSM results showed an anterior distribution for MM, a posterior distribution for IR, and both anterior and posterior distributions for AR. However, the circumstantial evidence provided by LORETA is consistent with SPSM of IR. These results indicated that the newly developed IR could provide increased accuracy; thus, we recommend IR for future ERP studies.

Descriptors: ERPs, Zero reference, Average reference, Mean mastoid, Audiovisual effects, Statistical parametric scalp mapping (SPSM)

Human sensory systems are interconnected to integrate stimuli in different modalities; thereby, they achieve unified and coherent percepts of environmental events. Event-related potentials (ERPs) with a high temporal resolution are very useful for determining which stage or stages of processing are influenced by multisensory perception. Using ERPs, researchers found that unisensory areas can be engaged in multimodal processing at both very early and late stages after stimulus onset (Giard & Peronnet, 1999; Meylan & Murray, 2007; Molholm et al., 2002; Murray et al., 2005; Talsma, Doty, & Woldorff, 2007).

However, the voltage waveforms (i.e., ERPs) are not unique because of their dependence on the choice of a reference. Furthermore, different voltage waveforms induced by reference effects could lead to a change in the observed scalp distribution of the observed significant difference between different conditions (i.e., the experimental effects). Different scalp distributions observed as experimental effects, which result from the choice of reference, could result in quite different conclusions for the same experiment. Therefore, the choice of reference is a critical issue for obtaining reliable ERPs when investigating cognitive processing. However, the current popular (nonzero) references, such as the mean mastoid reference (MM), the averaged reference (AR), and the vertex reference (Cz), might induce some unknown false fluctuations that destroy the genuine electroencephalogram (EEG) information, as confirmed in previous studies (Kayser & Tenke, 2010; Nunez, 2010; Qin, Xu, & Yao, 2010; Yao, 2001; Yao, Wang, Arendt-Nielsen, & Chen, 2007; Zhai & Yao, 2004).

To minimize the possible effect of different references in ERP studies of audiovisual effects, previous reports have implemented different reference schemes. The most popular choice is MM in the study of audiovisual effects. The potential reasons could be the following: (a) The ideal choice is a zero or neutral point; however, there is no such point on the human body surface (Nunez & Srinivasan, 2006; Yao, 2001), and any nonzero reference choice has both benefits and drawbacks. The best advice is usually to look at data with a few different references (Luck, 2005); (b) The MM effect would be the same across different laboratories, and the other popular choice (AR) in ERP studies (Bertrand, Perrin, & Pernier, 1985; Dien, 1998; Junghofer, Elbert, Tucker, & Rockstroh, 2000; Nunez, 1997; Nunez & Srinivasan, 2006; Tucker, 1993) could be different among different laboratories because they might actually adopt different electrode montages, including a different number of electrodes. However, these instances did not include a case in which there was a zero reference (Marzetti, Nolte, Perrucci, Romani, & Gratta, 2007; Yao, 2001). In fact, MM is challenged by methodological criticisms that, in turn, dramatically limit the neurophysiologic interpretability of the results. For example, our previous simulation showed that the power of MM significantly shifts to frontal and superficial positions (Yao et al., 2005). At the same time, our previous results also do not support AR as a common reference because the power and scalp network structure of EEG with AR can be distorted significantly (Qin et al., 2010).

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Moreover, there has also been some research adopting the nose as a reference because it is a long distance from the regions of interest (ROIs), such as visual- and auditory-related regions (Banerjee, Snyder, Molholm, & Foxe, 2011); however, some research has indicated that auditory cortex activity should also be reflected in a frontocentral ERP scalp topography because of a major contribution from a dipolar pair of sources in the superior temporal plane, pointing upward and slightly forward (Busse, Roberts, Crist, Weissman, & Woldorff, 2005).

In the present study, three references, that is, AR, MM, and the infinity zero reference (IR, realized by the software REST, www.neuro.uestc.edu.cn/rest), were comparatively investigated via ERP waveforms, voltage topographies, statistical parametric scalp mappings (SPSM), and electric tomography (LORETA). The considered measure is the spatial distribution of the electrodes, which have a significant ERP difference between attending audition (A) and attending vision (V) in a stimulation of the same audiovisual (AV) events. Unlike the traditional reference-related studies, which emphasized the different references that affect ERPs that were generated under one experimental condition, the present work first investigated the reference influence on the experimental effect (between two experimental conditions); thus, it is more similar to cognitive neuroscience research, which concerns the temporal aspects of the two ERPs that are evoked under two conditions. Although the spatial distribution pattern of ERPs (i.e., elicited by one condition) is reference free (Pascual-Marqui & Lehmann, 1993; Yao, 2001), the calculated experimental effects by subtraction (i.e., the ERP difference between two conditions) can be altered by different references, and such a phenomenon could induce different explanations of the underlying neural-cognitive functions. Therefore, the choice of reference is a crucial step.

Materials and Method

Participants

Twelve right-handed male subjects (20–23 years of age, mean age 21.4 years) participated in the experiment. All participants reported normal hearing and normal or corrected-to-normal vision. None had a history of mental or neurological problems. Informed consent was obtained prior to the study, and the participants received monetary compensation after the experiment. All of the materials and procedures were approved by the Institutional Review Board of Chongqing University of Posts and Telecommunications.

