Recent Advances in Resting-State Electroencephalography Biomarkers for Autism Spectrum Disorder—A Review of Methodological and Clinical Challenges

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ABSTRACT

BACKGROUND: Electroencephalography (EEG) has been used for almost a century to identify seizure-related disorders in humans, typically through expert interpretation of multichannel recordings. Attempts have been made to quantify EEG through frequency analyses and graphic representations. These “traditional” quantitative EEG analysis methods were limited in their ability to analyze complex and multivariate data and have not been generally accepted in clinical settings. There has been growing interest in identification of novel EEG biomarkers to detect early risk of autism spectrum disorder, to identify clinically meaningful subgroups, and to monitor targeted intervention strategies. Most studies to date have, however, used quantitative EEG approaches, and little is known about the emerging multivariate analytical methods or the robustness of candidate biomarkers in the context of the variability of autism spectrum disorder.

METHODS: Here, we present a targeted review of methodological and clinical challenges in the search for novel resting-state EEG biomarkers for autism spectrum disorder.

RESULTS: Three primary novel methodologies are discussed: (1) modified multiscale entropy, (2) coherence analysis, and (3) recurrence quantification analysis. Results suggest that these methods may be able to classify resting-state EEG as “autism spectrum disorder” or “typically developing”, but many signal processing questions remain unanswered.

CONCLUSIONS: We suggest that the move to novel EEG analysis methods is akin to the progress in neuroimaging from visual inspection, through region-of-interest analysis, to whole-brain computational analysis. Novel resting-state EEG biomarkers will have to evaluate a range of potential demographic, clinical, and technical confounders including age, gender, intellectual ability, comorbidity, and medication, before these approaches can be translated into the clinical setting.

Keywords: autism spectrum disorder, resting-state electroencephalography, EEG, biomarker

Introduction

Electroencephalography (EEG) has been used for almost a century to identify electrophysiological changes at the scalp in humans.1 Clinical “reading” of multichannel EEGs is typically performed by expert clinicians and neurophysiologists. Some attempts have been made to quantify and computerize EEG signals, typically through graphic representation of single-channel recordings, showing frequency analysis, hemispheric patterns, and so on.1 These “traditional” methods have not always been welcomed in clinical settings and have many methodological limitations.
Event-related potentials have been used in research settings for a few decades now but with no clear translation into clinical settings. In recent years, there has been a resurgence of interest in resting-state EEG signals as “data” for biomarker development to guide diagnosis, predict developmental outcomes, and monitor treatment response in neurodevelopmental disorders such as autism spectrum disorder. However, EEG data are nonlinear, dynamic and complex, comprise multiple channels, and have high dimensionality. The move towards a next generation of “whole-brain” EEG analysis therefore requires complex signal processing and mathematical modeling in the context of many clinical variables.

In a recent review, Jeste et al. highlighted the heterogeneity of neurodevelopmental disorders such as attention deficit hyperactivity disorder and autism spectrum disorder but suggested that continuous EEG measures, for instance, spectral power, coherence, and complexity analysis, may be able to identify those at risk of neurodevelopmental disorders, help to diagnose disorders, capture clinically meaningful subgroups within the broad diagnostic boundaries of such disorders, and combine with other variables to act as biomarkers for treatment response.

In spite of the potential for EEG to produce meaningful biomarkers, there are many limitations to existing “traditional” EEG biomarker analysis and numerous clinical confounders that may influence identification of methodologically robust and clinically meaningful novel biomarkers. Here, we review the three most recently published resting-state EEG biomarkers in autism spectrum disorder to draw attention to methodological and clinical considerations for biomarker development.

Search strategy and selection criteria

A systematic review was conducted using “resting state,” “EEG,” “biomarker,” and “autism,” contained in the title, keywords, or abstracts of articles in BioMed Central, PubMed, Scopus, ScienceDirect, and IEEE Xplore journals. We searched for all available publications until June, 30, 2014. We included only articles written in English. Primary articles identified were used to identify secondary literature sources regarding strengths and weaknesses of identified methods.

From traditional to novel EEG analysis methods

To date, no clear distinction has been made between commonly used quantitative EEG (qEEG) measures and more contemporary biomarker methodologies. Differences between the two main groups are outlined below and shown in Fig. 1.

