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Editorial

In search of the Rosetta Stone for scalp EEG: Converging on reference-free techniques

See Article, pages 1981–1991

Almost a hundred years have passed since Hans Berger's historic discovery that electrical brain waves can be recorded from the human scalp (Berger, 1929). Since that time, the electroencephalogram (EEG) has been recognized as a real-time, noninvasive measure of both tonic (e.g., at rest or during sleep) and phasic neuronal activity (e.g., as evoked responses to physical or cognitive events). Many approaches have been developed to identify, separate, quantify, and compare the temporal and spectral properties of the EEG, as evidenced in the pages of this journal over the past 60 years. The EEG remains a valuable and cost-effective tool for a wide range of clinical and basic research purposes, regardless of the recent numerous developments of complementary neuroimaging measures. In addition to an unparalleled temporal resolution, important technological advances, such as dense electrode arrays with over a hundred channels that allow an evenly-spaced scalp coverage, offer dramatically increased topographic capacities in a recording montage with improved data quality and reduced preparation time, owing to high impedance amplifiers and miniature preamplifiers located inside the scalp sensor. However, despite the impressive advances and continued promise of these methods, we still lack an universal key to decipher the functional meaning of the scalp-recorded EEG. One well-known problem in particular arises again and again, and often in forms that may be unrecognized at first: because an EEG signal must be quantified as a potential difference between any two sites, thereby yielding relative rather than absolute measures, the properties of the reference, whether determined by its physical location or its computational characteristics, will have a fundamental impact on the signal of interest. For example, if two sites are equipotential, no EEG activity is observed between them, no matter what the absolute potential may be. Another implication is that the information provided by a difference measure is unaffected by its direction, apart from its arbitrary sign (i.e., the selection of one of a pair as reference is inherently arbitrary).

Like other electrophysiological phenomena, the EEG is volume-conducted throughout the brain, skull, and body. Clinical applications deal with this basic biophysical fact by implementing simple and effective bipolar derivations (i.e., sequentially changing the reference) to isolate localized EEG abnormalities (e.g., Osselton, 1965). However, most electrophysiological research goals are pursued using unipolar recordings, which are by definition reference-dependent. Hence, it would be desirable to identify a "neutral" reference location, which, of course, does not exist anywhere on the body. This problem was therefore reexpressed as a search for a relatively neutral or "quiet" reference location, at least with respect to the signal of interest. For example, Wolpaw and Wood (1982) argued that a reference location below the neck, in contrast to nose, ear, mastoid, knee or ankle locations, will show minimal spatial and temporal voltage gradients during a duration of interest for auditory evoked potentials. The use of a sternovertebral reference, consisting of two sites anteriorly and posteriorly at the base of the neck, has also been recommended to minimize EKG artifacts (Stephenson and Gibbs, 1951). Of course, the orientation of the underlying generator (e.g., heart muscle position and functionality, visual evoked potentials) would require different reference locations for different processes as they unfold over space and time. Moreover, identifying the least active reference site on an individual basis, or adjusting the reference for different conditions or time periods would be both impractical and undesirable.

For all of these reasons, the search for a true electrically-neutral reference is generally appreciated as something akin to a Platonic ideal, more than as a serious clinical or research concern. Ideally, one would like to measure scalp potentials against the potential at infinity (i.e., a "true" potential of zero) but physical approximations of this ideal would lead to insurmountable problems, such as marked impedance differences between the reference at infinity and the volume-conductor of interest (i.e., the brain) and reduced signal quality because of increased non-physiological noise (e.g., Nunez and Srinivasan, 2006). Remote reference locations on the body will likewise increase physiological artifacts, thereby favoring a reference location on or near the scalp. As a result, the preferential use of different reference schemes (e.g., linked ear lobes, linked mastoids, nose, non-cephalic, average) has evolved for individual research teams and led to de facto standards or conventions for specific research fields or clinical practice.