Stimuli and Design

A fixation cross ($0.5° \times 0.5°$) was presented at the center of the display throughout the entire block. The stimuli included three conditions: The first stimulus condition was only an auditory stimulus, that is, a 1000 Hz pure tone (50 ms duration; 75 dB sound pressure level [SPL]), which was presented from a single JBL speaker located at the top of the monitor on which the visual stimulus was present. The second stimulus condition was a visual stimulus alone, which was a white horizontal raster ($0.75° \times 0.75°$) presented above a fixation cross. A third stimulus condition included an auditory and visual stimuli that were simultaneously presented. The auditory and visual stimuli were in close proximity, with the speaker placed on the top of the monitor, in vertical alignment with the visual stimulus.

Participants were required to fixate the cross and to minimize eye blinks and body motion during all of the experimental blocks. The eye position was monitored with horizontal and vertical electrooculogram (EOG) recordings. Participants were instructed to make a button-press response with their right index finger when an attended stimulus was detected, as quickly as possible without making errors. Three stimulus conditions were presented with equal probability, in random order. The stimulus onset asynchrony (SOA) varied randomly between 1,000 and 1,200 ms. Stimuli were blocked into sequences of 150 trials, and each subject completed a minimum of 6 blocks. Breaks were permitted between the blocks to maintain a high level of concentration and to prevent fatigue.

Each participant took part in two tasks: the attending to A task and the attending to V task. Half of the participants performed attending to A first, while the other half performed attending to V first. Each subject performed a total of 900 trials. Each task (attending to A or attending to V condition) consisted of 450 trials.

EEG Recording

EEG was recorded using a NeuroScan system (64-channel QuikCap, band pass: 0.05–100 Hz, sampling rate: 250 Hz, impedances < 5 kΩ). The 64 scalp electrode sites are FPz, Fz, FCz, Cz, CPz, Pz, POz, Oz, FP1/2, AF3/4, F7/5/3/1/2/4/6/8, FT7/8, FC5/3/1/2/4/6, T7/8, C5/3/1/2/4/6, M1/2, TP7/8, CB1/2, CP5/3/1/2/4/6, P7/5/3/1/2/4/6/8, PO7/5/3/4/6/8, O1/2. Cz was used as the reference. To monitor ocular movements and eye blinks, EOG signals were simultaneously recorded from four surface electrodes, one pair placed over the higher and lower eyelid and the other pair placed 1 cm lateral to the outer corner of the left and right orbit. Then, the EEG was divided into epochs (−100 ms pre- to 600 ms poststimulus onset). Trials with blinks and eye movement were rejected offline on the basis of the EOG. An artifact criterion of ± 60 μV was used at all of the other scalp sites to reject trials with excessive electromyographs (EMGs) or other noise transients. The data were rereferenced to the MM, the AR (computed as the average of all 64 channels), and the IR using the software REST (for details, see Appendix).

Data Analysis

EEG epochs were sorted according to stimulus conditions and were averaged from each subject to compute the ERP. The baseline was defined as the epoch from −100 ms to 0 ms poststimulus onset. Here, we chose only two conditions, attending A in AV (Av) and attending V in AV (aV), as an example for comparing the three references, with respect to the temporal-spatial (electrode) differences in the scalp voltages that were induced by the two different tasks.

The resulting ERPs were subjected to several analyses: (a) We initially conducted an explorative analysis for the multisensory effects, testing each sample point (4 ms) and electrode for an effect of the within-subjects factor stimulus type (i.e., aV vs. Av). These results were thresholded such that the effects were considered to be significant only when a p value of $<.05$ was found on at least two sequential samples at two or more neighboring channels. These criteria were chosen because they represented the minimum temporal and spatial thresholds necessary to remove some spurious results from our analyses. After this overall analysis, three post hoc tests were conducted, in which each of the individual levels of the factor stimulus type was tested against one of the other levels. The

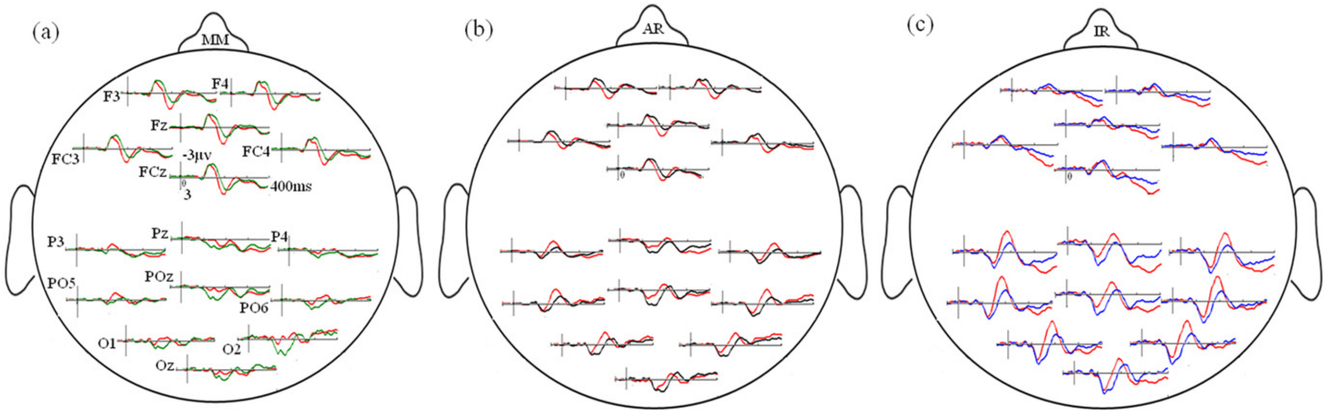

Figure 1. ERPs based on three references, at the anterior and posterior electrode sites. a: ERPs based on the MM reference. Thick line (red online): ERPs elicited by aV; thin line (green online): ERPs elicited by Av. b: ERPs based on the AR reference. Thick line (red online): ERPs elicited by aV; thin line (black online): ERPs elicited by Av. c: ERPs based on the IR reference. Thick line (red online): ERPs elicited by aV; thin line (blue online): ERPs elicited by Av; anterior electrode sites: F3, F4, Fz, FC3, FCz, and FC4; posterior electrode sites: O1, Oz, O2, PO5, POz, PO6, P3, Pz, and P4. MM = linked mastoid reference; AR = averaged reference; IR = infinite reference.