Traditional EEG analysis methods use a linear approach to analysis and rely on Fourier analysis to transform EEG data into the frequency domain and decompose the data into their constituent frequency bands (delta, theta, alpha, beta and gamma). The EEG is then quantified by describing absolute power, relative power, and coherence measures of the EEG. Absolute power reflects the amount of EEG activity in a specific frequency band (for instance, alpha waves), whereas relative power reflects the amount of EEG activity in a specific frequency band divided by the amount of activity in all frequency bands (in other words, it represents a “ratio”). Coherence provides a measure of the degree of synchronization between two EEG signals, on a frequency basis. Fundamental to these traditional analysis methods are the assumptions that EEG signals are inherently stochastic (or randomly distributed) and stationary.

Wang et al. conducted a review of resting-state EEG abnormalities in autism spectrum disorder, with the primary focus on Fourier analysis, which represents sums of simple trigonometric functions. The authors identified a U-shaped profile of EEG abnormalities, suggesting excessive power at the delta and theta (lower) frequency bands, reduced power in the alpha (middle) frequency band, and excessive power in the beta and gamma (higher) frequency bands in those with autism spectrum disorder. Second, the review identified enhanced power in the left hemisphere of the brain in autism spectrum disorder subjects, compared with the right hemisphere, noted across all EEG frequency bands. Third, overall local overconnectivity and long-range underconnectivity were observed, with the conclusion of abnormal functional connectivity. The review by Wang et al. offers further detail.

EEG signals are rich in information relating to the structure of neural networks in the brain because of their nonlinear, dynamic, and complex nature. Apart from the traditional qEEG methods, EEG biomarkers include methods in time series analysis that are able to account for the nonstationary, nonlinear, and complex nature of EEG signals and incorporate machine learning and data-driven strategies. The resulting features of these novel methods are often difficult to visualize and interpret, but may be more dynamic and flexible to predict diagnostic outcomes, define subgroups, and track treatment responses more accurately than traditional qEEG measures. Three primary methodological approaches to resting-state EEG biomarker development for early detection of autism spectrum disorder risk have been described to date: (1) modified multiscale entropy (MME), (2) coherence analysis (CA), and (3) recurrence quantification analysis (RQA). Fig 2 shows a simple illustration of traditional and novel methods of EEG analysis.
**Visual inspection**

EEG descriptors are identified through visual inspection and used to make a clinical diagnosis. The descriptors include the identification of spikes, waveforms, frequency, amplitude, distribution, phase, timing, persistence and reactivity.

**Quantitative electroencephalography**

EEG signals are decomposed into their constituent frequency bands using Fourier analysis. Features such as absolute power, and relative power are used to quantify the EEG in the frequency domain. A single plot is created per channel.

**Multiscale entropy analysis**

Coarse-grained time series are created through successive averaging of the original time series signal. Sample entropy is computed to estimate the complexity of the signal. This is calculated for each coarse-grained time series, up to scale n. This procedure is performed for each channel.

**Coherence analysis**

Coherence provides a measure of the degree of synchronisation between two EEG signals, on a frequency basis. This is computed for all possible channel combinations comprising two EEG signals.

**Recurrence quantification analysis**

Time series data are embedded to reconstruct the system dynamics in a phase space (shown by the attractor, indicating a set of numerical values toward which a system tends to evolve). A recurrence plot is then constructed and features such as recurrence rate, longest diagonal and vertical line length, are extracted. All channels are combined in a multivariate approach.
Novel resting-state EEG methods require automated signal processing. Automated EEG signal processing takes place across four steps as illustrated in Fig 3. Data acquisition entails the recording and storage of EEG signals from subject groups. Preprocessing entails the removal of artifacts or contaminants (i.e., undesirable potentials of physiological or nonphysiological origin) from the EEG signals. Feature extraction entails the extraction of characteristic information (termed “features”) from the signals to be used as predictor variables. In the final step (classification), pattern recognition is used to predict the class/group outcome of the input features. In the following sections, we will summarize the three resting-state EEG biomarker methods across the signal processing pipeline, before comparing their strengths and weaknesses.