Virtually every textbook and seminal paper on EEG methodology raises the issue that EEG measures are reference-dependent, and the need to clearly specify the EEG reference has been included among standard guidelines for EEG (Pivik et al., 1993) and event-related potential (ERP) research (Picton et al., 2000). Unfortunately, because this critical information is all too often reduced to a secondary methodological detail rather than regarded as a defining characteristic of the data, the absence of an universal reference standard has itself become a liability, raising issues of internal validity and across-study comparability. As an example, ERP components are often operationally defined by prominent waveform deflections (i.e., by their peak latency and topographical peak maximum); however, these "obvious" peaks may dramatically change in time, space, and

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polarity after choosing a different reference. A prototypical example is the polarity reversal and topographic shift of a visual N1, which has a neuronal origin in secondary visual cortex. It shows a prominent negative deflection at inferior-lateral sites when data are referenced to nose, but instead reveals a distinct positive deflection at mid-centroparietal sites when a linked-mastoids reference is used (cf. Kayser et al., 2007). In contrast, an auditory N1 generated in primary auditory cortex is largely unaffected by using a nose or linked-mastoids reference. In other words, the choice of EEG reference may severely impact on any comparisons in which the reference itself is differentially affected. It should be obvious that spectral measures (e.g., power and coherence) are equally affected by the choice of reference, although this may be even less intuitive given the complex transformations required. Thus, these reference-dependent variations affect the identification, nomenclature, quantification, and interpretation of any electrophysiologic measure, and thereby the outcome of any EEG study.

Over the years, there have been heated debates over the choice of a most appropriate or least biased reference (e.g., Desmedt et al., 1990; Pascual-Marqui and Lehmann, 1993), without any real resolution of this issue. Although it has been acknowledged that certain reference schemes maybe more advantageous than others for a given effect of interest, as they may maximize condition-dependent differences in the underlying generator (cf. Dien, 1998), in most instances the precise location and orientation of the underlying generator is unknown, and typically multiple generators overlapping in space and time contribute to EEG phenomena. A case has been made for the use of the average reference because the common average of all recorded EEG activity will approximate zero (i.e., an inactive reference) if the spatial sampling is both dense enough and sufficiently covers the signal space, that is, a closed surface containing all current within its volume (e.g., Nunez and Srinivasan, 2006). However, obvious practical limitations generally exclude recording from the ventral side of the brain case, rendering an incomplete estimate of a true zero potential, even with 128 or more EEG channels. Moreover, an uneven sampling across the current volume surface, as done by placing most EEG sensors on the scalp, will result in a bias toward that region, typically the vertex pole (Junghöfer et al., 1999). Furthermore, differences in EEG montage (i.e., total channel number and locations) may result in very different average waveforms, and therefore different recording references, despite their nominal identity as "the average reference". Notwithstanding these well-known limitations, a consensus has emerged among researchers relying on data from dense electrode arrays that the use of an average reference may still be considered as the "gold standard" for EEG analysis, as it seems to provide reasonable estimates of reference-independent potentials in simulation studies (Srinivasan et al., 1998). Regrettably, the use of a common average has sometimes even been incorrectly equated with a reference-free approach.

Spherical spline interpolation methods (Perrin et al., 1989) can be used to provide montage-independent common averages, to improve estimates for an undersampled montage, or to compensate for the systematic undersampling of inaccessible regions (e.g., the underside of the brain; Junghöfer et al., 1999; Scherg et al., 2002). Despite the adoption of a rather simplistic, although consistent, spherical head model, spherical splines have been found to yield greatly improved estimates of an infinite reference for different electrode montages (19, 65, or 129 channels), different dipole configurations representative of ERP (high signal-to-noise ratio) or EEG (distributed sources) signals, and different head tissue conductivity ratios when directly compared to the average reference (Ferree, 2006). Although increased montage density and more complete spatial sampling (i.e., covering a larger surface area) generally lead to more favorable results, improved estimates can nevertheless also be obtained from whole-head spherical splines when relying on more sparsely sampled electrode configurations if additional channels below the temporal plane are included (Scherg et al., 2002). Surprisingly, despite the comparatively simple implementation of a spherical spline algorithm, this suitable and practical resolution of the ubiquitous reference problem has not coaxed the research community from a reliance on referenced scalp data. On the other hand, the fast Fourier transform (FFT), which is a less intuitive and considerably more complex transformation, has paradoxically become a widely-used standard for the spectral analysis of EEG data.

Importantly, the *topographic* information in EEG signals is not altered by different reference schemes because the subtraction of a constant (i.e., a different reference) does not alter the relative values between recording sites per sample point (e.g., Osselton, 1965); however, this realization only became relevant after EEG was no longer restricted to a few recording sites. Variations in EEG/ERP topography form the basis for EEG source localizations methods (e.g., Michel et al., 2004), which can be considered reference-free methods. However, these approaches require additional biophysical assumptions to constrain the number of possible solutions (e.g., number, location, and direction of equivalent source dipoles) because an infinite number of inverse solutions can account for any observed surface potential topography, which is known as the inverse problem. In contrast, the forward solution of a surface potential topography from a known set of generator sources is unambiguous for a given head model. Almost a decade ago, Yao (2001) proposed a clever way to exploit these principles for estimating the theoretical EEG reference at infinity from the observed (i.e., referenced) surface potentials. The appeal of the proposed reference electrode standardization technique (REST) is that a non-unique equivalent dipole source solution is computed to provide a reference standardization matrix (i.e., the transfer matrix is independent of the actual neuronal generators), rather than to solve the EEG inverse problem. This matrix may then be used to rereference surface potentials of any given EEG montage to an (estimated) infinity reference, thereby rendering reference-free EEG data.