results from these tests were considered to be significant when they adhered to the same criteria as applied to the overall test (i.e., $p < .05$) on at least two sequential samples and two adjacent channels. Of major interest is the early multisensory effect, and it is reliable at 170–190 ms after the stimulus onset (see Figure 1). (b) We analyzed the SPSM of the N1 peaks (at 170–190 ms) and obtained a distribution of the significant difference effects from the scalp mapping. (c) To confirm a reasonable distribution of SPSM, LORETA (reference free) was used for localization of the distribution of the difference in the waves, from aV and Av, to provide circumstantial evidence in source space.

The LORETA algorithm was adopted to estimate the sources of specific components (Pascual-Marqui, Michel, & Lehmann, 1994). LORETA was conducted on individual ERPs for aV and Av trials over component peaks within a 20-ms window (Xu, Tian, Lei, Hu, & Yao, 2008). Paired *t* test was used to identify regions of differential activation for aV compared to Av trials with each time period for each voxel. All the calculations are conducted by LORETA-KEY software (http://www.uzh.ch/keyinst/NewLORETA/Software/Software.htm).

Results

Behavioral Measures

Table 1 shows the group mean reaction times (RTs) and standard deviations (*SD*) for the unisensory and multisensory conditions when attending to the different sensory stimuli. For attending A, RTs to the simultaneous condition were faster than those to the unisensory condition, $F(1,11) = 5.215$, $p < .05$. For attending V, RTs to the simultaneous trials were also faster than those to the visual alone trials, $F(1,11) = 9.149$, $p < .001$. RTs to the attending V in the simultaneous condition (aV) were faster than those to the attending A in the simultaneous condition (Av), paired *t* test: $t(11) = 2.597$, $p < .05$, indicating that the multisensory effect is stronger for detecting visual stimuli than for detecting auditory stimuli. Statistical analyses of missed responses and false alarms did not yield significant effects.

ERP Measures

ERP waveforms. For clarity, Figures 1a, b, and c present ERPs that were elicited by audiovisual stimuli when attending A (i.e., aV) or attending V (i.e., aV), based on MM, AR, and IR, respectively. Figure 2 shows the summary of the statistics (SPSM), which is the statistical significance of the multisensory interactions over all of the electrodes between 0 and 300 ms poststimulus for 12 subjects (correct trials only). As shown in Figure 2, we found that the significant difference between aV and Av focused on the N1 at 170–190 ms. Here, we can easily find that the three references showed different scalp distributions in the experimental effects at 170–190 ms. With MM (Figure 1a and Figure 2, MM), the significant difference was distributed at the anterior electrode sites such as F3, F4, Fz, FC3, FCz, and FC4 (all *p*s < .05). With AR

Table 1. *Behavioral Results*

Task	Sensory modal: Uni	Sensory modal: Multi	Difference Multi-Uni	Significance level
Attending audition	247 (± 13)	235 (± 9)	−12	$p < .05$
Attending vision	258 (± 13)	231 (± 9)	−27	$p < .001$
Attending (A-V) difference		−11	4	14
Significant level		*ns*	*ns*	$p < .05$

Note. Mean reaction times (standard deviations) are shown in milliseconds (ms) for the different sensory conditions when attending to the different sensory stimuli. Uni = unisensory; Multi = multisensory; Multi-Uni = multisensory-unisensory; *ns* = nonsignificant.