Comparison of three novel resting-state EEG methods

Bosl et al.6 investigated EEG complexity through a measure of entropy, reflecting the irregularity or unpredictability of a system, and found decreased EEG complexity in infants at high risk for autism spectrum disorder (defined as having an older sibling with autism spectrum disorder) versus typically developing (TD) infants. Duffy and Als8 investigated functional connectivity and found reduced short-distance and both reduced and increased long-distance coherences in the autism spectrum disorder group when compared with the TD group. The methodology followed by Pistorius et al.9 implemented recurrence analysis, which accounts for the complex, nonlinear, and dynamical nature of the brain.10,11 The RQA features yielded information relating to the complexity and determinism associated with the neural dynamics of each individual across all EEG electrodes, enabling autism spectrum disorder or TD group membership prediction.

The three different biomarker methodologies are outlined in Fig 4.

Data acquisition

All three studies captured resting-state EEG data, or nontask-related EEG data, during the eyes-open condition; the study by Pistorius et al.9 also investigated the analysis of eyes-closed data. In the study by Bosl et al.,6 infants were seated on their mother’s laps in a dimly lit room, and a research assistant attempted to engage the infants’ attention by blowing bubbles.8 Duffy and Als9 stated that EEG data were gathered in the “awake and alert” state but did not provide detail of the procedure. In the study by Pistorius et al.,9 participants were seated in an acoustic chamber and instructed to sit quietly during the eyes-open and eyes-closed EEG recordings.

Two important considerations stand out in this stage: (1) subject diagnosis and (2) sample size. The study by Bosl et al.6 investigated infants at high risk of autism, rather than infants with a confirmed diagnosis of autism spectrum disorder. In contrast, the data acquired by the other two studies used participants with confirmed clinical diagnoses. The biomarker of Bosl et al.6 thus needs to be evaluated in a follow-up investigation that incorporates the formal diagnoses of subjects. The three studies had very different sample sizes. Bosl et al. investigated subsamples ranging from 12 to 28 subjects with a single 20-second segment per subject; Duffy and Als8 investigated age-restricted subsamples ranging from 137 to 546 subjects, averaging multiple two-second segments per subject, whereas Pistorius et al.9 investigated a sample of 12 subjects with a single five-second segment per subject. Although the MME and the RQA biomarkers showed good classification performance, the small sample size clearly suggests the need for larger-scale replication.

Data preprocessing

The principal objective of the signal preprocessing stage is to enhance the signal-to-noise ratio of the signals before feature extraction is performed. Artifact handling is an essential step in EEG data preprocessing. This can be performed by applying artifact avoidance, artifact rejection, or artifact correction strategies (see Croft and Barry,12,13 and Fatourechi et al.14 for further detail on each strategy). Bosl et al. and Duffy and Als appear to have selected artifact-free segments by rejecting artifact-contaminated segments, whereas Pistorius et al.9 implemented a method of linear combination and regression. This entailed the estimation of the proportion of electro-oculographic activity that contaminated each EEG channel through backward propagation and the subsequent subtraction of this artifact contribution from each respective EEG channel.13,14 This method is associated with the potential loss of significant EEG data but allows a fast and automated method for selection of artifact-corrected segments.13,14

Independent component analysis, a blind source separation technique, may be more suitable for artifact correction; however, the implementation thereof requires expert knowledge for the identification of the relevant artifactual components. For reliable execution of this method, a specific minimum amount of data is required. Delorme and Makeig15 recommended that the number of data samples exceed at least a few times the square of the number of EEG channels used.

Feature extraction

All four steps in the signal processing pipeline indicated in Fig 3 are important, but in many respects, feature extraction is the most complicated and therefore most critical step. One of the reasons for this is the fact that we do not know what types of features would best represent the underlying physiological phenomena, i.e., we do not know how the brain activity of autism spectrum disorder and TD individuals may differ. The selection of a suitable EEG
feature extraction method depends on the nature of the data—whether stochastic or deterministic, linear or nonlinear, stationary or nonstationary—and whether one wishes to implement a univariate or multivariate approach.