In recent years, Yao and colleagues have demonstrated how this standardization technique favorably compares with other common reference schemes when analyzing EEG power spectra (Yao et al., 2005) or ERP topographies (Yao et al., 2007). In this issue, Qin, Xu, and Yao extend these evaluations to the concept of the default mode network as determined by EEG coherence and power. Not surprisingly, the authors demonstrated for real, high-density EEG that all traditional power bands (delta, theta, alpha, beta, gamma) revealed profound differences in EEG network configurations obtained with different references (left mastoid, linked mastoids, average reference, REST). This, by itself, underscores the equivocalness of any attempts to interpret EEG coherence and power findings obtained with a conventional reference. More importantly, this report also includes simulations of dipole-pair configurations when using different montage densities (20 versus 111 channels) and brain-to-skull conductivity ratios to compare the effectiveness of different reference schemes for reconstructing EEG connectivity. The results show that the residual error of coherence is minimal with infinity reference estimates (REST), but substantial for the conventional references, including the average reference derived from a dense electrode array. Although the residual error was affected by the conductivity ratio, this effect was negligible compared to the differences associated with different reference schemes. Overall, the findings are consistent with the notion that reference effects are a critical problem for all EEG measures, and an average reference is not the solution to this problem, even when using a high-density montage. In contrast, more suitable estimates of a "true" zero reference at infinity, such as evidently provided by the REST algorithm, can effectively resolve the reference problem.

The present comparison did not include improved estimates of an average reference that exploit spherical splines (cf. Ferree, 2006), or surface Laplacian (current source density, CSD) measures, which can also be based on spherical splines (e.g., Perrin et al., 1989) or even a simple linear derivation technique (e.g., Hjorth, 1975). As has long been recognized (e.g., Nunez and Srinivasan, 2006), CSD estimates are inherently reference-free by virtue of their computation (i.e., second spatial derivative), that is, they depend only on the topography of the EEG signal. For this reason, the application of REST to EEG/ERP data will not affect the derivation of CSD estimates, nor will it affect inverse solutions. We have championed CSD transformations of surface potentials as a core ingredient to a generic, reference-free approach for ERP/EEG data analysis (Kayser and Tenke, 2006; Tenke and Kayser, 2005). Ultimately, a direct comparison of surface potentials with an unbiased reference (i.e., REST) and their CSD counterparts will help determining whether the removal of volume-conducted contributions will be (more) useful for various EEG research purposes. Whereas all of these approaches are model- and montage-dependent, their empirical value will be determined by their convergence on accurate characterizations of the underlying neurophysiology.

The routine use of reference-free approaches for the analysis of scalp EEG is a long-desired, universal goal, not only because it would facilitate (rather, enable) the comparison and interpretation of study outcomes, but also because it would tremendously aid our understanding of brain activity. What is needed is global access to the REST procedures so it can be widely-used by the research community as an analytic tool, like FFT. As a first step, the authors have generously provided access to the REST software (see Supplementary material of Qin et al. (this issue)), which should enable motivated researchers to generate lead field transformation matrices for any given montage, and then compute reference-free EEG estimates for their own data. Independent validations and evaluations regarding the usefulness of the REST approach, along with identification of any limitations, are needed, and possible extensions of its applicability should be explored.

The ancient Egyptian Rosetta Stone was instrumental in deciphering the principles of hieroglyphic writing. It was neither a perfect solution nor a universal doctrine, but merely an unconfounded sample of parallel text in known and unknown languages. Its discovery only became important in the hands of diligent scholars, whose efforts transformed this archeological artifact into a critical key for a broader understanding of the unknown. In the study of the EEG, we have acquired a number of conventions, but we often struggle to extract generalizable meanings from our measures and subdisciplines. Reference-free EEG techniques represent a key to unravel the meaning of EEG spectra and coherence, and will significantly advance our understanding of tonic and phasic EEG measures. REST may be a critical link to connect various reference-free techniques, such as the surface Laplacian and inverse methods.

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