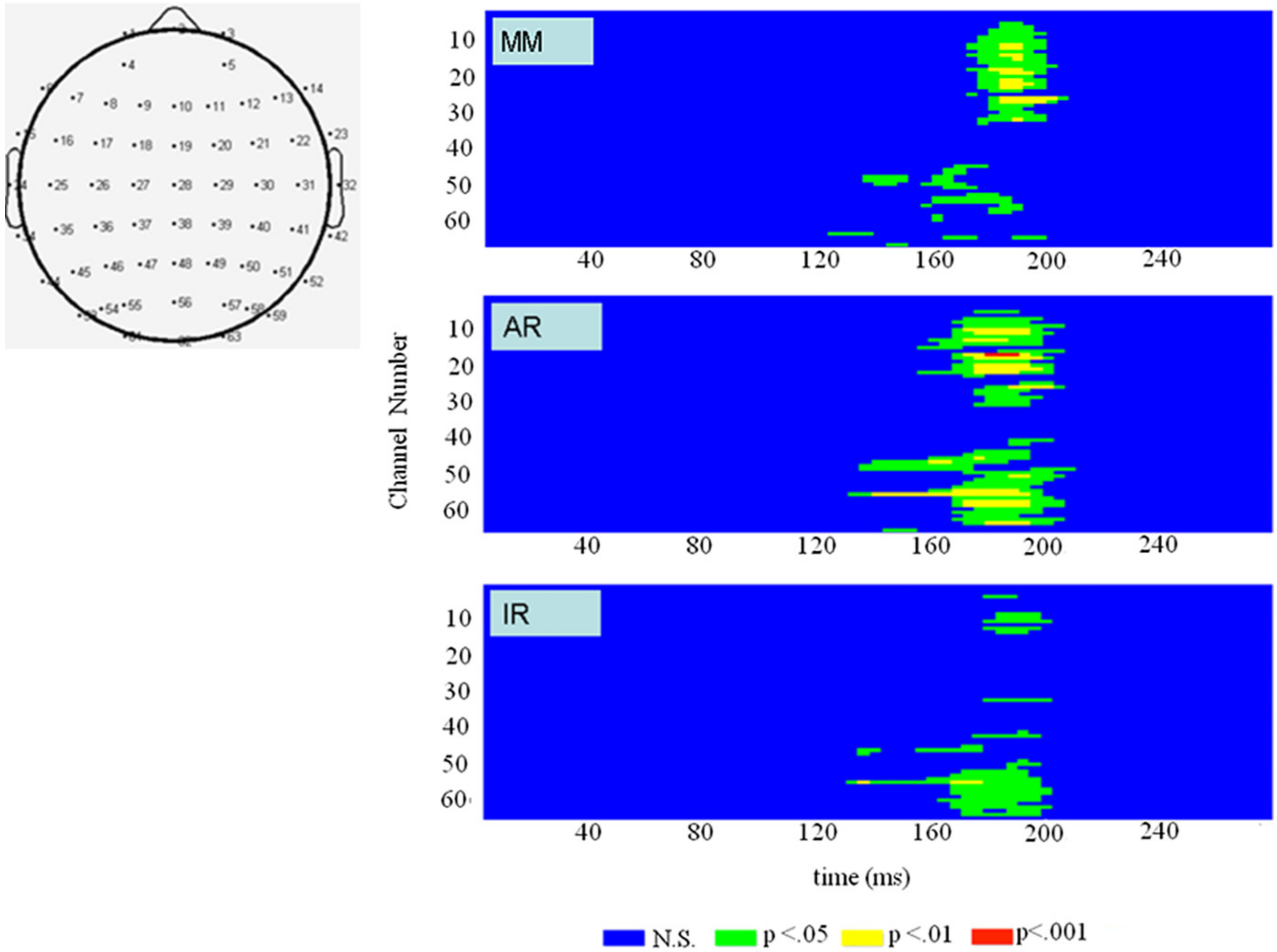

Figure 2. Summary of the statistics. Shown here is the statistical significance of the multisensory interactions over all of the electrodes (NeuroScan 64) between 0 and 300 ms poststimulus for 12 subjects (correct trials only). These results were thresholded such that the effects were considered to be significant only when a p value of $< .05$ was found on at least two sequential samples at two or more neighboring channels. These criteria were chosen because they represented the minimum temporal and spatial thresholds that were necessary to remove some spurious results from our analyses. Top left: the distribution of 64 electrodes with numbers.

(Figure 1b and Figure 2, AR), the significant difference was distributed at both the anterior electrode sites (e.g., F3, F4, Fz, FC3, FCz, and FC4) and posterior electrode sites (e.g., O1, Oz, O2, PO5, POz, PO6, P3, Pz, and P4) (all ps $< .05$). With IR, the significant difference was distributed at the posterior electrode sites (e.g., O1, Oz, O2, PO5, POz, PO6, P3, Pz, and P4) (all ps $< .05$).

Voltage Topographies and SPSM

Figure 3 presents voltage topographies (aV shown in Figure 3a; Av shown in Figure 3b) and SPSM (shown in Figure 3c) of the N1 (ranging from 170–190 ms) with different references, respectively. As shown in Figure 3a, the distributions of the voltage topographies on aV are similar among the three different references. The voltage topographies on Av (Figure 3b) also showed similar scalp distributions with different references. The only difference is a constant induced by the different references, which does not disturb the spatial distribution of the voltages (i.e., reference free) (Geselowitz, 1998; Pascual-Marqui & Lehmann, 1993; Yao et al., 2007), which is similar to how sea water can change the height above sea level of a mountain and not change its shape. However, the experimental effects (between aV and Av) showed a significant difference on the spatial distributions for different references, with MM indicating an anterior distribution, AR indicating anterior and posterior distributions, and IR indicating a posterior distribution (see Figure 3c). In other words, the experimental effect might show an anterior distribution (Figure 3c, left; MM), both an anterior and posterior distribution (Figure 3c, middle; AR), and a posterior distribution (Figure 3c, right; IR). These results revealed that, although the distribution (pattern) of voltage topography is reference free, the amplitude difference induced by a reference can disturb the difference in the experimental effects, and it could alter the final explanation.

LORETA Results

Figure 4 presents the neural sources that are activated by the experimental effects (i.e., aV vs. Av), as localized by LORETA. These results indicated that the sources are mainly located at the posterior bilateral sensory regions, such as the temporal and