Duffy and Als\textsuperscript{8} used a linear, univariate qEEG measure (CA) which is based on the assumption that the EEG data remain stationary.\textsuperscript{16} Stationarity necessitates that measured properties of the data do not change over time.\textsuperscript{17} A concession to the requirement of stationarity has been made in that segments of EEG data, 10 seconds or less in duration, captured under constant conditions, are assumed quasistationary for the purposes of an investigation.\textsuperscript{3} This assumption is, however, difficult to confirm experimentally as the state of an individual’s brain is constantly changing between different stages of sleep, eyes-closed resting, eyes-open resting, actively alert, a state of complex mental computing, or concentration, for instance.\textsuperscript{17} Time-frequency analysis, such as the short-time Fourier transform, has been developed to overcome this stationarity limitation\textsuperscript{21} but is associated with a time-frequency resolution trade-off.\textsuperscript{19} Long EEG segments, typically minutes in length, are required to obtain reliable coherence estimates.\textsuperscript{20} A trade-off thus exists between segment length and stationarity—the segment must be sufficiently long to yield good frequency resolution but also sufficiently short to satisfy the assumption of stationarity.\textsuperscript{21}

Coherence values provide an estimate of the amount of variance in one channel that may be explained by a linear transformation of the other channel.\textsuperscript{17} These values do not imply that a linear relationship exists in the underlying EEG dynamics; they only suggest that the underlying dynamics may be accounted for by a linear method.\textsuperscript{17} Blinowska and Żygierewicz\textsuperscript{3} emphasized that many systems with underlying nonlinear dynamics may exhibit an overall linear type of behaviour. Linear techniques may, however, fail to detect nonlinear interdependencies, in which case nonlinear techniques such as mutual information, nonlinear correlation coefficients, nonlinear Granger causality, and state-space measures may prove useful.\textsuperscript{16,21,22} Bosl et al.\textsuperscript{6} showed that 20-second epochs of spontaneous resting-state EEG were nonlinear, using the time irreversibility index. Nonlinear techniques, in comparison with their linear counterparts, generally exhibit the advantage of being less reliant on the condition of stationarity but also have the downfall of being less robust against noise.\textsuperscript{21}
The implementation of multivariate coherence estimation techniques, instead of only pairwise analysis of bivariate signals, has also been recommended to account for the complete covariance structure present in multichannel EEG recordings.\textsuperscript{3,21}

Bosl et al.\textsuperscript{5} and Pistorius et al.\textsuperscript{9} investigated the application of nonlinear time series analysis techniques. The MME feature vector (the proposed biomarker by Bosl et al.) was extracted using a univariate technique, i.e. these features were computed for each EEG channel on an individual basis. It is anticipated that features extracted using a multivariate feature extraction technique, i.e. from all EEG channels combined, will provide information relating to the system as a whole and will enable the extraction of more informative features that would enable better group discrimination. RQA is considered a robust technique for quantifying EEG dynamics because it is capable of univariate or multivariate (i.e. single- or multichannel EEG) time series analysis, can reliably analyze short and nonstationary segments, and can be applied to linear or nonlinear data.\textsuperscript{3,21,23}

The inherent ability of RQA to perform multivariate analysis enables a global approach to be taken that may allow one to detect underlying complex patterns within the data that may go undetected when using univariate techniques, as with MME\textsuperscript{5} and CA feature extraction.\textsuperscript{8,20}

Classification

The identification of a classification model is fairly straightforward. In this context a state-of-the-art supervised learning approach can be used, such as support vector machines or random forests that can reliably deal with sparse data (high-dimensional input vectors). The classification performance achieved will depend on the discriminative nature of the features, the selection of the optimal feature set, the choice of classifier implemented, and the optimal selection of classifier parameters. Another important factor to consider is the curse of dimensionality. As the dimensionality of the feature vector (or feature space) increases, the number of training samples required to adequately represent the respective classes within the data also increases significantly.\textsuperscript{3,24} Lotte et al.\textsuperscript{24} recommended the use of at least five to ten times as many training samples per class as the intrinsic dimensionality of the feature vector. Bosl et al.\textsuperscript{5} generated a total of 192 features per time scale, i.e. 192 features \times 20 time scales = 3840 features per subject; Duffy and Als\textsuperscript{8} generated 4416 features per subject, the dimensionality of the feature space was then reduced using PCA; Pistorius et al.\textsuperscript{9} extracted 10 features per subject and then used ANOVA feature inspection to reduce the feature set to retain only significant features that were able to distinguish autism spectrum disorder and TD classes irrespective of the training dataset implemented. The identification of a subset of features that contains the most discriminatory information for the classification task at hand is an essential step preceding classifier training. The smaller the feature set to be classified, the lower the complexity of the computational burden, which in turn may lead to improved classification performance.\textsuperscript{25}

A simple answer concerning which classifier would be best suited for the classification of resting-state EEG data is unfortunately not possible. Different classifiers may be better suited for the classification of different types of features. Generally, various linear and nonlinear classifiers are investigated in an attempt to identify the most appropriate classifier for a given application.