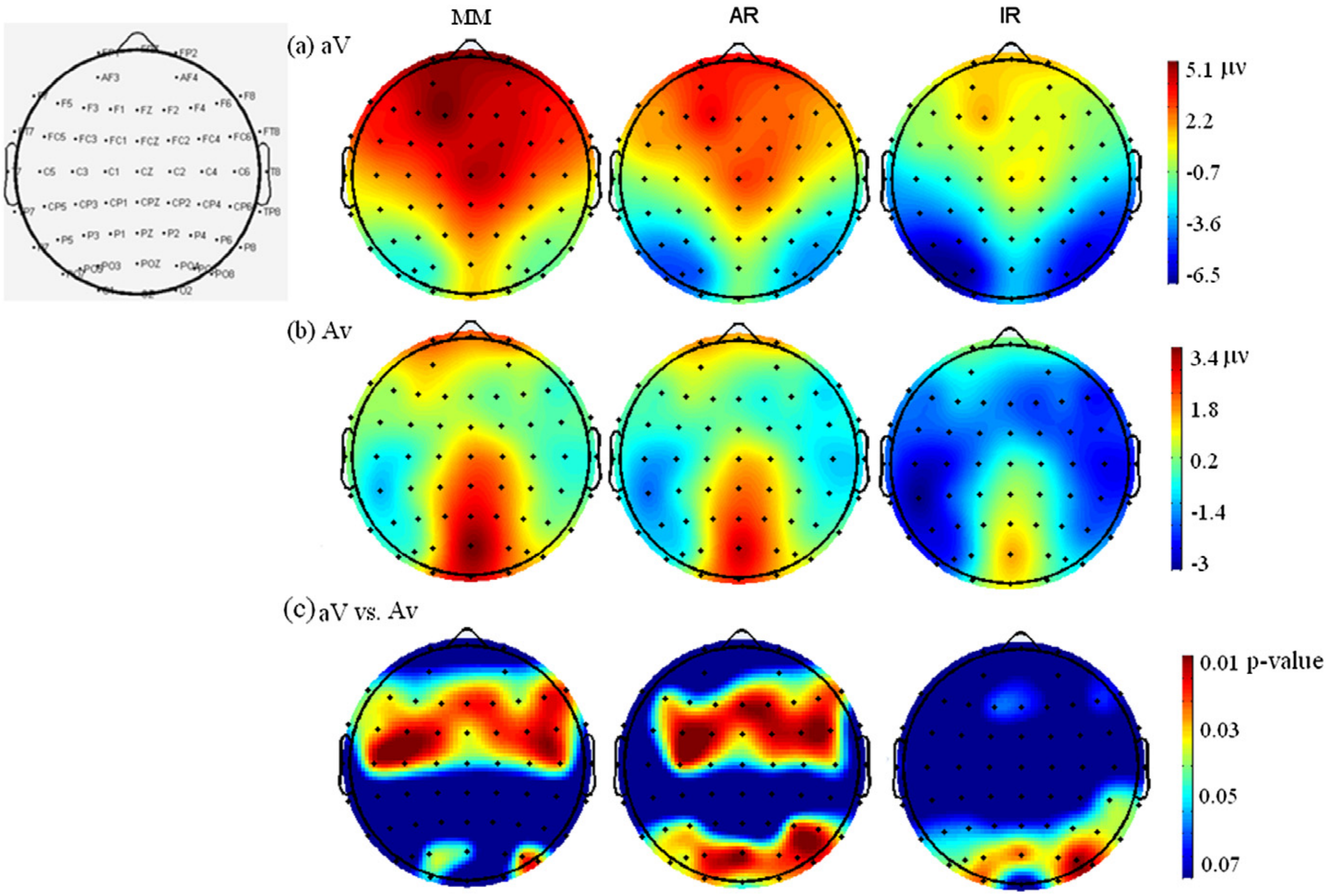

Figure 3. Voltage topographies and SPSM of N1 peaks (at 170–190 ms). a: Voltage topographies of attending V in an AV stimulus, i.e., aV, at 170–190 ms after the stimulus onset. b: Voltage topographies of attending A in an AV stimulus, i.e., Av, at 170–190 ms after the stimulus onset. c: SPSM (aV vs. Av) at 170–190 ms.

occipital cortex. The posterior parietal cortex (PPC) was also involved in aV and Av. More specifically, similar neural activities were elicited by the audiovisual simultaneous stimuli regardless of attending to A or attending to V. Moreover, the difference between aV and Av are also at the bilateral occipital-temporal regions more prominently over the right hemisphere; that is, a stronger activation at the occipital-temporal cortex appeared for aV compared to Av ($p < .05$).

Discussion

In the present study, three references, AR, MM, and IR, were comparatively investigated via the ERP waveforms (Figure 1), voltage topographies including SPSM (Figure 2 and Figure 3c), and LORETA (Figure 4), to reveal the most accurate spatial distribution of experimental effects between aV and Av. SPSM results demonstrated that MM showed an anterior distribution, AR showed both anterior and posterior distributions, and IR showed a posterior distribution (Figure 3). The LORETA results showed that aV- and Av-related activations were localized on bilateral occipital-temporal cortex and PPC (aV-related sources shown in Figure 4a; Av-related sources shown in Figure 4b). Experimental effects (aV vs. Av) were localized on bilateral occipital-temporal cortex slightly more to the right occipital-temporal cortex (Figure 4c).

With these results, three possible explanations can be suggested: (1) based on MM, the higher cognitive-related activations modulated the experimental effects; (2) based on AR, the likely explanation was that the higher cognitive-related neural sources modulated the multisensory information process and then re-entered the primary sensory cortex to enhance the discrimination of the stimuli; (3) based on IR, PPC activity was observed from the audiovisual stimuli regardless of attending to A or V. These results are highly similar to those reported previously for visual spatial attention (Banerjee et al., 2011; Foxe, McCourt, & Javitt, 2003; Kelly, Foxe, Newman, & Edelman, 2010; Tian, Chica, Xu, & Yao, 2011; Tian, Klein, Satel, Xu, & Yao, 2011; Tian & Yao, 2008), which supports that PPC plays an important role in supramodal deployment mechanisms (Farah, Wong, Monheit, & Morrow, 1989). We also found that the PPC and the bilateral sensory-related regions are activated by both aV and Av, and activations on the right parietal (or occipital) to temporal regions are larger for aV than for Av, indicating that some significant difference occurs when attending to V compared with attending to A. These facts indicate that both common and differential neural processes exist for top-down differentiation of the relevant sensory modality (Eimer, van Velzen, & Driver, 2002; Krumbholz, Nobis, Weatheritt, & Fink, 2009). Most critically, interpretation of study outcomes will depend on reference choice, and the reference choice that provided the closest approximation to the relevant literatures is IR.