A further concern to address is the limited sample size in the studies by Bosl et al.\textsuperscript{5} and Pistorius et al.\textsuperscript{9} Bosl et al.\textsuperscript{5} report achieving 100% accuracy in a sample of 12 males, aged nine months, using a ten-fold cross validation approach. Pistorius et al.\textsuperscript{9} report an accuracy of 83% in a sample of 12 subjects, aged 8 to 17 years, using a leave-one-out approach. Duffy and Als\textsuperscript{8} achieved accuracies of higher than 90% in the age-restricted subsamples ranging from 137 to 546 subjects, using a leave-one-out approach. The leave-one-out approach is valuable because it allows the simulation of a clinical scenario where an unseen subject is diagnosed. Typically, with cross-validation analysis, assuming that there are multiple resting-state EEG segments extracted from each subject, all data available per subject are usually allocated to a training and test data set according to a certain ratio. This would mean that a classifier is trained on a segment from a specific subject and then tested on another segment from the same subject, which is not the same as classifying a test segment from an unseen subject (as is the case when implementing a leave-one-out analysis). Implementation of a leave-one-out classification approach is recommended.

Summary of methodological challenges

As outlined previously, the three novel biomarker studies described to date in autism spectrum disorder have mixed strengths and weaknesses across the signal processing pipeline, from data acquisition, preprocessing, and feature extraction to classification. To date, no head-to-head comparison of these methods has been performed, and a definite conclusion about preference of one method over another is therefore not possible, but all three provide an advance from traditional qEEG methods.

Clinical challenges in development of resting-state EEG biomarkers for autism spectrum disorder

Scrutiny of the three potential biomarker methods reported to date suggests that binary categorical classification of individuals at high risk for autism spectrum disorder (screening) and differentiation between autism spectrum disorder and TD individuals (diagnostic classification) as proposed by Jeste et al.\textsuperscript{2} may be possible. However, many signal processing and analytical methods remain to be explored. These methods may also be refined by incorporating multivariate variables that may lead to identification of clinically meaningful subgroups or novel biomarkers to monitor treatment.

Apart from the not insignificant computational challenges to resting-state EEG in autism spectrum disorder as outlined previously, there is also a range of clinical considerations relating to biomarker development that have remained largely unexplored in the context of autism spectrum disorder. To date, all autism spectrum disorder biomarker studies compared data of individuals with nonsyndromal autism spectrum disorder to TD individuals, and no studies have examined biomarkers for autism spectrum disorder in relation to other neurodevelopmental
disorders, for instance, whether autism spectrum disorder can be differentiated from language disorders or from intellectual disability without autism spectrum disorder. No studies have investigated whether resting-state EEG can be used to perform categorical classification of autism spectrum disorder in individuals with syndromal autism spectrum disorder, such as fragile X or tuberous sclerosis complex (TSC) where most individuals have neurodevelopmental aberrations but only some have features of autism spectrum disorder. This is an important consideration given that a clinically useful biomarker should be able to do more than just differentiate between “typical” and “atypical.”

Peters et al. conducted a graph theoretical study of EEG connectivity measures in idiopathic autism spectrum disorder and TSC with and without a concurrent diagnosis of autism spectrum disorder and demonstrated decreased functional connectivity in both instances. A summary data-cloud plot of graph features, increased resilience for autism spectrum disorder versus altered network topology for TSC, revealed partial overlap among autism spectrum disorder, TSC, TSC + autism spectrum disorder, and control groups, but Peters et al. did not proceed to a classification step. That is, although the study was able to identify group-based differences, the study did not attempt to classify individual cases as “autism spectrum disorder,” “TSC,” “TSC + autism spectrum disorder,” or “control.” This classification step would be the key clinically meaningful contribution of a potential biomarker.

There is clear literature that has shown traditional qEEG methods to be sensitive to a range of sociodemographic, technical, and other clinical factors, including age, gender, socioeconomic status (SES), comorbidity, the use of medication, eyes-open versus eyes-closed condition, the number and location of electrodes, and test-retest reliability. Supporting evidence for the effects of these factors on EEG arise predominantly from the study of healthy subjects. No investigations of these factors in autism spectrum disorder subjects have been reported in the literature to date, except for the analysis of eyes-open versus eyes-closed in adults with autism spectrum disorder. In the following sections, we briefly discuss the evidence for these clinical and demographic confounders described to date (Fig 5). Empirical studies are required to determine whether novel resting-state EEG biomarkers will be sensitive to a similar range of factors.