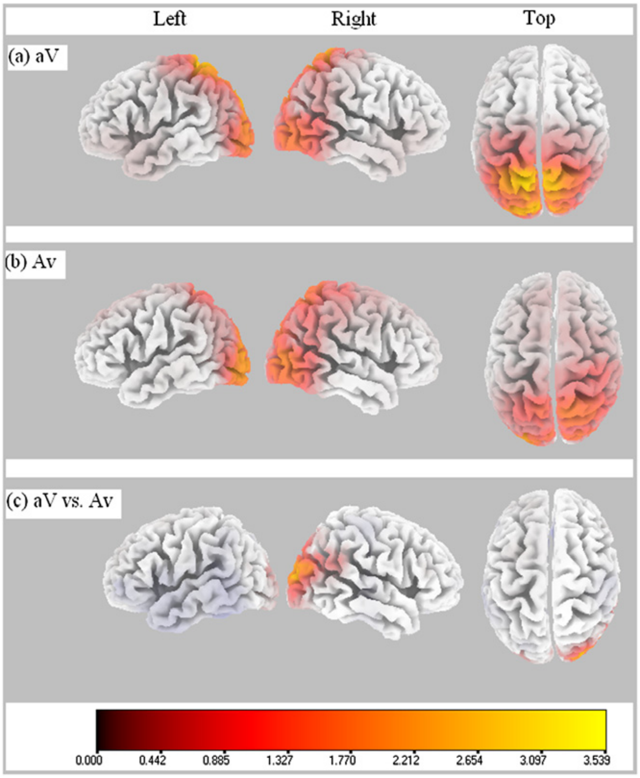

Figure 4. The sources localized by LORETA technology. a: aV condition, bilateral posterior sources, including bilateral occipital and temporal regions. b: Av condition, bilateral posterior sources, including bilateral occipital and temporal regions. c: aV vs. Av difference, bilateral posterior sources, including occipital regions. Specifically, the activation on the occipital cortex is stronger at aV than Av.

Why Can Different References Change the Experimental Effect?

Many ERP studies are based on the difference wave between two conditions, which is assumed to be the experimental effect (Luck, 2005). However, to our knowledge, investigators rarely check the effect of reference on the experimental outcomes. In theory, according to Figure 5, the effect of reference on the wave amplitudes of each experimental condition C1 or C2 may be transferred to an effect of reference on the spatial distribution of the distinct difference wave of two experimental conditions. In detail, Figure 5 illustrates a 2 (Conditions: C1 vs. C2) $\times$ 2 (References: R1 vs. R2) $\times$ 2 (Electrodes: e1 vs. e2) study; thus, there are eight ERP waveforms (C1R1e1, C1R2e1, C1R1e2, C1R2e2, C2R1e1, C2R2e1, C2R1e2, and C2R2e2). If one is only interested in the effect of reference on the wave amplitudes of each experimental condition C1 or C2, one can observe that the effect is a constant at both electrodes 1 and 2 for each time moment (Figure 5a). However, the constant is different for different time moments (t1), and thus may alter the spectra (Yao, 2001), the waveform-related coherence analysis (Marzetti et al, 2007; Thatcher, 2012), and the EEG network structure (Qin et al., 2010).

If one is interested in the effect of reference on the experimental effect between two experimental conditions, unlike the above-noted effect of reference on the waveform, one may further check the spatial distribution of the difference waveform. Based on R1 (Figure 5b, i), one can obtain four waveforms (C1R1e1, C2R1e1, C1R1e2, and C2R1e2). Using SPSM (Figure 5b, ii), one may obtain a significant difference between the two ERPs at the posterior region (related to e2). Thus, the possible neural-cognitive explanation is related to the functions of the posterior region. Based on R2 (Figure 5b, iii), one may obtain the other four waveforms (C1R2e1, C2R2e1, C1R2e2, and C2R2e2). Using SPSM

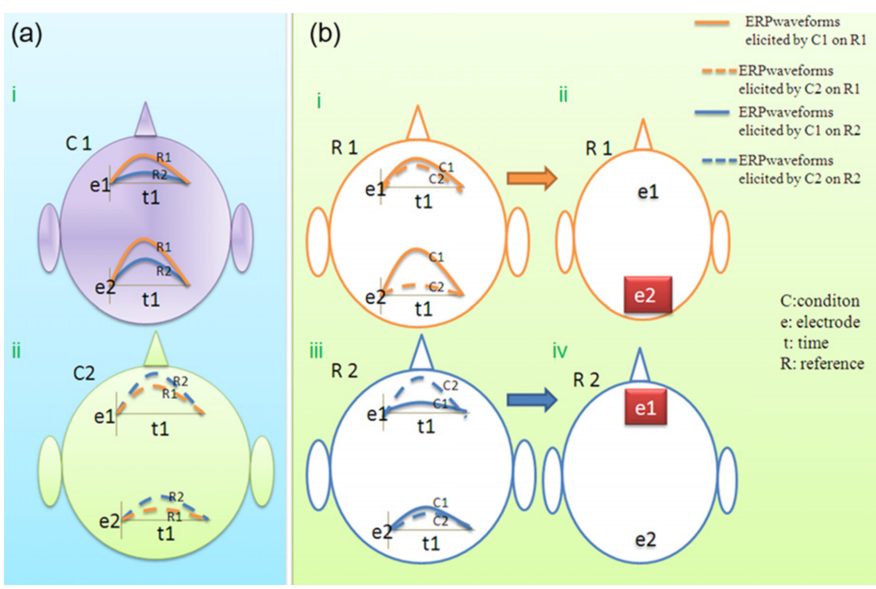

Figure 5. The illustration of the effect of reference on the distribution of the experimental effect deduced from two experiments. a: Effect of reference on wave amplitude. The waveforms at the two electrodes e1 and e2 are different when two different references R1 or R2 were adopted in experimental condition C1 (i) or experimental condition C2 (ii); however, the difference between R1 and R2 is a constant at e1 and e2 for each moment. b: Effect of reference on the experimental effect. As the experimental effect is the difference between two experimental conditions (C1–C2), a significant difference may appear at e2 when reference R1 is adopted (i–ii), and a significant difference may appear at e1 when R2 is adopted (iii–iv).

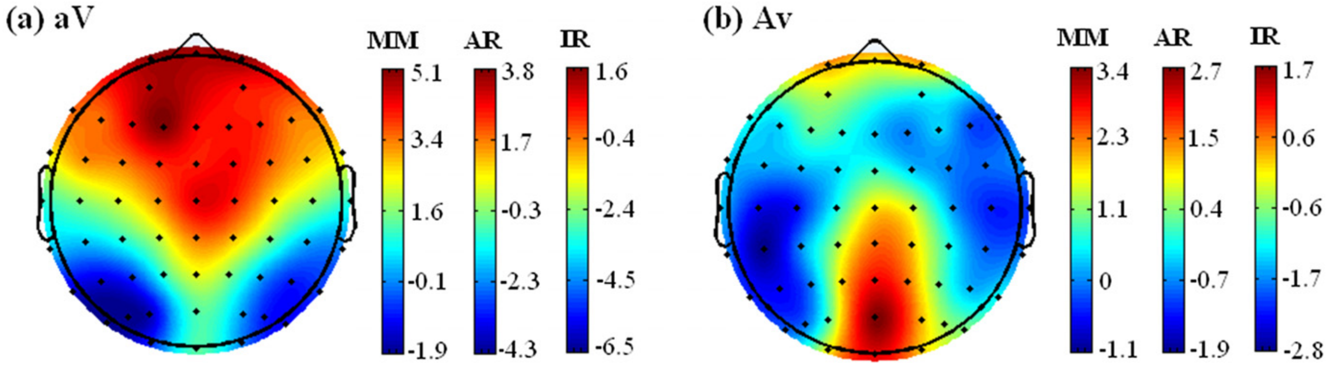

Figure 6. Same voltage topographies when different references are adopted for each experimental condition.

(Figure 5b, iv), one may obtain a significant difference between the two ERPs at the anterior region (related to e1). Thus, the possible neural-cognitive explanation is related to the functions of the anterior region. Apparently, one could obtain different results when using different references on the experimental effect.

What we find in this work is quite similar to the situation illustrated in Figure 5. Our results indicate that a nonzero reference can have a distinct effect on the difference wave, and thus could disturb the resulting psychological explanation. This result means that the choice of reference is a very important and fundamental issue because the difference wave (the experimental effect) is widely adopted in neurocognitive research.

Why Should SPSM-Based IR Be Trusted?

As noted previously, reference effects are similar to changing sea level; a sea level change can enlarge or reduce the difference between the top of a mountain and sea level, but it does not change the mountain's shape. For ERP/EEG, the scalp distribution pattern of voltages is not affected by the reference (i.e., reference free) (Figure 6), and thus the EEG inverse problem is independent of the scalp reference (Geselowitz, 1998; Pascual-Marqui & Lehmann, 1993; Yao, 2001; Yao et al., 2007). Based on this fact, the inner source distribution in the brain or cortical surface should be the same for all of the adopted references; thus, it can provide us with a relatively objective comparison to infer the relative utility of different references. In this work, we use LORETA to obtain the cortical surface equivalent sources and their differences between aV and Av. We argue that the difference at the source level should be properly reflected on the scalp if a zero reference is adopted. The results (Figure 4) confirmed that only the SPSM based on IR is consistent with the evidence provided by LORETA and is consistent with previous fMRI findings on audiovisual effects (Busse et al., 2005; Noesselt et al., 2007).

The Physical Difference Between AR and IR

Among the three references compared here, the MM is independent of the electrode montage, while the AR and IR are not. Furthermore, the physical bases of IR and AR are quite different. For AR, the best case is whole brain dense coverage; then, the average can converge to the theoretical zero-value—otherwise, it will not be zero. The underlying reason is the fact that the potential integration over a closed scalp surface is zero, which means that a dense sampling on a closed surface is the prerequisite for AR. Naturally, to have the average (integration) be zero, part of the scalp potential would be positive and the other negative. For practical upper head surface recordings, whether the average is zero strongly depends on the coverage, and only when the nonclosed coverage almost equally covers parts of the positive and negative surfaces, the average tends to zero. Now, for the assumed upper head surface electrode montage, the effectiveness obviously depends on the underneath true source distribution, especially the equivalent dipole orientation that determines the positive and negative potential distribution (Yao, 2001; Zhai & Yao, 2004). This fact means that the effectiveness of AR depends on not only the coverage and electrode number but also the underlying source distribution.

Why is AR recommended in the literature (e.g., Dien, 1998)? AR is the average of the whole electrode array. It is seemingly independent of any special electrode position, and looks fair to all of the electrodes. Some papers even call AR results reference free (e.g., Murray, Brunet, & Michel, 2008). The results of the present investigation indicate that these judgments may be based on an incomplete story about AR.