### Age

The standard observation in healthy individuals is age-related decreases in delta and theta power and increases in alpha and beta power. The amplitude and latency of the EEG response may be influenced by changes in synaptic density, myelination, skull thickness, and other physical maturational processes. The alpha rhythm is often used as an indicator for maturation. A TD child develops an 8 Hz alpha rhythm around the age of 3 years. The mean dominant frequency of the alpha rhythm gradually increases to approximately 10 Hz, from the age of 10 years through adulthood. In healthy elderly subjects, alpha activity may decrease up to 1 Hz every 10 years after the age of 50 years but always remains above 8 Hz, even beyond the age of 100 years.

### Gender

Various studies in TD populations report gender differences in structural brain development. Differences are also evident in EEG—the most common finding is earlier EEG maturation in males, which is commonly reflected by increased alpha and reduced delta or theta power in males during childhood. EEG maturation of females occurs at a higher rate during adolescence, bridging the age-related developmental gap between males and females at a later stage. A high male-to-female ratio in autism spectrum disorder prevalence is consistently noted throughout literature, typically in the range of 3:1 to 4:1. Variations in reports of this ratio may be due to differences in the level of functioning of the sample populations and the sampling characteristics but nevertheless remains an important potential covariate or confounder in resting-state EEG studies.

### Socioeconomic status

Children from families with low SES (i.e. having a very low income, low educational level and/or unemployment) revealed a maturational lag in comparison with children from families with a high SES—this is suggested by the increased absolute power and a higher percentage of delta and lower percentage of alpha in the EEG response of children from a low SES.

### Genetic and neurodevelopmental comorbidity

No resting-state EEG studies to date have investigated autism spectrum disorder in relation to the presence of co-occurring developmental, psychiatric, medical, and neurological disorders. It remains to be determined whether resting-state EEG may be useful in, for instance, distinguishing autism spectrum disorder from language disorders or from intellectual disability without autism spectrum disorder. Not only do 40% of individuals with autism spectrum disorder have epilepsy, but autism spectrum disorder is also associated with genetic syndromes such as TSC where high rates of epilepsy are seen. Seizures and epilepsy are therefore another important factor to control for.

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### Figure 5

Factors that may influence rsEEG biomarkers [Abbreviations: EC = eyes-closed; EO = eyes-open; SES = socioeconomic status; \( \theta \) = theta activity; \( \alpha \) = alpha activity; \( \beta \) = beta activity; \( \uparrow \) = increase; \( \downarrow \) = decrease].

| Age | EEG maturation accompanied by \( \downarrow \) in \( \delta \) and \( \theta \), and \( \uparrow \) in \( \alpha \) and \( \beta \) |
| Gender | Earlier EEG maturation in males during childhood |
| SES | Children from low SES show maturational lag: \( \uparrow \) in \( \delta \), \( \downarrow \) in \( \alpha \) |
| Comorbidity | In need of investigation |
| Medication | In need of investigation |
| EO vs. EC | \( \alpha \) suppression with visual stimulation |
| Electodes | \( \uparrow \) electrodes \( \uparrow \) source localization; \( \downarrow \) electrodes \( \downarrow \) costs |
| Reliability | Test-retest reliability > 0.7 = neuropsychological tests |
Medication

No data about the effect of medication on resting-state EEG signals in autism spectrum disorder have been identified in the literature to date. Most studies to date have enforced strict criteria in their study protocols to exclude potential participants who were taking medication at the time of the study; some studies, however, only prohibited the use of medication a few hours in advance of an EEG recording. The general expectation is that anticonvulsant and psychotropic medications which affect cognitive functioning may affect the EEG response. For further detail on the likely effects of central nervous system drugs on EEG, see. Interestingly, Landolt et al.58 found that even caffeine (relative to placebo) altered traditional EEG parameters, including reduced sleepiness and theta activity during a wakeful state, significant reduction in the EEG power in the 0.75–2 Hz band and enhanced power in the 11.25–20 Hz range.