For IR, the potential is reconstructed from actual recordings by an equivalent distributed source on the cortical surface (Yao, 2000a, 2001). Theoretically, a denser array is beneficial to the reconstruction. In our previous detailed simulation studies (Yao, 2001, Zhai & Yao, 2004), we evaluated the practical effect of the number of electrodes (e.g., 32, 64, and 128), and found that when the number is larger than 32, IR is distinctly better than AR and MM. In this study, we also investigated the case that had a smaller number of electrodes (e.g., 34 electrodes sparsely sampled from the original 64), and found that the basic conclusions are the same (detailed results omitted here).

IR(REST) is based on three facts: (1) actual scalp potential with any physical reference is produced by sources inside the brain, (2) the EEG inverse problem is independent of reference, and (3) the same scalp potential can be produced by different underlying sources (EEG inverse is nonunique). IR(REST) works like the Rosetta stone (Kayser & Tenke, 2010) or, say, a bridge, with one end linked to the actual scalp recordings with a physical reference and the other linked to the ideal scalp recordings with reference at infinity. There are many factors, such as the coverage, head model, equivalent source model, and inverse algorithm, which may affect the bridge effectiveness (See Appendix). Therefore, we may not be able to obtain the ideal "best" bridge that will result in a true zero reference. However, it is valuable from a practical standpoint if one can distinctly reduce the effect of nonzero reference, and we argue that the current IR(REST) is such a technique. On the one hand, IR is more complex than AR, thus more factors may affect its performance. On the other hand, it is these complexities that can compensate for information lacking in AR that allow us to make a step toward a "true" zero reference and illustrate its advantages over other references for various applications.

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Appendix

REST: Reference Electrode Standardization Technique

The physical principle behind REST. REST is a novel method that builds a bridge between a physical reference and the theoretical neutral reference at an infinity point (Yao, 2001; Yao et al., 2005). For an infinity reference, the forward EEG calculation is given by

$$V = GS \tag{1}$$

where G is the transfer matrix referenced at infinity, only dependent on the head model, source configuration, and electrode montage; S is the source; V is the scalp EEG recording with a reference at infinity generated by S. Scalp noise is not explicitly considered in this model.

For a physical reference such as the CZ referenced recordings V_{CZ}, we similarly have

$$V_{CZ} = G_{CZ}S \tag{2}$$

where G_{CZ} is the EEG lead-field matrix with CZ reference and V_{CZ} refers to EEG scalp recordings referenced at CZ. A solution for the source distribution S is given by

$$S = G_{CZ}^{-}V_{CZ} \tag{3}$$

where $(G_{CZ})^{-}$ may be the Moore-Penrose generalized inverse of matrix G_{CZ}. From Equations 2 and 3, we can see that the source S is the same, which reflects the fact that reference choice does not influence the source localization; that is, activated neural sources in the brain are not affected by the particular reference used (Pascual-Marqui & Lehmann, 1993). The potential with reference at infinity can thus be reconstructed as the following:

$$V_{REST} = G(G_{CZ}^{-}V_{CZ}) = UV_{CZ} \tag{4}$$

where $U = GG_{CZ}^{-}$ is the final transfer matrix simultaneously determined by the lead-field matrix G and G_{CZ}, where G is known, and G_{CZ} can be easily derived from G. In addition, recordings using any other single physical electrode as reference can be mathematically transformed to the infinity reference using a formula similar to Equation 4; the only difference is the use of a specific lead-field matrix corresponding to the adopted reference.

Implementation of REST. Because the potential produced by any actual sources can be equivalently produced by a source distribution enclosing the actual sources (Helmholtz, 1853, see also Luck, 2005; Yao, 2003; Yao & He, 2003), we may assume an equivalent source distribution (ESD) on the cortical surface that encloses all possible neural electric sources inside and assume that S in Equations 1 and 2 is the ESD, instead of the actual neural electric sources. Moreover, the ESD may be a closed radial dipole layer or a closed charge layer (Yao, 2000b; Yao & He, 2003). It may even be a series of equivalent multiple sources of the actual sources at the coordinate origin (Yao, 2000b; Thuraisingham, 2011). These three approaches are equivalent in producing the actual scalp potential (Yao, 2000b). Based on our experiences on these approaches, we adopted the equivalent dipole layer (Yao, 2003; Yao et al., 2001).

With the dipole layer approach, an assumed equivalent closed dipole layer was positioned in an assumed head model, then the EEG lead-field matrix G and G_{CZ} are determined by the electrode montage. The lead-field matrix is independent of the spontaneous mental states of EEG or the stimulus tasks in ERP studies. In another words, for an electrode montage and an assumed head model, the final transfer matrix U is the same for different EEG/ERP data of a subject.

The head model in REST may be a realistic head model (Zhai & Yao, 2004) or a three-concentric-sphere model (Yao, 2001). Taking into account all of the factors that affect the performance of REST—head model, configuration of the equivalent distributed source, numeric calculation method, individual difference of subjects, electrode montage, unknown recording noises, approximate equivalence in producing the scalp potential by different combinations of source model and head model, we recommend using the three-concentric-sphere model approach for general ERP/EEG studies. In this approach, the normalized radii of the three concentric spheres were 0.87 (inner radius of the skull), 0.92 (outer radius of the skull), and 1.0 (radius of the scalp). The normalized conductivities were 1.0, 0.0125, and 1.0 for the brain, skull, and scalp, respectively.

Free software of REST. Free software REST can be found at www.neuro.uestc.edu.cn/rest. Where both a realistic head model and three-concentric-sphere model or other head model can be introduced by using different forward models, even users themselves designed forward models.