Eyes-open versus eyes-closed recording

Resting-state EEG spectral measures computed during eyes-open (EO) versus eyes-closed (EC) conditions result in different powers and topographies.59 A decrease of global alpha activity with visual stimulation, when reverting to the EO condition, has been found in healthy children and adults.60,61 Mathewson et al.27 investigated regional EEG alpha power and coherence in adults with autism spectrum disorder during EO and EC resting state conditions and discovered that during the EC condition, the autism spectrum disorder and control groups showed no difference in alpha power or coherence, but during the EO condition the autism spectrum disorder group revealed less occipital alpha suppression. There are no studies that investigated EEG differences between EO and EC conditions in children with autism spectrum disorder.

Number and location of electrodes

Standard clinical low-density EEG systems comprise 19 channels, with electrode placement according to the international 10–20 system.61 Dense array EEG systems (comprising 64, 128 or 256 channels) were developed to enhance the spatial resolution, thereby enabling source localisation of, for instance, epileptiform discharges.62–64 Dense array systems are ideal for researchers attempting to solve the ‘inverse-problem’, i.e. to construct an accurate three-dimensional brain network representation of the origin of EEG signals from two-dimensional information obtained through scalp recordings. If source localisation is not the primary goal of an investigation, the standard 19 channel configuration, or perhaps even fewer channels, may be sufficient. A practical consideration when attempting to develop a low-cost EEG screening tool would be to keep the number of electrodes required to a minimum—the fewer the number of electrodes, the cheaper the equipment cost and associated processing power requirements. Further benefits may include reduction in preparation time, easier application with infants and individuals with tactile sensitivity on their heads, less technician time required, higher patient throughput in the clinic, and reduced costs of consumables, replacement electrodes and maintenance.

Test-retest reliability

Variability in EEG recordings can be controlled to some extent by ensuring that the experimental conditions, level of vigilance, use or non-use of medication, amongst other factors, remain the same. A large body of literature on test-retest reliability of resting-state EEG and event-related potentials data is available.28,45,65–78 McEvoy et al.72 demonstrated, in a sample of healthy adults, that certain components of the EEG are sensitive to task difficulty and changes in an individual’s cognitive state. Reliability estimates in this study were based solely on theta and alpha measures captured from midline channels Fz and Pz. A higher reliability (using the Pearson correlation coefficient, r) was obtained during task-related recordings of r > 0.9 during a working memory task and r > 0.8 during a psychomotor vigilance task, as opposed to r > 0.7 during the resting state condition. Task-compliance is considered to impose a more uniform level of alertness and mentation on the individual, imposing a more stabilising effect on the EEG.72 To put a test-retest reliability value of 0.7 for resting-state EEG into perspective—the reliability of neuropsychological measures implemented in practice and considered to be relatively reliable range from 0.7 to 0.9.74,79–81

Conclusions

The brain is a complex dynamic system that provides a snapshot of its functioning through its EEG signature. Ongoing advances in experimental design, equipment specifications, and data processing techniques may enable us to extract important physiological information from this signature that can be used to develop novel biomarkers and computer-based screening tools to identify infants, children and adults at risk of a range of neurodevelopmental disorders, such as autism spectrum disorder. Theoretically, these tools may have a clinical application as ‘screening’ tools for those at risk, as ‘monitoring’ tools for progress during interventions, or to identify ‘subtypes’ of individuals.

We propose that the advances in EEG analysis may be akin to the developments seen in neuroimaging, where we moved from visual inspection of a brain scan, to region-of-interest computational analysis, and now whole-brain computational analysis. In spite of the progress towards development of potential biomarkers for autism spectrum disorder, this review highlighted a number of analytical and clinical methodological factors that require significant further exploration before any of these theoretical approaches can be translated into a clinical setting. Future directions for electrophysiological biomarker research will require that factors such as age, gender, SES, comorbidity, the use of medication, eyes-open versus eyes-closed condition, the number and location of electrodes, and test-retest reliability be investigated in both normative, at-risk and affected populations. The sensitivity and specificity of proposed biomarkers need to be established before considering their implementation in clinical practice for evaluation of risk at an individual level.

It may also be important and helpful to do a head-to-head comparison of the three existing novel methods to
generate empirical evidence for their respective strengths and weaknesses. Such careful signal processing approaches may lead to improved, more robust biomarkers for autism spectrum disorder.

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